

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

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Abstract: The effect of leachate recirculation with cellulase augmentation on municipal solid waste (MSW) biostabilisation and landfill gas production was investigated using batch bioreactors to determine the optimal conditions of moisture content, temperature and nutrients. Experimentation was thereafter scaled-up in 7 L bioreactors. Three conditions were tested including (1) leachate recirculation only, (2) leachate recirculation with enzyme augmentation and (3) no leachate recirculation (control). Cumulative biogas production of the batch tests indicated that there was little difference between the leachate and control test conditions, producing on average 0.043 m³ biogas kg⁻¹ waste. However the addition of cellulase at 15·10⁶ U tonne⁻¹ waste doubled the biogas production (0.074 m³ biogas kg⁻¹ waste). Similar trend was observed with the bioreactors. Cellulase addition also resulted in the highest COD reduction in both the waste and the leachate samples (47% and 42% COD reduction, respectively). In both cases, the quantity of biogas produced was closer to the lower value of theoretical and data-based biogas prediction indicators (0.05-0.4 m³ biogas kg⁻¹ waste). This was likely due to a high concentration of heavy metals present in the leachate, in particular Cr and Mn, which are known to be toxic to methanogens.

The cost-benefit analysis (CBA) based on the settings of the study (cellulase concentration of 15×10⁶ U tonne⁻¹ waste) showed that leachate bioaugmentation using cellulase is economically viable, with a net benefit of approximately €12.1 million on a 5 Mt mixed waste landfill.

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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⁻¹ 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 m³ h⁻¹) 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.

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⁻¹ or 500-10 000 mg. L⁻¹) 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.

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

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

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

where: Cc = Field Capacity (kg H₂O /kg dry waste); Si = volume of water added to the bottle at the beginning of the test (L); Di = volume of water extracted from bottle (L); d = density of waste (kg L⁻¹); H = % MC of waste / 100; PV = weight density of solid waste; V = volume of bottle occupied by waste.

2.2.5. Metals analysis

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

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

2.3.1 Theoretical biogas

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

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

163 where L_o = 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); DOC_F = fraction of DOC decomposing under anaerobic conditions
166 (dimensionless); F = fraction of CH_4 in the landfill gas (dimensionless); 16/12 is the stoichiometric
167 factor to convert carbon into 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 N_2 gas to create anaerobic conditions before being incubated in a water
181 batch at 38°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 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 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- β -1,4-glucanase; 200 U of glucoamylase, α amylase
197 and pullulanase; 30,000 U of xylanase and 150,000 U of β -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) $\geq 300,000$ U/g;
- 221 • FPA filter paper assay (glucoamylase, α amylase and pullulanase) $\geq 20,000$ U/g ;
- 222 • Xylanase $\geq 3,000,000$ U/g;
- 223 • β-glucanase $\geq 15,000,000$ 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 m³). 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⁻³ was used as it
239 was found to be the highest possible density that would allow the desired recirculation rate before

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

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

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

2.5. Statistical analysis

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

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

A simplified CBA was conducted to evaluate the economic viability of cellulase addition to an existing leachate recirculation operation. The CBA was based on the CBA on leachate recirculation 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

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

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

311

3.2.2. Leachate characterisation

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

317 strength leachate ($21\,000\text{ mg L}^{-1}$) facilitated a three times higher biogas production than lower
318 strength leachate (3000 mg L^{-1}).

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 $8357\text{ }\mu\text{g L}^{-1}$ and Fe and Ni,
335 were above trace quantities at $38000\text{ }\mu\text{g L}^{-1}$ and $517\text{ }\mu\text{g L}^{-1}$, 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 $1927\text{ }\mu\text{g L}^{-1}$
339 while Cu, Pb, Zn and Cd were present at varying concentrations between 1 and $452\text{ }\mu\text{g L}^{-1}$. 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.

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

3.3.1. Theoretical biogas production

Theoretical biogas production for the reactors was calculated to assess whether the assumptions made in the literature compare well with empirical biogas production experiments. Based on the studies by Scarlat et al. (2015) and Aguilar-Virgen et al. (2014), an estimate of 0.05 m³ biogas per kg mixed waste was used as a conservative theoretical estimate of potential biogas production of the waste. Since each reactor hold a total of 3.8 kg waste, it was estimated that 0.19 m³ (190 L) biogas would be produced per reactor which equated to 0.05 m³ biogas kg⁻¹ waste.

3.3.2 BMP tests

Biochemical methane potential (BMP) tests were conducted on the organic and paper fraction of the waste samples to determine their biomethane potential. Results indicated that waste from the landfill site could potentially produce a volume of 0.00497 m³ kg⁻¹ under optimal conditions (Table 5). Considering each bioreactor held 3.8 kg waste, of which, 68 % was organic and paper (Table 2), this would potentially result in 0.012 m³ (12 L) of biogas produced or 0.0031 m³ kg⁻¹. Furthermore, the average methane content of the biogas was 28%, which is below the optimal 40-60 %. This suggested that even under optimal conditions, the methane yield from the MSW used was lower than expected.

