

1 **Evaluating leachate recirculation with cellulase addition to enhance waste biostabilisation and**  
2 **landfill gas production**

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4 Frank, R. R.<sup>1,2</sup>, Davies, S.<sup>3</sup>, Wagland, S.T.<sup>1</sup>, Villa, R.<sup>1</sup>, Trois, C.<sup>2</sup>, Coulon, F.<sup>1,2</sup>

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6 <sup>1</sup> School of Energy, Environment and Agrifood, Cranfield University, MK43 0AL, United Kingdom

7 <sup>2</sup> Centre for Research in Environmental, Coastal and Hydrological Engineering (CRECHE), School  
8 of Engineering, University of KwaZulu-Natal, Howard College Campus, Durban, 4041, South  
9 Africa.

10 <sup>3</sup> Viridor Waste management Ltd, Viridor House, Priory Bridge Road, Taunton, Somerset, TA1  
11 1AP.

12

13 \* Corresponding author: [f.coulon@cranfield.ac.uk](mailto:f.coulon@cranfield.ac.uk) ; Tel: +44(0)1234 754 981

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15

16 **Abstract:** The effect of leachate recirculation with cellulase augmentation on municipal solid waste  
17 (MSW) biostabilisation and landfill gas production was investigated using batch bioreactors to  
18 determine the optimal conditions of moisture content, temperature and nutrients. Experimentation  
19 was thereafter scaled-up in 7 L bioreactors. Three conditions were tested including (1) leachate  
20 recirculation only, (2) leachate recirculation with enzyme augmentation and (3) no leachate  
21 recirculation (control). Cumulative biogas production of the batch tests indicated that there was  
22 little difference between the leachate and control test conditions, producing on average 0.043 m<sup>3</sup>  
23 biogas kg<sup>-1</sup> waste. However the addition of cellulase at 15·10<sup>6</sup> U tonne<sup>-1</sup> waste doubled the biogas  
24 production (0.074 m<sup>3</sup> biogas kg<sup>-1</sup> waste). Similar trend was observed with the bioreactors. Cellulase  
25 addition also resulted in the highest COD reduction in both the waste and the leachate samples  
26 (47% and 42% COD reduction, respectively). In both cases, the quantity of biogas produced was  
27 closer to the lower value of theoretical and data-based biogas prediction indicators (0.05-0.4 m<sup>3</sup>  
28 biogas kg<sup>-1</sup> waste). This was likely due to a high concentration of heavy metals present in the  
29 leachate, in particular Cr and Mn, which are known to be toxic to methanogens.

30 The cost-benefit analysis (CBA) based on the settings of the study (cellulase concentration of  
31 15×10<sup>6</sup> U tonne<sup>-1</sup> waste) showed that leachate bioaugmentation using cellulase is economically  
32 viable, with a net benefit of approximately €12.1 million on a 5 Mt mixed waste landfill.

33

34 **Keywords:** leachate circulation; enzyme augmentation; waste biostabilisation; landfill bioreactor

35

## 36 **1. Introduction**

37 In recent years, advances in the field of integrated waste management and better understanding of  
38 landfill processes, such as municipal solid waste (MSW) decomposition, has led to a re-evaluation  
39 of traditional landfill management practices (Hettiaratchi et al., 2015; Warith, 2002). In particular,  
40 there has been focus on the improvement of existing landfill technologies from a  
41 storage/containment based operation towards more sustainable and resource efficient activities  
42 (Townsend et al., 2015; Warith, 2002). Several methods have been studied over the past years to  
43 facilitate and enhance waste degradation within a landfill site. These include waste shredding, waste  
44 compaction, pH adjustment, nutrient balance, sludge addition and leachate recirculation (Jayasinghe  
45 et al., 2011; Cirne et al., 2007; Sponza and Agdad, 2005).

46 In particular, the recirculation of leachate as part of the 'bioreactor landfill' model has received  
47 much attention due to its widespread success, in both small and large scale applications (Liu et al.,  
48 2014; Nair et al., 2014; Rastogi et al., 2014; Reinhart et al., 2002; Reinhart, 1996 a & b; Lagerkvist  
49 and Chen, 1993). The recirculation of leachate facilitates the rapid transformation and degradation  
50 of landfilled waste which promotes landfill space reduction and maximises biogas production.

51 These benefits can be further used as a source of renewable energy and reduces environmental  
52 disamenity (Nair et al., 2014; Liu et al., 2014; Rastogi et al., 2014; Reinhart et al., 2002; Clarke,  
53 2000). It further closes the resource loop allowing leachate to be used towards more economically  
54 and environmentally beneficial activities (Xu et al., 2014; Reinhart et al., 2002).

55 The degradation of the waste in a landfill site is facilitated by a consortium of microorganisms  
56 (Barlaz et al., 1990) and therefore any environmental modifications or bioengineered solutions need  
57 careful considerations. Leachate recirculation can affect the active microbial communities as the  
58 introduction of leachate can affect pH, temperature, oxidation/reduction potential as well as  
59 complex biochemical reactions necessary for microbial waste degradation (Mudhoo and Kumar,  
60 2013; Barlaz et al., 1990). Furthermore, the recirculation of leachate can also introduce a  
61 combination of heavy metals, contaminants and xenobiotics in varying amounts which affect

62 microbial communities (Chen et al. 2008; Bilgili et al., 2007a). This has been highlighted in a  
63 number of key studies (Zornoza et al., 2015; Mudhoo and Kumar, 2013; Frostegård et al., 1993).  
64 The most common heavy metals found in leachate are: iron (Fe), cadmium (Cd), chromium (Cr),  
65 copper (Cu), zinc (Zn), nickel (Ni) and lead (Pb) (Mudhoo and Kumar, 2013; Bilgili et al., 2007a).  
66 Fe has been reported to have stimulatory effects on microbial communities involved in waste  
67 degradation at concentrations below 8.1 mmol. L<sup>-1</sup> and be inhibitory at concentrations above  
68 (Gonzalez-Silva et al., 2009). Cu, Zn, Cd and Pb have been shown to be highly toxic to microbial  
69 biochemical reactions. They increase in their inhibitory effect as follows: Pb < Zn < Cu < Cd  
70 (Mudhoo and Kumar, 2013). Therefore, while the recirculation of leachate results increases  
71 moisture content required for optimal waste degradation, its introduction also requires stringent  
72 process control to minimise its associated deleterious effect on the active microbial community.  
73 Another important feature to take into consideration when evaluating technologies to facilitate  
74 waste degradation is the waste composition of landfill sites. Approximately 40-50% of landfill  
75 space is comprised of paper and cardboard, of which lignocellulose is a major component (Yuan et  
76 al., 2014; Kovács et al., 2009). Lignocellulose is composed of carbohydrate polymers, cellulose  
77 (most prominent) and hemicellulose as well as aromatic polymer, lignin (Yuan et al., 2014). Within  
78 a waste mass, lignocellulosic materials are considered recalcitrant as difficult to degrade under  
79 anaerobic conditions (Pareek et al., 2008). A technique to enhance the degradation of residual waste  
80 fractions, with particular application towards difficult to degrade materials, is the addition of  
81 enzymes (Zheng et al., 2014; Jayasinghe et al., 2012, 2011; Lin et al., 2010; Romano et al., 2009).  
82 In particular, degradation of cellulose to soluble sugars and glucose is catalysed by a group of  
83 enzymes called cellulases, which include: endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase and β-  
84 glucosidase. Industrial grade cellulases have been successfully used for lignocellulose degradation  
85 in many industries (Kudah et al., 2011).  
86 Enzymes, however, have historically been an expensive commodity which has hindered its  
87 application in waste management practices. Recent developments in biotechnology coupled with

88 reduced costs of manufacturing (particularly in China) have led to the use of enzyme to improve  
89 landfill gas production to be considered.

