

## Digested Sewage Sludge as Seed for Batch Test of Anaerobic Biodegradability

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### ABSTRACT

Research was carried out to optimize the use of digested sewage sludge (DSS) as a seed for batch tests of food waste anaerobic biodegradability. Digested sewage sludge was used as the seed because of its abundant supply and previous use as a seed. A seed volume of 1:1 g VS basis to the substrate provided a stable anaerobic process, however it inundated the 20 g food waste sample by 25 times more volume. In an attempt to reduce the seed volume, thus increasing the sample size, the seed was thickened via gravity settling. Although the solids content doubled to 2.4% TS with the concentrate, the percentage of methane was similar. About 120 g of food waste was an appropriate sample loading for 1.5 L of seed with methane production decreasing when sample size increased to 160, 200 or 240 g. The larger ratio of seed to substrate allows for a successful digestion without pH adjustment. The methane production of food waste was more consistent when 7 days old seed (DSS was adapted to test temperature for a week) was used compared to a fresh (1 day old) one. The 7 day-old seed also produced less methane, thereby improving the estimation of methane production from the substrate. A reduced acid concentration and reduced initial acid production rate with stored seed is likely to have led to these results. In conclusion, the use of 7 day old DSS is recommended to ensure a consistent anaerobic degradability test result with minimal methane background production.

### INTRODUCTION

The challenge for the 21<sup>st</sup> century in terms of a sound waste management strategy is the transformation of waste into resources for the future (Lens et. al., 2004). One way of achieving this is through anaerobic digestion, providing avenues to recover energy and compost whilst reducing waste at the same time. Operating and maintaining healthy anaerobic digesters requires understanding of the substrate biodegradability, gas yields, toxicity and other anaerobic problems. As such, plant designers often resort to laboratory scale investigations first, carefully avoiding a greater overall economic risk.

Bioassay techniques either operated batch or continuously are the most common laboratory methods, the latter being a closer simulation of a full-scale anaerobic

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operation; albeit more costly in terms of facilities, equipment, time and personnel. The batch technique is generally easier and cheaper to run, plus permits a wider range of variables for evaluation. An essential element to the anaerobic biodegradability testing is seeding as it supplements the initial bacteria population, without which the process might take months or years to establish.

A viable seed can be derived from the environments in which the anaerobic methanogenic decomposition of organic compounds occurs naturally, for example, anaerobic sewage digesters, anaerobic lake sediments or from animal feces. Because sewage commonly would have more fat and protein and less carbohydrate than food waste one might argue that sewage digestion would not provide the ideal seed source. However, past studies have shown that seed from a presumably more active cellulolytic environment such as the rumen (Chynoweth et. al., 1993), and anaerobic sediments containing decomposing kelp (Chynoweth, 1981) have not exhibited better decomposition rates compared to a sludge seed. Therefore, it seems reasonable that, for the case of anaerobic degradability testing where a standardized procedure is preferable, using seed sourced from a local wastewater treatment sludge digester would be suitable. Digested sewage sludge (DSS) contains a good broad spectrum inoculum, is abundant in supply and its successful use as seed has and is constantly being demonstrated (Owen et. al., 1979; and ISO 11734, 1995).

The volume of seed added is an important factor, as a low seed volume is desired to minimise biogas production which may blur the contribution from the substrate. A low seed volume also increases the allowable size of the substrate sample. However, a restricted seed amount can cause process overloading due to the overproduction of volatile acids (Angelidaki, 2004). For example, a seed to substrate by volume (v/v) ratio as high as 29:1 (2.3L seed to 78g substrate) resulted in a high maximum specific production per load (Raposo et. al. 2006). The maximum methane production per load can further be increased if more seed was added, as formation and stability of intermediate VFA products were already detected. Complete process failure was observed by Xu et. al. (2002) when using a seed to substrate ratio (by volume) of only 0.5:1. No methane was generated and pH dropped from 6.7 at start to 4.0 at Day 2 which remained acidic (<5.0) there after. Mathematical means for estimating seed volume have been derived but they require an assumption of substrate hydrolysis constants (Angelidaki and Sanders, 2004), which are not easily estimated.