3.4. Biogas improvement with leachate and enzymes

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

Batch tests were conducted to assess the effect of leachate addition on biogas and methane production under optimal conditions of moisture content, temperature and nutrients. Biogas production occurred almost immediately at the onset of the batch tests. The absence of lag phase was likely a result of the landfill site being in a methanogenic state which is supported by the alkaline pH of the waste and leachate. Furthermore the inoculation of sludge at a waste:sludge ratio 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

methane composition < 10%. While it is common that methane yield is sub-optimal, particularly during the early stages of the anaerobic degradation process, results from this study indicated that methane composition remained below expectation, even during the later stages of the batch tests. It is likely that the addition of cellulase resulted in increased degradation of cellulose, which led to excess formation of VFA. Since methanogens are sensitive to pH, it is believed that excessive production of VFA caused a reduction in the pH which affected methanogen function, as observed in Wang et al. (2015). This likely resulted in excessive production of H₂ and acetate by acetogens which thereafter cannot be converted to CH₄ by methanogens, as described in Clarke (2000). The pH and VFA composition was not tested during the part of the experiment to confirm this hypothesis. Unbalanced acidic conditions is however a common occurrence in anaerobic waste degradation as the growth of acidifying organisms is over ten times faster than acetogenic and acetoclastic methanogenic organisms (Clarke, 2000).

408

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

There was an approximately two week lag phase prior to the onset of biogas production (Figure 3). The occurrence of a lag phase in larger scale anaerobic digestion bioreactors is in agreement with literature (Ghatak and Mahanta, 2014; Hossain et al., 2008). The lag phase represents a distinct growth phase where the microbial populations adapt to the new environment before exponential growth (Hossain et al., 2008). The lag phase was followed by an exponential phase where biogas production steadily increased until approximately day 100. Results indicated that there was no significant difference in biogas production between the tests [$F(2,42) = 1.368$, $p = 0.266$]. Cumulative biogas production (Figure 3) indicated that biogas production was in good agreement with the batch tests (Figure 2), where there was little difference between the leachate only tests and control tests producing 0.40 and 0.43 L kg⁻¹ waste respectively throughout the duration of the experiment. It is interesting that the leachate only test, even at larger scale did not result in increased biogas production compared to the control, as often reported in the literature (Liu et al.,

2014; Nair et al., 2014; Rastogi et al., 2014; Chan et al., 2002). However, leachate augmented with cellulase improved biogas production by 50 %, resulting in a biogas volume of 0.6 L kg⁻¹ waste (Figure 3). This finding confirms that the use of cellulase can significantly improve the amount of biogas produced per mass of MSW. Moreover, the increase in biogas production as a result of enzyme addition exceeded results from other studies (Mao et al., 2015; Zheng et al., 2014) who showed potential biogas production improvements of 34% on account of enzyme addition. Notwithstanding this, the quantity of biogas produced in the bioreactors was lower than expected from the theoretical and BMP predictions. This was likely a result of contaminants in the leachate (i.e. presence of heavy metals) as observed in the batch bioreactors inhibiting microbial action biogas production coupled with sub-optimal waste compaction of 800 kg m⁻³ (Mudhoo and Kumar, 2013). High waste densities reduce the interaction between the solid and liquid phases, making waste more difficult to degrade (Hettiarachchi et al., 2007). The methane concentration in the biogas on average ranged between 10% and 45 % which was similar to the % observed in the batch tests. This lower methane content is likely due to the system parameters favouring the production and accumulation of VFA which altered the system biochemistry and resulted in CO₂ production rather than methane.

438

3.4.4. Waste and leachate characterisation of the bioreactors

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

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

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

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 ⁻¹)	544±82	437.5 ± 28.1
pH	7.6 ± 0.4	7.9 ± 0
Field Capacity (L kg ⁻¹)	0.60	-
Water Absorption	0.44 ± 0.15	0.30

687
688

689 Table 4: Leachate characterisation results

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

Core	Depth (m)	Sample no	L biogas kg ⁻¹ sample ^a	CH ₄ (%)
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
Average			4.97 ± 5.74	27.58 ± 14.46

