Optimizing volatile fatty acid production in partial acidogenesis of swine wastewater

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Abstract This research has been conducted based on the fact that acetic and butyric acids are favorable substrates for methanogens, and that a low level of propionic acid production during acidogenesis minimizes the inhibition effect on methanogenic growth. Raw swine wastewater was pretreated with ammonia stripping to enhance acidogenesis. The ammonia nitrogen concentration of less than 1.2 g/L did not significantly affect the biochemical acidogenic potential of swine wastewater. For acidogenesis of swine wastewater, a set of experiments were carried out to produce short chain volatile fatty acids (VFA) in laboratory-scale continuously stirred tank reactors. The production of acetic, propionic, and butyric acids associated with simultaneous changes in hydraulic retention time (HRT) and temperature was investigated. Response surface methodology was successfully applied to approximate the responses of the VFA productions. The optimum physiological conditions where the maximum acetic and butyric acids production occurred were 2.4 days HRT at 34°C and 2.1 days HRT at 35°C, respectively. The propionic acid production linearly increased as both HRT and temperature increased.

Keywords Acidogenesis; ammonia inhibition; anaerobic digestion; response surface analysis; swine wastewater

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Introduction

Anaerobic digestion provides a potential cost-effective solution for the treatment of high strength organic waste, such as swine wastewater, because of its methane formation and low sludge production. The anaerobic process is a multi-stage process in which complex organic components of the waste are broken down and fermented into intermediate products that are subsequently converted into methane, carbon dioxide, and microbial cell masses. The stages where these intermediate products are converted are referred to as hydrolysis or liquefaction, acidogenesis, and methanogenesis. These stages involve many different species of symbiotic microorganisms, which are broadly divided into two groups: acidogenic and methanogenic bacteria. These two groups of microorganisms differ widely in their physiology, biokinetics, and growth environment. Several researchers have claimed that optimization of each of these phases would enhance the overall rate of waste stabilization if the biphasic ecosystem could be maintained in separate digesters in a series; one for acid production and one for methane production (Nuri *et al*., 2001; Speece, 1996). It has been controversial, however, whether complete or partial acidification would improve treatment efficiency in an anaerobic process, as well as the way in which partial acidification should be achieved. Some reports show that two-phase processes with partial acidification of influent wastewater have advantages in organic loading rate and gas production over single-phase processes (Yang *et al*., 2003).

It has recently been suggested that overall process enhancement must be based on an understanding of the behavior of acidogens, especially optimization and mathematical modeling of acidogenesis (Hwang *et al*., 2001; Yang *et al*., 2003). Acidogens play the primary role in producing major substrates for methanogens. However, high concentrations

of short-chain organic acids can cause anaerobic digestion systems to fail. Methanogens utilize a limited number of substrates to form CH_4. Only a few of these limited substrates are thermodynamically favorable for conversion to methane gas. These substrates include hydrogen, carbon dioxide, methanol, ethanol, and acetic acid, which is a precursor for more than 70% of CH_4 formation in most anaerobic processes (Rittmann and McCarty, 2001). Butyric acid is another major intermediate that is rapidly converted to 2 moles of acetic acid per mole of butyric acid during anaerobic digestion.

Due to the high ammonia content in swine wastewater, ammonia inhibition has long been a serious problem in effective anaerobic digestion of the wastewater, thus limiting its economic feasibility (Bonmati and Flotats, 2003). For methanogenic cultures, ammonia inhibition has been observed to commence at a concentration of 1.5~2.5 g ammonia N/L in wastewater (Angelidaki and Ahiring, 1993). However, information on the effect of ammonia on acidogenesis of swine wastewater is lacking in the literature.

We hypothesized that the overall performance of a two-phase anaerobic system would be enhanced if the biodegradable components in swine wastewater were mainly converted to acetate and butyrate, which are known to be the best precursors of methane formation. Therefore, the objectives of this study were to (1) investigate the effect of different ammonia concentrations on acidogenic behavior, and (2) develop continuous response surfaces of acetic, propionic, and butyric acids production with swine wastewater using mathematical and statistical techniques. The production of propionic acid, which is another frequently found intermediate in anaerobic digestion, was also considered because of its toxicity to the anaerobic digestion system.

Materials and methods

Swine wastewater and acidogenic inoculum system

One batch (300 L) of swine wastewater screened with a 600 μm sieve was obtained from a local hog farm. The wastewater was homogeneously mixed and divided into smaller portions and frozen at –25°C for further use.

