2009

Innovative waste biomass derived organic acids synthesis, immobilization, and purification system

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Recommended Citation

Sawatdeenarunat, Chayanon, "Innovative waste biomass derived organic acids synthesis, immobilization, and purification system" (2009). *Graduate Theses and Dissertations*. 10705.
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Innovative waste biomass derived organic acids synthesis, immobilization, and purification system

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

Program of Study Committee:
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Iowa State University

Ames, Iowa

2009

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ABSTRACT

Carboxylic acid could be produced by anaerobic digestion of waste biomass and used as feedstocks for various chemical processes. The recovery of carboxylic acid and the optimization of organic acid producing are the challenges of this bioconversion. In this study, MCM-41 type amine functionalize mesoporous silica (MPS) was used as an adsorbent to separate carboxylic acids. The experimental data fitted with a modified Langmuir model. At an amine density of 3.14 mol N kg^{-1}, the highest acid adsorption capacity experienced was 3.38 mol kg^{-1}. The reaction was exothermic and favored at low temperature. More than 90% of maximum adsorption would be achieved at $pH < 4$. By increasing pH above 10.5 using alkali chemicals, the desorption/regeneration completed within 1 minute. The interferences such as ethanol, glucose and protein did not affect organic acid adsorption significantly. However, anions at the high concentration might compete acetic acid to adsorb at the same site of adsorbent. MPS has a high selectivity for carboxylic acid. The pseudo-second-order rate constant for acetic acid adsorption on MPS was 0.41 kg mol^{-1} min^{-1} which is higher than that of anion exchange resin and activated carbon. The maximum organic acid yield from serum-bottle test using corn stover as a sole substrate was 0.25±0.02 g/gVS and decreased when the substrate concentration increased. Acid inhibition might play an important role in this phenomenon. With dilution rate of 0.5 d^{-1} in leaching bed reactor study, the highest organic acid yield and productivity were 0.14 g/gVS and 0.70 g/L/d, respectively. On the other hand, the maximum organic acid yield from semi-batch reactor in this study was 0.056 g/gVS at substrate concentration of 70 gVS/L and hydraulic retention time of 5 days. The maximum organic acid productivity obtained from this study is 1.319 g/L/d at substrate

concentration of 70 gVS/L and hydraulic retention time of 2.5 days. The majority of acids in organic acids produced were acetic acid, propionic acid, and n-butyric acid.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

The majority of energy and organic chemicals in our society are derived from fossil fuels. However, fossil fuels resources are diminishing. Price of fossil fuel is on an upward trend due to the political instability of petroleum production countries and increasing fuel demand of developing countries. Also, excessive carbon dioxide emission from combustion of fossil fuels results in global warming. In the contrary, biomass derived chemicals are renewable and do not cause a net increase of carbon dioxide (Aliello-Mazzarri *et al*., 2006).

Biological conversion of waste biomass is a proven and sustainable method for chemical production. It is carried out at ambient temperatures and pressures and hence is less energy-intensive than thermochemical conversion technologies. Up to now, anaerobic digestion for methane production is one of the most popular bioconversion technologies. It has been adopted since a century ago with numerous full-scale installations worldwide. Anaerobic digestion is well-suited to digested non-sterile, diversified, complex of waste biomass. Also, the product, methane, can be easily separated and purified. However, methane is a low-value product (Bungay, 2004). Digester biogas has been generally used to fire a boiler as a heat source or an engine generator for generating electricity. Although it brings some saving in energy, it cannot satisfy the energy demand in a large scale. Therefore, the production of high-value chemicals from waste biomass might be more attractive than conventional methane production.

Useful chemicals (organic acids, glycerol, xylitol, lipase, protease, etc.) can be obtained by fermentation of organic materials in the waste biomass. However, pure- or co-culture fermentation is needed to produce such specific chemicals in most of the cases (Angenent *et al.*, 2004). Pure- or co-culture fermentation process might not be appropriate to non-sterile and complex waste biomass. However, a mixture of low-molecular weight carboxylic organic acids can be easily produced from various complex organics by an indigenous mixed microbial community. Moreover, the production of organic acids requires less alkalinity and reaction time compared to methane production.

For the selective separation of organic acids, several unit operations such as precipitation, adsorption, solvent extraction and ion-exchange have been tried (Wu *et al.*, 2003). However, the separation/purification is still regarded as the highest cost associating with harvesting organic acids from biomass (Angenent *et al.*, 2004). After being separated from mixed liquor, it could be converted to mixed alcohols; liquor fuels (Aliello-Mazzarri *et al.*, 2006). Moreover, individual organic acids themselves are feedstock of many chemical processes and are widely used in food industries (Wu *et al.*, 2003). Acetic acid is an important reagent in the production of polyethylene terephtalate, cellulose acetate and polyvinyl acetate. As an effective mold inhibitor, propionic acid is used for preservation of animal feed and human food (Nakano *et al.*, 1996). Various drugs have been derived from butyric acid, which has therapeutic effect for treating colorectal cancer and hemoglobinopathies (Huang *et al.*, 2002). Lactic acid is used in a variety of food stuffs to act as an acidity regulator (Yang *et al.*, 2006).

A novel method is proposed to separate and concentrate organic acids from the bulk solution with simple adsorption and desorption processes. ISU patented mesoporous silica (MPS) materials functionalized with various amine groups will be used as the regenerateable media to adsorb organic acids. The expected advantages of this method are described below.

a) Simplicity and low cost:

No additional chemical is needed to precipitate or extract organic acids from fermented liquor. No complicated multi-step physical/chemical separation, purification processes involved. MPS media is employed to separate organic acids by adsorption from fermented liquor. Concentrated organic acids are washed off during media regeneration and are further purified to produce final products. MPS media is reusable and synthesized from low cost materials, and is available with large quantity.

b) High surface area and homogeneity of MPS media:

MPS comes with different shapes and particle sizes ranging from nano- to mini-meter (Huh *et al.*, 2003). MPS media provides a matrix in which all of the surface area is accessible to the bulk solution (Huh *et al.*, 2004). The MPS media has extremely high surface area-to-mass ratio of more than 700 m^2/g which warrant a fast binding kinetics and a high adsorption capacity (Lin *et al.*, 2001; Huh *et al.*, 2003).

c) Ample selection of functional groups:

MPS allows a variety of function groups including various amines to be homogeneously incorporated onto the surface (Huh *et al.*, 2003). Amines have high affinity to organic acids and have been widely used to extract organic acids in solution (Yang *et al.*, 1991). The strong amine interactions with the organic acids allow for formation of acid-amine complexes. In addition, the high affinity of the organic base for the acid gives selectivity for the acid over non-acidic components in the bulk solution.

d) Efficient purification:

Low product concentration in fermentation bulk solution usually hinders the purification efficiency in fermentation (Nakano *et al*., 1996; Wu *et al*., 2003). But, in the new approach proposed, the adsorption step would act as the concentrating process to immobilize organic acids from the bulk solution to the media. This could significantly improve the efficiency of the subsequent purification step and make the overall process more economical.

The purposes of this research are to study an acid bioreactor for organic acids synthesis, an adsorption for organic acids immobilization using novel media and a recovery unit for organic acids purification and media regeneration.

Thesis Organization

This thesis is set up into five chapters. Chapter 1 and 2 include general introduction and review of literature to provide background of the research and general fundamental information for the study presented. Chapter 3 is a paper entitled “Recovery of Carboxylic Acids by Amine-Functionalized Mesoporous Silica” which is prepared for being submitted to Nano Letters Journal. Chapter 4 is also a paper named “Organic Acid Production from Corn Stover Using Cattle Manure as inoculum” and it will be submitted to The Environmental Science and Technology Journal. Chapter 5 provides the general conclusion and recommendation for future research.

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CHAPTER 2. LITERATURE REVIEW

Mesoporous Silica (MPS)

Mesoporous silica (MPS) is a nano material which was discovered by scientist of Mobil Corporation in 1992 (Trewyn *et al.*, 2007). After that, the researches studied and synthesized many types of MPSs such as MCM 41/48 (Beck *et al.*, 1992; Kresge et al., 1992), SBA-15 (Zhao et al., 1998), MSU-n (Bagshaw et al., 1995), KIT-1 (Ryoo *et al.*, 1996), and FSM-16 (Inagaki *et al.*, 1996). The MPSs could be functionalized with many kinds of compounds including amine group (Jun *et al.*, 2007). After functionalization, MPS could be used in many processes, for example, separation (Dai *et al.*, 1999; Lin et al., 2001; Yoshitake *et al.*, 2003; Hossin *et al.*, 2002), sensor design (Lin *et al.*, 2001), catalysis (Soler-Illia Galo *et al.*, 2002; Stein, 2003; Davis, 2002; Corma, 1997; Price *et al.*, 2000; Ying *et al.*, 1999; Sayari *et al.*, 1996; Moller and Bein, 1998) and drug delivery (Lai *et al.*, 2003). These are because of the particular properties of MPS such as large surface area, high chemical and thermal stability and easy functionalization of silica. The researchers from Department of Chemistry, Iowa State University invented amine-functionalized MPS for using as adsorbent. This MPS would be a potential adsorbent for organic acid recovery because of the high selectivity between amine groups and organic acids. The co-condensation synthesis method would alleviate non-homogenous surface coverage or distorted backbone structure at a high-functional-group density (Huh *et al.*, 2003; Algarra *et al.*, 2005). MPS was synthesized via co-condensation using a sodium hydroxide-catalyzed reaction of tetraethoxysilane (TEOS) with 3-aminopropyltrimethoxysilane (APTMS) in the presence of cetyltrimethylammonium bromide (CTAB) as the template.

The mixture of CTAB (2.0 g, 5.49 mmol), 2.0 M of NaOH (aq) (7.0 ml, 14.0 mmol), and H_2O (480 g, 26.67 mol) was heated at 80°C for 30 min. Then, TEOS (9.34 g, 44.8 mmol) and organoalkoxysilane (APTMS (0-40 mmol)) were injected sequentially. The solution was stirred at 550 rpm at 80°C for 2 hours. The products were isolated by a hot filtration, washed with copious amounts of water and methanol, and dried under vacuum. The dried material was added to a 1% (v/v) HCl-methanol solution and heated at 60°C for 6 hours to remove surfactant. The surfactant-removed solid products were filtered and washed with water and methanol, and then dried under vacuum.

The structural properties of amine-functionalized MPS are presented below.

Table 1. The structural properties of amine-functionalized MPS

N density (mol N/kg)	Structural properties		
	BET Surface area (m^2/g)	BJH Pore size (nm)	Pore volume (cm^3/g)
0	1043	2.5	0.91
0.84	755	2.7	0.52
1.38	531	2.8	0.36
3.14	502	2.6	0.33
3.49	337	2.6	0.24
3.65	330	2.2	0.18

BET (Brunauer, Emmett, and Teller) equation is famous for using to describe the internal surface area porous material such as activated carbon. This equation was published since 1938 in the journal of the American Chemical Society (Cooney, 1998).

$$\frac{q}{q_m} = \frac{b(P/P_s)}{(1-P/P_s)(1-P/P_s+bP/P_s)}$$

where P_s = the saturation vapor pressure at the temperature used in the test

q = the amount of substance adsorbed at any given pressure P

q_m = the amount of substance adsorbed when one complete monomolecular layer of surface coverage is attained

BJH (Barret, Joyner, and Halenda) pore size model which was developed in 1951 is a popular method to calculate the Particle Size Distribution (PSD) of mesoporous materials. This equation is based on Kelvin equation and modified to be suitable for multilayer adsorption.

The assumption of this method is assuming cylindrical pore geometry from nitrogen adsorption data. (Lu and Zhao, 2005)

Anaerobic Digestion

Anaerobic digestion is a complicate biochemical process which microorganisms convert biodegradable materials in absence of oxygen. This process is divided to four stages as shown in Figure 1 (van Haandel and Lettinga, 1994; Droste, 1997).

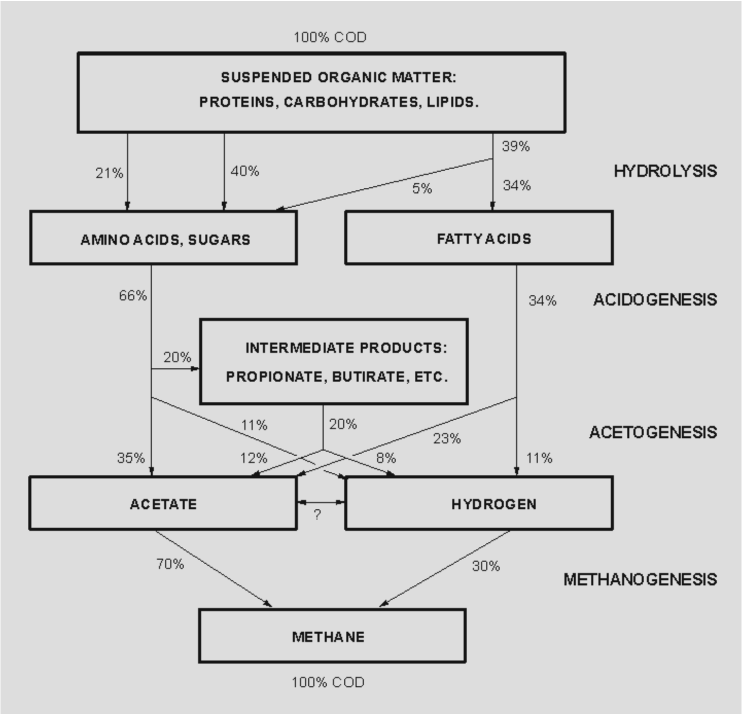

Figure 1. Schematic of anaerobic digestion

1. Hydrolysis stage

In this process, complex and high molecular weight molecules including suspended organic matters, proteins, carbohydrates and lipids are degraded into dissolved lower molecular weight compounds. The exo-enzymes from fermentative bacteria are required for this process.

2. Acidogenesis stage

Products from hydrolysis stage are converted to simple organic compound, such as fatty acids, alcohols, and lactic acids and mineral compounds including carbon dioxide gas, hydrogen gas, ammonia, and hydrogen sulfide gas.

3. Acetogenesis stage

The products from acidogenesis stage are converted to acetate, hydrogen gas, and carbon dioxide gas in this stage. The acid former microorganisms have an optimum pH range between 5.5 and 6.5.

4. Metanogenesis stage

Methane gas is produced in this stage either from acetate or the reaction between hydrogen and carbon dioxide by acetotrophic methanogenesis microorganism and hydrogenotrophic methanogenesis microorganism, respectively.

Environmental Variables

There are many physical and chemical variables effect the anaerobic microorganisms. These should be concerned in designing and operating anaerobic reactor (Droste, 1997; Ward *et al.*, 2008; Bouallagui *et al.*, 2003; Speece, 1996).

Temperature

There are three operating temperature ranges for anaerobic digestion: psychrophilic, mesophiclic and thermophilic, which have the temperature range below 15-20°C, 20-40°C, and 50-60°C, respectively (Droste, 1997). Most of reactors are operated at either mesophilic or thermophilic temperature (Ward *et al.*, 2008). However, anaerobic digestion might be operated in the temperature lower than 20°C or psychrophilic range with higher contact time (Bouallagui *et al.*, 2003; Speece, 1996).

pH and buffering capacity

The ideal optimum pH range for anaerobic digestion is between 6.8 and 7.2 (Speece, 1996). The methanogenic microorganism growth rate is significantly decreased with pH below 6.6 (Mosey, 1989). However, the optimum pH for acidogenesis and hydrolysis is between 5.5 and 6.5 so the buffering capacity of the system should be adequate enough to maintain pH in proper range (Droste, 1997). Typically, buffering capacity is related to the equilibrium of carbon dioxide and bicarbonate in the system. However, the other processes could increase this capacity including protein degradation and soap degradation (Speece, 1996).

Mixing

Mixing is one of the most important operational conditions in anaerobic reactors. It also plays an important role in distributing buffering agent all over reactor (Droste, 1997). Mixing could also help to reduce the matter size and liberate biogas from mixed liquor in reactor. Mixing could be implemented either mechanically by agitator or simply by gas recirculation (Chaoui and Richard, 2008).

Toxicity

There are many matters which could inhibit or be toxic to microorganisms in anaerobic process (Chen *et al.*, 2008). Inhibition might be indicated by the reduction of methane gas produced in steady stage. The matters which have potential to inhibit anaerobic microorganisms are ammonia, sulfide, light metal ions, heavy metals, and organic compounds (Chen *et al.*, 2008 and Droste, 1997).

Nutrients

Because anaerobic microorganism has much lower cell yield compared to aerobic microorganism, it requires lower amount of nutrients (Droste, 1997). Typically, activated sludge process requires the ratio between COD, Nitrogen (N), and Phosphorus (P) around 100:5:1 on a mass basis. Khanal, 2008 recommend the minimum ratios of COD, N, and P are 350:7:2 for highly loaded operation (0.8-1.2 kg COD/kgVSS-d) and 1000:7:1 for lightly loaded operation (<0.5 kg COD/kgVSS-d). Moreover, other nutrients including trace metals, vitamins, nickel, and cobalt are also required for the growth of microorganism (Khanal, 2008; Tchobanoglous *et al.*, 2004).

