DETERMINING WHETHER ESTIMATED SPORE RELEASE RATES FOR ASPERGILLUS FUMIGATUS ARE COMPATIBLE WITH THEIR MEASURED GROWTH RATES IN COMPOSTING SYSTEMS

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EXECUTIVE SUMMARY

The composting process like other waste management activities has the potential to generate large concentrations of bioaerosols which can be widely dispersed into the surrounding environment. There has been considerable interest in the literature of the effect of bioaerosols and in particular Aspergillus fumigatus on the health of plant operators and those living in close proximity to composting plants (Olver, 1994; Fischer et al., 1999; Fischer et al., 2000; Bunger et al., 200). Although bioaerosols can be generated through a range of operational procedures it has also been suggested that large numbers of *Aspergillus fumigatus* spores can be emitted from static compost piles through the action of natural air movements across the surface of the biodegrading material. Through the use of a portable wind tunnel apparatus researchers have determined the rate of emission of *Aspergillus fumigatus* spores as a result of air movement across the surface of the material (Taha et al., 2004; 2005; 2006 & 2007). The aim of this work was to use controlled laboratory experiments using compost samples and *Aspergillus fumigatus* spores to determine the sporulation rate of *Aspergillus fumigatus*. Using this data it would then be possible to verify whether bioaerosol emission rates from static compost windrows calculated and quoted in the literature can in fact be maintained over longer time periods.

A series of experiments were carried out using *Aspergillus fumigatus* spores on agar plates and small samples of green waste compost to determine the number of spores that could be generated by each existing spore over a seven day period. From the experiments the sporulation rate determined from the agar plates varied with averages of either 4.48x 10^4 or 2.83 spores/day depending upon the date set used and from the compost experiments it was 1.33 spores/day. Using this data and making a number of assumptions for moisture content (50%), bulk density (650 kg/m^3) and the wind penetration depth (10-25mm) the potential release rates were calculated. The data from the agar experiments yielded a potential release rate at 10mm up to 5 orders of magnitude in excess of that quoted by Taha et al. (2004 & 2005. Using the trimmed data set the agar release figures are between 6.17 x 10^3 and 1.23 x 10^4 cfu/m^2/s depending upon the wind penetration depth used and are comparable to those quoted in the literature. The release rate calculated using the compost experiments was slightly lower than the agar 2 data and consequently the release rates even at a wind penetration depth of 25mm are just outside the range quoted by Taha et al. (2004 & 2005).

Although the data presented in this paper were determined from controlled laboratory experiments they show that it is possible for *Aspergillus fumigatus* to generate spores at a sufficient rate to allow the release of significant numbers of spores. The calculated sporulation rates would allow spores to be released at the rates quoted in the literature and suggest that the fugitive release rates quoted in the literature would be able to be maintained over long periods of time.

However it is recognised that the data is affected greatly by the assumptions one makes, in particular the existing concentration of *Aspergillus fumigatus*. Despite the limitations the original intention of the work was to attempt to establish the long term applicability of the fugitive release rates for *Aspergillus fumigatus* from green waste compost quoted in the literature and it has achieved this objective.

1 INTRODUCTION

1.1 Background

Composting in the UK has become one of the most widely used processes for the treatment of solid and semi-solid organic materials. It is a natural process of aerobic thermophilic bioconversion of organic waste into a stabilised end product that is free of odours and pathogens, will not attract vermin and can be used as a valuable horticultural resource. However the composting process like other waste management activities has the potential to generate large concentrations of bioaerosols which in the case of open composting activities such as windowing can be widely dispersed into the surrounding environment. The bioaerosols generated at composting plants are principally airborne microorganisms and microbial constituents that are released from the process where the movement of material is involved (Sanchez-Monedero et al., 2003). Such activities with the potential to generate bioaerosols involve the movement and agitation of material and include the delivery of fresh material, shredding, turning of compost piles and screening.

