Cranfield University

School of Applied Sciences

PhD Thesis

Development and Evaluation of a Rapid Enzymatic Hydrolysis Test Method to Assess the Biodegradability of Organic Waste

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November 2008

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Abstract

The amount of biodegradable municipal waste (BMW) that can be disposed of in a landfill must be reduced, in accordance with the landfill allowance trading scheme (LATS) in England and Scotland (LAS in Wales). Biodegradability test methods are used to monitor the quantities of BMW diverted by waste treatment processes.

This research has outlined the requirements for timescale improvements on the currently used methods. The rapid (<24 h) enzymatic hydrolysis test (EHT) has been developed and the relationship of this with the long-term BM100 test has been compared with that of the established DR4 method. A range of untreated and treated organic waste materials taken from a number of treatment processes, and samples taken over a period of 9 months from a single treatment facility were analysed using each test method. The EHT is completed within 1 day, compared with 4 days for the current DR4 method, and was shown to possess a stronger correlation with the long-term BM100 test. This finding indicated the suitability of the EHT as an alternative short-term test method.

A humic substance extraction step was added to the EHT procedure, which was expected to provide a more accurate estimation of sample biodegradability. This technique was, however, found to be unsuitable for use in a short-term test method based on the results presented, although further understanding of the processes involved in the EHT has been discussed.

This thesis presents a new biodegradability test method, which has been developed, applied and evaluated. The processes of the EHT have been investigated, understood and discussed. Further developments are suggested based on the findings and observations throughout the thesis.

Acknowledgments

Defra provided the funding for the overall waste characterisation project undertaken by WRc plc, who in turn directly provided the funding required for this PhD research as part of that project. For this financial support and opportunity I am extremely grateful.

I would like to thank Dr Andy Godley of WRc plc, who as the industrial supervisor provided invaluable input right from the beginning of the PhD project. We have had many interesting discussions and debates about the issues covered in this thesis.

My sincere gratitude goes to Dr Sean Tyrrel, who has shown a great deal of interest in the project, and has provided valuable input and advice throughout. His enquiring and logical approach to understanding my ideas and thoughts enabled me to take a focused, yet, independent approach to my work.

Additionally I would like to thank Dr Gill Drew for the useful feedback on the written elements of this work, and Dr Phil Longhurst for the helpful advice and support given during my time at Cranfield. Gratitude also goes to Dr Emma Goslan, who provided assistance in the design of the humic extraction work described in this thesis. I would also like to thank the students of Water Sciences and the Centre for Resource Management and Efficiency for making the past three years so enjoyable.

I would also like to thank my parents who have always been very supportive, and proud, of everything I've done. Finally my utmost appreciation goes to Sarah, who has been incredibly supportive, encouraging and patient throughout my time at Cranfield University.

Acronyms

AD	Anaerobic digestion
BM100	Biochemical methane production over 100 days
BMP	Biochemical methane potential
BMW	Biodegradable municipal waste
COD	Chemical oxygen demand
DEFRA	Department of Environment, Food and Rural Affairs
DM	Dry matter
DOC	Dissolved organic carbon
DRI	Dynamic respiration index
EA	Environment agency
ECD	Enzymatic cellulose degradation (test)
EHT	Enzymatic hydrolysis test
ELWA	East London waste authority
FA	Fulvic acid
FI	Frog Island (waste treatment facility)
FT-IR	Fourier transform infra-red (spectroscopy)
HA	Humic acid
HS	Humic substances
LAS	Landfill allowance scheme

LATS	Landfill allowance trading scheme
LOI	Loss-on-ignition
MBT	Mechanical biological treatment
MSW	Municipal solid waste
P(1, 2 or 3)	Phase 1, 2 or 3 of the EHT
RI	Respiration index
SOUR	Specific oxygen uptake rate
SRF	Solid recovered fuel
SRI	Static respiration index
TOC	Total organic carbon

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Chapter One

Introduction

This chapter aims to provide a brief background on the topics that lead to the beginning of the research project. Here an overview is given of the project, including the detailed aims and objectives, the research questions and details of the research design.

1.1. Background

In 1999, the council of the European Union passed the Landfill Directive (1999/31/EC) (Council of the European Union, 1999). This directive provided European countries with landfill diversion targets in which the amount of biodegradable municipal waste (BMW) disposed of in landfill must be reduced.

These targets are progressive, to 75% of the 1995 baseline figure by 2006, 50% by 2008 and finally 35% by 2016 (Bench *et al.*, 2005; Council of the European Union, 1999; Price, 2001). For countries where landfill represents a predominant disposal route (e.g. England) these target years are extended to 2010, 2015 and 2020 respectively.

In 2005 the UK government set out the Landfill Allowances Trading Scheme (LATS) and the Landfill Allowance Scheme (LAS) in Wales. This scheme presents BMW landfill allowances (tonnes of BMW) to each local authority. These allowances are reduced each year, and so collectively the Landfill Directive targets can be fulfilled. The LATS targets are flexible, and so local authorities can trade allowances, in order to meet their requirements. This provides an additional incentive to exceed the diversion targets, allowing the sale of allowances to other local authorities.

BMW diversion is achieved in a number of ways. Primarily the amount of waste produced could be reduced, for example encouraging home recycling and composting. However, at least in the short term, there will be BMW produced, which must be diverted from landfill. The waste can be processed to produce a solid recovered fuel (SRF), which is used in energy from waste processes, such as incineration. Alternatively the biodegradable components can be treated, to reduce the biodegradable content, prior to disposal in landfill.

An increasingly common treatment process being used to divert BMW from landfill is mechanical biological treatment (MBT). MBT, however, is not a single technology, but a generic term assigned to various combinations of biological and mechanical treatment processes. The waste is mechanically shredded and sorted, and then biologically treated. The mechanical stage typically shreds the waste material, and removes the recyclable fraction of the waste, such as metals, glass and plastics. The biological treatment stage can occur prior to mechanical sorting. Biological treatment

is commonly anaerobic digestion (AD) or composting. Figure 1.1 provides a generic outline of MBT processes.

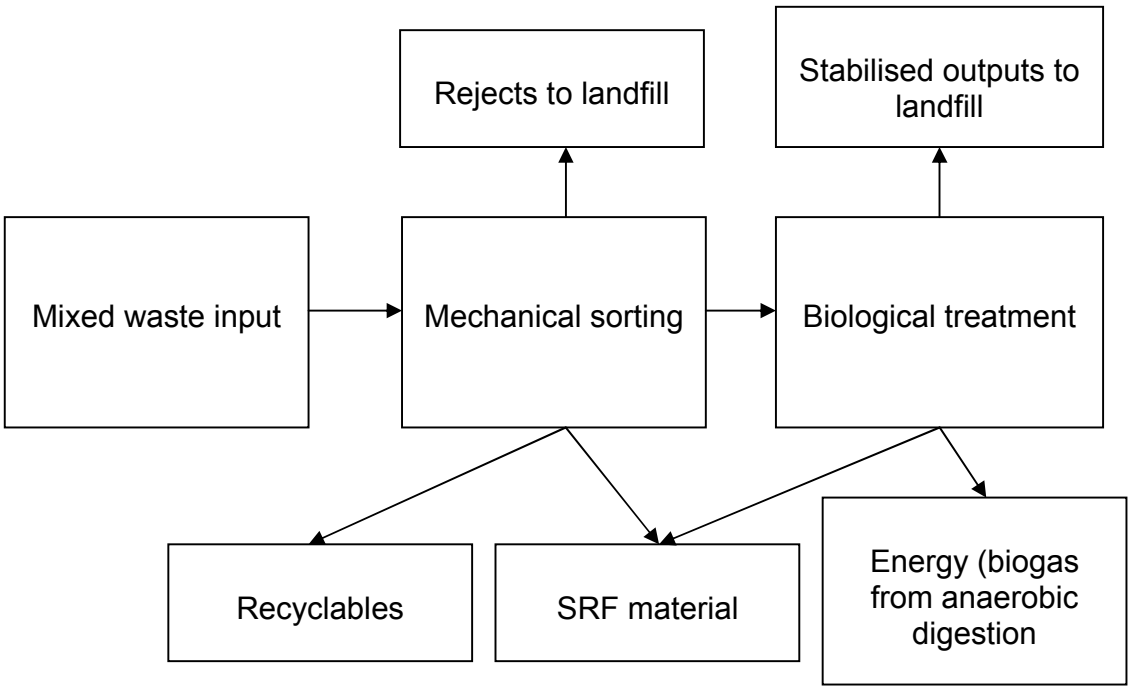

Figure 1.1. General flowchart of an MBT process.

The main objective of the EU landfill directive (1999/31/EC) is to reduce emissions of methane from landfills. Methane is a greenhouse gas, with a global warming potential of 21-23 times that of carbon dioxide over a 100 year period (IPCC, 1995; Lassey, 2007). Treating organic waste material by AD releases methane, however this is combusted to produce carbon dioxide, and electricity. AD can contribute to the reduction of methane emissions and provide a renewable source of energy. Composting is generally an aerobic process, and so methane release is reduced by instead oxidising organic waste to carbon dioxide. The carbon dioxide and methane productions are summarised in Figure 1.2.

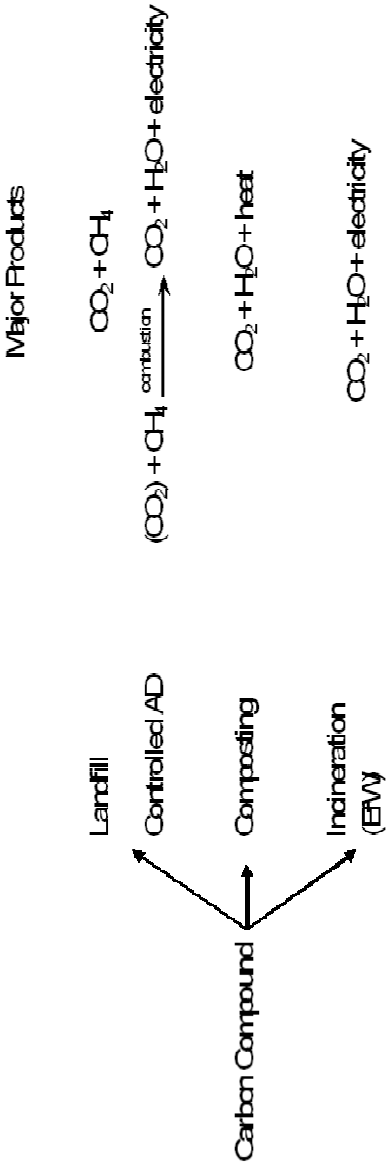

Figure 1.2. Basic overview of CO_2 and CH_4 emissions for waste disposal options.

The calculation of BMW diversion from landfill is outlined in the Environment Agency guidance on monitoring MBT and other pre-treatment processes for the landfill allowances trading scheme (Environment Agency, 2005). The biodegradable content of waste material taken from the input and output of the waste treatment process can

indicate the amount of biodegradable content removed by the process, and thus quantify how much BMW has been diverted from landfill.

The methods of biodegradability assessment are critically discussed in chapter 2. In England and Wales, the 4 day aerobic DR4 and 100 day anaerobic BM100 test method are the prescribed test methods for biodegradability assessment. In Scotland, the loss-on-ignition (LOI) test is used to assess the BMW diversion.

During the initial commissioning of a treatment process the BM100 method is used on a quarterly basis to calculate BMW diversion, with parallel DR4 analysis taking place to provide a correlation with BM100 data (Environment Agency, 2007a). As the DR4 correlation for that process is established and approved over a two year period, then the requirement for BM100 analysis becomes less frequent and the treatment operator is only obliged to use the shorter and therefore more cost-effective DR4 test method. The DR4 data can then be used to calculate BM100 data. The BM100 values are used to determine the amount of biogas (m^3) which would be produced under landfill conditions. The percentage reduction for the input and output samples is then related to the percentage of BMW diverted from landfill, and thus allows the calculation of mass (tonnes) diverted.

Biodegradability testing is therefore an important feature of waste treatment processing monitoring, providing information on the amount of BMW diverted. Under the LATS initiative, local authorities may sell surplus allowances, or they may be required to purchase some from another local authority, or borrow allowances from the

following year in the event that they are unable to meet the targets for that year. There is a financial incentive for local authorities to accurately quantify BMW diversion and avoid under-calculating the quantity, either to sell landfill allowances, or to avoid having to acquire any.

There is a significant financial benefit of a rapid biodegradability test method to waste treatment operators in that feedback on the process can be provided sooner, allowing optimisation of the process. For example, the process can be shortened if biostability is reached sooner, reducing the costs to the operator. Likewise the process can be lengthened if there were clear benefits in terms of a significant increase in BMW diversion. A more rapid biodegradability test method could also be more cost efficient, in that the labour time is significantly reduced- a saving which could be passed on to waste treatment operators if the analysis was done by commercial laboratories.

1.2. Basis of research project

Prior to this research, the demand for a more rapid and robust alternative biodegradability test method was recognised (Godley *et al.*, 2003). A test method based on the enzymatic hydrolysis of cellulose was initially described (Rodriguez *et al.*, 2003) and was evaluated as a possible alternative method (Godley *et al.*, 2004). The method used by Godley *et al.* (2004) was applied to a small range of organic waste material, indicating a correlation with a generic biochemical methane potential (BMP) method of r = 0.74 (11 samples). This indicated that a method based on enzymatic hydrolysis of cellulose could offer a potential rapid alternative methodology.

It was recognised by Godley *et al.* (2004) that this method required extensive further research to understand the characteristics of organic waste material, the biodegradation process and the results of such a test method. In addition to further understanding and research, refinement and validation of the test method was necessary if the test was to be adopted as an alternative method. The requirements and criteria for an alternative test method are discussed in later chapters.

The development of the enzymatic hydrolysis test (EHT) method is one component of a waste characterisation research program, sponsored by the Department of environment, food and rural affairs (Defra). The overall Defra project WR0110 (formerly WRT220) was a collaboration between Cranfield University, The Open University and WRc plc (project lead).

The fundamental difference between the currently used biodegradability test methods and the EHT is that the EHT is non-biological. As will be discussed in greater detail in Chapter 2, microbial based test methods such as the BM100 and DR4 are reliant on biological activity. This means that potentially there will be differences in microbial populations every time the test is used, leading to variation in the results produced. The EHT is not dependent on microbial populations, and so this is a potential advantage of the EHT over existing microbial methods.

This thesis covers the development of an alternative test method following early work by Godley *et al*. The test method was drafted based on typical hemicellulose and

cellulose proportions of organic waste material, and the enzyme ratio (hemicellulase to cellulase) determined accordingly.

1.3. Aims and objectives

The aim of this PhD project was to develop and evaluate an alternative test method of measuring the biodegradability of organic-based waste streams, such as municipal solid waste (MSW). Such a test method would enable the estimation of long term biodegradability through correlation with the long-term anaerobic BM100 test method. The test method developed should be cost-effective and be applicable to a wide variety of wastes, offering a significant timescale improvement to the currently available short-term biological DR4 test method outlined in the Environment Agency MBT monitoring guidance (Environment Agency, 2005).

The alternative test method will not be developed to measure the full extent of organic waste biodegradability, rather than to allow for the estimation of this through correlation with the BM100 method.

The project has the following objectives -

1. To produce a critical literature review of the existing methods used to measure the biodegradability of various waste streams, including MSW. To identify the advantages and disadvantages of each method, and discuss the requirements for an alternative test method, and how this can improve on current methods.

2. Using the current knowledge and principles, design a method based on the principle of enzymatic hydrolysis of hemicellulosic/cellulosic materials. Optimise the methodology with regards to the pH and temperature conditions.
3. Investigate the use of autoclaving and the effects of particle size on test reproducibility.
4. Apply the EHT to a wide range of untreated and treated organic waste samples using the optimum conditions determined, and compare with existing microbial methods.
5. Apply the EHT to a waste treatment process over an extended period of time.
6. Investigate the use of a humic extraction technique within the EHT procedure.

Specific hypotheses are described for each individual Chapter, however for the beginning of the PhD project the following overarching hypotheses were formulated-

"A method based on the enzymatic hydrolysis of organic waste materials would provide a rapid and reliable biodegradability test."

"The enzymatic hydrolysis test would have a stronger relationship with the long-term anaerobic BM100 method and offer a suitable alternative to the DR4 test method."

Following the initial development and application of the test method, further work was identified, and the following hypothesis formulated-

"The incorporation of a humic substance extraction method within the developed enzymatic hydrolysis test would enable a more accurate indication of sample biodegradability."

As discussed in later Chapters, the humic substance extraction method was a recently developed novel approach reported by Van Zomeren *et al* (2007). Therefore this thesis discusses the development of an innovative rapid biodegradability test, followed by the combination of the novel biodegradability test method with a novel humic substance extraction method. Therefore, this thesis demonstrates a series of significant scientific advancements in organic waste characterisation.

1.4. Thesis Overview

This thesis is presented in the format in which each experimental Chapter contains a methods, results and discussion section. Several parts of this thesis have been published or accepted for publication in journal and conference proceedings, each of which has been peer reviewed. These publications can be found in the Appendix section.

This chapter has provided the background topics and the objectives of the research, and the following Chapters provide a detailed account of the stages in the research, development and enhancement of the EHT method. This is followed by a final conclusions Chapter, which summarises the key findings of the thesis and emphasises the contribution to knowledge made by this work.

Figure 1.3 provides a basic outline of the PhD thesis.

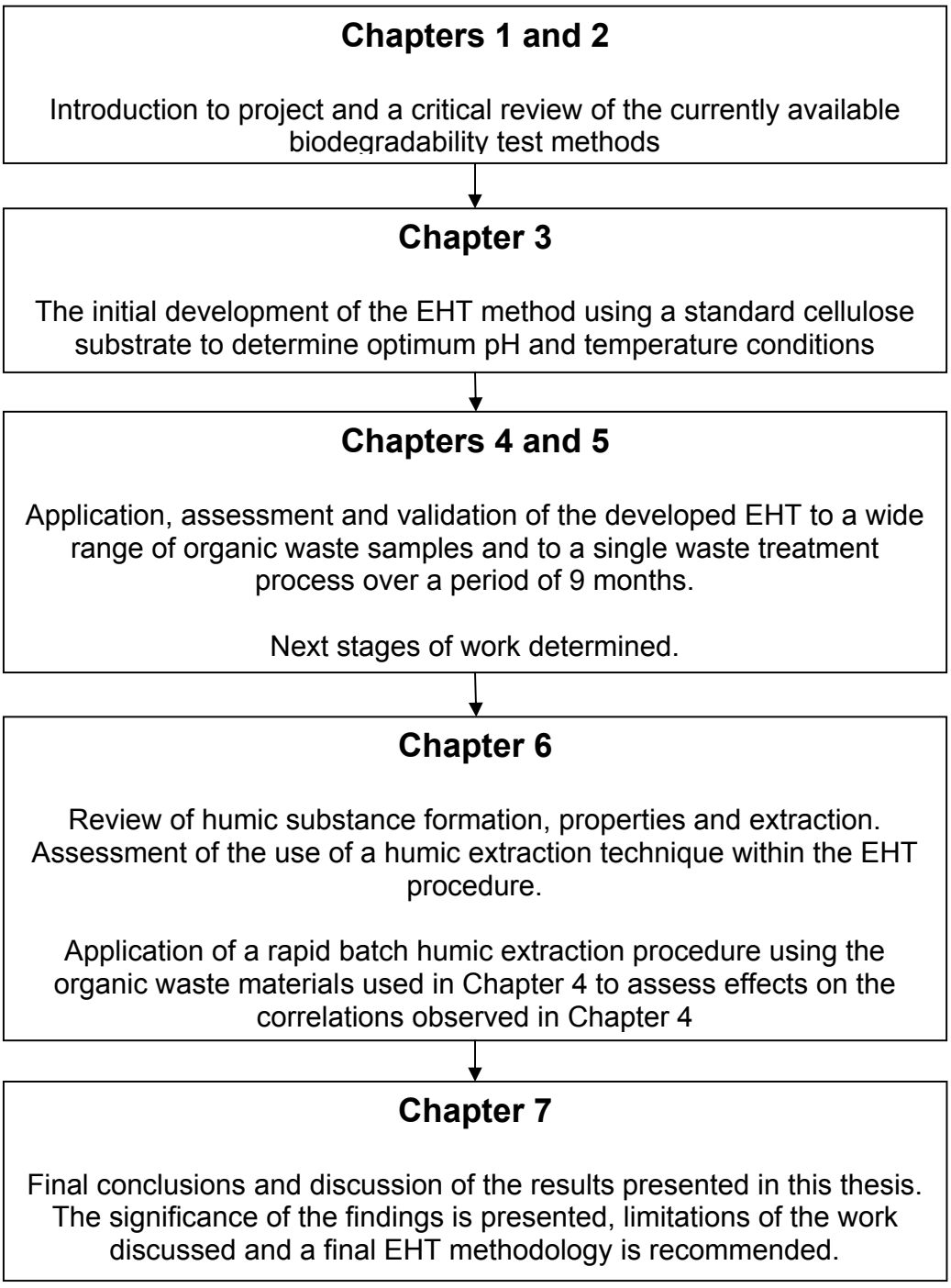

Figure 1.3. Basic overview of PhD thesis.

Chapter Two

Literature Review

This chapter critically reviews the available test methods available to assess the biodegradability of organic waste materials. The test methods are reviewed for the suitability to aid in the monitoring of biodegradable municipal waste (BMW) diversion from landfill. A modified form of this chapter has been published in the Waste Management journal (see Appendix F).

2.1. Introduction

The previous chapter discussed and introduced the context and aims of the overall project. The aim of this research is to develop and evaluate a rapid and reliable biodegradability test method. To achieve this aim it is necessary to review the currently available test methods used to assess the biodegradability of organic waste materials, and evaluate the limitations of such techniques. This chapter therefore provides a critical review of commonly used test methods.

Tests used to estimate biodegradability are an important part of organic waste characterization since they can be used for assessing the biological stability of wastes (Adani *et al.*, 2002; Iannotti *et al.*, 1993) or for assessing the diversion of

biodegradable waste from landfill (Godley *et al.*, 2003). A wide range of biological and non-biological test methods are available. Biological test methods based on the use of aerobic respiration indices have been recently reviewed for assessing the bio-stability of organic waste and/or compost materials (Gomez *et al.*, 2006). Suitability factors of the test methods include the timescale, applicability to a wide range of materials and ability to indicate the long-term biodegradability of organic waste samples.

The basic principle of tests to estimate biodegradability is to assess how much of the carbon can be mineralized and how quickly it will be degraded. The biodegradability tests can be used to assess the effectiveness of a certain treatment process. This is achieved by taking a representative sample of the waste before it goes through the treatment process, and then taking another sample post-treatment (Environment Agency, 2005). The degree to which the biodegradability of the waste is reduced indicates the effectiveness of the treatment process. Rapid and reliable methods are needed to allow for more frequent optimization of a treatment process (i.e. reduce the treatment time if applicable) offering financial benefits to the operator.

Several countries have preferred methods of measuring biodegradability, however each biodegradability test method has been developed for a specific purpose e.g. compost stability. This review focuses on the applicability of each test method to assess treatment process performance and aid in the quantitative evaluation of the diversion of BMW from landfill. A range of potentially applicable test methods are

discussed, with suggestions for further research and development being drawn from the overall conclusions.

2.2. Waste Biodegradability Test Methods

2.2.1. Anaerobic Test Methods

Anaerobic test methods measure the biodegradability of a substrate under anaerobic methanogenic conditions. This particular type of method measures the release of 'biogas' (CO_2 and CH_4), typically using a digester sludge seed as a source of microbes (Godley *et al.*, 2007b). Under anaerobic conditions, in the absence of oxygen, organic materials decompose in the presence of methanogenic bacteria, which release carbon dioxide and methane gas. This is shown in the following example for cellulose and hemicellulose respectively:

$$(C_6H_{10}O_5)n + nH_2O \rightarrow 3nCH_4 + 3nCO_2$$

$$2(C_5H_8O_4)n + 2nH_2O \rightarrow 5nCH_4 + 5nCO_2$$

Anaerobic test methods, such as the generic biochemical methane potential (BMP), are bioassays in which a sample is incubated in a temperature controlled system, where the nutrients and bacteria are also added to allow optimized microbial methanogenic conditions. Such anaerobic methods describe the bioassay procedures, with variations in the methods including the nutrients added and the method of biogas measurement. Table 2.1 provides a summary of the key aspects of

a number of anaerobic test methods. Although other methods are available in the literature, these are only slightly different to those cited that they have not been included in this review.

Method	Temp/ °C	Length of Test	Moisture	Seed	Sample Size	Sample Preparation	Biogas Measurement Method	Reporting Units	References
Owen *et al.*	35	30 days. Majority of gas production in first 20 days, however still activity after 30 days	No Moisture Adjustment	Mesophilic (35°C) digested sludge	Peat samples <2 g/L degradable COD content	30% CO_2 70%N_2 passed through at flow rate of 0.5 L/min for 15 min	Glass syringes equipped with 20 gauge needles	m^3 CH_4/kg COD m^3 CH_4/kg TS	(Owen, Stuckey *et al.*, 1979)
Shelton and Tiedje	35	8 weeks	Chemical compounds used, therefore no moisture adjustment	Sewage sludge from municipal digesters sparged with 10% CO_2 90% N_2	50 µg C/mL	Not waste samples, therefore no preparation	UniMeasure pressure transducer equipped with a P-8 bellows	Net gas production (mL) Percentage of theoretical gas production (%)	(Shelton and Tiedje, 1984)
Pagga and Beimborn	35	60 days	No moisture adjustment	1-3 g/L TS digested sludge	100 mg/l C (20 mg/L if sample is toxic)	Sparged with nitrogen. Adjusted to pH 7	Pressure measurement (mbar)	Total percentage of biodegradation, D_T (%)	(Pagga and Beimborn, 1993)
Stinson and Ham	35	60 days	No moisture adjustment	10% digested sludge solution prepared with inoculum	0.05 g non-lignin substrate (mass of sample = 0.05 g/ (1- lignin fraction)	Dried and screened to 2 mm Purged with N_2 gas to remove O_2	Gas samples collected from bottles following incubation measured using gas chromatography	Rate of cellulose decomposition calculated	(Stinson and Ham, 1995)
Kelly *et al.*	35	45 days	No moisture adjustment	Sludge from anaerobic digester 10% by volume inoculum	2 g MSW sample	Dried and shredded <10 mm	Gas samples taken at end of incubation period were analyzed using gas chromatography	Milliliters of methane per gram of dry MSW (mL/g)	(Kelly, Shearer *et al.*, 2006)
Incubation test GS90	40	90 days	Saturated to water holding capacity	No seed Sample is fresh and moist	1 kg DM plus water	Sieved to <20 mm	Pressure measurement by 'Eudiometer' Gas generation calculated to normal conditions (0°C, 1013 mbar)	NI /kg DS	(Binner and Zach, 1999)
Fermentation test GB21	35	21 days	50 g DS sample + 300 mL H_2O (200 mL in Austria)	Anaerobic digested sludge	50 g DS	Ground to <10 mm (<20 mm in Austria)	Gas production presses NaOH solution into a graduated measuring cylinder	mg /kg DS NI /kg DS	(Binner and Zach, 1999; Bockreis, Muller *et al.*, 2007)
Harries, Cross and Smith	35	3 months, some samples produced all gas within 2 months	Oven dried at 105°C	Laboratory maintained seed 'cultured' over several years, regularly fed with medium.	0.5 g	Dried at 105°C, grinded and sieved to <1 mm	Syringe via 3-way valve connected to a manometer. Syringe draws out gas until normal barometric pressure is reached	Cumulative gas production (mL) BMP value (m^3 CH_4/tonne DM)	(Harries, Cross *et al.*, 2001)
BM100	35	Up to 100 days (possibly longer)	Dried at 70°C to a DM content of 87-93%	Anaerobic digested sludge (3 g in 50 mL- 6% by DM)	20 g LOI 200 mL medium 50 mL seed	Non-BMW components removed, and percentage BMW recorded. Reaction mixture sparged with nitrogen	Biogas is collected in a graduated measuring cylinder filled with acidified water	NI /kg LOI l/kg LOI	(Godley, Lewin *et al.*, 2007)

Table 2.1. Anaerobic test method summary.

Whilst the recent studies into anaerobic test methods have focused on organic waste materials, some of the original methods were not prescribed for this material; for example the blue book method for determining biodegradability of anaerobic sewage sludge (Standing Committee of Analysts, 1977). As the interest in the biodegradable content of organic waste has increased, the anaerobic methods have been more widely applied to these materials.

An early test method was described by Owen, Stuckey *et al.* (1979). This method involved a simple glass set-up with an incubation temperature of 35°C (Owen *et al.*, 1979). These tests were run for 30 days using samples of peat material. Although there was still activity remaining at the end of the tests, the majority of gas production occurred in the first 20 days.

A test method used in a study by Shelton and Tiedje (1984) used a media which differed that used by Owen *et al*. The media used in the anaerobic tests is given in Table 2.2. The duration of this test was 56 days. Rather than using organic waste materials, the samples used by Shelton and Tiedje included ethanol and ρ-cresol (Shelton *et al.*, 1984).

Method	Medium/ Inoculum					References
Owen *et al.*	Resazurin KCl $Na_2MoO_4.2H_2O$ Folic acid Pantothenic acid	$(NH_4)_2HPO_4$ $MnCl_2.4H_2O$ $ZnCl_2$ Pyridine hydrochloride B_{12}	$CaCl_2.2H_2O$ $CoCl_2.6H_2O$ $FeCl_2.4H_2O$ Riboflavin ρ-aminobenzoic acid	NH_4Cl H_3BO_3 $Na_2S.9H_2O$ Thiamin Thioctic acid	$MgCl_2.6H_2O$ $CuCl_2.2H_2O$ Biotin Nicotinic acid	(Owen *et al.*, 1979)
Shelton and Tiedje	Phosphate Buffer KH_2PO_4 K_2HPO_4	Mineral Salts NH_4Cl $CaCl_2.2H_2O$ $MgCl_2.6H_2O$ $FeCl_2.4H_2O$	*Trace Metals* *$MnCl_2.4H_2O$* *H_3BO_3* *$ZnCl_2$* *$CuCl_2$*	*$Na_2MoO_4.2H_2O$* *$CoCl_2.6H_2O$* *$NiCl_2.6H_2O$* *Na_2SeO_3*	After Cooling $NaHCO_3$ $Na_2S.9H_2O$	(Shelton *et al.*, 1984)
Pagga and Beimborn	Resazurin KH_2PO_4 $Na_2HPO_4.12H_2O$ NH_4Cl	$CaCl_2.2H_2O$ $MgCl_2.6H_2O$ $FeCl_2.4H_2O$ $Na_2S.9H_2O$	*Trace Elements* *$MnCl_2.4H_2O$* *H_3BO_3* *$ZnCl_2$*	*$CuCl_2$* *$CoCl_2.6H_2O$* *$NiCl_2.6H_2O$* *Na_2SeO_3*	*$Na_2MoO_4.2H_2O$*	(Pagga *et al.*, 1993)
Harries, Cross and Smith	Resazurin NH_4Cl $KH_2PO_4.3H_2O$ NaH_2PO_4 $NaHCO_3$ $MgCl_2.6H_2O$	$FeCl_2.4H_2O$ Tryptose Yeast extract H_2O $FeS/CaCl_2$ Mercapto-ethane sulphonic acid (MES)		*Trace Elements* *$AlCl_3.6H_2O$* *$CoCl_2.6H_2O$* *$CuCl_2.2H_2O$* *H_3BO_3* *$MnCl_2.4H_2O$*	*$Na_2SeO_3.5H_2O$* *$Na_2WO_4.2H_2O$* *$(NH_4)_6Mo_7O_{24}.4H_2O$* *$NiCl_2.6H_2O$* *$ZnCl_2$*	(Harries *et al.*, 2001)
Godley *et al.*	KH_2PO_4 NH_4Cl $CaCl_2.2H_2O$ $MgCl_2.6H_2O$ or $MgSO_4.7H_2O$		*Trace Elements* *$FeCl_3.6H_2O$* *$CoCl_2.6H_2O$* *$MnCl_2.4H_2O$*	*$ZnCl_2$* *$NiCl_2$* *$Na_2MoO_4.2H_2O$* *$CuCl_2.2H_2O$*	*$Na_2WO_4.2H_2O$*	(Environment Agency, 2005)

Table 2.2. Media used in the anaerobic test methods.

A test method of 60 days was used in an investigation by Pagga *et al.* (1993), measuring chemical samples incubated at 35°C. Resazurin is used in the medium solution, however as an oxygen indicator (Owen *et al.*, 1979; Pagga *et al.*, 1993) and so is not used as a nutrient for methanogenic bacteria.

A small scale anaerobic test method was used in a study to investigate the effects of lignin on the anaerobic decomposition of cellulose (Stinson *et al.*, 1995). The mixture was incubated at 35°C for 2 months, and the released gas monitored using a syringe system, and measured using gas chromatography (GC).

A procedure modified from Stinson and Ham (1995) was applied more recently to landfill samples (Kelly *et al.*, 2006). Media was used modified from an earlier method (Shelton *et al.*, 1984), and anaerobic sludge added (10%) as inoculum. The test bottles were incubated at 35°C for 45 days, and the methane was measured using GC.

The incubation test (GS90) is a test method 90 days in duration used in Austria, using a moist fresh 1kg dry matter (DM) sample sieved to Ø 20 mm and saturated to water-holding capacity (Binner *et al.*, 1999b). The sample is then incubated at 40°C under anaerobic conditions. Whilst 90 days is the preferred length of test, the test duration can vary e.g. 240 days, known as GS240.

The fermentation test (GB21) method which is used in Germany uses a sample ground to <10 mm and filled to 300 ml with water (Bockreis *et al.*, 2007). The Austria

version of the GB21 method measures a 50 g DM sample, sieved to Ø 20 mm and 200 ml of water is added (Binner *et al.*, 1999b). Inoculum sludge (50 ml) is added, which in Austrian procedures is the leachate from the incubation test (GS90). This test can also vary in length (28 day test, GB28), although 21 days is the preferred length of time (Binner *et al.*, 1999b).

A small scale anaerobic test was used in a study by Harries *et al.* (2001). In the development of this method the seed is a laboratory maintained seed cultured over several years (Harries *et al.*, 2001). Here the sample is incubated with the inoculum and the seed at 35°C for 30 days.

For the BM100 method the waste sample is sorted and non-BMW components removed, with the percentage BMW recorded. The biogas produced is collected in a graduated measuring cylinder filled with water, which is displaced as the biogas enters the cylinder. The water is acidified to prevent dissolution of biogas (Environment Agency, 2005; Godley *et al.*, 2007b).

Other examples of anaerobic biodegradability tests have been described in the literature (Bogner, 1990; Hansen *et al.*, 2004; Zhang *et al.*, 2007), however the variations between these and those already described mostly involve the sample type and amount used, and for the overall purpose of this thesis are not described.

2.2.2. Aerobic Respirometric Methods

Aerobic respirometric methods are used to characterize organic waste samples by measuring the oxygen (O_2) consumption or carbon dioxide (CO_2) production of a sample. Biodegradable organic compounds are degraded under aerobic conditions as follows, using cellulose and hemicellulose respectively as an example:

$$(C_6H_{10}O_5)n + 6nO_2 \rightarrow 5nH_2O + 6nCO_2$$

$$(C_5H_8O_4)n + 5nO_2 \rightarrow 4nH_2O + 5nCO_2$$

There are advantages and disadvantages of measuring either O_2 consumption or CO_2 production. O_2 consumption measurements are often favored since oxygen is directly responsible for the oxidation of organic matter (Gomez *et al.*, 2005). Both measurement methods require specific instrumentation, although CO_2 measurements have been described as inexpensive (Adani *et al.*, 2001) and less sophisticated (Gomez *et al.*, 2005) than O_2 measurements. The calculation of O_2 consumed from the CO_2 produced assumes an O_2:CO_2 molar ratio of 1. In reality however, this ratio is dependent on the oxidation degree of the organic carbon (Adani *et al.*, 2001; Gomez *et al.*, 2005). The respiratory quotient (RQ) is the molar relationship between CO_2 produced and the oxygen consumed (Gea *et al.*, 2005), calculated using the following equation (Smars *et al.*, 2001).

$$RQ = \frac{(CO_2)_{produced}}{(O_2)_{consumed}}$$

The RQ values have been previously presented in studies of aerobic waste decomposition processes (Atkinson *et al.*, 1997; Gea *et al.*, 2004; Gea *et al.*, 2007; Smars *et al.*, 2001; Weppen, 2001). It was concluded from studies that RQ values are not suitable for indicating waste biodegradability or microbial activity (Gea *et al.*, 2004; Genc *et al.*, 2007). Example values of RQ from recent studies are given in Table 2.3. There is a wide range between these values, indicating that assuming an O_2:CO_2 molar ratio of 1 could be incorrect. This could lead to significant errors when CO_2 measurements are utilized in respirometric test methods.

Sample	Respiratory Quotient (RQ)	Reference
Pulp and paper mill primary sludge	0.92	(Atkinson, Jones *et al.*, 1997)
MSW	1.02	(Smars, Beck-Friis *et al.*, 2001)
Organic fraction of MSW (OFMSW)	1.24	(Gea, Barrena *et al.*, 2004)
Anaerobic digested sludge (ADS)	1.09	(Gea, Barrena *et al.*, 2004)
Dewatered raw sludge (RS)	1	(Gea, Barrena *et al.*, 2004)
Paper sludge (PS)	1.17	(Gea, Barrena *et al.*, 2004)
MSW and straw bulking agent	0.96	(Weppen, 2001)
Composted MSW	0.95	(Weppen, 2001)
Composted MSW amended with fat	0.87	(Weppen, 2001)

Table 2.3. Respiratory quotient (RQ) values for different organic waste materials.

There is a wide variety of respirometric methods; these can be classified as ‘dynamic’ or ‘static’. Dynamic methods are those where the sample is aerated throughout the assay, which minimizes problems associated with O_2 diffusion limitations (Gomez *et al.*, 2006). In static methods, the samples are not aerated. The O_2 transfer in a biological system is considered to be a limiting factor (Paletski *et al.*, 1995), a major disadvantage of static methods. Table 2.4 provides a summary of the key aspects of a number of aerobic test methods.

Method	Abbreviation	Dynamic /Static	Inoculum/ Seed	Nutrients	Moisture	Temp/°C	Length of Test	Sample Prep	Sample Size	Reporting Units	Test Purpose/ Common Use	References
Oxygen Uptake	O_2 Uptake	Static	None	None	50-55% w/w	37	16 h incubation 1 h assay	Samples sieved (<9.5mm) to remove glass, plastics, inerts and oversized materials	60 g dry weight	mg O_2/g VS/h	Compost stability, degree to which the biodegradable fraction in solid wastes has been diminished during composting	(Iannotti, Pang *et al.*, 1993)
Paletski and Young	N/A	Dynamic	None	None	None	35	48 h	Compost Samples	20 g wet weight	mg O_2/h/g DS	Compost stability/ performance of composting process	(Paletski and Young, 1995)
American Society for Testing and Materials	ASTM	Dynamic	Mature Compost	Nitrogen and Phosphor	50%	58	4 days		500 g		Compost stability	(ASTM, 1996)
Specific Oxygen Uptake Rate	SOUR	Static	None	Phosphate Buffer, $CaCl_2$, $FeCl_3$ and $MgSO_4$	Suspension	30	5-6 h	Composts Sample <9.5 mm	3-8 g wet weight (dependant on activity of sample)	mg O_2/g VS/h	Compost stability/ extent to which readily biodegradable material has decomposed.	(Lasaridi and Stentiford, 1998)
Cumulative Oxygen Uptake- 20h	OD_{20}				Suspension		20 h					(Lasaridi and Stentiford, 1998)
Specific Oxygen Uptake Rate in Solid State	DSOUR				None added		20 h					(Iannotti, Pang *et al.*, 1993; Lasaridi and Stentiford, 1998)
Dynamic Respiration Index	DRI	Dynamic	None	None	750 g kg^{-1}	Process	Maximum oxygen uptake over 24 h	Shredded <50mm Quartered	20-40 kg depending on bulk density w/w samples used	mg $O_2 kg^{-1} VS h^{-1}$	Degree of biological stability	(Adani, Confalonieri *et al.*, 2004; Adani, Lozzi *et al.*, 2001)
Real Dynamic Respiration Index	RDRI				None added		As DRI					(Adani, Lozzi *et al.*, 2001)
Potential Dynamic Respiration Index	PDRI				Optimal Water Content		As DRI					(Adani, Lozzi *et al.*, 2001)
Static Respiration Index	SRI	Static	None	None	750 g kg^{-1}	Process	Maximum oxygen uptake over 24 h			mg $O_2 kg^{-1} VS h^{-1}$		(Adani, Lozzi *et al.*, 2001)
Solvita ® Compost Maturity Test	Solvita ®	Static	None	None	Optimum/ saturation	Room Temp (20-25)	4 h (+ 48 h before to equilibrate if necessary)	Removal of stones and large stems and wood chips	To fill line of test jar	Solvita ® maturity scale (1-8, with 1 being very active, and 8 being very mature)	Compost maturity	(Changa, Wang *et al.*, 2003)
Dynamic Respiration over 4 Days	DR4	Dynamic	Mature Compost	Phosphor and Nitrogen	50% w/w	37	4 days	Non-BMW removed and % BMW recorded.	200-250 g DM	mg O/kg LOI	Monitoring performance of MBT and other treatment processes	(Godley, Muller *et al.*, 2005)
Respiration Index at Process Temperature	RI_T	Static	None	None	40-50% w/w	Process	4 h incubation 90 min assay	Large BMW shredded Samples sieved (<10mm) to remove glass, plastics and other inerts	250 ml (weight recorded)	mg O_2 $gOM^{-1}h^{-1}$	Evaluation of composting process/ stability of compost	(Gomez, Vazquez Lima *et al.*, 2005)
Respiration Index at 37°C	RI_{37}					37	18 h incubation 90 min assay					(Gomez, Vazquez Lima *et al.*, 2005)
German Static Respiration Index over 4 days	AT4 (Sapromat E)	Static	None	None	Saturation (~40-50%)	20	4 days	<20mm	30-40 g of wetted sample	mg O_2/g DS	Describe the biological activity of waste with respect to landfill regulations.	(Binner and Zach, 1999a; Binner and Zach, 1999b)
German Static Respiration Index over 7 days	AT7 (Sapromat E)						7 days					

Table 2.4. Aerobic respiration test methods summary.

Oxygen Uptake is a static method developed by Iannotti et al (1993). The sample was incubated for 16 h at 37°C, followed by a 1 h assay in which the decrease in oxygen level was monitored using a dissolved oxygen (DO) probe (Iannotti *et al.*, 1993). This test was designed to assess the biological stability of compost.

A dynamic test method used to assess compost stability has been described in which microbial seed, nutrients and moisture were not added to the sample (Paletski *et al.*, 1995).

The American Society for Testing and Materials (ASTM) method is a dynamic test used for determining the stability of compost by measuring oxygen uptake, with the sample being incubated at 58°C for 4 days (ASTM, 1996).

Specific Oxygen Uptake Rate (SOUR), like the O_2 uptake test, was designed to assess the biological stability of compost. This is different from other respirometric tests as the sample is suspended in water. Along with the SOUR test method, which is 5-6 h in length, there is a cumulative oxygen uptake method (OD_{20}) and SOUR in solid state method (DSOUR). OD_{20} is the same as the SOUR method, except the duration is 20 h. The DSOUR method is identical to the OD_{20} method, except no moisture is added to the sample (Lasaridi *et al.*, 1998).

The Dynamic Respiration Index (DRI) is a dynamic test method developed by Adani *et al* and designed to assess the degree of biological stability of waste

derived materials. There are three types of DRI reported; DRI, Real DRI (RDRI) and potential DRI (PDRI) (Adani *et al.*, 2004a). The moisture is adjusted to 750 g kg^{-1} for the DRI test with optimal water content for PDRI and a lack of moisture adjustment for the RDRI method.

The Static Respiration Index (SRI) test is the same as the DRI described by Adani *et al*. except that it is a static test method (Adani *et al.*, 2001).

The Solvita compost maturity test is a commercially available static method. A compost sample of optimum water content is placed in the supplied test jar up to the fill level and the sample is allowed to 'air' for an hour without the lid in place. The jar is then sealed and the sample is left to equilibrate if necessary. Gel-paddles are then inserted into the test jar without them coming into contact with the compost. The test jar is kept at room temperature and out of direct sunlight for 4 h and the results are based on the color of the paddles (1-8 Solvita scale), indicating the CO_2 concentration (Changa *et al.*, 2003).

Dynamic Respiration over 4 days (DR4) is a dynamic test method and is based on the ASTM method, differing by using a smaller sample size and a lower temperature (Environment Agency, 2005; Godley *et al.*, 2005). The samples are incubated at 37°C for 4 days. This test method was developed to monitor the performance of a waste treatment process.

Respiration Index (RI) is used to evaluate the stability of compost. There are two types of this static method, both described by Gomez et al. One is the RI_T, which is incubated at the *in situ* (process) temperature recorded at the time of sampling. The other is RI_{37} which is incubated at 37°C

The German Static Respiration Index (AT4 and AT7) methods last 4 and 7 days respectively. The CO_2 produced is measured as the CO_2 is absorbed by NaOH, and the pressure becomes negative, an oxygen generator produces O_2 until normal pressure conditions are restored. This set-up is commonly commercially known as the Sapromat method; however alternatives such as the Oxitop method exist. The O_2 production is then recorded. Similarly to the DR4 test method, these tests were developed to describe biological activity of waste with respect to landfill regulations.

In common with anaerobic test methods, main differences in aerobic methods are the amount of sample used, test conditions and test duration. A multitude of test methods have emerged in recent years as researchers have adapted previous methods to suit the practical needs of their studies, resulting in a modified method, which varies only slightly from other existing aerobic methods.

2.2.3. Temperature Increase Methods

In addition to the aerobic respirometric methods, there is also the Dewar self-heating test, which measures the heat produced by the sample under aerobic conditions, rather than the gases consumed or produced. The self-heating

tests have been investigated for the indirect estimation of respirometric activity (Koenig *et al.*, 2000) and maturity of compost material (Weppen, 2002).

The self-heating test is useful as it is very simple to operate, measuring the temperature increase due to sample activity.

2.2.4. Spectrographic Methods

Fourier Transform Infrared Spectroscopy (FT-IR) is a technique that is efficient in providing comprehensive information on chemical composition of heterogeneous materials. FT-IR characterizes the classes of chemical functional groups present in a sample (Chen, 2003), enabling the analysis of a complex mixture of chemicals found in waste materials. The technique has been applied to landfilled MSW during *in-situ* aeration (Tesar *et al.*, 2006), during composting (Castaldi *et al.*, 2005; Smidt *et al.*, 2005) and anaerobic digestion (Smidt *et al.*, 2007a).

The absorbance observed in the FT-IR spectra, along with the bands present is indicative of sample maturity. For example aliphatic methylene bands are weaker in landfill samples than in MBT waste material whilst a band assigned to aromatic amines is not observed in landfill samples, since these compounds decrease during biological treatment (Smidt *et al.*, 2007b).

2.2.5. Enzymatic Test Methods

Biodegradation of organic materials is a process performed by fungi and bacterial organisms, and as such the mineralization of the organic matter is through the action of extracellular enzymes (Pelaez *et al.*, 2004). A more extensive and comprehensive enzyme system is required as the substrate material becomes more complex (Tuomela *et al.*, 2000). The quantification of the activities of a range of enzymes within a waste material has been investigated as a means of assessing compost quality and maturity (Cayuela *et al.*, 2008; Mondini *et al.*, 2004; Pelaez *et al.*, 2004; Tiquia, 2005). The enzymes investigated in these studies included arylsulphatase, β-glucosidase, alkaline phosphatase and dehydrogenase, all of which are associated with the decomposition of the respective substrates found in waste material throughout composting.

The enzymatic cellulose degradation (ECD) method is a novel enzymatic approach to biodegradability measurement (Rodriguez *et al.*, 2005). A mixture of cellulase and xylanase (hemicellulase) enzymes were used in this study, which studied samples taken from a landfill site. The samples were mixed with a phosphate buffer (pH 5.5) at 40°C, and the monosaccharides liberated were recorded after the incubation period (40 h). The mass of the monosaccharides that are released by the MSW samples is reported to the initial mass of sample hydrolyzed in order to assess the biodegradability of waste samples.

2.3. Discussion

2.3.1. Method Practicality

The focus of this review is to assess the suitability of each method for the purpose of monitoring waste treatment process performance and the diversion of BMW from landfill. Here each method is critically discussed regarding sample size used (and hence the representation of overall waste material), timescale, reliability and applicability to a wide range of heterogeneous waste materials.

2.3.1.1. Anaerobic Tests

Several of the anaerobic methods (Owen et al, Pagga and Beimborn and Harries et al) are small scale tests. These tests use samples that are comparatively small in relation to the sample size of the GS90 test (1 kg DS) and the GB21 method (50 g DS). In general the larger the sample, the better representation that sample is of the overall waste batch. For example if a waste batch contained 10% cardboard, then this ideally needs to be reflected in a sample used in a test, and using 0.5 g of a sample (as with the Harries *et al* method) is unlikely to show a consistent 10% cardboard composition, and hence offer a reasonable representation of the waste. Shelton and Tiedje also used relatively small samples (50 µg/ml), although these were chemical samples. Considering other larger scale tests available, these small scale tests are less suitable for the monitoring of mixed solid waste. The use of smaller sample quantities would require rigorous sample preparation (grinding to a small particle size and thorough mixing) to provide a suitable representation of the overall waste material. Therefore, the larger scale test

methods, which use larger sample quantities, do not require such extensive sample preparation to provide an accurate representative of the waste material, and so provide more reliable and valid data.

The method of measuring biogas production varies between the methods. The use of syringes (Owen et al, Harries et al) should be restricted to smaller scale tests, as the size of syringe required for a larger sample (and therefore larger quantities of biogas produced) would be impractical. Alternatively, the syringes could be emptied on a more regular basis, but this could increase the measurement error. The BM100 method requires a 20 g LOI sample, which can produce as much as 15 liters of biogas over the 100 day period (Godley *et al.*, 2007b). This would be difficult to accurately capture in syringes. The GB21 method requires a 50 g DS sample, however biogas is only measured for 21 days, and so the biogas produced is unlikely to exceed 15 liters. Reported results include 10 liters of biogas released at 21 days (Bockreis *et al.*, 2007). The GS90 test method requires 1 kg DS so the expected biogas production is considerable. Measurements by Eudiometer (GS90) or by acidified water-filled cylinders (BM100) are therefore much more suited for the larger scale tests that are required for characterizing samples that are of a more representative size. The method for biogas production measurement has been previously discussed (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006). Manometric and volumetric measurements were found to have limitations. There is a limited range of accuracy associated with manometric analysis, whilst due to changes to the atmospheric pressure and evaporation of water in volumetric systems can

cause inaccuracies (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006).

It has therefore been recommended that gas analysis is performed using GC instead of volumetric gas release (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006), as used in studies such as Stinson and Ham (1995) and Kelly *et al* (2006). This technique would provide more accurate data for methane and carbon dioxide production; however this technique is more expensive than the other methods of gas production measurement.

Under anaerobic conditions, microbial growth efficiency is lower than in aerobic conditions. As a result the biogas release is an accurate representation of the microbial activity since a very small amount of the mineralized organic carbon is converted to new biomass. This is a major advantage of the anaerobic test methods. In aerobic conditions, the microbial growth efficiency is much higher, and so more of the mineralized organic carbon is converted to microbial biomass and therefore the carbon dioxide released is not an accurate representation of the organic carbon mineralization.

The major disadvantage of the larger scale anaerobic tests (GB21, GS90 and BM100) is that whilst the results produced are reproducible, the time scale for each test is not practical for routine analysis. Test durations of 21, 90 or 100

days have financial and operational implications for waste treatment and landfill operators if biodegradability data is delayed.

2.3.1.2. Aerobic Tests

The aerobic ASTM method operates at 58°C. This higher temperature could present some disadvantages for such a test method since the solubility of oxygen in water decreases with an increase in temperature. This may limit the oxygen transfer in the waste material which could result in lower results than expected. So whilst the high temperature may accelerate some reactions the biological activity will be hindered.

The static respiration methods generally give lower results that dynamic tests (Godley *et al.*, 2005), which indicates the advantage of aeration throughout the test duration.

The SOUR method uses a 3-8 g sample. This is a small sample, and so may not be a very good representation of the sample as a whole, thus reducing the reproducibility of the test. However, the aqueous suspension used in the method offers several advantages. One major advantage is that there is not a gas-liquid barrier on the surface of the substrate (Lasaridi *et al.*, 1998). This means that gaseous exchange can occur immediately due to the direct contact between the substrate material and the microbes, maximizing the diffusion of oxygen.

