DEFRA

WR0110 CHARACTERISATION OF RESIDUES FROM INDUSTRIAL PROCESSES AND WASTE TREATMENT

ANNEX H – ENZYMATIC HYDROLYSIS TEST

MAY 2009

FOREWORD

This Annex to the SID5 research project final report for project WR0110 provides further background to the enzymatic hydrolysis test.

The method development and testing was undertaken at Cranfield University as part of a PhD project by Stuart Wagland under the supervision of Dr Richard Smith and Dr Sean Tyrrel. A PhD thesis has been submitted.

H1 METHODOLOGY

The waste samples were sorted to remove glass, metals, plastics and inert materials and only the biodegradable material were 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 these samples using standard procedures (EN12879:2000).

There are many established biodegradability test methods, with a temporal range of just ~ 2 hours to over 100 days. These tests were designed for varying purposes (i.e. compost maturity, biogas potential etc.). Following a thorough literature review and evaluation of the current existing biodegradability test methods (Godley *et al*., 2003; Godley *et al*., 2004; Wagland *et al*., 2007; Wagland *et al*., 2009) a potential surrogate test method was formulated based on the enzymatic hydrolysis of cellulose.

Cellulose constitutes a large proportion of MSW, and contributes around 90% of all biogas released from a landfill site (Rodriguez *et al.*, 2005). Therefore it was concluded that a test method that utilised the readily available cellulose could potentially offer a more reliable, cost-effective and rapid testing method.

The enzymatic hydrolysis test was developed to provide an optimum pH of 3.75 at 50°C (Figure H1) using a standard α-cellulose sample (Wagland *et al.*, 2007).

The enzyme test method consists of three phases of measurement (Figure H1) and is described as follows.

Phase 1. 5 g of sample that had undergone LOI determination were placed in a 250 ml Erlenmeyer flask. 100 ml 0.37 M phosphate pH buffer was 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 sterilize 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 results were converted to DOC (dissolved organic carbon) (mg C/l) by assuming a COD/C ratio of 2.67 (based on the molecular formula of cellulose) and then expressed in terms of mg of carbon per kg of the sample (LOI) to provide the final values.

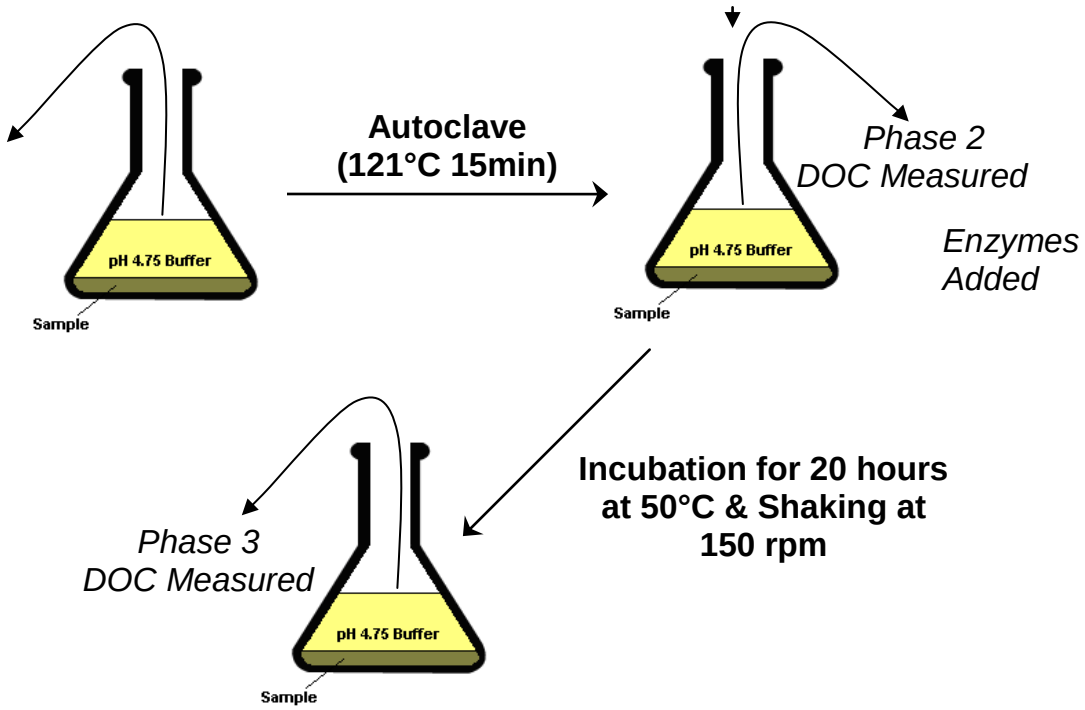

Figure H1. EHT Test method

H2 RESULTS

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 autoclaving. 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 H2.