The release of bioaerosols is one of the main health concerns raised by the public in relation to composting sites both from the viewpoint of the health effects upon the waste management workers but also the residence within the immediate vicinity. There has been considerable interest in the literature of the effect of bioaerosols on the health of plant operators and those living in close proximity to composting plants and the potential impacts of bioaerosols on health are being discussed from both allergological and toxicological points of view (Fischer et al., 1999; Fischer et al., 2000; Bunger et al., 2000). According to Bunger et al. (2000) the high exposure to bioaerosols of compost workers is significantly associated with a higher frequency of health complaints and diseases as well as higher concentrations of specific antibodies against moulds and actinomycetes. Herr et al (2003) reported for the first time that residents exposed to bioaerosol pollution were shown to report irritative respiratory complaints similar to mucous membrane irritation independently of perceived odours. The results of Heldal et al (2003) suggested that a moderate exposure to fungal spores, endotoxins, and [beta](1->3)-glucans during waste handling induced upper airway inflammation.

Aspergillus fumigatus has been the most widely studies bioaerosol due to its abundance within the composting pile and it potential negative effects upon human health (Olver, 1994). It is an opportunistic human pathogen which if subject to prolonged exposure can cause a range of relatively minor to extremely serious health effects. According to Milner et al. (1994) the main health effects associated with the inhalation of *Aspergillus fumigatus* range from the extremely serious invasive aspergillosis, through allergenic brochopulmonary aspergillosis, acute alveolitis, asthma induced by aspergillosis and aspergillus sinusitis down to a variety of allergies. There is no definitive dose-response information available for *Aspergillus fumigatus,* although exposure limit figures have been suggested for bacterial bioaerosol release (Malmros et al., 1992; Sigsgaard et al., 1990).

Unlike enclosed systems whereby the exhaust air can be directed into some kind of abatement/treatment system such as a scrubber or biofilter the emissions from an open windrow system cannot be controlled in the same way. In these cases there is reliance upon the natural air movements in and around the piles to dilute the bioaerosols during dispersal down to safe levels. Although bioaerosols can be generated through a range of operational procedures it has also been suggested that large numbers of *Aspergillus fumigatus* spores can be emitted from static compost piles through the action of natural air movements across the surface of the biodegrading material (Taha et al., 2004; 2005; 2006 & 2007). Watson et al. (2000) suggested that wind speeds as low as 0.19 m/s had the potential to entrain dust particles and transport them large distances from the source. In the case of dust entrained from composting material it is probable that this will contain viable *Aspergillus fumigatus* spores. Through the use of a portable wind tunnel apparatus researchers have determined the rate of emission of *Aspergillus fumigatus* spores as a result of air movement across the surface of the material (Taha et al., 2004; 2005; 2006 & 2007). The emission rate was calculated by Taha et al. (2004 & 2005) from the wind tunnel data using the following equation:

$$\text{SBER} = \frac{QxBC}{A}$$

Where:

SBER = the specific bioaerosol emission rate at ~ 3m/s the air velocity used in the wind tunnel (cfu/m^2/s)

Q = flow rate of air through the wind tunnel (m/s)
BC = bioaerosol concentration in the air (cfu/m^3)
A = area covered of material covered by the wind tunnel

In order to determine the corresponding emission rate at ground level wind velocities ($SBER_2$) the following equation was used;

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

Where:
$SBER_2$ = corresponding to ground level air velocity (cfu/m^2/s)
V_1 = air velocity inside the wind tunnel (m/s)
V_2 = actual ground level air velocity (m,/s)

Using this method Taha et al. (2004 & 2005) estimated fugitive *Aspergillus fumigatus* emission rates in the wind tunnel ($SBER_1$) were between 3.6 and 5.4 x10^3 cfu/m^2/s and corrected for ground level wind speed ($SBER_2$) were between 8 and 11 x 10^3 cfu/m^2/s. using the same methodology but carried out at a different location Taha et al. (2007) quoted specific bioaerosol emission rates figures for *Aspergillus fumigatus* and actinomycetes of between 100 - 1200 cfu/m^2/s.

