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Rapid Start-Up of Thermophilic Anaerobic Digestion with the Turf Fraction of MSW as Inoculum

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Abstract

This study aims to determine suitable start-up conditions and inoculum sources for thermophilic anaerobic digestion. Within days of incubation MSW at 55 ^{o}C, methane was produced at a high rate. In an attempt to narrow down which components of typical MSW contained the thermophilic methanogens, vacuum cleaner dust, banana peel, kitchen waste, and garden waste were tested as inoculum for thermophilic methanogenesis with acetate as the substrate. Results singled out grass turf as the key source of thermophilic acetate degrading methanogenic consortia. Within 4 days of anaerobic incubation (55 ^{o}C), anaerobically incubated grass turf samples produced methane accompanied by acetate degradation enabling successful start-up of thermophilic anaerobic digestion. Other essential start-up conditions are specified. Stirring of the culture was not conducive for successful start-up as it resulted specifically in propionate accumulation.

Key words: Turf, Rapid Start-up, Thermophilic Anaerobic Digestion, MSW, Inoculum

1. Introduction

Currently, thermophilic anaerobic digestion is of wide interest as a method to treat organic wastes due to its potential to increase volumetric biogas production (Griffin et al., 1998; Ahn and Forster, 2000) and facilitate pathogen reduction (Dugba and Zhang, 1999) when compared to mesophilic anaerobic digestion. Despite these advantages, full-scale thermophilic anaerobic digestion has had limited applications, confined to only some regions such as Europe (De Baere, 2000), due to the difficulties in control and start-up processes. Of these disadvantages, the start-up process is the most significant drawback of thermophilic anaerobic digestion. Due to the limited numbers of thermophilic anaerobic reactors, thermophilic inocula are not readily available. Without a source of thermophilic inoculum, the start-up period of thermophilic anaerobic digestion can be prolonged, with Ahring (1994) reporting up to one year for a reactor to reach steady state.

To decrease the duration of the start-up period, some researchers have succeeded in starting up thermophilic anaerobic digestion within a month by using anaerobic mesophilic sludge (Lepistö and Rintala, 1997; Boušková et al., 2005). However, during the transition of temperature from 37 ^{0}C to 55 ^{0}C, a proper strategy is necessary, to avoid sludge washout, as a direct result of temperature transition, resulting in a start-up period spanning several months (Fang and Lau, 1996). To start-up new commercial anaerobic reactors, it is still difficult to obtain a large amount of mesophilic anaerobic seed. This issue is particularly relevant in rural areas where anaerobic digestion is rarely in operation.

Despite the increasing interest in thermophilic anaerobic digestion, studies of thermophilic methanogenic sources remain limited. Inoculum source is a significant factor for the successful start-up of thermophilic anaerobic digestion. Hence, the generation of suitable thermophilic inocula, which are readily available in large quantity, is a worthwhile goal for a research project.

The last decade has witnessed an increase in the generation of municipal solid waste (MSW). In 2006, the amount of MSW produced globally was 2.02 billion tones, with this amount predicted to have increased by 37% between 2007 and 2011 (Global Waste Management Market Assessment 2007). This rapid increase in waste production has sparked enhanced interest in environmentally sustainable MSW processing and treatment.

The biological conversion of the organic fraction of municipal solid waste (OFMSW) to methane has been described to be able to be accomplished via thermophilic anaerobic digestion (Bolzonella et al., 2003; Walker et al., 2006a; Forster-Carneiro et al., 2007). In this process thermophilic methanogens are believed to be introduced into the reactor - at least in part – by the feedstock material (Charles et al., 2009). Based on the ready availability of MSW and the presence of thermophilic methanogens in MSW, MSW could be an alternative start-up seed for thermophilic anaerobic digestion.

The main purpose of this study was to find a source of thermophilic methanogens suitable for the rapid and sustained startup of thermophilic anaerobic digestion that is readily available in large quantities. We define "rapid sustained start-up" by the following parameters:

i. a short lag time before the production of methane

ii. significant methane production rate (>0.2 L/L/d) after the lag time

iii. the presence of acetate dependent methanogenesis

To succeed in rapid start-up of anaerobic digestion, the lag time has to be shorter than one week. In addition, acetate, the most significant methanogenic substrate must be degraded to ensure a sustainable operation. Other aims of this current study were to:

- quantify the effect of aerobic pretreatment of MSW on the start-up of thermophilic anaerobic digestion;
- investigate the effect of stirring on thermophilic start-up;
- identify the fraction of MSW responsible for the source of thermophilic methanogens.

