Waste Management, Volume 55, September 2016, Pages 61-70
DOI: 10.1016/j.wasman.2016.06.038

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 m^3 biogas kg^{-1} waste. However the addition of cellulase at $15 \cdot 10^6$ U $tonne^{-1}$ waste doubled the biogas production (0.074 m^3 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 m^3 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 15×10^6 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.

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

1. Introduction

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

In particular, the recirculation of leachate as part of the 'bioreactor landfill' model has received much attention due to its widespread success, in both small and large scale applications (Liu et al., 2014; Nair et al., 2014; Rastogi et al., 2014; Reinhart et al., 2002; Reinhart, 1996 a & b; Lagerkvist and Chen, 1993). The recirculation of leachate facilitates the rapid transformation and degradation of landfilled waste which promotes landfill space reduction and maximises biogas production. These benefits can be further used as a source of renewable energy and reduces environmental disamenity (Nair et al., 2014; Liu et al., 2014; Rastogi et al., 2014; Reinhart et al., 2002; Clarke, 2000). It further closes the resource loop allowing leachate to be used towards more economically and environmentally beneficial activities (Xu et al., 2014; Reinhart et al., 2002).

The degradation of the waste in a landfill site is facilitated by a consortium of microorganisms (Barlaz et al., 1990) and therefore any environmental modifications or bioengineered solutions need careful considerations. Leachate recirculation can affect the active microbial communities as the introduction of leachate can affect pH, temperature, oxidation/reduction potential as well as complex biochemical reactions necessary for microbial waste degradation (Mudhoo and Kumar, 2013; Barlaz et al., 1990). Furthermore, the recirculation of leachate can also introduce a combination of heavy metals, contaminants and xenobiotics in varying amounts which affect microbial communities (Chen et al. 2008; Bilgili et al., 2007a). This has been highlighted in a number of key studies (Zornoza et al., 2015; Mudhoo and Kumar, 2013; Frostegård et al., 1993). The most common heavy metals found in leachate are: iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni) and lead (Pb) (Mudhoo and Kumar, 2013; Bilgili et al., 2007a). Fe has been reported to have stimulatory effects on microbial communities involved in waste degradation at concentrations below 8.1 mmol. L^{-1} and be inhibitory at concentrations above (Gonzalez-Silva et al., 2009). Cu, Zn, Cd and Pb have been shown to be highly toxic to microbial biochemical reactions. They increase in their inhibitory effect as follows: Pb < Zn < Cu < Cd (Mudhoo and Kumar, 2013). Therefore, while the recirculation of leachate results increases moisture content required for optimal waste degradation, its introduction also requires stringent process control to minimise its associated deleterious effect on the active microbial community. Another important feature to take into consideration when evaluating technologies to facilitate waste degradation is the waste composition of landfill sites. Approximately 40-50% of landfill space is comprised of paper and cardboard, of which lignocellulose is a major component (Yuan et al., 2014; Kovács et al., 2009). Lignocellulose is composed of carbohydrate polymers, cellulose (most prominent) and hemicellulose as well as aromatic polymer, lignin (Yuan et al., 2014). Within a waste mass, lignocellulosic materials are considered recalcitrant as difficult to degrade under anaerobic conditions (Pareek et al., 2008). A technique to enhance the degradation of residual waste fractions, with particular application towards difficult to degrade materials, is the addition of enzymes (Zheng et al., 2014; Jayasinghe et al., 2012, 2011; Lin et al., 2010; Romano et al., 2009). In particular, degradation of cellulose to soluble sugars and glucose is catalysed by a group of enzymes called cellulases, which include: endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase and β-glucosidase. Industrial grade cellulases have been successfully used for lignocellulose degradation in many industries (Kudah et al., 2011).

Enzymes, however, have historically been an expensive commodity which has hindered its application in waste management practices. Recent developments in biotechnology coupled with reduced costs of manufacturing (particularly in China) have led to the use of enzyme to improve landfill gas production to be considered.

