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L. Ho, G. Ho

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Mitigating ammonia inhibition of thermophilic anaerobic treatment of digested piggery wastewater: use of pH reduction, zeolite, biomass and humic acid

L. Ho* and G. Ho

School of Environmental Science, Murdoch University, Perth, Western Australia

* *Corresponding author. Tel: + 673 3372251, E-mail: lilyho@tpg.com.au*

Abstract

High free ammonia released during anaerobic digestion of livestock wastes is widely known to inhibit methanogenic microorganisms and result in low methane production. This was encountered during our earlier thermophilic semi-continuously fed continuously-stirred tank reactor (CSTR) treatment of piggery wastewater. This study explored chemical and biological means to mitigate ammonia inhibition on thermophilic anaerobic treatment of piggery wastewater with the aim to increase organic volatile carbon reduction and methane production. A series of thermophilic anaerobic batch experiments were conducted on the digested piggery effluent to investigate the effects of pH reduction (pH 8.3 to 7.5, 7.0 and 6.5) and additions of biomass (10% v/v and 19% v/v anaerobic digested piggery biomass and aerobic-anaerobic digested municipal biomass), natural zeolite (10, 15 and 20 g/L) and humic acid (1, 5 and 10 g/L) on methane production at 55^{o}C for 9 to 11 days. Reduction of the wastewater pH from its initial pH of 8.3 to 6.5 produced the greatest stimulation of methane production (3.4 fold) coupled with reductions in free ammonia (38 fold) and total volatile fatty acids (58% TVFA), particularly acetate and propionate. Addition of 10 to 20 g/L zeolite to piggery wastewater with and without pH reduction to 6.5 further enhanced total VFA reduction and methane production over their respective controls, with 20 g/L zeolite producing the highest enhancement effect despite the ammonia-nitrogen concentrations of the treated wastewaters remaining high. Without pH reduction, zeolite concentration up to 20 g/L was required to achieve comparable methane enhancement as the pH-reduced wastewater at pH 6.5. Although biomass (10% v/v piggery and municipal wastes) and low humic acid (1 and 5 g/L) additions enhanced total VFA reduction and methane production, they elevated the residual effluent total COD concentrations over the control wastewaters (pH-unadjusted and pH-reduced) unlike zeolite treatment. The outcomes from these batch experiments support the use of pH reduction to 6.5 and zeolite treatment (10 to 20 g/L) as effective strategies to mitigate ammonia inhibition of the thermophilic anaerobic treatment of piggery wastewater.

Keywords: *Ammonia Inhibition, Piggery Wastewater, Thermophilic Anaerobic Digestion, Zeolite, Humic Acid, Biomass*

1. Introduction

Anaerobic digestion, particularly at thermophilic temperatures (55^{o}C) is a preferred technology for livestock wastes as it has higher volatile organics reduction and pathogens destruction efficiencies compared to its mesophilic counterpart (35-37^{o}C) (Angelidaki *et al.*, 2003; Ahn and Forster, 2002; Bendixen 1994; van Lier *et al.*, 1994; Lee *et al.*, 1989). However, at high reactor temperature and pH, the ammonia-nitrogen released during fermentation of nitrogen-containing materials such as urea and proteins exists largely as the unionised free ammonia (NH_3) which is more toxic to the methane-forming microorganisms than the ionised ammonium (NH_4^+) form as it diffuses more rapidly through the cell membrane of the microorganisms (Geraldi, 2006). Various levels of ammonia-nitrogen have been reported to inhibit biogas production. McCarty (1964) reported inhibition to occur at total ammonia (free ammonia plus ammonium) concentrations of 1.5 to 3.0 g-N/L at pH above 7.4 in the mesophilic range whilst above 3.0 g-N/L, ammonia inhibition occurred at all pH levels. At thermophilic conditions, Angelidaki and Ahring (1994) observed poor digester treatment efficiency of cattle manure at free ammonia concentration above 0.7 g-N/L at pH 7.4–7.9. Gallert and Winter (1997) reported 50% inhibition of methanogenesis at free ammonia concentration of 0.56-0.57 mg-N/L at pH 7.6. The differences in ammonia inhibition concentration are attributed to variable factors such as operational conditions (pH, temperature), type of substrates, inoculum source and microbial adaptation. Various means to reduce the ammonia inhibition of methane production from livestock wastes have been investigated by other researchers and their findings comprehensively reviewed in Yadvika *et al*. (2004) and Chen *et al.* (2008). These strategies included pH reduction of the reactor effluent with acid , dilution of the digester feedwater, recirculation of digested slurry to the reactor, process modification of the reactor design, gradual acclimatisation of the biomass with reduced organic loading rate and additions of inorganic additives such as zeolite, activated carbon, clay and iron to adsorb the inhibitory compounds (Kotsopoulos *et al*., 2008; Tada *et al*., 2005; Milan *et al*., 2001; 2003; Sánchez *et al*., 1995; Yadvika *et al*., 2004).

With our thermophilic digested piggery wastewater containing high level of inhibitory free ammonia (0.1 g N/L) at pH 8.3 and high percentage of undegraded soluble organics (32%), pH reduction, additions of natural zeolites and anaerobic piggery biomass were selected for investigations into their respective effectiveness in enhancing organics degradation to methane in post-batch thermophilic anaerobic treatment of the digested piggery wastewater. The investigations were extended to include municipal solid waste biomass and humic acid. It was perceived that the municipal biomass which had had prior exposure to a combined aerobic-anaerobic cycle of operation might contain microorganisms that were lacking in the anaerobic piggery biomass. For humic acid, it is found in humic substances (humus) which had been shown to possess electron-accepting capability in the anaerobic oxidation of organic carbon compounds and hydrogen (Scott *et al*., 1998; Lovley *et al*., 1999). The effects of both additives on methane production from piggery effluent have not been investigated to date.

Additional experimental studies were conducted to determine whether each of the additive (biomass, zeolite or humic acid) would perform better at the established optimum effluent pH compared to the unadjusted pH in mitigating ammonia inhibition and enhanced organics degradation to biomethane.

The main aim of these experimental studies was to find practical and effective ways to mitigate ammonia inhibition on thermophilic anaerobic conversion of organics in digested piggery wastewater to biofuel methane.

2. Materials and Method

2.1 Batch thermophilic anaerobic experiments

Digested piggery wastewater from our lab-scale thermophilic CSTR anaerobic semi-continuous reactor operating at 10-day of hydraulic retention time was used in the four batch vial thermophilic experiments. As the digested wastewater had been stored in the fridge at 4 deg C for up to three months prior to the experiments, 10 to 30% v/v raw piggery wastewater were added to ensure there were adequate degradable volatile fatty acids as carbon food source for the digested wastewater microorganisms. Table 1 lists some key physico-chemical characteristics of the raw (influent) and digested piggery wastewater (effluent) measured at the start and end of the semi-continuous reactor experiment respectively.

To account for changes in the physico-chemical and biological properties of the digested wastewater as a result of the different experimental start times and the added amount of raw piggery wastewater, baseline measurements at zero time were carried out at the start of each batch experiment to allow performance comparisons to be made of the varying concentrations of each treatment. Each batch experiment was carried out in duplicate with 50-ml of the digested piggery wastewater in 120-ml serum vials. The serum vials were capped with butyl rubber stoppers and crimped with aluminium seals. After degassing the headspace, the vials were incubated in a shaking water bath at 55^{o}C for between 9 and 11 days. Biogas volume was measured daily with plunger displacement of an air-tight glass syringe attached with metal hub needle whilst biogas composition was analysed simultaneously. At the end of the test period, triplicate samples were taken for a suite of chemical and molecular real-time PCR microbial analysis. In this paper, pH, total COD, VFA and ammonia-nitrogen (total and free) results were presented and discussed.

Table 1

2.1.1 Effect of pH reduction (Experiment 1). The aim of this experiment was to establish the optimum wastewater pH that produced the greatest enhancement in biodegradation of organic carbon compounds to methane. The pH of digested piggery wastewater was reduced from 8.3 to 7.5, 7.0 and 6.5 with concentrated hydrochloric acid.

