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**Effect of Pre-aeration and Inoculum on the Start-up of Batch Thermophilic Anaerobic  
Digestion of Municipal Solid Waste**

W. Charles\*, L. Walker, R. Cord-Ruwisch

Centre for Organic Waste Management, Murdoch University, South Street, Murdoch, WA.  
6150, Australia

(E-mail: [w.charles@murdoch.edu.au](mailto:w.charles@murdoch.edu.au))

**\*Corresponding Author:**

Dr. Wipa Charles

Research Fellow

Environmental Biotechnology CRC

<http://www.ebcrc.com.au>

Division of Science and Engineering

Murdoch University, South street, Murdoch,

Western Australia 6150

Phone +61(0)8 9360 7407

Fax +61(0)8 9360 4713

E-mail: [w.charles@murdoch.edu.au](mailto:w.charles@murdoch.edu.au)

## **Abstract**

In this study, a short pre-aeration step was investigated as pre-treatment for thermophilic anaerobic digestion of the organic fraction of municipal solid waste (OFMSW). It was found that pre-aeration of 48 hours generated enough biological heat to increase the temperature of bulk OFMSW to 60°C. This was sufficient self-heating of the bulk OFMSW for the start-up of thermophilic anaerobic digestion without the need for an external heat source. Pre-aeration also reduced excess easily degradable organic compounds in OFMSW, which were the common cause of acidification during the start-up of the batch system. Careful consideration however must be taken to avoid over aeration as this consumes substrate, which would otherwise be available to methanogens to produce biogas. To accelerate methane production and volatile solids destruction, the anaerobic digestion in this study was operated as a wet process with the anaerobic liquid recycled through the OFMSW. Appropriate anaerobic liquid inoculum was found to be particularly beneficial. It provided high buffer capacity as well as suitable microbial inoculum. As a result, acidification during start-up was kept to a minimum. With volatile fatty acids (VFAs- acetate in particular) and H<sub>2</sub> accumulation typical of hydrolysis and fermentation of the easily degradable substrates during start-up, inoculum with high numbers of hydrogenotrophic methanogens was critical to not only maximise CH<sub>4</sub> production but also reduce H<sub>2</sub> partial pressure in the system to allow VFAs degradation. In a lab scale bioreactor, the combined pre-aeration and wet thermophilic anaerobic digestion was able to stabilise the OFMSW within a period of only 12 days. The stabilised inert residual material can be used as a soil amendment product.

**Key words:** anaerobic digestion, thermophilic, municipal solid waste, pre-treatment, inoculum

## INTRODUCTION

The production of municipal solid waste (MSW) is continuously increasing. On a global scale, the amount of MSW generated worldwide in 2006 was 2.02 billion tonnes (Key Note, 2007) with production predicted to increase by 51 % between 2005 and 2025. In Australia, at least 5 million tonnes of food and garden organic waste were collected from household, commercial and industrial sources between 2003 and 2007. About one-third of this waste was recycled into soil, mulch and compost products (Chiodo, 2007) and the balance went to landfill. As landfill gas emissions are one of the largest anthropogenic sources of methane (Adhikari *et al.*, 2006) it is necessary to implement better management practice to prevent these uncontrolled emissions. Anaerobic digestion offers an alternative approach whereby the methane, which would otherwise be released to the environment, can be recovered for energy/electricity generation.

Anaerobic digestion of MSW may be performed in various ways; the leading concepts are dry continuous systems, dry/semi-dry batch systems, wet continuous systems, and co-digestion with other types of waste. The benefits and disadvantages of each concept were described by Lissens *et al.* (2001). Anaerobic digestion of solid material involves a series of metabolic reactions including hydrolysis, fermentation and methanogenesis. Hydrolysis is regarded as the rate-limiting step as the MSW substrate comprises complex organic solids. Various thermal, chemical, and enzymatic pre-treatment methods have been extensively studied to increase substrate solubility and subsequent methane production (Kim *et al.*, 2002; Kim *et al.*, 2005; Hartmann, 2005; Sonakya *et al.*, 2001).

Among biological methods, pre-aeration was found to improve the start-up and performance of dry anaerobic digestion of pulp mill sludge through methane yield and solids reduction (Capela *et al.*, 1999). Hasegawa and Katsura (1999) reported significant improvement in yield when sewage sludge was solubilised under slightly thermophilic aerobic conditions prior to anaerobic digestion. They suggested that the improvement was the result of external enzymes secreted by thermophilic aerobic bacteria. Recent work by Nguyen *et al.* (2007) showed a positive effect in methane production through micro-aeration, although it was unclear if the improvement was a result of hydrolysis enhancement.

Pre-aeration has also been used to remove the easy-degradable fraction of MSW prior to anaerobic digestion. It was found to improve methanogenic activities by reducing acidification, due to rapid degradation of easy-degradable organic compounds, during the start-up of batch anaerobic digestion (O'Keefe and Chynoweth, 2000; Parawira *et al.*, 2004).

In this study, a short aeration is used as a pre-treatment alternative prior to the batch thermophilic anaerobic digestion of MSW. Thermophilic digestion of solid waste is known to provide higher methane yields than their mesophilic counterpart (Nimmrichter and Kubler, 1999). However, thermophilic digestion involves greater demand for heating, which is in many cases approximates the excess energy yield (Mata-Alvarez *et al.*, 2000). One of the expected benefits of using pre-aeration is the self heating capacity due to aerobic respiration. Other benefits and disadvantages of pre-aeration on thermophilic anaerobic digestion of OFMSW were investigated. To optimise process performance, the anaerobic digestion in this study was operated as a wet process with

liquid recycling through OFMSW content. Anaerobic inoculum (liquid) was used for the start-up of the anaerobic digestion step. The benefits of inoculum on batch thermophilic anaerobic digestion of OFMSW are also studied and discussed.