1.2 Research objectives

As outlined above the literature has suggested that bioaerosols and in particular *Aspergillus fumigatus* can be released as a constant source from static compost piles through the action of natural air movements across the material. The data presented gives a snapshot measurement of emission rates and therefore the aim of this work was to use controlled laboratory experiments using compost samples and *Aspergillus fumigatus* spores to determine the sporulation rate of *Aspergillus fumigatus*. Using this data it would then be possible to verify whether bioaerosol emission rates from static compost windrows calculated and quoted in the literature can in fact be maintained over longer time periods.

2 METHODOLOGY

Two sets of controlled laboratory based experiments were performed in order to determine the sporulation rates of *Aspergillus fumigatus*. The first set involved the inoculation of sterile malt extract agar plates with known numbers of spores and subsequent incubation and enumeration of colonies and spores over time. The second set were carried out using green waste compost samples taken from a full scale green waste windrow composting plant. The compost was incubated and monitored for *Aspergillus fumigatus* spore concentrations over a set period of time.

2.1 Preparation of the *Aspergillus fumigatus* stock spore solution

In order to carry out the first set of experiments it was necessary to produce a stock solution containing viable *Aspergillus fumigatus* spores. This was done by inoculating ten sterile malt extract agar plates as described above with 0.1ml of a suspension prepared by shaking 10g fresh green waste compost with 100ml of sterile distilled water. After incubation at 40°C for 48 hours the growth on the gar plates was removed by repeated washing of the agar surface on all the plates using sterile distilled water and collecting the washings into a sterile 50ml bottle. The concentration of spores in the stock solution was then determined as described in section 2.2 below. From this data the number of spores in the inoculum used in the agar plate experiments could be determined. The stock spore solution was stored in the refrigerator until required.

2.2 Enumeration of *Aspergillus fumigatus* spores

Aspergillus fumigatus culturing and enumeration was carried out according to the method of Fisher et al. (1998). Malt extract agar was prepared using 20g l^{-1} of malt extract agar and 15 g l^{-1} of bacteriological agar dissolved in 1 litre of deionised water. After autoclaving two antibiotics (streptomycin, 50 mg l^{-1} and novobiocin, 10 mg l^{-1}) were added to the

cooled medium in order to suppress bacterial growth. After addition of the antibiotics the agar was poured into sterile 90mm petri dishes and allowed to cool and set completely, after which the plates were stored in the refrigerator until required.

Enumeration of *Aspergillus fumigatus* was carried out on liquid suspensions generated from agar plate washings or compost suspensions prepared as described in sections 2.3 or 2.4 below. Serial dilutions of the suspension were prepared in bottles containing 9ml of sterile ringers solution and for each dilution 0.1ml was plated out onto a sterile malt extract agar plate. The 0.1ml aliquot was spread evenly over the surface of the gar and allowed to dry before being incubated at 40°C for 48 hours. After incubation the number of *Aspergillus fumigatus* colonies were counted.

2.3 Determination of sporulation rate using inoculated agar plates

The first set of experiments were carried out using prepared sterile malt extract agar plates. A total of twenty four plates were each inoculated with an aliquot of stock spore solution calculated to contain 6 spores. The inoculum was spread evenly over the surface of the plate using a sterile glass spreader. After allowing the plates to dry they were placed into an incubator and incubated at 40°C until removed for enumeration. Every day for a total of 7 days three of the plates were removed from the incubator and the number of *Aspergillus fumigatus* colonies were counted. For the purposes of this work it was assumed that each of the colonies had been produced from the germination of a single spore. Once the colonies had been counted the surface of the agar plates was washed repeatedly as described in section 2.1. The number of *Aspergillus fumigatus* spores in the resulting solution was determined as described in section 2.2. This process was repeated for all three replicates for 7 days.