2.1.2 Effect of biomass, zeolite and humic acid additions to pH-unadjusted and pH-reduced piggery wastewater (Experiments 2, 3 and 4). In Experiment 2, two types of biomass at 10% and 19% v/v were tested for their digestion performances: 1) piggery biomass of the digested wastewater from a lab-scale thermophilic anaerobic reactor and 2) DiCOM (trade name) biomass from a local patented aerobic-anaerobic pilot digester that treats municipal solid wastes. Table 2 gives the solid contents of the two biomass. In Experiment 3, natural zeolite

(85% Clinoptilolite mineral) of particle size less than 1 mm from Castle Mountain, New South Wales was tested at concentrations of 10, 15 and 20 g/L. Table 3 gives the mineral composition of Castle Mountain natural zeolite. In Experiment 4, humic acid (Sigma-Aldrich, technical grade) was tested at concentrations of 1, 5 and 10 g/L. The objectives of these experiments were: 1) to compare their digestion performances with and without pH reduction to pH 6.5, and 2) to determine which concentration was most effective in mitigating ammonia inhibition to enhance thermophilic digestion performance.

Table 2

Table 3

2.2 Analytical methods

Determinations of pH and total chemical oxygen demand (TCOD) of the mixed liquor were performed according to the APHA Standard Methods (1998). Total ammonia nitrogen was measured using Hach Nessler Method on the centrifuged sample (13,000g for 5 min). Free ammonia concentration was calculated using the formula (Hansen *et al*., 1998): NH_3-N = (Total ammonium-nitrogen) x $(1 + 10^{-pH}/10^{-(0.09018 + 2729.92/T)})^{-1}$ where T = temperature (Kelvin). Volatile fatty acids (carbon-2 to 6 VFA) were analysed by gas chromatography Varian Star 3400 Model equipped with EC-1000 mega-bore column of 15 m x 0.53 mm x 1.2 µm and a flame-ionisation detector. Temperatures of the column, injector and detector were 80°C, 200°C and 250°C respectively with high purity nitrogen as the column carrier gas. VFA (C2 to C6) concentrations were determined from their respective four-point mixed VFA standard calibration graphs. Biogas composition (methane and carbon dioxide) was analysed using the same equipment equipped with Porapak Q 80/100 packed column and thermal conductivity detector. Temperatures of the column, injector and detector were set at 40°C, 120^{o}C and 120^{o}C respectively. Concentrations of methane and carbon dioxide were determined from their respective 4-point standard gas calibration graphs prepared from standard pure methane and pure carbon dioxide gas.

3. Results

3.1 Effect of pH reduction (experiment 1)

Reduction of the digested piggery wastewater pH from 8.3 to 7.5, 7.0 and 6.5 resulted in the largest increase in methane production from 200 ± 0 ml/L at pH 8.3 to 680 ± 0 ml/L (by 240%) at pH 6.5 which corresponded with the largest reduction in total VFA-COD (58%). ,(Table4). While total VFA concentrations had accumulated in the digested wastewaters at initial pH of 8.5 and 7.5 after ten days of batch digestion, methane production was still possible. With further pH reduction to 7.0 and 6.5, increased methane production and total VFA reductions were observed. The increases corresponded with significant decrease in initial free ammonia concentration of 916 ± 32 at initial pH 8.3 to 76 ± 0 at pH 7.0 and 24 ± 0 mg/L at pH 6.5. By the end of the 10-day batch experiment, all the pH-reduced vials showed increased pH of 0.6 to 1.3 units which corresponded with 3 to 18 fold increase in free ammonia concentrations. The largest increases in pH and free ammonia concentration were observed at the reduced pH of 6.5.

Table 4

Both the TVFA-COD reduction and methane production exhibited a strong negative correlation (R^2 = -0.98 and -0.97 respectively) with the final free ammonia concentration (Figure 1). The negative total VFA reduction reflects a build-up of total VFAs. Increased total VFA reduction positively correlated (R^2 = 0.99) with increased methane production as the pH was progressively reduced from 8.3 to 6.5 (Figure 2).

Gas chromatography analysis of the carbon-2 to -6 VFA components (Figure 3) revealed that without pH reduction of the digested wastewater at pH 8.3, acetate and propionate concentrations accumulated significantly as reflected by the negative percentage reductions at the end of the batch digestion. However, butyrate (i- and n-) and valerate (i- and n) concentrations were reduced to between 50% and 100%. When the initial pH of the digested wastewater was decreased from pH 8.3 to pH7.5 and 7.0, substantial acetate reductions of 37% and 66% respectively occurred compared to the final acetate concentration (1159 ± 6 mg/L) of the pH-unadjusted digested wastewater at pH 8.3. However, their final acetate concentrations were still 78% and 8% higher than the initial concentrations of 410 ± 10 mg/L and 368 ± 23 mg/L respectively. Reductions of propionate were minimal at both the reduced pH of 7.5 and 7.0. Decreasing the wastewater pH to 6.5 greatly stimulated acetate, propionate, i-butyrate and caproate degradation compared to the reduced pH of 7.0 and 7.5. (Figure 3). Both the acetate and propionate reductions displayed strong negative correlation (R^2 = -0.91 and -0.81 respectively) with their final free ammonia concentrations (Figure 4).

Figure 1

Figure 2

Figure 3

Figure 4

3.2 Effect of biomass additions (experiment 2)

Both piggery and DiCOM biomass additions greatly increased the initial total COD concentration (7481 ± 421 mg/L) of the pH-unadjusted digested piggery wastewater (Table 5). The increase was significantly greater with DiCOM biomass (by 91% and 166%) than piggery biomass (by 42% and 49%) at 10% v/v and 19% v/v respectively. Unlike the piggery biomass, DiCOM biomass also significantly increased the wastewater total VFA concentration (2132 ± 167 mg COD/L) by 151% with 10% v/v biomass and 275% with 19% v/v biomass.

With the addition of 10% v/v piggery biomass to the pH-unadjusted digested piggery wastewater(Figure 5a), methane yield increased by 73% from 74 ± 7 ml/g VS to 128 ± 20 ml/g VS with a concurrent 8% increase in TVFA reduction at the end of the experiment (Table5). The methane yield was comparable to the vials with 10% v/v (132 ± 3 ml/g VS) and 19% v/v (174 ± 40 ml/g VS) DiCOM biomass (Figure 5b) despite the DiCOM biomass stimulated significantly higher total VFA reductions of 52 ± 1% and 36% ± 1% respectively as opposed to the pH-unadjusted wastewater control. Similar with the pH reduction experiment (Table 4), methane production (74 ± 7 ml/g VS) was observed in the pH-unadjusted wastewater control with 26% elevated total VFA concentration of 2683 ± 31 mg COD/L. The observed drop in total VFA reduction at the higher DiCOM biomass (19% v/v) compared to the lower biomass (10% v/v) was also observed with the piggery biomass. Increasing the piggery biomass from 10% v/v to 19% v/v resulted in methane yield reduction of 61% from 128 ± 20 ml/g VS to 50 ± 25 ml/g VS and increased total VFA concentration of 44 ± 17%, particularly acetate and propionate (data not shown). This corresponded with a higher initial free ammonia level of 1103 ± 5 mg/L at 19% v/v biomass compared to 896 ±14 mg/L at 10% v/v biomass.

In the vials with pH-reduced digested wastewater (pH 6.5), adding 10% v/v and 19% v/v piggery biomass initially stimulated methane yields from 81±10 ml/g VS to 130 ± 4 ml/g VS (60%) and 100 ± 7 ml/g (23%) respectively during the first 3 days (Figure 5a). Methane production began to level off on day 4. By day 11, both methane yields were significantly lower than the vial without biomass addition (286 ± 20 ml/g VS) by 20% at 10% v/v biomass (228 ± 2 ml/g VS) and 37% at 19% v/v biomass (179 ± 2 ml/g VS) despite their higher total VFA reductions of 93 ± 1% compared to 71% in the pH-reduced wastewater control. Greater inhibition on biogas production was observed with 19% v/v than with 10% v/v piggery biomass in both the pH-unadjusted and pH-reduced piggery wastewaters . In contrast to piggery biomass, no obvious inhibitions were observed with DiCOM biomass (Figure 5b). However, methane yield was only increased slightly by 12% with 10% v/v DiCOM biomass (320 ± 3 ml/g VS) after an initial lag of 3 to 5 days. Increasing the amount to 19% v/v did not enhance the methane yield further (299 ± 11 ml/g VS).

Table 5

Figures 5a and 5b

Despite DiCOM biomass addition to the pH-reduced wastewater produced higher methane yield than the piggery biomass and the pH-reduced wastewater control (C2), it raised the wastewater residual organics level (total COD) significantly from 6667 ± 1258 mg/L to 9550 ± 953 mg/L at 10% v/v biomass and 11892 ± 900 mg/L at 19% v/v biomass at the end of the digestion period (Table5).