90 The waste used in this work comes from a site which has recorded declining biogas production over  
91 the past several years, even when taking into account the changes in waste composition prescribed  
92 by the Waste Framework Directive (2008/98/EC). The aim of the work was to investigate a cost  
93 effective and easy treatment to increase biogas output in landfill by examining the effect of leachate  
94 recirculation with and without a low-cost cellulase addition on waste stabilisation and biogas  
95 production. Additionally, a cost-benefit analysis (CBA) of leachate recirculation with enzyme  
96 addition was completed in order to inform commercial strategy. To the best knowledge of the  
97 authors, leachate recirculation with enzyme augmentation is a relatively new concept and to date  
98 there is little information available on its viability or commercial applicability at landfill site (Cirne  
99 et al., 2007; Lagerkvist and Chen, 1993).

100

## 101 **2. Methods**

### 102 **2.1. Waste and leachate origin and sampling procedure**

103 Ten municipal solid waste samples were collected from five drilled cores at depths of 10, 15, 20 and  
104 25 m from a landfill site in the UK opened in 1992 and closed in 2012. The age of the waste  
105 material used in the work ranges approximatively between 5 and 20 years old. Details of the landfill  
106 site are presented in Table 1. The site was selected on the basis that there has been declining biogas  
107 production at the site over the past several years (from 3000 to 2200 m<sup>3</sup> h<sup>-1</sup>) and the reason for this  
108 has been to date largely unaccounted for. The site therefore represented an opportunity to evaluate  
109 the influence of alternative site management strategies on biogas production. Untreated leachate  
110 used for recirculation was collected from the same landfill site in 2014 and was stored in a cold  
111 room at 4°C until use.

112

113 **2.2. Waste and leachate characterisation**

114 **2.2.1. Waste composition**

115 Waste composition was analysed according to international standard ASTM D 5231-92 (2003)  
116 (AbdAlqader and Hamad, 2012; Gidarakos and Ntzamilis, 2006). The composition of plastics,  
117 paper, organic, textiles, glass and metal and was determined by manually weighing each component  
118 of the total waste fraction using a kitchen scale.

119

120 **2.2.2. TS, TSS, VS, pH and sCOD**

121 To obtain a representative waste sample for characterisation, waste samples from all depths were  
122 combined, then cone and quartered according to Rubio and Ure (1993). Solid waste and leachate  
123 was characterised in terms of total solids (TS), volatile solids (VS), soluble chemical oxygen  
124 demand (sCOD) and pH according to Standard Analytical Methods published by the American  
125 Public Health Association (APHA, 2005). sCOD was conducted using Merck COD test kits (range  
126 100-1500 mg. L<sup>-1</sup> or 500-10 000 mg. L<sup>-1</sup>) in duplicate due to reliability of test kits while all other  
127 tests were conducted in triplicate. TS, VS, sCOD and pH were determined before and after  
128 completion of the pilot scale bioreactors experiment in order to understand the effect of leachate  
129 recirculation on the physicochemical conditions of the system. Total suspended solids (TSS) were  
130 determined by filtering a known amount of leachate through glass microfibre filter paper (70 mm  
131 diameter). The filter was then dried in an oven at 105°C for 24 hours and weighted.

132

133 **2.2.4. Field capacity**

134 Field capacity (FC) test was conducted to determine the amount of leachate that would be required  
135 to bring the waste mass to saturation. FC test was adapted from Orta de Velásquez et al. (2003).  
136 Briefly, 100 g mixed waste was placed into a 1 L bottle, to which 500 ml distilled water was added.  
137 The bottles were placed on a shaker for 24 hours. Water from the bottle was allowed to drain for 8  
138 hours into a measuring cylinder, until no excess water was observed. The amount of water

139 recovered from each bottle was recorded and the amount of water retained per unit waste was  
140 calculated. Experimentation was conducted in quadruplicate. FC was calculated according to  
141 Equation 1 (Orta de Velásquez et al., 2003).

142

$$143 \quad Cc = \frac{\left(\frac{H \times PV \times V}{100}\right) + (Si - Di) \times d}{PV \times V \times \left(1 - \frac{H}{100}\right)} \quad \text{Equation 1}$$

144

145 where: Cc = Field Capacity (kg H<sub>2</sub>O /kg dry waste); Si = volume of water added to the bottle at the  
146 beginning of the test (L); Di = volume of water extracted from bottle (L); d = density of waste (kg  
147 L<sup>-1</sup>); H = % MC of waste / 100; PV = weight density of solid waste; V = volume of bottle occupied  
148 by waste.

149

## 150 2.2.5. Metals analysis

151 The metals content of the leachate were analysed according to USEPA method 3015A. Specifically,  
152 Fe, Zn, Cu, Pb, Ni, Cd, Cr and Mn were determined by first pre-digesting 30 ml leachate with 1.5  
153 ml trace metal grade nitric acid and placing in a microwave (Type Mars Xpress) for 30 minutes. Fe  
154 was analysed using Atomic Absorption Spectroscopy (PerkinElmer Analyst 800 AAS instrument)  
155 and all other metals were analysed using an ICP-MS (PerkinElmer Elan 9000 AAS ICP-MS).

## 156

## 157 2.3. Theoretical and empirical (BMP) determination of biogas potential

### 158 2.3.1 Theoretical biogas

159 The potential biogas production was predicted prior to the experimentation using the studies of  
160 Scarlat et al. (2015), Aguilar-Virgen et al. (2014) and Zhou et al. (2011), based on the IPCC  
161 formula:

162

$$Lo = MCF \times DOC \times DOC_F \times F \times \frac{16}{12}$$

163 where  $L_0$  = methane generation potential ( $\text{m}^3 \text{CH}_4/\text{tonne waste}$ ); MCF=Methane Correction Factor  
164 (dimensionless); DOC=Degradable organic carbon in waste under aerobic conditions  
165 (dimensionless);  $\text{DOC}_F$  = fraction of DOC decomposing under anaerobic conditions  
166 (dimensionless);  $F$  = fraction of  $\text{CH}_4$  in the landfill gas (dimensionless); 16/12 is the stoichiometric  
167 factor to convert carbon into  $\text{CH}_4$  (dimensionless).

168

169 Biogas potential of MSW reported in the literature is highly variable. These studies found biogas  
170 production in MSW to be between  $0.05 - 0.40 \text{ m}^3 \text{ kg}^{-1}$  waste. For the purpose of this scenario the  
171 most conservative estimate of  $0.05 \text{ m}^3 \text{ kg}^{-1}$  waste was used.

