ACTI-ZYME (BIO-CATALYST) AS A SOLUTION FOR ENHANCED MUNICIPAL SEWAGE AND SEWAGE SLUDGE TREATMENT: EFFECT OF MESOPHILLIC AND THERMOPHILLIC DIGESTION

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Purpose: The purposes of this paper is to compare the effect of mesophillic and thermophilic digestion of municipal sewage sludge utilizing Acti-zyme as the bio-catalyst as a value addition strategy to sewage sludge management. The municipal sewage sludge was digested to biogas and bio-solids (digestate).

Experimental Design/Methodology/Approach: Raw sewage physicochemical properties were first characterized. Biogas and bio-solids was produced for anaerobic mesophillic conditions (37 ^oC) and thermophilic conditions (55 ^oC) for a retention period of 40 days in the bio-digester, Acti-zyme loadings of 0-70 g/m^3 and sewage sludge loadings of 5-10 g/L.day. Biogas composition was determined by a GC 5400 gas chromatography analysis whilst the The cumulative bio-solids (digestate) generated per day were recorded for possible use as biofertilisers. The nitrogen, phosphorous, and trace elements content was determined using Labtronics double beam ultra violet visible spectrophotometer (*uv-vis)*. The potassium content was determined using a Thermo Fisher flame atomization absorption spectrophotometer. The effect of the digestion conditions were statistically quantified using a t-test.

Findings: The biogas had a bio-methane (CH_4) composition of 72-78%, carbon dioxide (CO_2) composition of 16-20% and trace gases composition of 8-12%, whereas the bio-solids had a nitrogen, phosphorous and potassium composition of 8.17%, 5.84% and 1.32%. In overall biogas yield was more than 45% for all the sewage sludge loadings and Acti-zyme loadings digested at mesophillic conditions as compared to digestion at thermophilic conditions. This indicated that thermophilic catalyzed of municipal sewage sludge with Acti-zyme is not feasible as this result in less activity of the Acti-zyme hence less digestion occurs. In addition, the amount of bio-solids is about 40% higher in thermophilic digestion which can also result in landfilling problem.

Research Practical implications and value: Mesophillic digestion of municipal sludge catalyst by Acti-zyme can be utilized for sewage sludge management as a value addition strategy. This can be applied for minimizing landfill problems resulting from sewage sludge from sewage treatment.

Keywords: Acti-zyme, Biogas, Bio-solids, Mesophillic, Municipal sewage sludge, Thermophilic

Topic(s): Environmental Water and Water Resources or Domestic and Municipal Water and Sanitation

1. INTRODUCTION

Municipal sewage sludge management is increasingly becoming a problem in developing countries due to poor wastewater treatment methodologies. About 60% of the sewage sludge ends up in landfills. There is therefore need to manage this sewage sludge via value addition to products like biogas through anaerobic conditions employing bio-catalyzed routes such as Acti-zyme (Manyuchi *et al*., 2015a-c). Although sewage sludge digestion has been investigated at varying retention times, Acti-zyme loadings and sewage sludge loadings (Manyuchi *et al*., 2015c), the effect of temperature under mesophilic and thermophilic conditions still needs to be understood.

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Figure 1: Acti-zyme a bio-catalyst used in anaerobic sewage digestion

Mesophilic conditions are normally employed for wet substrates with TSS of less than or equal to 15%, residence times of 60-95 days and complete mixing is required whereas thermophilic conditions are required for wet substrates with TSS greater than or equal to 20% and residence times of 9-45 days (Vindis *et al*., 2009; Kardas *et al*., 2011). This work focused on sewage sludge digestion utilizing Acti-zyme a biocatalyst that enhances biogas production (Manyuchi *et al*., 2015a-c) focusing on the optimum temperature conditions. Municipal sewage biogas which is comprised of bio-methane (CH_4), carbon dioxide (CO_2) and trace gases is bio-catalyzed through hydrolysis, acidogenesis, acetogenesis and methanogenesis processes as indicated in Figure 2.