The minimization of diffusion rates is important since limited oxygen transfer through biomass layers into bacterial cells is typically considered to be the rate-limiting step (Adani *et al.*, 2004a; Paletski *et al.*, 1995).

Compositional methods such as dry matter or solids (DM or DS respectively) and loss-on-ignition or volatile solids (LOI or VS respectively) do not describe the biodegradability of a sample material. The LOI and VS analyses indicate organic carbon content, but not biodegradable carbon content. Not all carbon is amenable to biodegradation, and so the use of carbon content to indicate biodegradability, or BMW diversion from landfill, would result in an over-estimation of sample biodegradability.

Certain methods such as the aerobic AT4 or anaerobic GB21 methods use the DS content (mg O_2/g DS or mg/kg DS respectively). Considering the DS content, this would include the inorganic material, which is non-biodegradable, meaning that data presented in this form may not be comparable. Alternatively using LOI or VS only takes the organic fraction into account, which would allow for the varying inorganic content, allowing valid comparison between samples.

2.3.1.3. Alternative Tests

Temperature increase methods may not be suitable since temperature increase is due to chemical and biological reactions within the sample. Not all these exothermic reactions are related to the respiration reactions, such as acid hydrolysis. Some of the heat increase, to a certain degree, will not be

respirometric activity. The results are therefore unlikely to be accurate, considering that the moisture content of the sample will also affect the heating. Precise determination of bioactivity using the Dewar self-heating test is difficult under any condition, and additional factors such as packing density and humidity require careful consideration. The applicability of the Dewar self-heating test to fresh MSW samples has not been investigated to the authors' knowledge, and as such this may indicate that other methods may offer more suitable options.

Spectrographic techniques such as FT-IR have been shown to be efficient at assessing the chemical properties of waste material, indicating sample stability. However no research has indicated that this technique can quantify the amount of biodegradable material removed. This technique could be used in compost quality, or landfill acceptance criteria, based on the presence of certain bands indicating waste composition and whether the sample is stable or not. Aerobic and anaerobic test methods enable a simple calculation of biodegradability reduction from a waste treatment process, and so for the purpose of monitoring BMW diversion from landfill, FT-IR is perhaps not suitable, however further research could enable this technique to become suitable.

Measuring the enzymatic activity present in a waste material has been shown to be a suitable method for the characterization of compost stability (Cayuela *et al.*, 2008). The enzyme activity of the stabilized compost material varied depending on the starting substrate (Mondini *et al.*, 2004). There is a

necessity to perform several measurements to understand the activities of the wide range of enzymes, which is a disadvantage of this approach. Whilst measuring the enzyme activities may offer a good indication of compost maturity, this approach has not been applied to fresh MSW input samples, and the significantly different substrates available in fresh and treated waste samples would require a complex and possibly an individual analytical approach to each material. This would not allow simple and rapid monitoring of waste treatment processes, and so is unsuitable for the monitoring of BMW diversion from landfill.

2.3.2. Correlations with Anaerobic Methods

Assessing the complete biodegradability of the sample is ideal; however short-term tests cannot provide this and so are used to correlate with anaerobic tests. This allows for the prediction of long-term biodegradation potential in a shorter length of time, depending on a strong correlation of the tests. A summary of correlation coefficients from the evaluated studies is shown in Table 2.5.

Short-term Method	Long-term Method	Sample	Correlation coefficient (r)	Reference
AT4	GB21	MSW excavated from closed landfill (3 sites)	0.89	(Cossu *et al.*, 2008)
		Pre-treated residual waste	0.77	
AT7	GS90	Pre-treated residual waste	0.91	(Binner *et al.*, 1999b)
GB21	GS90	Pre-treated residual waste	0.97	(Binner *et al.*, 1999b)
DR4	BM100	MSW derived BMW	0.73	(Godley *et al.*, 2007)
ECD	BMP	MSW extracted from landfill (3 sites)	0.81 0.89 0.93	(Rodriguez *et al.*, 2005)

Table 2.5. Summary of correlation coefficients between short-term and long-term biodegradability test methods.

The German SRI (AT4 and AT7) tests have been investigated, along with an AT10 test (Binner *et al.*, 1999a; Binner *et al.*, 1999b). It was found that the AT10 results did not produce enough additional information to make the test more practical than the AT7 test, and as a result the AT7 test is recommended. The AT7 shows a strong correlation with the anaerobic incubation test (GS90) with correlation coefficients (r) of 0.94-0.95 (Binner *et al.*, 1999b). This relationship indicates that as the results of the AT7 increase, the results obtained from the GS90 are also expected to increase.

For routine testing, the length of the aerobic tests may still be too long, not providing results quickly enough and having financial implications for the treatment process operator.

The ECD method was shown to correlate with a classical BMP test, with correlation coefficients (r) between 0.81 and 0.93 (Rodriguez *et al.*, 2005). This relationship shows that as the ECD results increase, the expected BMP results will also increase (i.e. low biodegradable substrate gives low ECD and BMP values).

However, this method has the limitation that only the monosaccharides are measured. Cellulose can be hydrolyzed into cellodextrins and cellobiose molecules before glucose molecules are produced. As a result, the measurement of monosaccharides may not reveal the full extent of the cellulose hydrolysis. Organic waste material will also contain other enzyme hydrolysable substrates, such as proteins, and these may require further consideration.

The DR4 test correlates with the BM100 test method, allowing the estimation of BM100 data from DR4. A shorter anaerobic test, GS21 (fermentation test), also correlates well with the Austrian GS90 test (Binner *et al.*, 1999a). The AT4 is the recommended test method in Germany and Austria, with the allocated landfill acceptance criteria of 5 mg/g DM and 7 mg/g DM respectively (Muller *et al.*, 2005). The correlation of the DR4 method with BM100 data has been reported (Godley *et al.*, 2007b), whilst the correlation of the AT4 method with the GB21 method has also been investigated (Cossu *et al.*, 2008). The aerobic test methods have disadvantages such as the microbial growth efficiency, in which new biomass is produced efficiently from

the digestion of the waste material, and so the carbon dioxide produced doesn't sufficiently represent the degree of biodegradability. Aerobic test methods also have the disadvantage of measuring only the initial rate of biodegradability due to these tests measuring the readily biodegradable materials. The length of aerobic tests does not allow the measurement of slowly biodegradable material. Anaerobic test methods, such as the BM100 and GS90, give reliable results and measure the extent of biodegradability as the length of these tests is a clear disadvantage, but allows for the degradation of readily biodegradable and slowly biodegradable materials.

2.4. Conclusions

Biodegradability testing is an important part of monitoring BMW diversion from landfill in the EU. Data obtained from these tests assist the waste treatment operators in optimizing the waste treatment process and in the UK provide local authorities with information with which to calculate BMW diversion. Therefore there is a need for a rapid and cost-effective test method that would correlate with the reliable BM100 test method, which is not suitable for regular routine testing due to the duration.

The current biodegradability test methods have limitations, and no one test method is completely sufficient for routine biodegradability assessment. Further research is needed to develop the alternative and rapid test method that is required. From this review potential areas for further research include spectrographic FT-IR or enzyme-based approaches. As discussed in Chapter 1, and from the conclusions made in this chapter, an alternative

biodegradability test method based on the enzymatic hydrolysis of cellulose will be investigated in the following chapters.

Chapter Three

Development of the Enzymatic Hydrolysis Test (EHT)

This chapter covers the development of the EHT method, including the optimisation of pH and temperature conditions and the investigation of other test parameters.

3.1. Introduction

Following reviews of the current methods in the previous chapter it has been concluded that no single current test is sufficient for routine testing. There is a need for a rapid and cost-effective test method that would correlate with longer-term tests such as the anaerobic BM100 method. The BM100 test method is not suitable for regular routine testing due to the duration, however a short-term correlating test method would make routine testing viable. As discussed in Chapter 1, a method based on the enzymatic hydrolysis of cellulose was evaluated as a potential short term test method by Godley *et* al (2004). Cellulose and hemicellulose are hydrolyzed by cellulase and hemicellulase enzymes respectively and so an alternative method based on the enzymatic hydrolysis of cellulosic material could offer a suitable routine test method. This chapter therefore incorporates knowledge from previous studies and provides the development of the enzymatic hydrolysis test (EHT)

method.

The enzymatic hydrolysis of cellulose has previously been investigated (Adsul *et al.*, 2005; Adsul *et al.*, 2004; Saha *et al.*, 2005; Van Wyk, 1999b; Van Wyk, 2002; Van Wyk *et al.*, 2003). These studies investigated the bioconversion of cellulose to fermentable sugars using cellulase enzymes, for the application of obtaining bioenergy (such as ethanol) from biowaste. Such an approach could be utilized as a biodegradability test method, as a large proportion of organic MSW consists of lignocelluloses (lignin and cellulose) that can undergo biodegradation, with 30-50% of organic MSW consisting of cellulosic material (Rodriguez *et al.*, 2005). Agricultural crop waste and forestry residues consist of up to 75-80% cellulose and hemicellulose (Adsul *et al.*, 2005; Adsul *et al.*, 2004). However, up to 90% of the total methane produced in a landfill environment is from hemicellulosic/cellulosic material (Barlaz *et al.*, 1989; Rodriguez *et al.*, 2005). As a result hemicellulosic/cellulosic material is considered as the most important carbon source for methanogenesis. This highlights the importance of significantly reducing the hemicellulose and cellulose content of organic waste materials prior to disposal in a landfill.

An original biodegradability test had been developed using a mixture of cellulase and xylanase (hemicellulase) enzymes (Rodriguez *et al.*, 2001; Rodriguez *et al.*, 2003; Rodriguez *et al.*, 2005). This test method is known as the enzymatic cellulose degradation (ECD) test. Here, a mixture of cellulase and hemicellulase were used to analyze samples taken from a landfill site. The samples were sorted, removing the glass, plastics, metals and other inert

materials, dried for 5 days at 105°C and grinded to <5 mm (Rodriguez *et al.*, 2005). The samples were mixed with a phosphate buffer (pH 5.5) at 40°C, and the monosaccharides liberated were recorded after the incubation period (40 h) (Rodriguez *et al.*, 2005). The mass of the monosaccharides released by the MSW samples is related to the initial mass of sample hydrolyzed in order to assess the biodegradability of refuse samples (Rodriguez *et al.*, 2005). The ECD method was shown to have good correlation with a classical biochemical methane potential (BMP) test, with correlation coefficients (r^2) between 0.65 and 0.87 (Rodriguez *et al.*, 2005). However, the ECD has the possible limitation that only the monosaccharides are measured, which is a product of cellulose hydrolysis. Therefore other available substrates such as proteins and lipids that would otherwise be considered as biodegradable are not being taken into account.

The test method developed by Rodriguez *et al.* (2001) was evaluated by Godley *et al.* (2004) in a review of biodegradability test methods. Here an incubation time of 24 h was used, monitoring the production of dissolved organic carbon (DOC) (Godley *et al.*, 2004) rather than monosaccharide release. A good correlation with a classic BMP test for 11 samples was observed, further indicating the potential of an enzymatic approach, and forming the basis of this research project. A biodegradability test method based on the enzymatic hydrolysis of cellulose was proposed (Godley, 2005; Godley *et al.*, 2004) incorporating 3 distinct test stages, including a sterilisation procedure (autoclave).

The aim of this chapter is to present the development of the enzymatic hydrolysis test (EHT) following the initial work presented by Godley *et al* (2004).

Biogas (methane and carbon dioxide) released in a landfill environment is mostly from the biodegradation of hemicellulose and cellulose materials; however other organic materials such as proteins and lipids will also biodegrade under anaerobic conditions, releasing biogas. Therefore a crude enzyme mixture, along with the autoclave process is investigated as a potential biodegradability test method.

The objectives of this chapter are-

- Determine the optimum conditions for the crude enzyme mixture using a standard cellulose substrate.
- Investigate the use of a pure cellulase enzyme using a standard cellulose substrate.
- Investigate the effects of autoclave on DOC release using treated and untreated organic waste samples.

3.1.1. Determination of Optimum pH and Temperature Conditions

Previous studies into the enzymatic hydrolysis of organic waste materials show that the pH and temperature conditions have an effect on the degree of hydrolysis (Abraham *et al.*, 1997; He *et al.*, 2006; Van Wyk, 1999a). This investigation uses a solution of cellulase and hemicellulase enzymes,

therefore the effects of pH and temperature will be investigated for this particular mixture. The cellulase enzyme powder used is a crude mixture, also containing protease activity. Therefore under enzymatic hydrolysis cellulose and hemicellulose materials will be hydrolyzed as well as proteins, with the dissolved organic carbons (DOC) being measured, as opposed to exclusively monosaccharides which are included in the DOC. This investigation aims to determine the optimum conditions for the proposed test method using a crude cellulase, prior to applying the test on a range of organic waste samples.

3.1.2. Use of a Pure Cellulase Enzyme

In the previous section, a crude cellulase enzyme is introduced, which provides a source of cellulase from the fungus *Trichoderma Viride* species, but also possesses hemicellulase and protease activities. There are different types of cellulase enzymes, according to the reaction they catalyse (Walker *et al.*, 1991). These are as follows-

1. Endo-cellulase: breaks the intermolecular (hydrogen) bonds between cellulose polymer chains disrupting the crystalline structure, exposing individual cellulose chains.
2. Exo-cellulase: hydrolyses the bonds between monomeric units at the end of the cellulose chain, resulting in the production of cellobiose molecules.
3. Cellobiase: hydrolyses the cellobiose molecules produced by the exo-cellulase into monosaccharide (glucose) molecules.

The function of each cellulase enzyme type is shown in Figure 3.1.

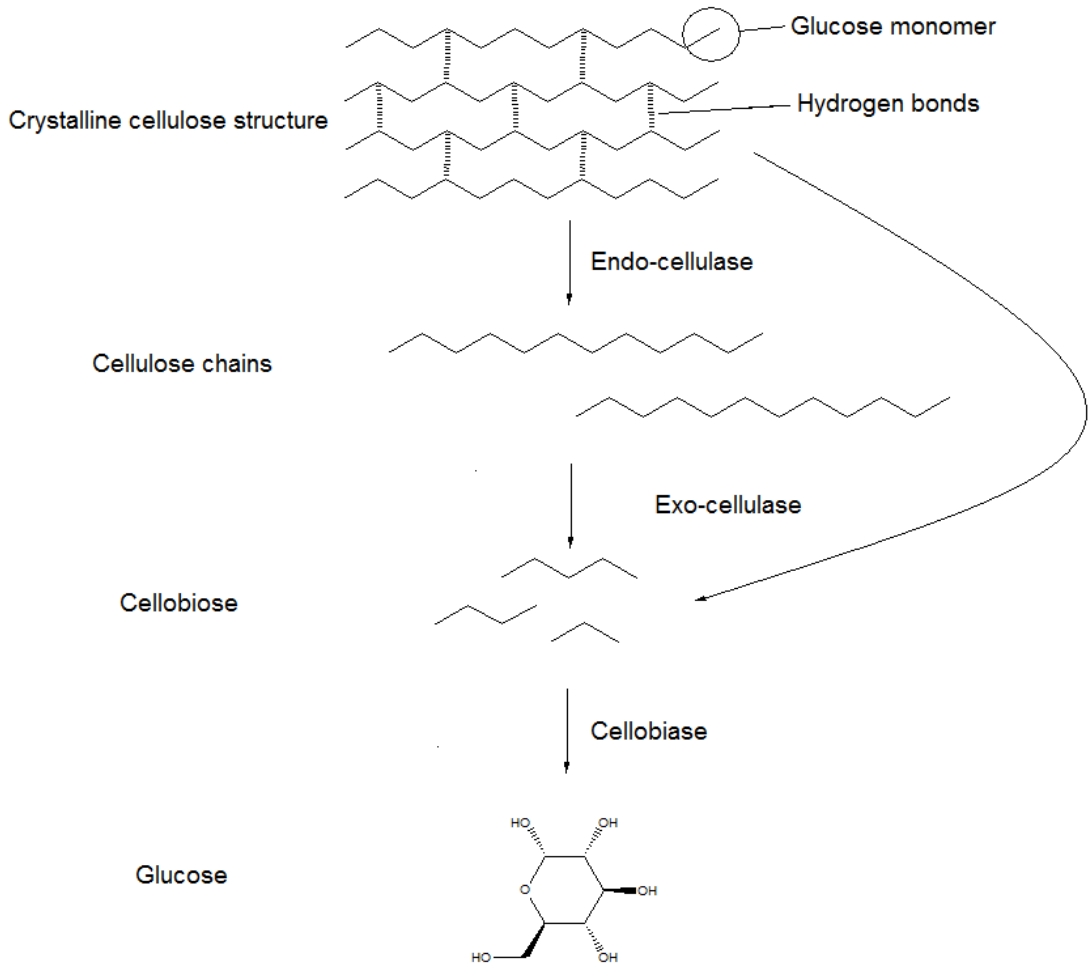

Figure 3.1. Function of cellulase enzyme types and the hydrolysis products.

Pure cellulase enzymes will not contain the hemicellulase and protease activities of a crude enzyme powder, and may also not contain different cellulase types as described. However, this investigation is to determine whether or not a pure cellulase enzyme is more efficient at hydrolysing a standard α-cellulose substrate than a crude cellulase enzyme.

3.1.2. The Effects of the Autoclave Process

Sterilisation of the waste material is required to prevent microbial growth on released DOC during the test, to ensure that the entire DOC released in the test is accounted for. Alternative sterilisation techniques to autoclave include dry heat, γ-irradiation, microwave and chemical methods (McNamara et al., 2003; Trevors, 1996). Dry heat sterilisation is impractical for a routine analysis tool, such as the EHT since the sample would need to be incubated for 1-2 days (Trevors, 1996). Irradiation is also impractical as this can only be carried out at a suitable facility, which is unlikely to be available in most standard waste characterisation laboratories. Microwave treatment is not a common sterilisation technique, and for the destruction of microorganisms microwave is less effective than autoclave or γ-irradiation (Trevors, 1996).

The use of chemicals (in gaseous form or otherwise) such as ethylene oxide has several problems for the EHT. Whilst the use of chemicals is an effective way to sterilise a sample, it would add DOC to the mixture which could possibly interfere with the enzymes added to the mixture in Phase 3 of the EHT. Therefore, considering the practicality of other sterilisation techniques, autoclave is a suitable method for the EHT. The high energy autoclave technique may adjust the physical chemical properties of the waste through thermal hydrolysis; however this may benefit the biodegradability measurement. In a recent study, waste samples from a landfill were measured using a dynamic respiration index (DRI) and biochemical methane potential (BMP) test (Tojo et al., 2007). Hydrothermal pre-treatment of the waste sample increased the biogas production in the BMP test, indicating that

a high energy pre-treatment causes the slowly biodegradable material to become more accessible and easier to decompose, suggesting that the use of a high energy treatment in the EHT is appropriate.

3.2. Materials and Methods

3.2.1. Sample

To optimize the test, a standard sample of Sigma α-cellulose was chosen. The dry matter (DM) and Loss-on-Ignition (LOI) were determined for this sample, using standard procedures (EN12879:2000), to be 94.0% and 99.7% respectively.

To investigate the effects of autoclaving on the DOC release during the EHT, a selection of waste samples were used, which included 3 untreated and 3 treated waste samples. These were fresh MSW input samples, composted MSW, anaerobic digestion treated MSW and composted kitchen and green waste. These samples were sorted to remove glass, metals, plastics and inert materials with the biodegradable material being retained and tested. Materials with large particle sizes were shredded to <10 mm before testing to ensure thorough mixing and a good representation of the sample as a whole. The dry matter (DM) and loss-on-ignition (LOI) of each sample was required to use consistent masses in the analysis (e.g. LOI indicates organic portion of the sample, therefore samples can be weighed out with consistent amounts of organic components). These were determined for each sample using standard procedures (EN12879:2000).

3.2.2. Enzyme Preparation

For each sample 25 mg of crude cellulase powder (Sigma) and 75 mg of hemicellulase powder (Sigma) were dissolved in 20 ml of distilled water, with 195 units' cellulase and 112.5 units' hemicellulase activities in each 20 ml of enzyme mixture (activities are in excess to allow for complete hydrolysis). This enzyme solution was then filtered through 0.22 µm Millipore membrane filters to sterilize the solution.

The crude cellulase enzymes also possessed some hemicellulase and protease activity, with the hemicellulase enzymes also having some cellulase activity (manufacturer specifications).

For the investigation using a pure form of the enzymes, cellulase from *Aspergillus* species was used. The cellulase batch was, unlike the crude enzymes, in liquid form and had a manufacturer specified activity of ≥1000 U/g. This was diluted to give the 200 units (equivalent of 200 µl) per 20 ml, which was equal to that used with the crude cellulase enzymes.
Hemicellulase powder was added in the same amounts as in the optimization study, 112.5 units (75 mg) per 20 ml distilled water.

3.2.3. Experimental Procedures

3.2.3.1. Determination of Optimum pH and Temperature Conditions

The α-cellulose was weighed out to 5 g LOI (based on the % LOI) and placed in a 250 ml Erlenmeyer flask. Phosphate buffer (100 ml) was added to the sample, and an initial 5ml sample was taken from the mixture for analysis.

The pH of the buffer solution was varied to determine the optimal pH for the enzymatic hydrolysis, using pH 4, 4.5, 5, 5.5 and 6. Each pH was carried out in triplicate, giving a total of 15 samples.

The flask was then autoclaved at 121°C for 15 min to sterilize the mixture and a further 5 ml sample was removed for analysis.

The prepared crude enzyme solution was then added to each of the flasks and the flask sealed with a neoprene bung. The flasks were placed in an Inovva shaking incubator at 150 rpm. The temperature of the incubator was varied with each experiment to determine the optimum temperature for enzyme hydrolysis. Temperatures used were 30, 40, 50 and 60°C.

Further samples were taken from the mixture at regular intervals for analysis as before.

3.2.3.2. Use of a Pure Cellulase Enzyme

The experimental procedure was the same as for the crude cellulase investigation described. However the pH of the buffer solution was varied to determine the optimal pH for the enzymatic hydrolysis, using pH 4, 4.5, 4.75, 5 and 5.5 (revised after the crude cellulase work). Each pH was carried out in triplicate, giving a total of 15 samples.

The prepared pure cellulase and hemicellulase enzyme mixture was added to each flask and placed in an Inovva shaking incubator at 150 rpm. The

temperature was set at 40°C. Based on the results discussed later, the investigation of other temperatures was not required.

Samples were taken from the mixture at intervals of 3, 9, 20 and 118 hours for filtration and chemical oxygen demand (COD) analysis.

3.2.3.3. Effects of the Autoclave Process

Each sample was weighed out in replicates of 6 to a mass of 5 g LOI (based on the % LOI) and placed in a 250 ml Erlenmeyer flask. Phosphate pH 4.75 buffer (100 ml) was added to the sample and a 5ml sample was taken from the mixture, filtered to remove any solids for COD analysis (phase 1).

Each of the 6 replicate flasks were then sealed and prepared for autoclave. However, 3 of the replicates were left to stand (i.e. not autoclaved), whilst the remaining 3 were autoclaved at 121°C for 15 min. Following the autoclave process, a 5 ml sample was taken from each of the 6 replicate flasks and filtered for COD analysis (phase 2).

The prepared crude enzyme solution was then added to each flask and sealed. The flasks were placed in an incubating shaker at 50°C with constant shaking at 150 rpm. After 20 h the flasks were removed, and a 5 ml sample was taken from each flask and filtered for COD analysis (phase 3).

3.2.4. Sample Analysis

The filtered samples removed from the mixture were analyzed for DOC using chemical oxygen demand (COD) test kits (Spectroquant). For practicality reasons, COD tubes were used to calculate DOC instead of a total organic carbon (TOC) analyser. The DOC is calculated based on the molecular formula of cellulose monomeric units (glucose), and the DOC is assumed to be glucose as this method is the enzymatic hydrolysis of cellulose into glucose. COD analysis measures the amount of oxygen used to oxidise the sample, i.e. the oxygen demand. Glucose reacts with oxygen as follows-

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

The relative molecular mass (RMM) of glucose is 180 g/mol, of which 72 g/mol is carbon. The RMM of oxygen is 192 g/mol. The molar ratio of carbon to oxygen is therefore 192/72= 2.67. The COD results are directly given as mg/l of oxygen, so to obtain the results as mg/l of carbon the molar ratio must be used. Carbon content in mg/l is calculated as follows-

$$\frac{COD}{2.67} = mg/l \ \ (carbon)$$

To obtain results in mg C/kg LOI, the volume of the sample mixture needs to be taken into account, along with the mass of sample used (g LOI), which is calculated using the % dry matter (DM) and % LOI of the sample as follows-

$$M = \frac{\%LOIx\left(\left(\frac{\%DM}{100}\right)xM_{moist}\right)}{100}$$

Where M_{moist} = moist mass of sample (g) and M = mass of sample (g LOI).

Also the mass of the sample (LOI) needs to be converted to kilograms. This is calculated as follows-

$$mgC/kgLOI = \left(\frac{C}{1000/V}\right)\left(\frac{1000}{M}\right)$$

Where C= mg/l carbon and V = volume (ml) of mixture at the time of removing DOC sample.

3.3. Results and Discussion

3.3.1. Determination of Optimum pH and Temperature Conditions

The DOC released from enzyme hydrolysis increases over time, with a fast initial hydrolysis rate, which decreases as available substrate is reduced. The purpose of this biodegradability test is for it to be rapid, and yet reliable, so the optimum conditions would be those that give the quickest end point, but also a high yield of DOC. The DOC increase over time at temperatures of 30, 40, 50 and 60°C are shown in Figures 3.2-3.5 respectively.

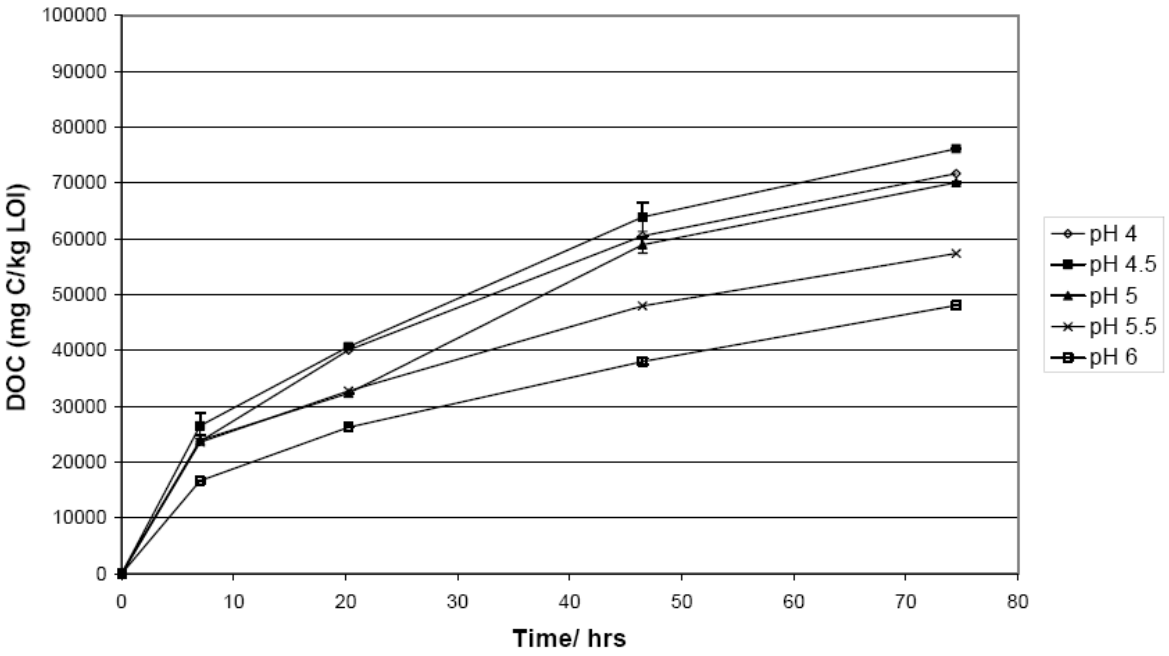

Figure 3.2. DOC (mg C/kg LOI) increase over time (Incubation temperature 30°C). Error bars shown as standard deviation (stdev).

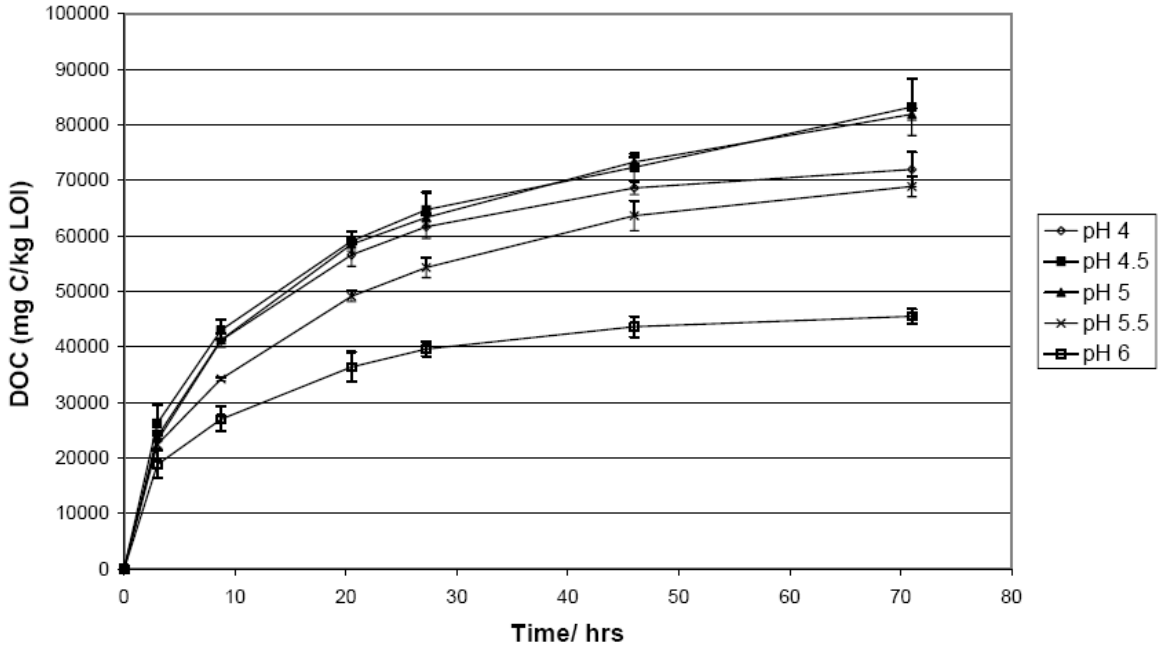

Figure 3.3. DOC (mg C/kg LOI) increase over time (Incubation temperature 40°C). Error bars shown as standard deviation (stdev).

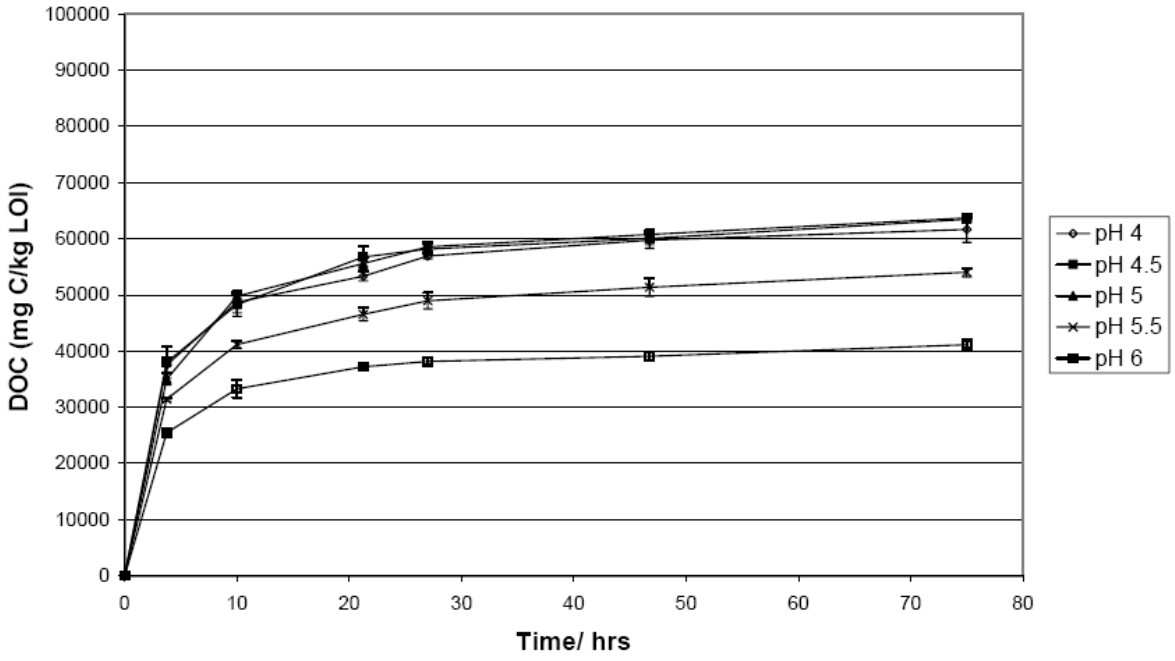

Figure 3.4. DOC (mg C/kg LOI) increase over time (Incubation temperature 50°C). Error bars shown as standard deviation (stdev).

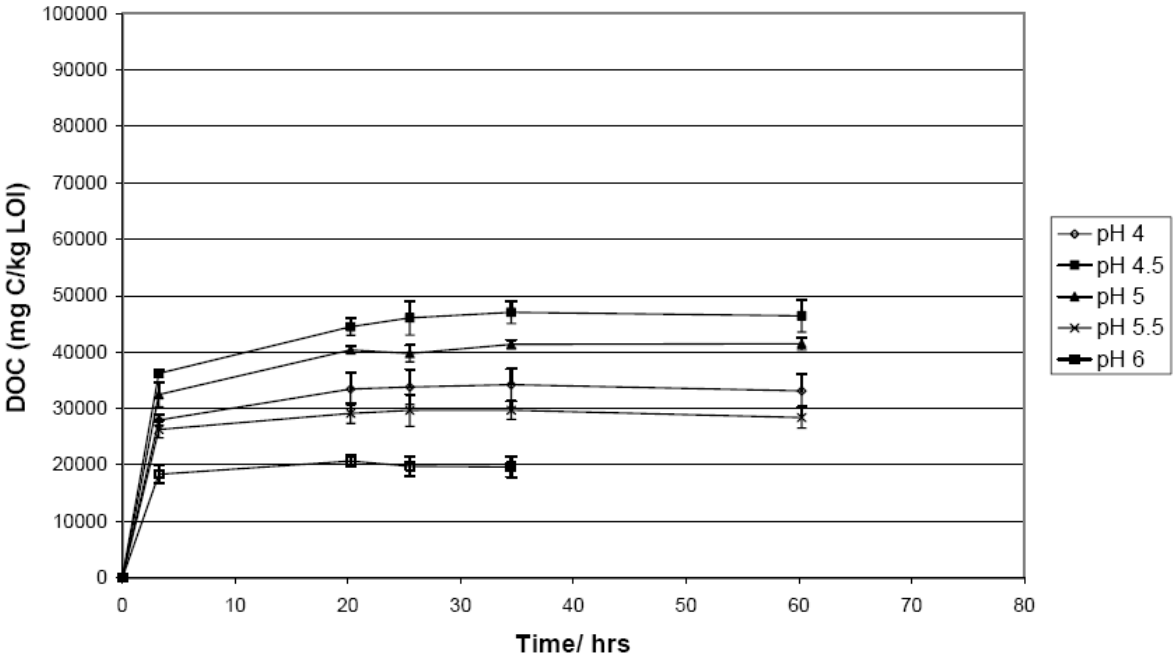

Figure 3.5. DOC (mg C/kg LOI) increase over time (Incubation temperature 60°C). Error bars shown as standard deviation (stdev).

The optimum pH was between 4.5 and 5 at each respective temperature as these pH levels gave the highest initial rate of hydrolysis and the highest

overall DOC yield (Figures 3.2-3.5). In each case there is very little difference between pH 4.5 and pH 5. The time for DOC release to cease was reduced as the temperature was raised, for example at 30°C (Figure 3.2) DOC release was still occurring after 75 h but at 60°C (Figure 3.5) the DOC release effectively stopped at around 20 h. At 50°C (Figure 3.4), the hydrolysis had almost ceased at around 20 h, and the DOC yield was higher at this time than at the same point in the 60°C test. This indicates that significant enzyme denaturation may have limited the amount of DOC released at 60°C. At 40°C (Figure 3.3), the hydrolysis rate was lower and DOC release was still occurring after 70 h, and although the highest DOC yield was at 40°C and pH 5 (90,285 mg C/kg LOI), it would take a long time to reach the end point, in comparison to 50 and 60°C. At 60°C the highest DOC yield was lower (pH 4.5- 47,000 mg C/kg LOI) than the highest DOC yield at 50°C (pH 4.5- 67,000 mg C/kg LOI). From the graph of 50°C, it is evident that about 85% of the total DOC released after 75 h is released after only 20 h. Therefore, although the amount of DOC released at 50°C is lower than at 40°C, the timescale is much more rapid.

The optimum temperature was 50°C, despite the higher yield at 40°C the rate of hydrolysis was higher at 50°C, and the reaction was completed sooner. The optimum pH is not clear as there was little difference between pH 4.5 and pH 5, but for the biodegradability test, pH 4.75 will be used as the optimum as this will then allow for any minor pH changes induced by the waste sample, if any.

The optimal test conditions chosen were therefore pH 4.75, temperature 50°C and a 20 hour incubation time as a compromise between enzyme denaturation and a rapid hydrolysis rate so that the test time-scale was reduced to less than a day and much lower than most biological tests. The optimum pH and temperature obtained in this study are comparable with previous studies into cellulose hydrolysis. The substrates used in these studies varied from sugarcane bagasse (Adsul *et al.*, 2005), various waste paper materials (Van Wyk, 1999a) and pre-treated tapioca waste (Abraham *et al.*, 1997). Adsul *et al* (2005), with samples of delignified sugarcane bagasse material used a citrate buffer at pH 4.5, with incubation temperatures of 30 and 50°C. The highest degree of hydrolysis for these samples was observed at 50°C (Adsul *et al.*, 2005). Van Wyk (1999) observed that the optimum pH differed for different substrates, with pH 4.5 yielding optimal hydrolysis for filter paper and microcrystalline cellulose. The optimum pH for newsprint and foolscap paper was pH 5 and pH 5.5 respectively (Van Wyk, 1999a). The optimum temperature also varied depending on the substrate, ranging between 45-55°C. Abraham and Kurup (1997) reported optimum pH between pH 4.6 and pH 5, with optimum temperatures between 40 and 50°C (Abraham *et al.*, 1997).

The optimum conditions observed in this study are therefore in the order of expected values. The range in optimum pH and temperature observed in past studies for different enzyme mixtures and substrates is narrow. The optimum pH and temperature are expected to vary depending on the substrate, however since this variation will be small, the extent of cellulose hydrolysis

should not be significantly affected. Therefore this test method could be suitable for the application to different substrates (i.e. organic waste material).

Running the test for longer has time constraints that are not desirable for a routine test method, and associated financial and resource costs. For this reason, a 40 hour test would be unfavourable, but still an improvement on the 4 days that the DR4 test method offers.

It would be expected that increasing the amount of enzymes used in each test would also increase the rate of hydrolysis and so the test would reach completion sooner. The DOC yield would not however increase as the substrate availability would be the same, and so is the limiting factor. Increasing the enzyme concentrations would increase the overall cost of the test, and without significantly increasing the test time this extra cost could not be justified.

To evaluate this test method, a study on a wide variety of organic waste samples is required with the samples undergoing analysis using the DR4 and BM100 methods, and the novel Enzymatic Hydrolysis Test (EHT) method. A correlation between the DR4 and BM100 results could then be compared with the correlation between the EHT and the BM100 results.

3.3.2. Use of a Pure Cellulase Enzyme

Following the addition of the enzyme mixture, the DOC release increases over time. This is shown in Figure 3.6.

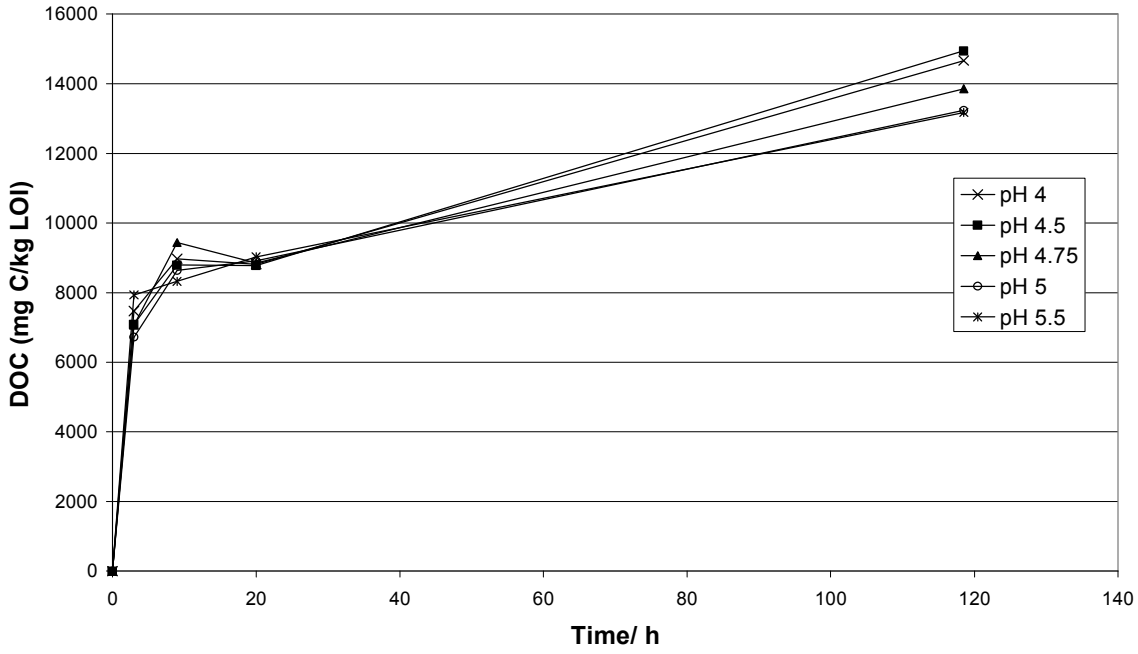

Figure 3.6. DOC (mg C/kg LOI) increase over time (Incubation temperature 40°C) using a pure cellulase enzyme. Error bars shown as standard deviation (stdev).

The initial rate of hydrolysis is comparatively rapid, with a gradual DOC release after 10 hours. The incubation temperature of 40°C was chosen as this temperature yielded the highest DOC release in the optimisation investigation using a crude cellulase enzyme. However, the DOC release using the pure cellulase enzyme is considerably lower than that observed for the crude enzymes. At 118 h the DOC values were between 13-15, 000 mg C/kg LOI, whereas with the crude cellulase enzymes at the same temperature, at 71 h the DOC values were between 45-83, 000 mg C/kg LOI. Due to this comparatively low DOC yield, it was concluded that no further investigation was required for the pure cellulase enzymes.

The low DOC yield may be due to the restricted types of enzyme present in the cellulase used (discussed in section 3.1.2). Ideally for complete hydrolysis of a cellulose substrate, each type of enzyme needs to be present. Therefore using a pure cellulase enzyme with a cellulose substrate could only allow for the partial hydrolysis to soluble carbons, indicated by the low DOC yield. Therefore a crude cellulase mixture is likely to be most suitable for this test method, particularly in cases where heterogeneous organic waste material will be analysed in later sections.

3.3.3. Effects of the Autoclave Process

A disadvantage of currently used biodegradability test methods, such as the DR4 and BM100 (discussed in previous Chapter), is that they are biological. This means that as they are dependent on biological activity, they are susceptible to variation. This is because ensuring the same biological activity in the seed material added is not possible, and can result in irreproducible data. The EHT is non-biological, and so is not dependent on biological activity, rather depends on the uniform addition of enzyme activity, which is easily achieved. To ensure that the DOC released in phase 3 of the EHT (after enzyme addition) is due to enzyme hydrolysis alone, the sample must be sterilised to remove biological activity.

The use of autoclave has been used in all investigations described in this Chapter as the method of sample sterilisation.

The autoclave process is an aggressive method of sterilising materials, using high temperature and pressure conditions to heat aqueous solutions above their boiling point. It is expected that there would be significant effects of the autoclave process on organic waste materials. The waste materials are mixed with a mild acid buffer, and so may undergo mild acid hydrolysis, aided by the high temperature conditions. Thermal decomposition of organic molecules may also occur, and so the molecular structures of the organic waste may be adjusted. For these reasons, the DOC release at phase 2 of the EHT is expected to be higher when the sample is autoclaved. The DOC released at each phase of the EHT for untreated and treated waste samples, with and without the autoclave step, is shown in Figure 3.7.

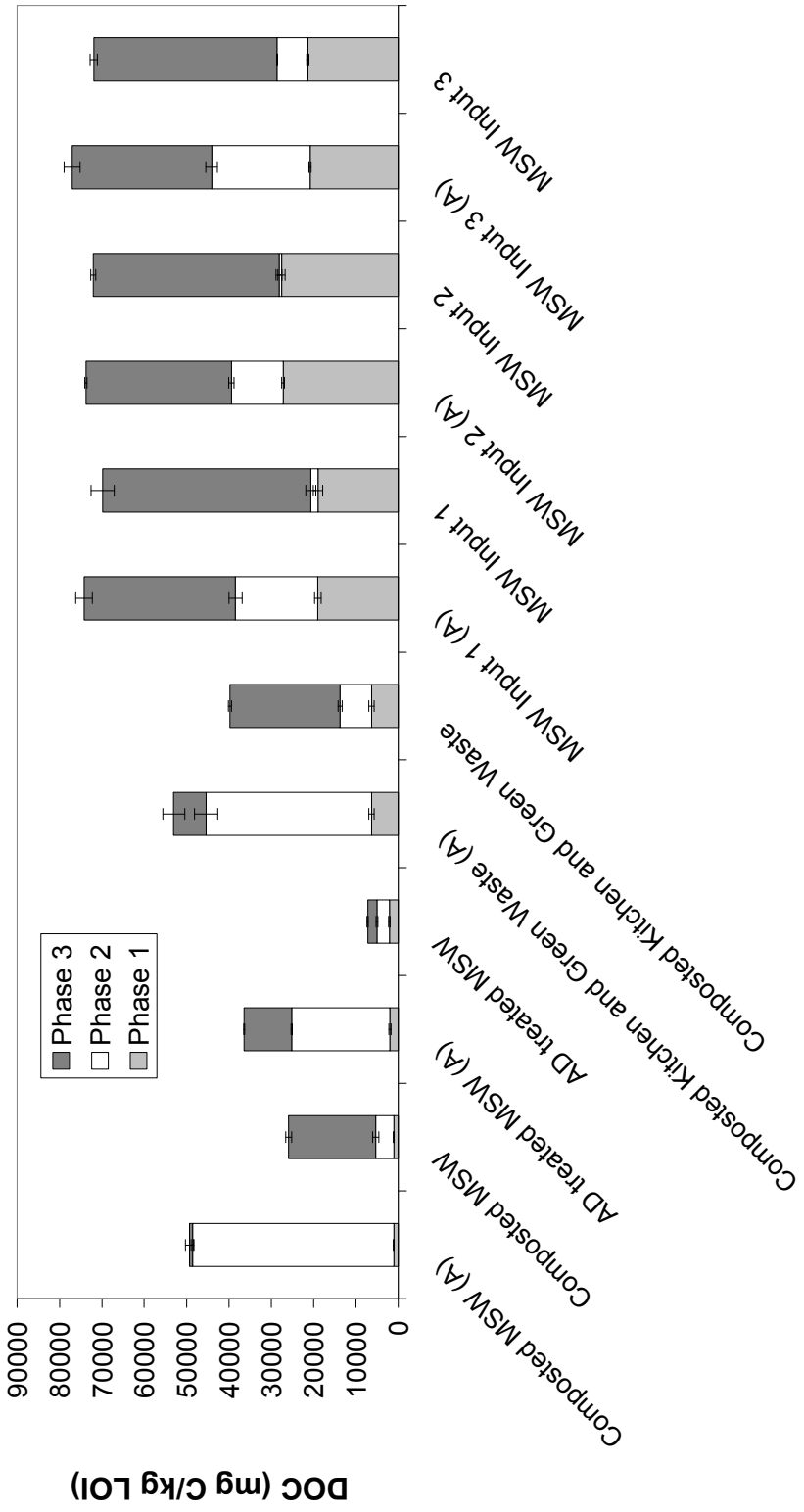

Figure 3.7. DOC release at each phase of the EHT with and without the autoclave step at phase 2 for untreated and treated waste samples.

As shown in Figure 3.7, the DOC released at phase 2 of the EHT is significantly higher for samples that are autoclaved. This suggests that the high temperatures may break down organic molecules and solubilise the products. High temperatures encourage solubilisation, and so a proportion of the DOC will be molecules that would otherwise have not dissolved in the time it takes to complete the autoclave cycle.

For 5 of the cases the DOC released in phase 3 (incubation with enzymes) is greater for samples which have not been autoclaved. Therefore organic molecules which would undergo mild-acid and thermal hydrolysis under autoclave conditions are instead hydrolysed by the enzymes added to the mixture. The anaerobic digestion treated MSW sample was an exception to this trend, and so suggests that the AD process removes such organic molecules, leaving those that are only hydrolysed by the enzymes added.

In each case the total DOC is greater for samples that were autoclaved, significantly so for the treated samples. Therefore organic molecules which would otherwise not dissolve are however solubilised as an indirect or direct result of the autoclave process.

The results indicate that the autoclave has a positive effect on the EHT method, increasing the total DOC release. Therefore the autoclave process is an important process of the EHT, as the benefits extend beyond sterilising the waste material.

3.4. Conclusions

The results of this investigation allow for the selection of the optimum conditions and timescales required for sufficient substrate hydrolysis. These conditions are pH 4.75, 50°C and an enzyme incubation time of 20 h.

The timescale of the test suggests an improvement to the 4 days of the DR4 test method currently used in England and Wales. It can be concluded from this investigation that the EHT has potential as an alternative short-term test method, however further evaluation and development are required.

The autoclave process sterilises the sample, eliminating potential error due to variation in biological activities, and thus ensures that the DOC released is from the standard enzyme solution added. The use of autoclave was found to be beneficial when the EHT was applied to organic waste materials, resulting in a greater DOC release.

Chapter Four

Comparison of the EHT with the DR4 and BM100 Methods

This chapter discusses the comparison of the EHT with the current microbial methods, the DR4 and BM100 tests. The three test methods are applied to a wide range of untreated and treated organic waste materials taken from a number of sources. The correlations of the short-term DR4 and EHT with the BM100 are compared to verify the use of the EHT as a short term biodegradability test method. A modified form of this chapter has been published in the Communications in Resource and Waste Management journal (see Appendix G).

4.1. Introduction

In the previous chapter the development of the enzymatic hydrolysis test (EHT) was described. Here it was concluded that the use of a crude cellulase enzyme was most suitable for the application to a wide range of heterogeneous organic waste materials. The optimum conditions for such a biodegradability test method were determined. The application of the EHT to organic waste samples is required to validate its use as a rapid alternative test method and to make a comparison with established biological test methods. In this chapter the EHT, DR4 and BM100 test methods are applied to 38 untreated and treated organic waste materials and the results are compared.

The majority of commonly used test methods used to determine waste biodegradability, as discussed in chapter 2, are biological methods and typically involve the use of live micro-organisms. These may be conducted over a few days to assess the initial organic matter decomposition rate i.e. the readily biodegradable material. Otherwise the tests can be conducted over many weeks until decomposition ceases, or slows to a negligible rate, and the full extent of degradation measured.

As discussed in previous chapters, the amount of biodegradable municipal waste (BMW) sent to landfill needs to be significantly reduced. Biodegradable content of waste materials can be reduced by waste treatment processes, such as composting or anaerobic digestion. The degree to which the rate of biodegradability of the waste is reduced by the treatment process, and the extent of decomposition achieved, can both be used as an indication of the performance and efficiency of the treatment process. The quantity of BMW diverted from landfill can be calculated from biodegradability data obtained from input and output materials in accordance with the landfill allowance trading scheme (LATS), which has been discussed in chapters 1 and 2.

