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STUDY OF CONCENTRATIONS OF VOLATILE FATTY ACIDS AND POTENTIAL METHANE DURING WASTEWATER SLUDGE DIGESTION

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ABSTRACT
Anaerobic degradation of organic matter in wastewater is one of the means of generating biogas (methane) which is a source of fuel. Studies have established that volatile fatty acids (VFAs) can be adopted as indicators of process stability during anaerobic digestion. This study therefore aims at investigating changes in the concentrations of volatile fatty acids at 25°C and at 37°C during the anaerobic reduction of domestic wastewater sludge (DWS). Five litre composite samples of two substrates, primary and secondary sludge, were obtained from various domestic wastewater treatment plants in Scotland. Digested sludge was used as source of anaerobic microbial biomass in some of the tests, after degassing for 48 hours by incubating at 37°C. Total solids, volatile solids, volatile fatty acids concentrations and pH were determined before and after the biochemical methane potential (BMP) tests. The results revealed higher reduction at 37°C than at 25°C for the volatile solids of both the primary and secondary sludge. Although the concentrations of VFAs in the tests with anaerobic biomass were substantially reduced compared to tests without anaerobic biomass, however, the tests without anaerobic biomass produced more methane per volatile solids reduced. The results indicate a more efficient conversion of the substrates to methane in anaerobic digesters with low anaerobic biomass to substrate ratio, provided the process is optimized to ensure efficient conversion of the available VFAs to methane.
Keyword: *Volatile fatty acids, methane, BMP, domestic waste sludge.*

INTRODUCTION
Anaerobic degradation of organic matter in wastewater occurs through three main stages: liquefaction (hydrolysis), acid formation and methane formation (Griffin 2012; Sanders 2001). During acid formation, the products of hydrolysis (dissolved sugars, long-chain fatty acids and amino acids) are converted to short-chain (volatile) fatty acids (mainly acetic, butyric, valeric and propionic acids), alcohols, hydrogen and carbon dioxide (Appels *et al.,* 2008). These short chain acids (propionic, butyric and lactic acids) are then converted to acetic acid, carbon dioxide and hydrogen. The acid formation stage is often the fastest step in the anaerobic process (Vavilin *et al.,* 2008), due to the availability of soluble molecules of degradable compounds, produced during liquefaction, as sources of energy and growth by microorganisms (Gallert and Winter 2005).

The fast rate of the acid phase, compared to the other stages in anaerobic digestion, in anaerobic reactors can result in sudden decline in pH due to accumulation of acids (Bassuney *et al.,* 2013). Ahring *et al.* (1995) concluded that volatile fatty acids (VFAs) can be adopted as indicators of process stability during anaerobic digestion. High concentrations of volatile fatty acids can affect the final phases of the anaerobic digestion, while low concentrations of acids will result in low biogas production. Siegert and Banks (as cited in Appels *et al.,* 2008) reported inhibition of the fermentation of glucose and the production of biogas when VFA concentrations were above 4 g/L and 8 g/L, respectively, while Angelidaki *et al.* (2005) reported stable operation of full scale biogas plants when VFA concentrations were below 1.5 g/L. According to Vavilin *et al.* (2008), intermediate compounds in anaerobic digestion can be possible inhibitors of the process, and any substantial accumulation of VFA within a reactor may inhibit the activity of methanogenic bacteria. Baere *et al.* (as cited in Veeken *et al.,* 2000) had proposed that 30 g/L is the maximum concentration of organic acids sustainable in anaerobic digestion. Brummeler *et al.* (as cited in Veeken *et al.,* 2000) observed that a VFA concentration of 33 g/L causes inhibition of the process but only if the pH is below 5.5.

The aim of this paper is to investigate changes in the concentrations of volatile fatty acids at 25°C and at 37°C during the anaerobic reduction of domestic wastewater sludge (DWS). The research focuses on the comparison of VFA concentrations for batch tests with anaerobic biomass against tests without anaerobic biomass.