2. Materials and Methods

2.1 Inoculum sources

Different potential sources of methanogens such as cow manure, MSW, turf were tested as a seed for start-up of thermophilic anaerobic digestion. The types of inocula used in each experiment are shown in Table 1. The MSW, collected from the Perth Metropolitan area, Western Australia, was mechanically sorted and the OFMSW (wet bulk density = 578 $kg.m^{-3}$; C:N = 18:1; total volatile solids = 0.56 g/g TS (total solids); moisture content = 55%) prepared as described by Walker et al. (2009).

Cow manure produced on the day was collected from the veterinary farm located on the Murdoch University campus, Perth, Western Australia and used immediately as inoculum. Fresh grass samples were taken from three different locations: a grass lawn at Murdoch University, a local Perth turf supplier, and from a local household in Perth.

2.2. Experimental design

Acetate, as a carbon source for methanogenesis, sodium bicarbonate ($NaHCO_3$) as buffer, and deionised water, to adjust working volume, were used in all experiments as shown in Table 1. Experiments were conducted in either 1 L Schott® bottles (section 3.1, 3.2 and 3.3) sealed with rubber stoppers or in 100 mL serum vials (Wheaton), performed in duplicate (section 3.4 and 3.5) and sealed with butyl rubber stoppers and aluminum crimps. To establish anaerobic conditions, the headspaces of all flasks were flushed with N_2/CO_2 (80%/20%) for 30 seconds. All samples were incubated at 55 ^{0}C in either a water bath (Paton, model RW 1812) with shaking (30 oscillations/minute (O.P.M.)) or, in section 3.3, a heating magnetic stirrer (RCT basic IKAMAG® control stirred at 800 rpm). Following initial set-up, all serum vials were depressurized to atmospheric pressure, after the first hour of incubation.

To measure methane production in the 1 L Schott® bottles, CO_2 was scrubbed from the biogas by passing it through sodium hydroxide solution (1 M). The gas stream (assumed to be methane) was measured by the downward displacement of oil (Dow Coming 200 Fluid 50 CS). In the case of - serum vials, the volume of biogas over-pressure produced was measured using a 50 ml glass syringe (popper & sons, Inc.). Aerobic pre-treatment of MSW samples (section 3.2) was performed as described by Walker et al. (2006a).

2.3 Analysis

The VFA concentrations of samples were analyzed by gas chromatography (GC) using a Varian Star 3400 equipped with a Varian 8100 auto sampler and a flame ionization detector (FID) as described by Walker et al. (2009). The methane concentration in biogas was analyzed by Varian Star 3400 gas chromatograph (GC) equipped with a thermal conductivity detector as described by Charles et al. (2009). Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were performed according to American Public Health Association (2005).

3 Results and Discussion

3.1 Comparison of thermophilic methanogenic activity in cow manure and MSW

The knowledge that cow manure is a good source of methanogens is well established in the literature (Chachkhiani et al., 2004). Moreover, composting of MSW has been reported to produce methane (Beck-Friis et al., 2000; Jäckel et al., 2005). This implies that methanogens may already be present in MSW. Also literature studies on the anaerobic digestion of MSW reported that the development of thermophilic methanogenesis occurred, without supplying an external inoculum such as anaerobic digester sludge (Charles et al., 2009).

To investigate the potential source of thermophilic methanogens, the following experiment aims at comparing the relative thermophilic methanogenic activity in cow manure with that in MSW. Because cow manure is deprived of readily degradable material such as acetate, 80 mM of acetate was added to the tests with cow manure, to ensure the availability of acetate as methanogenic substrate.

As methanogenic acetate conversion (encompassing direct acetoclastic methanogenesis and syntrophic acetate conversion) is critical for anaerobic digestion, it is relevant to establish whether acetate dependent methanogenesis is present or not. The thermophilic incubation of cow manure and MSW produced methane gas within 4 days in both cases (Fig. 1A). Over two weeks of incubation, methane from MSW was continuously produced until it reached 9.5 L/L compared to only about 1.0 L/L from cow manure. Furthermore, the batch reactor seeded with cow manure did not lead to acetate degradation (Fig. 1B). This showed that the thermophilic consortium in cow manure did not serve as a suitable inoculum for rapid thermophilic acetate degradation, while acetate degradation in the reactor seeded with MSW was obvious from the volatile fatty acids profile (Fig. 1B).