172

### 173 **2.3.2 BMP tests**

174 The biogas potential was determined empirically using the biochemical methane potential (BMP)  
175 tests according to WRAP guidelines (WRAP, 2010). Briefly the BMP tests were performed by  
176 mixing 20 g loss on ignition (LOI) equivalent of the organic and paper waste fraction with 40 g LOI  
177 equivalent of digested primary sludge (1:2 ratio) taken from the local wastewater treatment plant in  
178 Milton Keynes, UK. Sludge was used as the seed for the BMP tests to facilitate methane production  
179 as well as reduce the lag phase. The bottles were filled with water, leaving an adequate headspace  
180 of 200 ml, and flushed with  $\text{N}_2$  gas to create anaerobic conditions before being incubated in a water  
181 batch at  $38^\circ\text{C}$ . The volume of biogas was measured volumetrically daily until no more biogas was  
182 produced. The methane concentration of the biogas was measured using a gas analyser (Servomex  
183 1440 GA). Two control tests were conducted, which included: sludge alone and sludge + cellulose  
184 ( $10 \text{ g kg}^{-1}$ ) (both in the absence of waste). The biogas production of the inoculum was removed  
185 when calculating the amount of the biogas produced by the waste samples. All tests were conducted  
186 in duplicate and results were converted to standard temperature and pressure (STP).

187



## 188 **2.4. Biogas improvement with leachate and enzymes**

### 189 **2.4.1 Batch bioreactors: leachate addition (with and without enzymes)**

190 Batch tests were conducted to determine the effect of leachate addition with and without addition of  
191 cellulolytic enzymes on biogas production under optimal conditions (see Figure 1). Six bottles were  
192 setup into three test groups: (1) waste and leachate only; (2) waste, leachate and cellulase; (3) waste  
193 with no leachate, used as control. The amount of enzyme added was equivalent to 15 million U  
194  $\text{tonne}^{-1}$  waste as it was suggested that this is the upper enzyme concentration limit that can be used  
195 for leachate recirculation by Jayasinghe et al. (2012). In our case 10 mg of the enzyme were added  
196 to each bottle which contained 3000 U of endo- $\beta$ -1,4-glucanase; 200 U of glucoamylase,  $\alpha$  amylase  
197 and pullulanase; 30,000 U of xylanase and 150,000 U of  $\beta$ -glucanase.

198 200 g waste was shredded to a particle size of <10 mm, mixed with digested primary sludge at a 4:1  
199 (w/w) ratio and was placed in a 1 L bottle. This ratio was chosen to provide more realistic  
200 conditions compared to those provided for the BMP, the slower kinetics of these experiments aim at  
201 increasing the treatments impact. The bottles were filled up to 800 ml (200 ml headspace) with  
202 sterilised distilled water and flushed with nitrogen gas to set anaerobic conditions. They were  
203 secured with an air-tight rubber cap fitted with a single port for gas measurement. Bottles were  
204 incubated in a water bath at 38°C for 81 days. The quantity of biogas produced was measured  
205 weekly by capturing gas in a 2 L gas bag and measuring the volume of gas using a syringe. The  
206 methane content of the biogas was measured weekly using a Servomex 1440 GA gas analyser.

207

### 208 **2.4.2. Enzyme characteristics**

209 The lignocellulose material (paper, wood etc) contained in municipal waste is not so quick to  
210 degrade under anaerobic condition. Cellulose and hemicellulose are the two major components of  
211 this material, where cellulose represents generally about 40–50% of the biomass by weigh while  
212 hemicellulose represents 20–40% of the material by weight. Cellulase was therefore chosen to  
213 breakdown the major component of the material in a cost-effective manner. Cellulase CEL 30,

214 produced from *Trichoderma reesi*, was purchased from Sinobios (China). The optimum pH and  
215 temperature ranges were 4.0 to 6.0 and 40 to 60°C, respectively.

216 Cellulose hydrolysis involves the synergistic action of three types of cellulases including endo-β-  
217 1,4-glucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Cellulase  
218 CEL 30 is a feed grade preparation containing several of these enzymes with the following  
219 activities:

- 220 • Endo-β-1,4-glucanase (CMC) ≥300,000 U/g;
- 221 • FPA filter paper assay (glucoamylase, α amylase and pullulanase) ≥20,000 U/g ;
- 222 • Xylanase ≥3,000,000 U/g;
- 223 • β-glucanase ≥15,000,000 U/g.

224

#### 225 **2.4.3. Continuous bioreactors: leachate recirculation (with and without enzymes)**

226 Six water and gas-tight anaerobic bioreactors made from acrylic (PVC) cylinder were used in the  
227 study (see illustrative set-up in Figure 3). The dimensions of the columns were as follows: thickness  
228 = 8 mm, internal diameter = 110 mm and height = 0.75 m (volume = 0.00713 m<sup>3</sup>). The reactor  
229 consisted of three ports. One port (bottom) served as a leachate outlet pipe while the other two ports  
230 (top) served as a leachate inlet and gas outlet pipe, respectively. Approximately 0.2 m (7% of  
231 column volume) of gravel was layered at the bottom of column to prevent clogging of the leachate  
232 outlet pipe. Gravel with a particle size of 14-20 mm was placed at the bottom, and above that, 10  
233 mm and 2 mm gravel respectively. 0.5 m (67% of column volume) was packed with waste while 0.2  
234 m (25 % of column volume) was left as headspace for gas accumulation at the top of the column.  
235 Waste was mixed with digested sludge at a ratio of 4:1 prior to insertion into the column in order to  
236 introduce a consortium of active microorganisms, which would reduce the lag time for biogas  
237 production. Sludge digestate was obtained from the local wastewater treatment plant (Milton  
238 Keynes, UK) and was sampled 2 days prior to mixing. A waste density of 800 kg m<sup>-3</sup> was used as it  
239 was found to be the highest possible density that would allow the desired recirculation rate before

240 clogging occurred. Waste density at the landfill site was approximately  $900 \text{ kg m}^{-3}$  (Table 1) and  
241 therefore an attempt was initially made to simulate this waste density in the bioreactors. However  
242 significant leachate clogging was observed at all densities above  $800 \text{ kg m}^{-3}$ . Each bioreactor  
243 contained 3.8 kg of shredded waste (particle size  $<10 \text{ mm}$ ). The waste vertical profile according to  
244 depth of the drilled samples was simulated in each bioreactor to mimic the conditions of the landfill  
245 site.

246 Leachate was recirculated at  $200 \text{ ml d}^{-1}$  for a period of 130 days by being actively pumped from the  
247 main reservoir to the leachate inlet connection. Recirculation of leachate through the waste mass  
248 occurred by gravity until leachate exited through the leachate outlet connection, back into the  
249 airtight leachate reservoir.

250 The conditions tested for the bioreactors were the same as those of the batch tests. Six columns  
251 were divided into three groups each in duplicate, as follows: (1) leachate recirculation only; (2)  
252 leachate recirculation with the addition of cellulase (activity  $300 \text{ U mg}^{-1}$  at  $15 \times 10^6 \text{ U tonne}^{-1}$  waste);  
253 and (3) no leachate recirculation used as control.

254

## 255 **2.5. Statistical analysis**

256 The statistical difference in biogas production between the three conditions tested was evaluated  
257 using one-way analysis of variance (ANOVA) tests of the Statistical Package for Social Sciences  
258 (SPSS version 22). All statistical tests satisfied assumptions of normality using the Kolmogorov-  
259 Smirnov test and homogeneity of variance using the Levene's test as recommended in Lunney  
260 (1970). Significance level was set at 0.05.

261

## 262 **2.6. Cost-benefit analysis (CBA) of enzyme addition to leachate recirculation**

263 A simplified CBA was conducted to evaluate the economic viability of cellulase addition to an  
264 existing leachate recirculation operation. The CBA was based on the CBA on leachate recirculation  
265 described by Clarke (2000). Further to this, the recent works from Le et al. (2015) and Townsend et

266 al. (2015) were taken into account in developing the CBA scenario and costing. The CBA took into  
267 account the sum of increased biogas retrieval, landfill space savings, reduced environmental  
268 impacts and reduced post-closure costs minus capital and operational costs. The costs and benefits  
269 (including environmental benefits) were itemized and compared in order to assess opportunities and  
270 risks associated with the technology.

