Production of acetic acid and volatile fatty acids from lime-pretreated bagasse/chicken manure

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High-solids anaerobic fermentation of lime-pretreated Bagasse/Chicken manure (80: 20) were investigated to determine the feasibility of these feedstock for producing acetic acid and other volatile fatty acids. Batch and countercurrent fermentation were performed. The influence of solid loading rate (SLR), liquid retention time (LRT) and dilution rate (D) on the performance of the bioreactor was studied. The highest productivity of 1.49 g total acid / (L. of liquid .d) for a.SLR = 6 g VS/(L. of liquid .d) and D = 0.14 d^{-1}, was achieved. On the other hand, the highest acid concentration of 12.5 g total acid / L. of liquid and maximum yield (0.3 g total acid / g VS fed) for a SLR = 3 g VS / (L. of liquid .d) and D = 0.07d^{-1}, was achieved. The Continuum Particle Distribution Modeling (CPDM) method of Loescher was applied to Bagasse / Chicken manure fermentation. The model agreed with experimental final acid concentrations with standard error (0.62 – 8.26), and specific reaction rates with standard error (0.001).

تم دراسة إمكانية استخدام مصاصة القصب وروث الدواجن والذي سبق معالجتهم بالجير لإنتاج حمض الخليك والأحماض الدهنية المتطايرة . وذلك بالتخمر اللاهوائي لمخلوط من مصاصة القصب وروث الدواجن (٨٠ : ٢٠) . وكانت نسبة المواد الصلبة الداخلة للمخمر مرتفعة. تم دراسة تأثير معدل إضافة المواد الصلبة وزمن بقاء السائل وكذلك معدل التخفيف على أداء المخمر الذى يعمل بطريقة مستمرة . وكذلك تمت الدراسة على المخمر الذى يعمل بطريقة متقطعة . كانت أعلى إنتاجية للأحماض الكلية هي ١,٤٩ جرام / لتر . يوم لمعدل إضافة للمواد الصلبة يساوى ٧ جرام مواد متطايرة / لتر . يوم ومعدل تخفيف ٠,١٤ $يوم^{-1}$. وتم الحصول على أعلى تركيز للحامض وهو ١٢,٥ جرام / لتر . وأعلى كفاءة للإنتاج وهى ٠,٣ جرام أحماض كلية لكل جرام مواد متطايرة / لتر . يوم ومعدل تخفيف ٠,٠٧ $يوم^{-1}$. تم تطبيق نتائج هذا البحث على النموذج الرياضي CPDM لاستنتاج تركيز الحامض الكلى المنتج وكذلك معدل التفاعل النوعي . وكانت النتائج المستنتجة من النموذج الرياضي متقاربة جدا مع النتائج العملية .

Keywords: Acetic acid, Volatile fatty acids, Chicken manure, Bagasse, Fermentation.

1. Introduction

During the past thirty years, various ideas have emerged on how to obtain useful chemicals from waste biomass. Sterzinger [1] discusses gasifying biomass and then combusting the biogas in a turbine to produce electricity. Simultaneous saccharification and fermentation (SSF) is a method of producing ethanol from biomass. Methane production from the anaerobic fermentation of agricultural residue [1], municipal solid wastes [2], and sewage sludge [3] has been extensively studied. Currently, low cost of fossil fuels hinders the large-scale commercialization of these alternative fuel technologies.

Acetic acid has a higher value than fuel. If 50% digestibility is assumed for the biomass, theoretically one tone of biomass can yield 1000 pounds of acetic acid. Acetic acid is a commodity chemically used to produce a variety of products. ranging from latex to acetaminophen to octane enhancers for gasoline. It is currently produced by methanol carbonylation, a petrochemical-based process. Also, acetic acid can be produced via fermentation of sugars and waste biomass.

A mutant strain of *clostridium thermoaceticum* was studied in a cell-recycle membrane bioreactor for production of acetate from glucose [4]. Bioconversion of cellulose to acetate was accomplished with cocultures of two organisms. One was cellulolytic species *Ruminscoccus albus*. The other was a hydrogen using acetogen (HA). The major product of the fermentation by *R. albus* and HA coculture is acetate [5]. The production of volatile fatty acids by anaerobic fermentation

of municipal solid wastes was studied at pilot-plant level [6].