3.3 Effect of natural zeolite addition (Experiment 3)

Addition of natural zeolite to the digested piggery wastewater without pH reduction (C1) increased methane production significantly by 60 ± 4% at 20 g/L, 44 ± 3% at 15 g/L and 26 ± 7% at 10 g/L from the initial 0.72 ± 0.012 L/L at the end of the experiment (Figure 6). Total methane production (1.14 ± 0.028 L/L) at 20 g/L zeolite was comparable to the pH-reduced digested wastewater without zeolite (1.12 ± 0.008 L/L).

Figure 6

The increases in methane production corresponded with the increased total VFA-COD reduction, in particular acetate (Figure 7a) while there were no significant reductions in ammonium-nitrogen and free nitrogen concentrations (Table6). There was a strong positive correlation (0.98) between acetate degradation and zeolite concentration (Figure 8). While the applied zeolite concentrations were ineffective in reducing the elevated propionate concentration, they promoted small increases in n-butyrate and i-valerate degradation.

Addition of zeolite to the pH-reduced piggery wastewater (pH 6.5) increased the total methane production (1.12 ± 0.008 L/L) slightly by 10 ± 1% at 10 g/L and 15 g/L zeolite and 13 ± 0% at 20 g/L. The increases in methane production corresponded with increases in total VFA-COD degradation, particularly propionate (Figure 7b). There was a strong positive correlation (0.97) between propionate degradation and zeolite concentration (Figure 9). While small reductions of 17-19% in free ammonia concentrations were achieved at higher zeolite concentrations of 15 and 20 g/L, there were no reductions in ammonium-nitrogen concentrations.

Table 6

Figures 7a and 7b

Figure 8

Figure 9

3.4 Effect of humic acid addition (Experiment 4)

At 10 g/L of humic acid, methane production was greatly reduced particularly from the pH-unadjusted wastewater (C1) (Figure 10a). At 1 and 5 g/L, small improvements of 27-29% in methane production were observed from the pH-reduced wastewater (C2) (Figure 10b).

Table 7

Figures 10a and 10b

Reduction of TVFA-COD was marginally higher at 1 g/L (62 ± 3%) than at 5 g/L (57 ± 1%) humic acid addition (Table 7). Significant improvement in propionate degradation was observed at both concentrations while at 1 g/L addition, small improvements in acetate, i-valerate and caproate degradation were also observed (data not shown).

4. Discussion

4.1 Effect of pH

This batch vial experiment clearly demonstrated the effectiveness of pH reduction method in enhancing methane production from the digested piggery wastewater despite foaming being encountered due to the release of carbon dioxide during pH reduction with concentrated hydrochloric acid.

Without pH reduction of the digested piggery wastewater (pH 8.3), degradation of butyrate (i- and n-) and valerate (i- and n-) (Figure 3) as well as methane production (Figure 2) were still possible despite its high initial free ammonia concentration of 916 ± 32 mg N/L. The observed production of methane from the digested wastewaters with elevated total VFA concentrations at pH 8.3 and 7.5 (Table 4) was possibly from the conversion of hydrogen and carbon dioxide by the hydrogenotrophic microorganisms. VFA compositional results (data not shown) indicate that the butyrate-degrading acetogenic bacteria which oxidise butyrate as well as medium-chain (up to carbon-11) and long-chain fatty acids (up to carbon-18) to acetate, propionate and hydrogen were highly resilient to product inhibition unlike the acetoclastic methanogens and propionate-degrading acetogenic bacteria. These two groups of anaerobic microorganisms were severely inhibited as reflected by the build-up of acetate and propionate at the end of the batch digestion. Calli *et al.* (2005) and Pind *et al.* (2003) have also observed high butyrate degradation while propionate degradation was inhibited at free ammonia concentration above 200 mg/L and hydrogen level 5-6 times higher respectively.

Reducing the wastewater initial pH from 8.3 to 7.5, 7 and 6.5 greatly lowered the initial free ammonia concentration (916 ± 32 mg N/L) by more than 70%. This facilitated further degradation of proteinaceous organic materials as evidenced by the increases in pH and free ammonia concentration at the end of the experiment (Table 4). However, the accumulation of acetate and propionate at the initial reduced pH of 7.5 and 7 where the final free ammonia level was 643-686 mg N/L suggested that at this level, the acetoclastic methanogens and propionate-degrading acetogenic bacteria were progressively being inhibited. The accumulation of propionate in particular, suggested that the hydrogen-utilising microorganisms responsible for keeping the hydrogen level below the threshold of $<10^{-4}$ atmosphere (Harper and Pohland, 1987; Thauer *et al.*, 1977) for propionate degradation to occur were also progressively being inhibited. Wiegant and Zeeman (1986) reported propionate to accumulate when hydrogen-utilising methanogens were inhibited by high ammonia concentration. The strong negative correlations between acetate or propionate degradation and final free ammonia concentration (Figure 4) reflected the sensitivity of these microbial consortia to the final free ammonia value. Free ammonia values of 560-568 mg-N/L (Gallert and Winter, 1997) and 700 mg-N/L (Angelidaki and Ahring, 1994) have been reported to cause 50% inhibition of methanogenesis.

Reducing the initial wastewater pH from 8.3 to 6.5 produced the highest stimulatory effects on methane production and VFA reduction. While this pH value was at the upper end of the reported optimal pH range of 5.0 to 6.5 for acidogenic bacteria and below the optimal pH 6.7 to 7.2 for acetogens and methanogens (Angelidaki *et al*., 2003; Novaes, 1986), it was the most favourable pH for the piggery mixed anaerobic culture. The lower final acetate and propionate concentrations in comparison to their initial concentrations indicated that the acetoclastic methanogens and the syntrophic propionate-degrading acetogenic bacteria-hydrogenotrophic ±microorganisms were not inhibited at the final free ammonia concentration of 425 ± 22 mg/L. Real-time PCR analysis found significant increase in methanogen numbers with concurrent decrease in pathogenic *Clostridium perfringen* numbers as the piggery wastewater pH was reduced from 8.3 to 6.5 (Skillman *et al.*, 2009). Although the exact cause for the pathogen decay was unclear, microbial competition for common substrates and factors such as reactor temperature, retention period, pH and chemical interactions can contribute to pathogen decay during treatment of biowastes (Salsali *et al.*, 2008; Smith *et al.*, 2005).

4.2 Effect of biomass additions

In this study, it was demonstrated that adding 10% v/v piggery or DiCOM biomass to the pH-unadjusted wastewater (C1) was more effective than adding 19% v/v biomass in enhancing TVFA reduction and methane production. The 17% higher initial free ammonia level (1051 ± 10 mg/L) in the wastewater with 19% v/v piggery biomass addition compared to 10% v/v piggery biomass addition (896 ± 10 mg/L) could have contributed to the microbial inhibition (Table 5). Similar to the earlier pH reduction experiment, the observed methane production from the pH-unadjusted wastewater with elevated total VFA concentration possibly had come from the conversion of hydrogen and carbon dioxide by the hydrogenotrophic microorganisms. Although there was no increase in the initial free ammonia level (853 ± 3 mg/L) with 19% v/v DiCOM biomass addition compared to 10% v/v DiCOM biomass, its higher initial total VFA concentration could have overloaded the wastewater and resulted in the microbial conversion capability to be exceeded. The manifestation of elevated acetate and propionate levels (data not shown) suggested that the acetoclastic methanogens and syntrophic propionate-degrading acetogenic bacteria in conjunction with the hydrogenotrophic microorganisms were being inhibited.

With the pH-reduced piggery wastewater (C2), additions of 10% v/v and 19% v/v piggery biomass appeared to lead to early substrate limitation as observed in the levelling off in their methane yields after 4 days of batch digestion (Figure 5a). Their significantly higher total VFA reductions, particularly acetate and propionate (data not shown) over the control suggested that their microbial activities were much higher. The significantly lower methane yield at the higher biomass (19% v/v) compared to the lower biomass (10% v/v) addition could be due to higher non-methanogenic microbial activity. Sulfate reducing bacteria can out-compete methanogens for hydrogen, acetate and propionate due to their higher affinity for these electron donors (van Haandel *et al*., 2006; Gallert and Winter, 2005).