## MATERIALS AND METHODS

### Reactor Design and Operation

The reactor used in this experiment is based on the DiCOM<sup>®</sup> reactor (Walker *et al.*, 2006a) which combines composting and thermophilic anaerobic digestion in a single closed vessel under batch conditions. It allows both pre-aeration and anaerobic digestion of OFMSW in the same reactor.

The 7L cylindrical reactor is made from high temperature PVC and is insulated with closed cell elastomeric foam (Aeroflex<sup>®</sup>). It is fully computer controlled and equipped with probes and sensors as detailed in Figure 1. The external reactor temperature was maintained at a temperature equivalent to that of the reactor core, via a heat tape wrapped around the reactor to a maximum of 60°C.

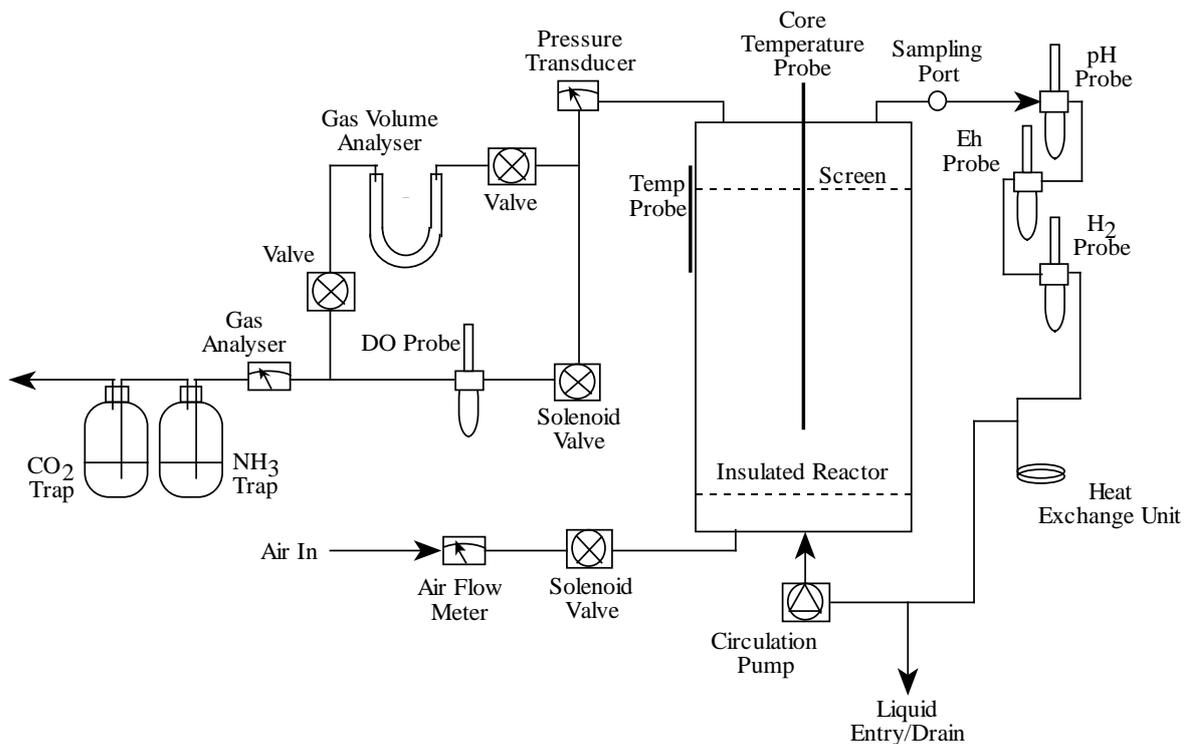


Fig. 1. Laboratory-scale DiCOM# reactor design

Pre-aeration: Pressurized air was introduced into the sealed reactor until the internal pressure was raised to a predetermined level, at which time inflow air was stopped and the internal pressure maintained before being released. This aeration regime was repeated every 10 minutes.

To produce pre-aerated OFMSW with different aeration periods, the internal volume of the reactor was divided into 6 equal vertical compartments using plastic dividers. The experiment initially filled only one compartment with OFMSW. For each of the following 5 days, a new compartment was filled with fresh OFMSW. At the end of day 6, aeration was stopped. The OFMSW, representing the different aeration periods, were then carefully removed from each compartment and used immediately.

Thermophilic anaerobic digestion: was performed by flooding the reactor with liquid (anaerobic inoculum) and re-circulated (maximum rate: 70mL/min) through an external heat exchange unit to maintain the temperature at 55°C. Liquid was withdrawn from the top of the reactor and reintroduced to the bottom of the reactor to minimize feed compaction and liquid channelling.

### **Logged Data**

O<sub>2</sub> concentration, airflow rate, internal pressure, core and outside reactor temperature, pH and biogas generation rate were logged by computer using a Labjack USB interface and National Instruments LabView 7 control software. O<sub>2</sub> concentration in the exit gas

during the pre-aeration was measured using a polarographic (Mettler Toledo InPro6100) O<sub>2</sub> sensor. Biogas production was determined by the downward displacement of oil (Dow Corning 200 Fluid 50CS) with real-time CO<sub>2</sub> and CH<sub>4</sub> concentrations in the exit gas logged by a gas analyser (Geotechnical Instruments GA 2000).