There are several established biological biodegradability test methods, in which the conditions are either aerobic or anaerobic, developed to meet specific objectives (such as compost maturity or landfill acceptance). In this chapter the non-microbial EHT method has been compared with a microbial based 100 day anaerobic test (BM100) and the 4 day aerobic test (DR4), the

latter two methods being specified in guidance for monitoring MBT processes in England and Wales (Environment Agency, 2005). The BM100 method has been reported to show good reproducibility between results (Godley *et al.*, 2003), but has the disadvantage of taking a long time (up to 100 days) to complete, thereby not providing rapid feedback on plant performance for commissioning, optimisation and routine monitoring purposes. Short-term (up to 7 days) aerobic methods including the DR4 test have other disadvantages such as preferentially decomposing the readily biodegradable components of the waste (Godley *et al.*, 2007b) and only measuring the initial biodegradation rather than actual extent of biodegradation. Therefore most current microbial based biodegradability test methods have limitations and no one test method is deemed suitable for the whole range of biodegradability testing requirements associated with monitoring MBT process performance and assessing organic waste bio-stability. A review of the current methods (Godley *et al.*, 2003) concluded that there is a need for a rapid and cost-effective test method that would mimic and correlate with longer-term tests such as the anaerobic BM100 method. The BM100 test method is not suitable for regular routine testing due to its duration, however a correlating method could make routine testing viable.

A large proportion of BMW consists of biopolymers (proteins, fats, polysaccharides and lignin) that undergo enzymatic hydrolysis to soluble monomers during the microbial decomposition process before the organic waste is utilised by the microbes as a carbon and energy source. Hemicellulosic/cellulosic material is considered as the most important carbon

source for methanogenesis in landfills as it contributes to 90% of the total biogas (CO_2 + CH_4) produced (Barlaz *et al.*, 1989; Rodriguez *et al.*, 2005). As a general rule, the higher the cellulose/hemicellulose content, the higher the biogas yield of the waste in anaerobic tests (Eleazer *et al.*, 1997). Therefore assessment of the waste cellulose and hemicellulose content may provide a non-biological test method for assessing biodegradability. However lignin (a complex aromatic based plant polymer) is closely associated with cellulose in native plant matter as lignocelluloses and this may comprise 30-50% of BMW (Rodriguez *et al.*, 2005). Lignin is also considered to be poorly biodegradable under anaerobic conditions (Chen *et al.*, 2004; Sjöberg *et al.*, 2004; Stinson *et al.*, 1995; Tuomela *et al.*, 2000). However the availability of the cellulose to enzyme hydrolysis can vary as the associated lignin can protect the cellulose from enzymatic decomposition. Direct chemical measurement of the cellulose and hemicellulose content of a waste sample could logically provide an estimate of the biodegradability of that sample. However this may be inappropriate as not all the cellulose is amenable to biodegradation when present as lignocellulose (Chen *et al.*, 2004).

Cellulose and hemicellulose are hydrolysed by cellulase and hemicellulase enzymes respectively and so the EHT method based on the enzymatic hydrolysis of cellulosic material could offer a suitable routine test method. This would mimic the natural microbial hydrolysis of organic matter and would be expected to take account of the impact of lignin on the availability of cellulose. A high concentration of enzyme can be added to the test which might be expected to hydrolyse all the potentially hydrolysable cellulose and therefore

more closely mimic the long term BM100 test rather than short-term DR4 tests.

In this study the DR4, BM100 and the enzymatic hydrolysis test (EHT) method have been applied to 38 organic treated and untreated waste samples from a range of sources in the UK. These samples were taken from a number of waste treatment processes, and include municipal solid waste (MSW), green waste and specific wastes such as pizza, fish and feather wastes. These samples were selected to provide a wide range of materials in order to validate the biodegradability test methods, and the applicability to a number of waste samples. The DR4 and EHT biodegradability test methods are compared and correlated with the longer-term BM100 method.

Due to the limitations of each test method, the waste samples may yield contrasting data. For example the cellulose substrate is known to yield a relatively low BM100 value (Godley *et al.*, 2007b). This is due to the acidification of the test mixture from the formation of acetic acids during anaerobic decomposition, causing methanogenesis to cease, leading to overall lower results. It has also been observed that the DR4 is sensitive to readily biodegradable content (Godley *et al.*, 2007b). Therefore a 'perfect' correlation with the BM100 method is very unlikely, since the EHT, DR4 and BM100 methods all possess limitations and measure different parameters. The EHT is a cellulase enzyme-based test method, and so in samples such as the turkey feathers there will not be a large amount of cellulose substrate present. However these samples may still be highly biodegradable, the EHT

may underestimate this due to the absence of the specific enzyme required to hydrolyse the substrate present in the waste material. As discussed in chapter 2, it is for this reason that a crude cellulase enzyme which possesses hemicellulase and protease activities is better suited for such a test than a pure cellulase enzyme.

This study aims to investigate whether the EHT is a more suitable short-term biodegradability test than the DR4 test method. This will be determined by considering the correlations of each test with the long-term BM100 test method. The overarching aim of developing an alternative short-term biodegradability test method is to provide an improved relationship with the BM100 from that of the DR4. The EHT is a more rapid test method than the DR4; however this chapter aims to test the hypothesis that the EHT will have a stronger relationship with the BM100 test method.

The objectives of this investigation are-

- Apply the EHT to untreated and treated organic waste materials and compare with existing microbial test methods.
- Investigate the DOC release in non-enzyme Phases of the EHT (Phases 1 and 2) for untreated and treated samples.
- Determine the required length of the enzyme incubation Phase, based on the correlations with the BM100 values.

In chapter 3, the optimum conditions were determined for a crude cellulase enzyme. Under these conditions stages of the hydrolysis could be observed,

which included the rapid initial rate, plateau region (point at which hydrolysis begins to cease) and the final steady and gradual rate. These three categories of reaction stage were assessed in this investigation, measuring the DOC release in Phase 3 at set benchmark times of 3, 20 and 40 h. These times correspond to the three reaction stages observed in chapter 3.

4.2. Materials and Methods

4.2.1. Samples

The organic waste samples were collected from a wide range of treatment processes and waste streams in the UK as part of Defra project WR0110 on waste characterisation. The samples included MSW derived samples, garden waste (partially treated in the short-term, stabilised and longer-term fully treated) and samples from specific waste streams such as fish, wood, pizza and feathers. Where possible the samples were collected pre-, during and post- treatment by either MBT or a mechanical thermal (autoclave) treatment. The biological treatment of the samples was either composting or anaerobic digestion.

The samples were collected from each source in 10 x 2 kg batches of the waste material, taken at the same time (Figure 4.1). These batches were then thoroughly mixed to make up the composite sample of the waste material and ‘coned and quartered’ and a 2-3 kg analytical sample was obtained. The 2-3 kg samples were frozen until required.

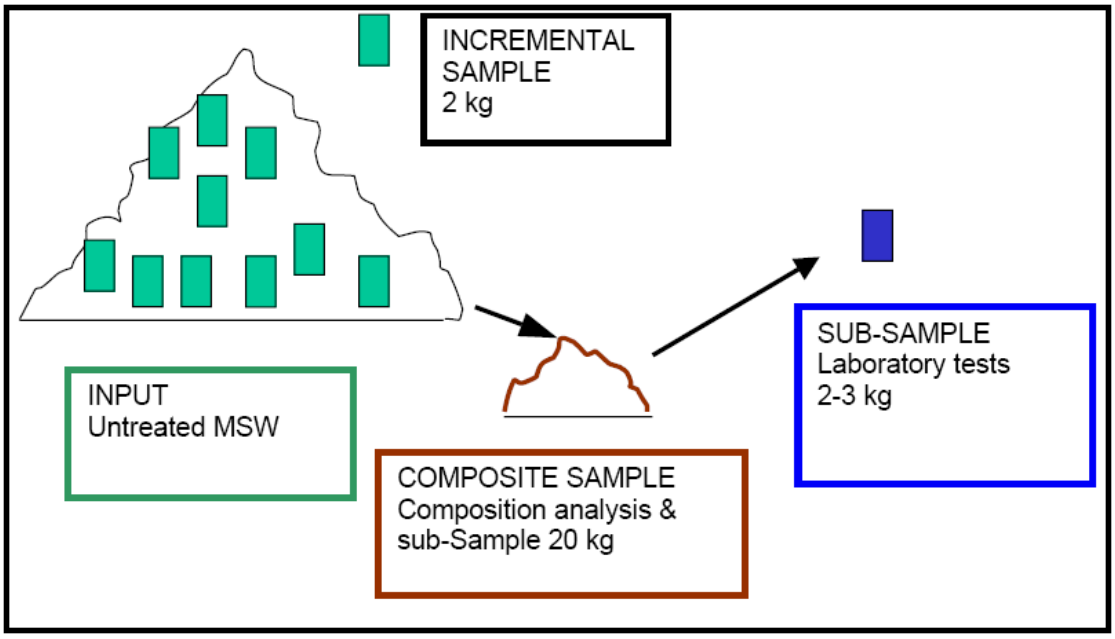

Figure 4.1. Guidance on obtaining a composite sample (Environment Agency, 2005).

Samples were sorted to remove glass, metals, plastics and inert materials with the biodegradable material being retained and tested. Materials with large particle sizes were shredded to <10 mm before testing to ensure thorough mixing and a good representation of the sample as a whole. The dry matter (DM) and loss-on-ignition (LOI) of each sample was required to use consistent masses in the analysis (e.g. LOI indicates organic portion of the sample, therefore samples can be weighed out with consistent amounts of organic components). These were determined for each sample using standard procedures (EN12879:2000).

4.2.2. UK Established Methods

Two established biodegradability test methods were used in this investigation. In a recent comparison of the two methods (Godley *et al.*, 2007b), it was stated that the 4 day aerobic test measures the rate of aerobic degradation,

whereas the 100 day anaerobic test measures the extent. The DR4 test method was carried out at the Open University, whilst the BM100 test was completed, partially by the author, at WRc plc.

4.2.2.1. Dynamic respiration over 4 days (DR4)

Biodegradability under aerobic conditions was determined using the DR4 test method (Environment Agency, 2005), which is adapted from the American Society for Testing and Materials (ASTM) method D5975-96 (ASTM, 1996; Godley *et al.*, 2007a; Godley *et al.*, 2007b). The test material (100 g DM) was prepared as outlined previously and mixed with the seed material, in this case mature green waste compost (100 g DM). Water and nutrients (nitrogen and phosphorus) were added to adjust to 50% w/w moisture content, based on the measured DM of the sample. The test mixture was placed in a reactor vessel at 35°C for 4 days, with constant aeration (500 ml/min (Environment Agency, 2005)) through the reactor vessel (Figure 4.2).

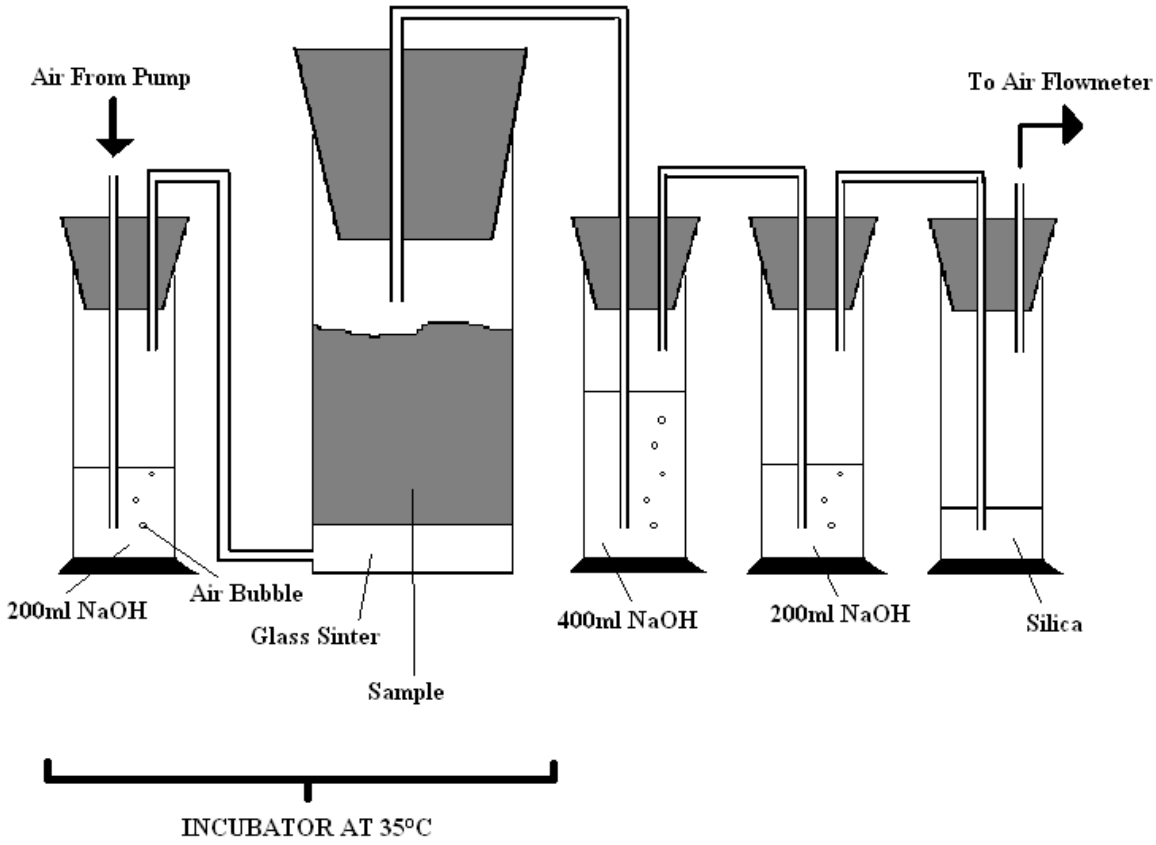

Figure 4.2. Set-up of the DR4 test method.

The air passed through the reaction vessel was then passed through NaOH (1M) carbon dioxide traps is a mild acid, and so neutralises the NaOH-

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

The NaOH is then titrated with 1M HCl with Phenolphthalein indicator (which change colour from pink to colourless in neutral conditions)-

$$NaOH + HCl \rightarrow H_2O + NaCl$$

The titration therefore allows for the calculation of NaOH reacted with CO_2, and so CO_2 production can be calculated. The CO_2 released over the 4 day period was measured and this data used to estimate O_2 consumption.

4.2.2.2. Biochemical methane potential over 100 days (BM100)

The BM100 test method (Environment Agency, 2005) is based on a sewage sludge digestion test (Godley *et al.*, 2007b; Godley *et al.*, 2003). The test material (20 g LOI) was placed in a glass container with microbial seed (digested sludge) and a nutrient mixture (shown in Table 2.2, in Chapter 2). The mixture was sealed and incubated at 35°C under anaerobic conditions and the release of CO_2 and CH_4 (biogas) was measured volumetrically until no further biogas was released (up to 100 days). Graduated glass cylinders were used to volumetrically measure biogas release. These cylinders were filled with acidified water (to prevent CO_2 dissolving) and the gas released displaced the liquid in the cylinder, enabling the direct reading of gas volume released. The BM100 set-up is shown in Figure 4.3.

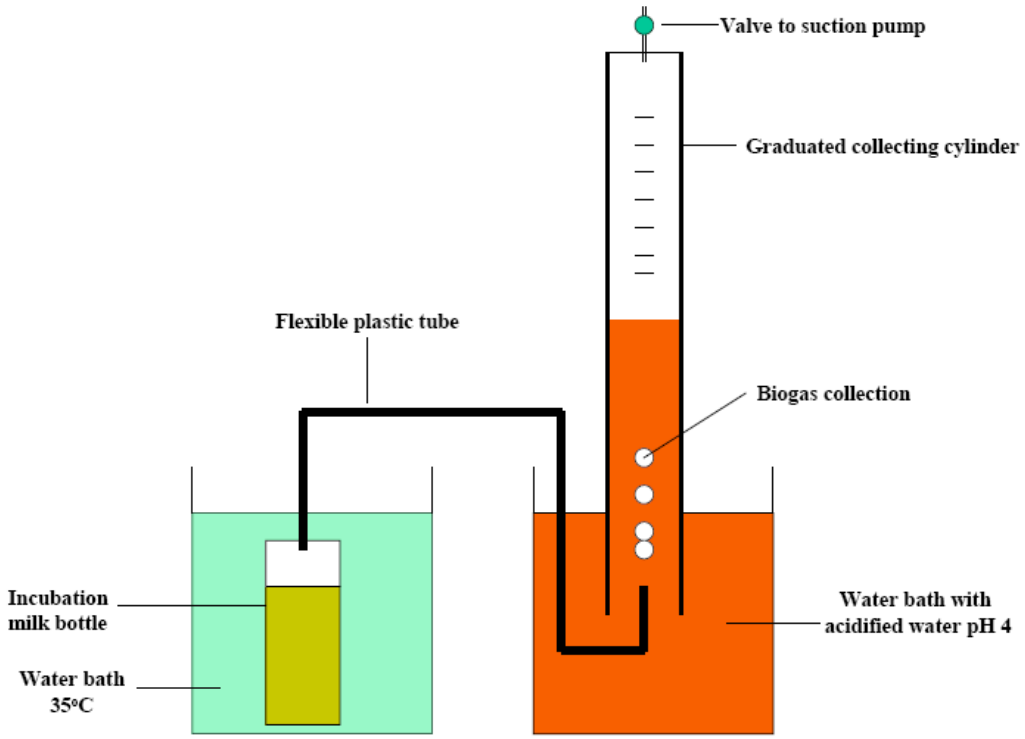

Figure 4.3. Set-up of the BM100 test method (Environment Agency, 2005).

4.2.3. Enzymatic Biodegradability Test Method

For each sample 25 mg of crude cellulase powder (Sigma) and 75 mg of hemicellulase powder (Sigma) were dissolved in 20 ml of distilled water, with 195 units of cellulase and 112.5 units of hemicellulase activities in each 20 ml of enzyme mixture. This enzyme solution was then filtered through 0.22 µm Millipore membrane filters for sterilisation.

The crude cellulase enzymes contain some hemicellulase and protease activity (within manufacturer specifications), with the hemicellulase enzymes also having some cellulase activity.

The test method consists of three Phases as shown in Figure 4.4.

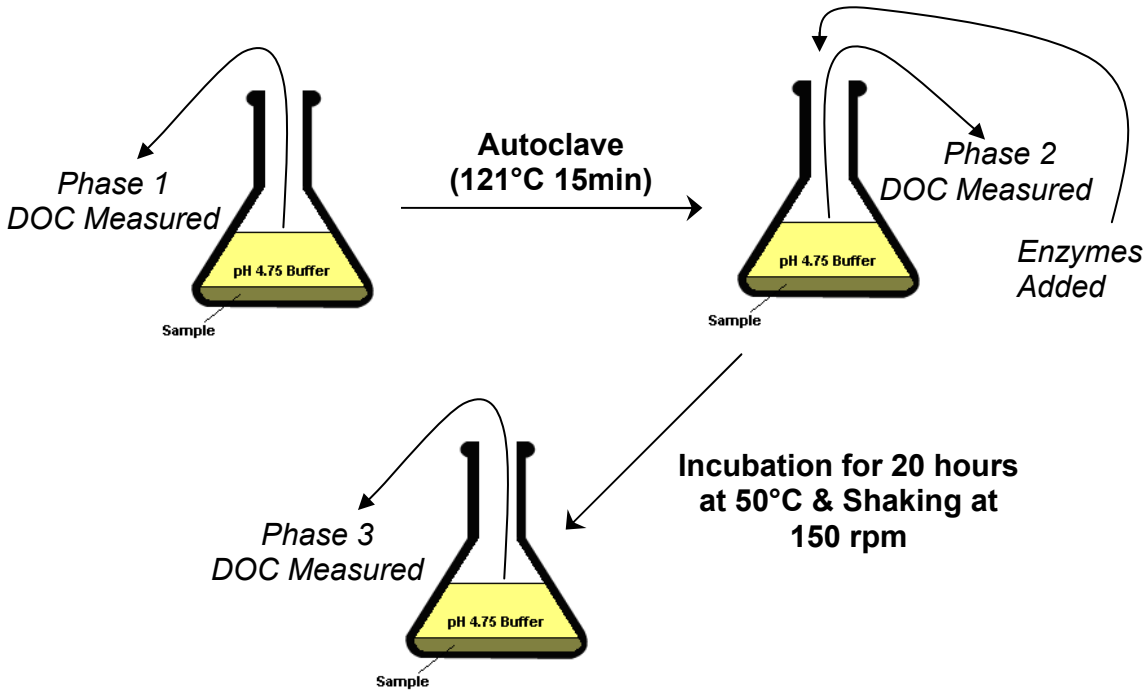

Figure 4.4. Schematic diagram of the EHT method.

- Phase 1. The waste sample (5 g LOI) was placed in a 250 ml Erlenmeyer flask. Phosphate pH buffer (100 ml 0.37 M) was then added to the flask. A 5 ml sample was removed and filtered (0.45 µm membrane filter) to remove any solids, and the filtered liquid was then analysed for chemical oxygen demand (COD) (Spectroquant COD test tubes).

- Phase 2. The sample mixture was then autoclaved at 121°C for 15 min to sterilise the mixture and a further 5 ml sample was removed and filtered for COD analysis.

- Phase 3. The prepared enzyme solution (20 ml) was then added to each of the flasks and the flask sealed with a neoprene bung. The flasks were placed in a shaking incubator at 150 rpm. A 5 ml sample was removed for COD analysis after 3, 20 and 40 h of incubation.

The amount of moisture in the waste sample and the removal of both the liquid and solids at each stage of sampling, along with the addition of liquid in Phase 3, were accounted for in the concentrations of carbon calculated. Soluble COD analysis results were converted to DOC (mg C/l) by assuming a COD/C ratio of 2.67 based on the relative molecular mass of cellulose monomeric units.

The error of the data is calculated as the standard error (SE), calculated from the standard deviation (SD) of the values as follows, where n is the number of samples-

$$SE = \frac{SD}{\sqrt{n}}$$

4.3. Results

4.3.1. DOC Release in Each Phase of the EHT

The EHT is a three-phased test method, and the DOC released at each Phase was measured. The DOC released in Phases 1, 2 and 3 of the EHT method varied between the samples. This is shown in Figures 4.5 and 4.6 for untreated and treated organic samples respectively.

The DOC released in Phase 3 (following enzyme hydrolysis) shown are following 20 h of enzyme incubation. Only this data is shown for clarity, but also due to the correlations with the BM100 observed at this incubation time, which are discussed later. The full dataset is shown in Appendix A.

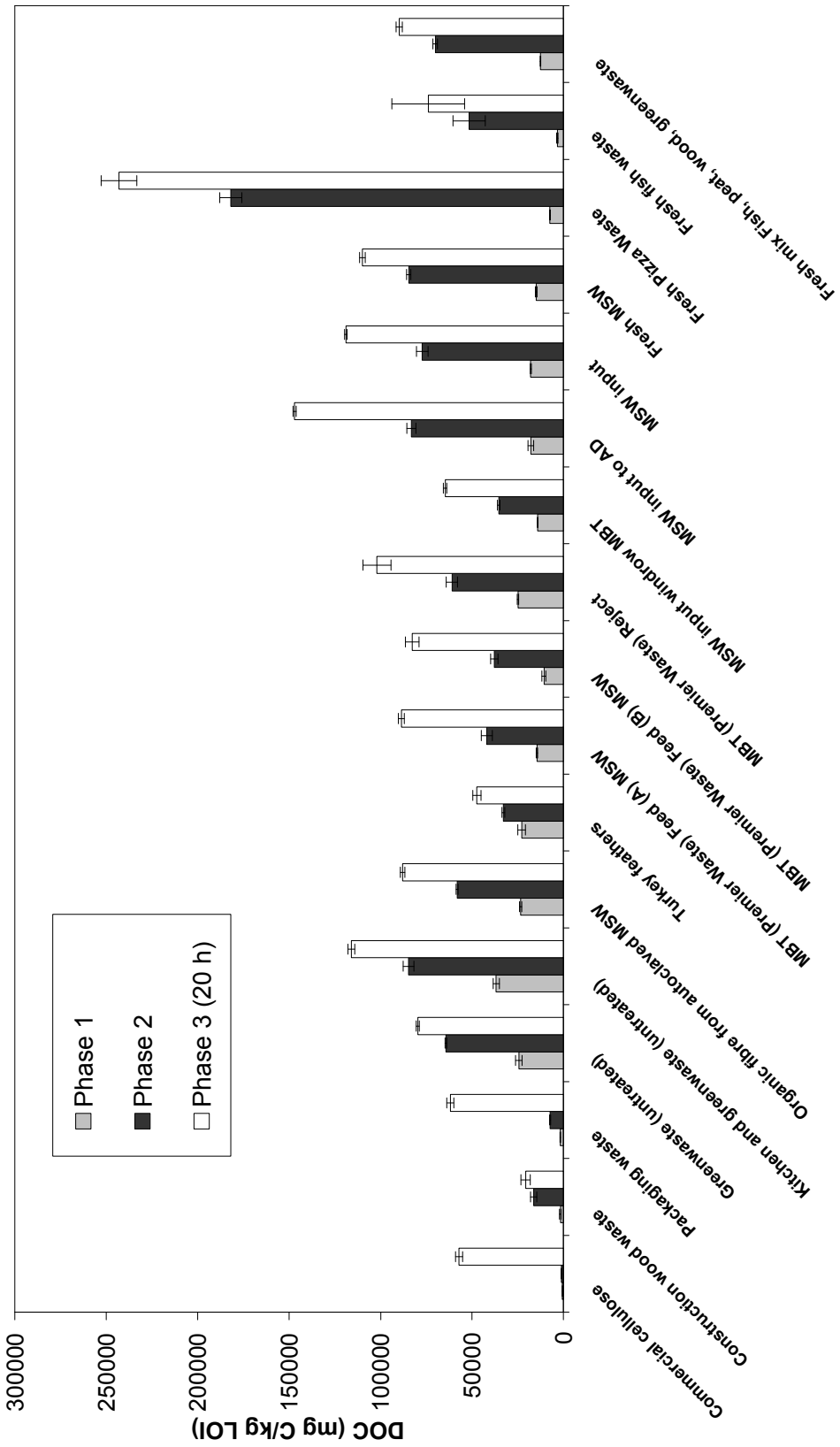

Figure 4.5. DOC released in Phases 1, 2 and 3 (20 h) for untreated organic samples. Error bars shown as the standard error.

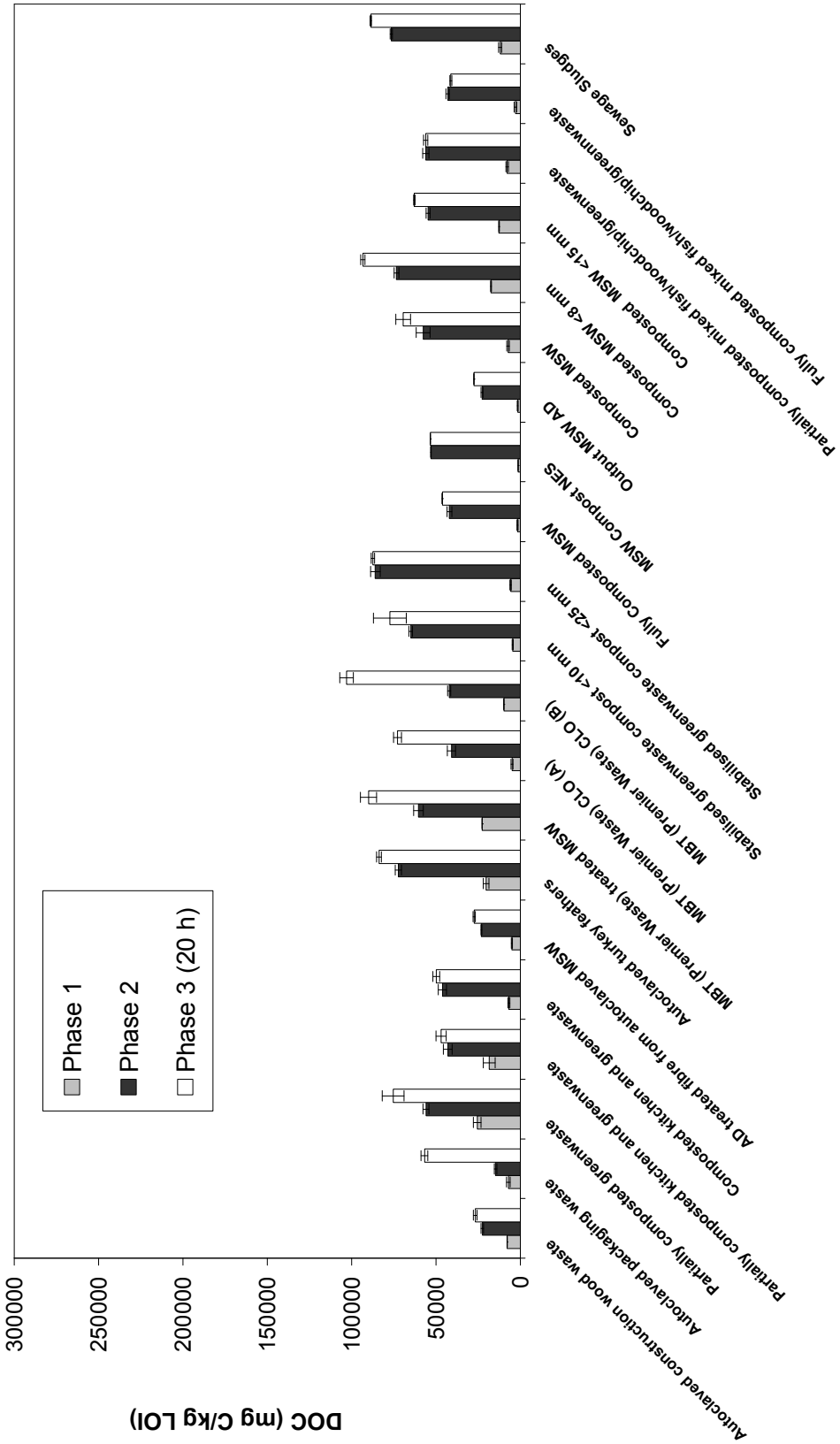

Figure 4.6. DOC released in Phases 1, 2 and 3 (20 h) for treated and stabilised organic samples. Error bars shown as the standard error.

As shown in figures 4.5 and 4.6, the DOC released in Phases 1 and 2 of the EHT varies between samples. Autoclaved turkey feathers shown in figure 4.6 also treated by anaerobic digestion.

Treatment of the organic materials generally results in lower amounts of DOC released in Phase 1. The DOC released in Phase 1 for untreated samples was on average 15,000 mg C/kg LOI, whereas for treated samples the Phase 1 DOC was 9,500 mg C/kg LOI on average.

After autoclave, in Phase 2, the average DOC of the untreated and treated samples was 58,000 mg C/kg LOI and 50,000 mg C/kg LOI respectively. However the highest increase in DOC was observed for treated samples. The average increase in DOC between Phases 1 and 2 was 834% for treated samples, whereas for untreated samples, this increase was 465%.

Following enzyme hydrolysis in Phase 3, the increase in DOC was generally highest for untreated samples. The average increase from Phase 2 to Phase 3 DOC was 37.5% for treated samples, whilst for untreated waste materials the average increase was 367%.

4.3.2. EHT Comparison with DR4 and BM100 Biodegradability Test Methods

Figures 4.7-4.13 show the relationship between the DR4, EHT and the BM100 data. For the EHT the total DOC (P3) is shown in figures 4.8-4.10 for 3, 20

and 40 h respectively. The DOC from enzyme hydrolysis alone (P3-P2) is shown in figures 4.11-4.13 for 3, 20 and 40 h respectively.

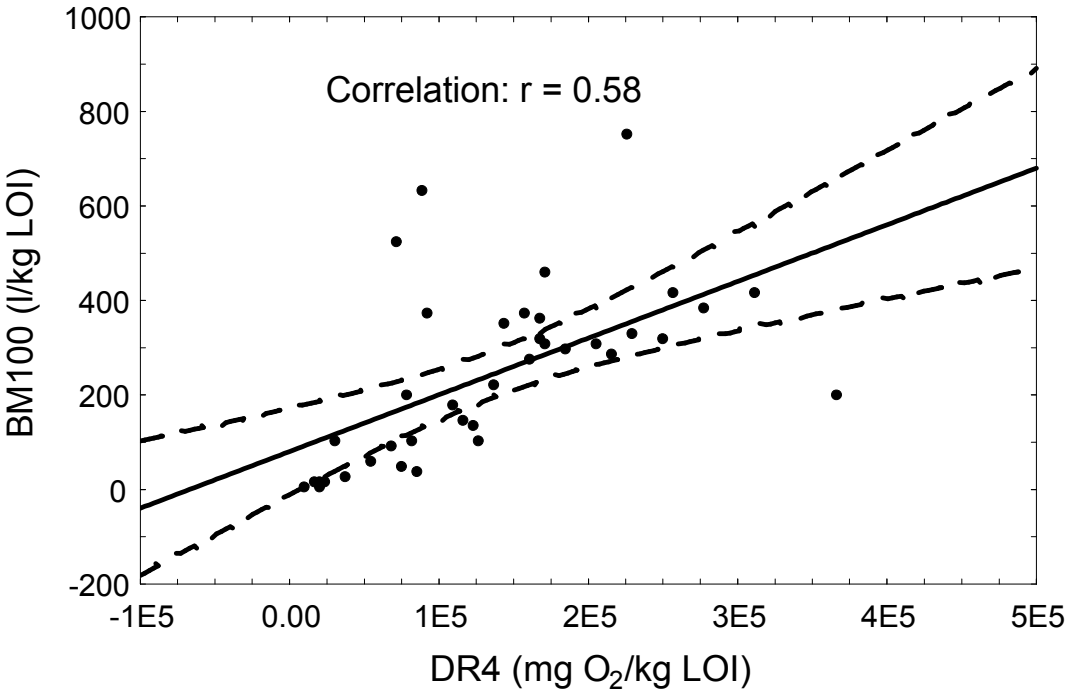

Figure 4.7. Correlation of DR4 with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

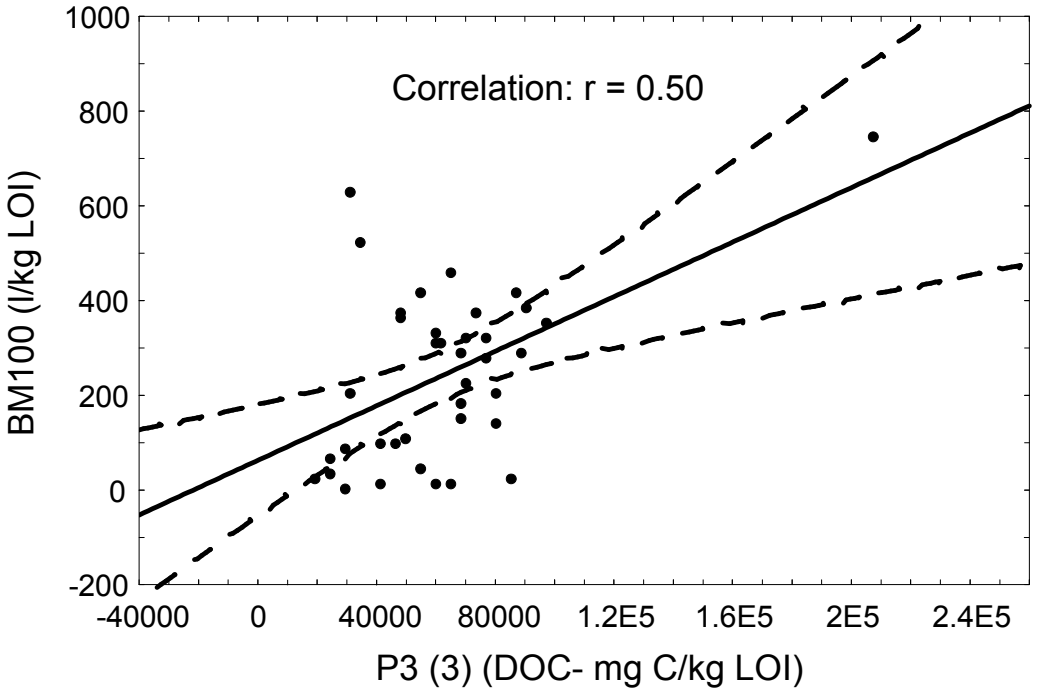

Figure 4.8. Correlation of EHT (total DOC at 3 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

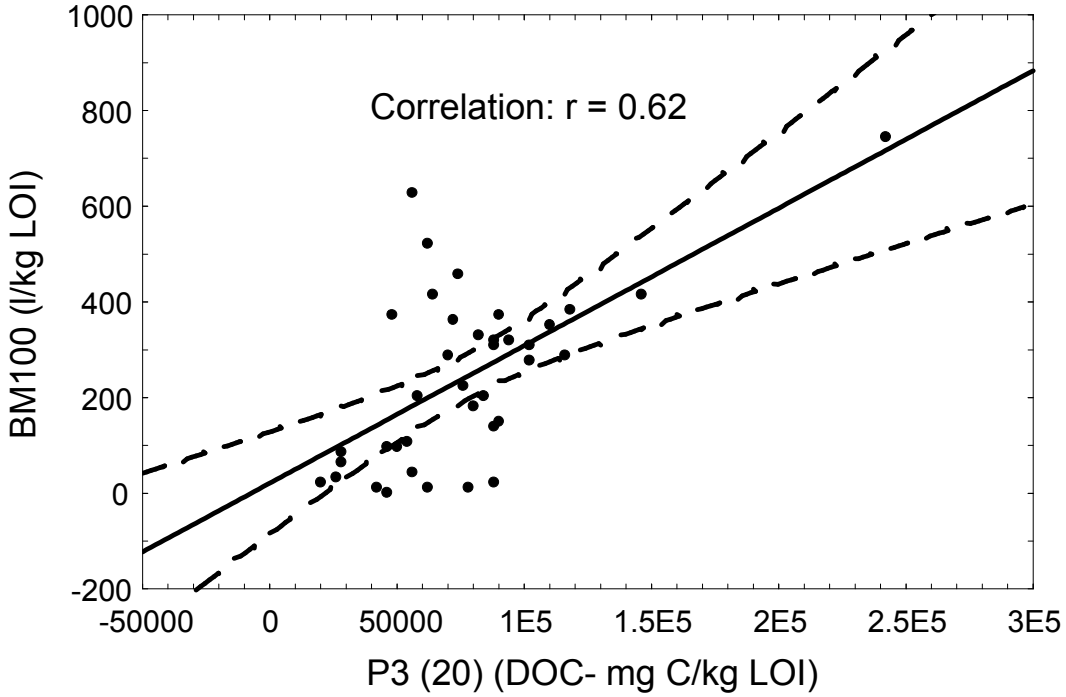

Figure 4.9. Correlation of EHT (total DOC at 20 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

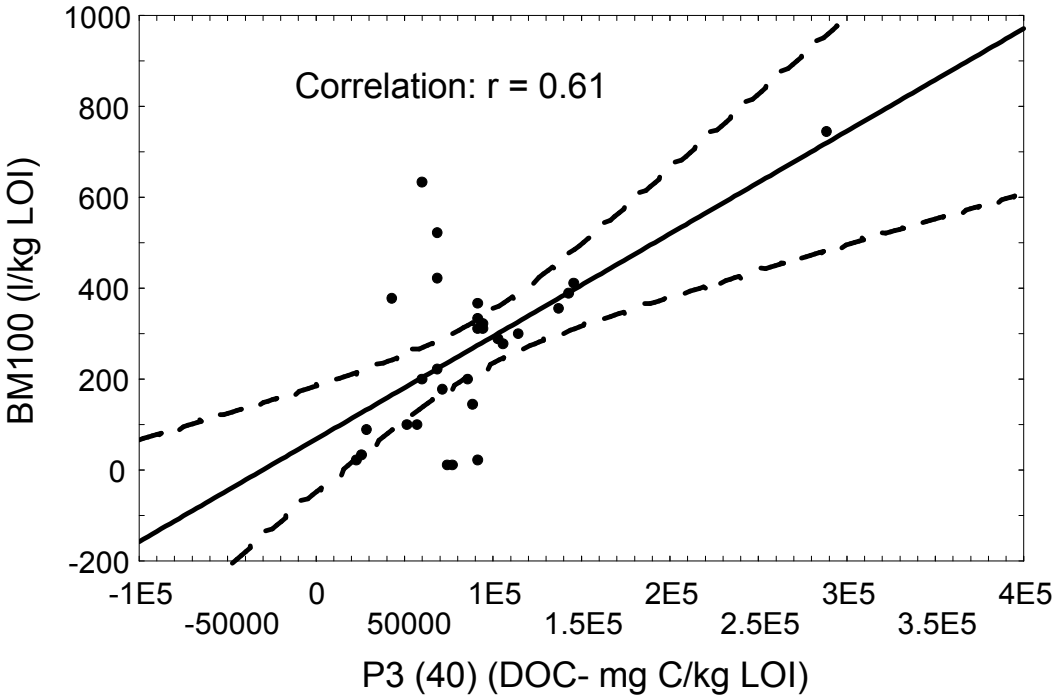

Figure 4.10. Correlation of EHT (total DOC at 40 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

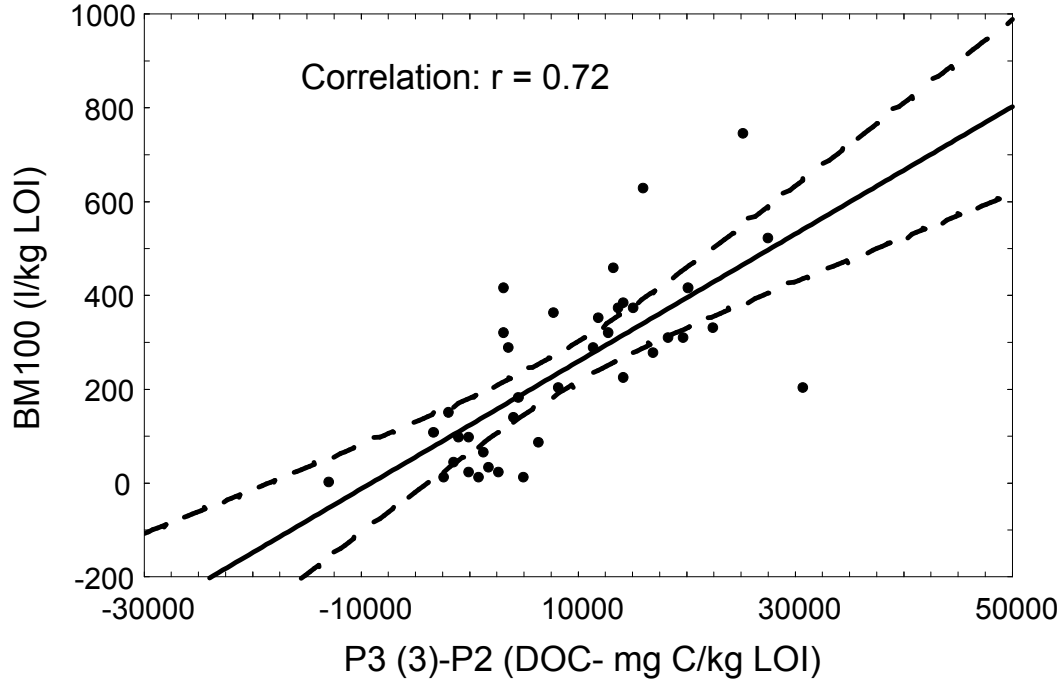

Figure 4.11. Correlation of EHT (DOC from enzyme hydrolysis at 3 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

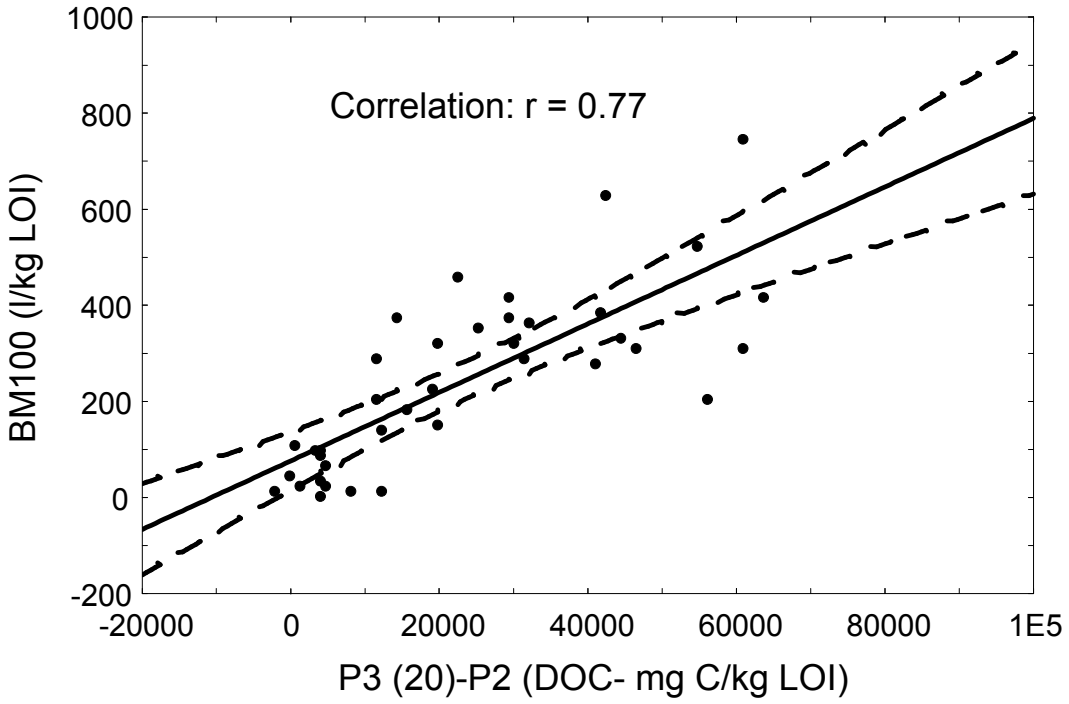

Figure 4.12. Correlation of EHT (DOC from enzyme hydrolysis at 20 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

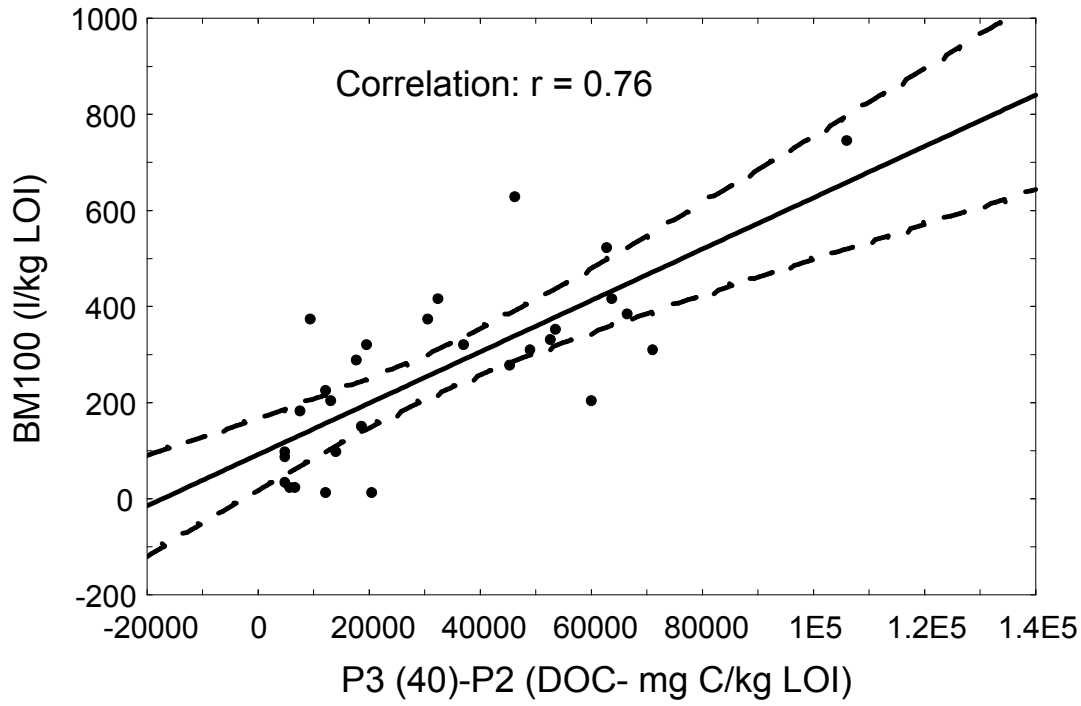

Figure 4.13. Correlation of EHT (DOC from enzyme hydrolysis at 40 h) with BM100 data for all samples. The dashed lines indicate region of 95% confidence.

There is a positive linear relationship observed for the DR4 and EHT with the BM100, indicated by the positive correlation coefficient (r) values shown in Figures 4.7-4.13.

From the correlations of the DR4 and EHT against the BM100 data, the EHT shows the greater linear correlation with the BM100, with the notable exception of the total DOC at 3 h. The correlation coefficients (r) for the EHT (total DOC) at 3, 20 and 40 h are highly significant ($p < 0.001$), as is the correlation coefficient of 0.58 for the DR4 ($p < 0.001$). The relationship between the EHT and BM100 data is stronger when only the DOC released from enzymatic hydrolysis is considered, giving a correlation of 0.72, 0.77 and 0.76 ($p < 0.001$) at 3, 20 and 40 h respectively.

4.4. Discussion

4.4.1. DOC Release in Each Phase of the EHT

The DOC released in Phases 1 and 2 are shown in Figures 4.5 and 4.6. The soluble carbon that is available prior to the addition of the enzyme solutions varies expectedly between sample types and treated and untreated samples. The Phase 1 DOC is likely to represent the low molecular weight readily soluble materials present in the waste. Therefore as expected these materials have been reduced by the treatment process, resulting in the lower Phase 1 DOC observed.

Autoclaving the waste material greatly increases the DOC release in Phase 2. Sterilisation of the waste material is required to prevent microbial growth on

released DOC during the test, to ensure that the entire DOC released in the test is accounted for. In the EHT procedure the waste material is mixed in a mild acid solution, and it has been observed previously that the hydrolysis of cellulose and hemicellulose are catalysed by mild acids (Jacobsen *et al.*, 2000). The pre-treatment of wood material with dilute acid has been investigated by Torget *et al* (1990) and Nguyen *et al* (1998) at high temperatures of 140-160°C and 200-230°C respectively. It was observed by Torget *et al* (1990) that the treatment of hardwood and herbaceous samples with dilute sulphuric acid at 140-160°C hydrolysed all hemicelluloses and small amounts (<15%) of lignin and cellulose (Torget *et al.*, 1990). Nguyen *et al* (1998) soaked softwood chips in dilute sulphuric acid, followed by steam treatment at 200-230°C. It was observed that 90-95% hemicellulose and up to 20% cellulose was hydrolysed (Nguyen *et al.*, 1998). The EHT involves the waste being mixed with a mildly acidic phosphorus pH buffer, rather than a dilute sulphuric acid solution. However the autoclave process will result in the mild acid hydrolysis of cellulose, lignin and particularly hemicellulose to an extent as described in previous studies.

The DOC released following the autoclaving process in Phase 2 represents soluble DOC following the thermal and mild acid hydrolysis of some of the polymeric components during autoclaving. Phase 2 DOC may also include soluble materials desorbed from the waste during autoclaving, along with more slowly soluble DOC.

The increase in DOC between Phases 1 and 2 is much greater for the treated waste samples. For untreated samples the average increase between Phase 1 and 2 was 465%, whilst for treated samples this increase was 834% on average. Significantly higher percentage increases were observed for untreated samples such as fish and pizza waste of 1450 and 2350% respectively. This is likely to be due to the higher levels of fats and proteins expected for such samples than for MSW or green waste materials. Whilst these compounds would solubilise without autoclaving, the higher temperature of autoclave encourages faster solubilisation, and so the P2 DOC increases significantly from the DOC values observed for P1. For the MSW compost NES (New Earth Solutions) sample a relatively low Phase 1 value was observed, which was just 2% of the DOC released in Phase 2, representing a 4100% increase.

The non-enzymatic DOC (Phases 1 and 2) for wastes that have undergone extended biological treatment (e.g. the fully composted green waste and composted MSW derived BMW samples), are likely to consist of significant amounts of humic substances resulting from the decomposition of lignin (Stevenson, 1994). These substances are not usually considered to be readily biodegradable, and so in these cases, the DOC due to enzymatic hydrolysis (Phase 3 only) may be indicative of sample biodegradability. Unlike the control polymeric cellulose, many of the untreated (raw or autoclaved) waste samples also showed significant amounts of DOC released during Phases 1 and 2. As these wastes have not been biologically treated it

is likely that much of the DOC released during Phases 1 and 2 will be inherently biodegradable.