Organic Acids Production by Anaerobic Digestion

Organic acids can be produced in the acidogenesis stage of anaerobic digestion. The low-molecular weight organic compounds, the product from hydrolysis stage, are converted to volatile fatty acid (VFA), alcohols, lactic acid, hydrogen gas and carbon dioxide gas (van Haandel and Lettinga, 1994). The end products of this stage are depending on the types of substrates (Khanal, 2008). The same groups of microorganism as hydrolysis stage play important role in this stage. Most of the microorganism is obligate anaerobic bacteria but facultative groups of bacteria can also produce organic acids.

The typical anaerobic microorganisms found in this process are in the family of Streptococcaceae and Enterobacteriaceae. The genera of *Bacteroide, clostridium, Butyrivibrio, Eubacterium, Bifidobacterium* and *Lactobacillus* are normally participating in this process (Khanal, 2008). At the end of this stage, some portions of organic acids with carbon more than 3 atoms, alcohols and inorganic compounds will be converted to acetate.

This process can also be called “acetogenesis stage”. The acetate will be the substrate for methanogenic microorganism in methanogenesis stage for producing methane as end product.

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CHAPTER 3. RECOVERY OF CARBOXYLIC ACIDS BY AMINE-FUNCTIONALIZED MESOPOROUS SILICA

A paper to be submitted to Nano Letters Journal

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Victor S.-Y. Lin

Abstract

Carboxylic acids can be fermented from biomass and used as feedstocks for various chemical processes. The feasibility of this bioconversion depends on the recovery of carboxylic acids from fermentation liquor. The authors used MCM-41 type amine-functionalized mesoporous silica (MPS) to separate carboxylic acids by adsorption. The adsorption isotherms were analyzed with an extended Langmuir model using an overloading term. The highest acid adsorption capacity deduced was 3.38 mol $kg^{-1}$for 1:1 complexation at an amine density of 3.14 mol N kg^{-1}. Positive isosteric heat showed the reaction was exothermic and favored at low temperature. The adsorption reaction was pH-dependent. Greater than 90% of maximum adsorption would be achieved at pH < 4. Desorption/regeneration by increasing pH to 10.5 was completed within 1 minute, and the regenerated MPS showed adsorption capacity equivalent to the original. MPS had a high selectivity for carboxylic acid over ethanol, glucose, and protein. The pseudo-second-order rate constant for acetic acid adsorption on MPS was 0.41 kg/mol.min, higher than that of an anion exchange resin (0.14 kg/mol.min) and activated carbon (0.06 kg/mol.min).

Keywords: amine-functionalized; carboxylic acid; MCM-41; mesoporous silica (MPS); regeneration

Introduction

The conversion of waste biomass into useful chemicals is an environmentally-sound, sustainable solution that can decrease fossil fuel dependency, reduce production of greenhouse gases and pollution in general (Angent *et al*., 2004). A mixture of low-molecular weight carboxylic acids such as acetic acid, propionic acid, butyric acid, lactic acid, etc. can be easily produced from various complex organics by an indigenous mixed microbial community (Thanakoses *et al*., 2003; Wang *et al*., 2006). The production of a mixture of carboxylic acids does not require a sterile environment, expensive tanks or enzymes. If organic acids could be separated from fermented liquor, the liquor could then be thermally- and chemically-converted to mixed alcohols and/or liquor fuels (Aliello-Mazzarri *et al*., 2006). Furthermore, fermentation of individual organic acids shows promise for biological conversion of waste biomass (Wu and Yang, 2003; Angent *et al*., 2004).

The economic feasibility of converting waste biomass to soluble chemicals is largely dependent on separating the products from the bulk solution. Costs for separation and purification are high for the selective separation of organic acids (Angent *et al*., 2004; Bungay, 2004). Precipitation, adsorption, solvent extraction and, ion-exchange and have all been evaluated (Wu and Yang, 2003).

The discovery of surfactant micelle-templated synthesis of mesoporous silica materials such as MCM-41/48, SBA-15, MSU-n, KIT-1, and FSM-16 enabled the use of the mesoporous materials for catalysis, sensors, and adsorbents (Huh *et al*., 2003). Amine-functionalized mesoporous silica (MPS) would be a potential adsorbent for organic acid recovery, owing to its strong and selective interaction between amine and organic acids (Jun *et al*., 2007; Huang *et al*., 2008) and high accessibility because of its homogenous pore structure (Algarra *et al*., 2005; Jun *et al*., 2007).

Here the authors report on the first attempt to use a series of MCM-41 type amine-functionalized MPS by co-condensation synthesis for carboxylic acid recovery. We studied carboxylic acid adsorption capacity at amine densities ranging from 0.84 to 3.65 mol N kg^{-1}; adsorption isotherms and kinetics at different pH and temperatures, and the suppression by coexisting organic/inorganic matter.

Experimental

Adsorption test

The highest capacity MPS was determined using adsorption isotherms of the six MPSs: 0, 0.84, 2.35, 3.14, 3.49, and 3.65 mol N kg^{-1} at 35°C and a final pH between 3.5 and 4.0. Acetic acid was used as the organic acid. The authors poured stock solutions of acetic acid (500 mM) into capped glass bottles at 35°C. Predetermined volumes of the stock solution for the target initial acetic acid concentration (3.3 to 500 mM) were transferred to 25-ml Erlenmeyer flasks in a gyratory water bath at 35°C (Model G76, New Brunswick Scientific Co. Inc., NJ). Flasks were filled to 10 ml with nanopure water and pH adjusted with 2 N HCl or 2 N NaOH. Then 0.05 g of MPS was added, and the flasks sealed using parafilm (Pechiney, WI). After shaking at 190 rpm for 2 h, the solution was filtered through a 0.2-µm filter and the filtrate evaluated for pH and soluble matter. The same procedures were used in isotherm studies for other organic acids (propionic, *n*-butyric, and lactic acids) and temperature ranges (20 and 55°C) using MPS with 3.14 mol N kg^{-1}.

The pH dependence (2.3 to 9.4, final value) of carboxylic acid adsorption was examined at the initial acetic acid concentration of 50 mM (Hwang *et al*., 2006). The pH dependence of carboxylic acid desorption and media regeneration was also studied in 15-ml centrifuge tubes at an initial acetic acid of 50 mM. After 2 hours of shaking at pH 4.0, the sample was removed from two tubes, and the pH of the other tubes was increased to 10.5 using 2 N NaOH. The tubes were then shaken again for 1 min. The sample was removed from another two tubes, and the remaining tubes were centrifuged at 2,000 rpm for 5 min to separate the supernatant and the regenerated MPS. Acetic acid addition, pH adjustment to 4.0, adsorption, desorption by pH adjustment to 10.5 and centrifugation were repeated three times.

The effects of coexisting matter in fermentation liquor were investigated at initial acetic acid levels of 10 to 50 mM. Chloride, sulfate, and phosphate were selected as representative anions. NaCl, Na_2SO_4, or $NaH_2PO_4 \cdot H_2O$ was added into acetic acid solution to provide Cl^- of 1.0 to 100.0 mM (35.5 to 3,550 mg L^{-1}), SO_4^{2-} of 0.5 to 100.0 mM (16.0 to 3,200 mg S L^{-1}), or PO_4^{2-} of 0.5 to 100.0 mM (15.5 to 3,100 mg P L^{-1}). Suppression by organic matter was studied using 26.7 to 133.3 mM (1.23 to 6.14 g L^{-1}) ethanol, 26.7 to 133.3 mM (4.8 to 24 g L^{-1}) glucose and 5.4 to 54.4 mM (0.8 to 8 g L^{-1}) glutamate, representing alcohols, carbohydrates, and amino acids, respectively, in the fermentation effluent of waste biomass.

Adsorption kinetics was examined at an initial acetic acid level of 50 mM. The 3.14 mol N/kg MPS and two conventional adsorbents (powdered activated carbon (PAC) (Aqua

Nuchar®, MeadWestvaco Co., VA) and a weak base anion exchange resin (Dowex® Marathon® WBA, Sigma-Aldrich, MO)) were used as adsorbents. The samples were taken at 0.5 to 120 min intervals over 4 hours.

Typical Langmauir isotherm as shown below had been used to describe the acetic acid adsorption process at initial concentration less than 100 mM.

$$Q_{EQ} = \frac{Q_{Max} bC_{EQ}}{1 + bC_{EQ}}$$

where Q_{EQ} (mol kg^{-1}) is the equilibrium amount of carboxylic acid adsorbed in the adsorbent, Q_{Max} (mol kg^{-1}) is the maximum adsorption capacity, C_{EQ} (mol L^{-1}) is the equilibrium concentration of unionized carboxylic acid, and b is the apparent equilibrium constant (L mol^{-1}).

Analysis

Carboxylic acid concentration was measured by a high-performance liquid chromatograph (GP40, Dionex, CA) with an absorbance detector (AD20, Dionex) and a 300 mm × 7.8 mm Metacarb 67H column (Varian, CA) using 0.05 M H_2SO_4 as mobile phase. Anions were measured by the same chromatograph with a conductivity detector (CD20, Dionex) and a 250 mm × 4.6 mm AN1 column (Varian, CA) using carbonate buffer (1.7 mM $NaHCO_3$ + 1.8 mM Na_2CO_3) as mobile phase.

Results and Discussion

Carboxylic acid adsorption isotherm

Figure 2 shows that the amine-functionalized MPS had a much higher adsorption capacity than pure MPS, implying that electron lone pairs on nitrogen atoms (Eq. (1)), rather than silica atoms (Eq. (2)), acted as the main adsorption sites (Do, 1998).

$$\{\text{-N:}\} + \text{HA} \rightarrow \{\text{-NH}^{+}\text{A}^{-}\} \qquad (1)$$

$$\{\text{-Si-OH}\} + \text{HA} \rightarrow \{\text{-Si-OH}_2^{+}\text{A}^{-}\} \qquad (2)$$

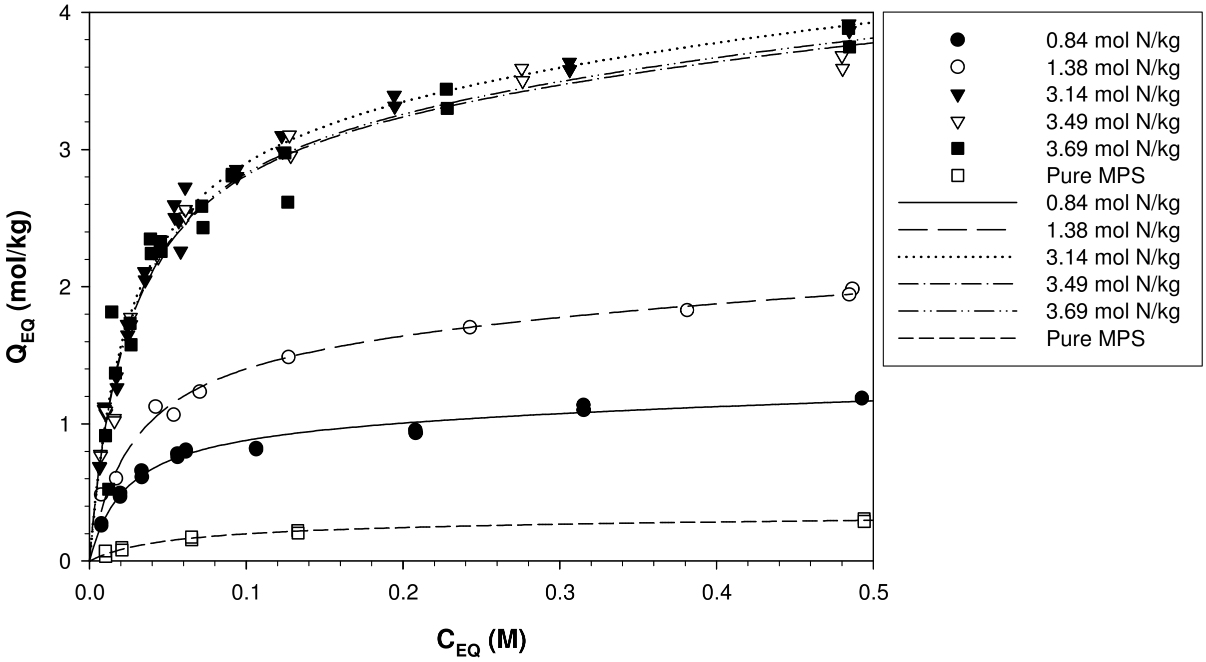

Figure 2. Acetic acid adsorption isotherms of MPS at 35°C (The symbol and line are experimental data and the extended Langmuir model, respectively.)

The experimental data resembled typical Langmuir-type isotherms. However, the calculated maximum adsorption capacities using the Langmuir model were larger than nitrogen densities of MPS (Table 3) due to the adsorption data at high equilibrium acid concentrations.

Table 2. Langmuir isotherm parameters for acetic acid adsorption at 35°C of amine-functionalized MPS and pure MPS

N density (mol N/kg)	Q_{Max}		b (L/mol)[a]	R^2
	(mol/kg)	(mol/mol N)[b]		
0	0.33	-	15.8	0.972
0.84	1.18	1.01	33.2	0.958
1.38	2.06	1.25	24.0	0.974
3.14	3.98	1.16	30.1	0.984
3.49	3.81	1.00	30.5	0.973
3.65	3.85	0.97	30.7	0.921

[a] Assuming that silica atoms in amine-functionalized MPS had same adsorption capacity of pure MPS.

Even assuming the silica atoms in amine-functionalized MPS have the same adsorption capacity of pure MPS (0.33 mol/kg), this could not explain the high adsorption capacities. It implied that more than one acetic acid molecule formed a complex with a basic site on MPS at high acetic acid load. Empirical approaches such as the Freundlich and Sips equations have been used to deal with the “overloading” phenomena (Kanazawa *et al.*, 2001; Srivastava *et al.*, 2007). However, the semi-empirical equations developed for adsorbent with complex pore and surface structure like activated carbon might not be appropriate to MPS with homogenous pore structure. Instead the authors used an extended Langmuir isotherm model as Eq. (3) based on 2:1 complexation between carboxylic acids and lone pairs. The model is based on the secondary hydrogen bonding of an additional acid molecule with the 1:1 complex (11), illustrated in Equation (3):

$$Q_{EQ} = \frac{Q_{Max}(b_1 C_{EQ} + 2b_2 C_{EQ}^{\ 2})}{1 + b_1 C_{EQ} + b_2 C_{EQ}^{\ 2}} \tag{3}$$

where Q_{EQ} (mol kg^{-1}) is the equilibrium amount of carboxylic acid adsorbed in the adsorbent, Q_{Max} (mol kg^{-1}) is the maximum adsorption capacity for 1:1 complexation, C_{EQ} (mol L^{-1}) is the equilibrium concentration of unionized carboxylic acid, b_1 is the apparent equilibrium constant (L mol^{-1}) for 1:1 complexation and b_2 is the apparent equilibrium constant for 2:1 complexation (overloading) (L^2 mol^{-2}). As shown in Table 3 and Figure 2, the extended Langmuir isotherm model explained the data with reasonable Q_{Max} values. Acid adsorption capacity increased with nitrogen densities up to 3.14 mol N kg^{-1}. The calculated maximum adsorption capacity for 1:1 complexation was 3.38 mol HAc kg^{-1}. At higher nitrogen densities, the acid adsorption did not increase. Table 3 also shows that the maximum capacity per surface area increased as nitrogen density increased, implying that decrease of surface area would not limit the efficiency. However, the ratios of adsorbed acetic acid to nitrogen content were maintained at no less than 0.83 mol HAc mol N^{-1}, because the accessibility of acid to nitrogen site was not significantly hindered. The mesoporous backbone structure was not seriously distorted at the high nitrogen density. The apparent equilibrium constants, b_1 and b_2, indicated how strong the adsorbate molecule is attached onto the primary and the secondary adsorption sites, respectively (Kanazawa *et al*., 2001).

Table 3. Structural properties and extended Langmuir isotherm parameters for acetic acid adsorption at 35°C of amine-functionalized MPS and pure MPS

N density (mol N/kg)	Structural properties			Extended Langmuir isotherm parameters					
	BET Surface area (m^2/g)	BJH Pore size (nm)	Pore volume (cm^3/g)	Q_{Max}, Maximum adsorption capacity for 1 to 1 complexation			Apparent equilibrium constant (L/mol)		R^2
				(mol/kg)	(mol/mol N)[a]	(mol/mm^2)	b_1, 1 to 1	b_2, 2 to 1	
0	1043	2.5	0.91	0.24	-	0.23	27.8	27.0	0.979
0.84	755	2.7	0.52	0.91	0.80	1.20	58.8	59.9	0.983
1.38	531	2.8	0.36	1.21	0.70	2.27	66.0	234.8	0.989
3.14	502	2.6	0.33	3.38	1.00	6.73	42.1	22.3	0.990
3.49	337	2.6	0.24	3.31	0.88	9.83	39.5	16.3	0.988
3.65	330	2.2	0.18	3.26	0.83	9.87	43.2	24.2	0.933

[a] Assuming that silica atoms in amine-functionalized MPS had same adsorption capacity of pure MPS.