Playne [7] studied the production of volatile fatty acids (VFAs) from Bagasse by non-sterile mixed-culture fermentation. In continuous culture, he achieved a total acid productivity of 4.9 g VFA / (L of liquid. d) at a concentration of 15.5 g/L VFA (9.5 g/L acetic acid), a pH of 6.8, and a yield of 0.25 kg of VFA/kg of bagasse. He concluded that improvements in fermentor productivity, final acid concentration, yield, and low pH tolerance (< 5.5) would be needed for the process to compete with traditional technology.

To increase rates and conversion, biomass can be made more easily digestible. Rapier [8] studied the pretreatment of municipal solid waste with $Ca(OH)_2$ which increased the in situ rumen rate and extent of digestion of the municipal solid waste by approximately 27%.

According to Wujcik's [9] data methane formed per unit solids is constant at a given acid concentration provided there is adequate mixing. Likewise, acetate formation per unit solids should be constant; hence, increasing the solids concentration can raise volumetric productivity.

In a typical fermentor, the biomass and liquid enter together which means that the easily fermentable soluble are quickly fermented to VFAs leaving the more difficult-to-digest components. To attain a high final product concentration and high conversion, the remaining recalcitrant components must now be slowly fermented.

Henk [10] discusses a somewhat similar problem in which an immobilized biocatalyst becomes deactivated with time in a fermentor. His solution is to flow the incoming substrate countercurrently to the immobilized biocatalyst.

The countercurrent idea is applied to a biomass fermentation. Presenting the most digested biomass with the lowest acid concentration allows for more complete digestion, fulfilling one Playne's requirements. Also, presenting the high-acid concentration fermentor with fresh biomass provides the best chance for further acid production.

Loescher [11] has developed a model that can simulate any continuous fermentor system based on data collected from batch experiments. The Continuum Particle Distribution Modeling, (CPDM) method predicted CSTR productivity in the fermentation of rye grass to VFAs to within 10% of the actual productivity. This model can be used to simulate the continuous countercurrent system.

Objectives of this study are : (1) to use methanogenic fermentation for low-energy waste treatment; (2) to improve the performance of the digester and maximize acetic acid production by using countercurrent fermentation system; and (3) to apply CPDM method to Bagasse / Chicken manure fermentation.

2. Materials and methods

2.1. Microorganism

Rumen fluid from a forage-fed fistulated steer (Texas A & M University Nutrition and Field laboratory), in addition to a different inocula from a variety of anaerobic environments. The inoculum samples were kept in an airtight container and were then used within three hours of collection.

2.2. Bioreactor

The batch and continuous processes were carried out in a centrifuge bottle fermentor (CFBR), which consists of a 1-L centrifuge bottle for the fermentor body (about 350 ml working volume) and a centrifuge bottle cap used to retain the # 11 EPDM stopper. A gas collection device and the stir bar (two pieces of 1/4-in stainless tubing) go through the # 11 stopper. The CFBR lies horizontally in a wheaton modular cell culture roller bottle apparatus (the apparatus consists of multiple decks of parallel rollers that rotate the bottles at approximately 2.0 rpm).

2.3. *biomass pretreatment*

Both sugar-cane bagasse and chicken manure were lime pretreated. When pretreating, the biomass was a fairly fluid slurry providing good dispersion of the lime in the biomass. The lime and liquid loading

during pretreatment were 0.05 g $Ca(OH)_2$ / dry g of biomass and 5 ml liquid / dry g biomass. The pretreatment condition was 121°C for 1 hour. The dried-pretreated-biomass was ground in a hammer mill (Forest Science Research Lab, Texas A & M University) fitted with a 3-mm screen.

2.4. *Countercurrent fermentation*

In all sugar-cane bagasse / chicken manure fermentations, the two substrates were combined in an 80/20 ratio. The volatile solid (VS) content of this combination was 87.277 wt%. The media used in all experiments was modified Caldwell and Bryant (C & B) as described by Loescher [11]. The liquid added to make up the media was deoxygenated water containing (g per liter of deairated water): Cysteine-HCl, 0.275; and $Na_2S \cdot 9H_2O$, 0.275.

