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Optimizing the Removal of Microbial Inhibitors From Steam Exploded Lignocellulosic Biomass to Improve Hydrogen Production

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By

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Abstract

Biohydrogen (bio-H_2) is a possible future alternative energy source. Hydrogen (H_2) derived from cheap agriculture feedstocks is a necessary requirement for economical full-scale production. Solubilizing cellulose, hemicellulose and other organic components in low value feedstocks is accomplished by processes such as acid treatment or steam explosion. During pretreatment, microbial inhibitors such as furfural and hydroxymethyl furfural (HMF) are generated from hexose and pentose sugars. A commercially available ion-exchange resin (XAD-4) was used to remove furan inhibitors from liquor derived from pretreating low value agriculture residues. A surface response model was used to predict inhibitor concentrations after 360 minutes of treatment. Experiments were conducted to demonstrate the impact of treating switchgrass liquor with XAD-4 resin on the H_2 yield. Treated and untreated switchgrass liquor generated maximum yields of 2.25±0.14 and 1.80±0.11 mol H_2/mol glucose, respectively. In comparison, a yield of 2.14±0.21 mol H_2/mol glucose was detected in cultures fed pure glucose.

Dedication

I dedicate this thesis to my loving and patient family: Rick, Susan, Mike and Meghan Kachler.

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Nomenclature

ADP	adenosine di-phosphate
ATP	adenosine tri-phosphate
Co-A	Coenzyme A
CSTRs	continuously stirred tank reactors
Fd	Ferrodoxin
GC	gas chromatography
HPLC	high pressure liquid chromatography
HRT	hydraulic retention time
HMF	hydroxymethyl furfural
IC	ion exchange chromatography
LA	linoleic acid
LCFA	long chain fatty acid
mol	moles
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
TCD	thermal conductivity detector
TSS	total suspended solids
VFA	volatile fatty acid
VSS	volatile suspended solids
µmol	micromol (10^{-6})

Chapter 1: Introduction

1.1 Background Information

Over the past century, fossil fuels have been used to meet the global energy demand. Currently, up to 90% of the world's energy demand is met by fossil fuels (B.P Statistical Review of World Energy, 2009). Fossil fuels are a relatively cheap energy sources when compared to other hydrocarbon sources such as bio-ethanol. Many disadvantages of using fossil fuels are associated with global climate change, pollution, ecology destruction and diseases (Bilgili, 2012).

Alternative renewable energy sources such as wind, solar and biomass are used in many countries to meet increasing energy demands. Hydrogen (H_2) is an emerging renewable energy source; however to date economical full-scale production is not feasible because additional research is required to develop efficient microbial processes as well as cheap easily degradable feedstocks. Hydrogen is an environmental friendly energy carrier with a high heat of combustion of 285.8 kJ/mol (David, 2003) and energy content per unit mass of 143 GJ/tonne (Boyles, 1984). During H_2 combustion harmful and toxic byproducts are not produced. Approximately 90% of H_2 produced industrially by non-biological processes utilizes fossil fuels (Das and Veziroglu, 2001).

1.2 Non-Biological Hydrogen Production

Non-biological H_2 production processes are classified as the following (Rosen and Scott. 1998):

1. Steam Reforming of Natural Gas: Methane and steam are mixed at 700-1000 °C in the presence of a metal catalyst to produce a gas mixture of H_2 and carbon monoxide.

2. Thermal Cracking of Natural Gas: Natural Gas is heated in the presence of a catalyst to produce a gas mixture of H_2 and carbon monoxide.
3. Partial Oxidation of Heavier Than Naphtha Hydrocarbons: In this process, H_2 is produced by the partial oxidation of hydrocarbons.
4. Coal gasification: Coal and water are heated and pressurized to produce H_2 and carbon monoxide.

Non-biological H_2 production is energy intensive because the mediation of chemical reactions requires elevated temperatures and pressures. Under these operating conditions, the process economics will likely yield an expensive end product. In addition, these processes are not environmentally friendly because they are associated with using fossil fuels.

Electrolysis, the use of electrical potential to split water into H_2 and oxygen, and thermochemical treatment, the breakdown of organic material under extreme conditions, are additional common H_2 production processes that do not require the use of fossil fuels (Hallenbeck and Benemann, 2002). However, electrolysis requires an input of 120 kJ to produce one mole of H_2 and the thermochemical treatment is also energy intensive and not environmentally friendly.