A lab-scale continuously stirred tank reactor (CSTR) with a working volume of 1.5 L was operated for acidogenic inoculum culture. Anaerobic seed sludge from a local municipal wastewater treatment plant was cultivated in the system in order to enrich a mixed population of acidogens by combining biokinetic and chemical controls (Hwang *et al.*, 2001). The system was operated using dilute whey wastewater with a chemical oxygen demand (COD) of 20.0 ± 0.5 g/L at 0.5 ± 0.02 days hydraulic retention time (HRT). A yeast extract was added at 1 g/L to provide trace minerals. Temperature and pH were maintained at 35°C and 6.0, respectively, using an automated heating controller and 6.0 N NaOH. The operating conditions remained constant throughout the experiment. The steady state effluent (2.7 L) was centrifuged at 6,000 g for 5 min to separate the liquid portion from the effluent. The collected biomass was used as a seed culture to give an initial biomass concentration of 2.4 g VSS/L for subsequent experiments to minimize any confounding effect that may be associated with the use of an inconsistent inoculum size.

Ammonia stripping and biochemical acidogenic potential test

Ammonia stripping operations consist of converting ammonia and ammonium nitrogen in wastewater to the gaseous phase and then dispersing the liquid in air, thus allowing transfer of the ammonia from the wastewater to the air. Because a sodium concentration of more than 5 g/L inhibits anaerobic microbial activity (Feijoo *et al.*, 1995), the pH of raw wastewater was adjusted to 10.25 using 6N NaOH. The final sodium concentration in the wastewater was 4.3 g/L. Swine wastewater was preheated to room temperature in a 12 L vessel followed by ammonia stripping. The vessel was aerated at a rate of 15 L/L/min using

a diffuser for 1, 2, 6, 24, 48, and 96 hours to give different final concentrations of ammonia nitrogen (NH_3/NH_4^+-N).

For the biochemical acidogenic potential (BAP) test, the wastewater sample was fermented under anaerobic conditions until the VFA concentration reached a stable maximum level. Three identical batch bioreactors with a working volume of 3 L, equipped with temperature and pH controllers, were used for the BAP test. Temperature and pH were maintained at 35°C and 7, respectively. The ammonia stripped swine wastewaters were used to evaluate the BAP at different ammonium nitrogen concentrations. Samples were periodically taken and analyzed until the VFA production reached equilibrium. Bromoethane sulfonate (BES) of 1 mM was added to inhibit methanogenesis (Ruel *et al*., 2002).

Selection of variables and optimization protocol

When selecting process variables in this experiment, inherent microbial activity and feasibility of the process were simultaneously considered. While HRT is a key variable used to control microbial activity of mixed populations in continuous anaerobic systems (Banerjee *et al*., 1998), selection of an operational range should be based on the types of reactors and characteristics of substrate used to treat. Based on the growth rate of anaerobes on pig originated wastes in a CSTR (Speece, 1996), the range of HRT was decided to be 1 to 3 days to give sufficient residence time for acidogenic activity. Activity of most mesophiles, including acidogens, exhibits a maximum temperature at higher than 30°C, and a sharp decrease at less than 25°C (Cha and Noike, 1997). Thus, the effect of temperature was investigated at a range of 25 to 35°C. Although a high concentration of ammonia nitrogen in either organic or inorganic form could be detrimental to microbial growth, alkalinity of the wastewater due to the ammonia is usually sufficient for anaerobic microbial activity to compensate for the acidity produced by acidogens. Furthermore, pH is not usually controlled for economic reasons in a full scale operation of anaerobic treatment of swine wastewater. Therefore, the effect of pH on acidogenesis of swine wastewater was excluded in the optimization study.

The central composite in cube (CCC) design, which consisted of an orthogonal 2^2 factorial design augmented by a center and axial points, was employed in this research. The orthogonal design is a unique class of experimental design techniques that minimize the variance of the regression coefficients (Montgomery, 2001; Lee *et al*., 2003). Response surface analysis (RSA), a collection of mathematical and statistical techniques for building empirical models (Box and Draper, 1987), was applied to approximate the continuous response of acetic, propionic, and butyric acids production within the design boundary. A sequential procedure of collecting data, estimating polynomials, and checking the adequacy of the model was used. The method of least squares was used to estimate the parameters in the polynomials.