By using non-radioactive polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) methods and 16S rDNA sequences, Chachkhiani et al. (2004) revealed that there were both *Methanosarcina thermophila* and *Methanoculleus thermophilus* in cow manure. Their initial biogas production rate was approximately 0.1 L/L/day after 3 days of cow manure's incubation and the methane content in the biogas reached 88% on day 10. The authors concluded that the methanogens present within the cow manure were suitable to start up thermophilic anaerobic digestion. By contrast the current study found that cow manure was not an appropriate thermophilic anaerobic inoculum due to lack of acetate degradation during methanogenesis. This indicates that cow manure does not always contain acetate degrading methanogens or synthophs . This was also found by the study by Shin et al. (2004), analyzing Archaea in cow rumen by PCR and 16S rDNA sequences, finding that the predominant Archaea belonged to the *Methanomicrobiaceae* family, which are hydrogen utilizing methanogens. This would make sense for the rumen environment, as VFA are the prime feed source for the animal.

3.2 The effect of aerobic pretreatment of MSW on methane production

The results above suggest that the MSW collected in the metropolitan area of Perth, Western Australia can be used as an inoculum for the rapid start-up of thermophilic anaerobic digestion. However MSW digestion can lead to overload conditions resulting in organic acid accumulation and acidification (Charles et al., 2009) and an unsuccessful start-up of anaerobic digestion. To avoid this, some researchers have applied an aerobic pretreatment to lower the level of easily-degradable carbon in MSW (O'Keefe and Chynoweth, 2000). As methanogens are strictly anaerobic microorganisms, their survival in such aerobic pretreatment remains unclear. To investigate whether aerobic pretreatment of MSW is beneficial to start-up of a subsequent thermophilic anaerobic digestion, fresh MSW was partially composted.

During two weeks of anaerobic incubation, the aerated MSW (3.7 L/L) produced two times less methane than untreated MSW (8.0 L/L) (Fig. 2). Nevertheless, the aerated MSW sample could still be used as an anaerobic inoculum to start-up thermophilic anaerobic digestion. The aerated pretreatment of MSW causes the oxidation of degradable organics by aerobic microbes and hence is expected to lower the methane production potential. This observation has been described for MSW during aerobic/anaerobic treatment and quantified by electron flow balance by Walker et al. (2006b).

The aeration pretreatment of 5 days tested here did not affect the viability of anaerobic acetate degraders in the MSW as the overall acetate degradation capacity was similar to the control without prior aeration treatment (Fig. 2). When using the initial rates of methane production as an indicator of the number of active methanogens present (Fig. 2A, days 5 to 8), it can be concluded that the aerobic pretreatment did not have a significant adverse effect on the survival of methanogens. In order to maintain stable digestion, the untreated reactor required approximately two times more (20.1 g/kg solid) sodium hydroxide addition than the aerobically pretreated reactor (12 g/kg solid) for neutralizing pH.

Although a mechanism of survival of oxygen sensitive methanogens during aerobic pretreatment is still unclear, a similar result was found in the literature. Charles et al. (2009) found that not only had acetoclastic methanogens survived in MSW during aerobic treatment but the amount of hydrogenotrophic methanogens had increased approximately 100-fold during aerobic pretreatment of MSW.

Overall results suggest that when microbes present in MSW serve as the sole source of methanogens, an aerobic pretreatment is an acceptable method to lower the easily degradable carbon in MSW, and reduce the risk of acidification, without adversely affecting the methanogenic inoculum present.

3.3 The effect of stirring during the initial start-up period

According to the literature, stirring improves anaerobic digestion, by

- providing uniform distribution of organisms and substrates (Lema et al., 1991)
- enhancing substrate and microbes contact (Smith et al., 1996).

However, it has also been recommended by some researchers to avoid intense stirring during the start-up phase (McMahon et al., 2001; Stroot et al., 2001; Hoffmann et al., 2008). To clarify these arguments, this experiment compared vigorously stirred and unstirred reactors during the start-up phase.

During the start-up phase of anaerobic digestion, stirring inside the reactor is an important operational process that needs to be considered. Figure 3 displays that vigorous stirring inhibited propionate degradation resulting in its accumulation. Similar results have been reported under both thermophilic (Kaparaju et al., 2008) and mesophilic conditions (Stroot et al., 2001). Therefore, to ensure a successful start-up process, vigorous stirring should be avoided as accumulated propionate (>20 mM) has been found to inhibit acetate degradation, especially at low pH (Barredo and Evison, 1991).