2.4 Determination of sporulation rate using green waste compost samples

The second set of experiments were carried out using samples of part composted green waste taken from the windrows of a full scale green waste composting facility. Approximately 500g of compost was placed into each of twenty four 500ml plastic beakers and each beaker was placed into a large plastic bag. The bag was then partially sealed using a twisted tie sufficient enough to prevent the compost drying out too much and stop the escape of spores from the sample but still allow oxygen to penetrate into the head space. The concentration of *Aspergillus fumigatus* in the compost was enumerated on a daily basis by taking a10g sample from each of the three replicates and suspending each in 100ml of sterile deionised water. The suspensions were shaken for 15 minutes and the concentration of *Aspergillus fumigatus* determined using the method described in section 2.2 above. In order to express the concentration in terms of per gram dry weight of compost the moisture content of the three replicates was also determined by drying at 105°C for 24 hours and determining the weight of moisture lost.

3 RESULTS AND DISCUSSION

3.1 Determination of sporulation rate using inoculated agar plates

Table 1 shows the results obtained during the first set of experiments using inoculated agar plates. Each plate was initially inoculated with six spores and three replicates each day were monitored for colony growth and further spore production. It can be seen that in terms of the number of extra colonies produced each day the number is small compared to the number of spores produced.

TABLE 1 **The number of spores and colonies on the agar plates during the seven day experimental period**

Time	Replicate 1			Replicate 2			Replicate 3		
(Days)	Colonies	Spores	Total	Colonies	Spores	Total	Colonies	Spores	Total
0	6	1.1E+06	1.1E+06	8	1.7E+06	1.7E+06	7	2.8E+06	2.8E+06
1	10	3.8E+09	3.8E+09	13	5.0E+09	5.0E+09	8	3.4E+09	3.4E+09
2	73	3.2E+09	3.2E+09	35	5.4E+09	5.4E+09	109	4.1E+09	4.1E+09
3	8	9.0E+09	9.0E+09	7	1.7E+10	1.7E+10	8	2.9E+10	2.9E+10
4	7	3.6E+09	3.6E+09	11	4.5E+10	4.5E+10	12	2.8E+11	2.8E+11
5	6	3.2E+11	3.2E+11	5	4.1E+10	4.1E+10	4	3.2E+10	3.2E+10
6	11	5.0E+10	5.0E+10	7	4.5E+10	4.5E+10	18	2.5E+12	2.5E+12
7	6	1.1E+06	1.1E+06	8	1.7E+06	1.7E+06	7	2.8E+06	2.8E+06

Taking this data the mean number of *Aspergillus fumigatus* including visible colonies and spores can be plotted (Figure 1). It is apparent that there is an almost exponential increase in numbers over the first two days followed by a much lower progressive increase over the remaining five days of the study. One would expect the increase over the first few days to be significantly higher than the remainder as the initial six spores produce colonies which in turn generate very high numbers of spores.