There may be opportunities to reduce the volume of sewage sludge used as seed by a simple, preliminary treatment. Digested sewage sludge (similar to undigested sewage sludge or mixed animal wastes) when left to settle typically separates out to three noticeable layers: a top floating scum layer, a watery middle layer followed by a bottom sludge layer. Kaparaju and Angelidaki (2008) optimized the phase separation when digested manure was settled for more than 24 hours and at a high temperature of 55°C. The total solids (TS) content of digested manure improved by 2.7 times for the top layer and 1.9 times for the bottom layer from the initial value of 3.9% TS. Ong et. al. (2000a and b) found that the methane potential of fresh cattle

manure was highest for the top layer which also has the highest total solids of 12.7%. The middle layer with only 2.8% TS had the lowest methane potential. The methane potential difference between the various layers was attributed to the higher amounts of degradable matter. Previous results indicate the possibility of reducing the amount of seed required for an anaerobic degradability test through the use of a concentrated or treated seed.

Static predigestion of sewage sludge has been tested as a simple treatment. This treatment involves maintenance of anaerobic conditions, incubation at an elevated temperature, and typically without mixing. In the ISO 11734 (1995) and Pagga and Beimborn (1993), predigestion of the sludge was recommended when preparing seed for use in an anaerobic assay test. Once collected from a local digester, the sludge inocula is allowed to digest in the laboratory at 35°C without the addition of any nutrients or substrates for up to seven days. Birch et. al. (1989) predigested the DSS seed for 2 and 4 weeks, and found that by predigesting the seed, especially for 5 days, the background gas production was reduced. No unacceptable increase in either the lag or incubation period was observed from the predigestion. More recently, Hansen et. al. (2004) also readapted seed from a thermophilic biogas plant to 55°C for three days before use in a batch anaerobic test. Further investigation of the effectiveness and suitability of predigesting the seed would be necessary before confirming its suitability over all substrates including solid organic samples.

Standardization of the batch bioassay technique for determining ultimate biodegradability of feedstocks suitable for anaerobic digestion has become the research focus of many researchers, but some questions related to the test procedures still remain. The objective of this research was to optimize the use of digested sewage sludge (DSS) as a seed for batch tests of anaerobic biodegradability, focusing on seed volume and the suitability of seed thickening and predigesting.

## **MATERIALS AND METHODS**

### **Batch test reactor**

An anaerobic respirometer developed at the Environmental Laboratory of the Civil Engineering Department, University of Canterbury was used as the batch test reactor. It was designed to cater for larger sample size of about 200 g fresh substrate, compared to only using 20-50 g dried, ground samples of 2 mm size which is a common method of testing organic solids. With a 200 g sample a more representative amount is attainable, which is especially important when analyzing a heterogeneous sample like food waste (Qamaruz-Zaman and Milke, 2007). The device was made of PVC pipe with 3600 ml capacity and a large opening to ease solid sample introduction. A detailed description of the device and its operating procedure is reported elsewhere (Qamaruz-Zaman and Milke, 2007). The reactors were maintained at 35°C in a temperature controlled room and manually mixed once daily for 20 seconds.

## **Substrate**

The feedstock for the batch test was simulated food waste prepared as per methods of Qamaruz-Zaman and Milke (2007). The prepared substrate consists of 59% (by wet weight) vegetable, 29% leftover starch and meat (e.g. spaghetti, bread, rice and mashed potato), 4% eggshells, and 8% teabags and coffee. An average food waste mixture had a pH of 4.8 and a total solids (TS) content of 21.6% where 87.4% is volatile. Feedstock was kept frozen in the laboratory refrigerator at 4°C and thawed overnight at 35°C for use the following day.

## **Seed**

The seed was sourced from a mesophilic digester (20 day residence time) of the Christchurch Wastewater Treatment Plant in New Zealand. For most of its use in the batch tests, the seed was first acclimated at laboratory conditions at 35°C for seven days. On average, a 7 day old DSS had a pH of 7.2, 1% TS with 69% being volatile.