Analytical methods

All analyses were duplicated and the results were quoted as mean values. A Hewlett-Packard gas chromatograph (Model 6890 plus) equipped with an Innowax capillary column and a flame ionization detector was used to determine the concentrations of volatile fatty acids (VFAs). Helium was the carrier gas at a flow rate of 2.5 mL/min with a split ratio of 10:1. The same gas chromatograph with an HP-5 capillary column (film thickness, 0.25 μm: length, 30 m; ID, 0.53 mm; phase ratio, 3) and a thermal conductivity detector was used to quantify methane in the biogas. Helium was the carrier gas at a flow rate of 8 ml/min with a split ratio of 70:0.

The nitrogen content was measured by the Kjeldahl method. The COD and solids concentrations were determined according to the procedures in Standard Methods (APHA,

1998). Sodium concentration in the sample was measured by using Dionex ion chromatographs (DX-120) with IonPac CS12A columns.

Results and discussion

Biochemical acidogenic potential of swine wastewater

The final concentrations of ammonia nitrogen (NH_3/NH_4^+-N) after aerating the swine wastewater for 0 (control), 1, 2, 6, 24, 48, and 96 hrs were 4.0, 3.0, 2.4, 1.5, 1.2, 0.9, and 0.8 g/L, respectively. Approximately 80% of initial ammonia nitrogen was removed after 96 hrs of air stripping. Variations in the concentrations of COD and solids before and after the aeration were less than 10% for all treatments (data not shown), which indicated that organic materials remained unchanged after the ammonia stripping.

Figure 1 represents the change in VFA concentrations as COD equivalent in the BAP test with different initial ammonia nitrogen concentrations. The absence or repression of methanogenic activity was verified by the lack of CH_4 production. Rapid acidogenesis of the swine wastewater in all trials occurred in approximately 48 hours of incubation and no significant increase in VFA production after this period was observed. This meant that the systems were likely to be in a stage of equilibrium with respect to the acidogenic activity. In comparison to the control, a maximum of 4.7 times more acidification was achieved during the treatment with the initial ammonia nitrogen concentration of 0.8 g/L. It should be noted, however, that the VFA production of all treatments was not proportional to the initial ammonia nitrogen concentrations. It can be clearly seen that VFA production increased as initial ammonia nitrogen concentrations decreased to 1.5 g/L. For the initial ammonia nitrogen concentration of less than 1.2 g/L, however, the VFA production did not increase significantly, but was 4.5 ± 0.2 times higher than control. The results agreed with previous reports that 1.5 g/L of ammonia nitrogen was an inhibitory level for anaerobes (Angelidaki and Ahring, 1993). Therefore, it can be concluded that 1.2 g/L of ammonia nitrogen was not an inhibitory level for acidogens. Subsequent RSA for acidogenesis of swine wastewater was conducted at this level of ammonia nitrogen.

Response surface analysis of acidogenesis of swine wastewater

A total of 10 trials were run to approximate the surface of the VFA production. Influent

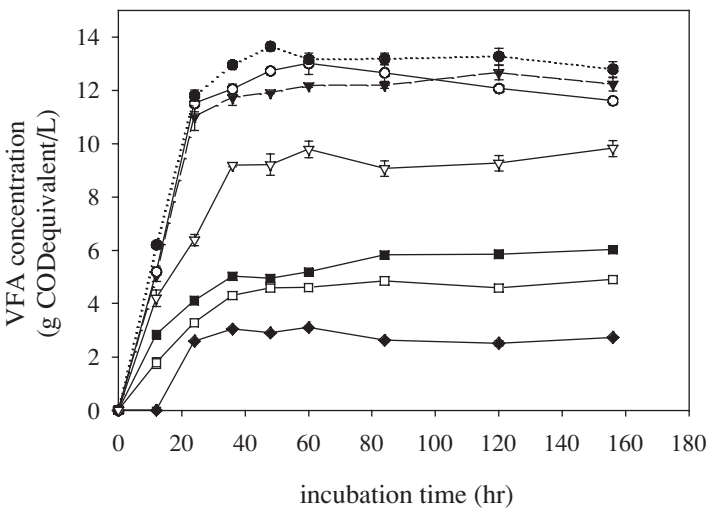

Figure 1 Production of short chain organic acids as COD equivalent at different concentrations of initial ammonia nitrogen (● 0.8 g/L, ○ 0.9 g/L, ▼ 1.2 g/L, □ 1.5 g/L, ■ 2.5 g/L, □ 3.0 g/L, ◆ 4.0 g/L)

substrate concentration was maintained at 80,000 mg COD/L for all trials and steady-state was assumed after five turnovers.