271

## 272 **3. Results**

### 273 **3.1. Waste composition**

274 The composition of MSW samples from the selected landfill site showed no clear trend associated  
275 to landfill depth or drilling core (Table 2). This indicated that there was an uneven distribution of all  
276 waste components throughout the landfill site.

277 While no other studies have assessed the vertical distribution of organic waste within a landfill site,  
278 it was expected that a higher amount of organic material would be present at the surface layers as  
279 waste closer to the top would be newer than waste obtained from greater depths and therefore has  
280 had less time to degrade.. The uneven distribution of organic waste throughout the landfill site  
281 coupled with a high organic fines and paper composition (between 50 and 87%) motivated the  
282 research aim to assess leachate recirculation for increasing waste degradation within the landfill  
283 site.

284

### 285 **3.2. Waste and Leachate characteristics**

#### 286 **3.2.1. Waste characterisation**

287 Waste used in the batch and bioreactor experiments was characterised in order to understand the  
288 nature of the waste and evaluate the physicochemical changes which will occur as a result of the  
289 treatments (Table 3). The MC of 37 % (wt) is considered slightly below sufficient, being > 40%, to  
290 promote waste degradation and biogas production (Emkes et al., 2015; USEPA, 2003). The ‘dry’  
291 conditions of the landfill site would therefore lend itself well to the assessment of leachate

292 recirculation strategies for biogas enhancement. This is because elevated levels of moisture allows  
293 volatile fatty acids (VFA), the intermediate products of organic waste degradation, to be diluted and  
294 therefore avoiding inhibition on the methanogenesis, thus resulting in an increased rate of biogas  
295 production (Qu et al., 2009).

296 The VS content of 32% was in agreement with typical ranges observed for MSW (Chiemchaisri et  
297 al., 2006). The determination of VS is particularly well suited for informing biological treatments,  
298 as it provides a first approach of the organic matter available to be biodegraded and furthermore its  
299 can be used as a process control parameter (Peces et al., 2014). The VS value of the waste therefore  
300 indicated that the waste had a sufficient organic strength to be further degraded which motivated the  
301 use of leachate recirculation strategies. The FC of the waste, indicating the amount of liquid that  
302 will be retained by the solid waste before saturation, was  $0.6 \text{ L kg}^{-1}$ . This finding was in good  
303 agreement with Orta de Velásquez et al (2003), which reported FC of MSW ranging between 0.55  
304 and  $2.84 \text{ L kg}^{-1}$ . They suggested that FC is inversely proportional to waste density, i.e. the higher  
305 the waste compaction, the less water was needed to satisfy FC (Orta de Velásquez et al, 2003).  
306 Understanding the FC of waste served as a process indicator, allowing for an informed decision to  
307 be made on leachate recirculation rates and waste density. The pH of the waste was slightly  
308 alkaline, being 7.6. This was however within the optimal range for methane production, which is  
309 between 6.0 and 8 (Emkes et al., 2015). The waste pH also indicates that the landfill site at the time  
310 of sampling was in a methanogenic state (Warith, 2002).

311

### 312 **3.2.2. Leachate characterisation**

313 Table 4 presents results from leachate characterisation. Leachate used in the study is considered  
314 relatively 'low strength' in terms of COD, being  $3219 \text{ mg L}^{-1}$ , and as a result would likely not  
315 promote optimal biogas production. Ghani and Idris (2009), in a study evaluating the effect of  
316 leachate COD strength on biogas production in leachate recirculation activities, found that higher

317 strength leachate (21 000 mg L<sup>-1</sup>) facilitated a three times higher biogas production than lower  
318 strength leachate (3000 mg L<sup>-1</sup>).

319 The leachate used for recirculation has a pH of 7.5 which confirmed that the landfill was relatively  
320 mature and likely in a methanogenic state. The pH of leachate is primarily influenced by landfill  
321 age, where leachate from younger landfills are typically more acidic (< 6.5) while leachate from  
322 older landfills are more alkaline (> 7.5) (Emkes et al., 2015). The relationship between leachate pH  
323 and landfill age is due to the accumulation of VFAs during the early stages of the anaerobic  
324 digestion process, causing the pH to become more acidic. Stabilised leachate shows little pH  
325 variation between 7.5 and 9 (Umar et al., 2010). When leachate pH is outside the optimal range, pH  
326 adjustment has been successfully utilised to promote biogas production (Jayasinghe et al., 2011; Liu  
327 et al., 2011; Warith, 2002). The heavy metals content of the leachate was analysed as these can have  
328 complex stimulatory, inhibitory, or toxic effect on the biochemical reactions mediated by the  
329 indigenous microbial communities of the landfill site (Mudhoo and Kumar, 2013). The effect of  
330 heavy metals on biochemical processes is directly correlated to the metal concentrations. The heavy  
331 metals considered were Fe, Zn, Cu, Pb, Ni, Cd, Cr and Mn as these are the most commonly  
332 occurring heavy metals in leachate (Mudhoo and Kumar, 2013). Mn, Fe and Ni enhanced biogas  
333 potential at trace quantities and are considered slightly toxic at elevated concentrations (Abdel-  
334 Shafy and Mansour, 2014). The concentration of Mn was high, being 8357 µg L<sup>-1</sup> and Fe and Ni,  
335 were above trace quantities at 38000 µg L<sup>-1</sup> and 517 µg L<sup>-1</sup>, respectively. The presence of these  
336 heavy metals therefore may be slightly toxic to microbial processes. Cr, Cu, Pb, Zn and Cd on the  
337 other hand are highly toxic heavy metals and are believed to severely inhibit microbial growth, even  
338 at low concentrations (Abdel-Shafy and Mansour, 2014). Cr was present at a concentration of 1927  
339 µg L<sup>-1</sup> while Cu, Pb, Zn and Cd were present at varying concentrations between 1 and 452 µg L<sup>-1</sup>. It  
340 is therefore possible that the high concentration of these heavy metals in the leachate used in this  
341 study created an environment toxic to the microorganisms, which would inhibit the biomethane  
342 production when used in recirculation activities.

### 343 **3.3. Theoretical and empirical (BMP) determination of biogas potential**

#### 344 **3.3.1. Theoretical biogas production**

345 Theoretical biogas production for the reactors was calculated to assess whether the assumptions  
346 made in the literature compare well with empirical biogas production experiments. Based on the  
347 studies by Scarlat et al. (2015) and Aguilar-Virgen et al. (2014), an estimate of 0.05 m<sup>3</sup> biogas per  
348 kg mixed waste was used as a conservative theoretical estimate of potential biogas production of the  
349 waste. Since each reactor hold a total of 3.8 kg waste, it was estimated that 0.19 m<sup>3</sup> (190 L) biogas  
350 would be produced per reactor which equated to 0.05 m<sup>3</sup> biogas kg<sup>-1</sup> waste.

351

#### 352 **3.3.2 BMP tests**

353 Biochemical methane potential (BMP) tests were conducted on the organic and paper fraction of the  
354 waste samples to determine their biomethane potential. Results indicated that waste from the  
355 landfill site could potentially produce a volume of 0.00497 m<sup>3</sup> kg<sup>-1</sup> under optimal conditions (Table  
356 5). Considering each bioreactor held 3.8 kg waste, of which, 68 % was organic and paper (Table 2),  
357 this would potentially result in 0.012 m<sup>3</sup> (12 L) of biogas produced or 0.0031 m<sup>3</sup> kg<sup>-1</sup>. Furthermore,  
358 the average methane content of the biogas was 28%, which is below the optimal 40-60 %. This  
359 suggested that even under optimal conditions, the methane yield from the MSW used was lower  
360 than expected.