Batch fermentations were initiated under anaerobic conditions by adding substrate (25% w/v), nutrients and inocula to prereduced media in one of the high-solids fermentors (CFBR). The incubation temperature was 40°C for all fermentations. The inhibitor concentration (2% Iodoform in ethyl alcohol) was added (40 μL) to each fermentor in all experiments. After the culture had time to be established in the fermentor (ca. 1 week), liquid and solid flow were started. Three trains (each train consisting of four fermentors) of fermentors A, B & C at varying liquid residence times and solids loading rates were conducted to determine the productivity of a countercurrent fermentation. The transfer procedure was made daily, and after each transfer session, the fresh solid (substrate) was added to fermentor # 1 and the fresh liquid (deoxygenated water) was added to fermentor # 4, also 2 g $CaCO_3$, 0.15 g urea (if pH < 7) and 40 μL iodoform solution were added to each fermentor. 3-ml samples of the supernatant liquid from fermentor ≠ 1 were taken and stored at 0°C, and the remaining liquids are stored at 0°C in a collection bottle. The solids removed from fermentor 4 are stored at 0°C in a collection bottle.

2.5. *CPDM method experimental procedure*

All batch experiments to obtain modeling data were conducted with CFBR fermentors. Five batch experiments were conducted to obtain modeling data. In the sets of batch experiments, the initial substrate concentrations were nominally 20, 40, 70, 100 and 100⁺ g/L. The 100 and 100⁺ fermentors had the same initial substrate concentrations, but the 100⁺ fermentor was initiated with approximately 20 g VFA/L of a 70 / 20 / 10 mixture of $CaAc_2$, $Ca\,Pr_2$ and $Ca\,Bu_2$ present in the media. The inoculum to all batch experiments was the supernatant liquid of the solids taken from well-established fermentors # 4 (from the countercurrent fermentation processes). The incubation temperature was 40°C for all experiments.

The batch experiments were performed for fourteen days using modified Caldwell & Bryant medium. Liquid samples were taken daily. The model parameters were determined according to the method described by Loesher [11], from the following rate equation.

$$\hat{r} = \frac{a\,(1-x)^b}{1 + c\,(x)^d + e\,(A_e)^f}$$

Where a, b, c, d, e and f are the model parameters

2.6. *Analytical methods*

Volatile solids (VS) was determined in the liquid product and solid residue by ashing the dried samples (at 105°C for two days) at 550°C for two hours. The volatile fatty acids determination in the liquid product (from fermentor # 1 in countercurrent fermentation or from batch fermentors) using gas chromatography procedure was described by Loescher [11]. The volume of gas produced was measured by displacing liquid in an inverted water-filled cylinder [12].

3. Results and discussion

3.1. Countercurrent fermentations

The results of the Bagasse / Chicken manure countercurrent fermentations are presented in Figs. 1-3.

The total acid and acetic acid concentrations of the product liquid exiting the fermentor train A as a function of time are presented in Fig. 1. As shown from the figure, the total acid concentration in the product liquid rose to 17.278 g total acid / L of liquid during the batch period and was 10.317 g total acid / L of liquid (at the quasi - steady state) during the countercurrent process with a productivity of 1.49 g total acid / (L of liquid. d) and the maximum acid yield was 0.248 g total acid / g VS fed. The LRT and SLR for fermentation A were 7 days and 6.0 g VS / (L of liquid. d), respectively and the dilution rate was 0.14 d^{-1}.

The total acid and acetic acid concentrations of the product liquid exiting the fermentor train B as a function of time are presented in Fig. 2. As shown from the figure, the total acid concentration in the product liquid rose to 13.684 g total acid / L of liquid during the batch period and was 10.168 g total acid / L of liquid (at the quasi - steady state) during the countercurrent process with a productivity of 1.02 g total acid / (L of liquid. d) and the maximum acid yield was 0.254 g total acid / g VS fed. The LRT and SLR for fermentation B were 11 days and 4 g VS / (L of liquid. d), respectively and the dilution rate was 0.1 d^{-1}.

From Fig. 3, the total acid concentration in the product liquid rose to 14.35 g total acid / L of liquid during the batch period and was 12.5 g total acid / L of liquid (at the quasi - steady state) during the countercurrent process with a productivity of 0.896 g total acid / (L of liquid. d) and the maximum acid yield was 0.3 g total acid / g VS fed. The LRT and SLR for fermentation C were 14 days and 3.0 g VS / (L of liquid. d), respectively and the dilution rate was 0.07 d^{-1}.

The VS for solid exiting the fermentor train A, B and C were 0.5292, 0.4731 and 0.4703 g/g biomass respectively. By comparing the above value with the initial VS (0.8727 g/g biomass), the digestion in fermentor train B and C are nearly the same and they were slightly higher than that in fermentor train A. These results explain why the maximum yield increased in fermentor train C compared with B and A.