## **Analytical Methods**

Analysis was done on both the feed as well as the treated effluent at the start and completion of the incubation. Solids analyses were based on Standard Methods (1998). The pH reading was taken using an EDT RE357 Microprocessor pH meter (Made in England) on raw sample. To determine the daily gas production, a 5L (1 cm = 246.6 ml) and/or 0.5 L gasometer operated on the water displacement concept was utilized. The produced biogas was first collected in a 1L, 5L or 10L Tedlar Bags and later emptied into the gasometer for volume determination. A landfill gas analyzer, GA2000Plus (Geotechnical Instruments, UK) was used to measure percentages of methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and oxygen (O<sub>2</sub>).

## **Experimental procedure**

### *Seed volume investigation*

A short preliminary experiment was conducted to establish a suitable seed volume for testing the anaerobic degradability of food waste. Two distinct ratios of food waste and seed (refer Table 1) were tested, with I<sub>500</sub>:S<sub>20</sub> having a larger seed volume compared to I<sub>250</sub>:S<sub>250</sub>. The tube with more inoculum was chosen to ensure a stable anaerobic process. On the other hand, the reduced seed volume in I<sub>250</sub>:S<sub>250</sub> allowed for a larger sample size.

### *Feasibility of seed thickening*

The required seed volume was expected to be reduced by using a thickened or concentrated sludge. For this, the collected DSS was allowed to separate out via gravity settling in a 500 ml glass cylinder without mixing for 3 hours. The resulting

middle watery portion or ‘supernatant’ and the settled bottom sludge termed ‘concentrate’, were then added to an amount of food waste and incubated for 6 days. Two trials were carried out as detailed in Table 2 and Table 3. The first trial had a similar food waste content of 20 g which was later repeated using a fixed amount of seed (250 ml). A larger sample size of 120 g in 1.5 L seed was also tested as O<sub>1500</sub>:S<sub>120</sub> which was a scale up of O<sub>250</sub>:S<sub>20</sub>

Table 1. Condition of batch test for seed volume study

Tube No.	I (ml)	S (g)	R <sub>I/S</sub> g VS	R <sub>I/S</sub> * g w/w	pH
I <sub>0</sub> :S <sub>500</sub>	0	500	0	0	4.8
I <sub>500</sub> :S <sub>20</sub>	500	20	1:1	25:1	7.3
I <sub>250</sub> :S <sub>250</sub>	250	250	0.04:1	1:1	6.3

I: inoculum; S: substrate; \* assuming 1ml~1g; g VS: g volatile solids basis; g w/w: g wet weight basis; pH: pH of mixture

Table 2. The batch test condition for DSS layers study with fixed substrate

Tube No.	St (ml)	Ct (ml)	O (ml)	S (g)	R <sub>I/S</sub> g VS	R <sub>I/S</sub> * g w/w	TS (%)
St <sub>500</sub> :S <sub>20</sub>	500			20	0.28	25	1
Ct <sub>100</sub> :S <sub>20</sub>		100		20	0.48	5	6
Ct <sub>50</sub> :S <sub>20</sub>		50		20	0.24	3	8
C <sub>250</sub> :S <sub>20</sub>		250		20	1.19	13	4
O <sub>500</sub> :S <sub>20</sub>			500	20	0.96	25	3
Seed blank			500	0			1

St: Supernatant; Ct: Concentrate; O: Original

Table 3. The batch test conditions for DSS layers study with fixed seed volume

Tube No.	St (ml)	Ct (ml)	O (ml)	S (g)	pH	TS (%)
St <sub>250</sub> :S <sub>20</sub>	250			20	7.15	1
Ct <sub>250</sub> :S <sub>20</sub>		250		20	7.29	4
Ct <sub>250</sub> :S <sub>20</sub>			250	20	7.40	3
O <sub>1500</sub> :S <sub>120</sub>			1500	120	7.40	3
Seed blank			250		7.72	1

St: Supernatant; Ct: Concentrate; O: Original

### *Feasibility of DSS pre-digestion*

A 20 L plastic jerry-can was used to collect the DSS seed from the wastewater treatment plant digester. It was filled up to the neck with minimal headspace (about 5 cm). Once at the laboratory, the inoculum was stored in a temperature control room

of 35 °C without mixing for a period of 1, 4, 7, 10 and 14 days. After each incubation period, 1.5 L of seed was added to 120 g of food waste and the effect of seed pre-digestion on the anaerobic batch test was determined. This experiment was repeated twice.