3.4 Quantification of viable thermophilic methanogenic activities in different fractions of MSW

The previous results revealed the presence of thermophilic methanogens in fresh MSW. While there is a significant variation in MSW, depending on culture, and season, it is generally recognized as comprising of similar types of domestic refuse including food, fruit and vegetables, and gardening wastes.

During the past decade, most studies relating to thermophilic methanogens in compost have focused on potential methane emissions, factors that lead to methane emissions, and thermophilic methanogenic communities in compost materials (Beck-Friis et al., 2000; Jäckel et al., 2005; Thummes et al., 2007). However, there has been no research investigating the origin of thermophilic methanogens in the start-up material such as

MSW. The aim of this experiment is to test for the presence of thermophilic methanogenic activity in the different fractions typical of MSW. Samples of fractions typically contained in MSW were collected and separately used as a potential source of methanogens for thermophilic anaerobic digestion.

Results clearly indicated that, of the samples tested (all containing 30 mM acetate and 200 mM bicarbonate), only the turf grass sample enabled the development of thermophilic methanogens in the presence of acetate and bicarbonate. Methane was not detected over a period of 30 days in any of the other samples consisting of mixed tree leaves, mixed tree bark, mixed vegetable waste (including banana peel, carrot, potato, mushroom), dust from a household vacuum cleaner, dry soil from 3 separate locations of Murdoch University campus (away from lawn).

Within a short incubation of about 4 days the turf grass sample initiated methane production. This suggests that the turf grass sample was a significant source of thermophilic methanogens in the synthetic MSW.

3.5 Comparison of methane production of turf grass samples from different sampling locations

The turf grass tested in the previous experiment was taken from a grass lawn on the campus of Murdoch University, Perth, Western Australia. To establish whether it is a general phenomenon that grass lawn contains a significant inoculum of thermophilic methanogens, samples from different grass lawns around Perth were tested. This included grass lawns located on campus, from a local turf supplier and from household gardens around the Perth metropolitan area.

Results showed that all turf grass samples displayed high thermophilic methanogenic activity within 7 days of incubation (Fig. 4) confirming previous results. Acetate analysis after 21 days showed that of the initial acetate concentration of 30 mM, less than 13 mM was left in all cases. This acetate degradation suggests that the turf grass samples contained acetate degrading methanogens or syntrophic bacteria, which are essential for stable proper start-up of anaerobic digestion.

To confirm the presence of acetate driven methanogenesis, VFA degradation profiles were recorded during the start-up of a larger 1 L reactor inoculated with turf. The time course of methane production (Fig 5A) and acetate degradation (Fig 5B) demonstrated the presence of acetoclastic methanogenesis or syntrophic acetate degraders. After an initial increase of acetate (day 1 to 9), explained by the fermentation of plant carbohydrates, acetate was rapidly methanized at 15 mM/L/day (Fig. 5B). Methane formation stopped when acetate was depleted.

The spike addition of additional acetate (Fig 5B, day 15) resumed acetate conversion to methane showing that a proper start-up of thermophilic anaerobic digestion associated with acetate conversion to methane was accomplished. The high methane production rate obtained (1 L/L/d) was in the order of well established full-scale anaerobic digesters.

Although methanogens have been found in various environments such as human dental plaque (Kulik et al., 2001), and rice paddy (Watanabe et al., 2006), the current study shows the first evidence of the systematic presence of thermophilic methanogens in grass lawn. To explain this finding, further study is required addressing questions such as below:

- Is the observation that thermophilic methanogens are present in grass lawn consistent globally?
- What part/s of turf grass: leaf and/or root and/or soil is/are the main source of methanogens?
- What are the species of methanogens in grass lawn?

4. Conclusions

The overall conclusions based on the results of this study can be summarized as follows: 1) MSW was a more effective thermophilic anaerobic methanogenic seed than cow manure. 2) Within the fractions of MSW, it was the turf, or grass clippings, that contained the source of thermophilic methanogens for fast digester start-up including acetate conversion. 3) During the start-up period, the provision of high buffer capacity and slow stirring is recommended. 4) To minimize the need for buffer or alkali addition during the start-up, aerobic pretreatment was a suitable method.