### **MSW source and characteristics**

Municipal solid waste (MSW) collected in the metropolitan area of Perth, Western Australia in March 2005 was passed through a mechanical sorting trommel with screen aperture of 50mm. Larger inert objects (plastic, metal, glass) in the sorted OFMSW were removed by hand before being further shredded so that the material would pass through a 25mm screen. The shredded OFMSW was sealed in plastic bags and frozen (-20°C) until required.

Prior to use, the OFMSW was thawed overnight to ambient temperature. To replace paper lost during the mechanical sorting process and provide a solid matrix, shredded paper (50g Hygenex<sup>®</sup> 2187951) and Jarrah wood chips (200g - trapped between 1 and 5mm screens) were added to the OFMSW (3kg, 1690g dry weight). De-ionised water (500mL) was added to provide sufficient moisture content of 55%. The C:N ratio of the mixed OFMSW was 18:1 and total volatile solids (TVS) was 0.56g/gTS. Fat, protein and carbohydrate contents were 4.5, 8.7, and 47.6 % of TS respectively. Throughout this study, this mixed OFMSW is referred to as OFMSW.

### **Anaerobic inoculum (liquid)**

Anaerobic inoculum used in this study was the anaerobic liquid drained from a lab-scale thermophilic anaerobic digester of OFMSW and stored at ambient temperature under an N<sub>2</sub> atmosphere until required. In preparation for use, the anaerobic liquid was heated in a water-bath from room temperature to 55°C over a 48 hour period and maintained at this temperature for 3 days prior to being introduced to the reactor.

### **Batch (serum vial) experiment**

All batch experiments were performed in 100mL serum bottles in triplicate. After the contents were introduced, the serum bottles were sealed with 1-cm thick butyl rubber stoppers and capped with aluminium crimp seals. Headspaces were flushed with 80% N<sub>2</sub> and 20% CO<sub>2</sub> before they were incubated in a 55°C shaking water bath. After the medium had equilibrated to 55°C, the bottles were vented, using a syringe needle, to atmospheric pressure. Daily gas production was measured by the downward displacement of water. CH<sub>4</sub>, H<sub>2</sub> and CO<sub>2</sub> concentrations were determined using a Varian Star 3400 Gas Chromatograph (GC) equipped with a thermal conductivity detector. The carrier gas was high purity nitrogen at a flow rate of 30 ml/min. Separation was performed on a Pora-PakQ packed column (2m x 5mm (internal diameter)) maintained at 40°C. Detector and inlet temperatures were maintained at 40 and 120°C respectively. Manual injections of 50 µl headspace samples were performed with a 100 µl gas-tight syringe (Hamilton).

### **Chemical Analysis**

A Varian Star 3400 gas chromatograph (GC) fitted with a Varian 8100 auto-sampler was used to analyse the VFA concentration of liquid samples. Samples were acidified

with formic acid (to 1% (v/v)) before 1 $\mu$ L samples were injected onto an Alltech ECONOCAP<sup>TM</sup> EC<sup>TM</sup> 1000 (15m x 0.53mm 1.2 $\mu$ m i.d.) column. The carrier gas (N<sub>2</sub>) was set at a flow rate of 30mL/min. The oven temperature was programmed as follows: initial temperature 80°C; temperature ramp 40°C/min to 140°C, hold for 1 min; temperature ramp 50°C/min to 230°C hold for 2 min. Injector and detector temperatures were set at 200 and 250°C respectively. The peak area of the Flame Ionisation Detector (FID) output signal was computed via integration using STAR Chromatography Software (© 1987-1995).

The moisture content of OFMSW was obtained by heating overnight at 105°C. Liquid extracts from the OFMSW samples were obtained to determine NH<sub>4</sub><sup>+</sup> content, pH and COD (Chemical oxygen demand) as per Australian Standard (AS 4454 - 2003). NH<sub>4</sub><sup>+</sup> and COD concentration in extracts was determined as described by American Public Health Association (2005).

Methanogen cell counts were estimated by 3-tube MPN according to a standard method (de Man, 1975). Based on information from molecular analysis, which identified *Methanosarcina sp.* and *Methanoculleus sp.* as the predominant acetoclastic and hydrogenotrophic methanogens respectively, anaerobic media (DSM 334 medium in DSMZ, 1983) were prepared with substrates in the following final concentrations: 80 mM acetate for acetoclastic methanogens and 10 mM acetate + H<sub>2</sub> + CO<sub>2</sub> for hydrogenotrophic methanogens.

For solid samples, 1 g of solid was mixed with 50 ml basal media, and then homogenized in a sterilized blender for 30 seconds. The mixed liquor (0.5 ml) was then used in a dilution series using 16.5-ml Hungate tubes containing 4.5 ml of the appropriate medium. Incubation temperature was 55°C. Growth was scored as positive when methane was produced. Microscopy was used to observe the morphology of the methanogens.