While both the 10% v/v and 19% v/v DiCOM biomass additions to the pH-reduced wastewater produced significantly higher methane yields after 11 days of batch digestion compared to the corresponding piggery biomass (Figure 5b), the 2.5 to 3.5 fold higher initial total VFA concentrations (Table5) indicated that the increased methane production could have come from the degradation of the increased VFA substrates present in the DiCOM biomass. Due to their high organic content, adding piggery or DiCOM biomass resulted in the wastewater initial and residual TCOD concentrations to increase over the pH-unadjusted and pH-reduced controls. With the methane yields of pH-reduced wastewater controls being 12% and 20% higher than with the addition of 10% v/v DiCOM and piggery biomass respectively as well as 54% higher than the pH-unadjusted wastewater with 10% v/v biomass additions, pH reduction of the digested piggery wastewater to 6.5 alone was clearly a more effective method than adding DiCOM or piggery biomass to enhance methane production.

4.3 Effect of zeolite addition

Natural zeolite has been demonstrated by several researchers to improve organic matter degradation and methane production in the anaerobic digestion of piggery manure at mesophilic (Montalvo *et al*., 2006, Milan *et al*., 2001; 2003; Sánchez *et al*., 1995) and thermophilic temperatures (Kotsopoulos *et al*., 2008) as well as sludge at mesophilic temperature (Tada *et al*., 2005). Its capacity to immobilise microorganisms as well as remove ammonia and ammonium ions through adsorption and ion-exchange on its reactive surface with the zeolite inorganic minerals were cited as some of the reasons for the process improvement.

This study found zeolite concentrations at 10, 15 and 20 g/L stimulated total VFA reduction and methane production from pH-unadjusted and pH-reduced piggery wastewaters, with 20 g/L zeolite promoting the greatest enhancement effect. The enhancement effect of zeolite doses was greater on the pH-unadjusted piggery wastewater (pH 8.1) than on the pH-reduced wastewater (pH 6.5), due probably to a change of NH_4^+-NH_3 equilibrium with pH (Gerardi, 2003; Milan *et al*, 2001) and/or ion-exchange competition between H^+ and NH_4^+ cations in the pH-reduced wastewater. The improvement particularly in propionate degradation in the pH-reduced wastewater (Figure 9) was indicative of a reduction in dissolved hydrogen concentration or partial pressure which at level above 10^{-4} atmosphere inhibits propionate degradation (Fox and Pohland, 1994). Despite the high ammonia concentration in the pH-unadjusted wastewater, the great improvement in acetate degradation with increasing zeolite concentration (Figure 8) demonstrated the effectiveness of zeolite in alleviating ammonia inhibition on acetoclastic methanogens.

These stimulatory zeolite concentrations observed in this study were much higher than the reported optimum concentrations of 2-4 g/L (Milan *et al.*, 2001) and 8 g/L (Kotsopoulos *et al.*, 2008) that enhanced mesophilic and thermophilic anaerobic digestion of swine manure wastewater respectively. It was also reported that beyond these optimum concentrations, methane production dropped sharply. The big differences in the effective natural zeolite concentrations amongst these studies can be attributed largely to the differences in the ammonium-nitrogen concentrations of the piggery wastewaters. Both the piggery wastewaters used in Milan *et al.* (2001) and Kotsopoulos *et al.* (2008) studies contained much lower total ammonium-nitrogen concentration of 410 mg NH_4^+-N/L and 275 mg NH_4^+-N/L respectively in contrast to the high concentration of 1740 mg NH_4^+-N/L present in our piggery wastewater. With high ammonium-enriched sludge (4500 mg-N/L), Tada *et al*. (2005) reported 50 g/L and 100 g/L mordenite natural zeolite to enhance methane production while at 200 g/L, methane production decreased significantly.

In all these studies, the final ammonium-nitrogen concentrations of the zeolite-treated wastewaters remained high relative to their controls. Milan *et al.* (2001) observed the ammonium-nitrogen concentration to increase with increasing zeolite doses from 0.2 to 10 g/L. The increase corresponded with a decrease in the wastewater organic nitrogen concentration and an increase in zeolites' ammonium-nitrogen concentration. Kotsopoulos *et al.* (2008) reported increasing pH and free ammonia concentration with increasing zeolite doses from 4 to 12 g/L while ammonium-nitrogen concentration showed slight reduction. In our study, free ammonia concentrations in the pH-unadjusted wastewater treated with zeolite were higher than the pH-unadjusted control while ammonium-nitrogen concentrations remained unchanged in the zeolite-treated pH-reduced wastewater after 10 days of batch digestion. These observations suggested that at the applied zeolite concentrations, they were inadequate in reducing the high ammonium-nitrogen and free ammonia concentrations to levels below the ammonia inhibition threshold (Angelidaki and Ahring, 1994; Gallert and Winter, 1997) through cation exchange. Nevertheless, they were still effective in enhancing methane production and VFA reduction. It is hypothesized that a combination of microbial immobilisation and stimulation by unknown exchanged cations released from the zeolite were likely factors that had contributed to the beneficial effects observed at these zeolite concentrations. Fernández *et al.* (2007) have provided strong scanning electron microscopy evidence of natural zeolite serving as microbial immobiliser in the anaerobic fluidised reactors treating vinasses. Calcium, sodium, magnesium, potassium, iron and nickel ions which are present in the mineral make-up of our natural zeolite (Table 3) have also been demonstrated to enhance methane production in other studies (in Yadvika *et al*., 2003 and

Chen *et al.*, 2008). However, the mechanism responsible for the improvements was unknown.

Based on the results of this study and others (Milan *et al.*, 2001; Tada *et al.*, 2005), it is reasonable to assume that further enhancement of methane production and reduction of ammonium or ammonia concentrations would be possible at zeolite concentrations above the applied maximum 20 g/L in this study. However, intensive testing will need to be conducted to establish the optimum zeolite concentration that produces maximum ammonium and free ammonia removal while not inhibiting methane production. In addition, an in-depth study into the mechanism responsible for zeolite enhancement effect should also be carried out to gain a better insight into its enhanced performance in wastewater treatment.

4.4 Effect of humic acid addition

The results from this study showed that humic concentration at 10 g/L adversely affected the methanogenic activity and resulted in reduced methane production (Figure 10a). The observed increased viscosity could in part have contributed to the retardation of nutrients transport to the microorganisms besides substrates competition by humics-reducing bacteria over methanogenic *archaea* (Cervantes *et al.*, 2008). Cervantes *et al.* (2000) have reported similar observation of methanogenesis inhibition with increased concentrations of anthraquinone-2, 6-disulfonate (AQDS), a model quinone analogue.

The small stimulatory effect on methane production rate at the lower doses of 1 and 5 g/L humic acid (Figure 10b) in conjunction with enhanced VFAs degradation particularly at 1 g/L (Table7) in the pH-reduced piggery wastewater clearly demonstrated that humic acid at the applied low concentrations had served as electron acceptors in the anaerobic degradation of volatile organic acids. Scott *et al*. (1998) have provided direct evidence using electron spin resonance (ESR) measurements that the soluble quinones within humic substances are the redox active reducible organic radicals that function as the electron-accepting moieties. A diversity of humic-reducing microorganisms has been shown by several researchers to transfer electrons derived from the oxidation of organic compounds and/or hydrogen to humic substances (Straub and Schink, 2003; Cervantes *et al.*, 2002; Lovley *et al*., 2000, 1996; Scott *et al.*, 1998; Benz *et al*., 1998).

The enhanced VFA degradation, particularly propionate which is the most difficult VFA to degrade, indicated an improvement in the redox potential or thermodynamic condition of the piggery wastewater treated with low humic acid concentrations. It implied that the high hydrogen concentration or partial pressure which inhibited the propionate from being degraded in the control wastewater was being scavenged by hydrogen-utilising microorganisms such as methanogens or sulphate-reducing bacteria (SRB) to a low level that allowed the propionate to be degraded. Humic-reducing bacteria also utilise hydrogen as electron donor. However, comparison of the thermodynamics of the microbial utilisation of hydrogen as an electron donor in Table 8 suggests that H_2-utilising SRB, methanogens and homoacetogens would be thermodynamically more competitive than humic-reducing bacteria in scavenging the hydrogen substrate by using sulphate or carbon dioxide as electron acceptors.