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

2. Methods

2.1. Waste and leachate origin and sampling procedure

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

2.2. Waste and leachate characterisation

2.2.1. Waste composition

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

2.2.2. TS, TSS, VS, pH and sCOD

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

2.2.4. Field capacity

Field capacity (FC) test was conducted to determine the amount of leachate that would be required to bring the waste mass to saturation. FC test was adapted from Orta de Velásquez et al. (2003). Briefly, 100 g mixed waste was placed into a 1 L bottle, to which 500 ml distilled water was added. The bottles were placed on a shaker for 24 hours. Water from the bottle was allowed to drain for 8 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).

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

where: Cc = Field Capacity (kg H_2O /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:

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

where Lo = methane generation potential (m^3 CH_4/tonne waste); MCF=Methane Correction Factor (dimensionless); DOC=Degradable organic carbon in waste under aerobic conditions (dimensionless); DOC_F = fraction of DOC decomposing under anaerobic conditions (dimensionless); F = fraction of CH_4 in the landfill gas (dimensionless); 16/12 is the stoichiometric factor to convert carbon into CH_4 (dimensionless).

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

2.3.2 BMP tests

The biogas potential was determined empirically using the biochemical methane potential (BMP) tests according to WRAP guidelines (WRAP, 2010). Briefly the BMP tests were performed by mixing 20 g loss on ignition (LOI) equivalent of the organic and paper waste fraction with 40 g LOI equivalent of digested primary sludge (1:2 ratio) taken from the local wastewater treatment plant in Milton Keynes, UK. Sludge was used as the seed for the BMP tests to facilitate methane production as well as reduce the lag phase. The bottles were filled with water, leaving an adequate headspace of 200 ml, and flushed with N_2 gas to create anaerobic conditions before being incubated in a water batch at 38°C. The volume of biogas was measured volumetrically daily until no more biogas was produced. The methane concentration of the biogas was measured using a gas analyser (Servomex 1440 GA). Two control tests were conducted, which included: sludge alone and sludge + cellulose (10 g kg^{-1}) (both in the absence of waste). The biogas production of the inoculum was removed when calculating the amount of the biogas produced by the waste samples. All tests were conducted in duplicate and results were converted to standard temperature and pressure (STP).

2.4. Biogas improvement with leachate and enzymes

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

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

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

2.4.2. Enzyme characteristics

The lignocellulose material (paper, wood etc) contained in municipal waste is not so quick to degrade under anaerobic condition. Cellulose and hemicellulose are the two major components of this material, where cellulose represents generally about 40–50% of the biomass by weigh while hemicellulose represents 20–40% of the material by weight. Cellulase was therefore chosen to breakdown the major component of the material in a cost-effective manner. Cellulase CEL 30, produced from *Trichoderma reesi*, was purchased from Sinobios (China). The optimum pH and temperature ranges were 4.0 to 6.0 and 40 to 60°C, respectively.

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

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

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

Six water and gas-tight anaerobic bioreactors made from acrylic (PVC) cylinder were used in the study (see illustrative set-up in Figure 3). The dimensions of the columns were as follows: thickness = 8 mm, internal diameter = 110 mm and height = 0.75 m (volume = 0.00713 m^3). The reactor consisted of three ports. One port (bottom) served as a leachate outlet pipe while the other two ports (top) served as a leachate inlet and gas outlet pipe, respectively. Approximately 0.2 m (7% of column volume) of gravel was layered at the bottom of column to prevent clogging of the leachate outlet pipe. Gravel with a particle size of 14-20 mm was placed at the bottom, and above that, 10 mm and 2 mm gravel respectively. 0.5 m (67% of column volume) was packed with waste while 0.2 m (25 % of column volume) was left as headspace for gas accumulation at the top of the column. Waste was mixed with digested sludge at a ratio of 4:1 prior to insertion into the column in order to introduce a consortium of active microorganisms, which would reduce the lag time for biogas production. Sludge digestate was obtained from the local wastewater treatment plant (Milton Keynes, UK) and was sampled 2 days prior to mixing. A waste density of 800 kg m^{-3} was used as it 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 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 15×10^{6} 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 al. (2015) were taken into account in developing the CBA scenario and costing. The CBA took into account the sum of increased biogas retrieval, landfill space savings, reduced environmental impacts and reduced post-closure costs minus capital and operational costs. The costs and benefits (including environmental benefits) were itemized and compared in order to assess opportunities and risks associated with the technology.