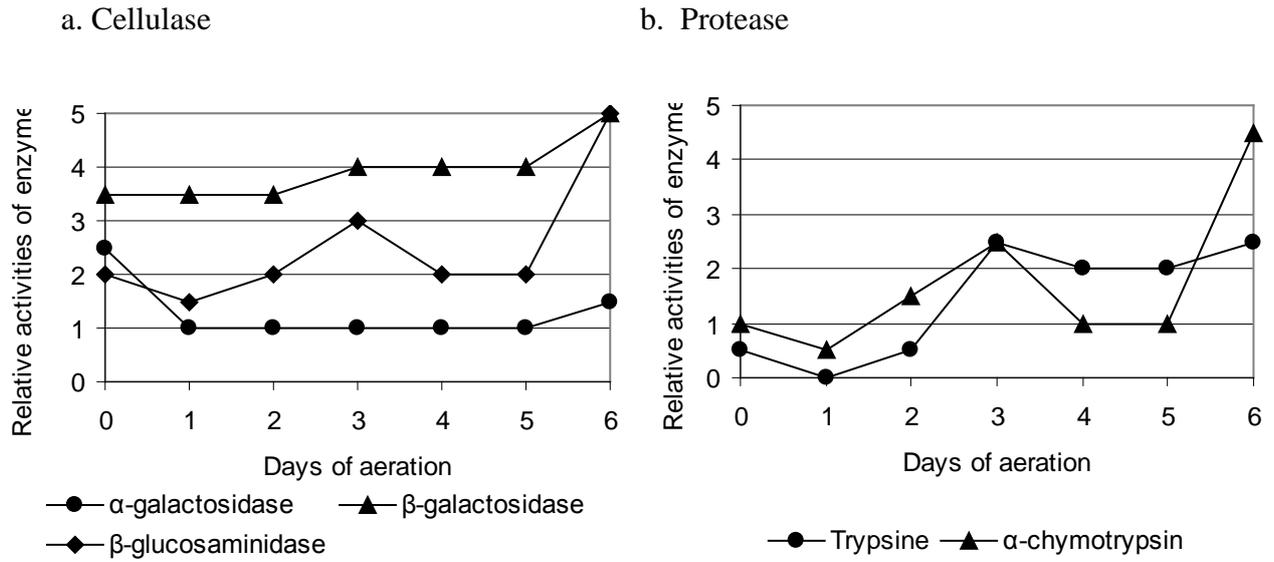
Extracellular enzymes were quantified according the enzyme assay method (Sonia *et al.*, 2001). Enzyme extracts were prepared by mixing 5 g of OFMSW sample with 50 ml of phosphate buffered water. The solution was shaken for 10 minutes using a stomacher (a shaker), allowed to settle for 10 min, and the supernatant was used for enzyme analysis. APYZYM™ strips (API Analytab Products, New York) were used to evaluate the extracellular enzyme profiles.

## RESULTS AND DISCUSSION

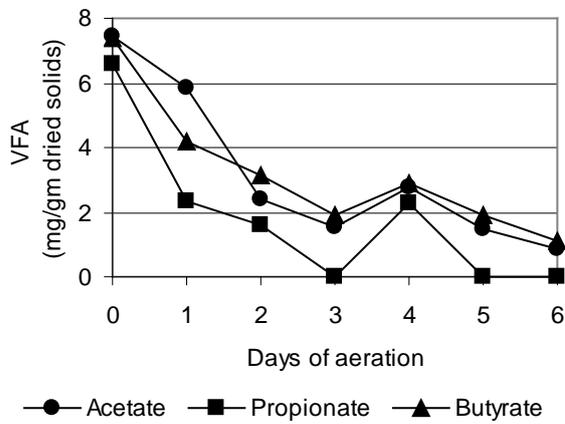
### Effect of pre-aeration on OFMSW characteristics

Due to the initially high microbial activity in OFMSW, the easily available organic compounds were rapidly converted into CO<sub>2</sub>, biomass and heat resulting in rapid heat generation within 48 hours of aeration, as is generally observed in composting (Epstein, 1997). This temperature increase coincided with a rapid increase in O<sub>2</sub> consumption. Thermophilic conditions (60 °C) were reached at the end of day 2. A total of 36 g of O<sub>2</sub> (1.12 mole) was consumed over the 2-day period. Assuming that complete oxidation liberates 14 kJ per gram of O<sub>2</sub> consumed (Finstein *et al.*, 1986) approximately 503 kJ of biological heat was produced, sufficient to self heat the bulk MSW to the thermophilic stage for subsequent anaerobic digestion without requiring an external heat source.

During pre-aeration, aerobic microbes hydrolyse organic solids by producing exoenzymes to solubilise the solid substrate. Once solubilised these substrates enter the cell to be degraded by endoenzymes, resulting in microbial growth and heat. In this study hydrolysis was apparent during pre-aeration, as both cellulase and protease exoenzyme activities were observed throughout the 5 days of pre-aeration (Figure 2).



**Figure 2:** Enzyme activities of OFMSW with different pre-aeration time (ApiZyme method).



**Figure 3:** VFAs of OFMSW with different pre-aeration time.

In contrast, the level of soluble COD and VFAs (Figure 3) drastically reduced during the first 2 days of aeration. As respiration degrades VFAs and COD while hydrolysis generates those substances, this indicates that respiration rates were significantly higher than hydrolysis rates (high conversion of substrate to CO<sub>2</sub>) during the initial aeration. Thereafter, the hydrolysis rates match the respiration rates resulting in stable dissolved COD levels.

