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Characterisation and Dispersal of Bioaerosols Emitted from Composting Facilities

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ABSTRACT

The role of sustainable and natural waste management processes such as composting are increasingly becoming more important in tackling the current environmental challenge of the amount of waste that is being produced. However a potential risk of composting facilities is the release and dispersal of bioaerosols that might result in adverse health effects in sensitive receptors. Therefore, environmental regulators request regulatory risk assessments from composting facilities that are within 250m of sensitive receptors to assess the risk posed by bioaerosols.

The prior art in compost related bioaerosol release and dispersal assessment is not extensive and gaps in the understanding of bioaerosols at source, on release from composting facilities and at receptor remain. Therefore, this research was undertaken to address some of these gaps in the current knowledge and to improve the understanding of the characterisation and dispersal of bioaerosols emitted from compost.

Therefore firstly two studies were completed in regards to the characterisation of bioaerosols emitted from compost, in particular in improving the understanding of their aggregation and size distribution. In this context, a novel methodology (the compost tumbler) was developed to release and measure bioaerosols in experimental conditions. Data was generated using a combination of culturing and scanning electron microscopy methods to characterise the aggregation and size distribution of bioaerosols emitted from compost. Secondly, site work was conducted to validate the results of these controlled experiments and characterise the aggregation and size distribution of bioaerosols emitted from composting facilities. These controlled experiments and site work showed evidence of aggregation in bioaerosols released from compost. However, the majority of these bioaerosols were in single cell units hence they are more likely to be dispersed for longer distances.

Following this, other studies were conducted in regards to the dispersal of bioaerosols emitted from compost, in particular in improving the understanding of bioaerosol concentration prediction by air dispersion modelling. Firstly preliminary air dispersion modelling was completed to assess the ability of a commercial air dispersion model,

ADMS 3.3, to predict bioaerosol emissions from composting facilities compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling. Folowing this, the sensitivities of ADMS 3.3 were analysed and the effect of different modelling parameters on predicted bioaerosol concentrations were assessed. Finally, a final assessment of the potential of ADMS 3.3 to predict bioaerosol emissions from composting facilities was conducted. The overall results from the modelling studies indicated that ADMS 3.3 was not able to consistently predict absolute downwind bioaerosol concentrations at composting facilities. However it was also concluded that ADMS 3.3 can be a useful tool for the initial screening and assessing relative changes of bioaerosols at a compost facility, provided that the detailed assessment of absolute bioaerosol emissions are made in conjunction with measurement of downwind bioaerosol concentrations.

The research presented in this thesis makes a significant contribution to knowledge in terms of improving the understanding of the characterisation and dispersal of bioaerosols emitted from composting facilities.

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ACRONYMS

ABP	Animal by-products
ACGIH	American conference of governmental industrial hygienists
AFM	Atomic force microscopy
ANOVA	Analysis of variance
BS	British standard
BSI	British standards institute
CFD	Computational fluid dynamics
CFU	Colony forming unit
COSHH	Control of substances hazardous to health
FLD	Farmer's lung disease
FM	Fluorescence microscopy
HSE	Health and safety executive
LPS	Lipopolysaccharide
MCE	Mixed cellulous ester
MEL	Maximum exposure limits
OEL	Occupational exposure limits
PCR	Polymerase chain reaction
SBER	Specific bioaerosol emission rate
SEM	Scanning electron microscope
SEPA	Scottish environment protection agency
SNIFFER	Scotland and northern Ireland forum for environmental research
TEM	Transmission electron microscopy
TLV	Threshold limit values
VBNC	Viable but nonculturable

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CHAPTER 1. INTRODUCTION

1.1. INTRODUCTION

One of the most important environmental challenges of our time is the large volume of waste going to landfill. The management of waste through landfill practices results in the release of greenhouse gases and polluting liquids through leachate (Tammemagi, 1999; Bell *et al.*, 2000). In addition, landfill sites are becoming increasingly scarce in parts of England and Wales and the volume of waste being diverted into disposal by landfill can be a wasted economic and environmental opportunity (DETR, 2000). In the UK, government legislation and waste strategies have set targets to address this (DETR, 2000), in line with the legal obligations to meet the EU Landfill Directive (1999/31/EC). Therefore the roles of potentially sustainable methods of waste management, such as recycling, refuse-derived fuel or composting, are becoming increasingly important.

Composting is the process of treatment of biodegradable, organic waste (e.g. proteins, fats, carbohydrates, celluloses and mineral matter) (Gilbert and Ward, 1998) by biological degradation. The process takes place under aerobic and controlled conditions to result in carbon dioxide, water and heat (Epstein, 1997). Composting also results in a humus-like product named compost (Epstein, 1997) which is a valuable resource with various applications in soil management (i.e. soil conditioning) (Lester and Birkett, 1999a). Development of thermophilic temperatures takes place due to the production of heat via biological processes (Swan *et al.*, 2002). The role of the micro-organisms present in compost is to accelerate the degradation process, reduce the emission of unwanted gases and destroy the pathogens present in the organic waste prior to composting (Beffa *et al.*, 1996; Hellmann *et al.*, 1997; Hassen *et al.*, 2001; Ngnikam *et al.*, 2001; Vinneras and Jonsson, 2003). The composting process is illustrated in Figure 1.1.

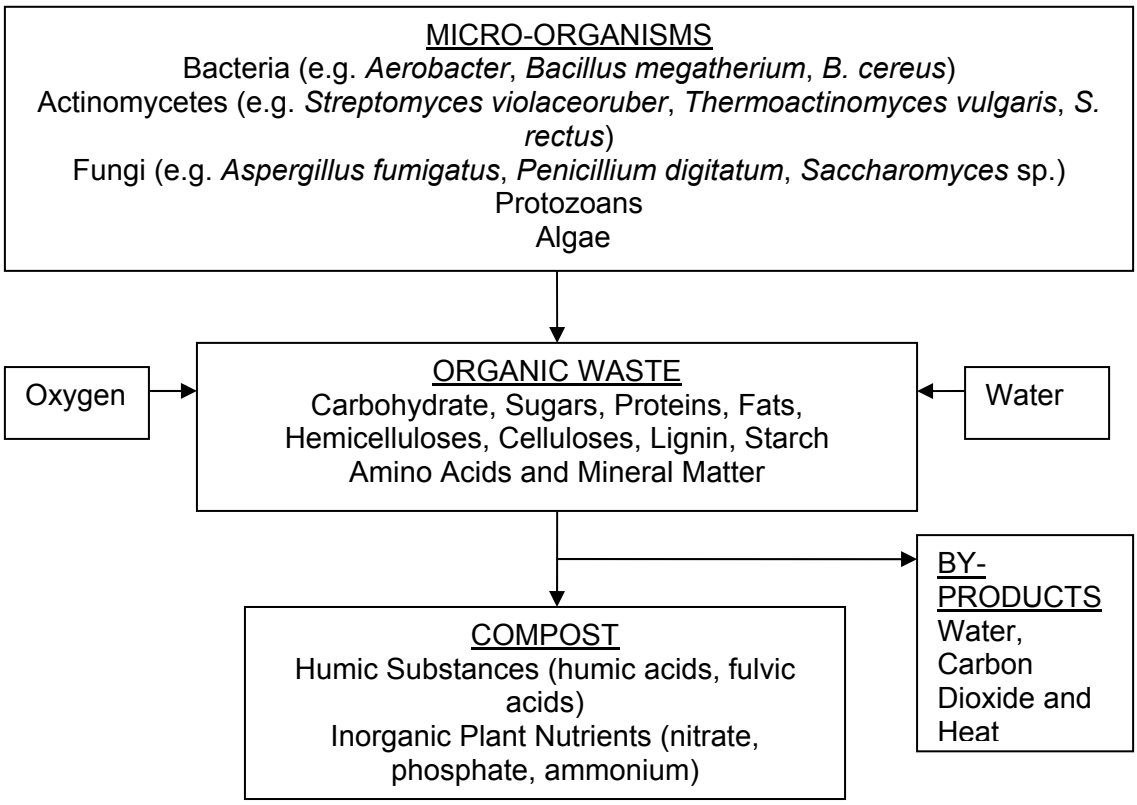


Figure 1.1 - Composting process (adapted from Epstein, 1997; Composting Association, 2001)

The operation of composting facilities should be a low hazard activity however poorly-operated compost facilities can pose risks to workers, public health and the environment (Environment Agency, 2004). As a result of such health concerns and the need for operators to demonstrate the safe operation of their facilities, the environmental regulator in England and Wales requests environmental risk assessments in support of planning consent and environmental permits, in particular, where facilities are within

250m of sensitive receptors (Environment Agency, 2001; 2007). These sensitive receptors can be residences, schools, hospitals and other public facilities. In return regulators use risk assessments to inform environmental permitting and the drafting of conditions within the operator's licences or permits to operate.

The activities (i.e. turning, agitation, screening, shredding) that take place within a composting facility result in the release of the micro-organisms present in the compost. Therefore a focus of these environmental risk assessments has been to quantify the exposure to these airborne micro-organisms or bioaerosols (Deportes *et al.*, 1995; Gilbert and Ward, 1998; Maritato *et al.*, 1992).

To assess to exposure of bioaerosols emitted from composting facilities, these environmental risk assessments need to take into account the source of the bioaerosol hazard (e.g. compost windrows), the pathway through which the hazard may reach the receptor (e.g. release, dispersal and deposition) and the characteristics of the receptor (e.g. differential sensitivity, sensitive receptors, health impacts) (Environment Agency, 2004; Pollard et al., 2006). As such, the understanding of factors that effect their behaviour at source, pathway and receptor is essential in analysing the risk of composting related bioaerosol exposure. However there are gaps in the scientific prior art of the processes which may effect the analysis of bioaerosols emitted from composting facilities. Therefore, this research was completed to address some of these gaps in the current knowledge and to improve the understanding of the characterisation and dispersal of bioaerosols emitted from compost.

The next chapter (Chapter 2) will discuss the research study rationale in detail and will present the overall research aims and objectives. Therefore the following literature review aims to 'set the scene' for this discussion. The literature review also aims to present and discuss the factors which may affect the quality of environmental regulatory risk assessments with respect to bioaerosols and composting. Finally, the gaps in knowledge of the processes which affect the analysis of bioaerosol exposure from composting facilities will be discussed.

1.2. BIOAEROSOLS

Bioaerosols are aerosols of biological origin (Cox and Wathes, 1995) ranging from 0.02 to 100 µm in size (Dowd and Maier, 2000; Ariya and Amyot, 2004). They are living micro-organisms such as bacteria, fungi, yeasts and protozoans or carry fragments of living micro-organisms (ADAS/SWICEB, 2005) and have the potential to pose serious health risks to humans.

Exposures to bioaerosols are common at composting plants, due to releases of fungi, bacteria, actinomycetes, endotoxins (found in cell walls bacteria), LPS (lipopolysaccharide), mycotoxins (secondary metabolites of fungi), and glucans (particularly (1→3)-β-D-glucan, found in cell walls of fungi and some bacteria) (Swan *et al.*, 2003). The adverse health effects related to industrial-scale composting have tended to concentrate on thermophilic actinomycetes and the fungi *Aspergillus fumigatus,* which occur naturally in the compost environment and are released into the air during agitation of the compost such as screening and turning operations (Composting Association, 2004).

Aspergillus fumigatus is a filamentous fungus that is commonly found in decaying organic matter such as composting vegetation, wood chip piles, municipal solid waste (MSW) compost, waste sludge compost and mouldy hay. It is an important micro-organism in the compost process with its capacity to degrade cellulose structures (Swan *et al.*, 2002). It thrives best between temperatures of 30°C and 52°C, with optimum growth at 37°C (Swan *et al.*, 2002), which enables it to grow at human body temperature.

Actinomycetes are a group of filamentous bacteria that resemble fungi, and are the dominant bacteria in the composting process (Lacey and Crook, 1988; Strom, 1985; Swan *et al.*, 2003). They are able to grow in high numbers in compost that is at the start of the self heating process and hence may used as micro-organisms to indicate bioaerosol presence in compost (Dutkiewicz, 1997; Swan *et al.*, 2003). Thermophilic actinomycetes have growth profiles of 30°C - 60°C (Swan *et al.*, 2002), which also enables them to grow at body temperature. The species grow as branching micro-

organisms with short chains of spherical hyphae (1 - 3 µm in diameter), which can become airborne if material that contains large colonies of the bacteria is disturbed (Swan *et al.*, 2002).

It is important to note that exposure to bioaerosols is not limited to composting facilities and there is a wide body of previous work that has examined bioaerosol exposure from various industries and applications (Dutkiewicz, 1997; Jacobs, 1997; Jager and Eckrich, 1997; Lacey, 1997; McNeel and Kreutzer, 1999; Nielsen *et al.*, 1997; Reponen *et al.*, 1998; Sánchez-Monedero and Stentiford, 2003; Seedorf *et al.*, 1998; Shelton *et al.*, 2002; SLR, 2006; Swan *et al.*, 2003). Examples of other industries and applications where bioaerosols and their components have been measured are presented in Table 1.1.

Table 1.1- Industrial and application sources of bioaerosols

Exposure Environment	Reference
Agriculture	Adhikari *et al*. (2004a); Dutkiewicz *et al.* (1989); Hameed and Khodr (2001); Lee *et al.* (2006a); Pande *et al.* (2000)
Farming	Melbostad and Eduard (2001); Sigurdarson *et al.* (2004)
Hop growing	Góra *et al. (*2004); Śpiewak *et al.* (2001)
Hardwood processing	Veilette *et al.* (2006)
Cotton mills	Christiani *et al.* (1999); Gokani *et al.* (1987); Ogden *et al.* (1993)
Textile plants	Su *et al.* (2002)
Sawmills	Dutkiewicz *et al.* (2001a)
Furniture, fiberboard and chipboard factories	Dutkiewicz *et al.* (2001b); Krysińska-Traczyk *et al.* (2002)
Hemp processing plant	Fishwick *et al.*(2001); Zuskin et al. (1990; 1992)
Grape stemming and crushing	Zollinger *et al.* (2005)
Livestock and swine industry	Adhikari *et al. (*2004b); Chang *et al.* (2001); Gibbs *et al.* (2004); Predicala *et al*. (2001); Seedorf *et al.* (1998)
Herb processing plants	Dutkiewicz *et al.* (2001c); Mackiewicz *et al.* (1999); Skórska *et al.* (2005)
Land application of biosolids and animal waste	Pillai and Ricke (2002)
Landfills	Huang *et al.* (2002)
Wastewater treatment	Brandi *et al.* (2000); Brooks *et al.* (2004); Fernando and Fedorak (2005); Lee *et al.* (2006b); Orsini *et a*l. (2002); Pascual *et al*. (2003); Tolvanen (2004)
Recycling	Gladding and Coggins (1997); Würtz and Breum (1997)
Waste Collection	Breum *et al.* (1996b); Heldal *et al.* (1997); Kiviranta *et al.*, (1999); Lavoie and Dunkerley (2002); Neumann et al. (2002; 2005); Nielsen *et al.* (1997); Thorn (2001); van Yperen and Rutten (1997); Williams (2002).

Bioaerosols are not only caused by micro-organism release from industries and applications. They also occur naturally in the environment (Borodulin, 2005; Bovallius *et al.*, 1978; Köck *et al*., 1998; Fang *et al.*, 2005; Mancinelli and Shulls, 1978; Spicer and Gangloff, 2005), are constantly released from sources such as soil dust and decay of vegetation (e.g. leaves) (Swan *et al.,* 2002) and hence are ubiquitous.

Indoor effects of bioaerosols are also evident in literature (Carrer *et al.*, 2001; Górny *et al.*, 2001; Stetzenbach, 1998; Grigorevski-Lima *et al.,* 2006). Studies suggest that 'Sick Building Syndrome' may be caused by the presence of undesirable bioaerosols (Bholah and Subratty, 2002; Helsing *et al.*, 1989; Mølhave, 1989; Rylander and Haglind, 1984; Wan and Li, 1998). Finally, bioaerosols have also been measured in:

- Domestic and office buildings (Baxter *et al.*, 2005; Boillard *et al.*, 2005; Garrett *et al.*, 1998; Hyvärinen *et al.*, 2002; Kalogerakis *et al.*, 2005; Koskinen *et al.*, 1999; Law *et al*., 2001; Lee and Jo, 2005; Lee *et al.*, 2006c; Lis and Pastuszka, 1997; Nevailanen *et al.*, 1991; Rautiala *et al.*, 1998; Reynolds *et al.*, 2001);
- Hospitals (Li and Hou, 2003; Streifel *et al.*, 1989);
- Shopping centres (Nunes *et al.*, 2005);
- Schools (Kalliokoski *et al.*, 2002; Rylander *et al.*, 1998; Ramanchandran *et al.*, 2005); and
- Day-care centres (Koskinen *et al.*, 1995).

1.3. SAMPLING AND ANALYSIS OF BIOAEROSOLS

The previous section has highlighted the occurrence of bioaerosols at composting facilities as well as from other industries and applications. To study these bioaerosols, there is an extensive variety of devices and methods used for their sampling and analysis.

At composting facilities, the choice of sampling device is based on the individual application (Dowd and Maier, 2000) and several factors need to be considered. These factors include the type of bioaerosol that is being investigated, flow rate and volume of

air being sampled, ease of transport of the device, and the environmental conditions of the sampling location.

The viability and culturability of the bioaerosol being analysed is also of importance when choosing a sampling device. A viable micro-organism is living and able to reproduce as it is metabolically active (Haas *et al.*, 1999a). Viable micro-organims are further divided into culturable and non-culturable micro-organisms. Culturable micro-organisms indicate those that are able to reproduce under controlled conditions however non-culturable micro-organisms are not able to reproduce under similar conditions (Jensen *et al.*, 1998) because the sampled micro-organisms might be stressed or injured due to effects such as environmental conditions (McFeters *et al.*, 1982). Common methods of collecting viable bioaerosols include impaction onto agar (e.g. slit agar impactors and multiple-hole impactors), centrifugation (e.g. wetted or dry cyclone samplers) and gravitational collection (e.g. direct deposition onto a culture medium) (Henningson and Ahlberg, 1994; Willeke and Macher, 1999; Dowd and Maier, 2000; Martinez *et al.*, 2004; Stetzenbach *et al.*, 2004).

Non-culturable micro-organisms are divided into dormant, viable but nonculturable (VBNC) and nonviable micro-organisms (Jjemba, 2004). A non viable micro-organism is one that is not capable of reproduction (i.e. metabolically inactive) and no longer living (Haas *et al.*, 1999a). The filtration method that is commonly used to collect viable micro-organisms can also be used for collecting non-viable micro-organisms. Filtration is based on drawing a certain volume of air through filters of specific sizes for collecting of biaoerosols and other particles such as dust. The collected micro-organisms can subsequently be analysed by being washed off the filter surface and cultured, using microscopy (Palmgren *et al.,* 1986) or other methods such as gene probes (Jensen *et al.*, 1998). Since the filter would not differentiate between culturable, non-culturable or non-viable micro-organisms, they are valuable for analysing total bioaerosol and particle counts in a sample. The efficiency of the filter is based on the face velocity of the filter and filters have been shown to have collection efficiencies of greater than 99% for particles of over 1 μm (Jensen *et al.*, 1998). However, loss of viability due to moisture

loss on the filter surface and poor recovery of micro-organisms collected by the filter can be the disadvantages of this filter method (Nielsen *et al.*, 1997).

Use of devices based on liquid impingement (e.g. all glass or three-stage impingers) and pollen, spore and particle impaction through devices such as the Burkard or Hirst spore trap (Levetin and Horner, 2002) onto glass slides or tape strips are also becoming more common (Sterling *et al.*, 1999). Both of these methods are designed for collection of both viable and non-viable micro-organisms.

Following the collection of the micro-organism, more traditional methods of bioaerosol identification and analysis are based on the morphological structure evaluation of the micro-organism colony after culturing of the sample. This method is reliant on the viability and the culturability of the micro-organisms. However following the collection of micro-organisms with methods such as filtration or pollen, spore and particle impaction, another common analysis method is microscopy. This method, in contrast to culturing methods, allows the determination of total number of micro-organisms in a sample (i.e. both culturable and non-culturable).

An example of such microscopy methods is the Scanning Electron Microscopy, used since the 1970s to study micro-organisms (Kormendy and Wayman, 1972). More recently, Scanning Electron Microscopes have been utilised to study moulds and actinomycetes in agricultural dusts (Karlsson and Malmberg, 1989), bioaerosols (Witmaack *et al.*, 2005), primary biological aerosol particle (Matthias-Maser and Jaenicke, 1995), environmental particles (i.e. biofilms) (Mavrocordatos *et al.*, 2004), aerosol particle size (Gwaze *et al.*, 2007); microbial aggregation (Borrego *et al.*, 2000) and fungal spores (Heikkilä *et al.*, 1988a; 1998b).

Other bioaerosol analysis methods used for analysing both non-viable and viable micro-organisms include techniques such as immunoassays (e.g. radioimmunoassay and fluorescence immunoassay), biochemical assays (e.g. endotoxin assay), chemical detection of bioaerosols (Spurny, 1994) and the utilisation of gene technology, such as the Polymerase Chain Reaction (PCR) (Mukoda *et al.*, 1994; West *et al.*, 2008).

New technologies of bioaerosol collection and analysis are in constant progress. One of these newer technologies is the capture of viable airborne micro-organisms through the use of a bubbling device using filters immersed into a liquid medium (Agranovski *et al.*, 1998; Agranovski *et al.*, 1999) and work described by Mainelis *et al*. (2002a) regarding a device that uses the electrical charge of airborne micro-organisms to capture them in an electrical field and then onto a growth media. This method is based on early technology developed in the sixties (Liu *et al.*, 1967). Following on from this, an electrostatic precipitator device that assesses the electrical charge of bioaerosols has been introduced (Lee *et al.*, 2004). The study claims that the method can differentiate between negatively and positively charged micro-organisms based on the fact that most micro-organisms carry electrical charges in their structure (Benninghof and Benninghof, 1982; Mainelis *et al.*, 2001), which would add a signature to the sampled micro-organisms to aid their identification. Sigaev *et al.* (2006) reports the development of a cyclone-based aerosol sampler that uses a re-circulating liquid film that is different in design from traditional cyclone samplers invented by Olenin *et al.* (1977). However, the wide use of such new technologies are not evident in literature.

1.4. HEALTH IMPLICATIONS OF BIOAEROSOL EXPOSURE

The previous sections have highlighted the occurrence of bioaerosols and some of the methods used for their sampling and analysis. At composting facilities, it is important to analyse the exposure to bioaerosols because they may pose serious health risks to composting facility operatives or sensitive receptors downwind of a composting facility.

1.4.1. Health Effects Associated with Fungi, Mycotoxins and *Aspergillus fumigatus*

Fungi are common allergen sources, are toxigenic, and cause infections and inflammatory reactions (Fischer and Dott, 2003). The fungal spores in the air, as well as their cell wall components, such as mycotoxins and (1→3)-β-D-glucans, have been proven to cause occupational asthma (Rylander and Lin, 2000). Eduard *et al.* (2001) have studied short term exposure of farmers to bioaerosols and have observed a high occurrence of eye symptoms (e.g. allergies) and coughing. These problems were linked to the occurrence of fungal spores. Some of the other environments in which fungus related asthma has been reported are mushroom cultivation, cheese manufacturing,

flour mills and food processing (Burge, 1995). Farmer's Lung Disease (FLD), which is a common form of allergic alveolitis, is linked to repeated exposure to moldy hay or straw (Reboux *et al.*, 2001).

Specifically for *A. fumigatus*, their conidia at 2-3 µm in size are capable of entering the lung and reaching the secondary bronchi (Raper and Fennell, 1965; Samson and Van Reenen-Hoekstra, 1988; Denning, 1998). Despite this capability, their inhalation rarely has any effect on healthy individuals because the conidia are eliminated by immune mechanisms (Latgé, 1999; Ziegler, 1993). Therefore the risk of potential infections arising from *A. fumigatus* exposure to healthy individuals is very low (Millner *et al.*, 1994). However *A. fumigatus* has been reported to cause severe effects on immuno-compromised patients (Andriole, 1993; Hibbard and Ferro, 1996; Bodey and Vartivarian, 1989; Denning, 1998; Dixon *et al.*, 1996), such as AIDS patients, those who have received organ transplants and also individuals who are atopic or asthmatic (Millner *et al.*, 1994).

In these patients, *A. fumigatus* can lead to a variety of Aspergillosis disease including Invasive Aspergillosis (i.e. an infection generally occurring in the lungs) (Denning, 1998; Latgé, 1999). Similarly, Pulmonary Aspergillosis has been detected in renal transplant patients who have been subjected to dust caused by hospital renovation (Arnow *et al.*, 1978). Gliotoxin, another metabolite of the *A. fumigatus* is also potentially linked to Aspergillosis and other *A. fumigatus* related diseases (Müllbacher and Eichner, 1984). Aspergillus infections have also been detected in cancer patients linked with fireproofing materials in a new hospital (Aisner *et al.*, 1976).

Despite the low occurrence of *A. fumigatus* related disease in healthy individuals, Millner (1994) found an increased likelihood of nonatopic individuals becoming sensitised to bioaerosols after constant exposure. In instances where bioaerosol exposure is very high for workers, such as those working with compost, the risk of adverse effects is increased (Millner *et al.*, 1994). In addition, when airborne fungal spores are inhaled into the bronchia and alveoli, and are then lysed by the immune system, the human body may be exposed to the primary and secondary metabolites,

such as the cell-wall component mycotoxins, which are known to be pathogens (Fischer and Dott, 2003).

1.4.2. Health Effects Associated with Actinomycetes, Endotoxins and LPS

As with *Aspergillus fumigatus*, actinomycetes are potentially capable of penetrating into the human lung because of their small size (Swan *et al.*, 2002). Prolonged inhalation exposure to actinomycetes has been linked to adverse health effects (Douwes *et al.*, 2003), including allergic alveolitis and other respiratory responses (Lacey and Crook, 1988; Lacey and Dutkiewicz, 1994; Lacey, 1997; Swan *et al.*, 2003).

Another group of bioaerosols that have been widely studied (Rylander, 2002) are endotoxins and LPS (lipopolysaccharide). Endotoxins are found in the cell walls of gram-negative bacteria and are linked to fever and the activation of the immune system in small doses, and to shock and even death in larger doses (Schlessinger and Schaechter, 1993). They have been linked to acute bronchoconstriction in healthy humans exposed to cotton dust (Castellan *et al.*, 1984). Douwes *et al.* (2000b) have shown that endotoxin exposure in sawmill workers was at sufficient levels to cause respiratory symptoms. LPS are the derivatives of endotoxins (Helander *et al.*, 1980; Michel *et al.*, 1997; Rylander and Haglind, 1984; Rylander, 1987) and their inhalation is known to cause inflammatory response in the lungs (Nightingale *et al*., 1998; Thorn and Rylander, 1998). Rylander *et al.* (1999) has found increased likelihood of airways inflammation in the workers of the paper industry caused by exposure to endotoxins and (1→3)-β-D-glucans. The latter is found in the cell walls of fungi and some bacteria (Swan *et al.*, 2003).

1.4.3. Health Impacts Associated with Biaoerosols in the Waste Industry

There has been a number of studies which have examined the risk of biaoerosol induced disease in the waste industry. Several studies (Gladding and Coggins, 1997; Gladding, 2002 and Gladding *et al.*, 2003) have found dry cough, nausea, nasal irritation as well as respiratory and gastrointestinal symptoms in the workers of materials recovery facilities. Malmros (1997) have noted diarrhoea, vomiting, nausea, fatigue and headache in garbage recycling workers.

There is a larger number of studies that have looked at the health impacts of bioaerosols on waste management workers and refuse handlers. Allmers *et al*. (2000) have studied the effect of fungi and especially *Aspergillus fumigatus* on garbage workers exposed to mouldy waste and found evidence of Allergic Bronchopulmonary Aspergillosis and hypersensitivity pneumonitis (extrinsic allergic alveolitis). Similar airway inflammatory response was found in waste handlers exposed to total bacteria, fungal spores, endotoxin and (1→3)-β-D-glucan by Heldal *et al.* (2003a, 2003b) and by Yang *et al.* (2001).

In addition to respiratory problems, other studies (Ivens *et al.*, 1997a; 1999; Kuijer and Frings-Dresen, 2004) have also noted gastrointestinal problems in waste collectors exposed to bacteria, fungal spores, endotoxin and (1→3)-β-D-glucans. Studies by Herr *et al.* (2004a; 2004b) have found skin-related complaints and diseases in residents of properties where there is indoor storage of organic domestic waste. The micro-organisms found in these properties were thermophilic actinomycetes, total bacteria and moulds. Examples of the health impacts related to bioaerosol exposure specifically for composting are presented in Table 1.2.

Table 1.2- Health impacts associated with bioaerosols emitted from compost

Type of Bioaerosol	Health impact	Affected Group	Reference
Fungi and bacteria	Significantly higher symptoms and diseases of the airways and skin	Biowaste collectors and Compost Workers	Bünger *et al.* (2000)
Actinomycetes and *A. fumigatus*	Respiratory difficulty, fatigue, cough, fever, chills and joint pain	Individual working with compost in his garden	Brown *et al.* (1995)
A.fumigatus and *Penicillium*	Fever, myalgia and dyspnea	Individual shovelling composted wood chips and leaves	Weber *et al.* (1993)
Endotoxin, glucans, fungi and total bacteria	Upper airway inflammation	Compost workers	Douwes *et al.* (1997, 2000a)
Dust, actinomycetes, bacteria and fungi	Gastrointestinal symptoms, itchy arms and eyes, dry throat and coughs	Compost workers	Wheeler *et al.* (2001)

A limited number of studies have examined the impact of biaoerosols emitted from compost on the residents living nearby a composting site. Herr *et al.* (2003) have found cases of health complaints including 'waking up due to coughing', 'bronchitis' and 'excessive tiredness' in the residents who were 150-200 m away from a composting site. These problems were attributed to the presence of thermophilic actinomycetes, total bacteria and moulds. However other studies were less conclusive. Browne *et al.* (2001) have studied bioaerosol health impacts in 63 residents living near a composting site and 82 controls in a control neighbourhood. No evidence of an association between *A. fumigatus* and increases in respiratory symptoms was noted however higher levels of *A. fumigatus* were observed in the neighbourhood close to the composting facility compared to those at the control neighbourhood. Cobb *et al.* (1995) have evaluated the health impacts of particulates (not bioaerosols) on the residents living 3000 feet away from a mushroom composting site. There was no evidence of a significant health hazard or any differences between exposed and control groups.

1.4.4. Dose-Response Relationship of Bioaerosol Induced Disease

As discussed in the previous sections, bioerosols are known to be associated with health effects. However the mechanisms of bioaerosol induced disease are not clear due to a lack of actual dose-response information in the literature regarding these health risks (Douwes *et al.*, 2003; Fischer and Dott, 2003; Folmsbee and Strevett, 1999; Gladding, 2002; Millner *et al.*, 1994; Swan *et al.*, 2003; Wheeler *et al*., 2001). Although it is argued that there is a cause and effect relationship in aeroallergen exposure and allergic disease, occupational exposure limits (OEL) and threshold limit values (TLV) for bioaerosols have not been introduced as legal regulations anywhere in the world (Burge, 1995; Gladding, 2002; Poulsen *et al.*, 1995a; 1995b; van Yperen and Rutten, 1997).

There have been numerous attempts to establish threshold levels and exposure limits for bioaerosols beyond which acute and chronic health effects have been observed, however these are only suggestions and are not widely accepted. Previous studies (Swan *et al*., 2003; Poulsen *et al.* (1995a; 1995b)) have reviewed and correlated observed health effects of endotoxins, organic dust, gram negative bacteria, fungi and

total viable bacteria with exposure levels found in various epidemiological studies. Similarly, Clark *et al.* (1983b) attempted to correlate a dose-response relationship between exposure to total bacteria, gram-negative bacteria, total fungi, *Aspergillus fumigatus* and endotoxins, and concurrent health effects.

More commonly threshold levels and exposure levels have been suggested for endotoxin and lipopolysaccharides (LPS) exposure. Such a study by Castellan *et al.* (1987) aimed to define a dose-response relationship between inhalation of cotton dust and lung disorders. The study was in line with research by Rylander (1987) examining dose-response relationships of endotoxin (in cotton dust) with fever, chest tightness, and reduction in air flow (bronchoconstriction) in cotton workers. Later studies on respiratory disorders and atopy in cotton, wool, and other textile mill workers in Denmark (Sigsgaard *et al.*, 1992) also investigated a dose-response correlation between endotoxin exposure and adverse health effects.

Work by Ivens *et al.* (1999) suggested an exposure-response relationship between nausea and endotoxin exposure, as well as between diahorrea and endotoxin, and viable fungi exposure. This study examined gastrointestinal problems of waste collectors. Mandryk *et al.* (2000) who studied sawmill workers, has also suggested a dose-response relationship in worker exposures to endotoxin, gram negative bacteria, fungi, (1→3)-β-D-glucan and respiratory symptoms including lung function. Work carried out on farms by Eduard *et al.* (2001) has attempted to correlate a dose dependent relationship to symptoms of the nose and eyes, as well as coughing. Another study (Lange *et al.*, 2003) analysed endotoxin exposure in farmers and suggested a dose-response relationship between endotoxin exposure and lung cancer risk. Some examples of endotoxin, LPS and glucan exposure benchmark values are presented in Table 1.3.

Table 1.3 - Suggested exposure benchmark levels for endotoxins, LPS and glucans. EU/m^3 denotes endotoxin units per cubic metre and ng/m^3 denotes nanograms per cubic metre.

Industry	Endotoxin, Glucan and LPS Exposure Benchmark Values	Health Effects	Reference
Cotton textile industry	Endotoxin 100 EU/m^3	Cough, wheezing, and obstructive ventilation patterns	Latza *et al.* (2004)
Paper mill	LPS 200 EU/m^3	Loss of lung function	Sigsgaard *et al*. (2004)
Wastewater	Endotoxin 50 EU/m^3	Lower respiratory, skin and flu-like and systemic symptoms	Smit *et al.* (2005)
Waste	Glucan 5-10 µg/m^3 Endotoxin 300-1000 EU/m^3	Various	Wouters *et al*. (2006)
Poultry work	Endotoxin 614 EU/m^3	Pulmonary lung function decrease	Donham *et al*. (2000)
General exposure	Endotoxin 2000 EU/m^3 Endotoxin 1000 EU/m^3 Endotoxin 100 EU/m^3	Toxic pneumonitis Systemic effects Airway inflammation	The International Committee on Occupational Health, Rylander (2002)
General exposure	50 EU/m^3 over an 8 hour exposure period with an in air endotoxin concentration of 4.5 ng/m^3	Various	Dutch Expert Committee on Occupational Standards (DECOS), Heedrick and Douwes (1997)
General exposure	Endotoxin 9 to 170 ng/m^3	No effect level	Heedrick and Douwes (1997)
Pig farming	Endotoxin 75 ng/m^3	Airway hyperresponsiveness and low lung function	Portengen *et al.* (2005)

A threshold dose for the exposure to a substance below which no effect or response will be measurable are termed as threshold limit values (TLV) developed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1991-1992) or maximum exposure limits (MEL) (Timbrell, 2002). Macher *et al.* (1999) suggest that a threshold limit value should have five primary components. These are:

- A scientific base for the standard;
- An established sampling method;

- An analytical method;
- A sampling strategy; and
- A limit value.

Despite attempts to establish threshold levels and exposure limits, sufficient information is not yet available on all these components (Macher *et al.*, 1999) for bioaerosols. In addition, the scientific base for bioaerosol health effects is limited. Apart from the guidelines published by the Composting Association (1999) and Association for Organics Recycling (formerly the Composting Association) (2009), there are no established standard sampling and analytical methods nor a standard sampling strategy from the Environment Agency or other government bodies in the UK. Such standard methods are currently emerging through the recently published British Standards (BS ISO 16000-16:2008 and BS ISO 16000-17:2008) (BSI, 2008a; 2008b) however these standards are for the detection and enumeration of moulds for indoor air and not for composting facilities. In Europe, similar standard methods for measurement of airborne micro-organisms and viruses in ambient air are available (VDI, 2004a; 2004b; 2007).

One reason for the lack of standard sampling and analytical methods has been discussed to be the fact that bioaerosols and their properties exhibit wide diversity and there are large differences in methods of microbiological air sampling and enumeration (Dutkiewicz, 1997). Furthermore tolerance to bioaerosols varies greatly between individuals, and for some such as immuno-compromised patients, even levels occurring in nature (i.e. background levels) might prove harmful. The human body has natural defences against bioaerosols built up by exposure to these naturally occurring ubiquitous bioaerosols, such as from decomposing leaves and viable airborne micro-organisms present in urban air (Mancinelli and Shulls, 1978). In addition to this, bioaerosols have also been commonly detected in indoor environments. Endotoxins and (1→3)-β-D-glucans have been detected in house dust (Douwes *et al.*, 1998), gram-positive and gram-negative mesophilic bacteria, fungi (Górny *et al.*, 1999) and actinomycetes (Grigorevski-Lima *et al.*, 2006) have been detected in the indoor air of human homes. Bacteria have been detected from air humidifiers (Strindehag and Josefsson, 1999) and in the indoor air of village houses (Hu and Liu, 1989).

Small amounts of bioaerosols are unlikely to have an ill effect on the body of a healthy individual. Problems arise when high concentrations of bioaerosols occur that overwhelm the body's natural defence mechanism (Wheeler *et al.*, 2001). The nature of human response can also change depending on past exposure to the agent and any sensitisation that may have occurred even in healthy individuals. If past exposure to bioaerosols has been to a high concentration, it is possible that the individual will be affected by much lower concentrations at the next exposure (Herr *et al.*, 2003). This is due to the fact that the individual has become sensitised to the bioaerosol. Individual susceptibility factors such as atopy, allergic sensitisation or immunodeficiency can be important factors in risk assessments carried out to assess health effects of bioaerosols (Herr *et al.*, 2003). Some studies suggest that the species of the bacteria being inhaled is as important as the dose of inhalation, when studying the severity of the adverse health effects (Helander *et al.*, 1980; Rylander and Lundholm, 1978). These factors introduce a great deal of uncertainty when studying dose-response relationships to bioaerosols. Therefore the lack of a dose-response relationship for composting related bioaerosol induced disease remains.

1.5. BIOAEROSOL AEROMICROBIOLOGICAL PATHWAY

The previous sections have discussed some of the methods used for the sampling and analysis of bioaerosols to account the source of a bioaerosol hazard and the potential adverse health effects caused by bioaerosols on any sensitive receptors. To analyse the pathway of a bioaerosol released from composting facilities, the understanding of the aerodynamics of bioaerosol dispersal is essential.

Currently the regulator in England and Wales requires a risk assessment for any facility that has a sensitive receptor (e.g. a home or office building) within 250 m of the site boundary. There are no studies to suggest a change to this limit at present, however a safe distance between compost facilities and sensitive receptors should be governed by the microbiological and aerodynamic properties of bioaerosols once released.

The aeromicrobiological pathway of a bioaerosol describes its initial release, dispersal and deposition (Dowd and Maier, 2000) and the aerodynamic behaviour of bioaerosol

particles are subject to the physical laws that also affect other aerosols. These include the effects of Brownian motion, gravitational fields, electrical forces, thermal gradients, electromagnetic radiation, turbulent diffusion and relative humidity. However in contrast to non-biological pollutants, bioaerosols also behave according to their unique biological properties (Cox, 1995). For example, the fungal spore release mechanisms are influenced by the species and their growth conditions (Sivasubramani *et al.*, 2004).

Bioaerosols are released instantenously or continuously from point sources (e.g. stack) as well as from linear and area sources (e.g. biosolid application in a field) (Dowd and Maier, 2000). Releases may be passive (e.g. by natural air movement) or by active mechanisms (e.g. by mechanical action of humans and animals) (Levetin, 1995). In indoor environments, their release is also dependent on air currents and is often enhanced by the activities of people or animals living on the premises (Madelin, 1994; McCartney, 1994; Reponen *et al.*, 1992). The growth and aerosolization rate for indoor bioaerosols may also depend on the material they originate from (Sivasubramani *et al.*, 2004). Górny (2004) reviewed the factors affecting the release of fungal fragments and spores from indoor contaminated surfaces. The factors examined included air velocity, moisture conditions and vibration. Moisture was shown to reduce fungal fragment release whilst vibration and high air velocity were shown to increase fragment release.

In outside environments, in addition to biological processes (i.e. fungal life cycle), weather factors such as wind, humidity, temperature and rain can also affect the release mechanisms of bioaerosols (Lloyd, 1969; McCartney, 1994; Muilenberg, 1995; Herrero and Zaldivar, 1997; Sivasubramani *et al.*, 2004). Regarding the effect of wind on spore release, the aerodynamic forces of the wind must overcome the forces that ensure the attachment of the spore to the surface (Aylor and Parlange, 1975). Studies that have examined this have concluded that wind speeds exceeding 0.5 m/s were needed to release *Erysiphe graminis* conidia (Hammett and Manners, 1974) and 5 m/s are needed to release *Helminthosporium maydis* conidia (Aylor, 1975).

Upon release, bioaerosols are dispersed in air horizontally or vertically (Dowd and Maier, 2000; Levetin, 1995). Dowd and Maier (2000) have shown that generally most

bioaerosols have limited ability to survive when suspended in the atmosphere and hence will most commonly undergo short periods and distances of travel (under 1 hour and under 1 km). However, they have also noted exception cases of spores travelling distances up to and over 100 km for days and longer. In contrast to this view, other studies (Levetin and Horner, 2002) have shown that airborne fungal spores are adapted to staying airborne and added that the period of time that a bioaerosol will remain airborne is dependent on several factors such as particle density, particle diameter and electrical charges. In line with this particle surface (i.e. hydrophobic or hydrophilic) has also been discussed to determine the type of air dispersal that will take place (i.e. by air or rain) (Levetin, 1995), as well as the time it will remain airborne once released (Muilenberg, 1995).

Levetin and Horner (2002) have also concluded that the period of time that a bioaerosol will remain airborne is dependent on ambient air conditions. These air conditions might include the effects of winds and updrafts that influence the dispersal distance of bioaerosols and have a varying effect according to particle size and settling velocities (Muilenberg, 1995). Frictional turbulence and thermal gradients are also known to affect the dispersal of spores (Fitt *et al.*, 1987).

Other studies (McCartney, 1994) have shown that individual spores upon release from a source will travel in different distances and paths due to the effects of wind eddies responsible for wind speed effects. However common to all spores and particles that are released, a plume of particles upon release from a source will be subject to a reduction in their concentration as the plume moves further away from the source (Gregory, 1973; McCartney, 1994).

Bioaerosols are subject to the same physical laws for other aerosols particles and these laws govern the processes through which bioaerosols are removed from the atmosphere (Cox, 1995; Muilenberg, 1995). Therefore following release and dispersal, the main mechanisms of particle deposition onto surfaces is sedimentation, Brownian diffusion and impaction/interception (Colls, 2002). Each of these processes are more effective depending on the size of the particle. For example, particles with small

diameters such as 0.1 μm would be expected to behave like gases and are transported through the effects of Brownian diffusion (Seinfeld and Pandis, 2006; Petroff *et al.*, 2008). As such as the particle diameter decreases, the Brownian diffusion coefficient approaches that of a gas (Sehmel, 1980). However for particles greater than 1 μm diameter, diffusion due to Brownian motion is less than gravitational settling (Cox, 1995). Therefore particles with diameters of 0.1 μm are transported less effectively than particles with diameters of <0.1 μm or larger than 1 μm (Cox, 1995; Ruijgrok et al., 1995).

In addition bioaerosols are deposited through gravitational settling, downward molecular diffusion, inertial impaction, surface impaction or dry, wet and electrostatic deposition (McCartney 1997a; Dowd and Maier, 2000; Pillai and Ricke, 2002). It is important to note that the deposition of particles is also affected by their shape (Colls, 2002). In this context, spores with non-spherical shapes would deposit more slowly compared to spores with spherical shapes even when both spores are of the same volume and density (McCartney, 1994).

1.6. EFFECT OF EPISODIC BEHAVIOUR ON BIOAEROSOL DISPERSAL

The understanding of the aerodynamic behaviour of bioaerosol in a composting site may be further complicated by their episodic behaviour. Such seasonal and daily peaks of bioaerosol spore levels are reported in many studies (Asan *et al.*, 2004; Corden and Millington, 2001; Fang et al., 2005; Hyvärinen *et al.*, 2001; Ivens *et al.*, 1997b; Mitakakis and Guest, 2001; Nielsen *et al.*, 1997; Nielsen *et al.*, 2000; Rahkonen *et al.*, 1990; Ramanchanran *et al.*, 2005; Rylander *et al.*, 1998; Thorn *et al.*, 1998; Thorn, 2001). It is important to note that naturally occurring bioaerosols also show episodic patterns. On a daily basis, highest levels of bioaerosols are observed around noon and on an annual basis, during winter and early spring (Lighthart and Shaffer, 1994). Levetin and Horner (2002) note that peaks of asexual fungi spores, such as those of *Aspergillus* species, are observed in the afternoon, but are low in early morning. Andreeva *et al.* (2001) examined the levels of live micro-organisms sampled in atmospheric aerosols in western Siberia. They found seasonal as well as altitudal differences in the concentrations of bioaerosols.

Outdoor bioaerosol occurrence patterns are influenced by the local climate and weather, resulting in seasonal cycles of emissions (Muilenberg, 1995). Therefore reported seasonal peak bioaerosol concentrations differ due to differences in topography, climate and weather of the study regions. Some distinct regional differences were observed even within studies (Köck et al., 1998; Shelton *et al.*, 2002) due to local climate and weather. Examples of the episodic patterns of bioaerosols in various industries are presented in Table 1.4.

Table 1.4- Episodic patterns of bioaerosol levels in various industries

Industry	Type of Bioaerosol	Seasonal Variation	Daily Variation	Reference
Outdoor metropolitan area	Total inhalable fungi	Higher levels in fall and summer	None observed	Adhikari *et al.* (2006)
Swine and dairy farms	Airborne micro-organisms including *Aspergillus* and actinomycetes species	Higher levels in summer	None observed	Lee *et al.* (2006a)
Wastewater treatment plants	Endotoxin	None observed	None observed	Lee *et al.* (2006b)
Building sites	Fungi	None observed	Variation between morning and afternoon	Spicer and Gangloff (2005)
Rural indoor dairy cattle shed	Total and culturable fungi	None observed	Higher levels in winter, late summer and the rainy season	Adhikari *et al.* (2004b)
Landfill	Culturable bacteria and fungi	Highest levels in winter	None observed	Huang *et al.* (2002)
Waste collectors	Total bacteria, endotoxins and molds	Highest levels in summer	None observed	Lavoie and Dunkerley (2002)
Refuse collectors	Endotoxin, total fungi and total bacteria	Highest in the summer months and midsummer	None observed	Neumann *et al.* (2002)
Indoor and outdoor levels	Fungi	Highest levels in the fall and summer months	None observed	Shelton *et al.* (2002)

In the composting industry, episodic release of bioaerosols are observed due to on-site activities, as well as seasonal or daily differences (Recer *et al.*, 2001; Sánchez-Monedero *et al.*, 2005; Taha *et al.*, 2006; Taha *et al.*, 2007a). Bioaerosol particles are released from the compost by gusty winds, rain drops and mechanical disturbance,

such as shredding or turning operations. The factors that influence bioaerosol dispersal are the composting material, composting period, composting facility size, operational characteristics and on-site activities (i.e. agitation frequency), moisture levels of the compost, local geography and meteorological conditions (Haas *et al.*, 1999b; Recer *et al.*, 2001).

Early studies of sewage sludge compost windrows revealed that *Aspergillus fumigatus* aerosols downwind of mechanically agitated windrows were significantly higher than those downwind of stationary windrows (Millner *et al.*, 1977; Millner *et al.*, 1980). The agitation was caused by the front-end loader moving and depositing the compost. Clark *et al*. (1983a) have shown that levels of *A. fumigatus* were higher in compost screening areas and where material was being handled, as opposed to control rooms and offices. Rautiala *et al.* (2003) have shown that farmers were exposed to higher levels of bioaerosols in composting in swine confinement buildings when the compost bed was being turned. Similar results are found in other studies (Jager *et al.*, 1994; Sánchez-Monedero *et al.*, 2005; Taha *et al.*, 2006) where agitation activities such as shredding, turning and screening resulted in higher bioaerosol levels. In line with this, endotoxin levels were higher in the air when the compost material was subjected to mechanical agitation compared to levels measured in the vicinity of a composting plant (Dannaberg *et al.*, 1997).

Epstein *et al.* (2001) examined *A. fumigatus* levels at a biosolids composting facility. The summer results indicate that the levels of this bioaerosol were greatest during the mechanical agitation activity of feedstock mixing. Hryhorczuk *et al.* (2001) studied bioaerosol emissions from a suburban yard waste composting plant, located in a woody area with a nearby river. They observed that both off-site and on-site concentrations of bioaerosols increased when activities such as shredding and turning were taking place. Other site activities, such as meadow grass mowing, also resulted in higher levels of bioaerosols. However, it is argued that the location of the composting facility may have also contributed to the bioaerosols in the ambient air.

Recer *et al.* (2001) investigated ambient levels of *A. fumigatus* and thermophilic actinomycetes in a residential neighbourhood near a yard-waste composting plant. They found a significant decrease in bioaerosol levels during the winter months of January to March. This variation was especially noticeable in levels of *A. fumigatus*. According to Recer *et al.* (2001) seasonal variations for *A. fumigatus* and other *Aspergillus* species have been observed in previous studies and peak emissions vary according to geographical location and the climate of the sampling area.

1.7. POTENTIAL OF AIR DISPERSION MODELS IN PREDICTING BIOAEROSOL DISPERSAL

The previous sections have discussed the aerobiological pathway of bioaerosols and the effect of episodic behaviour in analysing the pathway of bioaerosols released from composting facilities. Extensive knowledge of these pathway factors as well as any factors at the bioaerosol source and receptor are needed in high quality environmental regulatory risk assessments which aim to assess the impact of biaoerosol emissions. The prediction of bioaerosol concentrations downwind of a composting facility and close to sensitive receptors would also make an improvement to these risk assessments. Such prediction is currently made via bioaerosol sample collection at and downwind of composting facilities and their subsequent analysis. However the use of commercial air dispersion models might be a very useful and cost effective way of exploring different bioaerosol control situations and assessing bioaerosol emissions in a composting site. Therefore their potential in predicting bioaerosol dispersal merits discussion.

Air pollution dispersion models are mathematical models which have been developed to predict the dispersion of pollutants (e.g. particulates, odours, chemicals) usually in support of air quality regulatory requirements (Environment Agency, 2004). To this end some guidance has been published by the Royal Meteorological Society on the choice and use of air dispersion models (Britter *et al.*, 1995) and the Environment Agency (2000a) on the reporting of air dispersion modelling results. However the use of air dispersion models for predicting dispersal of bioaerosols from composting facilities is still in development and has not been adopted as official practice.

There are a large number of different types of air dispersion models, including box, Gaussian, Lagrangian, Eulerian and Dense gas models. Some of these dispersion models and their application for the dispersion of particles have been reviewed by Holmes and Morawska (2006). They have concluded that the current available models differ significantly in their capabilities and limitations. However a ranking comparison of different models was not possible as there has not been a single validation data set to enable such a comparison. Further difficulties were presented by the fact that most air dispersion models are not commercially available.

A study has reported modelling of airborne pollen concentrations patterns downwind from a maize crop using a Lagrangian Stochastic model. They have concluded that SMOP-2D was able to model the airborne pollen concentration pattern downwind however the model underestimated deposition rates up to 10 m downwind from the crop (Jarosz *et al*., 2004). Other dispersion models such as ISCST3, AUSPLUME, IN-PUFF 2 and WindTrax dispersion models were found to successfully estimate downwind odour concentrations for distance of 500m and 1000m, but not for 100m (Zhou *et al.*, 2005).

Several studies attempted to predict downwind concentrations of bioaerosols emittted from compost. The earliest of such studies is work completed by Millner *et al.* (1980) that have attempted to model the dispersion of *Aspergillus fumigatus* released from composting sewage sludge. They have estimated bioaerosol emission rates ranging from 2.3×10^4 – 6.7×10^{10} particles/second by fitting individual downwind concentrations (10-620 m downwind from source) into a Gaussian dispersion model (Pasquill, 1962). The pollutant was assumed to be gas and hence deposition effects were not taken into account. This study concluded that under unstable atmospheric conditions, the bioaerosols were able to travel 0.5 – 0.6 km downwind from source before reaching background concentrations.

Another such study was completed by Dannaberg *et al.* (1997) who measured concentrations of *A. fumigatus* downwind of a composting plant and used these to calculate emission rates. The emission rates were used to determine downwind

concentrations using two different models, the German Technical Instructions on Air Quality Control, TA-Luft and one developed to examine NOx emission from tall chimneys. The *A. fumigatus* emission rates were found to be similar to those calculated by Millner *et al.* (1980) and also in line with Millner *et al.* (1980) it was concluded that bioaerosols were able to travel 500 m before reaching background concentrations (500 CFU/m^3). A similar study modelled the dispersion from biosolid placement (Dowd *et al.*, 2000). They concluded that the results showed possibility of virus and bacteria induced risk to biosolid land placement workers but this risk was insignificant for population centers 1 x 10^4 m away from the biosolid land placement site.

There are currently no air dispersion models that have been specifically designed for modelling of bioaerosols. In addition the Environment Agency policy (Environment Agency, 2000b) on the use of air dispersion models states that:

"The Environment Agency does not favour or prescribe the use of any particular model. It is left to the operators/applicants to justify their choice of models (including the version)".

However, the use of air dispersion models such as the Gaussian model ADMS which are currently used for modelling pollutants such as particulates and odours that might have similar properties to bioaerosols (e.g. particle size), are being assessed for their potential in regulatory use in the UK (Hall *et al.*, 2002). In this context, there have been limited applications of ADMS to predict bioaerosol concentrations emitted from composting facilities (Drew *et al.*, 2005; ADAS/SWICEB, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a).

Holmes and Morawska (2006) have noted that several factors should be assessed when choosing a suitable air dispersion model, such as the complexity of the sampling environment and nature of the particle source. In this context, it is important to consider several reasons why the use of air dispersion models in predicting the emission of bioaerosols from composting facilities might prove to be problematic (Swan *et al.*, 2003). Firstly, it has been discussed that air dispersion models are developed to predict

pollutant release for distances of larger than 1 km and hence may be unsuccessful in predicting pollutant release at distances shorter than that (Swan *et al*., 2002). This might have implications for regulatory risk assessments as the current distance limit of importance for environmental regulators in England and Wales is 250m.

Secondly, there is a lack of description of bioaerosol source and subsequently of calculating bioaerosol emission rates (Swan *et al.*, 2003). Most studies to date have estimated bioaerosol emission rates by fitting the air dispersion model to individual downwind measurements (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Swan *et al.*, 2002) due to the lack of methods that enable the calculation of emission rates at source. Therefore such estimations are currently used to calculate biaoerosol emission rates from sources such as agitation activities, which are not physically well defined to allow the calculation of a flow rate or an emission rate.

Bioaerosol sources that can be defined as a stack with a known flow rate can be modelled as point sources and adapt the emission rate equations currently used for modelling of odours (Jiang and Kaye, 2001). Similarly Taha *et al.* (2005; 2006; 2007a) has used a wind tunnel traditionally used for measuring odours (Jiang and Kaye, 2001) to derive source term emissions from static compost windrows. However, there remains a lack of biaoerosol specific methods that can be used to determine biaoerosol emission rates from a source.

When compost is being agitated, the differences in the temperature between the inner compost windrow and the ambient temperature might lead to temperature gradients which might effect the dispersal of the bioaerosol. However, such effects on the dispersal of a bioaerosol when released from the compost source are not well defined (Swan *et al.*, 2002; Swan *et al.*, 2003). Hence factors such as these might complicate the use of air dispersion models in predicting the emission of bioaerosols from composting facilities.

Some of these factors which might complicate the modelling of bioaerosols were emphasised by a significant study completed by Wheeler *et al.* (2001) who monitored

bioaerosols, inhalable dust, VOCs, odour and noise from composting sites. Following this, attempts were made to predict downwind emissions of pollutant by the use of SCREEN3 (USEPA, 1995), a simple Gaussian screening model. Wheeler *et al.* (2001) has reported several problems with the use of SCREEN3 in estimating bioaerosol dispersion including the calculation of emission rates for bioaerosols emitted from compost. In addition to these it was concluded that the difficulties in fitting model curves to the data was also a result of the influence of bioaerosol aggregation and loss of microbial viability with time. They indicated that the incorporation of such data into the air dispersion model might improve model predictions. The potential effect of microbial aggregation and microbial viability on model predictions was further emphasised by other studies who have used air dispersion models to predict bioaerosol emissions from composting facilities (ADAS/SWICEB, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a). These properties will be discussed in further detail in the next two sections (Section 1.8 and 1.9).

1.8. BIOAEROSOL VIABILITY

The impact of bioaerosol viability on the dispersion and modelling of biaerosol emissions has been discussed by Wheeler *et al.* (2001). However the viability of airborne micro-organisms released from composting operations and their inactivation after take-off has not been widely studied (Swan *et al.*, 2003). It has been argued that the consequences of bioaerosol viability are not important (Swan *et al.*, 2003) because both viable and non-viable bioaerosol components (e.g. endotoxins, mycotoxins and glucans) can cause adverse health effects (mostly allergenic). Immune systems of the individual respond to the effects of these allergens whether they are ‘alive’ or ‘dead’ (Cox, 1995) and most allergenic spores are likely to retain their ability to cause adverse health effects and their toxicity even if they are no longer viable (Levetin, 1995; Swan *et al.*, 2003).

However, it is important to assess the viability of an airborne micro-organism to determine its specific potential to act as a human pathogen (Haas *et al.*, 1999a). Cox (1995) has emphasized that the ability of a bioaerosol to spread disease is dependent on their viability and infectivity following take-off from original source, as well as through

air transport and landing. Due to this, the loss of bioaerosol viability with time has been proven difficult to incorporate when modelling their dispersion (Wheeler *et al.*, 2001). These uncertainties may lead to conservative assumptions when assessing the release and dispersion of bioaerosols from composting facilities (Swan *et al.*, 2003; Wheeler *et al.*, 2001). Finally, further understanding of viability is important for establishing standardised procedures for the handling, sampling and assessment of bioaerosols (Griffiths and Stewart, 1998). This is because a great number of current bioaerosol sampling methods are known to cause desiccation stress and affect the viability of the samples obtained (Lange *et al.*, 1997; Lin *et al.*, 2000; Terzieva *et al.*, 1996).

The parameters that are of particular importance in assessing bioaerosol survival are temperature, relative humidity, oxygen, carbon monoxide, open air factors (mixture of factors produced when ozone and hydrocarbons react; closely linked to oxygen toxicity) and solar radiation (Anderson and Cox, 1967; Cox, 1995; Levetin, 1995; Lighthart, 1973; Lighthart and Mohr, 1987; Muilenberg, 1995; Dowd and Maier, 2000).

It has been stated that *"The ability of a micro-organism to remain viable in a bioaerosol is related to the organism's surface biochemistry"* (Dowd and Maier, 2000). In this context, gram negative bacteria survive best at low relative humidities and gram positive bacteria, such as the actinomycetes, survive best at high relative humidities. This is because the lipid-containing cell membranes of gram negative bacteria have a low thermodynamic stability which tends to be easily affected by changes in water content (Cox, 1995). In contrast, Holwill *et al.* (1998) showed a decrease in the culturability of the fungi *Penicillum expansum* and *Saccharomyces cerevisiae* spores at higher relative humidity. Other studies have concluded that relative humidity levels of lower than 20% and greater than 80% are stressful for airborne bacteria aerolized from distilled water (Webb, 1959).

High temperatures promote inactivation of bioaerosols due to desiccation and protein denaturation, although some micro-organisms lose viability when environmental conditions are close to freezing point (Dowd and Maier, 2000). Such biological

inactivation and viability loss is observed in gram negative bacteria at cooler temperatures (between -5ºC to +10ºC) (Cox, 1995).

A number of studies have considered the effect of engineered ultraviolet irradiation on microbial inactivation (Griffiths and Stewart, 1998; Ho *et al.*, 2005; Swan *et al.*, 2002; Wheeler *et al.*, 2001). They concluded that ionizing radiation had a negative effect on microorganism viability due to DNA damage caused by DNA helix distortion (Dowd and Maier, 2000). In addition, solar radiation was found to play a role in the inactivation of airborne micro-organisms (Herd *et al.*, 1993; Tong and Lighthart, 1997). Ulevičius *et al.* (1999) showed that solar radiation has a lethal effect on outdoor airborne fungal spores collected by the AGI-30. Solar sensitivity differed for the fungal spores depending on the time of day they were collected.

A study by Paez-Rubio and Peccia (2005) examined the effects of solar and non solar inactivation rates of airborne bacteria (*Mycobacterium parafortuitum* and *Escherichia coli*) released during the application of biosolids to land. An important finding was the influence of relative humidity on both solar and non solar inactivation rates of the airborne bacteria and, in contrast to Dowd and Maier (2000) and Webb (1959), it was found that the greatest inactivation rates were at moderate relative humidity levels (i.e. 50%).

Peccia and Hernandez (2004) have studied the effect of engineered ultraviolet irradiation as a means of inactivating *Mycobacterium bovis* bacteria in indoor environments (such as hospitals) to control microbial disease. This study is in line with previous studies on proposed methods to decrease the risk of microbial disease (Macher *et al.*, 1992; Miller and Macher, 2000; Nardell, 1993; Riley *et al.*, 1971; Stead *et al.*, 1996). However Peccia and Hernandez (2004) argue that the use of ultraviolet irradiation for the control of microbial disease has achieved little success due to the lack of design parameters such as the effect of relative humidity on ultraviolet irradiation (Peccia *et al.*, 2000; Peccia *et al.*, 2001).

The combination of oxygen (can lead to oxygen toxicity), open air factors and ions can inactivate many species (Dowd and Maier, 2000). These factors can promote desiccation of airborne micro-organisms by targeting membranes, proteins and nucleic acids (Cox, 1995).

A study by Yao *et al.* (2005) has shown that certain combinations of electrostatic field strength and exposure time can have a species specific effect on the inactivation of some micro-organisms such as *Pseudonomonas fluorescens* bacteria cells. However these cells were studied when deposited on a Mixed Cellulous Ester (MCE) filter surface and electrostatic fields were not effective for the inactivation of bacteria in an airborne state.

A similar study by Mainelis *et al.* (2002b) examines the effect of electrical charges and fields on the viability of airborne bacteria. They concluded that the recovery of *Pseudonomonas fluorescens* bacteria cells is affected by electrical charges and fields, unlike the other bacterium studied, *Bacillus subtilis* var. *niger*. This study also showed that bacterial cells depend on their membrane potential for metabolic activities. Therefore it was concluded that factors such as electrical charges and fields are likely to affect this membrane potential, and hence affect their viability.

1.9. AGGREGATION AND SIZE DISTRIBUTION OF BIOAEROSOLS

Microbial aggregation is the congregation of microbial cells in various media (e.g., liquid or air) to form a multicellular structure (Calleja *et al.*, 1984a; Calleja, 1984b). The number of microbial cells within an aggregate can range from a few cells to a billion and the aggregation mechanism may be natural, artificial, active or passive.

To date, microbial aggregation in water (Calleja *et al.*, 1984a; Calleja, 1984b; Wickman, 1994) or soil (Forster and Nicolson, 1981) has been more extensively studied than aggregation in air. It has been discussed that micro-organisms generated in water are found in aggregates in higher percentages than bioaerosols generated in other environments such as air. This is due to the adhesion of individual spores that are formed with a thin layer of moisture surrounding them (Wickman, 1994).

Aggregates have been observed as flocculation in brewer's yeast or in pellets of *Aspergillus niger* (Calleja *et al.*, 1984a; Calleja, 1984b). In industries such as brewing, fermentation and waste-water treatment aggregation of micro-organisms may determine process performance (Atkinson and Daoud, 1976; Bossier and Verstraete, 1996; Busch and Stumm, 1968; Furumai *et al.*, 1994; Mozes *et al*., 1994; Yang and Yang, 2005).

The importance of aggregation and particle size of bioaerosols emitted from compost has been discussed by Wheeler *et al.* (2001) who concluded that aggregation of biaoerosols would lead to their settling as particles instead of suspension in air (i.e. non-gaseous behaviour), which might complicate the modelling of their dispersion. In addition to this, the aerodynamic behaviour of a bioaerosol spore would also be determined by its shape, surface characteristics, size and the tendency of the spores to form 'aggregates' or 'clumps' (Levetin, 1995).

It is shown that in still air, the rate at which spores fall to the ground due to gravity is proportional to the square of the radius of the spore for a spherical particle (based on Stoke's law) (Gregory, 1973). However effects such as shape, surface characteristics and aggregation of the spore would affect this rate as nonspherical shape would increase surface drag, resulting in a delay in deposition (Lacey, 1991; Levetin, 1995). However, there is some debate as to the exact nature of the impact of aggregation behaviour on particle dispersion, as the increase in overall particle size could increase the deposition rate, but the increase in surface area associated with aggregates may lead to a delay in deposition (Lacey, 1991).

Another important reason for the study of microbial aggregation and overall biaoerosol size is their impacts on the efficiency of bioaerosol samplers, such as bioaerosol impactors. This is because bigger particles (> 2 μm) impact onto the collection media due to their weight related momentum whilst particles smaller than 1-2 μm might change direction before impaction on the media as they are affected more easily by airflow (West *et al.*, 2008). Therefore these devices are dependant on the particle size of the pollutant that they are aimed to collect (Reponen *et al.*, 2001) and any factors that might increase overall particle size such as aggregation would affect their performance

and collection efficiency (Trunov *et al.*, 2001). This may lead to under (Karlsson and Malmberg, 1989) or over-estimation of the bioaerosol particle count. Such effects on other sampling devices has been discussed by Eduard and Aalen (1988) who argued that aggregation introduced a large variation on filters when counting mould spores.

Finally microbial aggregation and particle size might effect not only the transport of the bioaerosol and the performance of the samplers used in bioaerosol collection, but also the amount of inhaled particles and consequently, their adverse health effects (Agranovski *et al.*, 2004; CIWM, 2002; Reponen *et al.*, 2001; Tham and Zuraimi, 2005; Venkataraman and Kao, 1999). According to Carrera *et al.* (2005), aerosols with a high number of individual micro-organisms might have increased infection potential. Similarly, the hygroscopicity of airborne fungal spores changes their aerodynamic diameter, making them larger, and resulting in a different deposition pattern on the human respiration system (Pasanen *et al.*, 1991; Reponen *et al.*, 1996). This is particularly important in the human airway where the relative humidity is almost 100% and the fungal spores are likely to grow larger (Li *et al.*, 1992).

Aggregation of micro-organisms in water or soil has been studied more broadly however microbial aggregation in air has been referred to in a number of studies. Lacey and Dutkiewicz (1976a) noted possible aggregates of bacteria during their experiments on the isolation of bioaerosols from mouldy hay in a sedimentation chamber. They have argued that *A. fumigatus* sedimented most slowly of the fungi and the bacteria sedimented faster than actinomycetes due to the fact that they were distributed in clumps.

Tham and Zuraimi (2005) studied the size relationship between airborne viable bacteria and particles in a controlled indoor environment study. They showed that for cell sizes between 3 and 7.5 μm, bacteria were found in aggregates, whilst at sizes of 1.0 and 2 μ m, they existed freely. Previously Ho *et al.* (2001) who developed an approach to estimate viable organisms in a single biological particle found that bacteria between the sizes of 2.5 and 4 μm contained 4.5 viable cells.

In contrast to these, Carrera *et al.* (2005) have studied the number of bacterial spores within aerosol particles and concluded that particles consisting of 1-2 cells accounted for 85% of overall generated particles. Similarly, Grinshpun *et al.* (1997) have attempted to study the aerosol characteristics of airborne actinomycetes and fungi and have found evidence of aggregation however these only accounted for 10-15% of all particles. They have noted that this number might have been in the range of 60% if a deagglomerating orifice was not used in the experimental set-up.

Jankoswka *et al.* (2000) have studied the collection of fungal spores on air ventilation filters. They noted that the difference between collection efficiencies for biological (*Penicillium*) and non-biological (potassium chloride) particles might be attributed to spore aggregation.

However there are only a limited number of studies (Reinthaler *et al.*, 1997; Pillai and Ricke, 2002) that have examined the particle size distribution in composting facilities and in particular there is a distinct lack of information on the aggregation of biaoerosols emitted from compost. In addition, the mechanisms of microbial aggregation in air are not clear.

Other studies (Calleja,1984a; 1984b) have shown that microbial aggregation is inducible through various factors:

- genetic conditions results in some species being more susceptible to aggregation than others;
- physiological state of the cell would induce cell aggregation, for example the end of the growth phase is the right time for the aggregation of species such as yeasts and myxobacteria; and
- environmental conditions such as light, temperature, agitation, aeration, nutrient, energy source, stimulators would all affect the clumping tendency of the cells.

In addition, it was discussed that several other factors can influence the particle size of micro-organism aggregates. One of these according to Amanullah *et al.* (2001) is

physico-chemical effects, such as hydrophobicity, in line with Tay *et al.* (2001) who argue that hydrophobicity may be the primary force for aggregation in aerobic granules due to increased cell to cell interaction. Similarly, Liao *et al.* (2004) argue that there are seasonal differences between fungal spore sizes resulting from changes of relative humidity and hygroscopicity.

Another factor in aggregation was shown (Calleja *et al.,*1984b) to be temperature. This is because the chemical reactions that cause aggregation occur at an increased rate at elevated temperatures. Finally Calleja (1984b) has shown the positive affect of the nutrient oxygen on the aggregation of *Aspergillus* species on spore aggregation.

1.10. CONCLUSIONS

The previous sections have provided an overview of the literature regarding the factors which may affect the quality of environmental regulatory risk assessments with respect to bioaerosols and composting. A risk assessment is based on hazard identification, exposure assessment, dose-response assessment and risk characterization (Gerba *et al.*, 2008). In this context, the quality of such a risk assessment aimed to address the bioaerosol hazard posed by a composting facility should be based on the understanding of the factors shown in Figure 1.2.

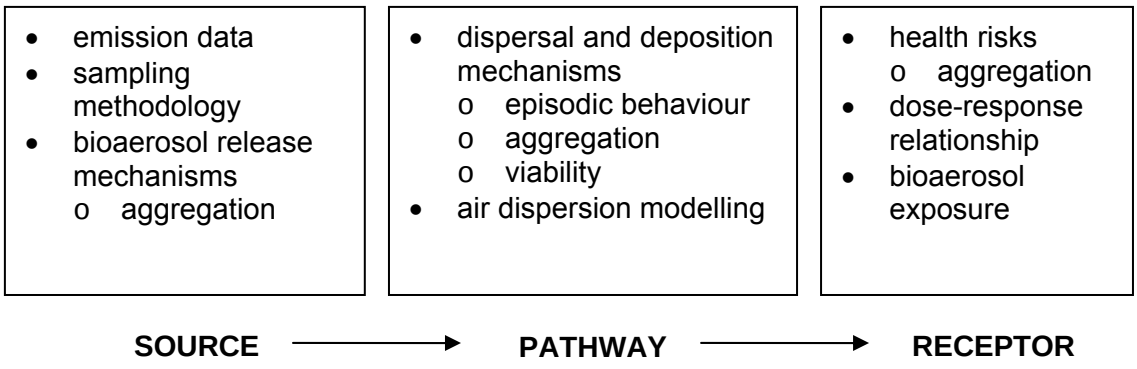


Figure 1.2 - Factors affecting the quality of an environmental regulatory risk assessment aiming to address bioaerosol hazards

However there are gaps in the understanding of these processes which may effect the assessment of the exposure of bioaerosols emitted from composting facilities.

1.10.1. Source Factors

There are a number of factors that might complicate the understanding behind bioaerosol hazard identification at source. Firstly there is a lack of description of bioaerosol source and subsequently of calculating bioaerosol emission rates to be used in air dispersion modelling of bioaerosols (Swan *et al.*, 2003). Source term data in composting facilities is often inadequate because of the practical and cost implications of airborne micro-organism analysis (Environment Agency, 2004; Pollard *et al.*, 2006). In addition, the lack of methods to calculate bioaerosol emission rates at source has resulted in most studies to date having to estimate bioaerosol emission rates by fitting the air dispersion model to individual downwind measurements (i.e. back-extrapolation) (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Swan *et al*., 2002).

Different methods of bioaerosol sampling and enumeration are used in different studies and these methods even when used in parallel yield differing results of bioaerosol counts (Köck *et al.*, 1998). Therefore, it is currently exceedingly difficult to compare and contrast the results of different studies and evaluate best practice. Apart from the guidelines published by the Composting Association (1999) and Association for Organics Recycling (2009), there are no guidelines from the Environment Agency or other government bodies in the UK as to the preferred sampling and analysis methods. Despite the numerous reviews and evaluations of bioaerosol collection techniques, there remains a need for standardised methods and procedures to ensure consistency of practices, especially in scientific research.

The levels of bioaerosols measured by the current bioaerosol sampling methods are often not representative of the actual long-term exposure levels. This is because the sampling periods are often very short-term (i.e. 15-45 mins) and do not take into account the episodic nature of bioaerosols emissions. A large number of current bioaerosol samplers rely on further culture-based enumeration that could result in under-estimates for species with low viability.

Further complications are added due to the fact that bioaerosols occur naturally in the environment (Andreeva *et al.*, 2001; Asan *et al.*, 2004; Jones and Cookson, 1983; Köck

et al., 1998; Passman, 1983). The implication of this is that it may be difficult to establish 'background' levels of bioaerosols at composting facilities. This is because it may not always be possible to determine if the bioaerosols sampled are emitted from composting sources or are ubiquitous in the atmosphere regardless of the composting source.

1.10.2. Pathway Factors

The physical and microbiological characteristics of bioaerosols determine their behaviour, transport and health effects in indoor and outdoor environments (Grinshpun *et al.*, 1997). Hence, the 'safe' distance between a sensitive receptor and a compost facility is governed by the aerodynamic and microbiological properties of bioaerosols once they are released from source. As revealed by the literature, there are gaps in the understanding of these characteristics.

The episodic nature of bioaerosol releases is important to consider when assessing the risks resulting from composting facilities. This is because conventional bioaerosol sampling methods may not represent the actual amounts of bioaerosols present in an environment due to episodic bioaerosol behaviour (Sivasubramani *et al.*, 2004), resulting in possible under-estimation of levels. In addition the literature shows that peak emissions of bioaerosols in composting facilities are associated with agitation activities and emissions of bioaerosols are higher downwind of mechanically agitated windrows (e.g. screening and turning) as opposed to static windrows with no activity. Therefore this should be a consideration when sampling for bioaerosols in a composting facility.

The use of air dispersion models for predicting dispersal of bioaerosols from composting facilities is still in development and has not been adopted as official practice. Therefore to date there has been limited applications of commercial air dispersion models in predicting bioaerosol dispersal. One such application has been completed by Wheeler *et al.* (2001) who has reported several problems when using SCREEN3 to estimate bioaerosol dispersion from compost. Along with other factors (e.g. emission rate determination, thermal effects), they concluded that the difficulties in fitting model curves to the data was also a result of the influence of bioaerosol aggregation and loss

of microbial viability with time. This study indicated that the incorporation of such data into the air dispersion model might improve model predictions.

There is a distinct lack of research regarding the inactivation and viability of airborne micro-organisms emitted from composting facilities. The effects of environmental factors (i.e relative humidity, temperature, radiation, oxygen levels, open air factors and ions, as well electrical charges and fields) on the viability of bioaerosols, should guide the direction of future composting bioaerosol research to improve the current knowledge in this field.

Finally, aggregation of bioaerosols in air or emitted from composting facilities has not been widely studied. However this is an important property that might lead to settling of particles instead of suspension in air (i.e. non-gaseous behaviour) on emission from composting facilities (Wheeler *et al.,* 2001). In addition to this the aerodynamic behaviour of a bioaerosol would also be determined by its tendency to form aggregates (Levetin, 1995). The prior art regarding aggregation mechanisms in water and soil is more extensively studied however it was shown that much about the aggregation process is still unknown due to the lack of knowledge on cell physical properties and their surface hydrophobicity (Dufrêne, 2000).

1.10.3. Receptor Factors

The health effects of bioaerosols vary depending on the type of bioaerosol in question, the industry they originate from and the receptor characteristics (worker or resident living close to the facility, previous exposure to bioaerosols and likely sensitisation, effect of smoking, age and gender). Evidence from the literature suggests that OEL and TLV values are used as benchmark numbers for comparisons of findings and thus enhance the understanding of the dose-response relationship. However the differences between various studies in terms of sampling procedures and analytical methods, individual variation of exposed subjects (e.g. smoker, pet owner, previous exposure to bioaerosol, lifestyle) and interpretation of results, introduce complications in making generalised correlations between exposure and adverse health effects.

There is a distinct lack of knowledge regarding the mechanisms of bioaerosol induced disease. In addition, a very poor association between the adverse health effects of bioaerosols and exposure data generated by air sampling exists (Sivasubramani *et al.*, 2004). Therefore a need to establish dose-response relationship between bioaerosol exposure and adverse health effects according to type of bioaerosol and exposure still remains. In addition, internationally recognised regulatory standards are required.

In conclusion, there are gaps in the current understanding of bioaerosols after release from composting facilities. The implication of this is the inaccurate estimation of the levels of bioaerosols in and around composting facilities. Therefore in order to improve the quality of composting bioaerosol risk assessments, bioaerosol studies in the composting industry need to address the gaps in the understanding of classification of bioaerosol source, bioaerosol emission and pathway and the implications of bioaerosol induced disease on the receptor.

CHAPTER 2. RESEARCH STUDY RATIONALE

2.1. RESEARCH AIMS AND OBJECTIVES

There is an increased need to divert municipal solid waste from landfill as a response to the UK waste strategies, the European Waste Framework and the Landfill Directive. Therefore there has been a consistent growth in composting in the UK over the last ten years as a more sustainable method of waste management (Composting Association, 2007). Operating a composting facility should be a low hazard activity however, these facilities do have the potential to cause pollution, harm to health and nuisance through odours, leachate, fires, dust, vermin and potentially harmful bioaerosols, if not operated properly (Environment Agency, 2004; Pollard *et al.*, 2006).

To address the public health concerns and the need for operators to demonstrate the safe and responsible operation of their facilities, environmental regulators request regulatory risk assessments in support of planning consent and environmental permits, in particular, where facilities are within 250m of sensitive receptors (Environment Agency, 2001; 2007).

These regulatory risk assessments allow operators to demonstrate that they understand the hazards associated with their processes, and can design and implement technical and management procedures to minimise unacceptable risks. Therefore for assessing bioaerosol risk posed by composting facilities, the environmental risk assessments require an understanding of:

- the mechanisms of release and source (e.g. agitation activities, compost windrows) of bioaerosols (the hazard);
- the receptors that the hazard affects (e.g. compost facility workers and sensitive receptors around a composting facility); and
- the pathway through which the bioaerosol hazard affects the receptor to assess the risks posed by composting facilities (Environment Agency, 2004).

As discussed in the previous chapter (Chapter 1) the behaviour of bioaerosols at source, pathway and receptor are determined by their physical and microbiological characteristics. However, there are gaps in the understanding of bioaerosols released from composting facilities. One such gap is the lack of information on the aggregation and size distribution of bioaerosols released from compost. This is an important property to consider as it has implications for bioaerosol behaviour at source, pathway and receptor. Consequently, addressing such gaps of knowledge would result in improved regulatory risk assessments.

For example, at source, the aggregation and overall size distribution of bioaerosols would have an impact on the collection efficiency of bioaerosol samplers (Jankowska *et al.*, 2000; Trunov *et al.*, 2001). The aggregation and size of a bioaerosol as well as other properties such as its shape and surface characteristics would also affect their behaviour upon release from composting facilities (Levetin, 1995; McCartney, 1994; McCartney *et al.*, 1997a). Hence, a larger particle might be subject to deposition velocities higher than for a small particle (Wheeler *et al.*, 2001; Swan *et al.*, 2003) suggesting that larger particles (e.g. bioaerosol aggregate), are more likely to settle out downwind of a bioaerosol source instead of suspension in air (Pillai and Ricke, 2002). As such, a larger particle might travel less distance downwind of a composting facility compared to a smaller particle. Finally, the understanding of bioaerosol aggregation and size distribution at receptor is important in predicting their inhalability and subsequent health effects (Agranovski *et al.*, 2004; CIWM, 2002; Reponen *et al.*, 2001; Tham and Zuraimi, 2005; Venkataraman and Kao, 1999).

Therefore, to address the gap in the knowledge of bioaerosol size distribution and aggregation, the first aim of this research project is to:

- Improve the current understanding of the aggregation and size distribution of bioaerosols emitted from compost.

The prediction of bioaerosol concentrations at various points downwind of a composting facility and close to sensitive receptors would also have a significant impact on the

improvement of composting regulatory risk assessments. This is currently done by collection of bioaerosol samples at composting facilities and the subsequent analysis of these samples. However, there are several difficulties posed by the collection of bioaerosol samples at a composting facility. Therefore using commercial air dispersion models might be a more useful and cost effective way of assessing bioaerosol exposure at a composting site and exploring different bioaerosol control situations.

The air dispersion models that are currently available are not designed for bioaerosols but for pollutants such as odours or particulates. As such, the use of air dispersion models for predicting dispersal of bioaerosols from composting facilities has not been adopted as official practice. However bioaerosols might behave as non-biological aerosols as well as possessing biological and specific properties hence the use of commercial air dispersion models for successfully predicting the emissions of bioaerosols from a composting source could be a possibility.

There are a limited number of studies (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Dowd *et al.*, 2000; Wheeler *et al.*, 2001; Taha *et al.*, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a) which have used such air dispersion models to predict downwind concentrations of bioaerosols emitted from compost sources. However these studies have not attempted to compare the actual measured on site downwind bioaerosol concentrations with those predicted by the model. Without such a comparison, it is not possible to verify the ability of an air dispersion model in predicting downwind bioaerosol concentrations.

Therefore the ability and sensitivity of such models to be able to predict downwind bioaerosol concentrations should be vigorously tested and verified. In addition, this should be done before the use of air dispersion models for assessing the emission of bioaerosols from composting facilities is adopted as official practice in support of composting regulatory risk assessments. It was also discussed that factors such as microbial aggregation and size distribution might complicate the modelling of the bioaerosol dispersion (Swan *et al.*, 2003; Wheeler *et al.*, 2001). Therefore the effect of

such factors and other parameters on the modelling of bioaerosols emitted from compost needs to be better understood.

In line with this, the second aim of this project is to:

- Improve the current understanding of the potential of a commercial air dispersion model to predict bioaerosol concentrations at composting sites.

In order to achieve these overall project aims, the following objectives were set:

1) Release and measure bioaerosols in experimental conditions and use the generated data to classify the overall size distribution and visual properties (i.e. aggregation, size and shape) of bioaerosols emitted from compost.
2) Complete site work to validate the results of such controlled experiments (objective 1) and classify the overall size distribution and visual properties (i.e. size, shape and aggregation) of bioaerosols emitted from compost at composting facilities.
3) Complete a preliminary assessment of a commercial air dispersion model, ADMS 3.3, in predicting bioaerosol emissions from composting facilities compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling.
4) Analyse the sensitivities of ADMS 3.3 and assess the effect of different modelling parameters on predicted bioaerosol concentrations.
5) Complete a final assessment of the potential of ADMS 3.3 in predicting bioaerosol emissions from composting facilities.