It should be noted that the overloading term, $b_2C_{EQ}^2$ was negligible at a low equilibrium acid concentration. For acetic acid adsorption onto MPS with 3.14 mol N L^{-1}, the difference between the two models was <5% at the equilibrium unionized acid concentration (<100 mM (6 g L^{-1})) as shown in supplementary material (Figure 3).

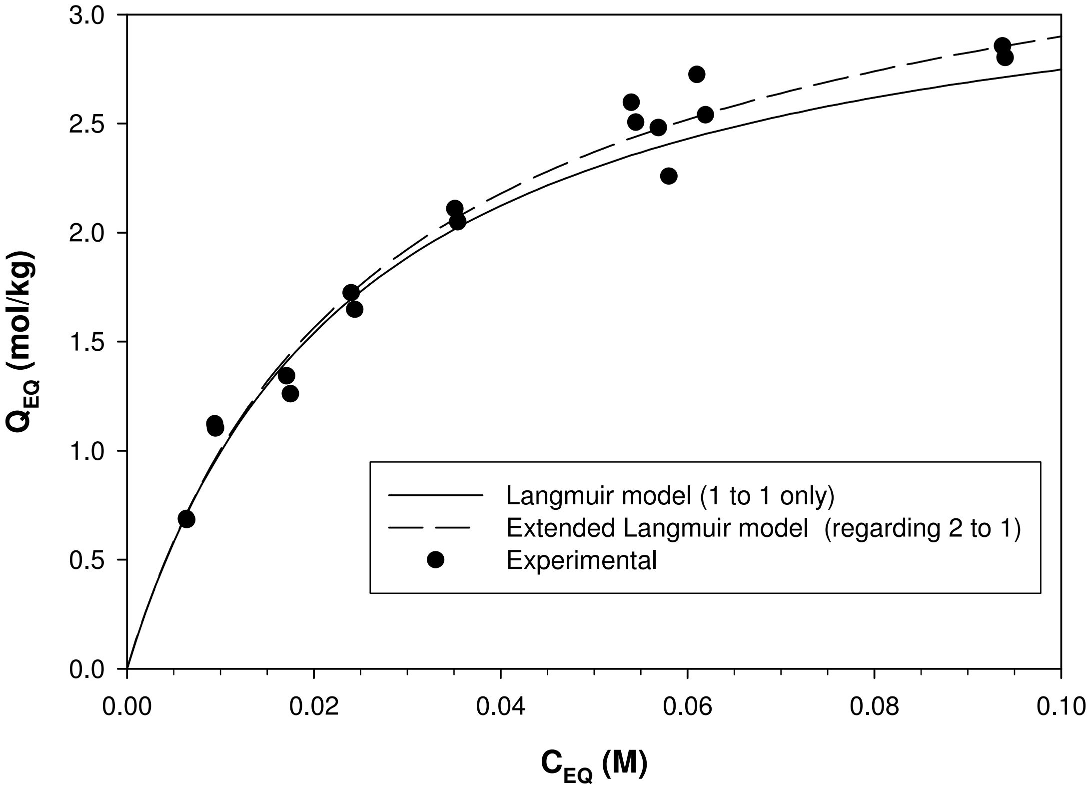

Figure 3. Comparison of Langmuir models with or without regarding 2 to 1 complexation for acetic acid adsorption onto the 3.14 mol N/kg MPS at 35°C

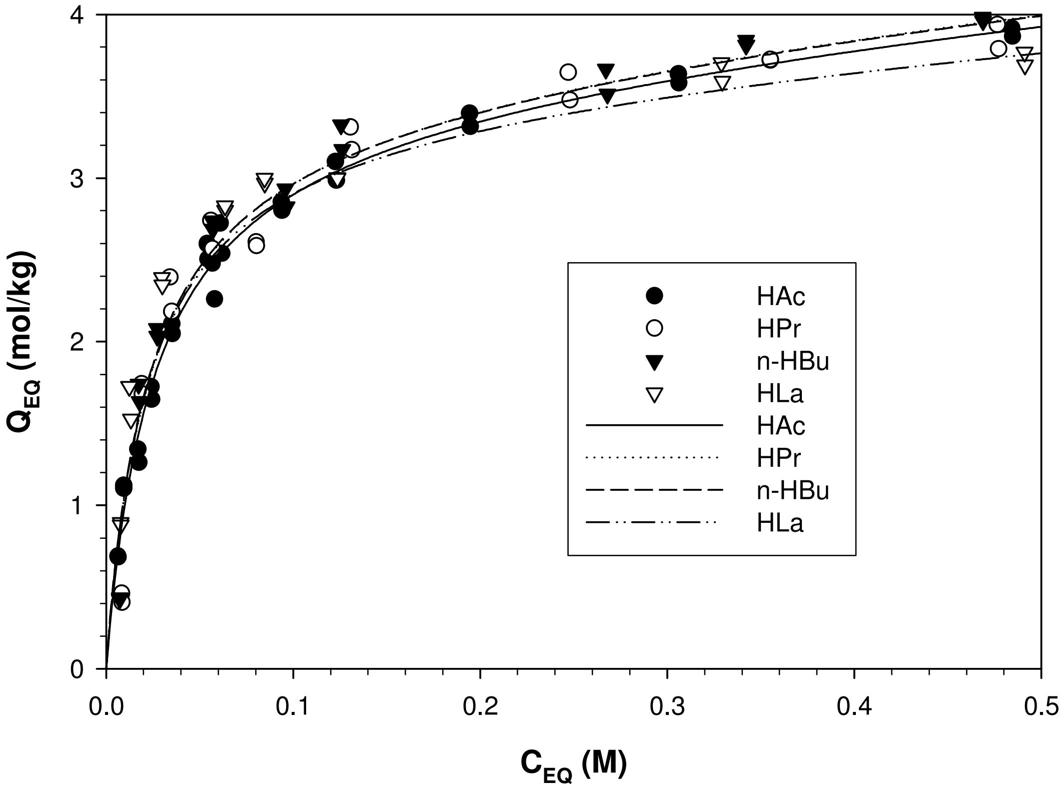

Figure 4. Adsorption isotherms of acetic, propionic, n-butyric, and lactic acids onto the 3.14 mol N/kg MPS at 35°C (The symbol and line are experimental data and the extended Langmuir model, respectively.)

Figure 4 depicts that isotherms of propionic, *n*-butyric, and lactic acids were not significantly different from that of acetic acid, indicating the carboxylic group would be the active site of adsorption to MPS. As acetic, propionic, *n*-butyric, and lactic acids are the main organic acids in acidogenesis of biomass, this result showed that the adsorption data obtained using acetic acid could be representative for the mixture of organic acids in acidogenesis (Thanakoses *et al.*, 2003; Wang *et al.*, 2006).

Acid adsorption was favored at low temperature (Figure 5). Isosteric heat of adsorption is one of the key thermodynamic variables for the design of an adsorption process (Xiao and Thomas, 2004). The isosteric heat was calculated using Clausius-Clapeyron equation as Eq (4).

$$-\Delta H_{ads} = -R\left.\frac{d(\ln C_{EQ})}{d(1/T)}\right|_{Q_{EQ}} \tag{4}$$

where ($-\Delta H_{ads}$) is the isosteric heat of adsorption (kJ mol^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the thermodynamic temperature (K).

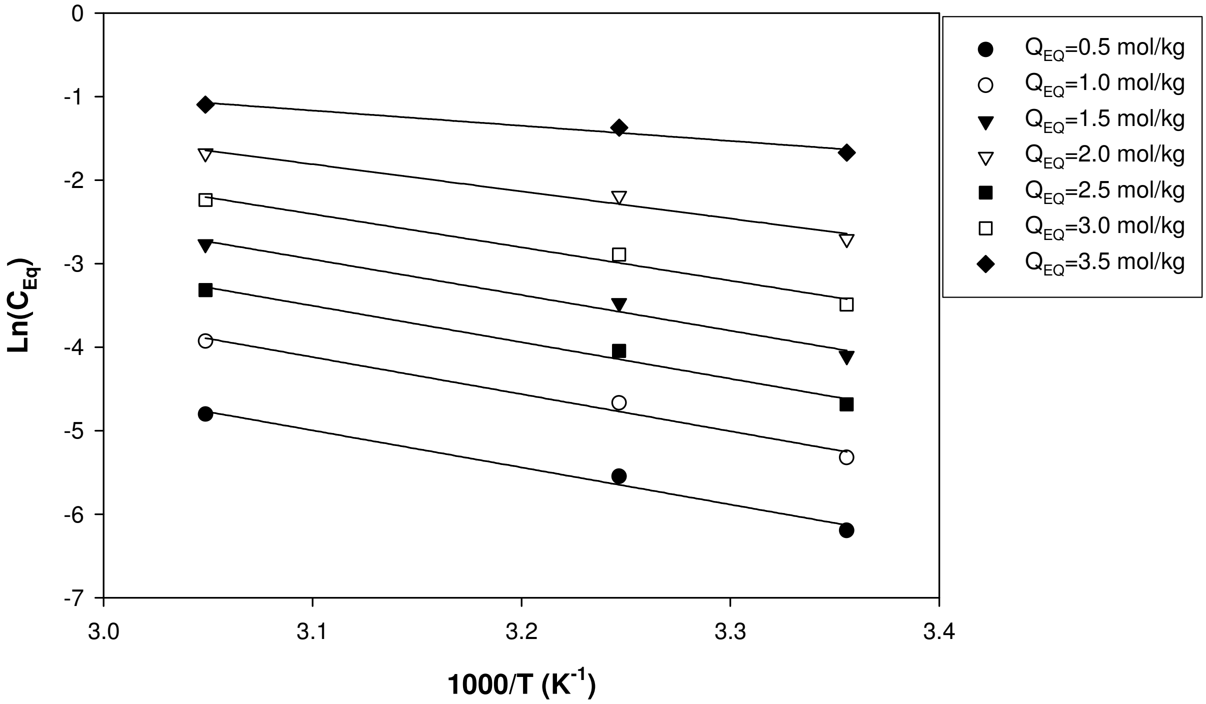

Figure 5. Effect of temperature on acetic acid adsorption onto the 3.14 mol N/kg MPS

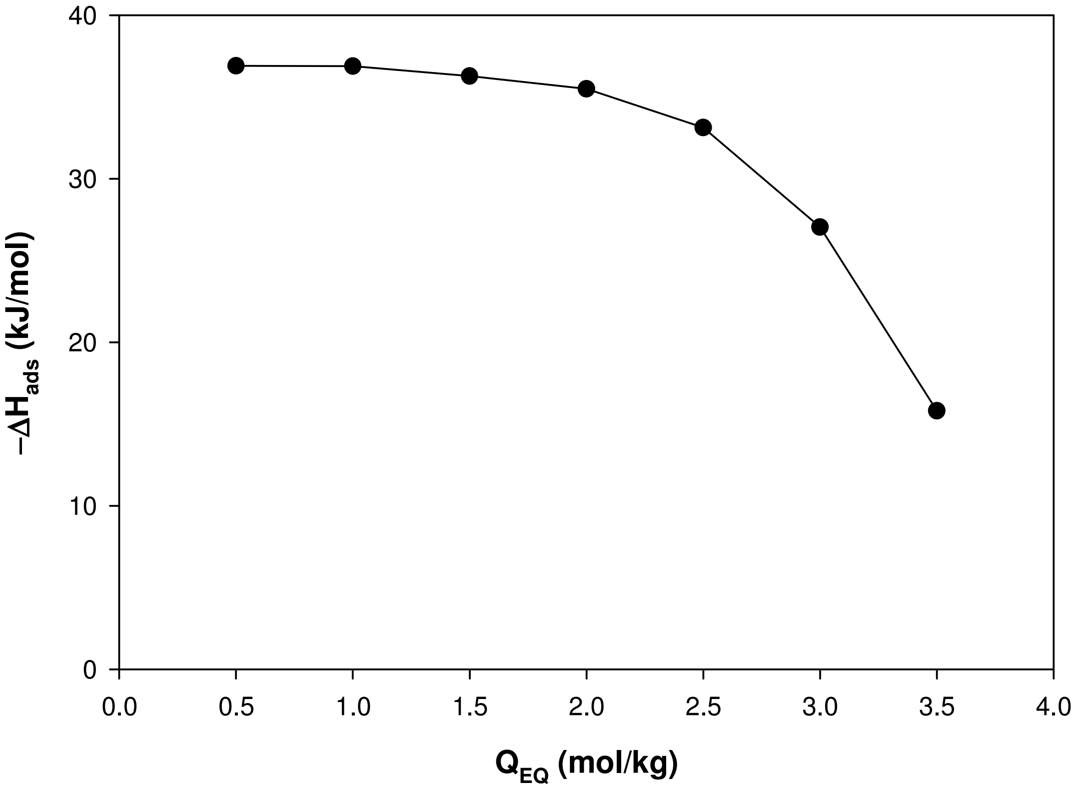

Figure 6. Isosteric heat of acetic acid adsorption onto the 3.14 mol/kg MPS

Figure 6 shows plots of the isosteric heats of adsorption with adsorbed amounts of acetic acid. The isosteric heat values varied from 36.9 to 15.8 kJ mol^{-1} with adsorbed acetic acid amounts from 0.5 to 3.5 mol HAc kg^{-1}. The positive isosteric heats (negative enthalpy) indicate that the adsorption reaction of acetic acid on MPS was exothermic. The heat values decreased with the adsorbed amount of acetic acid confirming that there were finite specific surface adsorption sites for acetic acid, an amine group in this case (Kanazawa *et al*., 2001).

Effect of pH on adsorption and desorption

Since the concentration of the unionized carboxylic acid molecules is a function of pH, adsorption would depend on pH. At low carboxylic acid loading where b_2C_{Eq} is negligible, the effects of pH on adsorption would be predicted using Eq. (5):

$$Q_{EQ} = \frac{Q_{Max}\, b_1 \frac{C_{Eq,\,T}}{1+10^{pH\text{-}pK_a}}}{1+b_1 \frac{C_{Eq,\,T}}{1+10^{pH\text{-}pK_a}}} = \frac{Q_{Max}\, b_1 \frac{C_{int}-rQ_{EQ}}{1+10^{pH\text{-}pK_a}}}{1+b_1 \frac{C_{int}-rQ_{EQ}}{1+10^{pH\text{-}pK_a}}} \qquad (5)$$

where $C_{EQ,\,T}$ (mol L^{-1}) is the equilibrium concentration of total (unionized + ionized) carboxylic acid, pK_a is carboxylic acid dissociation constant, C_{int} (mol L^{-1}) is the initial concentration of carboxylic acid, and r is the adsorbent-to-solution ratio (kg L^{-1}).

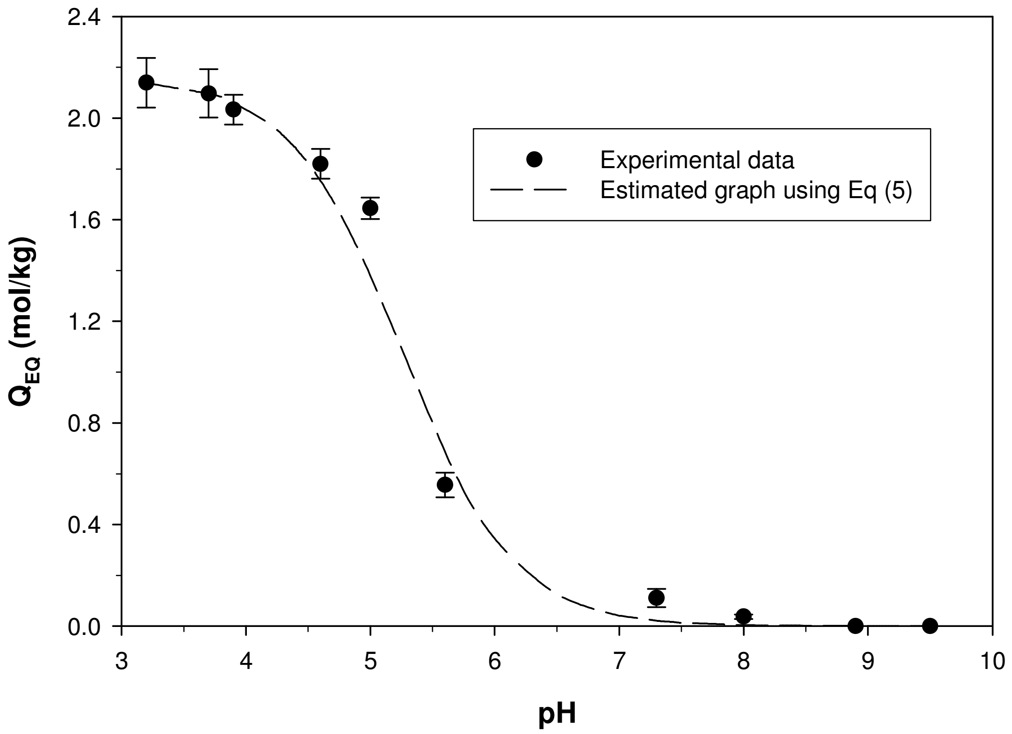

Figure 7. Effect of pH on acetic acid adsorption at initial acetic acid concentration of 50 mM

The prediction was well agreed with the experimental data at the initial acid concentration of 50 mM (R^2=0.9533, Figure 7). According to Eq. (5) and Figure 7, more than 90% of maximum adsorption would be warranted at pH < 4 in this study.