Coincident with the protease exoenzyme increase, NH<sub>4</sub><sup>+</sup> accumulation from protein degradation was evident. It gradually increased from 0.7 mg NH<sub>4</sub>-N/gmTS and peaked at 2.0 mg NH<sub>4</sub>-N/gmTS at the end of day 4. This was assumed to cause the increase in pH, from 6.7 to about 8.8 during the same period. The loss of NH<sub>4</sub><sup>+</sup> after day 4 (to 1.0 mg NH<sub>4</sub>-N/gmTS at the end of day 6) was due to volatilization as ammonia (NH<sub>3</sub>) facilitated by the alkaline pH (Osunadea *et al.*, 2008). This is in agreement with Sánchez-Monedero *et al.* (2001) who also found that the high degradability of MSW during the thermophilic step and high pH were responsible for the high ammonia volatilization losses. No nitrification took place at this stage as the temperature was much higher than the optimum temperature (32-35 °C) for nitrifying bacteria. This was also confirmed by the absence of nitrite and nitrate in the compost (Wong *et al.*, 2003).

### **Survival and significance of methane producing microbes in the pre-aerated OFMSW**

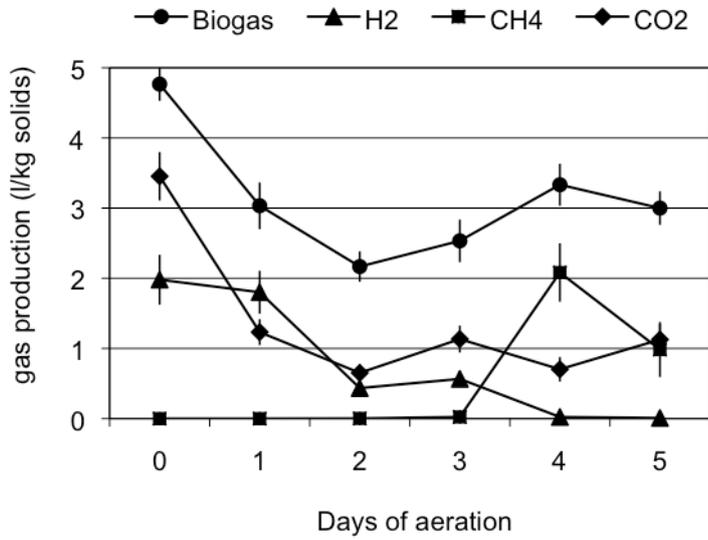
Although composting is generally considered an oxygen rich environment, a number of studies (Beck-Friis *et al.*, 2000, Jäckel *et al.*, 2005) observed methane emissions during the composting process. Recently, thermophilic methanogens in the compost material have also been detected (Thummes *et al.*, 2007a). Using single strand conformation

polymorphism (SSCP) and cloning analysis, Thummes *et al.* (2007b) indicated the presence of *Methanosarcina thermophila*, *Methanoculleus thermophilus*, and *Methanobacterium formicicum*. This indicates that the environmental conditions (low redox potential and high temperatures) during the early phase of composting induce enrichment of archaean methanogens.

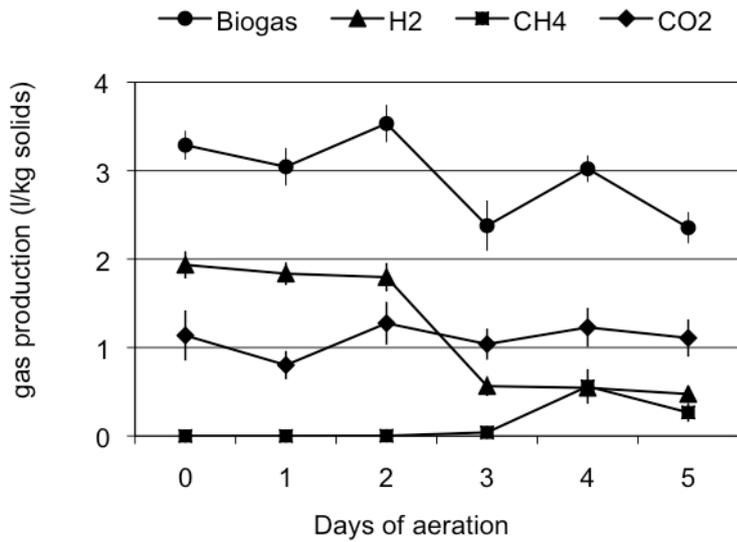
In this study, pulsed pressurized aeration was used and oxygen penetration was expected to be higher than in windrow composting and hence the survival or development of methanogenic bacteria during the aeration was expected to be limited. It was found from microscopical observations and Most Probable Number (MPN) assay that significant numbers of methanogens had survived or indeed grew under the pre-composting conditions. From the beginning, there were approximately  $10^3$  and  $10^5$  acetoclastic and hydrogenotrophic methanogens, respectively, present per gram of raw OFMSW. During the aeration, where temperature increased to 60°C and exit oxygen concentrations of more than 1 %, the methanogenic numbers did not decrease. While the acetoclastic methanogens were almost unaffected by the aerobic incubation, the number of hydrogenotrophic methanogens temporarily increased by almost 100-fold whilst oxygen partial pressure was less than 10 kPa. This implies that during pre-aeration, although oxygen in the environment was sufficient to wipe out the entire communities of methanogens, anoxic niches in the bulk OFMSW were able to maintain the anaerobic condition for these methanogens to survive. This result supports the finding of Jäckel *et al.* (2005) that the highly aerated environment of composting favors the development of anoxic niches during thermophilic phases, so that a basic requirement for thermophilic methanogenic activity is produced.