The DOC increase from Phase 2 following enzyme addition was significantly higher for untreated samples than for treated samples. This was expected since the content of biodegradable materials such as cellulose, hemicellulose, fats and proteins are likely to be lower following biological treatment of the waste material. Biological treatment reduces the biodegradable content of an organic waste material (Adani *et al.*, 2000; Binner, 2003; Cossu *et al.*, 2005; Cossu *et al.*, 2008). The DOC released by the enzymatic hydrolysis is therefore directly related to the readily biodegradable content of the waste, and so represents the available substrates that are amenable to hydrolysis. The enzyme mixture used in the test includes crude cellulase, hemicellulase and protease activities, and so the DOC release in Phase 3 is likely to represent the DOC released from the hydrolysis of cellulose, hemicellulose and protein.

4.4.2. EHT comparison with DR4 and BM100 test methods

The correlations obtained with the BM100 test method suggest that the EHT method is better suited to a wider range of waste types; particularly when considering the relationship of the DOC from enzyme hydrolysis and the BM100 (Figures 4.11-4.13). The correlations of the EHT with the BM100 values are summarised in Table 4.1.

Enzyme Incubation Time/ h	EHT Correlation (r) with BM100	
	Total DOC	Phase 3 DOC
3	0.50	0.72
20	0.62	0.77
40	0.61	0.76

Table 4.1. Correlations of the EHT with BM100 values.

The correlations of the DOC from enzyme hydrolysis with BM100 data indicate the strongest relationship at 20 h. However, the correlation at 40 h also indicates a similarly strong relationship. Despite this, there is no benefit of incubating the waste samples with the enzymes for 40 h. In the majority of cases the DOC released at 20 h is ≥80% of the final DOC released at 40 h. Therefore due to the typically smaller increases in DOC release after 20 h for the purpose of a rapid analytical tool incubating the waste sample for 40 h is not significantly beneficial.

The aim of the EHT is to correlate with the BM100 test method to provide a short-term estimate of BM100 values. Therefore incubating the waste samples with the enzymes for 40 h would provide no additional confidence in the relationship with BM100 data. The correlation at 3 h for DOC released from enzyme hydrolysis also suggests a strong relationship with the BM100 data. This therefore indicates the possibility of completing the short-term EHT method with just 3 h of incubation, presenting a significant improvement on the current 4 days of the DR4 method. However, this would require careful

consideration since the relationship with BM100 values is strongest for the EHT at 20 and 40 h.

A previous study of the DR4 correlation with BM100 determined a relationship up to a DR4 value of 150,000 mg O_2/kg LOI, above which there is no clear correlation. A relationship of 96 MSW derived BMW samples produced a correlation coefficient of R^2 = 0.54 (Godley *et al.*, 2007b). This is a correlation (r value) of 0.73, which is higher than that observed in this study of 38 mixed organic samples (r= 0.58). This indicates that the DR4 method may be better suited for MSW-derived samples only, rather than for a wide range of organic waste types such as in this study.

Several waste samples correspond to outlying plots on the graphs in Figures 4.7-4.13. The common outliers are shown in Table 4.2, which indicates the occurrence of that outlier.

Sample	BM100 Value l/kg LOI	DR4	EHT total DOC (P3)			EHT enzyme DOC (P3-P2)		
			3	20	40	3	20	40
Cellulose	200	✓	×	×	×	✓	✓	×
Packaging waste	527.4	✓	✓	✓	✓	×	×	×
Autoclaved packaging waste	629.9	✓	✓	✓	✓	✓	✓	✓
Pizza waste	748	✓	×	×	×	✓	✓	×
	Figure	**4.7**	**4.8**	**4.9**	**4.10**	**4.11**	**4.12**	**4.13**

Table 4.2. Common outliers observed in the correlations of the short term methods with the BM100 test method.

As expected the cellulose substrate yielded a relatively high EHT value, however for the BM100 test gave a relatively low value of 200 l/kg LOI (clearly seen in the far right of Figure 4.11, outside of the dashed lines). The cellulose substrate is a readily biodegradable material, and as a result has resulted in acidic conditions forming in the test vessel, preventing methanogenesis from occurring, or significantly slowing the process down.

The untreated and autoclaved packaging waste samples consisted mostly of cardboard, and so would be expected to contain a large proportion of cellulose. Therefore the biodegradability results obtained would be expected to be rather high relative to other samples. This was not the case for either sample in the DR4 test method. The DR4 is sensitive to readily biodegradable material (Godley *et al.*, 2007b), and the cardboard material will not consist of only readily biodegradable substrates, therefore the DR4 test method possibly underestimates the biodegradability of the untreated and

autoclaved packaging waste. This results in outlying data for the DR4 for these samples. The untreated and autoclaved packaging waste samples are outliers for the total DOC (P3) of the EHT against the BM100. For the enzyme-only DOC (P3-P2) of the EHT, only the autoclaved packaging waste sample is a clear outlier. It appears from the graphs that the EHT doesn't underestimate the biodegradability of these samples as much as the DR4, since for the EHT the values for these samples appears closer to the line of best fit, and so closer to the region of confidence. However the BM100 values for these samples are very high relative to the other samples, which results in the outlying plots for both the DR4 and the EHT.

The BM100 test method yielded a very high value for the pizza waste, which is undoubtedly a result of the very high fat content of this sample. The DR4 also gave a very high value, but this was not as high relative to the other samples compared to the BM100. Therefore this result was a clear outlier. Similarly, the pizza waste was an outlier for the EHT when the enzyme only DOC (P3-P2) is considered. The enzymes added to the EHT will not allow for the high fat content, and so the EHT would underestimate the biodegradability of this sample. When the total DOC (P3) of the EHT is considered, the pizza waste is not an outlier. As discussed in section 4.4.1 high quantities of DOC are released after autoclave, and due to the mild acid catalysed hydrolysis, the fat will be measured in the EHT prior to enzyme addition.

The total DOC release in the EHT was evaluated as a possible indication of sample biodegradability. However, since a proportion of Phase 2 DOC will

contain non-biodegradable carbon, the DOC released in Phase 3 only was also evaluated. In most samples, the biodegradability result is lower for the treated samples, considering the BM100 and DR4 values. This is expected since biological treatment of waste material removes biodegradable components (Adani *et al.*, 2004b; Gea *et al.*, 2004; Godley *et al.*, 2007b), producing a bio-stabilised material (such as a compost-like output, CLO).

The DOC released in Phase 3 results from the enzymatic hydrolysis of the material, and so may indicate the amount of additional biodegradable cellulose, hemicellulose and possibly proteinaceous material present. However, the DOC released at Phase 2 of the EHT is likely to consist of a mixture of biodegradable and non-biodegradable carbon. Therefore subtracting the Phase 2 DOC value from the final Phase 3 DOC value would eliminate biodegradable carbon from the overall DOC value. Similarly, if Phase 2 DOC is not subtracted, then the biodegradability determined for the waste sample would contain non-biodegradable carbon. To correct these potential errors it is necessary to characterise the Phase 2 DOC to differentiate between the biodegradable and non-biodegradable carbon to provide a more accurate biodegradability measurement.

The Phase 2 DOC is likely to contain varying concentrations of humic substances. The range of methods for the fractionation and quantification of humic substances has been previously described (Artiola Fortuny *et al.*, 1982; Stevenson, 1994; Thurman *et al.*, 1981; Van Zomeren *et al.*, 2007a). The use of a suitable extraction method may allow for a more selective deduction from

the final Phase 3 DOC of the EHT, considerably improving the biodegradability indication.

The quantification of non-biodegradable DOC released in Phase 2 would also aid the indication of biodegradability for samples of low cellulose content, such as the pizza waste. The pizza waste contained a high proportion of fat (24%) and a very low quantity of cellulose (0.1%)[1]. Therefore it would be expected for the EHT to yield a very low value, particularly when considering DOC from enzyme hydrolysis, since the enzymes added were predominantly cellulase enzymes. This is not the case as around 25% of the total DOC was released in Phase 3. However it is likely that during the incubation with the enzymes after Phase 2 that further DOC was released due to mild acid hydrolysis as discussed in section 4.4.1, rather than enzyme hydrolysis. However the non-biodegradable portion of the total DOC would be low, as this would consist largely of fats, which are biodegradable. In this case, the total DOC is most likely to provide the most suitable and accurate indication of sample biodegradability. The humic substance content of pizza waste is likely to be negligible, and therefore the selective deduction of humic content would result in little actually being deducted from the total DOC of the pizza waste.

An investigation into adding a humic substance extraction technique to the current EHT method is important, and will be described in a later chapter.

[1] Data obtained by the Open University as part of the Defra waste characterisation project (WR0110). Biochemical dataset in Appendix B.

The high energy autoclave technique may adjust the physical chemical properties of the waste; however this may benefit the biodegradability measurement. In a recent study, waste samples from a landfill were measured using a dynamic respiration index (DRI) and biochemical methane potential (BMP) test (Tojo *et al.*, 2007). Hydrothermal pre-treatment of the waste sample increased the biogas production in the BMP test, indicating that a high energy pre-treatment causes the slowly biodegradable material to become more accessible and easier to decompose, suggesting that the use of a high energy autoclave treatment in the EHT is appropriate. However the mild acid catalysed hydrolysis during the autoclave needs to be taken into account with regards to the use of total DOC (P3) or enzyme-only DOC (P3-P2) in the expression sample biodegradability.

4.4. Conclusions

The correlations observed with the BM100 for the total DOC (except at 3 h) and enzyme released DOC are stronger than the correlation observed for the DR4. This indicates that the EHT is better suited than the DR4 for the short-term biodegradability assessment of organic waste materials.

Based on the observed correlations for all EHT data it can however be concluded that using only the DOC from enzyme hydrolysis offers the best indication of sample biodegradability. The strongest relationship with the BM100 was observed for the data obtained after 20 h of incubation and is therefore considered to be the set test time for the EHT. However the relationship observed at 20 h was not significantly stronger than the

relationship for data obtained at 3 h. The correlation of the DOC released by enzyme hydrolysis after 3 h with the BM100 values was the weakest of the three times monitored. However despite this, there is a possibility that a 3 h version of the EHT could be used as an alternative to the DR4 test method.

Further work to quantify the proportion of humic substances in Phase 2 DOC is required. This would enable a selective deduction of the non-biodegradable DOC, and would therefore provide a more accurate indication of sample biodegradability. It is hoped that this would provide an improved correlation with the BM100 data. The EHT, like the DR4 and BM100 methods, has certain limitations. This further work will aim to address some of these potential errors. The work will determine the humic fractions of Phase 2 DOC, and assess the effects of a selective deduction on the correlations with the BM100 of the EHT at 3, 20 and 40 h.

Chapter Five

Monitoring of an Individual Waste Treatment Process

This Chapter describes and discusses the investigation of a single waste treatment process over a period of 9 months using three biodegradability test methods, including the enzymatic hydrolysis test (EHT).

5.1. Introduction

The previous Chapters have provided a background into the test methods used to assess organic waste biodegradability. Following a review of the currently available methods in Chapter 2, it was concluded that an alternative test method was required. The development of a test method based on the enzymatic hydrolysis of cellulose was discussed in Chapter 3, and this method was applied to a wide range of organic waste materials in Chapter 4, along with the aerobic DR4 and anaerobic BM100. In this Chapter, the three biodegradability test methods (EHT, DR4 and BM100) have been applied to a single waste treatment process over a period of 9 months. The aim of this study is to demonstrate the suitability of the EHT in the monitoring of individual waste treatment processes.

In the UK biodegradability test methods are used in the commissioning and monitoring of waste treatment processes, in compliance with the Environment Agency guidance on monitoring mechanical biological treatment (MBT) and other pre-treatment processes for the landfill allowance trading scheme (Environment Agency, 2005). The role of this guidance is to assist operators in the calculation of the biodegradable municipal waste (BMW) diversion from landfill, as a result of a treatment process. Under this guidance the operators of MBT should monitor the treatment process on a quarterly basis (Environment Agency, 2007a). This involves the analysis of the input and output materials using the DR4 and BM100 biological test methods, for the initial monitoring. Once a close correlation between the DR4 and BM100 has been established, $r^2 \geq 0.9$ over two years of monitoring (Environment Agency, 2007b), the on-going analysis is then by means of quarterly DR4 testing only (Environment Agency, 2007a). BM100 values can be calculated from the correlation using the following equation (Godley *et al.*, 2007b)-

$$\text{BM100 (l/kg LOI)} = \text{DR4 (mg } O_2\text{/kg LOI)} \times M$$

(Where M is the gradient of the straight line; this equation, also quoted in the Environment Agency MBT monitoring guidance, assumes an intercept, C, of 0)

Biogas production (m^3) is calculated from the following equation, considering the mass (tonnes, t) of MSW-

$$\text{Biogas } (m^3) = \text{tMSW} \times (\%\text{BMW}/100)(\%\text{DM}/100)(\%\text{LOI}/100) \times \text{BM100}$$

This equation takes into account the percentage dry matter (DM), and so the removal of moisture during treatment is taken into account. Also the percentage loss-on-ignition (LOI) is taken into account, thus removing all inorganic and non-biodegradable materials from the calculation.

The estimated biogas production, calculated from the previous equation, is an indication of how much biogas would be produced if the municipal solid waste (MSW) material was landfilled. This estimation can be used to calculate the BMW diversion from landfill considering the estimated biogas production of the input waste material and the output material, a percentage reduction can be calculated-

$$\%\text{ diverted from landfill} = 100 - ((\text{output biogas}/\text{input biogas}) \times 100)$$

The percentage diverted from landfill calculated from this equation provides the BMW diversion. For example, if it was calculated that the diversion was 75%, then the monitored treatment process will divert 75% of the BMW that is received from disposal to landfill.

Previous Chapters have reviewed the currently available test methods for assessing waste biodegradability, and consequently the development of the enzymatic hydrolysis test (EHT) method. This test method was applied to a wide range of organic waste materials in Chapter 4 where it was shown that

the EHT has a stronger relationship with the BM100 than the DR4 method. However to validate the use of the EHT in the monitoring of waste treatment processes, it is necessary to apply the EHT to a single process over an extended period of time. Since the BM100 and DR4 are used to monitor single waste treatment processes, it is necessary to determine whether the EHT is a suitable alternative to the DR4 as a short-term test method in the monitoring of a single waste treatment process. This Chapter describes the application of the BM100, DR4 and EHT to waste samples taken from an individual MBT process over a period of approximately 9 months.

The waste treatment process monitored was the Frog Island treatment facility operated by Shanks Waste Solutions on behalf of the East London Waste Authority (ELWA). This site uses the Ecodeco technology, which is designed to produce an SRF output, involving a 2 week composting process to 'bio-dry' the waste material before separating into individual fractions (i.e. glass, metal etc). The MSW material input is ground and placed in fully enclosed composting windrows for 2 weeks. The composting halls consist of a perforated floor and ductwork system, which allows air to be forced downwards through the waste. This aerates the waste material, and also provides the fully enclosed facility with a negative air pressure, which minimises the release of odours. The biological processes which occur in composting result in increased temperatures, between 50 and 60°C, which evaporate the water content of the material resulting in a mass reduction of approximately 25% (Ecodeco, 2001a). The bio-drying process provides a dried waste material, which allows for the separation of low mass material

(e.g. shredded paper, fabric etc) from the heavier glass and inert fractions. From the extraction hopper (Ecodeco, 2001b) fractions of metals (ferrous and non-ferrous), inert materials (glass, stones, brick etc), fines, and a solid recovered fuel (SRF) are separated. The SRF fraction consists of combustible material, such as paper, card, wood and fabric. The SRF can therefore be used in incinerators as a fuel. The fines components is removed by screening from <20 mm to <6 mm. This material consists of biodegradable materials (e.g. food) and fine materials such as soil, and is currently landfilled. A simplified overview of the Shanks Ecodeco process is shown in Figure 5.1.

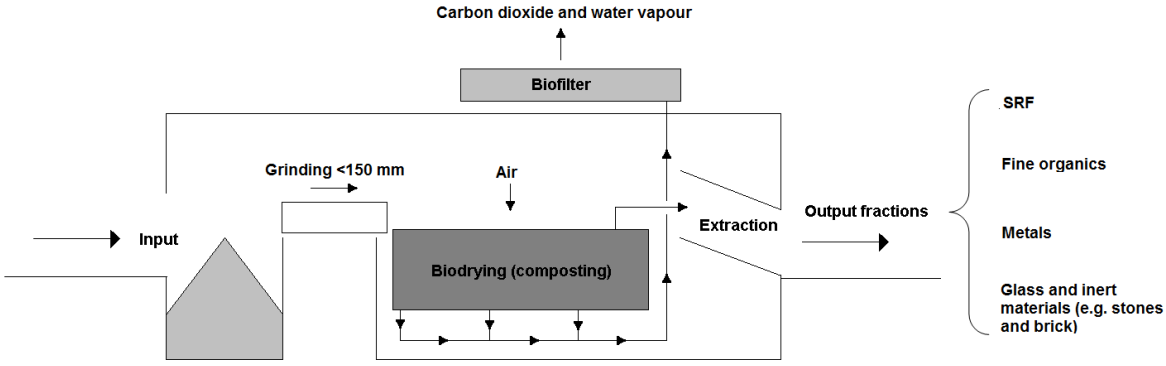

Figure 5.1. Schematic diagram of the Shanks Ecodeco process at the Frog Island waste treatment facility, East London (Ecodeco, 2001a; Ecodeco, 2001b).

The continuous monitoring of the MBT process was expected to indicate the variations in biodegradability of the different samples over time. For example, the biodegradability of the input material was expected to vary, since waste is a heterogeneous material, and the biodegradable components are very likely to change over the course of 9 months due to seasonal variations (Parfitt, 2002). It is therefore expected that the BM100, DR4 and EHT methods will

show similar trends, for example if the BM100 value increases one week, the DR4 and EHT values are also expected to increase.

In addition to monitoring the changes in biodegradability over time, the waste samples will be assessed using different sample sizes for the EHT. The surface area of the waste material is likely to affect the rate and extent to which the enzymes hydrolyse the substrate. It is hypothesised that grinding to smaller sample sizes will result in less variability between sample replicates, and so a smaller <2 mm particle size is used in addition to the standard <10 mm used in the DR4 and BM100 test methods. Also the increased surface area may result in a significantly higher dissolved organic carbon (DOC) release. Therefore this Chapter investigates the effect of sample size on the reproducibility of the EHT in addition to the 9 month monitoring of an MBT process.

5.2. Materials and Methods

5.2.1. Samples

In this study the samples were collected from the Shanks Ecodeco treatment facility at Frog Island and prepared by WRc plc. The waste treatment process uses an MSW input material and produces several output materials, including SRF, fines, dust and reject. The samples used in this study were the MSW input, SRF and fines output materials. The samples were collected on a fortnightly basis, in 10 x 2 kg batches, which were then thoroughly mixed to make up the composite sample of the waste material and 'coned and quartered' and a 2-3 kg analytical sample was obtained.

The samples were sorted to remove glass, metals, plastics and inert materials with the biodegradable material being retained and tested. The samples were dried at 70°C to 80-90% dry weight and shredded to <10 mm and <2 mm. The particle size of <10 mm was used for the EHT, DR4 and BM100 analysis, whilst the smaller <2 mm particle size was also used in EHT analysis. The samples were stored in sealed containers in a cold room until analysed.

Samples were analysed using both sample sizes, but due to limited sample stock, this was not possible for every sample.

5.2.2. Biodegradability Assessment

The DR4, EHT and BM100 test methods were used to assess the biodegradability of the prepared waste samples using the methods described in Chapter 4. The DR4 and BM100 analysis was carried out by WRc plc using the methods described in Chapter 4. The batch of cellulase enzymes used in the EHT had a greater activity of 9 units/ mg (manufacturer specifications) than the batch used in Chapter 4 (7.8 units/ mg). As a result of this, 21.67 mg of cellulase enzyme was used in each sample replicate (25 mg was used in Chapter 4).

The prepared sample size of <10 mm was used in all test methods, whilst the particle size of <2 mm was only used for EHT analysis.

The dissolved organic carbon (DOC) released during the EHT is measured using chemical oxygen demand (COD) kits as described in Chapters 3 and 4.

The error of the data is calculated as the standard error (SE), calculated from the standard deviation (SD) of the values as follows, where n is the number of samples-

$$SE = \frac{SD}{\sqrt{n}}$$

5.3. Results

5.3.1. Variation of Particle Size in the EHT

The particle size of the waste samples had an effect on the DOC released at each phase of the EHT. This is shown in Figures 5.2-5.4. The DOC at each phase is accumulative, therefore phase 2 (P2) also contains phase 1 (P1).

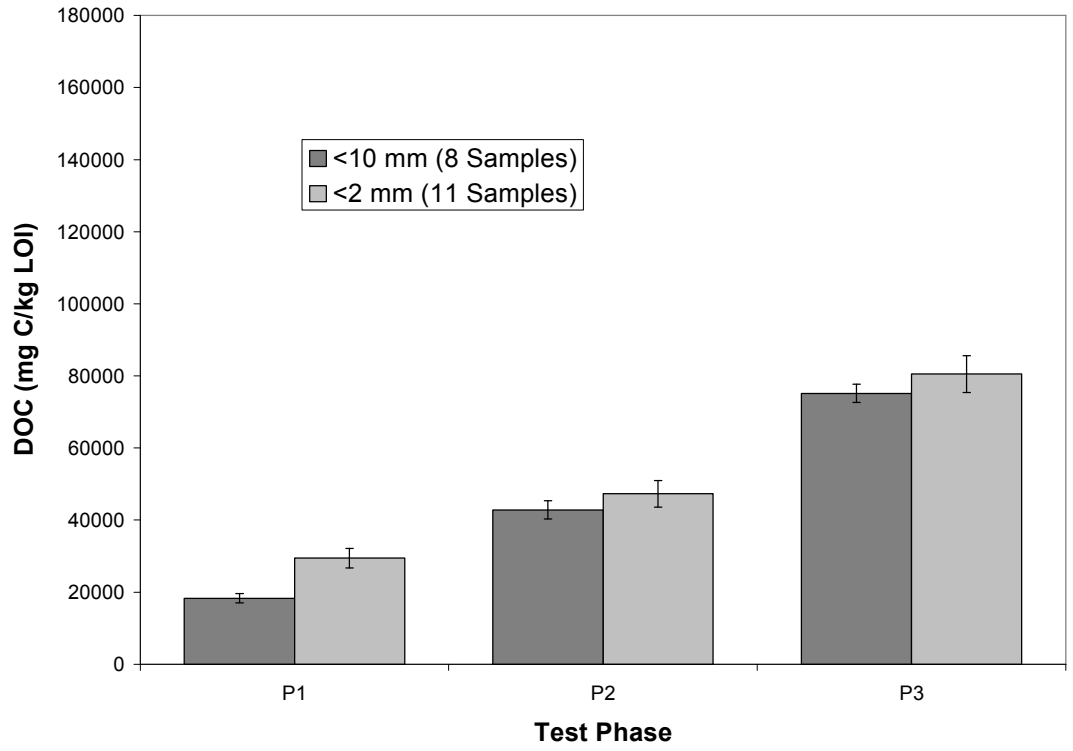

Figure 5.2. Average DOC release at each phase of the EHT for MSW input samples. Error bars shown as the standard error.

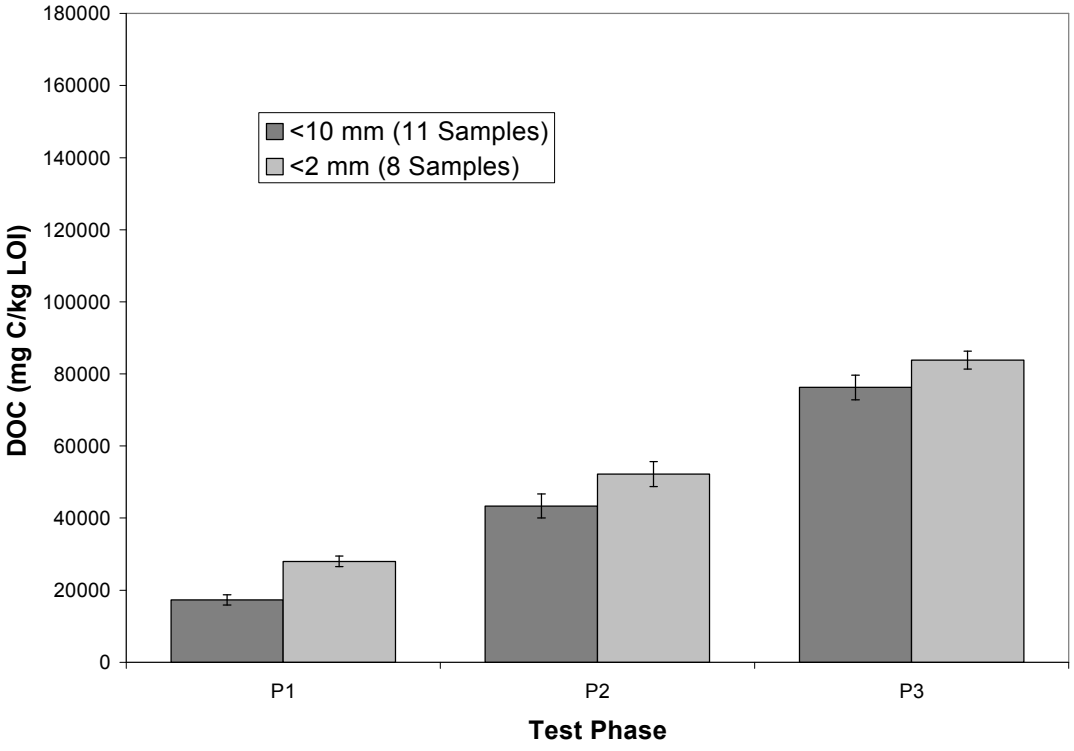

Figure 5.3. Average DOC release at each phase of the EHT for SRF output samples. Error bars shown as the standard error.

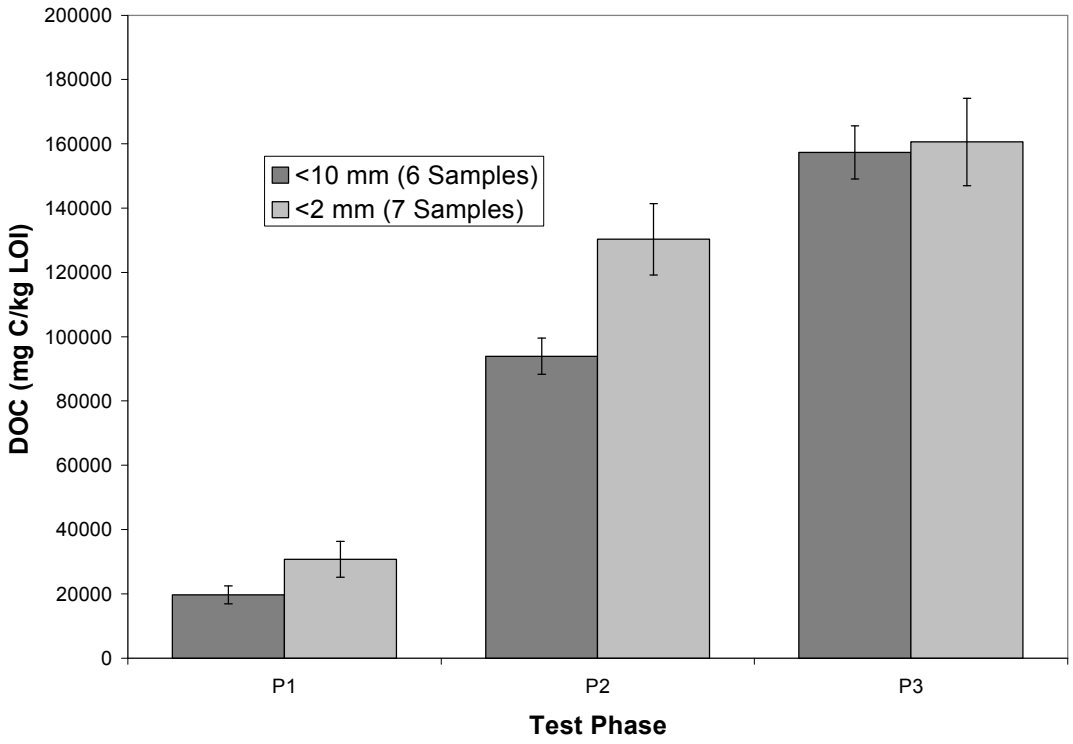

Figure 5.4. Average DOC release at each phase of the EHT for the fines output samples. Error bars shown as the standard error.

The DOC released over the course of the EHT method increases after each phase as the waste material is subjected to thermal and enzyme hydrolysis which is expected, based on findings discussed in Chapters 3 and 4. In terms of the total DOC (final phase 3 value) the fines material is the most biodegradable, and the SRF samples being on average slightly more biodegradable than the MSW input samples.

For each sample, at each phase of the EHT, the DOC released was higher for samples that are ground to <2 mm. The results obtained at each phase of the EHT also indicated a greater standard error for the <2 mm MSW and fines samples. However for the SRF samples at phase 3 of the EHT the greater variance was observed for the samples sizes of <10 mm.

5.3.2. Biodegradability of the Sample Fractions

The average biodegradability values obtained for the individual waste fractions for each of the biodegradability test methods are shown in Figures 5.5-5.8. The number of samples (n) for the MSW input, SRF and fines is 8, 11 and 6 respectively.

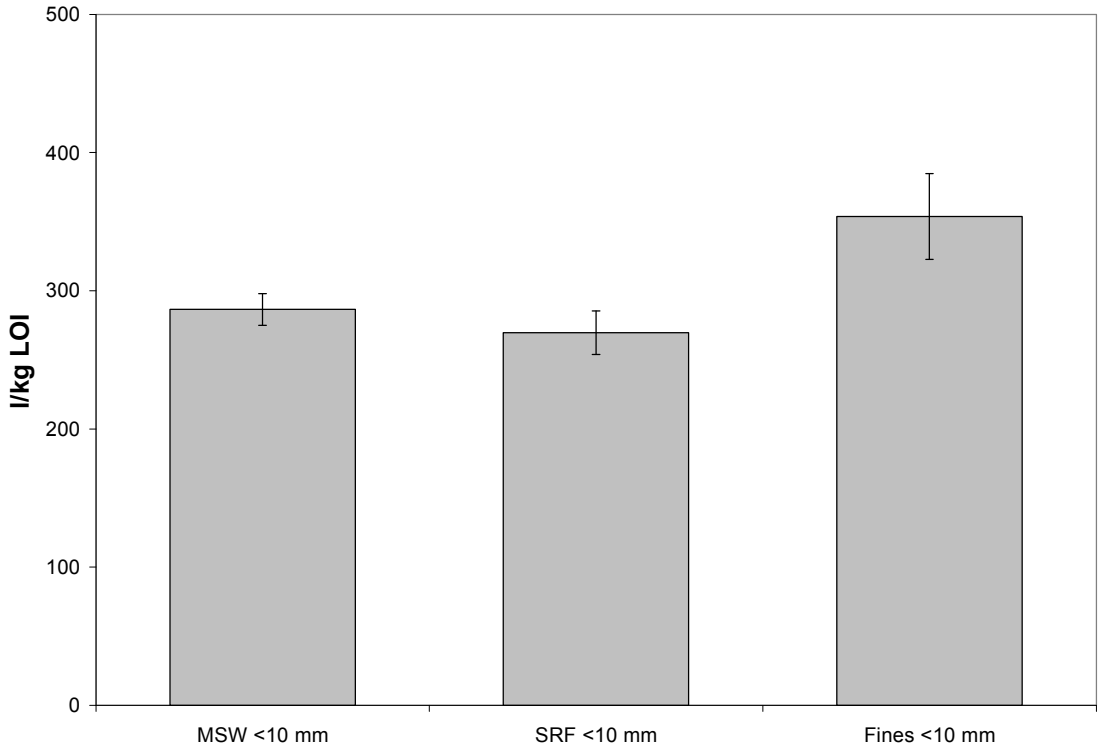

Figure 5.5. Average BM100 results for each of the waste fractions. Error bars shown as the standard error.

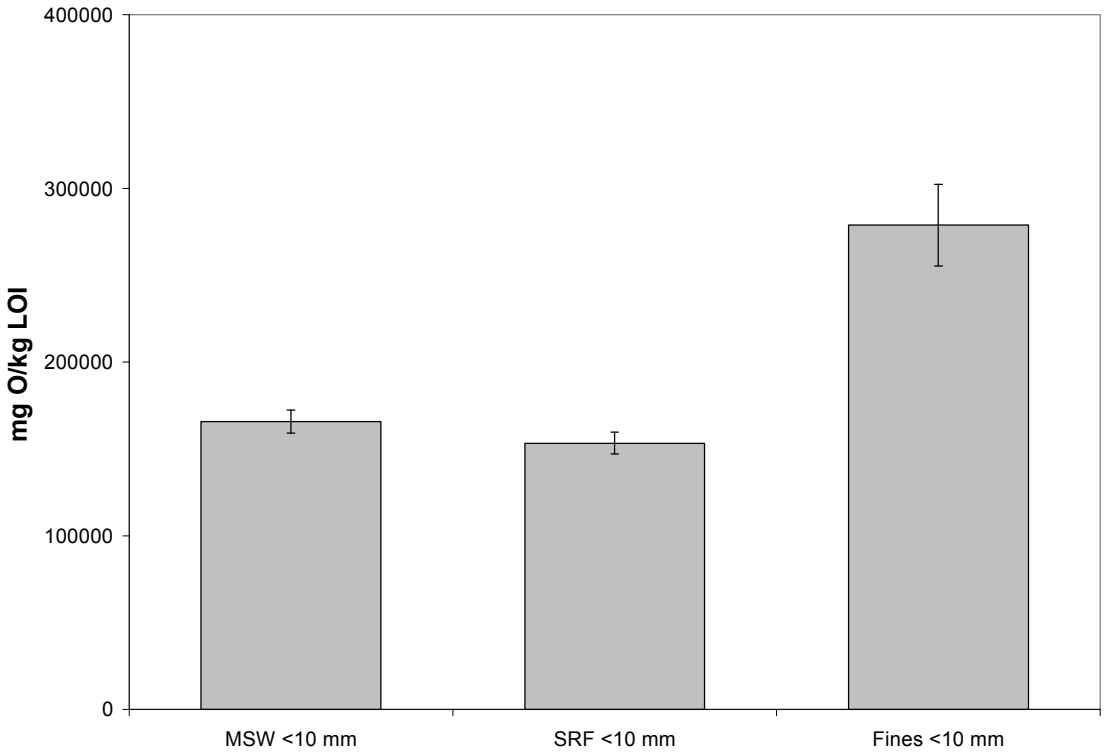

Figure 5.6. Average DR4 results for each of the waste fractions. Error bars shown as the standard error.

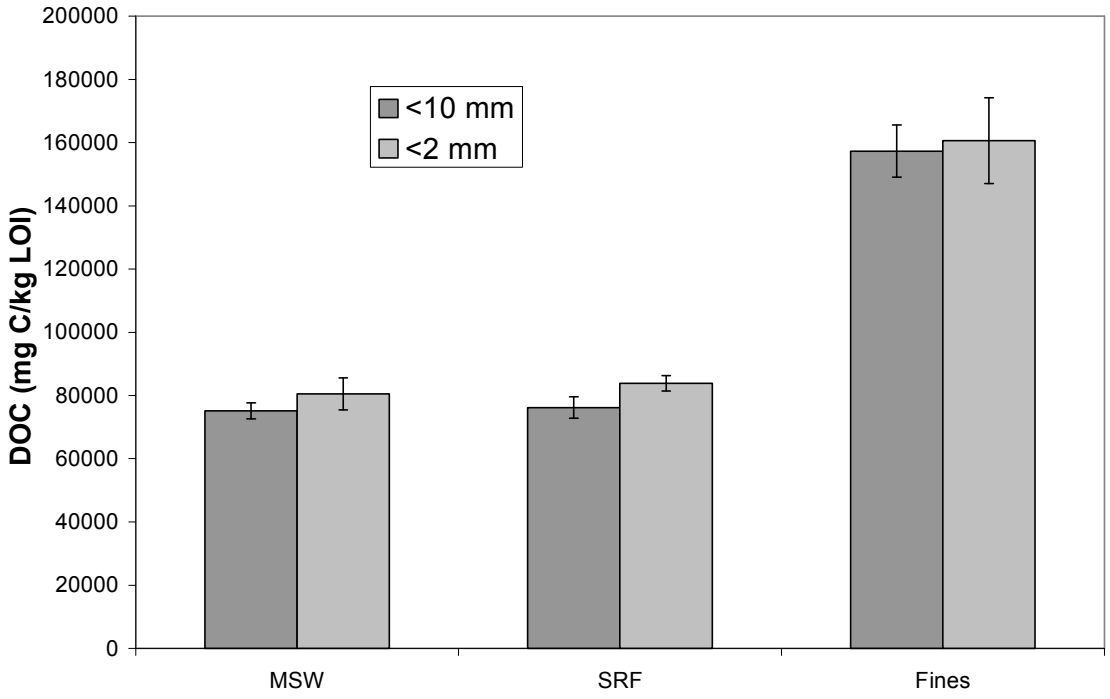

Figure 5.7. Average EHT (total DOC, P3) results for each of the waste fractions. Error bars shown as the standard error.

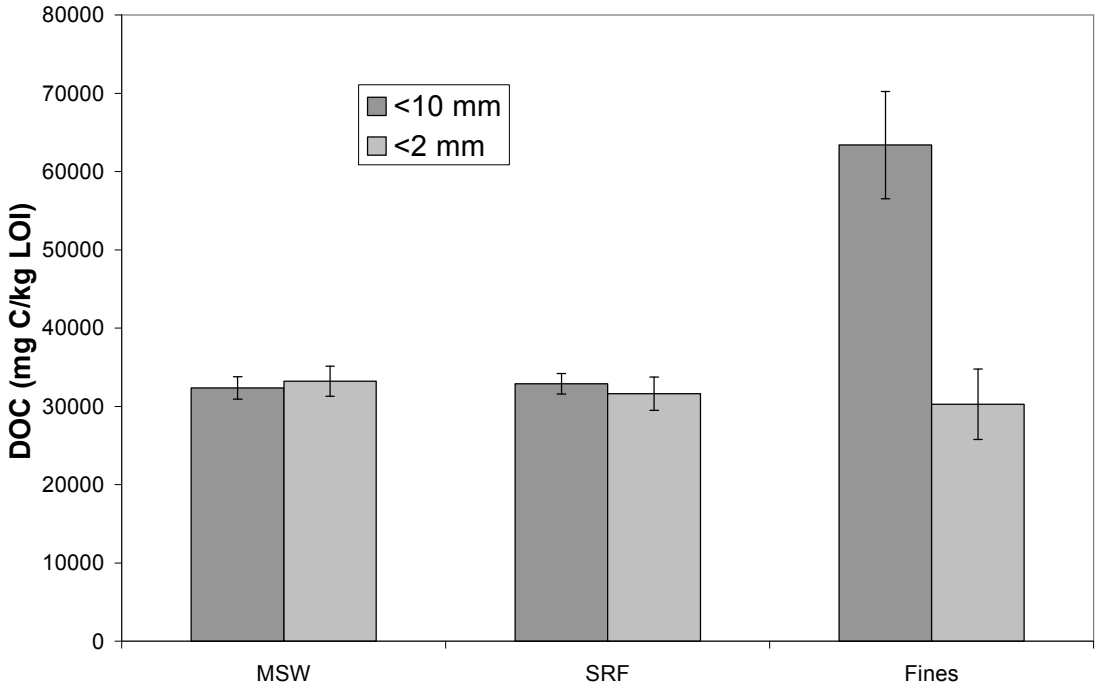

Figure 5.8. Average EHT (enzyme-only DOC, P3-P2) results for each of the waste fractions. Error bars shown as the standard error.

The values obtained from the BM100, DR4 and EHT test methods indicate that the fines fraction contains the most biodegradable material, with the notable exception of enzyme-only DOC for the EHT with particle sizes of <2 mm, which is similar in biodegradable content to that of the MSW input and SRF samples. Whilst the biodegradability values obtained for the fines samples are higher than the MSW and SRF samples for the BM100 method, for the DR4 and EHT the fines material was found to be significantly more biodegradable than the MSW input and SRF samples. For the EHT, the values obtained for the fines material are around two times that of the MSW input and SRF materials (except P3-P2 with samples of <2 mm). For the DR4, the values obtained for the fines material are almost twice that obtained for the MSW input and SRF materials.

The MSW input and SRF samples were in each case very similar in biodegradable content. The MSW input material is, however, slightly more biodegradable (+7.5% for the DR4, +6.25% for the BM100) than the bio-dried SRF material according to the DR4 and BM100 values, but not for the EHT when considering the total DOC (P3), or the enzyme-only DOC (P3-P2) with particle sizes of <10 mm. The enzyme-only DOC indicates that the MSW input is slightly more biodegradable than the SRF material when considering the particle size of <2 mm (33,200 mg C/kg LOI compared to 31,600 mg C/kg LOI, or 5% higher).

5.3.2. Biodegradable Content Variation over Time

The biodegradability values obtained from each test method vary over time. However no distinct trend is observed, for instance as the BM100 value increases, the DR4 or EHT values may or may not also increase. Therefore presenting the data for each sample type, for each particle size of the EHT (<10 mm and <2 mm) is not necessary. For this reason, the presentation of only the data from the BM100, DR4 and EHT (total DOC, P3, and enzyme-only DOC, P3-P2) for the MSW <10 mm sample is shown in Figure 5.9. The full dataset is provided in Appendices C, D and E. For comparison, the data is shown as the percentage of the mean average value for that dataset (i.e. the average of only the data shown). Presenting the data in this way normalises the values, as typically the DR4, BM100 and EHT results are expressed in different units, and so are not directly comparable in this way. The normalised values therefore show the biodegradability changes as a function of the mean average.

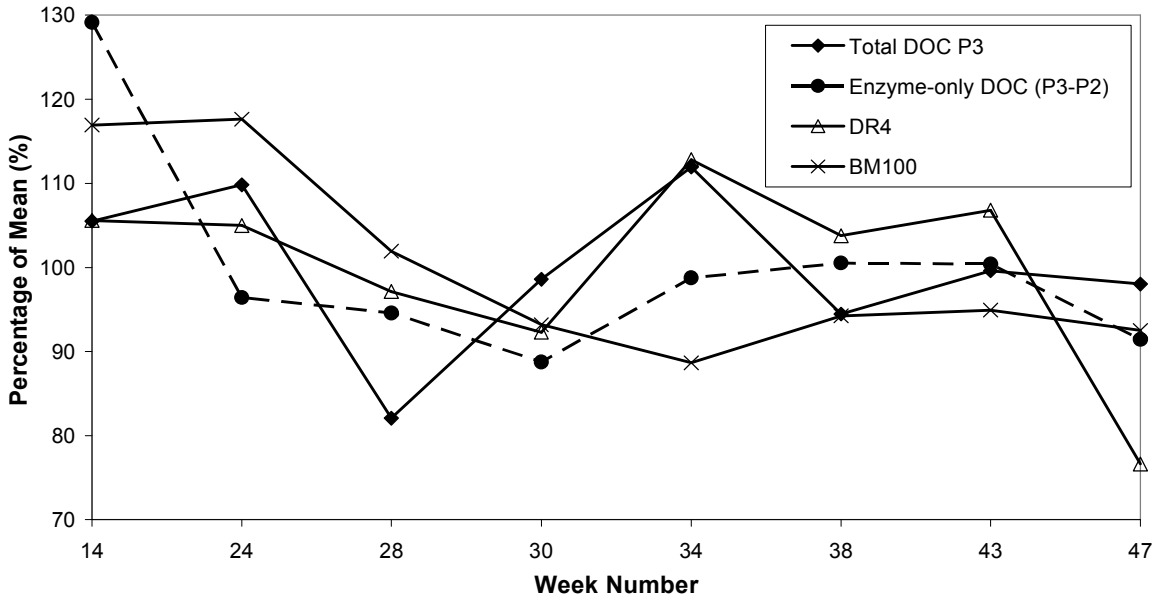

Figure 5.9. EHT (total DOC, P3, and enzyme only DOC, P3-P2), DR4 and BM100 data over time for the MBT input MSW-derived BMW samples. Data shown for the EHT are from samples of <10 mm.

The biodegradability values from the three test methods vary over time. The BM100 test values range from between 92-118% of the mean value, the DR4 values range from between 77-113%. For the EHT, the total DOC (P3) ranges from 82-112% and the enzyme-only DOC ranges from 89-129%. Each method indicates that the biodegradability content of the MSW input material changes over a period of time.

5.4. Discussion

5.4.1. Variation of Particle Size in the EHT

Grinding of the sample to a smaller size was expected to have an effect on the DOC yield and the variation observed between sample replicates. Grinding to <2 mm was expected to yield higher DOC release due to the increase in surface area of the substrate.

As shown in Figures 5.2-5.4 the DOC release at each phase is higher for samples of smaller particle size (<2 mm). This effect was expected since the increased surface area for the <2 mm samples would allow for an increased rate of soluble carbon leaching, and of mild acid, thermal and enzymatic hydrolysis. Particle size has been observed to yield higher rates of enzyme hydrolysis of cellulose in a previous study by Dasari and Berson (2007). In this study particle sizes of 33 µm to 850 µm were investigated, and up to 55% more glucose was produced from cellulase hydrolysis of the smallest particles than for the largest particle sizes (Dasari *et al.*, 2007). Whilst the particle sizes used in this chapter are considerably larger than those used by Dasari and Berson (2007), the same principle of surface area applies.

The use of a smaller sample particle size was also expected to allow a more uniform sample, and therefore provide lower variation between sample replicates. A greater surface area allows higher enzyme coverage, and therefore it is more likely that all available substrate will be hydrolysed in the given timescale (20 h). For larger sample sizes the enzymes would initially need to break down the larger particles to access the middle of the substrate, which would be achieved in the incubation time to a varying degree. This would be expected to result in variation, and so the standard error between the replicates would be expected to be higher in samples of larger particlesizes. This was however not the case, which, as can be seen in Table 5.1, the standard error is higher for the sample sizes of <2 mm. The exception to this is the <2 mm SRF sample at P3.

Sample	Size/ mm	Number of Samples	P1	P2	P3
MSW	**<10**	8	1275	2514	2516
	<2	11	2704	3675	5062
SRF	**<10**	11	1465	3305	3368
	<2	8	1493	3452	2449
Fines	**<10**	6	2815	5600	8244
	<2	7	5563	11128	13589

Table 5.1. Standard error between the triplicates for each phase of the EHT for the MSW, SRF and fines samples at <10 and <2 mm.

The use of samples of a smaller particle size yields a higher DOC release at each phase. This would suggest that the EHT benefits from further sample grinding from the <10 mm currently used in the DR4 and BM100 to <2 mm. However since the standard error for the samples of smallest particle size (<2 mm) is higher than that of the larger particle sizes (<10 mm), it is not more beneficial from the perspective of improved reproducibility. The biodegradable content of the waste samples varied over a period of time, as would be expected, and the test methods were sensitive to these changes (as shown in Figures 5.2-5.8). Therefore in order to obtain the most accurate and reliable indication of sample biodegradability, it is necessary to minimise the variation between the replicates. Hence, it is most suitable for a sample size of <10 mm to be used in the EHT method. This means that the sample preparation for the DR4 and BM100 methods is preferred, and therefore more suitable, for the EHT, meaning that no further sample preparation (grinding) is required.

Table 5.2 summarises the average DOC release at each phase of the EHT for each sample, and also provides the average enzyme-only DOC (P3-P2).

Sample	Size/ mm	Average P1 mg C/kg LOI	Average P2 mg C/kg LOI	Average P3 mg C/kg LOI	Average P3-P2 mg C/kg LOI
MSW	**<10**	18300	42800	75200	32300
	<2	29400	47300	80500	33200
SRF	**<10**	17300	43300	76200	32900
	<2	28000	52200	83900	31600
Fines	**<10**	19700	93900	157000	60700
	<2	30700	130000	161000	30300

Table 5.2. DOC release at each phase of the EHT for the MSW, SRF and fines samples at <10 and <2 mm.

As there is a higher surface area at <2 mm, a greater amount of DOC is released from the sample during autoclave. However, for the MSW input and SRF samples, the DOC release from enzyme hydrolysis is very similar for both <2 mm and <10 mm (Table 5.2). This indicates that following the autoclave step, the particle size has little effect on the DOC release. This supports the findings in Chapter 4, and in previous studies, where it was observed that the hydrolysis of hemicellulose and, to an extent, cellulose and lignin is catalysed by mild acid under high temperatures (Jacobsen *et al.*, 2000; Nguyen *et al.*, 1998; Torget *et al.*, 1990). The effects of a high energy pre-treatment process (such as autoclave) of waste material is also reported to cause the slowly biodegradable materials to be more accessible and easier to decompose (Tojo *et al.*, 2007). This again indicates that the additional grinding of the waste material from the <10 mm particle size to <2 mm is not required.

5.4.2. Biodegradability of the Sample Fractions

The biodegradability of the MSW input and the SRF materials were found to be very similar in biodegradability, according to the values obtained from each of the test methods. The biodegradable content of the MSW input is not reduced significantly due to the relatively short composting process, which is only designed to dry the waste material, and not to bio-stabilise it. It would, however, be expected that the MSW input material has a higher biodegradable content than the SRF material, which is not the case for the EHT (total or enzyme-only DOC).

The fines sample contains material that is not cardboard, paper and textiles (which forms the SRF material) and is not metals, glass and other inert material (other outputs of the MBT process). The fines sample is expected to be more biodegradable than the MSW input since this material has had the more slowly biodegradable materials removed (such as cardboard, wood and fabrics), and has become more concentrated with the readily biodegradable materials, such as food waste (vegetable peelings, meat residues etc). Therefore this material is expected to be the most biodegradable fraction, and this is observed in the EHT in terms of the total DOC (P3) for both particle sizes, and in terms of the <10 mm sample for the enzyme-only DOC (P3-P2). The DR4 and BM100 test method also indicate that the fines material is the most biodegradable fraction.

The total DOC (P3) for both particle sizes and enzyme-only DOC for <10 mm samples for the EHT, along with the DR4 values, suggest that the fines

material is significantly more biodegradable than the MSW input and SRF samples. The DR4 values for the fines material are 68% and 82% higher than the MSW input and SRF samples respectively, whilst for the total DOC (P3) of the EHT, the fines material is 109% and 106% higher than the MSW input and SRF samples respectively. The enzyme-only DOC (P3-P2) for the EHT suggests that the fines material is between 93 and 96% higher than the MSW input and SRF samples respectively. However the BM100 values for the fines material are 31% higher than the SRF samples, and 24% higher than the MSW input. This is likely to be because the BM100 test method measures the full extent of biodegradability (Godley *et al.*, 2007b; Wagland *et al.*, 2008), and so will completely hydrolyse a higher proportion of the more slowly biodegradable carbon (such as cardboard and wood) in the MSW input and SRF samples than the EHT and DR4 methods.

The DOC released in phase 2 for the fines samples is likely to consist mostly of biodegradable carbon, likewise for the MSW and SRF samples. Therefore the deduction of phase 2 DOC from the total DOC is unlikely to provide an accurate indication of sample biodegradability. As discussed in Chapter 4, there is a requirement to characterise the phase 2 DOC and quantify the non-biodegradable carbon content. If only the non-biodegradable DOC was to be deducted from the phase 3 DOC for the fines samples, then it is very likely that the selectively calculated value would be very similar to the total DOC (P3).

5.4.2. Biodegradable Content Variation over Time

It was expected that as the biodegradable content of a waste material, MSW input or an output material, changed from one week to the next, the test methods would all show a trend that indicates this. Generally there is some similarity, as for example, if the BM100 values increase, then the EHT values may also increase, although this is not very clear in many cases. It can therefore be concluded that there is not a direct relationship between the test methods. As the BM100 values increase and decrease, the EHT and DR4 values also increase and decrease, however equally common is that as the BM100 values increase, the DR4 and/or the EHT (total or enzyme-only DOC) will not follow the same pattern. This indicates that since the test methods are conducted under different conditions and measure different parameters.

Since each test method measures different parameters, the biodegradability trend over time perhaps should not be expected to be the same for each test method. If for example, the trend over time was measured using only anaerobic test methods, such as the BM100 and GS90 (discussed in Chapter 2), then similar trends would be expected in this case.

No correlation was observed between the short-term and long-term tests due to the biodegradability results, from all methods, being very similar which results in a cluster of data, through which no relationship is observed and no line of best fit can be confidently produced. And example if such a scenario is shown in Figure 5.10.