The results confirm that the volumetric VFAs productivity of the fermentation can be markedly increased by increasing SLR or raising the dilution rate. Thus, the highest productivity of 1.49 g total acid / (L. of liquid .d) was observed with D = 0.14 d^{-1} but only 39% of the VS in the feed was utilized.

3.2. Application of CPDM method to bagasse/ chicken manure fermentation.

The model predicted acid concentrations expressed as grams of acetate equivalents (Aceq) according to an empirical equation [11] describing the acid concentration as a function of time. This equation had the form

$$Aceq = b + \frac{ct}{1 + dt}.$$

Plots of the modeling experiment batch data versus model predictions for Aceq are included in Figs. 4-8. The standard error was calculated and it was found to be equal to 1.4, 0.62, 2.31, 6.34 and 8.25 for Bagasse / Chicken manure fermentation 20, 40, 70, 100 and 100+ respectively. Therefore, the model could be used to predict final acid product concentrations at a variety of initial substrate concentrations.

The overall, rate equation parameters were determined for a given batch experiment. Table 1 represents the overall rate equations for Bagasse/ Chicken manure fermentation 20, 40, 70, 100 and 100+. The standard error between the specific rate and model prediction of specific rate was calculated and it was found to be equal to 0.001 for all cases.

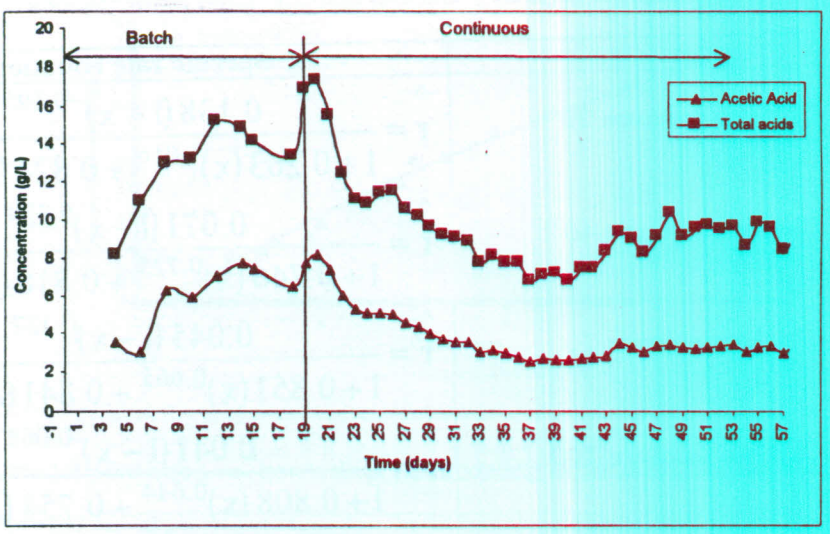

Fig. 1. Product acid concentrations for bagasse/chicken manure fermentation A.

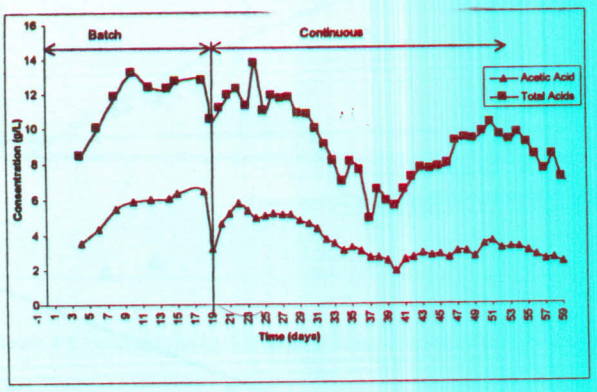

Fig. 2. Product acid concentrations for bagasse/chicken manure fermentation B.

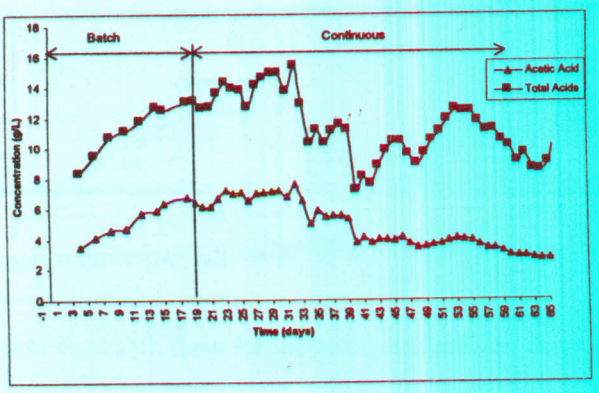

Fig. 3. Product acid concentrations for bagasse/chicken manure fermentation C.