In addition to recording the numbers of viable methanogens, their activity under substrate saturation was also evaluated by supplying acetate or formate for acetoclastic and hydrogenotrophic methanogens, respectively. For this purpose a series of batch experiment was set up using OFMSW solid samples, aerated from 0 to 5 days. The samples were flooded with water containing 30 mM acetate or formate (pH adjusted to 7). During 24 h of anaerobic incubation, all samples produced more than 2 L of biogas per kg solids. However, the gas was comprised largely of CO<sub>2</sub> and H<sub>2</sub> (Figure 4). Methane was only produced in samples that had been aerated for 4 days or more. Although methanogenic substrates were present, the methanogens did not produce methane. This is likely due to the acidification caused by the fermentation of easily degradable substrate available in non-aerated OFMSW, as evidenced by a pH drop to 5.5 in those trials that had been aerated for 0-3 days and pH 6 when aeration had been extended to 4-5 days. This suggested that microbial respiration during short aeration was beneficial as it reduced the risk of acidification during the start-up of subsequent anaerobic digestion of OFMSW.

a. Acetate



b. Formate



**Figure 4:** Composition of biogas produced from formate or acetate during 24 hrs of anaerobic incubation from OFMSW that was pre-aerated between 0 and 5 days.

## **Effect of anaerobic liquid on the start-up of batch thermophilic anaerobic digestion of OFMSW**

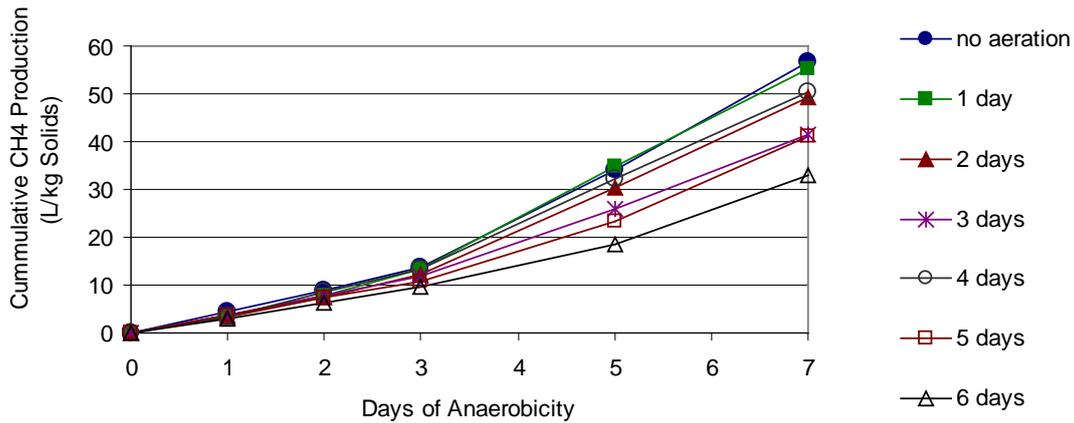
As the lack of methanogenic activity at the beginning of dry batch anaerobic digestion of OFMSW can be caused by acidification, the use of a liquid with higher buffer capacity would be expected to enhance methane formation. The idea of using recycled anaerobic liquid from the previous batch of an anaerobic digester of solid waste is not novel. The recirculation of leachate/anaerobic liquor has been shown to stabilize a reactor against acidification and enables a more rapid onset of methane production (El-Fadel, 1999; El-Mashad and van Loon, 2006; Walker *et al.*, 2006b).

In this study, anaerobic liquid was obtained from the drain of a laboratory-scale batch thermophilic anaerobic reactor treating OFMSW after methane production was being exhausted and VFAs were consumed. The liquid was found to contain high buffer capacity. It required around 150 mmole of  $H^+$  to lower the pH of 1 litre of anaerobic liquid from 8 to 6. Another expected benefit of recycling anaerobic liquid is inoculum. To clarify the role of anaerobic liquid in assisting in the start-up of anaerobic digestion of pre-aerated OFMSW, a series of serum vials were set up to differentiate between the buffering and inoculum effects of the anaerobic liquor. This was done by comparing the effect of anaerobic liquid (methane production) to a sterile-filtered anaerobic liquid where microbial inoculum was removed.

After 24 hr of incubation, the type of liquid added had no significant effect on the volume of gas produced. The different liquids used did however have a significant effect on the methane content in the biogas. The control that was flooded with water

showed only a small percentage of methane in the biogas. pH in this trial was 6. The buffer capacity in the sterile filtered anaerobic liquid buffered the pH to around 6.5, allowing more methane production. Significantly higher CH<sub>4</sub> production (26%) was however obtained in the trial using full anaerobic liquid (buffer + microbes). This shows that although the essential role of anaerobic liquid was its buffer capacity, the more important role was appropriate microbial communities essential for the rapid start-up of methanogenesis.

To determine the effect of duration of pre-aeration on subsequent thermophilic methanogenesis, pre-aerated OFMSW (0-6 days) were tested for their capacity to produce methane gas under thermophilic anaerobic conditions using the same anaerobic liquid. One could expect two effects of the aerobic pre-treatment: (a) aerobic hydrolytic exo-enzymes secreted by thermophilic aerobic bacteria provide methanogenic metabolites which would benefit methane production (Borowski and Szopa, 2007; Capela *et al.*, 1999; Hasegawa and Katsura, 1999; Miah *et al.*, 2005). (b) aerobic oxidation exhaust the easy-to-degrade component of the starting material (oxidation to CO<sub>2</sub>) which would otherwise have been used by anaerobic microbes to generate methane (Krzystek *et al.*, 2001). Results in Figure 5 clearly show the latter. Increased duration of aerobic pre-treatment limited the amount of methane gas formed rather than stimulating it.