MATERIALS AND METHODS
Materials for BMP Batch Tests
Five litre composite samples of two substrates, primary and secondary sludge, were obtained from various domestic wastewater treatment plants in Scotland, through the corporation responsible for sewerage services in Scotland, Scottish Water. Digested sludge was also sourced from the anaerobic digester of Hatton wastewater treatment plant in Arbroath, Scotland, and used as a source of anaerobic microbial biomass, after degassing for 48 hours by incubating at 37°C. The substrates and anaerobic biomass were characterized to determine their initial total solids (TS), volatile solids (VS), pH and volatile fatty acids (VFA) concentrations prior to initiation of the BMP tests. A nutrient medium, containing several minerals without any significant amount of organic carbon dissolved in distilled water (Angelidaki and Sanders, 2004), was added as a source of micronutrients and trace metals necessary for growth of microorganisms. The composition of the nutrient medium in this study was: 75 mg/L Ammonium Bicarbonate

(NH_4HCO_3), 400 mg/L Potassium Dihydrogen Phosphate (KH_2PO_4), 5.0 mg/L Magnesium Sulphate ($MgSO_4$), 5.0 mg/L Iron (III) Chloride ($FeCl_3$), 5.0 mg/L Calcium Chloride ($CaCl_2$), 5.0 mg/L Potassium Chloride (KCl), 1.0 mg/L Cobalt (II) Chloride ($CoCl_2$), 1.0 mg/L Nickel Chloride ($NiCl_2$) and 500 mg/L Sodium Bicarbonate ($NaHCO_3$).

Preparation of the BMP Batch Tests

Using standard methods (Angelidaki *et al.,* 2009), ten methane potential batch tests were prepared in duplicate 500 mL glass bottles (Table 1), and sealed with thick rubber septa and aluminium caps.

The pH values of the final mixtures were adjusted by carefully adding a few drops of a 10 M Sodium Hydroxide (NaOH) solution to each mixture until the pH reading was between 7.51 and 7.88. Then 350 mL of the mixtures were measured into labelled bottles, allowing for a headspace of 150 mL in order to avoid pressure build-up in the bottles once methane production started. The bottles were capped and the headspace was flushed with pure Nitrogen gas for 2 min to induce oxygen free conditions, and then placed in 25°C and 37°C cabinet incubators.

Collection of Samples from the BMP Tests

Samples were collected from the BMP tests through the septum cap using Plastipak® 2 mL disposable plastic hypodermic syringes and 21-guage needles (Fisher Scientific, UK). For parameter analysis, samples were collected from each test condition in five 2 mL volumes and mixed to make 10 mL composite samples.

Methods of Analysis

Total solids concentrations were determined based on recommended standard methods (American Public Health Association 1998), by drying the samples in an oven at 105°C over 24 hours, while the volatile solids concentrations were determined by igniting the dried samples in a furnace at 550°C for two hours. The measurements were performed in duplicate for each sample, and the average TS and VS was adopted. The pH of the samples was determined using a SensION3 pH probe and meter (Model No. 51750-18 Hach Company, Loveland Colorado U.S.A). VFA concentrations, expressed as acetic acid (mg/L HOAC) within the range of 27 - 2800 mg/L, were determined by spectrophotometry with the ferric hydroxamate method for determination of carboxylic esters (Hierholtzer *et al.,* 2013), also known as the Montgomery method, using a DR 5000 Hach Lange spectrophotometer (Hach Lange, Salford Manchester, UK).

The VFA analysis, defined as Method 8196 in the DR 5000 user manual (Hach Company 2005), was performed in triplicates for each sample, and the average of the three measurements was adopted as the VFA concentration for the sample. The methane gas concentrations were determined through gas chromatography (GC) with a Hewlett-Packard 5890 Series II gas chromatograph with dual thermal conductivity detector and an Alltech Heliflex® AT-Alumina stainless steel capillary column. Helium was used as a carrier gas at a flow rate of 7.0 mL/min, and the GC was operated according to methods described in Hierholtzer (2013).