Table 1 The overall rate equations for bagasse / chicken manure batch fermentations.

Batch	Specific rate equation
Bagasse / Chicken manure 20	$\hat{r} = \frac{0.138(1-x)^{-0.195}}{1+0.263(x)^{1.016}+0.824(A_e)^{1.22}}$
Bagasse / Chicken manure 40	$\hat{r} = \frac{0.071(1-x)^{0.329}}{1+0.746(x)^{0.725}+0.818(A_e)^{0.952}}$
Bagasse / Chicken manure 70	$\hat{r} = \frac{0.045(1-x)^{0.427}}{1+0.851(x)^{0.665}+0.841(A_e)^{0.568}}$
Bagasse / Chicken manure 100	$\hat{r} = \frac{0.041(1-x)^{-0.068}}{1+0.808(x)^{0.644}+0.754(A_e)^{0.201}}$
Bagasse / Chicken manure 100+	$\hat{r} = \frac{0.057(1-x)^{0.004}}{1+0.957(x)^{0.626}+1.178(A_e)^{0.101}}$

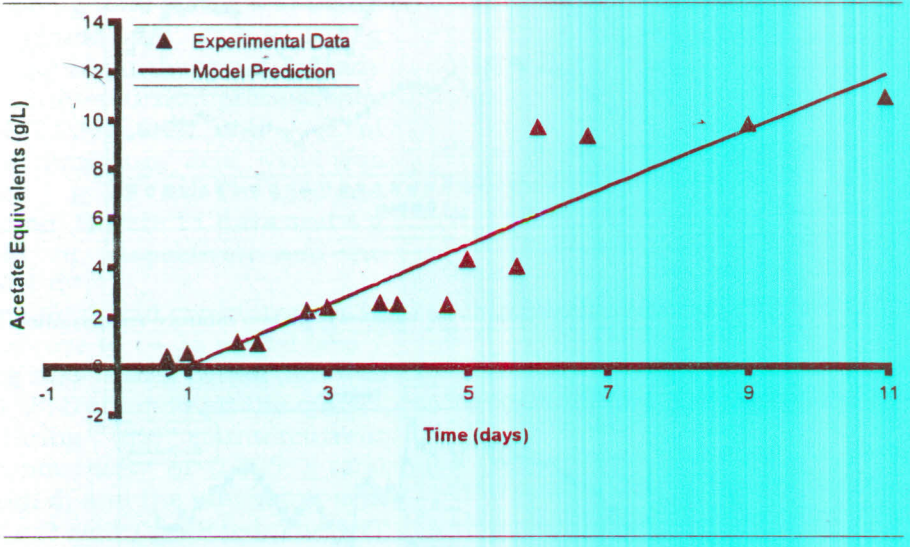

Fig. 4. Model prediction vs. batch data for modeling experiment Bagass/Chicken manure 20.

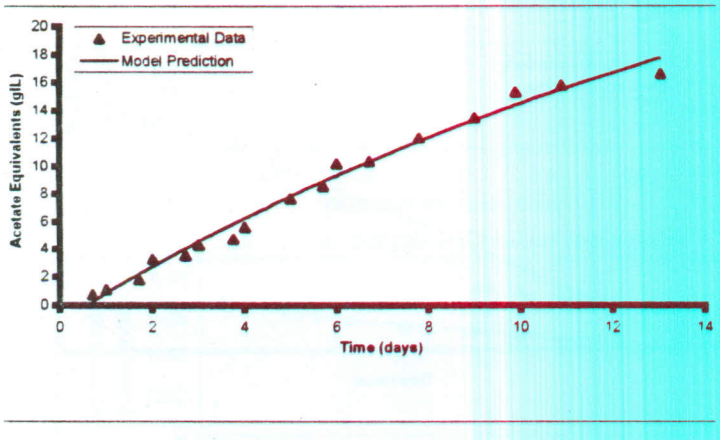

Fig. 5. Model prediction vs. batch data for modeling experiment Bagass/Chicken manure 40.