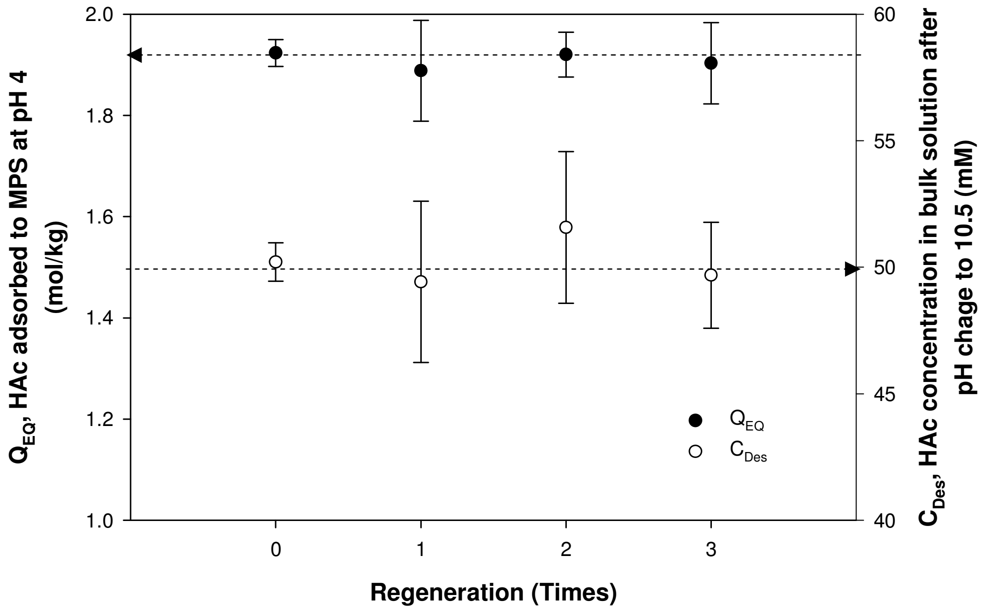

Figure 8. Acetic acid adsorption to regenerated MPS and desorbed acetic acid concentration by pH change

Figure 8 illustrates that MPS desorbed average more than 98% of adsorbed acetic (50 mM) acid in 1 min by pH adjustment to 10.5. Furthermore, the regenerated MPS showed adsorption capacity as high (>98%) as the original. This confirmed the regeneration of MPS and the extraction of adsorbed organic acid would be readily achieved by pH change.

Interference study

The suppression on carboxylic acid adsorption by coexisting matter in the bulk solution is another important factor in evaluating the applicability of adsorbents.

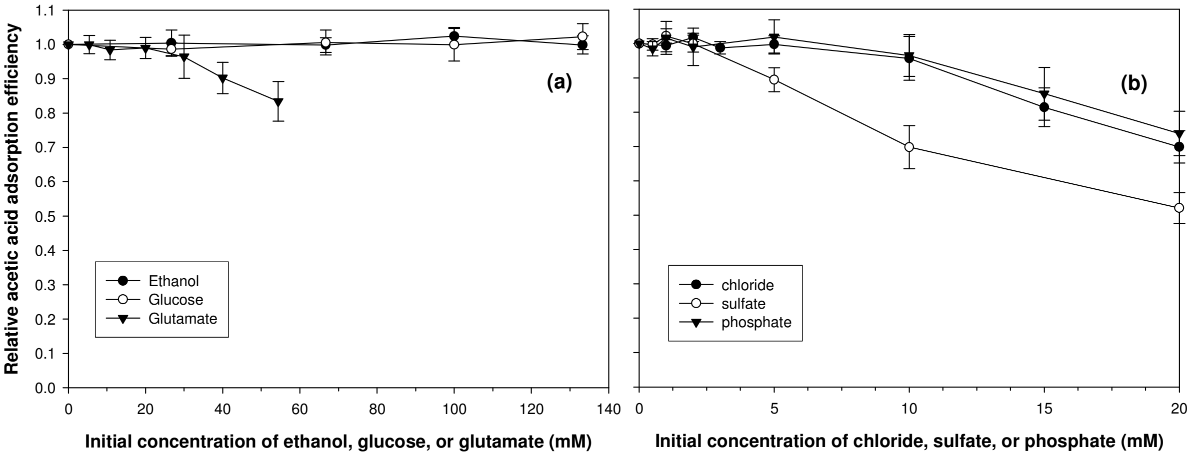

Figure 9. Effect of coexisting matters on acetic acid adsorption onto the 3.14 mol N/kg MPS at initial acetic acid concentration of 50 mM (a) ethanol, glucose, and glutamate; (b) chloride, sulfate, and phosphate

Figure 9 (a) shows the effect of common organic and inorganic fermentation matter on acetic acid adsorption at an initial acetic acid of 50 mM. MPS had high selectivity for carboxylic acid that was unaffected by the presence of other organic matters. Ethanol (at the initial concentration $\leq$133.3 mM or 6.1 g L^{-1}) and glucose (at the initial concentration $\leq$33.3 mM or 24.0 g L^{-1}) did not affect acetic acid adsorption. Glutamate, an amino acid having two carboxylic groups, slightly decreased acetic acid adsorption at an initial concentration $\geq$40 mM (5.9 g L^{-1}). At 54.4 mM (8.0 g L^{-1}), near the maximum solubility of the amino acid (0.1 g g^{-1} H_2O), the adsorption capacity was 84% of the control. The carboxylic group in the amino acid would be adsorbed onto the amine group of MPS. As glutamate is one of only two amino acids among 20 having two carboxylic groups, and the majority of carboxylic groups in protein are bound with amino groups by a peptide bond, the suppression effect of protein/amino acids in usual fermentation broth would be much less than that of glutamate.

The inorganic anions chloride (at the initial concentration $\geq$15 mM or 533 mg L^{-1}), sulfate (at the initial concentration $\geq$5 mM or 160 mg S L^{-1}), and phosphate (at the initial concentration $\geq$15 mM or 465 mg P L^{-1}) retarded acetic acid adsorption at an initial acetic acid level of 50 mM as illustrated in Figure 9 (b). The inorganic anions are regarded to be adsorbed onto an amine group as illustrated in Eq. (6).

$$\{\text{-N:}\} + H^+ + A^- \rightarrow \{\text{-NH}^+A^-\} \tag{6}$$

The authors used a competitive Langmuir adsorption isotherm as Eq. (7) to express the suppression by coexisting anions when b_2C_{Eq} was negligible.

$$Q_{EQ} = \frac{Q_{Max} b_1 C_{EQ}}{1 + b_1 C_{EQ} + b_i \gamma_i C_{EQ,i}} \tag{7}$$

where $C_{EQ,i}$ (mol L^{-1}) is the equilibrium activity of a competitor, b_i is the apparent equilibrium constant of a competitor, and γ_i is the activity coefficient of the competitor calculated using the extended DeBye-Hückel law.

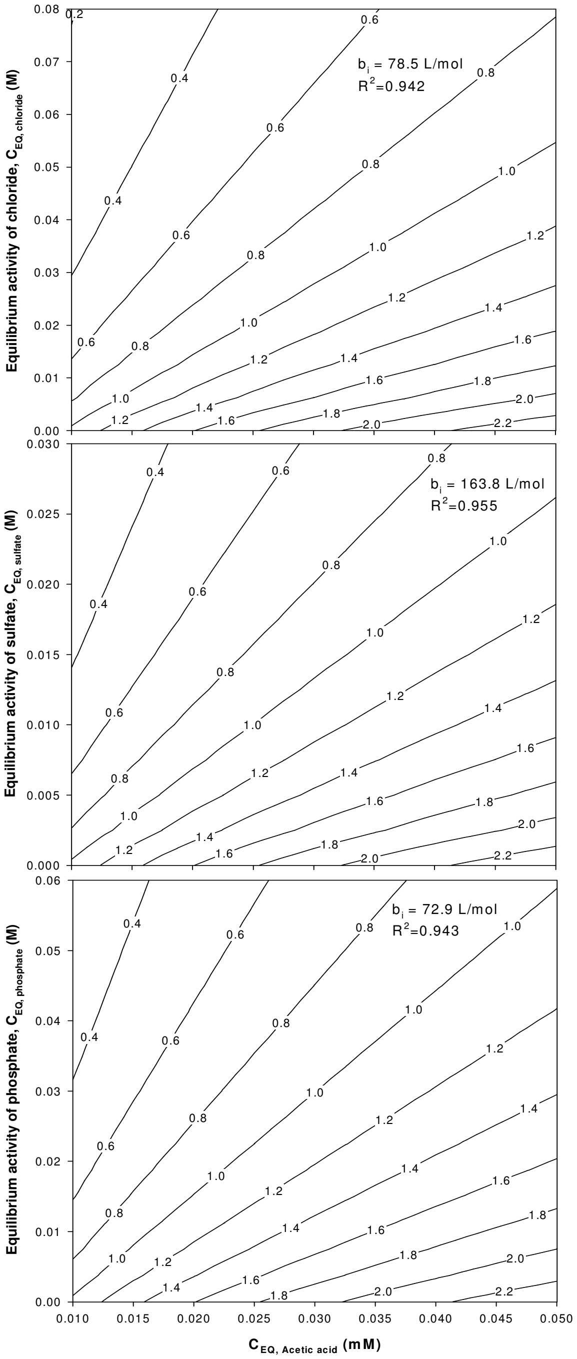

Figure 10. Contour lines of constant acetic acid adsorption onto the 3.14 mol N/kg MPS VS. equilibrium concentration of acetic acid and equilibrium activity of chloride, sulfate, and phosphate

The competitive Langmuir adsorption isotherm fitted experimental data with $R^2 >$ 0.942, implying that the inorganic anions would compete with carboxylic acids for the same adsorption sites. Apparent equilibrium constants of chloride, sulfate, and phosphate were 78.5, 163.8, 72.9 L mol^{-1}, respectively (Figure 10). The authors recommend considering the suppression effect when designing an adsorption process using MPS for fermentation liquor at high salt levels i.e., 20 mM of chloride, 20 mM of phosphate, or 10 mM of sulfate.

Kinetics of acetic acid adsorption

Absorption kinetics describing the adsorbate uptake rate is one of the important characteristics representing the adsorption efficiency of the adsorbent. The authors adopted the pseudo-second-order kinetic model (Eq. 8), which has been widely used for many chemisoportion reactions (Jang *et al.*, 2003).

$$Q_t = \frac{k_2 Q_{EQ}^2 t}{1 + k_2 Q_{EQ} t} \tag{8}$$

where Q_t (mol kg^{-1}) is the solid-phase loading of organic acids at time t, k (kg mol^{-1} min^{-1}) is the pseudo-second-order rate constant and t is time (min).

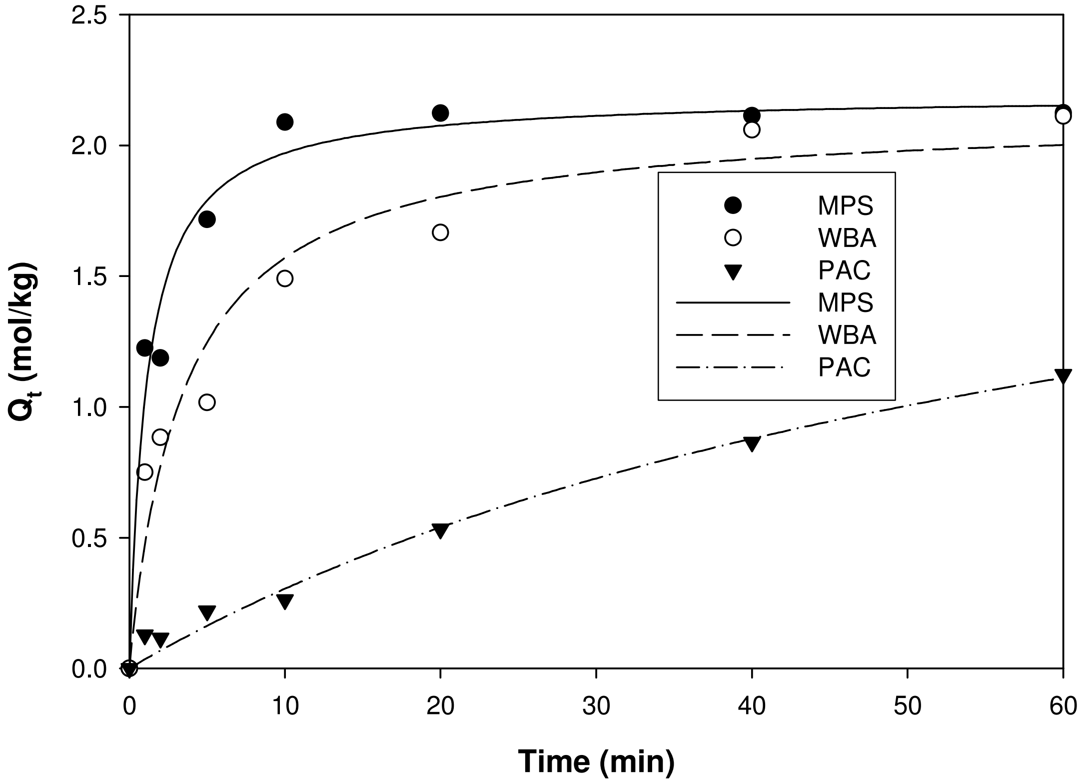

Figure 11. Kinetics of acetic acid adsorption at 35°C and initial acetic acid of 50 mM with the 3.14 mol N/kg MPS (MPS), a weak base anion exchange resin (WBA), and a powered activated carbon (PAC) (The symbol and line are experimental data and the pseudo-second-order kinetic model, respectively.)

Figure 11 shows the acetic acid adsorption kinetics for MPS with 3.14 mol N kg^{-1}, the weak base anion exchange resin, and the powdered activated carbon at initial acetic acid concentration of 0.05 M. The results were well-fitted to the pseudo-second-order model (R^2>0.89). For MPS with 3.14 mol N kg^{-1}, the adsorption reaction reached a steady state within 10 min. The pseudo-second-order rate constant of MPS was 0.41 kg mol^{-1} min^{-1}. It was higher than those of the anion exchange resin (0.14 kg mol^{-1} min^{-1}) and the activated carbon (0.06 kg mol^{-1} min^{-1}). The mesopore structure of MPS would result in the faster adsorption (Jang *et al*., 2004).

Conclusion

Carboxylic acids can be fermented from biomass and used as feedstocks for various chemical processes. The feasibility of this bioconversion depends on the recovery of carboxylic acids from fermentation liquor. The authors used MCM-41 type amine-functionalized mesoporous silica (MPS) to separate carboxylic acids by adsorption. The adsorption isotherms were analyzed with an extended Langmuir model using an overloading term. The highest acid adsorption capacity deduced was 3.38 mol kg^{-1} for 1:1 complexation at an amine density of 3.14 mol N kg^{-1}. Positive isosteric heat showed the reaction was exothermic and favored at low temperature. The adsorption reaction was pH-dependent. Greater than 90% of maximum adsorption would be achieved at $pH < 4$. Desorption/regeneration by increasing pH to 10.5 was completed within 1 min, and the regenerated MPS showed adsorption capacity equivalent to the original. MPS had a high selectivity for carboxylic acid over ethanol, glucose, and protein. The pseudo-second-order rate constant for acetic acid adsorption on MPS was 0.41 kg/mol.min, higher than that of an anion exchange resin (0.14 kg/mol.min) and activated carbon (0.06 kg/mol.min). The homogenous pore structure of MPS produces faster adsorption. The results suggest that using the amine-functionalized, MCM-41-type mesoporous silica is a favorable reclamation method for carboxylic acid fermentation.

Acknowledgement

This study was supported by a research grant (Grant number 07-02) from Iowa Energy Center (IEC), Ames, IA, USA.