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Figure 1: Comparison of methane production (A) and volatile fatty acid profile (B) during thermophilic anaerobic incubation of 100 g MSW or cow manure (+ 80 mM acetate) in a 1L batch reactor. The initial acetate concentrations were 80 mM for cow manure and 0 mM for MSW respectively. The initial bicarbonate concentrations were 100 mM and 250 mM for cow manure and MSW respectively. Legend (A): reactor seeded with MSW(●) and Cow manure (□). Legend (B): closed symbols: MSW, open symbols: cow manure. Acetate ●○, Propionate ■□, and Butyrate ▲△.

Figure 2: Effects of aerobic pretreatment of MSW on methane production (A) and VFA profiles (B) of thermophilic anaerobic batch digestion of 100 g MSW. Both reactors contained 100 mM bicarbonate. Legend (A): reactor seeded with untreated MSW (○) and aerated MSW (■). Legend (B): closed symbols: aerobically pretreated, open symbols: untreated; Acetate ●○, Propionate ■□, Butyrate ▲△.

Figure 3: Effects of reactor stirring on start-up of thermophilic anaerobic digestion of non-sterile MSW (A) and VFA profiles (B) of 200 g of MSW (unstirred and stirred reactor). Both reactors contained 250 mM bicarbonate. Legend (A): unstirred reactor (○) and stirred reactor (■). Legend (B): closed symbols: stirred reactor, open symbols: unstirred reactor; Acetate ●○, Propionate ■□, Butyrate ▲△.

Figure 4: Comparative methane production (A) of 15 g of turf grass samples taken from: Murdoch University (○), local Perth turf supplier (▲), and household located in East (◆) and South Perth (□) and acetate concentrations after four weeks incubation (B) in duplicated serum vials. All assays (40 mL working volume) contained 30 mM acetate and 250 mM bicarbonate.

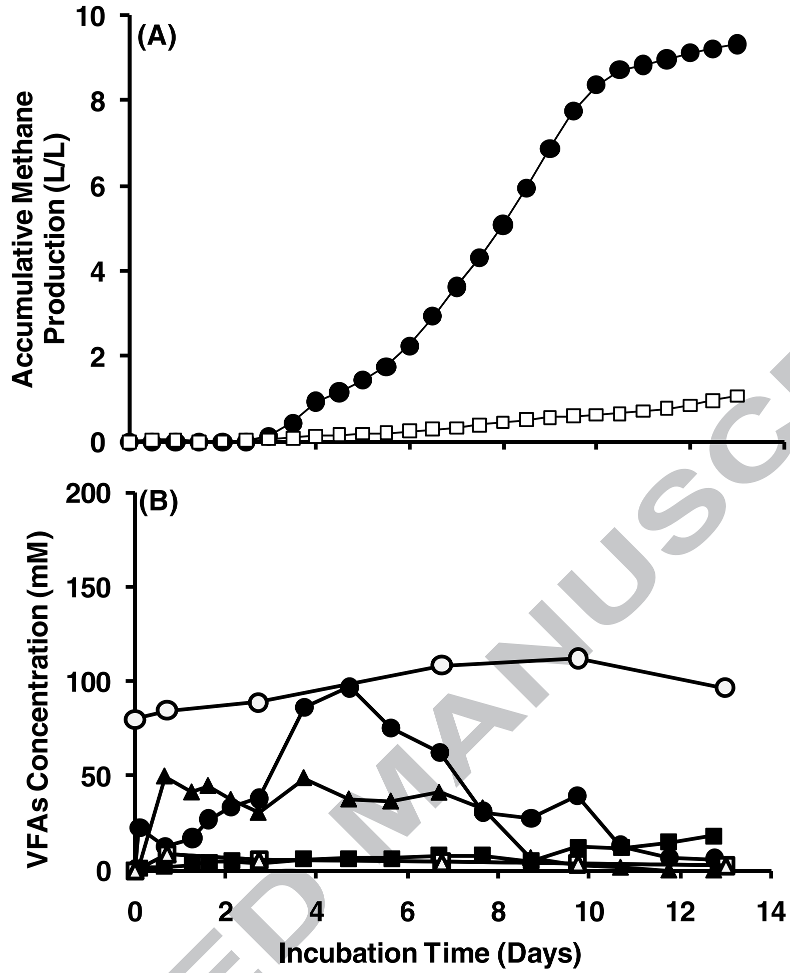
Figure 5: Methane production (A) and volatile fatty acid profile (B) during thermophilic anaerobic incubation of 200 g of grass turf in a 1 L batch reactor. The initial acetate and bicarbonate concentration were 100 mM and 250 mM respectively. Legend (B): Acetate (○), Propionate (□), Butyrate (△).