RESULTS AND DISCUSSION

The conversion of primary and secondary sludge to VFA and methane gas was monitored for a period of 40 days during the BMP tests. Table 2 presents the summary of the reduced fractions of the substrates and the methane produced during the experiment.

Table 1: 350 mL BMP tests for domestic wastewater sludge

TEST ID	Temp. (°C)	Substrate	Substrate volume (mL)	Anaerobic biomass volume (mL)	Nutrient solution volume (mL)
PS 25°C	25	Primary sludge	150	100	100
PS 37°C	37	Primary sludge	150	100	100
SS 25°C	25	Secondary sludge	150	100	100
SS 37°C	37	Secondary sludge	150	100	100
PS nol 25°C	25	Primary sludge	150	-	200
PS nol 37°C	37	Primary sludge	150	-	200
SS nol 25°C	25	Secondary sludge	150	-	200
SS nol 37°C	37	Secondary sludge	150	-	200
Blank	25	-	-	100	250
Blank	37	-	-	100	250

Footnote: PS = primary sludge; SS = secondary sludge; nol = no introduction of anaerobic biomass; °C = degrees centigrade; mL = millilitre

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Table 2: Reduction of substrates and methane production during 350 mL BMP tests

TEST ID	Temp (°C)	Anaerobic biomass	Initial VS (g)	VS reduced (g)	VS reduced (%)	Total methane produced (mL)	Methane produced (mL/g VS reduced)
PS 25°C	25	Yes	3.51	1.40	39.89	797.01	567.29
PS 37°C	37	Yes	3.51	1.97	56.13	1056.21	536.15
SS 25°C	25	Yes	3.89	1.25	32.13	105.08	84.06
SS 37°C	37	Yes	3.89	1.36	34.96	447.55	329.08
PS nol 25°C	25	No	3.31	0.73	22.05	671.05	919.25
PS nol 37°C	37	No	3.31	1.26	38.07	801.88	636.41
SS nol 25°C	25	No	4.22	1.27	30.09	201.10	158.35
SS nol 37°C	37	No	4.22	1.65	39.10	416.69	252.54

Footnote PS = primary sludge; SS = secondary sludge; nol = no introduction of anaerobic biomass; °C = degrees centigrade; mL = millilitre; g = grams

Substrate Reduction and Methane Production

The observed reduction of volatile solids of the substrates, Table 2, showed low reduction at 25°C compared to 37°C for the primary and secondary sludge irrespective of the presence of anaerobic biomass. For the primary sludge (PS) test with anaerobic biomass, 1.97 grams (55%) of the volatile solids were reduced at 37°C, while only 1.40 grams (40%) reduction was observed at 25°C (Table 2). For the secondary sludge (SS) test, 1.36 grams (33%) of the volatile solids were reduced at 37°C, while only 1.25 grams (22%) reduction was observed at 25°C. The results also indicated higher methane produced per volatile solids reduced (mL/g VS) for all the tests without anaerobic biomass compared to the tests with anaerobic biomass, except for secondary sludge test at 37°C. Figures 1 and 2 present the cumulative methane produced (mL/g VS added) against time (days) during the experiment.