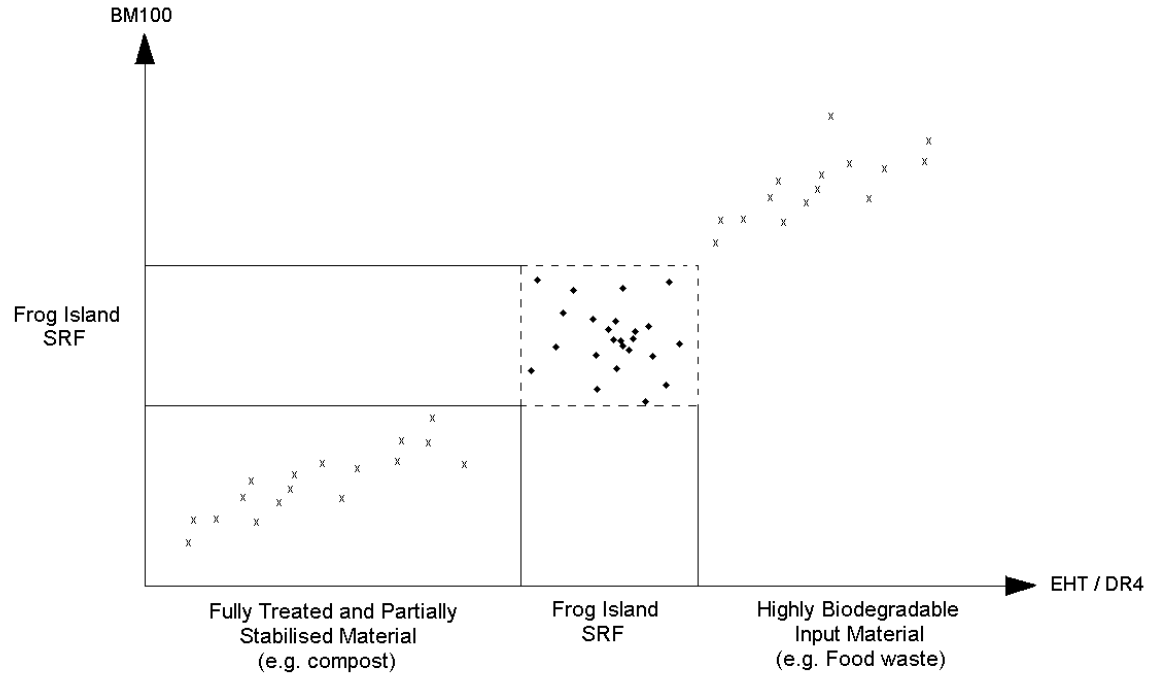

Figure 5.10. An example of data clusters for each variable, in this case the short-term and long-term biodegradability test methods.

Figure 5.10 demonstrates that it is possible to draw a line of best fit in almost any direction through the Frog Island data points, and there would be no confidence in the given linear relationship. However if input and output materials, containing a wide range of biodegradable content are monitored (shown in Figure 5.10), then it is clear that a positive linear relationship exists.

To obtain a linear correlation a wide range of data is required, ranging from low to high biodegradability (as shown in Figure 5.10). As such, an SRF producing waste treatment process which involves very little biological treatment will not produce these results. An investigation into a single biological treatment process (composting or anaerobic digestion) would allow samples to be taken throughout the whole process (i.e. input, then

subsequent weeks/months during, and the stabilised output material). A linear relationship between the long-term and short-term test methods would be expected in such a study, since a range of data would be obtained. Therefore as part of this research, it would have been more suitable to have monitored a treatment process which involves a bio-stabilisation process.

As shown in Chapter 4, the EHT and DR4 correlate, to varying degrees, with the BM100. However since each test method has limitations and measure different parameters, a correlation of r = 1.0 is very unlikely. The BM100 test is sensitive to highly biodegradable content, in which acidic conditions can inhibit methanogenesis, thus affecting the final results. The DR4 test method is also sensitive to readily biodegradable material, and as a result only measures the initial rate of biodegradation. The EHT doesn't have the biological disadvantages associated with the DR4 and BM100 methods, however may not measure the full extent of biodegradation in the given timescale (as indicated in Chapter 4). Also to allow for all possible biodegradable materials, a more complex enzyme mixture would be required, which allows for low cellulose content. This is highlighted for the pizza waste material (low cellulose content; very high fat content) in Chapter 4.

5.5. Conclusions

The variation between sample replicates for the EHT was significantly higher where sample sizes of <2 mm were analysed compared to sizes of <10 mm. However DOC release at each phase of the EHT was observed to be higher when using particle sizes of <2 mm. Despite the increased DOC yield at each

phase, the DOC released from enzyme hydrolysis was found to be similar for each particle size, with the exception of the fines samples. This was concluded to be due to the effects of the autoclave step, and the mild acid catalysed hydrolysis. Therefore it was not seen as necessary to grind the samples from the <10 mm used in the BM100 and DR4 methods to a smaller particle size of <2 mm.

The fines material was found to be significantly more biodegradable than the MSW input and SRF samples in all three test methods, particularly for the EHT and DR4 methods. It was found that the BM100 was more likely to have hydrolysed a higher proportion of the more slowly biodegradable compounds present in the MSW input and SRF samples, meaning that these samples were not as low comparatively to the fines samples.

The biodegradability of organic waste fractions have been found to vary slightly over time, as indicated by each of the three test methods. This is likely to be due to the changing composition, and seasonal variation, which has been described in a previous study (Parfitt, 2002). The variation observed between samples for each test indicates that the methods are sensitive to small changes in biodegradable content. However the tests methods measure different parameters, and so it was observed that they do not always follow the same trend. For example, if the BM100 values indicate an increase or decrease in biodegradable content for MSW input over a given period, the DR4 and/or EHT methods may, or may not, indicate the same trend. As a result, it can be concluded that there was very little trend evident.

It was observed that there was no correlation between the short-term DR4 and EHT methods with the long-term BM100 for this study. This was due to the similarity of the data points, which results in a cluster through which no clear line of best fit can be drawn, and as such no positive correlation can be confidently provided.

There is a requirement to characterise the DOC obtained from phase 2 of the EHT, based on the understanding gained from Chapter 4, and from results in this study. An investigation into quantifying the non-biodegradable content is necessary, and will be explored in the next Chapter.

Chapter Six

Humic Extraction in the Enzymatic Hydrolysis Test (EHT) Method

An investigation into the use of a humic substance extraction method as part of the EHT procedure is described in this Chapter. The aim of this work is to improve the biodegradability indication provided by adding an adapted extraction method to the EHT method, providing an innovative biodegradability test.

6.1. Introduction

In Chapter 4 the enzymatic hydrolysis test (EHT) method was compared with existing microbial degradation methods. From the results it was concluded that the dissolved organic carbon (DOC) released after autoclave at phase 2 (P2) of the EHT required further consideration. It was found that the strongest relationships with the long-term BM100 test method were observed when the P2 DOC was deducted from the final (phase 3, P3) DOC. It is known that biodegradable DOC would also be released following the autoclave process in P2 (since autoclave is a high temperature process), and therefore incorrectly deducted from the final P3 DOC value. From the DOC values discussed in Chapter 4 for samples of low biodegradability (e.g. composted green waste) it was concluded that the DOC would be composed largely of non-

biodegradable carbon. The DOC from enzyme hydrolysis (P3-P2) was relatively very low when compared to the total DOC (P3). As a result, there is a requirement to differentiate between the biodegradable and non-biodegradable DOC released in P2, which is expected to provide a more accurate measurement of biodegradability.

Humic substances are reported to be formed from the degradation of organic materials (Tuomela *et al.*, 2000), therefore the non-biodegradable DOC present in P2 is thought to be composed of large chain soluble humic substances molecules such as humic acid and fulvic acid.

The introduction of this Chapter aims to provide background on the formation and extraction of humic substances, and discuss how a humic extraction technique could remove a potential error of the EHT method.

6.1.1. Humic substances background

6.1.1.1. Formation of humic substances

Refractory organics which remain in landfill residues following the biodegradation of organic waste are mainly composed of humic substances (Xiaoli *et al.*, 2007). These substances are relatively inert, and can be considered as a major reservoir of organic carbon in soils and aquatic environments (Aiken, 1985).

Humic substances are a series of high molecular weight substances formed from the decomposition of lignocellulolytic materials (such as lignin,

polysaccharides and proteins) in a composting environment (Miikki *et al.*, 1997; Tuomela *et al.*, 2000). Humic substances are therefore formed in anaerobic landfill and aerobic composting environments, and as such would be expected to form during biological treatment of organic waste materials (Miikki *et al.*, 1997).

Although the definitive structure of humic substances is not known (Tuomela *et al.*, 2000), the substances have been divided into the following three groups (Aiken, 1985)-

- Humin
- Humic acid
- Fulvic acid

The formation of humic substances is not well understood and as such four potential formation pathways have been proposed (Stevenson, 1994). The four theories of humic substance formation are-

1. Lignin theory
2. Sugar-amine condensation
3. Polyphenol theory
4. Quinone mechanisms

As stated by Stevenson (1994), each of the pathways is likely to contribute to humic substance formation. These four pathways are illustrated in Figure 6.1.

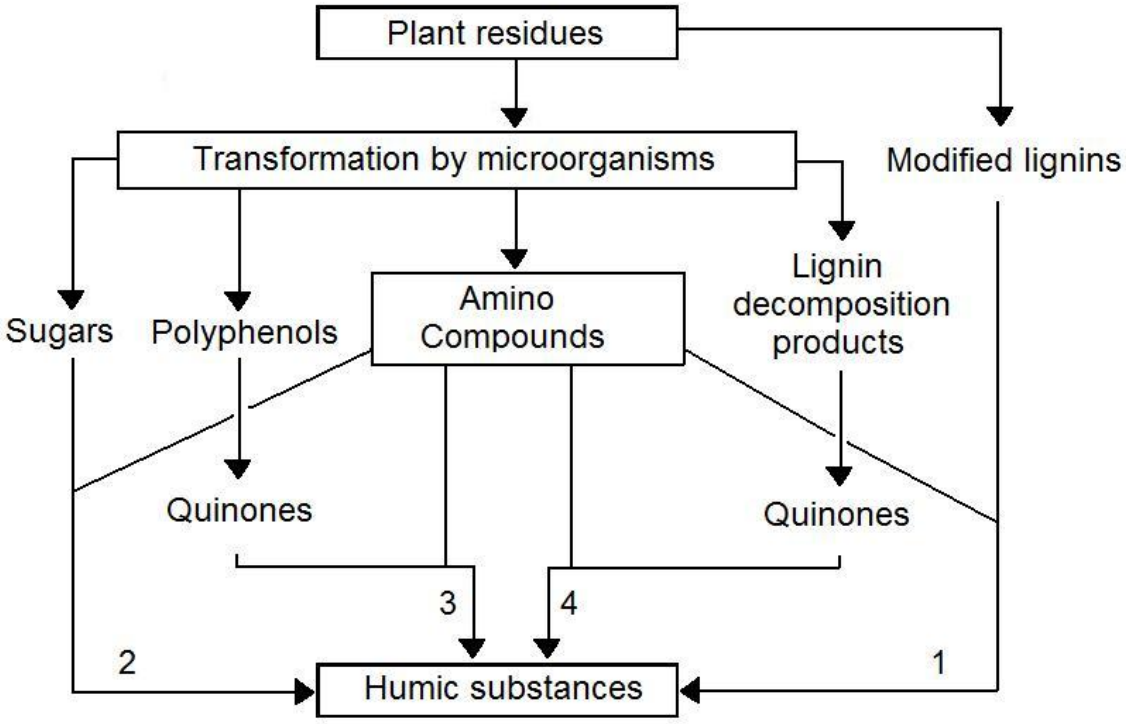

Figure 6.1. Pathways of humic substance formation (Stevenson, 1994).

The lignin route (1) of humic substance formation is an early theory presented in the 1932 by Waksman. In the lignin theory, the nitrogen contained in humic acids results from the condensation of modified lignin with protein (Waksman, 1932). The evidence cited by Waksman includes that lignins share many properties with humic acids, such as that they are both soluble in alkali. Also when lignins are warmed in alkali solution, they are transformed into methoxyl humic acids (Stevenson, 1994). A key feature of the lignin theory is that humic acid is formed before fulvic acid, which is contradicted in the other theories of humic substance formation. A schematic diagram of the lignin theory is shown in Figure 6.2.

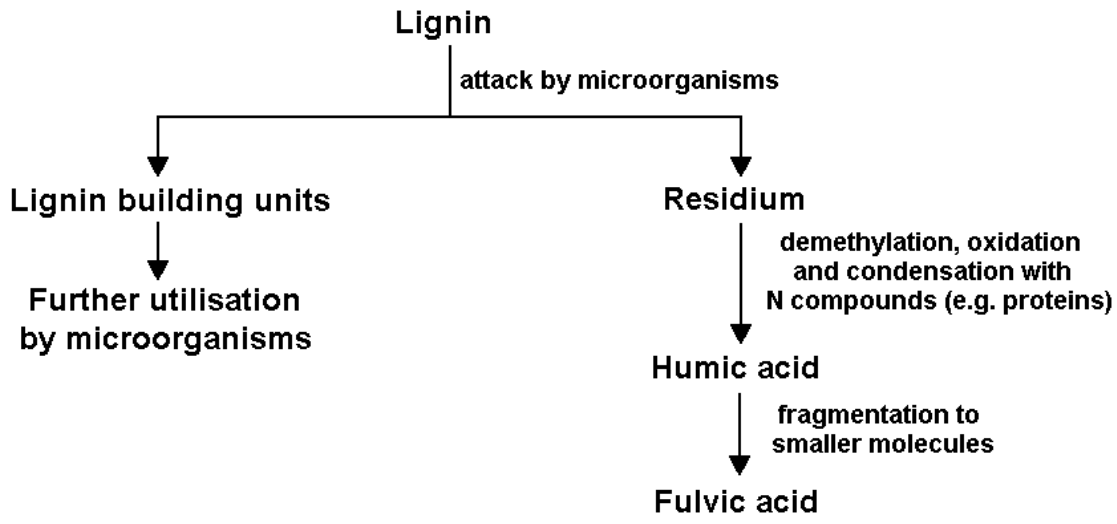

Figure 6.2. Lignin theory of humic substance formation (Stevenson, 1994).

The sugar-amine condensation route (2), first proposed in 1913 (Maillard, 1913), provides an explanation of humic substance formation from the non-enzymatic polymerisation of reducing sugars and amines (Stevenson, 1994). The initial reaction in sugar-amine condensation involves addition of the amine to the aldehyde group of the sugar to form the n-substituted glycosylamine, via a Schiff base reaction (Ishiwatari *et al.*, 1986; Stevenson, 1994). The glycosylamine subsequently undergoes the Amadori rearrangement to form the N-substituted-1-amino-deoxy-2-ketose (Ishiwatari *et al.*, 1986). This is subject to fragmentation, with the products being highly reactive and so readily polymerise, forming brown coloured polymers (humic substances).

The polyphenol (3) pathway is shown in Figure 6.3.

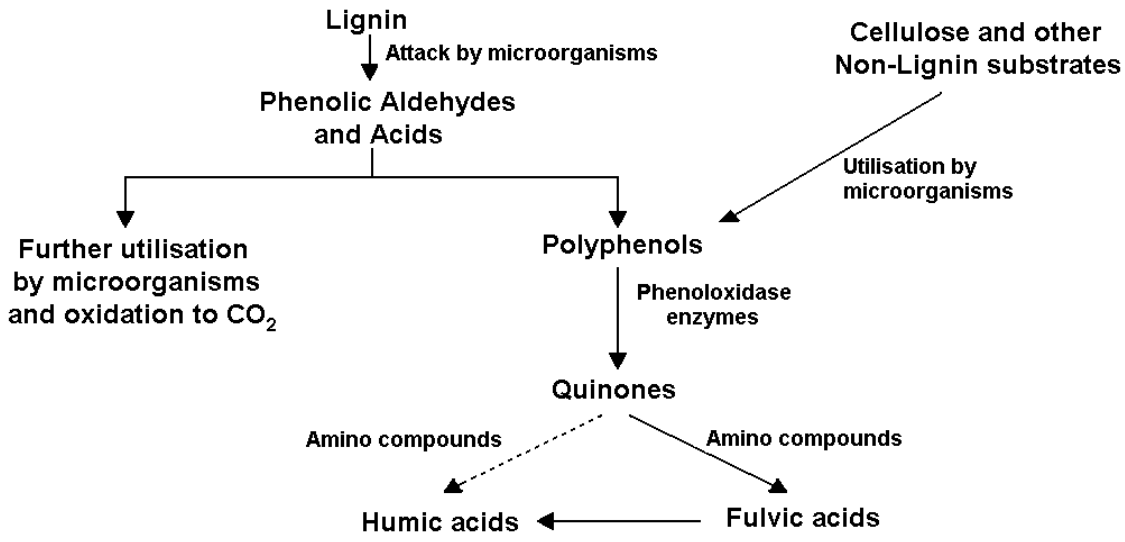

Figure 6.3. Polyphenol theory of humic substance formation (Stevenson, 1994).

The quinone theory (pathway 4) is very similar to the polyphenol theory, with the exception that polyphenols are produced from non-lignin carbon sources. In both routes, polyphenols formed are enzymatically oxidised to quinones, which form humic substances. Unlike the lignin theory, fulvic acids are formed before humic acids. This is supported in previous studies which found that fulvic acids were less polymerised forms of humic substances, and as such are formed before humic acid (Artiola Fortuny *et al.*, 1982; Christensen *et al.*, 1998; Riffaldi *et al.*, 1983; Varadachari *et al.*, 1984).

The polyphenol and quinone theories indicate that humic substances are formed from non-lignin materials. Therefore waste materials that contain no lignin can form humic substances. This is important, considering the treatment of waste materials such as fish and pizza waste as discussed in Chapter 4.

Humic substances are known to be refractory carbon molecules formed from biodegradable carbon sources (Miikki *et al.*, 1997; Tuomela *et al.*, 2000; Xiaoli *et al.*, 2007), and as such are not considered to be biodegradable. Previous studies have, however, indicated that humic acid and fulvic acid fractions of humic substances are not completely resistant to microbial degradation, but degrade very slowly (Gramss *et al.*, 1999; Qualls, 2004).

The understanding of humic substance formation can be applied to the biological treatment, such as composting, of organic waste materials. Humic substance formation has been observed during the composting of municipal solid waste (MSW) (Adani *et al.*, 1999; Inbar *et al.*, 1990; Miikki *et al.*, 1997). Humification is a very long process, occurring over a number of years (Stevenson, 1994), however it has been found that a significant amount of humic substances can form within the first 40 days of composting (Chen *et al.*, 1996; Inbar *et al.*, 1990). Therefore it can be expected that biological processes used to treat MSW, and other organic waste types, can result in a degree of humic substance formation during the timescale of the treatment process.

6.1.1.2. Extraction of humic substance fractions

Whilst the structural units of fulvic and humic acids may be similar (Varadachari *et al.*, 1984) the degrees of polymerisation is different (Aiken, 1985). The carboxylic acid groups decrease as polymerisation proceeds (Varadachari *et al.*, 1984) and as a result the acidity of humic substances will

decrease. Therefore fulvic acid is the most acidic type of humic substance. A summary of humic substance properties is shown in Figure 6.4.

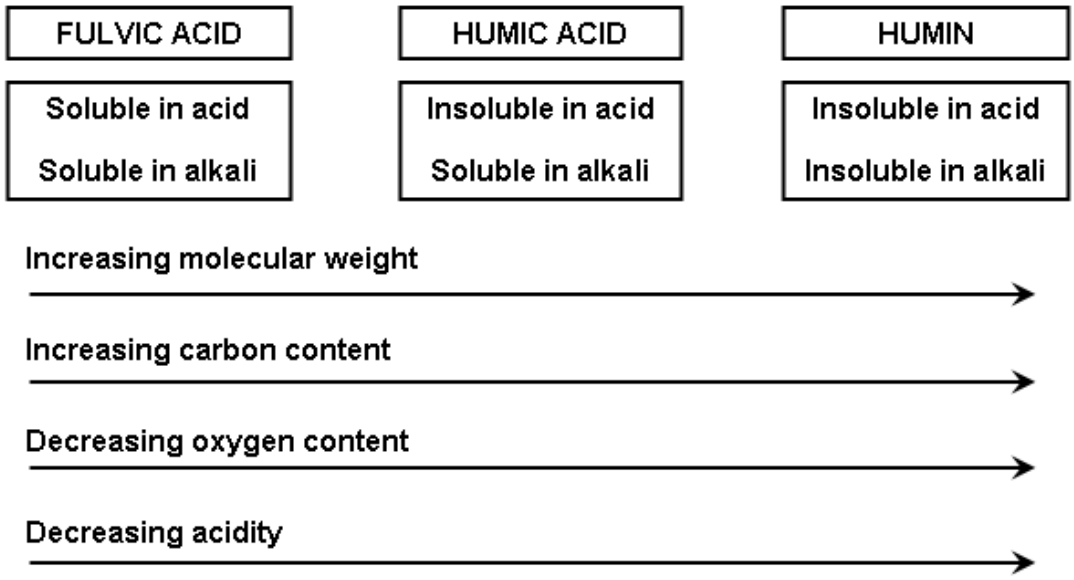

Figure 6.4. Properties of humic substance fractions (Aiken, 1985).

Humic substances can be fractionated based on the pH of the solution, such as the treatment of solid organic samples with alkali and acid solutions (Riffaldi *et al.*, 1983). Methods of fractionating aqueous samples have been presented (Artiola Fortuny *et al.*, 1982; Christensen *et al.*, 1998; Peuravuori *et al.*, 2002; Thurman *et al.*, 1981) in which the humic acid fraction is removed by acidification of the solution, followed by centrifugation. The remaining supernatant solution is passed through a fractionation column packed with XAD-8 resin, which adsorbs the hydrophobic carbon fraction (fulvic acid). The fulvic acid is then reverse eluted from the resin using a 0.1 M NaOH solution. A schematic of the humic substance isolation is given in Figure 6.5. The hydrophilic acids are non-humic compounds, and include biodegradable compounds such as glucose.

The humic acid, fulvic acid and hydrophilic acid are further purified separately as specified by Thurman and Malcolm (1981). These fractions are freeze dried and can be accurately weighed and further analysis carried out on the purified humic, fulvic and hydrophilic acids if required.

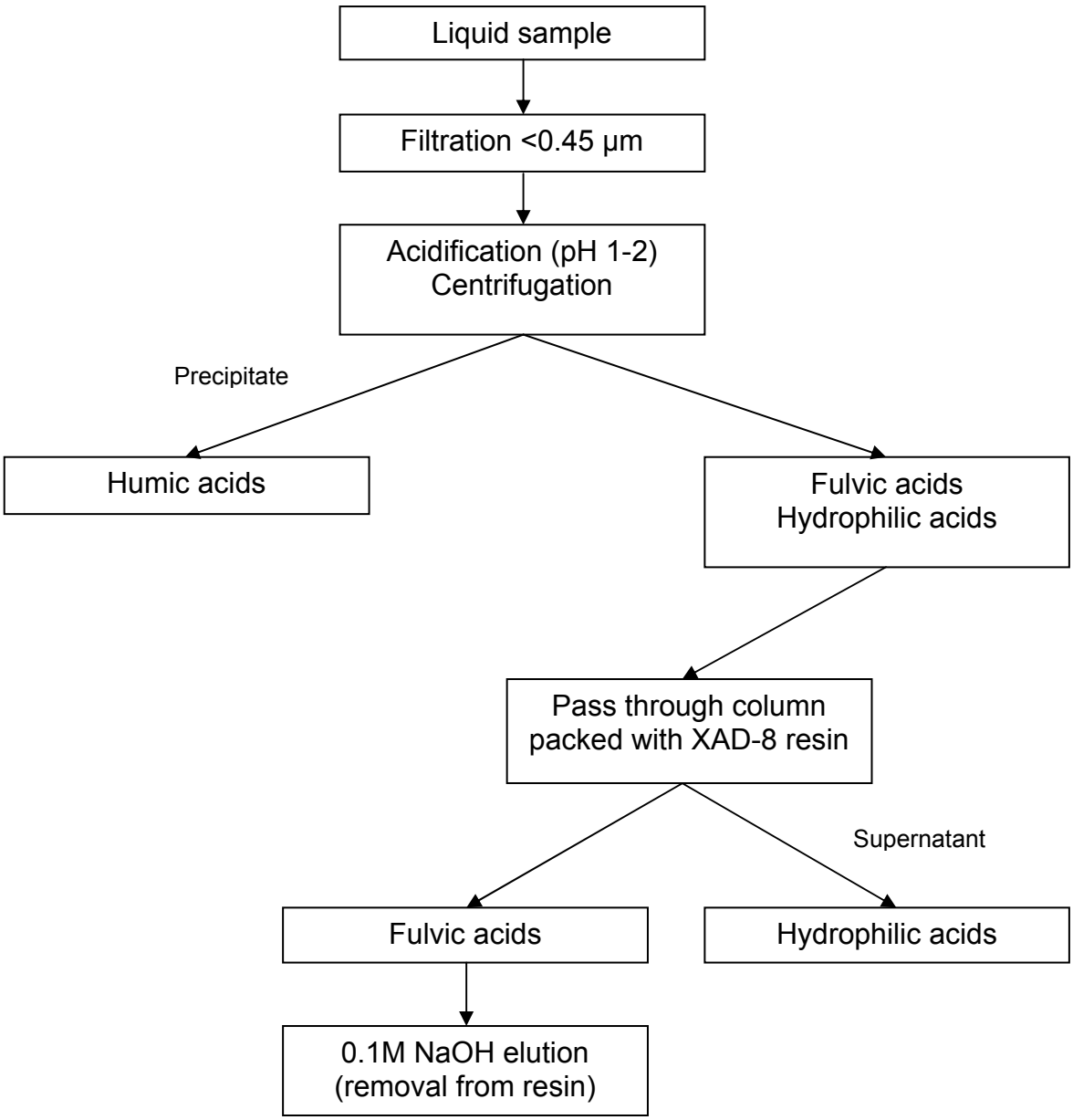

Figure 6.5. Isolation of humic substance fractions (Christensen et al., 1998).

Fractionation of humic substances using the method described in detail by Thurman and Malcolm (1981) is laborious. These methods are, as stated by Van Zomeren and Comans (2007), designed to isolate and purify sufficient quantities of each humic fraction for further analysis. A rapid batch procedure was developed (Van Zomeren *et al.*, 2007a) which is less laborious, and is more suitable for studies in which only the concentration of each humic fraction needs to be understood. As such, the rapid batch procedure does not physically isolate the solid humic fractions, rather it allows for the calculation of the concentration of each fraction in the initial sample. The rapid batch procedure, therefore, allows for more frequent analysis.

The non-ionic adsorbant XAD-8 resin used in previous studies is no longer commercially available, and as a result the rapid batch procedure is reported as using an alternative DAX-8 resin (Van Zomeren *et al.*, 2007a). The use of either resin is however valid, since they are reported to isolate humic solutes almost equally (Peuravuori *et al.*, 2002).

The rapid batch procedure is similar to previous humic extraction methods, with the notable exclusion of fractionation columns. The solid samples are treated as described by Riffaldi (1983), and the supernatant liquid acidified to pH 1-2. The solution is then left to stand overnight, and the precipitated humic acid fraction removed by centrifugation. The humic acid is dissolved in 0.1 M KOH and the total organic carbon (TOC) of the solution measured. The DAX-8 resin is then added directly to the remaining solution and left to equilibrate for 1 hour. The supernatant liquid (hydrophilic fraction) is filtered and the TOC

is measured to determine the hydrophilic acid concentration. The fulvic acid fraction is removed from the remaining resin using a 0.1 M KOH solution and the TOC of this solution is measured, which provides the fulvic acid concentration of the sample.

The humic fractions are not physically isolated and purified during the rapid batch procedure (although this is possible), as only the concentrations of each humic fraction in the sample are required. These fractions are calculated from the TOC measurements taken during the procedure.

6.1.2. Humic substances extraction within the EHT method

Due to the number of samples and replicates measured by the EHT it is not practical to measure each sample using fractionation columns as described in commonly used methods (Artiola Fortuny *et al.*, 1982; Christensen *et al.*, 1998; Peuravuori *et al.*, 2002; Thurman *et al.*, 1981). The rapid batch procedure of Van Zomeren and Comans (2007) therefore allows a number of samples to be analysed at the same time, and thus is less labour intensive. In this study an adapted method based on the rapid batch procedure described by Van Zomeren and Comans (2007) was investigated using the waste samples described in Chapter 4.

Humic substances are removed during the extraction process: humic acid is precipitated by acidification, whilst the fulvic acid fraction is adsorbed onto the surface on the XAD-8 resin as these molecules are hydrophobic. Therefore the DOC remaining is the hydrophilic compounds, which are non-humic

compounds, and represent the remaining DOC once the non-biodegradable fraction has been removed.

The aim of this investigation is to reduce a potential error in the EHT. The objective of reducing the potential error is to remove all humic substances (humic acid and fulvic acid) from the P3 DOC. Rather than calculating the biodegradability of the waste sample by deducting P2 from P3, it will instead be calculated by deducting the non-biodegradable DOC from P2 from P3. The revised EHT procedure is shown in Figure 6.6.

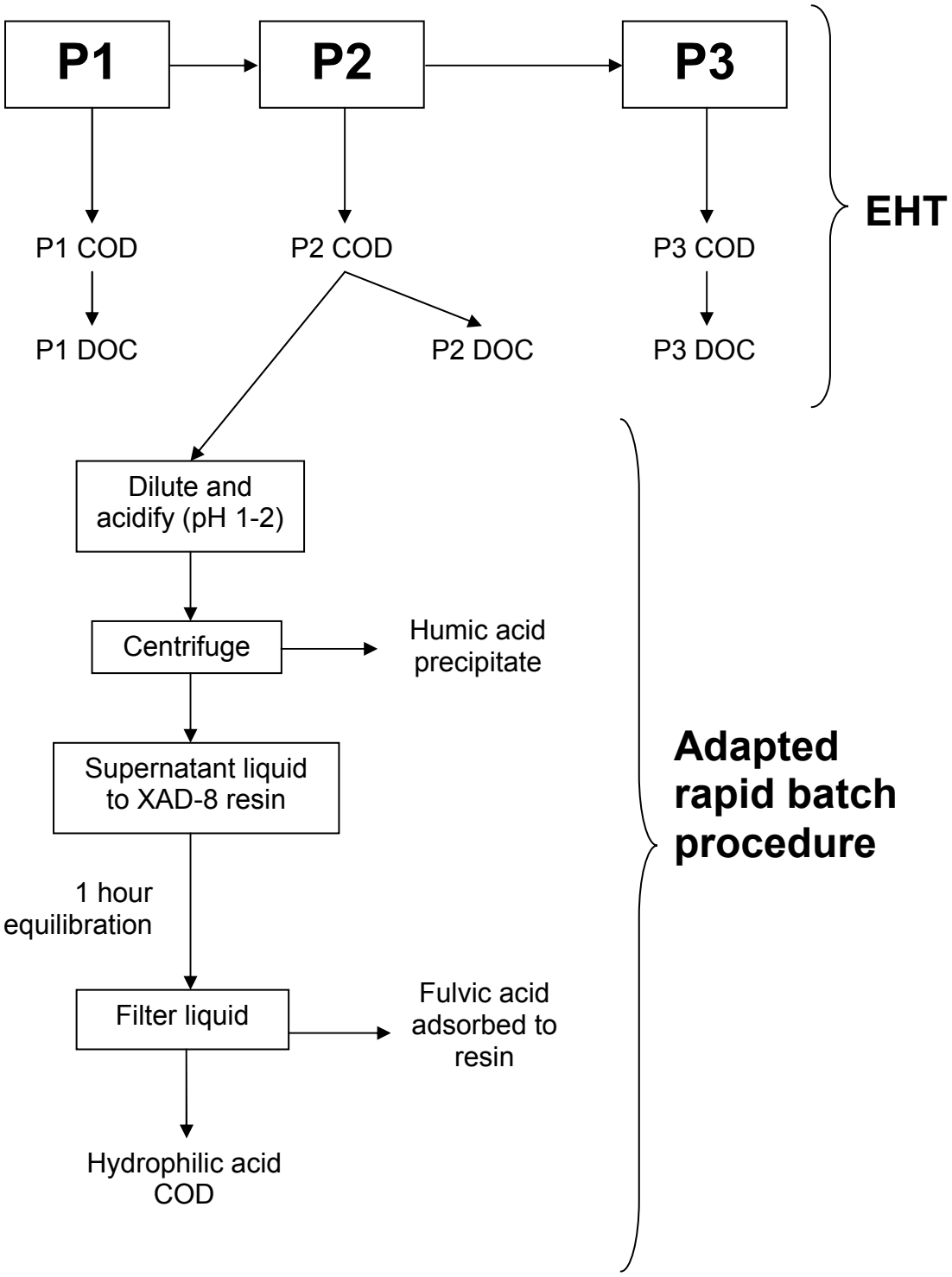

Figure 6.6. Schematic diagram of the revised EHT method.

Experimentally, the objectives of this investigation are as follows-

- To investigate the humic substance quantities present in the phase 2 DOC for each organic waste sample
- To assess whether the selective deduction of humic substances from the total DOC would enable a stronger correlation with the long-term BM100 method.

In this investigation, the samples analysed in Chapter 4 were used to quantify the amount of humic substances present in the DOC released by the autoclave step. Since humic substances are formed as the result of biodegradation the highest concentrations of humic substances in the P2 DOC would be expected to be observed in the treated waste samples (i.e. composted green waste). The correlations with the BM100 are compared for the previously obtained P3-P2 and total DOC values with the correlation obtained from a selective deduction from P3. The correlation of the DR4 method with the BM100 is also provided for comparison purposes.

6.2. Materials and Methods

6.2.1. Samples

The organic waste samples used in this investigation are those used in chapter 4, and as such were prepared in the same way.

Due to the limited amounts of sample available, not all of the samples could be used in this study. For this reason, 33 out of 37 samples were analysed.

As a result, the original correlations with the BM100 values discussed in Chapter 4 are adjusted for this Chapter.

6.2.2. Resin preparation

Although XAD-8 resin is no longer commercially available, this was the stocked resin, and so was used in this investigation. This resin is similar in humic adsorbance efficiency to DAX-8 resin (Peuravuori *et al.*, 2002), and as such is still valid for this study.

The XAD-8 resin (~200 mL in volume) was prepared by Soxhlet extraction for 24 h with methanol as described in previous research (Goslan *et al.*, 2002). This was then rinsed with 2 L HCl (0.1 M), followed by 2 L NaOH (0.1 M) and 2 L distilled water. The cleaned resin was then stored in a low acidity 5% HCl solution.

The resin was rinsed before use with distilled water of approximately 10 times the volume of resin using a Buchner funnel under vacuum to dry the resin.

6.2.3. Humic extraction procedure

The samples were weighed out in triplicates to 5 g LOI and placed in Erlenmeyer flasks. A pH 4.75 phosphate buffer (100 mL) was added, and the mixture was then placed in an autoclave at 121°C for 15 min. A 5 mL sample was then removed from the mixture and filtered (0.45 µm membrane filter) and the chemical oxygen demand (COD) measured (Spectroquant COD test

tubes). This represents up to phase 2 (P2) of the EHT as described in earlier Chapters.

A 2 mL aliquot of the removed sample was then placed in a centrifuge tube and diluted to 15 mL with distilled water. The COD was then measured. The solution was then acidified by adding 2 drops of HCl (5 M) and left to stand overnight to precipitate the humic acid fraction. The addition of 2 drops of 5 M HCl was observed to be adequate for lowering the pH to 1-2 in all samples, including blank distilled water.

The solution was then centrifuged at 3000 rpm for 10 min as described by Van Zomeren *et al* (2007). The liquid was decanted off, leaving the humic acid fraction in the centrifuge tube, and 10 mL was placed in a small beaker. The prepared XAD-8 resin (2 g) was then added and the beaker was left to stand for 1 h. The liquid was then filtered (0.45 µm membrane filter) using a syringe filter holder, and the COD was measured. This is shown schematically in Figure 6.6.

6.2.4. Humic content calculation

To accurately calculate the humic substance concentration in the solutions it is necessary to account for the addition of HCl, and of moisture from the XAD-8 resin. The correction factor (f) for the added volume of acid is calculated by the following equation-

$$f_1 = \frac{V_1 + V_2}{V_1}$$

Where V_1 is the volume of the sample solution and V_2 is the volume of acid added. In this investigation such small quantities of acid were used (2-3 drops) the volume of acid was calculated from the average mass of a drop of HCl.

The correction factor for the moisture added to the sample solution from the XAD-8 resin is calculated by the following equation-

$$f_2 = \frac{M_{XAD-8} x (100 - DM_{XAD-8}) x 0.01}{V} + 1$$

Where M_{XAD-8} is the mass of XAD-8 resin used, DM_{XAD-8} is the dry matter content (%) of the resin, and V is the volume of sample solution added to the resin.

The blank is calculated as follows-

$$Blank = B_2 \, x \, f_2 - B_1$$

Where B_1 is the COD of the distilled water used in the dilution with 2 drops of HCl and B_2 is the COD of the blank solution after standing with XAD-8 resin.

The COD of the supernatant hydrophilic liquid (COD_{Hy}) is calculated from the initial value ($iCOD_{Hy}$) using the correction factors and the blank values using the following equation-

$$COD_{Hy} = iCOD_{Hy} \, x \, f_1 \, x \, f_2 - Blank$$

The biodegradable DOC fraction is calculated from the COD_{Hy} value by determining the %DOC adsorbed onto the resin surface. The DOC adsorbed onto the resin is the fulvic acid (FA) fraction, and the humic acid (HA) fraction removed prior to resin addition by acidification. Biodegradable DOC is calculated for the revised EHT method (Figure 1) by the following equations-

$$\frac{COD_{Hy}}{P2 \quad COD} x100 = \%COD_{Hy} = \%DOC_{Hy}$$

$$100 - \%DOC_{Hy} = \%DOC_{HA/FA}$$

$$P3\ DOC - \left(\frac{\%DOC_{HA/FA}}{100} X\ P2 \quad COD \right) = Biodegradable\ DOC$$

The biodegradable DOC is therefore the P3 DOC value with the fraction of humic substances deducted.

6.3. Results

6.3.1. Humic content of P2 DOC

The percentage of the P2 DOC released during the EHT that is estimated to be humic content (Ha/Fa) is shown in Figure 6.7.

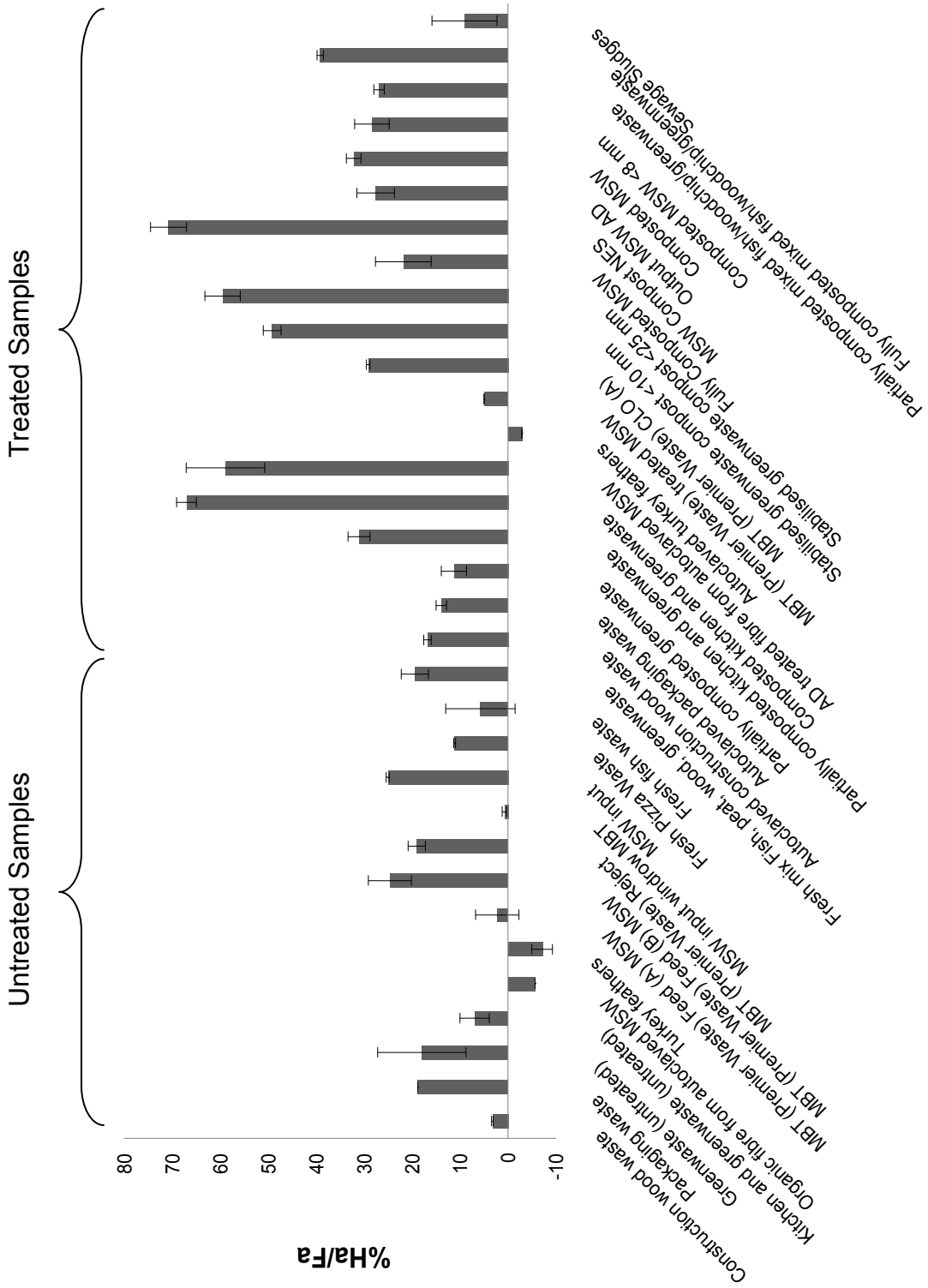

Figure 6.7. Average humic substance (%Ha/Fa) content of P2 DOC for untreated and treated organic waste samples. Error bars shown as standard error.

The humic content of the DOC released at P2 of the EHT varies significantly between the samples. The negative values on the graph will be discussed later. As expected, the highest humic content is observed for the treated samples, with values ranging from 5% up to 71%. The humic content in P2 DOC for the untreated samples ranged from <1% up to 25%.

6.3.2. Correlations of the modified EHT with the BM100

The correlation of the modified EHT is shown in Figure 6.8. The EHT data is P3 DOC minus the non-biodegradable proportion of P2 DOC. The percentage of humic substances (%$DOC_{HA/FA}$) is deducted from P2 DOC, and the product value is deducted from the final P3 DOC value.

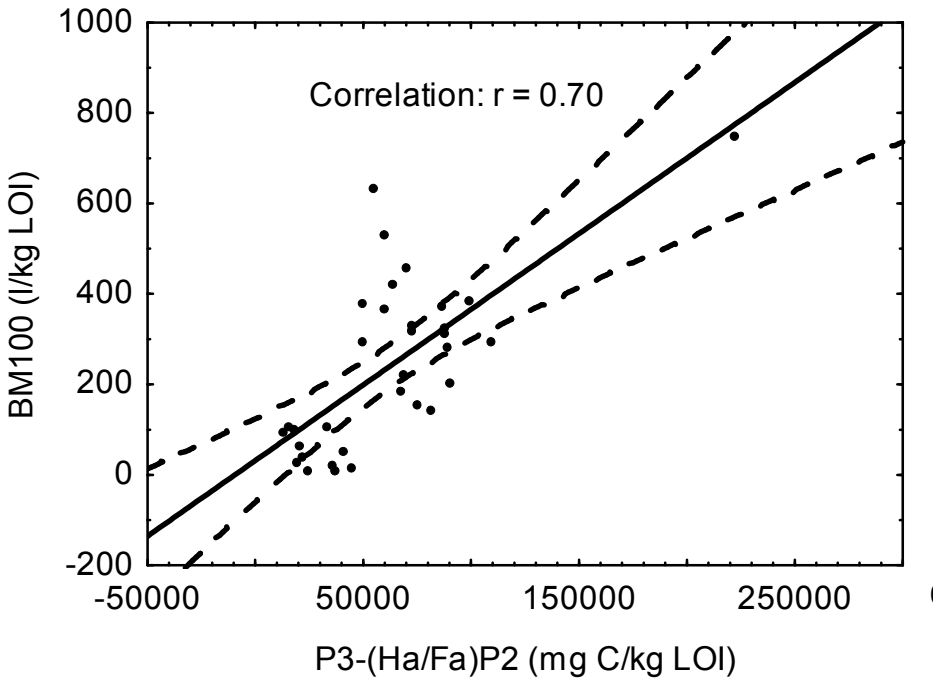

Figure 6.8. Correlation of EHT (P3 DOC –P2 $DOC_{HA/FA}$) with BM100 data for 33 organic waste samples. Dotted lines indicate region of 95% confidence.

The correlations for the enzyme only DOC (P3-P2) and total DOC (P3) are different from those quoted in Chapter 4 since a smaller number of samples

were used in this study. These correlations, along with the correlation of the DR4 with the BM100 are shown in Figures 6.9-6.11.

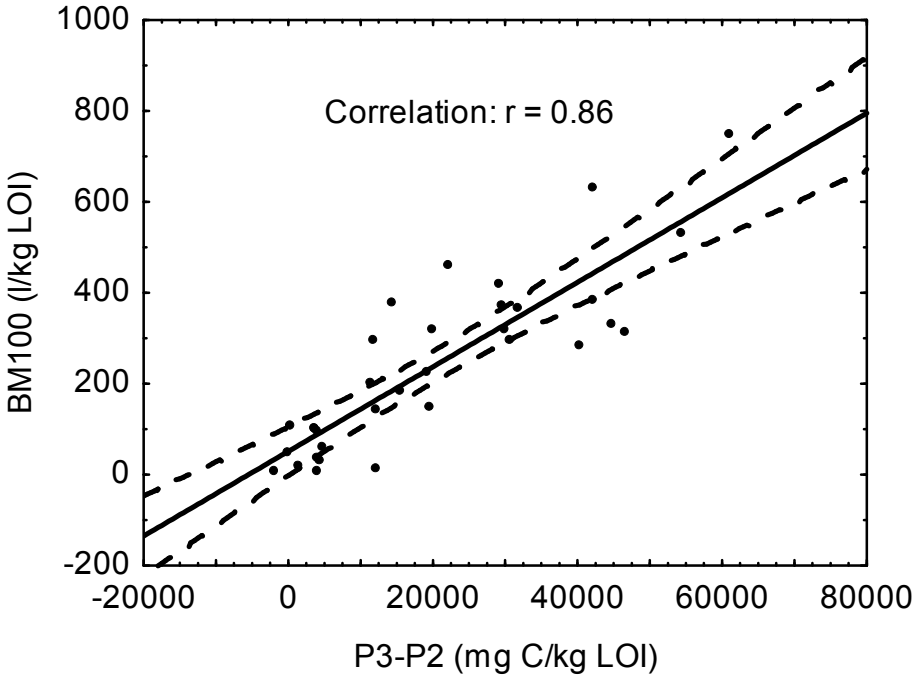

Figure 6.9. Correlation of EHT (P3 DOC – P2 DOC) with BM100 data for 33 organic waste samples. Dotted lines indicate region of 95% confidence.

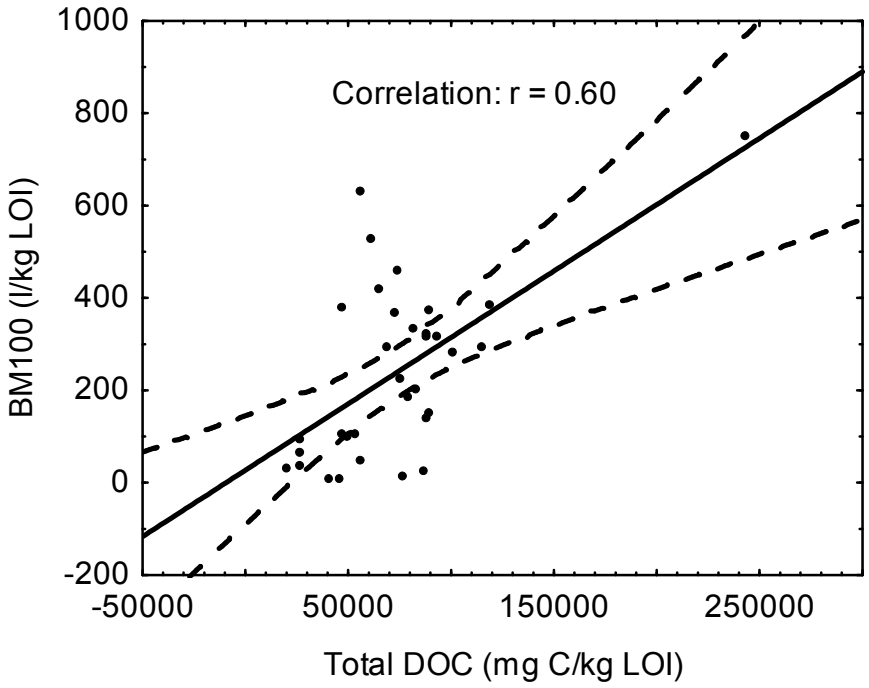

Figure 6.10. Correlation of EHT (total DOC, P3) with BM100 data for 33 organic waste samples. Dotted lines indicate region of 95% confidence.

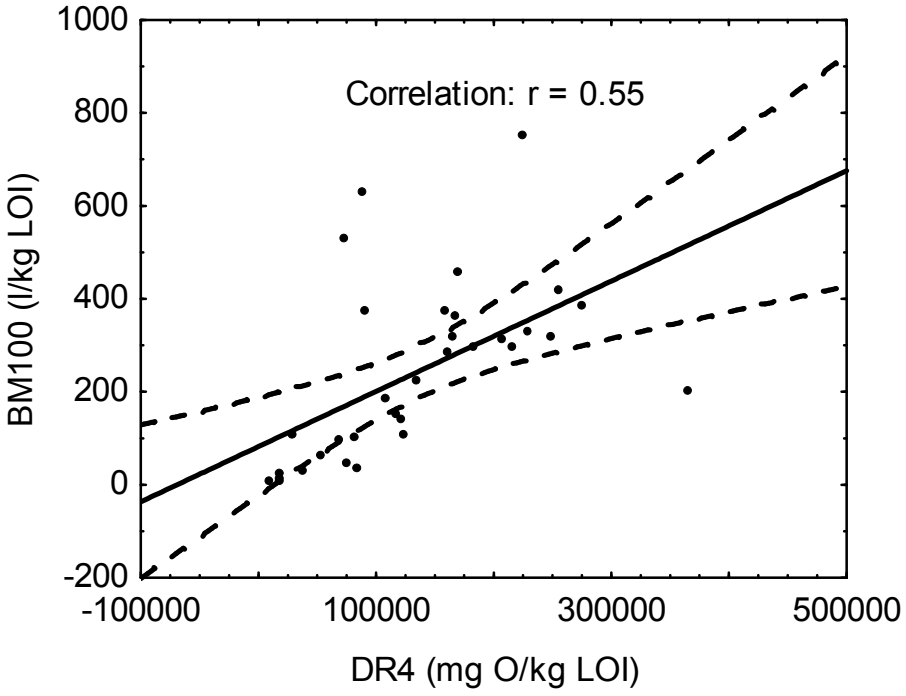

Figure 6.11. Correlation of DR4 with BM100 data for 33 organic waste samples. Dotted lines indicate region of 95% confidence.

The strongest relationship with the BM100 is observed for the enzyme only DOC (P3-P2), as indicated in Figure 6.8. The correlation (r) with the BM100 is 0.86, which is highly significant ($p < 0.001$), whilst for the total DOC $r = 0.60$ ($p < 0.001$). The correlation of the modified EHT, P3 DOC – P2 $DOC_{HA/FA}$, is shown in Figure 6.7 as $r = 0.70$ ($p < 0.001$). In each case the EHT exhibited a stronger correlation with the BM100 than the DR4 ($r = 0.55$, $p < 0.001$) as shown in Figure 6.10.

6.4. Discussion

6.4.1. Humic content of P2 DOC

The humic content of P2 DOC shown in Figure 6.6 indicated that the humic content was higher for treated samples. This is expected since humic substances are, as discussed earlier, formed from the breakdown products of lignin and other organic molecules. Therefore as the waste material undergoes biological treatment, humic substances would be expected to form towards the end of the treatment period (Adani *et al.*, 1999; Chen *et al.*, 1996; Inbar *et al.*, 1990; Miikki *et al.*, 1997). It was observed that greater quantities of humic acid precipitated from the samples which were least biodegradable, as shown in Figure 6.12 (after acidification, prior to centrifugation).