The non-enzymatic DOC (Phases 1 and 2) for wastes that have undergone extended biological treatment (i.e. 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 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-biological 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).

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 in the EHT is expected, and the actual values (Figure H2) 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 autoclaved MSW. Therefore the lower DOC from enzymatic hydrolysis (Phase 3) observed for the AD treated sample might then be expected.

The DR4 test results for the turkey feathers indicated that the autoclaving treatment of the feathers significantly increased the biodegradability results. This is also observed in the enzyme hydrolysis test; however, the BM100 showed that the autoclaving treatment reduced the biodegradability of the waste sample.

Table H1. DOC released at each phase in the EHT

Sample	DM % wet wt	LOI % DM	DOC (mg C/kg LOI) Phase 1	Phase 2	Phase 3
Commercial cellulose	94.0	99.7	585	1130	53658
Construction wood waste	77.5	91.4	1310	12610	16032
Autoclaved construction wood waste	63.9	90.7	4957	14414	17090
Packaging waste	42.6	93.6	764	3119	26362
Autoclaved packaging waste	40.4	93.1	2882	5936	22962
Green waste (untreated)	40.4	73.2	9828	25940	32179
Partially composted green waste	44.2	62.1	11290	24711	33293
Kitchen and green waste (untreated)	35.1	65.3	12934	29764	40583
Partially composted kitchen and green waste	38.3	68.0	7049	16470	17962
Composted kitchen and green waste	51.7	60.9	3526	23928	25774
Organic fibre from autoclaved MSW	50.5	76.9	11816	29361	44453
AD treated fibre from autoclaved MSW	29.2	72.5	1446	6736	7967
Turkey feathers	35.5	98.9	8128	11677	16837
Autoclaved turkey feathers	38.3	96.2	7757	27681	32027
MBT 1 feed MSW	56.0	71.6	8106	23469	49696
MBT 1 feed MSW	51.1	71.8	5461	19389	42291
MBT 1 treated MSW	57.1	77.2	12966	34494	51354
MBT 1 reject	58.0	72.5	14427	35403	58913
MBT 1CLO	51.9	57.7	2552	21139	37761
MBT 1 CLO	46.9	59.6	4597	19795	48515
Stabilised green waste compost <10 mm	71.9	29.2	3244	46636	61976
Stabilised green waste compost <25 mm	66.6	28.9	3763	57235	58235
MSW input windrow MBT	94.4	73.4	13330	33382	61125
Fully composted MSW	66.3	29.6	1115	27857	30621
MSW compost	69.3	26.7	880	36762	35836
MSW input to AD	96.5	58.7	17152	80277	141545
Output MSW AD	31.5	56.2	493	7154	8663
MSW input	95.5	65.8	17070	73769	114116
Composted MSW	50.3	58.3	3665	28954	34889
Composted MSW <8 mm	58.4	49.9	10146	42888	54517
Fresh MSW	94.4	39.3	14063	79844	104210
Composted MSW <15 mm	75.3	23.4	9515	41184	47288
Fresh pizza waste	43.1	95.2	3207	78532	104870
Fresh fish waste	38.1	73.2	1267	19648	28145
Fresh mix fish, peat, wood, green waste	97.1	58.4	12238	68049	87125
Partially composted mixed fish/woodchip/green waste	48.9	53.8	3785	27360	27435
Fully composted mixed fish/woodchip/green waste	65.0	34.3	1821	27949	25779
Sewage sludge	18.4	60.8	2193	14047	16265