3. Results

3.1. Waste composition

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

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

3.2. Waste and Leachate characteristics

3.2.1. Waste characterisation

Waste used in the batch and bioreactor experiments was characterised in order to understand the nature of the waste and evaluate the physicochemical changes which will occur as a result of the treatments (Table 3). The MC of 37 % (wt) is considered slightly below sufficient, being > 40%, to promote waste degradation and biogas production (Emkes et al., 2015; USEPA, 2003). The ‘dry’ 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).

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

The leachate used for recirculation has a pH of 7.5 which confirmed that the landfill was relatively mature and likely in a methanogenic state. The pH of leachate is primarily influenced by landfill age, where leachate from younger landfills are typically more acidic (< 6.5) while leachate from older landfills are more alkaline (> 7.5) (Emkes et al., 2015). The relationship between leachate pH and landfill age is due to the accumulation of VFAs during the early stages of the anaerobic digestion process, causing the pH to become more acidic. Stabilised leachate shows little pH variation between 7.5 and 9 (Umar et al., 2010). When leachate pH is outside the optimal range, pH adjustment has been successfully utilised to promote biogas production (Jayasinghe et al., 2011; Liu et al., 2011; Warith, 2002).The heavy metals content of the leachate was analysed as these can have complex stimulatory, inhibitory, or toxic effect on the biochemical reactions mediated by the indigenous microbial communities of the landfill site (Mudhoo and Kumar, 2013). The effect of heavy metals on biochemical processes is directly correlated to the metal concentrations. The heavy metals considered were Fe, Zn, Cu, Pb, Ni, Cd, Cr and Mn as these are the most commonly occurring heavy metals in leachate (Mudhoo and Kumar, 2013). Mn, Fe and Ni enhanced biogas potential at trace quantities and are considered slightly toxic at elevated concentrations (Abdel-Shafy and Mansour, 2014). The concentration of Mn was high, being 8357 µg L^{-1} and Fe and Ni, were above trace quantities at 38000 µg $L^{-1}$and 517 µg L^{-1}, respectively. The presence of these heavy metals therefore may be slightly toxic to microbial processes. Cr, Cu, Pb, Zn and Cd on the other hand are highly toxic heavy metals and are believed to severely inhibit microbial growth, even at low concentrations (Abdel-Shafy and Mansour, 2014). Cr was present at a concentration of 1927 µg L^{-1} while Cu, Pb, Zn and Cd were present at varying concentrations between 1 and 452 µg L^{-1}. It is therefore possible that the high concentration of these heavy metals in the leachate used in this study created an environment toxic to the microorganisms, which would inhibit the biomethane 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 m^3 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 m^3 (190 L) biogas would be produced per reactor which equated to 0.05 m^3 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 m^3 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 m^3 (12 L) of biogas produced or 000.31 m^3 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 indicated that there was a significant difference in biogas production between the tests [$F(2,30) = 3.2$, $p = 0.05$]. Cumulative biogas production suggested that there was little difference between the leachate only and the control, being 0.0040 m^3 biogas kg^{-1} waste compared to 0.0045 m^3 biogas kg^{-1} waste, respectively, over 81 days (Figure 2). The lack of increase in the biogas production as a result of leachate addition without enzyme can potentially be due to either the process of addition, the quantity of leachate added or the presence of heavy metals in the leachate. Previous lab-scale studies (Liu et al., 2014; Nair et al., 2014; Rastogi et al., 2014; Chan et al; 2002) and full-scale studies (Reinhart et al., 2002; Warith et al., 1999; Reinhart, 1996b) reported that increasing the moisture content to saturation was expected to improve biogas production. Also several studies reported the effects of heavy metals especially chromium, cadmium and nickel as stress factors on anaerobic digestion processes and biogas production (Mudhoo and Kumar, 2013). Differently from this, leachate addition with enzyme resulted in almost doubling the volume of biogas produced when compared to the leachate only and control test. Biogas showed exponential production for the first week, and thereafter continued steadily until day 60 (Figure 2). A total of 0.0076 m^3 biogas kg^{-1} waste was produced. Results suggest that cellulase was able to facilitate degradation of lignocellulosic material within the waste fraction resulting in elevated levels of biogas production. Furthermore, the alkalinity of the system (Tables 2 and 3) promotes cellulase activity (Cirne et al., 2007).