## RESULTS

### Seed volume

As shown in Table 4, the food waste decomposition started immediately when the seed was provided in a 1:1 g VS basis to the substrate. A higher methane percentage of 39.5% was recorded on Day 5, compared to only 2.6% when 1:1 wet weight basis was used. Evidently, a larger amount of seed provided enough buffer to raise the pH of the substrate from 4.8 to 7.3, compared to only pH 6.3 when the seed volume was restricted as in I<sub>250</sub>:S<sub>250</sub>.

Table 4. Gas composition of decomposing food waste using different seed volume

Tube No.	Day	CH <sub>4</sub> (%)	CO <sub>2</sub> (%)	O <sub>2</sub> (%)	Balance (%)
I <sub>0</sub> :S <sub>500</sub>	0	0.0	0.0	0.0	99.9
	1	1.3	29.0	0.0	69.7
	2	1.7	47.0	0.0	51.3
	3	4.2	81.1	0.0	14.7
	4	2.7	80.3	0.0	16.9
	5	1.2	77.6	0.0	21.2
I <sub>500</sub> :S <sub>20</sub>	0	0.0	0.0	0.0	99.9
	1	9.4	14.0	0.0	76.5
	2	21.2	22.3	0.0	56.2
	3	36.1	37.8	0.0	25.9
	4	38.5	30.3	0.0	31.0
	5	39.5	29.6	0.0	30.9
I <sub>250</sub> :S <sub>250</sub>	0	0.0	0.0	0.0	99.9
	1	1.8	38.7	0.0	59.4
	2	4.6	70.8	0.0	24.6
	3	5.6	88.6	0.0	5.7
	4	4.0	84.7	0.0	11.7
	5	2.6	79.2	0.0	18.0

### Seed thickening

Three distinctive layers of sludge were obtained after gravity settling for 3 hours. The supernatant and concentrate had a total solids content of 0.3% and 2.4%

respectively, of which 68% and 72.5% were volatile. The concentrate was 2.4 times thicker than the initial DSS values of 1% TS and 70% VS/TS.

From Figure 1, it can be seen that the highest food waste methane percentages were obtained when sludge was used in its original form ( $O_{500}:S_{20}$ ,  $C_{250}:S_{20}$  and  $O_{1500}:S_{120}$ ), whether as 500 ml or 250 ml volume. The concentrate also performs comparably with 250 ml volume ( $C_{250}:S_{20}$ ), but failed as the volume was reduced to 100 ml ( $C_{100}:S_{20}$ ) and 50 ml ( $C_{50}:S_{20}$ ). A low pH of 6.8 and 6.7, respectively, was recorded at Day 19. Using 500 ml supernatant ( $St_{500}:S_{20}$ ), an active anaerobic decomposition was observed. However, anaerobic activity declined by 50-60% as the supernatant volume was cut down to 250 ml ( $St_{250}:S_{20}$ ). Figure 1 also suggests the possibility of using a far larger sample size of 120 g for the batch reactor which warrants further investigation. Except for  $C_{100}:S_{20}$  and  $C_{50}:S_{20}$ , the neutral pH of  $7.5 \pm 0.2$  across all tubes suggests that the test conditions were suitable for anaerobic activity.