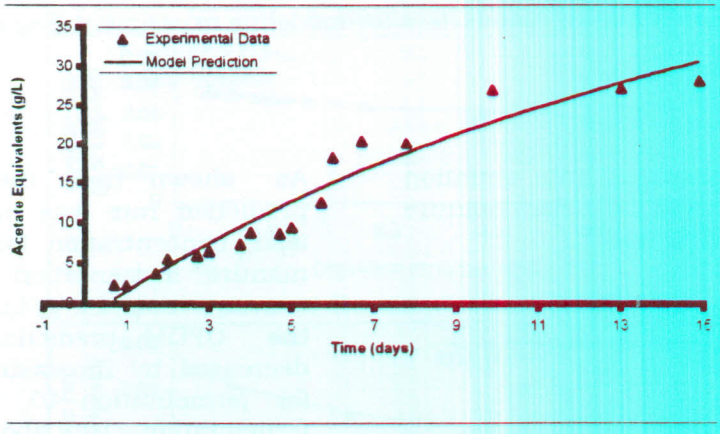

Fig. 6. Model prediction vs. batch data for modeling experiment Bagass/Chicken manure 70.

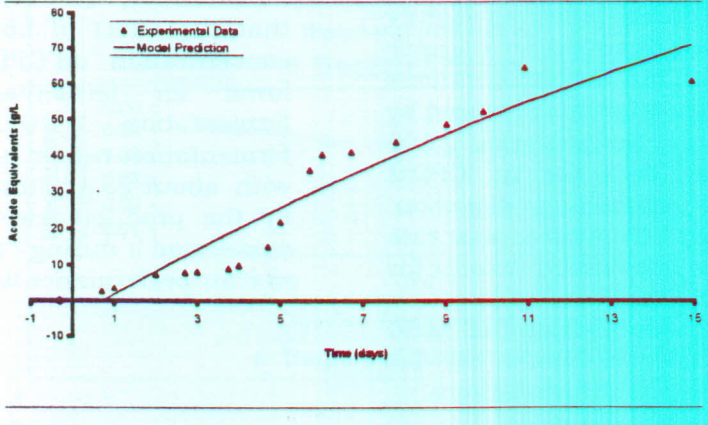

Fig. 7. Model prediction vs. batch data for modeling experiment Bagass/Chicken manure 100.

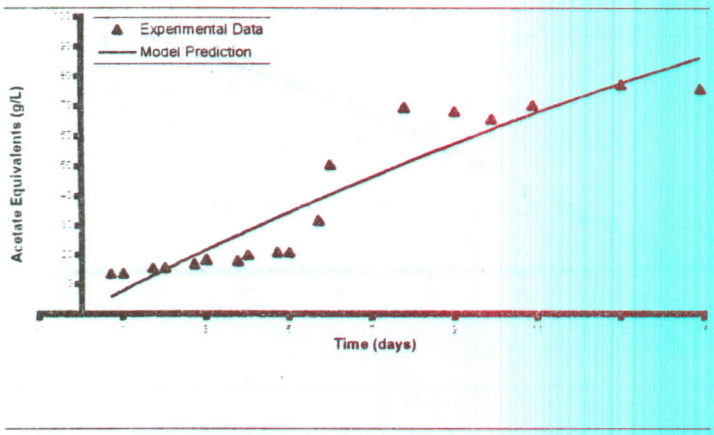

Fig. 8. Model prediction vs. batch data for modeling experiment Bagass/Chicken manure 100+.

Therefore, the governing rate equation obtained for the Bagasse / Chicken manure fermentation for Model fit was:

$$\hat{r} = \frac{0.07\,(1-x)^{0.099}}{1+0.725\,(x)^{0.735}+0.883\,(A_e)^{0.608}}.$$

Fig. 9 shows the effect of digestion on CPDM prediction rate for Bagasse / Chicken manure fermentations. As shown from the figure, the CPDM prediction rate was slightly increased by increasing digestion for Bagasse / Chicken manure 20, but for fermentations 40, 70 and 100+ were slightly decreased by increasing digestion. Fermentation 100 shows a markedly decrease in CPDM prediction rate by increasing digestion. Therefore, the model fit was a less rate reduction effect from conversion except for fermentation 100.

Fig. 10 shows the effect of acid concentration on CPDM prediction rate for Bagasse / Chicken manure fermentations.