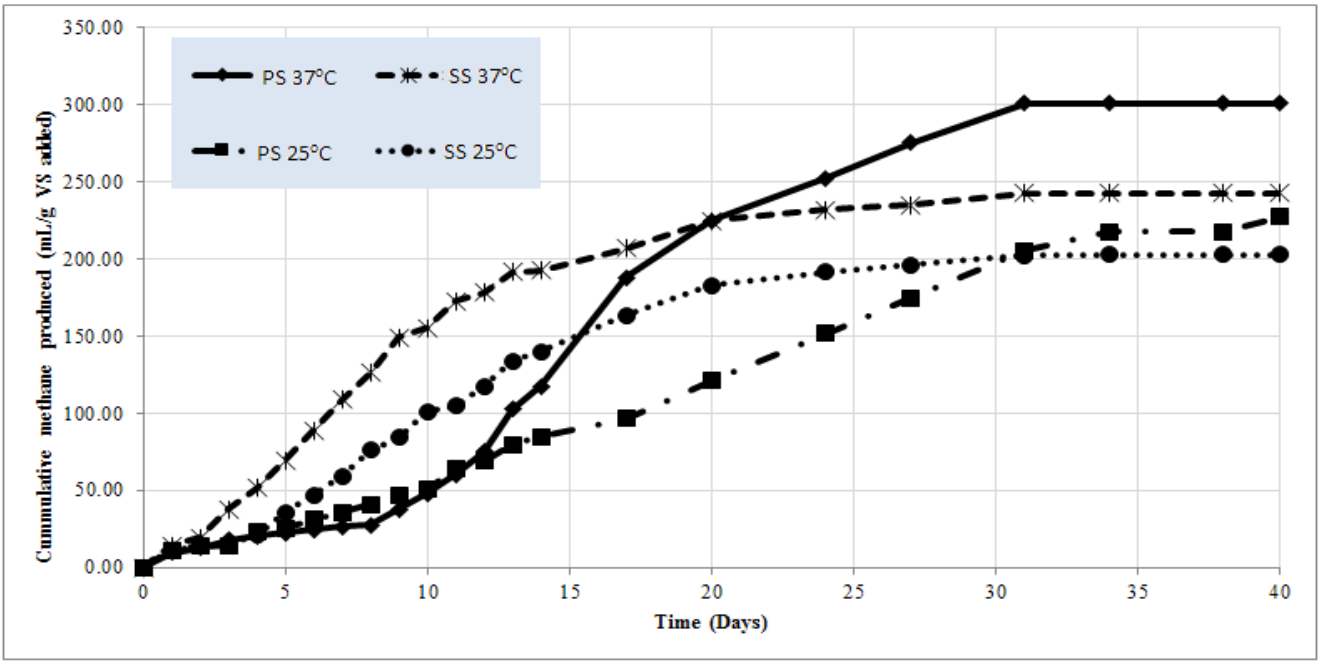

Figure 1: Cumulative methane produced (mL/g VS added) from the BMP test of domestic wastewater sludge with anaerobic biomass.