Table 8

As humic acid at 5 g/L greatly elevated the organic content of the piggery wastewater in terms of COD concentrations compared to the lower concentration of 1 g/L and the control, humic acid at 1 g/L was considered optimum in this study for methane production enhancement and reduction of the piggery wastewater organic matter. It is worth noting that while concentrations below 1 g/L humic acid were not tested in this study, it is conceivable that they might exert higher stimulatory effect on organics degradation and methane production. Quinone analogue, AQDS has been demonstrated to enhance ferric oxide reduction by acting as electron shuttle at concentration as low as 0.5 µM (0.2 mg/L) at hyperthermophilic conditions (Lovley *et al*., 1999) while concentrations above 5 mM (2 g/L) inhibited methanogenesis (Cervantes *et al.*, 2000). In view of the suppression of methanogenesis by humic-respiration (Cervantes *et al*., 2000, 2008), the effect of humic acid at concentrations below 1 g/L on anaerobic digestion of piggery wastewater warrants further study in order to establish the optimum concentration that would enhance organics degradation without inhibiting methanogenesis.

5. Conclusions

Based on the outcomes of methane production enhancement and the final quality of the digested wastewater, the following conclusions on the four studies are as follows: Reducing the pH of digested piggery wastewater from an initial pH 8.3 to 6.5 was the most effective in enhancing microbial degradation of the elevated total VFAs, particularly acetate and propionate. This facilitated enhanced methane production with the added benefit of decreasing the number of pathogenic *Clostridium perfringen* and increasing the methanogenic population.

For digested piggery wastewater without pH reduction, biomass at 10% v/v (piggery or DiCOM) was more effective than at 19% v/v in enhancing methane yield. For pH-reduced digested wastewater, Dicom biomass was more effective than piggery biomass.. . However, their high organic contents greatly elevated the wastewater residual TCOD concentration. Thus, pH reduction of the digested wastewater to pH 6.5 alone was a better option for enhancing methane production.

Zeolite treatments at 10 to 20 g/L zeolites were effective in stimulating methane production from the digested piggery wastewater with and without pH reduction to pH 6.5. Without pH reduction, up to 20 g/L zeolite was required to achieve comparable methane production enhancement as the pH-reduced wastewater. Further study into the mechanism responsible for the improvement is recommended.

Humic acid at 1 g/L concentration was more effective than 5 g/L in enhancing methane production from pH-reduced digested wastewater and with minimal elevation of the residual TCOD concentration. Further study into the electron-shuttling effect of humic acid at concentrations below 1 g/L is recommended

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Table 1. Some key physico-chemical characteristics of raw and digested piggery wastewater

Piggery wastewater	Raw	Digested
Analysis		
pH	7.3-7.7	8.2-8.5
Total alkalinity (mg $CaCO_3$/L)	4550-5595	5375-5650
NH_4^+-N (mg/L)	1800-2000	2104-2111
Free ammonia (mg/L)	4-10	916-920
Total COD (mg/L)	10593-13220	7134-7924
Soluble COD (mg/L)	6129-7010	3138-4889
Total VFA (mg COD/L)	5600-7687	1301-3452
Total solids (g/L)	8-8.2	7-7.5
Volatile solids (g/L)	5-5.2	3.6-4.2

Table 2. Solids content of anaerobic piggery and aerobic-anaerobic municipal solid waste Dicom biomass

Biomass	Total solid (g/L)	Volatile solid (g/L)
Anaerobic piggery	25.1	16.1
Aerobic-anaerobic municipal solid waste (Dicom)	50.6	33.1

Table 3. Chemical composition of Castle Mountain natural zeolite (< 1 mm particle size)

Mineral content	%	Trace elements	ppm	Type of minerals	%w zeolite
SiO_2 (silicon dioxide)	71.81	Ba (barium)	10	Clinoptilolite	85
Al_2O_3 (aluminium oxide)	12.10	Co (cobalt)	1.2	Mordenite	15
Fe_2O_3 (iron oxide)	1.14	Cr (chromium)	35	Quartz, Felspar, Montmorillonite	Minor
Na_2O (sodium oxide)	2.33	Se (Selenium)	<1		
K_2O (potassium oxide)	0.90	Cu (copper)	19		
CaO (calcium oxide)	2.60	Zn (zinc)	33		
MgO (magnesium oxide)	0.65	P (phosphorus)	187		
TiO_2 (titanium dioxide)	0.22				
MnO (manganese oxide)	0.03				
P_2O_5 (phosphorus pentoxide)	<0.01				
SrO (strontium oxide)	0.22				
Cation Exchange Capacity (CEC) meq/100 g zeolite powder	147				

Source: www.castlemountainzeolites.com.au

Table 4. Chemical composition and methane production at various initial pH at the start and end of the experiment (mean ± standard deviation)

Sample	Day	pH	NH_4^+-N (mg/L)	Free NH_3 (mg/L)	TVFA (mg COD/L)	Methane production ml CH_4/L
pH 8.3	0	8.3	2104 (73)	916 (32)	1301 (157)	
	10	8.1 (0.1)	2088 (85)	853 (101)	2059 (26)	200 (0)
pH 7.5	0	7.5	2291 (56)	249 (6)	1373 (8)	
	10	8.1 (0)	2097 (60)	686 (20)	1551 (29)	370 (14)
pH 7	0	7	2052 (0)	76 (0)	1281 (28)	
	10	8.1 (0.1)	2125 (56)	643 (50)	1102 (77)	500 (28)
pH 6.5	0	6.5	1976 (3)	24 (0)	1175 (22)	
	10	7.8 (0)	2171 (112)	425 (22)	497 (19)	680 (0)

Table 5. Chemical composition of the digested piggery wastewater before (C1) and after (C2) pH reduction with and without piggery (pb) and DiCOM (db) biomass additions (% v/v) at the start and end of experiment (mean ± standard deviation)

Sample	Day	pH	NH_4^+-N (mg/L)	Free ammonia (mg/L)	TCOD (mg/L)	TVFA-COD (mg/L)
C1 (pH 8.1)	0	8.1	2260 (22)	739(7)	7480 (421)	2132 (167)
C1 (pH 8.1)	11	8.3 (0)	2218 (70)	966 (31)	7568 (361)	2683 (31)
C1 + 10% pb	0	8.3	2064 (32)	896 (14)	10631 (729)	1977 (167)
C1 + 10% pb	11	8.4 (0)	2369 (95)	1167 (47)	9369 (669)	1829 (9)
C1 + 19% pb	0	8.4	2134 (19)	1051 (10)	11123 (557)	2046 (49)
C1 + 19% pb	11	8.4 (0)	2241 (10)	1103 (5)	11262 (569)	2948 (355)
C1 + 10% db	0	8.2	2248 (10)	853 (4)	14280 (365)	5365 (171)
C1 + 10% db	11	8.2 (0)	2475 (46)	940 (17)	13153 (857)	2602 (45)
C1 + 19% db	0	8.2	1982 (7)	853 (3)	19914 (748)	8000 (397)
C1 + 19% db	11	8.2 (0.1)	2590 (48)	984 (18)	15676 (1396)	5153 (100)
C2 (pH 6.5)	0	6.5	2174 (7)	26 (0)	7359 (631)	2118 (16)
C2 (pH 6.5)	11	7.9 (0.1)	2272 (111)	533 (26)	6667 (1258)	616 (9)
C2 + 10% pb	0	6.5	2169 (15)	26 (0)	9909 (811)	1883 (89)
C2 + 10% pb	11	7.8 (0)	2313 (92)	453 (18)	8919 (540)	135 (19)
C2 + 19% pb	0	6.6	1978 (27)	30 (0)	11487 (303)	1908 (3)
C2 + 19% pb	11	7.9 (0.1)	2361 (63)	554 (15)	9676 (388)	135 (19)
C2 + 10% db	0	6.5	2294 (36)	28 (0)	14887 (256)	4880 (215)
C2 + 10% db	11	7.9 (0)	2436 (44)	572 (10)	9550 (953)	266 (29)
C2 + 19% db	0	6.5	2285 (19)	28 (0)	20303 (607)	6736 (504)
C2 + 19% db	11	7.8 (0)	2513 (41)	492 (8)	11892 (900)	1084 (101)

Table 6. Chemical composition of the pH-unadjusted (C1) and pH-reduced (C2) digested piggery wastewater before and after zeolite addition (mean ± standard deviation)