361

### 362 **3.4. Biogas improvement with leachate and enzymes**

#### 363 **3.4.1. Batch bioreactors: leachate addition (with and without enzymes)**

364 Batch tests were conducted to assess the effect of leachate addition on biogas and methane  
365 production under optimal conditions of moisture content, temperature and nutrients. Biogas  
366 production occurred almost immediately at the onset of the batch tests. The absence of lag phase  
367 was likely a result of the landfill site being in a methanogenic state which is supported by the  
368 alkaline pH of the waste and leachate. Furthermore the inoculation of sludge at a waste:sludge ratio  
369 of 4:1 (w/w), contributed to the already present and active microbial community. Statistical analysis

370 indicated that there was a significant difference in biogas production between the tests [ $F(2,30) =$   
371  $3.2, p = 0.05]$ . Cumulative biogas production suggested that there was little difference between the  
372 leachate only and the control, being  $0.0040 \text{ m}^3 \text{ biogas kg}^{-1}$  waste compared to  $0.0045 \text{ m}^3 \text{ biogas kg}^{-1}$   
373 waste, respectively, over 81 days (Figure 2). The lack of increase in the biogas production as a  
374 result of leachate addition without enzyme can potentially be due to either the process of addition,  
375 the quantity of leachate added or the presence of heavy metals in the leachate. Previous lab-scale  
376 studies (Liu et al., 2014; Nair et al., 2014; Rastogi et al., 2014; Chan et al; 2002) and full-scale  
377 studies (Reinhart et al., 2002; Warith et al., 1999; Reinhart, 1996b) reported that increasing the  
378 moisture content to saturation was expected to improve biogas production. Also several studies  
379 reported the effects of heavy metals especially chromium, cadmium and nickel as stress factors on  
380 anaerobic digestion processes and biogas production (Mudhoo and Kumar, 2013). Differently from  
381 this, leachate addition with enzyme resulted in almost doubling the volume of biogas produced  
382 when compared to the leachate only and control test. Biogas showed exponential production for the  
383 first week, and thereafter continued steadily until day 60 (Figure 2). A total of  $0.0076 \text{ m}^3 \text{ biogas kg}^{-1}$   
384  $^1$  waste was produced. Results suggest that cellulase was able to facilitate degradation of  
385 lignocellulosic material within the waste fraction resulting in elevated levels of biogas production.  
386 Furthermore, the alkalinity of the system (Tables 2 and 3) promotes cellulase activity (Cirne et al.,  
387 2007).

388 It is also noteworthy to mention that while the addition of cellulase increased the volume of biogas  
389 produced, there was no effect on the methane concentration of the biogas, which remained below  
390 expectation (Figure 2). This indicated that cellulase facilitated a uniform increase in the production  
391 of all biogas constituents. The methane concentration, ranging between 15 and 25% was outside the  
392 expected range for biogas, which is typically between 40 and 60%. Several other studies have also  
393 observed a lower than expected methane composition. Manzur (2010) in an assessment of methane  
394 composition during landfill recirculation activities found methane gas yields between 15 and 28%.  
395 Sanphoti et al (2006) during the early acidogenic stages of leachate recirculation activities reported



396 methane composition < 10%. While it is common that methane yield is sub-optimal, particularly  
397 during the early stages of the anaerobic degradation process, results from this study indicated that  
398 methane composition remained below expectation, even during the later stages of the batch tests. It  
399 is likely that the addition of cellulase resulted in increased degradation of cellulose, which led to  
400 excess formation of VFA. Since methanogens are sensitive to pH, it is believed that excessive  
401 production of VFA caused a reduction in the pH which affected methanogen function, as observed  
402 in Wang et al. (2015). This likely resulted in excessive production of H<sub>2</sub> and acetate by acetogens  
403 which thereafter cannot be converted to CH<sub>4</sub> by methanogens, as described in Clarke (2000). The  
404 pH and VFA composition was not tested during the part of the experiment to confirm this  
405 hypothesis. Unbalanced acidic conditions is however a common occurrence in anaerobic waste  
406 degradation as the growth of acidifying organisms is over ten times faster than acetogenic and  
407 acetoclastic methanogenic organisms (Clarke, 2000).

408

#### 409 **3.4. 2. Continuous bioreactors: leachate recirculation (with and without enzymes)**

410 There was an approximately two week lag phase prior to the onset of biogas production (Figure 3).  
411 The occurrence of a lag phase in larger scale anaerobic digestion bioreactors is in agreement with  
412 literature (Ghatak and Mahanta, 2014; Hossain et al., 2008). The lag phase represents a distinct  
413 growth phase where the microbial populations adapt to the new environment before exponential  
414 growth (Hossain et al., 2008). The lag phase was followed by an exponential phase where biogas  
415 production steadily increased until approximately day 100. Results indicated that there was no  
416 significant difference in biogas production between the tests [F(2,42) = 1.368, p= 0.266].

417 Cumulative biogas production (Figure 3) indicated that biogas production was in good agreement  
418 with the batch tests (Figure 2), where there was little difference between the leachate only tests and  
419 control tests producing 0.40 and 0.43 L kg<sup>-1</sup> waste respectively throughout the duration of the  
420 experiment. It is interesting that the leachate only test, even at larger scale did not result in  
421 increased biogas production compared to the control, as often reported in the literature (Liu et al.,

422 2014; Nair et al., 2014; Rastogi et al., 2014; Chan et al., 2002). However, leachate augmented with  
423 cellulase improved biogas production by 50 %, resulting in a biogas volume of 0.6 L kg<sup>-1</sup> waste  
424 (Figure 3). This finding confirms that the use of cellulase can significantly improve the amount of  
425 biogas produced per mass of MSW. Moreover, the increase in biogas production as a result of  
426 enzyme addition exceeded results from other studies (Mao et al., 2015; Zheng et al., 2014) who  
427 showed potential biogas production improvements of 34% on account of enzyme addition.  
428 Notwithstanding this, the quantity of biogas produced in the bioreactors was lower than expected  
429 from the theoretical and BMP predictions. This was likely a result of contaminants in the leachate  
430 (i.e. presence of heavy metals) as observed in the batch bioreactors inhibiting microbial action  
431 biogas production coupled with sub-optimal waste compaction of 800 kg m<sup>-3</sup> (Mudhoo and Kumar,  
432 2013). High waste densities reduce the interaction between the solid and liquid phases, making  
433 waste more difficult to degrade (Hettiarachchi et al., 2007). The methane concentration in the  
434 biogas on average ranged between 10% and 45 % which was similar to the % observed in the batch  
435 tests. This lower methane content is likely due to the system parameters favouring the production  
436 and accumulation of VFA which altered the system biochemistry and resulted in CO<sub>2</sub> production  
437 rather than methane.