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CHAPTER 4. ORGANIC ACID PRODUCTION FROM CORN STOVER USING CATTLE MANURE AS AN INOCULUM

A paper to be submitted to The Environmental Science and Technology Journal

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Abstract

Agricultural residues such as corn stover and cattle manure have high potential to be substrates of organic acid production by anaerobic fermentation. Low-molecular weight carboxylic acids have been used in many processes and have higher value compared with methane gas. The maximum organic acid yield obtained from a serum-bottle test was 0.25±0.02 g/gVS and the yield decreased with increasing substrate concentration because of inhibition of end products. The highest organic acid yield and productivity from leaching bed reactor were 0.14 g/gVS and 0.70 g/L/d, respectively at dilution rate of 0.5 d^{-1}. For semi-batch reactor, organic acid yield showed the maximum of 0.056 g/gVS at substrate concentration of 70 gVS/L and hydraulic retention time of 5 days. The maximum organic acid yield and productivity were lower than the results from serum bottle tests. This might be caused by lack of adequate mixing in a non-homogenous high corn stover content reactor. Acetic acid, propionic acid, and n-butyric acid were the majority of organic acids in all the cases.

Keywords: anaerobic digestion; organic acid; corn stover; serum bottle test; leaching bed reactor; semi-batch reactor

Introduction

Waste biomass, which has been one of the major environmental concern in the world owing to grow environmental awareness and public health concerns, may become a plentiful and inexpensive substrate for various chemical productions. The conversion of waste into useful chemicals would be an environmental sound solution to decrease the dependency on fossil fuels, reduce the global warming and lower the pollution potential (Angenent *et al.*, 2004).

A mixture of low-molecular weight carboxylic organic acids such as acetic acid, propionic acid, butyric acid, lactic acid, etc. can be easily produced from various complex organics by an indigenous mixed microbial community (Thanakoses *et al.*, 2003; Wang *et. al.*, 2006). The production of a mixture of organic acids generally does not require sterile condition, expensive tanks or enzymes. Furthermore, individual organic acids themselves are feedstock of many chemical processes and are also widely used in food industries (Wu *et al.*, 2003).

In this research, corn stover and cattle manure were used as substrate and inoculum, respectively, for producing organic acid. Acidogenic (acid forming) bioreactor using high solid content substrate i.e., corn stover, could either be designed as a complete stirred tank reactor (CSTR) or a batch leaching bed reactor. Serum bottle tests, leaching bed and semi-batch reactors were studied to evaluate organic acid yields and productivities.

Materials and Methods

Cattle manure was obtained from Iowa State University Beef Nutrition Farm in Ames, IA. Corn stover was also gathered from the same farm at theoretical length of cut

(TLC) of 12.7 mm (Art's way MFG, IA). As collected from ground after harvest, the corn stover contained a small quantity of dirt. Volatile solid (VS) of corn stover and cattle manure were 87.6±0.6 and 13.2±0.1% (w/w), respectively. Table 4 shows characteristics of the corn stover and the cattle manure (inoculum).

Table 4. Characteristics of the corn stover and the cattle manure

Parameters	Corn stover	Cattle manure
TS (%, w/w)	87.6±0.6	13.2±0.1
VS (%, w/w)	76.7±1.6	10.7±0.1
COD (g/kg)	83.6±10.4	133.3±6.2
VSS (g/kg)	-	87.7±17.2
VFA (g/kg)	-	6.4±0.6
Alkalinity (g/kg as $CaCO_3$)	-	3.21±0.24

Serum bottle test

A part of corn stover was ground with a hammer mill (Speedy Jr. Winona Attrition Mill Co., MN) and passed a 2-mm screen sieve and used as "ground stover" in this research. The experiment was conducted using 24 serum bottles each with 250 ml at six (two by three) different conditions and they were quadruplicates of each condition. The VS concentrations of corn stover at 9, 18, and 45 g/L were tested as substrate for non-ground and ground conditions. Corn stover to cattle manure ratio was fixed as 9:1 by VS basis. Besides, twelve serum bottles were also used to serve as "blanks" containing only cattle manure of 1, 2, and 5 gVS/L in quadruplicates. Sodium bicarbonate of 4.8 g/L as $CaCO_3$ was maintained as a pH buffer after distilled water was added into the serum bottles to adjust the working volume to 200 ml. An initial pH was adjusted to 6.5 using 6 N HCl. Then, the bottles were purged with nitrogen gas, capped, and placed on a shaker rotating at 180 rpm in a 35°C incubator. Two milliliters of supernatant sample from two bottles of the quadruple was taken using syringe to be analyzed for organic acids and pH at 10 to 30 hours intervals during a 7-day test period. Sample withdrawals from bottles were alternated to minimize volume reduction so that the final working volume after the test was more than 190 ml (less than 5% reduction) at any case.

Leaching bed reactor

A Plexiglas leaching bed reactor is constructed with a working volume of 2.0 liters (liquid depth of 113 mm and inner diameter of 150 mm), operated temperature was maintained at 35±1°C. To collect the leachate, a perforated plate and glass beads were installed as an under drain system in the bottom part.

Corn stover and cattle manure were stacked alternately to obtain VS concentration of 53 g/L. The ratio of VS from corn stover and cattle manure was maintained to be 9:1 by VS basis. Tab water containing 0.5 or 1g $NaHCO_3$/L, was added to adjust the working volume to 2 liters. The headspace of the reactor was purged with nitrogen gas, capped and maintain at oxygen free condition for 6 hours. Then, dilution water was provided continuously at designed dilution rates of 0.5,1 and 2 d^{-1}. The effluent was sampled daily from the reactors for analysis.

Semi-batch reactor

The semi-batch reactors with a working volume of 0.5 liters (liquid depth of 80 mm and inner diameter of 90 mm), were conducted and operated in a gyratory water bath at 35±1°C. The reactors were filled with corn stover, cattle manure and distilled water for making the VS concentration to be 28, 49 and 70 g/L, then capped, purged with nitrogen gas and shaken at 200 rpm. The ratio of corn stover to cattle manure was maintained at 9:1 by VS basis. Predetermined amount of the reactor content was replaced by corn stover and cattle manure and purged with nitrogen gas once a day to maintain the designed hydraulic retention time (HRT). Buffering chemical 0.1 g $NaHCO_3$/g$VS_{substrate}$ was added with the substrate when pH was decreased below 5.5.

Analytical method

VS and TS contents were analyzed following Standard Methods for the Examination of Water and Wastewater (APHA *et. al.*, 1998). Liquid samples were filtered by 0.22 µm syringe filter tip before volatile fatty acid (VFA) analyzing. VFA was analyzed by high performance liquid chromatographer(GP40, Diomex CA) with an absorbance detector (AD 20, Dionex) and a 300 mm x 7.8 mm Metacarb 67H column (Varian, CA) using 0.05 M H_2SO_4 as mobile phase. pH of samples were measured by a dual channel pH/ion meter (AR25, Fisher Scientific).

Results and Discussion

Serum bottle tests

Organic acid production reached to the maximum within seven days as shown in Figure 12. Acetic, propionic, and n-butyric acids accounted for 55 to 63%, 22 to 28%, and 7 to 13% of total organic acids as weight basis, respectively. Isobutyric, n-valeric, isovaleric, formic, and lactic acids were also detected as shown in Figure 13. Methane was found after 2-4 days of incubation, but the amount was less than 0.015 g CH_4-COD/$gVS_{substrate}$.

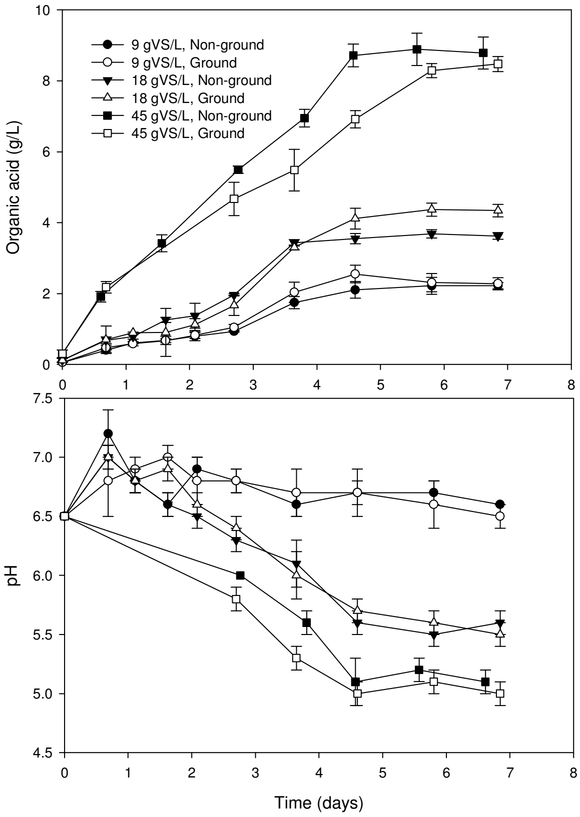

Figure 12. Organic acid concentration and pH versus incubation time in serum bottle test using corn stover and cattle manure (The VS concentrations in legends are for corn stover fed.)

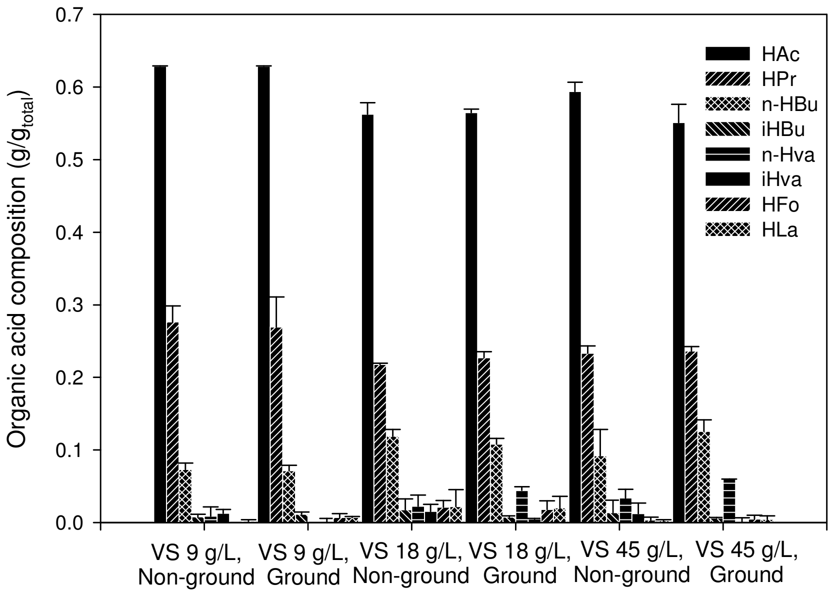

Figure 13. Organic acid compositions from corn stover/cattle manure in serum bottle tests

Table 5 summarized organic acid yields from serum bottle tests. The differences between the yields calculated for corn stover plus cattle manure and corn stover only were insignificant. The maximum yield, 0.26 $g/gVS_{corn\ stover}$, was achieved at 9 gVS/L using ground corn stover. The yield was equivalent to 64% of the highest reported values of 0.42 g/gVS (Thanakoses *et al.*, 2003; Hu *et al.*, 2005). However, the yield was achieved without any external fermentation medium except $NaHCO_3$, expensive terrestrial inoculum like rumen fluid, or chemical pretreatment, which were necessary in the previous studies. The current approach for corn stover acidogenesis by addition of animal manure would be a competitive way due to the costs saving in pretreatment. Organic acids yield decreased as substrate concentration increased. It would be caused by either pH drop or product inhibition of organic acids (Wu and Yang, 2003).

Another interesting finding is that acid fermentation yields of the non-ground corn stovers were above 82% of the yield of the ground one under the same VS concentration. Most existing cellulosic biomass fermentation technologies use grinding as a pre-processing to increase surface area for bacterial contact and to enhance hydrolysis. Anyway, grinding cost is one of the biggest burdens in cellulosic biomass use (Sokhansanj, 2006). The result of this study implied that excessive grinding might not be needed in organic acid production from corn stover.

Table 5. Organic acid yield of corn stover and cattle manure

Corn stover VS (g/L)	Corn stover type	Organic acid yield[a]	
		g/gVS$_{\text{corn stover}}$[b]	g/gVS$_{\text{total}}$[c]
9	Non-ground	0.22±0.01	0.22±0.01
	Ground	0.26±0.02	0.25±0.02
18	Non-ground	0.18±0.01	0.18±0.01
	Ground	0.22±0.01	0.22±0.01
45	Non-ground	0.18±0.01	0.18±0.01
	Ground	0.17±0.00	0.17±0.00

[a] n=4

[b] ((Maximum organic acid in a condition)-(Maximum organic acid in blank))/(gVS of corn stover fed)

[c] (Maximum organic acid in a condition)/((gVS of corn stover fed) + (gVS of cattle manure fed))

Leaching bed reactor

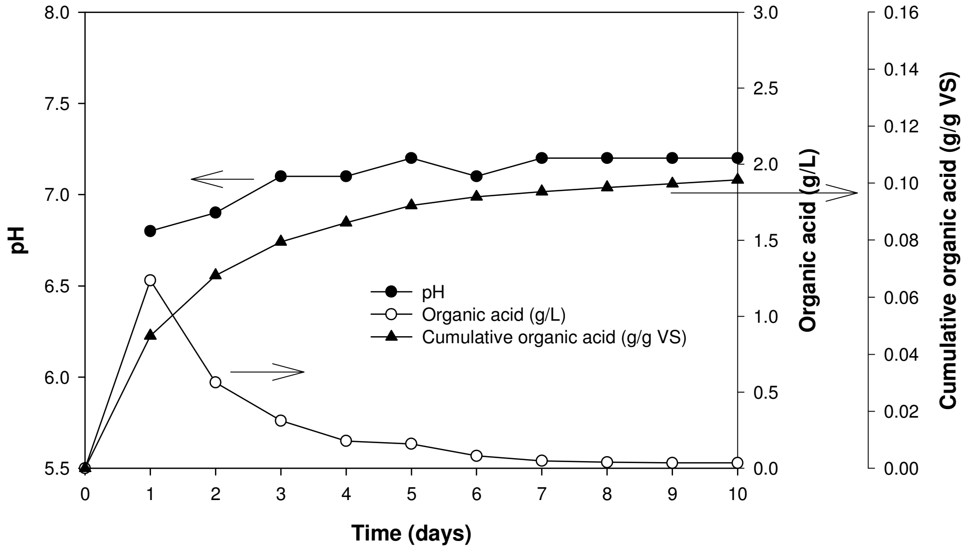

Figure 14. Variation of pH, organic acid concentration and the cumulative organic acid production in leaching bed at the dilution rate of 2 d^{-1}

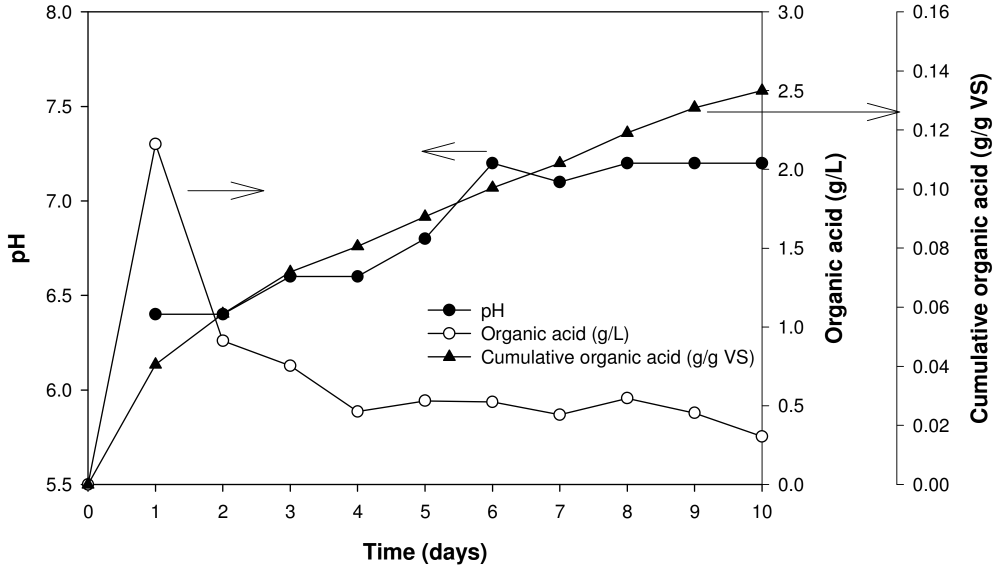

Figure 15. Variation of pH, organic acid concentration and the cumulative organic acid production in leaching bed at the dilution rate of 1 d^{-1}