693 ^a 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 ⁻¹)	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 ⁻¹)	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 ⁻¹)	7.4 ± 0.3	2.1 ± 0.1	2.5 ± 0.1	ND
Metals				
Fe (µg L ⁻¹)	38000 ± 3000	25000 ± 3000 (-34 %)	27000 ± 2000 (-29 %)	ND
Zn (µg L ⁻¹)	452 ± 32	371 ± 1 (-18%)	660 ± 3 (-20 %)	ND
Cu (µg L ⁻¹)	194 ± 15	439 ± 9 (+126 %)	312 ± 1 (+60 %)	ND
Pb (µg L ⁻¹)	101 ± 12	218 ± 2 (+116 %)	131 ± 0 (+30%)	ND
Ni (µg L ⁻¹)	517 ± 41	371 ± 1 (-28%)	324 ± 2 (-37%)	ND
Cd (µg L ⁻¹)	1 ± 0	0.70 ± 0 (-30 %)	0.80 ± 0 (-20 %)	ND
Cr (µg L ⁻¹)	1927 ± 179	380 ± 2 (-80 %)	550 ± 3 (-70 %)	ND
Mn (µg L ⁻¹)	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

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

703 ^a From product manufacturer

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705

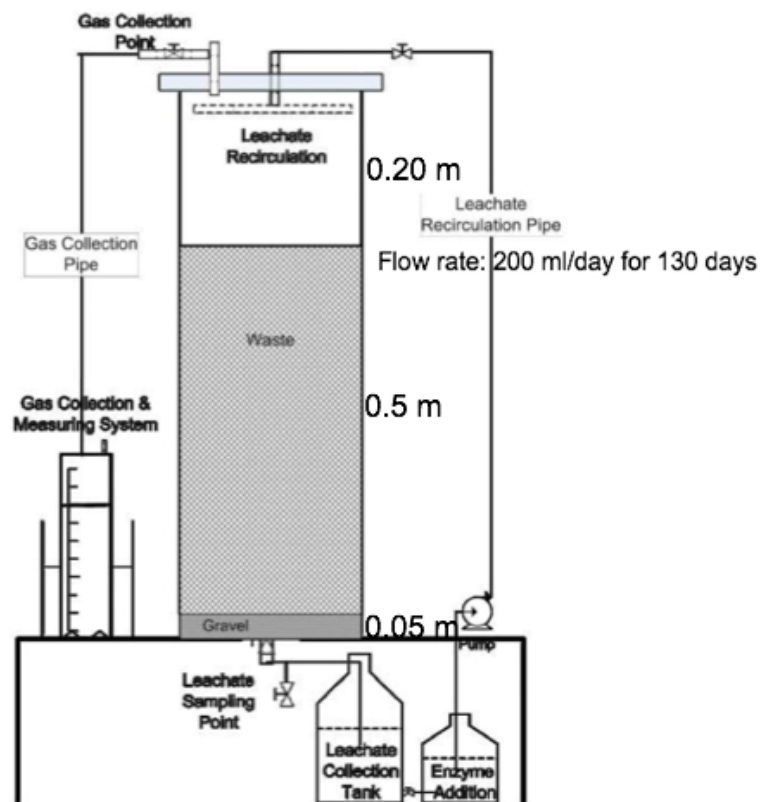


Figure 1: Diagrammatic representation of an anaerobic bioreactor landfill simulator (amended from Jayasinghe et al., 2012)