As shown from the figure, the CPDM prediction rate was increased by increasing acid concentration for Bagasse / Chicken manure fermentation 20 (maximum acid concentration 11 g/L). On the other hand, the CPDM prediction rate was slightly decreased by increasing acid concentration for fermentation 40, 70 and 100+ but for fermentation 100 about 60% reduction in rate was obtained by increasing the acid concentration to 60 g/L. By comparing fermentation 100 with 100+, it was found that the effect of both conversion and acid concentration on CPDM prediction rate were lower for fermentation 100+ than for fermentation 100. Therefore, the fermentation rate in a fermentor which starts with about 20 g/L total acid was not affected by the product acid concentration and the conversion during the process and the reactor performance was good.

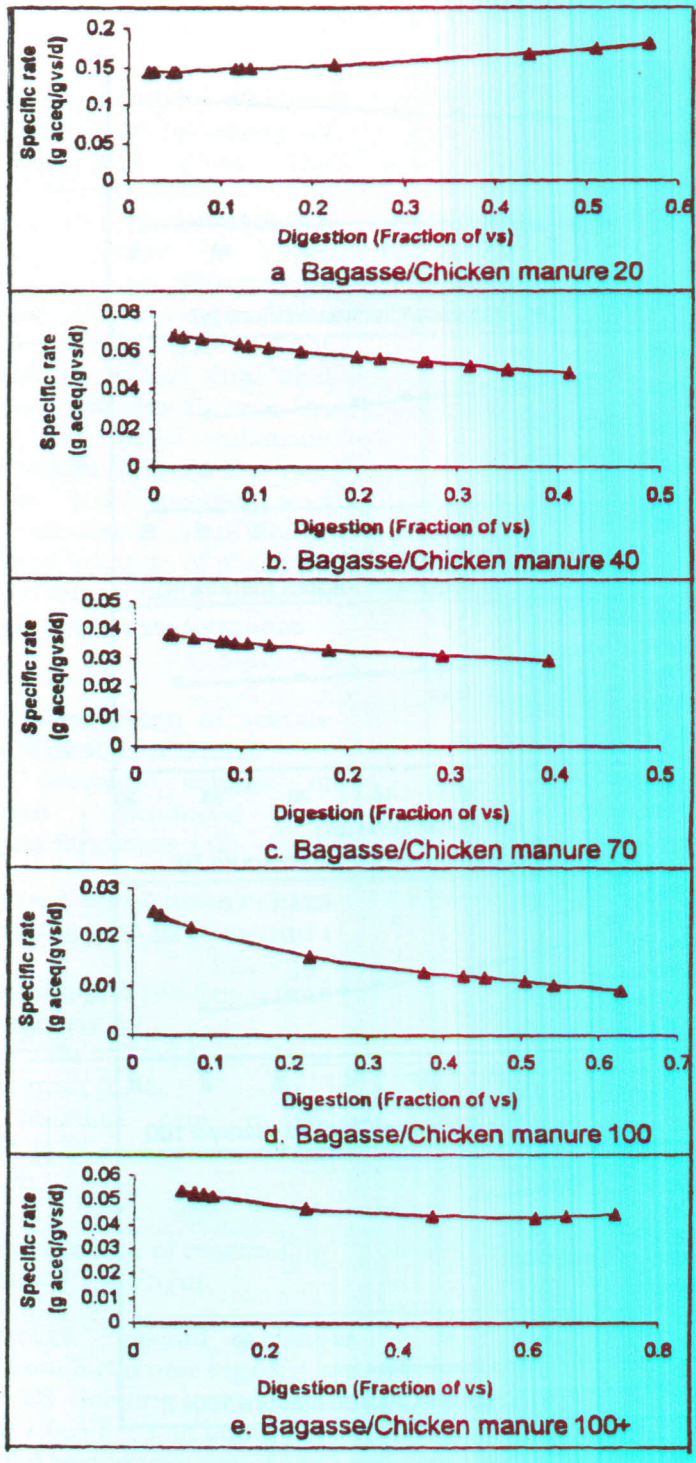

Fig. 9. The effect of digestion on CPDM prediction rate for Bagasse/Chicken manure batch fermentation.