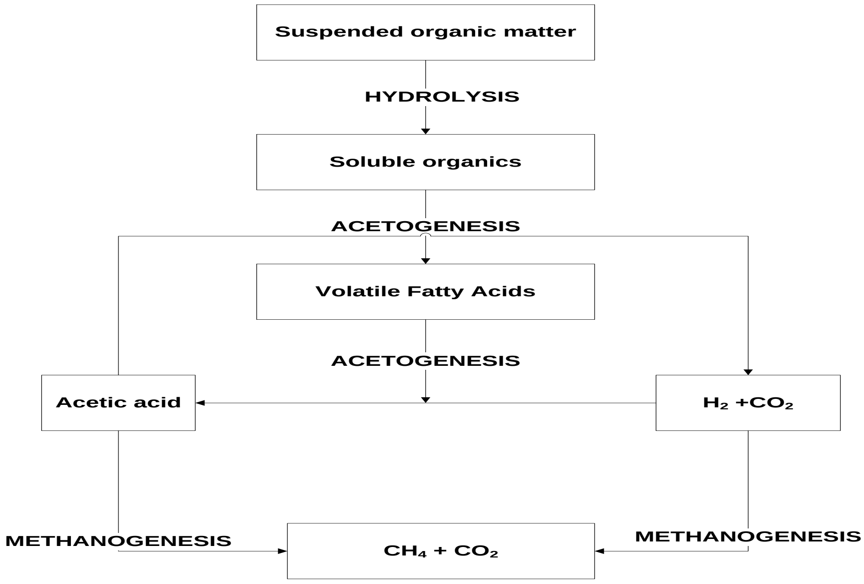

Figure 2: Biogas production processes from sewage sludge bio-catalyzed digestion using Acti-zyme

2. MATERIALS AND METHODS

2.1 Materials

Sewage sludge was obtained from a local used treatment plant which utilizes conventional sewage treatment methods.

Acti-zyme was obtained from AusTech in Australia. An Inco Therm Labotec Incubator was used as for maintaining the temperature constant at 37°C and 55°C for the 500 mL flasks were used as the digesters. The flasks were plugged with cotton wool then covered with aluminum foil paper to ensure anaerobic conditions were maintained.

2.2 Methods

2.2.1 Analysis of the sewage sludge

The sewage sludge was filtered and dried to 60-80% moisture content. Moisture content and volatile matter analyses were done using an AND moisture analyser. The %Moisture content (M) was determined by heating 5g of sample at 105◦C for 30 minutes and then recording the difference in weight. The %Volatile matter (VM) was determined by heating 5g of sample at 105◦C for 3 minutes and then recording the difference in weight. The %Ash content (AC) was determined by completely incinerating the 5g sample using a burner. The total %fixed carbon was determined as: 100% - %(M + VM + AC). pH and electrical conductivity measurements were done using a Hanna HI 9810 instrument. The total Kjeldahl nitrogen (TKN), total phosphates (TP), biological oxygen demand (BOD_5) and the chemical oxygen demand (COD) where measured in milligrams per litre (mg/L) using the standard titrimetric methods as indicated in Alpha (2012).

2.2.2 Biogas production

250 mL flasks representing the digesters were put in a water bath set at 37°C or 55°C to create mesophilic conditions at atmospheric pressure. Outlets were created to facilitate the collection and sampling of the biogas produced. Optimum Acti-zyme loading of 0-70 g/m^3 over retention period of 60 days batch wise were used in the digesters for biogas and bio-solids generation to ascertain the highest conditions that can be employed in sewage treatment using Acti-zyme (Manyuchi *et al*., 2015c). All experiments were replicated thrice and an average used. The pH in the digesters was between 6-7. Agitation in the digesters was fixed at 60 rpm using magnetic stirrers to ensure perfect mixing of sewage sludge and Acti-zyme.

The quantity of biogas produced from the sewage sludge was measured through the displacement of water in millilitres per day (mL/day) (Manyuchi *et al*., 2015c). The biogas generated was taken from the sampling points for composition analysis. A GC 5400 gas chromatography analysis was used to analyse the biogas content and the composition was expressed as a percentage.