2.2. THESIS STRUCTURE

This research was completed to improve the current knowledge of the understanding of the characterisation and dispersal of bioaerosols emitted from composting facilities and has two overall project aims as discussed in the previous section. As such, the thesis was divided into two themes and the chapters are presented in line with this. Some parts of this thesis have been published in a peer reviewed conference proceeding and previously presented as consultancy reports. Details of these publications as well as

other peer reviewed and published publications that the author has been involved in the production of are listed in Appendix A.

The summary of the thesis structure is presented in Figure 2.1.

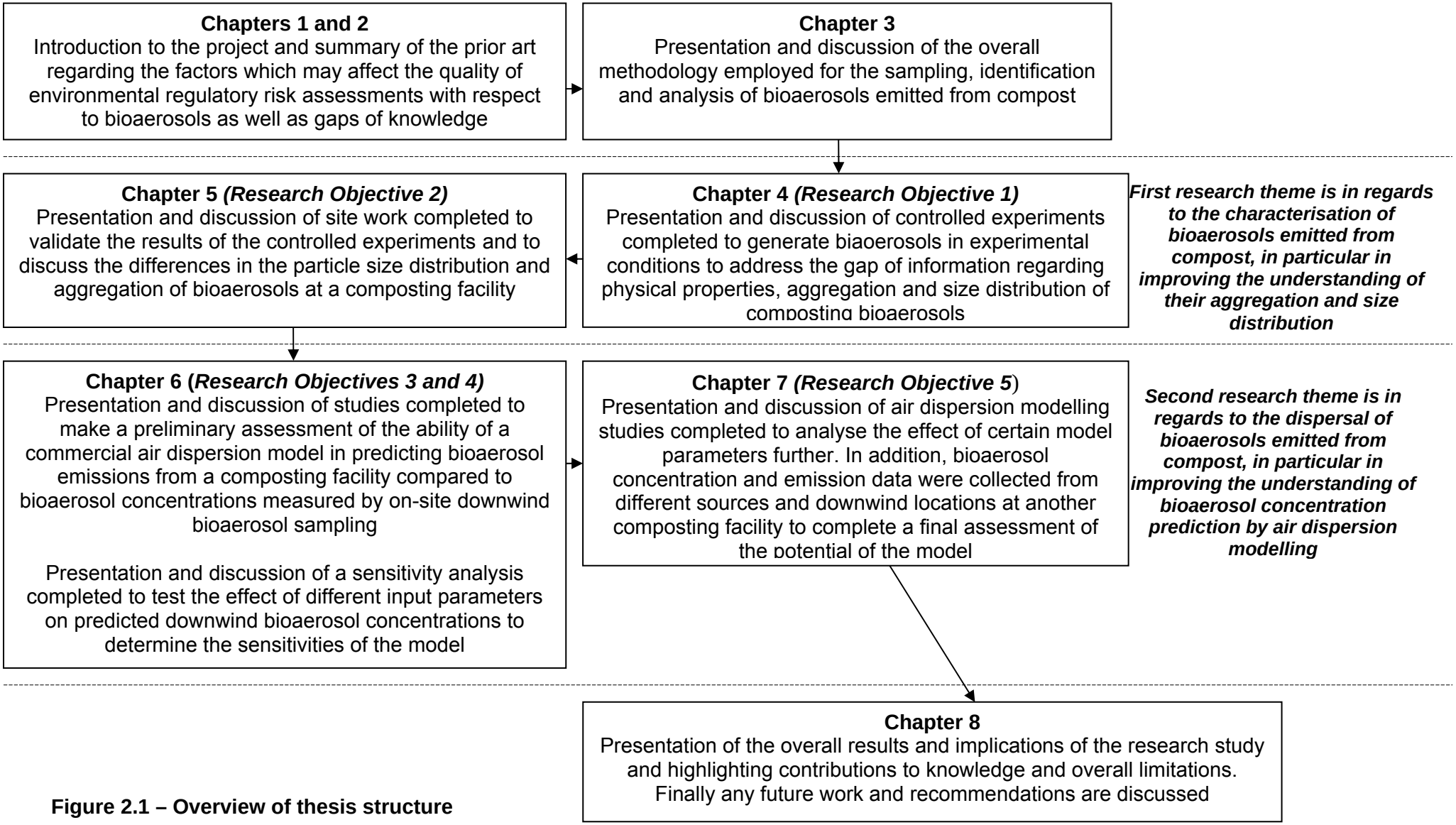


Figure 2.1 – Overview of thesis structure

CHAPTER 3. BIOAEROSOL SAMPLING, IDENTIFICATION AND ANALYSIS METHODOLOGY

3.1 INTRODUCTION

To achieve the overall project aims and objectives as discussed previously (Chapter 2), a combination of controlled experiments, site work and air dispersion modelling studies were employed. The methodology used for the air dispersion modelling studies will be discussed separately in Chapters 6 and 7. Therefore, this chapter describes the methodology deployed for the collection of bioaerosols by air filter sampling and the subsequent identification and analysis by two different methods; culturing and scanning electron microscopy. This chapter also includes details for all the composting sites visited for the site work, pre and post-sampling laboratory practices, expression of results and the health, safety and quality control measures taken.

The methodology for detection of bioaerosols following air sampling is based on standard filter based bioaerosol analysis as per previous studies (Taha *et al.*, 2005; Taha *et al.*, 2006; Taha *et al.*, 2007a). However, the SEM analysis methodology has been developed as a novel method for studying and analysing the aggregation and size distribution of biaoerosols emitted from compost.

3.2. COLLECTION OF BIOAEROSOLS BY AIR FILTER SAMPLING

Personal air filter samplers were used to collect bioaerosol samples (SKC; Figure 3.1), which draw a known volume of air through a filter medium where bioaerosols are captured. As previously discussed (Chapter 1, Section 1.3) there is a wide variety of methods for sampling of bioaerosols. The sampling device of choice must be chosen based on the required application and some factors that need consideration include the bioaerosol being sampled and the ease of the transport and use of the sampler (Dowd and Maier, 2000). The reasons why an SKC personal air filter sampler was used will be compared with the alternative use of an Andersen sampler (Andersen, 1958) because this is the sampler of choice in the only standardised protocol for the sampling and

enumeration of bioaerosols at composting facilities in the UK (Composting Association, 1999).

The main reason for the use of SKC personal air filter samples for use in this study was because the use of filters allowed for the simultaneous identification and analysis of the collected bioaerosols by two different methods, namely culturing and scanning electron microscopy. It would not be possible to do the same by using the Andersen impactor because in this sampler, the micro-organisms are collected directly onto the culture medium. This allows for easier culturability of samples compared to the filter from which the bioaerosols first need to be 'washed-off' however this means that it would not be possible to identify and analyse the bioaerosols collected by the Andersen impactor by subsequent scanning electron microscopy.

An Andersen sampler is often used for the size partitioning of the sampled bioaerosols and examination of the size distribution of bioaerosols was an important aspect of this study. However, filtration was used because it was not only size distribution of the bioaerosol that was of interest but also the physical characteristic that needed to be categorised such as the aggregation and shape of bioaerosols emitted from compost. This is because the response of a bioaerosol to the effects of forces such as Brownian motion or gravity are based on their shape as well as other physical properties (Pillai and Ricke, 2002).

In addition, the methodology for detection of bioaerosols following air sampling is based on standard filter based bioaerosol analysis as per previous studies (Taha *et al.*, 2005; Taha *et al.*, 2006; Taha *et al.*, 2007a) and these studies have all been based on the use of SKC personal filter samplers. Finally, filtration was used because of practical reasons such as the high portability and ease of handling of the sampler in line with a current study completed by the Environment Agency (Environment Agency, 2009).

To carry out the bioaerosol sampling, firstly the sampling cassette and filter were placed inside the IOM sampler head. The IOM sampler head (Figure 3.1) was connected to the pump by a 10mm internal diameter tygon tube. Polycarbonate filters (SKC) of 0.8 µm

pore size and 25 mm diameter were used (with the exception of the sampling completed at Lount as will be explained). The pump flow rate used for sampling was 2 ± 0.2 l/min as suggested by the sampler supplier (SKC, 2002). All equipment, including the filter cassettes, filter heads and IOM sampler heads, was sterilised using an autoclave (15 minutes, 121ºC) as recommended by Lester and Birkett (1999b) before being taken onto site. Pumps were calibrated before the start of sampling using a rotameter (variable area flowmeter, SKC).

Figure 3.1- Personal air sampler pump and 25 mm IOM sampling head (SKC Ltd)

3.2.1. Bioaerosol Sampling at Keenan Recycling

A set of site work and subsequent preliminary air dispersion modelling was completed at Keenan Recycling as will be discussed in Chapter 6. Keenan Recycling is an open windrow composting site in Aberdeenshire, Scotland and processes green waste received from local councils from kerbside collections and from local authority civic amenity centres. The estimated mass of processed waste is 19,000 tonnes per annum and the maximum age of the compost on-site is approximately 19 weeks.

At the time of the sampling, the site had an office, weighbridge and storage buildings. It is a family operated facility with the immediate family of the owner living in houses located adjacent to the composting facilities. A small stable is also located next to the houses for the horse and pony owned by the family. The site is surrounded by agricultural land and the nearest sensitive receptor is a farm located approximately

500m south of the site boundaries. There is also livestock in the fields outside the site boundaries, the nearest of which is at a distance of about 200m to the South/East. The schematic diagram of the site sampling locations is shown in Figure 3.2.

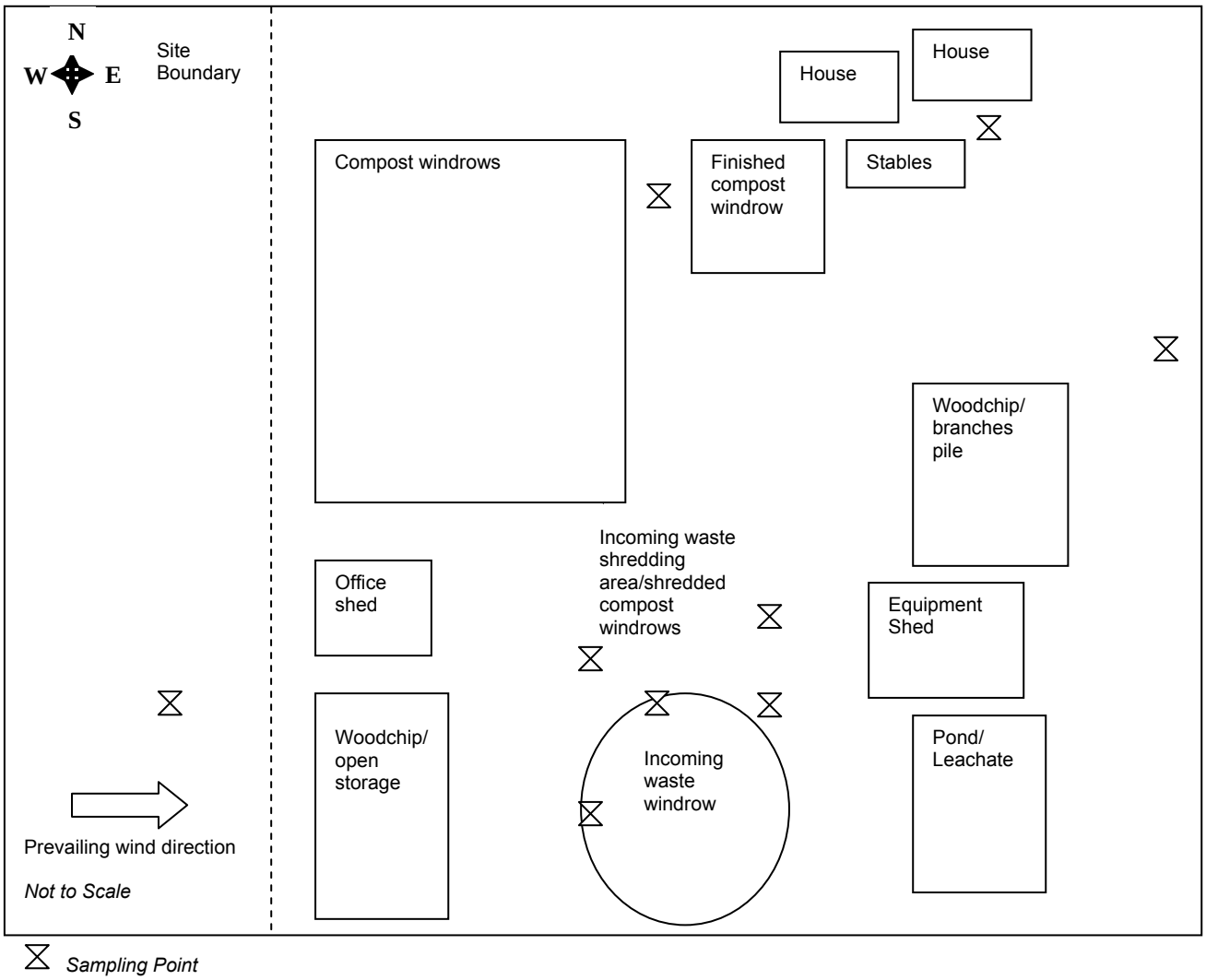


Figure 3.2- Layout of the sampling completed at Keenan Recycling

Throughout this site work a sampling duration of 30 minutes was used if the bioaerosol concentrations were expected to be quite high, and 45 minutes for lower measurements, such as background concentrations. Two pumps were used to take two simultaneous samples from each sampling location, with a third pump kept as standby. The average (arithmetic mean) of the two samples taken at each sampling location was used for analysis and reporting of results.

The background and on site sampling locations at Keenan Recycling were determined depending on the specific site details and the activities occurring during the site visit on the day. This meant that despite the consideration given to collect samples from the same location at each site visit to enable comparisons between different sampling dates, differences in specific sampling locations for different sampling days occurred. The sampling locations covered agitation activities such as shredding, turning, and screening (Figure 3.3).

Figure 3.3- Agitation activity at Keenan Recycling

A sampling hood (Figure 3.4, a) was used to capture bioaerosol emissions from the incoming waste compost windrow at Keenan Recycling for the first sampling day where sampling heads were placed in the top and bottom of the outlet of the sampling hood. The sampling hood was not available for the second and third sampling days hence the air samples for static sources such as compost windrows were taken by placing the sampling filters as close as possible to the bioaerosol source (Figure 3.4, b).

(a) **(b)**

Figure 3.4- Bioaerosol sampling at Keenan Recycling incoming waste compost windrow (a) using the sampling hood (b) placing the sampling filters on the compost windrow.

3.2.2. Bioaerosol Sampling at Donarbon Limited

A set of site work were completed at Donarbon Limited to:

- collect bioaerosol particle size and aggregation data (Chapter 5); and
- complete air dispersion modelling to assess the potential of air dispersion modelling in predicting bioaerosol concentrations downwind of a compost facility (Chapter 7).

Donarbon Limited is a composting site located in Waterbeach, Cambridgeshire and operates a composting system, refuse collection, rear end loaders and skip vehicles in addition to providing landfill and waste processing operations for 20,000 tonnes of waste for local councils. A wide variety of wastes are processed, including green waste and kerbside collected garden and kitchen waste.

The green waste is processed in a windrow technology and is matured until approximately 6 months of age before it is sold for use in agriculture, landscape and gardening. The kerbside collected waste is made up of a variety of biodegradable wastes including garden waste, catering waste, commercial growers' waste, paper and cardboard. This waste is processed in an Animal By-Products (ABP) compliant in-vessel system for the first two weeks where the oxygen levels and temperature are monitored

and controlled. This system ensures that all pathogens are killed. Following this, the compost is matured on concrete pads until approximately 6 months of age before it is used as a soil improver, top dressing or mixed with soils for utilisation in agricultural or restoration industry. The sensitive receptors surrounding this site are the Cambridge Research Park (offices) located approximately 400 m south of the facility and residential cottages and Chittering village approximately half a mile north east of the site. The schematic diagram of the bioaerosol sampling completed at this site is shown in Figure 3.5.

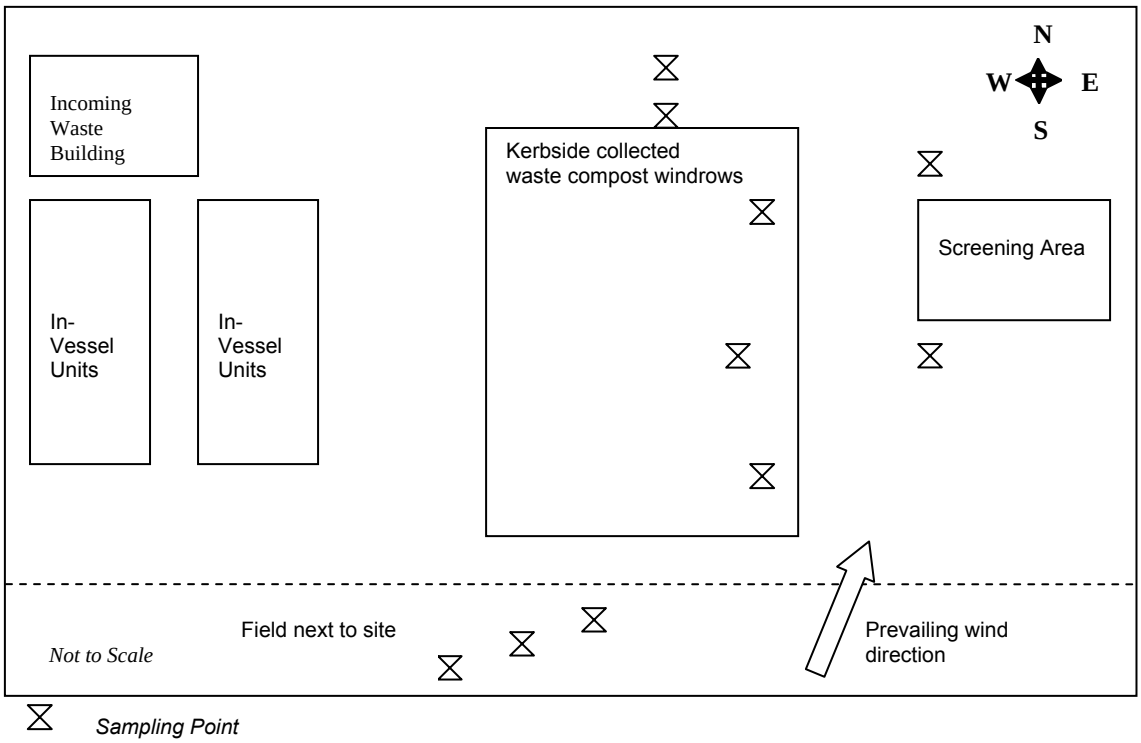


Figure 3.5- Layout of the sampling completed at Donarbon Limited

(Note: the plan only shows the kerbside collected waste processing section of the site where the sampling was carried out)

For the site work completed at Donarbon Limited, a sampling duration of 30 minutes was used. Three simultaneous samples were collected for Scanning Electron Microscopy (SEM) analysis whilst three samples were collected for culture based analysis of actinomycetes and *Aspergillus fumigatus*. A seventh pump was kept as a standby. When analysing and reporting results, the average of three replicated samples (two sets of three replicate samples for culture based and SEM analysis separately)

taken at each sampling location was used. Samples were taken upwind, at source and downwind from the source.

The source term data was derived from either composting agitation activities (i.e. screening) or from static emissions (i.e. compost windrows). A wind tunnel (Figure 3.6) was used to capture bioaerosol emissions from static compost windrows where three IOM sampling heads were located at the mixing chamber (outlet chamber) of the wind tunnel and one IOM sampling head was located at the inlet chamber of the wind tunnel. The overall biaoerosol concentration in the wind tunnel was calculated by subtracting the inlet bioaerosol concentration from the average (arithmetic mean) of the three outlet bioaerosol concentrations. The wind speed inside the sampling hood was measured with an anonemeter (Kestrel 3000 pocket size). The wind tunnel sampling was replicated for further locations to determine the range of bioaerosol concentrations across a compost windrow or across various compost windrows.

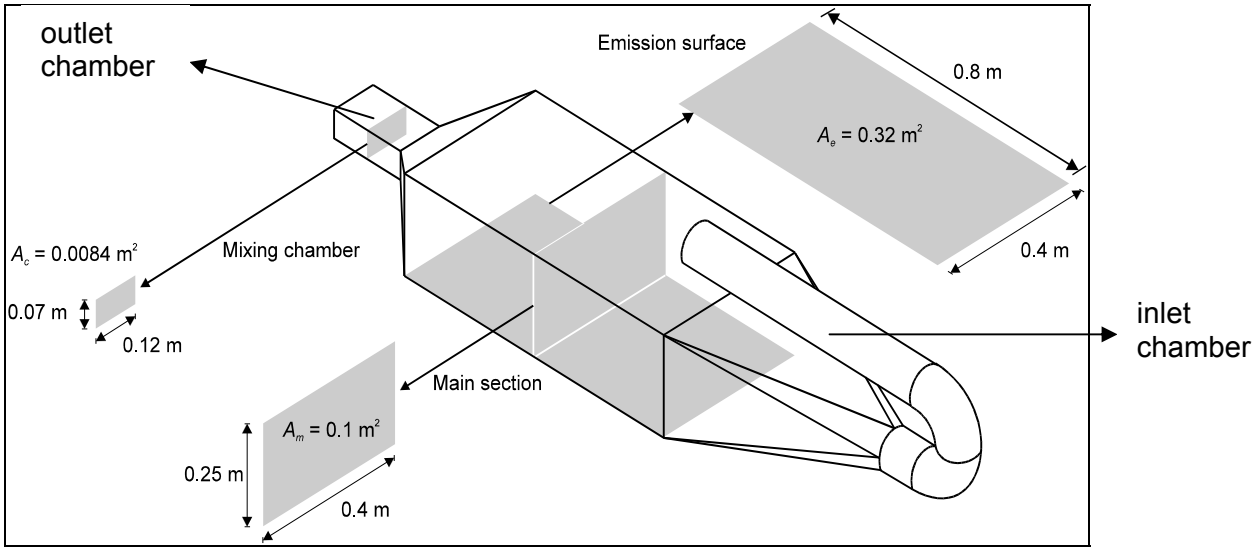


Figure 3.6- Schematic of wind tunnel used to take static samples (Jiang and Kaye, 2001; Taha *et al*., 2005)

3.2.3. Site Work at Lount

An opportunity was presented to do further site work in another site, Lount. The aim of this site work was to study the effect of different filter pore sizes on bioaerosol capture to re-create the particle size distribution obtained by the six stage Andersen sampler

(Deacon *et al.*, 2009). This opportunity was taken as this work allowed the researcher to capture further bioaerosol concentration and particle size distribution data as well as validate the data collected at Donarbon Limited (Chapter 5).

A common misconception in the use of filters for sampling aerosols is that the filters work as microscopic sieves which allow only particles that are smaller than the filter pores to get through. However, the passage of air through the filter is required and porous membrane filters such as the polycarbonate filters used in this study can have variable pore sizes which tend to restrict the flow rates going through the filter which in turn effects the amount of aerosols captured by the filter. Therefore the pattern of air flow through a filter is a key factor of the filter's efficiency (Hidy, 1982). In addition, the methods through which a particle can deposit on a porous membrane filter include diffusion to the walls of the pores, impaction and interception occurring at the inlet to the pores and gravitation settling (Hinds, 1982).

In the Andersen 6-stage sampler (Andersen, 1958), which allows the determination of the size distribution of the collected aerosol, the main principle of collection is by impaction of the collected aerosol (Vincent, 1989). The air is forced through a nozzle and ejected onto an impaction plate after which particles in the aerosol with sufficient inertia impact onto the plate (Hidy, 1984). As the aerosol flows through the stages of the sampler, the particles captured on a given stage represent those that are smaller than the cutoff size of the previous stage and larger than the cutoff size of the given stage (Hinds, 1982).

Therefore during this experiment two sets of samples were collected (Figure 3.7) using filter sampling (SKC) to test the effect of different filter sizes. The filter sizes that were tested were 0.65 µm, 1 µm, 2 µm, 3 µm, 5 µm and 8 µm to correspond to the size distribution of that represented by the Andersen 6-stage sampler (Andersen, 1958). The cut-off characteristics of the membrane filter and the 6-stage Andersen sampler are explained further as follows:

- Andersen Stage 1 is aimed to capture particles in the size range of 7-10 µm, therefore filter size 8 µm in this site work is aimed to replicate this;
- Andersen Stage 2 is aimed to capture particles in the size range of 4.7-7 µm, therefore filter size 5 µm in this site work is aimed to replicate this;
- Andersen Stage 3 is aimed to capture particles in the size range of 3.3-4.7 µm, therefore filter size 3 µm in this site work is aimed to replicate this;
- Andersen Stage 4 is aimed to capture particles in the size range of 2.1-3.3 µm, therefore filter size 2 µm in this site work is aimed to replicate this;
- Andersen Stage 5 is aimed to capture particles in the size range of 1.1-2.1 µm, therefore filter size 1 µm in this site work is aimed to replicate this;
- Andersen Stage 6 is aimed to capture particles in the size range of 0.65-1.1 µm, therefore filter size 0.65 µm in this site work is aimed to replicate this.

Due to time constraints, only a single set of the filter samples were analysed by SEM.

Figure 3.7- Bioaerosol sampling at Lount

Lount is a green waste composting facility located in Leicestershire, with a civic amenity facility located on the site. It receives up to 25,000 tonnes of green waste annually with an application in place for an increase to 31,000 tonnes. Approximately 60% of the waste comes from kerbside collections and 40% from civic amenity sites. After delivery, the waste is sorted and contaminants (e.g. plastics) are removed. Following this, improved homogeneity of the size and mix of waste material is achieved through shredding of the waste. Post-shredding, the waste is formed into compost windrows (3 m high, 3 m wide) and they are turned regularly to homogenise and aerate the material.

The moisture content of the compost windrows is kept between 30 and 60% and the compost is matured on concrete pads for 6-9 weeks before it is screened and passed to the consumer. The nearest sensitive receptor is the civic amenity facility that is located adjacent to the composting pad. The schematic diagram of the site sampling is shown in Figure 3.8.

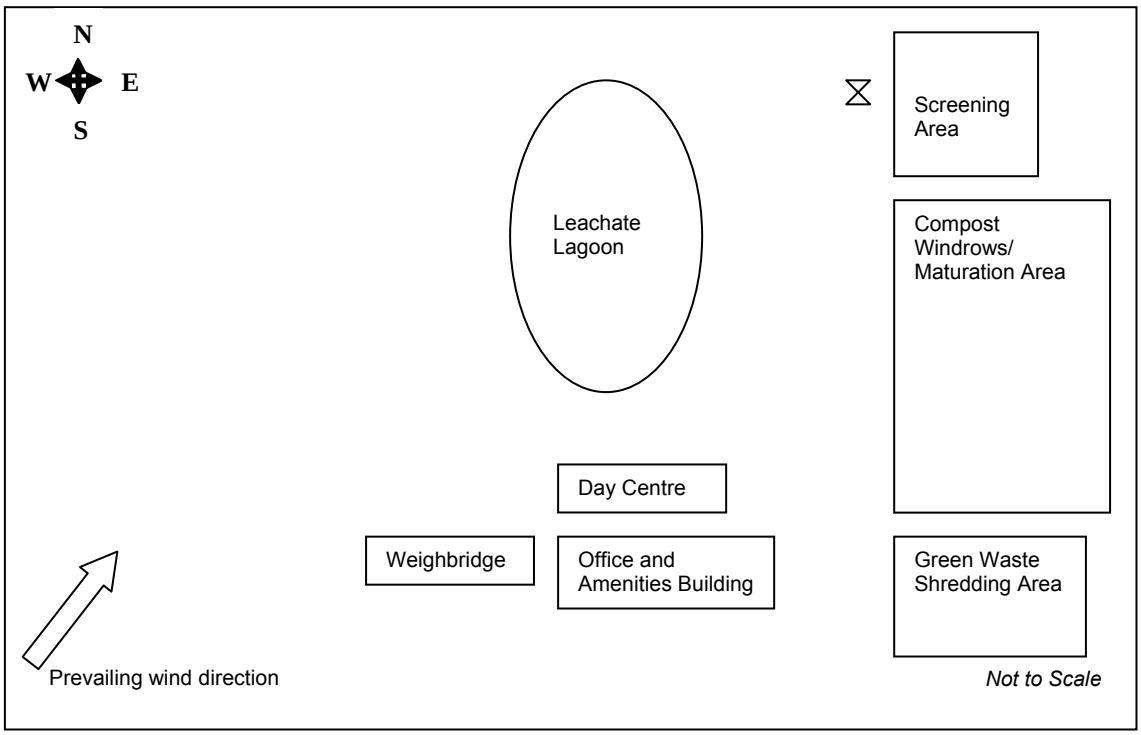


Figure 3.8- Layout of the sampling completed at the Lount Site

For all site work, sampling equipment was located at 1.8m above ground for downwind samples to represent the average height of a sensitive receptor. A Kestrel 3000 pocket size anemometer (Meterologica Ltd., Lancashire) was used to determine the temperature, relative humidity and wind speed for each site visit. General weather conditions, such as wind direction, rain or strong winds, were observed and recorded manually.

3.3. IDENTIFICATION AND ANALYSIS OF BIOAEROSOLS BY CULTURING

3.3.1. Pre-sampling Laboratory Practices

The micro-organisms measured in this study were *Aspergillus fumigatus* and actinomycetes. These micro-organisms were chosen as they are known to occur in large amounts during the composting process (Lacey, 1997; Fischer *et al.*, 1999), pose possible adverse health effects to sensitive receptors and have been widely studied in other research (Millner *et al.*, 1977; Millner *et al*., 1980; Clark *et al.*, 1983a; Gilbert and Ward, 1998; Hryhorczuk *et al.*, 2001; Recer *et al.*, 2001; Tolvanen *et al.*, 2005; Fischer *et al.*, 2008). Only viable micro-organisms were sampled to estimate the concentration of bioaerosols and in this context actinomycetes and *A.fumigatus* colonies were identified by visual inspection. Media preparation, inoculation, dilution and sterilization of all equipment used on site and in the laboratory were conducted in accordance with British Standard 5763 Part 0: General laboratory practices (BSI, 1996).

The actinomycetes were grown onto Petri dishes (Fisher Scientific, aseptic, polystyrene, 90 mm diamater and triple vent) containing two different agars, namely half strength nutrient agar (Oxoid) and a compost agar, developed by Taha *et al.* (2007b). This is agar-agar (Fisher Scientific, granular) mixed with a supernatant of 10% w/w loam-based compost (John Innes No. 1 compost). After preparation, the media were sterilised in an autoclave for 15 minutes at 121ºC and then allowed to cool to a temperature of 47ºC. Following this, 1% w/w cycloheximide (Acros Organics, 95%) in ethanol is added to the media to prevent the growth of fungi.

Aspergillus fumigatus was inoculated onto Petri dishes containing Malt Extract Agar (Oxoid) (Burge, 1992). The media was treated with 0.01% w/w antibacterial chloramphenicol (Sigma) before sterilisation, as chloramphenicol is temperature resistant. The media was sterilised in an autoclave for 15 minutes at 121ºC and then allowed to cool to a temperature of 47ºC as described for actinomycetes.

The dissolved, sterilised and treated media was poured into Petri dishes at around 2mm thickness (20 ml of agar per Petri dish) and left to solidify in a laminar-flow safety cabinet for 30 minutes with half open lids.

3.3.2. Post-sampling Laboratory Practices

Immediately after sample collection, the sampling cassettes containing the filter were placed and immersed in a 30ml vial (Nalgene) that contained a 10ml buffer solution (0.05% w/w Tween-80, 0.1% w/w NaCl and de-ionised water) and placed in an ice-box maintained at <4ºC. This solution is used to prevent cell osmosis of the collected air sample during transport to the laboratory. The de-ionised water and 0.1% w/w NaCl were sterilised at the same temperature and for the same length of time as described for the media. However the 0.05% w/w Tween-80 was added after the sterilisation took place as Tween-80 agent is heat sensitive.

On return to the laboratory, the filter was separated from the filter cassette and then shaken in the vial for 1 minute using a rotamixer (Hook and Tucker Instruments). The solution was then diluted in a common logarithmic order (10^0 (original sample), 10^{-1} and 10^{-2}) and used to inoculate the Petri dishes. This took place within 48 hours of the sample collection, due to the restrictions caused by the distance between the laboratory and the sites.

The Petri dishes were incubated for 7 days at 44ºC for actinomycetes and for between 3-5 days at 37ºC for A*spergillus fumigatus*, prior to counting by visual inspection. The incubation duration for *A. fumigatus* was variable as the petri dishes were kept in the incubation longer (up to 5 days) if no growth was observed after 3 days. The actinomycetes were identified by their white powdery appearance, as well as their characteristic “spider web-like” filamentous structure, depending on the stage of growth (Figure 3.9).

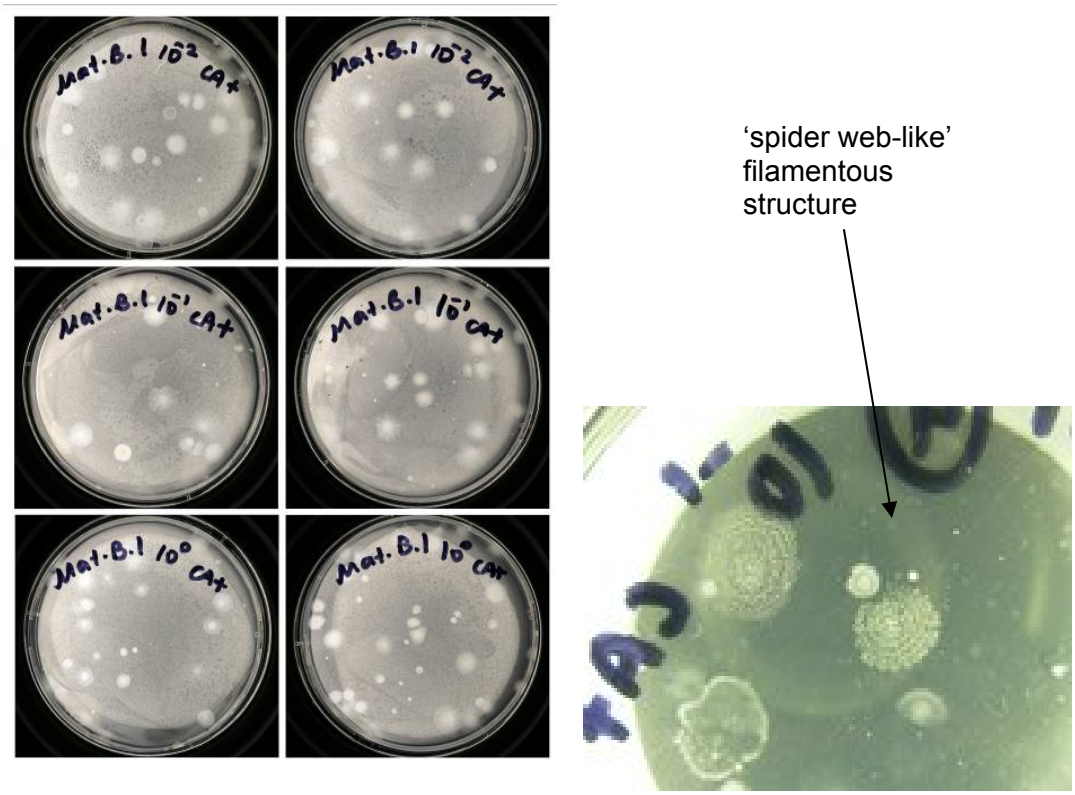


Figure 3.9- Actinomycetes culture plates

A. fumigatus was identified by its characteristic powdery blue-green appearance in the front and brown appearance at the back of the Petri dish (Figure 3.10).

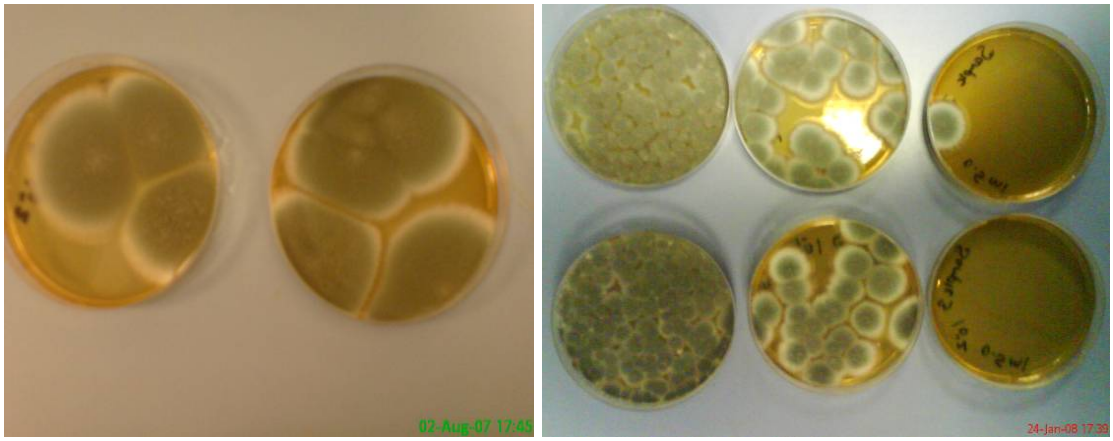


Figure 3.10- *A. fumigatus* culture plates

3.3.3. Expression of Results

Following incubation and counting of the colonies, the results were expressed using the equations from British Standard 5763 Part 0: General laboratory practices (BSI, 1996). If any of the inoculated plates had between 15 and 300 colonies, the concentration of

bioaerosols in the sampling solution (*N*) was calculated using the following equation as a weighted mean from two successive dilutions:

$$N = \frac{\sum C}{V \times \left[n_1 + (0.1 \times n_2)\right] \times d} \qquad \textit{Equation 3.1}$$

Where:

$\sum C$ is the sum of the colonies counted on all the dishes retained from two successive dilutions, and where at least one contains a minimum of 15 colonies (CFU);

V is the volume of inoculums applied to each dish (ml);

n_1 is the number of dishes retained at the first dilution;

n_2 is the number of dishes retained at the second dilution; and

d is the dilution factor corresponding to the first dilution retained [d = 1 when the undiluted liquid product (test sample) is used].

If there were no plates with more than 15 colonies, the estimated value of bioaerosols in solution (*N)* was calculated using the following equation:

$$N = \frac{\sum C}{V \times n \times d} \qquad \textit{Equation 3.2}$$

Where:

$\sum C$ is the sum of colonies counted on the two dishes (CFU);

V is the volume of the inoculums applied to each dish (ml);

N is the number of dishes retained (in this case, n = 2); and

d is the dilution factor corresponding to the dilution retained.

The calculated concentration of the solution (10ml liquid in 30ml vial) was then used to determine the concentration of bioaerosols in the sampled air in cfu/m^3 using the following equation:

$$B_{con} = \frac{N}{F_s \times t} \qquad \textit{Equation 3.3}$$

Where:

B_{con} is the sampled air bioaerosols concentration (cfu/m^3);

N is total number of bioaerosols in solution;

F_s is the air pumping or sampling flow rate (l/min); and

t is the sampling period (min).

3.4. IDENTIFICATION AND ANALYSIS OF BIOAEROSOLS BY SCANNING ELECTRON MICROSCOPY

The samples for SEM analysis were collected by drawing air through a personal sampler fitted with a polycarbonate filter (25 mm diameter, 0.8 µm pore size SKC). The filter had an effective diameter of 15 mm and a face velocity of 7.47 cm/s. Following collection, the filters were placed in pre-sterilised (autoclaved at 15 min at 121^{0}C) plastic vials (not filled with the buffer solution as for culture samples) for the purposes of transportation. On return to the laboratory, the filters were mounted onto a 25.3 mm (diameter) SEM stub before being gold coated within 24 hours of sample collection. The filters were gold-coated for the analysis (Polaron Equipment Ltd., SEM gold coating unit ES100) as preliminary experiments showed that non gold-coated filters resulted in very low resolution images. The filter was examined with high-resolution Scanning Electron Microscope (XL30SFEG, Phillips) in SEM mode (10-12 kV beam size, 3-4 spot size) according to standard SEM practices. The number of particles per sample were counted from a total of 100 viewing fields (as per Heikkilä *et al.*, 1988) at a magnification of x2000. On average, counting of particles per sample from a total of 100 viewing fields took 2-3 hours per filter and scanning the entire filter with the SEM would have been impractical.

3.4.1. Sampling Protocol

The first viewing field of the filter was located by using the co-ordinates for the bottom edge of the filter at a magnification of 30 (x= -6000, y= -6000). Magnification was increased to x2000, the scale at which it is possible to identify the shape, size and structure differences between individual particles. Ten viewing fields containing

'particles of interest' (i.e. particles larger than 0.5 µm in size and not pre-existing particles inherent in an non-exposed filter) were selected at this magnification. When a particle was identified, all details were recorded on the sampling proforma (Appendix B). 'Blank' field areas of the filter (areas without any particles in them) scanned at a magnification of x500, x1000 and x2000 were also recorded to calculate the total area of the filter that was scanned. The viewing fields on the sampling filter are schematically represented in Figure 3.11.

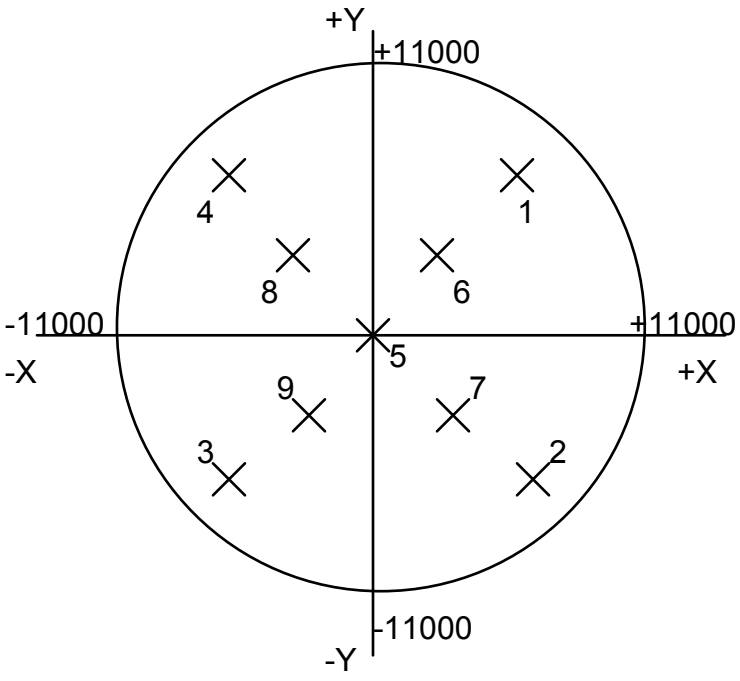


Figure 3.11-SEM sampling protocol co-ordinates where:
1) upper edge (x= 6000, y= 6000)
2) right edge (x= 6000, y= -6000)
3) bottom edge (x= -6000, y= -6000)
4) left edge (x= -6000, y= 6000)
5) middle (x=0, y=0)
6) upper middle (x= 3000, y= 3000)
7) right middle (x= 3000, y= -3000)
8) left middle (x= -3000, y= 3000) and
9) bottom middle (x= -3000, y= -3000) **totalling a 100 viewing fields (middle is scanned for 20 viewing fields). The circle denotes the sampling filter.**

This method was developed as it was decided that a systematic sampling regime would ensure the reproducible analysis of the air samples and avoid bias regarding areas of the filter that are concentrated with particular particles. However, it is important to note the limitations to this method which makes it difficult to identify areas of filter with high

counts (concentrated particles of interest areas). Furthermore, the classification of the shape and nature of particles of interest is based on subjective assessment.

Scans of blank filters showed that co-ordinates of x= -11000, x= 11000, y= -11000, y= 11000 represented left, right, top and left edges of the filter. However it was observed that the outer edges of the filters are high in inherent particles (Figure 3.12) which are probably pre-existing due to the filter production process. It was found easy to mistake these for genuine particles of interest, therefore the upper, bottom, left and right edge co-ordinates were chosen to avoid this.

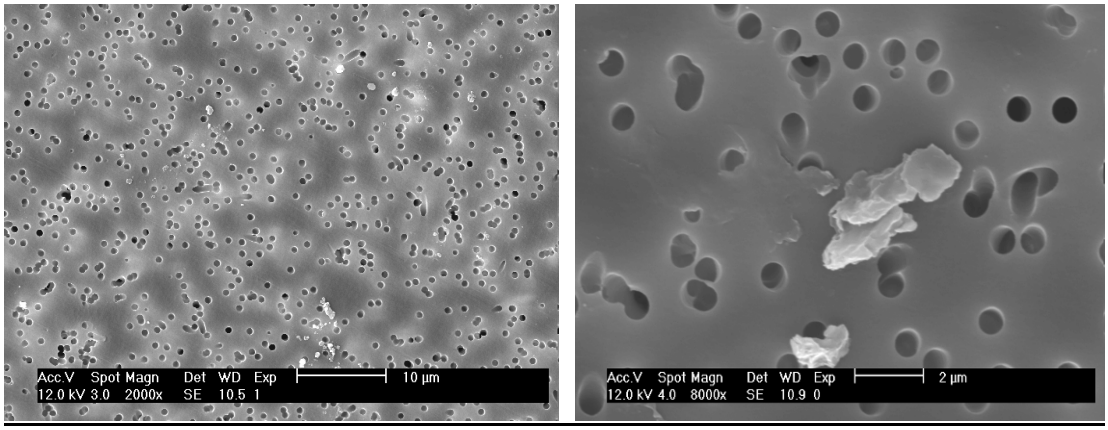


Figure 3.12- SEM images of pre-existing particles observed in filters which have not been used for analysis

Therefore the full SEM sampling protocol was developed and is presented as follows:

a) Take air sample with bioaerosol sampling filter (SKC 0.8 µm pore size, 25 mm diameter, polycarbonate) using SKC personal samplers.

b) Mount filter onto 25.3 mm (diameter) size SEM stubs.

c) Gold-coat the filter and prepare for SEM analysis (using standard SEM practices, 10-12 kV beam size, 3-4 spot size).

d) Place filter in the middle of the inspection stub (x=0, y=0).

e) Begin inspection of sample in SEM according to standard SEM analysis practices.

f) Record all 'blank' field areas of the filter (without particles of interest) scanned at a magnification of 500, 1000 and 2000.

g) Put co-ordinates for upper edge of the filter at a magnification of 30 (x=-6000, y=-6000).

h) Increase magnification to 2000 and pick 10 viewing fields containing "particles of interest" (i.e. particles larger than 0.5 µm in size and not pre-existing particles inherent in an non-exposed filter).

i) If the particle visual properties are unclear, analyse the particle at a higher magnification.

j) Record details (i.e. size, shape, type of particles and their aggregates) of all particles on the proforma sheet (Appendix B)

k) For each co-ordinate that represents the bottom, left, right edges and the middle areas of the filter; re-adjust magnification to 30.

l) Repeat steps h-j between each co-ordinate change.

m) Calculate the particle/m^3 counts of particles that were identified (will be explained in Section 3.4.3).

3.4.2. Sampling Image Guide

The collected filters were examined by SEM. The particles that were observed were divided into categories depending on shape and size. The number and structure of the particle along with experiment and image scanning details were recorded in the sampling proforma.

The presence of numerous types and sizes of particles that were deliberated to be bacterial, fungal and actinomycetes cells as well as their aggregates on the sampling filters were identified using SEM imaging. This assumption was based on previously published SEM images of fungal, bacterial and actinomycetes cells and spores (Kormendy and Wayman, 1972; Heikkilä *et al.*, 1988b; Karlsson and Malmberg, 1989; Chalupová, 1994; Prescott *et al.*, 1999a; Prescott *et al.*, 1999b; Klich, 2002; Wittmaack *et al.*, 2005) as well as SEM images of pure cultures of *A. fumigatus* and actinomycetes captured as part of the preliminary experiments. However identification and confirmation of the bioaerosol species was not within the scope of this study as visual properties of particle size, shape and aggregation of identified particles were of interest irrespective of the bioaerosol species. In addition, methods of sample preparation for SEM might

result in the dehydration of the sample which causes collapse and distortion of the image. This would present difficulties in identification of particular bioaerosol species. Other studies (Heikkilä *et al.*, 1988b) have reported similar difficulties where scanning electron microscopy classification at the species level was not possible due to morphological similarities between *Aspergillus* and *Penicillium.* Heikkila *et al.* (1988b) has advised that spore identification by SEM was only possible by preparation of pure culture spore images and comparison of the captured species with those. It is also important to note that for the purposes of this study, the term 'cell' will denote all bioaerosols released from compost and will refer to both spore and non-spore cells.

In addition to the bacterial and fungal cells, several other particles were also identified on the sampling filters. These particles were identified only occasionally and some of them have also been observed as pre-existing particles due to the structure of the unexposed filter. A small number of pollen-like cells were also identified by SEM on the sample filters analysed for Donarbon Limited and Lount site work. However, these particles are not included in the analysis of results and are listed in Appendix C. The full image guide of the particles analysed in the results are presented in Appendix D.

3.4.3. Expression of Results

Following examination and counting of the images by SEM, the results were expressed using equations based on British Standard, BS ISO 14966:2002, 'Ambient Air – Determination of numerical concentrations of inorganic fibrous particles – scanning electron microscopy method' (BSI, 2002). This is in accordance with other studies (Heikkilä *et al.*, 1988a, 1988b) that have used asbestos counting criteria to identify and count fungal spores. This method is suitable for determining concentrations of inorganic fibres such as asbestos particles for air quality purposes and has been suggested for use on non-spherical particles with a minimum aspect ratio of 3:1. This might not be applicable to bioaerosol particles and cells which might be spherical (i.e. aspect ratio of 1). However due to the lack of any standards specific to determining numerical concentrations of bioaerosols or cells, the principles of this method have been adapted for determining the numerical concentrations of particles observed by scanning electron

microscopy. Calculation of the numerical concentration for particle classification *i* is as follows:

$$c_i = \frac{n_i}{N.V_B} \qquad \textit{Equation 3.4}$$

Where,

$$V_B = \frac{4.F_B.V}{\pi.d_{eff}^2} \qquad \textit{Equation 3.5}$$

And

c_i is the numerical particle concentration of particle classification *i,* in particles per cubic metre;

n_i is the number of particles counted for particle classification *i*;

N is the number of image fields examined;

V_B is the sampled air volume, in cubic metres, per image field;

F_B is the area of the image field, in square millimetres;

V is the sampled air volume, in cubic metres;

d_{eff} is the effective filter diameter (diameter of the exposed circular filter area), in millimetres.

3.5. STATISTICAL ANALYSIS

For the data presented throughout Chapters 4-7, arithmetic mean values were determined to describe the data and where applicable, standard error was determined to measure variability. In addition, for some of the data presented in Chapter 4, a correlation analysis between different data groups was completed. The statistical tool used for these purposes was Microsoft Office Excel 2003.

For the data presented in Chapters 4 and 5, a one-factor analysis of variance (ANOVA) and where applicable a Fisher test was used to test the differences between independent data groups. The accuracy of the statistical analysis was first checked graphically and when the data group did not follow the normal distribution then the data were normalized by applying a square root or a natural logarithm transformation. The statistical tool used for this purpose was by STATISTICA 8 (StatSoft Ltd.).

3.6. HEALTH, SAFETY AND ANALYTICAL QUALITY CONTROL

Media preparation and inoculation of samples were conducted in a Class 2 laminar flow safety cabinet (Labcaire Systems) to prevent any cross-contamination from the laboratory environment and between different samples that were analysed. The safety cabinet was cleaned with 70% ethanol before and after use. All equipment and samples used were sterilised, clearly marked and care was taken to minimise the risk of cross-contamination.

A blank sample was taken for each sample to monitor any background contamination presented by the sampling environment. This sample was taken in the same fashion as described previously however the sampling pump was not running. The blank sample was analysed in the same way as all samples. In addition to these, empty petri dishes, petri dishes inoculated with only the autoclaved buffer solution and petri dishes inoculated with a buffer solution containing an autoclaved but unused filter were incubated to determine the levels of contamination in the laboratory environment. The total concentrations of contamination determined from these control petri dishes were deducted from the experimental results.

For SEM imaging, blank sampling filters taken from the container were analysed for any anomalies. In addition to this, filters that were autoclaved but not used for sampling were also analysed to determine any contamination presented by the laboratory environment. The transfer of sample filters onto SEM inspection stubs were conducted in a safety cabinet cleaned with 70% ethanol (before and after use) to avoid contamination from the laboratory. All equipment and samples used for the SEM analysis were sterilised, clearly marked and handled in a manner that minimised the risk of cross-contamination.

The health and safety requirements of the laboratory were strictly followed and COSHH (Control of Substances Hazardous to Health) assessments of hazardous chemicals were prepared and followed. The health and safety requirements of the specific site were adhered to during site visits which included practices such as wearing a reflective jacket, steel toed safety boots, safety goggles and safety helmets.

CHAPTER 4. AGGREGATION AND SIZE DISTRIBUTION OF BIOAEROSOLS EMITTED FROM COMPOST

4.1. INTRODUCTION

Understanding the physical and microbiological characteristics of bioaerosols is important when assessing their risks on emission from composting facilities. However, there are gaps in our understanding of bioaerosols after release from composting facilities. One such gap that has implications for bioaerosol behaviour at source, pathway and receptor is the lack of information on the aggregation and size distribution of bioaerosols released from compost as discussed previously (Chapter 1).

Assessment of bioaerosol behaviour following emission from source is dependent on the study of bioaerosol properties such as aggregation and size distribution. This is because microbial aggregation and overall biaoerosol size has an impact on the collection efficiency of bioaerosol samplers such as impactors (Jankowska *et al.*, 2000; Trunov *et al.*, 2001). As such, during air sampling carried out at a bioaerosol source, aggregated particles, because they are larger and hence heavier, might have higher inertia compared to single particles resulting in an increased likelihood of impact on collection media (Trunov *et al.*, 2001). Similarly Eduard and Aalen (1988) have attributed microbial aggregation to the non-random distribution of the spores on the filter samples they analysed. Such effects on the collection efficiencies of bioaerosol samplers may lead to under (Karlsson and Malmberg, 1989) or over-estimation of the bioaerosol particle count collected at source.

In addition to the lack of information regarding aggregation and size distribution of bioaerosols released from compost, there is a distinct gap of information regarding observed physical properties of cells and spores in general (Dufréne, 2000). The study of spores by collection onto membrane filters and subsequent SEM analysis to determine their physical properties has previously been carried out (Eduard *et al.*, 1988; Karlsson and Malmberg, 1989, Heikkilä *et al.,* 1988a and Heikkilä *et al.,* 1988b) for agricultural environments and saw mills but not for composting facilities.

It is important to note the difference between aerodynamic size and physical size of a bioaerosol. The physical size of a bioaerosol is determined by its width and length as measured under a microscope. The aerodynamic size takes the shape, surface structure and the density of the bioaerosol into account (Reponen *et al.*, 2001). Bioaerosol sizes reported in studies using samplers such as the Andersen or the aerodynamic particle sizer are based on the aerodynamic diameter of the bioaerosol.

The limited number of studies that have examined bioaerosol size distributions from composting facilities are based on data from size selective bioaerosol samplers (i.e. the Andersen sampler) or devices such as the optical particle counter (Reinthaler *et al.*, 1997; Pillai and Ricke, 2002; Byeon *et al.*, 2008). This methodology might provide information regarding bioaerosol aggregation as this would be linked to the overall bioaerosol size, however determination of the visual characteristics of a particulate contaminant is also important. This is because the response of a bioaerosol to the effects of forces such as Brownian motion, gravity, thermal gradients or relative humidity are based on their shape as well as other physical properties (Pillai and Ricke, 2002). For a spherically shaped spore, the rate that this spore falls to the ground due to gravitational forces is proportional to the square of its radius (Gregory, 1973). However non-spherically shaped spores might affect this rate due to an increased surface drag that would result in a delay in deposition (Lacey, 1991; McCartney, 1994; Levetin, 1995).

A recent study (Kanaani *et al.*, 2008) has explored the deposition rates of fungal spores in indoor environments compared to those for non-biological particles. They found that particle deposition rates for *Penicillium* and *Aspergillus,* which was attributed to their densities and shapes, were similar. The deposition rates of these fungi were also found to be similar to those for non-biological particles of canola oil and talcum powder which they believed indicated that aerosols of similar sizes behaved similarly regardless of their biological nature. In addition, devices such as the optical particle counter which has been used for determining the particle size distributions from composting facilities, were found to be non selective for bioaerosols (Kanaani *et al.*, 2008). Hence unless the use of

such devices is complemented by others that are selective for bioaerosols, they may not be suitable for determining the bioaerosol size distribution at composting facilities.

The behaviour of a bioaerosol after release from a source such as a compost windrow is governed by various physical (i.e. gravitational forces, Brownian motion) and environmental factors (i.e. wind speed, relative humidity and temperature) (Pillai and Ricke, 2002). However it is the properties of a bioaerosol cell such as the size, shape, surface characteristics and 'aggregation' tendency that also affect this behaviour (Levetin, 1995; McCartney, 1994; McCartney *et al.*, 1997a). For example, a larger particle might be subject to deposition velocities higher than for a small particle (Wheeler *et al.*, 2001; Swan *et al.*, 2003). Hence this might suggest that larger particles, such as a bioaerosol aggregate, released from compost are more likely to settle out downwind of a bioaerosol source instead of remaining suspended in air (i.e. non-gaseous behaviour) (Pillai and Ricke, 2002) and travel less far downwind of source compared to smaller particles. Such behaviour might complicate the modelling of bioaerosols emitted from composting sources (Wheeler *et al.*, 2001) and result in differences between simulated and measured downwind bioaerosol concentrations. Therefore, the particle size distribution of bioaerosols emitted from compost and their tendency to form aggregates may be important factors in predicting their dispersal (Madelin and Johnson, 1992).

Finally, the understanding of bioaerosol aggregation and size distribution at receptor is also important as it is the size of a bioaerosol that governs its inhalability predictability and the subsequent health effects. For example, particles that are smaller than 6 µm can be transported into the lung (Pillai and Ricke, 2002) and particles in the range of 1-2 µm can be retained in the alveoli (Randall and Ledbetter, 1966; Sattar and Ijaz, 1987). In contrast, larger particles (i.e. > 6 µm) are lodged only in the organs of the upper respiratory tract such as the nose (Pillai and Ricke, 2002).

Therefore controlled experiments were completed to classify the overall size distribution and visual properties (i.e. aggregation, size and shape) of bioaerosols emitted from compost. This was done to address the gap of information regarding physical

properties, aggregation and size distribution of compost related bioaerosols. A number of hypotheses were formulated for the controlled experiments.

The changes in the microbial population within a typical compost windrow is dependent on factors that also affect the quality of the compost such as C/N ratio, moisture, pH, substrate particle size (Singh *et al.*, 2006). This is especially the case for temperature as the stages in a compost windrow are linked to the temperature profile within it. Stage 1 is the mesophilic stage (ambient to 45^{o}C) where mesophilic bacteria and fungi dominate the composting process (IWM, 1994; Epstein, 1997). This stage has been reported to last for 2-3 days (IWM, 1994). Stage 2 is the further increase of temperature in the thermophilic stage (45-75^{o}C) starting around day 8 (Catton, 1983 from IWM, 1994) where at its peak, the microbial fauna consists only of thermophilic micro-organisms (IWM, 1994; Epstein, 1997). After around day 10, the compost windrow might have lost 40% of its bulk and the compost is pasteurised (IWM, 1994). This results in a decline in temperature which prompts re-population by other mesophilic micro-organisms (IWM, 1994) by day 20 (Catton, 1983 from IWM, 1994). However the rate of growth at this stage is slower than the start of the composting process (IWM, 1994). The third stage is the second mesophilic stage which can continue for months and temperature of the compost falls toward that of ambient (IWM, 1994). The biodiversity of micro-organisms at this stage is also reduced with only a few species becoming dominant (IWM, 1994). The final stage of the composting process is the maturation phase which can take six months or more with low levels of microbial activity (IWM, 1994). Similarly, Lacey (1997) has discussed that the concentrations of actinomycetes might decrease as a compost windrow matures and the water content of the compost decreases. Therefore based on this information, it is first hypothesised that *"total number of bioaerosols released from compost will decrease as the compost matures".*

Compost types such as green waste and kerbside collected garden and kitchen waste is produced by the decay of garden waste consisting of leaves, wood etc. The fibres present in this matter such as wood fibre might often be present in microscopic sizes in compost at even its final stages. Therefore, there is the likelihood that bioaerosol

particles released from compost might be attached to these fibres, which serve as a 'raft' to aid their travel following emission (Lighthart and Stetzenbach, 1994). In line with this, Lighthart (1997) has reported that 40% of the particles that contain bacteria in the atmosphere are larger than 7 µm because they are attached to debris. Therefore the second hypothesis is that *"bioaerosols released from compost will also be attached to wood fibres and other non-microbial matter"*.

Two of the most prevalent micro-organisms found in compost (i.e. actinomycetes and *A. fumigatus*) produce branching filamentous chain structures called mycelium (Lacey, 1997; Reynolds and Pepper, 2000; Papagianni, 2006). An actinomycete colony usually has an aerial mycelium (Figure 4.1) extending above the growth substrate forming asexual spores called conidia or conidiospores (Prescott *et al.*, 1999a).

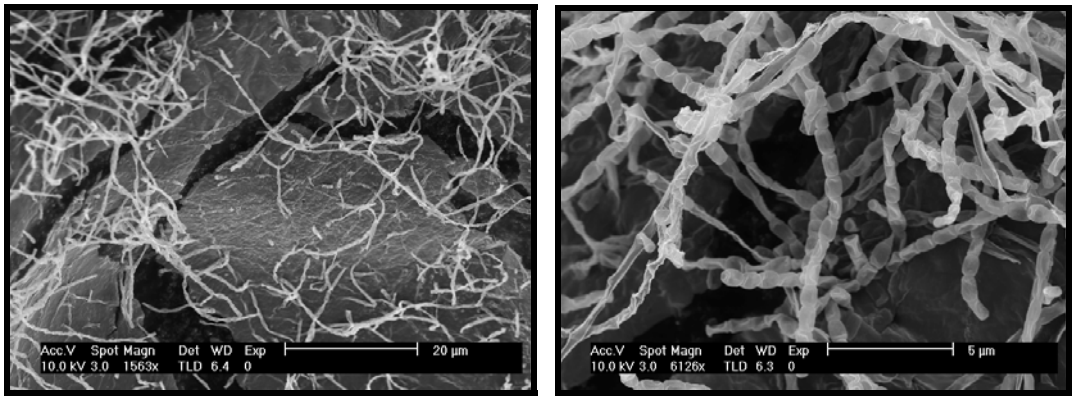


Figure 4.1 – Images of the actinomycetes branching filamentous mycelium structure

A. fumigatus dominantly produce asexual spores in flower-like structures called conidiophores with individual spores named conidiospores (Figure 4.2) (Prescott et *al.*, 1999b; Klich, 2002).

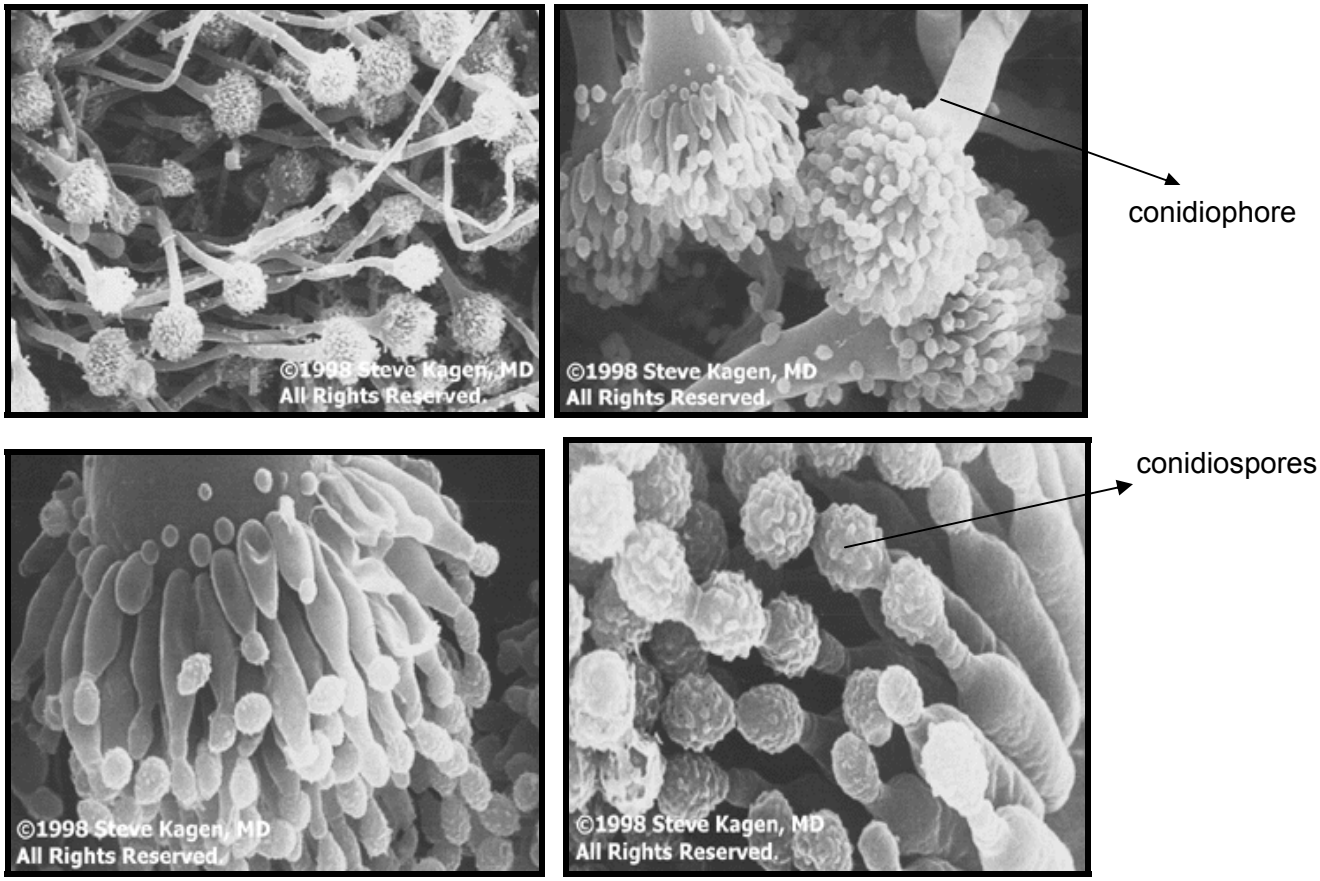


Figure 4.2 – Images of the *Aspergillus fumigatus* structure (from: Allernet, www.allernet.com/PHOTOS/m002a.html, accessed 10th February 2009).

Upon agitation of the compost, several scenarios would be expected regarding the release of such mycelia and conidiophore. The first scenario would entail the release of such filamentous chain structures such as those for *Penicillium* spores which are released in short chain structures when aerolised from their growth surface (Jankowska *et al.*, 2000). Similarly Madelin and Madelin (1995) have reported that pieces of mycelium can be blown away from contaminated surfaces. Therefore, in the first scenario, when compost is agitated or factors such as wind affects a static compost windrow, the mycelial structures present in compost would be expected to break off in smaller pieces and be released as aggregates (e.g. filamentous aggregates). However upon release it would be expected that environmental effects such as wind break up these aggregate structures into smaller cell or spore structures. This is because the filamentous aggregates are fragile in nature and easily susceptible to being broken into smaller fragments by mechnical forces (i.e. wind).

A second scenario entails the release of single spores from the mycelial structure or a conidiophore upon agitation of the compost. This might be the predominant form of release as micro-organisms that are released a single spores as opposed to aggregates might have a competitive dissemination advantage. In addition, it is also plausible that wind might spread single spores from structures such as a conidiophore in unagitated compost. Therefore it is thirdly hypothesised that *"the majority of bioaerosols released from compost will be in single cells".*

Microbial aggregation is the gathering of individual cells to make a larger particle/unit (Calleja, 1984b) and might also occur if cells that were originally dispersed clump together (Calleja *et al.*, 1984a). Therefore even if the majority of the bioaerosols released from compost are single spores or cells, these single spores emitted singly could aggregate after emission from compost. There is no previous literature which has examined the aggregation of biaoerosols released from compost. However other studies which have examined agricultural dust have claimed that fungal spores occur in aggregates made up of 2-6 spores when airborne (Karlsson and Malmberg, 1989; Lacey, 1991). Therefore in connection to the previous hypothesis (hypothesis three), it is predicted that "*if bioaerosol aggregates are observed to be emitted from compost, the number of aggregate structures made up of 2-6 units will be in a higher percentage than those made up of 7 or more units*".

There are no previous studies for composting facilities which have compared bioaerosol concentrations determined by culturing to those for particle concentrations determined by scanning electron microscopy. However there are a number of studies that have made similar comparisons for other industries. One such study by Heikkilä *et al.* (1988) has reported a 10- to 100- fold difference in culture based and SEM results when analysing fungal spores in cow barns and attributed this to the fact that only a small proportion of spores in the air were viable when collected for culture analysis methods. They have shown that spores are released as both single spores and aggregates of spores however an aggregate may form only one microbial colony when cultured (McCartney *et al.*, 1997a). Hence the fourth and final hypothesis is that *"compost*

related bioaerosol concentrations determined using by scanning electron microscopy analysis will be higher than those determined by culture analysis".

To test these hypotheses, the following chapter objectives were set:

- generate bioaerosols and particles in experimental conditions;
- test the viability of using SEM analysis to characterise the particle size distribution and aggregation of bioaerosols and particles emitted from compost;
- examine the effect of compost age on the concentrations, particle size distribution and aggregation of bioaerosols and particles emitted from two different feedstocks of compost;
- compare the differences in culture based and scanning electron microscopy based bioaerosol analysis.

To achieve these objectives, controlled experiments were completed to generate bioaerosols in experimental conditions and to study the size distribution and aggregation properties of bioaerosol particles released at source without interference from ubiquitous and background bioaerosols. The compost inside the experimental chamber was agitated to release the spores and particles similar to other studies which have used mechanical handling (Lacey and Dutkiewicz, 1976a), air currents (Madelin and Johnson, 1992) and swirling-flow dispenser (Reponen *et al.*, 1997; Reponen *et al.*, 1998) to release spores.

This chapter firstly discusses the controlled experiments methodology that was adopted. Following this, the results section presents the comparison of culture and scanning electron microscopy analysis for the controlled experiments and the scanning electron microscope results including details of total particle counts, particle classification and aggregation. Finally the results are discussed.

Related studies were completed at composting sites to validate the results presented in this chapter and to examine the size distribution and aggregation of bioaerosols on a composting site. These studies will be discussed in the next chapter (Chapter 5) which

will also aim to present key implications of the results and conclusions from both chapters (Chapter 4 and 5).

4.2. METHODOLOGY

4.2.1. Collection of the Samples for Controlled Experiments

A compost tumbler (Figure 4.3, Blackwall, Aerobic Compost Tumbler) was utilised for the controlled experiments to examine the effect of compost type and age on bioaerosol concentrations, size distribution and aggregation. The compost tumbler had a capacity of 200 L, and was made from plastic with a width of 690mm and height 1170mm.

The types of compost examined for these experiments were green waste (green/garden waste, commercial growers' waste) and kerbside collected kitchen and garden waste (variety of kerbside-collected organic waste including catering waste) that were 1 week, 5 weeks and 6 months old. The compost used for the experiments was collected from Donarbon Limited, Cambridgeshire (site description presented in Section 3.2.2 of Chapter 3). This site was chosen due to its proximity to Cranfield University where the controlled experiments and laboratory analysis took place. Donarbon Limited processes a wide variety of wastes including green waste and kerbside collected garden and kitchen waste. The 2005/2006 survey by the Composting Association (2007) has revealed garden waste (civic amenity plus kerbside collected) to account for 85% of all waste types to be composted followed by those for kerbside collected kitchen and garden waste at 11%. These figures were similar in the latest survey (Association for Organics Recycling, 2008) with 83% for garden waste and 10% for kerbside collected kitchen and garden waste. Therefore these waste types were chosen for the controlled experiments as they are the most common source of waste to be composted in the UK.

The ages of compost were chosen to give a representation of the compost at different stages as the microbial flora of compost is different at each stage (Hassen *et al.*, 2001; Michel *et al.*, 2002; Riddech *et al.*, 2002; Ryckeboer *et al.*, 2003; Ivanov *et al.*, 2004). This might mean that a possible variation in the bioaerosols and particles emitted from compost at different ages will be observed. The composting stage for the green waste

compost samples were based on compost age versus composting stage correlations previously reported for green waste compost (Catton, 1983 from IWM, 1994). Based on this principle, the one week old green waste compost will be assumed to be in the thermophilic stage, the 5 week old material will represent those in the second mesophilic stage and the six month old material will represent stabilized and mature compost.

The kerbside collected kitchen and garden waste is first processed in an Animal By-Products compliant in-vessel system for the first two weeks where the oxygen levels and temperature are monitored and controlled. This system ensures that all pathogens are killed. Following this, the compost is matured on concrete pads till approximately 6 months of age. The one week compost samples of kerbside collected kitchen and garden waste were taken from the in-vessel system however due to the high temperatures aimed for in an in-vessel system, this compost is likely to be already in the late thermophilic stage. Similarly, due to the differences in composting technologies, the 5 week old material might represent those in the maturation phase. However the six month old material should represent stabilized and mature compost. Due to these differences, the comparison of results from different compost feedstock will not be made.

The compost samples used for the experiments were taken at a depth of 20 cm of the compost windrow as per Riddech *et al.* (2002) on the morning of the experiment (no longer than four hours prior to the experiment). The samples were kept in a heavy duty refuse bag until experimental use to retain the compost windrow moisture and temperature conditions.

Figure 4.3-Images of the experimental chamber (Blackwall Aerobic Compost Tumbler) used for taking air samples for culture method and SEM analysis

At the start of the experiment, the compost was placed inside the compost tumbler and the screw top lid was closed. The compost tumbler was rotated 360^0 which manually forced agitation of the compost inside. The agitation was introduced to ensure that cells of the micro-organisms growing in the compost are released. This decision is based on previous studies that showed an increase of bioaerosol concentrations during site activities such as turning and screening, compared with background bioaerosol concentrations on site (Sánchez-Monedero *et al.*, 2005; Taha *et al.*, 2006; Taha *et al.*, 2007). 5 kg of compost was chosen as the amount for the experiments because it was not possible to agitate a larger amount of compost as this made the compost tumbler too heavy to rotate manually.

Subsequent to the rotation of the compost tumbler, the IOM sampler heads (Figure 3.1, Chapter 3) attached to 1m long metal rods needed to be put in place inside the compost tumbler through the holes located on its sides (Figures 4.4 and 4.5). This is because placing the IOM sampler heads through the top of the compost tumbler by removing the screw top lid would result in the loss of bioaerosols and particles released into the overhead space of the experimental chamber. Therefore approximately 2-3 minutes after the agitation of the compost which released the bioaerosols into the overhead space of the experimental chamber, air samples were taken inside the headspace using SKC personal air samplers as described previously (Chapter 3).

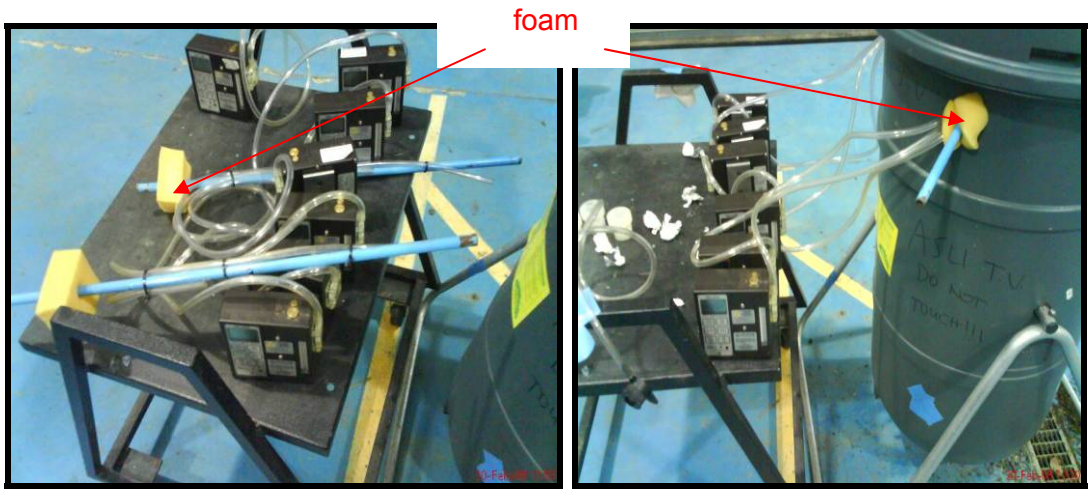


Figure 4.4-Images of the experimental set-up used for taking air samples for culture method and SEM analysis

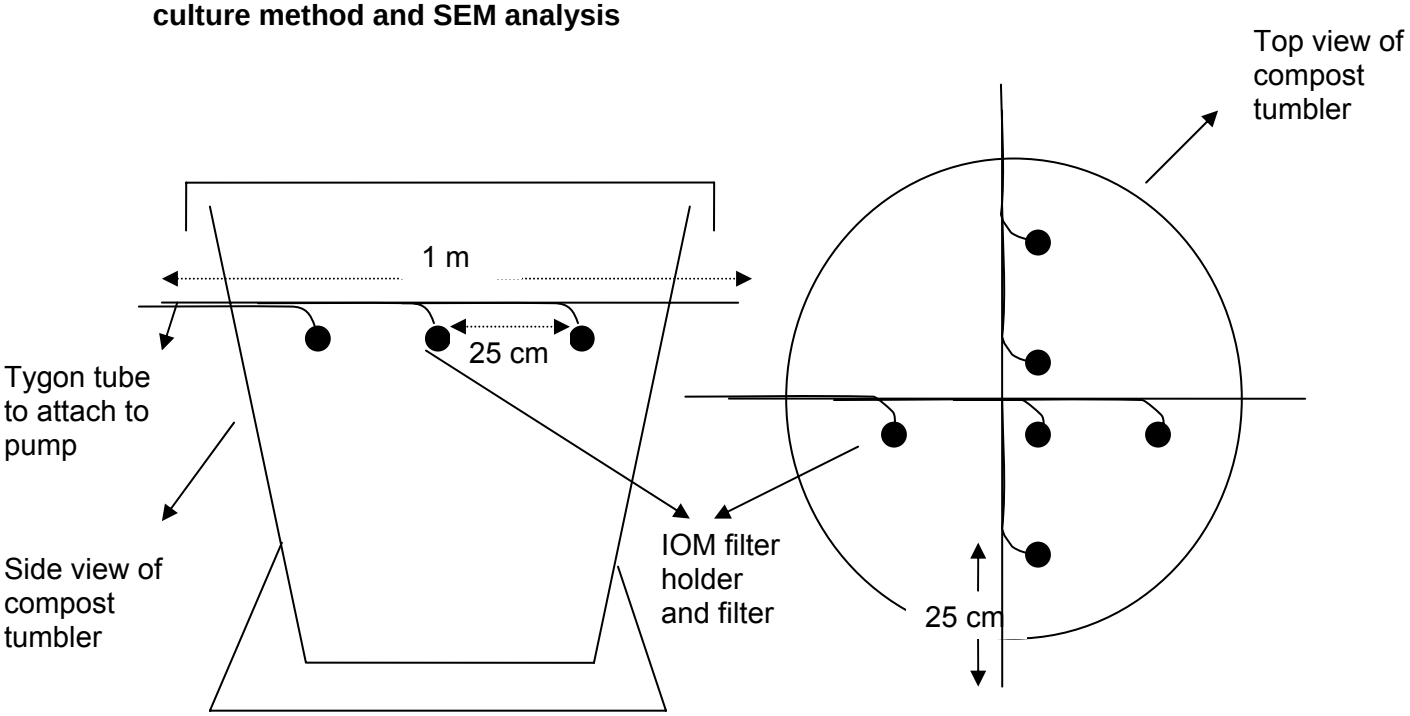


Figure 4.5-Schematic of the experimental set-up

Three samples were collected for Scanning Electron Microscopy (SEM) analysis whilst three samples were collected for culture based analysis of actinomycetes and *Aspergillus fumigatus* simultaneously. Filter sampling was the chosen method for the experiments as it allowed the simultaneous analysis of air samples by culture method and scanning electron microscopy as per previous studies (Pasanen *et al.*, 1989). A further sample was taken from the area where the compost tumbler is located (i.e. the pilot plant hall which is housed in a building) to determine the background levels of

bioaerosols present in the air around the compost tumbler by the culture based analysis. A total of seven air samples per experiment were collected.

The methodology, analytical quality control for culturing of actinomycetes and *A. fumigatus* and expression of results was carried out as described previously (Chapter 3).

4.2.2. Preliminary Experiments

Preliminary experiments were carried out to test the viability of using the SEM to visualise the nature of bioaerosols emitted from a known quality of compost and to test the use of the compost tumbler as a method for conducting controlled experiments. These consisted of experiments undertaken to determine:

- The most effective method of de-contamination between experiments;
- The optimum agitation duration per experiment that would result in the largest release of bioaerosols (i.e. highest concentrations of bioaerosols);
- The optimum sampling duration per experiment that would result in the largest release of bioaerosols.

The de-contamination method experiment was aimed to determine the effect of de-contamination of the compost tumbler between experiments by brushing only, rinsing by water or bleaching. It was hypothesised that bleaching of the compost tumbler would result in reduced bioaerosol concentrations and the results of the first experiment revealed this to be the case. This experiment was repeated a second time however this time the hypothesised result that bleaching of the container would result in reduced bioaerosol concentrations was not achieved and the lowest bioaerosol concentrations were achieved by brushing only after unloading of compost from the compost tumbler. However bleaching (sodium hypochlorite) of the compost tumbler between experiments was adopted as a way of de-contamination instead of cleaning by brushing or water only. This decision was taken as bleaching has been shown to reduce bacteria in previous studies (Rutala and Webber, 1997; Ikawa and Rossen, 1999), able to denature

Aspergillus antigens (Pacheco *et al.*, 2007) and be more effective against other cleaning agents such as ammonia, vinegar or liquid dishwashing detergent (Parnes, 1997).

The next preliminary experiment aimed to determine the optimum agitation duration to release bioaerosol cells into the compost tumbler chamber. Agitation durations of 2, 5 and 15 minutes were tested and it was expected that the longer agitation period of 15 minutes would result in the highest bioaerosol concentrations however it was found that lower agitation durations were as effective as longer ones. As a result of this an agitation time of 5 minutes was adopted for all the experiments as it was decided that a 5 minute agitation time was sufficient to release bioaerosol cells and particles into the overhead of the compost tumbler.

However these preliminary experiments were completed outdoors due to the lack of available indoor experimental space at the time close to the vicinity of a sewage works which are likely to have pre-existing bioaerosols in the air. This might have resulted in the contamination of the filters inside the compost tumbler.