It is also noteworthy to mention that while the addition of cellulase increased the volume of biogas produced, there was no effect on the methane concentration of the biogas, which remained below expectation (Figure 2). This indicated that cellulase facilitated a uniform increase in the production of all biogas constituents. The methane concentration, ranging between 15 and 25% was outside the expected range for biogas, which is typically between 40 and 60%. Several other studies have also observed a lower than expected methane composition. Manzur (2010) in an assessment of methane composition during landfill recirculation activities found methane gas yields between 15 and 28%. 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 H_2 and acetate by acetogens which thereafter cannot be converted to CH_4 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).

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 CO_2 production rather than methane.

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

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

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

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

A simplified CBA was conducted in order to identify the opportunities and benefits associated to the addition of cellulase to an existing leachate recirculation operation. Clarke (2000) conducted a cost-benefit analysis for leachate recirculation and quantified the benefits of waste digestion as a function of degradation time. Taking into account the sum of more rapid biogas retrieval, landfill space utilisation, reduced environmental impacts and reduced post-closure costs minus capital and operational costs, they determined that at waste degradation rates that could be achieved in a bioreactor landfill, the potential benefit would be between €7 and €9 per tonne of waste. For a 5 Mt landfill, this would equate to a €33 million. Based on results associated to enzyme addition, at 50% 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.

4. Conclusion

Results from the batch and pilot scale bioreactor studies indicated that leachate recirculation without enzyme addition did not improve biogas production neither under optimal or sub-optimal conditions. A significant increase in biogas production occurred however when leachate was supplemented with cellulase prior to recirculation. Our findings support the limited information currently available for the potential application of enzymes towards the bioreactor landfill model. The CBA of leachate addition to an existing recirculation operation indicated that the technology would be economically viable. This was an initial appraisal of the enzymatic process, more work needs to be done in identifying the optimal quantity of enzymatic addition and its recirculation potential, in terms of stability and activity. Furthermore, the viability of enzyme addition could be improved through more focused research to optimise the required enzyme concentration to promote waste degradation while minimising enzyme use. Bioengineering and biotechnology has already played a key role in the development of cellulosic biomass conversion technologies by dramatically reducing the cost of cellulase production. For continued progress and innovation for cost effective cellulose degradation, it is important for future biotechnology-based developments to also include improvement of cellulase production economics via microbe or plant based production systems. This will continue to add to the growing portfolio of innovative waste management practices promoting environmental sustainability and economic opportunity.

Acknowledgements:

The authors would like to thank the National Research Foundation (South Africa) and Viridor (UK) for research funding and support.

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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 2008 -2012	 3000 m^3 hr^{-1} 2200 m^3 hr^{-1}

Table 2: Waste composition of the ten MSW samples collected from the studied landfill site at 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

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

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

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

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**

[a] BMP test has been carried out in duplicate for each sample and the biogas concentration reported is the mean of duplicate measurements.

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±.01	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

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

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

Table 7: Tangible costs and benefits associated to enzyme addition to an existing leachate 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 Cost of transport Labour	Requires 250 tonnes enzyme at €17000 per tonne[a] = €4.25 million Negligible Negligible
Tangible benefits	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
Net benefit		€ 12.1 million