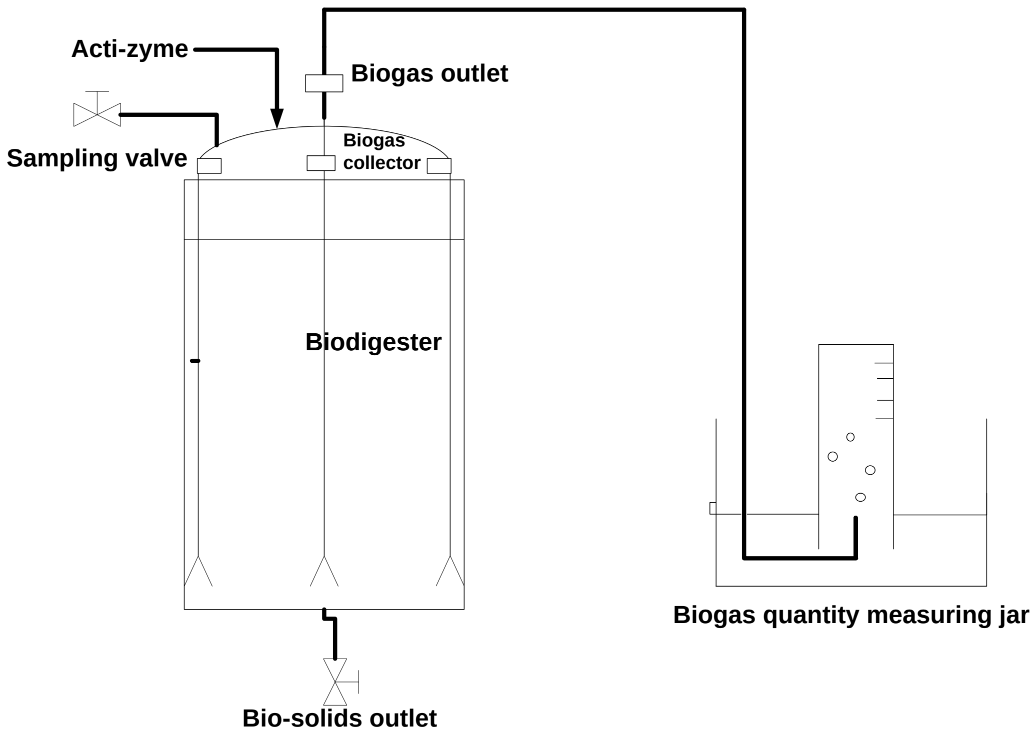

Figure 3: Municipal biogas collection system schematic

2.2.3 Bio-solids generation

The cumulative bio-solids (digestate) generated per day were recorded for possible use as bio fertilisers. The nitrogen, phosphorous, and trace elements content was determined using Labtronics double beam ultra violet visible spectrophotometer (*uv-vis)*. The potassium content was determined using a Thermo Fisher flame atomization absorption spectrophotometer.

2.3 Statistical analyses

Paired sample t-test was used to test the effect of temperature. Paired sample t-test is a statistical technique that is used to compare two population means in the case of two samples that are correlated. Paired sample t-test is used when the samples are the matched pairs, or when it is a case-control study as in this case where temperature is the controlled parameter. To calculate the paired sample t-test Equation 1 was used:

$$t = \frac{\bar{d}}{\sqrt{s^2/n}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

Where $\bar{d}$ is the mean difference between two samples, s^2 is the sample variance, n is the sample size and t is a paired sample *t-test* with n-1 degrees of freedom. Degrees of freedom is the number of values that are allowed to vary, in this case sample size minus one.

An alternate formula for paired sample t-test is represented in Equation 2:

$$t = \frac{\sum d}{\sqrt{\frac{n(\sum d^2) - (\sum d)^2}{n-1}}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(2)$$

A hypothesis was then developed and quantified with the t-test for the different amount of biogas, bio-methane, carbon dioxide, trace gases and bio-solids produced at the different temperatures at 95% confidence interval.

H_o = Digestion temperature does not have an effect on catalytic municipal sewage sludge digestion using Acti-zyme to biogas and bio-solids

H_1 = Digestion temperature have an effect on catalytic municipal sewage sludge digestion using Acti-zyme to biogas and bio-solids

The t-test was carried out at 95% level of significance. If the significant value (sig value) (p-value) is found to be less than 0.05 we reject H_0 otherwise we accept the hypothesis that the digestion temperature has no effect on municipal sewage sludge digestion utilizing Acti-zyme as bio-catalyst.

3. RESULTS AND DISCUSSION

The effect of mesophilic and thermophilic conditions on the various biogas constituents is discussed. Furthermore the impact of the mesophilic and thermophilic conditions on bio-solids generation is also discussed.