438

#### 439 **3.4.4. Waste and leachate characterisation of the bioreactors**

440 The VS and sCOD of the solid waste and leachate, indicative of the organic strength, decreased  
441 throughout the duration of all bioreactor tests (Table 6). The decrease in waste VS was relatively  
442 low and uniform throughout the tests and control, decreasing by 3% (Table 6) while the utilisation  
443 of COD corresponded to the biogas production in each bioreactor (i.e. highest decrease in COD  
444 corresponded to the leachate + cellulase test, followed by the control and thereafter the leachate  
445 only test, which were 47, 42 and 27% COD reduction, respectively). This result indicates that COD  
446 utilisation was directly correlated to biogas production, as also reported by Ghani and Idris (2009)  
447 and Timur and Ozturk (1999). However the COD utilisation in this study was lower than those

448 reported by Wang et al. (2006) who found a maximum COD reduction of >95% when leachate  
449 recirculation was used.

450 The pH of the solid waste and leachate increased slightly in the test conditions by the end of the  
451 experiment (Table 6). There was also a decrease in the total suspended solids (TSS) content of the  
452 leachate as a result of recirculation activities, which is an important beneficial consideration in  
453 leachate treatment. This finding is in good agreement with Kylefors and Lagerkvist (1997), Bilgili  
454 et al. (2007b) and Neethu and Anilkumar (2013) which reported that total solids concentration is  
455 expected to decrease as the leachate moves from acidogenic to methanogenic phases.

456 Heavy metals concentration including Fe, Zn, Ni, Cd, Cr and Mn decreased during the bioreactor  
457 tests as a result of metal precipitation into the waste mass (Table 6), which is common in anaerobic  
458 bioreactor landfill conditions, as reported by Bilgili et al. (2007a). In contrast, Cu and Pb  
459 accumulated during recirculation activities (Table 6). According to Mudhoo and Kumar (2013), Pb  
460 and Cu are two of the most toxic metals to biochemical reactions during waste stabilisation  
461 processes. Consequently, the accumulation of these metals would certainly have inhibited waste  
462 degradation and biogas production in the bioreactor experiment.

463

### 464 **3.5. Cost benefit analysis (CBA) of cellulase addition to leachate recirculation**

465 A simplified CBA was conducted in order to identify the opportunities and benefits associated to  
466 the addition of cellulase to an existing leachate recirculation operation. Clarke (2000) conducted a  
467 cost-benefit analysis for leachate recirculation and quantified the benefits of waste digestion as a  
468 function of degradation time. Taking into account the sum of more rapid biogas retrieval, landfill  
469 space utilisation, reduced environmental impacts and reduced post-closure costs minus capital and  
470 operational costs, they determined that at waste degradation rates that could be achieved in a  
471 bioreactor landfill, the potential benefit would be between €7 and €9 per tonne of waste. For a 5 Mt  
472 landfill, this would equate to a €33 million. Based on results associated to enzyme addition, at 50%  
473 increased biogas production and no significant improvement in methane concentration, the potential

474 benefit for a 5 Mt landfill would increase by approximately €16.4 million (Table 7), to  
475 approximately €49 million. The primary tangible cost associated with enzyme addition to leachate  
476 recirculation is the cost of cellulase. It was calculated that the cost of enzyme required for a landfill  
477 site containing  $5 \times 10^6$  tonnes (5 Mt) waste, ensuring an enzyme concentration of  $15 \times 10^6$  U tonne<sup>-1</sup>  
478 waste and with an enzyme cost of €17000 tonne<sup>-1</sup> would be €4.3 million (Table 7). The transport  
479 and additional labour costs were considered negligible. Therefore, the net benefit of enzyme  
480 augmentation to leachate recirculation at 50% increase in biogas production would be  
481 approximately €12.1 million, and thus the economic viability of the technology is supported.  
482 It is important to note that the generic quantification of economic values associated with the biogas  
483 production and the waste volume reduction is difficult as these benefits are dependent on numerous  
484 variables, such as methane yield, energy generation capacity, type of electricity generation, policy  
485 incentives such as among others, renewable energy certificates, tax credits and incentives,  
486 Renewable Energy bonds and GHG emissions trading. Findings from Clarke (2000) suggest that the  
487 benefits of introducing technologies that enhance landfill waste degradation on a per tonne basis are  
488 insensitive to the size of waste stream. However, the study suggests that costs reduce as waste  
489 stream size increases. Indeed a more detailed ad hoc cost analysis should be applied to individual  
490 projects where more detailed data are available. Since the enzyme concentration tested in this study  
491 is considered as the upper limit for enzyme addition in recirculation activities (Jayasinghe et al.,  
492 2012), further research is required to evaluate the optimal enzyme concentration to promote biogas  
493 production while minimizing its use in order to further promote the economic viability of the  
494 technology.

495

#### 496 **4. Conclusion**

497 Results from the batch and pilot scale bioreactor studies indicated that leachate recirculation  
498 without enzyme addition did not improve biogas production neither under optimal or sub-optimal  
499 conditions. A significant increase in biogas production occurred however when leachate was

500 supplemented with cellulase prior to recirculation. Our findings support the limited information  
501 currently available for the potential application of enzymes towards the bioreactor landfill model.  
502 The CBA of leachate addition to an existing recirculation operation indicated that the technology  
503 would be economically viable. This was an initial appraisal of the enzymatic process, more work  
504 needs to be done in identifying the optimal quantity of enzymatic addition and its recirculation  
505 potential, in terms of stability and activity. Furthermore, the viability of enzyme addition could be  
506 improved through more focused research to optimise the required enzyme concentration to promote  
507 waste degradation while minimising enzyme use. Bioengineering and biotechnology has already  
508 played a key role in the development of cellulosic biomass conversion technologies by dramatically  
509 reducing the cost of cellulase production. For continued progress and innovation for cost effective  
510 cellulose degradation, it is important for future biotechnology-based developments to also include  
511 improvement of cellulase production economics via microbe or plant based production systems.  
512 This will continue to add to the growing portfolio of innovative waste management practices  
513 promoting environmental sustainability and economic opportunity.

514

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518

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679

680 Table 1: General information on landfill sites

<b>Parameter</b>	<b>Values</b>
Landfill age	20 years
Waste tonnage	4.6 10 <sup>6</sup> tonnes
Average waste density	950 kg m <sup>-3</sup>
Average waste moisture content (MC)	37%
Average volatile solids (VS)	32%
Average Chemical Oxygen Demand (COD)	545 mg L <sup>-1</sup>
Methane content of landfill gas	40-49%
Landfill gas generation (average value)	
2000 - 2008	3000 m <sup>3</sup> hr <sup>-1</sup>
2008 -2012	2200 m <sup>3</sup> hr <sup>-1</sup>

681

682

683 Table 2: Waste composition of the ten MSW samples collected from the studied landfill site at  
 684 depths of 10, 15, 20 and 25 m

Core	Depth (m)	Plastic (%)	Paper (%)	Organic (%)	Textiles (%)	Glass & Metal (%)
Core 1	15	30	28	41	0.0	1
	25	34	12	54	0.0	0.6
Core 2	10	10	11	59	16	4
	15	51	10	37	0.0	2
	20	3	7	66	0.0	24
Core 3	10	19	27	23	31	0.4
	15	18	4	68	10	0.0
Core 4	20	24	28	44	2	2
Core 5	10	3	4	83	0.0	10
	20	19	23	51	2	5

685

686 Table 3: Characteristics of solid waste and solid waste + sludge

Characterisation of solid	Solid waste only	Solid waste and sludge (4:1)
Moisture content (MC) (%)	36.9	54.4
TS (%)	63.1 ± 1.8	45.6 ± 3.1
VS (%)	31.9 ± 1.7	31.6 ± 9.0
sCOD (mg L <sup>-1</sup> )	544±82	437.5 ± 28.1
pH	7.6 ± 0.4	7.9 ± 0
Field Capacity (L kg <sup>-1</sup> )	0.60	-
Water Absorption	0.44 ± 0.15	0.30