**Figure 5:** Cumulative CH<sub>4</sub> production from starting material with different pre-aeration time.

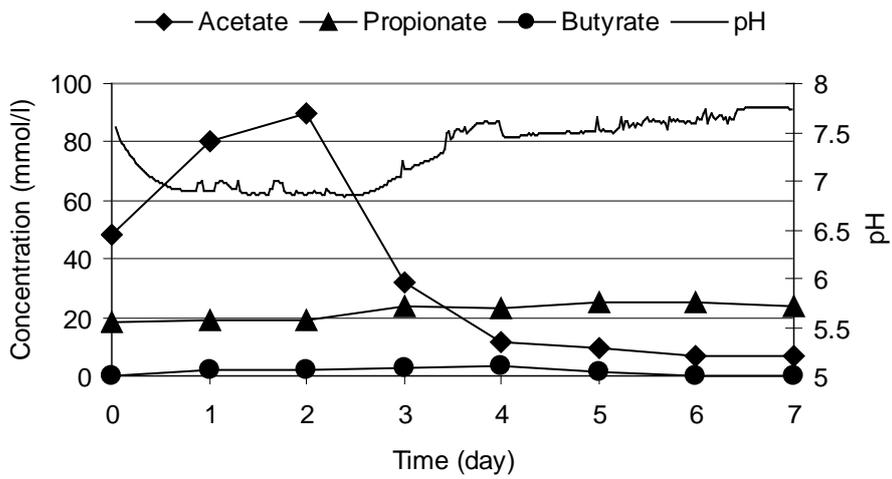
A carbon balance (Table I) showed that carbon lost from the OFMSW during the six days of aeration as CO<sub>2</sub> accounts for the reduction of CH<sub>4</sub> and CO<sub>2</sub> during subsequent anaerobic digestion. This suggests that prolonged aeration consumes substrates which would otherwise be available to methanogens to produce biogas. Therefore, if pre-aeration is employed to minimise acidification due to easily degradable substrate in MSW, careful consideration must be taken to avoid over aeration (optimise biogas production).

### **Lab-scale batch thermophilic anaerobic digestion of pre-aerated OFMSW**

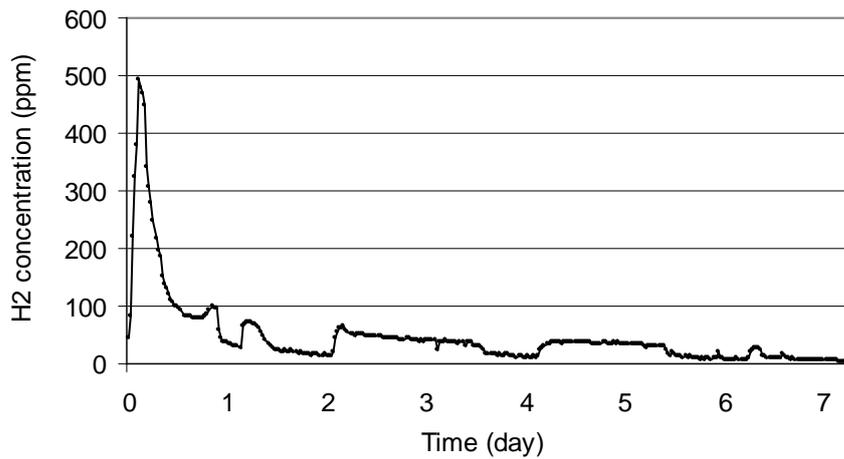
Based on the above information, a 7 L laboratory-scale batch reactor was set up. OFMSW was pre-aerated for 5 days, prior to thermophilic anaerobic digestion. The anaerobic digestion was started up by flooding the pre-aerated OFMSW (60 °C) with warm anaerobic liquid (55 °C). The anaerobic liquid was recycled to minimize feed compaction and liquid channelling. The added benefits of liquid recycling were mixing,

removal of localised inhibitory fermentation products, and improved buffer capability of acid (O'Keefe and Chynoweth, 2000; Ledakowicz and Kaczorek, 2002).

The VFA profiles in Figure 6 showed typical rapid acetate accumulation during the start-up of batch anaerobic digestion of solid waste (Brummeler, 2000; Han *et al.*, 2002; Maroun and Fadel, 2007). This is a clear indication of carbohydrate hydrolysis as carbohydrates were more readily degraded than nitrogenous materials and lipids (Eastman and Ferguson, 1981). The accumulation of acetate during the first 2 days coincided with the pH drop and high H<sub>2</sub> partial pressure (Figure 7) that indicated acidification during the start-up (despite pre-aeration). However, without additional buffer, the anaerobic liquid self-regulated to maintain the pH between 6.8 and 7.8, highlighting its significant buffer capacity. Butyrate concentration was very low throughout while propionate increased slightly. Ammonia accumulation was almost constant at around 4 mmole/L/day. As ammonia is the end product of protein degradation, this result indicated that protein degradation in the system was much more gradual than carbohydrate's. This result is inline with Jokela and Rintala (2003), which indicated that the proteins in MSW have a lower rate of degradation than cellulose.