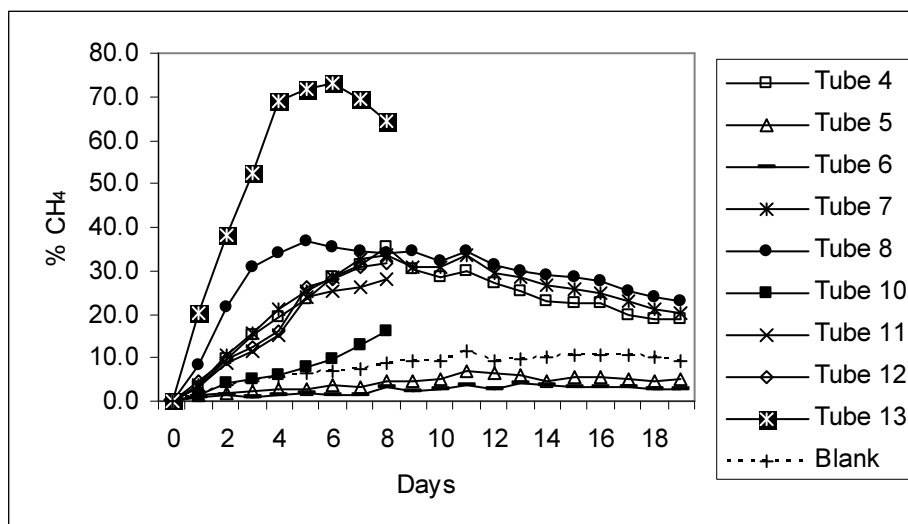


Figure 1. Daily methane percentage of food waste using different seed conditions; supernatant, original, and concentrate. Seed was 7 days old.

A high food waste methane production of 3618 ml over six days was observed when 120 g of food waste was added into 1.5 L of seed. The methane production drops to 2219, 1641 and 1322 ml when sample loading was increased from 120 g to 160 g, 200 g and 240 g, respectively (Figure 2). In addition, the methane production declined from Day 2 for the higher loadings (>120 g) which may have been upset by acidic conditions as observed by a low average pH of  $5.7 \pm 0.3$ , measured on Day 6.

### Inoculum predigestion

Figure 3 demonstrated that the gas production varied greatly for the 1 and 4 day predigested seed, and there were concerns about potential failure if additional

replicates had been tested. On the other hand, the 14 day predigested seed indicated lower methane production, which could impede finding results quickly. In addition, a greater background methane production was seen from fresh seed compared when the seed was pre-digested for seven days (refer Figure 4). As a result, it was decided that the 7 day predigested seed had a good combination of high methane production and robust performance.

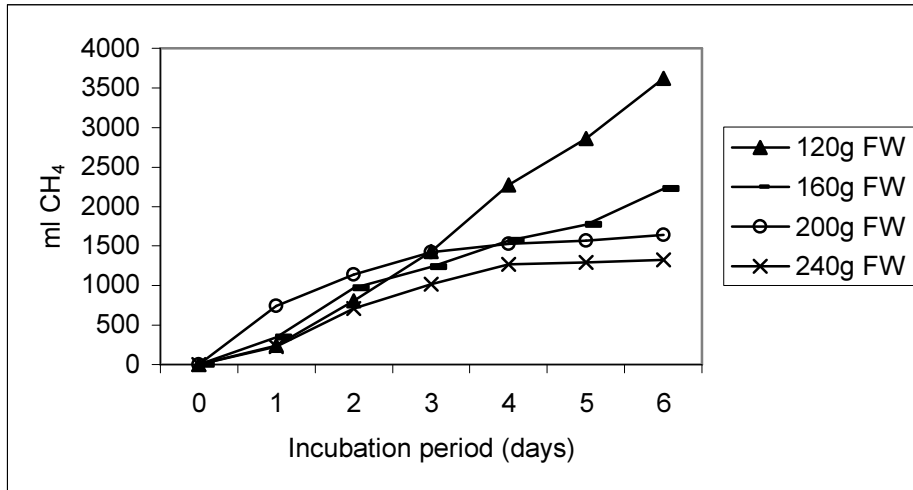


Figure 2. Cumulative methane production (after correction for seed blank) of different food waste loading with 7 days old digested sewage sludge.