Experimental conditions and data are shown in Table 1. The orthogonal design, a 2^2 factorial augmented by three center point runs, was used to collect data. The operating conditions at the center point were 2.0 days HRT and 30°C. Repeated observations at the center were used to estimate the experimental error. Seven trials were run initially. A first-order model, $\eta_i = \beta_o + \beta_1 x_1 + \beta_2 x_2$, was used to fit this data by least squares. The following models were obtained on each acid:

$$\eta_{\text{acetate}} = -3819 + 1147\, x_1 + 144\, x_2 \quad (1)$$

$$\eta_{\text{propionate}} = -681 + 399 x_1 + 17\, x_2 \quad (2)$$

$$\eta_{\text{butyrate}} = -733 + 239\, x_1 + 44\, x_2 \quad (3)$$

where η_i : the concentration of acid i (g i /L, where i = acetic, propionic, and butyric acids in order)

β_j : the coefficient values of the jth term (mg i /L/x_j, where β_o = constant; j = HRT and temperature in order)

x_j : the corresponding variable term (j = HRT and temperature in order)

The regression coefficient, the lack of fit of the first-order model, and p-values of parameter estimations were used to validate the models. The p-values of the lack of fit for acetic and butyric acids were, respectively, 0.003 and 0.002, which were significant at a 1% α level. The regression coefficient was significant only for propionic acid at a 0.1% α level. Therefore, the first-order model was an adequate approximation only for propionic acid production, which indicated the presence of curvature in the response of the acetic and butyric acids. The propionic acid production linearly increased as both HRT and temperature increased (Figure 2). This is in accordance with the previous results that propionate concentration increases as HRT increases (Speece, 1996).

At this point, additional trials were done to approximate the response of acetic and butyric acids more precisely. A second-order model could not be fitted using the data from trials 1 through 5 in Table 1 due to the lack of axial data points. Because there was an indication of curvature in the response of acetic and butyric acids, indicating that the operating conditions might be close to the condition for maximal production of the acids, additional trials for the acids were based on a modified orthogonal method. This procedure is based on moving a geometric shape through the parameter response space until the shape surrounds the best estimate (Haefner, 1996). Each trial was augmented with previous trials, and boundaries were continuously modified until a higher order model adequately approximated the response surfaces of acetic and butyric acids. Statistical analysis including p-values of regression, lack of fit, corresponding coefficients of the models, and residual plot analysis was done after each trial. The next trial conditions were determined based on the analysis. Finally, three additional trials, A-1 through A-3 (Table 1), were augmented with previous trials. Lack of fit was not significant but regression was significant at a 0.1% α level only for the quadratic model for both acids. This indicated that the model fitted the response surface. Therefore, the quadratic models were selected to describe the response surface of acetic and butyric acids within this region. Eqs (4) and (5) represent the models for acetic and butyric acid production, respectively.

Table 1 Conditions and results of the central composite design and extended trials

Trials	Conditions of variables		Concentration (g/L)		
	HRT (days)	Temperature (°C)	Acetic acid	Propionic acid	Butyric acid
1	1.0	25	0.6	0.1	0.5
2	1.0	35	1.4	0.3	1.1
3	3.0	25	1.9	0.9	1.7
4	3.0	35	4.0	1.2	2.8
5[a]	2.0	30	4.1 (0.1)	0.6 (0.0)	1.6 (0.1)
A-1[b]	1.0	30	1.0	0.2	0.9
A-2	2.0	35	4.2	0.8	1.7
A-3	3.0	30	3.9	1.0	2.6

a: Experiment was replicated 3 times and the response represented average values (standard deviation)
b: Additional trials outside the orthogonal design