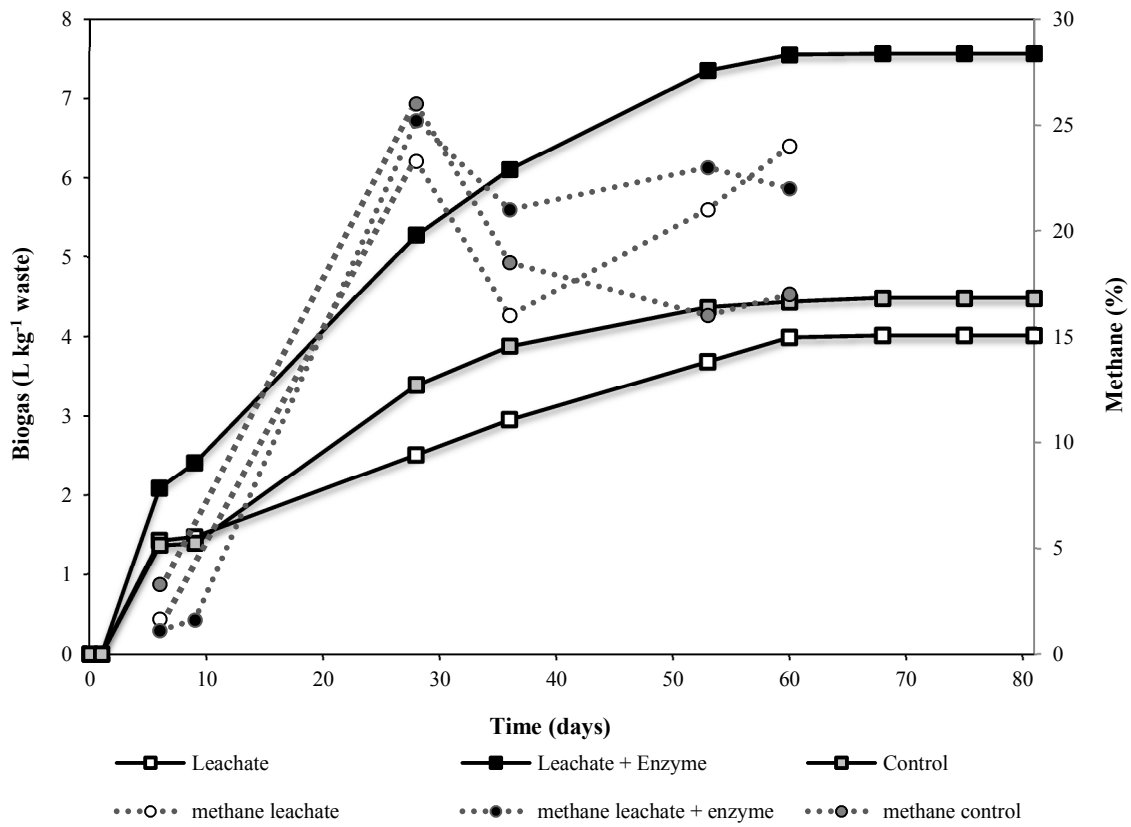


Figure 2: Cumulative biogas production (lines) and methane concentration (%) from the batch tests (dots).

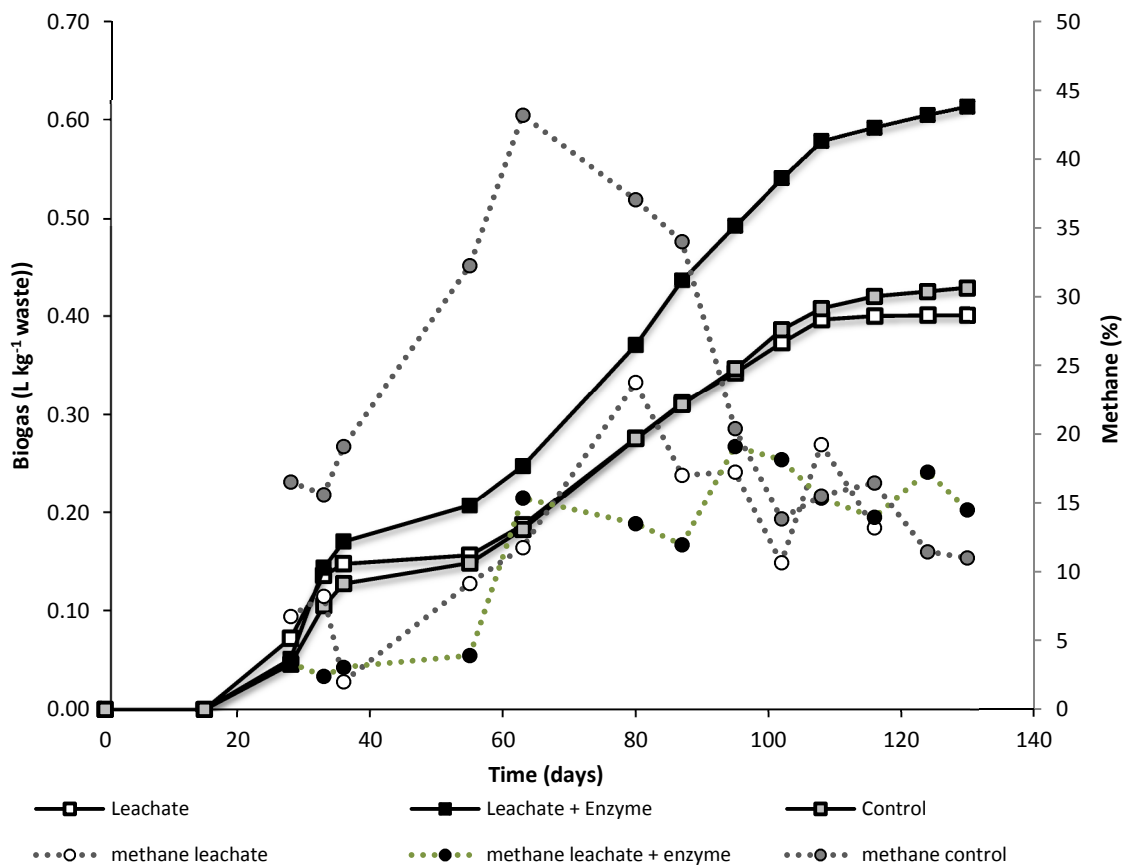


Figure 3: Cumulative biogas production (lines) and methane concentration (dots) in the bioreactors over 130 days.



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

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