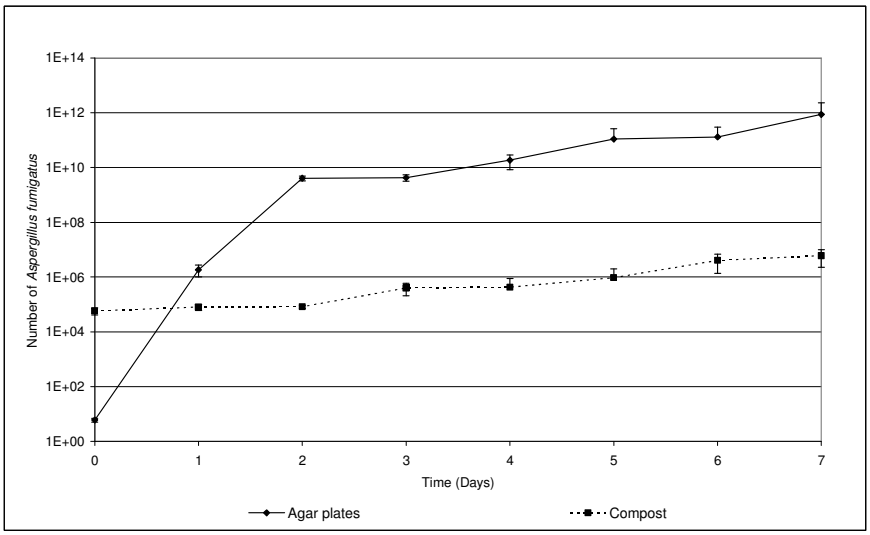

FIGURE 1 **The mean number of *Aspergillus fumigatus* present on the agar plates and in the compost samples during the seven day experimental period. (the error bars indicate one standard deviation)**

Figure 2 shows the daily increase in the number of *Aspergillus fumigatus* on the agar plates measured over the seven day period. It can be seen that approximately every 24 hours the number of additional spores generated ranged from 1.87×10^6 up to 7.34×10^{11} with a mean of 1.24×10^{11}. If this data is then related to the number of spores already on the plates then the number of spores generated per spores per day can be calculated and ranged from 0.04 up to 3.11×10^5 with a mean of 2.83. Therefore from this data it would appear that in a controlled experiment under ideal growth conditions each spore of *Aspergillus fumigatus* is capable of producing an additional 4.48×10^4 spores per day. However if the data from the first two days is omitted as being atypical then the average additional spores produced per existing spore is only 2.83.

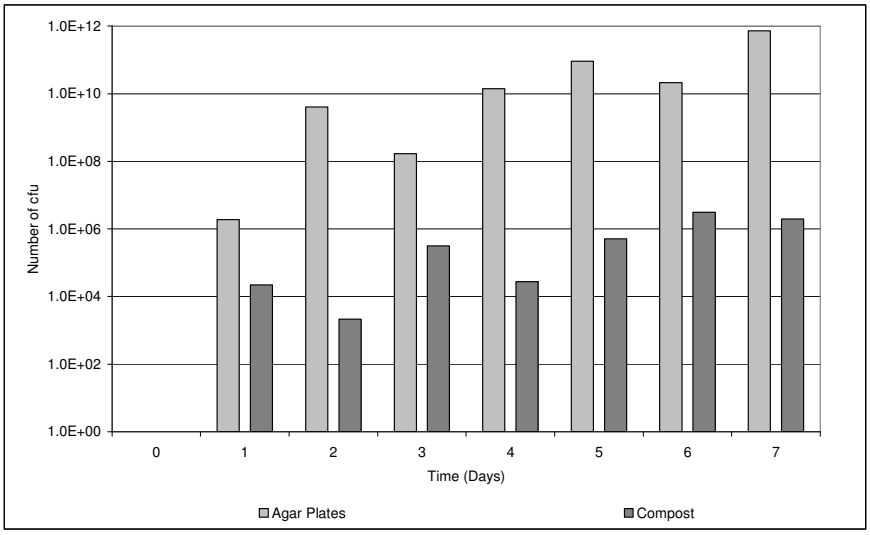

FIGURE 2 **Average daily increase in the number of aspergillus on agar plates and in compost samples**

3.2 Determination of sporulation rate using green waste compost samples

Table 2 shows the concentration of *Aspergillus fumigatus* in the compost samples over the experimental period of one week. This data was used to calculate the mean concentrations in the compost samples and is presented in Figure 1. It can be seen that the data is very different to that obtained on the agar plates with no exponential increase in the early days of the study due to the fact that there was a large existing population of *Aspergillus fumigatus* within the initial compost sample.