687

688

689 Table 4: Leachate characterisation results

<b>Leachate characteristics</b>	<b>Value</b>
Moisture content (MC) (%)	97.8
TS (%)	2.2±0
VS (% TS)	51.3±1
COD (mg L <sup>-1</sup> )	3219±30
pH	7.5±0
Total Suspended solids (mg L <sup>-1</sup> )	7.4±0.3
<b>Metals</b>	<b>Value</b>
Fe (µg L <sup>-1</sup> )	38000±3000
Zn (µg L <sup>-1</sup> )	452±32
Cu (µg L <sup>-1</sup> )	194±15
Pb (µg L <sup>-1</sup> )	101±12
Ni (µg L <sup>-1</sup> )	517±41
Cd (µg L <sup>-1</sup> )	1±0
Cr (µg L <sup>-1</sup> )	1927±179
Mn (µg L <sup>-1</sup> )	8357±804

690 Values presented are the mean of triplicate tests with ± standard deviation

691

692 Table 5: BMP test results on the landfill waste samples

<b>Core</b>	<b>Depth (m)</b>	<b>Sample no</b>	<b>L biogas kg<sup>-1</sup> sample<sup>a</sup></b>	<b>CH<sub>4</sub> (%)</b>
Core 1	15	1	6.59 ± 1.38	49.40 ± 4.4
	25	2	0.56 ± 0.79	33.90 ± 8.6
Core 2	10	3	0.98 ± 0.20	20.00 ± 2.6
	15	4	1.37 ± 0.33	14.00 ± 9.4
	20	5	6.06 ± 0.81	16.58 ± 0.2
Core 3	10	6	19.45 ± 0.72	18.45 ± 1.8
	15	7	0.83 ± 0.195	21.70 ± 13.2
Core 4	20	8	6.23 ± 0.05	11.70 ± 4.8
Core 5	10	9	6.43 ± 5.73	41.73 ± 3.9
	20	10	1.22 ± 0.26	48.35 ± 8.2
<b>Average</b>			<b>4.97 ± 5.74</b>	<b>27.58 ± 14.46</b>

693 <sup>a</sup> BMP test has been carried out in duplicate for each sample and the biogas concentration reported  
 694 is the mean of duplicate measurements.

695

696 Table 6: Waste and leachate characterisation pre- and post- lysimeter

	Pre-lysimeter characterisation	Post-lysimeter characterisation-leachate only test	Post-lysimeter characterisation-leachate + enzyme test	Post-lysimeter characterisation-control test
Characterisation of solid waste				
Moisture content (MC) (%)	54	63	63	50
TS (%)	46 ± 3.1	37 ± 7	37 ± 5	50 ± 3
VS (%)	32 ± 9.0	28 ± 1.0 *(-3 %)	28 ± 4.1 (-3 %)	28 ± 8 (-3 %)
sCOD (mg L <sup>-1</sup> )	438 ± 28	320 ± 9 (-27 %)	232 ± 26 (-47 %)	295 ± 47 (-42 %)
pH	7.9 ± 0	8.6 ± 0	8.0 ± 0	8.3 ± 0
Characterisation of leachate				
Moisture content (MC) (%)	98	99	98	98
TS (%)	2 ± 0	1 ± 0	2 ± 0	2 ± 0
VS (%)	51 ± 1	26 ± 0 (-25 %)	29 ± 1 (-22 %)	51 ± 1
COD (mg L <sup>-1</sup> )	3219 ± 30	2065 ± 57 (-35 %)	1843 ± 18 (-42 %)	2213 ± 30 (-32 %)
pH	7.5 ± 0	8.6 ± 0	8.2 ± 0	7.5
Total suspended solids (mg L <sup>-1</sup> )	7.4 ± 0.3	2.1 ± 0.1	2.5 ± 0.1	ND
Metals				
Fe (µg L <sup>-1</sup> )	38000 ± 3000	25000 ± 3000 (-34 %)	27000 ± 2000 (-29 %)	ND
Zn (µg L <sup>-1</sup> )	452 ± 32	371 ± 1 (-18%)	660 ± 3 (-20 %)	ND
Cu (µg L <sup>-1</sup> )	194 ± 15	439 ± 9 (+126 %)	312 ± 1 (+60 %)	ND
Pb (µg L <sup>-1</sup> )	101 ± 12	218 ± 2 (+116 %)	131 ± 0 (+30%)	ND
Ni (µg L <sup>-1</sup> )	517 ± 41	371 ± 1 (-28%)	324 ± 2 (-37%)	ND
Cd (µg L <sup>-1</sup> )	1 ± 0	0.70 ± 0 (-30 %)	0.80 ± 0 (-20 %)	ND
Cr (µg L <sup>-1</sup> )	1927 ± 179	380 ± 2 (-80 %)	550 ± 3 (-70 %)	ND
Mn (µg L <sup>-1</sup> )	8357 ± 804	277 ± 2 (-97%)	224 ± 1 (-97 %)	ND

697 \* numbers in brackets represent changes between pre and post lysimeter characterisation

698

699 (- = decrease; + = increase); ND = Not determined as leachate was not recirculated

700



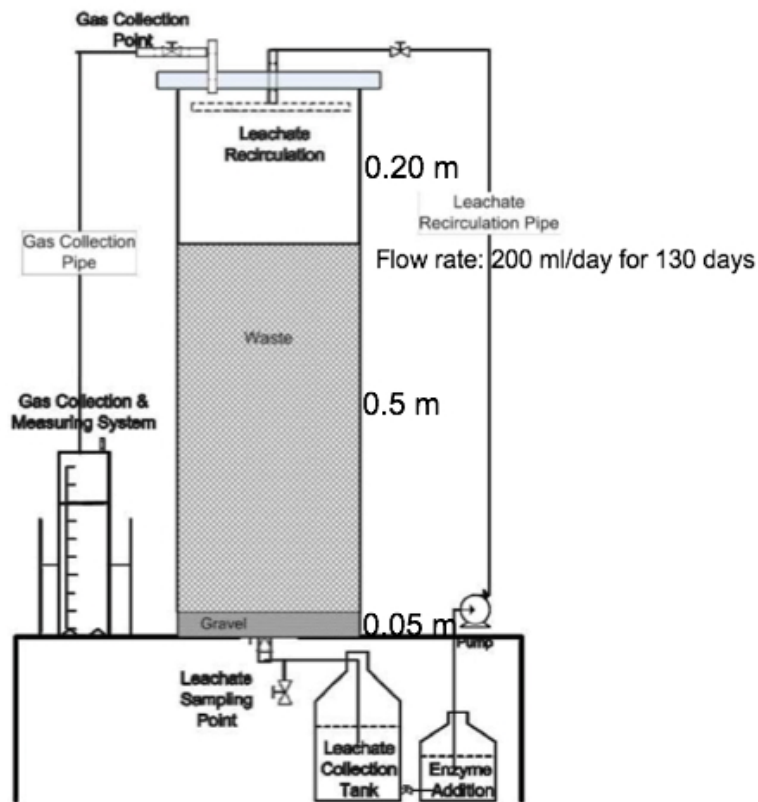
701 Table 7: Tangible costs and benefits associated to enzyme addition to an existing leachate  
 702 recirculation operation