Table 1: Summary of the types of inocula and initial bicarbonate and acetate concentrations used .

Experiment	Types of inocula	Bicarbonate concentration added (mM)	Acetate concentration added (mM)	Final working volume (mL)	Daily addition of NaOH to control pH

					at 7.0
3.1	100 g of cow manure, or	100	80	1000	No
	100 g of MSW	250	N.A.	1000	No
3.2	100 g of MSW, or	100	N.A.	1000	Yes
	100 g of pre-aerated MSW,	100	N.A.	1000	Yes
3.3	100 g of MSW	250	N.A.	1000	No
3.4	3 g of turf, mixed tree leaves, mixed tree bark, mixed dry soil (away from turf), mixed vegetable wastes, or dust from a household vacuum cleaner	100	30	50	No
3.5	15 g of turf from different sampling locations, or	250	30	40	No
	200 g of turf	250	100	1000	No

Remark N.A. = Not added. The initial pH of all experiments was between 8.0 and 8.4.



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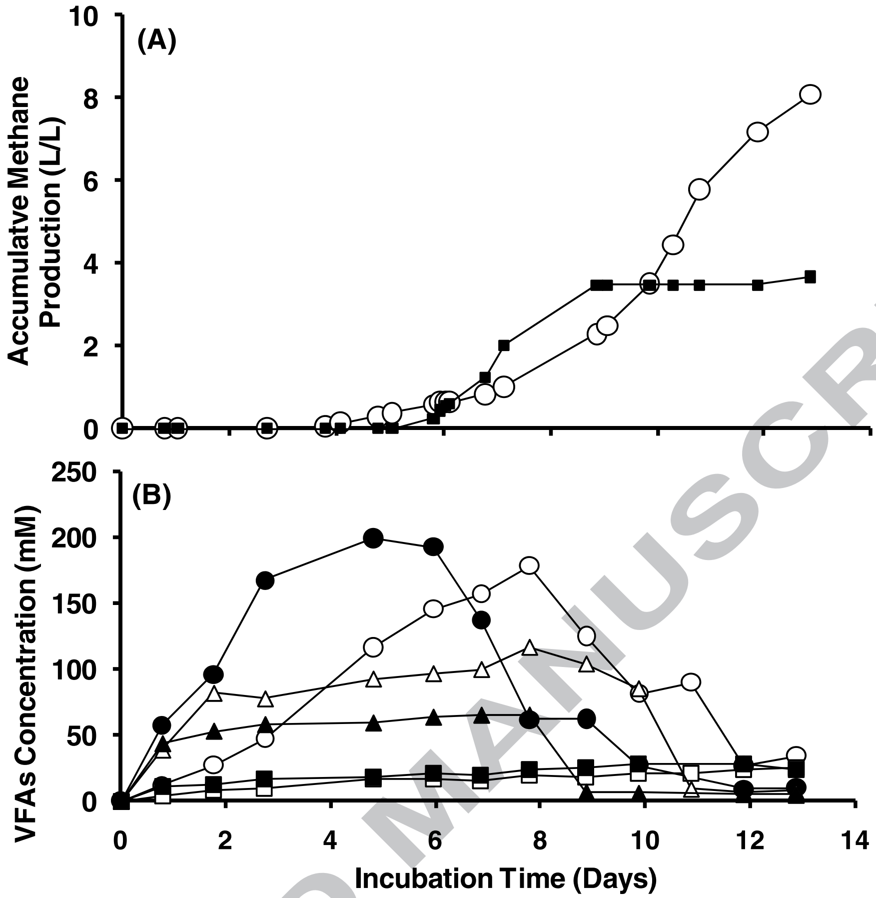

Figure 2: Effects of aerobic pretreatment of MSW on methane production (A) and VFA profiles (B) of thermophilic anaerobic batch digestion of 100 g MSW. Both reactors contained 100 mM bicarbonate. Legend (A): reactor seeded with untreated MSW (○) and aerated MSW (■). Legend (B): closed symbols: aerobically pretreated, open symbols: untreated; Acetate ●○, Propionate ■□, Butyrate ▲△.