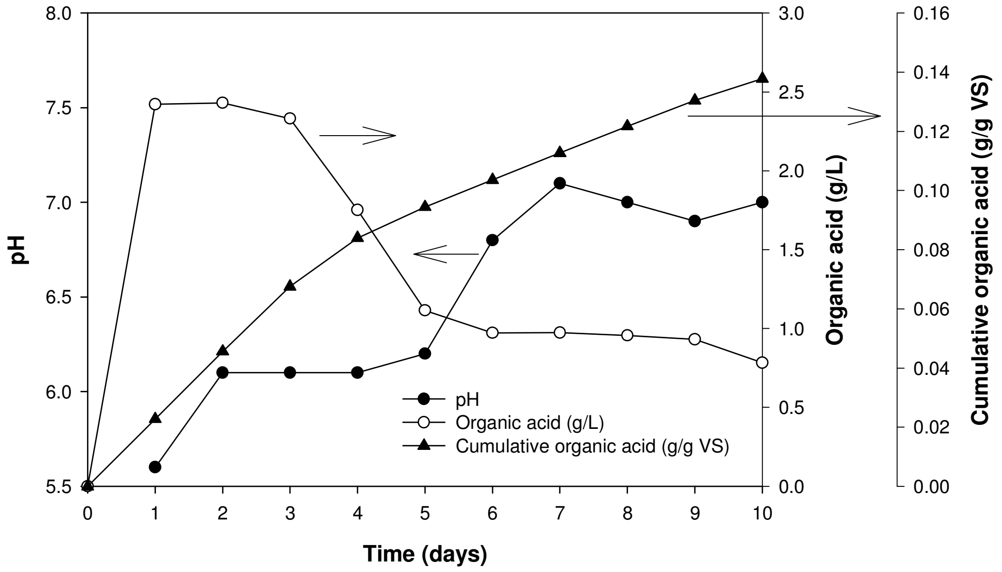

Figure 16. Variation of pH, organic acid concentration and the cumulative organic acid production in leaching bed at the dilution rate of 0.5 d^{-1}

Figures 14-16 show the variations of pH, organic acid concentration and the cumulative organic acid production during acidogenic fermentation at different dilution rates of 2, 1, and 0.5 d^{-1}. At the dilution rate of 2 d^{-1}, the highest VFA concentration of 1.23 g/L, was found at day 1. Organic acid concentrations continuously decreased after day 2 and reach to a steady production level after day 5. Organic acid yield and productivity were 0.10 g/gVS and 0.52 g/L/d, respectively. The value is lower than 0.26 g/gVS, the maximum value from batch operation. Unlike the serum bottle test, there is no mixing in reactor operation. The intimate of contact between substrate and microorganism might not be sufficient. As pH during the operation was above 6.8 (higher than 5.5-6.5 which is preferable pH range for acidogenic microorganism), it was assumed that the microbial washout was greater than the microbial proliferation (Han and Shin, 2002). With this hypothesis, when dilution rate was decreased to 1 and 0.5 d^{-1} the results show better efficiency. At the dilution rate of 0.5 d^{-1}, organic acid yield and productivity were increase to 0.14 g/gVS and 0.70 g/L/d, respectively. High organic acid concentration, more than 95% of maximum, was maintained within the first three days while pH was maintained over 5.6. In addition, continuous organic acid production lasted 10 days. When the amount of $NaHCO_3$ in dilution water was reduced to 0.5 g/L, organic acid yield did not change significantly as shown in Figure 17.

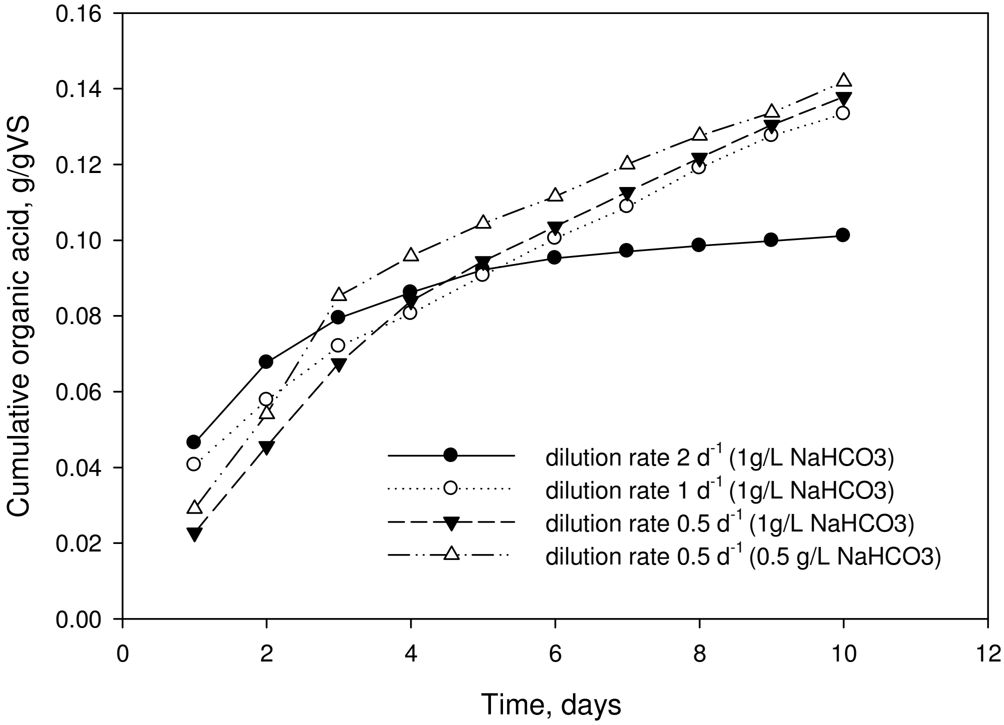

Figure 17. Cumulative organic acid production in leaching bed reactors

Semi-batch reactor

Table 6. Average organic acid yields and productivities in semi-batch reactor operations

VS	dilution rate	HRT	Average organic acid		
			concentration	yield	productivity
g/L	1/d	d	g/l	g/gVS added	g/L/d
28	0.2	5.0	1.072	0.038	0.214
28	0.4	2.5	0.899	0.032	0.360
49	0.1	10.0	2.701	0.055	0.270
49	0.3	3.3	2.324	0.047	0.697
49	0.5	2.0	1.875	0.038	0.937
70	0.2	5.0	3.953	0.056	0.791
70	0.4	2.5	3.297	0.047	1.319

Table 6 shows that the organic acid yield decrease with decreasing HRT and increase with increasing initial VS concentration. The results indicated that microorganism could be washed out at the low HRT and at VS 70 g/L, the substrate was not completely degraded. So base on this trend, higher substrate concentration should present the higher organic acid yield. However, due to the physical limitation of corn stover at high concentration, the higher substrate concentration could not be tested.

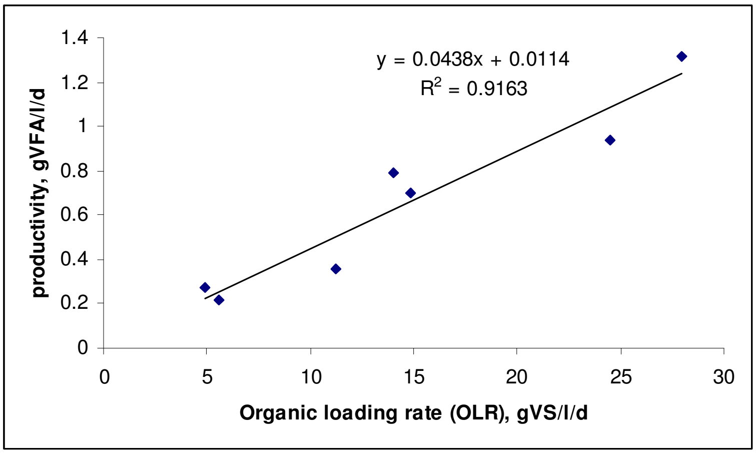

Figure 18. Relationship between organic loading rate and organic acid productivity

From Figure 18, the organic acid productivity shows a linear increasing trend with the organic loading rate (OLR). The maximum VFA productivity is 1.32 g/L/d which achieved at the run with initial substrate of 70 gVS/L and HRT of 2.5 days. pH of the mixed liquor fluctuated between 5.2 and 6.4 during the runs. Moreover, these were higher than literature suggested value of 0.89 g/L/d using corn stover as substrate (Thanakoses *et al*. 2003).

However, the results of this study showed the maximum VFA yield of semi-continuous batch reactor was much lower than those form serum bottles test (0.056 g/gVS and 0.22 g/gVS, respectively). This might be because the mixing intensity of semi-continuous batch reactors was much lower than serum bottle tests. The physical property of corn stover in the reactor could affect the efficiency of mixing and result in the low contact between substrate and microorganism.

Like the serum bottle tests and the leaching bed reactor test, the majorities of organic acids generated in semi-batch study were acetic acid, propionic acid and n-butyric acid.

Conclusion

For serum bottle tests, the maximum acid production occurred within 7 days. The amount of methane produced is negligible in this test. The maximum organic acid yield is 0.25±0.02 g/gVS with corn stover concentration of 9 gVS/L. However, organic acid yield decrease with increasing VS concentration. It might be caused by the end product inhibition. Moreover, the organic acid yield for non ground corn stover was not significant lower than the yield for ground corn stover. Therefore, the excessive grinding step might be neglected.

The results from leaching bed reactor operations showed that the higher organic acid production could be observed at lower dilution rate i.e., 0.5 d^{-1}. Because this operating condition could prevent short circuiting and washing out of microorganism. Nevertheless, organic acid yields found from leaching bed reactors were lower than maximum value from serum bottle test. It might be explained by the low mixing level of this kind of reactor was comparing serum bottle. So the contact between substrate and microorganism is much lower than serum bottle test.

The result form semi-batch reactors also showed the same trend. Even organic acid yield increase with increasing VS, the reactor could not be operated with VS higher than 70 gVS/L. Because physical limitation of the of corn stover which could cause the difficulty in the operation of this type of reactor. Organic acid productivity also increases in parallel to the increasing of VS concentration.

In all three studies, acetic acid, propionic acid, and n-butyric acid are the main organic acids in the effluent.

Acknowledgements

This study was supported by a research grant (Grant number 07-02) from Iowa Energy Center (IEC), Ames, IA, USA.

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Wu, Z., & Yang, S.-T. (2003). Extractive fermentation for butyric acid production from glucose by Clostridium tyrobutyricum. *Biotechnol. Bioeng., 82*, 93-102.

CHAPTER 5. GENERAL CONCLUSIONS

In this study, the amine-functionalized mesoporous silica (MPS) was used as adsorbent to study the optimization of carboxylic acid adsorption. The highest acid adsorption capacity was found in MPS with amine density of 3.14 mol N kg $^{-1}$. The adsorption isotherm could be well described using an overloading Langmuir model. The adsorption reaction did not depend on pH and favored at low temperature. MPS showed the outstanding desorption by adjusting pH to 10.5 within 1 minute. High concentrating of interferences including sulfate, phosphate, and chloride could affect the carboxylic acid adsorption process. Moreover, MPS showed the higher pseudo-second-order rate constant than an anion exchange resin and activated carbon. The optimum operating conditions for organic acid were studied using serum bottle tests, leaching bed reactors and semi-batch reactors. Corn stover and cattle manure, the agricultural residue, were used as substrate and inoculum, respectively. From the serum bottle test the maximum organic acid yield could be reached with in a week. The organic acid yields in leaching bed reactors were lower than the yields from serum bottle tests because of lack of adequate mixing of this kind of reactor. The low dilution rate operation showed the higher organic acid yield because of the lower microorganism washing out. For semi-batch reactor, the organic acid yield increase relating to the increasing of substrate VS concentration so did organic acid productivity.

Recommendations for Future Research

The synthesis wastewater was used to represent the effluent from anaerobic bioreactor in this study. However, in reality, the effluent might has much different characteristics compared with synthesis wastewater. The concentration of organic acids and other interferences would vary depending on the operating condition. So the studies with effluent from continuous acid reactor should be evaluated before scaling up to full scale adsorption column.

For leaching bed reactor, effluent recycling might improve mixing effectiveness which could enhance organic acid yield and productivity. Also dilution rate lower than 0.5 d^{-1} and $NaHCO_3$ lower than 0.5 g/L might be studied.

Because of the physical limitation of corn stover in this study, other types of reactor which have higher volume and more effective mixing i.e., mechanical agitator might be used in semi-batch reactor study instead of 0.5 liter-bottle and gyratory water bath. These might allow researchers studying with higher substrate concentrations.

APPENDIX A

The analysis results of MPS physical characteristics are presented in this part.

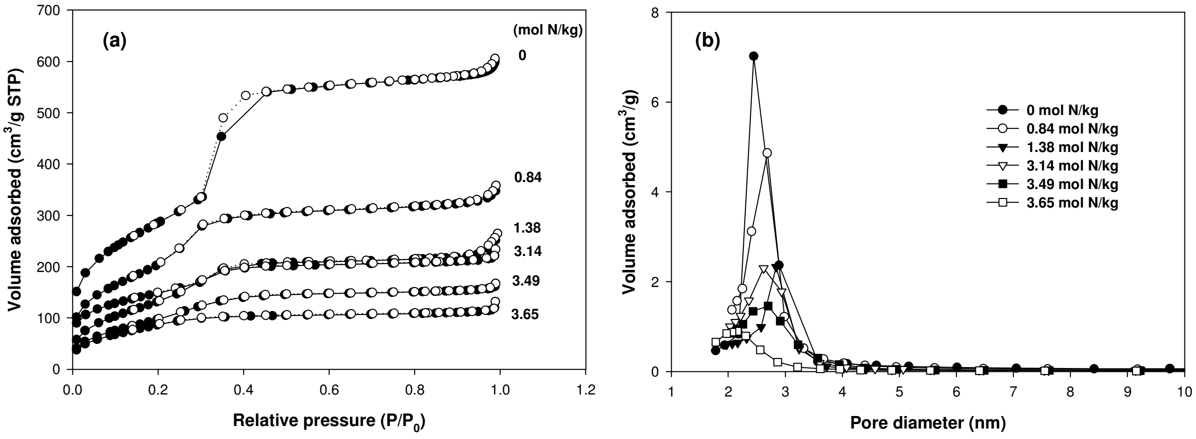

Figure A1. (a) BET isotherm and (b) BJH pore distribution of amine-functionalized MPS and pure MPS

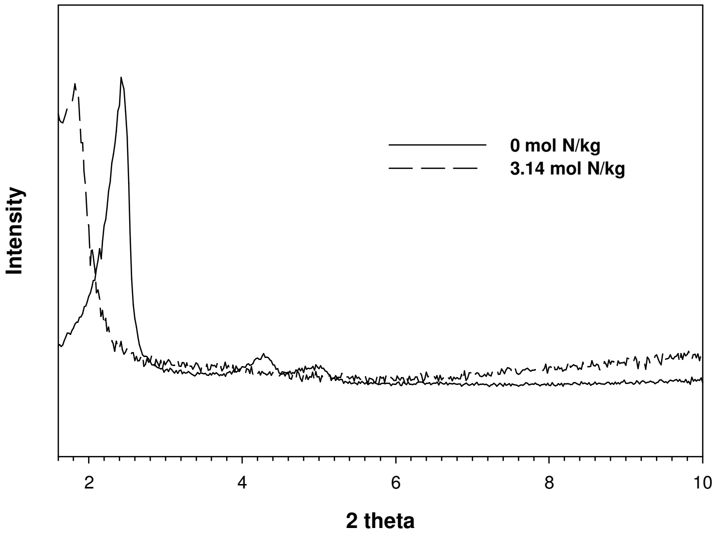

Figure A2. X-ray diffraction patterns of MPS before and after functionalization with amine group

APPENDIX B

Raw experimental data from leaching bed reactor and semi-batch reactor studies are presented in this part.