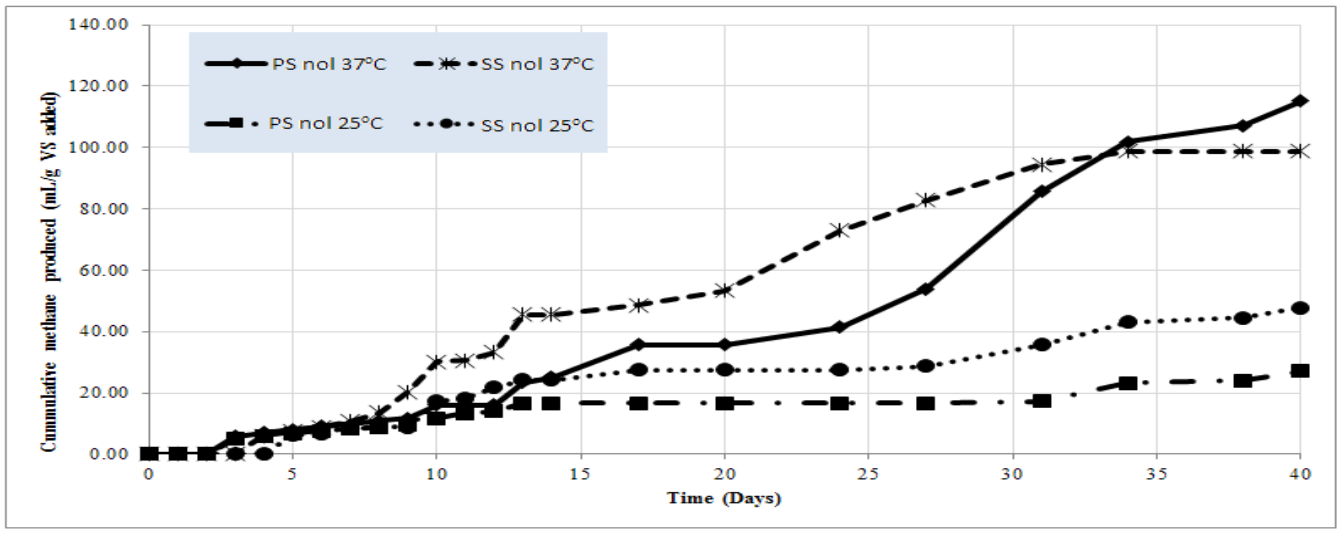

Figure 2: Cumulative methane produced (mL/g VS added) from the BMP test of domestic wastewater sludge without anaerobic biomass

Methane production was first observed after an 8-day lag period from the primary sludge tests with anaerobic biomass (Figure 1), while half of the cumulative methane produced from the secondary sludge tests was detected in the first ten days of the experiment. During the initial 10 days of the BMP experiment, no major changes were observed in the methane production for all the tests, except the secondary sludge tests with anaerobic biomass where the methane production recorded were observed to increase steadily from the first experimental day.

VFA Concentrations and pH

The acid producing phase was observed in the BMP tests based on the concentrations of VFA during the experiment, and the observed VFA concentrations and pH values against time (days) for the batch BMP tests are presented in Figures 3 - 6.