Figure 6.12. Precipitated humic acid fractions for (a) partially composted mixed fish, woodchip and green waste, (b) fully composted MSW (NES) and (c) stabilised green waste (<25 mm).

Humic acid, which is a dark brown polymer (Aiken, 1985), can be clearly seen in the stabilised green waste and composted MSW samples, but there is very little precipitate present in the partially composted sample. The $\%DOC_{HA/FA}$ for the partially composted sample was 27%, for the composted MSW this was 71%, and 60% for the stabilised green waste sample. It can be deduced from these values, and the observations, that the majority of the humic substances present in the partially composted P2 DOC was fulvic acid which remained in solution following the acidification step. However the humic acid concentration is likely to be significantly higher for the composted MSW. The humic acid concentration for the stabilised green waste is likely to be even higher, as expected, since this sample has undergone a longer stabilisation process. This indicates, as has been observed in previous studies (Artiola

Fortuny *et al.*, 1982; Christensen *et al.*, 1998; Riffaldi *et al.*, 1983; Varadachari *et al.*, 1984), that fulvic acids are formed before humic acid.

In partially treated waste samples, it is likely that some of the lignin present before treatment has been broken down into the smaller fractions (as discussed in section 6.1.1.1.). These lignin fractions may not have undergone the humification process at this stage, as indicated by the relatively low humic substance concentrations observed for untreated and partially treated waste samples. The theories of humic substance formation have been previously discussed, and a major function of this is the breakdown of lignin into smaller fractions, before larger chain molecules are formed. The result of this overall humification process (involving lignin) is that the slowly biodegradable lignin is broken down by the few microbes capable of this (Tuomela *et al.*, 2000) into smaller fractions which are more biodegradable than lignin. These smaller fractions, of which a higher number of microbes will be able to digest, will eventually undergo the polymerisation and processing to form humic substances. Therefore there may be an intermediate process involved in humification during a treatment process, which may release higher quantities of non-enzymatic DOC in EHT analysis than untreated samples due to the breakdown of lignin, but this DOC may not be humic-like substances. This places further emphasis on characterising the P2 DOC to understand the concentration of humic substances.

Some negative values were observed from the humic extraction analysis. This is likely to be due to very low quantities of humic substances being

present in the P2 DOC of the respective samples. Therefore the DOC of the solution would not decrease, since little will adsorb onto the surface of the XAD-8 resin. Some carbon may leach from the resin itself, which, despite a blank measurement used, could affect the final value, thus resulting in a negative value. Alternatively, the COD tubes used may be susceptible to small variations, which, at the dilutions used, could have significant effects on the final values.

The $\%DOC_{HA/FA}$ values obtained in this study are significantly lower than the values reported in a study by Van Zomeren *et al* (2007) for comparable samples. For example, in the study by Van Zomeren *et al* sewage sludges, fresh MSW and compost were reported to contain 75, 58-68 and 91% humic substances respectively (Van Zomeren *et al.*, 2007b). These values were as a percentage of all solubilised carbon. However in this study, values of 9, 1-25 and 22-71% respectively were observed. The rapid batch procedure involves acid and alkali extraction steps on solid samples to solubilise all the carbon in the sample (Van Zomeren *et al.*, 2007a). The lower values observed in this study are therefore due to the autoclave not solubilising all carbon. Full solubilisation would, however, render the autoclave process unsuitable for the EHT, since the EHT relies on DOC release from enzyme hydrolysis, which could not occur if all carbon was solubilised. As a result of this difference between the rapid batch procedure and the modified procedure added to the EHT, the $\%DOC_{HA/FA}$ values reported should not be considered to accurately represent the humic proportions of the solid samples. Rather the values reported in this study represent the humic-like substance

concentration in the P2 DOC. The purpose of adding a humic extraction procedure to the EHT was to enable an accurate representation of the non-biodegradable DOC released during the EHT, to enable a more accurate calculation of biodegradable DOC.

The values obtained in this investigation may not provide an accurate measurement of humic substance content in the solid samples, and this was not the aim of this investigation. The values, however, are what were expected relative to each other, in terms of the treated samples yielding a higher concentration of humic-like substances in the P2 DOC. This indicates that the rapid batch procedure adapted for these samples is valid, and provides an indication of the humic substance content of P2 DOC. This was the aim of adding a humic substance extraction method to the EHT, and the use of the less labour intensive rapid batch procedure is suitable for this purpose. Comparisons have made between the rapid batch procedure and the conventional procedure in a previous study (Van Zomeren *et al.*, 2007a). Such a study was not practical as part of this investigation, however if the modified rapid batch procedure is to be kept as part of the EHT method, then the conventional methodology should be considered for comparison.

6.4.2. Correlations of the modified EHT with the BM100

The samples used in Chapter 4 were also used in this study. However for practical reasons fewer samples were analysed and as such the correlations for the enzyme-only DOC (P3-P2), total DOC and DR4 with the BM100 vary slightly from those discussed in Chapter 4. These correlations, along with the

correlation obtained from the modified EHT, deducting only the humic portion of P2 DOC, are summarised in Table 6.1.

Short-term test method		Correlation (r) with BM100
EHT	P3-P2 $DOC_{HA/FA}$	0.70
	P3-P2	0.86
	Total DOC (P3)	0.60
	DR4	0.55

Table 6.1. Correlations of the three variants of the EHT and the DR4 with BM100 values (number of samples, n=33).

As was observed in Chapter 4, the enzyme-only DOC and total DOC exhibit a stronger relationship with the BM100 than the DR4. The modified EHT also provided a stronger relationship with the BM100 than the DR4; however this correlation (r = 0.70) is not as high as that observed for the enzyme-only DOC (r = 0.86). The addition of a humic extraction procedure to the EHT therefore does not result in the highest correlation with the BM100.

The aim of this investigation was to remove a potential error of the EHT, by deducting only the non-biodegradable DOC from the total DOC. The introduction of a humic substance extraction technique was expected to enable a more accurate indication of sample biodegradability. However as indicated from the correlations, the addition of the humic extraction technique to the EHT does not improve the relationship with the BM100.

It can be concluded from these results that the incorporation of a humic substance extraction method into the EHT provided an indication of the humic-like substance proportion of P2 DOC. However the enzyme-only DOC provided the strongest correlation, and is therefore most suitable as a short-term indicator of BM100 data.

All test methods used to assess the biodegradability of organic waste materials will possess certain limitations. Since the BM100, DR4 and EHT all measure different parameters, it is very unlikely that any short-term test method will correlate with the BM100 perfectly (i.e. r = 1.0). The BM100 possesses certain limitations, discussed in Chapter 2, and so will also contribute to outlying data points on a linear plot with a short-term test method such as the DR4 or EHT.

The purpose of the EHT is to provide a rapid estimation of BM100 values, as deducting only humic substances from the overall DOC was expected to provide a more scientifically valid biodegradability indication. Previous studies have reported that humic and fulvic acids are not completely biologically stable (Gramss *et al.*, 1999; Qualls, 2004). It was reported by Qualls (2004) that fulvic acid fractions were considerably more biodegradable than humic acid fractions. It is therefore possible that a proportion of the biogas released during long-term BM100 test method originates from the fulvic acid present within the original sample. Since the fulvic acid is removed from the modified EHT, this could account for the lower than expected correlation with the BM100. The aim of this investigation was to eliminate all non-biodegradable

DOC, leaving the biodegradable DOC. As such the addition of a humic substance extraction technique to the EHT method may therefore be too coarse and still result in the removal of biodegradable DOC.

These findings are from a wide range of untreated and treated organic materials, and so further studies into a single composting process using the rapid batch procedure within the EHT could yet result in an improved correlation over the standard enzyme-only approach. The individual concentration of humic acid and fulvic acid should also be observed, rather than simply calculating the concentration of humic substances such as in this investigation. This would investigate whether the deduction of only the humic acid fraction from the total DOC (P3) could result in an improved correlation with the BM100.

The EHT was developed as an alternative to the DR4 test method. To meet this objective the EHT was required to provide data in a shorter timescale than the DR4, alongside producing reliable and robust data. Without including sample preparation, the EHT is completed within 24 h, even with the humic extraction step, which is significantly less than the 4 days of the DR4. Based on the correlations discussed in this Chapter and Chapter 4 the EHT is more applicable to a wide range of untreated and treated waste materials. Therefore, as concluded in Chapter 4, the EHT is an appropriate alternative to the DR4 as a short-term test to the BM100. The most suitable method of representing EHT is as P3-P2 DOC; however total DOC and P3-P2 $DOC_{HA/FA}$

are also more suitable to the DR4. Therefore the EHT, in any form, is a more suitable short-term biodegradability test method to the DR4.

6.5. Conclusions

The use of a rapid batch procedure to extract humic content from the P2 DOC was successful based on the results obtained for each sample. In general these results were expected (i.e. higher $\%DOC_{HA/FA}$ for treated samples), although were lower than results obtained in a previous study, which was due to differences in obtaining the aqueous samples analysed. Since the adapted rapid batch procedure was effective at characterising the P2 DOC, a deduction of humic substance DOC from the total (P3) DOC released from the EHT method was possible. This was expected to result in a more scientifically valid indication of sample biodegradability, as the biodegradable DOC released in P2 were not excluded, whilst the non-biodegradable DOC (humic substances) were excluded. This was however not the case, as the correlation with the BM100 data did not reflect this.

The enzyme-only DOC, as discussed previously, has the potential limitation that proportions of biodegradable DOC released in P2 are deducted from the final DOC value. This method of expressing sample biodegradability still produces the strongest linear relationship with BM100 values. Therefore, for the purpose of the EHT as a short-term prediction tool for the BM100, the use of a humic extraction step within the EHT is not needed.

The use of a humic extraction procedure within the EHT should not be discarded completely, as for process specific studies, such as green waste composting, this may yet be the most suitable method of indicating sample biodegradability. It was also found that the fulvic acid fraction may be biodegradable, and may be measured in the BM100 test method, and therefore perhaps should not be deducted from the total P3 DOC. Therefore based on the findings of this Chapter, further research and consideration is required regarding the use of the rapid batch procedure within the EHT.

It is evident from this Chapter, and previous Chapters, that the EHT, regardless of biodegradability representation, is a more suitable short-term test method than the aerobic 4 day DR4 method.

Chapter Seven

Research Conclusions

This Chapter aims to summarise all of the findings from the research project, and highlight the significance of the work. The originality of the research is discussed, and recommendations for further development are made.

7.1. Introduction

The overall aim of this research project was to develop and evaluate an alternative test method to assess the biodegradability of organic waste materials. The alternative test method was required to be cost-effective and applicable to a wide range of organic waste streams.

The previous Chapters have covered the development of the enzymatic hydrolysis test (EHT) method. Each of these Chapters contained individual discussion and conclusion sections. The findings described in each of the previous Chapters resulted in the investigations described in subsequent Chapters. This Chapter aims to summarise the overall findings of the project, and critically assess the success and limitations of the research. This Chapter also aims to discuss how the previous Chapters meet the objectives set out

over the course of the project, and highlight the overall significance and originality of this work.

7.2. Research overview

The objectives of the project were stated in Chapter 1. These objectives and a discussion of how each was covered in the thesis are described as follows-

1. To produce a critical literature review of the existing methods used to measure the biodegradability of various waste streams, including MSW. To identify the advantages and disadvantages of each method, and discuss the requirements for an alternative test method, and how this can improve on current methods.

The literature review in Chapter 2 critically discussed a vast range of test methods that can be used to assess the biodegradability of organic waste material. The suitability of each test method to aid in the evaluation of the diversion of biodegradable municipal waste (BMW) from landfill was discussed. It was concluded that anaerobic test methods, such as the BM100, provided reliable data, however due to the timescales of the methods were unsuitable for frequent analysis. Short-term test methods are required to correlate with long-term anaerobic methods to enable the prediction of the anaerobic test data. Aerobic test methods were found to possess a number of limitations, and as a result an alternative to aerobic test methods were required. The conclusion was reached that no single test method was found to be completely sufficient based on the suitability criteria for assisting in the

BMW diversion from landfill. Enzyme-based approaches were highlighted as an area for further development.

2. Using the current knowledge and principles, design a method based on the principle of enzymatic hydrolysis of hemicellulosic/cellulosic materials. Optimise the methodology with regards to the pH and temperature

In Chapter 3 the EHT method was drafted and using a standard cellulose sample the optimum pH and temperature conditions were determined for use on organic waste materials. The dissolved organic carbon (DOC) release was monitored over an extended period of time, and from this data three benchmark enzyme incubation times were determined of 3, 20 and 40 hours. To validate the method this optimised test was needed to be applied to a range of organic waste materials and compared with existing microbial test methods.

3. Investigate the use of autoclaving various and the effects of particle size on test reproducibility.

The use of a pure cellulase enzyme and an investigation into the effects of the autoclave step were investigated in Chapter 3. The effect of particle size was investigated in a later study discussed in Chapter 5. The pure cellulase enzyme was found to be significantly less effective at hydrolysing the standard cellulose substrate. This was concluded to be due to the fact that a number of

different types of cellulase enzymes are required to break down cellulose, and as such the crude cellulase enzymes used in the initial optimisation study were more suitable. The autoclave process was found to have significant effects on the DOC released at P2 of the EHT. It was also found to have significant effects on the DOC released in the subsequent enzyme incubation phase (P3). The autoclave step was concluded to be beneficial to the EHT method, and that autoclaving was the most suitable method of sterilising the waste sample.

4. Apply the EHT to a wide range of untreated and treated organic waste samples using the optimum conditions determined, and compare with existing microbial methods.

The optimum conditions and benchmark times determined from Chapter 3 were applied to a range of untreated and treated organic waste samples in Chapter 4. The samples were also analysed using the 4 day aerobic DR4 and 100 day anaerobic BM100 test methods, which are specified in the Environment Agency guidance (2005). The correlations of the EHT and DR4 values with the BM100 were compared. It was concluded that the EHT correlated best with the BM100, and that an incubation time of 20 h was most suitable. This indicates that the EHT is a suitable alternative to the DR4 test method. It was also found that deducting the DOC released after autoclaving the waste sample (phase 2, P2, of the EHT method), leaving only the DOC released from enzyme hydrolysis, yielded the strongest relationship with the

BM100. The potential error of deducting the P2 DOC was discussed, and the requirements for further investigation were discussed.

5. Apply the EHT to a waste treatment process over an extended period of time.

The EHT method was applied to a single waste treatment process in Chapter 5. The biodegradability of the input and output samples was monitored over a period of 9 months. The effects of particle size were investigated as part of this study. The waste samples used in this study were also analysed using the DR4 and BM100 test methods, enabling a comparison similar to that discussed in Chapter 5.

No correlation between the EHT and DR4 with the BM100 was observed. This was due in part to the treatment process monitored, which was not intended to reduce biodegradable content, and therefore the biodegradability values observed for each test did not vary significantly. The tests methods measure different parameters, and so it was observed that they do not always follow the same trend. For example, if for MSW input the BM100 values indicate an increase or decrease in biodegradable content over a given period, the DR4 and/or EHT methods may, or may not, indicate the same trend.

The EHT was applied to samples ground to <10 and <2 mm, and the data obtained suggested that the most relevant grinding size is <10 mm. This conclusion was based on the variation observed between sample replicates

and the DOC release at each test phase. The sample preparation for the DR4 and BM100 involves grinding the sample to <10 mm, and this particle size is also most suitable for the EHT. Therefore the additional grinding of samples to particle sizes of <2 mm is not required.

6. Investigate the use of a humic extraction technique within the EHT procedure.

From the data obtained from the EHT in Chapter 4 it was concluded that the DOC released at P2 of the EHT required further characterisation. Deducting the DOC released at P2 (after autoclave) would remove the DOC values due to non-biodegradable carbon, leaving only the DOC released from enzyme hydrolysis. However since a variable proportion of the P2 DOC would contain biodegradable carbon, as discussed in Chapters 4 and 6, the deduction of P2 DOC from the total DOC could result in inaccuracies. Similarly, representing biodegradability as the total DOC would invariably contain non-biodegradable carbon. Therefore it was necessary to characterise the P2 DOC to enable the deduction of only the non-biodegradable DOC, without deducting biodegradable DOC.

Humic substances are often referred to as ‘non-biodegradable’, or very slowly biodegradable, and so the humic content of the P2 DOC was quantified. An adapted method based on a recently developed rapid batch procedure was used to measure the humic content of the P2 DOC. The samples used in Chapter 4 were used for this investigation. It was concluded, based on the

correlations with the BM100, that selectively deducting only the humic substances from the total DOC (P3) did not improve the relationship of the EHT with the BM100. This was found to be due to the fact that the addition of a humic substance extraction technique to the EHT method may therefore be too coarse and still result in the removal of biodegradable DOC. Fulvic acid has been previously reported to be slowly biodegradable, and so this could potentially be measured by the BM100 test method. Therefore removing the fulvic acid DOC from the total DOC (P3) may negatively affect the correlation of the EHT with the BM100.

The strongest correlation with the BM100 was again found to be when the EHT was expressed in terms of the enzyme-only DOC (P3-P2) used in Chapter 4. The three methods of expressing sample biodegradability using the EHT of total DOC, enzyme-only DOC and total DOC minus humic content of P2 were all found to have a higher correlation with the BM100 than the DR4. This indicated that the EHT, in any form, is a more suitable short-term biodegradability test method than the DR4.

The deduction of the humic substance content was expected a more accurate representation of sample biodegradability, but due to the correlations obtained the use of a humic extraction technique is not required for the EHT. The overall aim of the EHT is to provide an alternative short-term biodegradability test method to correlate with the BM100 and enable the prediction of long-term test data. Incorporating a humic substance extraction technique into the

EHT procedure does not provide the best method of predicting long-term test data.

7.3. Discussion of Final Method

7.3.1. The EHT Method

From the results presented in this thesis a final methodology can be provided. For the sample preparation, the metals, glass, plastics and other inert materials must be removed from the sample and the percentage BMW recorded. The remaining BMW is then dried and ground to <10 mm. The dry matter (DM) and loss-on-ignition (LOI) must then be recorded. The final EHT methodology is as follows-

- Phase 1. Place the prepared waste sample (5 g LOI) into a 250 ml Erlenmeyer flask. Analyse each sample in triplicate. Add the phosphate pH 4.75 buffer (100 ml 0.37 M) to the flask.

- Phase 2. Seal the flask with tin foil and secure tightly with autoclave tape, and place the sample mixture into the autoclave at 121°C for 15 min to sterilise the mixture. Remove a further 5 ml sample and filter for COD analysis, and in phase 1.

- Phase 3. Add the prepared enzyme solution (20 ml) to each of the flasks and seal with a neoprene bung. Then place the flasks in a shaking incubator at 150 rpm at a temperature of 50°C for 20 h. After 20 h of incubation remove a 5 ml sample and filter for COD analysis.

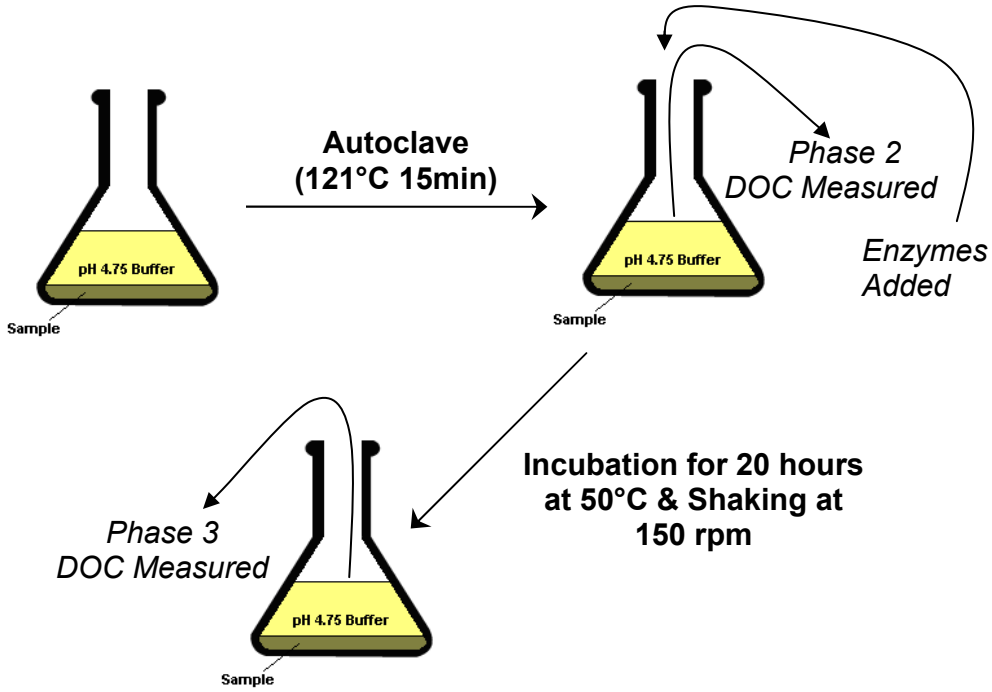

Figure 7.1. Schematic diagram of the final EHT method.

The calculation of DOC is provided in Chapter 3. The DOC is presented in units of mg C/kg LOI, and the final biodegradability value is obtained by deducting the DOC at P2 from the DOC obtained at P3.

The aim of this research was to develop a rapid and reliable biodegradability test method. The EHT was developed for this purpose, and has been applied to a wide range of treated and untreated waste materials, including those obtained regularly over a 9 month period (Chapter 5). From these studies, the correlations of the EHT with the BM100 have been determined, and were found to be stronger than those obtained from the DR4 test method. In Chapter 4, the EHT provided correlations (r) of 0.62 and 0.77 for total DOC (P3) and enzyme-only DOC (P3-P2) respectively at 20 h of enzyme

incubation, whilst the DR4 gave a correlation of r = 0.58. In Chapter 6, the samples from Chapter 4 were analysed, however fewer of the samples were analysed, and a humic extraction technique was incorporated into the EHT method to characterise the P2 DOC. In this investigation the EHT relationship with the BM100 were indicated by correlations (r) of 0.60, 0.70 and 0.86 for total DOC (P3), total DOC minus humic substance DOC (P3-$P2_{HA/FA}$) and enzyme-only DOC (P3-P2) respectively. Again the DR4 correlation with the BM100 was lowest, of r = 0.55. These relationships indicate that the EHT is a suitable alternative short-term test method to the DR4. The strongest correlation with the BM100 was observed for the EHT where the enzyme-only DOC (P3-P2) is considered. The overarching aim of the EHT is to correlate with the BM100, and to provide a short-term estimation of the long-term biodegradability of organic waste materials. For this purpose, the integration of a humic extraction technique is not required.

The application of each test method over a 9 month period, described in Chapter 5, indicates that the variability of each test method for different waste fractions over a period of time is similar. With values ranging, as a percentage from the average, from 82-112%, 89-129%, 77-113% and 92-118% for the EHT total DOC (P3), EHT enzyme-only DOC (P3-P2), DR4 and BM100 methods respectively.

7.3.2. Limitations of the Research

There are several limitations with the research project presented within this thesis. One such limitation is the measurement of DOC. The DOC released

during the EHT procedure was measured using chemical oxygen demand (COD) test kits, which were used for practical reasons. The calculation of DOC from the COD values is detailed in Chapter 3. To convert the COD values to TOC values, and subsequently DOC, the COD values measured must be assumed to be glucose (the monomeric unit of cellulose). This is due to requiring the relative molecular mass (RMM) of the carbon molecules to calculate the DOC. Therefore this calculation presents a potential error. The use of a TOC analyser would have by-passed this calculation, thus removing the error; however such equipment was not reliably available at the beginning of the research project. The investigations discussed in Chapters 3 and 4 would have needed to have been repeated to validate such a method change, which in the timescales of the project was not feasible.

The use of other enzymes has not been thoroughly investigated in this project. The crude cellulase enzyme used contained cellulase, hemicellulase and protease activities, and further hemicellulase was added to the enzyme mixture. This crude mixture was chosen as it provided a range of enzymatic activities, which would hydrolyse a large proportion of the biodegradable carbon present in organic waste materials. Whilst a pure cellulase enzyme was investigated in Chapter 3, further investigations on other cellulase enzymes, and the addition of extra enzyme types (such as protease and lipase enzymes) would have been beneficial to the research overall. Despite this limitation, the mild acid catalysed hydrolysis under autoclave conditions could mean that the use of additional enzyme types is unnecessary. But a further study should investigate this.

Another notable limitation of the research project is the treatment process monitored in Chapter 5. This facility did not involve a bio-stabilisation stage (rather a bio-drying stage) and so the biodegradability of the input and output solid recovered fuel (SRF) was very similar. As a result, the correlation between the test methods was very poor. The purpose of biodegradability testing, in the UK, is to quantify the BMW diverted from landfill. The SRF produced at the Frog Island Ecodeco facility is not landfilled; therefore biodegradability analysis of such samples may not be required for calculations in the LATS targets discussed in Chapter 1. Therefore for monitoring a single waste treatment process, it would have been more suitable to monitor a composting or anaerobic digestion facility over the various stages of the process. This would have also allowed a more comprehensive study into the use of a humic substance extraction technique as discussed in Chapter 6. However, the use of an alternative facility was not possible due to funding limitations, though should be considered in future work.

Each of the biodegradability test methods used as part of this research project measure different parameters, and, as discussed previously, each test method possesses specific limitations. The result of this is that obtaining a close correlation between the short-term and long-term test methods is very difficult. Whilst previous researchers have reported strong relationships, these investigations have typically used fewer samples and samples from a similar source. For example, MSW samples of different stages of degradation from landfills.

The use of a humic extraction technique within the EHT method was investigated to provide a more accurate representation of sample biodegradability. The aim of humic extraction was to allow only the soluble humic substances released in the EHT to be deducted from the total DOC (P3). This would ideally result in only biodegradable DOC remaining in the solution. However it has been reported in previous studies that humic substances, particularly fulvic acid, are not in fact 'non-biodegradable'. This means that deducting all humic substances may also deduct biodegradable DOC, which is not the intended result. Humic acid is significantly more bio-stable than fulvic acid, and as a result is unlikely to release biogas in the BM100 test method. Therefore the deduction of only the humic acid fraction and not the humic and fulvic acid fractions together, may be more applicable to biodegradability assessment by the EHT. The humic extraction method investigated may therefore be too coarse for intended purpose.

The EHT presented in this thesis has been shown to be a suitable alternative to the DR4. As such, in accordance with the Environment Agency guidance on monitoring BMW diversion from landfill (2005), the EHT can be considered as an alternative short-term correlating method with the BM100. However, the BM100 is not without its limitations and so failure to attain a correlation of r = 1.0 should not be completely attributed to the short-term test method used.

7.3.3. Further Work

From the discussions and conclusions provided in each chapter, further work can be recommended.

An investigation into the addition of extra enzymes (such as protease and lipase) to the enzyme mixture used in the EHT is recommended. It would be necessary to determine optimum pH and temperature conditions for a new enzyme mixture. Such an investigation should aim to assess whether it is necessary to add extra enzymes to the mixture, or whether the mild acid hydrolysis under autoclave conditions is adequate for the hydrolysis of protein and fat substrates.

A potential future investigation could involve monitoring a single treatment process such as composting over a period of time. Input and output samples could be analysed using the EHT and the BM100. Additionally samples taken during the treatment sample, for example, after 2, 4 and 10 weeks of composting, depending on the timescale of the treatment process. This would allow for a range of biodegradability results to be obtained. The humic content would be expected to increase during the composting process. Therefore such a study would be suitable to further investigate the addition of a humic extraction procedure into the EHT method. The use of a humic extraction was included into the EHT procedure to provide a more accurate representation of biodegradable DOC released. It was concluded that the removal of all humic substances may result in the removal of slowly biodegradable carbon (notably fulvic acid) which would be measured by the

BM100. For the purpose of obtaining the best possible correlation, it is necessary to avoid the deduction of biodegradable DOC which would be represented in the BM100 data. Therefore if this technique is applied to a large number of specific samples humic extractions within the EHT may provide an improved correlation with the BM100. This further work would therefore investigate the EHT when applied to a single treatment process, which involves bio-stabilisation, over a period of time. Additionally this work would investigate whether the humic extraction technique within the EHT is more suited for specific samples, rather than a wide range of waste materials as used in Chapter 6.

Understanding the fate of compounds (specifically humic substances) in the long-term anaerobic test methods would provide further understanding of the biodegradation process, and therefore the measurement of biodegradability. Subsequently the development of short-term test methods, such as the EHT, which could estimate the anaerobic results, could potentially provide more accurate results.

The method of measuring DOC has previously been discussed. The suitability of using a TOC analyser instead of COD tubes should be investigated, with the overarching aim of removing the calculation assumption, and additionally to reduce the costs of the EHT.

7.4. Contribution to knowledge

The work presented in this thesis makes a significant contribution to knowledge in terms of the development of a new rapid and reliable biodegradability test method, and also from the knowledge acquired regarding the processes which occur during the EHT.

This test method offers a significant timescale improvement on the currently used short-term DR4 test method. The DR4 test method is completed in 4 days, whilst the EHT is completed in less than 24 hours. The sample preparation of the two methods is the same, since it was observed that additional grinding from <10 mm to <2 mm increased the variability of the EHT data. Therefore grinding to <10 mm is more suitable.

The EHT also has a stronger relationship with the BM100 than the DR4. This means that BM100 data can be more reliably predicted using the EHT.

The development of the EHT is significant to the waste industry since this method will enable the analysis of sample biodegradability sooner, which would have financial benefits to waste treatment operators. The improved reliability of the EHT would also allow for more accurate calculations of BMW diversion, and as a result could be financially beneficial in terms of the LATS targets.

Although a test method based on the cellulase hydrolysis of organic waste was previously reported by Rodriguez *et* al (2005), the development of the EHT is original research for the following reasons-

- The EHT measures DOC rather than monosaccharide release, meaning that the EHT allows for other biodegradable carbon molecules, such as amino and fatty acids.
- The EHT is the only biodegradability test method to include an autoclave step, which results in an overall higher DOC release.
- The EHT has been applied to a wide range of organic waste materials, such as pizza, fish, turkey feathers and wood waste. This is in addition to the MSW-derived BMW samples used in this project. Such a broad waste characterisation project has not been previously reported.
- No other biodegradability test method has incorporated a humic substance extraction technique.

The EHT was developed as part of a large collaborative waste characterisation project in collaboration with Cranfield University, the Open University and WRc plc. The waste characterisation project was funded by Defra, and overall has resulted in further understanding of waste composition and waste biodegradability.

The EHT has been considered as an alternative short-term biodegradability test method in an Environment Agency consultation on the revised guidance for monitoring MBT processes (Environment Agency, 2007a). The outcome of this was that the DR4 is now no longer the definitive short-term test method,

and that other short-term test methods which can be scientifically justified, such as the EHT, are acceptable.

From the development of the EHT, the understanding of the processes which occur over the course of the test have been developed from the thorough analysis of the results obtained and relating the data to findings reported by other researchers.

The effects of the autoclave process on the DOC release at phases 2 and 3 of the EHT have been investigated and evaluated. The autoclave process was found to result in significantly higher DOC release at phase 2, as discussed in Chapter 3. This was concluded to be the result of the mild acid catalysed hydrolysis of the waste material under the high temperature conditions. These conditions were found to hydrolyse cellulose, lignin and particularly hemicellulose in previous studies, and therefore it is likely that the hydrolysis of these compounds is occurring to an extent in the EHT at phase 2 during autoclave.

It was hypothesised that large amounts of humic substances are solubilised during the autoclave process for samples that had been biologically treated. The humic content was investigated in Chapter 6, and found that the humic content of the P2 DOC was highest for treated samples. This was expected since humic substances are formed from the breakdown products of lignin and other organic molecules.

The DOC from enzymatic hydrolysis (P3-P2) was greater for samples of high biodegradability content. The P3 DOC from the EHT indicates the readily hydrolysable cellulosic material. Since readily biodegradable materials such as hemicellulose and cellulose are reduced during biological treatment (such as anaerobic digestion or composting) the P3 DOC from the EHT was highest for untreated samples.

As indicated from the correlations of the enzyme-only DOC (P3-P2) with the BM100 values, the enzyme-only DOC is suitable for indicating the biodegradable content of organic materials. However biodegradable carbon such as fats and proteins are released in phase 2, particularly for samples which have not undergone biological treatment. Quantifying the non-biodegradable DOC present in the P2 DOC was attempted by measuring the humic substance (humic acid and fulvic acid) fraction. The use of the humic substance extraction technique did not improve the correlation with the BM100. It is concluded that the humic extraction technique within the EHT is too coarse, and that fulvic acids are slowly biodegradable (as reported in previous research). Therefore it was proposed that these substances are biodegraded, to an extent, in the long-term BM100 test method, and that the removal of them from the EHT total DOC (P3) has an adverse effect on the correlation with the BM100 values.

The contribution to scientific knowledge provided in this thesis is the development of a new biodegradability test method, which is a more rapid and reliable alternative to the currently used short-term DR4 test method. In

addition to the development of the test, the understanding of the scientific processes occuring during the test method is discussed. These understandings have enabled a critical evaluation of the EHT, in which the limitations of the test method have been discussed and recommendations for further research have been made.

7.5. Conclusions

This project aimed to develop an alternative biodegradability test method. The result was the EHT method, which is more rapid and reliable than the DR4. The investigations discussed in the Chapters of this thesis have provided significant evidence of this.

This Chapter has highlighted how the project aims and objectives have all been achieved, and has also discussed the testing of the research hypotheses. This Chapter also presented the final recommendations for the final methodology.

The originality of this research lies in-

- The development and application of a new biodegradability test method.
- The application of a developed technique (humic extraction) to a purpose not previously investigated (within a biodegradability test).

Further work has been identified based on the findings of this research. Such research could result in further improvements being made to the EHT, and in

general the overall understanding of waste characterisation and biodegradability measurement.

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Appendix

Data Appendices

A- Chapter 4 EHT, DR4 and BM100 biodegradability data.

B- Chapter 4 biochemical data.

C- Chapter 5 EHT data for <10 mm samples.

D- Chapter 5 EHT data for <2 mm samples.

E- Chapter 5 DR4 and BM100 data.

Publications Appendices

Part 1- Journal Publications

F- (2008) Review article: test methods to aid in the evaluation of the diversion of biodegradable municipal waste (BMW) from landfill. Waste Management. **In Press**. (Modified from Chapter 2)

G- (2008) Comparison of a novel enzymatic biodegradability test method with microbial degradation methods Communications in Waste and Resource Management **9**(3), 80-86. (Modified from Chapter 4)

Part 2- Peer-reviewed Conference Publications

H- (2007) Development and application of an enzymatic hydrolysis test to assess the biodegradability of organic waste material. Proceedings

Sardinia 2007, Eleventh International Waste Management and Landfill Symposium, S. Margherita di Pula, Cagliari, Italy, CISA.

I- (2008) The development of a novel biodegradability test method based on the enzymatic hydrolysis of cellulose. Waste 2008, Stratford-upon-Avon.

Appendix A

Chapter 4 EHT, DR4 and BM100 biodegradability data.

			DR4	BM100	EHT- DOC (mg C/kg LOI)				
Sample	**%DM**	**%LOI**	**mg O/kg LOI (see note 1)**	**l/kg LOI (see note 2)**	**P1**	**P2**	**P3(3)**	**P3(20)**	**P3(40)**
Commercial cellulose	94.0	99.7	80000	200	622	1200	32000	57100	61300
Construction wood waste	77.5	91.4	37600	27	1690	16300	18800	20700	21800
Autoclaved construction wood waste	63.9	90.7	84700	35	7760	22600	24400	26700	27200
Packaging waste	42.6	93.6	73000	527	1790	7320	34900	61900	69900
Autoclaved packaging waste	40.4	93.1	90000	630	7130	14700	30900	56800	61100
Greenwaste (untreated)	40.4	73.2	108000	182	24400	64300	68600	79700	72200
Partially composted greenwaste	44.2	62.1	136000	221	25500	55900	70000	75300	68200
Kitchen and greenwaste (untreated)	35.1	65.3	217000	292	36800	84700	88300	116000	102000
Partially composted kitchen and greenwaste	38.3	68.0	125000	103	18400	43000	42000	46900	57000
Composted kitchen and greenwaste	51.7	60.9	82000	99	6810	46200	46100	49800	50900
Organic fibre from autoclaved MSW	50.5	76.9	249000	319	23400	58100	70700	88000	95000
AD treated fibre from autoclaved MSW	29.2	72.5	69000	92	4960	23100	29300	27300	28300
Turkey feathers	35.5	98.9	91000	375	22900	32900	47800	47400	42700
Autoclaved turkey feathers	38.3	96.2	366000	199	20300	72300	80300	83700	85100
MBT (Premier Waste) Feed (A) MSW	56.0	71.6	207000	312	14500	41900	60400	88700	90900
MBT (Premier Waste) Feed (B) MSW	51.1	71.8	230000	330	10700	37900	60500	82700	90500
MBT (Premier Waste) treated MSW	57.1	77.2	159000	372	22700	60400	74200	89900	91200
MBT (Premier Waste) Reject	58.0	72.5	161000	281	24900	61000	77800	102000	106000
MBT (Premier Waste) CLO (A)	51.9	57.7	168000	364	4920	40800	48500	72800	74200
MBT (Premier Waste) CLO (B)	46.9	59.6	171000	306	9790	42200	61800	103000	113000

			DR4	BM100	EHT- DOC (mg C/kg LOI)				
Sample	**%DM**	**%LOI**	**mg O/kg LOI (see note 1)**	**l/kg LOI (see note 2)**	**P1**	**P2**	**P3(3)**	**P3(20)**	**P3(40)**
Stabilised greenwaste compost <10 mm	71.9	29.2	18000	12	4510	64900	65900	77300	77400
Stabilised greenwaste compost <25 mm	66.6	28.9	19000	21	5650	85900	86000	87400	92300
MSW input windrow MBT	94.4	73.4	256000	418	14100	35400	55700	64700	67600
Fully Composted MSW	66.3	29.6	9000	5	1680	42000	29200	46200	48500
MSW Compost NES	69.3	26.7	30000	105	1270	53000	49900	53300	54000
MSW input to AD	96.5	58.7	313000	413	17800	83200	86500	147000	147000
Output MSW AD	31.5	56.2	54400	62	1570	22700	24000	27500	27600
MSW input	95.5	65.8	276000	385	17900	77200	91300	119000	144000
Composted MSW	50.3	58.3	184300	293	7290	57600	69000	69400	69200
Composted MSW <8 mm	58.4	49.9	167000	316	17400	73400	76300	93300	93300
Fresh MSW	94.4	39.3	144000	354	14900	84600	96600	110000	138000
Composted MSW <15 mm	75.3	23.4	22000	16	12600	54700	59700	62800	74900
Fresh Pizza Waste	43.1	95.2	226000	748	7440	182000	207000	243000	288000
Fresh fish waste	38.1	73.2	170000	457	3330	51600	64800	73900	81300
Fresh mix Fish, peat, wood, greenwaste	97.1	58.4	117000	150	12600	70100	68200	89800	88700
Partially composted mixed fish/woodchip/greenwaste	48.9	53.8	75000	47	7740	56000	54500	56100	60800
Fully composted mixed fish/woodchip/greennwaste	65.0	34.3	19000	8	2800	43000	40700	41200	46800
Sewage Sludges	18.4	60.8	122000	140	11900	76500	80400	88600	88400

Note 1- DR4 values obtained by the Open University, and are reported to LOI data obtained by the Open University

Note 2- BM100 values obtained by WRc plc, and are reported to LOI data obtained by WRc plc

Appendix B

Chapter 4 biochemical data.

Sample	Fat %	Solubles %	Hemicellulose %	Cellulose %	Lignin %
Commercial cellulose	0.3	11.8	18.2	68.3	0.4
Construction wood waste	2.0	14.1	20.3	36.0	23.2
Autoclaved construction wood waste	2.1	15.1	16.5	34.9	27.4
Packaging waste	2.7	7.1	11.3	42.6	34.0
Autoclaved packaging waste	2.4	8.1	8.0	48.7	29.5
Greenwaste (untreated)	1.5	25.9	16.0	19.8	19.7
Partially composted greenwaste	1.8	27.7	11.7	14.8	17.3
Kitchen and greenwaste (untreated)	3.1	33.9	14.5	19.1	15.5
Partially composted kitchen and greenwaste	1.0	26.6	13.3	22.2	19.3
Composted kitchen and greenwaste	1.0	33.4	7.9	16.1	16.1
Organic fibre from autoclaved MSW	3.0	34.9	8.1	26.2	18.9
AD treated fibre from autoclaved MSW	0.6	26.8	10.5	11.9	40.2
Turkey feathers	3.4	12.5	64.0	3.7	15.6
Autoclaved turkey feathers	2.9	62.5	24.6	4.4	3.9
MBT (Premier Waste) Feed (A) MSW	1.8	30.4	9.7	17.8	20.9
MBT (Premier Waste) Feed (B) MSW	4.8	30.9	9.8	29.8	15.4
MBT (Premier Waste) treated MSW	3.3	23.3	10.8	39.3	14.7
MBT (Premier Waste) Reject	3.2	23.3	10.5	39.1	15.7
MBT (Premier Waste) CLO (A)	2.1	22.3	11.7	23.2	16.3
MBT (Premier Waste) CLO (B)	5.7	36.0	7.3	27.3	12.3

Sample	Fat %	Solubles %	Hemicellulose %	Cellulose %	Lignin %
Stabilised greenwaste compost <10 mm	2.4	29.9	6.6	4.7	8.1
Stabilised greenwaste compost <25 mm	0.4	27.5	6.2	5.1	9.2
MSW input windrow MBT	3.2	31.0	11.1	21.7	10.9
Fully Composted MSW	0.2	27.1	5.1	3.9	10.9
MSW Compost NES	0.5	24.2	8.7	5.2	6.2
MSW input to AD	2.9	31.6	9.0	25.9	9.6
Output MSW AD	0.4	29.4	10.8	17.4	20.9
MSW input	8.3	41.1	10.1	14.5	9.1
Composted MSW	1.6	39.4	10.1	19.1	15.6
Composted MSW <8 mm	0.8	40.7	9.6	11.1	11.5
Fresh MSW	2.9	50.1	8.3	7.3	8.1
Composted MSW <15 mm	1.1	39.1	6.9	4.9	12.0
Fresh Pizza Waste	24.2	71.2	3.4	0.1	0.5
Fresh fish waste	7.3	45.2	13.5	13.0	16.1
Fresh mix Fish, peat, wood, greenwaste	1.7	45.3	9.9	17.0	18.9
Partially composted mixed fish/woodchip/greenwaste	0.7	45.3	7.1	18.9	12.3
Fully composted mixed fish/woodchip/greennwaste	0.1	34.9	7.6	9.6	9.7
Sewage Sludges	1.0	62.9	14.9	2.7	10.8

Appendix C

Chapter 5 EHT data for <10 mm samples.

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
MSW Input- 10 mm	M-W14-10	14	17700	37500	79300	41800
	M-W24-10	24	13800	51400	82500	31100
	M-W28-10	28	17300	31100	61700	30600
	M-W30-10	30	13700	45400	74100	28700
	M-W34-10	34	24600	52300	84100	31800
	M-W38-10	38	19000	38500	71000	32500
	M-W43-10	43	19300	42400	74800	32400
	M-W47-10	47	20900	44100	73700	29600
		Average	**18288**	**42838**	**75150**	**32313**

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
SRF- 10 mm	S-W6-10	6	6880	30400	64700	34300
	S-W8-10	8	13000	39200	77100	37900
	S-W14-10	14	20000	44800	78700	33900
	S-W16-10	16	16100	37400	67300	29900
	S-W18-10	18	17100	47000	79900	32900
	S-W22-10	22	19000	40800	76800	36000
	S-W24-10	24	16200	39800	71100	31300
	S-W26-10	26	20200	43400	72900	29500
	S-W28-10	28	26300	72400	100000	27600
	S-W32-10	32	16200	34100	60800	26700
	S-W41-10	41	19600	47600	88700	41100
		Average	**17325**	**43355**	**76182**	**32827**

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
Fines- 10 mm	F-W8-10	8	9850	72500	124000	51500
	F-W18-10	18	17200	87900	154000	66100
	F-W20-10	20	17800	93200	181000	87800
	F-W28-10	28	26200	111000	161000	50000
	F-W32-10	32	29000	107000	164000	57000
	F-W53-10	53	18100	92400	144000	51600
		Average	**19692**	**94000**	**154667**	**60667**

Appendix D

Chapter 5 EHT data for <2 mm samples.

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
MSW Input- 2 mm	M-W2-2	2	29300	45800	74200	28400
	M-W6-2	6	53500	77600	127000	49400
	M-W10-2	10	20400	28400	61000	32600
	M-W14-2	14	27500	41900	79000	37100
	M-W16-2	16	24000	44200	76200	32000
	M-W20-2	20	27200	55900	81300	25400
	M-W24-2	24	32300	50100	87500	37400
	M-W28-2	28	25100	42500	71700	29200
	M-W30-2	30	22600	48100	81900	33800
	M-W34-2	34	34600	46400	75700	29300
	M-W38-2	38	27200	39400	70500	31100
		Average	**29427**	**47300**	**80545**	**33245**

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
SRF- 2 mm	S-W12-2	12	26000	43200	82100	38900
	S-W14-2	14	23500	44100	73300	29200
	S-W16-2	16	24100	47800	82300	34500
	S-W18-2	18	30000	46400	80300	33900
	S-W20-2	20	28400	54200	88700	34500
	S-W22-2	22	30300	58900	95300	36400
	S-W26-2	26	25400	50400	78700	28300
	S-W28-2	28	36300	72600	91900	19300
		Average	**28000**	**52200**	**84075**	**31875**

	Sample	Week Number	Average P1 DOC (mg C/kg LOI)	Average P2 DOC (mg C/kg LOI)	Average P3 DOC (mg C/kg LOI)	P3-P2
Fines- 2 mm	F-W8-2	8	13300	130000	176000	46000
	F-W16-2	16	54100	180000	224000	44000
	F-W18-2	18	21300	86900	110000	23100
	F-W20-2	20	29700	117000	135000	18000
	F-W28-2	28	18600	114000	151000	37000
	F-W32-2	32	33100	133000	156000	23000
	F-W41-2	41	45100	151000	173000	22000
		Average	**30743**	**130271**	**160714**	**30443**

Appendix E

Chapter 5 DR4 and BM100 data.

Sample	Week Number	DR4 mg O/kg LOI	BM100 l/kg LOI
MSW Input	2	173000	302
	6	204000	427
	10	176000	317
	14	175000	335
	16	172000	324
	20	173000	363
	24	174000	337
	28	161000	292
	30	153000	267
	34	187000	254
	38	172000	270
	43	177000	272
	47	127000	265
	Average	***171077***	***310***

Sample	Week Number	DR4 mg O/kg LOI	BM100 l/kg LOI
SRF Output	6	139000	264
	8	132000	281
	12	160000	313
	14	162000	203
	16	165000	326
	18	132000	253
	20	158000	250
	22	165000	273
	24	166000	217
	26	117000	283
	28	182000	239
	32	150000	237
	41	176000	390
	Average	***154154***	***271***

Sample	Week Number	DR4 mg O/kg LOI	BM100 l/kg LOI
Fines Output	8	319000	403
	16	289000	480
	18	278000	424
	20	252000	421
	28	312000	350
	32	178000	250
	41	224000	383
	53	334000	275
	Average	***273250***	***373***

Appendix F

(2008) Review article: test methods to aid in the evaluation of the diversion of biodegradable municipal waste (BMW) from landfill. Waste Management. In Press.

(Modified from Chapter 2)

Waste Management xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Test methods to aid in the evaluation of the diversion of biodegradable municipal waste (BMW) from landfill

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ARTICLE INFO

Article history:
Accepted 28 August 2008
Available online xxxx

ABSTRACT

A wide range of waste characterization methods are available, each developed for a specific purpose such as determining compost stability, or for landfill acceptance criteria. Here test methods have been evaluated for the purpose of assessing waste treatment process performance and monitoring the diversion of biodegradable municipal waste (BMW) from landfill. The suitability factors include the timescale of the method, applicability to a wide range of materials and ability to indicate the long-term biodegradability of organic waste samples.

The anaerobic test methods, whilst producing reliable results, take at least several weeks to complete, therefore, not allowing for regular routine analysis often required for diversion assessments. Short-term tests are required which can correlate with, and, therefore, estimate, values obtained from long-term anaerobic methods. Aerobic test methods were found to offer a significantly improved timescale compared with anaerobic test methods; however, they have limitations due to not measuring the full extent of sample biodegradability.

No single test method was found to be completely sufficient for routine biodegradability analysis suitable for monitoring the BMW diversion from landfill. Potential areas for further research include spectrographic FT-IR or enzyme-based approaches such as the ECD or EHT methods.

Contents

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0956-053X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.wasman.2008.08.024

1. Introduction

Tests used to estimate biodegradability are an important part of organic waste characterization since they can be used for assessing the biological stability of wastes (Adani et al., 2002; Iannotti et al., 1993) or for assessing the diversion of biodegradable waste from landfill (Godley et al., 2003). A wide range of biological and non-biological test methods are available. Biological test methods based on the use of aerobic respiration indices have been recently reviewed for assessing the bio-stability of organic waste and/or compost materials (Gomez et al., 2006). Suitability factors of the test methods include the timescale, applicability to a wide range of materials and ability to indicate the long-term biodegradability of organic waste samples.

The basic principle of tests to estimate biodegradability is to assess how much of the carbon can be mineralized and how quickly it will be degraded. The biodegradability tests can be used to assess the effectiveness of a certain treatment process. This is achieved by taking a representative sample of the waste before it goes through the treatment process, and then taking another sample post-treatment (Environment Agency, 2005). The degree to which the biodegradability of the waste is reduced indicates the effectiveness of the treatment process. Rapid and reliable methods are needed to allow for more frequent optimization of a treatment process (i.e. reduce the treatment time if applicable), offering financial benefits to the operator.

Several countries have preferred methods of measuring biodegradability; however, each biodegradability test method has been developed for a specific purpose, e.g. compost stability. This review focuses on the applicability of each test method to assess treatment process performance and aid in the quantitative evaluation of the diversion of BMW from landfill. A range of potentially applicable test methods are discussed, with suggestions for further research and development being drawn from the overall conclusions.

2. Waste biodegradability test methods

2.1. Anaerobic test methods

Anaerobic test methods measure the biodegradability of a substrate under anaerobic methanogenic conditions. This particular type of method measures the release of 'biogas' (CO_2 and CH_4), typically using a digester sludge seed as a source of microbes (Godley et al., 2007). Under anaerobic conditions, in the absence of oxygen, organic materials decompose in the presence of methanogenic bacteria, which release carbon dioxide and methane gas. This is shown in the following example for cellulose and hemicellulose, respectively:

$$(C_6H_{10}O_5)n + nH_2O \rightarrow 3nCH_4 + 3nCO_2$$

$$2(C_5H_8O_4)n + 2nH_2O \rightarrow 5nCH_4 + 5nCO_2$$

Anaerobic test methods, such as the generic biochemical methane potential (BMP), are bioassays in which a sample is incubated in a temperature controlled system, where the nutrients and bacteria are also added to allow optimized microbial methanogenic conditions Such anaerobic methods describe the bioassay procedures, with variations in the methods including the nutrients added and the method of biogas measurement. Table 1 provides a summary of the key aspects of a number of anaerobic test methods. Although other methods are available in the literature, they are only slightly different to those cited, and so they have not been included in this review.

Whilst the recent studies into anaerobic test methods have focused on organic waste materials, some of the original methods were not prescribed for this material; for example the blue book method for determining biodegradability of anaerobic sewage sludge (Standing Committee of Analysts, 1977). As the interest in the biodegradable content of organic waste has increased, the anaerobic methods have been more widely applied to these materials.

An early test method was described by Owen et al. (1979). This method involved a simple glass set-up with an incubation temperature of 35 °C (Owen et al., 1979). These tests were run for 30 days using samples of peat material. Although there was still activity remaining at the end of the tests, the majority of gas production occurred in the first 20 days.

A test method used in a study by Shelton and Tiedje (1984) used a media which differed that used by Owen et al. The media used in the anaerobic tests is given in Table 2. The duration of this test was 56 days. Rather than using organic waste materials, the samples used by Shelton and Tiedje (1984) included ethanol and ρ-cresol.

A test method of 60 days was used in an investigation by Pagga and Beimborn (1993), measuring chemical samples incubated at 35 °C. Resazurin is used in the medium solution; however, as an oxygen indicator (Owen et al., 1979; Pagga and Beimborn, 1993) and so is not used as a nutrient for methanogenic bacteria.