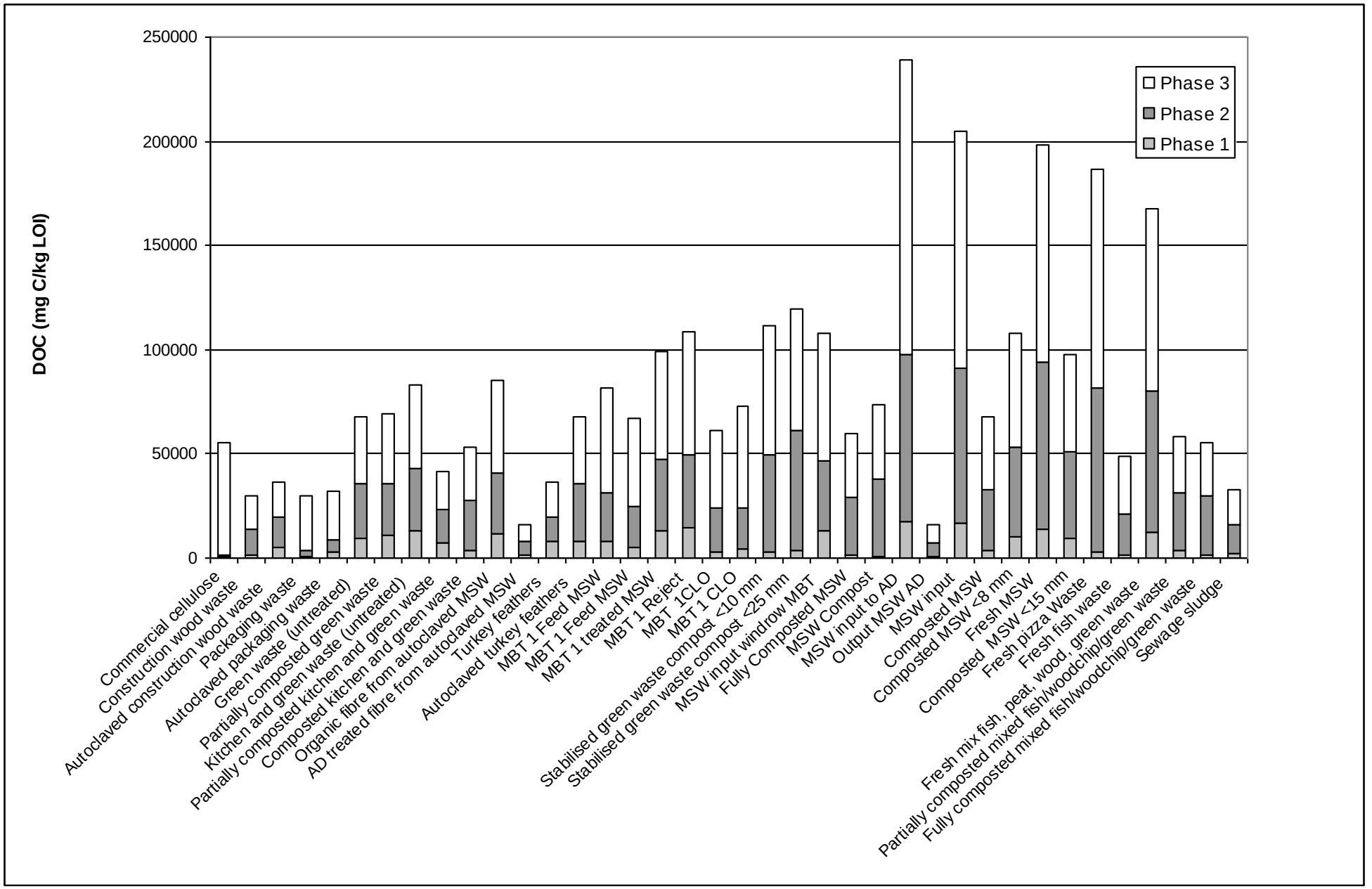

Figure H2. DOC released at each phase of the EHT

If the Enzymatic Hydrolysis Test is to be accepted as a suitable routine test method, then correlation with the reliable BM100 test method is considered necessary.

As discussed previously, there is no clear manner to represent the data in terms of using the entire DOC released, or the DOC released through enzymatic hydrolysis alone. However there is no correlation found when the entire DOC is used, whereas the correlation from the enzyme hydrolysis DOC gives a clear linear correlation (Figure H3). As the commercial cellulose yields unexpectedly low results in the BM100, this data has been excluded from the correlation.