Conclusions were drawn from preliminary experiments completed to determine optimum sampling time. Sampling durations of 30 and 45 minutes have been used for the site work (Chapter 3) and in other studies which have carried out on-site sampling of bioaerosols with personal air filters (Dillon *et al.*, 2006; Taha *et al*., 2006; Taha *et al.*, 2007a). Some studies have used even longer sampling durations of up to 90 minutes for sampling by filtration (Rautiala *et al.*, 2003). Collection of bioaerosols on filters is the method chosen by the German Commission on Air Pollution and Prevention of VDI and DIN (VDI, 2004a) and the recommended sampling time is given as between 10 minutes and 24 hours.

However it was determined that because of the limited air capacity inside the compost tumbler (200 L), air from the outdoor environment might infiltrate into the headspace of the compost tumbler if the sampling activity is carried out for a long duration. In this context, a sampling duration of 30 minutes with the pump flow rate of 2.2 L/min for 6 pumps would result in a total sampled volume of 396 L. This might result in

contamination of the air samples taken inside the headspace of the compost tumbler by the outdoor environment air. Therefore in addition to 30 and 45 minutes sampling times, a shorter sampling time of 15 minutes calculated in the light of the above (15 minutes sampling time with a pump flow rate of 2.2 L/min for 6 pumps resulting in 200L) as well as the effect of 5 minutes were also tested. The results are presented (Figure 4.6)

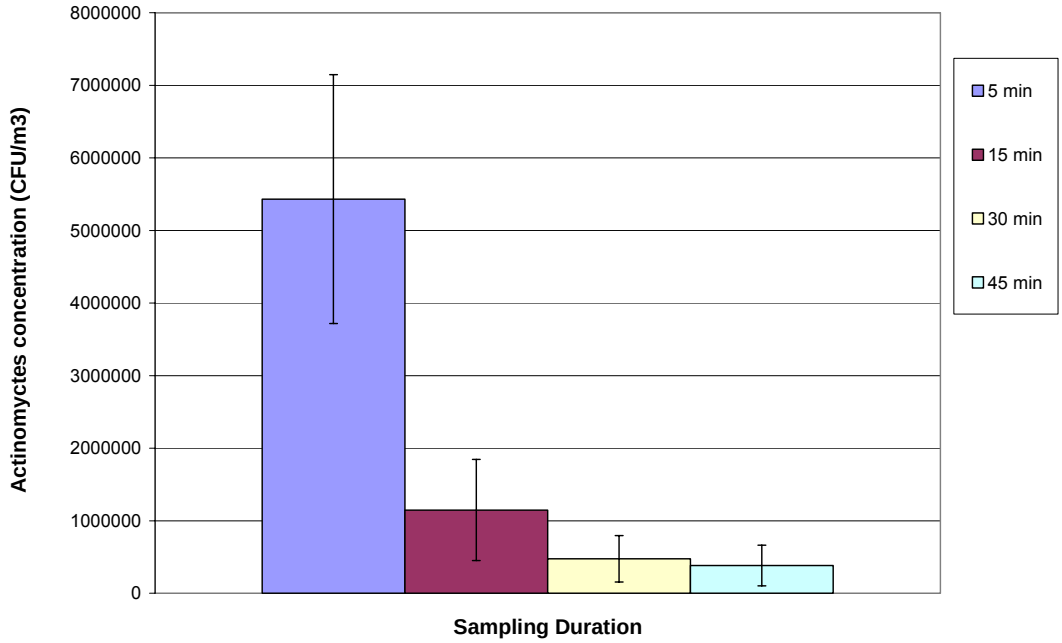


Figure 4.6- Effect of sampling durations of 5, 15, 30 and 45 minutes on actinomycetes concentrations collected by the air filter samples. The bars denote the arithmetic mean (n=3), the error bars denote standard error.

The results showed that the 5 minute sampling duration resulted in the highest concentrations of bioaerosols. The subsequent one factor ANOVA analysis and further Fisher LSD tests also revealed that 5 minute sampling resulted in significantly higher concentrations of bioaerosols ($p = 0.007666$) compared to sampling durations of 15, 30 and 45 minutes and these latter sampling durations were homogeneous in the 95% confidence limit interval. This experiment was repeated a second time to test the effect of shorter sampling times of 1 and 2 minutes as shown in Figure 4.7.

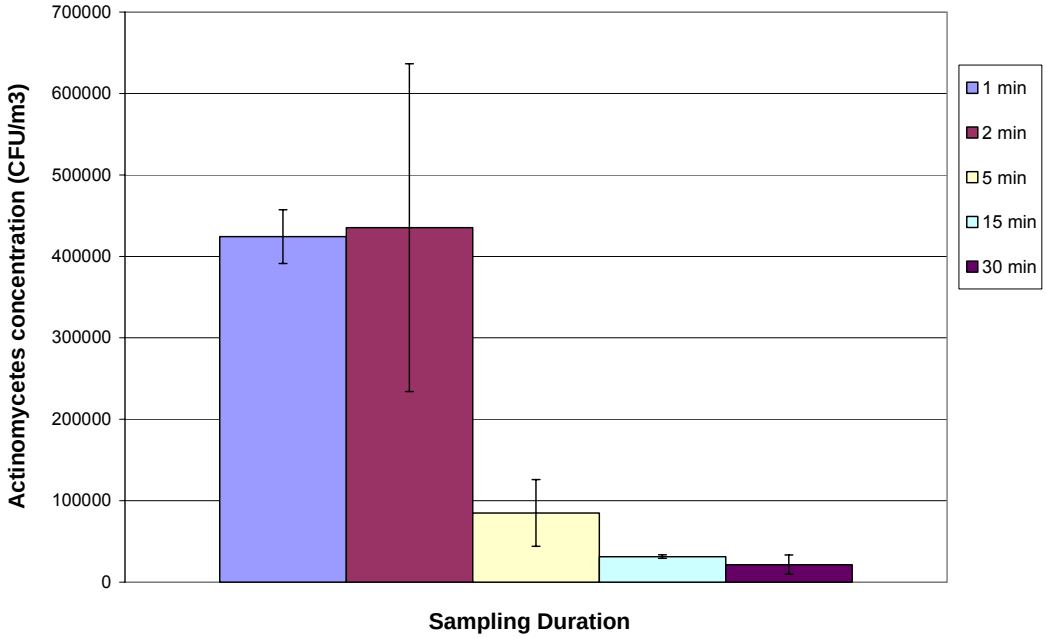


Figure 4.7- Effect of sampling durations of 1, 2, 5, 15 and 30 minutes on actinomycetes concentrations collected by the air filter samples. The bars denote the arithmetic mean (n=3), the error bars denote standard error.

The results showed that a shorter sampling duration of 2 minutes resulted in the highest level of bioaerosols collected from the compost tumbler environment with higher concentrations of bioaerosols compared to all other sampling durations. Statistical analysis showed that the bioaerosol concentrations for 1 and 2 minute sampling durations were significantly higher than those for 5, 15 and 30 minute sampling durations ($p<0.00154$) however were homogenous in the 95% confidence limit interval. However these results were taken from only one experiment and it was not possible repeat this particular experiment whose results are shown in Figure 4.7. Therefore it was felt that a sampling duration of 1 minute might not be sufficient to capture all the bioaerosol particles in the overhead space of the compost tumble and hence a more conservative sampling duration of 2 minutes was chosen for all controlled experiments.

In the light of the findings of the preliminary work, the full sampling protocol was developed and is presented as follows:

a) Prepare the SKC personal samplers for 2 minute sampling.

b) Attach the IOM filter holders to two metal rods (1 m long, needs to be able to fit through the holes of the compost tumbler) using standard cable ties (as shown in Figure 4.3). Place three IOM samplers on each metal rod and place them with the filter side down to enable particles to be drawn in to the filter rather than being deposited on the filter. Take one sample outside the compost tumbler to determine any background contamination in the experiment environment.

c) Place aluminium foil over the IOM filter holders until the compost agitation activity is carried out to prevent any particles depositing on the filter before the commencement of the sampling.

d) Tape all holes on side of the compost tumbler with standard heavy duty tape to prevent the escape of compost through the holes when compost is being agitated as well as to prevent contamination of the air inside the compost tumbler by the outside air.

e) Manually agitate the compost tumbler for 5 minutes by turning the compost tumbler by 360^0

f) Following agitation, remove the foil over the IOM sampling heads and remove the tape from the top side of the compost tumbler.

g) Insert the metal rod with the IOM sampling heads attached into the compost tumbler through the non-taped off holes without taking off the screw on compost tumbler lid. Ensure that the flexible foam placed at the end of the metal road covers the compost tumbler holes so the compost being agitated inside the tumbler is not able to penetrate outside the compost tumbler headspace.

h) Complete the air sampling and remove the samples.

4.3. RESULTS

4.3.1. Image Density of Sample Filters

The total area of the sampling filters scanned by the SEM was recorded. This is the area occupied by 100 viewing fields containing particles of interest (i.e. particles larger than 0.5 μm in size and not pre-existing particles inherent in a non-exposed filter) as described previously and the 'blank' image fields empty of particles of interest (as explained in Section 3.4.1., Chapter 3). Since it is not practical to scan the whole filter for SEM analysis, all subsequent SEM results relate to this portion of the sampling filter.

If the particles of interest on a sample filter are located sparsely (Figure 4.8), this would result in the recording of a high number of 'blank' viewing fields in between the particles of interest. Since each sample filter needs to be scanned for 100 viewing fields containing particles of interest, this would result in a higher percentage of the overall sample filter being analysed. However, a sample filter that is heavily populated with particles of interest (Figure 4.9) would result in a smaller number of 'blank' viewing fields being recorded. This would mean that a lower percentage of the overall sample filter is scanned.

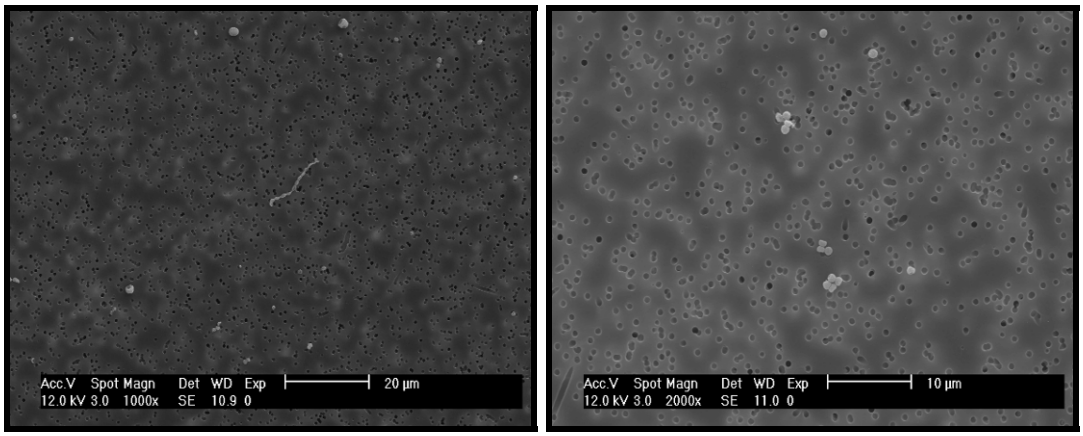


Figure 4.8- SEM Images of Sampling Filters Sparsely Populated with Particles

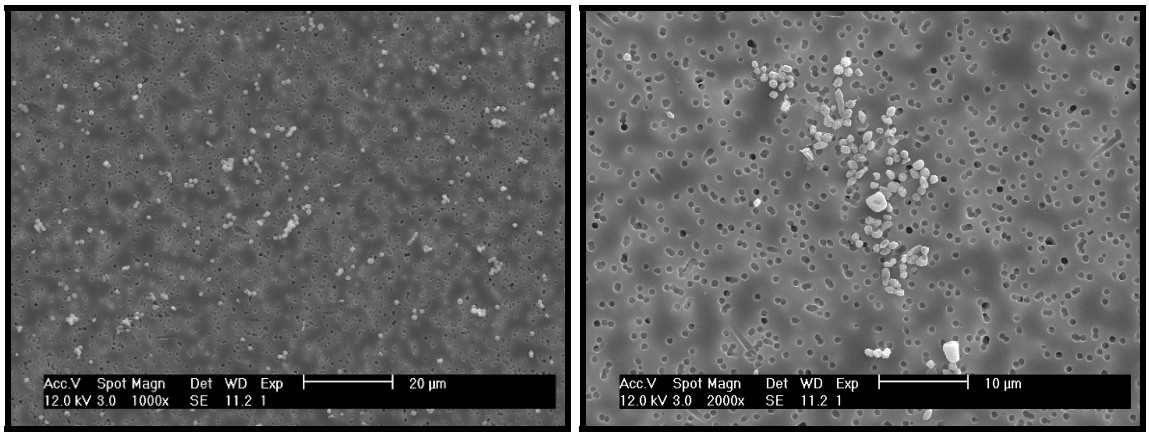
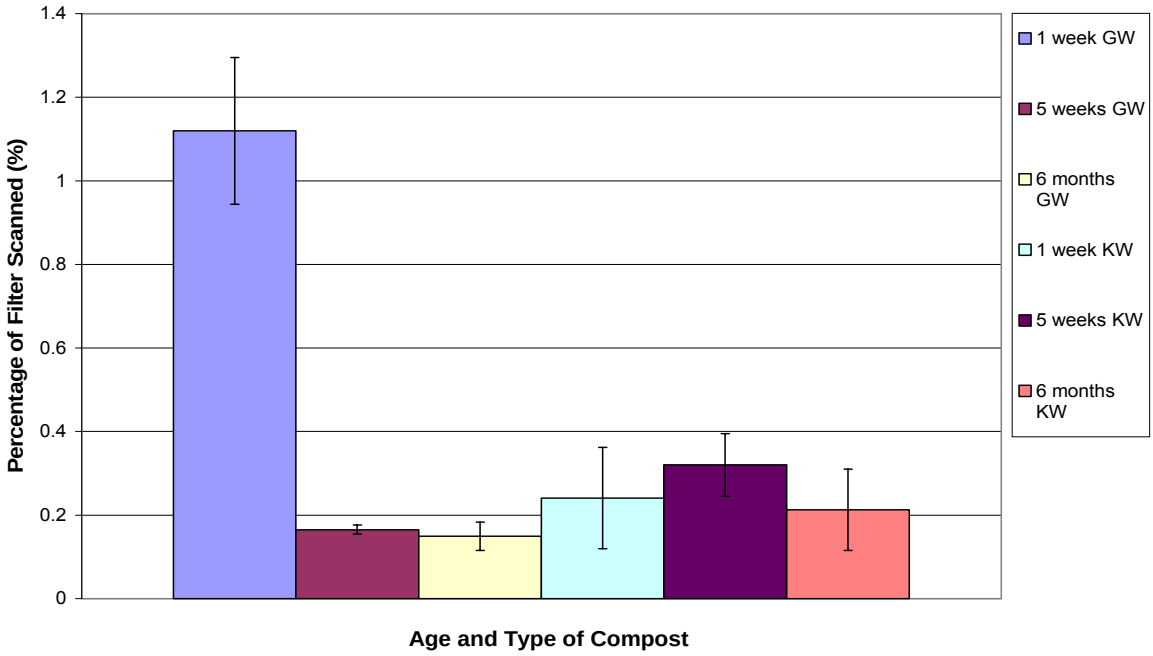


Figure 4.9- SEM Images of Sampling Filters Heavily Populated with Particles

The results of the percentages of filter scanned per experiment for the controlled experiments are presented in Figure 4.10.



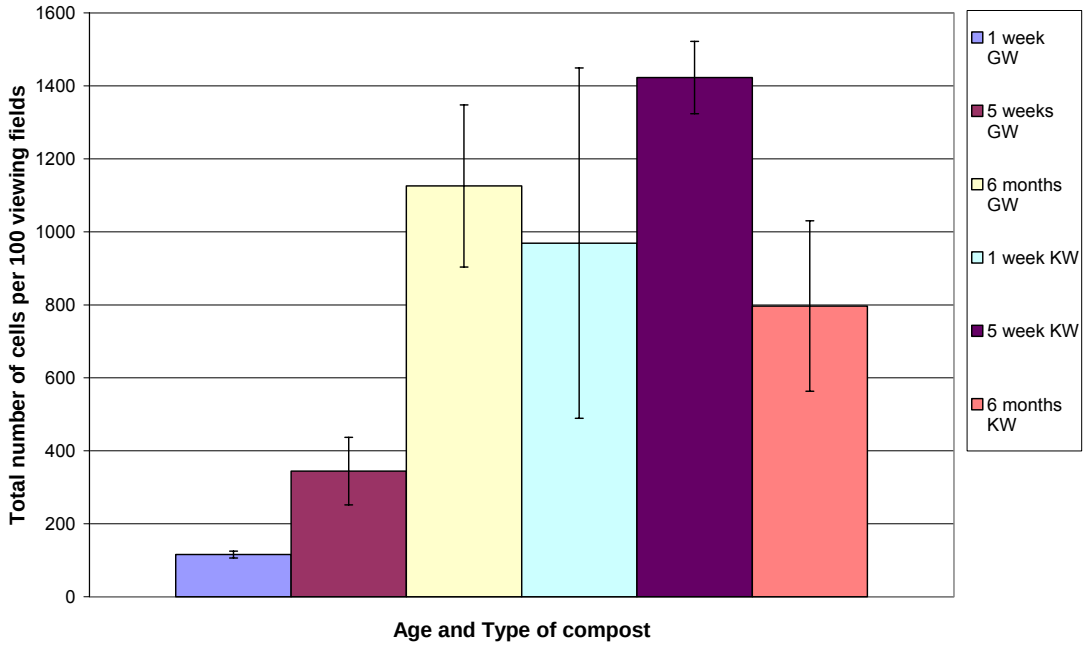
Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste
Figure 4.10 - Results of the percentage of filter scanned for controlled experiments. The bars indicate arithmetic mean (n=3) and error bars indicate standard error.

The results show that for all sample filters taken during the controlled experiments, on average 0.37% of the total sampling filter was scanned by SEM. It would have been expected that the 6 month old compost for both feedstocks would be least heavily populated with particles of interest (i.e higher percentage of filter scanned) as this

compost represents stabilised and mature compost which should have lower populations of micro-organisms. However this is not the case for either compost types.

4.3.2. Total Cell Counts

The total number of numerous types and sizes of particles that were deliberated to be bacterial, fungal and actinomycetes cells as well as their aggregates on the sampling filters were identified using SEM imaging. These were classified as large cells, large cell aggregates, small cells, small cell aggregates, mixed large cells and mixed small cells (see Appendix C for the image guide). Particles that are observed very occasionally such as filamentous particles or rod shaped particles (see Appendix B for the image guide) and unstructured particles were not taken into account for these results as these were possible pre-existing particles resulting from the structural defects of the filters. It is important to note that these total cell counts per sample filter represent the counts per 100 viewing fields which is equivalent to an area of 0.252 mm^2 of the filter as opposed to a total filter area of 490.8 mm^2 or an effective filter area of 176.7 mm^2. The results are presented in Figure 4.11.



Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste

Figure 4.11 - Total number of cells per 100 viewing fields for the samples taken during the controlled experiments. The bars indicate arithmetic mean (n=3) and error bars indicate standard error.

It would be expected that a higher total number of cells per 100 viewing fields would have been captured for composts aged 1 and 5 weeks. The results for “kerbside collected green and kitchen waste” (KW) showed this trend and the total number of cells per 100 viewing fields was lower for the 6 month old compost. However this was not the case for “green waste” (GW) compost and no common trend for compost age between the two compost types in terms of the total number of cells were observed. There was a higher number of total cells observed from KW compost compared to GW compost. The statistical analysis has confirmed these results where the total number of cells counts between the GW and the KW were significantly different ($p = 0.034873$). However the effect of compost age was not significant ($p = 0.580170$).

4.3.3. Cell Classification and Aggregation

The SEM analysis of the sample filters revealed two sizes of cells released from the compost samples. The large size cells were 2-3 μm in diameter (Figure 4.12) whilst the small size cells were 0.5-1 μm in diameter (Figure 4.13). Previous studies have reported physical sizes of some fungi spores or conidia as 2.5-3 μm for *Aspergillus fumigatus*, 3.5-5 μm for *Aspergillus niger* and 3-4.5 μm for *Penicillium brevicompactum* (Samson *et al.*, 1995; Latgé, 1999; Menetrez *et al.*, 2007). Most bacterial spores are reported to be within the physical size range of 0.5 -1 μm in diameter (Matthias-Maser and Jaenicke, 1995; Reynolds and Pepper, 2000), specifically 0.7-1 μm for *Streptomyces albus* (Madelin and Johnson, 1992) and 0.5-1.5 μm for *Thermoactinomyces vulgaris* (Lacey, 1989). Therefore based on these size ranges, the comparison of SEM images previously reported in literature and observed during the controlled experiments and the site work, it is assumed that the large cells belong to fungal species and the small cells belong to actinomycetes or other bacterial species.

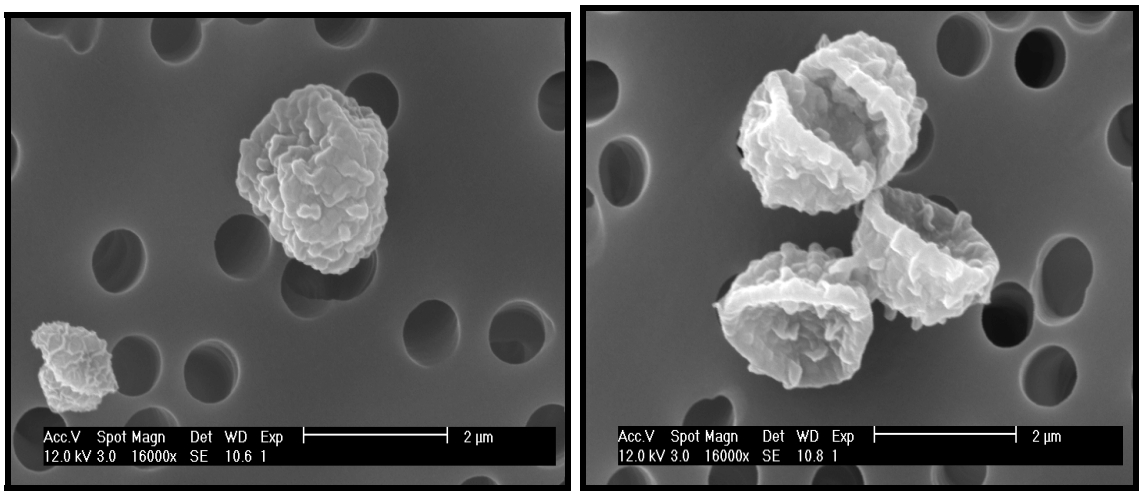


Figure 4.12 - SEM Images of Large Single Cells and Large Cell Aggregates (Type B)

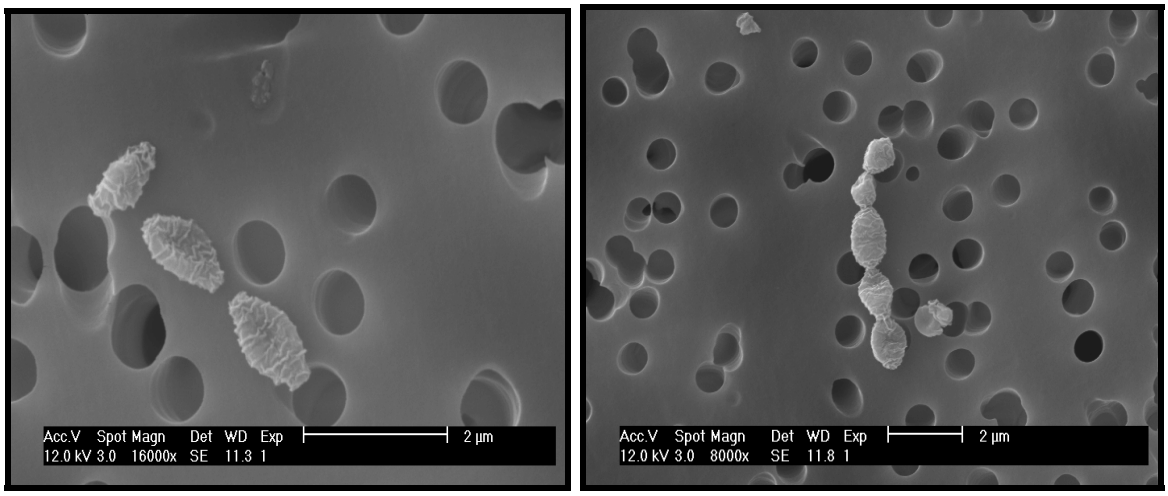
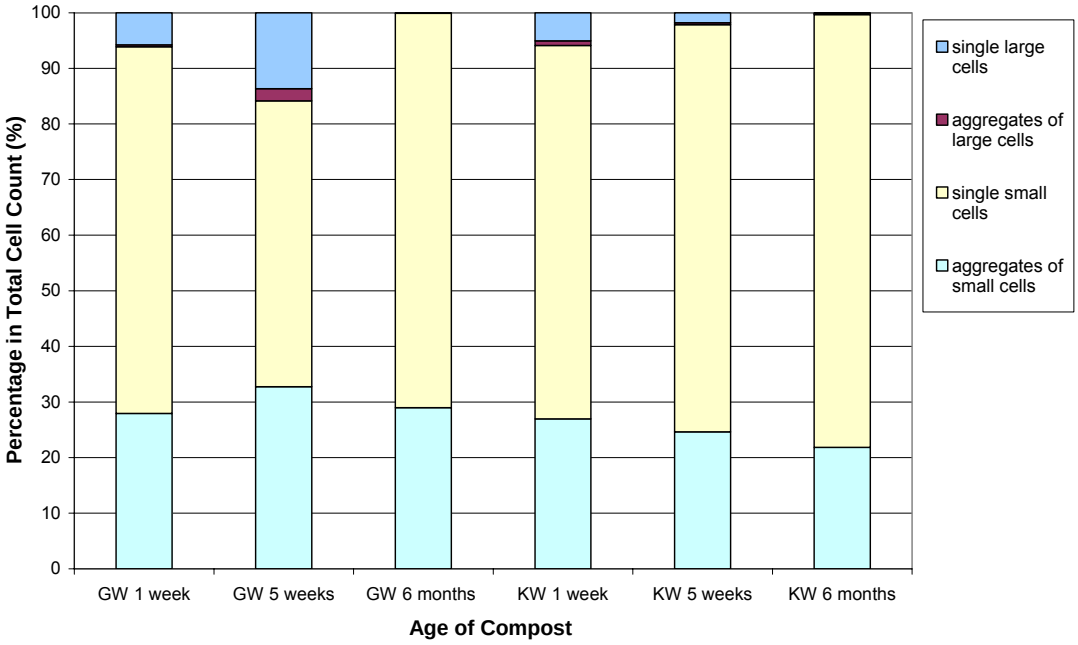


Figure 4.13 - SEM Images of Small Single Cells and Small Cell Aggregates (Type B)

Based on these, the distributions of single and aggregate cells for small and large cells for controlled experiments are presented in Figure 4.14.



Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste
Figure 4.14 - Cell classification and aggregation for controlled experiments. The bars indicate arithmetic mean (n=3).

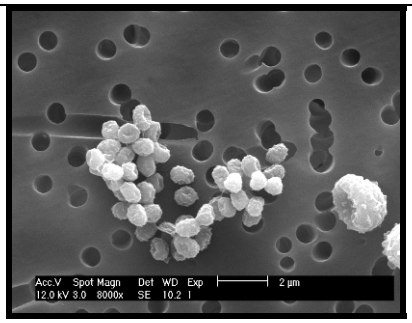
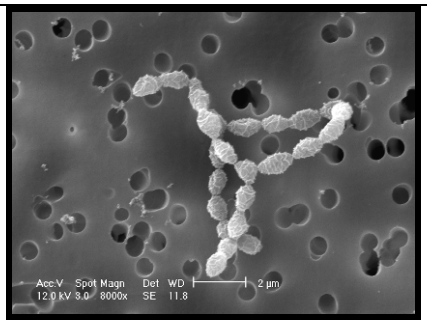
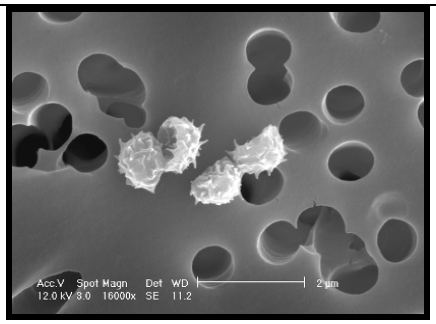
The results show that for all compost types and ages, the majority of cells were single small cells (51-78%) followed by their aggregates (22-33%). The percentages of single large cells and their aggregates were much lower (0.1-14% and 0.2-2.2% respectively). Large cell aggregates were not identified for 6 month old green waste compost samples. This might indicate that cells of micro-organisms such as bacteria or actinomycetes were found in greater numbers in bioaerosol structures. In addition, the cells of these micro-organisms might be more likely to be in aggregates when airborne than those of larger cells of micro-organisms such as fungi.

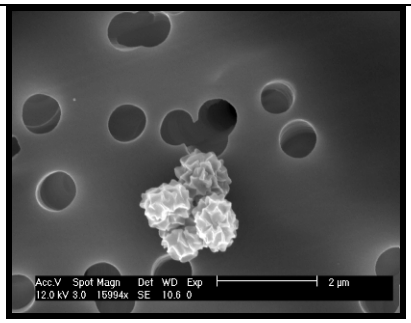
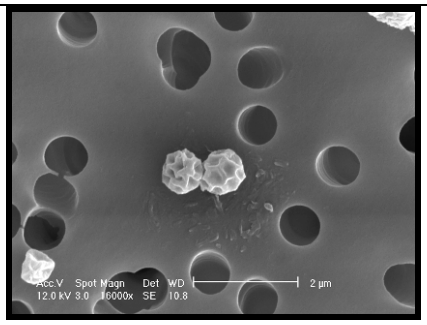
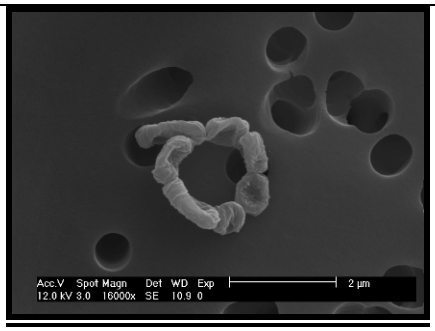
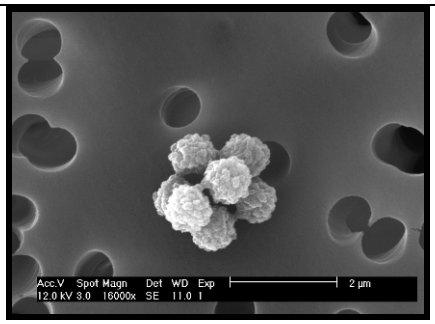
In addition to these, the culturing results for controlled experiments (presented in Section 4.3.9) show that on average, the concentrations of *A. fumigatus* were lower than those for actinomycetes. Hence it would be expected that the percentage of small cells which indicate bacteria and actinomycetes would be higher than large cells which indicate fungi. In addition to this lower numbers of both cell types would be expected to be observed for composts aged 6 months. However the results did not show significant differences ($p>0.05$) for the small and large cells measured for different compost ages.

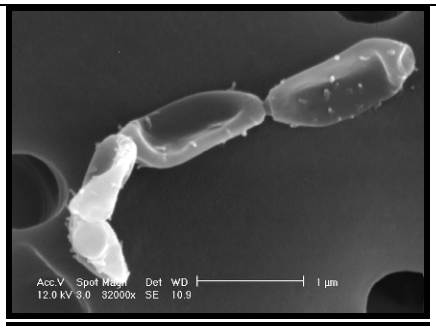
4.3.4. Small Cell Aggregate Classification

As previously discussed (Section 4.3.2), numerous types of small size cells and their aggregates were identified by SEM analysis (Table 4.1).

Table 4.1 –Cell description and image examples of small size cells and their aggregates. Observed size for all cells 0.5-1 µm length

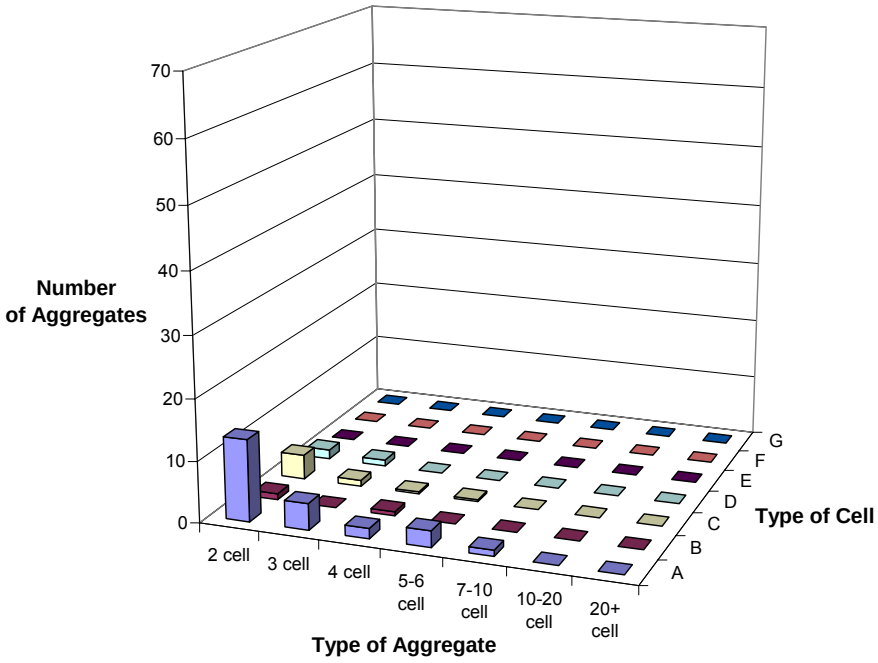
Cell Type	Cell Description	Example of Aggregate Structure and Number
A	Round particles with smooth surface or with small bumps and occasional 'Raspberry-like' structure.	 *20+ cell aggregation*
B	Oval shaped particles with ridges	 *20+ cell aggregation*
C	Round particles with visible spikes	 *2 cell aggregation*

D	Round particles with ridges and a 'flower-like' structure	 *4 cell aggregation*
E	Round particles with dents	 *2 cell aggregation*
F	Oval shaped particles with 'shrivelled' appearance. Mostly occurring in 'chain' structures	 *7 cell aggregation*
G	Round particles with prominent bumps, 'cauliflower-like' appearance and 'scar'	 *5-6 cell aggregation*

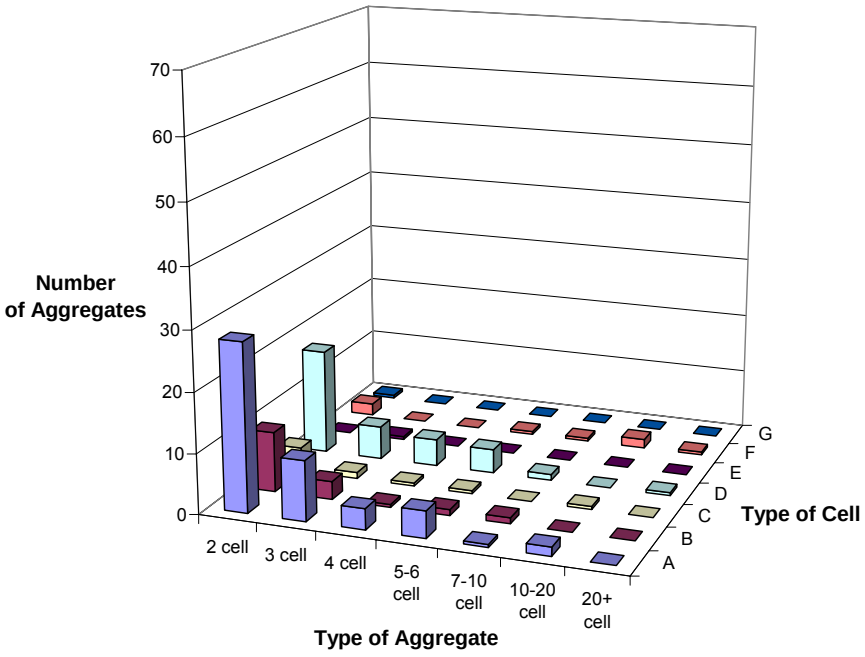
H	Oval shaped particles with small 'warts'	 *4 cell aggregation*

SEM images from previous studies suggest small cell type E to be *Thermoactinomyces sacchari* (Prescott *et al.*, 1999) and small cell types C and F to be *streptomyces sp.* (Skujiņš *et al.*, 1971; Prescott *et al.*, 1999), which would be consistent with the types of micro-organisms identified in compost (Epstein, 1997). However as explained previously, the determination of the cell species was not within the scope of this study.

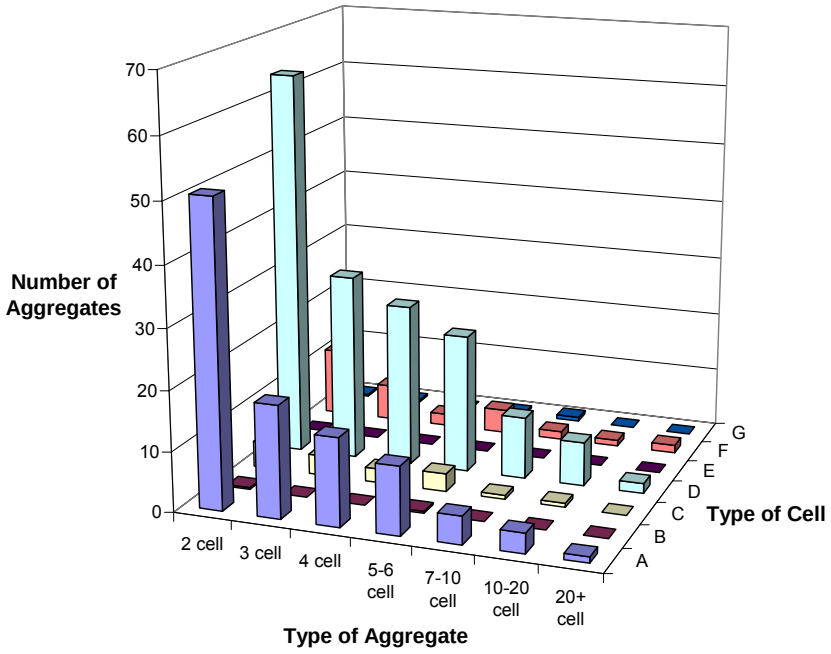
The number of aggregates observed per type of small cell and aggregate for controlled experiments are presented in Figures 4.15 and 4.16 for GW and KW compost respectively. The aggregates consisting of 5-6, 7-10, 10-20 cells and bigger aggregates were classified together as it was not always possible to distinguish the exact number of cells in an aggregate. Small cell type H has only been identified once (KW, 5 week old) hence are not represented in the following results.


70
60
50
40
30
20
10
0
Number of Aggregates
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(a)


70
60
50
40
30
20
10
0
Number of Aggregates
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(b)

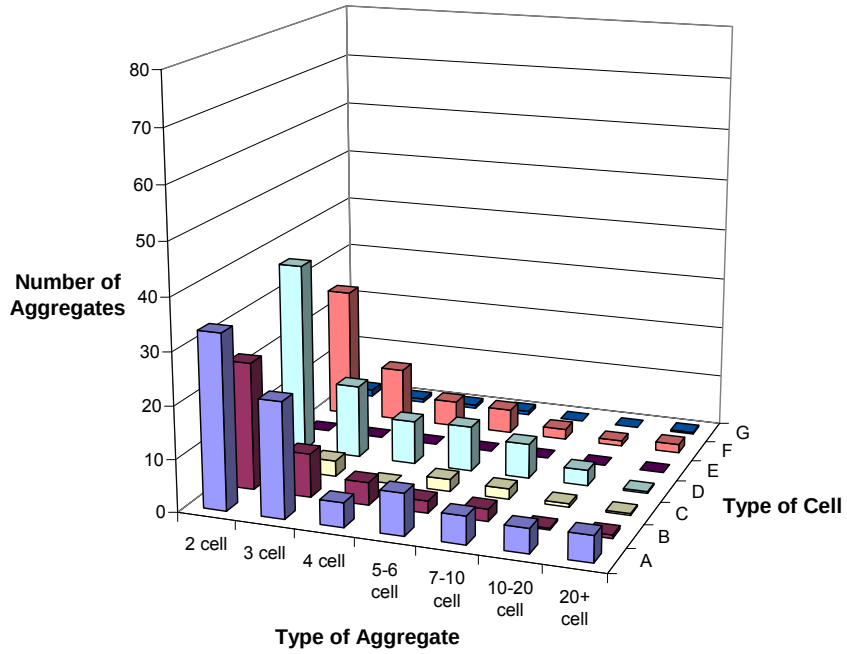


(c)

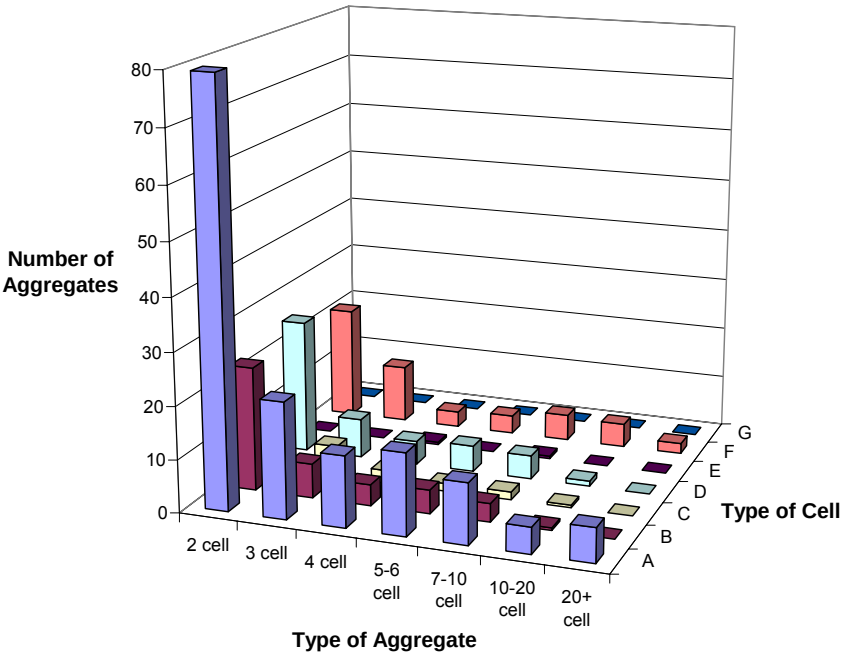
Figure 4.15- Small cell aggregate distribution for green waste compost aged (a) 1 weeks, (b) 5 weeks and (c) 6 months. The bars indicate arithmetic mean (n=3).

For sample filters of all ages of green waste compost, aggregates made up of 2 cells were in the majority compared to aggregates made up of more cells. For the 1 and 5 week old green waste compost samples, small cell type A aggregates consisting of 2 cells were dominant. The dominant species through different stages of composting is different with changing compost age. Therefore in line with this, for the 6 month old compost samples, the highest aggregate type was for small cell spore type D aggregates consisting of 2 cells. Higher number of aggregates made up of 3 or more cells was observed for the 6 month old compost compared to those for 1 week and 5 week old compost. This is an interesting result as per the first hypothesis, the total number of bioaerosols and particles and hence aggregates were expected to reduce as compost matures however this is not the case.

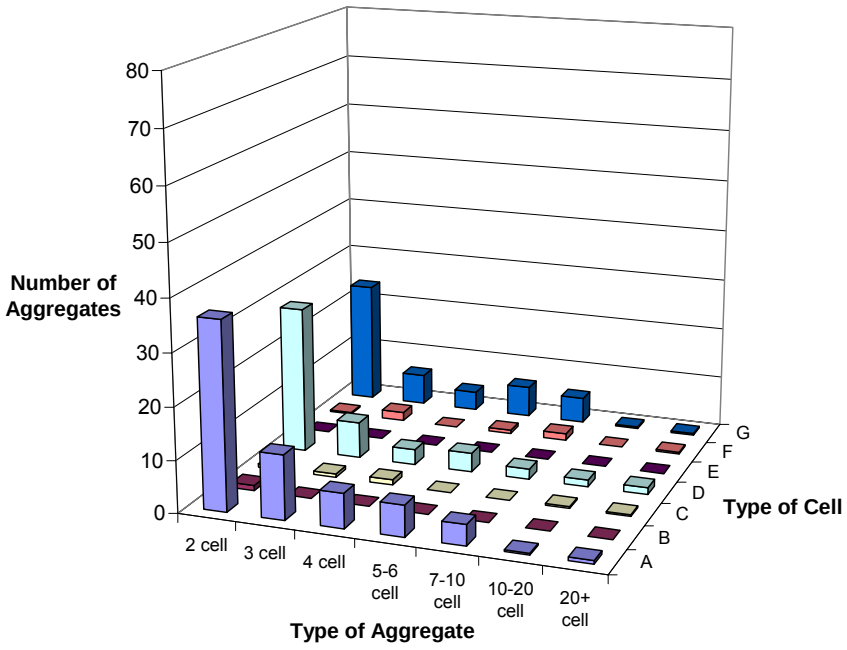
The number of aggregates observed per type of cells and type of aggregate for different ages of KW compost is presented in Figure 4.16


80
70
60
50
40
30
20
10
0
Number of Aggregates
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(a)


80
70
60
50
40
30
20
10
0
Number of Aggregates
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(b)



(c)

Figure 4.16- Small cell aggregate distribution for kerbside collected garden and kitchen waste compost aged (a) 1 week, (b) 5 weeks and (c) 6 months. The bars indicate arithmetic mean (n=3).

Similar to the green waste compost results, aggregates made up of 2 cells were in the majority compared to aggregates made up of more cells. The 1 week old compost sample filters had similar overall numbers for small cell aggregates for types A, B, D and F but the highest number of 2-cell aggregates were for small cell type D at 33. Overall number of aggregates including those consisting of 3 or more cells were lower for the 6 month old compost compared to 1 week and 5 weeks old compost. These results were in line with what was expected which would be the reduction in the number of bioaerosols and particles (and hence aggregates) with an increase in compost age. Finally, the summary of the dominant aggregate and cell types for aggregate for the controlled experiments are presented in Table 4.2.

Table 4.2 – The dominant aggregate and cell types for all controlled experiments

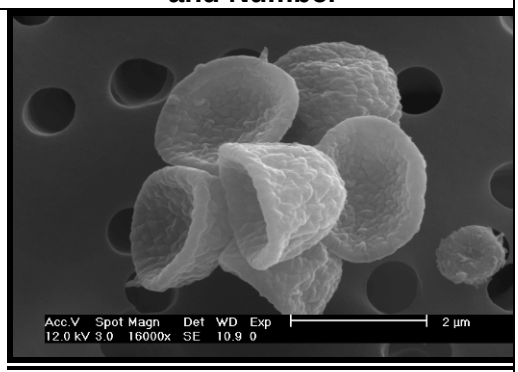
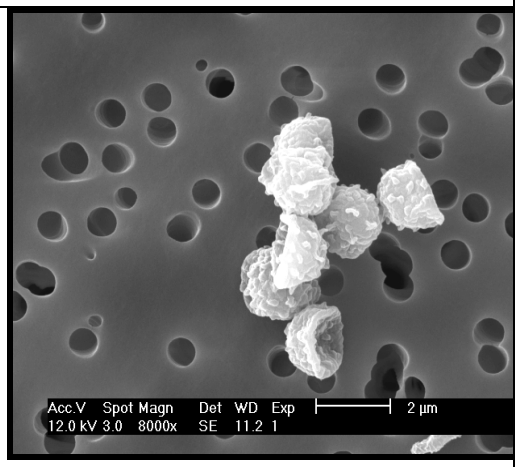
Type and Age of Compost	Dominant Cell Type	Dominant Aggregate
GW 1 week	A	2 cell
GW 5 weeks	A	2 cell
GW 6 months	D	2 cell
KW 1 week	D	2 cell
KW 5 weeks	A	2 cell
GW months	A	2 cell

Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste

4.3.5. Large Cell Aggregation

For the controlled experiments, two major types of large size cells and their aggregates were identified by SEM analysis (Table 4.3). A third type of large size cell (Spore Type C) was only observed occasionally hence will not be included in the analysis of the large size cells.

Table 4.3 – Cell description and image examples of large size cells and their aggregates. Observed size for all cells is 2-3µm length.

Cell Type	Cell Description	Example of Aggregate Structure and Number
A	Round particles with bumpy or smooth surface and 'raspberry-like' structure	 *6 cell aggregation*
B	Round particles with small spikes	 *7 cell aggregation*

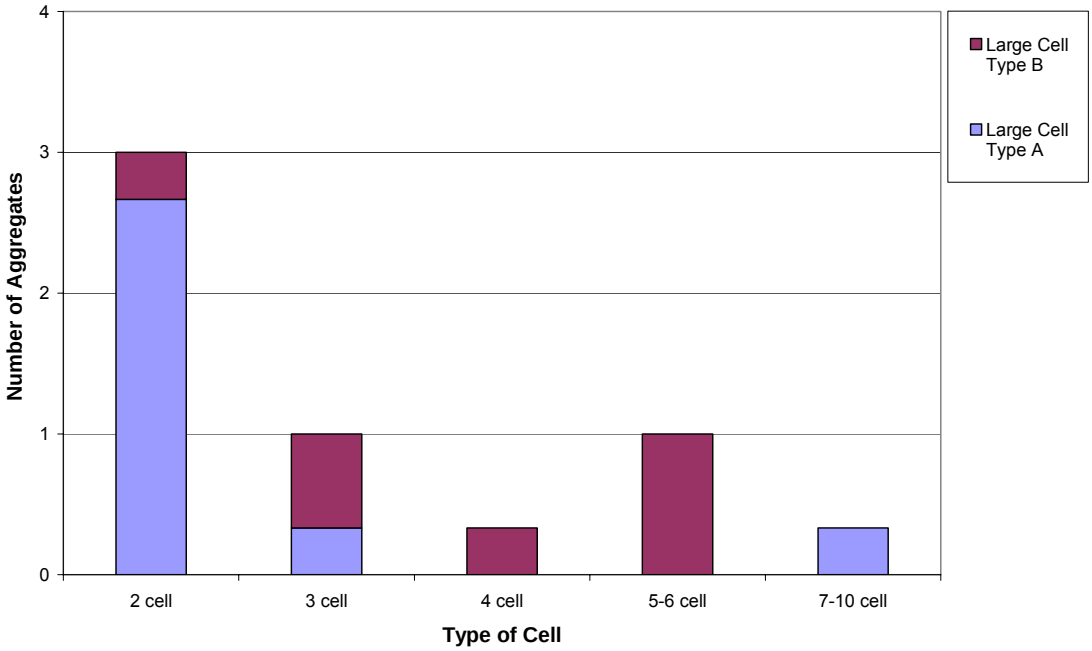


Figure 4.17- Large cell aggregate distribution for green waste compost aged 5 weeks. The bars indicate arithmetic mean (n=3).

The results (Figure 4.17) shown for 5 week old green waste compost are the largest number of large cell aggregates observed for any controlled experiment. The number of large cell aggregates observed for the other controlled experiments were low and are summarised as follows:

- For 1 week old green waste compost, there was only a single 2 cell Type A aggregate observed for one sampling filter;
- There were no large cell aggregates for any type observed for the 6 month old green waste compost;
- For both 5 week and 1 week old kerbside collected waste compost, on average (n=3), one 2 cell Type A aggregate, one 2 cell and one 4 cell Type B aggregates were observed;
- For 6 month old kerbside collected waste compost, on average (n=3), one 3 cell Type A aggregate was observed.

4.3.6. Aggregate Structure

Two different types of aggregates were observed on the sample filters analysed by SEM. The first type was classed as 'chain aggregates' as shown in Figure 4.18 where cells are aggregated resulting in a long chain-like structure. The second type of aggregate was classed as a 'cluster aggregate' as shown in Figure 4.19 where cells are aggregated in a shorter bunched structure.

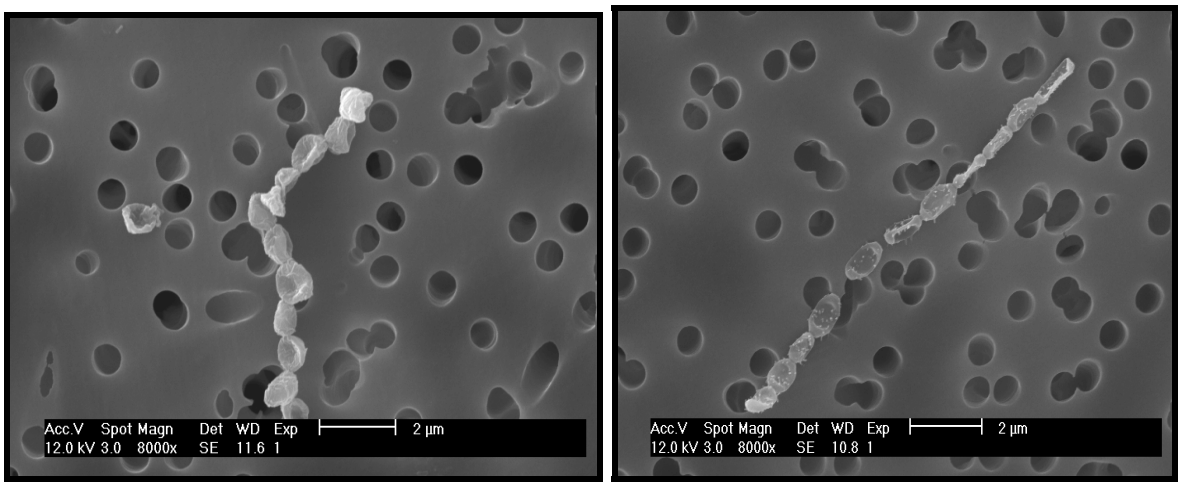


Figure 4.18- SEM Images of chain aggregates

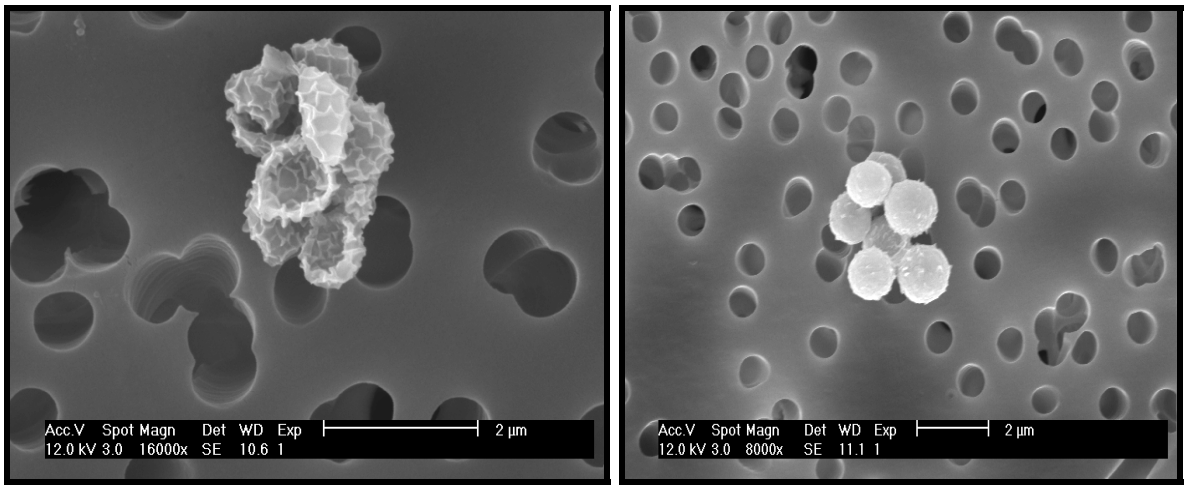
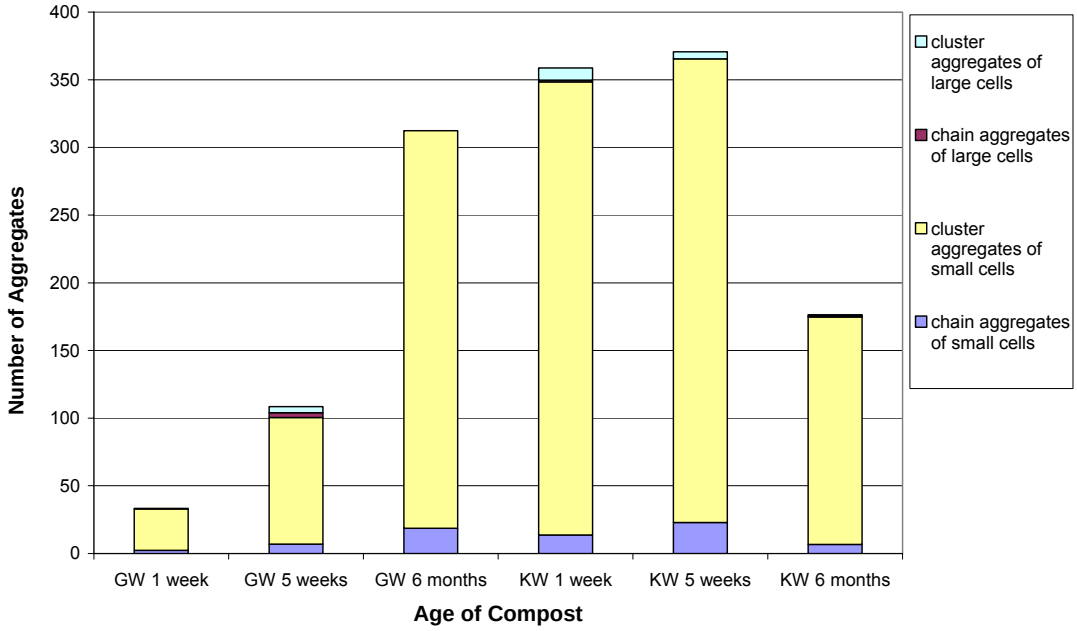


Figure 4.19- SEM Images of cluster aggregates

The classification of aggregates for small and large cells for controlled experiments is presented in Figure 4.20.



Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste
Figure 4.20- Small and large cell aggregate distribution for controlled experiments. The bars indicate arithmetic mean (n=3)

In line with hypothesis three, the chain aggregates are probably the fragments of filamentous mycelial structures present in compost which have broken into smaller pieces on release from compost. However the cluster aggregates are more likely to be either aggregates of micro-organisms in compost which do not grow in filaments or are single spores which have aggregated upon release. Based on these, the results show that for all compost types and ages, the cluster aggregates for both types of cell sizes were in the majority indicating that either a larger percentage of non-filamentous micro-organism aggregates are being aerolised or that cells are clustering into aggregates upon release from compost .

4.3.7. Size Distribution

The 2D image dimensions (width and length) of the small and large cell aggregates observed with SEM were noted as shown in Figure 4.21. The cell aggregates were classified according to the dimension which is the greatest. Therefore, for instance, a cell aggregate with the dimensions of 2 μm (width) and 10 μm (length), is classified as having an aggregate diameter of 10 μm.

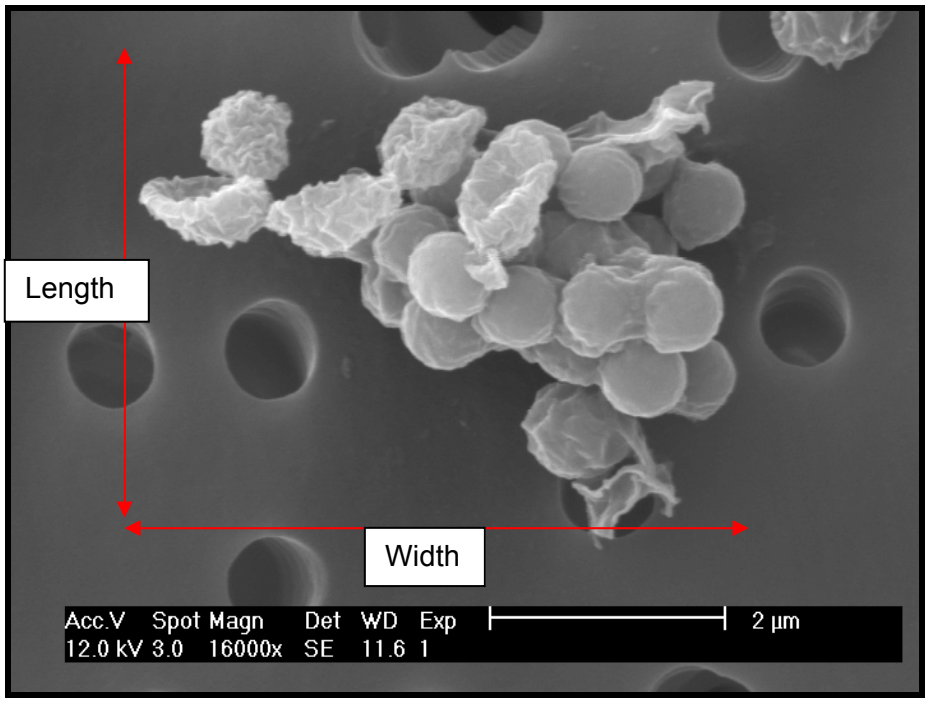
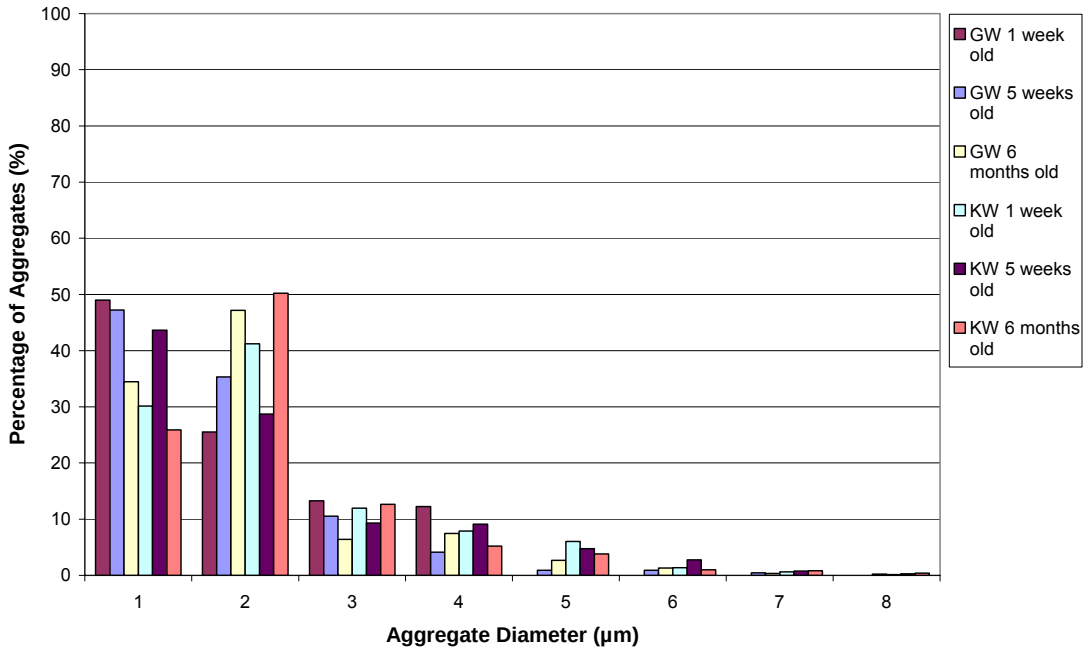
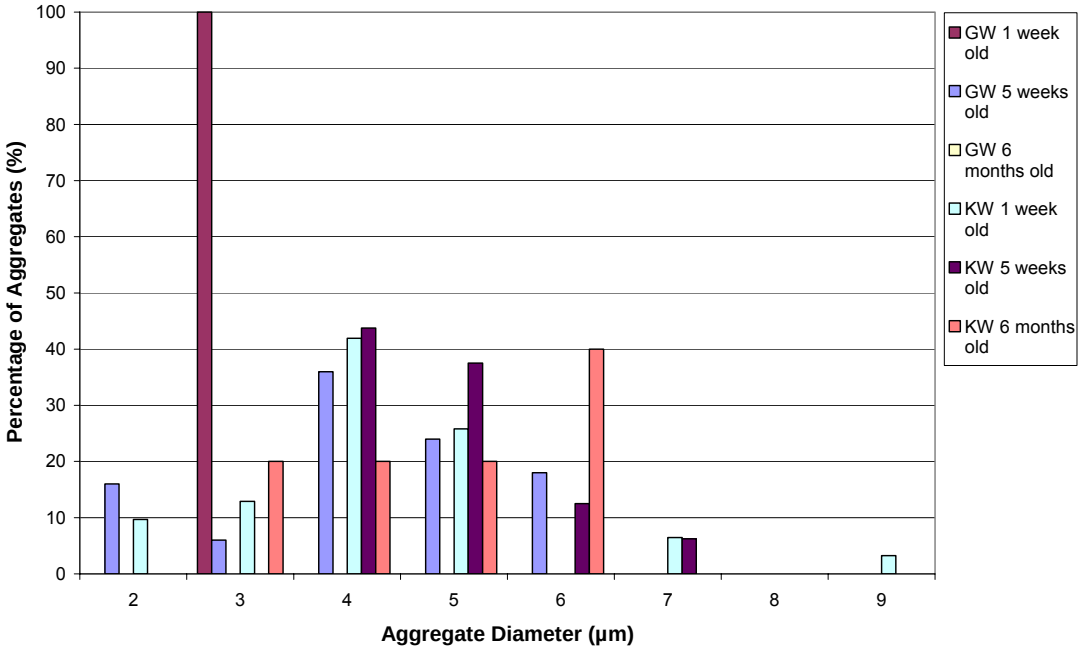


Figure 4.21 - 2D Dimensions of SEM cell aggregate images

The small and large cell size aggregate distributions for controlled experiments are shown in Figure 4.22.



(a)



(b)

Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste

Figure 4.22- Small cell (a) and large cell (b) aggregate size distribution for controlled experiments. The bars indicate arithmetic mean (n=3)

The aggregate size distribution for small cells for all the filter samples taken for controlled experiments show that aggregates of 1 and 2 µm in diameter were the most profuse at an average of 50% of all aggregates observed. Since the size of a single small cell is 0.5-1 µm, this would equate to 2-3 cell aggregates hence these are in line with the results presented in Section 4.3.4. The aggregate size distribution for large cells show that, on average, aggregates of 4 and 5 µm in diameter were dominant with the exception of samples of 1 week old green waste compost and 6 months old kerbside collected kitchen and garden waste compost. The size of a single large cell is 1-2 µm, therefore this would equate to 2-4 spore aggregates.

In addition to these results, the largest aggregate particle observed per sample for the controlled experiments and site work was analysed. Based on this, the following tables show the number of individual units for the largest aggregate of small and large cells for controlled experiments (Tables 4.4 and 4.5).

Table 4.4 – The number of individual units for the largest aggregate of small cells identified per sample for the controlled experiments. The values within the brackets denote the length and height of the observed aggregate in µm.

Compost Age	Green Waste			Kerbside Waste		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1 week	4 (3,4)	6 (4,4)	6 (4,2)	110 (15,8)	150 (12,11)	60 (10,10)
5 weeks	7 (6,3)	*No sample due to pump malfunction*	13 (11,10)	12 (11,8)	100 (13,8)	14 (8,11)
6 months	13 (7,7)	30 (6,7)	100 (8,8)	22 (4,5)	75 (13,14)	18 (6,5)

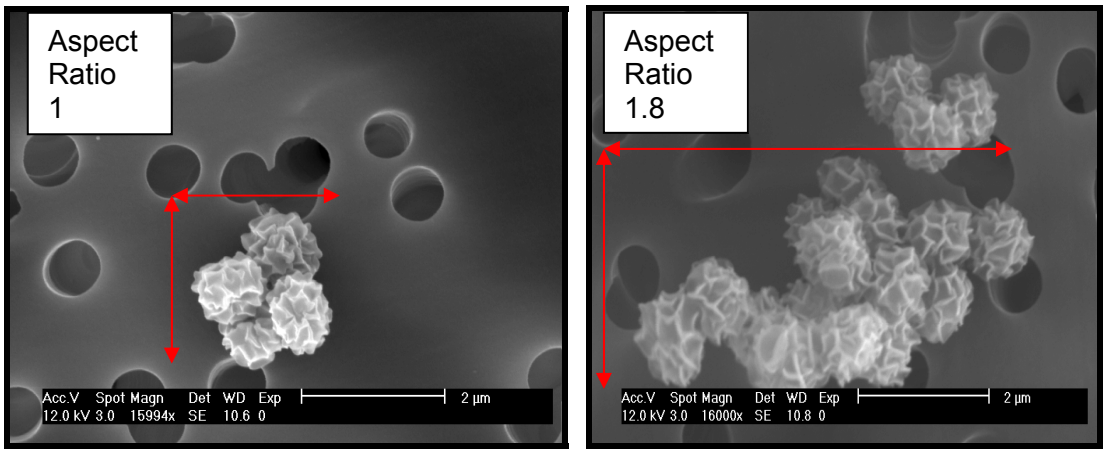
Table 4.5 – The number of individual units for the largest aggregate of large cells identified per sample for the controlled experiments. The values within the brackets denote the length and height of the observed aggregate in µm.

Compost Age	Green Waste			Kerbside Waste		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
1 week	None	None	2 (3,3)	4 (5,7)	4 (5,5)	3 (4,4)
5 weeks	3 (4,4)	*No sample due to pump malfunction*	7 (5,6)	4 (5,6)	7 (6,7)	3 (5,5)
6 months	None	None	None	None	4 (3,6)	3 (5,4)

The results show that small cells are more likely to form aggregates consisting of 7 or more aggregates and the particle diameter of these aggregates increased considerably (e.g. 10 µm) compared to the size of a single cell (0.5-1 µm). However there is no evidence of age or compost feedstock related trends.

4.3.8. Particle Shape Distribution

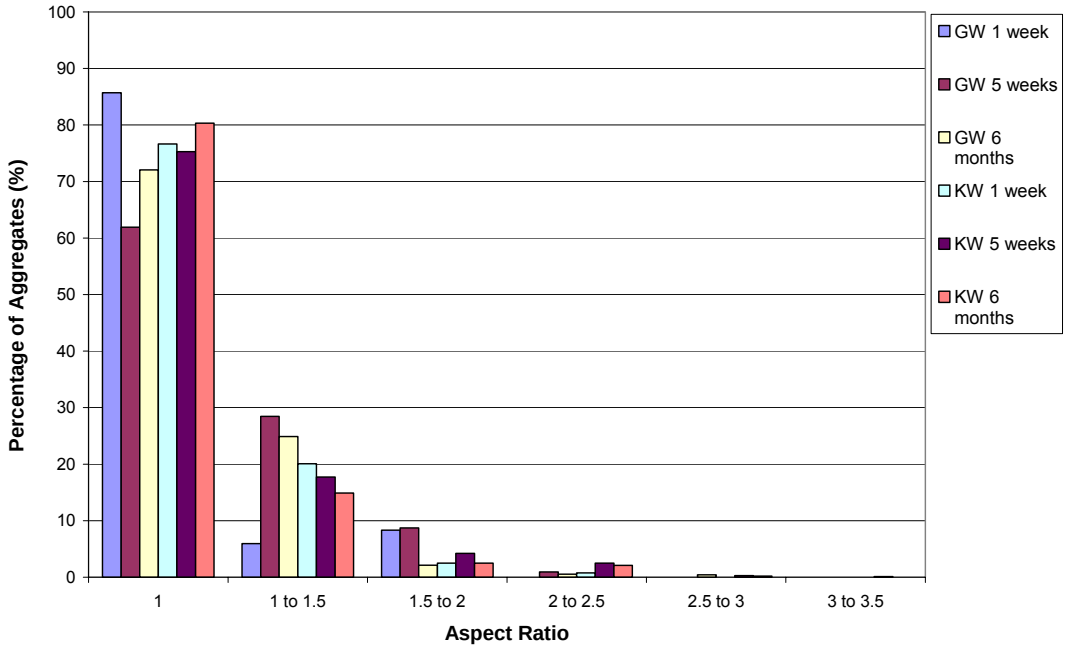
The aspect ratios of aggregate structures for the small and large cell aggregates were observed with the SEM. Some aggregate structures had an aspect ratio of 1:1 as shown in Figure 4.23 (a). Alternatively, aggregate structures also showed aspect ratios other than 1:1 as shown in Figure 4.23 (b). Therefore the analysis presented in this section will help to determine the percentage of particles with different aspect ratios because such particle shape characteristics will have implications in terms of their aerodynamic behaviour.



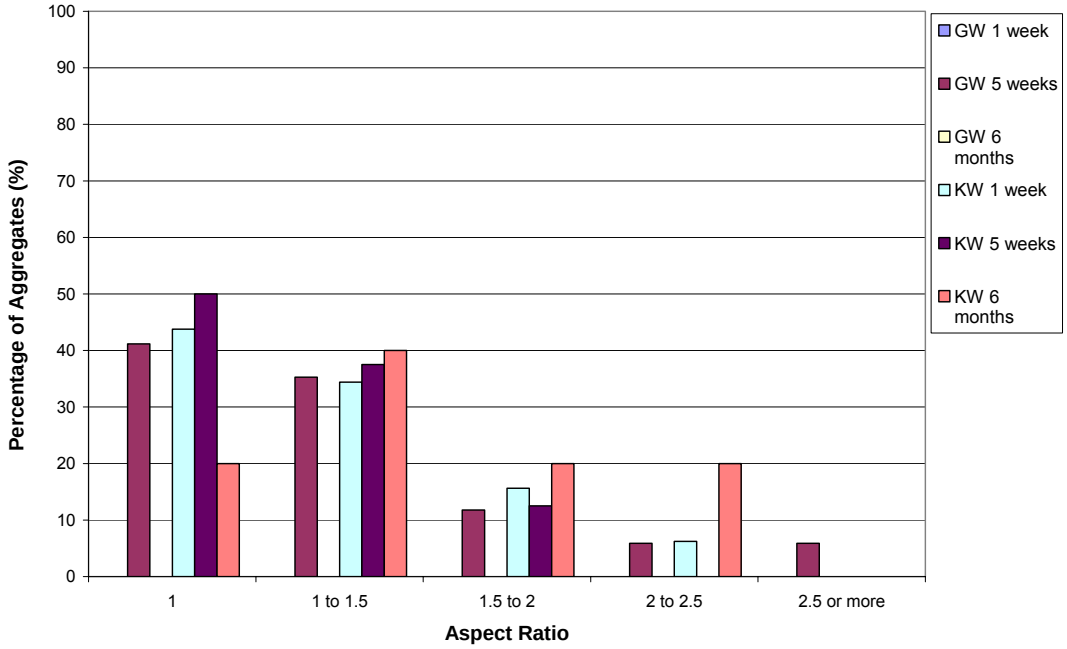
(a) **(b)**

Figure 4.23- SEM images of aggregate structures with an aspect ratio of 1 (a) and aggregate structures with an aspect ratio other than 1 (b)

The small and large cell size particle shape distributions for controlled experiments are shown in Figure 4.24



(a)



(b)

Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste
Figure 4.24- Aspect ratio distribution for small cell (a) and large cell (b) aggregates for controlled experiments. The bars indicate arithmetic mean (n=3)

The results for all samples show that the majority of small cell aggregates had an aspect ratio of 1 (63% to 86%). Similar results for large cell aggregates were observed where the majority of all aggregates also had an aspect ratio of 1. However the number of aggregates with an aspect ratio between 1 and 1.5 were higher for larger cell aggregates compared to small cell aggregates.

4.3.9. Comparison of Culturing and SEM Results

The concentrations (in CFU/m^3) of *A. fumigatus* and actinomycetes calculated by the culture method are compared with the concentrations (in particle/m^3) of large and small cells and their aggregates. To enable this comparison, the assumption is made that large cells and their aggregates represent *A. fumigatus* counts whilst small cells and their aggregates represent actinomycetes counts as explained previously (Section 3.4.2., Chapter 3).

Previous studies (McCartney *et al.*, 1997a) have shown that an aggregate may form only one microbial colony when cultured. However it is likely that these aggregates

might shatter on impact with a sampling filter and result in an increased number of colonies resulting from one aggregate. The results shown in the previous sections have shown the presence of aggregates on the sampling filters following impact hence aggregates do not always shatter on impact with a sampling filter. However, the culturing methodology involves the rigorous mixing of any aggregates which would result in them breaking into single cells. Therefore for the purposes of the particle concentration determination, the assumption has been made that an aggregate when cultured will result in the same number of colonies as the individual number of cells in that aggregate. Hence, for example 2-spore aggregate will result in two colonies when cultured and a 6-spore aggregate will result in six colonies when cultured. Based on this, Tables 4.6 and 4.7 presents the concentrations of micro-organisms and bioaerosols detected in green and kerbside collected kitchen and garden waste compost by culture and SEM analysis for *A.fumigatus* and actinomycetes respectively.

Table 4.6 – Controlled experiments *Aspergillus fumigatus* and large cell and aggregates concentrations (arithmetic mean of 3 samples ± standard error)

	Green Waste Compost Bioaerosol Concentrations		**Kerbside Collected Waste Compost Bioaerosol Concentrations**	
Compost Age	**Culture (*CFU/m*^{3})**	**SEM (particle/*m*^{3})**	**Culture (*CFU/m*^{3})**	**SEM (particle/*m*^{3})**
1 week	$3.9\text{x}10^4$ ± $5.7\text{x}10^2$	$1.1\text{x}10^5$ ± $3.0\text{x}10^4$	$1.3\text{x}10^6$ ± $3.0\text{x}10^5$	$4.1\text{x}10^5$ ± $1.8\text{x}10^5$
5 weeks	$1.1\text{x}10^6$ ± $5.3\text{x}10^5$	$1.2\text{x}10^5$ ± $5.0\text{x}10^4$	None detected	$1.7\text{x}10^5$ ± $5.4\text{x}10^4$
6 months	None detected	$5.9\text{x}10^3$ ± $3.0\text{x}10^2$	None detected	$4.4\text{x}10^5$ ± $2.0\text{x}10^4$

The correlation analysis of the results of culturing vs SEM of these results revealed a weak relationship between the *A. fumigatus* concentrations determined by culture methods and the total (i.e. single and aggregate) large cell particle concentrations determined by the SEM method ($r^2 = 0.0801$).

Table 4.7 – Controlled experiments actinomycetes and small cell and aggregates concentrations (arithmetic mean of 3 samples ± standard error)

	Green Waste Compost Bioaerosol Concentrations		Kerbside Collected Waste Compost Bioaerosol Concentrations	
Compost Age	**Culture (*CFU/m*^{3})**	**SEM (particle/*m*^{3})**	**Culture (*CFU/m*^{3})**	**SEM (particle/*m*^{3})**
1 week	$2.9 \times 10^5 \pm 5.3 \times 10^4$	$1.5 \times 10^6 \pm 4.4 \times 10^5$	$1.8 \times 10^6 \pm 5.0 \times 10^5$	$1.0 \times 10^7 \pm 3.0 \times 10^6$
5 weeks	$9.4 \times 10^5 \pm 3.9 \times 10^5$	$1.8 \times 10^6 \pm 1.9 \times 10^5$	$2.4 \times 10^6 \pm 9.0 \times 10^4$	$1.0 \times 10^7 \pm 2.0 \times 10^6$
6 months	$2.7 \times 10^7 \pm 3.3 \times 10^6$	$7.9 \times 10^6 \pm 2.1 \times 10^6$	$3.7 \times 10^6 \pm 5.2 \times 10^5$	$6.0 \times 10^7 \pm 2.0 \times 10^6$

The correlation analysis of the results of culturing vs. SEM methods for actinomycetes also revealed a weak relationship between the results calculated by the culture and SEM methods (r^2 = 0.0043). Following this, a further analysis was completed to determine the ratios of scanning electron microscopy particle concentrations to culture based microbial concentrations (Table 4.8).

Table 4.8 - Quantitative comparison of culture and SEM results for the controlled experiments

Sample Type or Location	*A.fumigatus* SEM/CFU ratio	Actinomycetes SEM/CFU ratio
GW 1 week old	2.8	4.9
GW 5 week old	0.1	1.9
GW 6 month old	No result	0.3
KW 1 week old	0.3	5.1
KW 5 week old	No result	5.3
KW 6 month old	No result	15.5

Note: GW denotes Green Waste and KW denotes Kerbside Collected Kitchen and Garden Waste

A ratio value of 1.0 would denote that the culture concentrations for actinomycetes are equal to particle per metre cube concentrations for small cells and their aggregates. Ratio values higher than 1 denote that the microbial concentrations calculated by culture methods were less than particle concentrations calculated by scanning electron microscopy methods for the same sample. Likewise, ratio values smaller than 1 denote

that microbial concentrations calculated by culture methods are higher than the particle concentrations by scanning electron microscopy methods for the same sample.

Based on this, for all experiments, on average, large cell and large cell aggregate concentrations were 1.1 times higher than *A. fumigatus* concentrations. For the same set of experiments, small cell and small cell aggregate concentrations, on average, were 5.5 times higher than those for actinomycetes concentrations.

4.4. DISCUSSION

Physical properties of a bioaerosol spore such as its size, shape, surface characteristics and their tendency to aggregate affects the aerodynamic behaviour of the bioaerosol (Levetin, 1995; McCartney, 1994; McCartney *et al.*, 1997a) and the understanding of this behaviour is essential for analysing their release from composting facilities.

The efficiency of bioaerosol samplers, such as bioaerosol impactors, is also dependant on the particle size (Reponen *et al.*, 2001) and particle aggregation affects their performance and collection efficiency (Trunov *et al.*, 2001). This may lead to under (Karlsson and Malmberg, 1989) or over-estimation of the bioaerosol particle count or large variations on filters when counting spores (Eduard and Aalen, 1988).

Furthermore, particle size and aggregation of bioaerosol particles has an impact on the amount of inhaled particles and consequently, their adverse health effects (Morrow, 1980; Venkataraman and Kao, 1999; Reponen *et al.*, 2001; CIWM, 2002; Agranovski *et al.*, 2004; Tham and Zuraimi, 2005;). Allergic alveolitis resulting from infiltration into the alveolar space of the lung (Houman and Morgan, 1977) has been observed to be caused by particles with a diameter of 5 µm. According to Carrera *et al.* (2005), aerosols with a high number of single micro-organisms might have increased infection potential however aerosols with a high number of aggregated micro-organisms might have increased survival.

Fungal spores such as *Cladosporium, Penicillum* and other airborne micro-organisms of epidemiological interest often occur as aggregates when aerosolized (Bell *et al.*, 2000;

Lacey, 1991; Levetin, 1995; Trunov *et al.*, 2001; Zollinger *et al.*, 2005), in clumps or chains (Madelin and Johnson, 1992) or attached to non-viable particles (Gregory, 1973; Akers and Won, 1979). Tham and Zurami (2005) have reported the presence of viable bacteria in clumps of 2-5 µm and Lacey and Dutkiewicz (1976a; 1976b) have found aggregate structures consisting of 5 spores in their study.