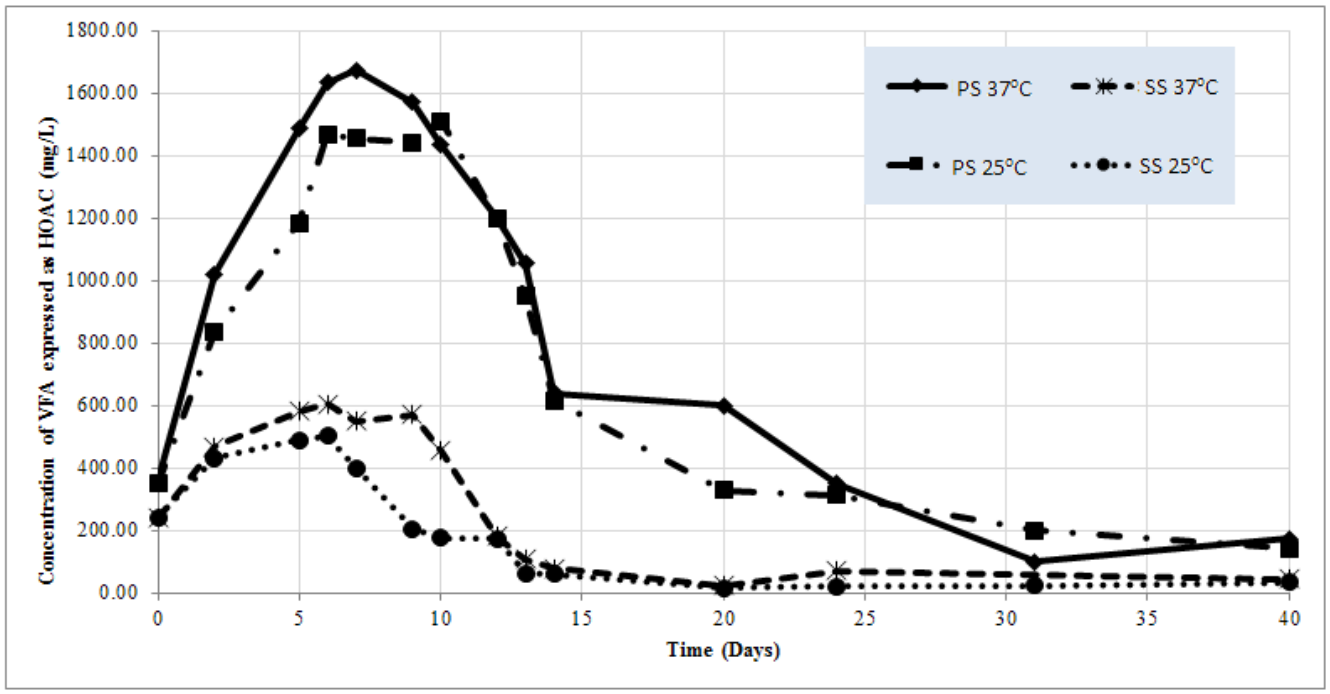

Figure 3: Volatile fatty acids concentrations (mg/L) from the BMP test of domestic wastewater sludge with anaerobic biomass

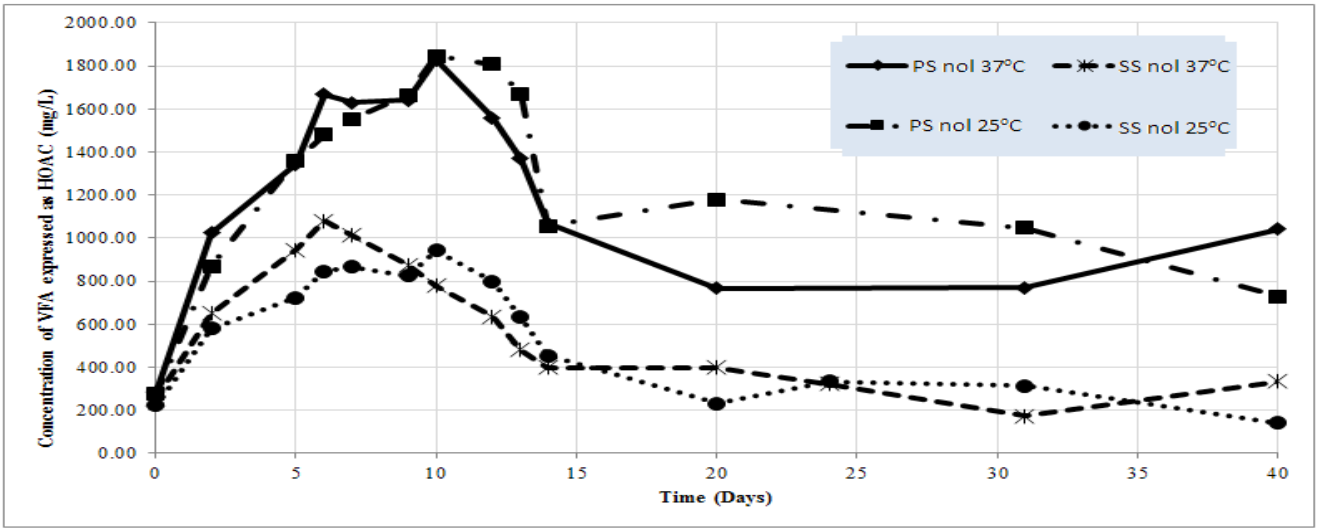

Figure 4: Volatile fatty acids concentrations (mg/L) from the BMP test of domestic wastewater sludge without anaerobic biomass

