

1 **INVESTIGATION OF THE APPLICATION OF AN ENZYME-BASED**
2 **BIODEGRADABILITY TEST METHOD TO A MUNICIPAL SOLID WASTE**
3 **BIODRYING PROCESS**

4

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1 **Abstract**

2 This paper presents a study to evaluate the recently developed enzymatic
3 hydrolysis test (EHT) through its repeated application to a waste treatment process. A
4 single waste treatment facility, involving a biodrying process, has been monitored using
5 three different methods to assess the biodegradable content of the organic waste fractions.
6 These test methods were the anaerobic BMc, aerobic DR4 and the EHT, which is a
7 method based on the enzymatic hydrolysis of the cellulosic content of waste materials.
8 The input municipal solid waste (MSW) and the output solid recovered fuel (SRF) and
9 organic fines streams were sampled over a period of nine months from a single
10 mechanical biological treatment (MBT) facility. The EHT was applied to each stream
11 following grinding to <10 mm and <2 mm, in order to investigate the effect of particle
12 size on the release of dissolved organic carbon (DOC) from enzyme hydrolysis. The
13 output organic fines were found to more biodegradable than the MSW input and SRF
14 output samples in each of the test methods, significantly ($p < 0.05$) for the EHT and DR4
15 methods, on the basis of DOC released and oxygen consumed respectively. The variation
16 between sample replicates for the EHT was higher where sample sizes of <2 mm were
17 analysed compared to sizes of <10 mm, and the DOC release at each phase of the EHT
18 was observed to be higher when using particle sizes of <2 mm. Despite this, additional
19 sample grinding from the <10 mm to a smaller particle size of <2 mm is not sufficiently
20 beneficial to the analysis of organic waste fractions in the EHT method. Finally, it was
21 concluded that as similar trends were observed for each test method, this trial confirms
22 that EHT has the potential to be deployed as a practical operational biodegradability
23 monitoring tool.

1 **Keywords-** Biodegradability, Enzymatic Hydrolysis, Waste Characterization, Waste
2 Treatment, Landfill Diversion, Organic Waste

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4 **1. Introduction**

5 In accordance with the EU Landfill Directive, the amount of biodegradable
6 municipal waste (BMW) disposed of in landfill needs to be dramatically reduced
7 (Council of the European Union, 1999). The BMW proportion of municipal solid waste
8 (MSW) can be reduced via treatment of the waste material in processes such as
9 mechanical biological treatment (MBT) which involve the separation of solid recovered
10 fuel (SRF) and biological treatments such as composting or anaerobic digestion (Archer
11 et al., 2005). Methods of assessing the biodegradable content of input and output
12 materials of the treatment processes can provide important information on process
13 performance and the diversion of BMW from landfill (Wagland et al., 2009). There is a
14 general acceptance that all test methods have their advantages and limitations but the
15 suitability of the available test methods for routine operational use remains the subject of
16 academic debate (Sánchez, 2009; Wagland and Tyrrel, 2010), suggesting a requirement
17 for further research and development into alternative methods. One such method is the
18 enzymatic hydrolysis test (EHT) (Wagland et al., 2009). This procedure uses a mixture
19 of hemicellulase and cellulase enzymes, under optimum conditions, to hydrolyze the
20 biodegradable substrate (Wagland et al., 2007). These enzymes are used as BMW
21 consists of 30-50% lignocellulosic material (Godley et al., 2007a; Rodriguez et al., 2005;
22 Wagland et al., 2008), and hemicellulose/cellulose can contribute to up to 90% of the

1 total biogas (CO₂/CH₄) produced under anaerobic conditions, such as landfill (Barlaz et
2 al., 1989).

3 In the recent study by Wagland *et al* (2008) the BM100, DR4 and EHT methods
4 were applied to a wide range of untreated and treated organic waste materials including
5 MSW, garden waste, food waste and sewage sludge. The BM100 is an anaerobic test
6 method which measures the biogas (CO₂ and CH₄) release over a period of 100 days; and
7 the DR4 is a dynamic 4 day aerobic test which measures the oxygen consumption of
8 biodegradable material under aerobic conditions (Wagland et al., 2009). The correlations
9 of the short-term EHT and DR4 methods with the long-term BM100 test method were
10 compared. The EHT generated a stronger correlation with the BM100 than that of the
11 DR4 ($r = 0.77$ and 0.58 respectively) indicating that the method has some potential and
12 should be subject to further testing. The use of the EHT test remains debatable, however,
13 due to concerns that the test will not register the biodegradable content of wastes with a
14 relatively low composition of polysaccharides (Wagland et al., 2008). Biological
15 methods are commonly recognized as suitable approaches, capable of high correlations
16 with long-term anaerobic methods for specific waste streams and treatment processes
17 (Cossu and Raga, 2008; Ponsá et al., 2008; Sánchez, 2009).