Sample	Day	pH	TCOD (mg/L)	TVFA (mg COD/L)	NH_4^+-N (mg N/L)	Free NH_3 (mg N/L)
C1 (pH 8.1)	0	8.1 (0)	8310 (281)	3240 (125)	1740 (11)	569 (4)
C1 (pH 8.1)	10	8.2 (0)	7157 (225)	2389 (165)	2015 (15)	765 (6)
C1 + 10 g/L	10	8.3 (0)	7077 (195)	1848 (19)	1952 (48)	850 (21)
C1 + 15 g/L	10	8.3 (0)	7793 (112)	1546 (146)	2165 (4)	942 (18)
C1 + 20 g/L	10	8.3 (0.1)	6600 (210)	1326 (6)	1908 (11)	778 (79)
C2 (pH 6.5)	0	6.6 (0)	8509 (337)	2602 (162)	1721 (6)	26 0)
C2 (pH 6.5)	10	7.9 (0)	6322 (57)	1462 (78)	1830 (13)	430 (3)
C2 + 10 g/L	10	7.8 (0)	6521 (325)	1193 (60)	2017 (290)	395 (57)
C2 + 15 g/L	10	7.8 (0.1)	6096 (368)	1101 (43)	1988 (102)	355 (29)
C2 + 20 g/L	10	7.8 (0.1)	6481 (334)	999 (48)	1934 (22)	346 (42)

Table 7. Total COD and total VFA concentrations of the thermophilic piggery wastewater before and after humic acid treatment at the start and end of the experiment (mean data ± standard deviation)

Sample	Day	TCOD (mg/L)	TVFA (mg COD/L)
C2 (pH 6.5)	0	8168 (169)	2667 (209)
C2 (pH 8.1)	9	6096 (56)	1515 (10)
C2 + 1 g/L	0	9203 (395)	2823 (13)
C2 + 1 g/L	9	7371 (169)	1069 (93)
C2 + 5 g/L	0	13864 (0)	3528 (106)
C2 + 5 g/L	9	12350 (338)	1517 (51)

Table 8. Thermodynamic comparison of various anaerobic microbial groups using hydrogen as electron donors

Reaction	Δ G^o (kJ/mol)
$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$ (by homoacetogens)	-95.0
$4H_2 + 2CO_2 \rightarrow CH_4 + 2H_2O$ (by hydrogenotrophic methanogens)	-131.0
$4H_2 + SO_4^{2-} \rightarrow S^{2-} + 4H_2O$ (by sulphate-reducing bacteria)	-151.0
H_2 + AQDS $\rightarrow$ AH_2QDS (by humics-reducing bacteria)	-44.4

(Sources: Cervantes *et al.*, 2000; Brock *et al*., 1993, Thauer *et al.*, 1977)

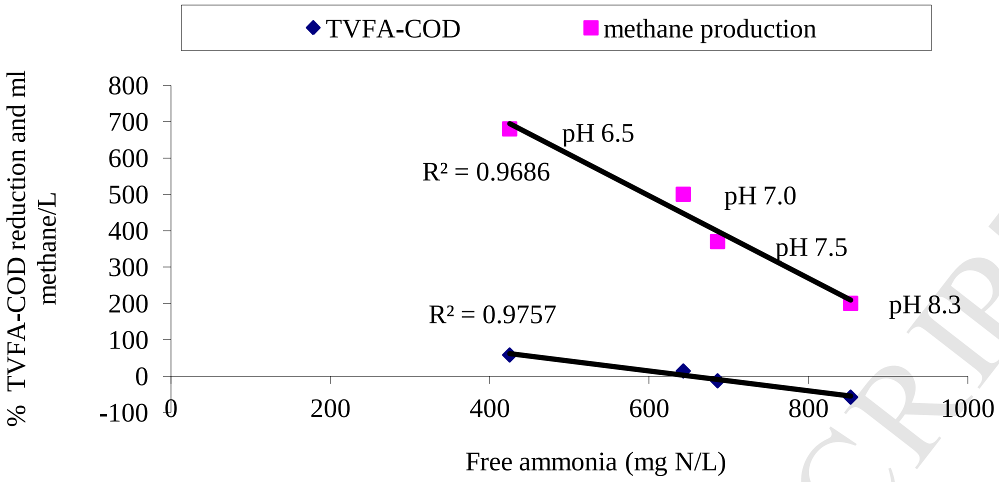

Figure 1. Relationships between final free ammonia, methane production and total VFA-COD reduction in piggery wastewater at different initial pH

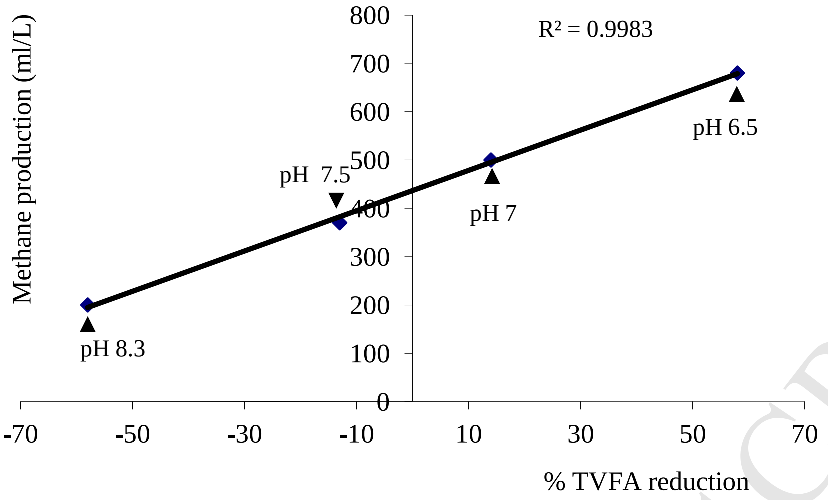

Figure 2. Relationship between total VFA-COD degraded and methane production from piggery wastewater at different initial pH

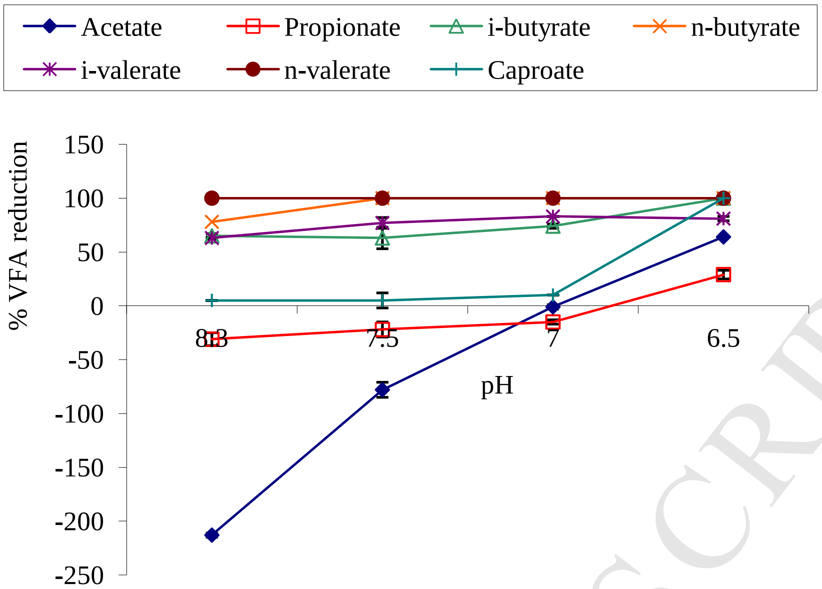

Figure 3. VFA degradation as a function of piggery wastewater initial pH (error bars indicate standard deviations) after 10 days of batch digestion

Figure

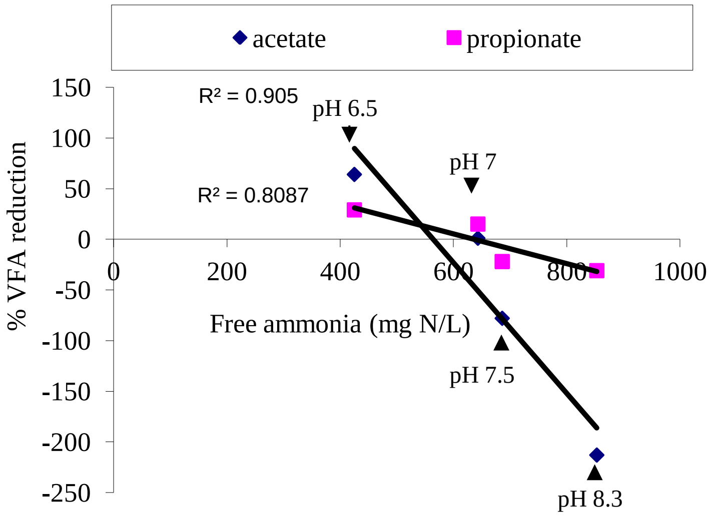

Figure 4. Relationship between final free ammonia and acetate or propionate reduction in piggery wastewater at different initial pH