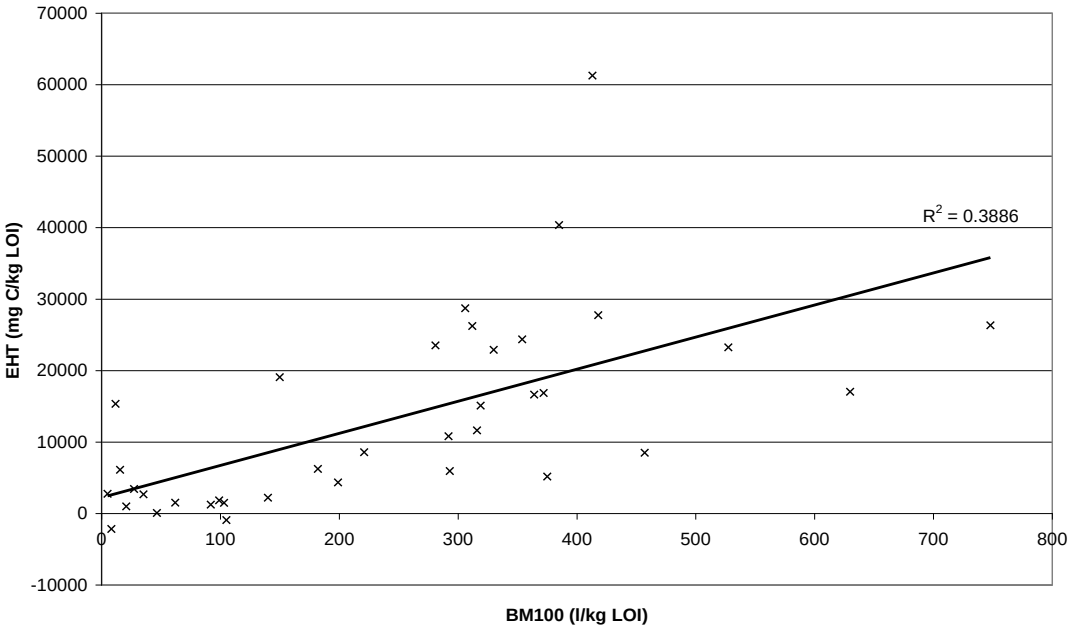

Figure H3. DOC released in the enzyme hydrolysis phase of the EHT against the results of the BM100 test.

Using the SPSS software, the data can be statistically analysed. The correlation coefficient calculated (r^2= 0.39) for the complete dataset (N=37) suggests that both measures are significantly correlated (Students *t*-Test for 99% significance and $\rho<0.001$).

However, when comparing the MSW-derived samples (N=20) (Figure H4) a correlation coefficient of r^2=0.57 is calculated, which can also be assumed to be a significant correlation (Students *t*-Test for 99% significance and $\rho<0.001$).

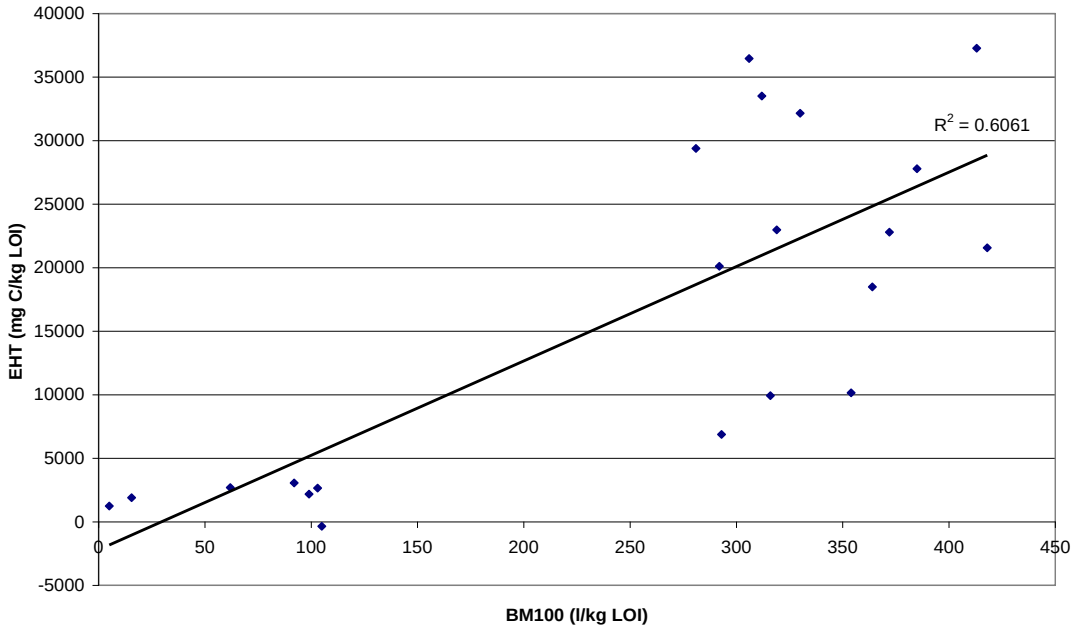

Figure H4. DOC released in the enzyme hydrolysis phase of the EHT against the results of the BM100 test (BMW-derived samples only).

Conclusion

The EH test has been used to measure the amount of DOC released for a range of treatment process wastes.

A correlation between the quantities of DOC released during EHT and BM100 tests has been established for these waste samples and this has been compared with the correlation between the DR4 and the BM100 test. The EHT provided a better correlation with the BM100 than the DR4, and provided biodegradability data in much shorter time scales (24 hours) than other tests.

Further development of the EHT method is required to ensure its robustness and versatility.

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