[a] From product manufacturer

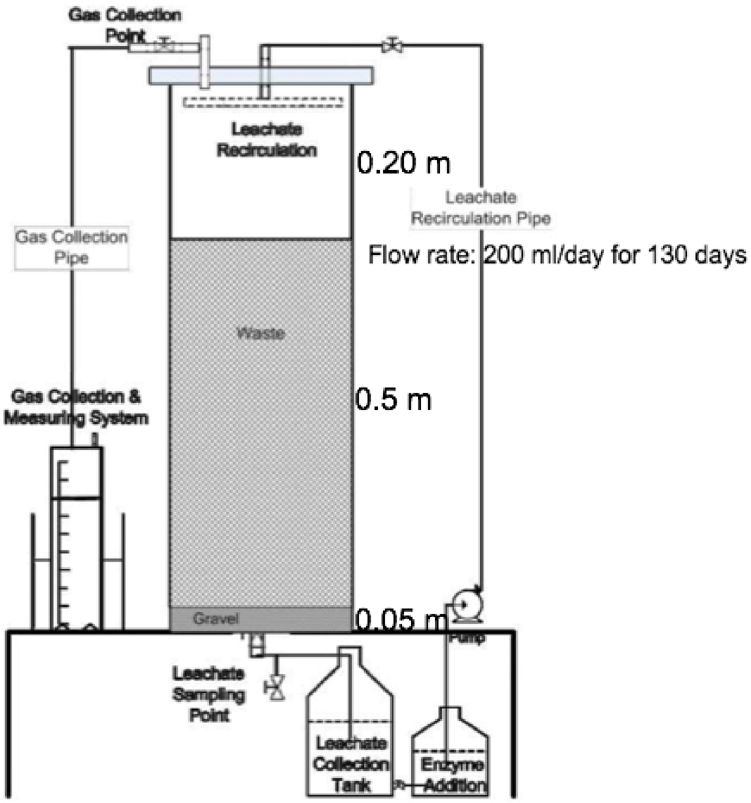

Figure 1: Diagrammatic representation of an anaerobic bioreactor landfill simulator (amended from Jayasinghe et al., 2012)