3.1 Characterization of the raw sewage sludge

The sewage had total solids of 1143 mg/L and the pH changed from being acidic to alkaline during the digestion process with Acti-zyme. The other raw sewage sludge physico-chemical characteristics are indicated in Table 1.

Table 1: Raw sewage characteristics

Parameter	**Value**
pH	6.3-8.3
COD	750±12.5 mg/L
TS	1143±14.35 mg/L
VS	2.5±0.05%
AC	15±5%
Moisture content	60±20%
TKN	245±5.1 mg/L
TP	52.5±2.7 mg/L
BOD_5	557±2.5 mg/L

3.2 Biogas production

The biogas had a bio-methane (CH_4) composition of 72-78%, carbon dioxide (CO_2) composition of 16-20% and trace gases composition of 8-12%, whilst the bio-solids had a nitrogen, phosphorous and potassium composition of 8.17%, 5.84% and 1.32%, respectively (Manyuchi *et al*., 2015c). Biogas production increased with increase in Acti-zyme loading for both the mesophilic and thermophilic conditions. However, maximum biogas was achieved at mesophilic conditions with about 50% higher as compared to thermophilic conditions (Figure 4). This can be related due to the Acti-zyme activity which is optimum at temperatures around 37 ^{o}C and decreases with increase in temperature. The biogas trend was unlike in other bio-catalyst free municipal sewage digestion reactors whereby the cumulative biogas amount actually increased by more than four times at thermophilic conditions (Vindis *et al*., 2009; Kardas *et al*., 2011).

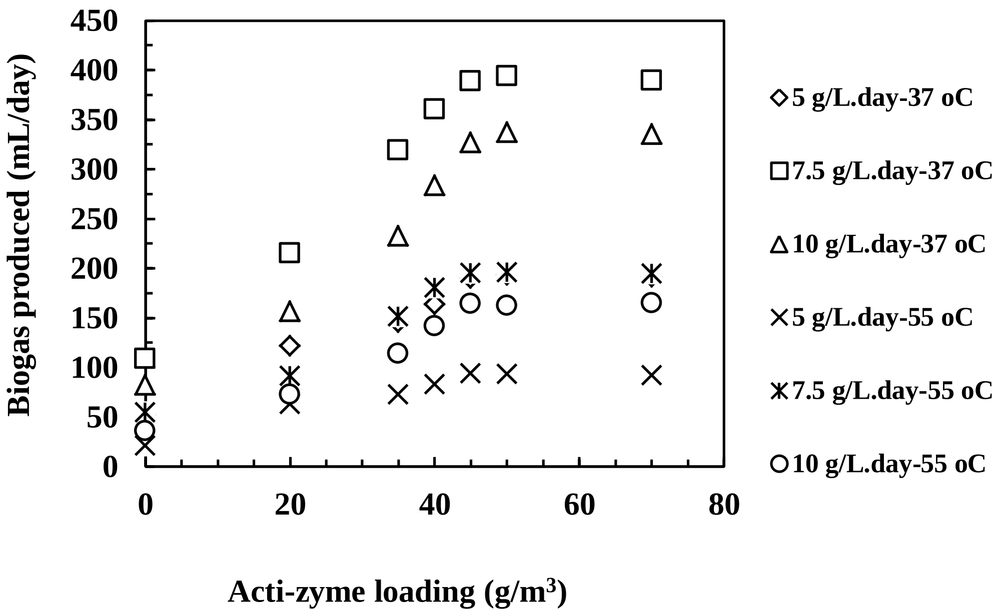

Figure 4: Effect of mesophilic and thermophilic temperature on biogas production at varying Acti-zyme loading and retention time of 40 days

Paired sample t-test results shown in Table 2 showed t (20) = 10.27, $p<0.0005$ and therefore it was concluded that there is a significant difference in the production of biogas at temperatures of 37 °C and 55 °C, since p is less than 0.05.