18 The BM100 test for monitoring BMW diversion from landfill has been
19 superseded and is now referred to as the biodegradability under methanogenic conditions
20 (BMc) (Environment Agency, 2005; Turrell et al., 2009). Therefore, currently in the UK
21 the aerobic DR4 and BMc test methods are used to monitor BMW diversion from landfill
22 (Environment Agency, 2005; Godley et al., 2007b; Turrell et al., 2009). In this study the
23 EHT, DR4 and BMc methods were applied to a series of samples taken over a nine month

1 period from a single MBT facility which employs a 2 week biodrying process. The
2 principal aim was to evaluate the performance of EHT as a biodegradability test when
3 applied in the context of the routine monitoring of a waste treatment facility. In addition
4 to monitoring the changes in biodegradability, the waste samples were assessed using
5 different particle sizes for the EHT. The surface area of the waste material is likely to
6 affect the rate and extent to which the enzymes hydrolyze the substrate. It was
7 hypothesized that grinding to smaller sample sizes would result in less variability
8 between sample replicates, and so a smaller <2 mm particle size was used in addition to
9 the standard <10 mm used in the DR4 and BMc test methods. Also the increased surface
10 area: particle volume ratio may result in a significantly higher dissolved organic carbon
11 (DOC) release, which has been observed previously (Dasari and Eric Berson, 2007).
12 Therefore this study investigated the effects of particle size on variation and DOC yield
13 for the EHT in addition to the monitoring of an MBT process using UK-established
14 biodegradability test methods, to indicate the suitability of the EHT method for assessing
15 the biodegradable content of MSW-derived material.

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17 **2. Methods**

18 ***2.1. Samples***

19 The samples were collected from a single MBT facility located in the south of
20 England. This facility receives general mixed MSW collected from the local area. The
21 waste material is shredded and placed in a large composting hall for 2 weeks where it is
22 dried using the heat generated by microbiological activity (biodrying) before passing
23 through a complex separation process (Figure 1).

1 The samples were sorted to remove glass, metals, plastics and inert materials with
2 the biodegradable material being retained and tested (Environment Agency, 2005). The
3 samples were dried at 70°C to 80-90% dry weight and shredded using an adjustable
4 grinder to <10 mm and <2 mm. The standard particle size of <10 mm (Environment
5 Agency, 2005) was used for the EHT, DR4 and BMc analysis, whilst the smaller <2 mm
6 particle size was only used in EHT analysis as part of an exploration to assess the effects
7 of particle size on the DOC yield and variation between sample types. The samples were
8 analysed immediately, or otherwise stored in sealed containers in a cold room (<4°C)
9 until required. Each of the samples was subsampled and tested in triplicate, and the
10 results expressed are the mean values obtained.

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12 ***2.2. Aerobic DR4***

13 Biodegradability under aerobic conditions was determined using the DR4 test
14 method (Environment Agency, 2005; Godley et al., 2007b; Godley et al., 2005). The test
15 material (100 g dry matter (DM)) was mixed with a seed material (100 g DM), which was
16 a mature green waste compost. Water and nutrients (nitrogen, as 2 M ammonium
17 chloride, and phosphorus, as 1 M potassium phosphate) were added to adjust to 50% w/w
18 moisture content, based on the measured %DM of the sample. The test mixture was
19 placed in a reactor vessel at 35°C for 4 days, with constant aeration (500 ml/min
20 (Environment Agency, 2005)) through the test material. The O₂ consumed during the 4
21 days was estimated from the amount of CO₂ released, which was measured by using 1 M
22 NaOH solutions to 'trap' CO₂ and then titrated against 1 M HCl (Turrell et al., 2009).
23 The volatile solids (VS) content, referred here as loss on ignition (LOI) (European

1 Committee for Standardisation, 2005) for each sample was determined; and the results
2 expressed in terms of the LOI content of the test material (mg O/kg LOI) (Environment
3 Agency, 2005).