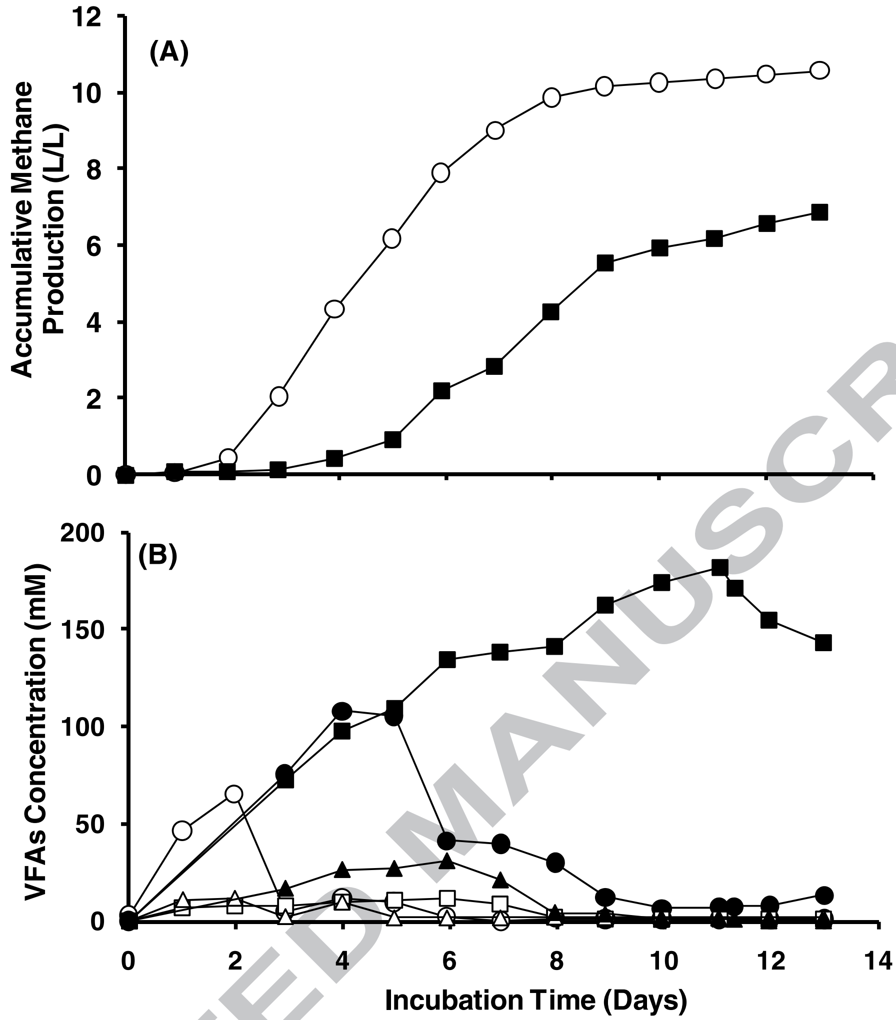

Figure 3: Effects of reactor stirring on start-up of thermophilic anaerobic digestion of non-sterile MSW (A) and VFA profiles (B) of 200 g of MSW (unstirred and stirred reactor). Both reactors contained 250 mM bicarbonate. Legend (A): unstirred reactor (○) and stirred reactor (■). Legend (B): closed symbols: stirred reactor, open symbols: unstirred reactor; Acetate ●○, Propionate ■□, Butyrate ▲△.

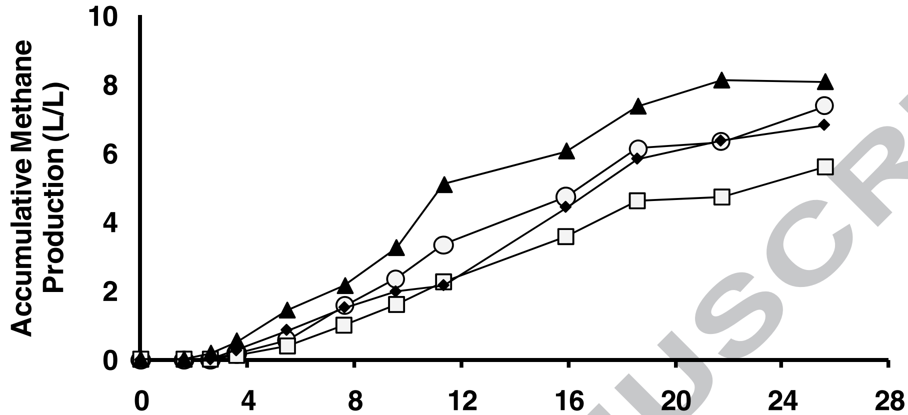

Figure 4: Comparative methane production of 15 g of turf grass samples taken from: Murdoch University (○), local Perth turf supplier (▲), and household located in East (◆) and South Perth (☐). All assays (40 mL working volume) contained 30 mM acetate and 250 mM bicarbonate.