Figure

Figure 5a. With piggery biomass

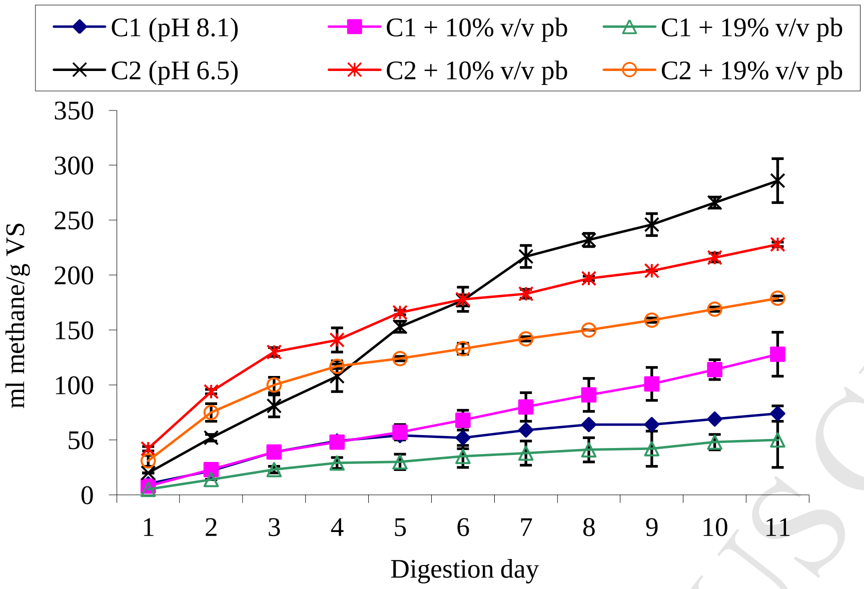

Figure 5a. Methane yields of pH-unadjusted (C1) thermophilic piggery wastewaters supplemented with piggery biomass (pb) biomass (db) (error bars indicate standard deviations)

Figure 5b. With DiCOM biomass

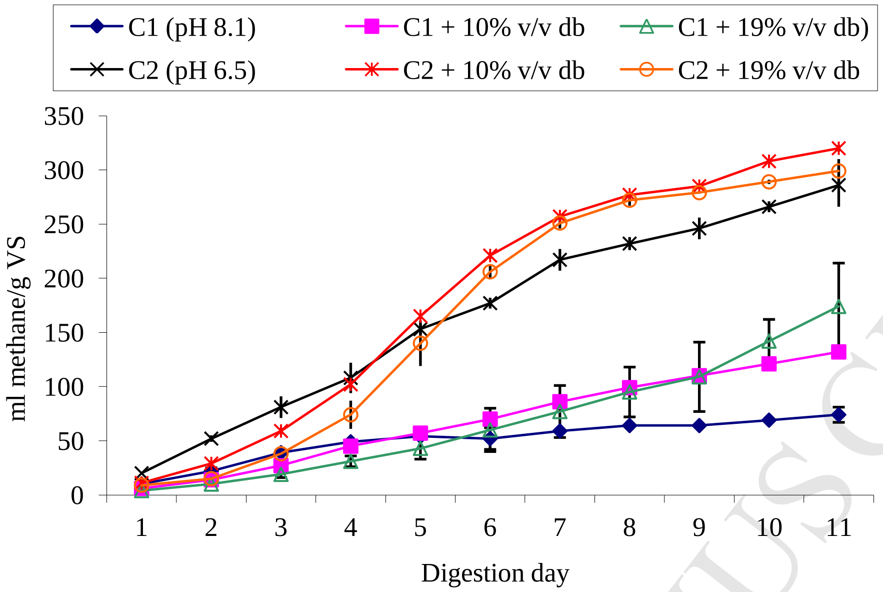

Figure 5b. Methane yields of pH-reduced (C2) thermophilic piggery wastewaters supplemented with DiCOM biomass (db) (error bars indicate standard deviations)

Figure

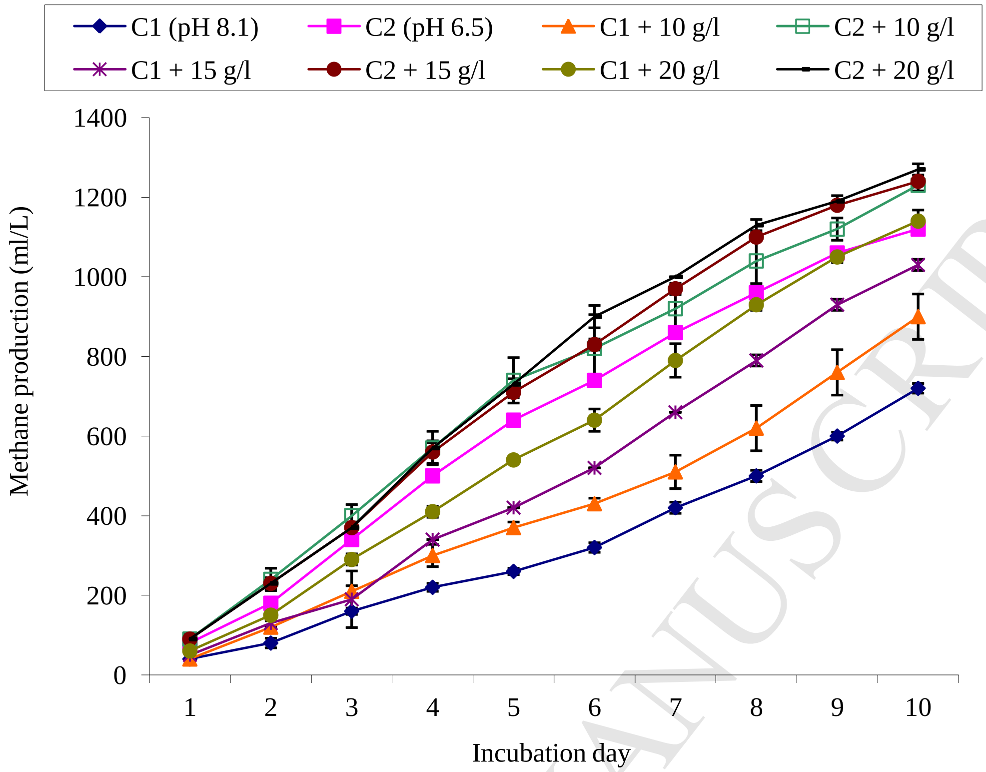

Figure 6. Effect of zeolite concentrations on cumulative methane production from thermophilic piggery reactor effluent (error bars indicate standard deviations)

(7a)

Figure 7a. Thermophilic digested piggery wastewater without pH reduction

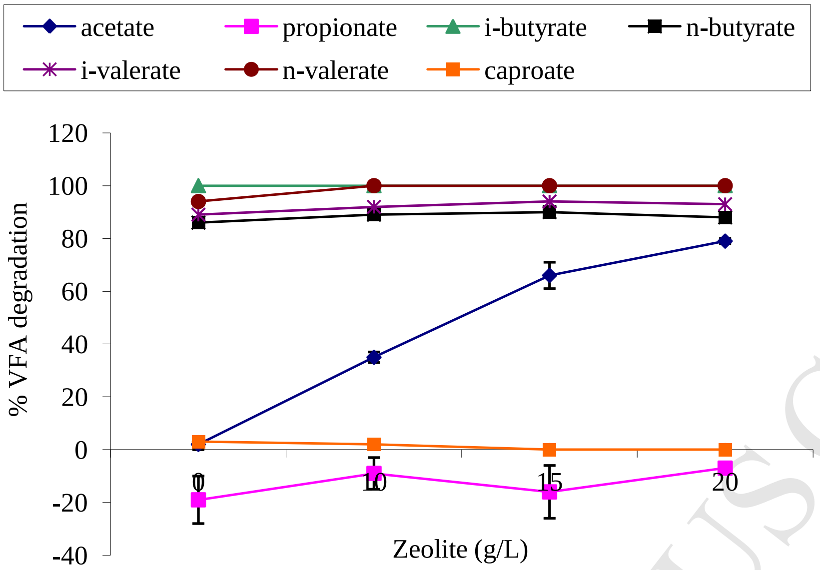

Figure 7a. Effect of zeolite concentrations on VFA degradation in thermophilic digested piggery wastewater without pH reduction (C1) (error bars indicate standard deviations) after 10 days of batch digestion

(7b)