However literature review of previous research (Chapter 1) has revealed a distinct gap of information regarding the size, shape, surface characteristics and aggregation of bioaerosols released from composting facilities. Byeon *et al.* (2008) who have studied the size distribution of micro-organisms in a municipal composting facility found that the size of the microorganisms detected (*Bacillus*, *Staphylococcus* and *Streptomyces*) in their study were larger than those reported by previous studies and they have attributed this to the likely aggregation of the bioaerosols within the composting facility. However they have also discussed a distinct lack of data regarding total airborne particles and bioaerosols from municipal composting facilities. Therefore the overall research objective of this chapter was to release bioaerosols in experimental conditions and use the generated data to characterise the overall size distribution and visual properties (i.e. aggregation, size and shape) of bioaerosols emitted from compost. In order to achieve this, a set of hypothesis and chapter objectives were set. Each hypothesis will be discussed separately in light of the results.

4.4.1. Effect of Compost Age on Bioaerosols Released from Compost

The first hypothesis stated that the *"total number of bioaerosols released from compost will decrease as the compost matures".* This hypothesis was based on previous studies which have discussed declines in micro-organisms found in compost as compost age increases for different compost types and technologies including the windrow composting of biosolids and bark (Epstein,1997) (with data taken from Walke (1975)), composting of source separated household waste (i.e. vegetable, fruit and garden waste with paper) in a 200-l insulated composting bin (Ryckeboer *et al.,* 2003), composting of biowaste composts (i.e. vegetable, fruit, garden waste with paper and cardboard) in reactors with a capacity of 170-l (Lemunier *et al.*, 2005) and composting of municipal solid waste (Hassen *et al.*, 2001).

To test this hypothesis, firstly the image density analysis of the sample filters taken during the controlled experiments was analysed. The sample filters taken for 1 and 5 week old compost were expected to be more heavily populated with particles of interest (i.e. particles larger than 0.5 µm in size and not pre-existing particles inherent in an non-exposed filter) compared to those for 6 month old compost. However as shown by the results presented in Figure 4.10 (Section 4.3.1) this was not the case for either of the compost types. Secondly, the total cell counts (per 100 viewing fields) per filter were analysed. In contrast to expectations, the 6 month old sample for green waste compost showed the highest number of total cells as shown in Figure 4.11 (Section 4.3.2). The results for the kerbside collected kitchen and garden waste were in line with the expected and the number of total cells for 6 month old compost were the lowest of all three ages of compost. However this difference was not statistically significant ($p>0.05$). Therefore the hypothesis was rejected and the results showed that the number of bioaerosols released from different types of compost is not dependent on compost age. This indicates that even stabilised and mature compost (i.e. 6 months old) which would be expected to pose minimal risk in terms of bioaerosol emissions is likely to emit a high number of bioaerosols which will have implications in terms of health impacts on sensitive receptors. This result is in line with a study by Millner *et al.* (1977) who has examined concentrations of *A. fumigatus* in sewage sludge compost. The compost was formed into windrows covered with a layer of cured compost and subjected to forced aeration for 3 weeks before being disassembled and left untreated for curing (4 weeks). The cured compost was subsequently screened and stored further for up to 6 months. High levels of *A. fumigatus* were found in cured and screened compost. This was attributed to re-inoculation of previously *A. fumigatus* free patches of the compost when the cured compost was agitated. In contrast, stationary storage of *A. fumigatus* for a month or longer caused a decline in the *A. fumigatus* concentrations in the compost.

One possible reason for these results might be due to the limitations caused by the fact that it was not possible to determine the exact stage of composting for the compost samples. Assumptions were made on composting stage based on compost age however the real determination of the compost stage will be based on the monitoring of compost properties such as temperature. This is because the growth temperatures of

active micro-organisms in compost are used to describe the composting stages (e.g. mesophilic, thermophilic) (IWM, 1994). The compost samples were taken at a depth of 20 cm and the temperature of material collected at this depth might be closer to that of ambient. This might mean that the compost sample collected might be at a different stage than those in the middle of the compost windrow.

In summary, the results indicate that the number of bioaerosols released from different types of compost was not dependent on compost age and the agitation of mature compost may also be a source of bioaerosols.

4.4.2. Nature of Bioaerosols Released from Compost

Previous studies (Eduard and Aalen, 1988; Karlsson and Malmberg, 1989; Lacey, 1991; Reponen *et al.*, 1996; Swan *et al.*, 2003; ADAS/SWICEB 2005) have reported that particles are released from their growth source as a combination of single cells, aggregate of cells or cells attached to dust particle/wood fibres. Similarly Lighthart and Stetzenbach (1994) have discussed that biaoerosols released will be attached to particles such as wood fibres which would serve as a 'raft' to aid their travel on emission. Another study (Lighthart, 1997) which has analysed particles in alfresco atmosphere reported that 40% of the particles containing bacteria were greater than 7 µm. This was attributed to the attachment of the bacteria to debris.

Therefore it was next hypothesised that *"bioaerosols released from compost will also be attached to wood fibres and other non-microbial matter"*. However there was no evidence of cells attached to dust particle/wood fibres for any samples and the hypothesis was rejected. This indicates that the release mechanisms for the bioaerosols in compost is not dependent on the aeropathway of inorganic matter such as wood fibres. However it was only possible to examine a small section of each filter hence it is possible that such occurrences might have been on parts of the filter that were not looked at. Also, it is likely that the filters lacked sufficient face velocity to be able to retain other inorganic matter such as wood fibres. The effect of this is that the low face velocity of the suction at the filter face may not be sufficient to overcome the inertia of

some larger sized particles (i.e. wood fibres) as they settle. As a result, particles such as wood fibres would not be able to be retained by the filter.

The third hypothesis set concerning the nature of bioaerosols from compost was that *"the majority of bioaerosols released from compost will be in single cells".* This was based on the argument that the most prominent micro-organisms in compost, such as actinomycetes, have a filamentous mycelium structure with spores that become easily airborne when disturbed in agitation activities such as turning of compost, releasing individual spores (Lacey, 1997). In addition, even if shorter filamentous mycelium structures are released into the atmosphere, they would be expected to break up due to environmental effects such as wind speed (Pillai and Ricke, 2002). This hypothesis was supported as shown by the results presented in Section 4.3.3 (Figure 4.14). Variations between each sample was observed however the general trend for all samples was that the percentage of single cells released from compost compared to their aggregates was higher. For all compost types and ages, the majority of small cells were single cells at 71.6% for all small single and aggregate cell counts. In contrast, the percentage of small cell aggregates were 28.4%. Similar results were observed for the large cells, where the percentage of single cells were 92% compared to those for large cell aggregates at 0.8%.

One factor which might affect the release of bioaerosols from compost is moisture content of both the compost and the cells released from it. The moisture content of the spores affects both the aerodynamic and physical sizes of a cell (Lacey, 1991; Liao *et al.*, 2004; Madelin and Johnson, 1992; Meklin *et al.*, 2000; Pasanen *et al.*, 1991; Reponen *et al.*, 1996; Reponen *et al.*, 2001). Madelin and Johnson (1992) have discussed that an increase in moisture content might cause an increase in the aerodynamic diameter of the spore which leads to the breakage of structures consisting of spore chains. Such a scenario might indicate that the high moisture contents found within a typical compost environment might induce an increase of the aerodynamic diameter of the individual spores in a chain which might result in the breakage of the spore chains and lead to the release of single spores into the atmosphere. However, in contrast, Górny *et al.* (2002) have discussed that adhesion forces such as those caused

by moisture might reduce the release of fungal propagules. This may suggest that composts with a higher moisture content might have an increase in adhesion forces and will have a reduced number of particles being released from it compared to a drier compost.

The moisture levels of the composts used for the controlled experiments (Table 4.9) was calculated by the following equation.

$$MC = \frac{(W_w - W_d)}{W_w} x100 \qquad \textit{Equation 4.1}$$

Where;

MC is the moisture content of compost (%);

Ww is the wet weight of compost (g); and

Wd is the dry weight of compost (g).

Table 4.9- Moisture contents of the compost material used for the controlled experiments. Values show mean contents (n=6)

Type of Compost	Age of Compost	Moisture Content
Kerbside Collected Kitchen and Garden Waste	1 week	64.4%
	5 weeks	62.6%
	6 months	41.5%
Green Waste	1 week	48.7%
	5 weeks	57.8%
	6 months	42.4%

Therefore, testing the argument based on the study by Madelin and Johnson (1992) the material with the highest moisture content for garden waste and kerbside collected kitchen and garden waste compost (i.e. aged 5 weeks and 1 week respectively) might have the higher percentage of single cells released from the compost. However the results shown in Section 4.3.4 (Figure 4.14) show that this is not the case for small size cells released from either of the compost types and a higher percentage of aggregates are released from this material. This means that the argument based on the study by Górny *et al.* (2002) may be applicable to these results and might indicate that a greater

release of small size cells (i.e. bacteria or actinomycetes) are released from composts with lower moisture content.

However for large size cells the argument based on the study by Madelin and Johnson (1992) was applicable as higher percentages of single cells were observed for the materials with the highest moisture content. Hence this might indicate that high moisture contents of the compost might break the mycelial chain structure of micro-organisms such as fungi and result in an increase in cell release.

4.4.3. The Evaluation of Bioaerosol Aggregate Structures

In connection with hypothesis three, it was also expected that "*if bioaerosol aggregates are observed to be emitted from compost, the number of aggregate structures made up of 2-6 units will be in a higher percentage than those made up of 7 or more units*". This was based on previous studies which have examined agricultural dust (Karlsson and Malmberg, 1989; Lacey, 1991).

The results presented in Section 4.3.4. showed that the most abundant aggregate type for small cells was a 2-cell aggregate at a percentage range of 51% for all small cell aggregates. In contrast, the percentage of three to six small cell aggregates were 38% and seven or more small cell aggregates were 11%. Aggregates of large cells were less often observed (Section 4.3.5.) however on average 2-cell aggregates for this cell size were also more abundant. Therefore in the light of these findings this hypothesis was supported. Similar results were found in previous studies such as that by Carrera *et al.* (2005) who attempted to determine the number of bacterial spores within aerosol particles generated by a small pressurized metered-dose inhaler. They found that the percentage of aerosol particles consisting of one spore was 70%, consisting of two spores was 15%, three to five spores was 11% and six or more spores was 4%.

Another finding from the results that are shown in Figure 4.20 (Section 4.3.6) was in terms of the types of bioaerosol aggregation observed. Micro-organisms dominant in compost such as actinomycetes have a filamentous mycelium structure. Specifically species such as *promicromonospora*, *saccharopolyspora*, *actinomadura* and

amycolatposis have been reported to occur in short or long chains which can break into fragments of different sizes and shapes (Lacey, 1997). Other studies (Latgé, 1999) have observed such chains of conidia of 20-30 µm for *Aspergillus fumigatus* species and *A. fumigatus* is also dominant through the composting process. When compost is agitated or environmental factors such as wind effects a compost windrow, these mycelial structures would be expected to break off into smaller pieces. Therefore on emission from compost, these might be released in 'chain aggregates' such as those shown in Figure 4.18 (Section 4.3.6). On the other hand the 'cluster aggregates' shown in Figure 4.19 (Section 4.3.6) might represent the aggregates of other micro-organisms in compost that do not grow in filaments but have aggregated within the compost before release. Alternatively they might represent the clustering of spores when airborne.

The results (Figure 4.20, Section 4.3.6) have revealed that the number of cluster aggregates for all compost types and ages was much higher than those for chain aggregates. The results have also revealed that the differences between chain aggregates for different compost ages (for either compost type) were not significant ($p>0.05$). This might indicate that a higher number of micro-organisms that do not grow in filaments are forming aggregates or that cells are forming aggregates after release from compost regardless of compost age.

Finally, it is important to note that the cluster aggregates emitted from compost consisted of the same cell type to indicate single species of micro-organisms rather than a mixture of different cells types. This indicates that the cluster aggregates are more likely to have formed when the bioaerosol was suspended in air rather than on impact with the filter surface.

4.4.4. Size Distribution of Bioaerosols Released from Compost

The results presented in Section 4.3.7 show that the majority of all bioaerosols (single or aggregates) emitted from compost were smaller than 3 µm. This was in line with previous studies (Kamilaki and Stentiford, 2001; Reinthaler *et al.*, 1997; Byeon *et al.*, 2008) that have reported the particle size distribution of bioaerosols in composting facilities using an Andersen 6 stage sampler. They found that the numbers of particles

less than 3.3 µm in diameter were higher compared to others. As previously discussed, the majority of bioaerosols emitted from compost are single cells that are 0.5-1 µm for small size cells and 1-2 µm for large size cells. The size distribution for aggregates were based on either the width or length of the aggregate whichever was the highest. Since the majority of the aggregates were spherical or almost spherical, the width and length for most aggregates were equal and would represent the diameter of the particle. The majority of small and large cell aggregates for all controlled experiment samples were in the vicinity of 1-2 µm and 4-5 µm respectively.

To the author's current knowledge, this is the first study that has classified bioaerosols emitted from compost according to shape, size and number. One other study (Byeon *et al.*, 2008) which has examined total airborne particles and bioaerosols in a municipal composting facility have reported total airborne particle concentrations sized 0.3 µm to be the majority of all particles examined at 10^8 particles/m^3. This was captured near the screening process. Another study (Kamilaki and Stentiford, 2001) has reported that 80% of all *A. fumigatus* colonies monitored in a composting plant were captured in the Stages 3, 4 and 5 of the Andersen 6 stage sampler corresponding to spores and particulates in the size range of 1.1 to 3.3 µm. Similar results were found by Reinthaler *et al.* (1997) who have also used an Andersen 6 stage sampler found that 56-73% of all particles sampled were smaller than 3.4 µm.

These diameters reported in studies that have used an Andersen 6 stage sampler are based on the aerodynamic diameter of the bioaerosols. Other studies (Madelin and Johnson, 1992; Reponen *et al.*, 1996; Reponen *et al.*, 1998; Trunov *et al.*, 2001) that have used an aerodynamic particle sizer have also reported spore size in aerodynamic diameter terms. *The aerodynamic diameter of a particle is the diameter of a sphere of unit density (i.e.* ρ_o *= 1000 kg/*cm^3 *) which has the same settling velocity (i.e.* v_g *) of the particle* (Griffiths *et al.*, 1984). It was not within the scope of this project to calculate the density of the bioaerosols emitted from compost however it has been reported that the average density of a spore is heavier than water at 1.1 g/cm^3 or 1.2 g/cm^3 (Gregory, 1973). Therefore assuming the latter density and that all bioaerosols are spherical, the aerodynamic diameter range for the small cell aggregates are calculated to be 1.1 – 2.2

μm. With the same assumptions, the aerodynamic diameter range for the large cell aggregates are calculated to be 4.4 – 5.5 μm by using the following equation from Colls (2002).

$$d_a = \sqrt{\frac{\rho_{part}}{1000}} \times d_{part} \qquad \textit{Equation 4.2}$$

Where,

d_a is the aerodynamic diameter of the particle (μm);

ρ_{part} is the density of the particle (kg/cm^3); and

d_{part} is the physical diameter of the particle (μm).

Therefore despite the fact that the aerodynamic diameter range of 4.4 – 5.5 μm for large cell aggregates are higher than the aerodynamic diameters reported in other studies (Kamilaki and Stentiford, 2001; Reinthaler *et al.*, 1997; Byeon *et al.*, 2008), the majority of all cells detected represent small size cells and their aggregates with the aerodynamic diameter range of 1.1 – 2.2 μm in line with these studies.

4.4.5. Shape and Aspect Ratio of Bioaerosols Released from Compost

There are no previous studies that have examined the physical shape and aspect ratio range of bioaerosols emitted from compost. However there is a wide diversity of micro-organisms growing in compost (and possibly released from compost) such as those identified by Michel *et al.* (2002) where over 42 species of micro-organisms were identified in 29 day old green waste compost, and over 94 species of micro-organisms were identified in 64 day old green waste compost. Similarly Epstein (1997) have listed 16 species of bacteria, 16 species of actinomycetes and 35 species of fungi identified in compost. Therefore it would be expected for the bioaerosols released from compost to exhibit a wide range of physical characteristics (e.g. the presence of ridges on cell surface) due to a wide range of micro-organisms naturally present in compost. The results were in line with this (Section 4.3.4) where eight types of small size cells were identified. In contrast, only two major large cell types were identified (Section 4.3.5).

However, despite a range in observed physical characteristics, the results for both cells showed that (Section 4.4.4), the majority of bioaerosols emitted from compost are single cells that are 0.5-1 μm for small size cells and 1-2 μm for large size cells. The physical shapes of the single cells and their aggregates were also expressed in terms of aspect ratios (Section 4.3.8). The majority of the single small cell types that were identified had an aspect ratio of 1. The other half had aspect ratios at 1.4-1.5. However the results (Section 4.3.4) showed that on average for all controlled experiments and site work, small cells with an aspect ratio of 1 such as types A and D were dominant compared to other small cell types. Both of the large cell types that were identified also had an aspect ratio of 1. The range of bioaerosol aggregates were more varied with varying aspect ratios however the majority of the small cell aggregates had an aspect ratio of 1. For larger cell aggregates a higher percentage of particles with an aspect ratio of 1 to 1.5 were observed. However in conclusion, for all cell types and all samples the majority of single and aggregated particles observed to be emitted from compost had an aspect ratio of 1.

4.4.6. Differences in Culture and SEM Analysis

Previous studies have analysed air samples from agricultural dusts by scanning electron microscopy (SEM), fluorescence microscopy (FM) and the culture method (Karlsson and Malmberg,1989). They have found that the average CFU count was one sixth of the total count estimated by SEM or FM and attributed this to slow growth or high aggregating tendencies. Hence it was expected that "*bioaerosol concentrations determined by scanning electron microscopy analysis will be higher than those determined by culture analysis*". This hypothesis was supported based on the results of the controlled experiments and site work as presented in Section 4.3.8. On average, large spore and large spore aggregate concentrations detected using SEM methods were 1.1 times higher than *A. fumigatus* concentrations detected using culture methods. The difference between small spore and small spore aggregate concentrations compared to those for actinomycetes concentrations were even higher by 5.5 times.

Similarly, Heikkilä *et al.* (1988a; 1988b) reported a 10- to 100- fold difference in culture based and SEM results (i.e. the SEM results were higher) when analysing fungal spores

in cow barns and attributed this to the fact that only a small proportion of spores in the air was viable when collected for culture analysis methods. Some airborne spores are more likely to lose their viability quickly upon release from a source (Levetin and Horner, 2002) hence the dead spores captured with the sampling filters are not able to colonise on the culture plates. It is still possible to visualise these spores with the SEM method hence the number of total particles visualised with SEM should be higher than those captured with the culture method.

4.4.7. Limitations of the Controlled Experiments

SEM is able to provide accurate and detailed information on particle surface and physical particle size however the samples are prepared and scanned under vacuum conditions, which causes dehydration, collapse and distortion of particles that might introduce bias on the actual size and surface characteristic information of the particle (Heywood, 1969; Skujiņš *et al*., 1971; Gwaze *et al*., 2007). Therefore it is not possible to identify some small spores with the SEM as the size of these spores might result in the user not being able to identify the distinct morphological features of the species (Levetin and Horner, 2002). This is the case for some large cells where distinction between morphologically similar species such as *Aspergillus* and *Penicillium* is difficult by microscopy. Accurate distinction of cell characteristics requires the use of high magnifications that present time limitations on examination of samples. Subsequently, this results in microscopic samples being only a representation and not absolute values of the overall bioaerosol concentrations and proportions of release. Therefore firstly, it is important to analyse all results in the context that a very small percentage of the overall filter was analysed due to the time consuming nature of the method.

The classification of the shape and nature of particles of interest was based on subjective assessment. Similar limitations for Atomic Force Microscopy (AFM) and SEM techniques were discussed in previous studies that have reported the tendency of the operator to neglect some particles and consider more interesting particle features (Gwaze *et al.*, 2007; Shekunov *et al.*, 2007) and difficulties in distinguishing between species of similar morphology such as *Penicillium* and *Aspergillus* (Wittmaack *et al.*, 2005). However similar difficulties might exist for identification of micro-organisms

through different microscopy techniques. For example, Lacey and Dutkiewicz (1976b), during their experiments examining mouldy hay have noted that actinomycete spores were not able to be distinguished microscopically from bacterial spores.

Madelin and Johnson (1992) have shown that spore chain and aggregates shatter on impact in line with Trunov *et al.* (2001) who have noted that whilst aggregate particles are more likely to impact on a collection media, they may also deaggregate during impaction. Such an effect might have an implication in analysing the results because it might indicate that airborne aggregates are shattering on impact with the filter. This would mean that the number of airborne aggregates in air at the time of sampling may be higher than those that are captured on the filters.

Microscopic examination is needed to analyse the results of the air samples collected by other methods such as the Burkard spore trap. The counting methods of the microscopic slides commonly used for the Burkard spore trap are 12 vertical sweeps (Figure 4.25, a), single longitudinal sweep (Figure 4.25, b) or 3 or 4 longitudinal sweeps across the slide (Figure 4.25, c) (Levetin and Horner, 2002).

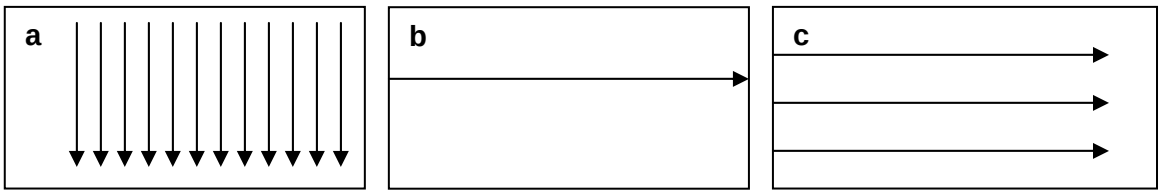


Figure 4.25 – Counting methods frequently used for Burkard spore trap slide analysis (from Levetin and Horner, 2002)

Levetin and Horner (2002) note other studies that have assessed the accuracy of different methods for counting of Burkard spore trap slides when compared with total slide counts. They have reported that a study (Kapyla and Penttinen, 1981), which had assessed the accuracy of different methods concluded that the method shown in Figure 4.25, a resulted in reliable estimates of daily airborne pollen compared to the method shown in Figure 4.25, b. This was in line with another study (Comtois *et al.*, 1999) who studied patterns of pollen and concluded that the method shown in Figure 4.25, a and c had smaller percentages of error compared to Figure 4.25, b. Finally a study completed

by Sterling *et al.*, (1999) examined the results for methods shown in Figures 4.25 b and a and concluded that method a was slightly advantageous in providing better approximations.

The SEM sampling protocol that was developed is a systematic way of ensuring reproducible analysis of the samples. The methodology developed for scanning a circular sample filter combines tranverse as well as longitudinal sweeps of the filter. Hence based on the argument for Burkard spore trap slide counting, it should present a good approximation of the presence of various spores and other particles emitted from compost. However, it is important to note that these are still indications of the outdoor bioaerosol concentrations rather than absolute values. In addition, the filter method collects air samples for 30 minutes at a time as opposed to continuous sampling with samplers such as the Burkard spore trap.

The protocol aims to avoid bias regarding areas of the filter that are concentrated with particular particles however, this very factor might have introduced a limitation to the methodology. Therefore if areas of the filter with high counts (concentrated particles of interest areas) are included in the analysis this might increase the particle concentrations predicted by SEM.

SEM has been previously used as a technique for characterising morphological properties of small particles (Friedbacher and Grasserbauer, 1995), examination of actinomycetes spores (Williams, 1970) and agricultural spores (Hiranuma *et al.*, 2008) and the study of actinomycetes in soil (Skujins *et al.*, 1971). Pasanen *et al.* (1989) used SEM to study fungi in farm houses whilst Borrego *et al.*, (2000) have studied microbial aggregation in mycobacterium using electron microscopy. Karlsson and Malmberg (1989) have noted that the study of microbial aggregation using SEM or optical microscopy is preferable to detection by Fluorescence Microscopy (FM). They have attributed this to the fact that SEM or optical microscopy allows direct examination of the original collection filter as opposed to collecting the bioaerosols on a second filter for FM detection by washing them off from the original collection filter.

Dead or unviable spores might still have health implications in terms of their potential to be allergens (Levetin and Horner, 2002). Therefore it is important to capture a combination of viable and non-viable bioaerosol spores in order to assess their health impacts. Based on this, a major advantage of the use of microscopic methods such as SEM in assessing the bioaerosol emissions is that microscopy allows to determine the number of viable as well as non-viable particles emitted from compost (Donham *et al.*, 1986; Karlsson and Malmberg, 1989; Levetin and Horner, 2002). In addition, use of SEM methods would allow the study of the presence of other particle in the air spora such as pollen which might be important when assessing the full health impact of the total air spora.

Advantages of SEM over other microscopy methods such as the light microscope is the ability of SEM to provide higher magnifications for the study of small particles, the 'life-like' images that are produced, greater image resolution and also the ease and practicality of sample preparation (Heywood, 1969; Hawker, 1971; Locci, 1972). Transmission Electron Microscopy (TEM) is another method used for the analysis of environmental particles however SEM has been discussed to be the superior technique (Mavrocordatos *et al.*, 2004). Atomic Force Microscopy (AFM) which is also based on the scanning of the sample surface is slower than SEM and the sample preparation methodologies are much more complicated than those for SEM (Shekunov *et al.*, 2007). However regardless of technique, any air samples taken only reflect a portion of the air spora collected at that point in time (Levetin and Horner, 2002).

The main advantage of the controlled experiments was that they were easy to conduct and allowed for a greater number of experimental repetition within research time constraints compared to the preparation and conduct of experiments in a composting site. Hence, they were a very useful and novel way of a thorough analysis of the nature of bioaerosols emitted from compost.

However, there were some possible concerns regarding the controlled experiments. The main concern was due to the collection of the compost sample used for the controlled experiments as discussed previously. As the sample was collected from a

depth of 20 cm, there is a possibility that it might not be representative of the material in the core of the compost windrow. Precautions were taken to use the material within an hour or so of collection and keep it sealed to ensure that the temperature and relative humidity of the sample were not compromised. However some changes of temperature and relative humidity might still have occurred.

The sealing of the environmental chamber was an important consideration to minimise interference from bioaerosols outside the chamber and to ensure that the spores released from the compost represent compost source data. Any holes inherent to the compost tumbler were sealed with heavy duty tape as per Kanaani *et al.* (2008). However the metal rod to which the IOM sampling heads were attached to had to be inserted into the compost tumbler through the non-taped off holes without taking off the screw on compost tumbler lid to ensure that no bioaerosols and particles escaped outside the compost tumbler. Flexible foam had to be placed at the end of the metal rods to cover the non-taped off holes to ensure that compost being agitated inside the tumbler is not able to penetrate outside the compost tumbler headspace (Figure 4.4). It is considered that some air from the outside environment might have penetrated through foam. However the use of foam was justified as a flexible material was needed and the effects of any possible contamination should be reduced by the use of the short sampling duration of 2 minutes.

Finally, subsequent to the agitation of the compost inside the compost tumbler, the procedure of inserting the metal rod to which the IOM sampling heads were attached to into the compost tumbler through the non-taped off holes without taking off the screw on compost tumbler lid took between 2-3 minutes. Therefore even though the results show that the use of the compost tumbler successfully released bioaerosols in the compost sample into the tumbler overhead, some of the bioaerosols released might have settled back into the compost within these 2-3 minutes. In addition, the amount of compost used for the experiments was small compared to a typical compost windrow. Hence based on these, in a composting facility, the numbers of bioaerosols emitted from compost might be higher than those studied in a controlled environment.

As a result of these possible concerns, the requirement to ensure that any site work produces results comparible to those for the controlled experiments arose. Therefore the methodology developed for the study of size distribution and aggregation emitted from compost as discussed in this chapter was used for further site work (Chapter 5) to examine the size distribution and aggregation of bioaerosol and particles emitted at a composting site.

These studies were also completed to validate the results of the controlled experiments and also to study the differences in the particle size distribution and aggregation of bioaerosols at a composting source and downwind from source. Therefore, the results presented in this chapter will be discussed in validation of the trends shown in the site work studies (Chapter 5) with emphasis on key implications. Finally, the conclusions for all studies completed to study the size distribution and aggregation of bioaerosols released from compost (Chapters 4 and 5) will be presented and discussed in the next chapter.

CHAPTER 5. AGGREGATION AND SIZE DISTRIBUTION OF BIOAEROSOLS EMITTED FROM COMPOSTING FACILITIES

5.1. INTRODUCTION

The gaps in the understanding of bioaerosols after release from composting facilities has been previously discussed (Chapter 1). One such gap with implications for bioaerosol behaviour at source, pathway and receptor is the lack of information on the aggregation and size distribution of bioaerosols released from compost. Due to this, controlled experiments were completed to generate bioaerosols in experimental conditions and to study the size distribution and aggregation properties of bioaerosol particles released from compost (i.e. source) (Chapter 4).

The controlled experiments had a number of advantages, including the fact that they were easy to conduct and repeat. However there were also some potential concerns regarding these experiments discussed in Chapter 4 including:

- the possibility of the compost sample collected for the experiments not being a representative of the material in a commercial compost windrow;
- the possibility of some bioaerosols released from the compost to settle in the 2-3 minutes it took to start the sampling;
- the possibility of the overhead space inside the compost tumbler being contaminated by other bioaerosols and
- the amount of compost sample used in the experiments being smaller compared to a commercial compost windrow.

Therefore it was felt that a requirement to ensure that any site work produces results comparible to those for the controlled experiments arose. As such, the developed methodology was used for further site work discussed in this chapter to examine the size distribution and aggregation of bioaerosols and particles emitted at a composting site. This was completed to validate the results of the controlled experiments discussed previously (Chapter 4) and classify the overall size distribution and visual properties (i.e. size, shape and aggregation) of bioaerosols emitted from compost at composting facilities. In addition, a number of additional composting site specific hypotheses were

formulated as these hypotheses were not possible to test in previous controlled experiments.

It was previously discussed (Chapter 4) that two of the dominant micro-organisms in compost (i.e *A. fumigatus* and actinomycetes) grow in branching filamentous structures (Lacey, 1997; Reynolds and Pepper, 2000; Papagianni, 2006) that might be released with activities (e.g. agitation of the compost) in a composting facility. However upon release, environmental effects such as wind would break up these aggregate structures into single cells. The results of the controlled experiments have supported this and the majority of the bioaerosols released from compost were in single cells.

Despite the low percentage of bioaerosol aggregates compared to the single cells, the results of the controlled experiments showed some evidence of aggregates in bioaerosols emitted from compost. However in a composting facility, even if a bioaerosol is released as an aggregate from compost, as this aggregate travels further downwind, it would be expected to break up further into single cells due to environmental effects. In addition, any larger particles such as aggregates present at source are likely to settle out with increasing distances downwind from source. Therefore it is hypothesised that *"if bioaerosol aggregates are observed to be emitted from compost in a composting facility, the percentage of aggregates compared to single cells will decrease with increasing distances downwind from bioaerosol source".*

As discussed previously (Chapter 4), the number of aggregate structures made up of 2-6 units were hypothesised to be in higher percentage than those made up of 7 or more units. In light of the results, this hypothesis was supported. If a bioaerosol aggregate travelling downwind from a compost source does not break into single cells, it will definetely be expected to break into smaller aggregates. Therefore in line with this and the noted hypothesis, it would also be expected that *"the size distribution of bioaerosols at a composting facility will change with increasing distances downwind from bioaerosol source as the larger particles settle".*

Based on these, the studies presented and discussed in this chapter were completed to examine the particle size distribution and aggregation of bioaerosols emitted upwind, at source and downwind at a composting facility (Donarbon Limited as previously discussed in Section 3.2.2, Chapter 3).

Finally, an opportunity was presented to take part in a study completed to examine the size distribution of bioaerosols in a composting site (Lount as previous discussed in Section 3.2.3, Chapter 3) and re-create the air sampling results that would typically be achieved by an Andersen six stage bioaerosol sampler by using different size air sampling filters. This opportunity was taken to collect further site work data to validate the developed SEM methodology and to gain further understanding of the size distribution and aggregation of biaoerosols emitted from compost.

The methodology used for the studies discussed in this chapter have previously been presented (Chapter 3). Therefore this chapter presents the results of the site work including details of total particle counts, particle classification and aggregation details. It is important to note that clear comparisons between the two sets of results from the different composting facilities cannot be made. The samples taken at Donarbon Limited were taken from various locations including upwind, downwind, agitation and static compost sources. It might only be possible to compare the agitation data collected at Donarbon Limited to the data collected at Lount as both sets of samples were taken in the vicinity of an agitation activity at a composting facility however variations through the use of different size filters are introduced at the site work completed at Lount. Therefore the results will be presented to reflect this and despite some comparisons in the analysis of the results, the data sets will be treated separately.

As this chapter aims to validate the hypotheses set and the results presented in the previous chapter (Chapter 4), the trends from all sets of data (i.e. controlled experiments and all site work) will also be discussed with emphasis on implications of the results. Finally, the key conclusions from this chapter and the previous chapter (Chapter 4) will be presented.

5.2. RESULTS - DONARBON LIMITED

5.2.1. Image Density of Sample Filters

The importance of the image density of the sample filters has previously been explained (Section 4.3.1., Chapter 4). Based on these, the results of the percentages of filter scanned per sampling location for the site work completed at Donarbon Limited are presented in Figure 5.1.

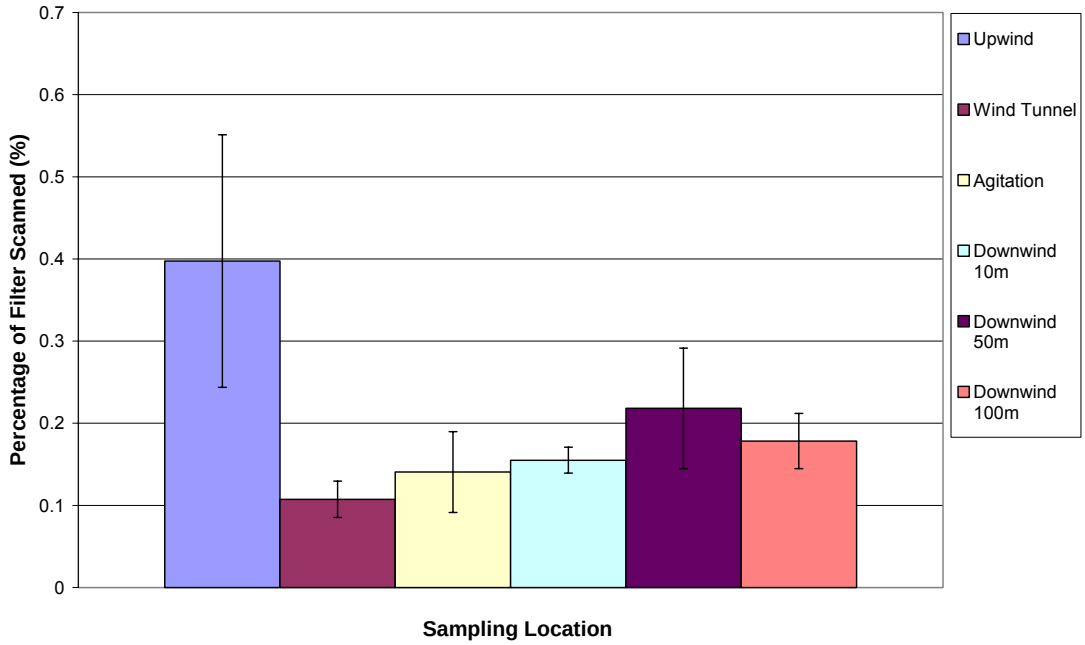


Figure 5.1- Results of the percentage of filter scanned for site work at Donarbon Limited. The bars indicate arithmetic mean (n=3) and error bars indicate standard error.

The filters taken at the upwind sampling location would not be expected to be heavily populated as these represent the background pollutant concentrations. In contrast, the filters taken at source (i.e. wind tunnel and agitation) would be expected to be most heavily populated with particles of interest as these capture the concentrations of the actual pollutant. However the filters taken at the agitation activity would be expected to be more densely populated with particles of interest as the agitation activity releases bioaerosols within static compost as well as other particles such as wood fibres or plant material. The image density of the filters would be expected to reduce as the pollutant plume travels downwind due to the pollutant being distributed due to effects such as wind and thermal diffusion.

As would be expected, the results show that the filters that were most sparsely populated were those for the upwind sampling and 0.39% of the overall sampling filter was scanned. Filters most heavily populated were those for the compost windrow wind tunnel sampling and 0.11% of the overall sampling filter was scanned. This is an unexpected result as the filters taken at the agitation activity were expected to be more densely populated with particles of interest compared to the ones taken at the static compost windrow using the wind tunnel. Of all downwind samples, the filters collected 50m downwind from the source were the least heavily populated with particles compared to those for the 10m and 100m downwind sampling locations this difference was not statistically significant ($p= 0.160120$). Finally for all sample filters taken during the site work at Donarbon Limited, on average 0.19% of the total sampling filter was scanned by SEM.

5.2.2. Total Cell Counts

The total number of particles that are assumed to be bacterial, fungal and actinomycetes cells and their aggregates counted with the scanning electron microscope were noted for the site work completed at Donarbon Limited. The same particles observed for the controlled experiments (see Appendix D) were also observed for the sampling completed at Donarbon Limited. Therefore these were classified as explained previously (Section 4.3.2., Chapter 4) and the results are presented in Figure 5.2.

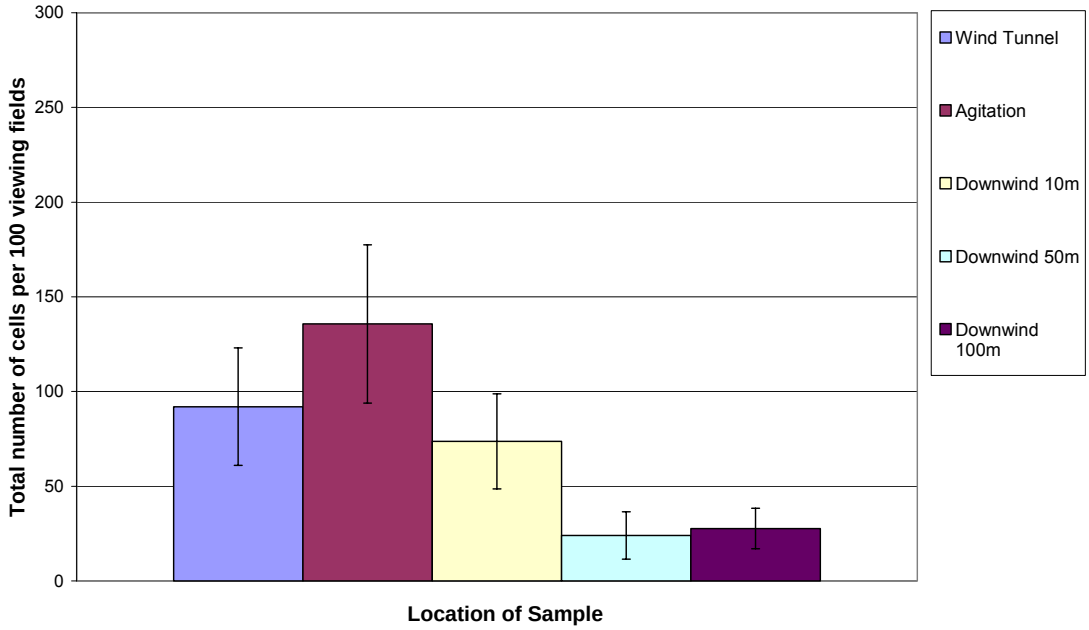


Figure 5.2 - Total number of cells per 100 viewing fields for the samples taken during the site work at Donarbon Limited. The bars indicate arithmetic mean (n=3) and error bars indicate standard error.

The filters taken at source (i.e. wind tunnel and agitation activity) would be expected to have the highest count of total cells at this site followed by those downwind. The results are in line with this and show that the highest number of cells were counted on the filters analysed at source for the agitation activity, followed by the filters analysed for the compost windrow wind tunnel. As expected, downwind total cell counts were lower than those measured at source. However the statistical analysis revealed the total number of cells sampled at different sampling locations were not significantly different than each other (p= 0.071011).

No cells were observed on the upwind sampling location air sample filter which might indicate low or no background concentrations of cells in line with the culturing results for Donarbon Limited (presented in Section 5.2.8.). However it is important to keep in mind that these results represent the cells observed on 100 viewing fields therefore it is possible that cells were present on other viewing fields on the upwind sampling location air sample filter.

5.2.3. Cell Classification and Aggregation

Similar to the results presented for the controlled experiments (Chapter 4), the SEM analysis of the sample filters taken at Donarbon Limited revealed two sizes of cells observed on the samples. These were classified as explained previously (Section 4.3.3., Chapter 4). As such, the distribution of single and aggregate cells for small and large cells for site work completed at Donarbon Limited is presented in Figure 5.3.

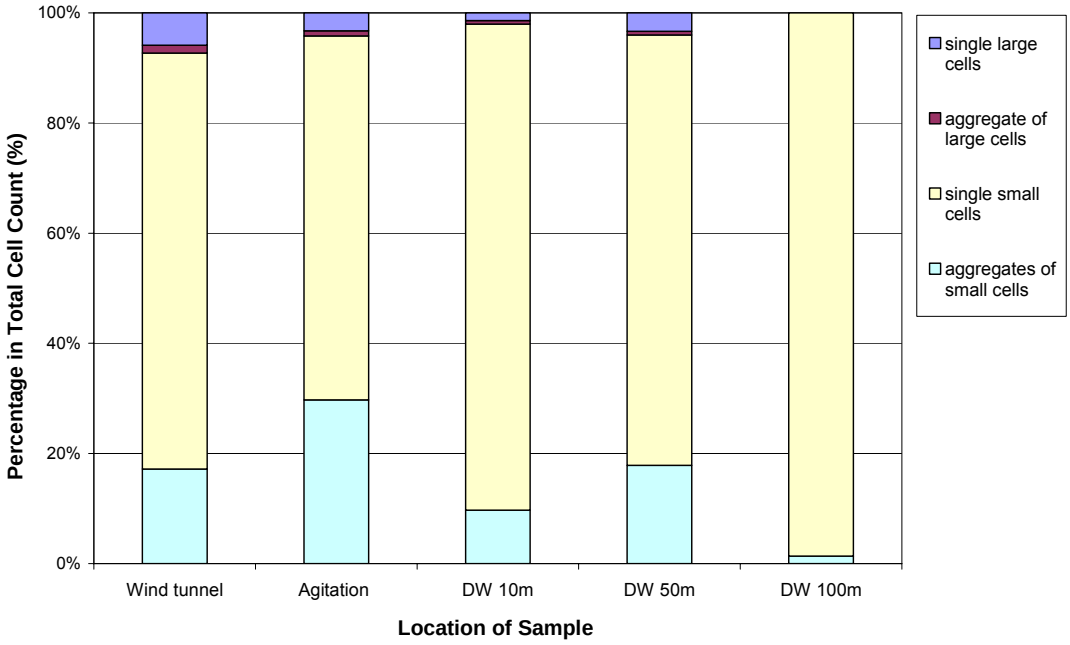


Figure 5.3 – Cell classification and aggregation for site work at Donarbon Limited. The bars indicate arithmetic mean (n=3).

At Donarbon Limited, it was expected that the sample collected at source (i.e. wind tunnel and agitation) would have the highest number of aggregate structures. However the cells released at source would either be expected to be broken up into single cell structures or deposited due to the increase in particle size and mass. Therefore the number of aggregate structures would be expected to decrease with increasing distance downwind from the source.

In summary, the results of the samples collected at Donarbon Limited showed that the majority of spores observed for all sampling locations were single small cells at 66-99% followed by their aggregates at 1.4-30%. In contrast, the percentage of single large cells and their aggregates are 1.3-6% and 0.7-1.4% respectively. Since it was not possible to

grow at *A. fumigatus* this might be indicative of other fungal cells that are aerolised. In line with this, no single large cells were identified 100m downwind at Donarbon Limited.

In terms of aggregation trends, the results are as expected in that the number of total cell (i.e. large and small size) aggregates at agitation are the highest followed by those at the static compost windrow. The results at 100 m downwind from source are also as expected as the majority of cells identified in this location were single small cells. The one way ANOVA results revealed that the variation across large aggregates between different sampling locations was not statistically significant ($p = 0.601810$) however the variation across small aggregates between different sampling locations were statistically significant ($p = 0.005390$).

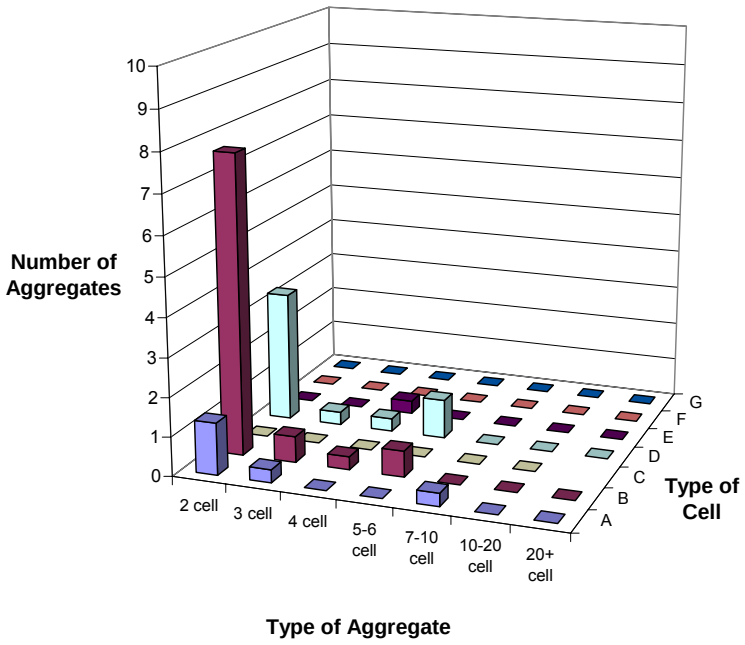
The percentage of small cell aggregates 50 m downwind would be expected to be less than those at 10 m downwind, however the results do not support this. Further analysis by Fisher LSD test also revealed that the small cells aggregate percentages at 50m and 100m downwind from source were not homogenous at the 95% confidence interval. Finally no cells of any type were identified upwind of compost source at Donarbon Limited hence this location was not presented in the results.

In summary, similar to the results presented for the controlled experiments, the results for all the site work completed at Donarbon Limited show that single small cells at 0.5-1 μm size range are the dominant cell type followed by their aggregates.

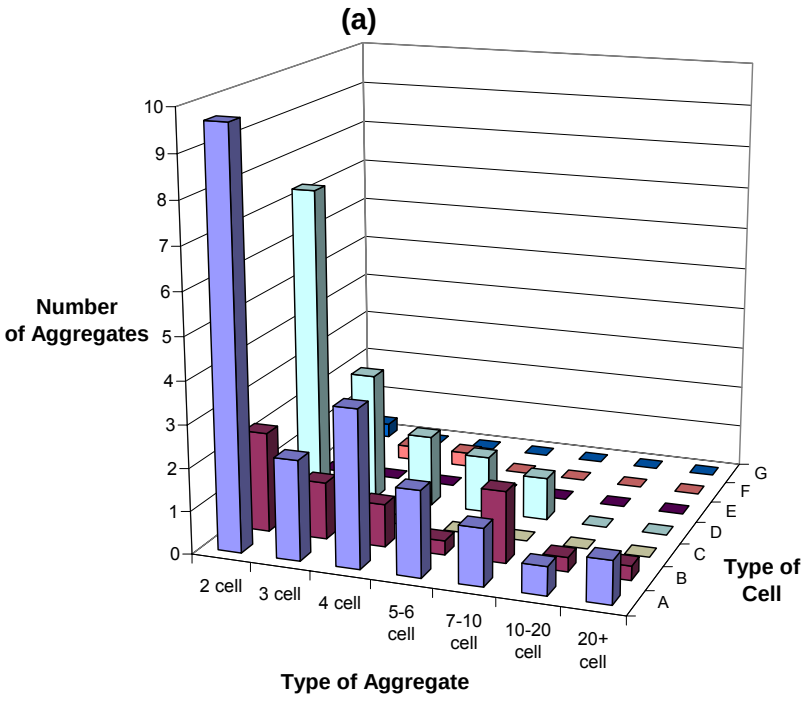
5.2.4. Small and Large Cell Aggregation

The same types of small size (Section 4.3.4., Chapter 4) and large size (Section 4.3.5., Chapter 4) cells and their aggregates listed previously for the controlled experiments were also observed for the samples taken during the site work at Donarbon Limited. For the small size cells, as in line with the controlled experiments results (Sections 4.3.4. and 4.3.5., Chapter 4), the aggregates consisting of 5-6, 7-10, 10-20 cells and bigger aggregates were classified together as it was not always possible to distinguish the exact number of cells in an aggregate. Based on this, the small cell aggregate distributions for site work completed at Donarbon Limited are presented in Figure 5.4. In

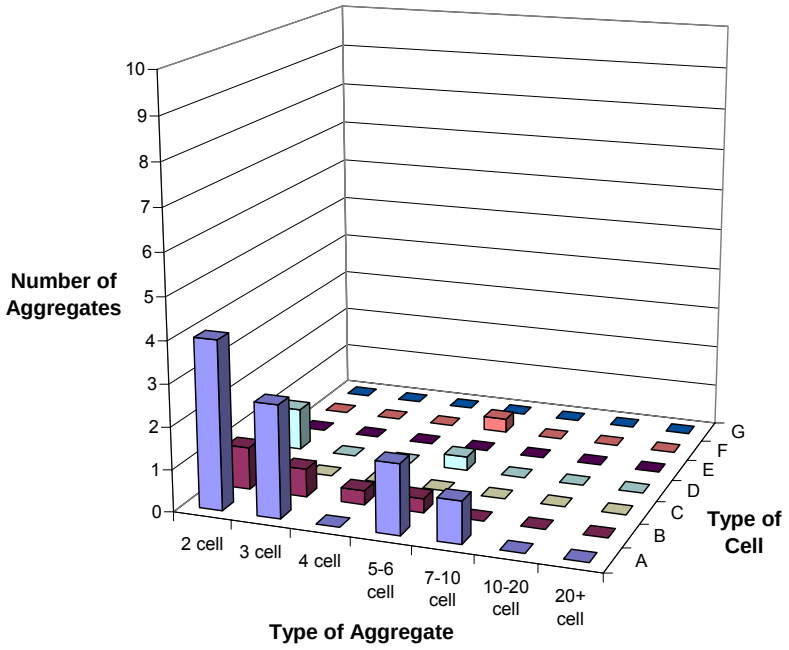
contrast to the results of the controlled experiments where small spore type H was occasionally identified, small spore type H was not identified for any of the samples hence are not represented in the following results.



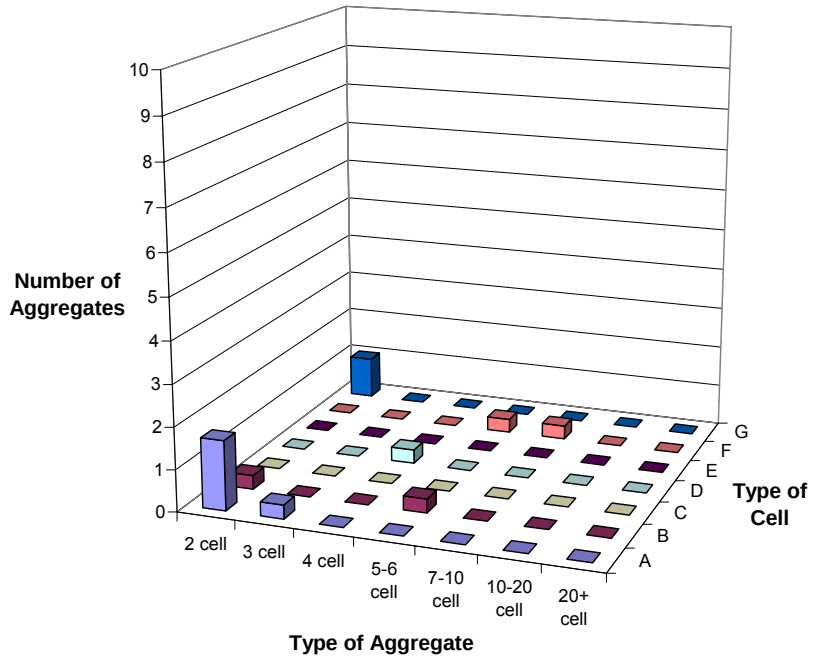
(a)



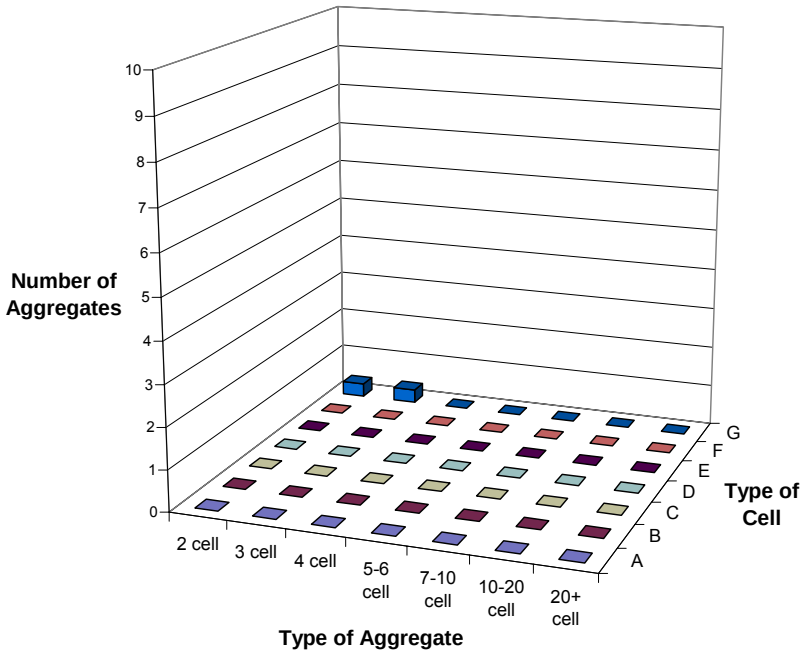
(b)


Number of Aggregates
10
9
8
7
6
5
4
3
2
1
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(c)


Number of Aggregates
10
9
8
7
6
5
4
3
2
1
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20+ cell
Type of Aggregate
G
F
E
D
C
B
A
Type of Cell

(d)



(e)

Figure 5.4- Small cell aggregate distribution for Donarbon Limited at (a) wind tunnel, (b) agitation, (c) downwind 10m, (d) downwind 50m and e) downwind 100m sampling locations. No small cell aggregates were observed for upwind sampling location. The bars indicate arithmetic mean (n=3).

The results show that similar to controlled experiments, the most abundant aggregate type for all sampling locations was a 2-cell aggregate. The types of aggregate structures made up of 3 or more cells at source were higher compared to the data from downwind sampling locations. The number of multi-celled aggregates were the highest for filters taken in the vicinity of the agitation activity compared to those taken with the wind tunnel at the static compost source. The agitation activity releases a large number of biaoerosols than those that might be sampled from a static compost source (as shown in the results presented in Section 5.2) hence this might account for this difference.

The results for the cell types show that small cell types B and D aggregates were dominant for wind tunnel compost windrow sample filters, in contrast to the agitation source sample filters where the small cell type A and D aggregates were dominant. For sample filters collected 10m and 50m downwind from source, small cell type A aggregates were higher in number however at 100m downwind small cell type G aggregates were in higher numbers. These indicate that on average, in line with the

results presented for the controlled experiments, particles with an aspect ratio of 1 are aerolised from compost.

The number of large cell aggregates observed at Donarbon Limited were much lower than those for small cell aggregates. Upwind, 50m downwind and 100m downwind from source, no large cell aggregates of any type were observed. For wind tunnel, agitation and 10m downwind sampling locations, on average (n=3), only one 2 cell Type A aggregate was observed. Therefore these results will not be shown in a graph.

5.2.5. Aggregate Structure

Similar to the trends observed for the controlled experiments, two different types of aggregates were observed on the sample filters analysed by SEM as discussed previously for the controlled experiments (Section 4.3.6, Chapter 4). As such, the classification of aggregates for small and large cells for the site work at Donarbon Limited are presented below in Figure 5.5.

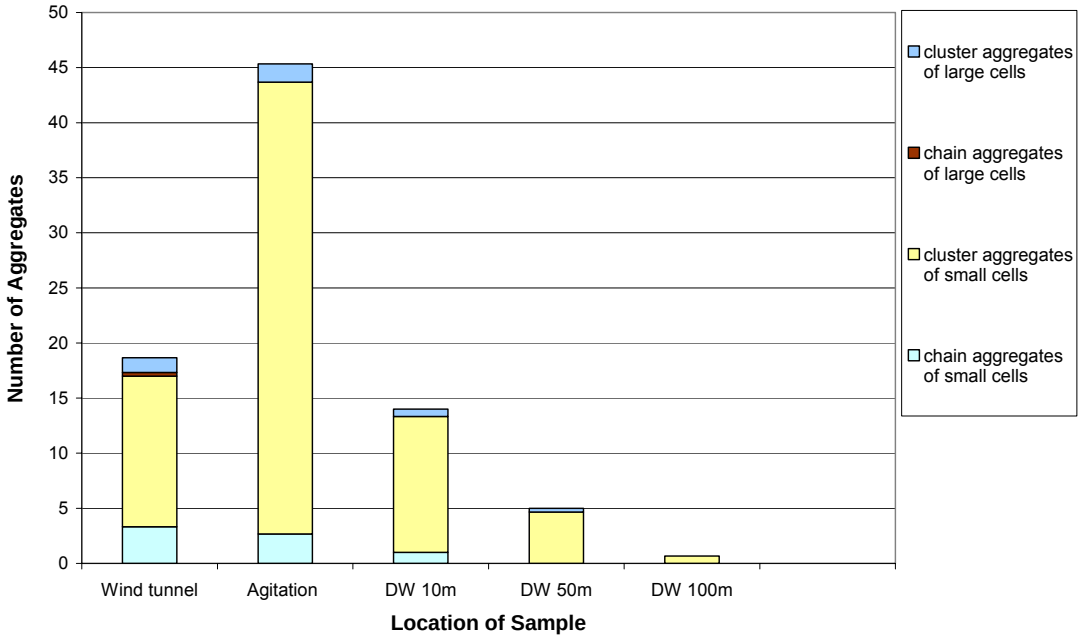


Figure 5.5- Small and large cell aggregate distribution for site work at Donarbon Limited. The bars indicate arithmetic mean (n=3)

The results show that the number of chain aggregates at source (i.e. wind tunnel and agitation) are higher. This is an expected result as chain aggregates are probably fragments of filamentous mycelial structures present in compost which have broken into

smaller pieces on release from compost. However the majority of the aggregates seen on the filters collected at Donarbon Limited are cluster aggregates. Therefore, similar to the results presented for the controlled experiments, the results indicate that either a larger percentage of non-filamentous micro-organism aggregates are being aerolised or that cells are clustering into aggregates upon release from compost .

5.2.6. Size Distribution

The 2D image dimensions (width and length) of the small and large cell aggregates were observed with SEM as explained previously (Section 4.3.7, Chapter 4). Based on this, the small cell size aggregate distribution for the site work completed at Donarbon Limited is shown in Figure 5.6.

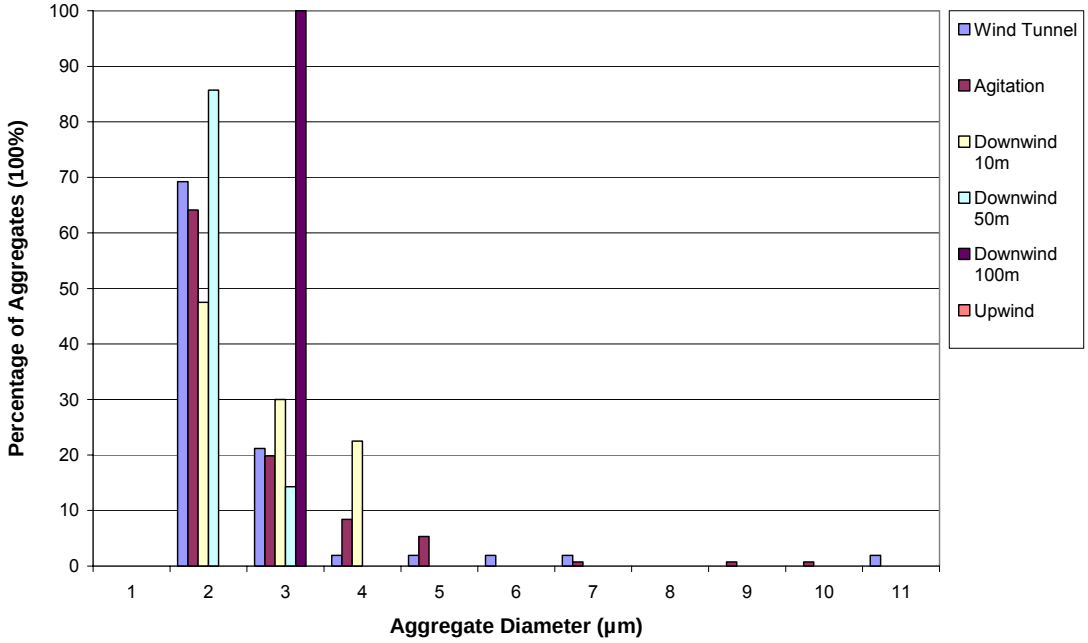


Figure 5.6- Small cell aggregate size distribution for site work at Donarbon Limited. The bars indicate arithmetic mean (n=3)

The small cell aggregates observed at Donarbon Limited with numbers of aggregates of 2 µm in diameter were highest followed by aggregates of 3 µm in diameter. Since the size of a single small cell is 0.5-1 µm, this would equate to 2-4 cell aggregates in line with the results presented in Section 5.2.4.

The aggregate size distribution for large cells were more varied where the majority of large cell aggregates for the agitation activity sampling location were 9 µm in diameter followed by 2 and 3 µm in diameter. The other sampling location where large cell aggregates were observed was the wind tunnel sampling location and the majority of the cell aggregates for this location were 2 µm in diameter. However the actual number of large cell aggregates were very low compared to small cell aggregates.

In addition to these results, the following table shows the number of individual units for the largest aggregate of small and large cells for site work at Donarbon Limited (Table 5.1).

Table 5.1 – The number of individual units for the largest aggregate of small and large cells identified per sample at Donarbon Limited. The values within the brackets denote the length and height of the observed aggregate in µm.

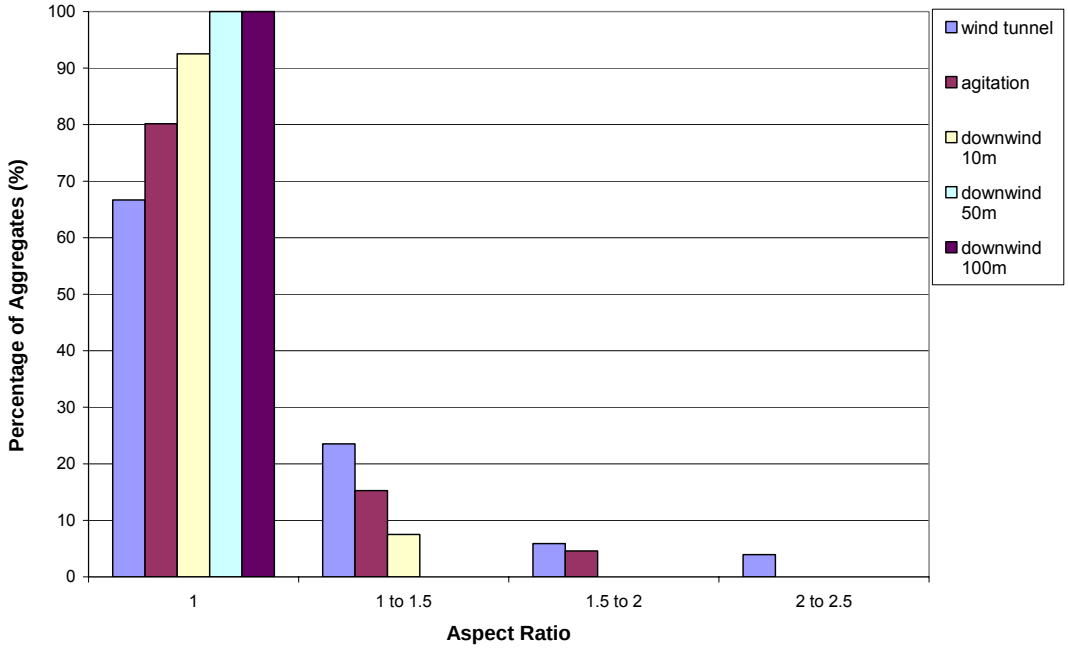
Sampling Location	Sample 1		Sample 2		Sample 3	
	Large cells	Small cells	Large cells	Small cells	Large cells	Small cells
Wind Tunnel	2 (5,4)	5 (5,4)	3 (3,3)	6 (7,3)	None	9 (6,4)
Agitation	10 (5,5)	40 (7,6)	2 (2,3)	50 (10,7)	None	11 (5,3)
Downwind 10m	2 (4,3)	6 (4,4)	None	7 (4,4)	None	7 (3,3)
Downwind 50m	None	5 (3,3)	None	9 (3,3)	None	2 (2,2)
Downwind 100m	None	None	None	3 (3,3)	None	None

The results show expected trends where the number of individual units per aggregate as well as the overall size of the aggregate is reduced with increasing distances downwind from bioaerosol source. Also in line with the expectations, the size of aggregates formed during the agitation activity are the biggest for all sampling locations. Surprisingly the aggregates sampled at the static compost windrow (wind tunnel) are lower in the number of individual units compared to the agitation activity however the overall size of the aggregate particle is similar to the aggregates observed for the agitation activity.

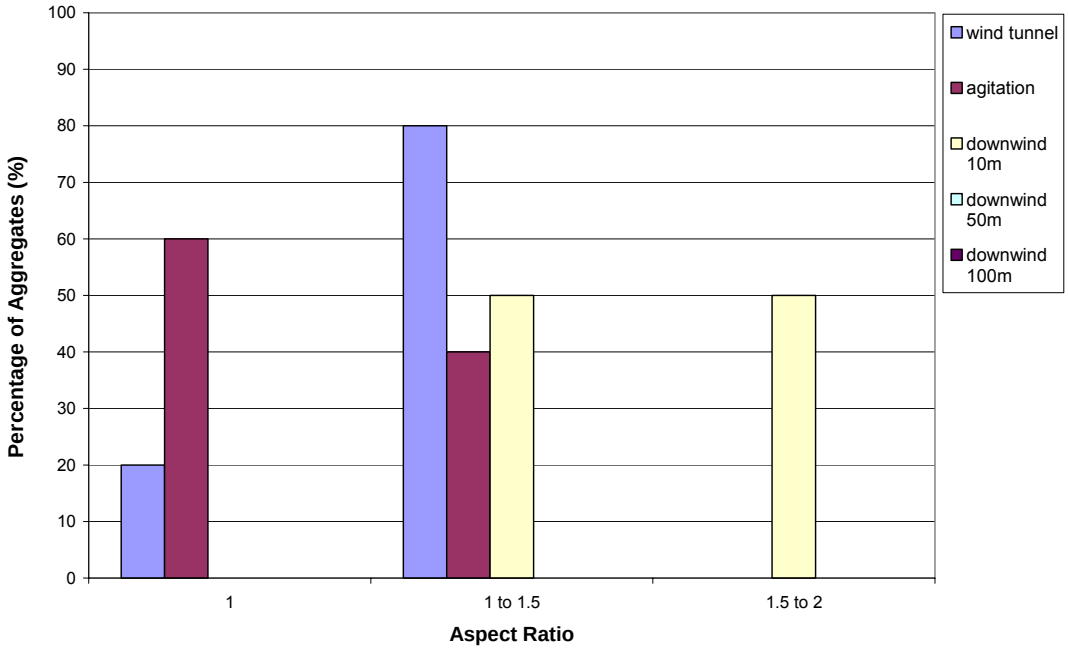
5.2.7. Particle Shape Distribution

The aspect ratios of aggregate structures for the small and large cell aggregates were observed with the SEM as explained previously (Section 4.3.8, Chapter 4). Based on

this, the small and large cell size particle shape distributions for the site work completed at Donarbon Limited are shown in Figure 5.7.



(a)



(b)

Figure 5.7- Aspect ratio distribution for small cell (a) and large cell (b) aggregates for site work at Donarbon Limited. The bars indicate arithmetic mean (n=3)

The results show that, similar to the results presented for the controlled experiments, the majority of small cell aggregates had an aspect ratio of 1. However a distinct peak of aggregates with an aspect ratio of 1 for the agitation activity samples was observed. The aspect ratios for larger cell aggregates were more varied where for the wind tunnel sample, the aggregates with an aspect ratio of 1 to 1.5 was higher than those with an aspect ratio of 1.

5.3. RESULTS - LOUNT

5.3.1. Image Density of Sample Filters

The importance of the image density of the sample filters has previously been explained (Section 4.3.1., Chapter 4). Based on this, the results of the percentages of filter scanned per sample for the site work completed at Lount is presented in Figure 5.8.

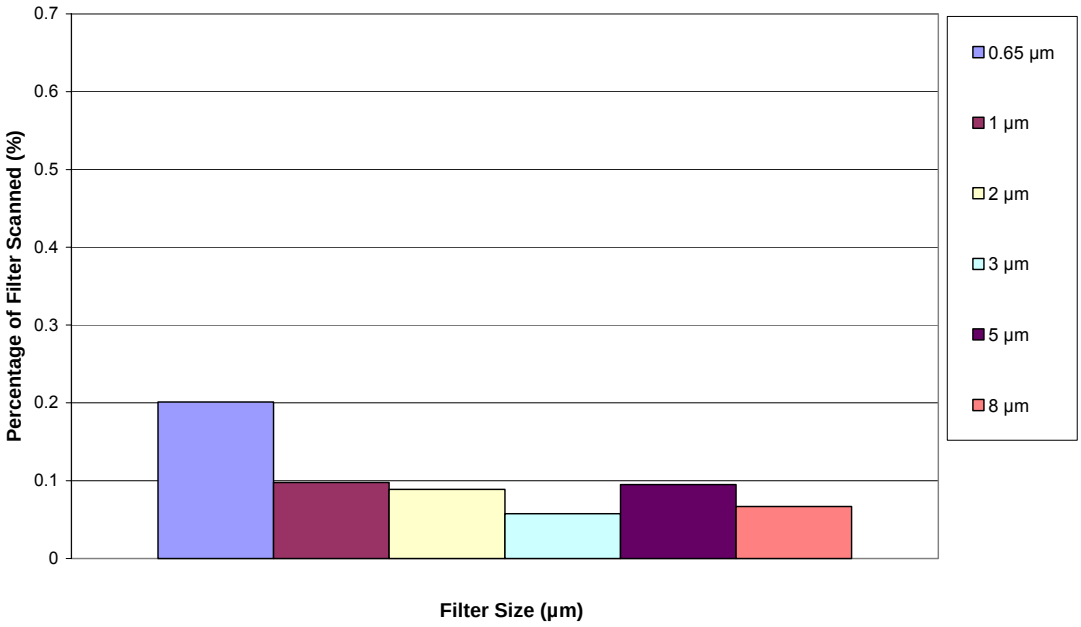


Figure 5.8- Results of the percentage of filter scanned for site work at Lount. The bars are the results from one filter.

The sample filters collected at Lount were all taken at the same location at an agitation source. Therefore as expected the sampling filters collected at Lount were more heavily populated with particles of interest when compared to the sampling filters collected at the controlled experiments or at site work completed at Donarbon Limited (0.1%). The results showed that the sample with a filter pore size of 0.65 µm was most sparsely

populated and the sample filter pore size at the mid range of 3 µm was most heavily populated with particles of interest. However it is important to note that these results are from one filter per sample and hence a statistical analysis of the results was not possible.

5.3.2. Total Cell Counts

The total number of particles that are assumed to be bacterial, fungal and actinomycetes cells and their aggregates counted with the scanning electron microscope were noted for the site work completed at Lount, in line with the results presented previously for the controlled experiments and the site work at Donarbon Limited. These were classified as explained previously (Section 4.3.2., Chapter 4) and the results are presented in Figure 5.9.

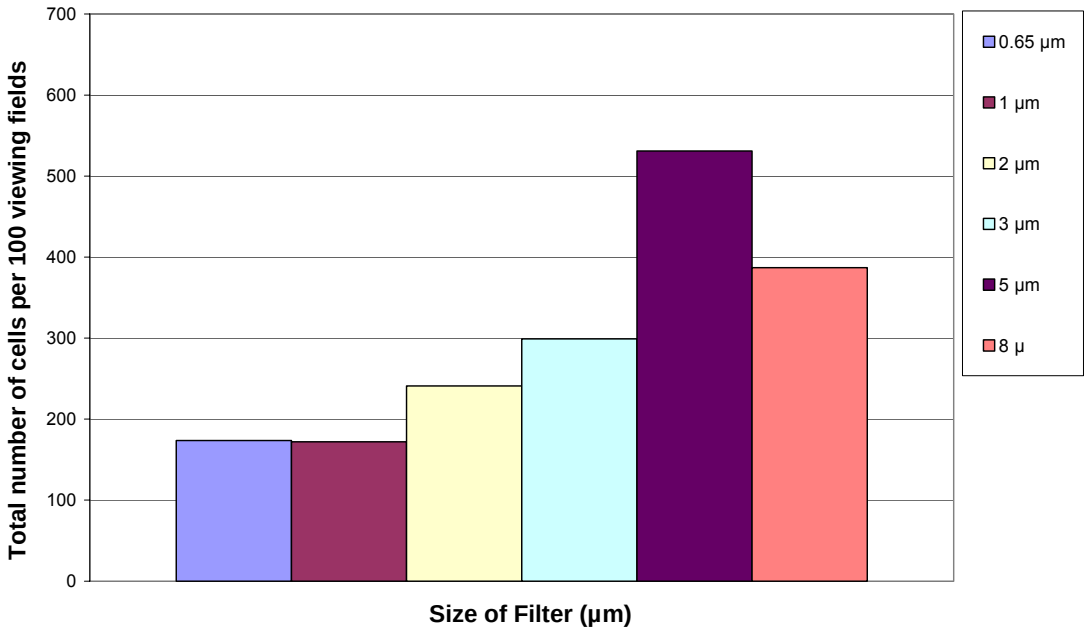


Figure 5.9 - Total number of cells per 100 viewing fields for the samples taken during the site work at Lount. The bars for are the results from one filter.

The results show that the highest number of total cells are counted on 5 µm filters followed by those counted on 8 µm filters. The lowest number of cells were counted on 0.65 and 1 µm size filters. These results show that filters with larger holes are able to retain a higher number of total cells as well as filters with smaller holes. This might indicate that the bioaerosols are sticking to the filter and not permeating through the large holes despite the flow of air through the filter.

5.3.3. Cell Classification and Aggregation

Similar to the results presented for the controlled experiments and the Donarbon Limited site work, the SEM analysis of the sample filters at Lount also revealed two sizes of cells observed on the samples.These were classified as explained previously (Section 4.3.3., Chapter 4). In line with this, the distribution of single and aggregate cells for small and large cells for site work completed at Lount is presented in Figure 5.10.

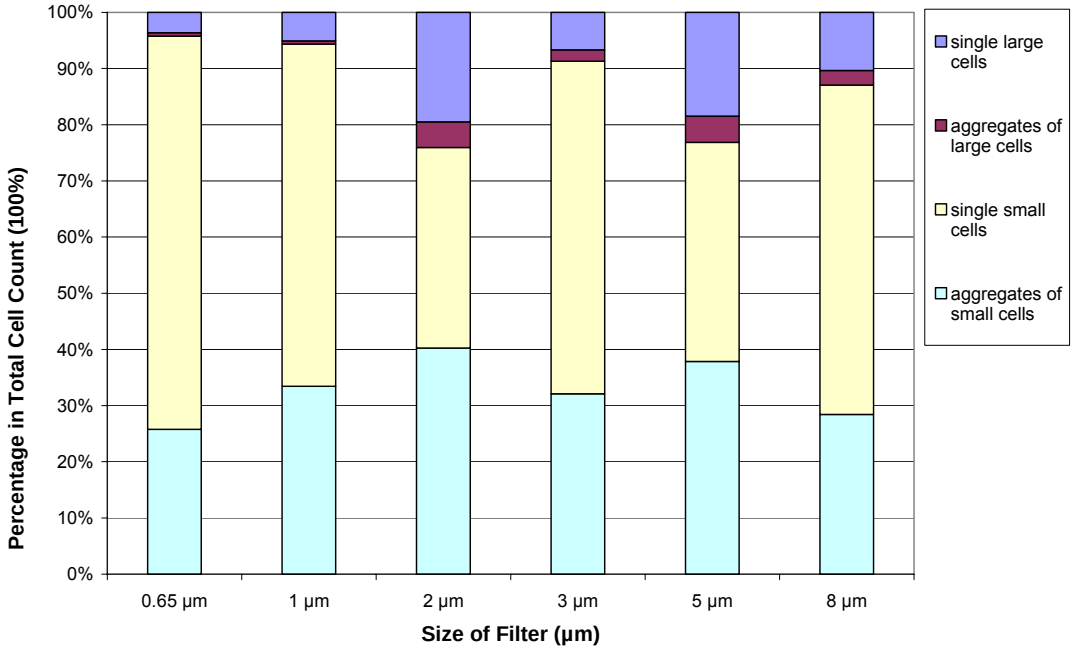


Figure 5.10 - Cell classification and aggregation for site work at Lount. The bars indicate results from one filter.

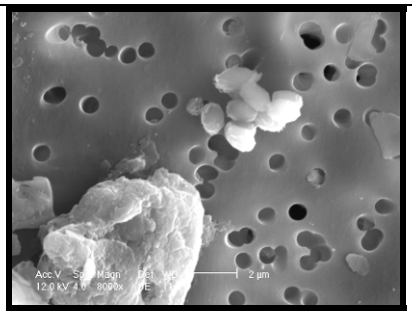
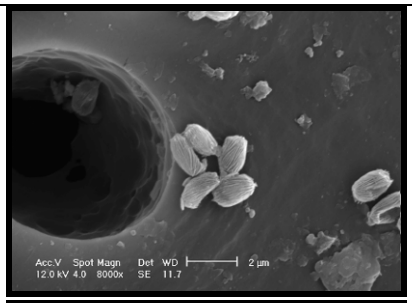
The majority of cells observed for filter sizes were single small cells at 36-70% followed by their aggregates at 26-40%. In contrast, the percentage of single large cells and their aggregates are 4-20% and 0.1-5% respectively. In terms of individual filter pore sizes, no clear trend in microbial aggregation was observed. The largest percentages of aggregates were observed for filter sizes 2 and 5 µm with the smallest percentages aggregates observed for filter sizes of 0.65 and 8 µm.

5.3.4. Small and Large Cell Aggregation

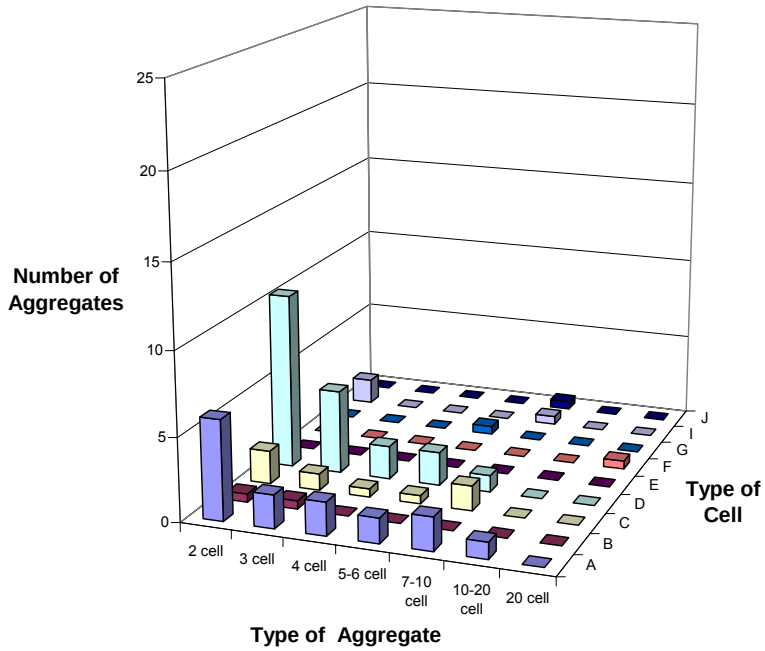
The same types of small size (Section 4.3.4., Chapter 4) and large size (Section 4.3.5., Chapter 4) cells and their aggregates listed previously for the controlled experiments and Donarbon site work were also observed for the samples taken during the site work at Lount. However in addition to these, two other types of small size cells and their

aggregates were also observed for the samples taken at Lount and these are listed in Table 5.2.

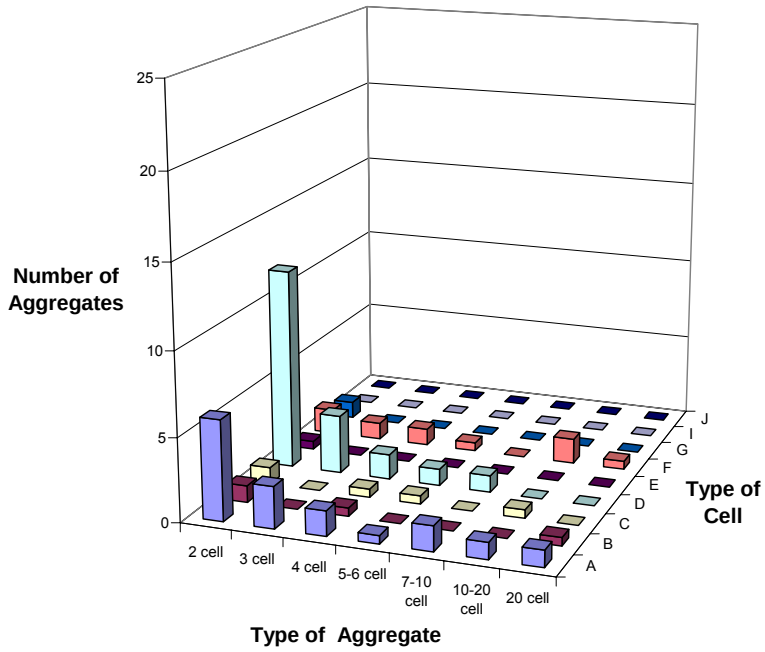
Table 5.2 –Cell description and image examples of new small size cells and their aggregates observed at Lount. Observed size for all cells 0.5-1 µm length

Cell Type	Cell Description	Example of Aggregate Structure and Number
I	Oval shaped particles with smooth appearance	 *8 cell aggregation*
J	Oval shaped particles with 'ridged' appearance	 *5 cell aggregation*

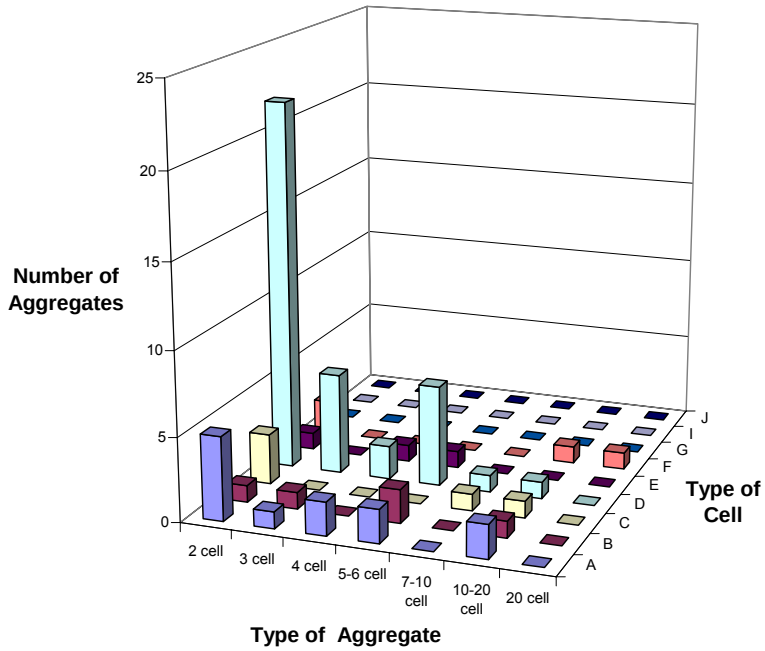
For the small size cells, as in line with the controlled experiments results (Sections 4.3.4., Chapter 4), the aggregates consisting of 5-6, 7-10, 10-20 cells and bigger aggregates were classified together as it was not always possible to distinguish the exact number of cells in an aggregate. The small cell aggregate distributions for the sample filters collected during the site work at Lount are presented in Figure 5.11. Similar to the Donarbon Limited site work results, small cell type H was not identified for any of the samples taken at Lount hence are not represented in the following results.