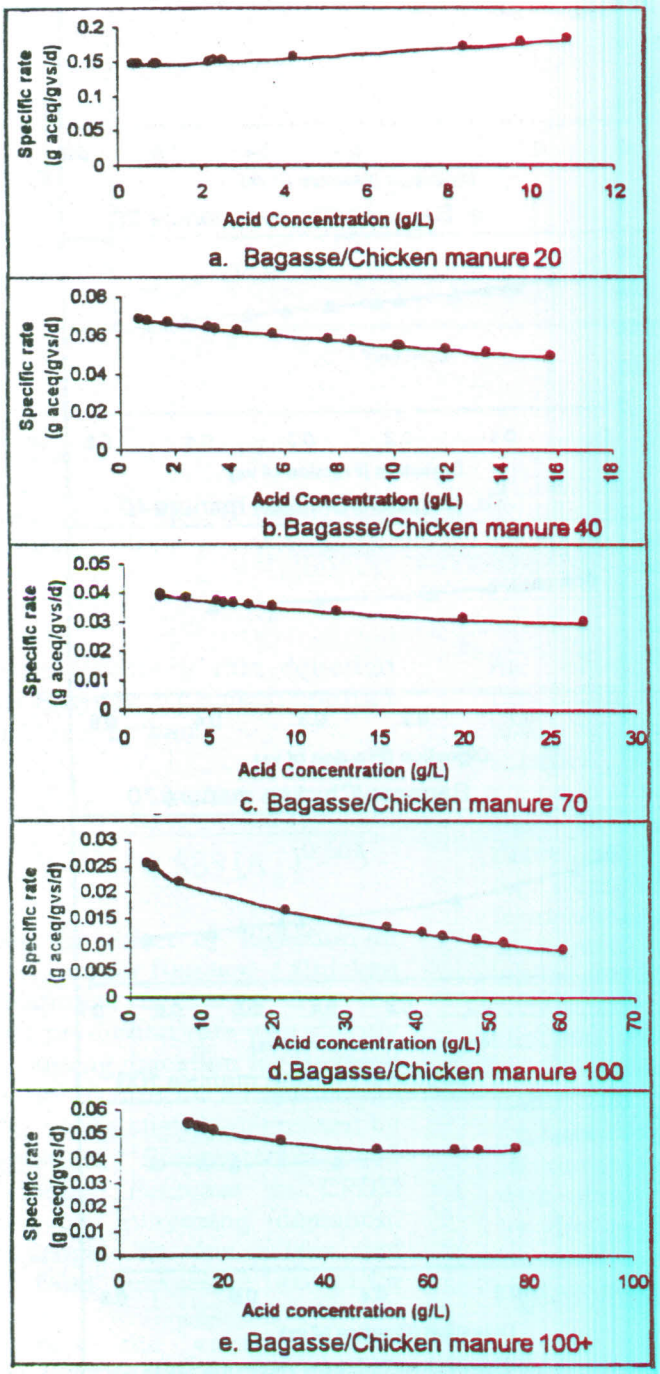

Fig. 10. The effect of acid concentration on CPDM prediction rate for Bagasse/Chicken manure batch fermentation.

4. Conclusions

The lime-pretreated Bagasse / chicken manure could be a favorable substrate for production of acetic and VFAs. The countercurrent fermentation is recommended for high-solids fermentation of Bagasse / chicken manure to VFAs. Increasing SLR or raising the dilution rate can markedly increase the volumetric VFAs productivity of fermentation. The CPDM model could be used to predict final acid product concentrations and specific reaction rate at a variety of initial substrate concentrations. The model fit has a less rate reduction effect from both product acid concentrations and conversion in all except for one case. The initial addition of about 20 g/L total acid to the fermentation media is recommended for high reactor performance.

Nomenclature

Ae	is the concentration of acetate equivalents (mol/L of liquid).
Fi	is the average volume of supernatant produced by centrifuging fermentor i (L).
$\bar{K}_i$	is the average liquid mass of cake after centrifugation in fermentor i (g).
LRT_{tot}	is the total liquid residence time of all fermentors (d).
Q	is the flow rate of liquid out of the fermentor train (L/d).
r_i	is the reaction rate in ith fermentor (g of acid produced / L of liquid. d).
$\hat{r}$	is the specific rate of reaction, (g acid produced / g VS/d).
$\bar{S}_i$	is the average liquid mass removed from fermentor i (g/d).
SLR	is the VS loading rate into fermentor 1 (g VS / L of liquid .d)
SRT_i	is the solid residences time of an individual fermentor i (d).
SRT_{tot}	is the overall solid residence time of a fermentor train (d).
TLV	is the total liquid volume of all fermentors (L).
w	is the average liquid fraction of centrifuged cake in fermentor i (L liquid / g wet cake).
x	is the fractional conversion of biomass digestion (dimensionless).

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