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5 **2.3. Anaerobic BMc**

6 The BM100/BMc test method (Environment Agency, 2005; Turrell et al., 2009) is
7 based on a sewage sludge digestion test (Godley et al., 2007b; Godley et al., 2003). The
8 test material (20 g LOI) was placed in a 350 ml glass container with 50 ml/l microbial
9 seed (digested sludge) and a nutrient mixture. The mixture was sealed and incubated at
10 35°C under anaerobic conditions and the release of CO₂ and CH₄ (biogas) was measured
11 volumetrically until no further biogas was released (up to 100 days). The results are
12 expressed as the volume (litres) of biogas generated per kg of LOI of the test material
13 (l/kg LOI) (Environment Agency, 2005).

14

15 **2.4. Enzyme hydrolysis test**

16 The EHT was applied as described in previous studies (Wagland et al., 2008;
17 Wagland et al., 2007). For each sample 25 mg of crude cellulase powder (Sigma,
18 C9422) and 75 mg of hemicellulase powder (Sigma) were dissolved in 20 ml of distilled
19 water. According to the manufacturer's specification, each 20 ml of enzyme mixture
20 possessed approximately 175 units of cellulase and 112.5 units of hemicellulase activity.
21 According to the manufacturer's specification, the crude cellulase powder was expected
22 to exhibit some hemicellulase and protease activity, and the hemicellulase enzymes some

1 cellulase activity. To sterilise the enzyme solution it was then filtered through a 0.22 μm
2 Millipore membrane

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4 The test method consists of three phases as follows:

- 5 i. The test material (5 g LOI) was placed in a 250 ml Erlenmeyer flask.
6 Phosphate pH buffer (100 ml 0.37 M) was then added to the flask. A 5 ml
7 sample was removed and filtered (0.45 μm membrane filter) to remove any
8 solids, and the filtered liquid was then analysed for chemical oxygen demand
9 (COD) (Spectroquant COD test tubes).
- 10 ii. The sample mixture was then autoclaved at 121°C for 15 min to sterilise the
11 mixture and a further 5 ml sample was removed and filtered for COD analysis.
- 12 iii. The prepared enzyme solution (20 ml) was then added to each of the flasks
13 and the flask sealed with a neoprene bung. The flasks were placed in a
14 shaking incubator at 150 rpm for 20 h at 50°C. A final 5 ml sample was then
15 removed for COD analysis.

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

22 To assess the effect of particle size on DOC yield and variation between replicates
23 the following was considered-

- 24
- Post-autoclave DOC [P2];

1 t-test) but not for the difference in biodegradability between the fines and the SRF. The
2 MSW input and SRF samples were in each case very similar in biodegradable content.
3 For each of the methods, the difference in biodegradability between MSW and SRF was
4 not statistically significant ($P>0.1$, two-tailed t-test).

5 **3.2. Effect of Particle Size in the EHT**

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7 The particle size of the waste samples had an effect on the DOC released at each
8 phase of the EHT. This is shown in Figure 2.

9 As expected, the DOC released over the course of the EHT method increased after
10 each phase of the process. In terms of the total DOC (final phase 3 value) the fines
11 material was the most biodegradable ($P<0.05$, two-tailed t-test), whereas the MSW input
12 and SRF output samples were not significantly different ($P>0.1$, two-tailed t-test).

13 The coefficient of variation (C_v) for each set of results was calculated from the
14 following equation-

$$15 \quad C_v = \frac{\sigma}{\mu} \quad \text{Equation 1}$$

16 Where C_v is the coefficient of variation, σ is the standard deviation and μ is the
17 mean. C_v is useful since this is a normalised statistic allowing comparison between the
18 three methods used. The C_v for each sample at each phase of the EHT is shown in Table
19 2.

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1 **Table 2.** Coefficient of variation at each phase of the EHT for MSW, SRF and organic
 2 fines samples.

		P2 (Post-autoclave)	P3 (Total)	P3-P2 (Enzyme-only)
MSW	(≤10 mm)	0.17	0.09	0.12
	(≤2 mm)	0.26	0.21	0.19
SRF	(≤10 mm)	0.25	0.15	0.13
	(≤2 mm)	0.19	0.08	0.19
Organic Fines	(≤10 mm)	0.15	0.13	0.26
	(≤2 mm)	0.23	0.22	0.39

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 4 The C_v was consistently higher for the <2 mm samples of MSW and organic
 5 fines, whereas the C_v was lower for samples <2 mm for the SRF materials.