TABLE 2 **The concentration of Aspergillus fumigatus in the compost samples during the seven day experimental period (cfu/gdw)**

Time (Days)	*Aspergillus fumigatus* cfu/gdw		
	Replicate 1	Replicate 2	Replicate 3
0	4.30E+04	5.50E+04	7.60E+04
1	8.10E+04	6.00E+04	9.90E+04
2	9.18E+04	8.36E+04	7.10E+04
3	2.03E+05	5.85E+05	4.11E+05
4	2.04E+05	9.48E+05	1.30E+05
5	3.83E+05	2.12E+06	3.29E+05
6	9.41E+05	5.49E+06	5.75E+06
7	8.76E+06	7.65E+06	1.73E+06

Figure 2 shows the daily increase in the number of *Aspergillus fumigatus* within the compost samples measured over the seven day period. It can be seen that approximately every 24 hours the number of additional spores generated ranged from 2.13 $x10^3$ up to 73.12 x 10^6 with a mean of 8.56 x 10^5. If this data is then related to the concentration of Aspergillus fumigatus already contained within the compost then the number of spores generated per spores per day can be calculated and ranged from 0.03 up to 3.87 with a mean of 1.33. Therefore from this data it would appear that when a sample of green waste compost is incubated in this manner each spores of the population of *Aspergillus fumigatus* within the compost is capable of producing an additional 1.33 spores per day.

Using the sporulation rate data generated during the experiments it should be possible to calculate the number of spores available for release from a green waste windrow making a number of assumptions for moisture content, bulk density and the depth of the contributing compost layer and to relate this to the figures measured by Taha et al. (2004, 2005 & 2007). The experiments yielded three figures for sporulation rate, two from the agar plates 4.48x 10^4 spores per/spore/day and 2.83 spores /spore/day, and one from the compost experiments at 1.33 spores/spore/day.

Assuming a bulk density of 650 kg/m^3 and a moisture content of 50% and using the *Aspergillus fumigatus* concentration from the original green waste compost used in the experiments the potential release rates were calculated. This data is presented in Table 3 for a range of wind penetration depths and using the three sporulation rates determined from the experiments.

TABLE 3 **Calculated concentration of *Aspergillus fumigatus* spores available for release from a green waste compost pile using different wind penetration depths**

Wind Penetration Depth (mm)	Number of Aspergillus fumigatus available for release (cfu/m^2/s)		
	Agar 1 (4.48 x 10^4)	Agar 2 (2.83)	Compost (1.33)
10	9.76E+07	6.17E+03	2.90E+03
15	1.48E+08	9.38E+03	4.41E+03
20	1.95E+08	1.23E+04	5.80E+03
25	2.44E+08	1.54E+04	7.25E+03

Taha et al. (2004 & 2005) found using spot measurements that between 8 and 11 x 10^3 cfu/m^2/s were being released during his study. Comparing this data to that in Table 3 shows that using the whole data set from the agar plate experiments to calculate the sporulation rate yields a potential release rate up to 5 orders of magnitude in excess of that

quoted by Taha et al. (2004 & 2005) even at only 10mm wind penetration depth. If on the other hand the first two days data are disregarded then the calculated release rates are between 6.17 x 10^3 and 1.23 x 10^4 cfu/m^2/s depending upon the wind penetration depth used. This data is comparable to that quoted by Taha et al. (2004 & 2005) assuming between 15 and 20mm wind penetration. The release rate calculated using the compost experiments was slightly lower than the agar 2 data and consequently the release rates even at a wind penetration depth of 25mm are just outside the range quoted by Taha et al. (2004 & 2005).

4 CONCLUSIONS

Although the sporulation data presented in this paper were determined from controlled laboratory experiments they show that it is possible for *Aspergillus fumigatus* to generate spores at a sufficient rate to allow the release of significant numbers of spores every second. The calculated sporulation rates would allow spores to be released at the rates quoted in the literature without depleting the concentration of *Aspergillus fumigatus* remaining in the compost material. This suggests that the fugitive release rates quoted in the literature would be able to be maintained over long periods of time.

However it is recognised that the data is affected greatly by the assumptions one makes regarding moisture content, bulk density etc. and in particular the existing concentration of *Aspergillus fumigatus* in the compost material. Despite the limitations the original intention of the work was to attempt to establish the long term applicability of the fugitive release rates for *Aspergillus fumigatus* from green waste compost quoted in the literature and it has achieved this objective.

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