**Figure 6:** VFA concentrations during batch anaerobic digestion of OFMSW.



**Figure 7:** H<sub>2</sub> concentration in the anaerobic liquid during batch anaerobic digestion of OFMSW.

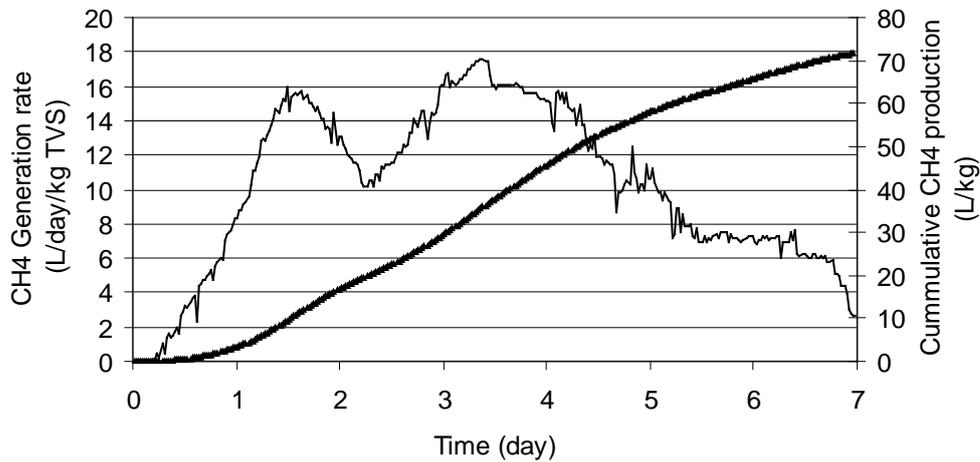
The onset of methanogenesis was rapid and commenced within hours of the anaerobic liquid being introduced into the reactor. The absence of a lag phase of methanogenesis indicates sufficient inoculum in the system. The maximum biogas and methane production rate occurred when acetate consumption was most rapid (day 3-4). During this stage the % methane in the biogas rose from 30% at the beginning to 65%. The mean methane and biogas production rate was determined to be 10.2 and 18.2 L/day/kg of TVS respectively, a lower value than the continuous (Bolzonella *et al.*, 2003; Sinclair and Kelleher, 1995; Krzystek *et al.*, 2001) and batch (Krzystek *et al.*, 2001) anaerobic digestion systems reported in the literature. However, these systems expose the microorganisms to non-aerated feeds containing easily degradable substrates that would provide greater availability of substrate for biogas generation.

After only 7 days of anaerobic incubation, VFA accumulation (Figure 6) and methane production (Figure 8) were near completion, indicating the easily biodegradable components of OFMSW were exhausted. Total solids degradation was 41% of TVS. This is considered low compared to volatile solid destruction of 43.8-72.5% and 34-78% achieved by Elefsiniotis and Oldham (1994) and Yu *et al.* (2003), respectively. These studies, however, used sewage sludge where biodegradability and surface area would be different from OFMSW used in our study. To complete conversion of the OFMSW to biogas (the ultimate anaerobic biodegradation), the process would require up to 200 days (Rao *et al.*, 2000) with a minimum of 20 days to convert 95% of the degradable fraction to biogas (Napharatana *et al.*, 2007). With the aim to only stabilise OFMSW rather than total degradation, the process was considered to reach conclusion. The stabilised end-product, which contains residual inert material, such as lignin,

microbial cells and un-degraded cellulose, can be recycled as a useful soil amendment. By combining pre-aeration and wet thermophilic anaerobic digestion as described in this study, the OFMSW could be stabilised within 12 days. This is significantly shorter than the 21 days reported for traditional batch anaerobic digestion to stabilise the OFMSW (Brummeler, 2000).

Two distinct peaks in methane production rate (at day 1.5 and day 3) (Figure 8) were observed in repeated experiments. The second peak corresponded with rapid acetate consumption. However, the first peak coincided with acetate accumulation. This observation couples with H<sub>2</sub> and VFA profiles (Figure 7 and 6 respectively), suggesting that unlike continuously fed anaerobic digesters (operating at steady state, feed and all intermediates), the batch anaerobic digestion of solids waste systems (such as MSW) creates a dynamic change of the conditions. The shock load of easily degradable organic compounds during start-up created acidification condition and a rush of H<sub>2</sub> production. Since methanogenesis from acetate (both acetoclastic and syntrophic co-culture) was limited in the presence of high H<sub>2</sub> partial pressure (Ferguson and Mah, 1983; Lee and Zinder, 1988), it is most likely that methane formed at this stage originated purely from H<sub>2</sub> and CO<sub>2</sub> produced by the fermentation of organic material, rather than from acetate (Eastman and Ferguson, 1981). In a separate experiment, where isotope methane (<sup>13</sup>CH<sub>4</sub>) was tracked following the spiking of isotope acetate (C1 and C2) into the system (results not shown), also confirmed that there was no conversion of acetate to methane during the first 2 days. High numbers of hydrogenotrophic methanogens in the inoculum were therefore critical during the start-up to maximise CH<sub>4</sub> production from the high H<sub>2</sub> level and ease H<sub>2</sub> partial pressure in the system. Only when the H<sub>2</sub> partial pressure was

reduced, its inhibitory effect on the methanogenesis of acetate diminished. The condition then allowed acetate to be utilised by methanogens (day 2 and 3-Figure 6), which was observed as the second methane production peak (Figure 8).



**Figure 8:** CH<sub>4</sub> production rate (thin line) and cumulative CH<sub>4</sub> production (bold lines) during batch anaerobic digestion of OFMSW.

This observation highlighted the uniqueness of batch anaerobic digestion of solid waste (OFMSW in particular). Short aeration followed by wet thermophilic anaerobic digestion proposed by this study proved to be able to maximise and accelerate methane production within a minimum retention time of only 12 days.

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