Number of Aggregates
25
20
15
10
5
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20 cell
Type of Aggregate
J
I
G
F
E
D
C
B
A
Type of Cell

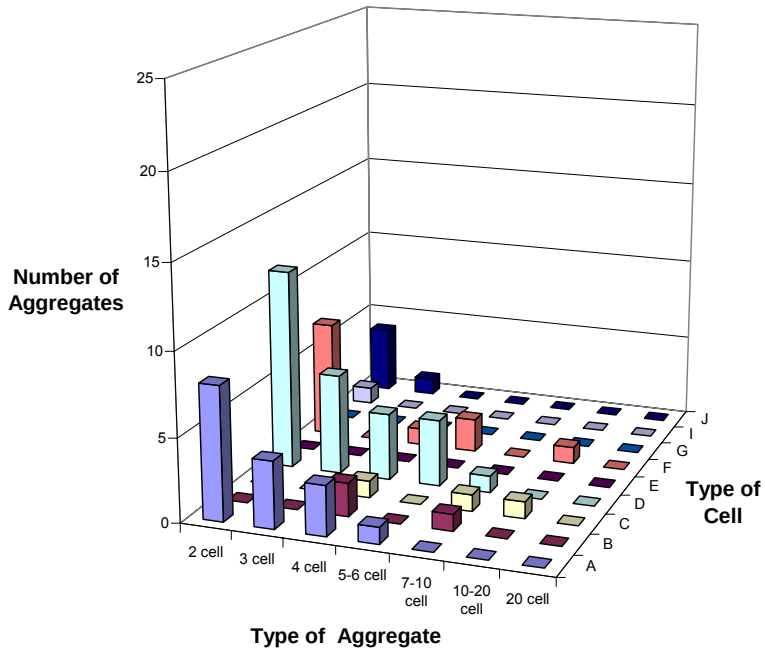
(a)


Number of Aggregates
25
20
15
10
5
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20 cell
Type of Aggregate
J
I
G
F
E
D
C
B
A
Type of Cell

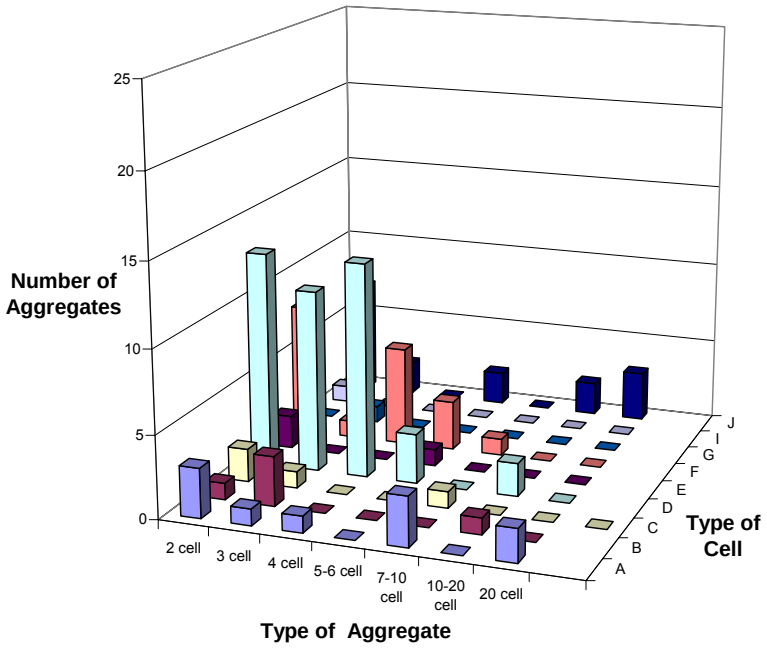
(b)


Number of Aggregates
25
20
15
10
5
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20 cell
Type of Aggregate
J
I
G
F
E
D
C
B
A
Type of Cell

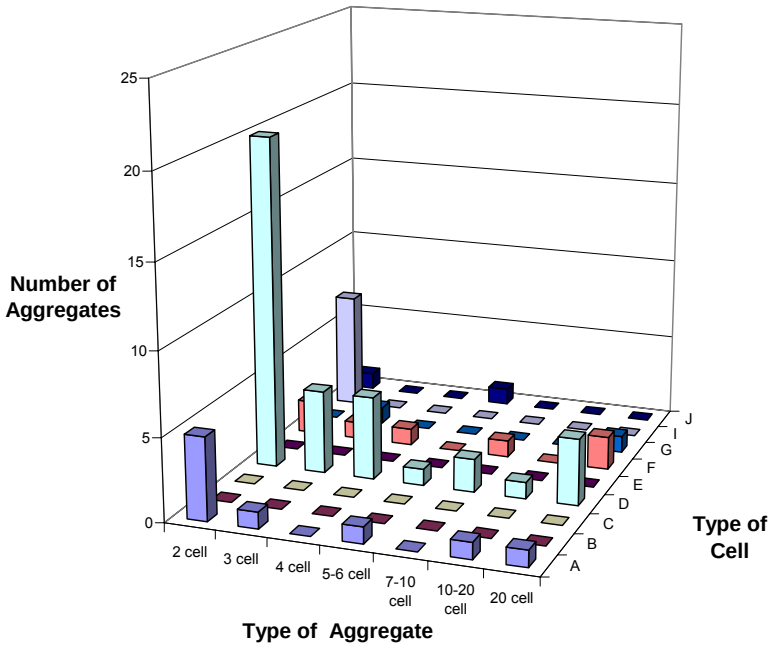
(c)


Number of Aggregates
25
20
15
10
5
0
2 cell
3 cell
4 cell
5-6 cell
7-10 cell
10-20 cell
20 cell
Type of Aggregate
J
I
G
F
E
D
C
B
A
Type of Cell

(d)



(e)



(f)

Figure 5.11 – Small cell aggregate distribution for Lount Site work for (a) 0.65 µm (b) 1 µm, (c) 2 µm (d) 3 µm, (e) 5 µm and (f) 8 µm filter sizes. The bars for are the results from one filter.

The results show overall trends that are similar to controlled experiments and Donarbon Limited site work, the most abundant aggregate type for all sampling locations was a 2-cell aggregate and for all filter sizes, small cell type D aggregates. However apart from

this, no clear trends such as those that might be expected are evident. Finally, in terms of the number of aggregates, the average number of 2-cell aggregates counted on the Lount sampling filters is 15 aggregates compared 5 aggregates for Donarbon Limited sample filters and 40 aggregates for controlled experiment sample filters.

The highest number of large cell aggregates overall for any experiments or site work were observed at Lount and these are shown in Figures 5.12 and 5.13 for spore types A and B respectively.

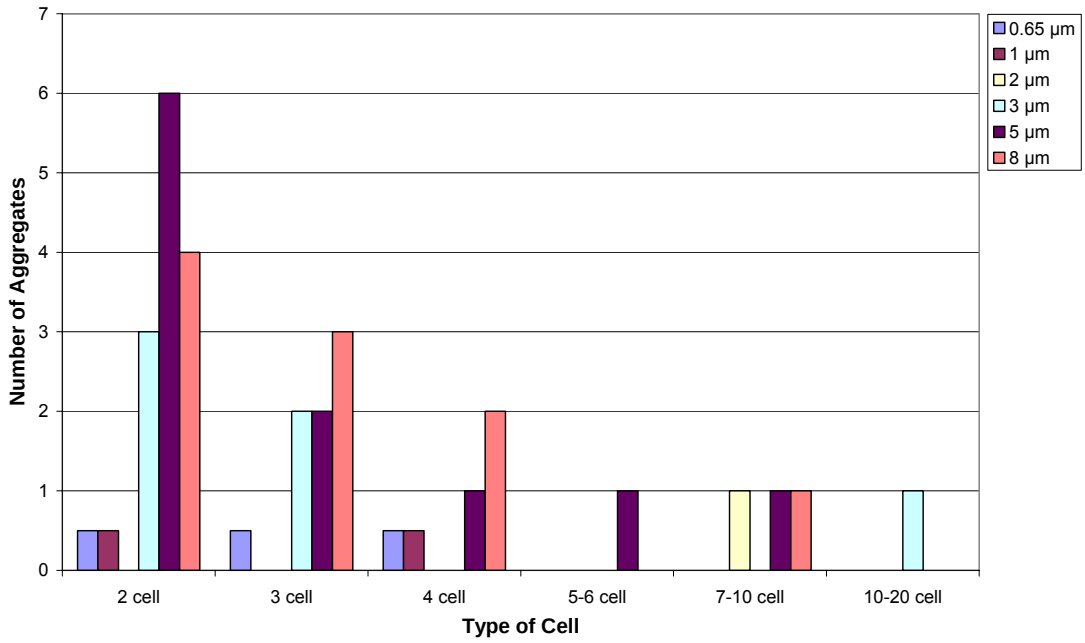


Figure 5.12- Large cell type A aggregate distribution for site work at Lount. The bars for are the results from one filter.

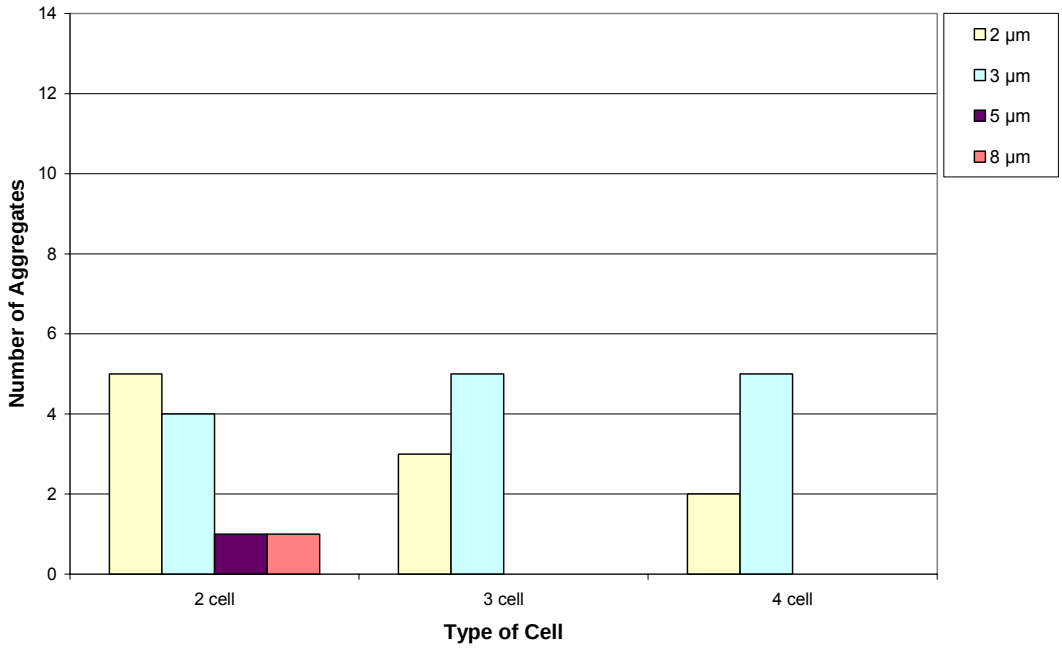


Figure 5.13- Large cell type B aggregate distribution for site work at Lount. The bars for are the results from one filter.

The results expected for the sampling filters taken at Lount in terms of large cell aggregate structures would be the same for those observed for the small cell aggregate structures. However the results show that for large cell type A, the number of smaller aggregate structures (2 and 3 cells) were higher for filter sizes of 3, 5 and 8 µm. No large cells of type B were observed on filter sizes of 0.65 and 1 µm and the majority of aggregate structures were on filter sizes 2 and 3 µm.

5.3.5. Aggregate Structure

Similar to the trends observed for the controlled experiments and Donarbon Limited site work, two different types of aggregates were observed on the sample filters analysed by SEM as discussed previously (Section 4.3.6, Chapter 4). Based on this, the classification of aggregates for small and large cells for the site work at Lount are presented below in Figure 5.14.

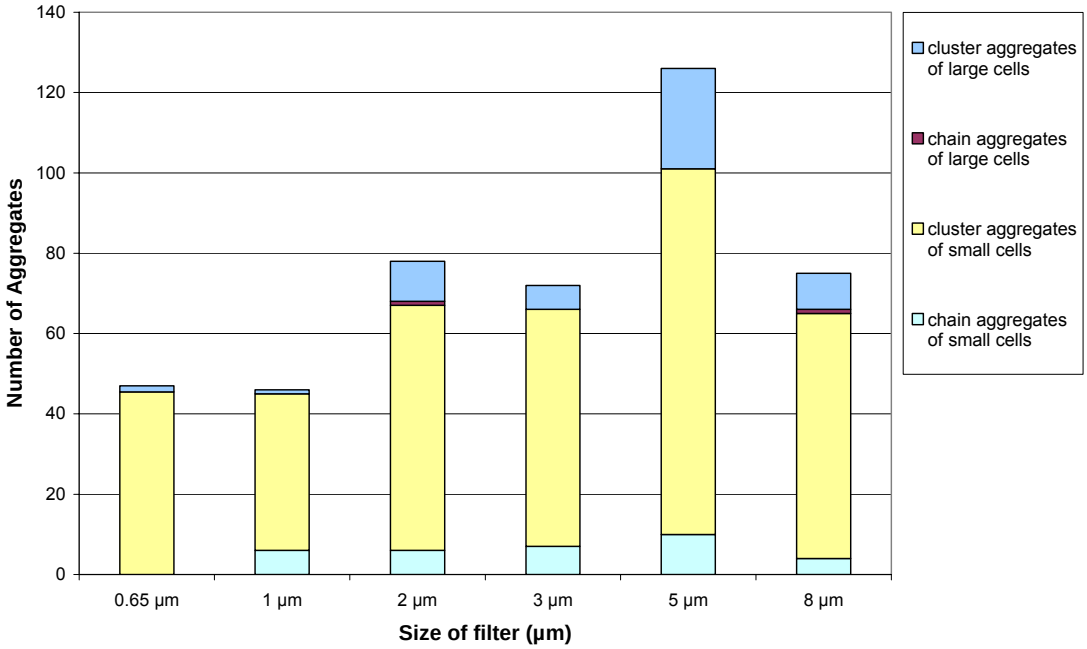
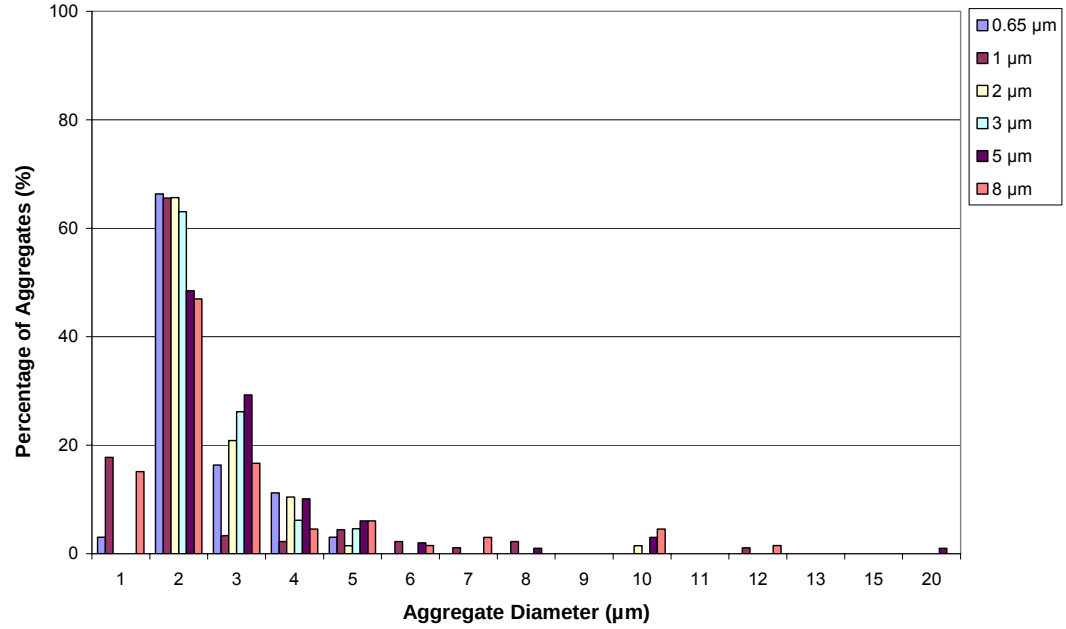


Figure 5.14- Small and large cell aggregate type distribution for site work at Lount. The bars for are the results from one filter.

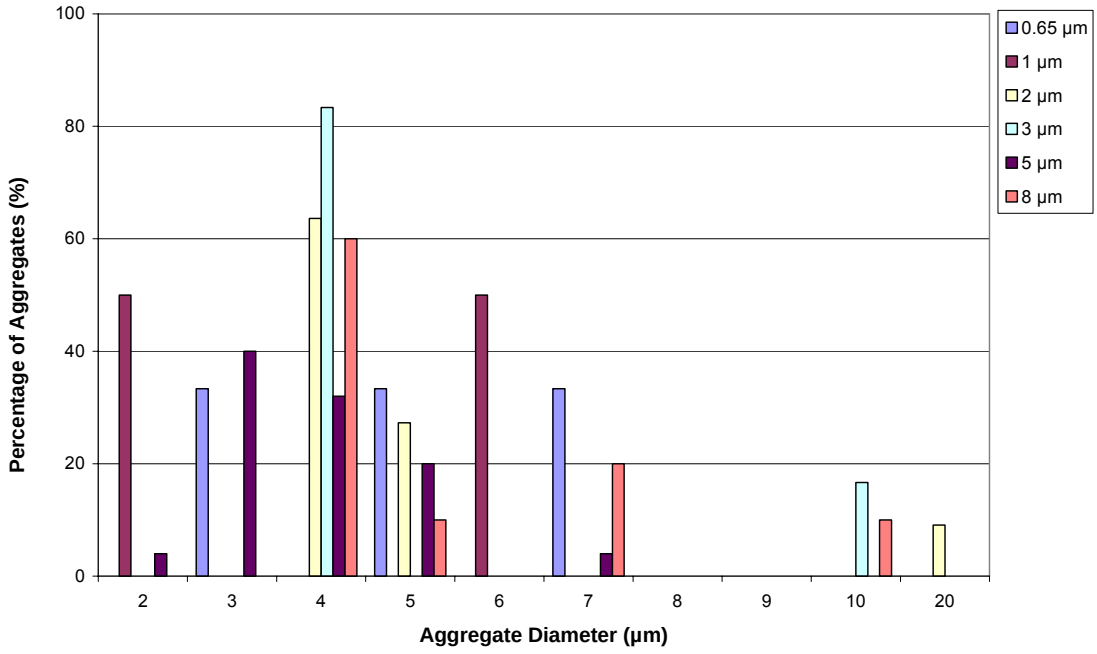
The results for Lount are similar to those for controlled experiments and Donarbon where cluster aggregates are the most abundant type of aggregate for both small and large cells.

5.3.6. Size Distribution

The 2D image dimensions (width and length) of the small and large cell aggregates were observed with SEM as explained previously (Section 4.3.7., Chapter 4). As such, the small and large cell size aggregate distributions for the site work completed at Lount are shown in Figure 5.15.



(a)



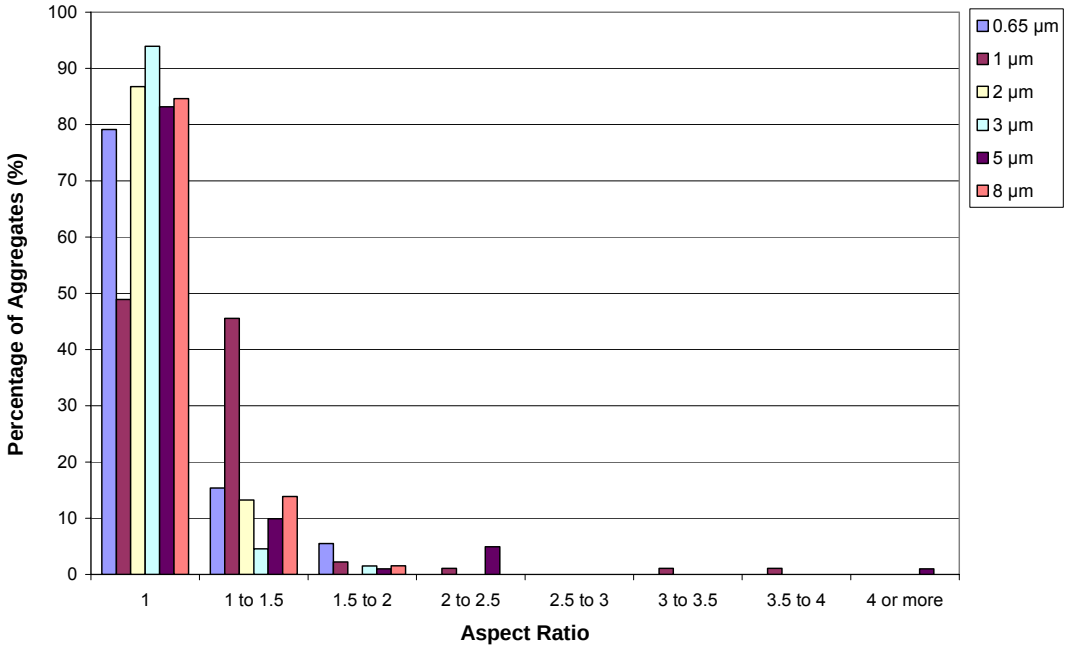
(b)

Figure 5.15- Small cell (a) and large cell (b) aggregate size distribution for site work at Lount. The bars for are the results from one filter.

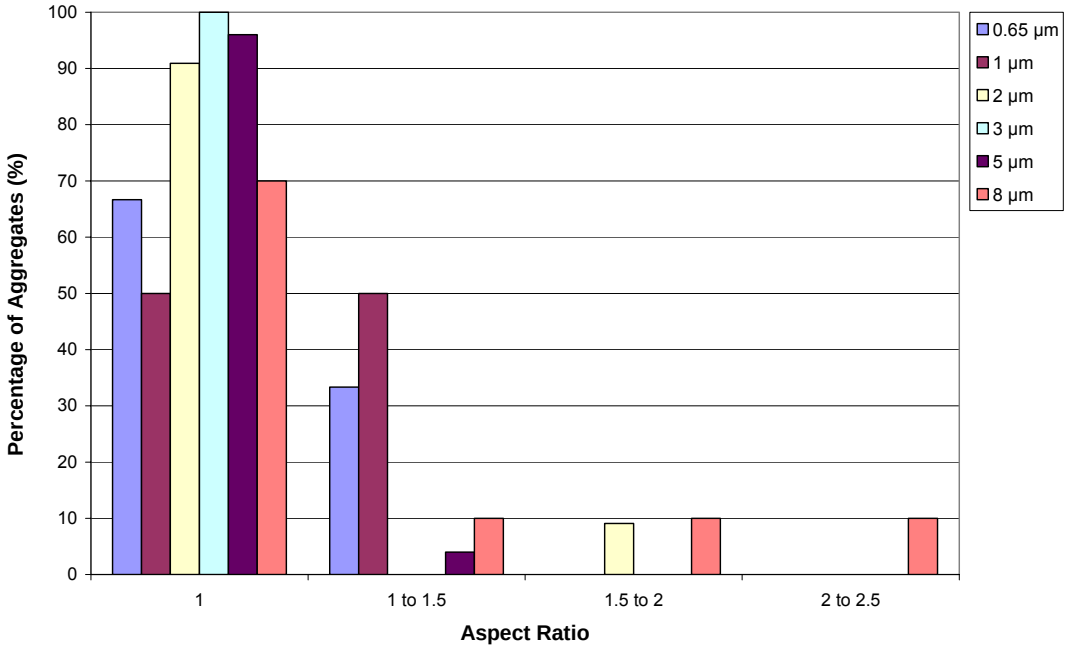
The numbers of aggregates for small spores of 2 and 3 µm overall diameter were the highest. The number of small spore aggregates with overall diameters of 10 µm or more were limited. This trend is the same as those observed previously for controlled experiments and for Donarbon Limited site work.

5.3.7. Particle Shape Distribution

The aspect ratios of aggregate structures for the small and large cell aggregates were observed with the SEM as explained previously (Section 4.3.8, Chapter 4). Based on this, the small and large cell size particle shape distributions for the site work completed at Lount are shown in Figure 5.16.



(a)



(b)

Figure 5.16- Aspect ratio distribution of small cell (a) and large cell (b) aggregates for site work at Lount. The bars for are the results from one filter.

The results show that the majority of small cell aggregates had an aspect ratio of 1 in line with the trends shown for the controlled experiments and the Donarbon Limited site work. However, the aspect ratio distribution was very distinct for large cells where the majority of all aggregates had an aspect ratio of 1 whilst for the controlled experiments there was a larger percentage of large cells with an aspect ratio of 1 to 1.5.

5.4. DISCUSSION

The previous chapter (Chapter 4) has presented and discussed the results of the controlled experiments designed to release bioaerosols into an experimental chamber. This novel methodology allowed for quick conduct of experiments and allowed the researcher to collect repetitive data. However some potential concerns were identified which identified the need to validate the results for the controlled experiments with those at a composting facility. Therefore, the site work presented in this chapter were completed to validate the methodology and results presented in the previous chapter and also to study the study the aggregation and size distribution of bioaerosols emitted from composting facilities. Firstly the results presented in this chapter will be discussed

in validation of the trends shown throughout the previous chapter and overall implications will be presented. In addition, a set of hypothesis which were not possible to test within the controlled experiments were set. Therefore following the discussion of key implications, each hypothesis and objective will be discussed separately in light of the results. Finally the key conclusions from both chapters (Chapters 4 and 5) will be presented.

5.4.1. The Validation of the Controlled Experiments

Due to a number of potential concerns regarding the controlled experiments, similar studies were completed at two different composting sites to ensure that any site work produces results comparible to those for the controlled experiments. Therefore the comparison of the general trends shown for the controlled experiments and site work would help to validate the methodology developed for the controlled experiments.

However it is important to discuss the potential differences between the methodology of data collection for the three set of studies as the results should be analysed in this context. The methodology developed for the controlled experiments was designed to ensure that bioaerosols were released into the experimental chamber. The turning of the compost tumbler was introduced based on the argument that that a higher number of bioaerosols are released from agitation activities compared to static compost windrows. Hence the results of the controlled experiments might be comparable to those at an agitation activity at a composting site and comparisons between the results at Donarbon Limited and the controlled experiments will be made on this basis. However there are some potential differences between the pollutant plume for the two methods. The plume released into the compost tumbler headspace represents those micro-organisms that are in the source term only and a closed environment was chosen for the experiments to ensure that there was minimal interference from other background micro-organisms. However the plume in an agitation activity taking place at a composting site might also include background micro-organisms as well as those released from other agitation activities at the compost facility.

The studies at Donarbon Limited and Lount were both conducted on site however there are some distinct differences between the two. The samples taken at Donarbon Limited were taken from various locations including upwind, downwind, agitation and static compost sources. In contrast, the data collected at Lount were taken in the vicinity of an agitation activity. Therefore it might only be possible to compare the agitation data collected at Donarbon Limited to the data collected at Lount, however it is important to remember that other variations are introduced through the use of different size filters. In the light of these, the trends for key conclusions are presented and discussed.

Image Density

For all controlled experiments and site work the percentage of the sample filter that was scanned was analysed to provide an indication of how populated a sample filter was. In this context, if the particles of interest on a sample filter were located sparsely this would result in the recording of a high number of 'blank' viewing fields in between the particles of interest. Since each sample filter needs to be scanned for 100 viewing fields containing particles of interest, this would result in a higher percentage of the overall sample filter being analysed. However, if a sample filter was heavily populated with particles of interest, this would result in a smaller number of 'blank' viewing fields being recorded resulting in a lower percentage of the overall sample filter being scanned.

The results showed that for all sample filters taken during the controlled experiments (Figure 4.10), the range of the percentage of the total sampling filter scanned by SEM was 0.19 -1.1%. In contrast, the sample filters taken at the agitation activity for Donarbon Limited (Figure 5.1) were slightly more populated with particles of interest at a lower percentage of the total sampling filter being scanned by SEM at 0.13%. The results for Lount (Figure 5.18) were similar to those at Donarbon Limited and, on average, 0.1% of the total filter was scanned. This comparison shows that the controlled experiments are able to produce overall results comparable to those at a composting site. It might have been expected that the sample filters in the compost tumbler might have experienced overloading on the filter due to the close proximity of the filter to the compost but the results show that this is not the case. In addition, the results show that the sample filters collected for the controlled experiments were not contaminated inside

the experimental chamber by a scenario such as the compost falling onto the sampling filter which might have resulted in sampling filters heavily populated with particles of interest. One potential problem in the compost tumbler might have been that bioaerosols would settle on the compost filter instead of being captured by the filter and resulting in data not representative of sampling of bioaerosols at a composting site. This is the reason why the sampling filters were placed upside down in the compost tumbler. However the results show that this is not the case and provides further validation of this methodology.

Total Cell Counts

The total number of cells counted on the agitation sampling filters collected during the site work at Donarbon Limited (Figure 5.2) were on average 1-log lower than those collected for the controlled experiments (Figure 4.11). This indicates that the methodology developed for the controlled experiments were effective at releasing bioaerosols from a compost source. This is despite the fact that the bioaerosols sampled at Donarbon Limited were released from an industrial compost windrow as opposed to those released from a 5 kg sample for the controlled experiments. Hence the potential concerns regarding the amount of compost used in the controlled experiments or the possibility of aerolised bioaerosols settling back into the compost after tumbling may no longer be considered potential concerns. In line with the results collected at Donarbon, the total number of cells counted on the sample filters collected during the site work at Lount (Figure 5.9) were also lower than those collected during the controlled experiments.

Cell Classification and Aggregation

The results of the samples collected during agitation at Donarbon Limited (Fig 5.3) showed that the majority of all observed cells were single small cells at 70% followed by their aggregates at 25%. These results were similar to those for the controlled experiments (Fig 4.14) where the majority of all observed cells were single small cells at a range of 56-77% followed by their aggregates at 23-31%. The results of the samples collected at Lount (Figure 5.10) were also similar to those for the controlled experiments and site work completed at Donarbon Limited. In general for all controlled experiments

and site work, there were a smaller percentage of single large cells and their aggregates observed compared to those for small cells and their aggregates. Hence these results suggest that the use of compost tumbler releases cells from compost in a similar way to those observed in composting facilities.

Small Cell Aggregate Classification

The results for the controlled experiments (Figures 4.15 and 4.16) show that for both green waste compost and kerbside collected garden and kitchen waste compost, small cell types A and D predominate. Similarly, for the site work completed at Lount (Figure 5.11), the most abundant aggregate type for all sampling locations was the small cell type D aggregate. The results of the Donarbon site work for the cell types (Figure 5.4) show that small cell type A and D aggregates were dominant for the agitation source sample filters, however, for the wind tunnel compost windrow sample filters, B and D aggregates were dominant. These results show that the controlled experiments are able to generate the same type of cells that would be generated during an agitation activity at a composting facility however the cells generated by a static windrow might differ somewhat from the results of the controlled experiments.

More importantly, the trends for all controlled experiments and site work regarding the number of aggregates were similar where the 2-spore aggregate type was the most dominant for all sampling filters. The average number of 2-spore aggregates counted on the Lount sampling filters was 15 aggregates compared 5 aggregates for Donarbon Limited sample filters and 40 aggregates for controlled experiment sample filters. The samples taken at Lount are all from the agitation activity where a higher number of overall particles including aggregates would be expected as opposed to the samples taken at Donarbon where only one of the samples represented the agitation activity. These results also suggest that the controlled experiments might be generating a higher number of 2-cell aggregates compared to those found on site however the general trend for bioaerosol release for all controlled experiments and site work is that a higher number of single cells are released compared to any type of bioaerosol aggregate.

Aggregate Structure

The general trends for the aggregate structure (i.e. cluster versus chain) were similar for all controlled experiments and site work where cluster aggregates are the most abundant type of aggregate both small and large cells. However the actual figures for the number of small cell cluster aggregates were more variable. The number of small cell cluster aggregates at the agitation activity at Donarbon (Figure 5.5) was 40 and in contrast, the number of cluster aggregates for the controlled experiments (Figure 4.20) was higher and ranged from 40-350 small cells. The results taken at Lount (Figure 5.14) showed that the number of small cell cluster aggregates ranged from 40-90. In addition, contrasting with the results of the controlled experiments and site work, the number of large cell cluster aggregates were higher at Lount than those observed for the controlled experiments and Donarbon site work. These results suggest that for all controlled experiments and site work, there is a possibility that cells are clustering into aggregates upon release from compost.

Particle Shape and Size Distribution

For all controlled experiments (Figure 4.24) and site work at Donarbon Limited (Figure 5.7) and Lount (Figure 5.16), on average, the observed single cell bioaerosols and bioaerosol aggregates had an aspect ratio of 1. Similarly for all controlled experiments and site work, on average, there was a higher percentage of small cells compared to large cells observed on release from compost. Therefore the size distribution of bioaerosols emitted from compost will be analysed in terms of the small cell size. The individual results show that for the controlled experiments (Figure 4.22), the percentage of small cells with an aggregate diameter of 1 and 2 μm were dominant compared to other aggregate diameters. The size of the small cell aggregates observed for the agitation activity at Donarbon Limited (Figure 5.6) were also dominantly of 2 μm in diameter however, in contrast to the controlled experiments, there were no aggregates observed with a diameter of 1 μm. The small cell aggregates observed at Lount (Figure 5.15) showed a small percentage of aggregates at 1 μm diameter however more dominantly they were sized 2 μm. The results show that the trends in aggregate size for small cells throughout the controlled experiments are similar to those for site work

however the compost tumbler seems to release a higher number of small cell aggregates of a smaller size compared to those for the site work.

However it is important to remember that for all controlled experiments and site work overall, the majority of bioaerosols observed were single small cells and the overall trends between individual studies are very similar. This is demonstrated by the following graph (Figure 5.17).

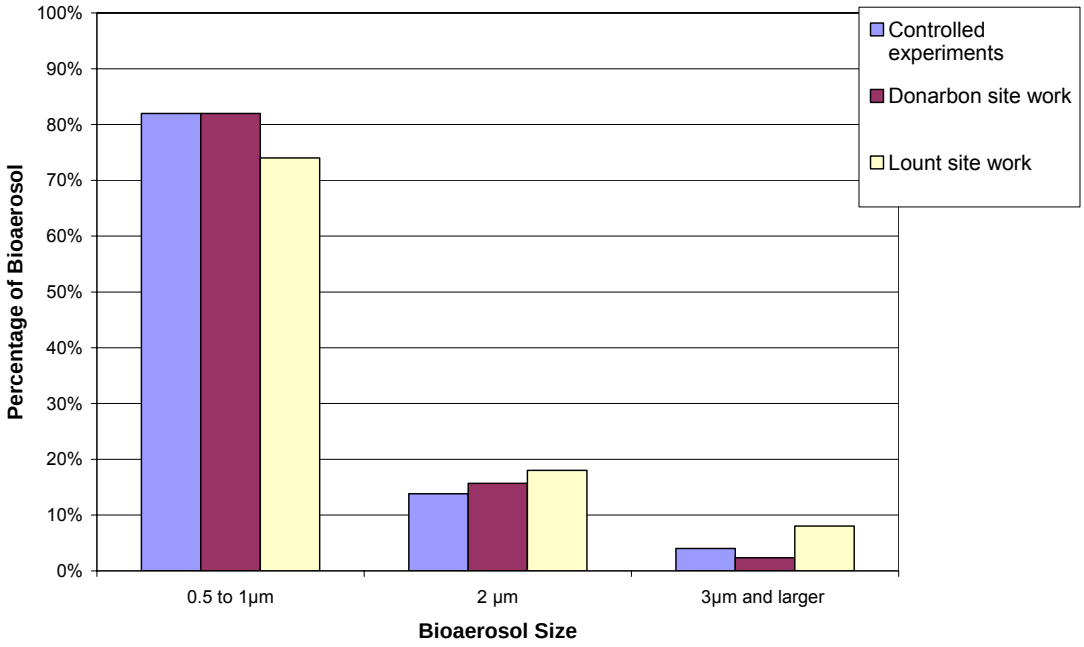


Figure 5.17- The bioaerosol size distribution summary for all controlled experiments and site work.

Therefore in conclusion, there are some exceptions between different studies conducted in a controlled chamber and on site however in general, similar trends are observed. This indicates that the methodology developed for the controlled experiments is able to generate data similar to those shown in composting sites with regards to the characterisation of bioaerosols emitted from compost. In addition, it was shown that the potential concerns presented regarding the controlled experiments did not have an impact on the results of the controlled experiments. Hence the controlled experiment methodology might be used in future studies using other compost types to generate further data for characterisation of bioaerosols released from compost. The controlled experiments are easy to conduct and repeat and hence would have distinct advantages

as a method of generating bioaerosol characterisation data compared to those for site work.

5.4.2. The Implications of Common Trends

As discussed previously (Section 5.4.1.), the trends concerning the size, shape and aggregation of biaoerosols shown in the previous chapter (Chapter 4) were similar to the trends shown for the site work completed at Donarbon Limited and Lount. The implications of these common trends are discussed as follows.

The Implications in Terms of Bioaerosol Dispersal and Deposition

There was evidence of biaoerosol aggregation in the samples taken for all studies however in general, the majority of all bioaerosols emitted were single cells. Aerosols containing aggregate structures will be larger and heavier than aerosols consisting of single cells. Therefore these results indicate that the bioaerosols released from compost are more likely to be dispersed in air for longer distances than if the majority of the bioaerosols observed were in aggregate structures.

In addition to this, for all cell types and all samples the majority of single and aggregated cells observed to be emitted from compost had an aspect ratio of 1. It has been shown that for a spherically shaped spore, the rate that this spore falls to the ground due to gravitational forces is proportional to the square of its radius (Gregory, 1973). However non-spherically shaped spores might affect this rate due to an increased surface drag that would result in a delay in deposition (Lacey, 1991; McCartney, 1994; Levetin, 1995). Balazy and Podgórski (2007) have studied the agglomeration of aerosols of non-biological origin such as diesel exhausts and concluded that the deposition efficiency of nonspherical fractal-like aggregates were significantly different from those for the spherical particles. Since the majority of the single and aggregated cells observed in this study have aspect ratios of 1 to 1.5, this might implicate that the effects of surface drag on the bioaerosols would be minimal.

Implications in Terms of Cell Viability

The majority of any aggregate structure observed for all studies consisted of 2 cells and an implication of this is in regards to cell viability. Recent studies (Duncan and Ho, 2008) have discussed that micro-organisms might be more likely to be dispersed as aggregates due to an ecological survival advantage. This was in line with Marthi *et al.* (1990) who have found that larger particles of bacteria (i.e. aggregates) had higher viability compared to smaller particles. Similarly, it has been reported that bacterial cells survive better in particles of 7 μm compared to particles of 1.1 μm due to effects of temperature, solar radiation and relative humidity (Lighthart and Schaffer, 1997; Tong and Lighthart, 1997; Carrera *et al.*, 2005). This was attributed to the aggregation of the bacterial cells within the 7 μm particle therefore, there was a higher chance of the overall particle surviving due to increased number of individual cells. Therefore the outer layer of the aggregate may act as a protective 'blanket' for the inner layer of the cells in the aggregate (Thomas *et al.*, 2008). Based on these arguments, since the majority of aggregate structures emitted from compost consisted of 2 cells, this might mean that even if they are dispersed for further distances downwind, there might be less protection from the effects of temperature, solar radiation and relative humidity. Hence this might result in reduced cell viability. It is also important to note that the majority of all bioaerosols emitted from compost were in single cells. Hence such lack of aggregation protection in terms of cell viability is valid for the majority of all bioaerosols emitted from compost .

Aerosols of *Bacillus atrophaeus* have been shown to exist in aggregates ranging from 1-9 μm (Ho *et al.*, 2001) however it has been shown that only a percentage of the individual cells in this aggregate may be viable (Duncan and Ho, 2008). This would have implications in terms of bioaerosol aggregate dispersal on release from composting facilities as aggregates are more likely to be deposited due to their weight and size compared to single aerosols. However the effect of factors such as radiation, temperature or oxygen might have a reduced impact on aggregated particles compared to those for single cells.

Implications in Terms of Infectivity and Health Impacts

Another implication of micro-organism aggregation is in terms of their infectivity (Duncan and Ho, 2008). Thomas *et al.* (2008) showed that since an aggregated particle contains a higher number of individual cells compared to a non-aggregate particle, a lower number of aggregate particles need to be inhaled to result in the dose that is needed to initiate a health impact. Therefore the aggregation of bioaerosols emitted from compost might also have important implications in determining a dose-response relationship for bioaerosols. However since the number of single cells emitted from compost were in majority compared to those emitted as aggregates, this would indicate that larger doses of single cells might also initiate an adverse health impact.

Air pollution studies have shown that particles smaller than 3 μm are able to penetrate the secondary and terminal bronchi (Spengler and Wilson, 1996). A finding common to all studies was that the majority of all bioaerosols (single or aggregates) emitted from compost were smaller than 3 μm which might suggest that bioaerosols emitted from compost are able to penetrate into these regions as well as the bronchioli of the lung. In addition bioaerosol aggregates with a size of 10 μm or more were very limited. Therefore these results might indicate that the majority of bioaerosols detected in controlled experiments and site work are able to penetrate the alveolar space and cause allergic alveolitis because they are smaller than 5 μm (Houman and Morgan, 1977; Palmgren et al., 1986). Air pollution studies (Dockery *et al.*, 1993; Schwartz *et al.*, 1996; Levy *et al.*, 2000) on the health impacts of particulate pollutants have discussed that particles that are <2.5 μm are more likely to have adverse health outcomes compared to coarse particles. Hence the results presented in all studies (i.e. controlled experiments and site work) show that size of bioaerosols emitted from compost are in the range that might result in adverse health impacts. The results have also indicated that the majority of the bioaerosols are single cells which indicated that they might be able to travel for distances longer than if they were all in aggregate structures. Despite this, it is not possible to make any conclusions on the possible health impacts of biaoerosols emitted from composting facilities on sensitive receptors living within 250 m of the facility. However, the dispersal of bioaerosols which were studied might affect the

health of the operatives inside the composting facility who are in high proximity to compost and hence exposure to biaoerosols.

5.4.3. The Aggregation and Size Distribution of Bioaerosols Emitted at a Composting Facility

The first hypothesis set for the site work was that *"if bioaerosol aggregates are observed to be emitted from compost in a composting facility, the percentage of aggregates compared to single cells will decrease with increasing distances downwind from bioaerosol source".* This hypothesis was supported by the results presented in Figure 5.3 (Section 5.2.3) where 100m downwind from the compost source, the percentage of small cell aggregates was at less than 5% compared to over 95% for small single cells.

In line with this hypothesis, it was also expected that *"the size distribution of bioaerosols at a composting facility will change with increasing distances downwind from bioaerosol source as the larger particles settle".* The results of Figure 5.6 (Section 5.2.6) show that for small cell aggregates, the number of aggregates with a diameter of 2 , 3 and 4 µm were lower at 10m downwind compared to those detected at the wind tunnel (i.e. static compost windrow) and agitation activity. These aggregates were at even lower numbers (<5) at 50m downwind and only one aggregate was detected at 100m downwind. This is an interesting result because in terms of the percentages of small spore aggregates as discussed previously (Figure 5.4) the percentage of these aggregates was higher at 50m downwind compared to 10m and 100m downwind. Therefore this might indicate that even though there are a larger percentage of small spore aggregates 50m downwind compared to 10m downwind, the actual number of aggregates and the size distribution of aggregates at this sampling location is lower. Therefore in light of these findings, this expectation was also supported.

Other results Figure 5.4 (Section 5.2.4) presented in this chapter show that aggregate structures emitted from compost measured downwind from the source were on majority made of up <6 cells. In addition, the number of aggregates made up of 2 or more units decreased with increasing downwind distances from source. In specific for source

samples, the number of aggregates made up of 6 or more units for the agitation activity were the highest however for the compost windrow sample taken with the wind tunnel the number of aggregates made up of 6 or more units is also low.

Aggregate structures consisting of a large number of individual cell units will be larger and heavier than those consisting of a smaller number of aggregates or single cells. The implications of this is that larger particles would settle out of the airstream faster than smaller particles (Dowd and Maier, 2000; Pillai and Ricke, 2002). In addition, this increase in weight and size of the particle will indicate that they are more likely to be impacted onto surfaces (Gregory, 1973). In the absence of air movement, the effects of gravitational pull are described by Stokes Law which is calculated by (Dowd and Maier, 2000).

$$v = \frac{\rho \times g \times d^2}{18\eta} \qquad \textit{Equation 5.1}$$

Where

v is the terminal velocity of the particle (cm/s);

ρ is the particle density (g/cm^3);

g is acceleration due to gravity (cm/s^2);

d is the particle diameter and

η is the viscosity of air (g/cm-s)

The results of the size distribution analysis (Table 5.1) presented in Section 5.2.6 showed that the largest aggregate structure observed for compost source at Donarbon Limited was an aggregate comprising of 50 individual cells sampled during the agitation activity. This aggregate structure had an observed length of 10 µm and width of 7 µm. Assuming that the particle diameter is 10 µm, the particle density of 1.2 g/cm^3 (Gregory, 1973), under normal gravitational acceleration (981 cm/s^2) and under normal air viscosity (at 18^{o}C, the average surface temperature during the actual sampling day was 20.5^{o}C) at 1.8 x 10^{-4} g/cm-s (Dowd and Maier, 2000), the terminal velocity of this particle would be 0.4 cm/s. Experiments by Gregory *et al.* (1961) on *Lycopodium* spore

plumes showed that only 13.5% of the spores liberated at a wind speed of 0.83 m/s (this wind speed is within the range to those found in this study as shown in Table 5.3) and height of liberation of 1 m were deposited within 2.5-10 m from source (Gregory, 1973). The terminal velocities of such larger spores (i.e. *Lycopodium sp.*) have been reported to be similar to that calculated in the previous page (i.e. 0.4 cm/s) and range from 0.7-3.5 cm/s for a 32 μm particle size (Gregory, 1950; Chamberlain 1967 from Sehmel, 1980). Hence this would indicate that only a small percentage of any large aggregated particles of 10 μm observed in this study would deposit within 10 m from source. In addition, the results also showed that the majority of all aggregates were in 2-3 spore structures and smaller than 10 μm therefore this might imply that these particles are likely to be dispersed in air for even longer distances than heavier particles.

The effect of gravitational settling on microbial particles subjected to winds above 2.2 m/s may be negligible (Dowd and Maier, 2000). Table 5.3 summarises the weather conditions measured on site at Donarbon Limited on the day of the sampling. Therefore based on the above argument, the higher wind speeds observed on top of the compost windrow where the wind tunnel sampling took place might cause the dispersal of any bioaerosols and particles released from top of the compost windrow instead of any released particles being settled back into the compost. In line with this logic, the low wind speeds observed at 10m and 50 m downwind from the compost source might have also influenced the deposition of any aggregates due to gravitational forces.

Table 5.3 – Weather conditions measured on site at Donarbon Limited

Type of Sample	Sampling Location	Relative Humidity (%)	Wind Speed (m/s)	Temperature (^{o}C)
Background	Upwind	37.9	0.9	20.3
Source	Top of compost windrow (average *n*=*3*)	43.6	3.0	20.3
	Agitation activity	53.5	1.1	19.7
Background	Downwind 10m	35.5	0.9	20
	Downwind 50m	37.8	1.8	22.2
	Downwind 100m	38	2.2	20.9

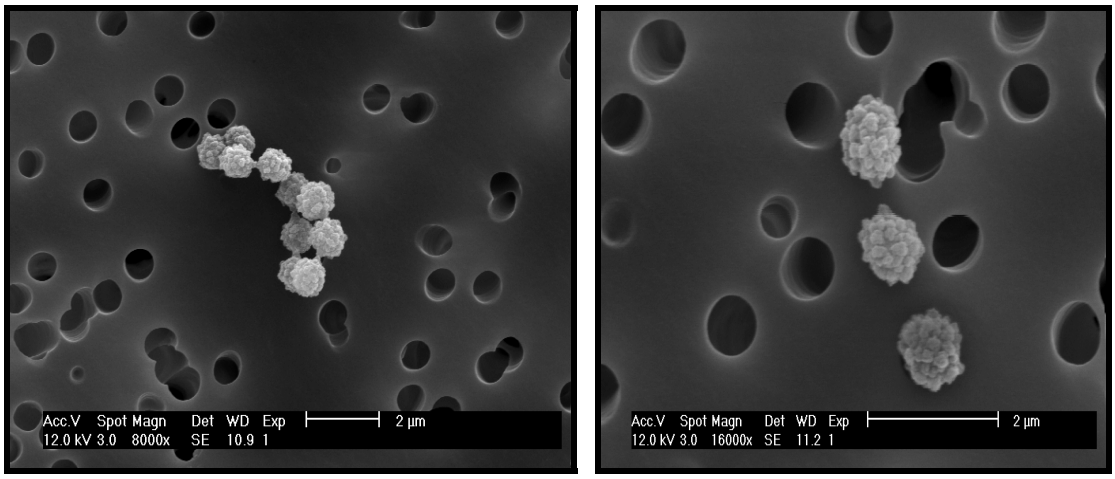
Such environmental factors might also have implications for breaking of aggregates into individual spores. A recent study (Thomas *et al.*, 2008) has discussed that large environmental aerosols (> 20 μm) are likely to contain water which might evaporate to result in dried smaller particles of a diameter such as 5 μm (Nicas *et al.*, 2005). This rate of evaporation has been attributed to the constituents of the particle as well as environmental factors such as the relative humidity, temperature and wind effects (Thomas *et al.*, 2008). Surface temperature might also affect the density of airborne spore particles (Madelin and Johnson,1992) and it was concluded that increasing the temperature from 20^{o}C to 38^{o}C resulted in an increase in aerodynamic size of the spores and breaking of the chains of spores. Such a high temperature increase from 20^{o}C to 38^{o}C in a single day at a composting facility might be unusual however this might still suggest that changes in temperature in different locations in a composting facility might result in such changes in aerodynamic size of spores and breaking of the chains of spores resulting in an increased number of single particles.

At Donarbon Limited the highest percentage of single small spores with respect to small aggregate spores was observed 100m downwind. The temperatures measured at this location are slightly higher than those for source sampling locations however the highest surface temperature observed during the sampling day was at 50m downwind and the percentage of aggregates at this point compared to single spores was higher than those at 100m. Therefore this implies that the effect of temperature on the breaking of aggregate structures downwind from a compost source was not evident. This is confirmed by the results presented in Figure 5.3.

5.4.4. The Evaluation of Bioaerosol Aggregate Structures at a Composting Facility

As discussed previously (Chapter 4) the chain aggregates that represent mycelially growing micro-organisms growing in compost would be expected to break up as transported downwind. Therefore it would be expected that in a composting facility, the number of chain aggregate structures for bioaerosols measured at source to be higher than those measured downwind from source. The results presented in Figure 5.5 (Section 5.2.5) are in line with this and show that the number of chain aggregates for the compost windrow (i.e. wind tunnel) and agitation sampling locations representing

compost source are higher than those at 10m downwind from source. In addition, as would be expected no chain aggregates were seen 50m and 100m downwind which suggests that mycelial chain structures for aggregates are broken into single spores as the particles travel further downwind such as represented in Figure 5.17 in which (a) might indicate a mycelial chain aggregate at a compost source however (b) might indicate single cells of the same micro-organism as they are dispersed further downwind.



(a) **(b)**

Figure 5.18- SEM Image of (a) mycelial chain aggregate for small spore type G (b) single cells for small spore type G

However the number of cluster aggregates for all sampling locations at Donarbon were much higher compared to the number of chain aggregates indicating that single spores are aggregating after release from compost or aggregates of non-filamentous micro-organisms present in compost are released in higher numbers.

5.4.5. Factors Affecting Bioaerosol Release from Compost at a Composting Facility

The results shown in Figure 5.2 (Section 5.2.2) show that the highest number of cells were counted on the filters analysed at source for the agitation activity. This was an expected result as higher number of bioaerosols are expected to be emitted from the agitation activity compared to those emitted from a static compost windrow. This is because comparisons of bioaerosol concentrations during compost agitation activities (i.e. turning or shredding) with static compost concentrations (i.e. compost windrows)

have shown the former to be higher than the latter with differences of up to 3-log (Sánchez-Monedero *et al.*, 2005; Taha *et al.*, 2006; Taha *et al.*, 2007). However in contrast, the results shown in Figure 5.1 (Section 5.2.1) show that the filters most heavily populated were those for the compost windrow wind tunnel sampling and not for the agitation activity although this suggests that a larger number of particles of interest other than cells were collected on the filter taken with the wind tunnel on the compost windrow.

The compost windrow samples were taken from three different compost windrows which were joined together to represent the overall emission of bioaerosols and particles from the compost windrow structure on site. Therefore the material represented a mixture of 2, 3 and 4 week old compost. In contrast the compost that was agitated consisted only of 4 week old compost. It was previously shown (Chapter 4) that compost age was not a factor in the number of bioaerosols released. Therefore this is probably not a factor in these results. However it is important to note that the differences between sampling locations for both set of results were not statistically significant ($p>0.05$).

Previous studies (Gregory and Lacey, 1963; Pasanen *et al.*, 1991; Górny *et al.*, 2001) have reported that the release of fungal spores increase with increasing air velocities above the contaminated surface. The wind speed measured near the agitation activity was 1.1 m/s which is much lower than the air velocity inside the wind tunnel that ranged between 2.5-3.4 m/s for replicate samples. However it was not possible to measure the air velocity created by the agitation activity because the filter samples taken within the agitation activity were collected 1 m downwind of the agitation activity due to health and safety precautions. The air velocities inside the agitation plume might have been much higher. Therefore determining the typical air velocity created by an agitation activity and comparing this with the air velocity in the wind tunnel might provide further analysis of the effect of air velocity in spore release from compost.

However the effect of air velocity for particles smaller than 1.6 µm might be negligible. This is based on studies by Górny *et al.* (2002) on the release of fungal fragments in an aerolization chamber by using either an agar plate or a ceiling tile as the contaminated

material. Different air velocities were applied to contaminated surfaces to represent indoor air, outdoor air and ventilation duct air velocities. The results showed that the release of fungal fragments from smooth agar surfaces did not increase with the increasing air velocity. This was attributed to differing release mechanisms for single spores and fragments. SEM images of the fungal fragments reported in Górny *et al.* (2002) were not available for a comparison however if some of the small spores identified in this study are fungal fragments this might indicate that the mechanisms of release for these particles and those of large size spores would be different.

5.4.6. Effects of Filter Size on the Capture of Bioaerosols Emitted from Compost

Finally, the effects of filter size on the capture of bioaerosols emitted from compost are discussed. In terms of total 'particles of interest', the results in Figure 5.8 (Section 5.3.1) showed that the sample with a filter pore size of 0.65 µm was most sparsely populated and the sample filter pore size at the mid range of 3 µm was most heavily populated with particles of interest. Similar results were shown for total spore counts where the number of spores on the largest filter sizes were the highest. This might indicate that the bioaerosols and particles captured on these filters are sticking to them and not permeating through as shown in Figure 5.18. However, this sample was taken at close proximity to the agitation activity therefore a bioaerosol overloading on the filter might also account for this result. In addition these results are only from one sample and in the light of this, the results are inconclusive. Therefore repeated site work would need to be completed to study these effects further.

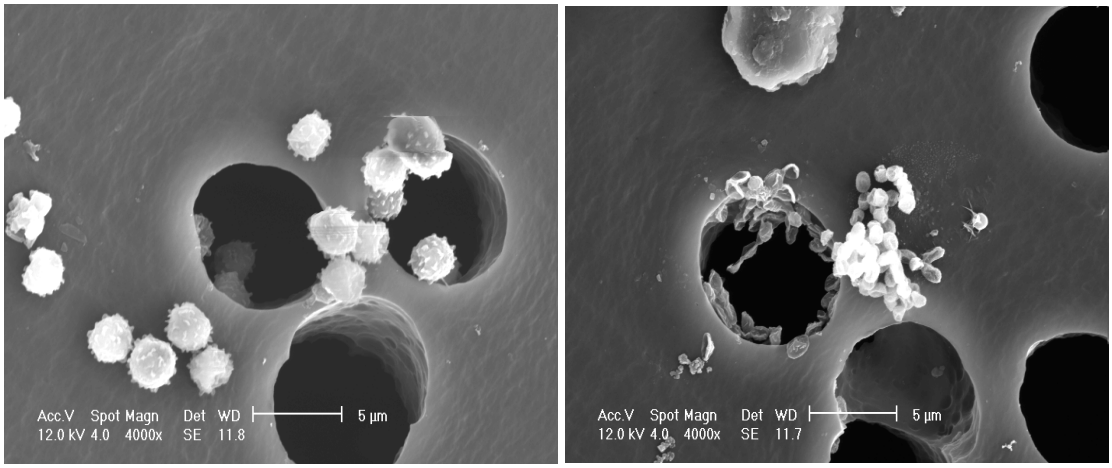


Figure 5.19 - SEM Images of 'stickiness' of spores captured on filter size 8 µm.

5.5. CONCLUSIONS

A set of hypothesis and objectives were set to address the gap of information regarding microbial aggregation and bioaerosol particle size distribution. Despite some potential concerns, scanning electron microscopy (SEM) was proven to be a viable method for visualising the size, shape, particle surface and aggregation characteristics of bioaerosol and particles emitted from compost.

The results suggest the following statements regarding the release and dispersal of bioaerosols and particles from compost for both controlled experiments and on composting sites.

- 7-9 different types of small (0.5 – 1 μm) cells and 2 different types of large (1-2 μm) cells and their aggregates are released from both static (i.e. compost windrow) and active (i.e. agitation) compost sources regardless of compost age or feedstock.

- The majority of these bioaerosols are single cells with an aspect ratio of 1. This would implicate that these cells are more likely to be dispersed in air for longer distances than if they were in aggregate structures (i.e. heavier units). In addition the effects of surface drag in dispersal would be minimal. However, the bioaerosols were not attached to particles such as wood fibres that might have aided their dispersal.

- The majority of all aggregates were in 2-3 cell structures and smaller than 10 μm. This might imply that these cells are more likely to be dispersed in air for longer distances than heavier cells. However this could also indicate that even if they are dispersed for further distances downwind, there might be less protection from the effects of temperature, solar radiation and relative humidity due to the lack of ‘blanket’ protection offered by the outer cells to the inside cells in a larger aggregate. This would mean that these cells would lose their viability quicker however its important to remember that sometimes a non-viable bioaerosol is still able to cause adverse health effects.

- The aggregate structures that are released from static and active compost sources are in clusters as opposed to chains. This might indicate that a higher number of micro-organisms that do not grow in filaments are forming aggregates or that cells are forming aggregates upon release from compost. However any mycelial chain structures for aggregates are broken into single cells as the particles travel further downwind.

- A decrease in aggregates is observed within 10m from the compost source boundary. The percentage of small cell aggregates at 50m downwind (in relation to single cells at this location) is higher compared to the same result for 10m downwind. However the actual number of aggregates and the size distribution of aggregates 50m downwind is lower than 10m downwind.

- There are no aggregate structures observed at 100m downwind from compost source. Since non-viable aggregates would still be captured on the filter and visualised by SEM, this might suggest that aggregates drop out from the pollutant plume.

Therefore in conclusion, there was evidence of aggregation in biaoerosols released from compost and that these aggregate structures drop out of the pollutant plume by 100m downwind from source. However despite this, the majority of these bioaerosols were in single cell units. This indicates that they are more likely to dispersed for longer distances.

The studies discussed in this chapter and the previous chapter (Chapter 4) have improved the understanding of the release of biaoerosols emitted from compost (i.e. source). It has been previously discussed that (Chapter 1) such improvements of knowledge would allow for improved regulatory risk assessments.

The prediction of bioaerosol concentrations at various points downwind of a composting facility and close to sensitive receptors would also have a significant impact on the improvement of composting regulatory risk assessments. This is currently done by

collection of bioaerosol samples at composting facilities and the subsequent analysis of these samples. However, there are several difficulties posed by the collection of bioaerosol samples at a composting facility including factors such as the costly and time consuming nature of bioaerosol sampling and analysis. Therefore one potential method of predicting bioaerosol dispersal is the use of commercial air dispersion models in assessing the risks of bioaerosols released from composting facilities. The use of such models might be a very useful and cost effective way of exploring different bioaerosol control situations and assessing bioaerosol emissions in a composting site.

The air dispersion models that are currently available are not designed for bioaerosols but for other aerosol pollutants such as odours or particulates. However, the results shown imply that bioaerosols might behave as non-biological aerosols according to their size, shape and aggregation tendencies as well as possessing biological properties. Therefore the use of commercial air dispersion models for successfully predicting the emissions of bioaerosols from a composting source might be a possibility.

A limited number of studies (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Dowd *et al.*, 2000; Taha *et al.*, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a) have used such air dispersion models to predict downwind concentrations of bioaerosols emitted from compost sources. However, some of these studies have indicated that factors discussed in study such as biaoerosol aggregation and size distribution might complicate their modelling (Swan *et al.*, 2003; Wheeler *et al.*, 2001; ADAS/SWICEB, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a). Therefore the next two chapters will discuss the potential of one air dispersion model, ADMS 3.3, in predicting downwind bioaerosol concentrations at composting sites. In addition, the sensitivities of the model to parameters such as microbial aggregation and size distribution will also be explored.

6. PRELIMINARY AIR DISPERSION MODELLING AND SENSITIVITY ANALYSIS OF THE MODEL

6.1. INTRODUCTION

There are several gaps in the assessment of bioaerosol related risk in composting facilities as previously discussed (Chapter 1). Gaps in the understanding of these bioaerosols such as the lack of sufficient knowledge on their viability or aggregation properties as well as the deficiency of a dose response relationship in bioaerosol induced disease have significant impacts in composting regulatory risk assessments. In addition, there are several difficulties posed by the collection of bioaerosol samples at a composting facility. Some of these problems which have been encountered throughout the studies in this thesis include:

- costly and time consuming nature of bioaerosol sampling and analysis (e.g. up to 1 week enumeration period between sample collection and confirmation of concentration results);
- practical difficulties of bioaerosol sampling on site (e.g. unexpected loss of power in sampling pump due to problems with battery);
- unpredictable weather conditions at a composting facility on the day of sampling (e.g. unexpected heavy weather);
- unplanned variation in site practices at a composting facility on the day of sampling (e.g. unexpected or lack of agitation activities);
- sampling limitations posed by on-site or surrounding area topography (e.g. downwind samples);
- difficulties in source apportionment of compost related bioaerosols (e.g. presence of ubiquitous bioaerosols).

In addition to these, as previously discussed (Chapter 1) there is lack of a single standardised bioaerosol sampling method or bioaerosol specific methods for determining their emission rate upon release. These problems might cause difficulties in collecting bioaerosol emission data at a composting facility for regulatory purposes. As such the collection of adequate downwind bioaerosol concentration data at a

composting facility to better assess the risk of bioaerosols close to sensitive receptors may be difficult. Air dispersion models have been in use for assessing the impacts of other pollutants such as chemicals or particulates for air quality purposes for many years. Therefore the use of air dispersion models in predicting bioaerosol concentrations at various points downwind of a composting facility has a very big potential in improving composting regulatory risk assessments.

The potential of air dispersion models in predicting bioaerosol dispersal in composting facilities has previously been discussed (Section 1.6, Chapter 1). Despite the fact that such use of commercial air dispersion models has not been adopted as official practice by environmental regulators, successful prediction of the emissions of bioaerosols from a composting source is a plausible possibility. This is because bioaerosols may have similar properties (e.g. density, shape or size) (Kanaani *et al.*, 2008) to other more traditional and non-biological pollutants (i.e. particulates) that the current commercial air dispersion models are used for. In line with this, the studies presented in the last two previous chapters (Chapters 4 and 5) have improved the understanding of the release of bioaerosols emitted from compost and showed that bioaerosols emitted from compost show certain size, shape and aggregation characteristics.

Therefore if the currently available air dispersion models can be proved to be successful in predicting the concentrations of bioaerosols at a composting site, they can be very useful tools in predicting bioaerosol concentrations downwind of a composting facility. Such model outputs would enable new composting facilities to assess the risks of bioaerosols released from their operations and would subsequently accompany the composting facility risk assessments required by the regulators. In addition, air dispersion models would be useful for use in existing composting facilities which are planning to make significant changes on their site such as changing their composting technology or composting operations. Hence the model would enable the facility to predict and assess the relative changes in downwind bioaerosol concentrations resulting from different operating scenarios. The current methods of bioaerosol sampling and analysis are generally time consuming and costly. Therefore finally, the

use of air dispersion models in estimating risk of bioaerosols released from composting facilities might be a cheaper and faster option.

A limited number of studies (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Dowd *et al.*, 2000; Taha *et al.*, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a) have used such air dispersion models to predict downwind concentrations of bioaerosols emitted from compost sources. However these studies have not compared the actual measured on site downwind bioaerosol concentrations with those predicted by the model. Without such a comparison, it is not possible to verify the ability of an air dispersion model in predicting downwind bioaerosol concentrations.

Therefore to the authors' current knowledge, this is the first type of study which completes a preliminary assessment of the ability of a commercial air dispersion model to predict bioaerosol emissions from a composting facility compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling. If the model is able to predict the bioaerosol concentrations measured downwind, then this might indicate a potential for the use of the model in support of composting regulatory risk assessments. The preliminary dispersion modelling studies discussed in this chapter have been previously presented in two other publications (Tamer Vestlund *et al.*, 2007; SEPA/SNIFFER, 2006) which also focused on predicted versus measured bioaerosol concentrations from two in-vessel systems as well as an open windrow composting site. However this thesis focuses on windrow composting, so only the studies completed at the open windrow composting site, namely Keenan Recycling, are discussed.

The potential reasons why the estimation of bioaerosol dispersal by air dispersion models might prove problematic has been previously discussed (Section 1.6, Chapter 1). In addition, properties of bioaerosols such as their aggregation and size distribution (as studied previously in Chapters 4 and 5) might further complicate their modelling (Swan *et al.*, 2003; Wheeler *et al.*, 2001). Throughout the bioaerosol air dispersion modelling studies completed to date (Millner *et al.*, 1980; Dannaberg *et al.*, 1997; Wheeler *et al.*, 2001), bioaerosols have been assumed to be gas pollutants due to their small size and bioaerosol aggregation has not been considered. However the clumping

of bioaerosols results in the increase of their overall size and hence might indicate that they behave as a non-gaseous pollutant instead (Drew *et al.*, 2006). This means that a bioaerosol aggregate which has a larger size than a single bioaerosol is more likely to settle out downwind upon release and travel shorter distances downwind of source compared to the single bioaerosol which is more likely to be suspended in air (Pillai and Ricke, 2002).

The effect of such factors on the modelling of bioaerosols emitted from compost needs to be better understood. Therefore, following the preliminary air dispersion modelling, a sensitivity analysis of the model was completed to enable this.

In addition, the sensitivity analysis provides an examination of any possible uncertainties posed by the preliminary dispersion modelling by testing the effect of different input parameters on predicted downwind bioaerosol concentrations to determine which modelling parameters the model is sensitive to. Therefore the studies presented in this chapter were also completed to analyse the sensitivities of a commercial air dispersion model and assess the effect of different modelling parameters on predicted versus measured bioaerosol concentrations. In order to fulfill this, the following objectives were set for the sensitivity analysis:

- determine which parameters in a composting facility (e.g. source, pollutant, meteorology) the model is most sensitive to and
- determine if the model is most sensitive to adjusting the pollutant size and aggregation parameters or if other parameters such as source definition (i.e. point or area source) are just as important.

Firstly the methodology that was adopted is explained including details of the air dispersion model that was used for the studies and the determination of the bioaerosol emission rate. Following this, the results of the preliminary air dispersion modelling of the site work completed at Keenan Recycling are presented and discussed. Finally the results of the sensitivity analysis are presented and discussed. The next chapter (Chapter 7) will incorporate the findings from this chapter in order to assess the

potential of the model in determining the concentrations of bioaerosols emitted from composting facilities. Therefore, the key conclusions for the overall air dispersion modelling studies will be discussed in the next chapter.

6.2. MODELLING THEORY

The range of dispersion models available is large (as discussed in Chapter 1) but for the modelling of bioaerosol emission from composting facilities, certain model types would be more advantageous than others.

The air dispersion model that is most frequently used for assessment of pollutants at the local scale (i.e. up to tens of km) is the Gaussian dispersion model (Petts and Eduljee, 1994). The Gaussian model theory works on the principle of a 'plume' (i.e. instantaneous release of pollutant from a source) moving downwind along the wind direction away from its source (Colls, 2002) as shown in Figure 6.1. As the pollutant 'plume' travels away from its source it expands in volume and becomes diluted in the air surrounding it subject to other random movements in the air surrounding it, due to air turbulence. This would enable the concentration of the pollutant to be calculated at any point downwind from its source. The Gaussian model assumes that the plume and the pollutants in the plume are horizontally and vertically distributed in a Gaussian curve (i.e. normal distribution).

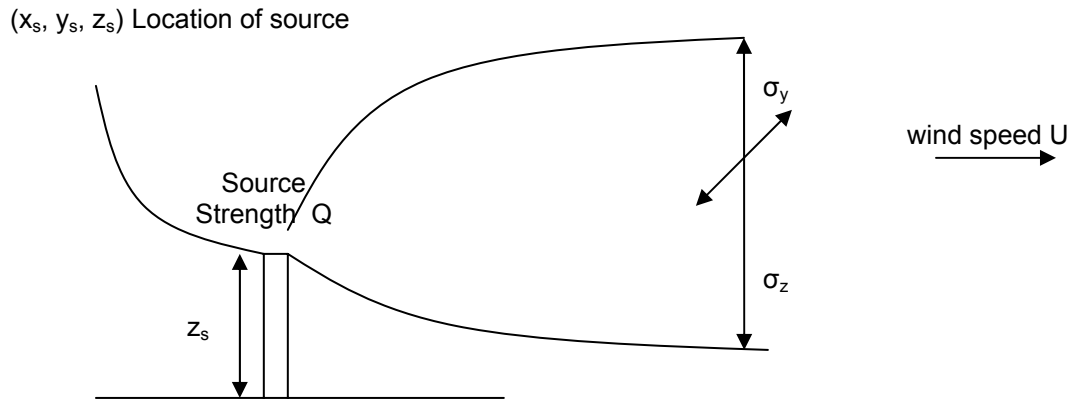


Figure 6.1- Pictorial of a Gaussian plume (adapted from Carruthers, 1998)

Based on this theory, the Gaussian plume diffusion equation is described as follows (Carruthers, 1998):

$$C = \frac{Q}{2\pi U \sigma_y \sigma_z} \exp\left\{-\frac{1}{2}\left[\frac{(y-y_s)^2}{\sigma_y^2} + \frac{(z-z_s)^2}{\sigma_z^2}\right]\right\} \qquad \textit{Equation 6.1}$$

Where:

C	is the concentration distribution (units/ m^3) when pollutant is continously emitted at a constant rate from a single point source into a uniform flow with speed *U*, and spread due to turbulence;
Q	is the rate of discharge of the pollutant (mass units/s);
x_s, y_s, z_s	is the source location.
σ_z, σ_y	is the standard deviation of the horizontal or vertical spread or dimension of the plume.

To enable the calculation of downwind pollutant concentrations, Gaussian models take the effects of wind direction, wind speed and atmospheric stability into account (McCartney, 1994). The pollutant concentration heavily depends on the wind speed (i.e. U) and the dimension of the plume (i.e. σ_z, σ_y) (Carruthers, 1998). These further depend on how the atmospheric boundary layer (i.e. layer in which the dispersion of the pollutant occurs and is affected by the earth's surface) is characterised, therefore the characterisation of this boundary layer can significantly affect the model predictions of the pollutant concentrations (Carruthers, 1998). Traditionally these dispersion models express the degree of atmospheric stability using Pasquill (Pasquill, 1962) stability classes that range from a class of A (very unstable) to G (very stable) with the Pasquill stability class D having the highest occurrence in the UK (Colls, 2002). The neutral condition (i.e. stability class D) represents medium to strong wind speeds and vigorous mixing of the atmospheric boundary layer is observed (CERC, 2004).

However more modern models (e.g. ADMS) describe the state of the atmospheric boundary layer by employing recent stability theories in which the Monin-Obukhov

length (Gostelow *et al.*, 2001) and the boundary layer depth are the added parameters (McHugh *et al.*, 1997). Monin-Obukhov length is the '*height at which buoyancy and wind generated turbulence are equal'* and is derived from friction velocity and heat flux (Gostelow *et al.*, 2001). The implications of this is that in neutral and stable conditions, a vertical Gaussian concentration distribution is assumed however in unstable conditions a skewed-Gaussion distribution is assumed (McHugh *et al.*, 1999) which is a more realistic representation of the atmospheric boundary layer (Carruthers, 1998).

Gaussian plume dispersion models are advantageous over other model types (Carruthers, 1998). For example, box models which are based on the *conservation of mass and assuming uniform mixing within single or multiple boxes whose height are estimated by the mixing depth or height of the atmospheric boundary layer* (Carruthers, 1998) are not able to model the detailed structure of the pollutant. However, as discussed in previous studies (Wheeler *et al.*, 2001), the structure of a bioaerosol pollutant (e.g. size or aggregation) might have a significant impact on the predicted downwind concentrations. Therefore, box models would not be suitable for use in predicting bioaerosol emissions.

On the other hand, particle models that '*simulate' the mean flow and turbulence for weather conditions allowing the tracking of individual particles of pollution* (Carruthers, 1998) are too demanding for use in regulatory purposes. Similarly computational fluid dynamics (CFD) models that *use the full or partial solutions of motion equations to determine the flow field and dispersion of a pollutant* (Carruthers, 1998) are too expensive and difficult to run for regulatory purposes. However, a model which will have potential to be used for predicting the concentrations of biaoerosols emitted from composting facilities would need to be user-friendly and cost-effective to run to be adopted as a common tool by the environmental regulators.

Similar observations were made by Riddle *et al.* (2004) who evaluated the performance of ADMS compared with FLUENT which uses Computational Fluid Dynamics (CFD) software. ADMS and FLUENT were set up to simulate the dynamics of a basic atmospheric boundary layer and the prediction of gas dispersion from a single stack.

Although the CFD simulations were satisfactory, it was concluded that ADMS was a more appropriate method for normal atmospheric dispersion studies. This was due to the larger run times and greater complexity that were needed to set up to run the FLUENT model.

ADMS was therefore chosen as the commercial air dispersion model to be used for the air dispersion modelling studies in this project. ADMS is an advanced steady state, Gaussian plume dispersion model which has been developed by CERC (Carruthers *et al.*, 1994; CERC, 2003) and the UK Meteorological Office. ADMS is used for predicting the dispersion of gases and particulate emissions into the atmosphere. There is a choice of modelling the effects of plume rise, wet and dry deposition, radio-active decay, variable roughness terrain, coastal regions and buildings (Carruthers *et al.*, 1994). It is one of the four models in common use (Carruthers, 1998) along with AERMOD, ISC3 and models based on the R-91 algorithms.

This model is currently used by government regulatory authorities such as the UK Health and Safety Executive (HSE), Environment Agency of England and Wales, Scottish Environmental Protection Agency (SEPA) in Scotland, UK Food Standards Agency and Environment and Heritage Service in Northern Ireland. It is in use by some local authorities in the UK for managing urban air quality (Arciszewska and McClatchey, 2001). In addition to this, the model is used by a variety of industries including power generation companies, consultants, light and heavy industry and academics (McHugh *et al.*, 1997). As previously mentioned, the use of air dispersion modelling for predicting bioaerosol concentrations at composting facilities has not been accepted officially. However there have been limited applications of ADMS to predict bioaerosol concentrations emitted from composting facilities (Drew *et al.*, 2005; ADAS/SWICEB, 2005; Taha *et al.*, 2006; Drew *et al.*, 2006; Taha *et al.*, 2007a). Therefore the wide application of ADMS indicates that it is currently the air dispersion model of choice for various applications and studies in the UK and this has been the most important determining factor in choosing it for the studies discussed here.

In addition to this, the model was also chosen because it allowed the user to define multiple pollutant sources. This is a more realistic estimation of the possible compost sources at a composting site because at a typical composting site, various sources of bioaerosols would be expected to be present, for example compost windrows as well as the agitation activity area for an open compost windrow site. This means that, in reality a combination of bioaerosol sources would contribute to the downwind bioaerosol concentrations at a composting site rather than single bioaerosol sources.

The model also allows the user to consider dry deposition. As previously discussed the aggregation of a bioaerosol might complicate its modelling due to increased particle size (Wheeler *et al.*, 2001; Swan *et al.*, 2003). In limited bioaerosol air dispersion modelling studies to date, bioaerosols have been modelled as gases however an increase in particle size might require them to be modelled as particles. However to study the effects of this, the air dispersion model being used needs to enable the user to consider dry deposition.

6.3. PRELIMINARY AIR DISPERSION MODELLING

6.3.1. Methodology

The bioaerosol concentrations measured at Keenan Recycling (see Section 3.2.1, Chapter 3 for site description) were modelled as a combination of area and point sources. Pollutant sources such as static compost windrows were modelled as area sources and agitation activities such as screening or shredding observed on site were modelled as point sources.

The air dispersion modelling completed for Keenan Recycling aimed to complete a preliminary assesment of ADMS 3.3 in predicting the concentrations of bioaerosols emitted from an open windrow composting facility. Hence for simplicity reasons, no model options (e.g. dry deposition) were used and ADMS 3.3 default values were used where appropriate. A site surface roughness of 0.2 was chosen to denote an agricultural area which was decided as the best description of the area surrounding this open windrow composting facility.

The parameters used for the source (e.g. source height, diameter, geometry or temperature) represented the measurements taken on site. The model output represented bioaerosol concentrations at a height of 1.8 m which was the height at which bioaerosol samples were collected on site. Exit velocity was chosen as the efflux format and the model default values to represent air were used for the parameters defining the release material, because the release material is predominantly air. The bioaerosol pollutant was assumed to be gas and no deposition velocity or washout coefficient was defined. For meteorological data, stability class D (neutral conditions) (Pasquill, 1961; 1962) of the ADMS 3.3 file R91A-G was used for all modelling exercises, as this represents the most frequently occurring atmospheric state in the UK (Colls, 2002). The model output is presented in terms of short or long term concentrations. The long term modelling option is useful for assessing any percentiles in concentration or exceedence values for comparison with regulatory standards and is generally used with hourly sequential meteorological data. However for the air dispersion modelling at Keenan Recycling, since it was a preliminary assessment of the model and the ADMS 3.3 file R91A-G was utilised instead of hourly sequential meteorological data recorded on site, the short term modelling option was used. In addition, the preliminary site work represents bioaerosol emissions from Keenan Recycling throughout only a single sampling day as opposed to representing bioaerosol emissions from a composting facility for a longer time limit (i.e. one year).

The air dispersion modelling studies for Keenan Recycling aimed to make a preliminary assessment of the model in predicting bioaerosol concentrations. Therefore to reduce the number of variables and for simplicity reasons, several simplifying assumptions were made as per Taha *et al.* (2005; 2006; 2007a):

- The modelled surface is flat, hence the effects of terrain have not been taken into account;
- The effects of building downwash have not been taken into account;
- Wind velocity and direction are constant over the modelled time and distance;
- Bioaerosol size distribution and aggregation was not taken into account.

The bioaerosol emission rates and a list other modelling parameters used for Keenan Recycling modelling studies is presented in Appendix E. The methods used for calculating all emission rates (Chapters 6 and 7) are described below.

a) Estimation of the Bioaerosol Emission Rate for an Area Source

An area source is defined as a release over a specified height over a horizontal convex polygon (e.g. sewage tank) (CERC, 2003). Therefore the compost windrow from which bioaerosol samples were collected during the first sampling date was assumed to be an area emission source and the sampling hood method was employed to directly measure the emission rates based on previous studies by Taha *et al.* (2005). The basis of this method is to isolate a section of the emission surface and to force air to flow over this surface. Two sets of bioaerosol samples were collected using the sampling hood placed on both sides of a compost windrow, with an approximate size of 22m x 10m x 4m (length, width, height) (Chapter 3, Section 3.2.1, Figure 3.4). However for modelling purposes, the bioaerosol emissions from both sides of the compost windrow were used as an average. This is because the contribution of downwind bioaerosol concentrations from a windrow is likely to be the combined emission from the entire windrow.

The average (arithmetic mean) of the bioaerosol concentrations measured at the bottom and top of the outlet of the sampling hood were used to calculate the net bioaerosol concentration in the sampling hood. The wind speed inside the sampling hood was measured with an anonemeter (Kestrel 3000). Following this the air velocity inside the sampling hood was calculated using the following equation (Taha *et al.*, 2005):

$$V_1 = V_2 x A_2 / A_1 \qquad \textit{Equation 6.2}$$

Where:

V_1 is the air velocity the main section of wind tunnel (m/s);

A_1 is the area of the main section of wind tunnel (m^2);

V_2 is the air velocity of the mixing chamber, where sampling is carried out (m/s); and

A_2 is the area of the mixing chamber where sampling is carried out (m^2).

The specific bioaerosol emission rate (SBER) is the quantity of bioaerosols emitted per unit time from a unit surface area. The equation used (Equation 6.3) is adapted from odour measurement calculations to calculate the bioaerosol emission rate inside the wind tunnel chamber (Jiang and Kaye, 2001).

$$SBER = \frac{Q \times BC}{A}$$ *Equation 6.3*

Where:

SBER is the specific bioaerosol emission rate (cfu/m^2/s);

Q is the flow rate through the wind tunnel (m^3/s);

BC is the bioaerosol concentration in air (cfu/m^3); and

A is the area covered by the wind tunnel (m^2).