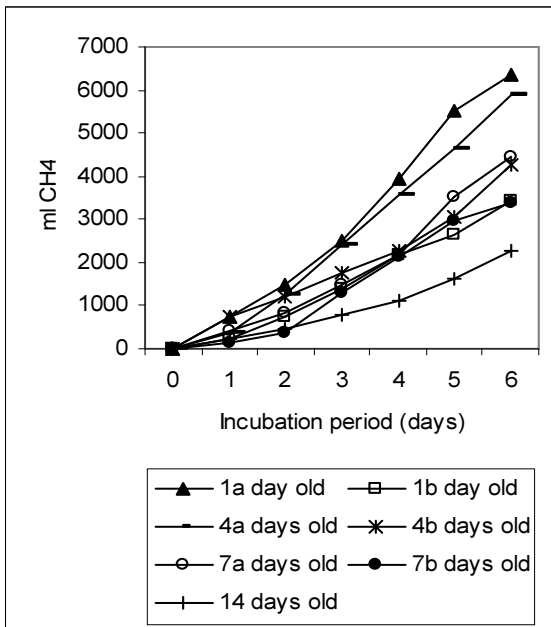


Figure 3. Cumulative methane production of substrate (after seed blank correction) from use of various aged seed

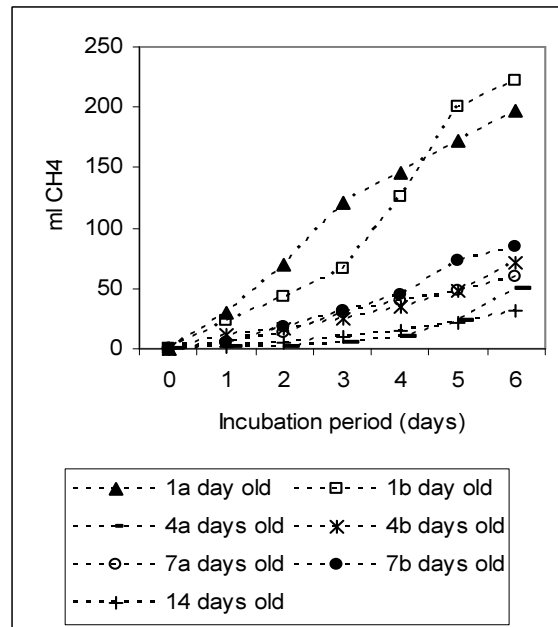


Figure 4. Cumulative methane production from DSS blank varying in pre-digestion age



## **DISCUSSION**

An inoculum to substrate ratio of 1.0 (g VS basis) or greater is the common feed rate for the BMP test as was developed by Owen et.al. (1979) and later used by Chynoweth et. al. (1993) in their anaerobic studies. In the ISO 11734 (1995), adding aliquots of the inoculum until a total solids between 1 g/l and 3 g/l is reached in all reactors was suggested. However the volume of seed was about 25 times more than amount of substrate under evaluation (refer Table 1). The large seed volume is a restriction to solid samples hence the small sample size requires excessive homogenization of the original substrate. In order to increase the sample size, a reduction in seed volume was seen as an option through the use of an active and concentrated seed source.

The similar methane production between original and concentrated DSS both at 250ml suggests that although the seed can be thickened, the use of various fractions has no significant effect on methane production.

The high seed volume may have provided buffering capacity to the mixture where an optimal pH range around 7.0 (neutral) is favored for the growth of methane forming bacteria. Supplementary nutrients provided by the higher seed volume may also have been a contributing factor.

A reduced initial acid production rate with stored seed seems to have contributed to the consistent degradation. Fresh digested sewage sludge, when mixed with degradable food waste, appears to support a very high acid production rate. On the other hand, holding the materials for seven days at an elevated temperature appears to reduce the initial acid production rate while not compromising the long-term methane production capability.

## **CONCLUSION**

The main issue encountered with seed, is the seed quality which varies between each use. Such factors like wastewater treatment plant operation, sampling time and condition, seed storage and age may be influencing the seed quality. As some of these factors are beyond the researchers' control, the seed variation issue can be addressed by consistently using the same aged seed e.g 7 days and to do a seed blank each time. The collected seed can be stored at incubation temperature for 4 to 7 days without any ill effects to anaerobic decomposition. The use of 7 day old DSS as seed seems to have two benefits:

- (i) a more stable substrate degradation, and
- (ii) a smaller amount of methane which would not blur the result of methane production from the test material.

It is crucial to prevent volatile fatty acids accumulation within the seed particles which may affect the adaptive methanogenic competence of the seed, especially so in a batch test. The use of a large inoculum appears to counteract the acidification allowing for a successful digestion without pH adjustment.

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