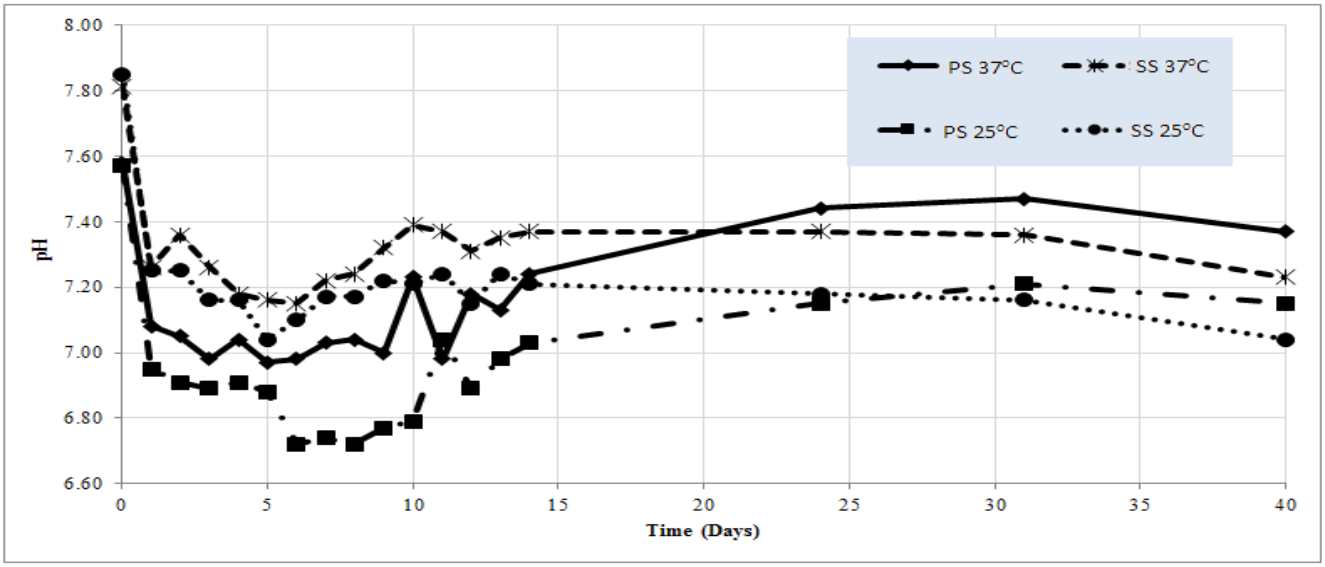

Figure 5: Observed pH values from the BMP test of domestic wastewater sludge with anaerobic biomass

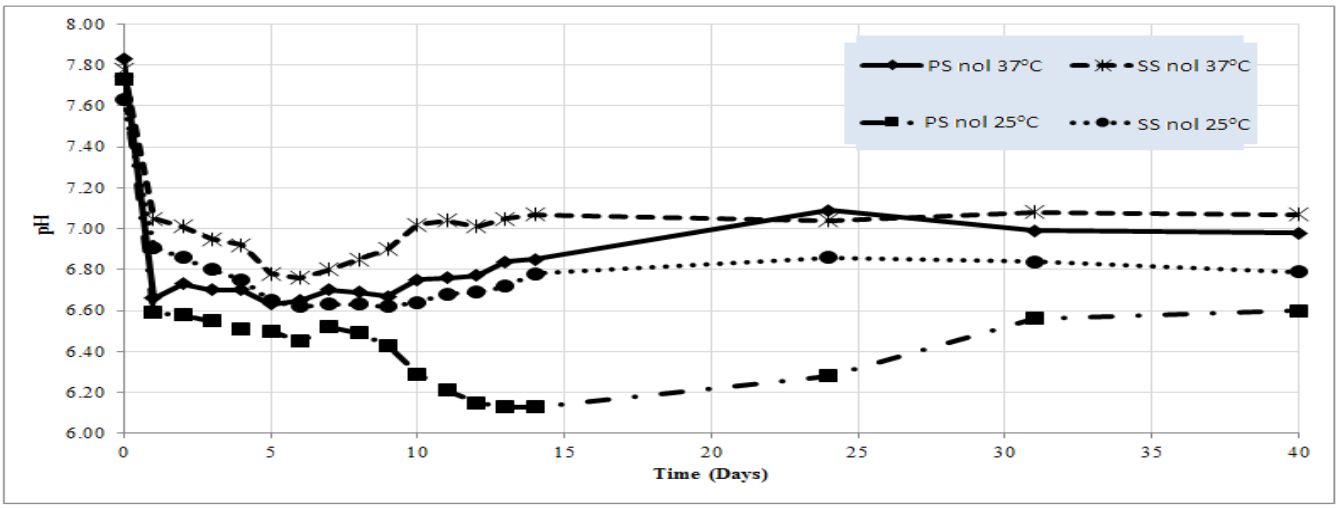

Figure 6: Observed pH values from the BMP test of domestic wastewater sludge without anaerobic biomass