Following this, the specific bioaerosol emission rate corresponding to ground level air velocity was estimated using the following adapted from Jiang and Kaye, 2001:

$$SBER_2 = SBER_1 \times \left(\frac{V_2}{V_1}\right)^{0.5}$$ *Equation 6.4*

Where:

$SBER_1$ is the surface bioaerosols emission rate measured using the wind tunnel (cfu/m^2/s);

$SBER_2$ *is the* surface bioaerosols emission rate corresponding to ground level air velocity (cfu/m^2/s);

V_1 is the air velocity inside wind tunnel (m/s); and

V_2 is the ground level air velocity (m/s).

For the first sampling date, no agitation activities were observed hence the incoming waste compost windrow emissions were assumed to be the sole contributor to the downwind bioaerosol concentrations.

b) Estimation of the Bioaerosol Emission Rate for a Point Source

The agitation activities of shredding and screening (Chapter 3, Section 3.2.1, Figure 3.3) captured at Keenan Recycling during the second and third sampling days were modelled as point sources. It was not possible to directly measure the bioaerosol emissions rates at the point of release due to health and safety measures that must be taken to minimise risk of injury from agitation processes. Furthermore, as these are activities, and not static sources (i.e. compost windrows), it was not possible to use a wind tunnel or sampling hood to collect source term data. Therefore the bioaerosol emission rate was estimated by performing a back-extrapolation using the air dispersion model ADMS 3.3 (CERC, 2003) as described by Taha *et al.* (2005) based on the known bioaerosol concentrations measured at 2m and 10m downwind of the agitation activity. Various emission rates were tested as inputs to ADMS 3.3, together with the measured mean temperature, wind speed and the height of sampling. The size of the dust cloud created by agitation was observed and the dimensions were estimated to be 3m x 3m x 3m in line with Taha *et al.* (2005). These dimensions were also entered as model inputs.

At a typical composting site, multiple sources of bioaerosols would be expected to be present. As such, the definition of the source term data for the preliminary air dispersion modelling subsequent to the initial site work completed at Keenan Recycling was based on a combination of potential biaoerosol souce term data per sampling day per site rather than defining each bioaerosol source separately and modelling them individually. However it was not possible to capture source term data from every possible bioaerosol source at the compost site throughout a sampling day due to practical difficulties of bioaerosol sampling (as discussed previously in Section 6.1). In addition, the sampling hood was not available for the second and third sampling days hence it was not possible to calculate a bioaerosol emission rate for any static bioaerosol emission

sources (i.e. compost windrows) on those days. Subsequently these sources could not be included in the air dispersion modelling. In the light of these, Table 6.1 summarises the combined bioaerosol source term data used for the preliminary air dispersion modelling for the emission from each site per sampling day.

Table 6.1 – Combined source term data used for the preliminary air dispersion modelling per sampling day at Keenan Recycling

Sampling Day	Source Term Data Used for the Modelling	Number of Total Sampling Points at Source (2 per sampling location)
First	Compost windrow left Compost windrow right	n = 4
Second	Agitation activity	n = 2
Third	Agitation activity	n = 2

This also means that even though individual measured concentrations at source are from two sampling replicates only (i.e. n = 2), the overall source term data is a combination of various sources.

6.3.2. Results

a) Area Source Modelling

Figure 6.2 shows the results of the ADMS 3.3 modelling of *A. fumigatus* and actinomycetes emitted from one compost windrow (i.e. the incoming waste compost windrow as per Figure 3.2, Chapter 3) at Keenan Recycling during the first sampling day. The material in this windrow was less than 5 days old and this was the only sampling location in which it was possible to collect bioaerosol source samples and hence determine a bioaerosol emission rate (as shown in Table 6.1). Bioaerosols were sampled approximately 50m downwind of all site operations, which equates to 500m downwind of the incoming waste compost windrow where the bioaerosol source sampling took place. Comparing the measured downwind concentrations of *A. fumigatus* and actinomycetes with the model predictions at 500m downwind distance can give some idea of how accurate the predictions of ADMS 3.3 are.

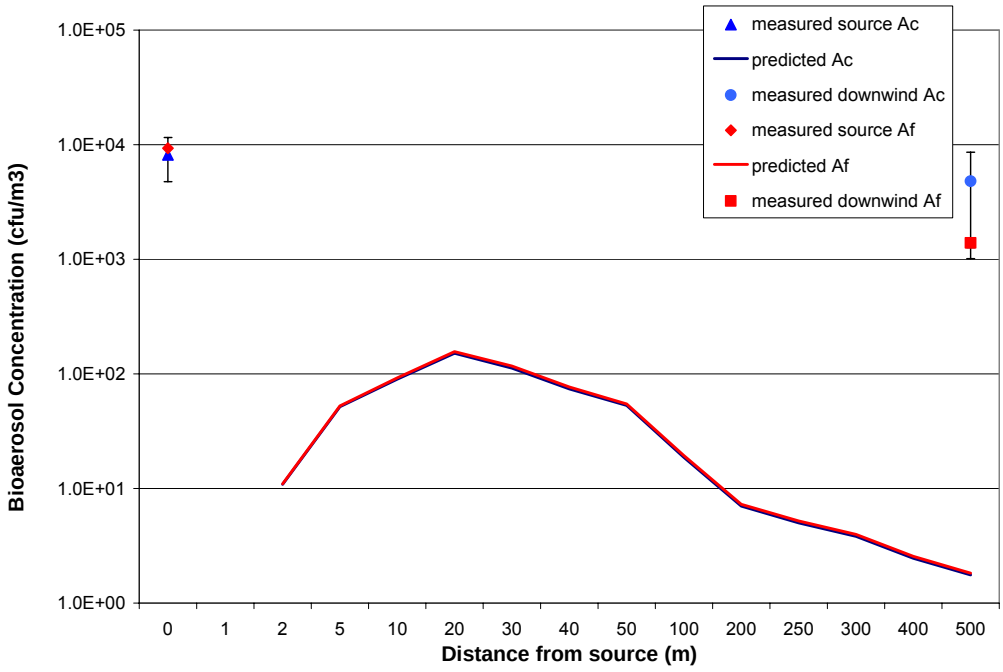


Figure 6.2- Predicted vs. measured downwind concentrations of bioaerosol emissions from incoming waste compost windrow (sampling hood) at Keenan Recycling site during the first sampling day. (Af is *A.fumigatus*, Ac is actinomycetes, the y axis is in logarithmic scale and the bars denote standard error for measured bioaerosol source and downwind concentrations)

The model predictions for 1 m downwind were at 0 CFU/m^3 ground level concentration therefore the results are shown from 2 m downwind only. In addition, the emission curve for both micro-organisms were the same order of magnitude hence they appear to be as one line. This is because the emission rates for both micro-organisms used in the modelling are also of the same order of magnitude.

The model emission curve showed an initial increase in ground concentrations for both micro-organisms at approximately 20m downwind from source before a steady decrease. The results showed that the model under predicts the concentrations of both species by up to 4-log. The source term data was the average of 4 sampling locations on the compost windrow as shown in Table 6.1 and a standard error between these concentrations was shown. The downwind bioaerosol concentration was the average of 2 replicate samples and a standard error between these concentrations was also shown. However considering the standard error for the bioaerosol concentrations

measured on site for source and downwind sampling locations did not have an effect on the scale of this difference.

There are three possible explanations for the differences between the predicted and measured downwind concentrations. Firstly, bioaerosol concentrations measured at source are also approximately 3-log higher than the first prediction of the model at 2m downwind from source. Hence because the model is not able to make accurate predictions close to the bioaerosol source as shown by these results, it would be expected that this would have an impact on the bioaerosol concentrations predicted further downwind.

During this sampling day, it was only possible to calculate an emission rate for bioaerosol release from the incoming waste compost windrow due to practical bioaerosol sampling difficulties. However as shown on the site diagram (Chapter 3, Figure 3.2), this was not the only possible bioaerosol source on this site. Other sources included compost windrows during various stages of maturity as well as compost agitation activities that take place on site (as shown in Figure 3.2, Chapter 3). In addition, the owners of the composting facility reside on-site and kept horses in stables. These were likely additional contributors to the downwind bioaerosols. Therefore secondly, these results might indicate that other bioaerosol sources that are not reflected in the model input have contributed to the downwind bioaerosol concentrations measured on site. Previous studies have found increased levels of bioaerosol concentrations for agitation activities (Taha *et al.*, 2005; Taha *et al.*, 2006; Crook *et al.*, 2006; Taha *et al.*, 2007a) and reported that biaoerosol concentrations during site operations are approximately 2-log higher than background bioaerosol concentrations measured at composting facilities (Sanchez-Monedero and Stentiford, 2003). However there were no noted agitation activities during this sampling day hence this could not account for the elevated downwind bioaerosol concentrations. Therefore it is likely that the other compost windrows or the activities of the residents (i.e. keeping of horses) on the site have contributed to the downwind bioaerosol concentrations.

Upwind background concentrations of *A. fumigatus* were detected during the first sampling day and they were in the same order of magnitude as the bioaerosol concentrations detected downwind and in the vicinity of the houses and stables located on site. Therefore this might suggest that an unidentified upwind source of *A. fumigatus* was present during this sampling day. Hence for *A.fumigatus* this might have been the major contributor to the bioaerosols measured downwind irrespective of the biaoerosols generated within the composting site.

In contrast, no concentrations for actinomycetes were detected upwind or in the vicinity of the on site residencies hence there are no background concentrations of actinomycetes for the summer sampling that might have contributed to the levels of actinomycetes detected downwind. However, it is possible that bioaerosol plumes from other compost windrows might contribute to the downwind actinomycetes levels.

b) Point Source Modelling

Two further site visits were completed at Keenan Recycling. Due to changing site practices between different site visits, it was not possible to take samples at the same locations at each site visit. For both sampling days, bioaerosols were sampled approximately 35m downwind of all site operations, which equated to about 40m and 50m downwind of where the agitation activities were taking place for second and third sampling days respectively. The modelling results of *A. fumigatus* and actinomycetes emissions for the second (compost screening) sampling day at Keenan Recycling site is shown in Figure 6.3.

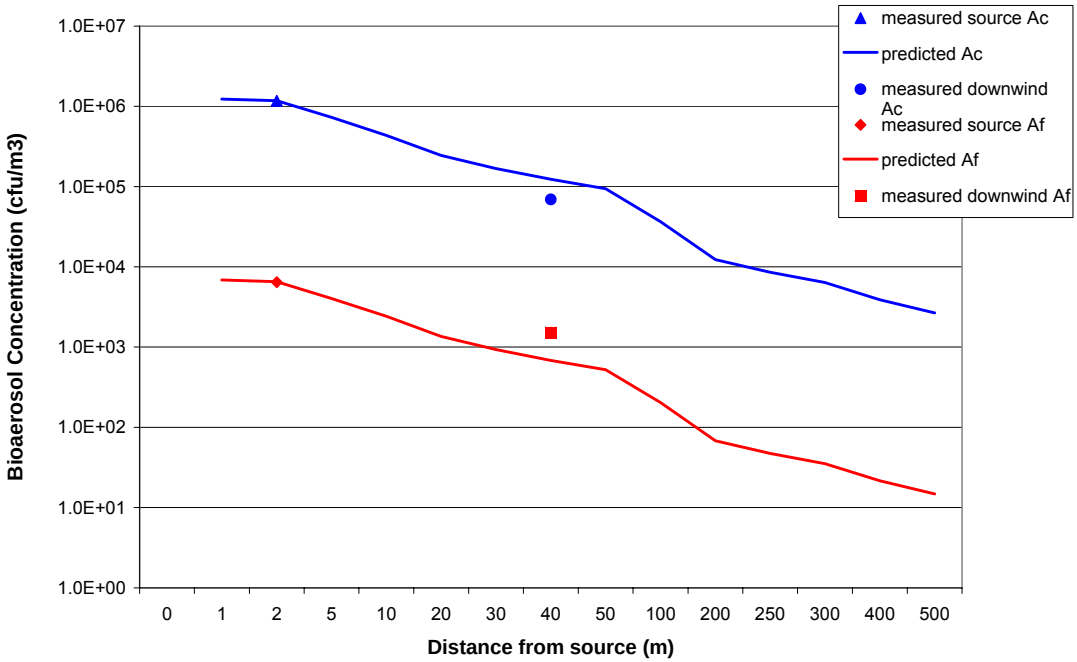


Figure 6.3- Predicted vs. measured downwind concentrations of bioaerosol emissions from agitation activities at Keenan Recycling site during the second sampling day (Af is *A.fumigatus*, Ac is actinomycetes and the y axis is in logarithmic scale)

The results presented for the second sampling date show that the model predictions of the bioaerosol concentrations for agitation activity were much closer (less than 1-log) to the bioaerosol concentrations measured on site than the model predictions of the bioaerosol concentrations for compost windrows as discussed previously (Section 6.2.2.1). Similar results were observed for the model predictions for the agitation activity during the third sampling day where the difference between predicted and modelled actinomycetes concentrations were less than 1-log. This might suggest that for both sampling days the major contribution to downwind bioaerosol concentrations are from the agitation activities.

It was not possible to estimate the bioaerosol emission rate from the incoming waste compost windrow during the second and third sampling days to the lack of the sampling hood. However air samples were taken 5 m downwind from this compost windrow. Therefore as a final analysis of the effect of point and area source modelling, the back-extrapolation methodology was repeated for the 5 m downwind actinomycetes concentrations to estimate a bioaerosol emission rate for this area source. The emission rates estimated by back-extrapolation were then used to generate emission curves in

ADMS 3.3 for the incoming waste compost windrow modelled as an area source. The results of this analysis are presented in Figure 6.4.

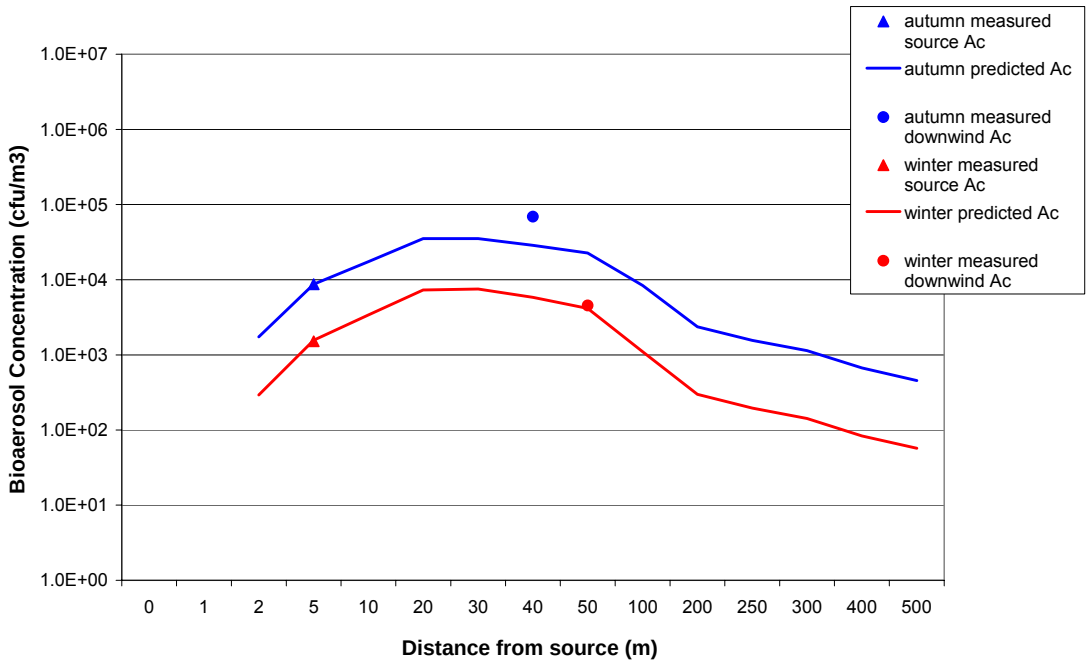


Figure 6.4- Predicted vs. measured downwind concentrations of actinomycetes emissions from incoming compost windrow at Keenan Recycling site during the second and third sampling days (Ac is actinomycetes and the y axis is in logarithmic scale)

The results presented in Figure 6.4 also showed that similar to the emission curves generated by using the agitation activity emission rates for the second and third sampling dates, the model was able to predict downwind bioaerosol concentrations within a difference of less than 1-log. However the estimated actinomycetes emission rates from the incoming compost windrow using the back-extrapolation methodology were 103,000 cfu/m^2/s and 13,000 cfu/m^2/s for the second and third sampling days as opposed to the actinomycetes emission rates determined for the same compost windrow by using the sampling hood in the first sampling day being at a considerably lower at 121 cfu/m^2/s and 606 cfu/m^2/s. The difference between these values is due to the differing methods of emission rate calculation where odour emission rate equations (Equations 6.2 to 6.4) were used for the determination of the emission rate for the first sampling day but a back-extrapolation estimation method was used for the second and third sampling days. The implications of this is that the actual bioaerosol emission rate has a profound effect on the overall emission curves. However, the back-extrapolation

method is just an estimation of the bioaerosol emission rate as opposed to the method which uses the sampling hood which is a standard method for determining the emission rate for another common air quality pollutant. Hence the validity of this analysis should be assessed in this context.

It is also interesting to note that even though the model predicted distinct differences between the shape of the emission curves generated by point and area source modelling (Figures 6.4 and 6.5 as opposed to Figure 6.6), the differences between predicted and measured downwind bioaerosol emissions remain for both source type modelling results. This indicates that even though the model appears to be more successful at predicting individual bioaerosol concentrations downwind of a compost source by using the back-extrapolation bioaerosol emission rate methodology, the fit of other downwind bioaerosol concentrations to the model predictions might differ significantly depending on how the source is defined. However it was not possible to test this further due to a major limitation in the site work methodology which was the lack of multiple downwind sampling points.

6.3.3. Discussion

The objective of the air dispersion modelling studies discussed so far was to complete a preliminary assessment of the ability of a commercial air dispersion model, ADMS 3.3, in predicting bioaerosol emissions from a composting facility compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling.

Two different methods were employed to determine the bioaerosol emission rates at the open windrow site, Keenan Recycling. One of these methods was the determination of the bioaerosol emission rate by back-extrapolating the known bioaerosol concentrations downwind of a bioaerosol source as per the methodology outlined in Taha *et al.* (2005). This resulted in a difference of less than 1-log between measured versus predicted bioaerosol concentrations regardless of the bioaerosol source being defined as a point or area source for agitation activity or compost windrow respectively. In contrast, the use of the sampling hood methodology outlined in the same study (Taha *et al.*, 2005) for determining bioaerosol emission rates from a compost windrow defined as an area

source resulted in the under prediction of the measured downwind concentrations by up to 4-log.

For the model to have the potential for use in support of composting regulatory risk assessments, it should be able to make an approximate match of bioaerosol concentrations measured on a composting site. However the results discussed in this chapter lack consistency and show a wide variety of bioaerosol concentrations and emission curves predicted by the model depending on how the bioaerosol source is defined (i.e. point or area source). This indicated that the differences in ADMS 3.3 predictions for different sources at a composting facility might be due to the variation in the use and determination of bioaerosol emission rates.

However another reason for the discrepancy in the modelling output might also be the limitations of the initial site work and preliminary air dispersion modelling studies completed at Keenan Recycling. The emission curves shown in Figures 6.3 and 6.4 showed that the model might be able to successfully predict individual bioaerosol concentrations downwind of a compost source by using the back-extrapolation bioaerosol emission rate methodology. However the main limitation to the methodology discussed throughout this chapter is regarding the determination of the bioaerosol emission rates used for the air dispersion modelling of point sources (i.e. back-extrapolation method). The method of bioaerosol emission rate determination by use of wind tunnels is based on standard methods of odour emission calculations (Jiang and Kaye, 2001) as well as other studies which have used a wind tunnel to determine the emission rate of a bioaerosol (Taha *et al.*, 2005). The determination of a bioaerosol emission rate by back-extrapolation has also been previously used by other studies (Millner *et al*., 1980; Dannaberg *et al.*, 1997; Swan *et al.*, 2002; Taha *et al.*, 2007a) however is only an estimation of the bioaerosol concentration at source rather than a standardised method. Hence the result of more successful model predictions of the back-extrapolation technique rather than the wind tunnel methodology should be approached with caution and hence no definitive conclusions can be drawn on the advantages of one method over the other.

Another important limitation of this study is the lack of multiple downwind sampling points. The bioaerosol concentrations measured downwind of site to which the predicted model concentrations are compared with represent those for one sampling location only however the model cannot be validated without other downwind sampling locations. There are distinct differences in the shape of the emission curves predicted for a point or area source hence the fit of other downwind bioaerosol concentrations to the model predictions might differ significantly depending on how the source is defined. Therefore if other downwind sampling locations were available, it would be possible to analyse the differences between measured and predicted downwind bioaerosol concentrations for different source definitions. Finally for downwind concentrations, it is important to remember that the measured concentrations are from two sampling replicates only and at some instances from only one sampling point with no replicates. Hence this would also have been expected to affect the validity of the results and analysis. The limitations of the sampling strategy were presented due to the practical difficulties of bioaerosol sampling at a composting facility such as those discussed in Section 6.1

The analysis of upwind and other background bioaerosol concentrations per site indicated the possibility of other sources of bioaerosols at Keenan Recycling site. This site is mainly surrounded by fields so it is likely that a constant source of bioaerosols upwind is present due to sources such as soil dust and decay of vegetation (e.g. leaves) (Swan *et al.,* 2002). In addition, bioaerosols are ubiquitous and are present in nature (Borodulin, 2005; Bovallius *et al.*, 1978; Köck *et al.*, 1998; Fang *et al.*, 2005; Mancinelli and Shulls, 1978; Spicer and Gangloff, 2005). Therefore another major limitation of the initial site work is the likelihood that the bioaerosol concentrations at the downwind location to which the model predictions are compared to do not only represent the bioaerosol sources that were used for determining the bioaerosol emission rates. For example, for the preliminary site work completed at Keenan Recycling, it was not possible to determine an emission rate for a majority of the composting windrows and hence they were not included in the modelling studies. On-site agitation of the compost has previously been shown to be the major contributor to bioaerosol emissions from composting sites (Taha *et al.*, 2005; 2006; 2007a) which indicates that modelling the

agitation activity at a composting facility might be sufficient to determine the approximate overall bioaerosol emissions from a composting site. However, compost windrows would also be expected to be emission sources due to the effects of wind blowing on a compost windrow as well as thermal convection of bioaerosols as they move from an environment of higher temperature (i.e. compost windrow) to lower temperatures (i.e. ambient). Hence a static bioaerosol source such as a composting windrow should also be considered when determining the bioaerosol emission from a composting facility along with the consideration of emission from active sources such as agitation activities.

Therefore in the light of these limitations and the lack of further analysis of model predictions for more downwind sampling locations, the effect of model parameters on the prediction of downwind bioaerosol concentrations was not clear. In addition the results of the preliminary dispersion modelling studies indicated that the definition of the source term (i.e. area or point source) might have a significant effect on the output concentrations predicted by the model. As such, the next section of this chapter will analyse the sensitivities of ADMS 3.3 to determine which input parameters the model is most sensitive to.

6.4. SENSITIVITY ANALYSIS

6.4.1. Methodology

In model development, a sensitivity analysis of a model might be conducted for various reasons including the determination of parameters which need further research for validation of the model or the determination of insignificant parameters for elimination from the model (Hamby, 1994). Once a model is commercially available, a sensitivity analysis can be used to determine how a model reacts to any changes in input parameters and to evaluate the parameters with the biggest effect on modeling output (Hamby, 1994). Such an analysis would differ according to the purpose that the model is used for.

Air dispersion models are used to simulate very complex physical and environmental phenomena (Hamby, 1994) and there may be a level of uncertainty in the

concentrations predicted by the model (Tilden and Seinfeld, 1982). An additional level of uncertainty is presented for modelling of bioaerosols emitted from composting facilities because currently there are no dispersion models developed specifically for this purpose. Therefore a sensitivity analysis of the model was completed to analyse these uncertainties in ADMS 3.3 by testing the effect of different modelling parameters on predicted downwind bioaerosol concentrations to determine which modelling parameters the model is sensitive to.

Throughout the preliminary air dispersion modelling completed for Keenan Recycling, the bioaerosol pollutant was assumed to exhibit gas like properties, hence particle size distribution and particle aggregation was not taken into account. However, it has been discussed that (Wheeler *et al.*, 2001) dispersion modelling of bioaerosols from composting facilities was difficult due to the influence of factors such as aggregation of bioaerosol spores to form larger particles (which would cause them to exhibit particle like properties instead of exhibiting gas like properties). Therefore the incorporation of these properties into the air dispersion model parameters is important to assess the potential improvement in model predictions compared to bioaerosol concentrations measured on site.

Based on this, the sensitivity analysis involved establishing a base model created by using parameters measured previously at Keenan Recycling to represent realistic parameter values measured at a composting facility. Then these parameters were increased by certain factors to create adjusted models. Finally the differences between the model output generated by the base and adjusted models were analysed using a simple quantitative analysis. This analysis method was adapted from performance measurement assessments for different dispersion models in previous studies (Petts and Eduljee, 1994; Beychok, 2001; Hanna *et al.*, 2001) and compared ratios of base and adjusted bioaerosol concentrations. The basis of the quantitative analysis is that if a ratio of base and adjusted bioaerosol concentration is smaller than 1.00, this indicates that the model under predicts downwind concentrations compared to those predicted by the base model. If this ratio is higher than 1.00, this indicates that the model over predicts downwind concentrations compared to those predicted by the base model. A

ratio of 1.00 indicates that the model is successfully able to simulate the measured downwind bioaerosol emissions from source.

The model was not expected to perfectly (i.e. ratio of 1.00) simulate downwind bioaerosol concentrations for each study and some error margin was expected. Therefore an initial arbitrary 20% range (as per Hanna *et al.*, 1999) within 1.00 (i.e. 0.80 to 1.20) was also set as a ratio range which is considered for the model to successfully simulate the measured downwind actinomycetes emissions from source. This range enables an easy understanding of the distinction between parameters which are successfully able to match predicted versus measured downwind bioaerosol concentrations.

In addition to this, the numerical differences between the calculated ratios and 1.00 were also determined. This was completed to analyse the effect of a certain adjusted parameter on the downwind bioaerosol concentrations relative to other adjusted parameters. A higher difference between the ratio and 1.00 indicates the scenario with the most effect on the downwind bioaerosol concentrations and a lower difference indicates the parameter which has the least effect on the downwind bioaerosol concentrations.

For sensitivity analysis of source and meteorological parameters, the pollutant was assumed to be a gas and dry deposition effects were not taken into account for simplicity reasons. However, for sensitivity analysis of pollutant parameters, effects of both gaseous and particulate pollutants were explored using the dry deposition option. This is because the model needs to take the effect of dry deposition into account to study the effects of particulate pollutant parameters. Otherwise the dispersion of the particles in the atmosphere is treated in the same way as it will be for gases.

The sensitivity analysis discussed in this study was based on varying one parameter at a time (changed parameters are presented in detail in Appendix G) and some model parameters were kept constant. The details of these parameters are presented in

Appendix F. Further details of methodology used for sensitivity analysis of source, pollutant and meteorological parameters are discussed below.

a) Effect of Source Parameters on Downwind Concentrations

For the sensitivity analysis of the source parameter, three different source types were considered. These were the point source which represents source term data derived from agitation activities and the area source which represents source term data derived from wind tunnel sampling of the compost windrows, as explained previously.

Compost windrows have been modelled as two dimensional area sources for the preliminary air dispersion modelling at Keenan Recycling and this was repeated for the air dispersion modelling studies discussed in this chapter. However compost windrows are three dimensional structures and hence may be defined instead as a volume source as per Wheeler *et al.*, (2001). Therefore, a third source was tested for the sensitivity analysis which assumed that a compost windrow was a volume source as opposed to an area source. The model manual (CERC, 2003) describes a volume source as "*a release from an area source with vertical extent but no plume rise*". A volume source emission rate is described as "*area emission rate divided by the vertical extent of the volume source*". Therefore, to calculate the emission rate when the compost windrow is assumed to be a volume source the average wind tunnel emission rate calculated for experiment three was divided by 3 m as the vertical extent of the volume source and the resulting emission rate was used for the sensitivity analysis of the volume source.

For point and area sources, all parameters that could be adjusted (limited by the model) were adjusted and these parameters were source height, source temperature and source velocity. In addition to these, for a point source, source diameter could also be adjusted. The parameters that could be adjusted for a volume source were the vertical dimension of source and mid height of volume source (i.e. vertical dimension of source by two) above ground.

The base parameters for all sources were those measured on site (Appendix F) to represent the realistic parameter values usually measured on a composting site. These

parameters were then increased by factors of 10 and 100 to create adjusted model scenarios used to test the sensitivity of the model to different source parameters (Table 6.2). Instead of adjusting the base parameters by a factor of for example, 20% or 50% increase, multiplying the parameters by factors of 10 and 100 allowed to explore a wider range of parameter values allowed by the model (i.e. maximum and minimum parameter values permitted by model). In addition, multiplying the base parameters by factors of 10 were applicable to other parameters changed (i.e. pollutant and meteorological) hence allowed comparisons to be made between different parameter groups.

Table 6.2- Details of adjusted model scenarios for source parameter sensitivity analysis

Type of Source	Adjusted Model Scenario Label	Description of Model Scenario
Point	A	Source height
	B	Source temperature
	C	Source velocity
	D	Source diameter
Area	E	Source height
	F	Source temperature
	G	Source velocity
Volume	H	Mid height of the volume above ground
	I	Vertical dimension of volume source

b) Effect of Pollutant Parameters on Downwind Concentrations

A sensitivity analysis study was completed to test the sensitivity of ADMS 3.3 to changing the pollutant parameters and to further examine the effect of bioaerosol aggregation and size distribution data into the model. Similar to the sensitivity analysis completed for source parameters, all parameters that could be adjusted (limited by the model) were adjusted. In addition, especially the parameters adjusted for a particulate pollutant such as particle density, particle diameter or particle mass fraction assumed to denote aggregation will determine the behavior and deposition of the pollutant when airborne. These concepts have been previously discussed in detail in Chapters 4 and 5.

Base parameters for particulate and gaseous pollutants for an average pollutant were chosen based on values given for gas and particulate properties (Gregory, 1950; Gregory, 1973; Chamberlain, 1967; Lacey and Dutkiewicz, 1976a; Sehmel, 1980; Cox,

1995; Muilenberg, 1995; Tisa *et al.*, 1982; and Carrera *et al.*, 2008) as well as the results presented previously (Chapters 4 and 5) to represent the realistic parameter values which would usually be measured for a bioaerosol particle. These properties then were increased by factors of 10, 100 and 1000 to create the adjusted models as described below for the sensitivity analysis (Table 6.3). The reason for increasing the pollutant parameters by these factors were the same as those discussed previously by source parameters. In addition, the parameter range for pollutant parameters allowed to increase the base parameters by an additional factor of 10 (i.e. 1000). Hence this was also explored to further analyse the effect of parameter factor increase on the model output.

Table 6.3- Details of adjusted model scenarios for pollutant parameter sensitivity analysis and parameterisation

Type of Pollutant	Adjusted Model Scenario Label	Description of Model Scenario
Particulate	J	Particle deposition and terminal velocity
	K	Particle density
	L	Particle diameter
	M	Particle mass fraction*
Gaseous	N	Deposition velocity

** Note: This parameter was changed by factors of 2, 2.5, 5 and 10 because changing this parameter by factors of 100 and 1000 were beyond the range allowed by the model.*

c) Effect of Meteorological Parameters on Downwind Concentrations

Finally, a sensitivity analysis was completed to test the sensitivity of ADMS 3.3 to changing the meteorological parameters. Wind speed is a major factor in generation and dispersing of bioaerosols from composting facilities (Aylor and Parlange, 1975; Crook *et al.*, 2006) and environmental conditions such as temperature and relative humidity as well as wind or rain are important in spore release (Muilenberg, 1995) and dispersal mechanisms (McCartney, 1994). Therefore wind speed, relative humidity and surface temperature were chosen as the meteorological parameters which would be adjusted to study the effect on predicted actinomycetes concentrations.

The base parameters for meteorological factors described in Appendix F (chosen to represent the realistic parameter values usually measured on a composting site) were

changed by factors of 0.1 and 10 (as limited by the model), to create the adjusted model scenarios as described below for the sensitivity analysis:

- **Adjusted Model Scenario O:** Wind speed.
- **Adjusted Model Scenario P:** Relative humidity.
- **Adjusted Model Scenario Q:** Surface temperature.

6.4.2. Results

a) Effect of Source Parameters on Downwind Concentrations

The results of the quantitative analysis for point, area and volume sources are summarised in Tables 1, 2 and 3 of Appendix H. Results for all source types showed that any increases in parameter values result in under prediction of the model output generated for the base model (i.e. running the model with the realistic parameters detected on site).

a.1) Point Source

The analysis of most and least sensitive parameter and increase factors are shown in Figure 6.5.

Downwind Distance	Most Sensitive → Least Sensitive						
1 m	Height x 10 Height x 100 Velocity x 100		Diameter x 33	Velocity x 10	Diameter x 10	Temp x 100	Temp x 10
10 m	Height x 10 Height x 100 Velocity x 100		Diameter x 33	Velocity x 10	Diameter x 10	Temp x 100	Temp x 10
100 m	Height x 100 Velocity x 100	Height x 10	Diameter x 33	Temp x 100	Diameter x 10	Temp x 10	Velocity x 10
250 m	Height x 100	Velocity x 100	Diameter x 33	Height x 10	Diameter x 10	Temp x 100	Temp x 10	Velocity x 10
500 m	Height x 100	Velocity x 100	Diameter x 33	Diameter x 10	Temp x 100	Height x 10	Temp x 10	Velocity x 10

Figure 6.5 – Analysis of most and least sensitive parameter and increase factors for point source parameters
Note: 'Temp' denotes 'Temperature'

The analysis of the results showed that increases in parameter values affected the model differently depending on distance downwind from source. However, a common parameter and increase factor for all downwind distances was source height and running the model when using a source height of 300 m (highlighted in yellow). This made the most difference between predicted downwind actinomycetes concentrations compared to running the model with a base source height of 3 m as measured on site.

The model in general was less sensitive to varying source temperature for all downwind distances (highlighted in two shades of blue) and the results also showed that for a point source the model was least sensitive to varying the source temperature by a factor of 10 at 1 and 10m downwind. Similarly, the model was less sensitive to varying the source velocity by a factor of 10 at 100, 250 and 500m downwind even though for the same distances the model was more sensitive to varying this same parameter by a factor of 100.

Increasing some parameters with certain factors had no difference on the model output when compared with the base model output (ratio 1.00 ± 20%) as highlighted in Table 1 of Appendix H. These are listed as follows:

- Source velocity x 10 (100, 250 and 500m)
- Source temperature x 10 (1m)

a.2) Area Source

The analysis of the most and least sensitive parameter and increase factors for the area source were similar to those shown for the point source (Figure 6.5). As such increases in parameter values affected the model differently depending on distance downwind from source.

The two common parameters and increase factors for all downwind distances was increasing both the source height and source velocity by a factor 100. Therefore similar to point source modelling, running the model using a source height of 300 m made the most difference between predicted downwind bioaerosol concentrations compared to running the model with a base source height of 3 m as measured on site.

Running the model with a source velocity of 120 m/s also had a large effect on the difference between predicted downwind bioaerosol concentrations compared to running the model with a base source velocity of 1.2 m/s as measured on site. This was similar to results for a point source where the model was sensitive to increasing the source velocity by a factor 100.

The results also showed that for an area source (the same as a point source), the model was least sensitive to varying the source temperature by a factor of 10 at 1 and 10m downwind. Similarly, the model was least sensitive to varying the source velocity by a factor of 10 at 100, 250 and 500m downwind. However the model was more sensitive to varying the source height for an area source by a factor 10 at 100, 250 and 500m downwind. This was in contrast to varying the source temperature by a factor of 10 as it was for a point source. Increasing the source velocity by 10 at 100, 250 and 500m downwind of source had no difference on the model output when compared with the base model output (ratio 1.00 ± 20%).These ratios are highlighted in Table 2 of Appendix H.

a.3) Volume Source

The analysis of most and least sensitive parameter and increase factors for a volume source are shown in Figure 6.6.

Downwind Distance	Most Sensitive → Least Sensitive		
1 m	Mid Height x 100 Vertical Dimension x 100	Vertical Dimension x 10	
10 m	Mid Height x 10 Mid Height x 100 Vertical Dimension x 100	Vertical Dimension x 10	
100 m	Mid Height x 100 Vertical Dimension x 100	Mid Height x 10	Vertical Dimension x 10
250 m	Mid Height x 100 Vertical Dimension x 100	Mid Height x 10	Vertical Dimension x 10
500 m	Mid Height x 100 Vertical Dimension x 100	Mid Height x 10	Vertical Dimension x 10

Figure 6.6 – Analysis of most and least sensitive parameter and increase factors for volume source parameters

It was not possible to compare these results with those for point and area sources as different parameters are used for volume source modelling. However, similar to point and area sources, the model was less sensitive to smaller increases in parameters (i.e. increasing a parameter by a factor of 10 as opposed to a factor of 100). The model (CERC, 2003) defines a volume source as *"a release from an area source with vertical extent but no plume rise".* Hence the volume source would be expected to behave similarly to an area source and might explain this outcome.

The analysis showed that for all downwind distances, changing both parameters for a volume source by a factor of 100 had the largest output change. The only exception to this was that changing the mid height of the source above ground by a factor of 10 at 10m downwind from source to which the model was also most sensitive. The least output change for all downwind distances was detected when the vertical dimension of volume source was increased by a factor of 10 (hence at 30m).

Increasing the vertical dimension of volume source by a factor of 10 at 100, 250 and 500m downwind from source had no difference on the model output when compared with the base model output (ratio 1.00 ± 20%). These ratios are highlighted in yellow in Table 3 of Appendix H.

b) Effect of Pollutant Parameters on Downwind Concentrations

b.1) Particulate Pollutant Parameters

Two separate quantitative analyses were completed following the particulate pollutant sensitivity analysis. The first quantitative analysis (Appendix H, Table 4) aimed to analyse the sensitivity of the model to increasing particle parameters from the chosen base parameters (for a realistic pollutant) when modelling the pollutant as a particulate and considering the effects of dry deposition. The summary of the first quantitative analysis is shown in Table 6.5 for a point source. This quantitative analysis showed that changing the particle mass fraction (denoting particle aggregation) of the pollutant has no effect on the modelling output regardless of the source type (i.e. ratio is equal to 1.00). Therefore this parameter was not analysed further in Table 6.4.

Table 6.4 – Point source modelling particle pollutant sensitivity analysis summary; N denotes 'no', Y denotes 'yes', NR denotes 'no-result', UP denotes ' under prediction', OP denotes 'over prediction'.

Parameter	Change Factor	Is the Model Sensitive? (at downwind distances, m)					Over or under prediction? (at downwind distances, m)				
		1	10	100	250	500	1	10	100	250	500
Deposition and Terminal Velocity	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
	X 100	N	N	N	N	N	NR	NR	NR	NR	NR
	X 1000	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
Particle Density	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
	X 100	N	N	N	N	N	NR	NR	NR	NR	NR
	X 1000	N	N	N	N	N	NR	NR	NR	NR	NR
Particle Diameter	X 10	Y	N	Y	Y	Y	UP	NR	UP	UP	UP
	X 100	Y	N	Y	Y	Y	UP	NR	UP	UP	UP
	X 1000	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP

The results showed that the model was most sensitive to changing the particle diameter of a particle pollutant as opposed to other properties such as deposition/terminal velocity, particle density and particle mass fraction. Similar results were observed for the pollutant modelling of area and volume source types.

A second quantitative analysis was also completed (Appendix H, Table 5) to compare the effects of various particle pollutant parameter values for all source types between two scenarios, first of which assumed the pollutant to be a particulate and second that assumed the pollutant to be a gas (i.e effects of dry deposition not taken into account). The results of this analysis were similar to those described for the first quantitative analysis. They showed that:

- The model was not sensitive to differences in modelling the pollutant as a particulate aggregate with a defined particle mass fraction or as a gas;
- The model was not sensitive to differences in modelling the pollutant as a particle with a defined particle density or modelling the pollutant as a gas (only exception when an unrealistic particle density of 1,000,000 kg/m^3 is assumed for a pollutant emitted from a volume source at which instance the average ratio is an under prediction of 0.64 as an average of all downwind distances);

- The model was not sensitive to differences in modelling the pollutant as a particle with a defined deposition/ terminal deposition or modelling the pollutant as a gas (only exception when an unrealistic deposition/terminal velocity 0.01 m/s is assumed for the pollutant emitted from a point source, under prediction of 0.01 as an average of all downwind distances).

The model was sensitive to changing the particle diameter only. This had an effect on the model output when compared to modelling the pollutant as a gas with overall ratios of 0.73, 0.82 and 0.57 (under prediction) for all downwind distances for point, area and volume sources respectively. The ratios at which the parameter is noted to have no effect on the output (0.8 to 1.2) are also highlighted in Tables 4 and 5 in Appendix H.

b.2) Gaseous Pollutant Parameters

Two separate quantitative analyses were completed following the gas pollutant sensitivity analysis and parameterisation. The first quantitative analysis (Appendix H, Table 6) was completed to analyse the effect of changing the gas deposition velocity when modelling the pollutant as a gas and considering the effects of dry deposition. This is summarised in Table 6.5. Please note that the only parameter adjusted was gas pollutant deposition velocity.

Table 6.5 – Gas pollutant sensitivity analysis summary for first quantitative analysis; N denotes 'no', Y denotes 'yes', NR denotes 'no-result', UP denotes ' under prediction', OP denotes 'over prediction'.

Source Type	Change Factor	Is the Model Sensitive? (at downwind distances, m)					Over or under prediction? (at downwind distances, m)				
		1	10	100	250	500	1	10	100	250	500
Point	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
	X 100	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	X 1000	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
Area	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
	X 100	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	X 1000	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
Volume	X 10	N	N	Y	Y	Y	NR	NR	UP	UP	UP
	X 100	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	X 1000	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP

The results showed that for all source types, increasing the deposition velocity of the gas pollutant by factors of 100 and 1000 resulted in the highest change for all downwind distances. In contrast increasing the deposition velocity of the gas pollutant by a factor of 10 resulted in the least change for all downwind distances with the exception of gas pollutant emitted from a volume source. This means that increasing the deposition velocity of a gas pollutant from the base parameter of 0.003 m/s to 0.3 m/s and 3 m/s has a profound effect on the predicted downwind bioaerosol concentrations.

The second quantitative analysis was carried out (Appendix H, Table 7) to analyse the difference between modelling the pollutant as a gas (no model effects) and modelling the pollutant as a gas with defined deposition velocity (dry deposition). This is summarised in Table 6.6. Please note that the only parameter adjusted was gas pollutant deposition velocity.

Table 6.6 – Gas pollutant sensitivity analysis summary for second quantitative analysis; N denotes 'no', Y denotes 'yes', NR denotes 'no-result', UP denotes ' under prediction', OP denotes 'over prediction'.

Source Type	Value (m/s)	Is the Model Sensitive? (at downwind distances, m)					Over or under prediction? (at downwind distances, m)				
		1	10	100	250	500	1	10	100	250	500
Point	0.003	N	N	N	N	N	NR	NR	NR	NR	NR
	0.03	Y	N	Y	Y	Y	UP	NR	UP	UP	UP
	0.3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
Area	0.003	N	N	N	N	N	NR	NR	NR	NR	NR
	0.03	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	0.3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
Volume	0.003	N	N	N	N	N	NR	NR	NR	NR	NR
	0.03	N	Y	Y	Y	Y	NR	UP	UP	UP	UP
	0.3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	3	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP

The results of the second quantitative analysis were similar to those described for the first quantitative analysis and they showed that:

- The model was not sensitive to increasing the deposition velocity of the gas pollutant by a factor of 10;
- The model was sensitive to increasing the deposition velocity of the gas pollutant by a factor of 100 or 1000.

The ratios at which the parameter is noted to have no effect on the output (0.8 to 1.2) are highlighted in yellow in Tables 6 and 7 in Appendix G.

c) Effect of Meteorological Parameters on Downwind Concentrations

Two separate quantitative analyses were completed following the sensitivity analysis of meteorological parameters. The first quantitative analysis (Appendix H, Table 8) was completed to analyse the effect of increasing the meteorological parameters with factors of 10 and 100. The parameters were increased from base meteorological parameters which are those measured on site. This is summarised in Table 6.7.

Table 6.7 – Meteorological parameter sensitivity analysis summary for first quantitative analysis; N denotes 'no', Y denotes 'yes', NR denotes 'no-result', UP denotes ' under prediction', OP denotes 'over prediction'.

Source Type	Parameter	Change Factor	Is the Model Sensitive? (at downwind distances, m)					Over or under prediction? (at downwind distances, m)				
			1	10	100	250	500	1	10	100	250	500
Point	Wind Speed	X 10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
		X 100	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	Relative Humidity	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
		X 100	N	N	N	N	N	NR	NR	NR	NR	NR
	Surface Temp	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
		X 100	N	Y	Y	Y	Y	NR	OP	OP	OP	OP
Area	Wind Speed	X 10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
		X 100	Y	Y	Y	N	Y	UP	UP	OP	NR	UP
	Relative Humidity	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
		X 100	N	N	N	N	N	NR	NR	NR	NR	NR
	Surface Temp	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
		X 100	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Volume	Wind	X 10	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

	Speed	X 100	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
	Relative Humidity	X 10	N	N	N	N	N	NR	NR	NR	NR	NR
		X 100	N	N	N	N	N	NR	NR	NR	NR	NR
	Surface Temp	X 10	Y	Y	Y	Y	Y	UP	UP	UP	UP	UP
		X 100	N	N	Y	Y	Y	NR	NR	OP	OP	OP

It was not possible to run the model with a wind speed of 0.5 m/s for any of the source types as the minimum wind speed value allowed by the model was 0.5 m/s. In addition, it was not possible to run the model with a surface temperature of 50^0C for an area source hence these are represented as NA and highlighted in grey in Table 6.7.

The results showed that increasing the relative humidity did not have an effect for any of the downwind distances with a ratio of 1.00 for all source types. On average for all source emission types, the model was most sensitive to changing the wind speed by a factor of 100 (from 5 m/s to 50 m/s) with under predictions observed with the exception of an over prediction for 100m downwind distance for an area emission source type. The model was also sensitive to increasing the surface temperature by a factor of 100 for point and volume emission source types with over predictions of outputs observed when results were compared to running the model with parameters measured on site.

In addition to this, a second quantitative analysis was carried out (Appendix H, Table 9) which compared modelling the effects of various meteorological parameters (as described for the first quantitative analysis) for all source types and modelling using the ADMS 3.3 meteorological file R91A-G which represents the effects of different Pasquill stability classes (the Pasquill stability class D was chosen as discussed previously). The results of the this analysis showed that:

- for all source types and at all downwind points, modelling using a wind speed of 50 m/s had the most effect compared to modelling with Pasquill stability class D.
- For both volume and point sources, the least effect was observed when modelling with a surface temperature of 50^0C compared to modelling with Pasquill stability class D. In contrast for an area modelling using a surface

temperature of 5^0C had the least effect compared to when modelling with Pasquill stability class D. However, it should be noted that it was not possible to model for an area source using a surface temperature of 50^0C.

- In addition for an area source, modelling with any relative humidity (1, 10 or 100%) and using a wind speed of 5 m/s also had a small effect compared to when modelling with Pasquill stability class D.

The ratios at which the parameter is noted to have no effect on the output (0.8 to 1.2) are highlighted in yellow in Tables 8 and 9 in Appendix H.

6.4.3. Discussion

The sensitivity analysis studies were completed to improve the understanding of the sensitivity and limitations of the model. In order to achieve this aim, objectives were set to:

- determine which parameters characteristic of a composting facility (e.g. source, pollutant, meteorological) the model is most sensitive to and
- determine if the model is most sensitive to adjusting the pollutant size and aggregation parameters or if other parameters such as source definition (i.e. point or area source) are just as important.

Sensitivity analysis of source, pollutant and meteorological parameters was carried out by assigning a set of base parameters for each parameter which were based on parameters measured during the site work (Appendix E) or other values which have been noted by previous studies (Appendix G). The emission curves and downwind concentration data were generated for this base model. Then these base parameters were changed by a set of factors to create adjusted models and the emission curves and downwind concentration data were generated for these adjusted model scenarios. The quantitative analysis was completed which determined ratios of the adjusted to base model output to analyse and determine parameters which have the most and least effect on the bioaerosol concentrations predicted by the base model.

Currently the regulator in England and Wales requires a risk assessment for any facility that has a sensitive receptor (e.g. a home or office building) within 250 m of the site boundary (Environment Agency 2001; 2007). Therefore the following summary (Figure 6.7) of the quantitative analysis results for the sensitivity analysis of emission source, pollutant and meteorological parameters is based on model predictions at 250 m from source.

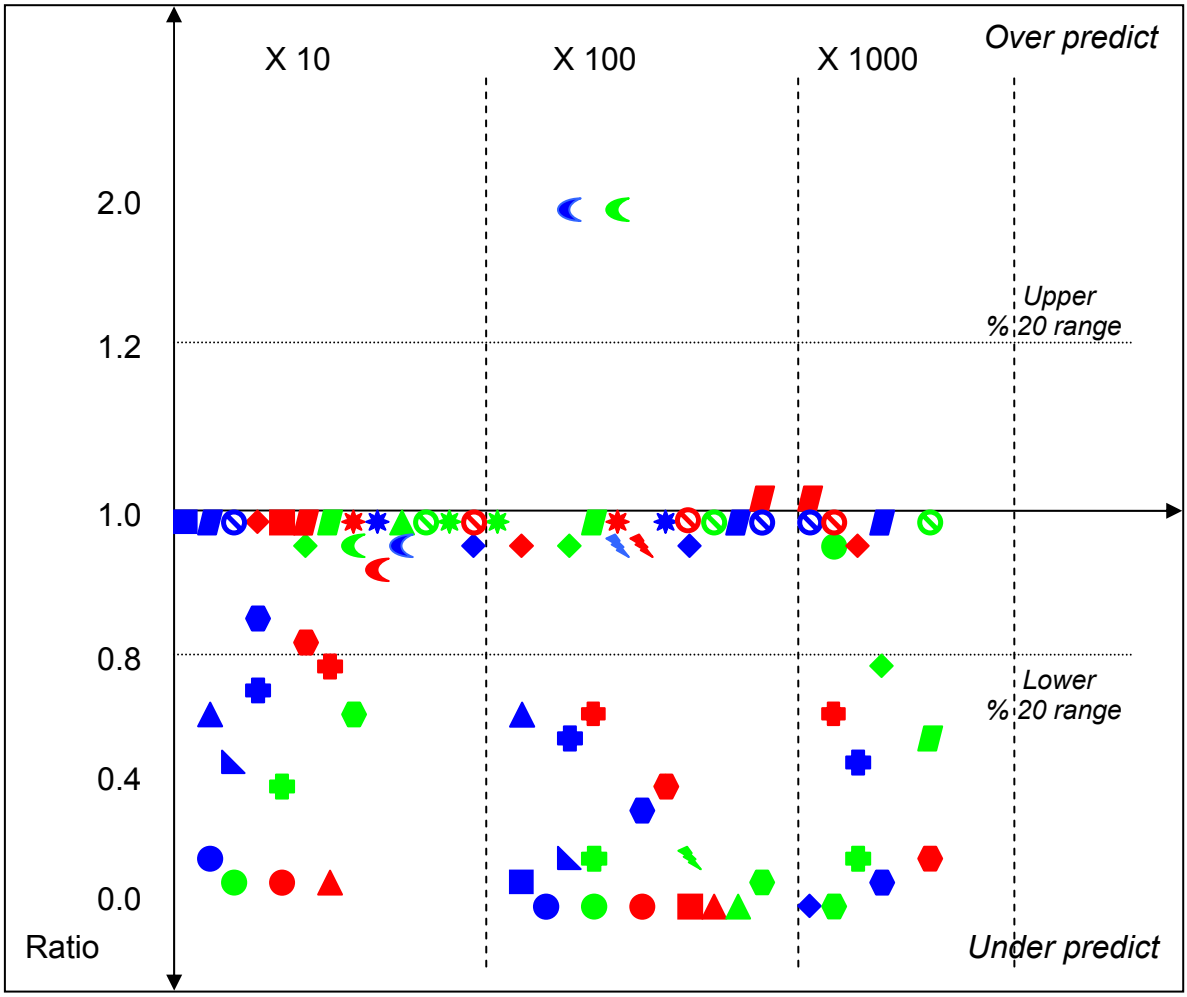


Figure 6.7- Summary of source, pollutant and meteorological parameter change analysis. Blue symbols denote point source parameters, red symbols denote area source parameters and green symbols denote volume source parameters.

Note: Legend for Symbols

Parameter Type	Parameter Name	Base Value	Symbol
Emission Source	Point Source Height	3 m	●
	Point Source Velocity	1.1 m/s	■
	Point Source Temperature	19.7°C	▲
	Point Source Diameter	3 m	◣

	Area Source Height	3 m	
	Area Source Velocity	1.2 m/s	
	Area Source Temperature	25.6°C	
	Volume Source Mid height above ground	1.5 m	
	Volume Source Vertical dimension	3 m	
Particulate Pollutant	Deposition/Terminal Velocity (Point Source)	0.00001 m/s	
	Particle Density (Point Source)	1000 kg/m^3	
	Particle Diameter (Point Source)	0.000001 m	
	Particle Mass Fraction (Point Source)	0.1	
	Deposition/Terminal Velocity (Area Source)	0.00001 m/s	
	Particle Density (Area Source)	1000 kg/m^3	
	Particle Diameter (Area Source)	0.000001 m	
	Particle Mass Fraction (Area Source)	0.1	
	Deposition/Terminal Velocity (Volume Source)	0.00001 m/s	
	Particle Density (Volume Source)	1000 kg/m^3	
	Particle Diameter (Volume Source)	0.000001 m	
	Particle Mass Fraction (Volume Source)	0.1	
Gas Pollutant	Deposition Velocity (Point Source)	0.003 m/s	
	Deposition Velocity (Area Source)	0.003 m/s	
	Deposition Velocity (Area Source)	0.003 m/s	
Meteorological	Wind Speed (Point Source)	5 m/s	
	Relative Humidity (Point Source)	10 %	
	Surface Temperature (Point Source)	5°C	
	Wind Speed (Area Source)	5 m/s	

	Relative Humidity (Area Source)	10 %	
	Surface Temperature (Area Source)	5°C	
	Wind Speed (Volume Source)	5 m/s	
	Relative Humidity (Volume Source)	10 %	
	Surface Temperature (Volume Source)	5°C	

The sensitivity analysis for emission source, pollutant and meteorological parameters as summarised above (Figure 6.7) revealed the parameters which have the least and most overall change on the bioaerosol concentrations predicted by the base model and the factors by which these changes are observed at 250m downwind from source.

One objective of the sensitivity analysis was to analyse the effect of source definition (i.e. point or area source) on model output. Hence all parameters were tested separately for a point and area source (shown by blue and red symbols respectively). The definition of the source resulted in a difference between model output for a point or area source for a limited number of model parameters. For example, increasing the particle deposition/terminal velocity by a factor of 1000 had the largest effect for a point source but did not have any effect for an area source. However, in general, the results (Figure 6.7) showed that model output for the parameterisation of different parameters did not differ for a point or area source.

The results (Figure 6.7) also showed that common to all source types, the parameter which had the most effect on the predicted downwind concentrations was increasing the source height by a factor of 100 (shown by blue, red and green filled circles). This would mean that modelling the source as one that is 300m high as opposed to 3m high which was taken as the base parameter. This was in line with previous studies which have reported that exposure of bioaerosols emitted from sewage was modulated by season as well as the height at which these waste was agitated (Nielsen *et al.*, 1997). The height of a composting windrow (bioaerosol source) for all compost windrows at all sampling days was determined to be 3m. The height of an agitation cloud had also been observed to be 3m as reported in previous studies (Taha *et al.*, 2006). Therefore,

increasing the source height parameter which denotes the bioaerosol source in a composting facility by a factor of 100 would result in a value that will never be likely or practical in a composting facility.

For both point and area sources, increasing the source velocity by a factor of 100 also had a big effect on the predicted downwind concentrations (shown by blue and red filled squares). The base value taken for this parameter is 1.2 m/s which was the wind speed measured on top of a composting windrow or inside the agitation cloud which would denote the velocity by which bioaerosols are dispersed from source. A wind speed of 120 m/s might occur in hurricane-tornado environments (McCaul, 1991) but would be very unlikely to occur in a composting facility.

The model describes particle mass fraction as *the mass fraction of the particles with specified velocities/diameters which must add up to 1* (CERC, 2003). This parameter is used for particle emissions and allows the user to model particulate mixtures where up to 10 different particle size/density combinations can be specified. For the purposes of this study, the particle mass fraction was assumed to denote bioaerosol aggregation as per previous studies (Drew *et al.*, 2006). Therefore a number of particle pollutants were defined in terms of their deposition parameters with their mass fraction adding up to 1 representing a whole aggregate. It is also important to note that the sensitivity analysis discussed in this study was based on varying one parameter at a time hence the particle size and density was kept constant whilst different particle mass fractions were tested. The results showed that for particle pollutant parameters, changing the particle mass fraction of the pollutant did not have an effect for any source type or any increase factor.

Changing the particle density of a particle pollutant by any factor for point and area sources and by 10 and 100 for a volume source also did not have an effect on the model output. In addition changing the particle terminal/deposition velocities by any factor for area and volume sources resulted in no effect on the model output. Therefore ADMS 3.3 was not sensitive to any of these pollutant parameters for a particulate pollutant.

The model was not sensitive to increases in particle deposition/terminal velocity for an area source. In contrast, increasing the particle deposition/terminal velocity by a factor of 1000 had the largest effect for a point source. However this change would indicate a parameter which is equivalent to $1x10^{-2}$ m/s deposition/terminal velocity. The base value for the deposition and terminal velocities of a particle pollutant was $1x10^{-5}$ m/s. This parameter value was chosen in the range of model minimum, maximum and various velocities listed in previous literature (Table 6.8).

Table 6.8- Velocities of various microorganisms

Microorganism	Type of Velocity	Value of Velocity	Reference
thermoactinomycetes	Terminal	$5x10^{-5}$ m/s	Gregory, 1973
A.fumigatus		$5x10^{-4}$ m/s	
Bacteria	Settling	$1x10^{-4}$ m/s	Muilenberg, 1995
A. *fumigatus*	Sedimentation	$2.9x10^{-4}$ m/s	Lacey and Dutkiewicz, 1976a
Penicillium		$3.1x10^{-4}$ m/s	
Thermoactinomyces vulgaris		$1x10^{-5}$ m/s	
Micropolyspora faeni		$1.1x10^{-4}$ m/s	
Saccharomonospora viridis		$1x10^{-4}$ m/s	
Nocardia sp		$1.3x10^{-4}$ m/s	
Bacteria		$2.1x10^{-4}$ m/s	

In addition to these Chamberlain (1967) has noted terminal velocities of spheres with a unit density of $3.5x10^{-5}$ and $1.3x10^{-4}$ for 1 and 2 μm spheres. Therefore a deposition/terminal velocity of $1x10^{-2}$ m/s would be unlikely to occur for actinomycetes, bacterial or fungal species. However the terminal velocities of bigger particles such as Lycopodium spores have been reported to range from $0.7\text{-}3.5x10^{-2}$ m/s for a 32 μm spore size (Gregory, 1950; Chamberlain 1967 from Sehmel, 1980), $1.5 – 4.5x10^{-2}$ m/s for a spore size of 20 μm; $3.3\text{-}9.9x10^{-2}$ m/s for a spore size of 32-35 μm and $2\text{-}6x10^{-1}$ m/s for a spore size of 90-100 μm (Raynor, 1976 from Sehmel, 1980). Therefore even though the model was sensitive to incorporation of the deposition/terminal velocity of a particulate pollutant, the results show that it is sensitive to the deposition/terminal velocity rates of larger particles such as a Lycopodium spore (i.e. 32 μm). Therefore, if a

bioaerosol forms an aggregate in a size similar to that of a Lycopodium spore, then the model would be able to account for this. However, the results shown in Chapters 4 and 5 have revealed that the majority of bioaerosols emitted from compost are in single cells and even in aggregates, their overall size is not bigger than approximately 15 μm in length hence this result does not have implications in the use of the model for bioaerosol modelling.

The base value for the particle diameter used was $1x10^{-6}$ m which was chosen in the range of model maximum, minimum and the results of SEM analysis of controlled experiments and site work (Chapters 4 and 5) which observed particles within the size ranges of $5x10^{-7}$ to $1x10^{-6}$ m (0.5-1 μm) for small spores and $2x10^{-6}$ to $3x10^{-6}$ m (2-3 μm) for large spores. The model was sensitive to parameterisation of particle size for all source types and for all multiplication factors of 10. In addition, the definition of a larger particle size (≥$1x10^{-6}$ m equating to ≥10 μm) such as that for a bioaerosol aggregate resulted in the under prediction of the model output compared to the scenario where the pollutant was assumed to be gas. This might indicate that the model is able to consider the effect of a larger pollutant settling out compared to that for a smaller pollutant. Hence even if the model was not sensitive to definition of particle mass fraction (i.e. used for modelling particulate mixtures equalling to an overall particle mass fraction of 1) which was assumed to denote aggregation, these results might indicate that the model is sensitive to defining the bioaerosol as an aggregate due to its sensitivity to larger particle sizes. However, it is important to remember that the model only appears to be sensitive to particle sizes ≥10 μm compared to modelling the pollutant as a gas. The results of the controlled experiments and site work showed evidence of larger bioaerosols emitted from compost however the largest of such particles had a length of 15 μm and width of 8 μm and in general, the bioaerosols emitted from compost were in single cells. As such, there is no definitive evidence to suggest that bioaerosols should be re-defined as particles when modelling their dispersion with ADMS 3.3.

For gas pollutant parameters, it was only possible to analyse the sensitivity of the deposition velocity. For all source types, increasing the deposition velocity by 10 had the least effect and increasing the deposition velocity by 1000 had the most effect on

the model output. The base deposition velocity parameter for a gas pollutant was taken as 0.003 m/s for a non reactive gas for CO_2 (Hill, 1971 from Sehmel, 1980). Therefore ADMS 3.3 was sensitive to only using an unlikely deposition velocity of 3.0 m/s. As such, there is no evidence to show that when bioaerosols are modelled as gases, their dry deposition should be taken into account.

The composition, concentration and size of microbial populations in the atmosphere are expected to change with the changes in the environmental conditions (Cox, 1987) as well as changes in bioaerosol source (Pillai, 2002; Pillai and Ricke, 2002). Therefore the model needs to be able to reflect changes in meteorological conditions such as wind speed, temperature or relative humidity against predicted bioaerosol concentrations.

For meteorological parameters, for all source types, parameterisation of relative humidity had no effect on the predicted downwind concentrations. This is in contrast to previous studies (Lighthart et al., 1987; Muilenberg, 1995, Stetzenbach, 1997) which have noted that relative humidity is an important parameter which would effect the transport of bioaerosols in air and low relative humidity was discussed to cause daily peaks of some fungal spores such as Basidiospores and Ascospores whose diurnal rhythm requires atmospheric moisture for spore release (Levetin and Horner, 2002).

The parameter which had the most effect for a point and area source was increasing the wind speed by 100 and increasing the surface temperature by a factor of 100 had the most effect for a volume source. The effect of wind speed on the predicted downwind actinomycetes concentrations are in line with previous studies (Gostelow *et al.*, 2001; Colls, 2002) which have discussed the effect of wind speed on the dispersion of bioaerosols. In addition, daily peaks of some fungal spores such as Cladosporium, Alternaria and Epicoccum have been discussed to occur at high temperatures and wind speeds (Levetin and Horner, 2002). This is because these spores are released passively and their dispersal is dependent on weather effects such as the wind speed. However, increasing the base speed 0.5 m/s by a factor of 100 would result in a wind speed of 50 m/s which is not a realistic value for a wind speed usually observed in a composting facility. Similarly, increasing the surface temperature by a factor of 100

would result in a surface temperature of 1970^{o}C which will not occur in a composting facility.

As discussed previously, the methodology developed for the sensitivity analysis involved the use of base parameter values used to generate the base models which were those measured on site or reported in previous studies for particle or gas pollutants. Therefore, it was important to analyse the results of the sensitivity analysis with regards to the parameter values which would occur in nature and a composting facility. This is because even if changing a certain parameter in ADMS 3.3 by a certain factor might result in improved predicted downwind concentrations in a composting site, if that final parameter is one that would not occur in real-life then this would not be a real improvement in the ability of the model to predict bioaerosol dispersion.

The results lead to the conclusion that when the model was sensitive to a parameter, the value of this parameter was generally unlikely to occur in nature or in a composting facility. However, one potential reason for this might also be due to the limitations of the sensitivity analysis approach that remits discussion.

The sensitivity analysis approach involved establishing a base (i.e. baseline scenario) model created by using parameters measured previously at a composting site or in previous studies to represent realistic parameter values measured at a composting facility. Then these parameters were increased by 10 and its multiplication factors (e.g. 100, 1000) to create adjusted models (i.e. scenarios). This approach was taken as the multiplication of base parameters by factors such as 10 and 100 allowed the exploration of a wider range of parameter values allowed by the model (i.e. maximum and minimum parameter values permitted by model) compared to using smaller factors such as an increase by x2 or x5. In addition, multiplying the base parameters by multiplication factors of 10 were applicable to other parameter groups (i.e. pollutant and meteorological) that were explored simultaneously and hence allowed comparisons to be made between different parameter groups. The approach of dividing the base parameter by values such as 0.1 or 0.01 was not feasible to achieve across a higher

number of parameters because the range of parameter values allowed by the model was limited.

However, multiplication of the base parameter by factors such as 10 and higher values results in the creation of parameters which are unlikely to occur in nature or a composting facility. This is a limitation of the sensitivity analysis approach rather than a critique of the model. The sensitivity analysis was one of the many different studies explored within this thesis and hence was constrained by time limitations. As such, some model parameters were kept constant and other model parameters which were deemed to have an increased likelihood on model output were explored. However, for future studies, it would be recommended that, for parameters which the model has been shown to be sensitive to at a multiplication factor of 10, the approach should be further refined to explore smaller changes in model parameters. Hence a factor such as the parameterisation of the particle diameter which for a point and area source the model was sensitive to at a factor of 10 (blue and red filled plus sign as shown in Figure 6.7) should be further tested to analyse its effect on the model output at a smaller factor of x 2 or x 5 which would further represent values likely to occur in a composting facility in line with the results shown in Chapters 4 and 5. Such an analysis might have implications in the definition of the pollutant (i.e. particulate or gas) for future studies.

An arbitrary range of 20% within 1.00 (i.e. 0.80 to 1.20) was set as a ratio range which was considered for the model to successfully simulate the measured downwind actinomycetes emissions from source. However, the results shown in Figure 6.7 show that a lower arbitrary range such as 10% within 1.00 (i.e. 0.90 to 1.10) might be more suitable for the data range. In addition, in line with the above argument for the multiplication factors, this might further refine the sensitivity analysis approach.

However, on average, the model was not sensitive to a larger number of parameters than the number of parameters it was sensitive to. Therefore the further refinement of the model to test the effect of lower factors on a large number of parameters is unlikely to result in a significantly different outcome.

6.5. CONCLUSIONS

Preliminary air dispersion modelling completed at Keenan Recycling was discussed. The bioaerosol sources at this open windrow site were defined as either point or area sources. The determination of the bioaerosol emission rate by back-extrapolating the known bioaerosol concentrations downwind of a bioaerosol source as per the methodology outlined in Taha *et al.* (2005) resulted in a difference of less than 1-log between measured versus predicted bioaerosol concentrations for Keenan Recycling agitation and compost windrow sampling locations. In contrast, the use of the sampling hood methodology outlined in the same study (Taha *et al.*, 2005) for determining bioaerosol emission rates from a compost windrow defined as a bioaerosol source resulted in the under prediction of the measured downwind concentrations by up to 4-log. The results of the preliminary dispersion modelling studies therefore indicated that the definition of the source term (i.e. point or area source) might have a significant effect on the output concentrations predicted by the model and the differences in model predictions for different sources might be due to the variation in the use and determination of bioaerosol emission rates.

Following this, an analysis was completed to analyse the sensitivities of ADMS 3.3 to determine which parameters that the model is most sensitive to. In addition, it was aimed to explore if the model was most sensitive to adjusting the pollutant size and aggregation parameters as discussed in other studies (Wheeler *et al.*, 2001) or if other parameters such as source definition (i.e. point or area source) as implied by the results of the preliminary dispersion modelling were just as important. The results showed that, in general, the model output for the parameterisation of different parameters did not differ for a point or area source.

In conclusion, the model was not sensitive to a larger number of parameters than the number of parameters it was sensitive to. Some of the key parameters that the model was sensitive to included source height (change by x10 and x100), source velocity (change by x100), particle diameter and wind speed (change by x100). In contrast, the model was not sensitive to a number of key parameters including particle mass fraction

(denoting aggregation), particle density, gas deposition velocity (change by x10), relative humidity and surface temperature.

Previous studies have discussed the possibility of bioaerosols emitted from composting facilities forming aggregates that might result in overall particle sizes exhibiting non-gaseous behaviour (Wheeler *et al.*, 2001; Swan *et al.*, 2002). In line with this, the results of the sensitivity analysis showed that the model was not sensitive to changes in particle mass fraction which was assumed to denote defining the particulate pollutant as an aggregate. However the model was found to be somewhat sensitive to definition of a particulate pollutant size. The method developed for the sensivity analysis was based on varying one parameter at a time hence the particle pollutant size and density was kept constant whilst the particle mass fraction was changed. Hence this might also explain why the model was not sensitive to mass fraction but somewhat sensitive to pollutant size. However the results of the controlled experiments and site work (Chapters 4 and 5) showed that despite the evidence of aggregation, the majority of the bioaerosols emitted from compost were in single cells. Hence even though the model is sensitive to the definition of particle pollutant size, there was no conclusive evidence to re-define bioaerosols to be modelled as particulates. Therefore in line with previous studies (Drew et al., 2006; Taha *et al.*, 2007a), the principle that bioaerosol cells are of a sufficiently small size to justify the assumption of them acting as gaseous pollutants remains.

The next chapter (Chapter 7) discusses the further assessment of the potential of ADMS 3.3 in determining the concentrations of bioaerosols emitted from composting facilities. In addition, the next chapter also aims to present and discuss the key conclusions for the overall air dispersion modelling studies.

7. POTENTIAL OF ADMS 3.3 TO PREDICT BIOAEROSOL CONCENTRATIONS AT A COMPOSTING FACILITY

7.1. INTRODUCTION

The preliminary air dispersion modelling completed at Keenan Recycling (Chapter 6) showed that modelling of bioaerosol sources at an open windrow site, resulted in a difference of up to 4-log between measured and predicted bioaerosol concentrations for point and area sources. The results of the preliminary dispersion modelling studies indicated that the definition of the source term (i.e. point or area source) may have a significant effect on the output concentrations predicted by the model.

Therefore, a sensitivity analysis of the model was completed to determine which parameters that the model is most sensitive to. In addition, it was aimed to explore if the model was sensitive to adjusting the pollutant size and aggregation parameters as discussed in other studies (Wheeler *et al.*, 2001) or if other parameters such as source definition are just as important. The results showed that, in general, the model output for the parameterisation of different parameters did not differ for a point or area source. In addition, in general, the model was sensitive to only a limited number of parameters.

The results of the sensitivity analysis also showed that the model was not sensitive to defining the particulate pollutant as an aggregate however was somewhat sensitive to the particulate pollutant size parameter. However the results presented in the previous chapters (Chapters 4 and 5) have showed that despite the evidence of aggregation, the majority of the bioaerosols emitted from compost were in single cells. Hence even though the model is sensitive to the definition of particle pollutant size, it was felt that there was no conclusive evidence to re-define bioaerosols to be modelled as particulates.

The preliminary air dispersion modelling studies presented and discussed in Chapter 6 also indicated that the bioaerosol emission rate was a factor which affected the ability of the model to successfully predict pollutant concentrations. Therefore site work was undertaken with the objective to analyse the effect of source definition further and to

collect bioaerosol concentration and emission data from different sources and downwind locations at another composting facility. The initial site work completed previously at Keenan Recycling had various limitations including the lack of multiple downwind bioaerosol concentrations to compare with the bioaerosol concentrations predicted by the model and a lack of an emission rate for some bioaerosol sources due to practical bioaerosol sampling difficulties. Therefore the studies completed in this chapter were designed to address some of the limitations encountered during the previous site work.

Firstly the methodology that was adopted is explained including details of the bioaerosol sampling and air dispersion modelling studies. Following this, the culturing results of the bioaerosol concentrations detected on site are compared with the results of the air dispersion modelling for varying scenarios and discussed. Finally, the key conclusions for all air dispersion modelling studies (Chapter 6 and this chapter) are presented and recommendations are made on the best use of the model in predicting bioaerosol concentrations at a composting site.

7.2. METHODOLOGY

7.2.1. Bioaerosol Sampling

The details of the bioaerosol sampling completed at Donarbon Limited including site description, sampling methodology, expression of results and the health, safety and quality control precautions were discussed previously (Chapter 3, Section 3.2.2). However, it is important to separately discuss the design of the sampling methodology to enable generation of data in support of the model evaluation. The site work completed at Keenan Recycling was aimed to complete a preliminary assessment of the ability of a commercial air dispersion model, ADMS 3.3, in predicting bioaerosol emissions from a composting facility compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling. However a series of limitations were encountered throughout the initial site work visits at Keenan Recycling as discussed previously (Chapter 6) including:

- the lack of multiple downwind sampling points;
- the likelihood that the bioaerosol concentrations at the downwind location to which the model predictions are compared to not solely representing the bioaerosol sources that were used for determining the bioaerosol emission rates; and
- the lack of an emission rate determined for a majority of the composting windrows.

Therefore, the site work discussed in this chapter was designed to generate specific data to further improve the understanding of the ADMS 3.3 model in predicting downwind bioaerosol concentrations. The composting site, Donarbon Limited, was evaluated beforehand to locate the most suitable location to complete such experiments in light of the above limitations. The kerbside collected waste compost windrow maturation area with adjacent compost agitation activity was therefore deemed as this location. This is because at the time of sampling, this area was not surrounded by other possible sources of bioaerosols, allowed the researcher to determine an emission rate from both compost windrows and agitation activities and to take multiple downwind samples.

During the first and second sampling days, the prevailing wind direction was north easterly and the downwind sampling points were evaluated according to this. However, during the third sampling day, the prevailing wind direction was in the opposite direction hence the downwind sampling points were not the same as those for the first and second experiments.

In addition, it was not possible to take downwind bioaerosol samples at a distance of further than 100m downwind due to the constraints presented by the site geography. It was also considered important to collect downwind concentration data at distances close to the emission source to reduce the effect of other bioaerosol sources on the downwind concentrations. In the light of these, the sampling points for each sampling day are shown in the schematic (Figure 7.1).

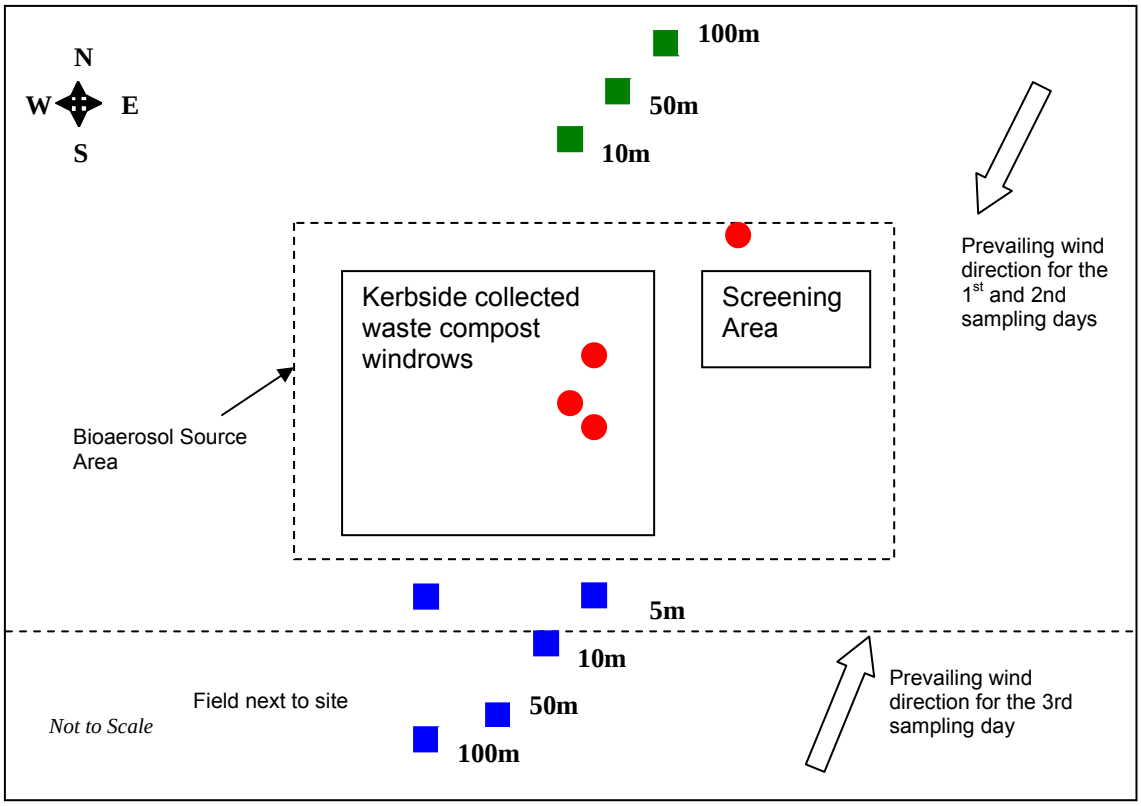


Figure 7.1. Layout of the sampling completed at Donarbon Limited Site.

Key:

Symbol	Meaning of Symbol
●	***bioaerosol samples taken at source***
■	***downwind bioaerosol sampling points for the 1st and 2nd sampling days***
■	***bioaerosol sampling points for the 3rd sampling day***

Note: It was not possible to obtain actinomycetes concentration data for the third sampling day at 5m downwind from bioaerosol source

7.2.2. Air Dispersion Modelling

Following the collection of source and downwind bioaerosol concentration data, air dispersion modelling was completed using the air dispersion model ADMS 3.3.

The compost source term static emissions were modelled as area sources as in previous studies (Taha *et al.*, 2005; Taha *et al.*, 2007a) and as per the preliminary dispersion modelling completed in the previous chapter (Chapter 6). These emissions

were determined by taking bioaerosol samples collected using a wind tunnel placed on a compost windrow (Figure 7.2).

Figure 7.2. Static source bioaerosol sampling by wind tunnel

The air velocity inside the wind tunnel, the specific bioaerosol emission rate (SBER) and the specific bioaerosol emission rate corresponding to ground level air velocity were calculated using equations 6.2, 6.3 and 6.4 as described previously (Chapter 6).

The agitation activity of screening captured at Donarbon Limited was modelled as a point source as described in the previous chapter (Section 6.3.1). The bioaerosol emission rate was estimated by performing the back-extrapolation using ADMS 3.3 as previously described in Section 6.3.1 and was based on the known bioaerosol concentrations measured at 15m downwind of the agitation activity.

Agitation activities were observed for the first two sampling days however, it was not possible to capture agitation activity bioaerosol concentrations on these dates due to unforeseen changes in site practices on the day. Hence the emission rate calculated from the data gathered from the third sampling day was used for modelling of the data from the previous two experiments.

The results presented in Chapters 4, 5 and 6 indicated that there no conclusive evidence to re-define bioaerosols to be modelled as particulates. Therefore, similar to

the preliminary air dispersion modelling completed at Keenan Recycling, the bioaerosol pollutant was assumed to be gas and no deposition velocity or washout coefficient was defined (i.e. no model option). As per the preliminary modelling completed at Keenan Recycling, the parameters used for the source (e.g. source height, diameter, geometry or temperature) represented the measurements taken on site. The results of the sensitivity analysis also showed that the model was sensitive to meteorological parameters such as wind speed and surface temperature however not at values likely to occur in a composting facility. Therefore stability class D (neutral conditions) (Pasquill, 1961) of the ADMS 3.3 file R91A-G was used for all modelling exercises representing the most frequently occurring atmospheric state in the UK (Colls, 2002). Finally, the same simplifying assumptions as listed in Chapter 6 (Section 6.3.1) were made. Based on these, the bioaerosol emission rates and a list other modelling parameters used for the Donarbon Limited modelling studies are presented in Appendix I.

Previous air dispersion modelling subsequent to the preliminary site work at Keenan Recycling was completed to test the ability of ADMS 3.3 in predicting downwind bioaerosol concentrations from various sources at a composting site to assess the potential of the model for use in support of regulatory risk assessments. To this end, data collected during the preliminary site work were assumed to represent 'typical' bioaerosol regulatory risk assessment data that might be collected at a compost site. However, since the site work discussed in this chapter are aimed to analyse the effect of source definition further, it was decided to test different scenarios to determine the effect of these scenarios in the ability of the model to predict downwind bioaerosol concentrations.

Therefore the aim of testing the first two scenarios was to determine if modelling three different compost windrow locations as a separate source term is better at predicting measured downwind concentrations compared to modelling three different compost windrows as one single source term. The kerbside composting windrows at Donarbon that were used consisted of compost of different ages which might result in differing bioaerosol emissions from each windrow, however the resulting downwind bioaerosol emission would be emitted from a combination of these windrows as a source area.

In addition to this, two different scenarios were tested when calculating the agitation activity emission rate. The first scenario assumed the temperature of the material being agitated to be equal to ambient temperature. However, the temperature range needed to optimise the rate of biodegradation of waste material inside a compost windrow are between 45-55^{o}C (Stutzenberger, F.T., 1970; Kane and Mullins, 1973; Stentiford, 1996) with temperatures rising up to 70-80^{o}C (Lacey *et al.*, 1996). Therefore, when a compost windrow is subjected to activities such as turning or screening, the agitation of the material inside the compost windrow which is at an elevated temperature might give rise to the release of hot material and air. This might cause an increase in heat flow rate on the surface of a windrow (Swan *et al.*, 2003). Therefore the second scenario assumed the temperature of the material being agitated to be 55^{0}C and two different emission rates were calculated and used for the initial air dispersion modelling. The scenarios tested for the air dispersion modelling are as described in Table 7.1.

Table 7.1 – ADMS 3.3 Site Modelling Scenarios

Scenario	Name of Scenario	Description of Scenario
1	Multiple Source	Modelling wind tunnel bioaerosol emissions from three different compost windrows locations as 3 separate sources
2	Single Source	Modelling an average (arithmetic mean) for the wind tunnel bioaerosol emissions as one single source
3	Ambient Temperature	Modelling a combination of wind tunnel and agitation activities. This scenario best represents the conditions observed on site. The agitation activity source temperature is equal to that of ambient temperature
4	High Temperature	Modelling a combination of wind tunnel and agitation activities. This scenario best represents the experiment conditions observed on site. The agitation activity source temperature is 55^{o}C.