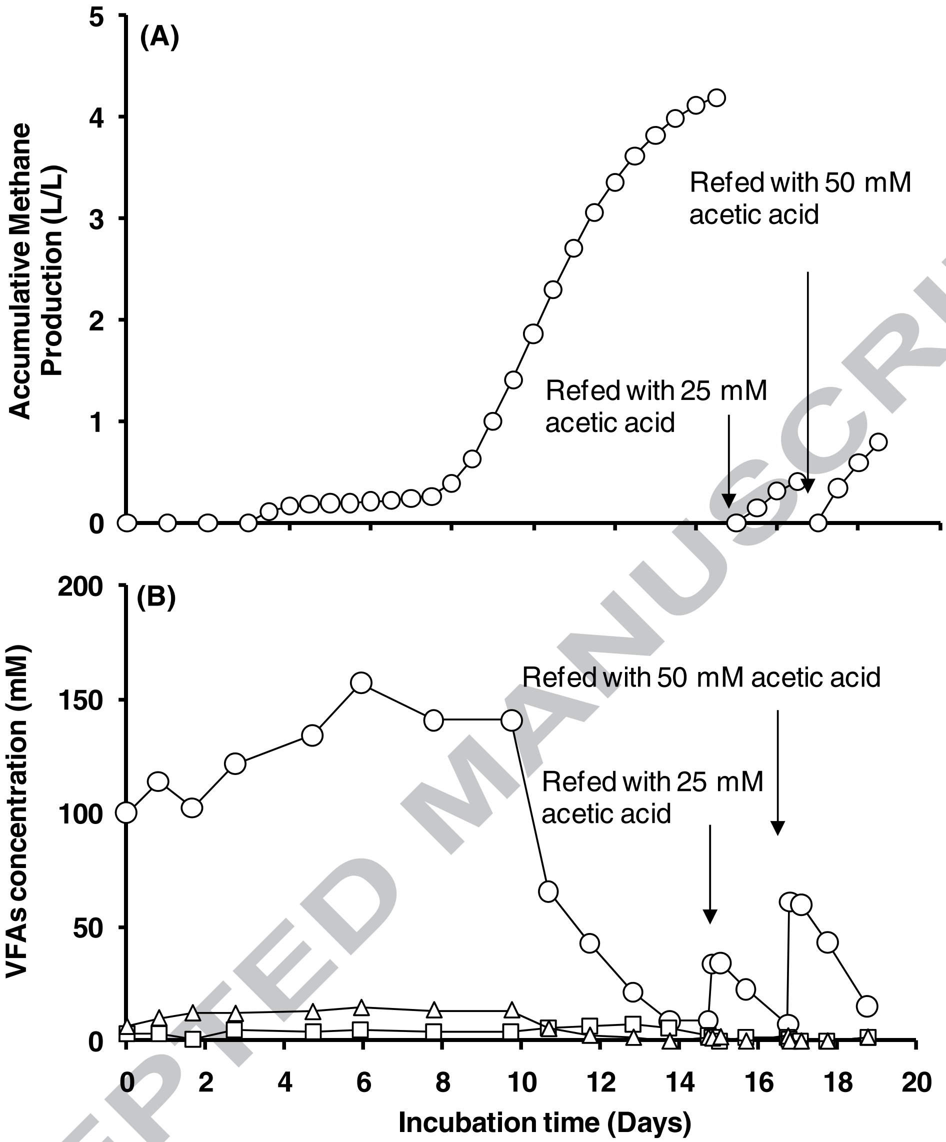

Figure 5: Methane production (A) and volatile fatty acid profile (B) during thermophilic anaerobic incubation of 200 g of grass turf in a 1 L batch reactor. The initial acetate and bicarbonate concentration were 100 mM and 250 mM respectively. Legend (B): Acetate (○), Propionate (□), Butyrate (△).

Highlights of Manuscript entitled: Rapid Start-up of thermophilic anaerobic digestion with the turf fraction of MSW as inoculum

- Turf grass present in MSW is a key source of thermophilic methanogens.
- MSW can be an inoculum for rapid start-up of thermophilic anaerobic digestion.
- Thermophilic methanogens from turf grass include acetoclastic methanogens.