<b>Parameters</b>	<b>Description</b>	<b>Monetary costs (where available)</b>
<b>Tangible costs</b>	Cost of cellulase: Based on: -5 Mt waste in landfill -Enzyme concentration of 15 10 <sup>6</sup> U/tonne  Cost of transport Labour	Requires 250 tonnes enzyme at €17000 per tonne <sup>a</sup> = €4.25 million  Negligible Negligible
<b>Tangible benefits</b>	Income from increased waste degradation leading to improved biogas/methane production (based on 50% increase in biogas production)  Landfill space savings  Reduced environmental impacts  Reduced post-closure requirements	Estimated € 16.4 million direct benefit
<b>Net benefit</b>		€ 12.1 million

703 <sup>a</sup> From product manufacturer

704

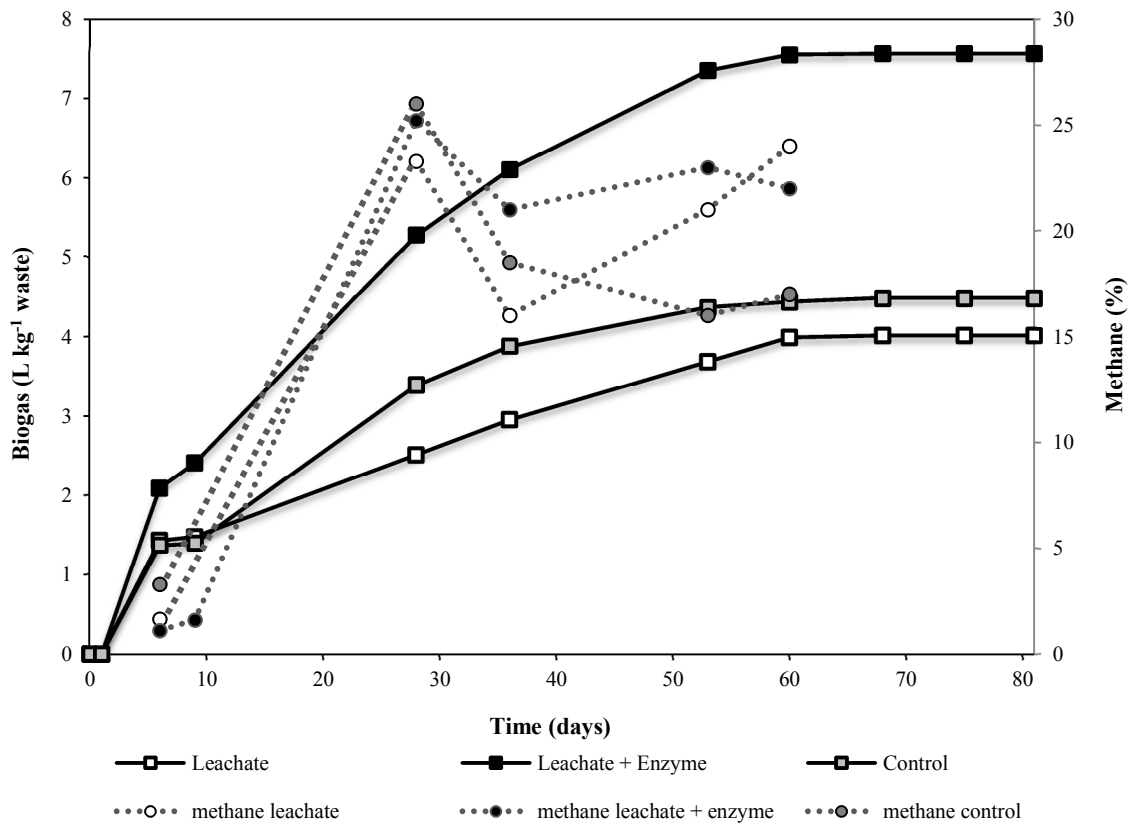
705



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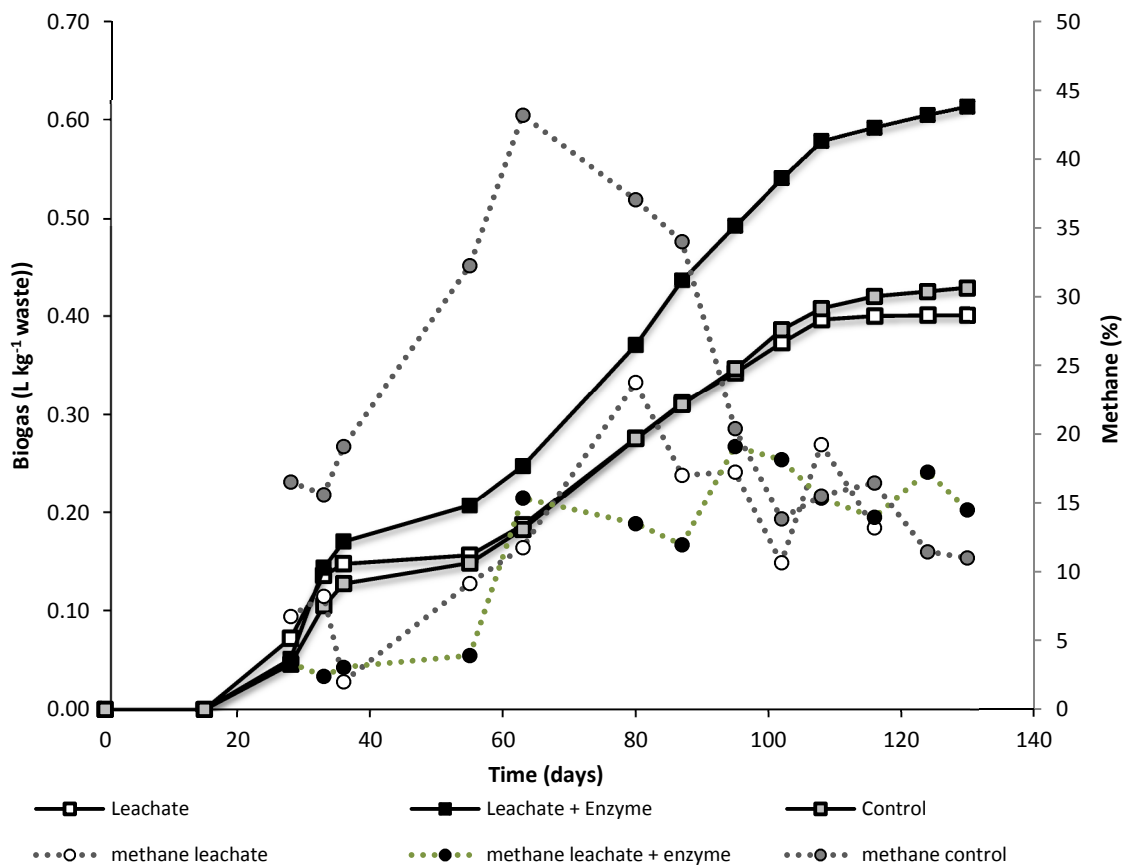
707 Figure 1: Diagrammatic representation of an anaerobic bioreactor landfill simulator (amended from  
 708 Jayasinghe et al., 2012)

709



710  
 711 Figure 2: Cumulative biogas production (lines) and methane concentration (%) from the batch tests  
 712 (dots).

713  
 714



715

716 Figure 3: Cumulative biogas production (lines) and methane concentration (dots) in the bioreactors  
 717 over 130 days.

718

719

720

# Evaluating leachate recirculation with cellulase addition to enhance waste biostabilisation and landfill gas production

Frank, R. R.

2016-07-05

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