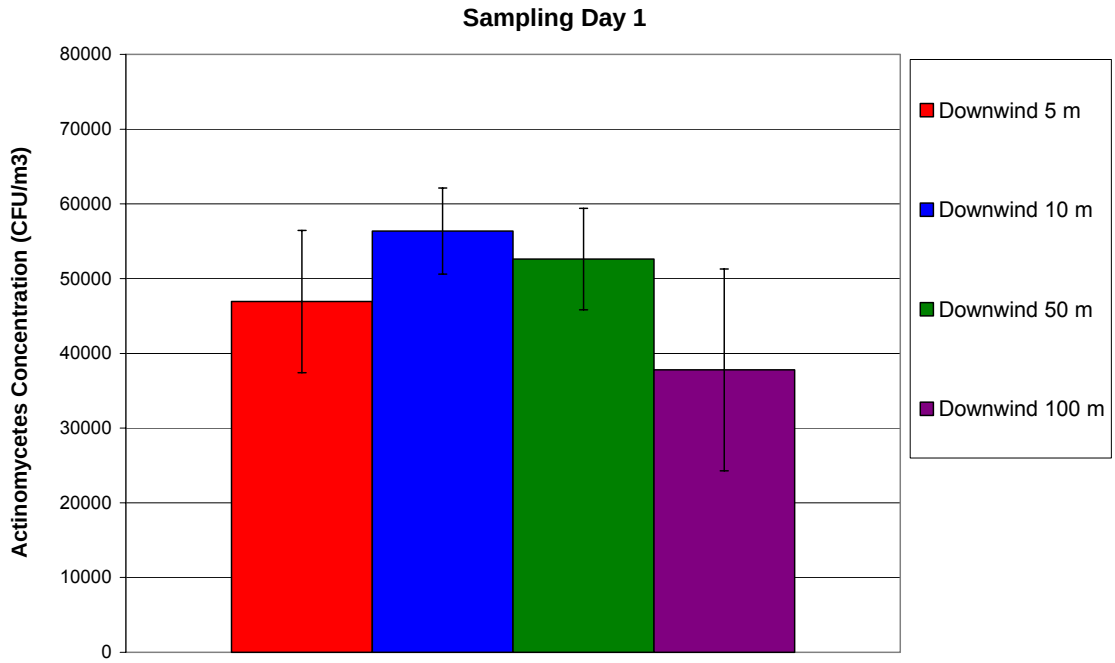
A quantitative analysis was completed following this study which compared the modelling outputs from different scenarios as described previously in Section 6.3.1 (Chapter 6).

The preliminary air dispersion modelling site work discussed in Chapter 6 revealed that bioaerosol emission rate might be an important factor in the likelihood of ADMS 3.3 in predicting of bioaerosol concentrations. Therefore, as a final study the effect of different emission rates on model output were tested to attempt to match the predicted downwind bioaerosol concentrations to those measured on site.

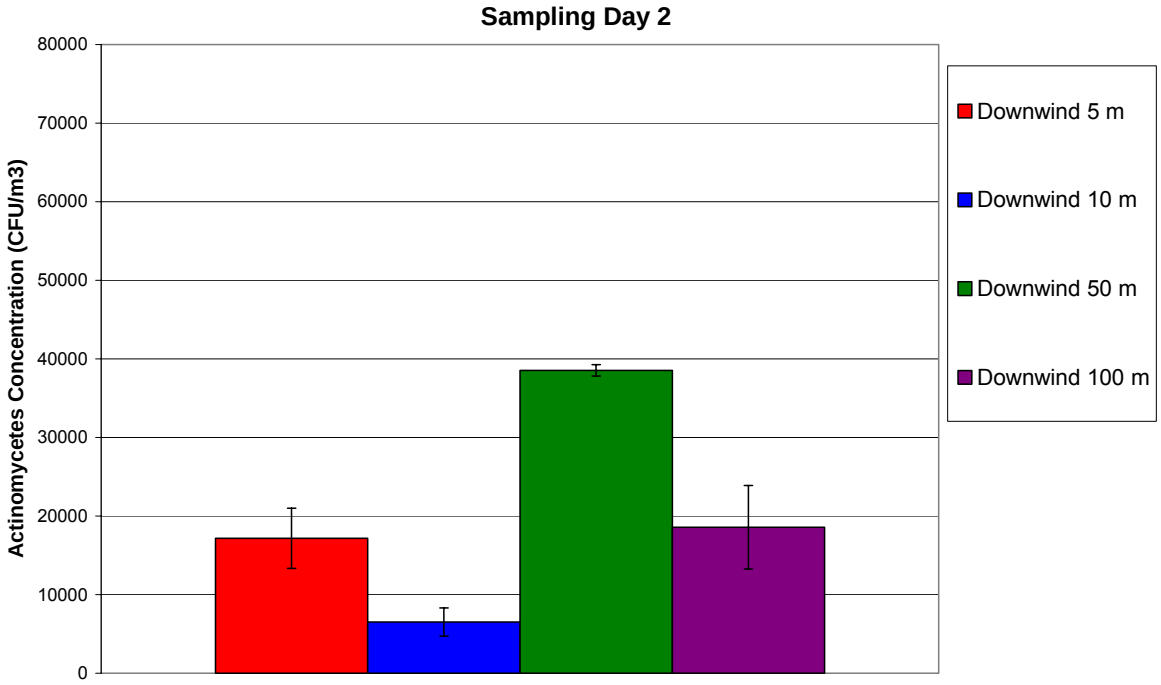
7.3. RESULTS

7.3.1. Downwind Bioaerosol Concentrations

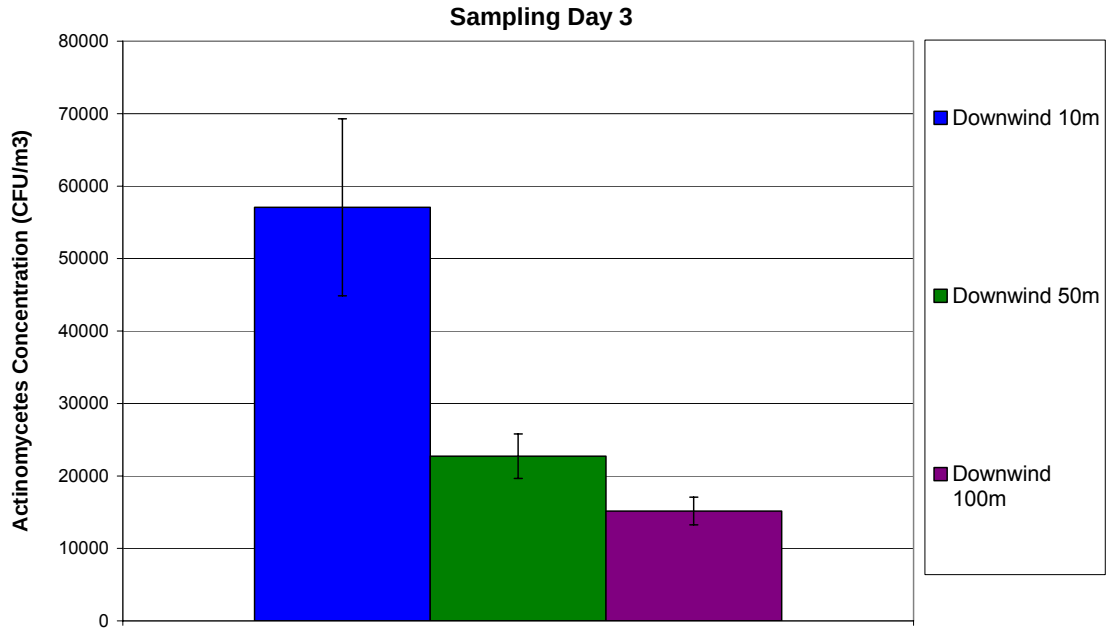
It was not possible to culture *Aspergillus fumigatus* from the site work carried out at Donarbon Limited hence concentrations for actinomycetes only are presented and modelled. The actinomycetes concentrations detected downwind from the bioaerosol source (i.e. compost windrow or agitation activity) for all sampling dates are presented in Figure 7.3.



(a)



(b)



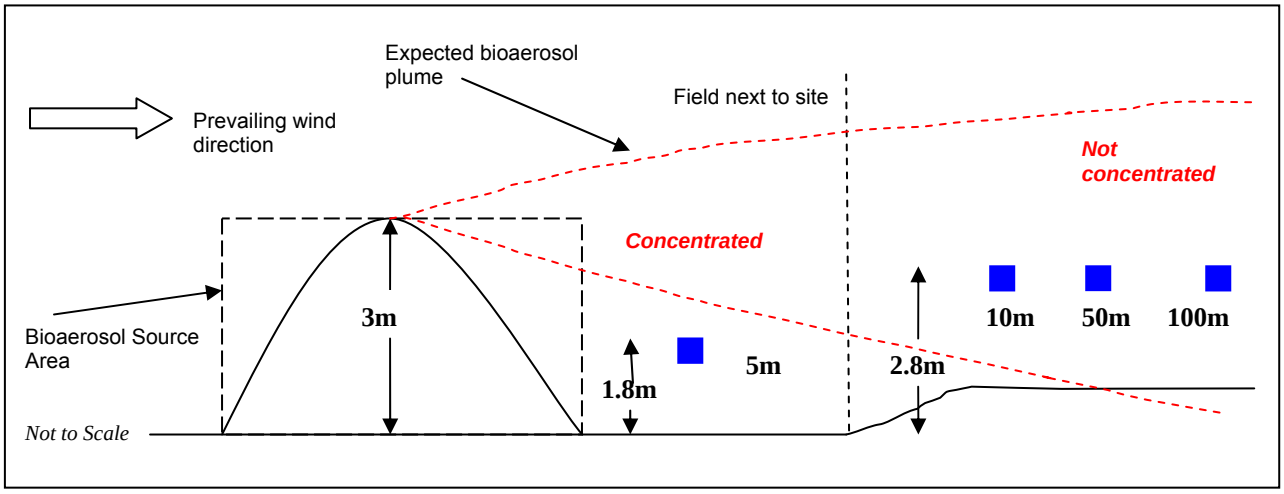
(c)

Figure 7.3- Culture results for downwind actinomycetes concentrations for all site work completed at Donarbon Limited (a)sampling day 1; (b) sampling day 2; (c) sampling day 3

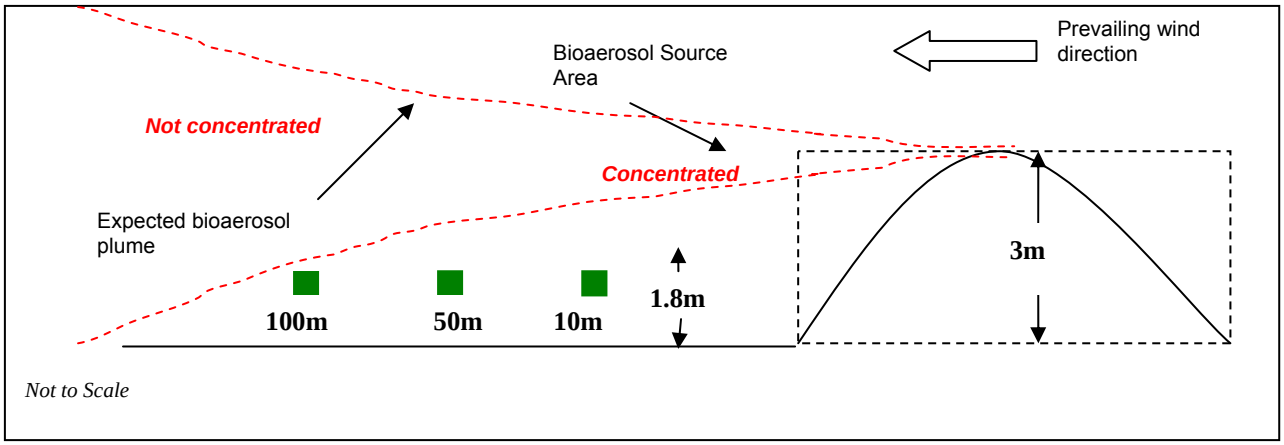
The downwind actinomycetes concentrations showed an inconsistent emission pattern for the first two sampling days. At downwind distances closer to the bioaerosol emission

source (i.e. static compost windrows and agitation activity), the bioaerosol concentrations would be expected to be the highest (i.e. at 5 m). However it would be expected for the bioaerosol concentrations tend to decline with distance and time from source, due to dispersion within the atmosphere and dilution (Composting Association, 2004; Taha *et al.*, 2006). The final sampling day showed a downwind concentration pattern which would be as expected with highest concentrations measured 10 m downwind from source and decreasing steadily.

The prevailing wind direction for the first and second sampling days were the same but in the opposite direction of that detected for the third sampling day. Hence this might account for the differences in patterns of bioaerosol emission. It is also important to note that the downwind sampling points at 10, 50 and 100m for the first and second experiments were taken at the nearby field due to site geography constraints. This field was elevated by approximately a meter compared to the site where the sampling took place. Therefore this might also have accounted for the differences in bioaerosol emission patterns as demonstrated in Figure 7.4.



(a)



(b)

Figure 7.4- Relationship between downwind sampling points and expected bioaerosol emission patterns for (a)sampling days 1 and 2 ; (b) sampling day 3.

However, for sampling days 1 and 3, the highest downwind actinomycetes concentrations were both detected at 10 m downwind from source despite differences in the prevailing wind direction. In addition to these, actinomycetes concentrations at static compost windrow source were determined as presented in Table 7.2.

Table 7.2 - Actinomycetes concentrations detected at Donarbon Limited Static Compost Windrow Bioaerosol Source

Sampling Day	Sample Location	Actinomycetes Concentrations (*cfu/m*^{3})
1	Wind Tunnel 1	86,226
	Wind Tunnel 2	53,030
	Wind Tunnel 3	52,617
2	Wind Tunnel 1	4,040
	Wind Tunnel 2	3,535
	Wind Tunnel 3	18,572
3	Wind Tunnel 1	4,293
	Wind Tunnel 2	3,283
	Wind Tunnel 3	1,894

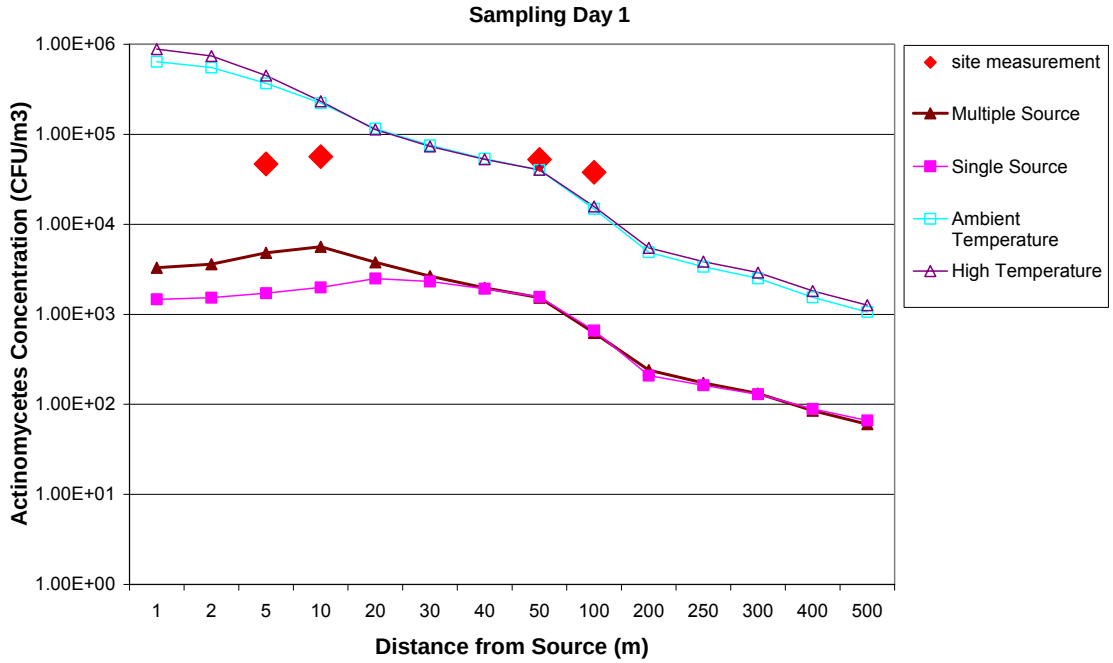
These results showed that for the second and third sampling days, the average actinomycetes concentrations were approximately 1-log lower than those detected for the first sampling day. In addition for the third sampling day, the source actinomycetes concentrations were approximately 1-log lower than those for downwind measurements in contrast to the results of the first and second sampling days.

The bioaerosol concentrations measured at a static source (compost windrow) using a wind tunnel were lower than those measured downwind. This might also indicate that a combination of bioaerosol sources (agitation and compost windrow) contribute to downwind bioaerosol concentrations as opposed to compost windrows only. In addition, for the third sampling day, the highest actinomycetes concentration was detected for the agitation activity at 148,232 cfu/m^3. This is also the highest actinomycetes concentration detected overall for all three sampling days, however it is important to remember that it was not possible to calculate this source concentration in previous experiments due to unforeseen changes in site practices on the day. This value is in the range reported by previous studies for agitation activity concentrations for bioaerosols (Wheeler *et al.*, 2001; Taha *et al.*, 2006).

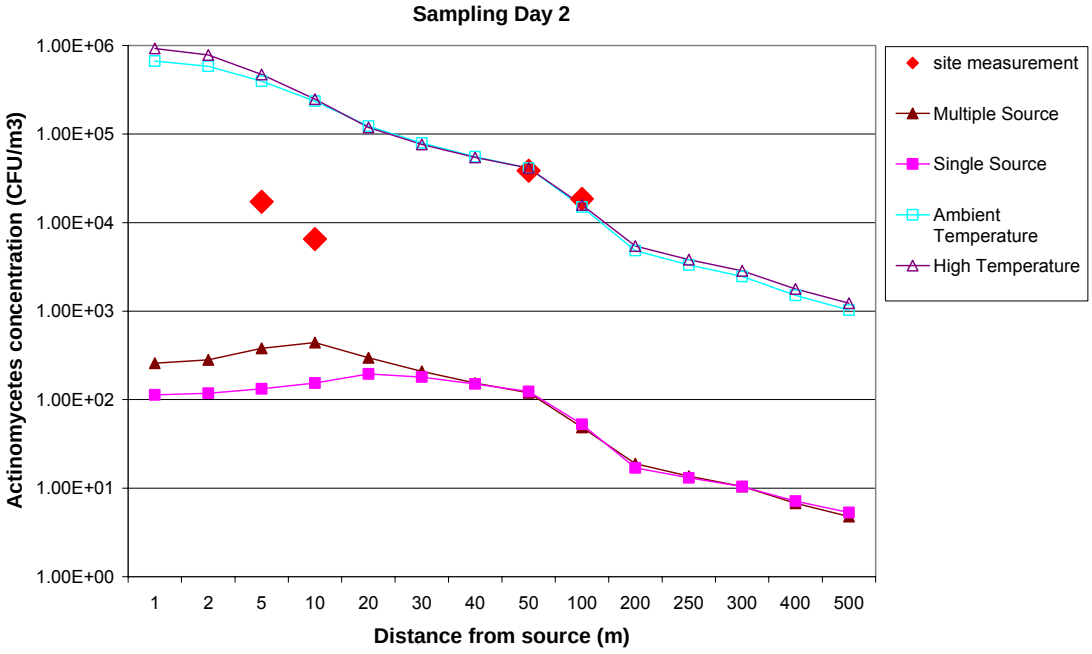
Upwind (background) concentrations measured for sampling days 2 and 3 were both the lowest overall bioaerosol concentrations detected (2,847 cfu/m^3 and 1,515 cfu/m^3 respectively). The downwind concentrations did not reduce to background measurements however the furthest downwind concentration measured was at 100m from source. Hence the downwind concentrations might have reduced to background measurements for further downwind actinomycetes sampling points however it was not possible to collect further downwind actinomycetes concentration data. This was because of practicality constraints presented by the sampling methodology and the site geography. It was also considered important to collect downwind concentration data at distances close to the emission source to reduce the effect of other bioaerosol sources on the downwind concentrations. These results are consistent with Crook *et al.* (2006) where the thermophilic actinomycetes concentrations measured at 125m downwind at various composting sites were higher than those measured for upwind (background).

7.3.2. Air Dispersion Modelling

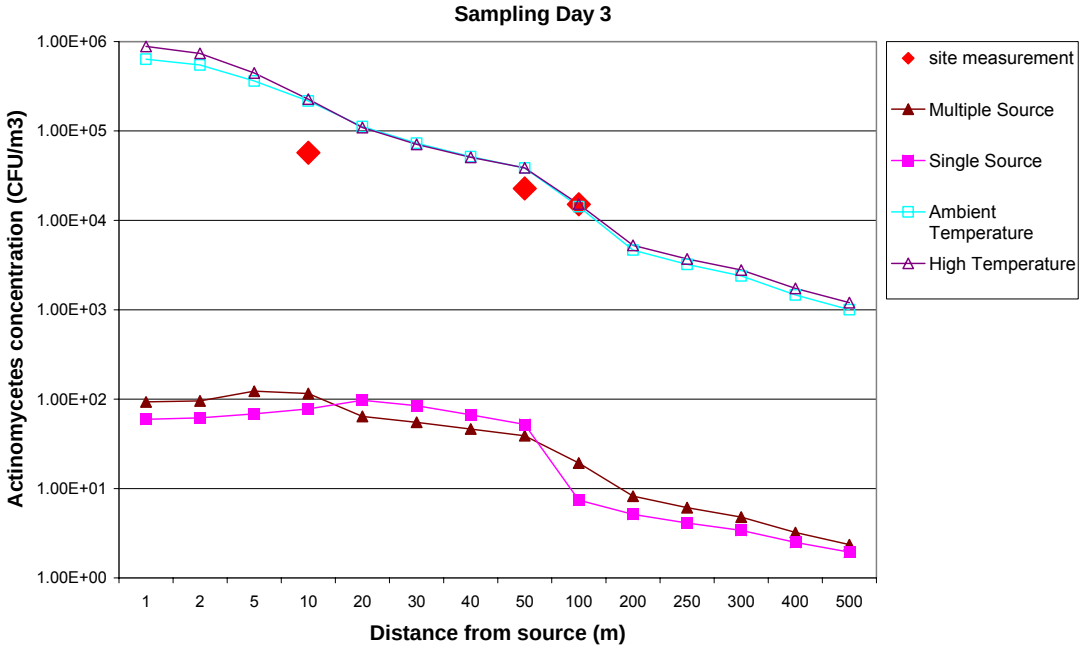
The air dispersion modelling results for all sampling days completed at Donarbon Limited are presented below (Figure 7.5).


Sampling Day 1
Actinomycetes Concentration (CFU/m3)
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1
2
5
10
20
30
40
50
100
200
250
300
400
500
Distance from Source (m)
site measurement
Multiple Source
Single Source
Ambient Temperature
High Temperature

(a)


Sampling Day 2
Actinomycetes concentration (CFU/m3)
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1
2
5
10
20
30
40
50
100
200
250
300
400
500
Distance from source (m)
site measurement
Multiple Source
Single Source
Ambient Temperature
High Temperature

(b)



(c)

Figure 7.5 –Modelling results for all site work completed at Donarbon Limited (a) sampling day 1; (b) sampling day 2; (c) sampling day 3.

The results showed that, on average, modelling wind tunnel bioaerosol concentrations as the only source term data (Scenarios 1 and 2) under predicted downwind concentrations measured on site by a factor of up to 3-log. However modelling wind tunnel bioaerosol concentrations combined with the agitation activity (Scenarios 3 and 4) provided better predictions. This was also confirmed by the results of the quantitative analysis (Table 7.3) where the ratios highlighted in yellow indicated scenarios which were successfully able to predict downwind bioaerosol concentrations when compared to values measured on site. However the emission curves as well as the results of the quantitative analysis also showed that differences between Scenarios 3 and 4 were more distinct after 10m downwind from source. Similarly for scenarios 1 and 2, differences between scenarios were more distinct after 50m downwind from source.

The quantitative analysis results (Table 7.3) showed that modelling three different compost windrow locations as separate source term data do not have any difference to modelling them as an average one single source term data. These similarities in ratios between Scenario 1 and 2 are highlighted in green. Likewise the assumption of the

agitation activity source temperature being equal to that of ambient temperature did not have a significant (with ratios numerically similar) difference to the assumption of the agitation activity source temperature being 55^{o}C and not equal to that of ambient temperature.

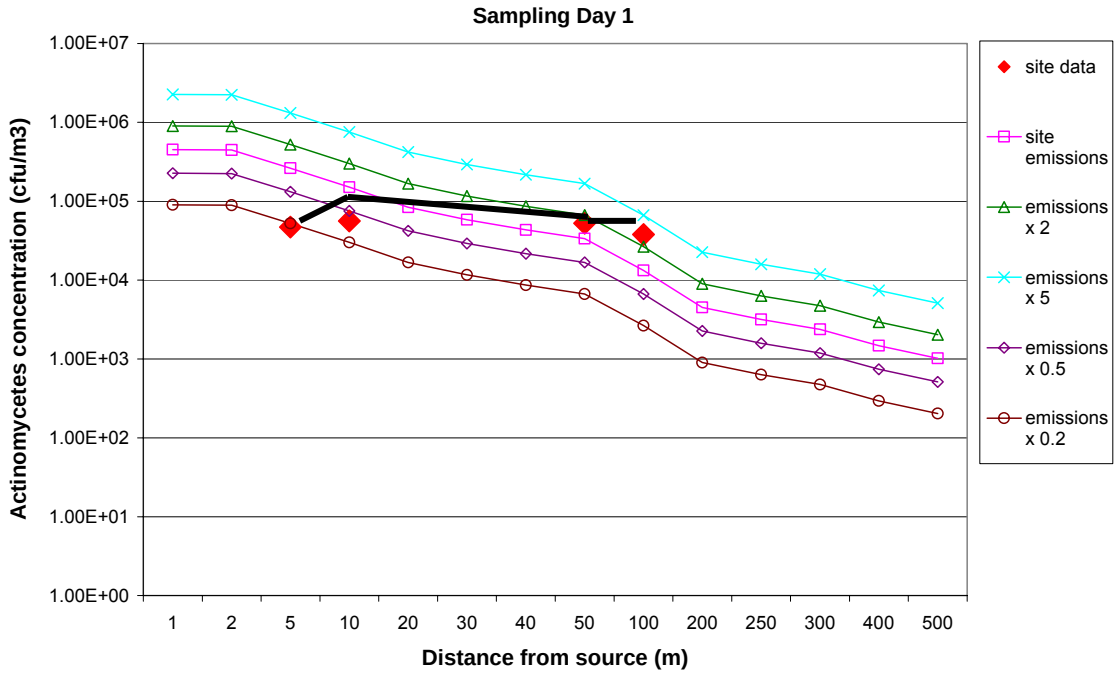
Table 7.3- Quantitative analysis of the results of the four different scenarios for initial ADMS modelling

Sampling Day	Scenario	Ratio of predicted/measured actinomycetes concentrations at downwind distances			
		5m	10m	50m	100m
1	1	0.10	0.10	0.03	0.02
	2	0.04	0.04	0.03	0.02
	3	7.83	3.94	0.76	0.39
	4	9.59	4.13	0.77	0.42
2	1	0.02	0.07	0.00	0.00
	2	0.01	0.02	0.00	0.00
	3	23.03	36.08	1.07	0.81
	4	27.52	38.11	1.07	0.86
3	1	N/A	0.00	0.00	0.00
	2	N/A	0.00	0.00	0.00
	3	N/A	3.79	1.69	0.94
	4	N/A	3.98	1.70	1.00

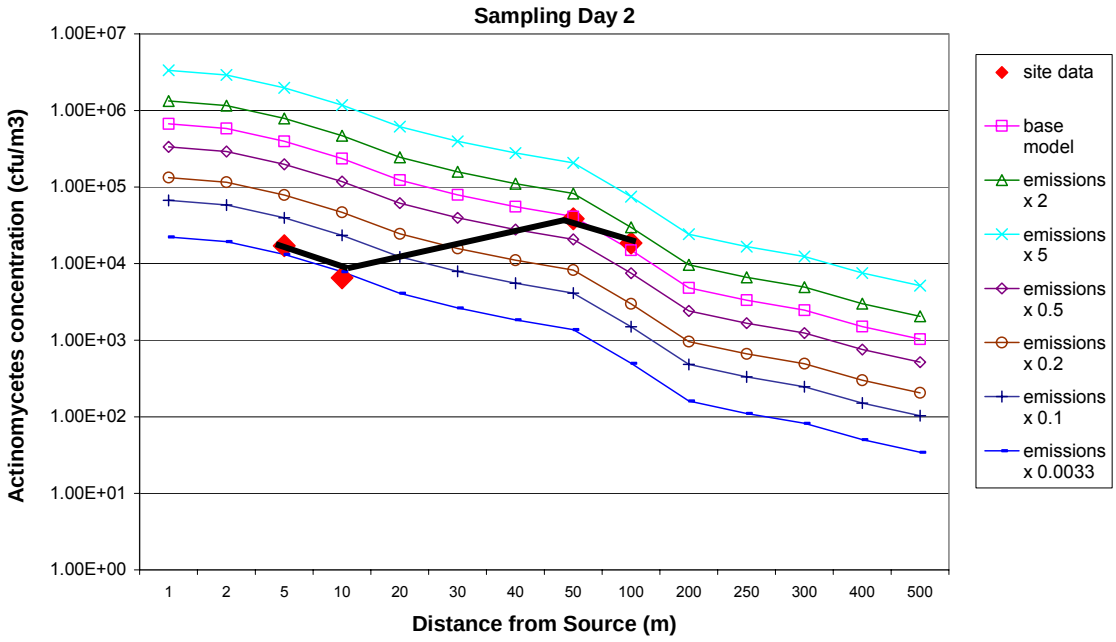
Note: The ratios highlighted in yellow denote those within the arbitrary 20% range of 1.00

7.3.3. Effect of Emission Rates on Downwind Concentrations

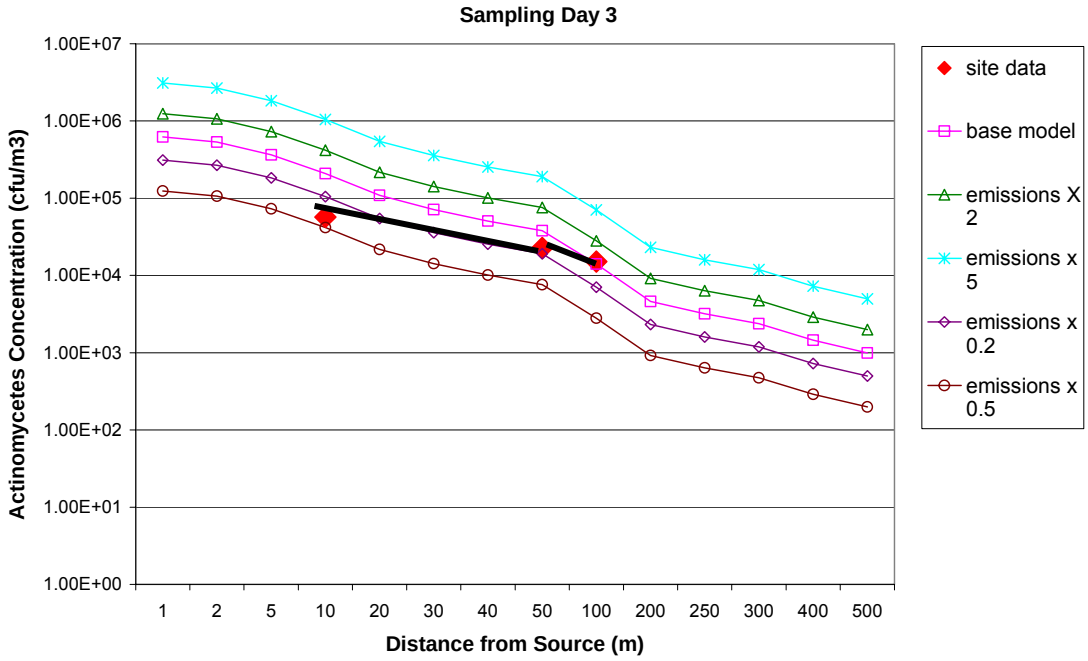
The effect of changing the bioaerosol emission rates for static (compost windrow) and agitation source term data was also explored. The aim of this exercise was to determine the approximate factor by which the emission rates need to be changed to match the downwind actinomycetes concentrations measured on site. The results of these are presented below (Figure 7.6).


Sampling Day 1
Actinomycetes concentration (cfu/m3)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1
2
5
10
20
30
40
50
100
200
250
300
400
500
Distance from source (m)
site data
site emissions
emissions x 2
emissions x 5
emissions x 0.5
emissions x 0.2

(a)


Sampling Day 2
Actinomycetes concentration (cfu/m3)
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
1
2
5
10
20
30
40
50
100
200
250
300
400
500
Distance from Source (m)
site data
base model
emissions x 2
emissions x 5
emissions x 0.5
emissions x 0.2
emissions x 0.1
emissions x 0.0033

(b)



(c)

Figure 7.6 – The effect of emission rate on the prediction of downwind bioaerosol concentrations for all site work completed at Donarbon Limited (a) sampling day 1; (b) sampling day 2; (c) sampling day 3. The bold lines show the dispersal curves derived from the actinomycetes concentrations measured on site. The non-bold lines show the dispersal curves predicted by the model. The y axis is in logarithmic scale.

A comparison of the bold and non-bold lines show that the model is not successfully able to replicate the dispersal curves for downwind actinomycetes concentrations. Therefore the results show that by changing the emission rate by a known factor, it was possible to match the modelled downwind bioaerosol concentrations to those measured on site however only for one downwind bioaerosol concentration point at a time. The following table (Table 7.4) explores this argument further in which the factors that are needed to multiply the emission rates are listed to match the predicted and measured downwind actinomycetes concentrations. An ideal multiplication factor would be the same for all downwind distances.

Table 7.4 - Emission rate multiplication factors needed to match predicted and measured actinomycetes concentrations

Sampling Day	Approximate factor for downwind distances			
	5m	**10m**	**50m**	**100m**
1	0.2	0.5	2	2
2	0.03	0.03	None*	None*
3	N/A	0.5	0.2	None*

*Note: *This indicates that the base model matches the actinomycetes concentration measured on site*

For sampling day one, the calculated actinomycetes emission rate would need to be reduced by a factor of 0.2 to match the predicted and measured downwind concentrations at 5m downwind. The emission rate would need to be reduced by half to match the predicted and measured downwind concentrations at 10m however would need to be doubled to match the predicted and measured downwind concentrations at 50m and 100m. For the second sampling day, the model was successfully able to predict downwind actinomycetes concentrations measured at 50m and 100m downwind. However the actinomycetes emission rate would need to be reduced by a factor of 0.033 to match the predicted and measured concentrations at 5m and 10m.

For the third sampling day, the model was successfully able to predict downwind actinomycetes concentrations measured at 100m downwind. However the actinomycetes emission rate would need to be reduced by factors of 0.5 and 0.2 to match the predicted and measured concentrations at 10m and 50m respectively. It was not possible to measure the actinomycetes concentrations at 5m for the third sampling day. Therefore, in conclusion, it was not possible to match the predicted actinomycetes concentrations to those measured on site by changing the actinomycetes emission rate by the same factor for all downwind distances for any experiment.

The results of further quantitative analysis (Appendix J, Table 1) revealed that for all sampling days, when emission rates are changed by a certain factor, the ratio of actinomycetes concentration of adjusted to a base model also changes by that certain factor for all downwind distances that were examined. This is because the emission rate (rate of discharge of the pollutant) is directly proportional to the concentration distribution of the pollutant as shown in Equation 6.1 (Section 6.2). As such, this was

different from the results of the sensitivity analysis completed for source term, meteorological and pollutant parameters as presented previously (Chapter 6) in which the factors by which parameters were changed did not match the factors by which the output concentrations changed.

7.4. DISCUSSION

7.4.1. Modelling of Bioaerosol Emissions at Donarbon Limited

Pollutants such as odours (McIntyre, 2000; Sarkar *et al*., 2005; Sheridan *et al.*, 2004) and fine particulates that may have similar properties to those of bioaerosols have previously been successfully modelled using air dispersion models (Silibello *et al.*, 2008). In addition models have been developed to examine the dispersion of bioaerosols emitted from agricultural operations (Lighthart, 1984) and spread of biosolids (Dowd *et al.*, 2000).

However, the use of air dispersion models for bioaerosol emissions from composting facilities is relatively new. Several previous studies (Millner *et al.*, 1980; Wheeler *et al.*, 2001; ADAS/SWICEB, 2005; Drew *et al.*, 2005; Taha *et al.*, 2006) have tested the applicability of Gaussian air dispersion models for use in modelling of bioaerosols from composting facilities. More recent studies (Drew *et al.*, 2006; Taha *et al.*, 2007a) have examined the applicability of ADMS 3.3 in predicting dispersion of bioaerosols emitted from composting facilities. However these studies have not made a comparison of predicted versus measured downwind concentrations of bioaerosols.

Therefore, preliminary site work was completed at an open windrow composting site to improve the understanding of the applicability of ADMS 3.3 for modelling dispersion of bioaerosols emitted from composting sites (Chapter 6; SEPA/SNIFFER, 2007; Tamer Vestlund *et al.*, 2007). The results showed that the model was not consistent in predicting downwind bioaerosol concentrations across different sampling days and showed a wide variety of bioaerosol concentrations and emission curves predicted by the model depending on how the bioaerosol source was defined.

Previous air dispersion modelling studies of bioaerosols from composting sources used the principle that bioaerosol spores were of a sufficiently small size to justify the assumption of them acting as gaseous pollutants (Drew et al., 2006; Taha *et al.*, 2007a). However, bioaerosols emitted from composting facilities might form aggregates that would result in overall particle sizes which would exhibit non-gaseous behaviour (Wheeler *et al.*, 2001; Swan *et al.*, 2002). Previous studies have shown that a larger particle such as an aggregate might be subject to higher deposition velocities than those for smaller particles (Wheeler *et al.*, 2001; Swan *et al.*, 2003). This might suggest that on release from compost, a bioaerosol aggregate is more likely to settle out downwind of a bioaerosol source instead of remaining suspended in air (i.e. non-gaseous behaviour) (Pillai and Ricke, 2002). The results of the model sensitivity analysis (Chapter 6) showed that the model is somewhat sensitive to the definition of particle pollutant size. However the controlled experiments and site work discussed previously (Chapters 4 and 5) showed that the majority of bioaerosols emitted from compost were in single cell units despite the evidence of bioaerosol aggregation. Therefore since the results show that the majority of the bioaerosols released from compost were in single cell units, this indicates that the majority of these bioaerosols might travel longer distances downwind of source compared to a situation where the majority of released bioaerosols were in aggregates.

These results (Chapter 4 and 5) also showed that the majority of these bioaerosols had an aspect ratio of 1 or 1.4-1.5. For non-spherically shaped spores, an increase in surface drag might result in a delay in deposition (Lacey, 1991; McCartney, 1994; Levetin, 1995). Therefore these results suggest that the majority of bioaerosols emitted from compost were less likely to disperse for longer distances because there will not be any such delays in deposition. In addition the results also suggested that aggregate structures drop out of the pollutant plume by 100m downwind from source. As such, there was no conclusive evidence that showed a need to define bioaerosols as particles rather than gases.

The preliminary air dispersion modelling studies presented and discussed in Chapter 6 also indicated that the bioaerosol emission rate was a factor which affected the ability of

the model to successfully predict pollutant concentrations. Therefore to explore the importance of bioaerosol emission rate and to further improve the understanding of the model, a set of site work were completed to collect bioaerosol emission data from bioaerosol sources. Subsequently further air dispersion modelling studies were completed to test the effect on predicted versus measured concentrations. This work was similar to the air dispersion modelling studies discussed in Chapter 6 and was based on the same simplifying assumptions.

The results of these studies (at Donarbon Limited) were in line with the preliminary air dispersion modelling completed for Keenan Recycling and showed that:

- Modelling the emissions from compost windrows as the only source of bioaerosols under predicted measured actinomycetes downwind concentrations by up to 3-log whether the individual compost windrow concentrations were modelled as single sources or combined as one source.
- When the compost windrow bioaerosol emissions and the agitation activity are modelled together, the model was more successful at predicting measured downwind concentrations and was able to match the downwind bioaerosol concentrations at some of the downwind sampling points.

Transport of bioaerosol particles is affected by thermal gradients where they would be expected to move from higher to lower temperatures (Pillai and Ricke, 2002). On this principle, it might be expected that the downwind concentrations of bioaerosols at a composting site would be significantly different in different scenarios which assume the source temperature to be equal to that of ambient or higher. However, the model output suggests that the model is not able to reflect this as the results were very similar whether the agitation source temperature is assumed to be equal to ambient temperature or equal to 55^0C. However, to calculate the starting emission rate for an agitation activity, source temperature had to be taken into account and the calculated emission rates were different for the two different scenarios. Hence the effects of thermal gradients might be reflected in how the model is used to estimate the bioaerosol emission rate.

The ADMS 3.3 depletion curves for all sampling days showed a steady decline from source to downwind. However, air flow patterns in outdoor environments are rarely laminar or constant (Pillai and Ricke, 2002) which is likely to be the reason why an inconsistent pattern of decline of actinomycetes concentrations were observed in the culture results for the first two sampling days. Unpredictable weather conditions at a composting facility on the day of sampling are one of the problems posed by sampling for bioaerosols at a composting site. In line with this, for all sampling days, slight changes in wind direction were observed throughout the day. Other studies are in line with this where ADAS/SWICEB (2005) report that the pattern of bioaerosol concentration downwind from source varied considerably on a sampling day. However, in general, an expected pattern of high levels at 25m downwind, lower levels at 75m downwind and background levels at 125m downwind from the boundary of the composting facility was observed. In contrast Crook *et al.* (2006) found an inconsistent pattern of decline in bioaerosol concentrations for downwind locations and argued that this might be due to unpredictable influences on bioaerosol concentrations such as wind current patterns, particle size distributions and variations in site topography (Colls, 2002) and elevation. Hence, it would be very difficult for a commercial air dispersion model to account for such changes in a sampling day.

Other reasons for the inconsistent pattern of decline of actinomycetes concentrations in the culture results for the first two sampling days may be as follows. The 10m, 50m and 100m downwind actinomycetes concentrations for the first two sampling days were taken at the field next to the Donarbon Limited site (Figure 5.4) however in the last experiment all downwind concentrations were collected on site due to changes in the prevailing wind direction for different sampling days. These downwind locations measured during the first two sampling days were on a slope compared to the downwind locations measured during the last experiment.

Finally, the presence of other bioaerosol sources such as those naturally occurring in the environment (Borodulin, 2005; Bovallius *et al.*, 1978; Köck *et al.*, 1998; Fang *et al.*, 2005; Mancinelli and Shulls, 1978; Spicer and Gangloff, 2005; Swan *et al.,* 2002) might

have added complications to the bioaerosol concentrations measured downwind from source.

In conclusion, the results of the air dispersion modelling from three different site visits showed that the model is not able to match the overall emission curves that were measured on site. However it is also possible that the inherent difficulties of bioaerosol sampling practices at a composting site also make it difficult to collect valid data to which the model output can be compared.

7.4.2. Bioaerosol Emission Rates

Calculation of bioaerosol emission rates as an input into air dispersion models has been recognized as a main challenge in air dispersion modelling studies (Wheeler *et al.*, 2001) and the air dispersion models are only as good as the data input to the model. Fixed emission rates are used for modelling studies and in reality, the emission rate might be inconsistent because of the changes in the source throughout the sampling day (Gostelow *et al.*, 2001). In addition, there are no direct methods to measure bioaerosol emission from point sources such as agitation activities at present and the emission rates are based on back-extrapolation of downwind bioaerosol concentrations (Millner *et al.*, 1980; Dannaberg *et al.*, 1997; Swan *et al.*, 2002; Taha et al., 2006). Therefore a final study was completed to examine the effect of changing different emission rates on the predicted downwind bioaerosol concentrations.

The results of this study showed that for all sampling days, when emission rates are changed by a certain factor, the ratio of actinomycetes concentration of adjusted to the base model also changes by that certain factor. This is in contrast to the results of the previous sensitivity analysis studies for source, pollutant and meteorological parameters (Chapter 6) in which the factors by which parameters were changed did not match the factors by which the output concentrations changed. The model defines a pollutant by its mass emission rate (i.e. plume) (CERC, 2003) which indicates that the definition of the emission rate is a parameter directly influencing the model output. Hence this might be the reason for these results.

In addition to this, a comparison of the downwind depletion curves for culture based measured concentrations and those for predicted concentrations showed differences between the two. Hence in conclusion, by changing the emission rate by a known factor, it was possible to match some of the modelled downwind bioaerosol concentrations to those measured on site. However such a match was possible only for one downwind bioaerosol concentration point at a time and it was not possible to match the overall emission curves.

7.4.3. Limitations of Modelling

The results of the studies discussed in this and the previous chapter (Chapter 6) showed that there are many limitations to air dispersion modelling which should be considered when analysing the results of air dispersion modelling studies.

Firstly, the results suggest that ADMS 3.3 was not capable of dealing with changes in site measurements from one sampling day to another. This is in line with Carruthers (1998) who has shown that in Gaussian models, if the weather conditions in a day are highly variable, then this might have problems in terms of the ability of the model to predict pollutant concentrations. However there are also problems when sampling for bioaerosols at a composting site (previously mentioned in Section 6.1 of Chapter 6) which may result in the data collected by bioaerosol sampling not representing what is really happening at the composting site. In addition, in a compost site, there are likely to be a wide range of variations between different sampling days in factors such as weather conditions, prevailing wind direction or site practices. Hence when comparing results of measured versus modelled concentrations, the capability of a commercial air dispersion model in assessing the risks of bioaerosols at composting facilities should be analysed in this context.

Bioaerosols possess biological as well as physical properties. ADMS 3.3 is not designed for use on bioaerosols however the representation of the physical properties of bioaerosols (i.e. size, density, aggregation) might allow for them to be successfully modelled by ADMS 3.3. The model was somewhat sensitive to the definition of pollutant size however was not sensitive to the definition of other pollutant parameters such as

aggregation or density. In addition, the variabilities in bioaerosol occurrence due to their biological properties are not represented by ADMS 3.3. This includes properties such as spore shape or aggregate structure discussed in Chapters 4 and 5.

Other variabilities such as the variability of spore presence in the atmosphere and spore discharge have been mentioned by previous studies (Stetzenbach and Lighthart, 1994; Levetin and Horner, 2002). This variability has been attributed to diurnal rhythms of spore discharge as well as weather and seasonal effects. Another study (Levetin and Horner, 2002) has reported an increase of spore levels from 27,000 to 144,000 spores/m^3 in a matter of 2 hours in atmospheric spore levels in Tulsa. They have attributed this to increases in wind speed. Therefore an air dispersion model that aims to predict bioaerosol emissions may need added algorithms to reflect such biological variability to ensure accurate predictions of bioaerosol concentrations.

The use of Gaussian models also presents some limitations. These models have been shown to be less applicable for prediction of emissions closer to the source term, at low wind speeds and when predicting emissions in varied terrain (Environment Agency, 2004). The results presented for all modelling studies are in line with this where the prediction of bioaerosol emissions closer to source term was weak. Other limiting assumptions are made when Gaussian models such as ADMS 3.3 are used (Stetzenbach, 1997), including (Colls, 2002):

- The pollutant release is steady state.
- Timescales of hours rather than minutes are implied;
- Pollutant is chemically stable;
- Pollutant is <20 μm hence the effects of sedimentation from plume is negligible.

However as discussed within this chapter, some of these assumptions are rarely true in real life (Colls, 2002). In a composting facility, the pollutant (i.e. bioaerosols) would mostly be expected to be released in steady state (e.g. constant bioaerosol plume released from a compost windrow) however compost related bioaerosols also exhibit episodic behaviour due to site practices or daily differences (Recer *et al.*, 2001;

Sánchez-Monedero et al., 2005; Taha *et al.*, 2006; Taha *et al.*, 2007a). Secondly, the sampling duration used at Donarbon Limited was 30 min (as previously presented in Chapter 3, Section 3.2.2) which only provides a snapshot of the bioaerosol concentrations at a composting facility at a certain sampling point. This is a limitation of bioaerosol sampling completed in composting facilities. However, if in a Gaussian model timescales of hours rather than minutes are implied, this would indicate that the concentrations predicted by the model might differ than snapshot concentrations measured on site. Hence this might also account for some of the discrepancies between the predictions by the model and concentrations measured on site.

The results of the previous chapters (Chapters 4 and 5) have shown that the majority of the bioaerosols emitted from compost are smaller than <20 μm hence this limiting assumption is correct for modelling bioaerosols emitted from composting facilities. However the chemical stability of the bioaerosol as well as their properties such as inactivation are not clearly understood. These have been previously discussed in Chapter 1.

It is important to remember that air dispersion modelling is only an estimate of the behaviour of a pollutant in the atmosphere and it is not possible to capture the effects of slight changes in the atmospheric conditions throughout the sampling day (Gostelow *et al.*, 2001) which has been shown by the results presented in this chapter. McIntyre (2000) showed that air dispersion models do not provide absolute predictions of pollutants as there are too many variables in the models themselves. In addition, there are emission variations of pollutant from source and difficulties in obtaining accurate data such as those discussed within this study.

McIntyre (2000) mentions that successful agreement between measured and modelled values in odour emissions is an exception rather than the norm. Validation studies completed for ADMS for various data sets including a volume release in a field and a power plant stack in flat terrain also concluded that for the highest predicted and observed concentrations, ADMS under predicted on average by 20% (Hanna *et al.*, 1999). Therefore the tendency of the model to under predict concentrations indicates

that ADMS 3.3 has the potential to be used as a screening tool to assess relative changes in the emission of bioaerosols from the compost site, however the results should preferably be compared with the downwind bioaerosol concentrations measured on a composting site to assess the percentage of model under prediction.

7.5. CONCLUSIONS

The results of the preliminary air dispersion modelling at Keenan Recycling showed that the model was not able to consistently predict the bioaerosol concentrations downwind of a composting source. It was also shown that there were differences between the measured and predicted bioaerosol concentrations depending on the bioaerosol emission rate and how the biaoerosol source was defined (i.e. point or area). Previous studies (Wheeler *et al.*, 2001) had suggested that the incorporation of bioaerosol aggregation into such dispersion models might improve the fit of the model to concentrations measured on site. The results of the sensitivity analysis showed that the model was not sensitive to defining the particulate pollutant as an aggregate but was somewhat sensitive to the particulate pollutant size parameter. However the results presented in the previous chapters (Chapters 4 and 5) showed that despite the evidence of aggregation, the majority of the bioaerosols emitted from compost were in single cells. Therefore it was felt that there was no conclusive evidence to re-define bioaerosols to be modelled as particulates. In addition, in general, the model was sensitive to only a limited number of parameters.

There were a number of limitations identified in the preliminary air dispersion modelling studies. Therefore based on this, further studies were designed to analyse the effect of source definition and to collect bioaerosol concentration and emission data from different sources and downwind locations at another composting facility. Several scenarios were tested and the results showed that modelling only the emissions measured on the compost windrows at Donarbon Limited resulted in a difference of up to 3-log between predicted and measured concentrations (regardless of modelling individual compost windrow concentrations were modelled as single sources or combined as one source). In contrast, modelling both bioaerosol sources (compost windrow and agitation activity) at Donarbon Limited was found to be more successful at

predicting measured downwind concentrations and was able to match the downwind bioaerosol concentrations at some of the downwind sampling points.

The results of the culturing results for the studies completed at Donarbon Limited showed an inconsistent pattern of decline in downwind actinomycetes concentrations for the first two sampling days. Therefore, ADMS 3.3 was not able to successfully predict the downwind source depletion curve for these experiments. In contrast, the model was more successful at predicting the source depletion curves for sampling day three. This might indicate that the model is not capable of dealing with changes in site measurements from one sampling day to another. However the culturing results might also be indicative of the inherent difficulties of bioaerosol sampling practices at a composting site. Such difficulties make it difficult to collect valid data to which the model output can be compared. Therefore when comparing results of measured versus modelled concentrations, the capability of a commercial air dispersion model in assessing the risks of bioaerosols at composting facilities should be analysed in this context. Finally, it was shown that it was possible to match the individual measured downwind concentrations by changing the actinomycetes emission rates however it was not possible to match the overall depletion curve.

Therefore the overall results from the modelling studies indicate that ADMS 3.3 was not able to consistently predict absolute downwind bioaerosol concentrations at a composting facility. However air dispersion models have been useful (Carruthers *et al.*, 1998) for testing the effect of different emission scenarios which might be beneficial in completing regulatory risk assessment for composting facilities. Hence if an existing composting facility is planning changes to their operations, ADMS 3.3 might be a valuable tool for predicting and assessing relative changes in downwind bioaerosol concentrations resulting from different operating scenarios. In addition, air dispersion models might be the only way to explore different bioaerosol control situations and to assess bioaerosol emissions in a composting site. Therefore despite any limitations of using air dispersion models in assessing bioaerosol exposure, models such as ADMS 3.3 may provide a useful overview of emissions in a composting site (Environment Agency, 2008). Therefore further research should be employed to improving the understanding of these models for modelling downwind concentrations of bioaerosols in

a composting facility. Such research should aim to complete extensive validation work to compare the measured versus modelled bioaerosol concentrations at different composting sites.

Another area which needs extensive research in improving the prediction of bioaerosol concentrations by air dispersion models is the generation of further bioaerosol emission data. This is because air dispersion models are only as good as the data input to the model and one of the main challenges in air dispersion modelling studies is the calculation of bioaerosol emission rates as an input into air dispersion models (Wheeler *et al.*, 2001). The results of all modelling studies presented in this research (Chapters 6 and 7) and previous studies (Millner *et al.*, 1977; Millner *et al.*, 1980; Clark *et al*.,1983a; Jager *et al.*, 1994; Sánchez-Monedero *et al.*, 2005; Taha *et al.*, 2006) show that agitation activity at a composting site is likely to be the major contributor of bioaerosols emitted from composting sites. However, there are no direct methods to measure bioaerosol emission from point sources such as agitation activities at present and the emission rates are based on estimation by back-extrapolation of downwind bioaerosol concentrations (Millner *et al.*, 1980; Dannaberg *et al.*, 1997; Swan *et al.*, 2002; Taha *et al.*, 2006).

As such, it is not possible to recommend a definitive emission rate for bioaerosols emitted from composting facilities. However the variation of bioaerosol emission rates estimated for *Aspergillus fumigatus* and actinomycetes in various sites for this study are compared with the emission rates estimated by Taha (2005) in two different open windrow green waste composting facilities (Table 7.5). From this, a range of bioaerosol emission rates can be recommended to be used in future modelling exercises in the absence of any other site specific bioaerosol emission data. However, it is emphasised that any future studies should prioritise the collection of further bioaerosol emission data to validate any such existing emission rates .

Table 7.5 – Variation of bioaerosol emission rates at various open windrow green waste composting facilities

Type of bioaerosol	Range of estimated emission rate (cfu/s)	Detail of collection site
Aspergillus fumigatus	$0.75 – 4.7 \times 10^6$	Keenan Recycling (Chapter 6)
	$0.2 – 890 \times 10^6$	Site 1 (Taha *et al.*, 2005)
	$0.055 -16 \times 10^6$	Site 2 (Taha *et al.*, 2005)
Actinomycetes	$22 -135 \times 10^6$	Keenan Recycling (Chapter 6)
	5.77×10^6	Donarbon Limited (Chapter 7)
	$0.5 – 860 \times 10^6$	Site 1 (Taha *et al.*, 2005)
	$0.0048 – 11 \times 10^6$	Site 2 (Taha *et al.*, 2005)

Based on the emission rates per individual bioaerosol as presented above (Table 7.5), when modelling the dispersal of *A. fumigatus* and actinomycetes from point sources (i.e. agitation activity), 'high', 'medium' or 'low' range of emission rates may be used to represent worst case, medium or best case scenarios at a composting site. Hence for actinomycetes, a best case emission rate of 4.8×10^3 cfu/s, a worst case emission rate of 8.6×10^8 cfu/s or a medium emission rate of 14.8×10^7 cfu/s (i.e. arithmetic mean for all actinomycetes rates) may be used.

In the light of the site work and subsequent air dispersion modelling studies (Chapters 6 and 7), a set of recommendations are made for assessing the risk of bioaerosols at an open windrow compost site. It may not always be possible to collect bioaerosol sampling data at a composting site prior to attempts at air dispersion modelling especially for sites which are assessed for bioaerosol exposure prior to construction. Hence the first set of key recommendations (Table 7.6) give general advice regarding the set up of the air dispersion model in the absence of any data collected on site. However ideally, any air dispersion modelling studies should be accompanied by bioaerosol sampling data collected on site. Therefore the second set of recommendations (Table 7.7) focuses on the collection of data for input into ADMS 3.3 for assessing the downwind concentrations of bioaerosols at a composting facility. The model input parameters recommended in Table 7.6 may be used as general parameters subsequent to modelling of bioaerosol emission data collected at a composting facility. However, the model predictions (i.e. output) are recommended to be analysed in the

context of any data measured on site. In addition, the use of data collected on site specific to the site and sampling day (e.g. meteorological or source data) rather than generic data should always be preferred if possible.

Table 7.6 – Key recommendations for model set-up

•	The bioaerosol source should be classified as a point source (to represent an agitation activity) . 'High', 'medium' or 'low' range of bioaerosol emission rates (Table 7.4) may be used to represent worst case, medium or best case scenarios at a composting site
•	The pollutant should be represented as a gas pollutant with no model option unless the use of deposition or other model options are specifically required.
•	Stability Class D of ADMS 3.3 file R91A-G can be used for meteorological data

Table 7.7 – Recommendations for model input data collection

•	An upwind sample should be taken in close proximity to the site, ideally downwind of any sensitive receptors. It is important to remember that this upwind sample might be the downwind sample at another sampling day if the prevailing wind direction changes.
•	The compost site should be assessed before sampling to define the bioaerosol source area (i.e. compost windrow or agitation activity) which will be assumed to contribute to downwind bioaerosol concentrations.
•	The determination of on site background bioaerosol concentrations such as those upwind of the bioaerosol source area (different from the site upwind sampling location) or other sources of bioaerosols in the compost site should be noted and included in the analysis.
•	Multiple samples downwind of the bioaerosol source area should be taken ideally at 4-5 separate sampling locations to ensure the comparison of the model output with the downwind bioaerosol concentrations measured on site
•	Changes in direction of the prevailing wind direction and wind velocity throughout the day should be noted and included in the subsequent assessment.
•	If agitation activities (i.e. screening, shredding, turning) are taking place on the sampling day, the biaoerosol emission rate determined from this (i.e. back-extrapolation method) is sufficient representation of bioaerosols released into the air from the compost source area. This should be represented as a point source.
•	However if there are no agitation activities taking place on the sampling day, the bioaerosol emission rates should be determined from a representation of the compost

	windrows on site (i.e different ages). In the absence of a more appropriate method, the emission rate should be determined by using the back-extrapolation method and these should be represented as area sources.
•	The weather conditions during the sampling day should be noted for each sampling location and input into ADMS 3.3 to define the meteorological parameters. However, if it is not possible to measure the weather conditions on the sampling day, then it is recommended that 'real-time' meteorological data specific to the sampling site available from the Met Office is used.

It is important to bear in mind that these recommendations can only be made for an open windrow compost site with compost sources comprising of compost windrows or agitation activities only. The definition of other bioaerosol sources such as in-vessel composting facilities as a source parameter should be assessed based on the type of in-vessel facility. In addition, the determination of the biaoerosol emission rate for such facilities is likely to differ from those for an open windrow compost site. An initial attempt at modelling two different in-vessel facilities was presented by Tamer Vestlund *et al.* (2007) and SEPA/SNIFFER (2006). However without a detailed sensitivity analysis of the ability of the model to predict bioaerosol emissions from in-vessel facilities, it is not currently possible to make recommendations for this composting technology.

The previous chapters (Chapters 4 and 5) have improved the understanding of the release of bioaerosols emitted from compost and have assessed the indications of the results with respect to their dispersal in order to fulfill the first aim of the overall project. The chapters which discussed modelling of bioaerosols (Chapters 6 and 7) have assessed the potential of ADMS 3.3 to predict downwind bioaerosol concentrations at composting sites. In addition, the sensitivities of the model were explored. These chapters have fulfilled the second aim of this project, namely to improve the current understanding of the potential of a commercial air dispersion model to predict bioaerosol concentrations at composting sites. The next chapter will present the overall research conclusions as well as highlighting contributions to knowledge.

8. CONCLUSIONS

8.1 RESEARCH BACKGROUND SUMMARY

The amount of waste that is currently produced is an important environmental challenge that needs to be addressed as a matter of priority. To this end, it is important to develop the role of more sustainable and natural processes such as composting. However there are potential risks from poorly operated composting facilities (Environment Agency, 2004; Pollard *et al.*, 2006), including the release and dispersal of bioaerosols with the potential to result in adverse health effects in sensitive receptors. Therefore, to assess the bioaerosol risk posed by composting facilities, environmental regulators request regulatory risk assessments in support of planning consent and environmental permits from facilities that are within 250m of sensitive receptors (Environment Agency, 2001).

There is a large scientific base regarding composting processes and compost quality. In contrast, the prior art in compost related bioaerosol release and dispersal assessment is not extensive. Previous research has improved the quality of source term data used in regulatory risk assessment (Taha *et al.*, 2005; 2006; 2007a). However gaps in the understanding of bioaerosols at source, on release from composting facilities and at receptor remain. Addressing these gaps of knowledge would allow for improved regulatory risk assessments.

Therefore, this research was undertaken to address some of these gaps in current knowledge and to improve the understanding of the characterisation and dispersal of bioaerosols emitted from compost. Two overall project aims were set both of which were achieved by the research conducted:

- Improve the current understanding of the aggregation and size distribution of bioaerosols emitted from compost.
- Improve the current understanding of the potential of a commercial air dispersion model to predict bioaerosol concentrations at composting sites

The thesis was divided into two themes based on these two aims and research conclusions will be presented in line with this.

8.2 RESEARCH CONCLUSIONS

8.2.1. Research Theme 1

The first theme of this project, in line with the first research aim, was in regards to the characterisation of bioaerosols emitted from compost, in particular in improving the understanding of their aggregation and size distribution. To fulfill this aim, two objectives were set.

The first research objective set was to release and measure bioaerosols in experimental conditions and use the generated data to classify the overall size distribution and visual properties (i.e. aggregation, size and shape) of bioaerosols emitted from compost. To fulfill this objective, a novel methodology (the compost tumbler) was developed to release bioaerosols in experimental conditions. Data was generated using a combination of culturing and scanning electron microscopy methods to classify the overall size distribution and visual properties of bioaerosols emitted from compost.

The second research objective set was to complete site work to validate the results of the controlled experiments and classify the overall size distribution and visual properties (i.e. size, shape and aggregation) of bioaerosols emitted from compost at composting facilities. The methodology developed to fulfill objective 1 was used to generate further data regarding the classification of the size distribution and visual properties of bioaerosols at a composting source and downwind from source.

The results from the controlled experiments and site work showed that the methodology developed for the controlled experiments was able to generate data similar to those shown in composting sites with regards to the characterisation of bioaerosols emitted from compost. As such trends concerning the size, shape and aggregation of bioaerosols shown in the controlled experiments were similar to the trends shown for the site work. In line with this, a summary of the main findings for Objectives 1 and 2 as well as key implications are presented in Table 8.1

Table 8.1 - Summary of main findings and implications for the first research theme

	Main Findings and Implications
Objective 1 *Release and measure bioaerosols in experimental conditions and use the generated data to classify the overall size distribution and visual properties (i.e. aggregation, size and shape) of bioaerosols emitted from compost*	• 7-9 morphologically-distinct types of small (0.5 – 1 μm) cells and 2 morphologically-distinct types of large (1-2 μm) cells and their aggregates were released from compost regardless of compost age or feedstock. This indicates that agitation of mature compost is likely to be a source of bioaerosols. Spore morphological characteristics indicate that the small spores are actinomycetes and the large spores are fungi. • The majority of the bioaerosols released from compost were in single cells with an aspect ratio of 1. This would implicate that these cells are more likely to be dispersed in air for longer distances than if they were in aggregate structures (i.e. heavier units). In addition the effects of surface drag in dispersal would be minimal. • The bioaerosols released from compost were not attached to particles such as wood fibres so their dispersal could not be aided by such particles acting as a 'raft'. • Aggregates of cells were less commonly found than single cells. Where aggregates were detectable, the majority were in 2-3 cell structures and smaller than 10 μm. This might imply that these particles are more likely to be dispersed in air for longer distances than heavier particles. However this may also indicate that even if they are possibly dispersed for further distances downwind, there might be less protection from the effects of temperature, solar radiation and relative humidity due to the lack of 'blanket' protection offered by the outer cells to the inside cells in a larger aggregate. This would mean that these particles might lose their viability quicker although non-viable bioaerosols can still cause adverse health effects. • The aggregate structures released from static and active compost sources were found to be in clusters as opposed to chains. This might indicate that cells are forming aggregates upon release from compost. • The majority of the bioaerosols released from compost were in the respirable range and hence had an increased likelihood of causing adverse health effects.
Objective 2 *Complete site work to validate the results of*	• There were some exceptions between different studies conducted in a controlled chamber and on site however in general, similar trends were observed. This indicated that the methodology developed for the controlled

controlled experiments and classify the overall size distribution and visual properties (i.e. size, shape and aggregation) of bioaerosols emitted from compost at composting facilities.	experiments was able to produce data comparable to those shown in composting sites with regards to the characterisation of bioaerosols emitted from compost. • In line with this, the same conclusions listed for Objective 1 were also observed for this objective regarding the aggreation, size distribution and visual properties of bioaerosols. • A decrease in any cell aggregates was observed within 10m from the compost source boundary. • There were no aggregate structures observed at 100m downwind from compost source. Since non-viable aggregates would still be captured on the filter and visualised by SEM, this suggests that aggregates drop out from the pollutant plume.

In conclusion, the controlled experiments and site work showed evidence of aggregation in bioaerosols released from compost. However, the majority of these bioaerosols were in single cell units hence they are more likely to be dispersed for longer distances.

8.2.2. Research Theme 2

The second theme of this project, in line with the second research aim, was in regards to the dispersal of bioaerosols emitted from compost, in particular in improving the understanding of bioaerosol concentration prediction by air dispersion modelling. To fulfill this aim, three objectives were set and these are discussed as follows.

The third research objective was to complete a preliminary assessment of the ability of a commercial air dispersion model, ADMS 3.3, to predict bioaerosol emissions from composting facilities compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling. To fulfill this objective, preliminary air dispersion modelling was completed using data collected during site work conducted at an open windrow composting site.

The fourth research objective was to analyse the sensitivities of ADMS 3.3 and assess the effect of different modelling parameters on predicted bioaerosol concentrations. To

fulfill this objective, a sensitivity analysis of ADMS 3.3 was completed to analyse the sensitivities of the model to the following parameter categories:

- Emission source (point, area and volume source)
- Pollutant (particulate and gas)
- Meteorology

The fifth and final research objective was to complete a final assessment of the potential of ADMS 3.3 to predict bioaerosol emissions from composting facilities. A set of site work was completed to collect bioaerosol concentration and emission data from different sources and downwind locations at another composting facility to be used as model input data. The summary of the main findings and implications for air dispersion modelling studies are presented in Table 8.2.

Table 8.2 - Summary of main findings and implications for the second research theme

	Main Findings and Implications
Objective 3 *Complete a preliminary assessment of a commercial air dispersion model, ADMS 3.3, in predicting bioaerosol emissions from composting facilities compared to bioaerosol concentrations measured by on-site downwind bioaerosol sampling.*	• The determination of the bioaerosol emission rate from agitation of compost by back-extrapolating the known bioaerosol concentrations downwind of the bioaerosol source resulted in a difference of less than 1-log between measured versus predicted bioaerosol concentrations. • The use of the sampling hood methodology for determining bioaerosol emission rates from a static compost windrow defined as a bioaerosol source resulted in the under prediction of the measured downwind concentrations by up to 4-log. • The results of the preliminary dispersion modelling studies therefore indicated that the definition of the source term (i.e. point or area source) has a significant effect on the output concentrations predicted by the model and the differences in model predictions for different sources might be due to the variation in the use and determination of bioaerosol emission rates.
Objective 4 *Analyse the sensitivities of ADMS 3.3 and assess the*	• The results showed that, in general, the model output for the parameterisation of different parameters did not differ for a point or area source • In general, the model was sensitive to only a limited number of

effect of different modelling parameters on predicted bioaerosol concentrations.	parameters. • The results of the sensitivity analysis showed that the model was not sensitive to defining the particulate pollutant as an aggregate and was somewhat sensitive to definition of a particulate pollutant size. However the results of the controlled experiments and site work (Chapters 4 and 5) showed that despite the evidence of aggregation, the majority of the bioaerosols emitted from compost were in single cells. Hence even though the model appears to be sensitive to the definition of particle pollutant size, there was no conclusive evidence to re-define bioaerosols to be modelled as particulates. • Therefore when using this model, the principle that bioaerosol cells are of a sufficiently small size to justify the assumption of them acting as gaseous pollutants remains.
Objective 5 *Complete a final assessment of the potential of ADMS 3.3 in predicting bioaerosol emissions from composting facilities.*	• Modelling only the emissions measured on the compost windrows resulted in a difference of up to 3-log between predicted and measured concentrations (regardless of modelling individual compost windrow concentrations were modelled as single sources or combined as one source). • Modelling both bioaerosol sources (static compost windrow and agitation activity) was more successful at predicting measured downwind concentrations and was able to match the downwind bioaerosol concentrations at some of the downwind sampling points. These results further highlight that source definition and the use and determination of bioaerosol emission rates are important factors in air dispersion modelling of bioaerosols. • It was shown to be possible to match the individual measured downwind concentrations by changing the bioaerosol emission rates however it was not possible to match the overall depletion curve. • The results of the culturing results for the studies showed an inconsistent pattern of decline in downwind bioaerosol concentrations for the first two sampling days. Therefore, ADMS 3.3 was not able to successfully predict the downwind source depletion curve trend for these experiments. In contrast, the model was more successful at predicting the source depletion curves for sampling day three. This might indicate that the model is not capable of dealing with changes in site measurements from one

	sampling day to another. However the culturing results might also be indicative of the inherent difficulties of bioaerosol sampling practices at a composting site. Such difficulties make it difficult to collect valid data to which the model output can be compared. Therefore when comparing results of measured versus modelled concentrations, the capability of a commercial air dispersion model in assessing the risks of bioaerosols at composting facilities should be analysed in this context.

Therefore the overall results from the modelling studies indicate that ADMS 3.3 was not able to consistently predict absolute downwind bioaerosol concentrations at composting facilities. However ADMS 3.3 still has potential in testing the effect of different emission scenarios which might be beneficial in completing regulatory risk assessment for composting facilities. Hence if an existing composting facility wish to make changes to their operations (e.g. expansion of the facility), the model might be a valuable tool for predicting and assessing relative changes in downwind bioaerosol concentrations resulting from different operating scenarios. Therefore, it is concluded that ADMS 3.3 can be a useful tool for the initial screening and assessing relative changes of bioaerosols at a compost facility however detailed assessment of absolute bioaerosol emissions should be made in conjunction with measurement of downwind bioaerosol concentrations.

8.3. CONTRIBUTIONS TO KNOWLEDGE

The research presented in this thesis makes a significant contribution to knowledge in terms of improving the understanding of the characterisation and dispersal of bioaerosols emitted from composting facilities.

As previously discussed, the understanding of factors that effect bioaerosol behaviour at source, pathway and receptor is essential in analysing the risk of composting related bioaerosol exposure. However there are gaps in the scientific prior art of the processes (e.g. aggregation and size distribution) which may affect the analysis of bioaerosols emitted from composting facilities. As such, to the author's current knowledge, this is the first study that has aimed to close the gap in one of these processes and hence has developed a novel methodology to characterise the size distribution and visual

properties (i.e. size, shape, and aggregation) of bioaerosols emitted from compost. This methodology was used to generate extensive data on the characterisation of the aggregation, size distribution and other physical properties of bioaerosols emitted from compost. To the author's current knowledge, this is the first such library of data generated for compost related bioaerosols. Furthermore this novel methodology was shown to produce results comparable with those that would be seen in a composting facility. Hence, in line with this, another novel aspect of the study has been the classification of the aggregation and size distribution of bioaerosols emitted from composting facilities.

The use of air dispersion modelling to predict bioaerosol concentrations at composting facilities has not been adopted as official practice. As previously discussed, there are a limited number of studies which have used air dispersion models to predict downwind concentrations of bioaerosols emitted from compost sources. However these studies have not attempted to compare the actual measured downwind bioaerosol concentrations with those predicted by the model. Therefore another novel aspect of this research was the completion of a number of studies which have compared predicted bioaerosols emissions to those measured on site and downwind. In addition, this is the first study of its kind which has completed a detailed assessment (including a sensitivity analysis specific to bioaerosols) of the potential of the air dispersion model, ADMS 3.3 to predict bioaerosol emissions from composting sites. Finally, a set of novel recommendations have been made for assessing the risk of bioaerosols at an open windrow compost site.

In conclusion, the results achieved by this research have introduced new insights to the current understanding of the characterisation and dispersal of bioaerosols emitted from composting facilities. As such, these new insights would be expected to make a significant contribution to the needs of new and existing composting facilities in quantifying bioaerosol site exposures to meet increased regulatory requirements as well as the needs of the regulatory body, Environment Agency, in evaluating the exposure risks of bioaerosols from composting facilities.

8.4. LIMITATIONS AND RECOMMENDATIONS FOR FUTURE WORK

The specific limitations for each stage of the research have been discussed individually throughout the previous chapters. However, in addition to these, a number of general limitations were identified. These limitations represent additional studies which might have improved the findings of this research project but could not be completed due to time restrictions. They are as follows:

- Exploring the effect of seasonal differences or other weather related episodic behaviour on the characterisation of the properties of bioaerosols emitted from composting facilities. This would have provided additional insight into the mechanisms of characterisation and dispersal of bioaerosols. Therefore future studies are recommended to explore the effect of seasonal differences or other weather related episodic behaviour.

- The characterisation (i.e. aggregation, size distribution) of bioaerosols at sampling locations in the vicinity of sensitive receptors. This would have provided additional understanding of bioaerosol exposure risk at receptor. Therefore the methodology developed in this research project for completing bioaerosol classification studies is recommended for use at sampling points in the vicinity of sensitive receptors within 250 m of composting facilities.

This research was completed to improve the current knowledge of the understanding of the characterisation and dispersal of bioaerosols emitted from compost. However other gaps in the understanding of bioaerosol behaviour at source, pathway and receptor and hence the assessment of bioaerosol risk from composting facilities remain. These gaps need to address the lack of knowledge on their viability and inactivation on release from compost and use this understanding to assess the pathway of a bioaerosol. In this context, the effect of environmental factors (relative humidity, temperature, radiation, oxygen levels, open air factors and ionisation) on the viability of bioaerosols should be considered.

The release, dispersal and deposition of bioaerosols from composting facilities are significantly affected by the regional, environmental, geographical and meteorological factors. Therefore it is important to know and understand these parameters to be able to make site based judgements when analysing the results of sampling and modelling from composting sites.

There remains a significant gap in establishing dose-response relationships between bioaerosol exposure and adverse health effects. To this end international regulation standards need to be introduced to establish this relationship according to type of bioaerosol and exposure.

The scope of this study was to visually characterise the bioaerosols emitted from compost and species determination was not included. However future studies should complete visual characterisation of bioaerosols released from compost on a species level to determine the effect of their biological properties on bioaerosol release and dispersal. In line with this, studies which account for the species and concentrations of bioaerosols in the compost flora (i.e. not airborne) with respect to those released from it (i.e. airborne) should be considered.

Further research should be employed to improving the understanding of ADMS or other air dispersion models for modelling downwind concentrations of bioaerosols in a composting facility. In addition, extensive validation work should be completed to compare predicted versus measured bioaerosol concentrations at a number of other composting facilities.

Bioaerosols behave according to their unique biological properties as well as being influenced by physical laws that other non-biological aerosols are subject to. This introduces an extra challenge in understanding their aeromicrobiological behaviour. In this context, future studies which aim to improve the understanding of bioaerosol release and dispersal should combine the understanding of species specific behaviour (e.g. fungal life cycle) with those for aerosol physical laws.

The potential of optical detection tools such as LIDARS for determining the structure of the atmosphere for bioaerosol plume detection has been explored by numerous studies (Yee *et al.*, 1992; Roy and Roy, 2008). Other studies have attempted to predict the limits of buoyant plume rise in a well-mixed boundary layer using LIDAR technology (Bennett, 1995). Bennett and Hunter (1997) have compared the estimates of peak ground-level concentrations of LIDAR with those by ADMS and an agreement between the two was found depending on the meteorological data that were used. There is no evidence of any studies that use these technologies in assessing the bioaerosol plume emitted from a compost source. Therefore as a final recommendation, the use of such technologies should be considered for future studies that aim to analyse bioaerosol plumes emitted from compost sources. In addition, the use of LIDAR and other such technologies should be used to validate the results of any future air dispersion modelling studies that attempt to predict the emission of bioaerosols from compost.

In conclusion, future bioaerosol studies need to tackle these issues and explore the use of new technologies in order to improve the quality of composting risk assessments.

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LIST OF APPENDICES

PUBLICATIONS LIST

- M.P.M. Taha, G.H. Drew, **A. Tamer Vestlund**, D. Aldred, P.J. Longhurst and S.J.T Pollard.(2007). Enumerating actinomycetes in compost bioaerosols at source – use of soil compost agar to address plate 'masking'. *Atmospheric Environment*, 41, p. 4759-4765.

- M.P.M. Taha, G.H. Drew, **A. Tamer**, G. Hewings, G.M. Jordinson, P.J. Longhurst and S.J.T Pollard. (2007). Improving bioaerosol exposure assessments of composting facilities – comparative modeling of emissions from different compost ages and processing activities. *Atmospheric Environment*, 41, p. 4504-4519.

- SEPA/SNIFFER. (2007). Bioaerosol and odour monitoring from three composting sites. Prepared by G.H. Drew, **A. Tamer Vestlund,** S.F. Tyrrel, P.J. Longhurst, and S.J.T. Pollard. SNIFFER Research Project UKPIR12. *Available online at http://www.sniffer.org.uk.*

- **A. Tamer Vestlund**, G.H. Drew, B. Jehlickova, P. Olsen, R. Sneath, S.F. Tyrrel, P.J. Longhurst, G.M. Jordinson, and S.J.T. Pollard. (2007). Bioaerosol and odour emissions: A comparison of three composting sites in Scotland. Paper presented at the Eleventh International Waste Management and Landfill Symposium, Sardinia, 1-5 October 2007.

- G.H. Drew, **A. Tamer Vestlund**, G.M. Jordinson, R. Smith, S. Tyrrel, P.J. Longhurst , and S.J.T. Pollard. (2007). Developing a Best Practice Method for Modelling Dispersion of Bioaerosols from Composting Facilities. Paper presented at the Eleventh International Waste Management and Landfill Symposium, Sardinia, 1-5 October 2007.

- Defra. (2007). Health Impact Assessment of Alternate Week Collections of Biodegradable Waste. Prepared by G.H. Drew, **A. Tamer**, R. Hough, S. Chackiath, P.J. Longhurst, M. Broomfield, N. Bellamy and J. Davies for Wycombe District Council. DEFRA Waste Implementation Programme Report.

- G.H. Drew, **A. Tamer**, R.L. Hough, S.J. Chakiath, M. Broomfield, and P.J. Longhurst. (2007). Health Impact Assessment of Alternate Week Waste Collections. Paper presented at The Chartered Institution of Wastes Management Conference and Exhibition, Torbay, 12-15 June 2007.

- G.H. Drew, **A. Tamer**, M.P.M Taha, R. Smith, P.J. Longhurst, R. Kinnersley, and S.J.T. Pollard. (2006). Dispersion of bioaerosol from composting facilities. Paper presented at the Waste 2006 conference, Stratford, 19-21 September 2006.