6 **4. Discussion**

7 **4.1. Biodegradability of the Sample Fractions**

8
 9 The aim of this study was to investigate the suitability of the EHT to monitor a
 10 waste treatment process over a prolonged period of time by comparison with standardised
 11 biodegradability tests. Each of the three methods produced comparable results which
 12 indicated that the MSW input and SRF output samples were similar in terms of their
 13 biodegradability whereas the fines fraction was consistently more biodegradable. The
 14 extent of variation between samples was comparable in each of the tests (Table 3)
 15 indicating that the tests produce consistent measures of biodegradability over an extended
 16 sampling period and suggesting that the waste fractions tested were also consistent over
 17 the monitoring period.

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3 **Table 3.** Coefficient of variation of the BMc, DR4 and EHT (P3 DOC) for each of the
4 samples (<10 mm).

	BMc	DR4	EHT
MSW (n=8)	0.11	0.11	0.09
SRF (n=11)	0.19	0.14	0.15
Organic Fines (n=6)	0.21	0.21	0.13

5

6 The biodegradability of the MSW input and the SRF output materials was found
7 to be very similar. It was originally expected that the MSW input material would be more
8 biodegradable than the SRF material. However, it is apparent from these results that the
9 biodegradable content of the MSW input is not reduced significantly ($P>0.1$) due to the
10 relatively short composting period employed in the biodrying process, which is only
11 designed to dry the waste material, and not to bio-stabilise it.

12 In spite of the biodrying process, the fines output sample was found to be more
13 biodegradable than the MSW input since this material has had the more slowly
14 biodegradable materials removed (such as cardboard, wood and fabrics), with the readily
15 biodegradable materials, such as food waste (vegetable peelings, meat residues etc)
16 effectively becoming more concentrated. The DOC released during the EHT, along with
17 the DR4 values, suggest that the fines material is significantly ($P<0.05$) more
18 biodegradable than the MSW input and SRF output materials. The DR4 values for the
19 fines material were 68% and 82% higher than the MSW input and SRF samples
20 respectively, whilst for the total DOC (P3) of the EHT, the DOC output from the fines

1 material was 109% and 106% higher than that generated from the MSW input and SRF
2 samples respectively. However the difference in biodegradability between the fines
3 fraction and the other fractions was lower for the BMc compared to the DR4 and EHT
4 tests. The BMc value for the fines material was 31% higher than the SRF output
5 ($P<0.05$), and 24% higher than the MSW input ($P<0.1$). This difference in relative
6 biodegradability between fractions is likely to be because the BM100/BMc test method
7 measures the full extent of biodegradability (Godley et al., 2007a; Godley et al., 2007b;
8 Wagland et al., 2008), and so will completely hydrolyse a higher proportion of the more
9 slowly biodegradable carbon (such as cardboard and wood) in the MSW input and SRF
10 samples than the EHT and DR4 methods.

11 **4.2. Effect of Particle Size in the EHT**

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13 Grinding of the sample to a smaller size was expected to have an effect on the
14 DOC yield and the variation observed between sample replicates. Grinding to <2 mm
15 was expected to yield higher DOC release due to the increase in surface area: volume
16 ratio of each particle.

17 As shown in Figure 2 the DOC release at each phase proved to be largely
18 unaffected by particle size with the exception of the fines fraction (P2). Reductions in
19 particle size have been observed to yield higher rates of enzyme hydrolysis of cellulose in
20 a previous study by Dasari and Berson (2007). In their study particle sizes of $33\ \mu\text{m}$ to
21 $850\ \mu\text{m}$ were investigated, and up to 55% more glucose was produced from cellulase
22 hydrolysis of the smallest particles than for the largest particle sizes (Dasari and Eric
23 Berson, 2007). Whilst the particle sizes used in this study were considerably larger than

1 those used by Dasari and Berson (2007), the same principle would be expected to apply.
2 This suggests that the enzymes used in the EHT test were able to access biodegradable
3 substrate even in the centre of 10 mm particles.

4 The use of a smaller sample particle size was also expected to generate a more
5 uniform sample, and therefore provide lower variation between sample replicates. A
6 greater surface area: particle volume ratio allows higher enzyme coverage, and it was
7 postulated that for larger particle sizes the enzymes would be able to access the middle of
8 the substrate to varying degrees in the relatively short incubation time, and that it was
9 more likely that all available substrate will be hydrolysed in the given timescale (20 h) for
10 the smaller particles. This however did not prove to be the case.