Table 2: Paired samples test for effect of mesophilic and thermophilic conditions on biogas production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference: Lower	95% Confidence Interval of the Difference: Upper			
Pair 1 37°C-55°C	121.48	54.23	11.83	96.79	146.16	10.27	20	0.000

3.3 Bio-methane generation

Bio-methane (CH_4) production was maxima at mesophilic conditions by more than 40% compared to thermophilic conditions since this is where Acti-zyme activity is at its peak (Figure 5). CH_4 yield was around 78% for the mesophilic conditions whilst it was around 40% for thermophilic conditions at sewage sludge loadings of 7.5 g/L.day. This clearly indicated mesophilic conditions are favorable for Acti-zyme catalyzed biogas production from municipal sewage sludge as a value addition strategy.

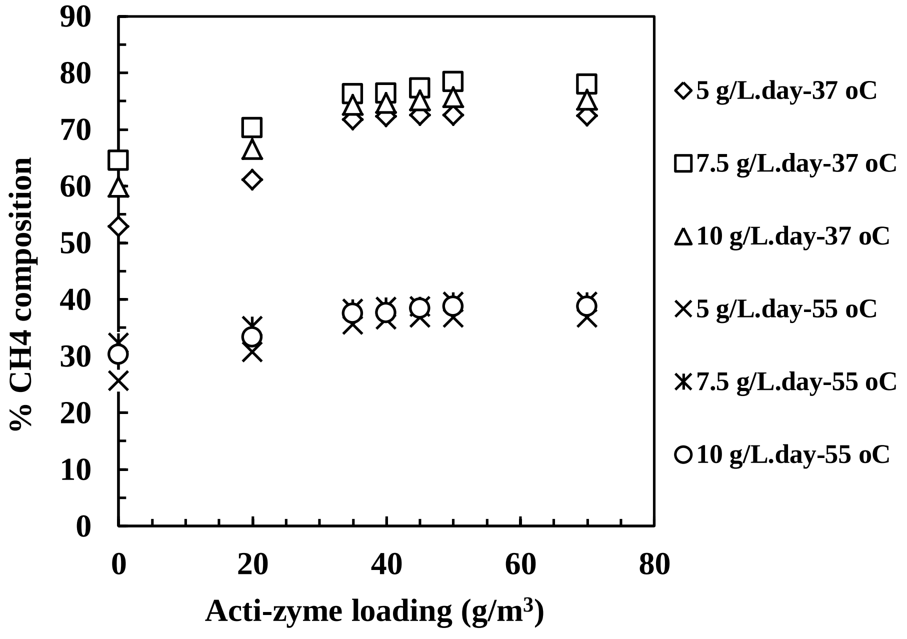

Figure 5: Effect of mesophilic and thermophilic temperature on bio-methane production at varying Acti-zyme loading and retention time of 40 days

The t-test results in Table 3 showed that there is a significant difference in the bio-methane at varying temperatures since a p-value is less than 0.005 that is $t\ (20) = 53.27$, $p\text{-value} < 0.0005$

Table 3: Paired samples test for effect of mesophilic and thermophilic conditions on bio-methane production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 37°C-55°C	35.29	3.04	0.66	33.91	36.67	53.27	20	0.000

3.4 Carbon dioxide composition

The CO_2 produced was 45% higher in mesophilic conditions as compared to thermophilic conditions (Figure 6). This was generally attributed to the decreased Acti-zyme activity and other naturally existing micro-organisms during the digestion process resulting in the municipal sewage sludge being partially digestion. However, for both mesophilic and thermophilic conditions, the CO_2 produced significantly decreased due to the Acti-zyme activity which enhances bio-methane production and hinders production of other gases.