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
10/31/08	1	6.79	0.57	0.57	0.53	0.34	0.12	0.08	0.03	0.00	0.00	0.01	0.00	58.75	20.63	14.15	4.77	0.00	0.00	1.71	0.00
11/1/08	2	5.95	1.03	1.61	0.99	0.62	0.27	0.10	0.03	0.00	0.00	0.00	0.01	60.36	26.02	9.36	2.82	0.00	0.00	0.00	1.44
11/2/08	3	5.42	1.45	3.06	1.34	0.83	0.41	0.10	0.01	0.08	0.00	0.00	0.02	57.35	28.32	6.60	0.82	5.49	0.00	0.00	1.42
11/3/08	4	6.56	0.95	4.00	0.92	0.58	0.28	0.06	0.01	0.02	0.00	0.00	0.00	61.15	29.25	6.25	1.15	1.70	0.00	0.00	0.50
11/5/08	6	5.06	1.33	5.33	1.33	0.90	0.35	0.08	0.00	0.00	0.00	0.00	0.00	67.81	26.10	6.08	0.00	0.00	0.00	0.00	0.00
11/6/08	7	5.71	0.93	6.26	0.89	0.59	0.24	0.06	0.01	0.03	0.00	0.00	0.00	63.55	26.14	6.16	1.43	2.72	0.00	0.00	0.00
11/7/08	8	5.15	1.00	7.26	1.00	0.71	0.23	0.06	0.00	0.00	0.00	0.00	0.00	71.04	23.21	5.75	0.00	0.00	0.00	0.00	0.00
11/8/08	9	5.70	0.92	8.18	0.92	0.65	0.22	0.05	0.00	0.00	0.00	0.00	0.00	70.87	23.76	5.37	0.00	0.00	0.00	0.00	0.00
11/9/08	10	5.19	1.28	9.47	1.23	0.84	0.32	0.07	0.00	0.05	0.00	0.00	0.00	65.12	25.12	5.27	0.27	4.17	0.00	0.00	0.06
11/10/08	11	5.99	0.84	10.31	0.83	0.57	0.22	0.04	0.00	0.01	0.00	0.00	0.00	67.38	26.70	4.37	0.00	1.55	0.00	0.00	0.00
11/11/08	12	5.28	0.89	11.19	0.85	0.58	0.24	0.04	0.01	0.02	0.00	0.00	0.01	65.18	27.04	4.08	1.16	1.79	0.00	0.00	0.76
11/12/08	13	6.01	1.19	12.38	1.19	0.83	0.31	0.05	0.00	0.00	0.00	0.00	0.00	69.53	25.99	4.48	0.00	0.00	0.00	0.00	0.00
11/13/08	14	5.61	0.89	13.27	0.84	0.58	0.23	0.04	0.01	0.04	0.00	0.00	0.00	65.32	25.53	3.97	1.09	4.09	0.00	0.00	0.00
11/14/08	15	5.32	0.80	14.07	0.80	0.55	0.21	0.04	0.00	0.00	0.00	0.00	0.00	68.30	26.27	5.43	0.00	0.00	0.00	0.00	0.00
11/15/08	16	6.17	0.70	14.77	0.68	0.46	0.19	0.04	0.00	0.02	0.00	0.00	0.00	66.05	26.48	5.08	0.21	2.19	0.00	0.00	0.00
11/16/08	17	5.52	0.99	15.76	0.99	0.66	0.26	0.06	0.00	0.00	0.00	0.00	0.00	67.39	26.23	6.38	0.00	0.00	0.00	0.00	0.00
11/17/08	18	6.40	1.23	16.99	1.23	0.84	0.31	0.07	0.00	0.00	0.00	0.00	0.00	68.55	25.48	5.98	0.00	0.00	0.00	0.00	0.00
11/18/08	19	5.66	1.05	18.04	0.98	0.67	0.24	0.07	0.02	0.05	0.00	0.00	0.00	63.91	23.27	6.51	1.49	4.81	0.00	0.00	0.00
11/19/08	20	5.46	1.05	19.08	0.95	0.65	0.23	0.08	0.02	0.08	0.00	0.00	0.00	61.97	21.54	7.60	1.55	7.33	0.00	0.00	0.00

Fig B1 Results of semibatch operation at VS 28 g/L and HRT 2.5 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
1/21/08	1	6.48	1.04	1.04	1.02	0.74	0.24	0.03	0.00	0.00	0.00	0.00	0.02	71.68	23.09	3.13	0.00	0.00	0.00	0.00	2.10
1/22/08	2	6.15	1.34	2.37	1.29	0.93	0.32	0.04	0.00	0.03	0.00	0.00	0.02	69.53	23.82	3.05	0.00	2.10	0.00	0.00	1.51
1/23/08	3	5.76	1.35	3.73	1.35	0.98	0.33	0.04	0.00	0.00	0.00	0.00	0.01	72.06	24.18	3.21	0.00	0.00	0.00	0.00	0.54
1/24/08	4	5.59	1.46	5.19	1.40	1.02	0.32	0.05	0.00	0.05	0.00	0.00	0.02	70.01	22.16	3.24	0.00	3.41	0.00	0.00	1.18
1/25/08	5	5.42	1.41	6.60	1.36	0.98	0.32	0.06	0.00	0.04	0.00	0.00	0.01	69.40	22.44	4.03	0.00	3.09	0.00	0.00	1.04
1/26/08	6	5.76	1.28	7.88	1.24	0.89	0.29	0.05	0.00	0.04	0.00	0.00	0.00	69.76	22.98	3.91	0.00	3.27	0.00	0.00	0.08
1/27/08	7	5.35	1.40	9.28	1.40	1.00	0.33	0.06	0.00	0.00	0.00	0.00	0.00	71.62	23.77	4.61	0.00	0.00	0.00	0.00	0.00
1/28/08	8	5.90	1.36	10.64	1.36	0.97	0.33	0.06	0.00	0.00	0.00	0.00	0.00	71.20	24.21	4.59	0.00	0.00	0.00	0.00	0.00
1/29/08	9	5.62	1.21	11.85	1.21	0.88	0.28	0.06	0.00	0.00	0.00	0.00	0.00	72.27	23.08	4.65	0.00	0.00	0.00	0.00	0.00
1/30/08	10	6.11	1.49	13.35	1.48	1.06	0.36	0.07	0.00	0.00	0.01	0.00	0.00	70.60	24.07	4.43	0.26	0.00	0.65	0.00	0.00
1/31/08	11	5.69	1.45	14.79	1.45	1.04	0.35	0.06	0.00	0.00	0.00	0.00	0.00	71.77	24.05	4.18	0.00	0.00	0.00	0.00	0.00
2/1/08	12	5.49	1.36	16.16	1.36	0.97	0.34	0.06	0.01	0.00	0.00	0.00	0.00	70.76	24.73	4.09	0.42	0.00	0.00	0.00	0.00
2/2/08	13	6.02	1.45	17.60	1.44	1.00	0.37	0.06	0.01	0.00	0.00	0.00	0.00	69.35	25.82	4.36	0.48	0.00	0.00	0.00	0.00
2/3/08	14	5.77	1.32	18.92	1.31	0.89	0.36	0.06	0.00	0.00	0.01	0.00	0.00	67.52	27.70	4.33	0.00	0.00	0.45	0.00	0.00
2/4/08	15	5.64	0.96	19.88	0.92	0.61	0.29	0.02	0.04	0.00	0.00	0.00	0.00	63.85	30.56	1.91	3.68	0.00	0.00	0.00	0.00
2/5/08	16	6.20	1.38	21.25	1.37	0.83	0.47	0.07	0.01	0.00	0.00	0.00	0.00	60.20	34.01	5.13	0.66	0.00	0.00	0.00	0.00
2/6/08	17	6.14	1.24	22.49	1.23	0.69	0.48	0.07	0.01	0.00	0.00	0.00	0.00	55.42	38.82	5.29	0.46	0.00	0.00	0.00	0.00
2/7/08	18	6.00	1.14	23.63	1.08	0.50	0.51	0.07	0.00	0.06	0.00	0.00	0.00	44.24	44.66	5.79	0.00	5.32	0.00	0.00	0.00
2/8/08	19	6.02	0.78	24.41	0.77	0.30	0.43	0.05	0.01	0.00	0.00	0.00	0.00	37.95	54.94	6.38	0.73	0.00	0.00	0.00	0.00
2/9/08	20	5.75	1.05	25.46	1.04	0.44	0.53	0.06	0.00	0.00	0.00	0.00	0.00	42.39	51.04	6.13	0.44	0.00	0.00	0.00	0.00

Fig B2 Results of semibatch operation at VS 28 g/L and HRT 5 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
11/21/08	1	6.25	1.26	1.26	1.08	0.64	0.26	0.18	0.01	0.01	0.02	0.11	0.03	51.06	20.60	14.05	0.92	1.10	1.20	8.52	2.55
11/22/08	2	5.58	2.48	3.74	2.30	1.43	0.69	0.18	0.04	0.07	0.00	0.00	0.08	57.67	27.84	7.10	1.42	2.87	0.00	0.00	3.10
11/23/08	3	5.07	2.03	5.77	2.02	1.17	0.58	0.27	0.00	0.01	0.00	0.00	0.00	57.54	28.68	13.11	0.00	0.61	0.00	0.00	0.07
11/24/08	4	6.14	2.73	8.50	2.64	1.58	0.75	0.30	0.01	0.03	0.01	0.00	0.04	57.77	27.60	11.13	0.40	1.01	0.46	0.00	1.65
11/25/08	5	5.23	2.28	10.79	2.15	1.33	0.58	0.24	0.01	0.11	0.00	0.00	0.01	58.07	25.29	10.69	0.44	4.93	0.00	0.00	0.58
11/26/08	6	5.98	2.27	13.06	2.19	1.37	0.59	0.24	0.02	0.06	0.00	0.00	0.01	60.18	25.78	10.37	0.74	2.56	0.00	0.00	0.36
11/27/08	7	5.70	2.53	15.59	2.48	1.62	0.64	0.22	0.02	0.04	0.00	0.00	0.00	63.99	25.16	8.74	0.63	1.48	0.00	0.00	0.00
11/28/08	8	5.21	1.97	17.56	1.89	1.24	0.47	0.18	0.01	0.07	0.00	0.00	0.00	63.20	23.82	9.05	0.55	3.38	0.00	0.00	0.00
11/29/08	9	6.07	2.26	19.82	2.16	1.46	0.52	0.18	0.01	0.08	0.01	0.00	0.00	64.40	23.01	7.95	0.58	3.65	0.41	0.00	0.00
12/1/08	11	5.17	2.32	22.13	2.18	1.44	0.56	0.18	0.03	0.11	0.00	0.00	0.00	62.32	24.01	7.69	1.10	4.88	0.00	0.00	0.00
12/2/08	12	6.18	1.80	23.93	1.72	1.11	0.48	0.13	0.02	0.04	0.02	0.00	0.00	61.75	26.77	6.98	1.09	2.35	1.06	0.00	0.00
12/3/08	13	5.43	1.73	25.65	1.67	1.04	0.50	0.13	0.01	0.05	0.00	0.00	0.00	60.01	29.11	7.55	0.67	2.66	0.00	0.00	0.00
12/4/08	14	6.36	2.02	27.67	1.92	1.22	0.58	0.12	0.02	0.05	0.02	0.00	0.00	60.61	28.57	6.04	0.90	2.70	1.19	0.00	0.00
12/5/08	15	5.70	2.16	29.83	1.99	1.24	0.62	0.13	0.01	0.13	0.02	0.00	0.00	57.67	28.97	5.87	0.60	5.87	1.02	0.00	0.00
12/6/08	16	5.20	1.68	31.50	1.63	1.03	0.47	0.13	0.00	0.05	0.00	0.00	0.00	61.50	28.17	7.49	0.00	2.84	0.00	0.00	0.00
12/7/08	17	6.31	1.24	32.75	1.23	0.81	0.34	0.08	0.00	0.01	0.00	0.00	0.00	65.25	27.00	6.69	0.00	1.05	0.00	0.00	0.00
12/8/08	18	5.40	1.16	33.90	1.16	0.77	0.32	0.07	0.00	0.00	0.00	0.00	0.00	66.50	27.29	6.20	0.00	0.00	0.00	0.00	0.00
12/9/08	19	6.60	0.96	34.87	0.96	0.67	0.25	0.05	0.00	0.00	0.00	0.00	0.00	69.11	26.13	4.76	0.00	0.00	0.00	0.00	0.00
12/10/08	20	5.65	1.56	36.43	1.48	0.98	0.41	0.08	0.00	0.08	0.01	0.00	0.00	62.96	26.46	5.07	0.00	4.94	0.57	0.00	0.00

Fig B3 Results of semibatch operation at VS 49 g/L and HRT 2 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
10/31/08	1	6.75	1.46	1.46	1.32	0.83	0.29	0.21	0.11	0.00	0.02	0.01	0.00	56.59	19.90	14.09	7.38	0.00	1.48	0.56	0.00
11/1/08	2	6.14	1.98	3.44	1.85	1.16	0.54	0.15	0.06	0.02	0.00	0.00	0.04	58.91	27.25	7.47	3.19	1.11	0.00	0.00	2.07
11/2/08	3	5.46	2.34	5.77	2.27	1.41	0.73	0.14	0.02	0.00	0.00	0.00	0.04	60.22	31.08	5.89	1.07	0.00	0.00	0.00	1.74
11/3/08	4	6.15	1.85	7.62	1.84	1.18	0.53	0.12	0.01	0.00	0.00	0.00	0.00	63.79	28.69	6.72	0.80	0.00	0.00	0.00	0.00
11/4/08	5	5.59	2.28	9.90	2.18	1.38	0.58	0.22	0.04	0.03	0.02	0.00	0.00	60.43	25.38	9.77	1.85	1.53	1.04	0.00	0.00
11/5/08	6	5.02	2.00	11.90	1.96	1.26	0.54	0.16	0.02	0.00	0.00	0.00	0.02	63.25	26.85	8.16	0.88	0.00	0.00	0.00	0.85
11/6/08	7	5.90	3.14	15.05	3.10	2.05	0.77	0.27	0.02	0.00	0.01	0.00	0.02	65.35	24.42	8.73	0.59	0.00	0.32	0.00	0.59
11/7/08	8	5.18	2.06	17.11	2.05	1.39	0.46	0.19	0.00	0.00	0.00	0.00	0.01	67.58	22.47	9.41	0.00	0.00	0.00	0.00	0.54
11/8/08	9	5.63	2.54	19.64	2.47	1.67	0.55	0.24	0.01	0.06	0.00	0.00	0.00	65.84	21.85	9.60	0.37	2.34	0.00	0.00	0.00
11/9/08	10	5.19	2.59	22.23	2.52	1.69	0.57	0.26	0.02	0.05	0.00	0.00	0.00	65.45	21.84	9.99	0.74	1.98	0.00	0.00	0.00
11/10/08	11	5.74	2.65	24.88	2.47	1.61	0.61	0.25	0.03	0.13	0.02	0.00	0.00	60.66	23.16	9.52	1.15	4.85	0.66	0.00	0.00
11/11/08	12	5.33	2.75	27.63	2.50	1.60	0.64	0.26	0.04	0.17	0.02	0.00	0.01	58.37	23.34	9.43	1.53	6.29	0.72	0.00	0.31
11/12/08	13	5.88	2.34	29.96	2.21	1.45	0.55	0.22	0.04	0.06	0.02	0.00	0.00	61.97	23.49	9.26	1.62	2.76	0.90	0.00	0.00
11/13/08	14	5.61	2.49	32.45	2.25	1.44	0.60	0.22	0.05	0.15	0.02	0.00	0.01	57.78	24.08	8.67	1.97	6.18	0.95	0.00	0.37
11/14/08	15	5.38	2.09	34.54	1.90	1.20	0.52	0.19	0.04	0.12	0.02	0.00	0.00	57.38	24.72	8.99	2.10	5.81	0.99	0.00	0.00
11/15/08	16	6.13	1.61	36.16	1.53	0.96	0.43	0.14	0.03	0.03	0.02	0.00	0.00	59.53	26.54	8.84	1.75	2.15	1.20	0.00	0.00
11/16/08	17	5.60	2.20	38.35	2.07	1.29	0.59	0.19	0.05	0.05	0.03	0.00	0.00	58.55	27.02	8.63	2.12	2.27	1.42	0.00	0.00
11/17/08	18	6.40	2.36	40.72	2.16	1.34	0.65	0.17	0.05	0.12	0.03	0.00	0.00	56.64	27.47	7.33	2.15	5.11	1.30	0.00	0.00
11/18/08	19	5.87	2.47	43.19	2.26	1.42	0.67	0.17	0.05	0.14	0.02	0.00	0.00	57.33	27.24	6.87	2.12	5.58	0.86	0.00	0.00
11/19/08	20	5.57	2.02	45.21	1.91	1.17	0.58	0.15	0.04	0.06	0.02	0.00	0.00	58.22	28.64	7.66	1.94	2.78	0.76	0.00	0.00

