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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.

Keywords: Biodegradability, waste, characterization, 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 10 million 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 i.e. 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 as 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 (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. 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 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 (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) 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 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 sterilised by filtration through a 0.22 μm 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 (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 (0.45μm membrane filter) to remove solids, and the filtrate analysed for chemical oxygen demand (COD) (Spectroquant COD test tubes).
- **Phase 2**. The sample mixture was sterilised by autoclaving at 121°C for 15 min and a further 5 ml sample was removed and filtered for COD analysis.
- **Phase 3**. The prepared enzyme solution (20 ml) 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 5 ml sample was removed for COD analysis after 20 h of incubation.

The moisture content of the waste sample; the removal of the liquid and solid at each stage of sampling; 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 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 (e.g. 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 (i.e. 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 $R^2 = 0.54$ 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.

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