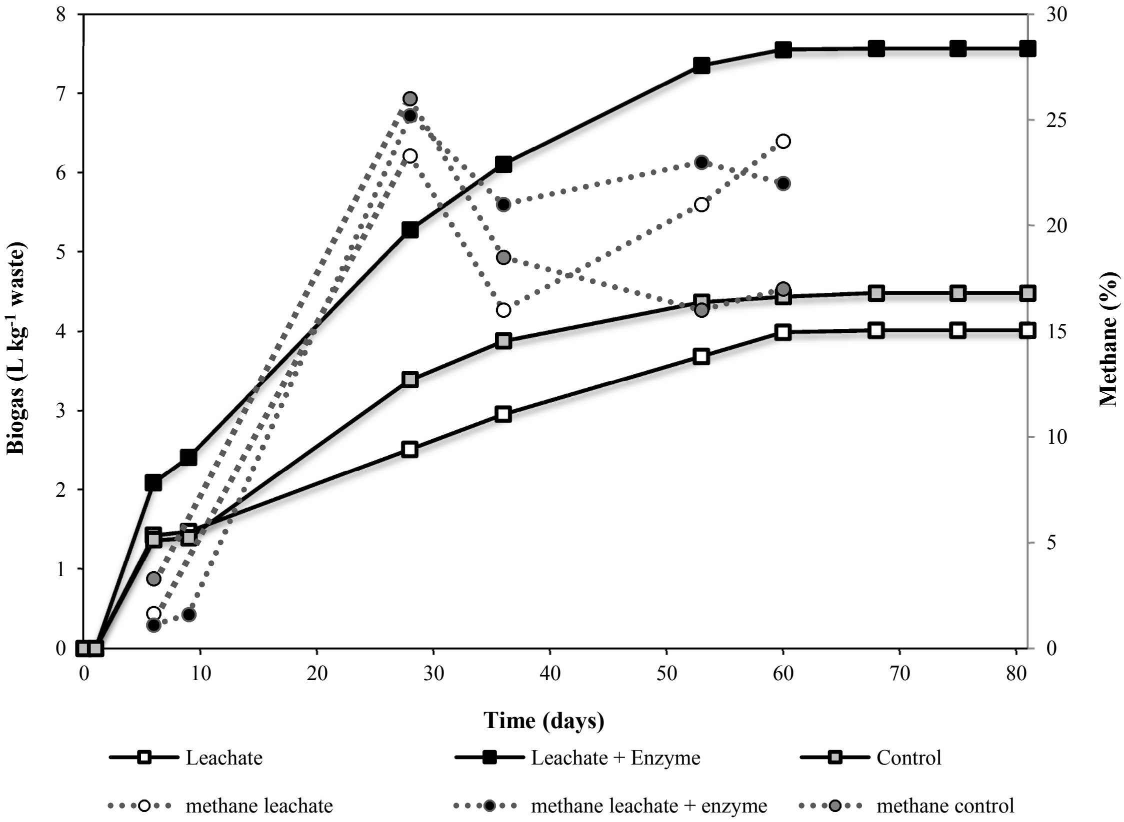

Figure 2: Cumulative biogas production (lines) and methane concentration (%) from the batch tests (dots).

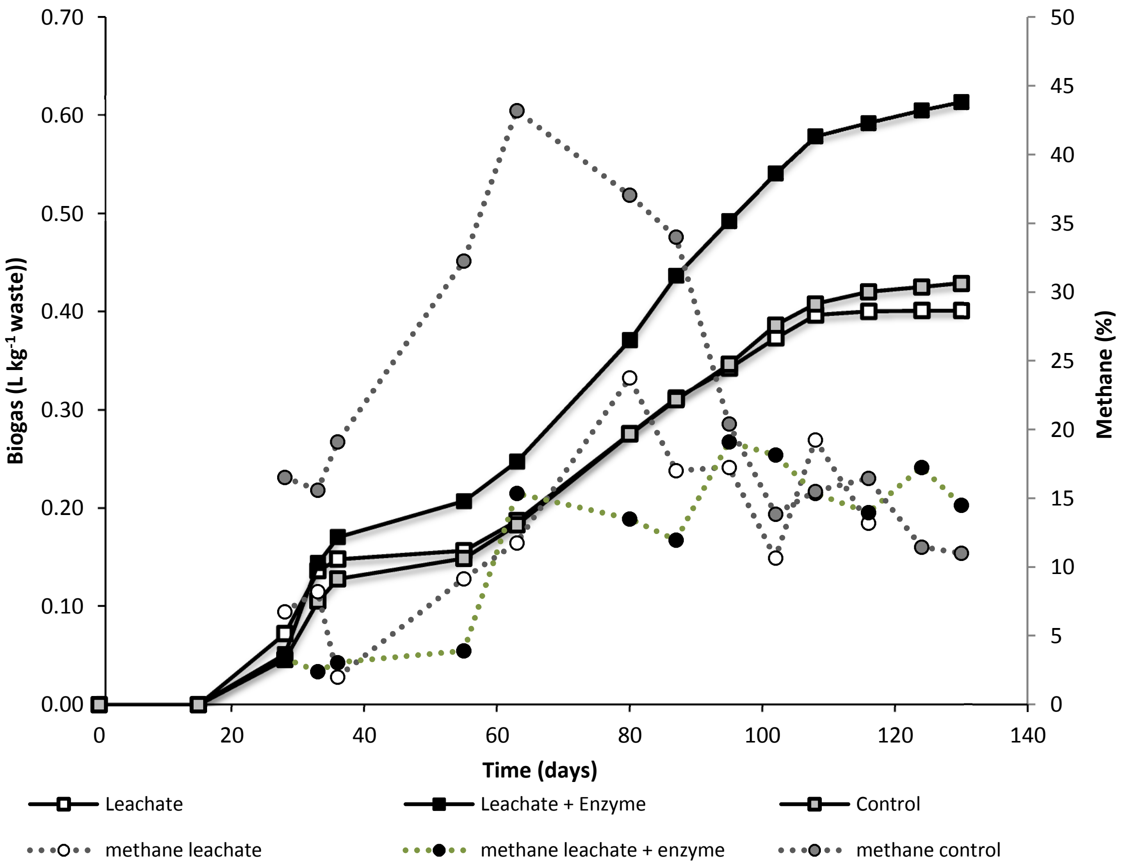

Figure 3: Cumulative biogas production (lines) and methane concentration (dots) in the bioreactors over 130 days.