11 The use of samples of a smaller particle size resulted in a higher DOC release at
12 each phase, however in each case (except fines P2) the differences between DOC release
13 between <10 mm and <2 mm were not statistically significant ($p \geq 0.1$). The difference
14 between DOC release at P2 for the Fines sample at <10 mm and <2 mm was significant
15 ($p < 0.05$), however the difference at P3 was not ($p > 0.1$). This would suggest that the EHT
16 would not benefit from further sample grinding from <10 mm (currently the DR4 and
17 BMc requirement) to <2 mm. As shown in Table 2, since the coefficient of variation (C_v)
18 for the samples of smallest particle size (<2 mm) is higher than that of the larger particle
19 sizes (<10 mm), there is no benefit in terms of improved consistency. This means that
20 the sample preparation currently used for the DR4 and BMc methods (grinding to <10
21 mm) is suitable for the EHT.

22 Whilst not statistically significant, for the MSW and SRF materials a greater
23 amount of DOC was released from the sample during autoclaving for the <2 mm samples

1 than for <10 mm. This supports the findings in previous studies, where it was observed
2 that the hydrolysis of hemicellulose and, to an extent, cellulose and lignin is catalysed by
3 mild acid under high temperatures (Jacobsen and Wyman, 2000; Nguyen et al., 1998;
4 Torget et al., 1990). The effects of a high energy pre-treatment process (such as
5 autoclave) of waste material was also reported to cause the slowly biodegradable
6 materials to be more accessible and easier to decompose (Tojo et al., 2007). However as
7 the difference resulting from additional grinding was not statistically significant, this
8 extra sample preparation is not necessary for the EHT method.

9 As shown by Wagland *et al* (2008) the EHT and DR4 correlate, to varying
10 degrees, with the BM100/BMc. However since each test method has limitations and
11 measures different parameters, a correlation of $r = 1.0$ is very unlikely. The BMc test is
12 sensitive to highly biodegradable substrates, in which acidic conditions can inhibit
13 methanogenesis (Environment Agency, 2005), thus affecting the final results. The DR4
14 test method is responsive to readily biodegradable material, but due to its short duration
15 can potentially underestimate the presence of slowly biodegradable materials. The DR4
16 therefore only measures the initial rate of biodegradation (Godley et al., 2007a; Godley et
17 al., 2007b). The EHT doesn't have the biological disadvantages associated with the DR4
18 and BMc methods, however may not measure the full extent of biodegradation in the
19 given timescale because of the inherent limitations associated with providing a suitably
20 diverse range of enzymes and conditions to ensure their sustained activity. As discussed
21 by Wagland *et al* (2008), the DOC released at P2 may contain varying quantities of DOC
22 comprising biodegradable and non-biodegradable fractions, likewise P3 may contain
23 DOC of both biodegradable and non-biodegradable natures, and therefore further

1 investigation is required to sufficiently determine only the biodegradable DOC. All
2 currently available test methods have their limitations. However, this extended
3 comparison with accepted methods suggests that the EHT is able to produce comparable
4 and consistent results and therefore shows promise as an operational monitoring tool.
5 Further development of the test is needed, for instance the use of a more complex enzyme
6 mixture to ensure that the biodegradability of a wide range of materials including fats and
7 proteins is measured.

8

9 **4. Conclusions**

- 10 • Each of the biodegradability methods used in this study generated consistent values of
11 relative biodegradability for the three sample types tested.
- 12 • The fines material was found to be significantly more biodegradable than the MSW
13 input and SRF output materials in all three test methods. It was found that the BMc
14 test indicated a smaller difference in MSW and SRF biodegradability relative to the
15 fines samples. This was attributed to the likelihood that the BMc was more likely to
16 have hydrolysed a higher proportion of the more slowly biodegradable compounds
17 present in the MSW input and SRF samples
- 18 • The use of particles of <2 mm in the EHT test did not release appreciably higher
19 amounts of DOC from the waste samples tested. The variation between sample
20 replicates for the EHT was significantly higher where sample sizes of <2 mm were
21 analysed compared to sizes of <10 mm. Therefore it is not necessary to grind the
22 samples from the <10 mm used in the BMc and DR4 methods to <2 mm.