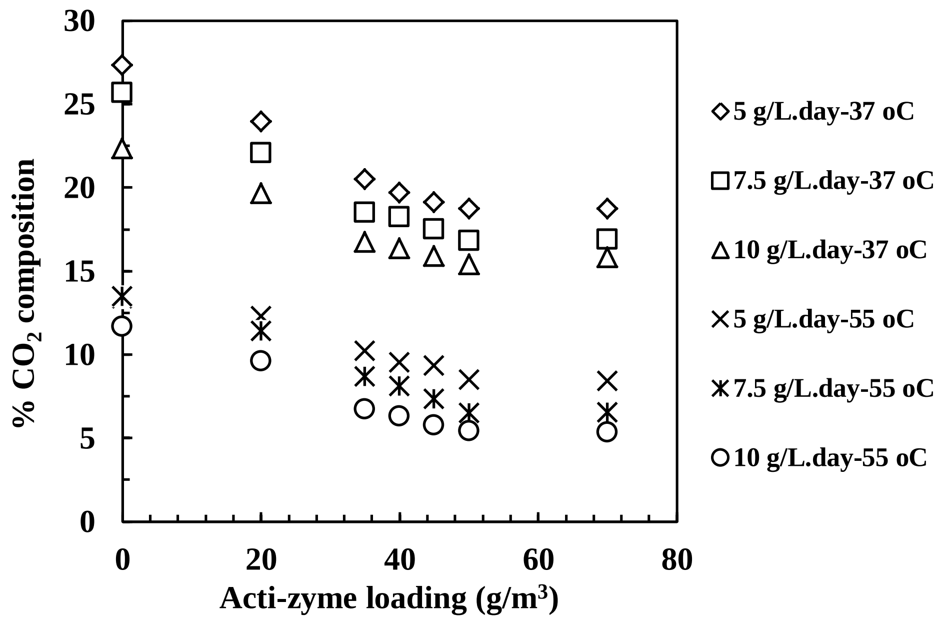

Figure 6: Effect of mesophilic and thermophilic temperature on CO_2 production at varying Acti-zyme loading and retention time of 40 days

Paired sample t-test results shown in Table 4 showed t (20) = 6.22, p<0.0005 and therefore it was concluded that there is a significant difference in the production of CO_2 at temperatures of 37 ^oC and 55 ^oC, since p is less than 0.05

Table 4: Paired samples test for effect of mesophilic and thermophilic conditions on carbon dioxide production

	Paired Differences							
				95% Confidence Interval of the Difference				
Pair 1	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
37°C-55°C	7.10	5.22	1.14	4.72	9.47	6.22	20	0.000

3.5 Trace graces composition

Trace gases included trace amounts of H_2S, ammonia and some water. The amount of trace gases produced was more than 48% in mesophilic conditions as compared to thermophilic conditions (Figure 7). During thermophilic conditions, the Acti-zyme becomes inactive hence minimal gas is produced.

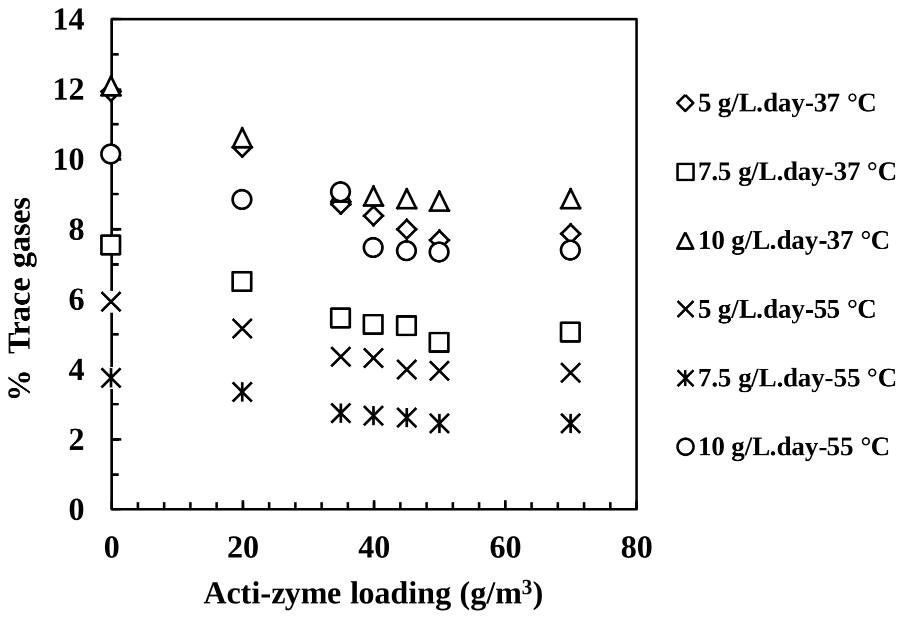

Figure 7: Effect of mesophilic and thermophilic temperature on trace gases production at varying Acti-zyme loading and retention time of 40 days

Paired sample t-test results shown in Table 5 showed t (20) = 5.12, $p<0.0005$ and therefore it was concluded that there is a significant difference in the production of trace gases at temperatures of 37 °C and 55 °C, since p is less than 0.05.