The VFA concentrations indicate three stages in the process (Figures 3 and 4), with the first stage as a period during which a continuous increase in intermediate compounds concentrations was observed along with a decrease in pH values (Figures 5 and 6), lasting for up to 10 days for all the batches. This stage was followed by the depletion of acids concentrations over a short period of time, between 4 - 8 days (Figures 3 and 4). The third stage is identified by a relatively stable concentration of VFA until the end of the experiment (Figures 3 and 4). After the initial 10 days of the experiment, the observed stability in pH values and the decrease in VFA concentrations were probably as a result of established methanogenesis and therefore the conversion of the VFA to methane.

From Figure 3, concentrations of volatile fatty acids were observed to decline in the tests without anaerobic biomass after the first 10 days of the experiment. This decline corresponds to recorded increase in methane production as shown in Figure 2, but the decline in VFA concentrations ceased after the 15th day of the experiment even though high concentrations of VFA remained in the tests (greater than 600 mg/L for the PS nol. 25^{o}C, 300 mg/L for SS nol. 37^{o}C, 150 mg/L for SS nol. 25^{o}C, and 1000 mg/L for the PS nol. 37^{o}C after day 40). The pH values in the tests were within the same range of 6.0 - 8.0 for all the tests indicating there was no inhibition of methanogenesis due to accumulation of VFA, and consequently decline in pH levels in the tests. For the tests with anaerobic biomass, observed VFA concentrations after the 40th day of the experiment were less than 200 mg/L (Figure 3).

Ahring (2003) described the anaerobic digestion process as consisting of two final methane producing pathways from available hydrogen and acids, mainly acetate, propionate, butyrate and other VFAs. The availability of VFAs indicates hydrolysis and fermentation processes are progressing, and producing hydrogen and VFAs in the tests. However, the thermodynamics of the reactions for VFA degradation requires low hydrogen concentrations in order to progress efficiently (Ahring 2003). High hydrogen concentrations tend to reduce the accessibility of the volatile fatty acids to the microorganisms that will utilize them and convert them to acetate and eventually biogas. The observed high concentration of unconverted acids in the tests without anaerobic biomass could be as a result of a deficiency in hydrogen utilizing organisms, which would have been available in the anaerobic biomass. This potentially explains why the results from the tests with anaerobic biomass indicate substantial reduction of VFAs, while the results from the tests without anaerobic biomass indicate poor reduction of VFAs.

CONCLUSIONS

The results revealed higher reduction at 37°C than at 25°C for the volatile solids of the primary sludge, where over 55% of the volatile solids were reduced at 37^{o}C, while only 40% reduction was observed at 25^{o}C for the primary sludge (PS) test. For the secondary sludge (SS) test, over 33% of the volatile solids were reduced at 37^{o}C, while only 22% reduction was observed at 25^{o}C. The concentrations of volatile fatty acids were observed to decline after the first 10 days of the experiment. However, this decline ceased after the 15th day of the experiment in the tests without anaerobic biomass even though high concentrations of VFA remained in the tests until the end of the experiment. The retained high VFA concentrations in the tests without anaerobic biomass could potentially be due to a deficiency of hydrogen utilizing methanogens. There is the potential that the hydrogen utilizing organisms were unable to adequately establish in the tests without anaerobic biomass, whereas they were able to establish in the tests with anaerobic biomass, and consequently the concentrations of VFAs in the tests with anaerobic biomass were substantially reduced. The results indicate a more efficient conversion of the substrates to methane in anaerobic digesters with low anaerobic biomass to substrate ratio, provided the process is optimized to ensure efficient conversion of the available VFAs to methane through further research.

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