Figure 7b. Thermophilic digested piggery wastewater with pH reduction

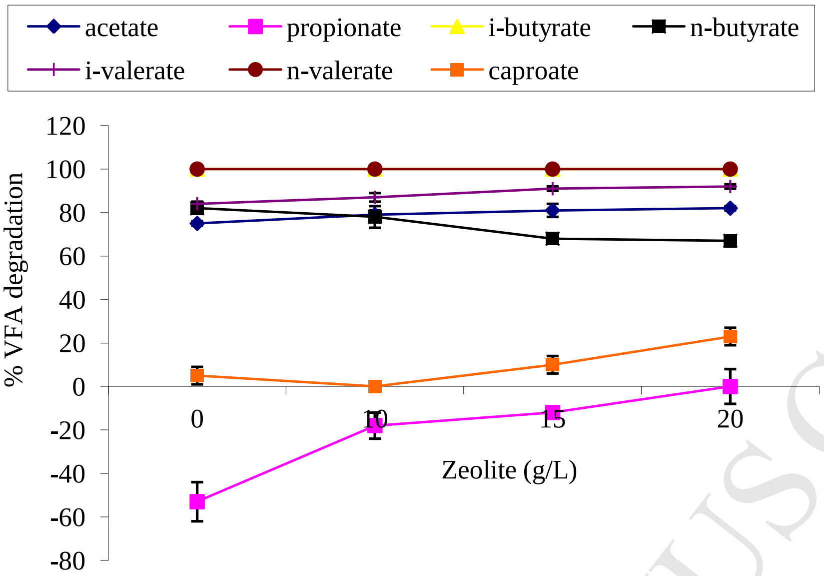

Figure 7b. Effect of zeolite concentrations on VFA degradation in thermophilic digested piggery wastewater with pH reduction (C2) (error bars indicate standard deviations) after 10 days of batch digestion

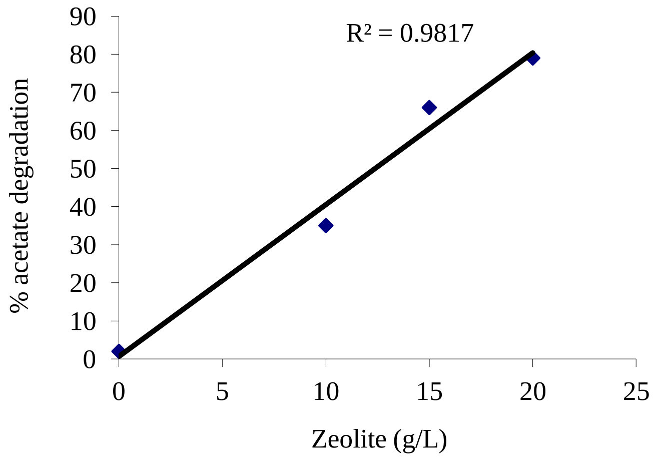

Figure 8. Relationship between percentage acetate degradation and zeolite concentrations in thermophilic digested piggery wastewater without pH reduction (C1)

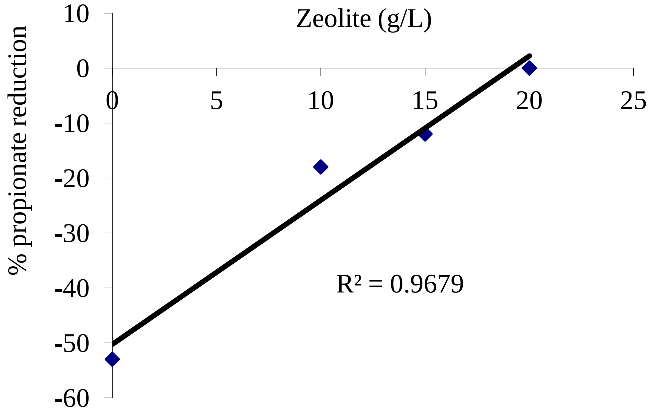

Figure 9. Relationship between percentage propionate degradation and zeolite concentrations in thermophilic digested piggery wastewater with pH reduction (C2)

Figure 10a. High humic acid concentraion

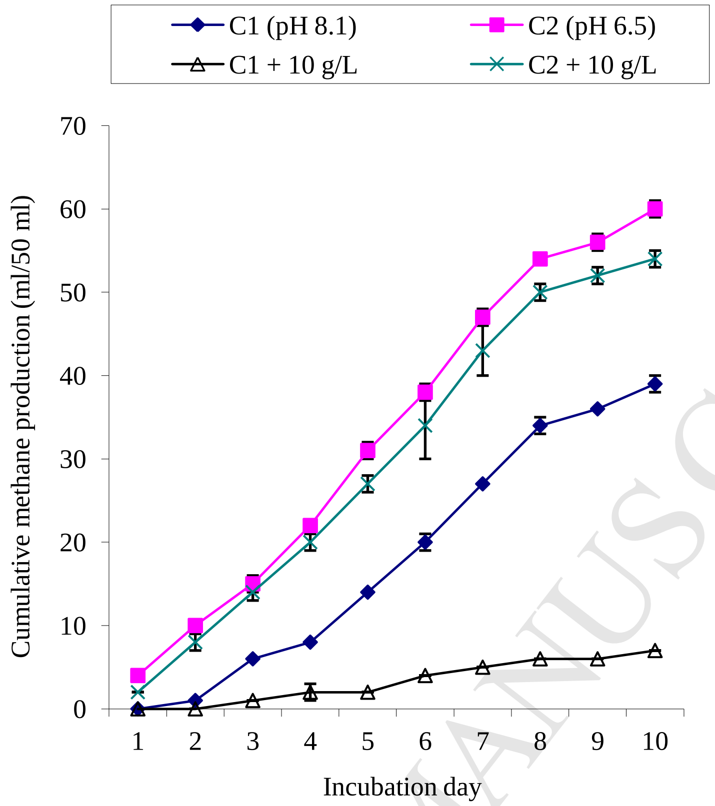

Figure 10a. Effect of high (10g/L) humic acid concentration on methane production from thermophilic piggery wastewater without pH reduction (C1) (error bars indicate standard deviations of duplicate vials)

Figure 10b. Low humic acid concentrations

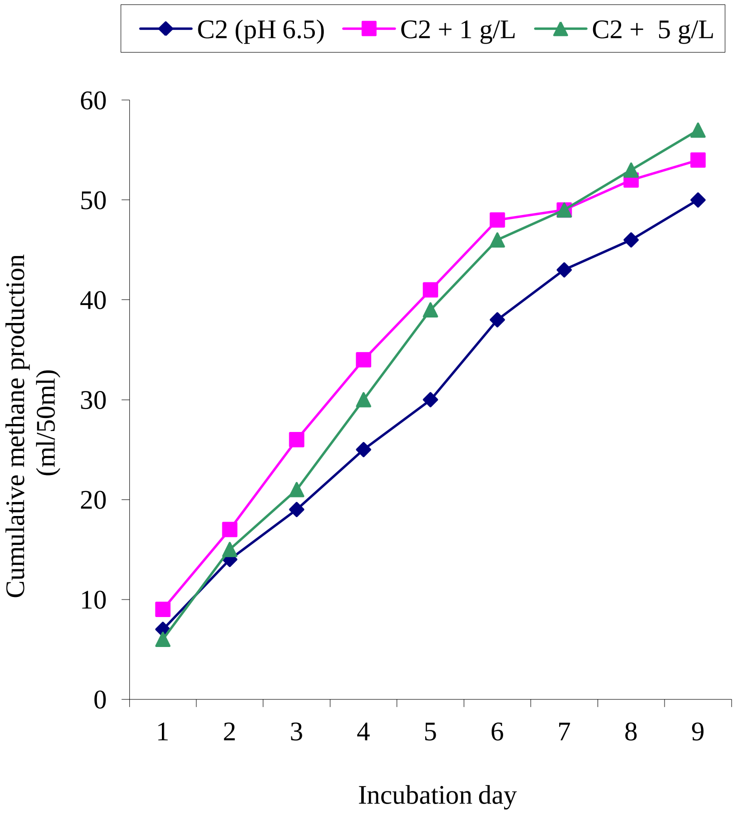

Figure 10b. Effect of low (1 and 5 g/L) humic acid concentrations on methane production from thermophilic piggery wastewater with pH reduction (C2) (error bars indicate standard deviations of duplicate vials)

Research Highlights

- pH reduction, zeolite, biomass and humic acid were evaluated for ammonia mitigation
- pH reduction to 6.5 and zeolite addition greatly stimulated methane production
- 20 g/L zeolite produced the highest methane enhancement effect
- Biomass (10%) and humic acid (1 & 5 g/L) additions elevated the effluent TCOD level