23

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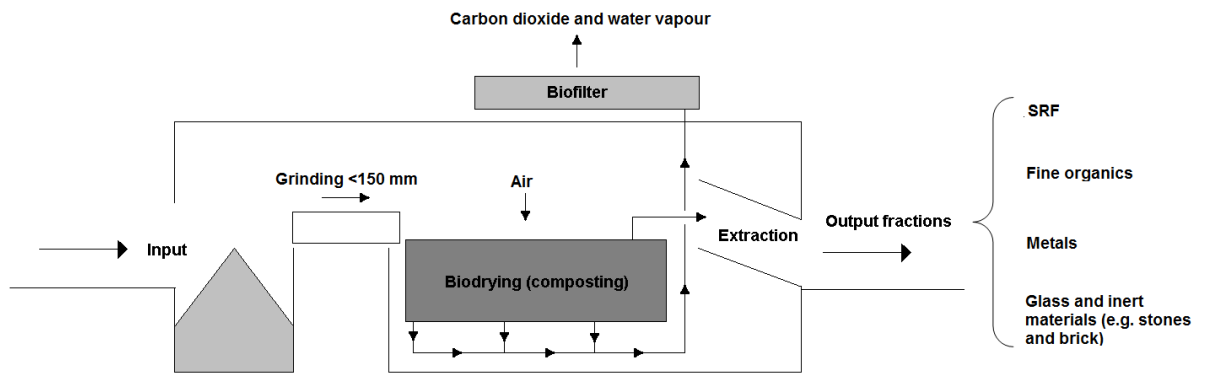
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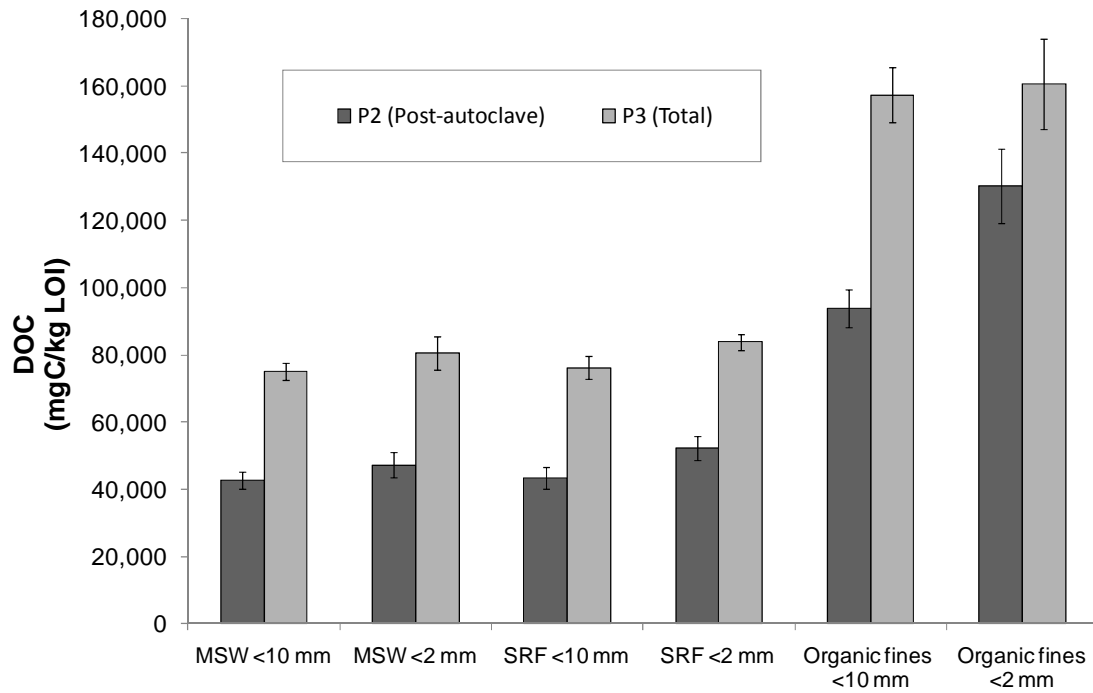
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Figure 1. Schematic diagram of MBT process



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3 **Figure 2.** Average EHT results for each of the waste fractions, indicating post-autoclave,
 4 total and enzyme-only DOC. Error bars shown as the standard error.

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