A small scale anaerobic test method was used in a study to investigate the effects of lignin on the anaerobic decomposition of cellulose (Stinson and Ham, 1995). The mixture was incubated at 35 °C for 2 months, and the released gas monitored using a syringe system, and measured using gas chromatography (GC).

A procedure modified from Stinson and Ham (1995) was applied more recently to landfill samples (Kelly et al., 2006). Media modified from an earlier method was used (Stinson and Ham, 1995), and anaerobic sludge was added (10%) as inoculum. The test bottles were incubated at 35 °C for 45 days, and the methane was measured using GC.

The incubation test (GS90) is a test method 90 days in duration used in Austria, using a moist fresh 1 kg dry matter (DM) sample sieved to Ø 20 mm and saturated to water-holding capacity (Binner et al., 1999b). The sample is then incubated at 40 °C under anaerobic conditions. Whilst 90 days is the preferred length of the test, the test duration can vary, e.g. 240 days, known as GS240.

The fermentation test (GB21) method which is used in Germany uses a sample ground to <10 mm and filled to 300 ml with water (Bockreis et al., 2007). The Austria version of the GB21 method measures a 50 g DM sample, sieved to Ø 20 mm and 200 ml of water is added (Binner et al., 1999b). Inoculum sludge (50 ml) is added, which in Austrian procedures is the leachate from the incubation test (GS90). This test can also vary in length (28 days test, GB28), although 21 days is the preferred length of time (Binner et al., 1999b).

A small scale anaerobic test was used in a study by Harries et al. (2001). In the development of this method, the seed is a laboratory maintained seed cultured over several years (Harries et al., 2001). Here the sample is incubated with the inoculum and the seed at 35 °C for 30 days.

For the BM100 method, the waste sample is sorted and non-BMW components removed, with the percentage of BMW recorded. The biogas produced is collected in a graduated measuring cylinder filled with water, which is displaced as the biogas enters the cylinder. The water is acidified to prevent dissolution of biogas (Environment Agency, 2005; Godley et al., 2007).

Other examples of anaerobic biodegradability tests have been described in the literature (Bogner, 1990; Hansen et al., 2004; Zhang et al., 2007); however, the variations between these and those already described mostly involve the sample type and

Please cite this article in press as: Wagland, S.T. et al., Test methods to aid in the evaluation of the diversion of biodegradable ..., Waste Management (2008), doi:10.1016/j.wasman.2008.08.024

Table 1
Anaerobic test methods summary

Method	Temp/ (°C)	Length of test	Moisture	Seed	Sample size	Sample preparation	Biogas measurement method	Reporting units	References
Owen et al.	35	30 days. Majority of gas production in first 20 days; however, still activity after 30 days	No moisture adjustment	Mesophilic (35 °C) digested sludge	Peat samples <2 g/L degradable COD content	30% CO_2 70% N_2 passed through at flow rate of 0.5 L/min for 15 min	Glass syringes equipped with 20 gauge needles	m^3 CH_4/kg COD m^3 CH_4/kg TS	Owen et al. (1979)
Shelton and Tiedje	35	8 weeks	Chemical compounds used, therefore, no moisture adjustment	Sewage sludge from municipal digesters sparged with 10% CO_2 90% N_2	50 µg C/mL	Not waste samples, therefore, no preparation	UniMeasure pressure transducer equipped with a P-8 bellows	Net gas production (mL) Percentage of theoretical gas production (%)	Shelton and Tiedje (1984)
Pagga and Beimborn	35	60 days	No moisture adjustment	1–3 g/L TS digested sludge	100 mg/l C (20 mg/L if sample is toxic)	Sparged with nitrogen Adjusted to pH 7	Pressure measurement (mbar)	Total percentage of biodegradation, D_T (%)	Pagga and Beimborn (1993)
Stinson and Ham	35	60 days	No moisture adjustment	10% digested sludge solution prepared with inoculum	0.05 g non-lignin substrate (mass of sample = 0.05 g/(1−lignin fraction)	Dried and screened to 2 mm Purged with N_2 gas to remove O_2	Gas samples collected from bottles following incubation measured using gas chromatography	Rate of cellulose decomposition calculated	Stinson and Ham (1995)
Kelly et al.	35	45 days	No moisture adjustment	Sludge from anaerobic digester 10% by volume inoculum	2 g MSW sample	Dried and shredded <10 mm	Gas samples taken at end of incubation period were analyzed using gas chromatography Pressure measurement by 'Eudiometer'	Milliliters of methane per gram of dry MSW (mL/g)	Kelly et al. (2006)
Incubation test GS90	40	90 days	Saturated to water-holding capacity	No seed Sample is fresh and moist	1 kg DM plus water	Sieved to <20 mm	Gas generation calculated to normal conditions (0 °C, 1013 mbar)	Nl/kg DS	Binner and Zach (1999a)
Fermentation test GB21	35	21 days	50 g DS sample + 300 mL H_2O (200 mL in Austria)	Anaerobic digested sludge	50 g DS	Ground to <10 mm (<20 mm in Austria)	Gas production presses NaOH solution into a graduated measuring cylinder	mg/kg DS Nl/kg DS	Binner and Zach (1999a); Bockreis et al. (2007)
Harries, Cross and Smith	35	3 months, some samples produced all gas within 2 months	Oven dried at 105 °C	Laboratory maintained seed 'cultured' over several years, regularly fed with medium	0.5 g	Dried at 105 °C, grinded and sieved to <1 mm	Syringe via 3-way valve connected to a manometer. Syringe draws out gas until normal barometric pressure is reached	Cumulative gas production (mL) BMP value (m^3 CH_4/tonne DM)	Harries et al. (2001)
BM100	35	Up to 100 days (possibly longer)	Dried at 70 °C to a DM content of 87–93%	Anaerobic digested sludge (3 g in 50 mL – 6% by DM)	20 g LOI 200 mL medium 50 mL seed	Non-BMW components removed, and percentage BMW recorded. Reaction mixture sparged with nitrogen	Biogas is collected in a graduated measuring cylinder filled with acidified water	Nl/kg LOI l/kg LOI	Godley et al. (2007)

Table 2
Media used in the anaerobic test methods

Method	Medium/inoculum					References
Owen et al.	Resazurin	$(NH_4)_2HPO_4$	$CaCl_2 \cdot 2H_2O$	NH_4Cl	$MgCl_2 \cdot 6H_2O$	Owen et al. (1979)
	KCl	$MnCl_2 \cdot 4H_2O$	$CoCl_2 \cdot 6H_2O$	H_3BO_3	$CuCl_2 \cdot 2H_2O$	
	$Na_2MoO_4 \cdot 2H_2O$	$ZnCl_2$	$FeCl_2 \cdot 4H_2O$	$Na_2S \cdot 9H_2O$	Biotin	
	Folic acid	Pyridine hydrochloride	Riboflavin	Thiamin	Nicotinic acid	
	Pantothenic acid	B_{12}	ρ-aminobenzoic acid	Thioctic acid		
Shelton and Tiedje	Phosphate buffer	Mineral salts	Trace metals	$Na_2MoO_4 \cdot 2H_2O$	After cooling	Shelton and Tiedje (1984)
	KH_2PO_4	NH_4Cl	$MnCl_2 \cdot 4H_2O$	$CoCl_2 \cdot 6H_2O$	$NaHCO_3$	
	K_2HPO_4	$CaCl_2 \cdot 2H_2O$	H_3BO_3	$NiCl_2 \cdot 6H_2O$	$Na_2S \cdot 9H_2O$	
		$MgCl_2 \cdot 6H_2O$	$ZnCl_2$	Na_2SeO_3		
		$FeCl_2 \cdot 4H_2O$	$CuCl_2$			
Pagga and Beimborn	Resazurin	$CaCl_2 \cdot 2H_2O$	Trace elements	$CuCl_2$	$Na_2MoO_4 \cdot 2H_2O$	Pagga and Beimborn (1993)
	KH_2PO_4	$MgCl_2 \cdot 6H_2O$	$MnCl_2 \cdot 4H_2O$	$CoCl_2 \cdot 6H_2O$		
	$Na_2HPO_4 \cdot 12H_2O$	$FeCl_2 \cdot 4H_2O$	H_3BO_3	$NiCl_2 \cdot 6H_2O$		
	NH_4Cl	$Na_2S \cdot 9H_2O$	$ZnCl_2$	Na_2SeO_3		
Harries, Cross and Smith	Resazurin	$FeCl_2 \cdot 4H_2O$		Trace elements	$Na_2SeO_3 \cdot 5H_2O$	Harries et al. (2001)
	NH_4Cl	Tryptose		$AlCl_3 \cdot 6H_2O$	$Na_2WO_4 \cdot 2H_2O$	
	$KH_2PO_4 \cdot 3H_2O$	Yeast extract		$CoCl_2 \cdot 6H_2O$	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	
	NaH_2PO_4	H_2O		$CuCl_2 \cdot 2H_2O$	$NiCl_2 \cdot 6H_2O$	
	$NaHCO_3$	$FeS/CaCl_2$		H_3BO_3	$ZnCl_2$	
	$MgCl_2 \cdot 6H_2O$	Mercapto-ethane sulphonic acid (MES)		$MnCl_2 \cdot 4H_2O$		
Godley et al.	KH_2PO_4		Trace elements	$ZnCl_2$		Environment Agency (2005)
	NH_4Cl		$FeCl_3 \cdot 6H_2O$	$NiCl_2$	$Na_2WO_4 \cdot 2H_2O$	
	$CaCl_2 \cdot 2H_2O$		$CoCl_2 \cdot 6H_2O$	$Na_2MoO_4 \cdot 2H_2O$		
	$MgCl_2 \cdot 6H_2O$ or $MgSO_4 \cdot 7H_2O$		$MnCl_2 \cdot 4H_2O$	$CuCl_2 \cdot 2H_2O$		

amount used, and for the overall purpose of this paper are not described.

2.2. Aerobic respirometric methods

Aerobic respirometric methods are used to characterize organic waste samples by measuring the oxygen (O_2) consumption or carbon dioxide (CO_2) production of a sample. Biodegradable organic compounds are degraded under aerobic conditions as follows, using cellulose and hemicellulose, respectively, as an example:

$$(C_6H_{10}O_5)n + 6nO_2 \rightarrow 5nH_2O + 6nCO_2$$

$$(C_5H_8O_4)n + 5nO_2 \rightarrow 4nH_2O + 5nCO_2$$

There are advantages and disadvantages of measuring either O_2 consumption or CO_2-production. O_2 consumption measurements are often favored since oxygen is directly responsible for the oxidation of organic matter (Gomez et al., 2005). Both measurement methods require specific instrumentation, although CO_2 measurements have been described as inexpensive (Adani et al., 2001) and less sophisticated (Gomez et al., 2005) than O_2 measurements. The calculation of O_2 consumed from the CO_2 produced assumes an O_2:CO_2 molar ratio of 1. In reality; however, this ratio is dependent on the oxidation degree of the organic carbon (Adani et al., 2001; Gomez et al., 2005).

The RQ values have been previously presented in studies of aerobic waste decomposition processes (Atkinson et al., 1997; Gea et al., 2004, 2007; Smars et al., 2001; Weppen, 2001). It was concluded from studies that RQ values are not suitable for indicating waste biodegradability or microbial activity (Gea et al., 2004; Genc and Yonsel, 2007). There is a wide range between these values, indicating that assuming an O_2:CO_2 molar ratio of 1 could be incorrect. This could lead to significant errors when CO_2 measurements are utilized in respirometric test methods.

There is a wide variety of respirometric methods; these can be classified as 'dynamic' or 'static'. Dynamic methods are those where the sample is aerated throughout the assay, which minimizes problems associated with O_2 diffusion limitations (Gomez et al., 2006). In static methods, the samples are not aerated. The O_2 transfer in a biological system is considered to be a limiting factor (Paletski and Young, 1995), a major disadvantage of static methods. Table 3 provides a summary of the key aspects of a number of aerobic test methods.

Oxygen Uptake is a static method developed by Iannotti et al. (1993). The sample was incubated for 16 h at 37 °C, followed by a 1 h assay in which the decrease in oxygen level was monitored using a dissolved oxygen (DO) probe (Iannotti et al., 1993). This test was designed to assess the biological stability of compost.

A dynamic test method used to assess compost stability has been described in which microbial seed nutrients and moisture were not added to the sample (Paletski and Young, 1995).

The American society for testing and materials (ASTM) method is a dynamic test used for determining the stability of compost by measuring oxygen uptake, with the sample being incubated at 58 °C for 4 days (ASTM, 1996).

Specific oxygen uptake rate (SOUR), like the O_2 uptake test, was designed to assess the biological stability of compost. This is different from other respirometric tests as the sample is suspended in water. Along with the SOUR test method, which is 5–6 h in length, there is a cumulative oxygen uptake method (OD_{20}) and SOUR in solid state method (DSOUR). OD_{20} is the same as the SOUR method, except the duration is 20 h. The DSOUR method is identical to the OD_{20} method, except no moisture is added to the sample (Lasaridi and Stentiford, 1998).

The dynamic respiration index (DRI) is a dynamic test method developed by Adani et al. and designed to assess the degree of biological stability of waste derived materials. There are three types of DRI reported; DRI, real DRI (RDRI) and potential DRI (PDRI) (Adani et al., 2004). The moisture is adjusted to 750 g kg^{-1} for the DRI test with optimal water content for PDRI and a lack of moisture adjustment for the RDRI method.

The static respiration index (SRI) test is the same as the DRI described by Adani et al. except that it is a static test method (Adani et al., 2001).

Table 3
Aerobic respiration test methods summary

Method	Abbreviation	Dynamic/ static	Inoculum/ seed	Nutrients	Moisture	Temp/ (°C)	Length of test	Sample preparation	Sample size	Reporting units	Test purpose/common use	References
Oxygen uptake	O_2 uptake	Static	None	None	50–55% w/ w	37	16 h incubation 1 h assay	Samples sieved (<9.5 mm) to remove glass, plastics, inerts and oversized materials	60 g dry weight	mg O_2/g VS/h	Compost stability, degree to which the biodegradable fraction in solid wastes has been diminished during composting	Iannotti et al. (1993)
Paletski and Young	N/A	Dynamic	None	None	None	35	48 h	Compost samples	20 g wet weight	mg O_2/h/g DS	Compost stability/performance of composting process	Paletski and Young (1995)
American society for testing and materials	ASTM	Dynamic	Mature compost	Nitrogen and phosphor	50%	58	4 days		500 g		Compost stability	ASTM (1996)
Specific oxygen uptake rate	SOUR				Suspension		5–6 h					Lasaridi and Stentiford (1998)
Cumulative oxygen uptake – 20 h	OD_{20}	Static	None	Phosphate buffer, $CaCl_2$, $FeCl_3$ and $MgSO_4$	Suspension	30	20 h	Composts sample	3–8 g wet weight (dependant on activity of sample)	mg O_2/h/g DS	Compost stability/extent to which readily biodegradable material has decomposed	Lasaridi and Stentiford (1998)
Specific oxygen uptake rate in solid state	DSOUR				None added		20 h	<9.5 mm				Iannotti et al. (1993); Lasaridi and Stentiford (1998)
Dynamic respiration index	DRI				750 g kg^{-1}	Process	Maximum oxygen uptake over 24 h					Adani et al. (2004, 2001)
Real dynamic respiration index	RDRI	Dynamic	None	None	None added	Process	As DRI	Shredded 50 mm	20–40 kg depending on bulk density w/w samples used	mg O_2 kg^{-1} VS/h	Degree of biological stability	Adani et al. (2001)
Potential dynamic respiration index	PDRI				Optimal water content		As DRI	Quarterly				Adani et al. (2001)
Static respiration index	SRI	Static	None	None	750 g kg^{-1}	Process	Maximum oxygen uptake over 24 h			mg O_2 kg^{-1} VS/h		Adani et al. (2001)
Solvita® compost maturity test	Solvita®	Static	None	None	Optimum/ saturation	Room temp (20–25)	4 h (+48 h before to equilibrate if necessary)	Removal of stones and large stems and wood chips	To fill line of test jar	Solvita® maturity scale (1–8, with 1 being very active, and 8 being very mature)	Compost maturity	Changa et al. (2003)
Dynamic respiration over 4 days	DR4	Dynamic	Mature compost	Phosphor and nitrogen	50% w/w	37	4 days	Non-BMW removed and % BMW recorded	200–250 g DM	mg O/kg LOI	Monitoring performance of MBT and other treatment processes	Godley et al. (2005)
Respiration index at process temperature	RI_T	Static	None	None	40–50% w/ w	Process	4 h incubation 90 min assay	Large BMW shredded	250 ml (weight recorded)	mg O_2 gOM^{-1}/h	Evaluation of composting process/stability of compost	Gomez et al. (2005)
Respiration index at 37 °C	RI_{37}					37	18 h incubation 90 min assay	Samples sieved (<10 mm) to remove glass, plastics and other inerts				Gomez et al. (2005)
German static respiration index over 4 days	AT4 (Sapromat E)	Static	None	None	Saturation (~40–50%)	20	4 days	<20 mm	30–40 g of wetted sample	mg O_2/g DS	Describe the biological activity of waste with respect to landfill regulations	Binner and Zach (1999a); Binner et al. (1999b)
German static respiration index over 7 days	AT7 (Sapromat E)						7 days					

The Solvita compost maturity test is a commercially available static method. A compost sample of optimum water content is placed in the supplied test jar up to the fill level and the sample is allowed to 'air' for 1 h without the lid in place. The jar is then sealed and the sample is left to equilibrate if necessary. Gel paddles are then inserted into the test jar without them coming into contact with the compost. The test jar is kept at room temperature and out of direct sunlight for 4 h, and the results are based on the color of the paddles (1-8 Solvita scale), indicating the CO_2 concentration (Changa et al., 2003).

Dynamic respiration over 4 days (DR4) is a dynamic test method and is based on the ASTM method, differing by using a smaller sample size and a lower temperature (Environment Agency, 2005; Godley et al., 2005). The samples are incubated at 37 °C for 4 days. This test method was developed to monitor the performance of a waste treatment process.

Respiration index (RI) is used to evaluate the stability of compost. There are two types of this static method, both described by Gomez et al. One is the RI_T, which is incubated at the *in situ* (process) temperature recorded at the time of sampling. The other is RI_{37}, which is incubated at 37 °C.

The German static respiration index (AT4 and AT7) methods last 4 and 7 days, respectively. The CO_2 produced is measured as the CO_2 is absorbed by NaOH, and the pressure becomes negative; an oxygen generator produces O_2 until normal pressure conditions are restored. This set-up is commonly commercially known as the Sapromat method; however, alternatives such as the Oxitop method exist. The O_2 production is then recorded. Similarly to the DR4 test method, these tests were developed to describe biological activity of waste with respect to landfill regulations.

In common with anaerobic test methods, main differences in aerobic methods are the amount of sample used, test conditions and test duration. A multitude of test methods have emerged in recent years as researchers have adapted previous methods to suit the practical needs of their studies, resulting in a modified method, which varies only slightly from other existing aerobic methods.

2.3. Temperature increase methods

In addition to the aerobic respirometric methods, there is also the Dewar self-heating test, which measures the heat produced by the sample under aerobic conditions, rather than the gases consumed or produced. The self-heating tests have been investigated for the indirect estimation of respirometric activity (Koenig and Bari, 2000) and maturity of compost material (Weppen, 2002).

The self-heating test is useful as it is very simple to operate, measuring the temperature increase due to sample activity.

2.4. Spectrographic methods

Fourier transform-infrared spectroscopy (FT-IR) is a technique that is efficient in providing comprehensive information on chemical composition of heterogeneous materials. FT-IR characterizes the classes of chemical functional groups present in a sample (Chen, 2003), enabling the analysis of a complex mixture of chemicals found in waste materials. The technique has been applied to landfilled MSW during *in situ* aeration (Tesar et al., 2006), during composting (Castaldi et al., 2005; Smidt et al., 2005) and anaerobic digestion (Smidt and Meissl, 2007a).

The absorbance observed in the FT-IR spectra, along with the bands present, is indicative of sample maturity. For example, aliphatic methylene bands are weaker in landfill samples than in MBT waste material whilst a band assigned to aromatic amines is not observed in landfill samples since these compounds decrease during biological treatment (Smidt et al., 2007b).

2.5. Enzymatic test methods

Biodegradation of organic materials is a process performed by fungi and bacterial organisms, and as such the mineralization of the organic matter is through the action of extracellular enzymes (Pelaez et al., 2004). A more extensive and comprehensive enzyme system is required as the substrate material becomes more complex (Tuomela et al., 2000). The quantification of the activities of a range of enzymes within a waste material has been investigated as a means of assessing compost quality and maturity (Cayuela et al., 2008; Mondini et al., 2004; Pelaez et al., 2004; Tiquia, 2005). The enzymes investigated in these studies included arylsulphatase, β-glucosidase, alkaline phosphatase and dehydrogenase, all of which are associated with the decomposition of the respective substrates found in waste material throughout composting.

The enzymatic cellulose degradation (ECD) method is a novel enzymatic approach to biodegradability measurement (Rodriguez et al., 2005). A mixture of cellulase and xylanase (hemicellulase) enzymes were used in this study, which studied samples taken from a landfill site. The samples were mixed with a phosphate buffer (pH 5.5) at 40 °C, and the monosaccharides liberated were recorded after the incubation period (40 h). The mass of the monosaccharides that are released by the MSW samples is reported to the initial mass of sample hydrolyzed in order to assess the biodegradability of waste samples.

An enzymatic approach based on the ECD method has been developed and applied to a wide range of organic waste materials including untreated and treated MSW derived BMW, food, wood and garden wastes (Wagland et al., 2008, 2007). The enzymatic hydrolysis test (EHT) uses a mixture of crude cellulase and hemicellulase enzymes (which also exhibit protease activities). The samples are mixed with a phosphate buffer at pH 4.75 and autoclaved to sterilize the mixture. The dissolved organic carbon (DOC) is measured and a prepared enzyme mixture added. The mixture is incubated at 50 °C for 20 h, and the DOC is measured. The DOC released from the autoclave is deducted from the final value to provide an indication of sample biodegradability.

3. Discussion

3.1. Method practicality

The focus of this review is to assess the suitability of each method for the purpose of monitoring waste treatment process performance and the diversion of BMW from landfill. Here each method is critically discussed regarding sample size used (and hence the representation of overall waste material), timescale, reliability and applicability to a wide range of heterogeneous waste materials.

3.1.1. Anaerobic tests

Several of the anaerobic methods (Owen et al., 1979; Pagga and Beimborn, 1993; Harries et al., 2001) are small scale tests. These tests use samples that are comparatively small in relation to the sample size of the GS90 test (1 kg DS) and the GB21 method (50 g DS). In general the larger the sample, the better representation that sample is of the overall waste batch. For example if a waste batch contained 10% cardboard, then this ideally needs to be reflected in a sample used in a test, and using 0.5 g of a sample (as with the Harries et al. (2001) method) is unlikely to show a consistent 10% cardboard composition, and hence offer a reasonable representation of the waste. Shelton and Tiedje (1984) also used relatively small samples (50 μg/ml), although these were chemical samples. Considering other larger scale tests available, these small scale tests are less suitable for the monitoring of mixed

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solid waste. The larger scale test methods use sample sizes more representative of the waste material, and so provide more reliable and valid data.

The method of measuring biogas production varies between the methods. The use of syringes (Owen et al., 1979; Harries et al., 2001) should be restricted to smaller scale tests, as the size of syringe required for a larger sample (and, therefore, larger quantities of biogas produced) would be impractical. Alternatively, the syringes could be emptied on a more regular basis, but this could increase the measurement error. The BM100 method requires a 20 g LOI sample, which can produce as much as 15 L of biogas over the 100-days-period (Godley et al., 2007). This would be difficult to accurately capture in syringes. The GB21 method requires a 50 g DS sample; however, biogas is only measured for 21 days, so the biogas produced is unlikely to exceed 15 L. Reported results include 10 L of biogas released at 21 days (Bockreis et al., 2007). The GS90 test method requires 1 kg DS so the expected biogas production is considerable. Measurements by Eudiometer (GS90) or by acidified water-filled cylinders (BM100) are, therefore, much more suited for the larger scale tests that are required for characterizing samples that are of a more representative size. The method for biogas production measurement has been previously discussed (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006). Manometric and volumetric measurements were found to have limitations. There is a limited range of accuracy associated with manometric analysis; changes to the atmospheric pressure and evaporation of water in volumetric systems can cause inaccuracies (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006).

It has, therefore, been recommended that gas analysis is performed using GC instead of volumetric gas release (Anaerobic Biodegradation Activity and Inhibition (ABAI) Task Group, 2006), as used in studies such as Stinson and Ham (1995); Kelly et al. (2006). This technique would provide more accurate data for methane and carbon dioxide production; however, this technique is more expensive than the other methods of gas production measurement.

Under anaerobic conditions, microbial growth efficiency is lower than in aerobic conditions. As a result, the biogas release is an accurate representation of the microbial activity since a very small amount of the mineralized organic carbon is converted to new biomass. This is a major advantage of the anaerobic test methods. In aerobic conditions, the microbial growth efficiency is much higher, and so more of the mineralized organic carbon is converted to microbial biomass and, therefore, the carbon dioxide released is not an accurate representation of the organic carbon mineralization.

The major disadvantage of the larger scale anaerobic tests (GB21, GS90 and BM100) is that whilst the results produced are reliable, the time scale for each test is not practical for routine analysis. Test durations of 21, 90 or 100 days have financial and operational implications for waste treatment and landfill operators if biodegradability data is delayed.

3.1.2. Aerobic tests

The aerobic ASTM method operates at 58 °C. This higher temperature could present some disadvantages for such a test method since the solubility of oxygen in water decreases with an increase in temperature. This may limit the oxygen transfer in the waste material, which could result in lower results than expected. So whilst the high temperature may accelerate some reactions, the biological activity will be hindered.

The static respiration methods generally give lower results than dynamic tests (Godley et al., 2005), which indicates the advantage of aeration throughout the test duration.

The SOUR method uses a 3–8 g sample. This is a small sample, and so may not be a very good representation of the sample as a whole, thus reducing the reproducibility of the test. However, the aqueous suspension used in the method offers several advantages. One major advantage is that there is no gas–liquid barrier on the surface of the substrate (Lasaridi and Stentiford, 1998). This means that gaseous exchange can occur immediately due to the direct contact between the substrate material and the microbes, maximizing the diffusion of oxygen.

The minimization of diffusion rates is important since limited oxygen transfer through biomass layers into bacterial cells is typically considered to be the rate-limiting step (Adani et al., 2004; Paletski and Young, 1995).

Compositional methods such as dry matter or solids (DM or DS, respectively) and loss-on-ignition or volatile solids (LOI or VS, respectively) do not describe the biodegradability of a sample material. The LOI and VS analyses indicate organic carbon content, but not biodegradable carbon content. Not all carbon is amenable to biodegradation, and so the use of carbon content to indicate biodegradability, or BMW diversion from landfill, would result in an over-estimation of sample biodegradability.

Certain methods such as the aerobic AT4 or anaerobic GB21 methods use the DS content (mg O_2/g DS or mg/kg DS, respectively). Considering the DS content, this would include the inorganic material, which is non-biodegradable, meaning that data presented in this form may not be comparable. Alternatively, using LOI or VS only takes the organic fraction into account, which would allow for the varying inorganic content, allowing valid comparison between samples.

3.1.3. Alternative tests

Temperature increase methods may not be suitable since temperature increase is due to chemical and biological reactions within the sample. Not all these exothermic reactions are related to the respiration reactions, such as acid hydrolysis. Some of the heat increase, to a certain degree, will not be respirometric activity. The results are, therefore, unlikely to be accurate, considering that the moisture content of the sample will also affect the heating. Precise determination of bioactivity using the Dewar self-heating test is difficult under any condition, and additional factors such as packing density and humidity require careful consideration. The applicability of the Dewar self-heating test to fresh MSW samples has not been investigated to the authors' knowledge, and as such this may indicate that other methods may offer more suitable options.

Spectrographic techniques such as FT-IR have been shown to be efficient at assessing the chemical properties of waste material, indicating sample stability. However, no research has indicated that this technique can quantify the amount of biodegradable material removed. This technique could be used in compost quality, or landfill acceptance criteria, based on the presence of certain bands indicating waste composition and whether the sample is stable or not. Aerobic and anaerobic test methods enable a simple calculation of biodegradability reduction from a waste treatment process, and so for the purpose of monitoring BMW diversion from landfill, FT-IR is perhaps not suitable. However, further research could enable this technique to become suitable.

Measuring the enzymatic activity present in a waste material has been shown to be a suitable method for the characterization of compost stability (Cayuela et al., 2008). The enzyme activity of the stabilized compost material varied depending on the starting substrate (Mondini et al., 2004). There is a necessity to perform several measurements to understand the activities of the wide range of enzymes, which is a disadvantage of this approach. Whilst measuring the enzyme activities may offer a good indication of compost maturity, this approach has not been applied to fresh

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Table 4
Summary of correlation coefficients between short-term and long-term biodegradability test methods

Short-term method	Long-term method	Sample	Correlation coefficient (R^2)	Reference
AT4	GB21	MSW excavated from closed landfill (3 sites)	0.80	Cossu and Raga (2008)
		Pre-treated residual waste	0.60	
AT7	GS90	Pre-treated residual waste	0.83	Binner et al. (1999b)
GB21	GS90	Pre-treated residual waste	0.95	Binner et al. (1999b)
DR4	BM100	MSW derived BMW	0.54	Godley et al. (2007)
ECD	BMP	MSW extracted from landfill (3 sites)	0.65	Rodriguez et al. (2005)
			0.79	
			0.87	
EHT	BM100	MSW derived BMW and specific waste materials	0.59	Wagland et al. (2008)

MSW input samples, and the significantly different substrates available in fresh and treated waste samples would require a complex and possibly an individual analytical approach to each material. This would not allow simple and rapid monitoring of waste treatment processes, and so is unsuitable for the monitoring of BMW diversion from landfill.

3.2. Correlations with anaerobic methods

Assessing the complete biodegradability of the sample is ideal; however, short-term tests cannot provide this and so are used to correlate with anaerobic tests. This allows for the prediction of long-term biodegradation potential in a shorter length of time, depending on a strong correlation of the tests. A summary of correlation coefficients from the evaluated studies is shown in Table 4.

The German SRI (AT4 and AT7) tests have been investigated, along with an AT10 test (Binner and Zach, 1999a; Binner et al., 1999b). It was found that the AT10 results did not produce enough additional information to make the test more practical than the AT7 test, and as a result the AT7 test is recommended. The AT7 shows a strong correlation with the anaerobic incubation test (GS90) with correlation coefficients (r^2) of 0.88–0.906 (Binner et al., 1999b). This relationship indicates that as the results of the AT7 increase, the results obtained from the GS90 are also expected to increase.

For routine testing, the length of the aerobic tests may still be too long, not providing results quickly enough and having financial implications for the treatment process operator.

The ECD method was shown to correlate with a classical BMP test, with correlation coefficients (r^2) between 0.65 and 0.87 (Rodriguez et al., 2005). This relationship shows that as the ECD results increase, the expected BMP results will also increase (i.e., low biodegradable substrate gives low ECD and BMP values).

However, this method has the limitation that only the monosaccharides are measured. Cellulose can be hydrolyzed into cellodextrins and cellobiose molecules before glucose molecules are produced. As a result, the measurement of monosaccharides may not reveal the full extent of the cellulose hydrolysis. Organic waste material will also contain other enzyme hydrolysable substrates, such as proteins, and these may require further consideration.

A relationship was observed for the EHT with the BM100 with a correlation coefficient (r^2) of 0.59. This method indicates the availability of enzyme hydrolysable substrates such as proteins (unlike the ECD). However, this method has the limitation of potentially excluding biodegradable DOC released in the autoclave procedure (Wagland et al., 2008). The carbon released in the autoclave procedure would contain biodegradable and non-biodegradable carbon, and, therefore, careful consideration and further development is required.

The DR4 test correlates with the BM100 test method, allowing the estimation of BM100 data from DR4. A shorter anaerobic test, GS21 (fermentation test), also correlates well with the Austrian GS90 test (Binner and Zach, 1999a). The AT4 is the recommended test method in Germany and Austria, with the allocated landfill acceptance criteria of 5 mg/g DM and 7 mg/g DM, respectively (Muller and Bulson, 2005). The correlation of the DR4 method with BM100 data has been reported (Godley et al., 2007), whilst the correlation of the AT4 method with the GB21 method has also been investigated (Cossu and Raga, 2008). The aerobic test methods have disadvantages such as the microbial growth efficiency, in which new biomass is produced efficiently from the digestion of the waste material, and so the carbon dioxide produced does not sufficiently represent the degree of biodegradability. Aerobic test methods also have the disadvantage of measuring only the initial rate of biodegradability due to these tests measuring the readily biodegradable materials. The length of aerobic tests does not allow the measurement of slowly biodegradable material. Anaerobic test methods, such as the BM100 and GS90, give reliable results and measure the extent of biodegradability as the length of these tests is a clear disadvantage, but allows for the degradation of readily biodegradable and slowly biodegradable materials.

4. Conclusion

Biodegradability testing is an important part of monitoring BMW diversion from landfill in the EU. Data obtained from these tests assist the waste treatment operators in optimizing the waste treatment process and in the UK provide local authorities with information with which to calculate BMW diversion. Therefore, there is a need for a rapid and cost-effective test method that would correlate with the reliable BM100 test method, which is not suitable for regular routine testing due to its duration.

The current biodegradability test methods have limitations, and no one test method is currently sufficient for routine biodegradability assessment. Further research is needed to develop the alternative and rapid test method that is required. From this review, potential areas for further research include spectrographic FT-IR or enzyme-based approaches such as the ECD or EHT methods.

Acknowledgements

This research is part of a wider programme of research on waste characterisation sponsored by the Department for Environment, Food and Rural Affairs (Defra), from whom the authors are grateful for permission to publish. Views expressed are those of the authors' alone. The authors would also like to thank Dr. M.W. Milke for the invaluable feedback provided.

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Please cite this article in press as: Wagland, S.T. et al., Test methods to aid in the evaluation of the diversion of biodegradable ..., Waste Management (2008), doi:10.1016/j.wasman.2008.08.024

Appendix G

(2008) Comparison of a novel enzymatic biodegradability test method with microbial degradation methods Communications in Waste and Resource Management 9(3), 80-86. (Modified from Chapter 4)

Comparison Of A Novel Enzymatic Biodegradability Test Method With Microbial Degradation Methods

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ABSTRACT

A novel enzymatic hydrolysis test (EHT) has been evaluated as a surrogate for conventional microbial biodegradability methods, using 37 assorted organic waste samples collected from diverse sources. The results of the EHT method are compared with those obtained from two conventional tests; the 4 day aerobic DR4 and 100 day anaerobic BM100 test methods currently applied in England and Wales. The EHT is based on the enzymatic hydrolysis of cellulosic materials and can be completed in less than 24 hours. Linear regression for 37 samples against the BM100 data showed the DR4 provided a correlation coefficient of r = 0.58; the EHT method gave a correlation of r = 0.62 for the total DOC release; and r = 0.77 for the DOC released from enzymatic hydrolysis. The correlations suggest that the EHT method may be better suited to a wider range of waste types when correlating with anaerobic BM100 test results since it more closely mimics the full extent of decomposition rather than that from the readily biodegradable fraction.

KEY WORDS

Biodegradability, waste, characterisation, organic, enzymatic hydrolysis, landfill

1. INTRODUCTION

In Europe, the Landfill Directive (Council of the European Union, 1999) sets specific requirements for the design and operations of landfill sites, including the types of waste that can be accepted into these landfills. One of the aims of the Landfill Directive is to reduce the amount of biodegradable municipal waste (BMW) sent to landfill (Godley *et al.*, 2004). To achieve these reductions, the Directive sets targets for member states to progressively reduce the amounts of BMW landfilled to 75% of the 1995 baseline amount by 2006, 50% by 2008, and finally 35% by 2016 (Bench *et al.*, 2005; Council of the European Union, 1999). In England, typically 68% of municipal solid waste (MSW) sent to landfill was found to be BMW (Parfitt, 2002). For countries in which landfill represents the predominant disposal route (≥80% MSW) there is a temporal extension to the deadlines. This derogation applies to the UK, in which the amount of BMW sent to landfill must be reduced to 75% of the 1995 baseline by 2010, then 50% by 2015 and then finally to 35% by 2020 (Price, 2001). The landfill allowance trading scheme (LATS) allocations in England indicate targets for a 10m tonne reduction in the amount of BMW landfilled before 2020 from the 1995 baseline figure (Defra, 2005).

Organic waste can be treated to reduce the BMW content in processes such as mechanical-biological treatment (MBT), a generic term to describe the process of mechanically sorting and shredding the waste followed by biological treatment by composting or anaerobic digestion (Archer *et al.*, 2005). Mechanical sorting can also occur after biological treatment. Monitoring such processes is an important aspect in assisting operators to achieve desired performance criteria, optimise the process and to estimate the amount of BMW diverted from landfill resulting from treatment (Environment Agency, 2005).

This may include analysing waste samples for biodegradability using appropriate methods.

Biodegradability tests typically involve the use of live micro-organisms and may either be conducted over a few days to assess the initial organic matter decomposition rate ie the readily biodegradable material, or be conducted over many weeks until decomposition ceases and the full extent of degradation measured. The degree to which the rate of biodegradability of the waste is reduced by the process, and the extent of decomposition achieved, can both be used as an indication of the performance and efficiency of the treatment process.

In this study we have compared the novel non-microbial EHT method with a microbial based 100 day anaerobic test (BM100) and the 4 day aerobic test (DR4), the latter two methods being specified in guidance for monitoring MBT processes in England and Wales (Environment Agency, 2005). The BM100 method has been reported to show good reproducibility between results (Godley *et al.*, 2003), but has the disadvantage of taking a relatively long time to complete, thereby not providing rapid feedback on plant performance for commissioning, optimisation and routine monitoring purposes. Shorter-term aerobic methods, including the DR4 test, have other disadvantages such as preferentially decomposing the readily biodegradable components of the waste (Godley *et al.*, 2007b) and therefore may not indicate potential long-term biodegradability.

Therefore most current microbial based biodegradability test methods have limitations and no one test method is deemed suitable for the whole range of biodegradability testing requirements associated with monitoring MBT process performance and assessing organic waste bio-stability. Following a review of the current methods (Godley *et al.*, 2003) it has been concluded that there is a need for a rapid and cost-effective test method that would mimic and correlate with longer-term tests such as the anaerobic BM100 method.

A large proportion of BMW consists of biopolymers (proteins, fats, polysaccharides and lignin) that undergo enzymatic hydrolysis to soluble monomers during the microbial decomposition process. Hemicellulosic/cellulosic material is considered to be the most important carbon source for methanogenesis in landfills as it contributes to as much as 90% of the total biogas (CO_2 + CH_4) produced (Barlaz *et al.*, 1989; Rodriguez *et al.*, 2005). As a general rule, the higher the cellulose/hemicellulose content, the higher the biogas yield of the waste in anaerobic tests (Eleazer *et al.*, 1997). Therefore assessment of the waste cellulose and hemicellulose content may provide a non-biological test method of assessing biodegradability. However lignin is closely associated with cellulose in native plant matter as lignocelluloses and this may comprise 30-50% of BMW (Rodriguez *et al.*, 2005). Lignin is also considered to be poorly biodegradable under anaerobic conditions (Chen *et al.*, 2004; Sjöberg *et al.*, 2004; Stinson *et al.*, 1995; Tuomela *et al.*, 2000). The availability of the cellulose to enzyme hydrolysis can therefore vary as the associated lignin can protect the cellulose from enzymatic decomposition. Therefore, although direct chemical measurement of the cellulose and hemicellulose content of a waste sample could logically provide an estimate of the biodegradability of that sample, this may be inappropriate as not all the cellulose is amenable to biodegradation when present as lignocellulose (Chen *et al.*, 2004).

Cellulose and hemicellulose are hydrolysed by cellulase and hemicellulase enzymes respectively and so the novel EHT method based on the enzymatic hydrolysis of cellulosic material could offer a suitable routine test method. This would mimic the natural microbial hydrolysis of organic matter and would be expected to take account of the impact of lignin on the availability of cellulose. A high concentration of enzyme can be added to the test which might be expected to hydrolyse all the potentially hydrolysable cellulose and therefore more closely represent the long term BM100 test rather than shorter term DR4 tests. In this study the DR4, BM100 and the novel enzymatic hydrolysis test (EHT) methods have been applied to 37 waste samples from a range of sources in the UK. The DR4 and EHT biodegradability test methods are compared and correlated with the longer-term BM100 method.

2. MATERIALS AND METHODS

2.1. Samples

The organic waste samples were collected from a wide range of treatment processes and waste streams in the UK as part of Defra project WRT220 on waste characterisation. The samples included MSW derived samples, garden waste (partially treated in the short-term, stabilised and longer-term fully treated) and samples from specific waste streams such as fish, wood, pizza and feathers. Where possible the samples were collected pre-, during and post- treatment by either MBT or a mechanical thermal (autoclave) treatment. The biological treatment of the samples was either composting or anaerobic digestion.

Samples were sorted to remove inert materials with the biodegradable material being retained and tested. Materials with large particle sizes were shredded to <10 mm before testing. The dry matter (DM) and loss-on-ignition (LOI) was determined for each sample using standard procedures (EN12879:2000).

2.2. UK established methods

Two established biodegradability test methods were used in this investigation. In a recent comparison of the two methods (Godley *et al.*, 2007b), it was stated that the 4 day aerobic test measures the rate of

aerobic degradation, whereas the 100 day anaerobic test measures the extent.

1. Dynamic respiration over 4 days (DR4). Biodegradability under aerobic conditions was determined using the DR4 test method (Environment Agency, 2005; Godley *et al.*, 2007a; Godley *et al.*, 2007b). The test material (100g DM) was prepared as outlined previously and mixed with the seed material, in this case mature green waste compost (100 g DM). Water and nutrients (nitrogen and phosphorus) were added to adjust to 50% w/w moisture content. The test mixture was placed in a reactor vessel at 35°C for 4 days, with constant aeration (500ml/min (Environment Agency, 2005)) through the reactor vessel. The CO_2 released over the 4 day period was measured and this data used to estimate O_2 consumption.

2. Biochemical methane potential over 100 days (BM100). The BM100 test method (Environment Agency, 2005) is based on a sewage sludge digestion test (Godley *et al.*, 2007b; Godley *et al.*, 2003). The test material (20g LOI) was placed in a glass container with microbial seed (digested sludge) and a nutrient mixture. The mixture was sealed and incubated at 35°C under anaerobic conditions and the release of CO2 and CH4 (biogas) measured volumetrically until no further biogas was released (up to 100 days).

2.3. Novel biodegradability test method

A novel biodegradability test method based on the enzymatic hydrolysis of cellulosic materials has been developed (Godley *et al.*, 2004; Wagland *et al.*, 2007). For each sample 25mg of crude cellulase powder (Sigma) and 75mg of hemicellulase powder (Sigma) were dissolved in 20ml of distilled water, with approximately 175 units of cellulase and 112.5 units of hemicellulase activities in each 20ml of enzyme mixture. This enzyme solution was sterilised by filtration through a 0.22µm Millipore membrane.

The crude cellulase enzymes exhibited some hemicellulase and protease activity, with the hemicellulase enzymes also having some cellulase activity (within manufacturer specifications).

The test method consists of three phases (Figure 1) as follows:

Phase 1. The waste sample (5g LOI) was placed in a 250ml Erlenmeyer flask. Phosphate pH buffer (100ml 0.37 M) was then added to the

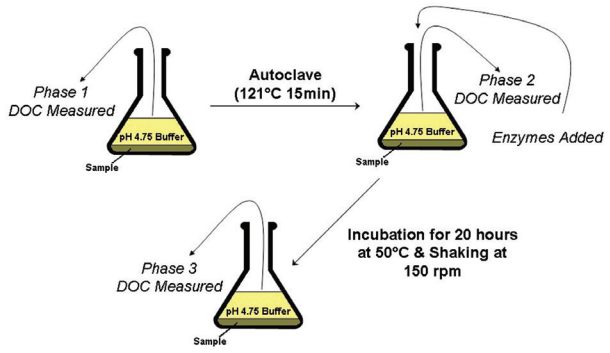

Figure 1: schematic diagram of the EHT method

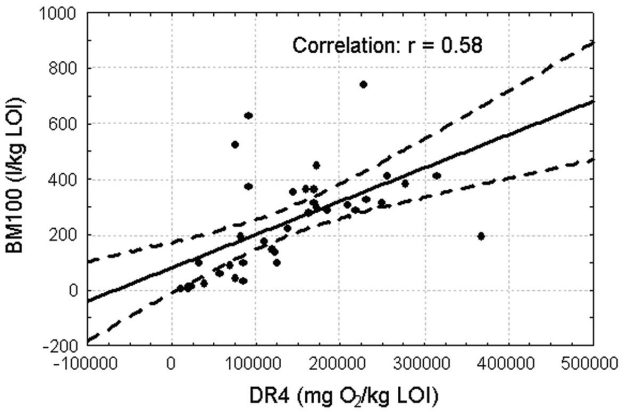

Figure 2: correlation of DR4 with BM100 data for all samples

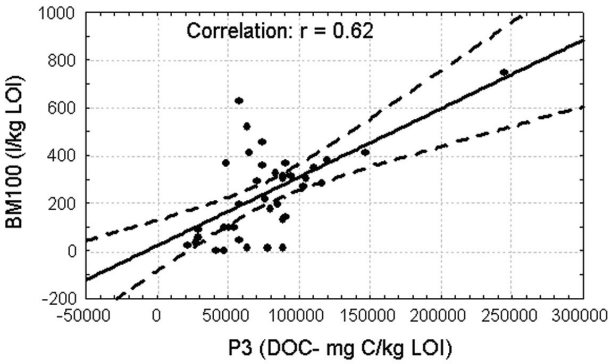

Figure 3: correlation of EHT (total DOC) with BM100 data for all samples

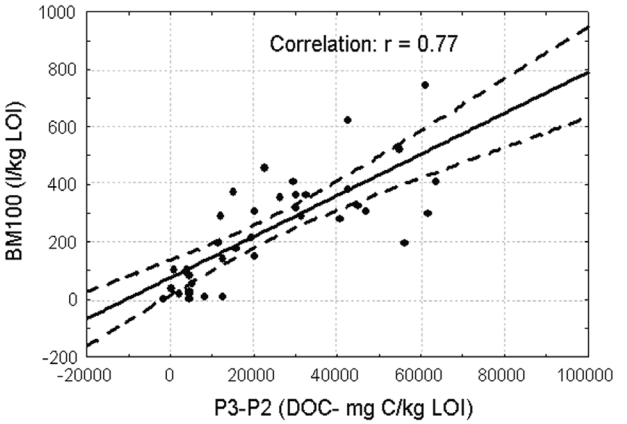

Figure 4: correlation of EHT (DOC from enzyme hydrolisis) with BM100 data for all samples

	DM	LOI	EHT (DOC- mg C/kg LOI)			Enzymatic DOC	BM100	DR4
Sample	% wet wt	% DM	Phase 1	Phase 2	Phase 3	(P3- P2)	l/kg LOI	mg O/kg LOI
Commercial cellulose	94.0	99.7	622	1200	57100	55900	200	80000
Construction wood waste	77.5	91.4	1690	16300	20700	4400	27.4	37600
Autoclaved construction wood waste	63.9	90.7	7760	22600	26700	4100	35.2	84700
Packaging waste	42.6	93.6	1790	7320	61900	54580	527.4	73000
Autoclaved packaging waste	40.4	93.1	7130	14700	56800	42100	629.9	90000
Greenwaste (untreated)	40.4	73.2	24400	64300	79700	15400	182	108000
Partially composted greenwaste	44.2	62.1	25500	55900	75300	19400	221	136000
Kitchen and greenwaste (untreated)	35.1	65.3	36800	84700	116000	31300	292	217000
Partially composted kitchen and greenwaste	38.3	68.0	18400	43000	46900	3900	103	125000
Composted kitchen and greenwaste	51.7	60.9	6810	46200	49800	3600	99	82000
Turkey feathers	35.5	98.9	22900	32900	47400	14500	375	91000
Autoclaved turkey feathers	38.3	96.2	20300	72300	83700	11400	199	366000
Stabilised greenwaste compost <10 mm	71.9	29.2	4510	64900	77300	12400	11.7	18000
Stabilised greenwaste compost <25 mm	66.6	28.9	5650	85900	87400	1500	20.8	19000
Fresh Pizza Waste	43.1	95.2	7440	182000	243000	61000	748	226000
Fresh fish waste	38.1	73.2	3330	51600	73900	22300	457	170000
Fresh mix Fish, peat, wood, greenwaste	97.1	58.4	12600	70100	89800	19700	150	117000
Partially composted mixed fish/ woodchip/ greenwaste	48.9	53.8	7740	56000	56100	100	46.5	75000
Fully composted mixed fish/ woodchip/ greenwaste	65.0	34.3	2800	43000	41200	-1800	8.3	19000
Sewage Sludges	18.4	60.8	11900	76500	88600	12100	140	122000

Table 1: biodegradibility test results for specific waste samples

solution (20ml) was added to each of the flasks and the flask sealed with a neoprene bung. The flasks were placed in a shaking incubator at 150 rpm. A 5ml sample was removed for COD analysis after 20 hours of incubation.

The moisture content of the waste sample; the removal of the liquid and solid at each stage of sampling; and the addition of liquid in phase 3 were accounted for in the concentrations of carbon calculated. Soluble COD was converted to DOC (mg C/l) by assuming a COD/C ratio of 2.67 based on the relative molecular mass of cellulose monomeric units.

3. RESULTS AND DISCUSSION

3.1. EHT comparison with DR4 and BM100 biodegradability tests

The results from the biodegradability test methods are shown in Table 1 for specific waste streams, and Table 2 for MSW-derived BMW samples. The DOC released at each phase of the EHT varied greatly. Phase 1 DOC is likely to represent the low molecular weight readily soluble materials present in the waste. The DOC released in Phase 2 may represent DOC solubilised by mild acid hydrolysis of polymers during autoclaving. Phase 2 DOC may also include soluble materials desorbed

	DM	LOI	EHT (DOC-mg C/kg LOI)			Enzymatic DOC	BM100	DR4
Sample	% wet wt	% DM	Phase 1	Phase 2	Phase 3	(P3- P2)	l/kg LOI	mg O/kg LOI
Organic fibre from autoclaved MSW	50.5	76.9	23400	58100	88000	29900	319	249000
AD treated fibre from autoclaved MSW	29.2	72.5	4960	23100	27300	4200	92	69000
MBT Feed MSW (a)	56.0	71.6	14500	41900	88700	46800	312	207000
MBT Feed MSW	51.1	71.8	10700	37900	82700	44800	330	230000
MBT treated MSW	57.1	77.2	22700	60400	89900	29500	372	159000
MBT Reject	58.0	72.5	24900	61000	102000	41000	281	161000
MBT CLO	51.9	57.7	4920	40800	72800	32000	364	168000
MBT CLO (a)	46.9	59.6	9790	42200	103000	60800	306	171000
MSW input windrow MBT (b)	94.4	73.4	14100	35400	64700	29300	418	256000
MSW Compost (b)	69.3	26.7	1270	53000	53300	300	105	30000
Fully Composted MSW (b)	66.3	29.6	1680	42000	46200	4200	5	9000
MSW input to AD	96.5	58.7	17800	83200	147000	63800	413	313000
Output MSW AD	31.5	56.2	1570	22700	27500	4800	62	54400
MSW input	95.5	65.8	17900	77200	119000	41800	385	276000
Composted MSW	50.3	58.3	7290	57600	69400	11800	293	184300
Composted MSW <8 mm	58.4	49.9	17400	73400	93300	19900	316	167000
Fresh MSW	94.4	39.3	14900	84600	110000	25400	354	144000
Composted MSW <15 mm	75.3	23.4	12600	54700	62800	8100	15.5	22000

Table 2: biodegradability test results for MSW-derived BMW samples

from the waste during autoclaving. The DOC released in Phase 3 results from the enzymatic hydrolysis of the material, and so may indicate the amount of additional biodegradable cellulose, hemicellulose and possibly proteinaceous material present.

The non-enzymatic DOC (Phases 1 and 2) for wastes that have undergone extended biological treatment (eg the fully composted green waste and composted MSW-derived BMW samples), are likely to contain significant amounts of humic substances resulting from the decomposition of lignin (Tuomela *et al.*, 2000). These substances are not usually considered to be readily biodegradable, and so in these cases, the DOC due to enzymatic hydrolysis (Phase 3 only) may be indicative of sample biodegradability. Unlike the control polymeric cellulose, many of the untreated (raw or autoclaved) waste samples also showed significant amounts of DOC released during Phases 1 and 2. As these wastes have not been biologically treated it is likely that much of the DOC released during Phases 1 and 2 will be inherently biodegradable.