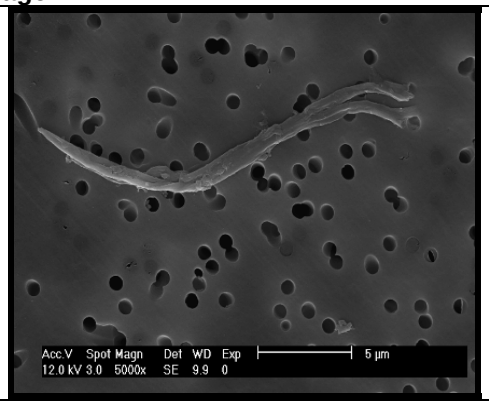
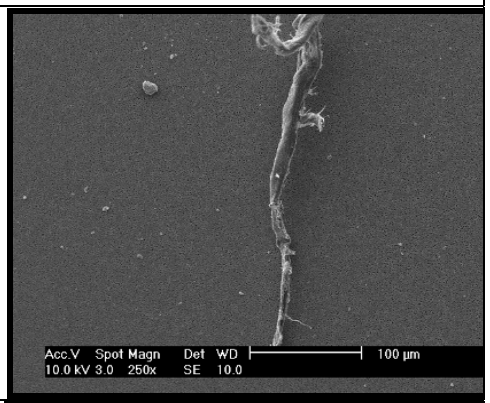
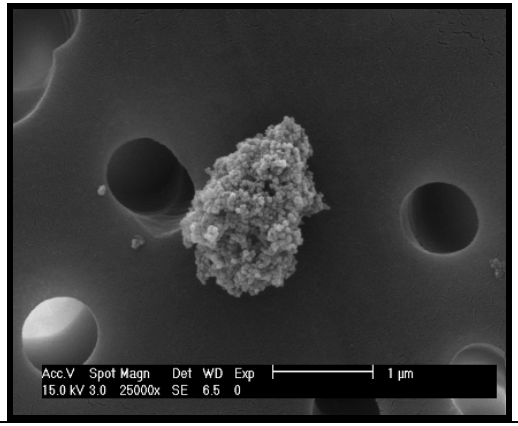
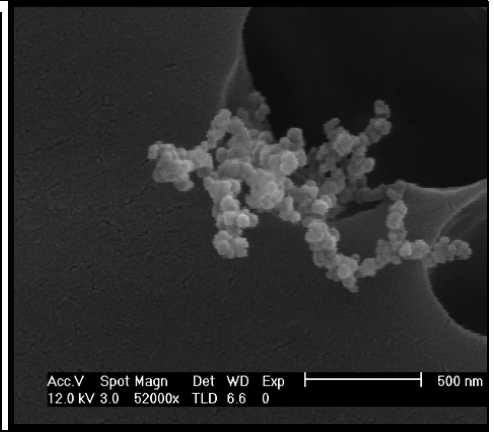
Sample Name: Sampling Date:	Scanning Started At: (AM/PM) Scanning Finished At: (AM/PM)
TYPE OF PARTICLE	**NUMBER OF PARTICLES**
Large Cell Type A	
Large Cell Type B	
Large Cell Type C	
Large Cell Aggregate Type A (Specify number of cells, total aggregate size and type)	
Large Cell Aggregate Type B (Specify number of cells, total aggregate size and type)	
Large Cell Aggregate Type C (Specify number of cells, total aggregate size and type)	
Large Cell Mixed Aggregates	
Mixed Large and Small Cells	
Small Cell Type A	
Small Cell Type B	
Small Cell Type C	
Small Cell Type D	
Small Cell Type E	
Small Cell Type F	
Small Cell Type G	
Small Cell Type H	

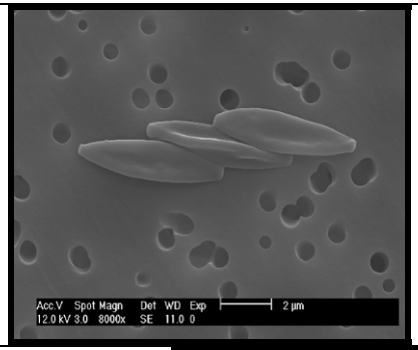
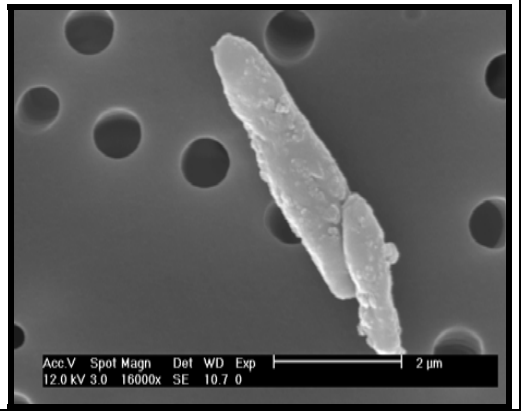
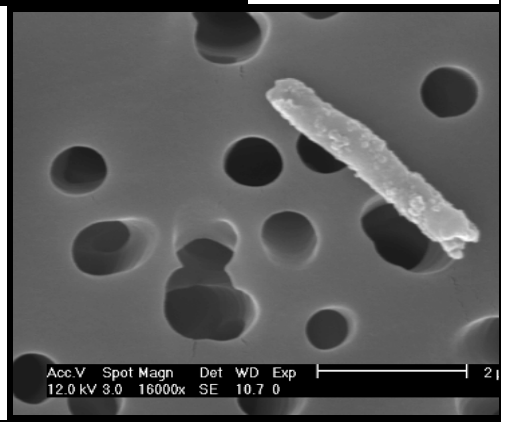
Small Cell Type I	
Small Cell Type J	
Small Cell Aggregate Type A (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type B (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type C (Specify number of cells, total aggregate size and type)	
Small Spore Aggregate Type D (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type E (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type F (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type G (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type H (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type I (Specify number of cells, total aggregate size and type)	
Small Cell Aggregate Type J (Specify number of cells, total aggregate size and type)	
Small Cell Mixed Aggregates	

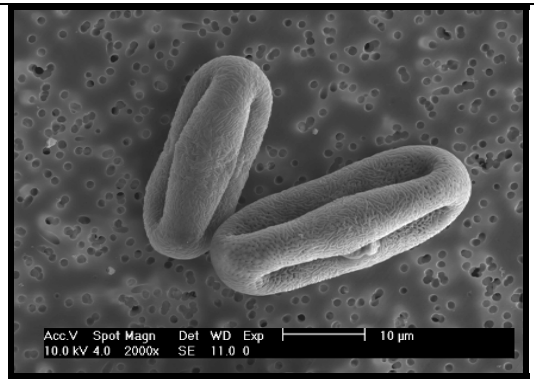
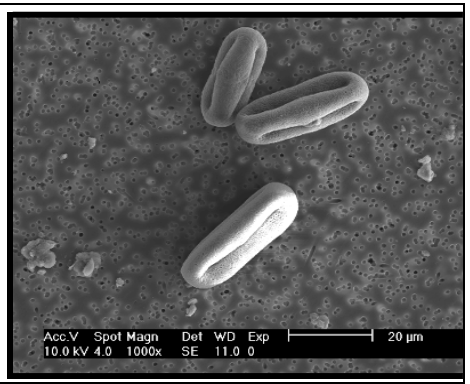
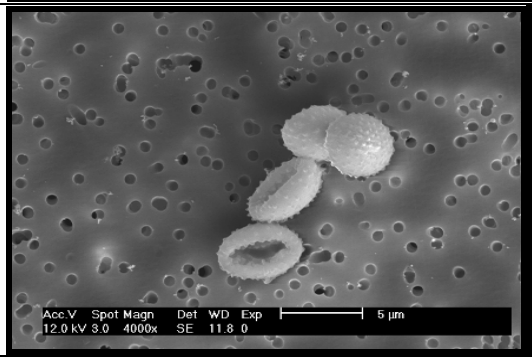
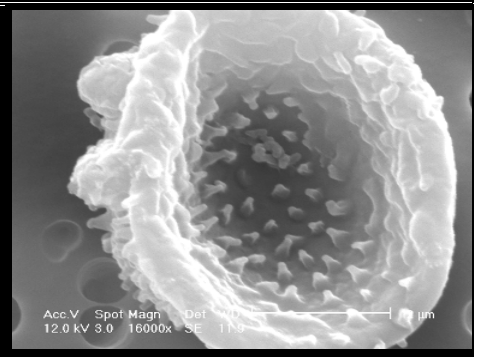
Large Cell-like Particles	
Plant-like Cell Particle	
Filamentous Particle	
Rod Shaped Particles	
Crystal Cluster Particles	
Unstructured Particles	
Pollen-like Particles (Specify Type)	

Fields with Particles of Interest at 2000 mag:	
Blank Fields at 500 mag:	
Blank Fields at 1000 mag:	
Blank Fields at 2000 mag:	

Notes:

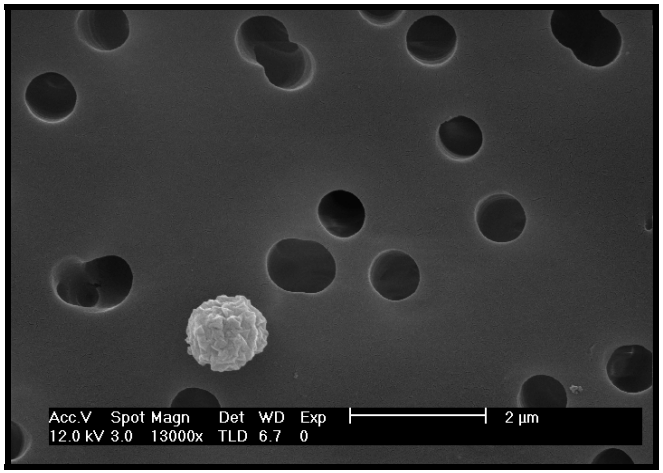
Name of Particle	Description	Size	Image
Filamentous particles	Particles with 'fibre-like' structure	Physical size range of 1 to 100 µm or larger (length)	 
Crystal cluster particles	Aggregates of crystal-like particles	Physical size range of size 1-3 µm or larger (length). The individual crystal-like particles are smaller than 0.5 µm	 

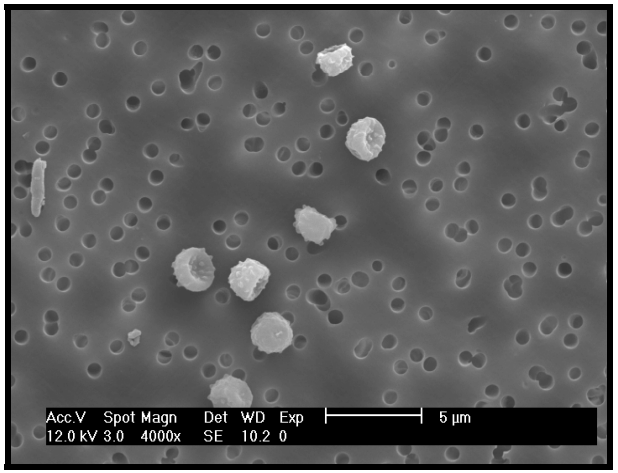
Large cell-like particles	Smooth particles with an ellipsoidal shape, 3-D structure	Physical size range of 7-8 µm (length)	
Rod shaped particles	Rod shaped particles with small bumps on surface	Physical size range of 1-3 µm (length)	 

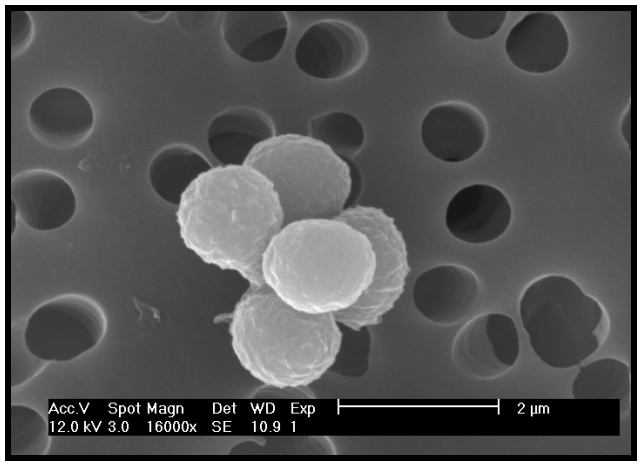
Pollen-Like Particles Type 1	Oval particles with textured surface and with prominent '2 ridges' structure	Physical size of approximately 30 µm (length).	 
Pollen-Like Particles Type 2	Round particles with 'worty' surface	Physical size of approximately 6-7 µm (length)	 

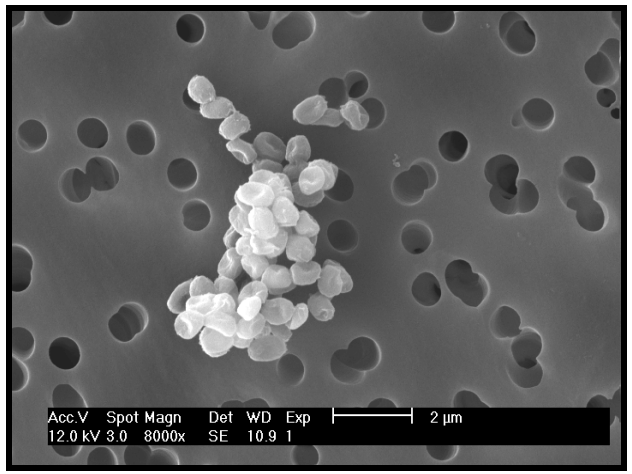
Small Cell Type A and Aggregates:

Round particles with smooth surface or with small bumps. Some have characteristic 'Raspberry-like' structure. Physical size range of 0.5-1 µm (length).



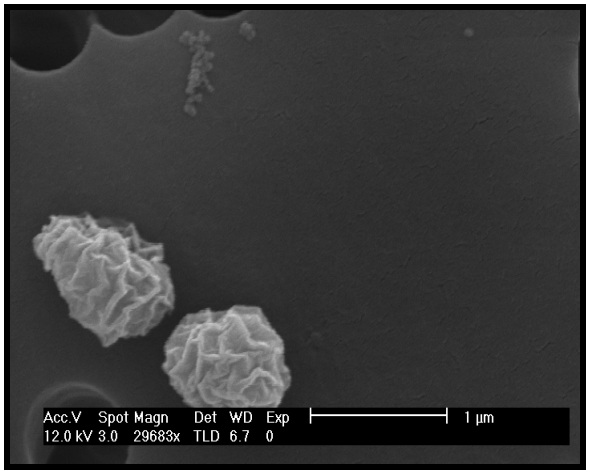


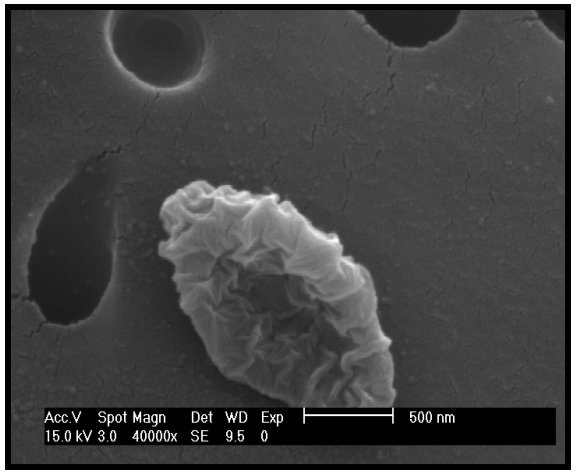


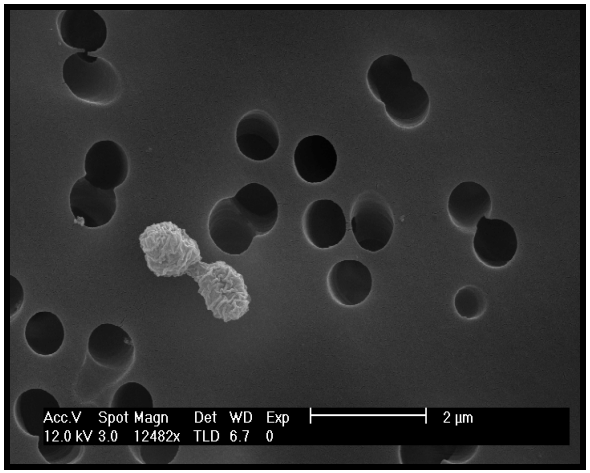


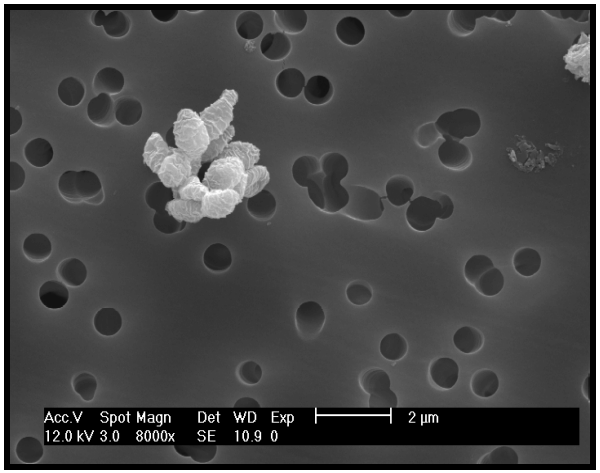
Small Cell Type B and Aggregates:

Oval shaped particles with ridges. Physical size range of 0.5-1 µm (length).



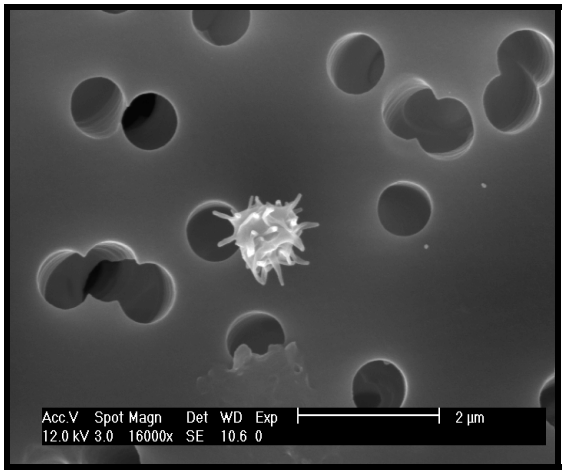


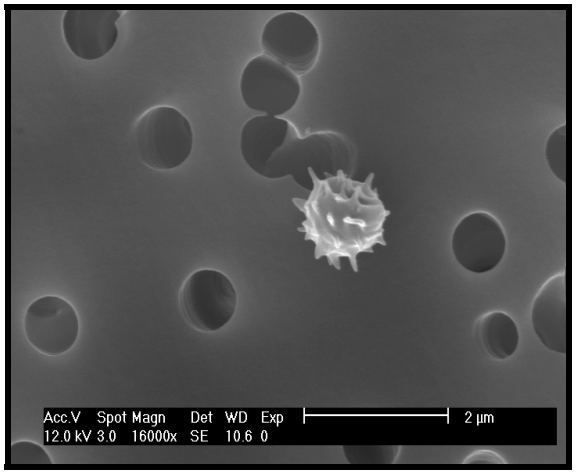


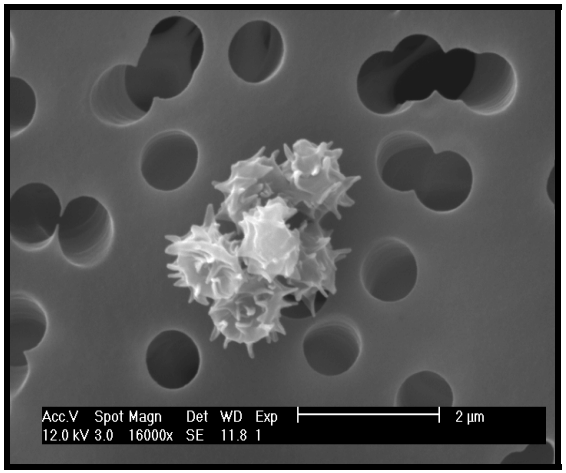


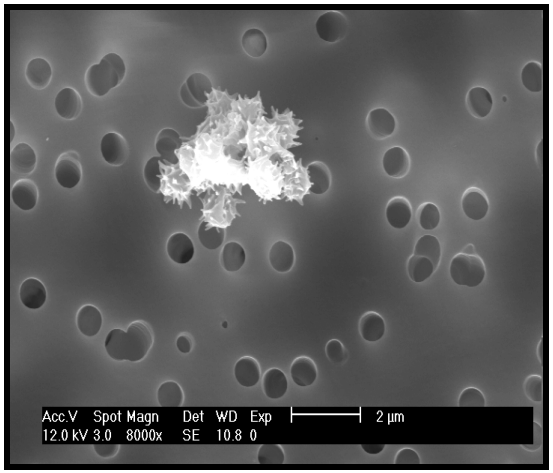
Small Cell Type C and Aggregates:

Round particles with visible spikes. Physical size range of 0.5-1 µm (length).



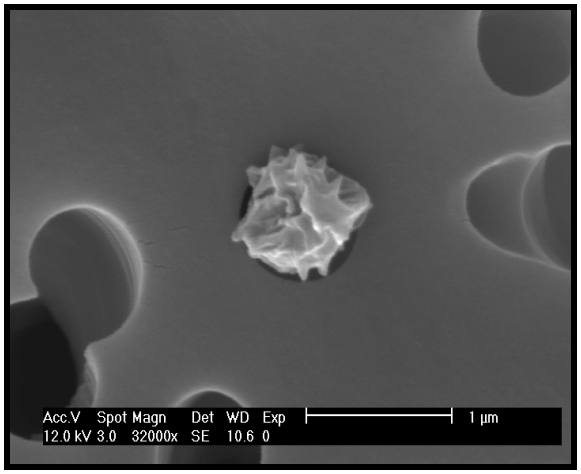


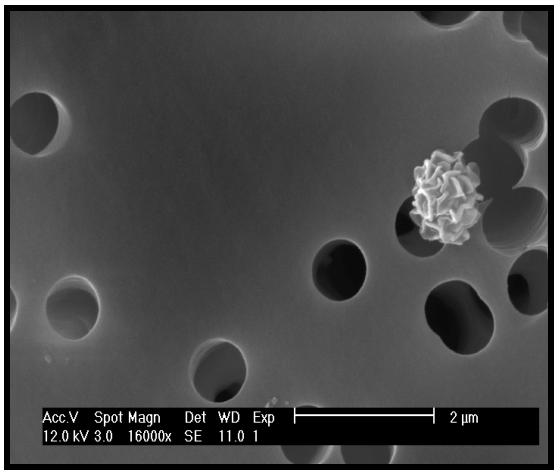


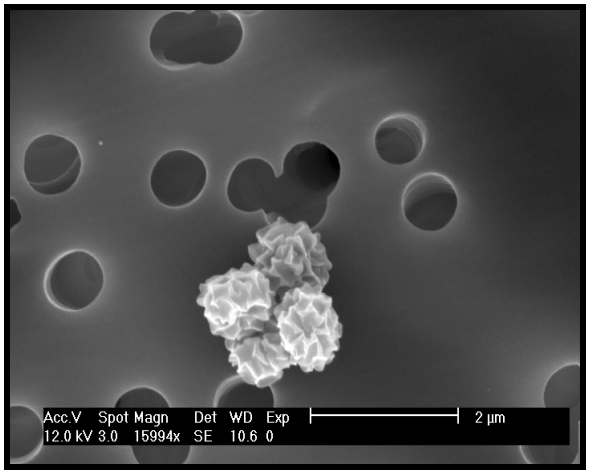


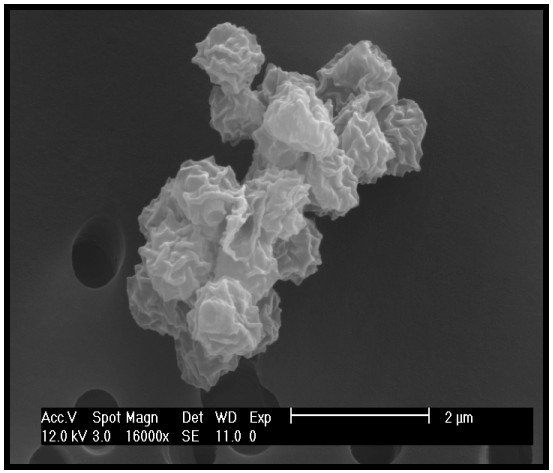
Small Cell Type D and Aggregates :

Round particles with ridges and a ‘flower-like’ structure. Physical size range of 0.5-1 µm (length).



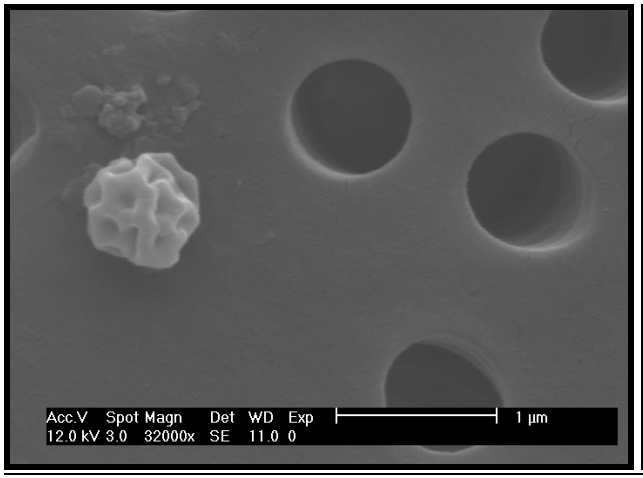


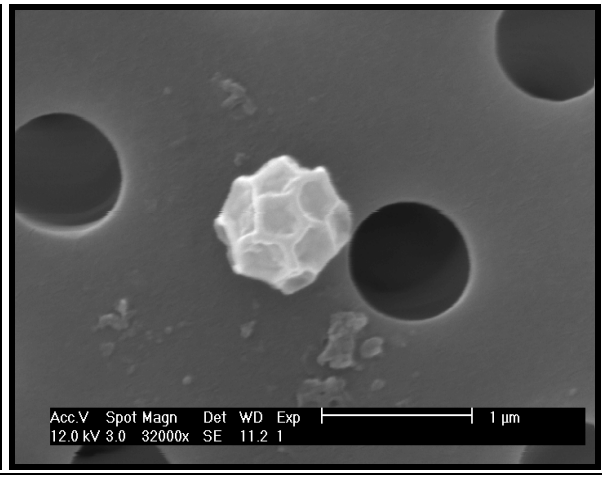


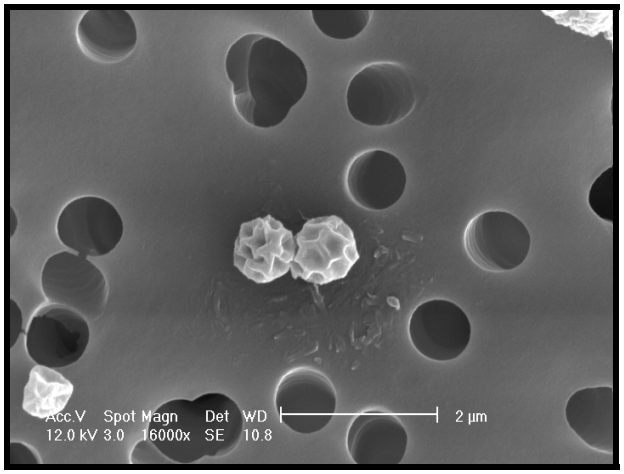


Small Cell Type E and Aggregates:

Round particles with dents. Physical size range 0.5-1 μm (length).

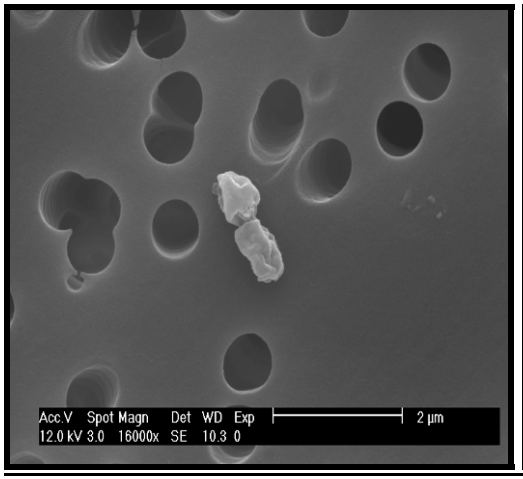


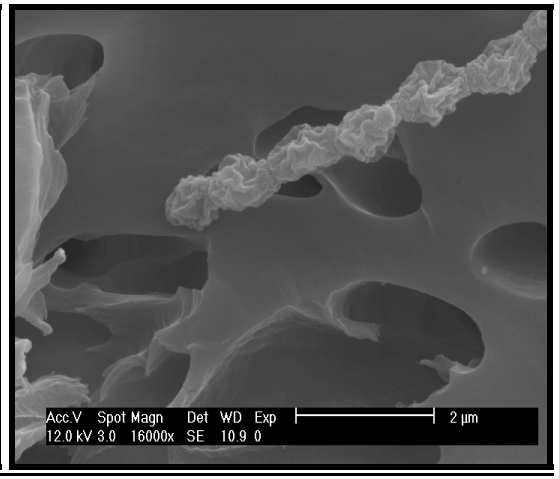


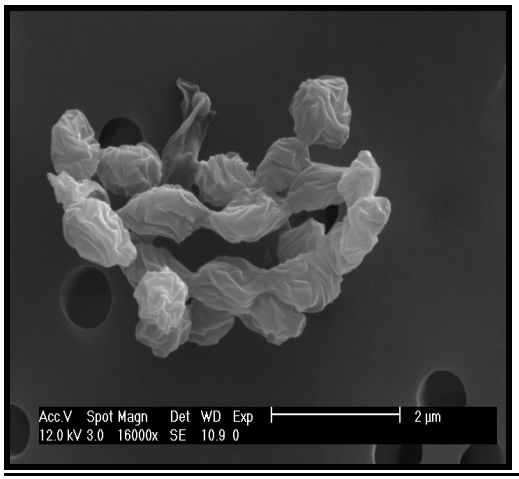


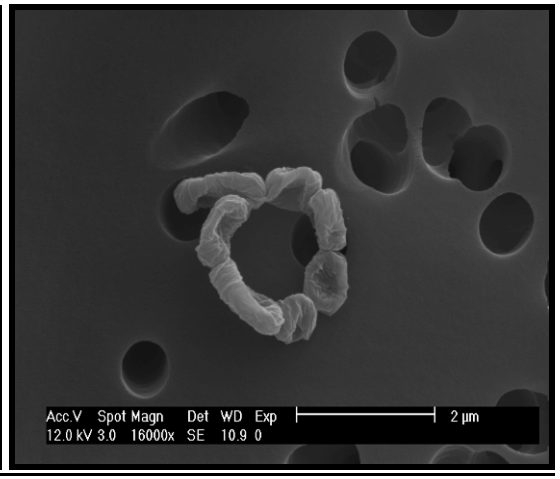
Small Cell Type F and Aggregates:

Oval shaped particles with ‘shrivelled’ appearance. Mostly occurring in ‘chain’ structures. Physical size range 0.5-1 µm (length).



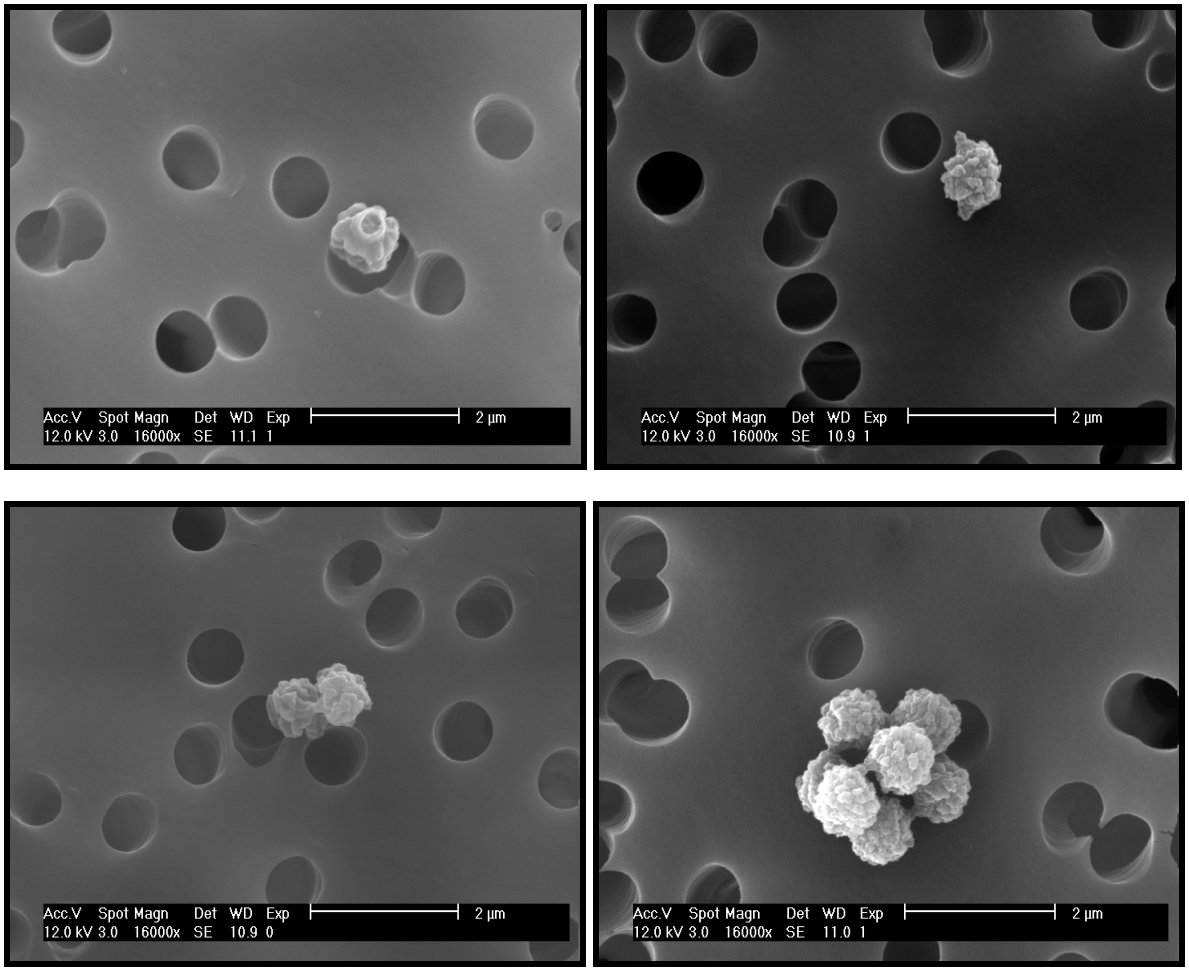






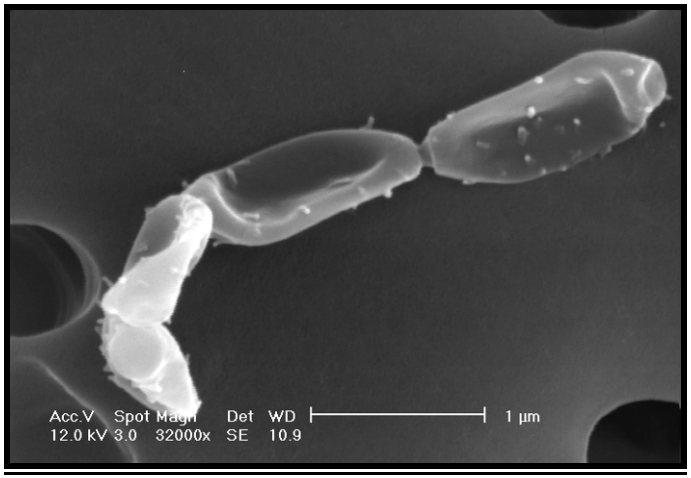
Small Cell Type G and Aggregates:

Round particles with prominent bumps, ‘cauliflower-like’ appearance and ‘scar’. Physical size range 0.5-1 µm (length).



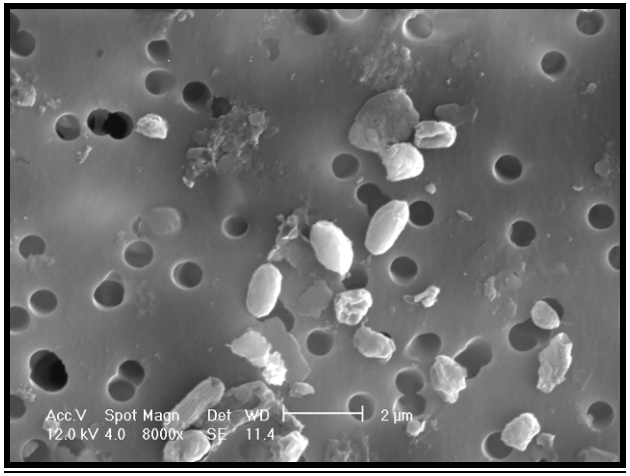
Small Cell Type H and Aggregates:

Oval shaped particles with small ‘warts’. Physical size range 0.5-1 µm (length).



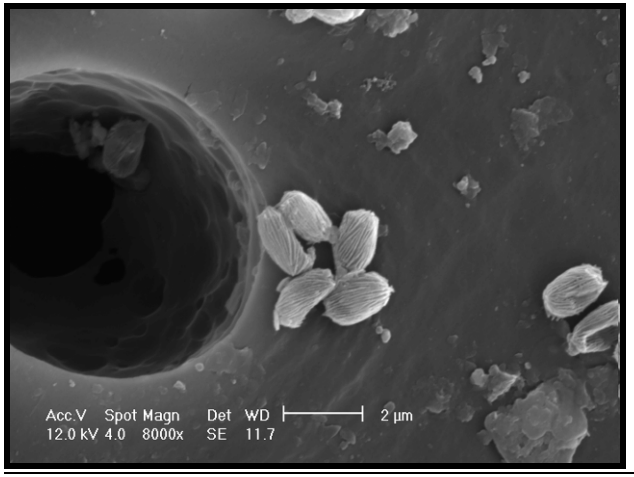
Small CellType I and Aggregates:

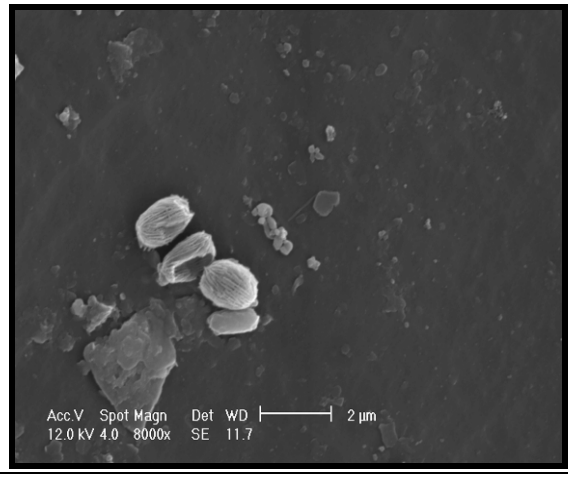
Oval shaped particles with smooth appearance. Physical size range 0.5-1 μm (length).



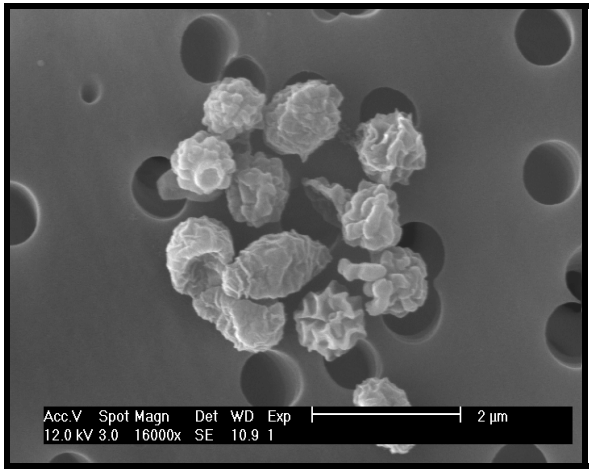
Small Cell Type J and Aggregates:

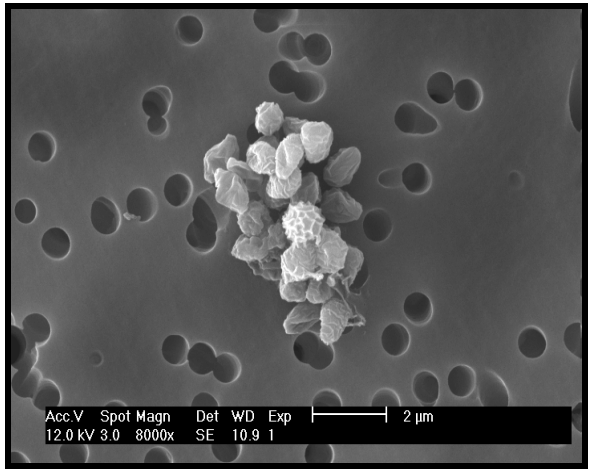
Oval shaped particles with 'ridged' appearance. Physical size range 0.5-1 µm (length).





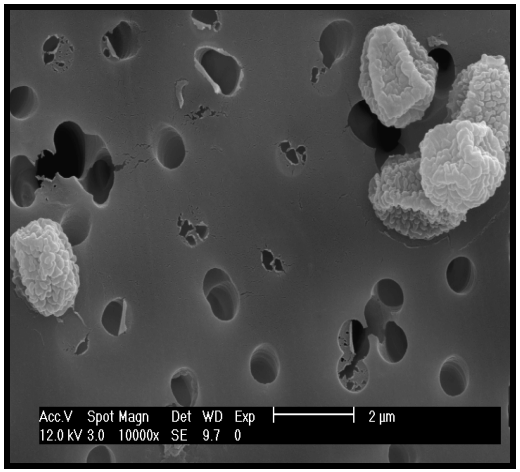
Small Size Mixed Aggregates:

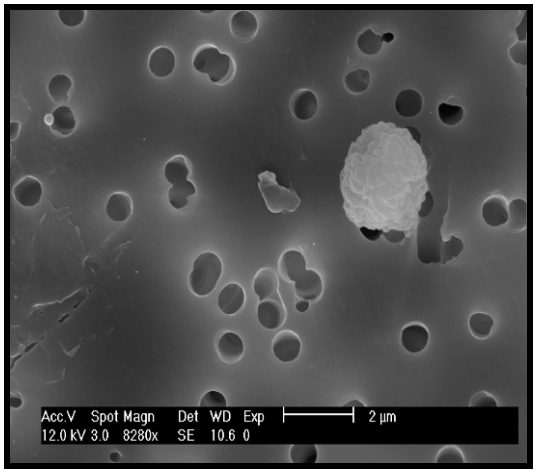


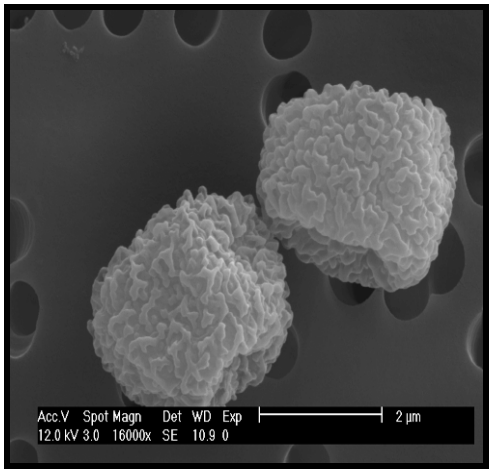


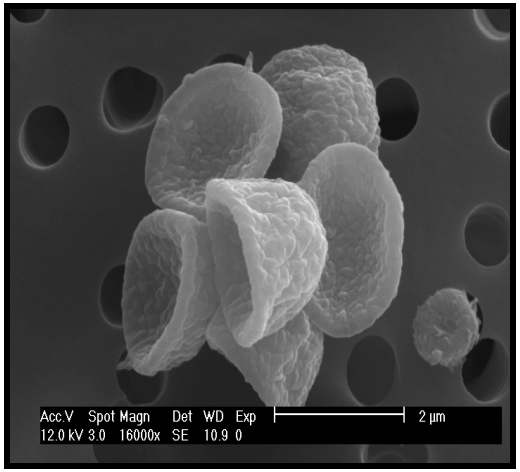
Large Cell Type A and Aggregates:

Round particles with bumpy or smooth surface and 'raspberry-like' structure. Physical size range of 2-3 µm (length).



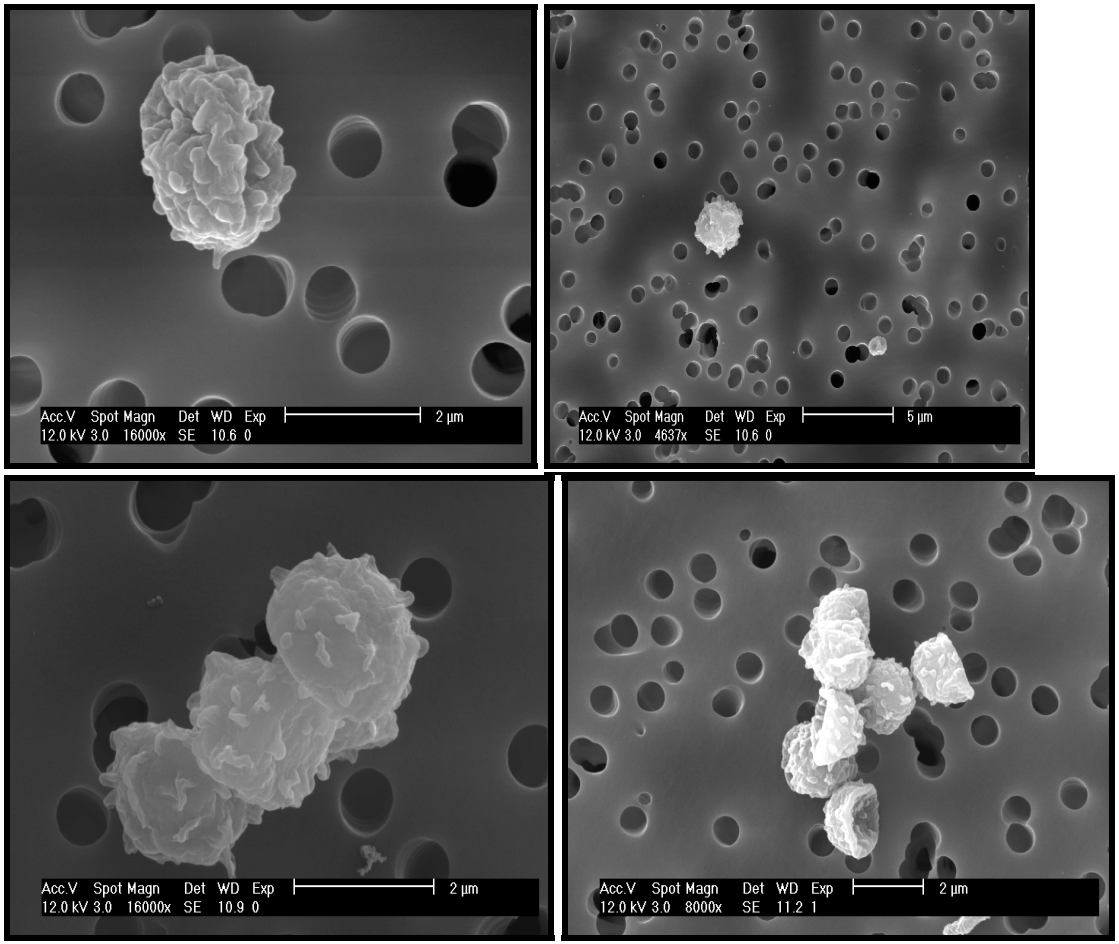






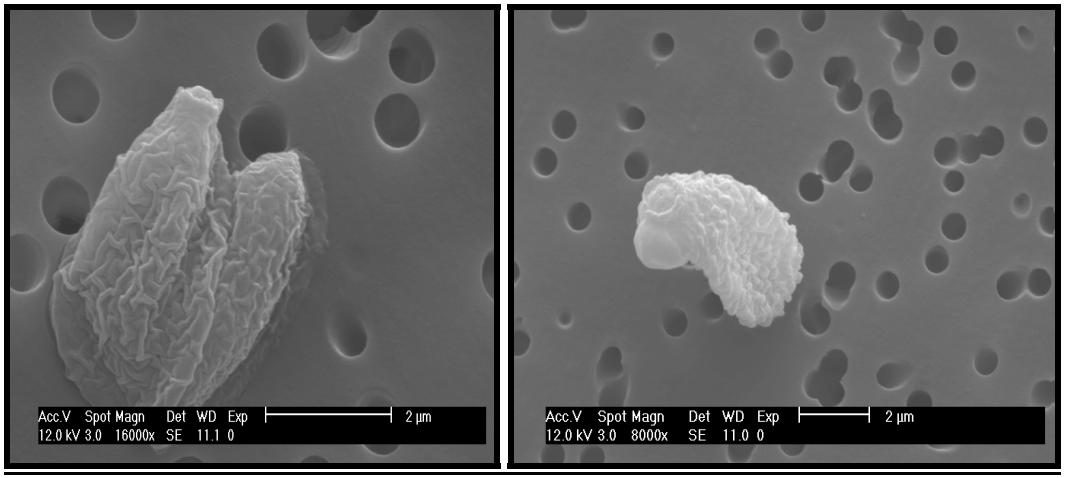
Large Cell Type B and Aggregates:

Round particles with small spikes. Physical size range of 2-3 µm (length).

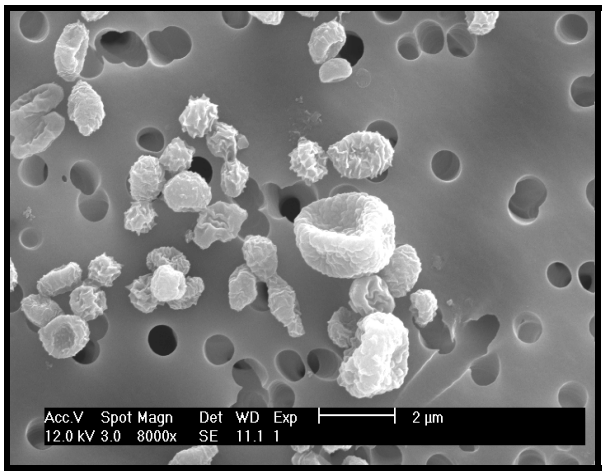


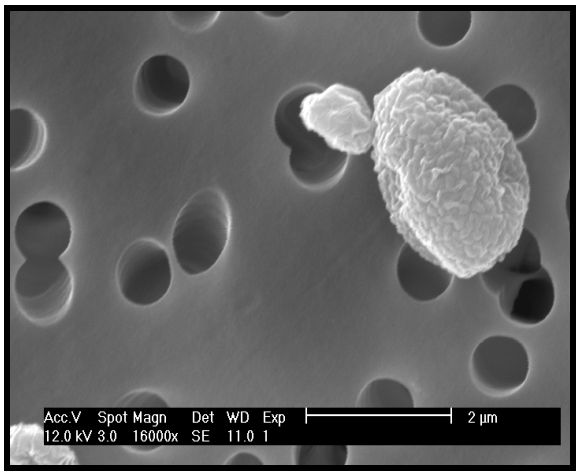
Large Cell Type C and Aggregates:

Oval particles with bumpy surface and with prominent ‘scar’. Physical size range of 2-3 µm (length).



Large and Small Cell Aggregates





	FIRST SAMPLING DAY		**SECOND SAMPLING DAY**	**THIRD SAMPLING DAY**
ADMS Parameter/Sampling Location	On incoming waste compost windrow wind tunnel 1	On incoming waste compost windrow wind tunnel 2	Agitation Screening	Agitation Shredding
Site Surface Roughness (m) (denotes agricultural area)	0.2	0.2	0.2	0.2
Site Latitude (^{0}) (default)	52	52	52	52
Source type	Area	Area	Point	Point
Pollutant emission rate for *A.fumigatus*	350 cfu/m^2/s	410 cfu/m^2/s	750,000 cfu/s	4,700,000 cfu/s
Pollutant emission rate for actinomycetes	121 cfu/m^2/s	606 cfu/m^2/s	135,000,000 cfu/s	22,000,000 cfu/s
Pollutant type (deposition velocity not specified)	Gas	Gas	Gas	Gas
Source height (m)	3	3	3	3
Source diameter (m)	22	22	3	3
Source exit velocity (m/s)	1.1	1.3	N/A	N/A
Ambient temperature (^{0}C)	24.2	27	11.5	5
Ambient velocity (m/s)	0.9	0.6	1.7	3.6
Source specific heat capacity-Cp (J/kg/^{0}C) (default)	1012	1012	1012	1012
Source molecular weight (g/mol) (default)	28.966	28.966	28.966	28.966
Efflux	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate
Stability class	D	D	D	D

Parameter type	**Parameter name and chosen value**	**Allowed model range**	**Model default**	**Notes**
Set-Up	Site Surface Roughness: 0.2 m	10^{-7}m to 10 m	0.1 m	Chosen value represents agricultural areas
	Site Latitude: 52^{O}	-90^{O} to 90^{O}	52^{O}	
Source	Geometry of Source for area and volume source (four corners):	N/A	**X** / **Y**: -21 / -23; -21 / 23; 21 / 23; 21 / -23	Source geometry kept at 0,0 for point source. The X,Y co-ordinates used for the area source reflect the actual compost windrow measurements taken on site.
	Known Efflux Format: Exit Velocity	See notes	Exit Velocity	Model allows Efflux choice of Vol: volumetric flow rate, Fm, Fb: momentum flux and heat release rate and Mass: mass flow rate. Exit velocity is chosen as that of the efflux is known.
	T, RHO or Ambient: T	See notes	T	T is chosen when the temperature of the release is known, RHO is entered when the density of release is known. Ambient is to be entered when release is at ambient temperature and density. T chosen as the temperature of the release is known.
	Actual or NTP: Actual	See notes	Actual	Chosen value means that emission parameters are to be entered at actual temperature and pressure. NTP would be 1 atm and 273 K.
	Molecular mass of the release material: 28.966 g	1 to 300 g	28.966 g	This is the typical value for air. This value is chosen as the release material is predominantly air
	Specific heat capacity of the release, Cp: 1012 $J/C^{o}/kg$	1 to 10^{5} $J/C^{o}/kg$	1012 $J/C^{o}/kg$	This is the specific heat capacity of air. This value is chosen as the release material is predominantly air
Meteorology	For source and pollutant parameter sensitivy analysis, ADMS File R91A-G is used. Stability Class D (neutral) values chosen. For meteorological parameter sensitivity analysis, the following parameters were kept constant: wind angle: 270^{0} (this angle allows to user to align the specified points with the wind and hence they would be downwind of the source), year: 2006, julian day number: 168, local time: 13, cloud cover: 4.			

Grid	Output Grid Information: Cartesian co-ordinate system chosen (refers to a point by a pair of x,y co-ordinates as opposed to radius and angle as for Polar co-ordinate system), specified points analysed as below 14 Specified X(m) points of 1, 2, 5, 10, 20, 30, 40, 50, 100, 200, 250, 300, 400, 500. Y(m) points kept at 0 and Z(m) points at 1.8 m. (Z(m) point refers to the height above ground at which output is calculated. 1.8m is chosen to represent the average height of sensitive receptor).
Output	Short Term Average Results (1 hr averaging time). Short term average results denote the output for the first 24 lines of meteorological data only. 1 hr averaging time is the model default.

Parameter Type	**Parameter name, chosen and adjusted values**	**Allowed model range**	**Model default**
Set-Up	Model Option: None and Dry Deposition	Dry deposition, wet deposition, radioactive decay, plume visibility, odours, chemistry, buildings, hills, coastline, puff and fluctuations. *Note: No model option denotes concentrations for a continuous (plume) release in flat terrain. Dry deposition denotes the loss of material from plume at the surface of ground with specified deposition velocity. Other model options are not applicable.*	N/A
Source	**Area and Point Source:** Height (m): **3** (site measurement for area or point source), 30, 300	0 to 2000 m	50 m
	Area Source: Temperature of Release (^{o}C): **25.6** (site measurement), 256, 2560	-100 to 5000^{o}C	14 ^{o}C
	Area Source: Vertical velocity of release at source exit (m/s): **1.2** (site measurement), 12, 120	0 to 1000 m/s	15 m/s
	Point Source: Internal diameter of the source (m): **3** (site measurement), 30, 100	0 to 100 m	1 m
	Point Source: Temperature of Release (^{o}C): **19.7** (site measurement), 197, 1970	-100 to 5000^{o}C	14 ^{o}C
	Point Source: Vertical velocity of release at source exit (m/s): **1.1** (site measurement), 0, 11, 110	0 to 1000 m/s	15 m/s
	Volume Source: Mid height of the volume source above ground (m): **1.5** (site measurement), 15, 150.	0 to 2000 m	50 m
	Volume Source: Vertical dimension of volume source (m): **3** (site measurement), 0.001, 30, 300	0.001 to 1000 m	1 m
Meteorological	Wind Speed: **5** (m/s), 0.5, 50	0.5 to 50 m/s	5 m/s
	Relative Humidity: **10** (%), 0.1, 100	0 to 100%	50%
	Surface Temperature: **5** (^{o}C), 0.5, 50.	-80 to 80 ^{o}C	15 ^{o}C
Pollutant	Gas Pollutant Deposition Velocity (m/s): **3 x 10^{-3}** , 3 x 10^{-2}, 3 x 10^{-1},	0 to 10 m/s	0 m/s

	3.0		
	Pollutant Deposition and Terminal Velocity (m/s): **1×10^{-5}** , 1×10^{-4}, 1×10^{-3}, 1×10^{-2}	0 to 10 m/s *Note: Deposition velocity parameter is required for both gases and particles. Terminal velocity parameter is required for particles only. In order to determine the effect of deposition velocity, dry deposition model option needs to be enabled*	0 m/s
	Pollutant Particle Diameter (m): **1×10^{-6}** (observed particle size for actinomycetes) 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}	1×10^{-2} to 1×10^{-9} m	1×10^{-5} m (10 µm) for PM10 particles and 1×10^{-6} m (1µm) for user defined particles
	Pollutant Particle Mass Fraction: 1, 0.5, 0.25, 0.2, **0.1** (no aggregate, 2 aggregate, 4 aggregate, 5 aggregate and 10 aggregate)	0.0000000001 to 1	1
	Particle with defined particle density (kg/m^3): **1000** (density of water), 10000, 100000, 1000000)	1 to 1000000 kg/m^3 *Note: The base parameter value used was 1000 kg/m^3 which is the density of water. This value is also within the range model minimum, maximum and densities of various species measured by previous studies. Some of these are the reported density range of bioaerosols at 900-1300 kg/m^3 (Cox, 1995) measured density of fungi at 560 -1440 kg/m^3 (Gregory, 1973), the dry densities of Bacillus species at 1400-1520 kg/m^3 (Carrera et al., 2008) and the dry densities of bacterial species including B. cereus and B. subtilis at 1050-1500 kg/m^3 (Tisa et al., 1982).*	1000 kg/m^3

For all tables, values in brackets indicate the numerical deviation of ratio from 1.00. Higher values indicate parameters which have the most effect on the model output compared to the base model output.

Table 1 - Quantitative Analysis for Point Source Parameters

Model Scenario	Adjusted Parameter	Adjusted Parameter Value	Change Factor	Bioaerosol Concentration Ratio of Adjusted Model/Base Model at various downwind distances				
A	Point Source Height	Base Parameter: 3 m		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
		30	10	0.00 (1.00)	0.00 (1.00)	0.01 (0.99)	0.26 (0.74)	0.62 (0.38)
		300	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
B	Point Source Velocity	Base Parameter: 1.1 m/s						
		11	10	0.04 (0.96)	0.04 (0.96)	1.00 (0)	1.00 (0)	1.00 (0)
		110	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.01 (0.99)	0.03 (0.97)
C	Point Source Temperature	Base Parameter: 19.7^{o}C						
		197	10	0.94 (0.06)	0.41 (0.59)	0.52 (0.48)	0.64 (0.36)	0.72 (0.28)
		1970	100	0.77 (0.23)	0.16 (0.84)	0.32 (0.68)	0.49 (0.52)	0.59 (0.41)
D	Point Source Diameter	Base Parameter: 3 m						
		3	10	0.07 (0.93)	0.06 (0.94)	0.36 (0.64)	0.47 (0.53)	0.54 (0.46)
		100	33*	0.02 (0.98)	0.01 (0.99)	0.06 (0.94)	0.15 (0.85)	0.24 (0.76)

**change factor limited by model parameter range*

Table 2 - Quantitative Analysis for Area Source Parameters

Model Scenario	Adjusted Parameter	Adjusted Parameter Value	Change Factor	Bioaerosol Concentration Ratio of Adjusted Model/Base Model at various downwind distances				
E	Area Source	Base Parameter: 3 m		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
		30	10					
				0.01	0.01	0.05	0.09	0.44

	Height			(0.99)	(0.99)	(0.95)	(0.91)	(0.56)
		300	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
F	Area Source Velocity	Base Parameter: 1.2 m/s						
		12	10	0.23 (0.77)	0.19 (0.81)	1.00 (0)	1.00 (0)	1.00 (0)
		120	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
G	Area Source Temperature	Base Parameter: 25.6^{o}C						
		256	10	0.74 (0.26)	0.33 (0.67)	0.01 (0.99)	0.01 (0.99)	0.02 (0.98)
		2560	100	0.07 (0.93)	0.04 (0.96)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)

Table 3 - Quantitative Analysis for Volume Source Parameters

Model Scenario	**Adjusted Parameter**	**Adjusted Parameter Value**	**Change Factor**	**Bioaerosol Concentration Ratio of Adjusted Model/Base Model at various downwind distances**				
H	Mid height of the volume source above ground	Base Parameter: 1.5 m		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
		15	10	N/A	0.00 (1.00)	0.02 (0.98)	0.07 (0.93)	0.09 (0.91)
		150	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
I	Vertical dimension of volume source	Base Parameter: 3 m						
		30	10	0.03 (0.97)	0.03 (0.97)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		300	100	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)

Note: N/A indicates where modelling was not possible

Table 4 - Quantitative Analysis for Particle Pollutant Parameters

Source Type	**Model Scenario**	**Adjusted Parameter**	**Adjusted Parameter Value**	**Change factor**	**Bioaerosol Concentration Ratio of Adjusted Models/Base Model at various downwind distances**				
Point	J	Deposition and Terminal	Base Parameter: 0.00001		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
			0.0001	10					
					1.00	1.00	1.00	1.00	1.00

		Velocity in (m/s)			(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
			0.001	100	0.99 (0.01)	1.00 (0.00)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)
			0.01	1000	0.00 (1.00)	0.01 (0.99)	0.01 (0.99)	0.01 (0.99)	0.02 (0.98)
	K	Particle Density in (kg/m^3)	Base Parameter: 1000						
			10000	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100000	100	1.00 (0.00)	1.01 (0.01)	1.00 (0.00)	0.99 (0.01)	0.99 (0.01)
			1000000	1000	1.01 (0.01)	1.05 (0.05)	0.99 (0.01)	0.94 (0.06)	0.89 (0.11)
	L	Particle Diameter in (m)	Base Parameter: 0.000001						
			0.00001	10	0.77 (0.23)	0.88 (0.12)	0.79 (0.21)	0.73 (0.27)	0.67 (0.33)
			0.0001	100	0.69 (0.31)	0.81 (0.19)	0.66 (0.34)	0.56 (0.44)	0.49 (0.51)
			0.001	1000	0.69 (0.31)	0.79 (0.21)	0.66 (0.34)	0.55 (0.45)	0.48 (0.52)
	M	Particle Mass Fraction (unitless)	Base Parameter: 0.1						
			0.2	2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.25	2.5	1.00 (0.00)	1.000 (0)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.5	5	1.00 (0.00)	1.000 (0)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Area	J	Deposition and Terminal Velocity in (m/s)	Base Parameter: 0.00001						
			0.0001	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.001	100	0.99 (0.01)	0.99 (0.01)	1.00 (0.00)	0.99 (0.01)	0.99 (0.01)
			0.01	1000	0.90 (0.01)	0.92 (0.08)	0.99 (0.01)	0.97 (0.03)	0.96 (0.04)
	K	Particle	Base Parameter: 1000						

		Density in (kg/m^3)	10000	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100000	100	1.00 (0.00)	1.00 (0.00)	1.02 (0.02)	1.01 (0.01)	1.01 (0.01)
			1000000	1000	0.99 (0.01)	1.00 (0.00)	1.15 (0.15)	1.07 (0.07)	1.04 (0.04)
	L	Particle Diameter in (m)	Base Parameter: 0.000001						
			0.00001	10	0.68 (0.32)	0.71 (0.29)	0.79 (0.21)	0.78 (0.22)	0.76 (0.24)
			0.0001	100	0.54 (0.46)	0.57 (0.43)	0.65 (0.35)	0.63 (0.37)	0.61 (0.39)
			0.001	1000	0.53 (0.47)	0.56 (0.44)	0.64 (0.36)	0.63 (0.37)	0.61 (0.39)
	M	Particle Mass Fraction (unitless)	Base Parameter: 0.1						
			0.2	2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.25	2.5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.5	5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Volume	J	Deposition and Terminal Velocity in (m/s)	Base Parameter: 0.00001						
			0.0001	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.001	100	1.00 (0.00)	0.99 (0.01)	0.98 (0.02)	0.97 (0.03)	0.96 (0.04)
			0.01	1000	0.96 (0.04)	0.95 (0.05)	0.79 (0.21)	0.71 (0.29)	0.66 (0.34)
	K	Particle Density in (kg/m^3)	Base Parameter: 1000						
			10000	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100000	100	1.00 (0.00)	0.99 (0.01)	0.97 (0.03)	0.96 (0.04)	0.95 (0.05)
			1000000	1000	0.92 (0.08)	0.89 (0.11)	0.62 (0.38)	0.47 (0.53)	0.39 (0.61)

	L	Particle Diameter in (m)	Base Parameter: 0.000001						
			0.00001	10	0.88 (0.12)	0.83 (0.17)	0.46 (0.54)	0.34 (0.66)	0.27 (0.73)
			0.0001	100	0.79 (0.21)	0.73 (0.27)	0.25 (0.75)	0.15 (0.85)	0.10 (0.90)
			0.001	1000	0.79 (0.21)	0.72 (0.28)	0.24 (0.76)	0.14 (0.86)	0.09 (0.91)
	M	Particle Mass Fraction (unitless)	Base Parameter: 0.1						
			0.2	2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.25	2.5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			0.5	5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)

Table 5 - Quantitative Analysis of the Effect of Particle Pollutant Parameters on Particle versus Gas Pollutant Modelling

Source Type	**Changed Parameter**	**Parameter Value**	**Bioaerosol Concentration Ratio of Pollutant Model/Gas Model at various downwind distances**				
			1 m	**10 m**	**100 m**	**250 m**	**500 m**
Point	Deposition and Terminal Velocity in (m/s)	0.00001	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.0001	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.001	0.99 (0.01)	1.00 (0.00)	1.00 (0.00)	0.99 (0.01)	0.99 (0.01)
		0.01	0.00 (1.00)	0.00 (1.00)	0.01 (0.99)	0.01 (0.99)	0.02 (0.98)
	Particle Density in (kg/m^3)	1000	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)	0.98 (0.02)
		10000	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)	0.98 (0.02)
		100000	0.98 (0.02)	1.00 (0.00)	0.99 (0.01)	0.99 (0.01)	0.98 (0.02)
		1000000					
			0.99	1.04	0.98	0.92	0.88

			(0.01)	(0.04)	(0.02)	(0.08)	(0.12)
	Particle Diameter in (m)	0.000001	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)	0.98 (0.20)
		0.00001	0.76 (0.24)	0.88 (0.12)	0.79 (0.21)	0.72 (0.28)	0.66 (0.34)
		0.0001	0.68 (0.32)	0.80 (0.20)	0.66 (0.34)	0.55 (0.45)	0.48 (0.52)
		0.001	0.68 (0.32)	0.79 (0.21)	0.65 (0.35)	0.54 (0.46)	0.47 (0.53)
		0.01	0.68 (0.32)	0.79 (0.21)	0.65 (0.35)	0.55 (0.45)	0.47 (0.53)
	Particle Mass Fraction (unitless)	0.1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.25	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Area	Deposition and Terminal Velocity in (m/s)	0.00001	1.00 (0.00)	1.000 (0)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.0001	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.001	0.99 (0.01)	0.99 (0.01)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.01	0.90 (0.10)	0.92 (0.08)	0.99 (0.01)	0.97 (0.03)	0.96 (0.04)
	Particle Density in (kg/m^3)	1000	0.98 (0.02)	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)
		10000	0.98 (0.02)	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.01)
		100000	0.98 (0.02)	0.98 (0.02)	1.01 (0.01)	1.00 (0.00)	0.99 (0.01)
		1000000					
			0.98	0.98	1.14	1.06	1.03

			(0.02)	(0.02)	(0.14)	(0.06)	(0.03)
	Particle Diameter in (m)	0.000001	0.98 (0.02)	0.98 (0.02)	0.99 (0.01)	0.99 (0.01)	0.99 (0.011)
		0.00001	0.67 (0.33)	0.70 (0.30)	0.78 (0.22)	0.77 (0.23)	0.76 (0.24)
		0.0001	0.53 (0.47)	0.56 (0.44)	0.64 (0.36)	0.63 (0.37)	0.61 (0.39)
		0.001	0.52 (0.48)	0.55 (0.45)	0.63 (0.37)	0.62 (0.38)	0.60 (0.40)
		0.01	0.52 (0.48)	0.55 (0.45)	0.64 (0.36)	0.62 (0.38)	0.60 (0.40)
	Particle Mass Fraction (unitless)	0.1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.25	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
Volume	Deposition and Terminal Velocity in (m/s)	0.00001	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.0001	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.001	1.00 (0.00)	0.99 (0.01)	0.98 (0.02)	0.97 (0.03)	0.96 (0.04)
		0.01	0.96 (0.04)	0.95 (0.05)	0.79 (0.21)	0.71 (0.29)	0.65 (0.35)
	Particle Density in (kg/m^3)	1000	0.99 (0.01)	0.99 (0.01)	0.97 (0.03)	0.95 (0.05)	0.95 (0.05)
		10000	0.99 (0.01)	0.99 (0.01)	0.97 (0.03)	0.95 (0.05)	0.94 (0.06)
		100000	0.99 (0.01)	0.99 (0.01)	0.94 (0.06)	0.92 (0.08)	0.89 (0.11)
		1000000					
			0.91	0.89	0.60	0.45	0.37

			(0.09)	(0.114)	(0.40)	(0.55)	(0.63)
	Particle Diameter in (m)	0.000001	0.99 (0.01)	0.99 (0.01)	0.97 (0.03)	0.95 (0.05)	0.95 (0.05)
		0.00001	0.87 (0.13)	0.82 (0.18)	0.44 (0.56)	0.32 (0.68)	0.25 (0.75)
		0.0001	0.79 (0.21)	0.72 (0.28)	0.24 (0.76)	0.14 (0.86)	0.09 (0.91)
		0.001	0.79 (0.21)	0.72 (0.28)	0.24 (0.76)	0.13 (0.87)	0.09 (0.91)
		0.01	0.79 (0.21)	0.72 (0.28)	0.24 (0.76)	0.13 (0.87)	0.09 (0.91)
	Particle Mass Fraction (unitless)	0.1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.2	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.25	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		0.5	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
		1	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)

Table 6 - Quantitative Analysis for Gas Pollutant Parameters

Source Type	**Model Scenario**	**Adjusted Parameter**	**Adjusted Parameter Value**	**Change factor**	**Bioaerosol Concentration Ratio of Adjusted Model/Base Model at various downwind distances**				
Point	N	Deposition Velocity	Base Parameter: 0.003 m/s		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
			0.03	10	0.85 (0.15)	0.93 (0.07)	0.89 (0.11)	0.84 (0.16)	0.81 (0.19)
			0.3	100	0.63 (0.37)	0.67 (0.33)	0.45 (0.55)	0.33 (0.67)	0.25 (0.75)
			3	1000	0.58 (0.42)	0.48 (0.52)	0.18 (0.82)	0.08 (0.92)	0.05 (0.95)
Area	N	Deposition Velocity	Base Parameter: 0.003 m/s						
			0.03	10	0.80 (0.20)	0.82 (0.18)	0.88 (0.12)	0.87 (0.13)	0.87 (0.13)

			0.3	100	0.37 (0.63)	0.39 (0.61)	0.42 (0.58)	0.40 (0.60)	0.38 (0.62)
			3	1000	0.20 (0.80)	0.20 (0.80)	0.12 (0.88)	0.09 (0.91)	0.08 (0.92)
Volume	N	Deposition Velocity	Base Parameter: 0.003 m/s						
			0.03	10	0.93 (0.07)	0.90 (0.10)	0.65 (0.35)	0.55 (0.45)	0.49 (0.51)
			0.3	100	0.69 (0.31)	0.59 (0.41)	0.08 (0.92)	0.03 (0.97)	0.01 (0.99)
			3	1000	0.56 (0.44)	0.45 (0.55)	0.01 (0.99)	0.00 (1.00)	0.00 (1.00)

Table 7 - Quantitative Analysis of the Effect of Gas Pollutant Parameters on Dry Deposition versus Non Dry Deposition Modelling

Source Type	**Changed Parameter**	**Parameter Value**	**Bioaerosol Concentration Ratio of Dry Deposition versus Non Dry Deposition Model at Various Downwind Distances**				
Point	Deposition Velocity in (m/s)		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
		0.003	0.97 (0.03)	0.99 (0.01)	0.99 (0.01)	0.98 (0.02)	0.97 (0.03)
		0.03	0.82 (0.18)	0.93 (0.07)	0.87 (0.13)	0.82 (0.18)	0.76 (0.24)
		0.3	0.61 (0.39)	0.66 (0.34)	0.45 (0.55)	0.32 (0.68)	0.25 (0.75)
		3	0.56 (0.44)	0.48 (0.52)	0.40 (0.60)	0.08 (0.92)	0.04 (0.96)
Area	Deposition Velocity in (m/s)	0.003	0.97 (0.03)	0.97 (0.03)	0.98 (0.02)	0.98 (0.02)	0.98 (0.02)
		0.03	0.77 (0.23)	0.80 (0.20)	0.87 (0.13)	0.86 (0.14)	0.85 (0.15)
		0.3	0.36 (0.64)	0.38 (0.62)	0.42 (0.58)	0.40 (0.60)	0.37 (0.63)
		3	0.19 (0.81)	0.19 (0.81)	0.12 (0.88)	0.09 (0.91)	0.07 (0.93)
Volume	Deposition Velocity in (m/s)	0.003	0.99 (0.01)	0.99 (0.01)	0.95 (0.05)	0.93 (0.07)	0.92 (0.08)
		0.03	0.92 (0.08)	0.89 (0.11)	0.62 (0.38)	0.51 (0.49)	0.44 (0.56)

		0.3	0.68 (0.32)	0.58 (0.42)	0.08 (0.92)	0.03 (0.97)	0.01 (0.99)
		3	0.56 (0.44)	0.45 (0.55)	0.01 (0.99)	0.00 (1.00)	0.00 (1.00)

Table 8 - Quantitative Analysis for Meteorological Parameters

Source Type	**Model Scenario**	**Adjusted Parameter**	**Parameter Value**	**Change Factor**	**Bioaerosol Concentration Ratio of Adjusted Model/Base Model at various downwind distances**				
Volume	O	Wind Speed	Base Parameter: 5 m/s		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
			0.5	10	N/A	N/A	N/A	N/A	N/A
			50	100	0.11 (0.89)	0.11 (0.89)	0.17 (0.83)	0.23 (0.77)	0.27 (0.73)
	P	Relative Humidity	Base Parameter: 10 %						
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100	100	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
	Q	Surface Temperature	Base Parameter: 5^{o}C						
			0.5	10	0.99 (0.01)	0.99 (0.01)	0.95 (0.05)	0.93 (0.07)	0.92 (0.08)
			50	100	1.09 (0.09)	1.11 (0.11)	1.33 (0.33)	1.99 (0.99)	2.28 (1.28)
Area	O	Wind Speed	Base Parameter: 5 m/s						
			0.5	10	N/A	N/A	N/A	N/A	N/A
			50	100	0.31 (0.69)	0.36 (0.64)	1.93 (0.93)	0.90 (0.10)	0.62 (0.38)
	P	Relative Humidity	Base Parameter: 10 %						
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100	100	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
	Q	Surface Temperature	Base Parameter: 5^{o}C						
			0.5	10	0.98 (0.02)	0.96 (0.04)	0.81 (0.19)	0.85 (0.15)	0.89 (0.11)
			50	100	N/A	N/A	N/A	N/A	N/A

Point	O	Wind Speed	Base Parameter: 5 m/s						
			0.5	10	N/A	N/A	N/A	N/A	N/A
			50	100	0.15 (0.85)	0.22 (0.78)	0.20 (0.80)	0.23 (0.77)	0.26 (0.74)
	P	Relative Humidity	Base Parameter: 10 %						
			1	10	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
			100	100	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.00 (0.00)
	Q	Surface Temperature	Base Parameter: 5°C						
			0.5	10	0.99 (0.01)	0.95 (0.05)	0.94 (0.06)	0.94 (0.06)	0.93 (0.07)
			50	100	1.17 (0.17)	1.92 (0.92)	2.02 (1.02)	2.04 (1.04)	2.28 (1.28)

Table 9 - Quantitative Analysis of the Effect of Meteorological Parameters on Site Met Data vs ADMS Pasquill Stability Class D Modelling

Source Type	**Adjusted Parameter**	**Adjusted Parameter Value**	**Bioaerosol Concentration Ratio Site Met Data/ ADMS Pasquill Stability Class D Model at various downwind distances**				
Volume			**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
	Wind Speed in (m/s)	0.5	N/A	N/A	N/A	N/A	N/A
		5	0.90 (0.10)	0.89 (0.11)	0.58 (0.42)	0.43 (0.57)	0.37 (0.63)
		50	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)
	Relative Humidity in (%)	1	0.90 (0.10)	0.89 (0.11)	0.58 (0.42)	0.43 (0.57)	0.37 (0.63)
		10	0.90 (0.10)	0.89 (0.11)	0.58 (0.42)	0.43 (0.57)	0.37 (0.63)
		100	0.90 (0.10)	0.89 (0.11)	0.58 (0.42)	0.43 (0.57)	0.37 (0.63)
	Surface Temperature in (°C)	0.5	0.89 (0.11)	0.88 (0.12)	0.55 (0.45)	0.40 (0.60)	0.34 (0.66)
		5	0.90 (0.10)	0.89 (0.11)	0.58 (0.42)	0.43 (0.57)	0.37 (0.63)
		50	0.98	0.98	0.95	0.86	0.84

			(0.02)	(0.02)	(0.05)	(0.14)	(0.16)
Area	Wind Speed in (m/s)	0.5	N/A	N/A	N/A	N/A	N/A
		5	0.92 (0.08)	0.85 (0.15)	0.78 (0.22)	0.73 (0.27)	0.70 (0.30)
		50	0.29 (0.71)	0.31 (0.69)	1.51 (0.51)	0.66 (0.34)	0.43 (0.57)
	Relative Humidity in (%)	1	0.94 (0.06)	0.88 (0.12)	0.98 (0.02)	0.86 (0.14)	0.79 (0.21)
		10	0.94 (0.06)	0.88 (0.12)	0.97 (0.03)	0.86 (0.14)	0.79 (0.21)
		100	0.94 (0.06)	0.88 (0.12)	0.97 (0.03)	0.86 (0.14)	0.79 (0.21)
	Surface Temperature in (^{o}C)	0.5	0.92 (0.08)	0.85 (0.15)	0.79 (0.21)	0.73 (0.27)	0.70 (0.30)
		5	0.94 (0.06)	0.88 (0.12)	0.97 (0.03)	0.86 (0.14)	0.79 (0.21)
		50	N/A	N/A	N/A	N/A	N/A
Point	Wind Speed in (m/s)	0.5	N/A	N/A	N/A	N/A	N/A
		5	0.82 (0.18)	0.70 (0.30)	0.59 (0.41)	0.49 (0.51)	0.40 (0.60)
		50	0.13 (0.87)	0.16 (0.84)	0.12 (0.88)	0.11 (0.89)	0.11 (0.89)
	Relative Humidity in (%)	1	0.82 (0.18)	0.70 (0.30)	0.59 (0.41)	0.49 (0.51)	0.40 (0.60)
		10	0.82 (0.18)	0.70 (0.30)	0.59 (0.41)	0.49 (0.51)	0.40 (0.60)
		100	0.82 (0.18)	0.70 (0.30)	0.59 (0.41)	0.49 (0.51)	0.40 (0.60)
	Surface Temperature in (^{o}C)	0.5	0.82 (0.18)	0.66 (0.34)	0.56 (0.44)	0.46 (0.54)	0.37 (0.63)
		5	0.82 (0.18)	0.70 (0.30)	0.59 (0.41)	0.49 (0.51)	0.40 (0.60)
		50	0.97 (0.03)	1.34 (0.34)	1.20 (0.20)	1.00 (0.00)	0.92 (0.08)

	Sampling Day 1			
ADMS Parameter/Sampling Location	On compost windrow wind tunnel 1	On compost windrow wind tunnel 2	On compost windrow wind tunnel 3	On compost windrow average wind tunnel
Site Surface Roughness (m)	0.2	0.2	0.2	0.2
Site Latitude (^{0})	52	52	52	52
Source type	Area	Area	Area	Area
Pollutant emission rate for actinomycetes	6385.2 cfu/m^2/s	4594 cfu/m^2/s	4339.5 cfu/m^2/s	5236 cfu/m^2/s
Pollutant type	Gas	Gas	Gas	Gas
Source height (m)	3	3	3	3
Source exit velocity (m/s)	0.8	0.7	0.9	0.8
Source exit temperature (^{0}C)	24.7	20.2	19	21.3
Source specific heat capacity-Cp (J/kg/^{0}C)	1012	1012	1012	1012
Source molecular weight (g/mol)	28.966	28.966	28.966	28.966
Efflux	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate
Stability class	D	D	D	D

	Sampling Day 2			
ADMS Parameter/Sampling Location	On compost windrow wind tunnel 1	On compost windrow wind tunnel 2	On compost windrow wind tunnel 3	On compost windrow average wind tunnel
Site Surface Roughness (m)	0.2	0.2	0.2	0.2
Site Latitude (^{0})	52	52	52	52
Source type	Area	Area	Area	Area
Pollutant emission rate for actinomycetes	321.4cfu/m^2/s	259 cfu/m^2/s	657 cfu/m^2/s	419 cfu/m^2/s
Pollutant type	Gas	Gas	Gas	Gas
Source height (m)	3	3	3	3
Source exit velocity (m/s)	0.8	0.9	0.9	0.9
Source exit temperature (^{0}C)	22	18.9	21.8	20.9
Source specific heat capacity-Cp (J/kg/^{0}C)	1012	1012	1012	1012
Source molecular weight (g/mol)	28.966	28.966	28.966	28.966
Efflux	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate
Stability class	D	D	D	D

	Sampling Day 3					
ADMS Parameter/Sampling Location	On compost windrow wind tunnel 1	On compost windrow wind tunnel 2	On compost windrow wind tunnel 3	On compost windrow average wind tunnel	Agitation source temp= ambient temp	Agitation source temp= 55degC
Site Surface Roughness (m)	0.2	0.2	0.2	0.2	0.2	0.2
Site Latitude (^{0})	52	52	52	52	52	52
Source type	Area	Area	Area	Area	Point	Point
Pollutant emission rate for actinomycetes	406 cfu/m^2/s	321 cfu/m^2/s	890 cfu/m^2/s	256 cfu/m^2/s	57,700,000 cfu/s	81,000,000 cfu/s
Pollutant type	Gas	Gas	Gas	Gas	Gas	Gas
Source height (m)	3	3	3	3	3	3
Source diameter (m)	N/A	N/A	N/A	N/A	3	3
Source exit velocity (m/s)	1.5	1.7	0.5	1.2	1.1	1.1
Source exit temperature (^{0}C)	22.5	28.5	25.9	25.6	19.7	55
Source specific heat capacity-Cp (J/kg/^{0}C)	1012	1012	1012	1012	1012	1012
Source molecular weight (g/mol)	28.966	28.966	28.966	28.966	28.966	28.966
Efflux	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate	Volumetric flow rate
Stability class	D	D	D	D	D	D

The values in brackets indicate the numerical deviation of ratio from 1.00. Higher values indicate parameters which have the most effect on the model output compared to the base model output.

Table 1 - Effect of Emission Rates on Downwind Comnentrations (Actinomycetes Concentration Ratio of Adjusted Model to Base Model)

Experiment Number	Adjusted Parameter	Adjusted Parameter Value	Change Factor	Actinomycetes Concentration Ratio of Adjusted Model/Base Model at Various Downwind Distances				
1	Emission Rate	Agitation emission rate **(A)**: 5.77×10^7 cfu/s Wind tunnel emission rate **(W)**: 5.24×10^3 cfu/m^2/s		**1 m**	**10 m**	**100 m**	**250 m**	**500 m**
		A: 1.15×10^8 **W:** 1.05×10^4	2	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)
		A: 2.89×10^8 **W:** 2.62×10^4	5	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)
		A: 2.89×10^7 **W:** 2.62×10^3	0.5	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)
		A: 1.15×10^7 **W:** 1.05×10^3	0.2	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)
2	Emission Rate	Agitation emission rate **(A)**: 5.77×10^7 cfu/s Wind tunnel emission rate **(W)**: 4.19×10^2 cfu/m2/s						
		A: 1.15×10^8 **W:** 8.38×10^2	2	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)
		A: 2.89×10^8 **W:** 2.1×10^3	5	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)
		A: 2.89×10^7 **W:** 2.1×10^2	0.5	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)
		A: 1.15×10^7 **W:** 8.38×10^1	0.2	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)
		A: 5.77×10^6 **W:** 4.19×10^1	0.1	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)	0.10 (0.90)
		A: 1.92×10^6 **W:** 1.40×10^1	0.03	0.03 (0.97)	0.03 (0.97)	0.03 (0.97)	0.03 (0.97)	0.03 (0.97)

3	Emission Rate	Agitation emission rate **(A)**: 5.77×10^{7} cfu/s Wind tunnel emission rate **(W)**: 2.56×10^{2} cfu/m2/s						
		A: 1.15×10^{8} **W:** 5.12×10^{2}	2	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)	1.99 (0.99)
		A: 2.89×10^{8} **W:** 1.28×10^{3}	5	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)	5.01 (4.01)
		A: 2.89×10^{7} **W:** 1.28×10^{2}	0.5	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)	0.50 (0.50)
		A: 1.15×10^{7} **W:** 5.12×10^{1}	0.2	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)	0.20 (0.80)

** these extra values also investigated to match the concentrations measured on site*