Fig B4 Results of semibatch operation at VS 49 g/L and HRT 3.33 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
10/31/08	1	6.75	1.46	1.46	1.32	0.83	0.29	0.21	0.11	0.00	0.02	0.01	0.00	56.59	19.90	14.09	7.38	0.00	1.48	0.56	0.00
11/1/08	2	6.14	1.98	3.44	1.85	1.16	0.54	0.15	0.06	0.02	0.00	0.00	0.04	58.91	27.25	7.47	3.19	1.11	0.00	0.00	2.07
11/2/08	3	5.46	2.34	5.77	2.27	1.41	0.73	0.14	0.02	0.00	0.00	0.00	0.04	60.22	31.08	5.89	1.07	0.00	0.00	0.00	1.74
11/3/08	4	6.15	1.85	7.62	1.84	1.18	0.53	0.12	0.01	0.00	0.00	0.00	0.00	63.79	28.69	6.72	0.80	0.00	0.00	0.00	0.00
11/4/08	5	5.59	2.28	9.90	2.18	1.38	0.58	0.22	0.04	0.03	0.02	0.00	0.00	60.43	25.38	9.77	1.85	1.53	1.04	0.00	0.00
11/5/08	6	5.02	2.00	11.90	1.96	1.26	0.54	0.16	0.02	0.00	0.00	0.00	0.02	63.25	26.85	8.16	0.88	0.00	0.00	0.00	0.85
11/6/08	7	5.90	3.14	15.05	3.10	2.05	0.77	0.27	0.02	0.00	0.01	0.00	0.02	65.35	24.42	8.73	0.59	0.00	0.32	0.00	0.59
11/7/08	8	5.18	2.06	17.11	2.05	1.39	0.46	0.19	0.00	0.00	0.00	0.00	0.01	67.58	22.47	9.41	0.00	0.00	0.00	0.00	0.54
11/8/08	9	5.63	2.54	19.64	2.47	1.67	0.55	0.24	0.01	0.06	0.00	0.00	0.00	65.84	21.85	9.60	0.37	2.34	0.00	0.00	0.00
11/9/08	10	5.19	2.59	22.23	2.52	1.69	0.57	0.26	0.02	0.05	0.00	0.00	0.00	65.45	21.84	9.99	0.74	1.98	0.00	0.00	0.00
11/10/08	11	5.74	2.65	24.88	2.47	1.61	0.61	0.25	0.03	0.13	0.02	0.00	0.00	60.66	23.16	9.52	1.15	4.85	0.66	0.00	0.00
11/11/08	12	5.33	2.75	27.63	2.50	1.60	0.64	0.26	0.04	0.17	0.02	0.00	0.01	58.37	23.34	9.43	1.53	6.29	0.72	0.00	0.31
11/12/08	13	5.88	2.34	29.96	2.21	1.45	0.55	0.22	0.04	0.06	0.02	0.00	0.00	61.97	23.49	9.26	1.62	2.76	0.90	0.00	0.00
11/13/08	14	5.61	2.49	32.45	2.25	1.44	0.60	0.22	0.05	0.15	0.02	0.00	0.01	57.78	24.08	8.67	1.97	6.18	0.95	0.00	0.37
11/14/08	15	5.38	2.09	34.54	1.90	1.20	0.52	0.19	0.04	0.12	0.02	0.00	0.00	57.38	24.72	8.99	2.10	5.81	0.99	0.00	0.00
11/15/08	16	6.13	1.61	36.16	1.53	0.96	0.43	0.14	0.03	0.03	0.02	0.00	0.00	59.53	26.54	8.84	1.75	2.15	1.20	0.00	0.00
11/16/08	17	5.60	2.20	38.35	2.07	1.29	0.59	0.19	0.05	0.05	0.03	0.00	0.00	58.55	27.02	8.63	2.12	2.27	1.42	0.00	0.00
11/17/08	18	6.40	2.36	40.72	2.16	1.34	0.65	0.17	0.05	0.12	0.03	0.00	0.00	56.64	27.47	7.33	2.15	5.11	1.30	0.00	0.00
11/18/08	19	5.87	2.47	43.19	2.26	1.42	0.67	0.17	0.05	0.14	0.02	0.00	0.00	57.33	27.24	6.87	2.12	5.58	0.86	0.00	0.00
11/19/08	20	5.57	2.02	45.21	1.91	1.17	0.58	0.15	0.04	0.06	0.02	0.00	0.00	58.22	28.64	7.66	1.94	2.78	0.76	0.00	0.00

Fig B5 Results of semibatch operation at VS 49g/L and HRT 10 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
1/21/08	1	7.02	2.72	2.72	2.64	1.91	0.58	0.15	0.03	0.00	0.02	0.00	0.04	70.20	21.47	5.39	0.95	0.00	0.61	0.00	1.37
1/22/08	2	6.25	3.48	6.20	3.33	2.31	0.83	0.18	0.02	0.05	0.03	0.00	0.06	66.39	23.94	5.28	0.45	1.48	0.74	0.00	1.71
1/23/08	3	5.42	3.60	9.79	3.46	2.41	0.83	0.22	0.00	0.06	0.02	0.00	0.06	66.88	23.08	6.20	0.00	1.59	0.59	0.00	1.66
1/24/08	4	6.02	3.01	12.80	2.91	2.02	0.73	0.17	0.00	0.04	0.01	0.00	0.04	67.04	24.17	5.60	0.00	1.45	0.30	0.00	1.44
1/25/08	5	5.46	2.80	15.60	2.74	1.91	0.66	0.16	0.00	0.03	0.00	0.00	0.03	68.35	23.73	5.65	0.00	1.03	0.00	0.00	1.24
1/26/08	6	5.81	2.76	18.36	2.68	1.86	0.67	0.15	0.00	0.05	0.00	0.00	0.03	67.40	24.22	5.42	0.00	1.83	0.00	0.00	1.13
1/27/08	7	5.30	3.39	21.75	3.31	2.31	0.79	0.21	0.00	0.05	0.01	0.00	0.02	68.25	23.17	6.13	0.00	1.41	0.31	0.00	0.73
1/28/08	8	6.09	4.32	26.07	4.13	2.77	1.12	0.24	0.00	0.13	0.03	0.00	0.02	64.17	26.03	5.53	0.00	3.08	0.62	0.00	0.57
1/29/08	9	5.41	2.87	28.94	2.85	1.92	0.76	0.17	0.00	0.01	0.01	0.00	0.00	66.83	26.36	5.92	0.00	0.48	0.41	0.00	0.00
1/30/08	10	6.49	3.40	32.34	3.30	2.24	0.88	0.18	0.03	0.05	0.02	0.00	0.01	65.73	25.84	5.31	0.77	1.40	0.69	0.00	0.26
1/31/08	11	5.76	3.80	36.14	3.72	2.52	1.00	0.20	0.01	0.06	0.02	0.00	0.00	66.18	26.36	5.38	0.19	1.46	0.42	0.00	0.00
2/1/08	12	5.32	2.39	38.53	2.34	1.57	0.63	0.14	0.00	0.04	0.01	0.00	0.00	65.79	26.27	5.81	0.00	1.54	0.59	0.00	0.00
2/2/08	13	6.40	3.74	42.27	3.59	2.39	0.98	0.22	0.02	0.08	0.04	0.00	0.00	63.99	26.19	5.88	0.56	2.23	1.16	0.00	0.00
2/3/08	14	5.55	3.63	45.90	3.48	2.25	0.98	0.25	0.00	0.11	0.04	0.00	0.00	62.08	26.96	6.91	0.00	2.95	1.10	0.00	0.00
2/4/08	15	5.20	3.31	49.21	3.17	1.98	0.91	0.28	0.00	0.12	0.03	0.00	0.00	59.61	27.32	8.60	0.00	3.53	0.94	0.00	0.00
2/5/08	16	6.33	3.07	52.29	2.92	1.80	0.83	0.29	0.02	0.11	0.03	0.00	0.00	58.46	27.01	9.37	0.51	3.57	1.08	0.00	0.00
2/6/08	17	5.76	3.60	55.89	3.43	2.10	0.98	0.35	0.02	0.12	0.03	0.00	0.00	58.40	27.27	9.60	0.45	3.39	0.89	0.00	0.00
2/7/08	18	5.23	2.93	58.82	2.81	1.71	0.80	0.31	0.02	0.07	0.03	0.00	0.00	58.32	27.22	10.56	0.55	2.45	0.91	0.00	0.00
2/8/08	19	6.25	3.02	61.84	2.84	1.70	0.85	0.29	0.02	0.12	0.04	0.00	0.00	56.24	28.17	9.47	0.79	4.10	1.23	0.00	0.00
2/9/08	20	5.74	3.72	65.56	3.52	2.14	1.04	0.33	0.02	0.13	0.04	0.00	0.00	57.69	27.96	9.00	0.60	3.61	1.14	0.00	0.00

Fig B6 Results of semibatch operation at VS 70 g/L and HRT 2.5 days

Date	Time (d)	pH	Concentration, g/L											%							
			Total OA	Cumulative	HAc+HPr+Hbu	9.7	11.5	14.3	13.1	21	16.7	9.0	8.2								
						HAc	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*	*HAc*	*HPr*	*HBu*	*i-HBu*	*HVa*	*i-HVa*	*HFo*	*HLa*
11/21/08	1	6.83	1.70	1.70	1.54	0.93	0.32	0.28	0.00	0.00	0.01	0.13	0.03	54.39	19.08	16.72	0.00	0.00	0.86	7.40	1.56
11/22/08	2	6.80	3.32	5.02	3.21	2.08	0.87	0.27	0.04	0.00	0.02	0.00	0.04	62.62	26.09	8.01	1.23	0.00	0.74	0.00	1.31
11/23/08	3	5.81	3.32	8.34	3.06	1.91	0.89	0.26	0.03	0.18	0.00	0.00	0.05	57.44	26.88	7.85	0.77	5.47	0.00	0.00	1.59
11/24/08	4	5.41	3.64	11.98	3.43	2.10	0.99	0.33	0.03	0.14	0.00	0.00	0.04	57.78	27.24	9.15	0.88	3.95	0.00	0.00	1.01
11/25/08	5	5.83	4.44	16.42	4.20	2.57	1.20	0.44	0.03	0.19	0.01	0.00	0.00	57.91	26.96	9.90	0.78	4.17	0.28	0.00	0.00
11/26/08	6	5.55	3.68	20.10	3.48	2.08	0.96	0.45	0.03	0.17	0.00	0.00	0.00	56.54	26.05	12.14	0.75	4.52	0.00	0.00	0.00
11/27/08	7	5.38	3.67	23.76	3.46	2.06	0.89	0.50	0.02	0.18	0.00	0.00	0.00	56.34	24.36	13.61	0.57	5.00	0.12	0.00	0.00
11/28/08	8	5.38	3.66	27.42	3.42	2.01	0.83	0.58	0.03	0.20	0.02	0.00	0.00	54.82	22.70	15.81	0.70	5.33	0.63	0.00	0.00
11/29/08	9	5.68	4.01	31.43	3.83	2.22	0.92	0.69	0.03	0.12	0.04	0.00	0.00	55.27	23.06	17.11	0.73	2.91	0.92	0.00	0.00
12/1/08	11	5.30	4.37	35.80	4.19	2.37	1.01	0.81	0.03	0.11	0.03	0.00	0.00	54.20	23.19	18.53	0.78	2.50	0.79	0.00	0.00
12/2/08	12	5.84	3.82	39.63	3.68	2.06	0.93	0.68	0.04	0.08	0.04	0.00	0.00	53.80	24.44	17.89	0.98	1.97	0.92	0.00	0.00
12/3/08	13	5.65	4.25	43.88	4.10	2.27	1.10	0.72	0.05	0.07	0.03	0.00	0.00	53.31	25.99	17.05	1.11	1.75	0.80	0.00	0.00
12/4/08	14	5.41	3.87	47.74	3.72	2.02	1.06	0.64	0.04	0.07	0.03	0.00	0.00	52.38	27.36	16.47	1.12	1.83	0.83	0.00	0.00
12/5/08	15	5.67	4.48	52.23	4.24	2.30	1.25	0.69	0.05	0.16	0.03	0.00	0.00	51.29	27.94	15.30	1.12	3.59	0.77	0.00	0.00
12/6/08	16	5.37	3.96	56.19	3.73	2.03	1.13	0.56	0.05	0.16	0.03	0.00	0.00	51.28	28.60	14.24	1.20	3.96	0.72	0.00	0.00
12/7/08	17	5.95	3.86	60.04	3.61	1.98	1.14	0.49	0.05	0.15	0.05	0.00	0.00	51.46	29.53	12.69	1.23	3.90	1.19	0.00	0.00
12/8/08	18	5.51	3.79	63.84	3.57	1.98	1.14	0.45	0.04	0.14	0.04	0.00	0.00	52.33	30.00	11.82	1.14	3.63	1.07	0.00	0.00
12/9/08	19	5.90	4.23	68.07	3.95	2.17	1.30	0.47	0.05	0.19	0.05	0.00	0.00	51.24	30.81	11.21	1.23	4.42	1.08	0.00	0.00
12/10/08	20	5.67	3.48	71.55	3.24	1.76	1.08	0.40	0.04	0.17	0.04	0.00	0.00	50.49	30.90	11.51	1.12	4.80	1.18	0.00	0.00

Fig B7 Results of semibatch operation at VS 70 g/L and HRT 5 days

Table B8. Results from leaching bed reactor: dilution rate 2d^{-1} and $NaHCO_3$ 1 g/L

Time	pH	VFA (g/L)	VFA (g/l/d)	cumulative VFA (g/g VS)	VFA productivity (g/L/d)
1	6.90	1.2355	2.4710	0.0464	2.3828
2	6.80	0.5642	1.1284	0.0677	1.7354
3	7.10	0.3126	0.6252	0.0794	1.3579
4	7.10	0.1792	0.3584	0.0861	1.1048
5	7.20	0.1603	0.3207	0.0922	0.9457
6	7.10	0.0808	0.1616	0.0952	0.8141
7	7.20	0.0488	0.0975	0.0970	0.7112
8	7.20	0.0387	0.0775	0.0985	0.6316
9	7.20	0.0350	0.0700	0.0998	0.5689
10	7.20	0.0351	0.0703	0.1011	0.5188

Table B9. Results from leaching bed reactor: dilution rate 1d^{-1} and $NaHCO_3$ 1 g/L

Time	pH	VFA (g/L)	VFA (g/L/d)	cumulative VFA (g/gVS)	VFA productivity (g/L/d)
1	6.40	2.1606	2.1606	0.0406	2.0834
2	6.40	0.9122	0.9122	0.0578	1.4815
3	6.80	0.7542	0.7542	0.0719	1.2301
4	6.80	0.4624	0.4624	0.0806	1.0341
5	7.30	0.5313	0.5313	0.0906	0.9297
6	7.50	0.5230	0.5230	0.1004	0.8588
7	7.50	0.4425	0.4425	0.1088	0.7971
8	7.70	0.5474	0.5474	0.1191	0.7634
9	7.80	0.4538	0.4538	0.1276	0.7272
10	7.80	0.3043	0.3043	0.1333	0.6839

Table B10. Results from leaching bed reactor: dilution rate 0.5 d^{-1} and $NaHCO_3$ 1 g/L

Time	pH	VFA (g/L)	VFA (g/L/d)	cumulative VFA (g/gVS)	VFA productivity (g/L/d)
1	5.60	2.4213	1.2107	0.0228	1.1674
2	6.10	2.4302	1.2151	0.0456	1.1696
3	6.10	2.3309	1.1655	0.0675	1.1543
4	6.10	1.7516	0.8758	0.0840	1.0769
5	6.20	1.1143	0.5572	0.0944	0.9689
6	7.10	0.9725	0.4862	0.1036	0.8856
7	6.80	0.9731	0.4865	0.1127	0.8261
8	6.50	0.9558	0.4779	0.1217	0.7804
9	7.40	0.9312	0.4656	0.1305	0.7436
10	6.90	0.7833	0.3916	0.1378	0.7070

Table B11. Results from leaching bed reactor: dilution rate 0.5 d^{-1} and $NaHCO_3$ 0.5 g/L

Time	pH	VFA (g/L)	VFA (g/L/d)	cumulative VFA (g/gVS)	VFA productivity (g/L/d)
1	5.26	3.0925	1.5462	0.0291	-
2	5.30	2.6622	1.3311	0.0541	2.77
3	5.15	3.3151	1.6575	0.0852	2.19
4	4.70	1.1163	0.5581	0.0957	1.64
5	4.56	0.9219	0.4610	0.1044	1.34
6	4.80	0.7584	0.3792	0.1115	1.14
7	4.65	0.9037	0.4518	0.1200	1.03
8	5.16	0.8049	0.4025	0.1276	0.94
9	5.45	0.6465	0.3232	0.1337	0.86
10	5.40	0.8676	0.4338	0.1418	0.81
11	5.43	0.6297	0.3149	0.1477	0.76
12	5.42	0.6903	0.3452	0.1542	0.72
13	5.62	0.7034	0.3517	0.1608	0.69
14	5.60	0.6853	0.3426	0.1673	0.66
15	5.65	0.6797	0.3399	0.1737	0.64
16	5.50	0.6203	0.3102	0.1795	0.61

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my thanks to those who helped me with various aspects of conducting research and the writing of this thesis. First and foremost, I thank Dr. Shihwu Sung for his guidance, patience and support throughout this research and the writing of this thesis. His insights and words of encouragement have often inspired me and renewed my hopes for completing my graduate education. I would also like to thank my committee members for their efforts and contributions to this work: Dr. Hans van Leeuwen and Dr. Shang-Yi Lin. I would additionally like to thank Dr. Sang-Hyon Kim for his guidance throughout the research work and Assoc. Prof. Prasert Rerkkriangkrai, Energy Research and Development Institute, Chiang Mai University, Thailand, for his supports during my study. Finally, I would like to thank my family in Thailand especially Mr. Chainun, my father and Mrs. Kaneungsuk, my grand mother. Without them, I could not have this greatest chance in my life, studying at Iowa State University.