The total DOC release in the EHT is evaluated as an indication of sample biodegradability. However, since a proportion of Phase 2 DOC will contain non-biodegradable carbon, the DOC released in Phase 3 only is also evaluated. In most samples, the biodegradability result is lower for the treated samples, considering the BM100 and DR4 values. This is expected since biological treatment of waste material removes biodegradable components, producing a bio-stabilised material (such as a compost-like output, CLO).

Figures 2, 3 and 4 show the relationship between the DR4, EHT and the BM100 data. For the EHT the total DOC is shown in Figure 3, and the DOC from enzyme hydrolysis alone is shown in Figure 4. The correlation coefficient (r) of 0.62 for the EHT (total DOC) is highly significant ($p < 0.001$), as is the correlation coefficient of 0.58 for the DR4 ($p < 0.001$). The relationship between the EHT and BM100 data is stronger when only the DOC released from enzymatic hydrolysis is considered (ie P3-P2 shown in Tables 1 and 2), giving a correlation coefficient of 0.77 ($p < 0.001$). The correlations suggest that the EHT method is better suited to a wider range of waste types, particularly when considering the relationship of the DOC from enzyme hydrolysis and the BM100 (Figure 4).

Whilst each test method measures a different parameter, each of these parameters is indicative of sample biodegradability, and comparison between the parameters is possible by means of linear correlation. A previous study of 96 MSW-derived BMW samples indicated a linear relationship of $[R2] = 0.54$ for the DR4 correlation with BM100 (Godley *et al.*, 2007b).

The findings of this study suggest that the EHT method has potential as a rapid measure of biodegradability. However several limitations need to be addressed.

The DOC released at Phase 2 of the EHT is likely to consist of a mixture of biodegradable and non-biodegradable carbon. Therefore subtracting the Phase 2 DOC value from the final Phase 3 DOC value would eliminate biodegradable carbon from the overall DOC value. Similarly, if Phase 2 DOC is not subtracted, then the biodegradability determined for the waste sample would contain non-biodegradable carbon. This implies that the Phase 2 DOC needs to be characterised to differentiate between the biodegradable and non-biodegradable carbon to provide a more accurate biodegradability measurement. The use of a suitable extraction method may allow for a more selective deduction from the final Phase 3 DOC of the EHT, considerably improving the biodegradability indication.

Autoclaving the waste material greatly increases the DOC release in Phase 2 (Tables 1 and 2 indicate several examples of this). Sterilisation of the waste material is required to prevent microbial growth on released DOC during the test, to ensure that the entire DOC released in the test is accounted for. Alternative methods of sterilisation need to be evaluated.

Batches of commercially available enzymes may contain varying levels of activities which may contribute to variations in the EHT procedure. This issue may be less significant than the error associated with the use of microorganisms, and the use of a high purity grade cellulose substrate as a standard material would enable the quantification of such variation.

4. CONCLUSIONS

The EHT is a suitable alternative routine biodegradability test method, offering a reduction on the timescales of the DR4 test method. The EHT is completed in less than 24 hours compared to 4 days for the DR4. The EHT is being considered as an alternative test method incorporated in a consultation to revise the MBT monitoring guidance for England and Wales (Environment Agency, 2005). Correlations of the EHT with the BM100 show the significance of the EHT method as an alternative short-term test method. However further research is in progress aimed at improving the versatility and validity of the EHT method.

ACKNOWLEDGEMENTS

This work was funded by the Department of Environment, Food and Rural Affairs (Defra) as part of project WR0110, from whom the authors are grateful for funding and permission to publish. The opinions expressed are the authors' alone.

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Appendix H

(2007) Development and application of an enzymatic hydrolysis test to assess the biodegradability of organic waste material. Proceedings Sardinia 2007, Eleventh International Waste Management and Landfill Symposium, S. Margherita di Pula, Cagliari, Italy, CISA.

DEVELOPMENT AND APPLICATION OF AN ENZYMATIC HYDROLYSIS TEST TO ASSESS THE BIODEGRADABILITY OF ORGANIC WASTE MATERIAL

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SUMMARY: A novel and rapid biodegradability test method has been developed based on the enzymatic hydrolysis of cellulose. The test method consists of three phases, in which the first two phases consist of the pH buffer addition, and then autoclaving of the mixture and the final phase is the addition of the enzyme mixture and incubation. An initial investigation was carried out to determine the optimum conditions for the enzymes using standard commercial cellulose as the substrate. The optimised test was then applied to a wide range of organic waste samples including untreated and treated MSW derived mixed BMW, and specific wastes such as waste wood, packaging waste (cardboard), turkey feathers and green waste. The DOC released by enzymatic hydrolysis indicates that this could give an indication of the sample biodegradability. However the DOC released in phases 1 and 2 may also contain some biodegradable components (depending on the extent of biological treatment applied to the waste sample) and these would need to be differentiated from the non-biodegradable DOC and used together with the DOC from phase 3 to give the best possible biodegradability indication.

1. INTRODUCTION

The EU Landfill Directive 31/1999/EC requires that the amount of biodegradable municipal waste (BMW) disposed in landfill be progressively reduced. In the UK the amount of BMW sent to landfill must be reduced to 75%, 50% and 35% of the 1995 baseline by 2010, 2015 and 2020 respectively (Council of the European Union 1999).

Organic waste can be treated to reduce the BMW content in processes such as mechanical-biological treatment (MBT), which is a generic term to describe the process of mechanically sorting and shredding the waste, and then biologically treating the waste by means of composting or anaerobic digestion (Archer, Baddeley *et al.* 2005). Monitoring such processes may be required to assist with maintaining optimal performance and possibly determination of the amount of BMW diverted from landfill following treatment (Environment Agency 2005). This may include monitoring the input and output waste samples for biodegradability using

Proceedings Sardinia 2007, Eleventh International Waste Management and Landfill Symposium
S. Margherita di Pula, Cagliari, Italy; 1 - 5 October 2007

suitable biodegradability tests.

Such tests may either be conducted over a few days and assess the initial organic matter decomposition rate or be conducted over many weeks until decomposition ceases and the extent of decomposition is measured. The degree in which the rate of biodegradability of the waste is reduced by the process, and the extent of decomposition achieved, can both be used as an indication of the performance and efficiency of the treatment process.

Guidance on the monitoring of MBT processes has been provided for England and Wales (Environment Agency 2005). The Environment Agency specifies two biodegradability test methods, the 100 day anaerobic test (BM100) and the 4 day aerobic test (DR4). The anaerobic test method has been reported to show good reproducibility between results (Godley, Lewin *et al.* 2003), but has the disadvantage of taking a very long time to complete. Short-term aerobic methods like the DR4 test have other disadvantages such as preferentially decomposing the readily biodegradable components of the waste (Godley, Lewin *et al.* 2007) and high microbial growth efficiency such that much of the decomposed organic matter is transformed to microbial biomass rather than mineralized. Therefore most current biodegradability test methods have limitations, and no one test method may be suitable for the whole range of biodegradability testing requirements such as monitoring MBT process performance and assessing organic waste biostability.

A large proportion of BMW consists of biopolymers (proteins, nucleic acids, fats and polysaccahrides) that undergo enzymatic hydrolysis to soluble monomers during the microbial decomposition process before the organic waste is utilized by the microbes as a carbon and energy source. Lignin is an aromatic based polymer that is degraded by oxidative enzymes before utilization by microbes. Lignin is closely associated with cellulose in native plant matter as lignocelluloses and this may comprise 30-50% of organic MSW (Rodriguez, Hiligsmann *et al.* 2005). Agricultural crop waste and forestry residues consist of up to 75-80% cellulose and hemicellulose (Adsul, Bastawde *et al.* 2005). Hemicellulosic/cellulosic material is considered as the most important carbon source for methanogenesis in landfills as it contributes to 90% of the total biogas (CO_2 + CH_4) produced (Rodriguez, Hiligsmann *et al.* 2005). As a general rule, the higher the cellulose/hemicellulose content, the higher the biogas yield of the waste in anaerobic tests (Eleazer, Odle III *et al.* 1997), although the availability of the cellulose can vary as the associated lignin can 'protect' the cellulose from chemical or enzymatic decomposition. Therefore assessment of the waste cellulose and hemicellulose content may provide a non-biological test method of assessing biodegradability.

Direct chemical measurement of the cellulose and hemicellulose content of a waste sample might be considered to give an estimate of the biodegradability of that sample, however this is inappropriate as not all the cellulose is amenable to biodegradation when present as lignocellulose (Chen, Knappe *et al.* 2004). The resistance of lignin to biological and chemical degradation allows it to protect cellulose (Stinson and Ham 1995), and so not all the cellulose picked up in a direct measurement will be biodegradable cellulose. In the lignocellulosic material, lignin may present a physical barrier preventing cellulolytic enzymes from hydrolyzing the cellulosic material (Chen, Knappe *et al.* 2004). Lignin is also considered to be poorly biodegradable under anaerobic conditions (Chen, Knappe *et al.* 2004; Sjöberg, Nilsson *et al.* 2004; Stinson and Ham 1995; Tuomela, Vikman *et al.* 2000).

Following reviews of the current methods (Godley, Lewin *et al.* 2004; Wagland, Tyrrel *et al.* 2007) is has been concluded that there is a need for a rapid and cost-effective test method that would correlate with longer-term tests such as the anaerobic BM100 method. The BM100 test method is not suitable for regular routine testing due to its duration, however a correlating method could make routine testing viable. Cellulose and hemicellulose are hydrolyzed by cellulase and hemicellulase enzymes respectively and so a novel method based on the enzymatic hydrolysis of cellulytic material could offer a suitable routine test method.

Here the development of the novel enzymatic hydrolysis test (EHT) method is described, and the method is applied to a wide range of organic waste samples.

2. METHODS

2.1 Waste samples

Organic waste samples were collected from a wide range of treatment processes and specific waste streams in the UK as part of the Defra sponsored R&D Waste characterisation project WRT220 (Table 1). The samples ranged from general household waste (BMW), garden waste, wood waste and packaging waste. Where possible the samples were collected pre-, during and post- treatment by either MBT or a mechanical thermal (autoclave) treatment.

The waste samples were sorted to remove glass, metals, plastics and inert materials and only the biodegradable material retained and tested. Materials with large particle sizes were shredded to <10 mm before testing. The dry matter (DM) and loss-on-ignition (LOI) was determined for this sample using standard procedures (EN12879:2000).

2.2 Enzymatic Hydrolysis Test

The enzyme test method consists of three phases of measurement as follows:

- Phase 1- 5 g of LOI is placed in a 250 ml Erlenmeyer flask. 100 ml 0.37 M phosphate pH buffer is then added to the flask and mixed. A 5 ml sample was removed and filtered to remove any solids, and the filtered liquid was then analysed for chemical oxygen demand (COD).
- Phase 2- The sample mixture was then autoclaved at 121°C for 15 min to sterilise the mixture and a further 5 ml sample was removed, and filtered, for COD analysis.
- Phase 3- 20 ml of the prepared enzyme solution was then added to each of the flasks and the flask sealed with a neoprene bung. The flasks were placed in a shaking incubator at 150 rpm. A 5 ml sample was removed for COD analysis, at times specified in later sections.

The amount of moisture in the waste sample and the removal of both the liquid and solids at each stage of sampling, along with the addition of liquid in phase 3, were accounted for in the concentrations of carbon calculated. Soluble COD analysis results were converted to DOC (mg C/l) by assuming a COD/C ratio of 2.67 and then expressed in terms of mg of carbon per kg of the sample (LOI) to give the final values.

2.2.1 Enzyme Preparation

For each sample, 25 mg of crude cellulase powder (Sigma) and 75 mg of hemicellulase powder (Sigma) were dissolved in 20 ml of distilled water, with approximately 175 units' cellulase and 112.5 units' hemicellulase activities in each 20ml of enzyme mixture. This enzyme solution was then filtered through 0.22 μm Millipore membrane filters to sterilise the solution.

The crude cellulase enzymes also possessed some hemicellulase and protease activity, with the hemicellulase enzymes also having some cellulase activity (manufacturer specifications).

2.2.2 Optimisation of the Enzymatic Hydrolysis Test

A commercially available cellulose preparation was chosen for test optimisation (α-cellulose, Sigma). The pH of the buffer solution was varied at values of 4, 4.5, 5, 5.5 and 6 to determine the optimal pH for the enzymatic hydrolysis. Tests at each pH were carried out in triplicate and the reported results are the mean values.

The effect of temperature was also investigated at each pH value to determine the optimum

temperature for enzyme hydrolysis. Temperatures used were 30, 40, 50 and 60°C.

During the Phase 3 enzymic hydrolysis flasks were incubated for at least 60 hours and 5 ml samples taken from the mixture at regular intervals for analysis.

2.2.3 Sample Analysis

The opimal enzymic hydrolysis test conditions of pH 4.75 and temperature 50^{o}C determined using the commercial cellulose (see Section 3.1) was then applied to the collected organic waste samples. In Phase 3 of the test the enzymic hydrolysis incubation period was stopped after 20 h.

3. RESULTS AND DISCUSSION

3.1 Optimisation of the Enzymatic Hydrolysis Test

For the optimisation work, only the DOC released from the enzymic hydrolysis Phase 3 was considered as the amount of DOC released during Phases 1 and 2 for the commercial cellulose was low and amounted to only about 3% of the DOC released by enzyme hydrolysis. Hence Phase 2 results are deducted from Phase 3 values to give only the DOC released by enzymes.

The rate of DOC released from enzyme hydrolysis is initially rapid but then declines until the DOC release stabilises. The purpose of this test is to mimic a biological test and the shapes of the curves is similar to BM100 biogas production curves. The implications being that the EHT may have released a similar amount of DOC that would be decomposed in biological tests suggesting that the test may mimic biological tests that determine the extent of biodegradation. Whether this is the case and whether the initial hydrolysis rate mimics short term biodegradation tests such as the DR4 method (which also measures a rate) is under further evaluation.

Ideally the DOC released in the enzymic test should occur rapidly and be reproducible in order to provide a rapid alternative to long-term tests such as the BM100 method. This may depend on the optimum pH of the enzyme and the impact of temperature on the enzyme activity. Figures 1-4 indicate that the optimum pH was between 4.5 and 5 at each respective temperature as these pH levels gave the highest initial rate of hydrolysis and the highest overall DOC yield. The time for DOC release to cease was reduced as the temperature was raised, for example at 30°C (Fig. 1) DOC release was still occuring after 75 h but at 60°C (Fig. 4) the DOC release effectively stopped at around 20 h. At 50°C (Fig. 3), the hydrolysis had almost ceased at around 20 h, and the DOC yield was higher at this time than at the same point in the 60°C test. This indicates that significant enzyme denaturation may have limited the amount of DOC released at 60°C. At 40°C (Fig. 2), the hydrolysis rate was lower and DOC release was still occurring after 70 h, and although the highest DOC yield was at 40°C and pH 5 (90285 mg C/kg LOI), it would take a long time to reach the end point, in comparison to 50 and 60°C. At 60°C the highest DOC yield was lower (pH 4.5- 47000 mg C/kg LOI) than the highest DOC yield at 50°C (pH 4.5- 67000 mg C/kg LOI). From the graph of 50°C, it is evident that about 85% of the total DOC released after 100 h is released after only 20 h. Therefore, although the amount of DOC released at 50°C is lower than at 40°C, the timescale is much more rapid. The optimal test conditions chosen were therefore pH 4.75, temperature 50°C and a 20 hour incubation time as a compromise between enzyme denaturation and a rapid hydrolysis rate so that the test time-scale was reduced to less than a day and much lower than most biological tests.

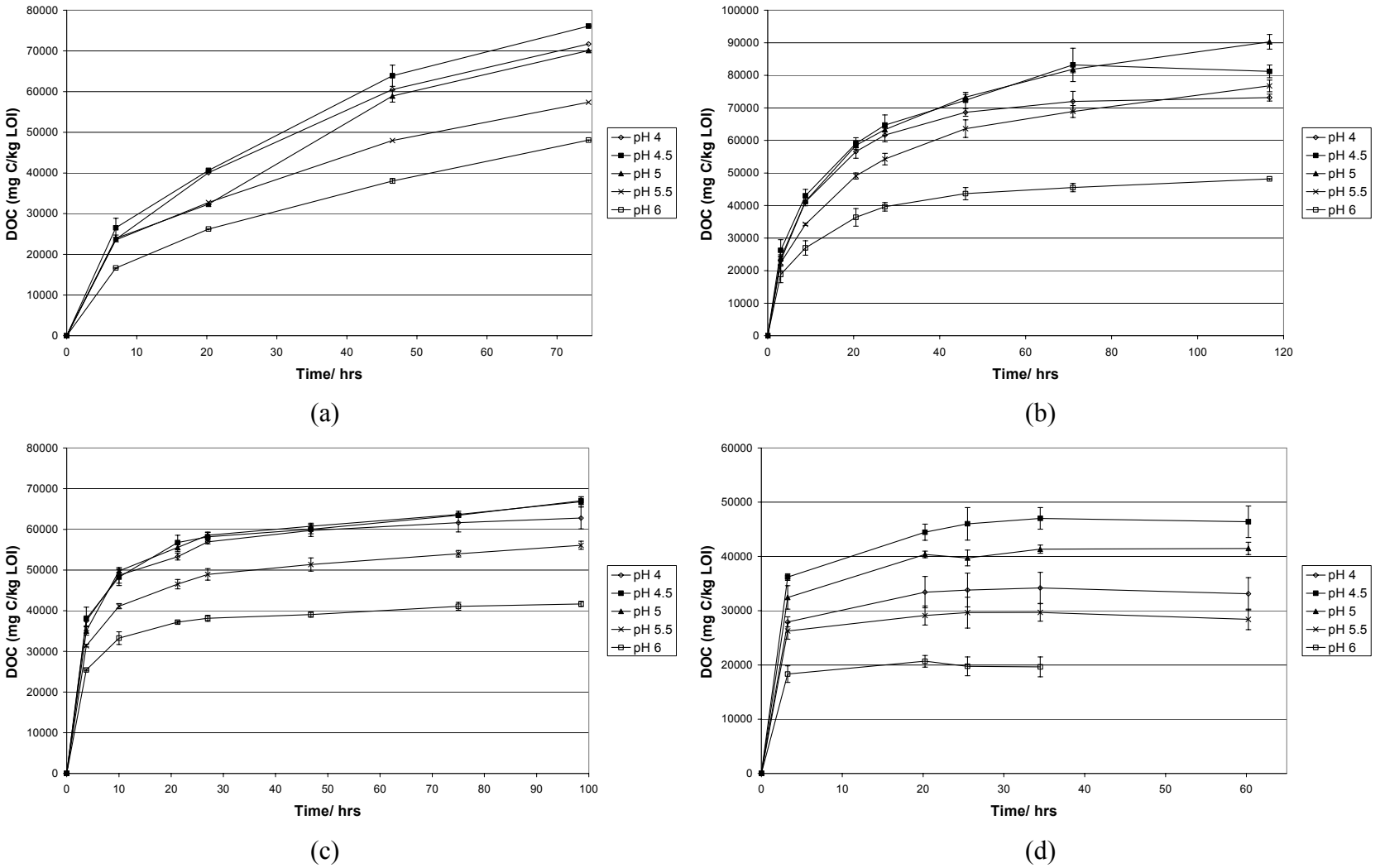

Figure 1. DOC (mg C/kg LOI) increase over time at incubation temperature 30°C (a), 40°C (b), 50°C (c) and 60°C (d). Error bars shown as standard deviation (stdev).

3.2 Waste Samples

The waste samples selected for this study included samples before and after MBT treatment (composting and anaerobic digestion), composting and autoclave treatment to demonstrate the effects of treatment to the DOC obtained in the test at each stage. The samples included packaging waste (cardboard), waste wood, garden waste, household waste (mixture of kitchen and garden waste), turkey feathers and MSW derived mixed BMW.

The cumulative DOC released after each phase of the test varied greatly between the samples (Table 1). The results are expressed as mean values of the three replicates analysed. Much more DOC was released during Phases 1 and 2, before the enzyme hydrolysis Phase 3, in many of the waste samples compared with commercial cellulose. Phase 1 DOC may represent the low molecular weight readily soluble materials present in the waste, whilst the DOC released in Phase 2 may represent soluble DOC following mild acid hydrolysis of some of the polymeric components during autoclave. Phase 2 DOC may also include soluble materials desorbed from the waste during the autoclaving. Finally the DOC released in Phase 3 is due to the enzymatic hydrolysis of the material, and so may indicate the amount of additional biodegradable cellulose, hemicellulose and possibly proteinaceous material present. The DOC released at each phase is shown graphically in Figure 5.

Table 1. Results of DM, LOI and the Enzymatic Hydrolysis Test (DOC released at the end of each phase).

	DM	LOI	DOC (mg C/kg LOI)		
Sample	**% wet wt**	**% DM**	**Phase 1**	**Phase 2**	**Phase 3**
Commercial cellulose	96.5	98.4	779	1550	53500
Construction wood waste	77.5	91.4	1500	13000	16000
Autoclaved construction wood waste	63.9	90.7	5380	15900	17800
Packaging waste	42.6	93.6	960	3540	26300
Autoclaved packaging waste	40.4	93.1	3080	6350	22900
Greenwaste (untreated)	40.4	73.2	10000	26400	32100
Partially composted greenwaste	44.2	62.1	11500	25100	33200
Kitchen and greenwaste (untreated)	35.1	65.3	11300	26000	34800
Partially composted kitchen and greenwaste	38.3	68.0	7250	16900	17900
Composted kitchen and greenwaste	51.7	60.9	3870	25400	26800
Organic fibre from autoclaved MSW	50.5	76.9	12000	29800	44400
AD treated fibre from autoclaved MSW	29.2	72.5	1640	7160	7900
Turkey feathers	33.7	99.3	8290	12000	16700
Autoclaved turkey feathers	38.3	96.2	7950	28100	32000
Stabilised greenwaste compost <10 mm	71.9	29.2	3450	47100	55500
Stabilised greenwaste compost <25 mm	66.6	28.9	3970	57700	58200
MSW input windrow MBT	94.4	73.4	13500	33800	61100
Fully Composted MSW	66.3	29.6	1310	28300	30600
MSW input to AD	96.5	58.7	17400	80800	141000
Output MSW AD	31.5	56.2	685	7570	8600
Fresh MSW	94.4	39.3	14300	80300	104000
Composted MSW <15 mm	75.3	23.4	9710	41600	47200

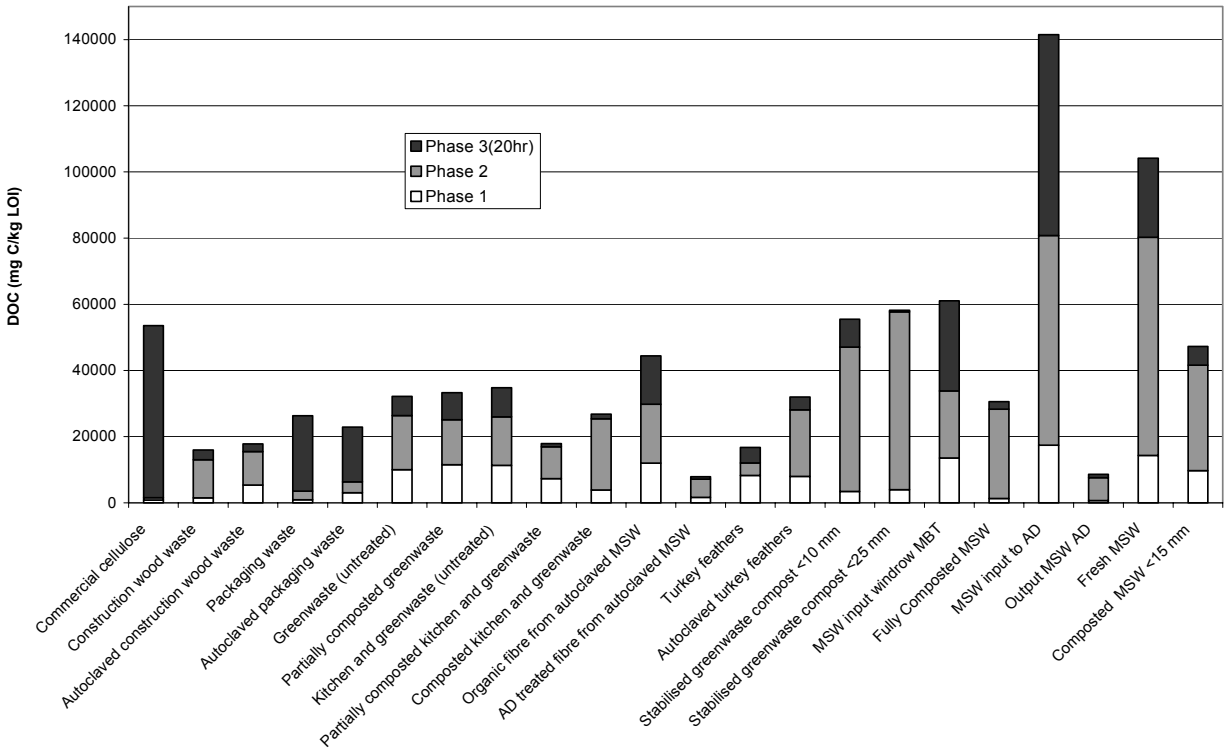

Figure 5. DOC released at each phase of the Enzymatic Hydrolysis Test.

The non-enzymatic DOC (Phases 1 and 2) for wastes that have undergone extended biological treatment (e.g. the fully composted greenwaste and composted MSW derived BMW samples), are likely to consist of significant amounts of humic substances resulting from the decomposition of lignin (Stevenson 1994). These substances are not usually considered readily biodegradable, and so in these cases, the DOC due to enzymatic hydrolysis (Phase 3 only) may be indicative of the sample biodegradability. Unlike the control cellulose, many of the non-biologically treated (raw or autoclaved) waste samples also showed significant amounts of DOC released during Phases 1 and 2. As these wastes have not been biologically treated it seems reasonable to assume that much of the DOC released during Phases 1 and 2 will be inherently biodegradable. Therefore a key question regarding this data is whether the test result should include either the entire DOC released, or the DOC released through enzymatic hydrolysis alone (Phase 3 only).

As part of Defra project number WRT220, BM100, DR4 and biochemical data is available (Godley, Frederickson *et al.* 2007; Godley, Lewin *et al.* 2007) for the majority of these samples. A full interpretation of these results is in preparation, but preliminary analysis of the data indicates that the EHT test method shows good correlation wth the BM100 test results for many of the samples.

For example, the packaging waste and autoclaved packaging waste both have a high proportion of cellulose (42.6 – 46.2% of dry matter) and gave high biogas production values in the BM100 test (527 – 630 l/kg LOI). In the EHT the major fraction of DOC was released during Phase 3 in both samples, which is expected given the high cellulose content of these samples.

The organic fibre from autoclaved MSW and the AD treated fibre from MSW both contain a relatively high fraction of solubles (35% and 27% respectively). Therefore the high DOC contribution observed for Phases 1 and 2 (Fig. 6) in the EHT is expected, and the actual values (Fig. 5) are consistent with the soluble fraction differences. The AD treated fibre from MSW has

under half the cellulose fraction and over double the lignin faction of the organic fibre from autclaved MSW. Therefore the lower DOC from enzymatic hydrolysis (Phase 3) observed (Fig. 6) for the AD treated sample might then be expected.

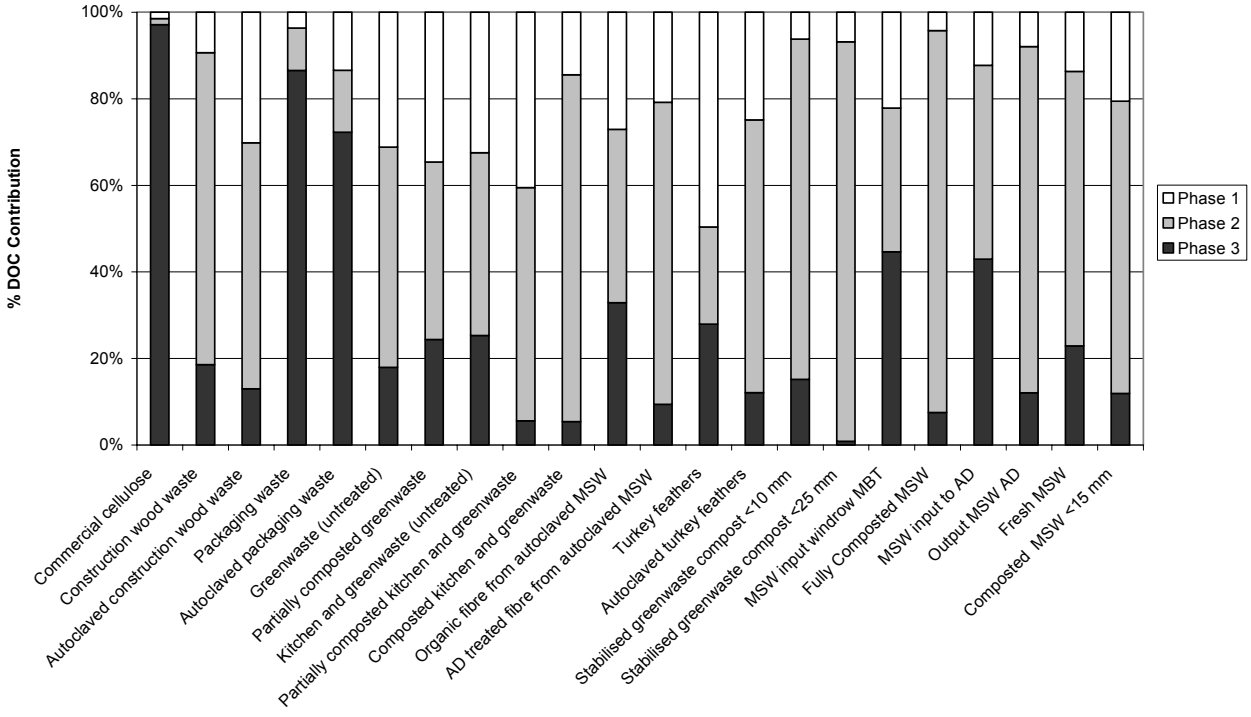

Figure 6. Percentage contribution of each phase to the total DOC released.

In general the results indicate that significant amounts of DOC are released by the EHT test during Phases 1 and 2 before the enzyme is introduced into the tests. For untreated waste samples much of this material may be biodegradable and for fully biologically treated wastes this material may be recalcitrant. The DOC released during Phases 1 and 2 for partially biologically treated materials may be composed of mixtures of biodegradable and recalcitrant materials. Therefore in order to successfully assess the biodegradability of a sample the DOC due to enzymatic hydrolysis alone is not sufficient, although it may give a good indication. The biodegradable DOC from Phases 1 and 2 may need to be differentiated from the non-biodegradable DOC and used together with the DOC from Phase 3 to give the best possible biodegradability indication.

4. CONCLUSIONS

From this work it is concluded that the enzymatic hydrolysis test shows good potential as a novel and rapid (sub 24 hour) biodegradability test method. Further work is progressing to fully evaluate this test method, including consideration of the impact of pre-biological treatment on the biodegradability of DOC released during Phases 1 and 2 of the test and the application of the test to a wider range of samples and comparison with the results obtained from the BM100 and DR4 test methods.

ACKNOWLEDGEMENTS

This research is part of a wider programme of research on waste characterisation sponsored by the Department for Environment, Food and Rural Affairs (Defra), from whom the authors are grateful for permission to publish. Views expressed are those of the authors' alone.

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Appendix I

(2008) The development of a novel biodegradability test method based on the enzymatic hydrolysis of cellulose. Waste 2008, Stratford-upon-Avon.

THE DEVELOPMENT OF A NOVEL ENZYMATIC BIODEGRADABILITY TEST METHOD

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SUMMARY: This enzymatic hydrolysis test (EHT) has been developed over the past three years at the Centre for Resource Management and Efficiency (CRME) at Cranfield University. It is part of a Defra funded waste characterisation project in collaboration with WRc plc and the Open University. This paper summarises key findings from the research project and presents early data from a periodic monitoring of a mechanical biological treatment (MBT) process. Here the UK established BM100 and DR4 test methods, and the EHT, were applied to input municipal solid waste (MSW) samples taken over a period of approximately 9 months. The biodegradability of the samples varied significantly over the time period, and in general the biodegradability values obtained from the three test methods showed a similar trend over time. Results suggest that the EHT represents biodegradability more closely than the DR4 test method. This is shown by the stronger correlation with BM100 values, which was r = 0.75 for the EHT, compared to r = 0.61 for the DR4. Further work aimed at improving the current EHT method has been discussed and is progressing to the end of the 3 year research programme.

1. INTRODUCTION

In accordance with the EU Landfill Directive, the amount of biodegradable municipal waste (BMW) disposed of in landfill need to be dramatically reduced (Council of the European Union, 1999). In the UK, these targets are assigned to local authorities by means of the landfill allowance trading scheme (LATS), or the landfill allowance scheme (LAS) in Wales (Defra, 2005). The BMW proportion of municipal solid waste (MSW)

Proceedings Waste 2008: Waste and Resource Management – a Shared Responsibility
Stratford-upon-Avon, Warwickshire, England, 16-17 September 2008

can be reduced via treatment of the waste material, in processes such as mechanical biological treatment (MBT) involving separation of solid recovered fuel (SRF) or through biological treatments such as composting or anaerobic digestion (Archer, Baddeley *et al.*, 2005). To quantify the amount of BMW diverted away from landfill disposal as a result of waste treatment the biodegradability of the input and output materials need to be measured.

Biodegradability of organic wastes can be measured in a number of ways, and in the UK the Environment Agency preferred test methods are the aerobic 4 day DR4 and the anaerobic 100 day BM100 test methods (Environment Agency, 2005). The BM100 method has been reported to show good reproducibility between results (Godley, Lewin *et al.*, 2003), but has the disadvantage of taking a long time to complete and, as such, is of limited value in routine operational monitoring of waste treatment processes. Short-term aerobic methods like the DR4 test have other disadvantages such as preferentially decomposing the readily biodegradable components of the waste (Godley, Lewin *et al.*, 2007) and high microbial growth efficiency. In this case the decomposed organic matter is used for microbial growth and formation of new biomass, rather than being metabolised. Therefore most current biodegradability test methods have limitations, and no one test method may be suitable for the whole range of biodegradability testing requirements such as monitoring MBT process performance and assessing organic waste biostability.

A novel biodegradability test method based on the enzymatic hydrolysis of cellulosic material has previously been reported (Godley, Lewin *et al.*, 2004; Wagland, Tyrrel *et al.*, 2007). This test method involves incubating organic waste material with a formulated mixture of cellulase and hemicellulase enzymes and measuring the dissolved organic carbon (DOC) released as a result of the enzymatic hydrolysis of the waste material. Hemicellulosic/cellulosic material is considered to be the most important carbon source for methanogenesis in landfills as it contributes as much as 90% of the total biogas (CO_2 + CH_4) produced (Barlaz, Ham *et al.*, 1989; Rodriguez, Hiligsmann *et al.*, 2005). As a general rule, the higher the cellulose/hemicellulose content, the higher the biogas yield of the waste in anaerobic tests (Eleazer, Odle III *et al.*, 1997), although the availability of the cellulose can vary as the associated lignin can 'protect' the cellulose from chemical or enzymatic decomposition. Therefore assessment of the waste cellulose and hemicellulose content would provide a non-biological test method of assessing biodegradability. Direct chemical measurement of the cellulose and hemicellulose content of a waste sample could be considered as a possible method to allow an estimate of sample biodegradability. However not all the cellulose is amenable to biodegradation when present as lignocellulose (Chen, Knappe *et al.*, 2004). The resistance of lignin to biological and chemical degradation allows it to protect cellulose (Stinson and Ham, 1995), and so not all the cellulose determined in a direct measurement will be biodegradable cellulose, over-estimating sample biodegradability.

The measurement of *available* cellulose and hemicellulose content therefore offers a suitable representation of sample biodegradability. The enzymatic availability of cellulose in organic waste has been investigated (Rodriguez, Hiligsmann *et al.*, 2001) and identified as a potential surrogate biodegradability test method (Godley, Lewin *et al.*,

2004). Based on these initial findings the enzymatic hydrolysis test (EHT) was developed and optimised at Cranfield University.

The EHT method was investigated to determine the optimum pH and temperature conditions using a standard α-cellulose sample (Wagland, Tyrrel *et al.*, 2007). Applying these optimum conditions to the EHT, the method has since been compared with the DR4 and BM100 methods following the application to a wide range of untreated, partially treated and treated organic waste materials from a wide range of waste treatment processes. This study reported that the EHT indicated a stronger correlation with the long term BM100 test method than the short term 4 day DR4 test (data not shown- in press). The EHT has been shown to be a suitable alternative short-term test method to the DR4 method when applied to a wide variety of organic waste samples (Wagland, Tyrrel *et al.*, 2007).

Here early data is presented following a comprehensive study of a single waste treatment process. This study aims to further evaluate whether the EHT is a suitable alternative to the currently used DR4 method, with the focus on test reliability when applied to a single waste treatment process. The EHT, BM100 and DR4 test methods were applied to each of the waste samples, which were taken over a ~9 month period, and the standardised data obtained from the EHT and DR4 with the BM100 are compared.

2. MATERIALS AND METHODS

2.1 Waste Samples

MSW samples from a mechanical biological treatment (MBT) process were obtained at regular intervals over a 9 month period. The samples were sorted to remove glass, metals, plastics and inert materials providing an MSW-derived BMW sample, and dried overnight. The samples were ground to <10 mm for the BM100 and DR4 analysis, and <2 mm for the EHT method. The grinding size variation is due to the EHT being a rapid analytical test method, and is likely to be sensitive to particle size differences; whereas this has been observed to not be the case with the DR4 and BM100 test methods. The samples were analysed in triplicate for each of the test methods, including the dry matter (DM) and loss-on-ignition (LOI) which were determined for each waste sample using standard procedures (EN12879:2000).

Here only the MSW derived BMW input data is presented, with the data for output samples currently in progress.

2.2 UK Established Test Methods

The waste samples were analysed using the BM100 and DR4 test methods at WRc plc as previously described (Godley, Lewin *et al.*, 2007).

Biodegradability under aerobic conditions was determined using the DR4 test method (Environment Agency, 2005), which is adapted from ASTM D5975-96 (ASTM, 1996; Godley, Frederickson *et al.*, 2007; Godley, Lewin *et al.*, 2007). The test material (100 g DM) was prepared as outlined previously and mixed with the seed material, typically a

mature green waste compost (100 g DM). Water and nutrients (nitrogen and phosphorus) were added to adjust to 50% w/w moisture content. The test mixture was placed in a reactor vessel at 35°C for 4 days, with constant aeration (500 ml/min (Environment Agency, 2005)) through the reactor vessel. The CO_2 released over the 4 day period was measured and this data is used to estimate O_2 consumption.

The BM100 test method was used to assess the biodegradability under anaerobic conditions, and is based on a sewage sludge digestion test (Godley, Lewin *et al.*, 2007; Godley, Lewin *et al.*, 2003). The test material (20 g LOI) was placed in a glass container with microbial seed (digested sludge) and a nutrient mixture. The mixture was sealed and incubated at 35°C under anaerobic conditions and the release of CO_2 and CH_4 (biogas) was measured volumetrically until no further biogas was released (up to 100 days).

2.3 Enzymatic Hydrolysis Test

The enzyme test method consisted of three phases of measurement as follows. The procedure is summarized in figure 1.

- **Phase 1.** The waste sample (5 g LOI) was placed in a 250 ml Erlenmeyer flask. Phosphate pH buffer (100 ml 0.37 M) was then added to the flask. A 5 ml sample was removed and filtered (0.45 µm membrane filter) to remove any solids, and the filtered liquid was then analysed for chemical oxygen demand (COD) (Spectroquant COD test tubes).
- **Phase 2**. The sample mixture was then autoclaved at 121°C for 15 min to sterilise the mixture and a further 5 ml sample was removed and filtered for COD analysis.
- **Phase 3**. The prepared enzyme solution (20 ml) was then added to each of the flasks and the flask sealed with a neoprene bung. The flasks were placed in a shaking incubator at 150 rpm. A 5 ml sample was removed and filtered for COD analysis after 20 h of incubation.

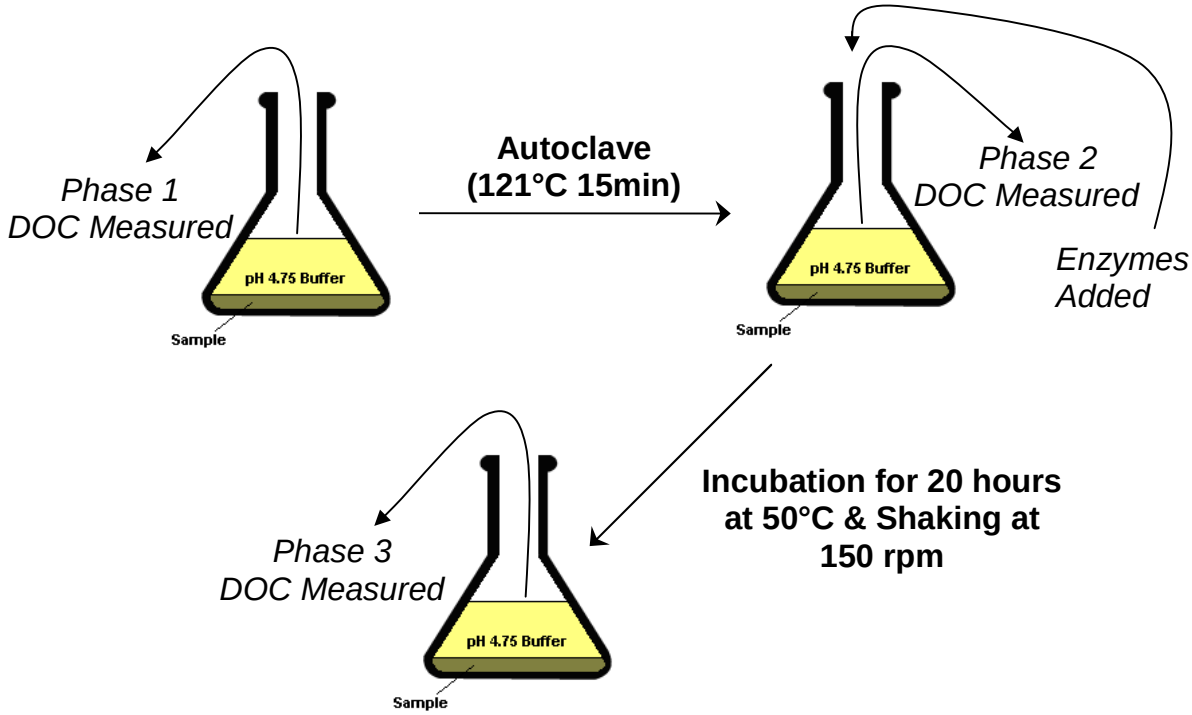

Figure 1. Schematic diagram of the EHT method.

For each sample 25 mg of crude cellulase powder (Sigma) and 75 mg of hemicellulase powder (Sigma) were dissolved in 20 ml of distilled water, with approximately 175 units of cellulase and 112.5 units of hemicellulase activities in each 20ml of enzyme mixture. This enzyme solution was then filtered through 0.22 µm Millipore membrane filters to sterilise the solution. The crude cellulase enzymes exhibited some hemicellulase and protease activity, with the hemicellulase enzymes also having some cellulase activity (within manufacturer specifications).

The amount of moisture in the waste sample and the removal of both the liquid and solids at each stage of sampling, along with the addition of liquid in phase 3, were accounted for in the concentrations of carbon calculated. Soluble COD analysis results were converted to DOC (mg C/l) by assuming a COD/C ratio of 2.67 and then expressed in terms of mg of carbon per kg of the sample (LOI) to give the final values.

In a previous study (Wagland, Tyrrel *et al.*, 2007) it was found that deducting the Phase 2 DOC yielded the strongest relationship with the BM100. This was due to observation that for treated waste samples the autoclave step releases non-biodegradable carbon and so deducting this Phase 2 DOC from the final Phase 3 DOC correct for this effect. For fresh untreated waste material there is likely to be very little non-biodegradable DOC released by the autoclave step, and so deducting Phase 2 DOC would be eliminating biodegradable carbon, and so would hinder the accuracy of the data presented. For this reason, the data presented here is the total DOC released (i.e. the Phase 3 DOC) as there is likely to be very little non-biodegradable carbon released in the EHT since the waste material has not been biologically treated.

3. RESULTS AND DISCUSSION

The data presented is normalised for comparative purposes between the test methods. Each data point is shown as the percentage of the mean of the data for each respective test method. The trends in the graph are identical to that if the original data had been shown, however due to the difference in method units and values the data points would not allow for ease of comparison between the results. The results for the EHT, DR4 and BM100 analysis is shown in figure 2. Each of the test method datasets is shown over a period of approximately 9 months.

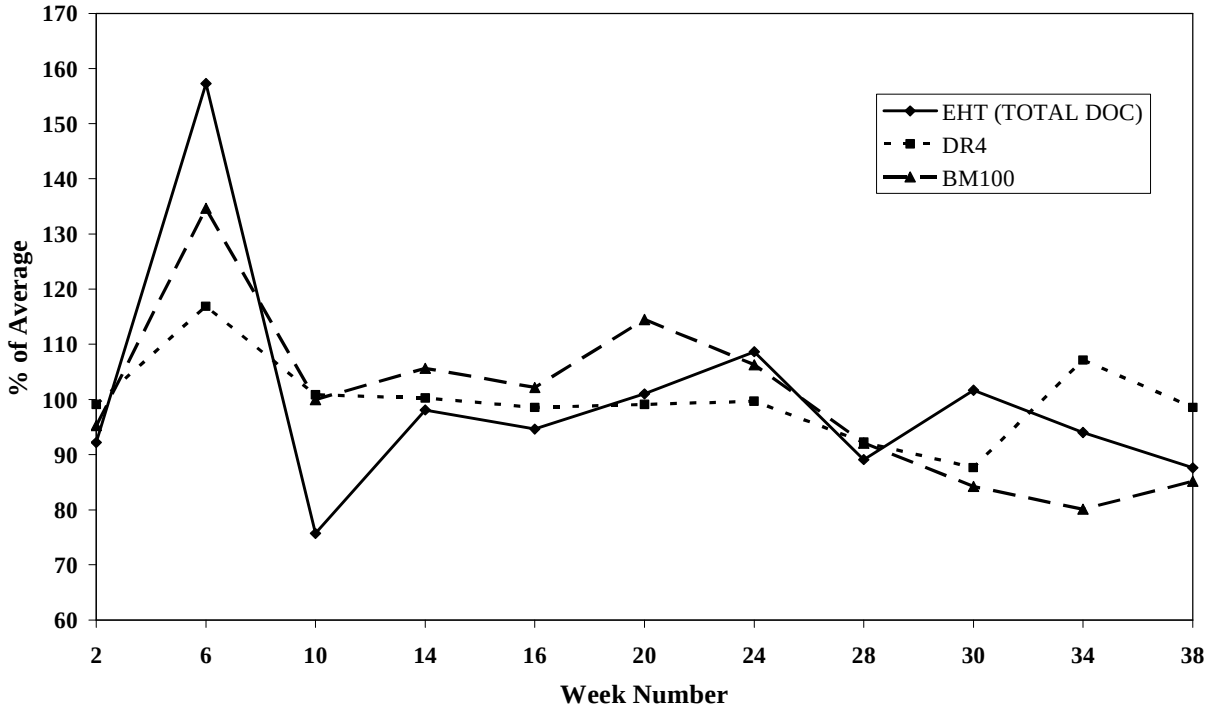

Figure 2. Biodegradability results (normalised as a percentage of the respective mean average) from the EHT, DR4 and BM100 test methods for the MBT input MSW-derived BMW samples.

The graph shows that the biodegradability values of MSW derived BMW input samples vary significantly over time. This trend is observed for the EHT, DR4 and BM100 values. In general as the BM100 values increase, so do the EHT and DR4 values, demonstrating that there is a relationship between the test methods with regards to the data that is produced.

Biodegradability is influenced by the content of readily biodegradable materials, such as monosaccharides, starch and lipids and the more resistant biodegradable materials such as cellulose. In a typical waste sample, some non-biodegradable (or very slowly biodegradable) organic materials, such as lignin, may exist. Therefore the biodegradable content of the waste can vary, depending on the content of different organic materials (i.e.

paper, cardboard etc.).

Initially the variations around the long-term average for the EHT method are more pronounced than the DR4 and BM100. However the changes in DR4 values are less significant than for the EHT and BM100, with the majority of results being close to the average value (100% on the graph). The sample at week 6 is significantly more biodegradable than later samples. This is indicated by the data of all three test methods being above average (>100%). At week 10, the DR4 and BM100 indicate that the waste sample is of average biodegradability, whilst the EHT indicates lower than average values. The biodegradability data is relatively consistent after these points, with all three biodegradability tests indicating similar trends. At week 34 however, the EHT indicates that the sample is of average biodegradable content, whilst the DR4 values are above average and the BM100 is below average.

The DR4 preferentially degrades the readily biodegradable carbon, whilst the BM100 measures the full extent of biodegradability (readily and slowly biodegradable material). This data is from fresh waste material, in which there will be a relatively high amount of readily biodegradable material. The variation in BM100 values may indicate that the DR4 method has mineralised only the readily biodegradable material. In contrast the BM100 data will include all biodegradable carbon, which would lead to a greater variation between the sample biodegradability. The variation in biodegradability indicated by the EHT method could be due to the EHT mineralising a comparable amount of carbon to the BM100, or at least more so than the DR4. This could indicate that the EHT is a more suitable test method to predict the sample biodegradability.

The correlation of the DR4 and EHT with the BM100 values also indicates that the EHT has a stronger relationship with the BM100 than the DR4. A correlation of $r = 0.61$ was observed for the DR4 values, however a correlation of $r = 0.75$ was observed for the EHT (total DOC) values. The DOC from enzyme hydrolysis alone (Phase 3 minus Phase 2) gave a correlation of $r = 0.64$. The variation in EHT correlations could be due to the effect of deducting Phase 2 DOC (discussed previously) considered to consist of a differing proportion of carbon that is both inherently biodegradable and non-biodegradable (Wagland, Tyrrel *et al* 2007). In the case of MSW derived BMW input material which has not undergone biological treatment; the DOC released would mostly consist of biodegradable DOC. However if a treated waste material is considered, then the DOC released in Phase 2 may consist of a significant amount of non-biodegradable material, such as humic substances. Therefore whilst the deduction of Phase 2 DOC from the final Phase 3 value may be more accurate for mature stabilised waste, this may be less accurate for fresh waste material.

The non-biodegradable DOC is likely to consist largely of humic substances, and so the quantification of these would enable a more selective deduction from the final Phase 3 DOC. For mature waste samples, the resultant deduction would be similar to that of deducting all of Phase 2, whilst for fresh material the deduction of humic substances would be similar to that of not deducting Phase 2 DOC at all (i.e. use the total DOC).

Humic substances can be easily quantified using standard humic extraction procedures

(Artiola Fortuny and Fuller, 1982; Thurman and Malcolm, 1981), however a more recent rapid batch procedure has been used to measure humic and fulvic acid concentrations (Van Zomeren and Comans, 2007). The quantification of humic substances has been incorporated into the EHT methodology and will be applied to each waste sample presented here, and all samples currently undergoing analysis. To validate the humic extraction step, this procedure will be carried out on the stored samples from the previous study to test the hypothesis that the deduction from the total DOC will be large with mature waste samples, and relatively small for fresh samples.

The analysis of the output and remaining input samples of the MBT process are currently progressing, along with the incorporation of the humic substance extraction and quantification step into the EHT method.

4. SUMMARY

- The research project has resulted in a novel biodegradability test being developed, optimised and applied to a wide variety of organic waste samples.

- Previous data has allowed for the conclusion that the EHT method is a possible alternative to the DR4 test method, exhibiting a stronger correlation with the long-term BM100 test method when applied to a range of organic waste material. The reported correlation also suggests that the EHT is suitable for the application to a wide range of organic waste materials from a variety of waste treatment processes.

- Data presented in this paper offers early evidence that the EHT is a suitable alternative test method for monitoring a single MBT process; however further data from the both the input and output materials are required to ascertain this.

ACKNOWLEDGEMENTS

The authors are grateful to the East London Waste Authority (ELWA) for the additional financial assistance and samples provided for this study, and also to Defra for permission to publish the outcomes of this research funded as part of project WR0110. Views expressed are those of the authors' alone.

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