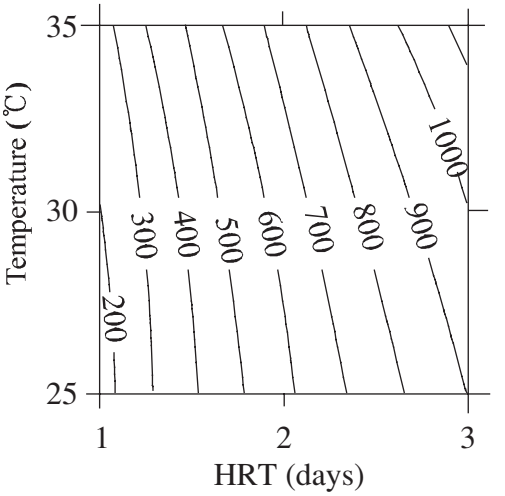

Figure 2 Two dimensional contour plot of the first order model for propionic acid production with respect to HRT and temperature

$$\eta_{\text{acetate}} = -22{,}716 + 5{,}818\,x_1 + 1{,}180\,x_2 + 64\,x_1x_2 - 1{,}650\,x_1^2 - 19\,x_2^2 \quad (4)$$

$$\eta_{\text{butyrate}} = -6{,}258 + 3{,}241\,x_1 + 247\,x_2 - 4\,x_1x_2 - 724\,x_1^2 - 3\,x_2^2 \quad (5)$$

The residual plots of the model were randomly distributed without any pattern, which was another indication of the adequacy of the fit of the model (data not shown). Two-dimensional response surfaces of the corresponding models for acetic and butyric acid production are shown in Figures 3 and 4, respectively. The response surfaces of both acids showed rounded ridges, running diagonally on the plot from left to right. From statistical inspection of the coefficients of the models, the effects of HRT on acid production were more significant than that of temperature at the 1% confidence level. These effects are clearly seen as regions of elongated ellipses in the response surfaces of the acid concentrations.

The conditions that maximized the production of acetic and butyric acids were calculated by setting the partial derivatives of the functions to zero with respect to the corresponding variables. The conditions for maximal acetic and butyric acid production were 2.4 days HRT at 34°C and 2.1 days HRT at 35°C, respectively. The calculated model outputs at the optimal conditions were 4.7 ± 0.7 g acetic acid/L and 1.7 ± 0.1 g butyric acid/L. The unbounded polynomial models that defined the response surface could approximate the response well outside the design boundary. However, since the polynomial

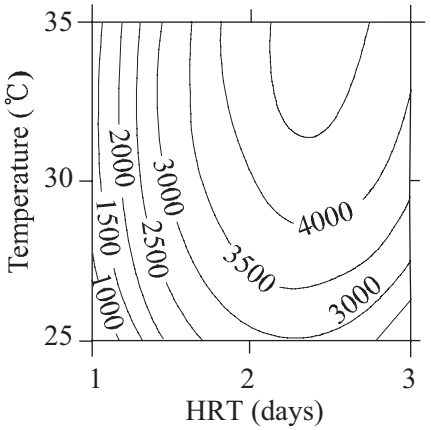

Figure 3 Two dimensional contour plot of the quadratic model for acetic acid production with respect to HRT and temperature

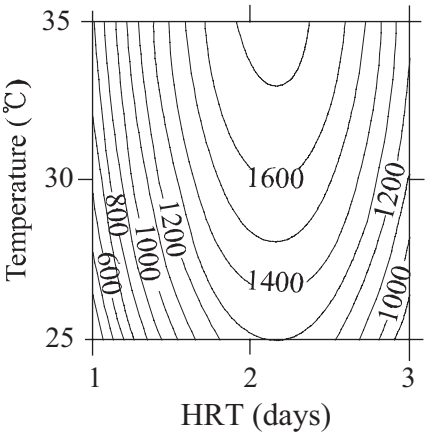

Figure 4 Two dimensional contour plot of the quadratic model for butyric acid production with respect to HRT and temperature

models extend to infinity, the models used to predict the response surface here should be restricted within specified boundaries.

Conclusions

In this study, acidogenesis of swine wastewater with different initial concentrations of ammonia nitrogen was investigated. Response surface methodology was successfully applied to determine the optimum physiological conditions with respect to the HRT and pH where the maximal acetic and butyric acid production occurred. These conditions were 2.4 days HRT at 34°C and 2.1 days HRT at 35°C, respectively. Therefore, it was concluded that the enhanced acidification process to manage swine waste should be operated in the range of 2.1 to 2.4 days HRT at 34.5 ± 0.5°C with an ammonia nitrogen concentration of less than 1.2 g/L.

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