Table 5: Paired samples test for effect of mesophilic and thermophilic conditions on trace gases production

	Paired Differences							
				95% Confidence Interval of the Difference				
Pair 1	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
37°C-55°C	1.53	1.36	0.30	0.90	2.143	5.12	20	0.000

3.6 Bio-solids production

Bio-solids are generated as digestate during the Acti-zyme catalysed digestion of sewage sludge (Figure 8). These bio-fertilisers can be utilised as an alternative source of bio fertilisers. The bio-solids generated had nitrogen, phosphorous and potassium composition of 8.17±0.15%, 5.84±0.03% and 1.32±0.02% respectively (Manyuchi *et al.*, 2015c). The bio-solids also contained copper (0.0073±0.0002%), iron (0.0087±0.0003%), calcium (0.0079±0.002%) and magnesium (0.016±0.0021%) which are essential for plant growth.

Figure 8: Bio-solids generated as digestate during sewage sludge digestion

The amount of bio-solids generated was lowest at 5-10 g/L.day at 37 °C due to increased digestion due to Acti-zyme activity which was absent at 55 °C (Figure 10). As the temperature increased from mesophilic to thermophilic conditions, the amount of bio-solids produced observed an exponential decay trend for all the sewage sludge loadings and Acti-zyme loadings. However, if the target main product is bio-solids thermophilic conditions can be encouraged since they promote pathogen reduction (Willis and Schafer, 2006).

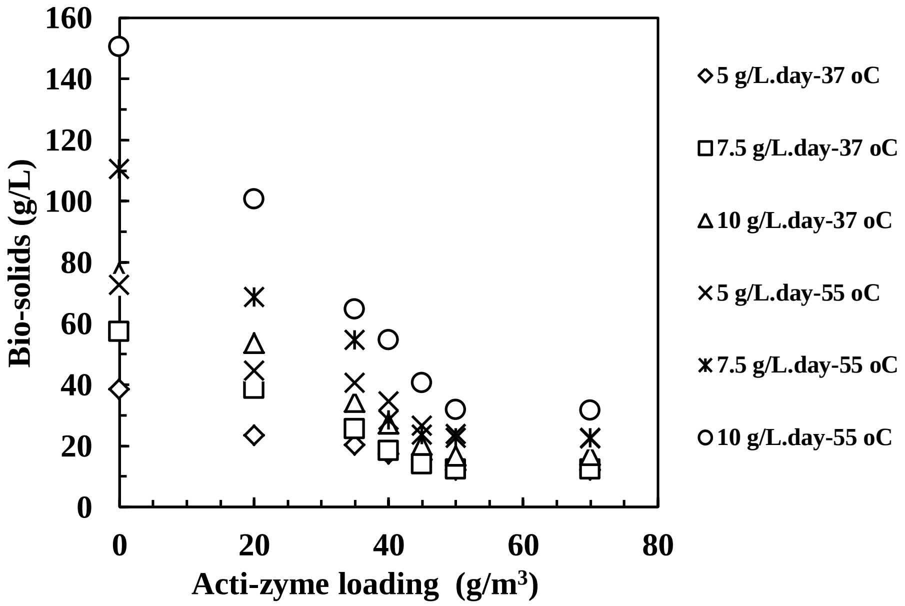

Figure 9: Effect of mesophilic and thermophilic temperature on bio-solids production at varying Acti-zyme loading and retention time of 40 days

Paired sample t-test results shown in Table 6 showed t (20) = -6.59, $p<0.0005$ and therefore it was concluded that there is a significant difference in the production of bio-solids at temperatures of 37 °C and 55 °C, since p is less than 0.05.

Table 6: Paired samples test for effect of mesophilic and thermophilic conditions on bio-solids production

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 37°C-55°C	-24.15	16.80	3.67	-31.80	-16.50	-6.59	20	0.000

CONCLUSION

Mesophilic digestion of municipal sewage sludge using Acti-zyme as biocatalyst is more efficient and should be utilized for biogas digestion from municipal sewage sludge. A rich bio-methane is produced during mesophilic conditions due to the stability of the bio-catalyst as well as increased Acti-zyme loading. Acti-zyme technology employing mesophilic conditions is therefore applicable in municipal sewage sludge management.

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