1.3 Fermentative and Non-Fermentative Biological Hydrogen Production

Biological processes are environmental friendly as they require lower operating temperatures and product distribution is more specific when compared to non-biological processes. Producing H_2 using biological methods can be accomplished using pure and mixed microbial cultures (Table 1.1). Numerous pure culture studies have primarily used *Clostridium sp*. to produce H_2 from glucose. However, culture contamination and the

requirement for expensive and sterile feedstocks are major disadvantages hindering process development.

Naturally occurring mixed anaerobic communities are a useful source of fermentative microorganisms. These organisms are robust and able to degrade a wide variety of substrates under optimum conditions. Anaerobic digestion has been used to produce a methane rich gas. Organic effluents from many industrial sectors as well as sewage contain substrates that can be converted into methane using anaerobic digestion (Table 1.1).

Table 1.1: Biological reactions involved in H_2 production

Process	Reaction	Microorganism
Two-Phase H_2 + CH_4	$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$	Fermentative bacteria +
Fermentations	$2CH_3COOH \rightarrow 2CH_4 + 2CO_2$	Methanogenic bacteria
	$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$	Fermentative bacteria
Direct biophotolysis	$2H_2O$ + light $\rightarrow 2H_2 + O_2$	Microalgae
Photo-fermentations	$CH_3COOH + 2H_2O$ + light $\rightarrow 4H_2 + 2CO_2$	Purple bacteria, Micro-algae
Indirect biophotolysis	$6H_2O + 6CO_2$ + light $\rightarrow C_6H_{12}O_6 + 6O_2$	Microalgae, Cyanobacteria
	$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2$	
	$2CH_3COOH + 4H_2O$ + light $\rightarrow 8H_2 + 4CO_2$	
Water Gas Shift Reaction	$CO + H_2O \rightarrow CO_2 + H_2$	Fermentative bacteria

Anaerobic bioreactors operating under methanogenic conditions consist of numerous microorganisms, which ferment complex organic chemicals into methane. This microorganism mixture contains hydrolytic microorganisms, acidogens, acetogens and methanogens. Hydrogen producers (acetogens) and H_2 consumers (acetogens and methanogens) survive in a syntrophic relationship, and inhibiting H_2 consumption leads to H_2 accumulation. Operational as well as reactor engineering design parameters and

chemical inhibitors are used to control whether H_2 or methane is the main byproduct from the fermentation of complex organic substrates. Environmental factors include pH and temperature while a reactor operational parameter such as hydraulic retention time (HRT) can be used to control the growth of H_2 consumers.

Dark fermentation processes are able to utilize a variety of low value organic substrates including those from municipal waste, agricultural residues and industrial waste. Converting low value agriculture materials into energy, faster reaction rates and utilizing non-sterile feedstocks are major drivers that could lead to dark fermentation as a much more attractive H_2 production route (Tanisho *et al.,* 1994).

Unfortunately, H_2 yields from dark fermentation are not yet at the point where the process can be economically commercialized. Inhibiting H_2 consumers such as hydrogenotrophic methanogens has been shown to increase the H_2 yield (Philpot, 2011; Reaume, 2009; Ray *et al.,* 2008; Chowdhurry, 2005). Long chain fatty acids (LCFAs) are environmentally friendly methanogenic inhibitors (Lalman and Bagley, 2000) that are present effluents from food processing facilities as well as from vegetable oil manufacturing. 2-bromoethanesulfonic acid (BES) is a synthetic methanogenic inhibitor that has been shown to inhibit methanogens (Philpott, 2011; Zinder *et al*., 1984; Liu *et al.,* 2011). Other chemicals inhibitors include ethylene, chloroform, propionic acid, nitroethane and ethyl trans-2-butenoate (Xu *et al.,* 2010).

Although numerous publications related to fermentative H_2 production have been reported, little progress has been made towards developing a viable industrial application (Hallenbeck and Benemann, 2002). Hence, additional research is required to develop an economical commercial fermentative H_2 production process.

Other H_2 production processes include those that rely on light (Table 1.1). Hydrogen can be produced by photosynthetic bacteria using light to split water. However, light dependency and low conversion efficiencies are major constraints affecting process development (Hallenbeck and Benemann. 2002). Light mediated reactions are classified as direct, indirect and photofermentation reactions. These reactions are depicted in Table 1.1.

1.4 Substrates

A large fraction of studies to date have been conducted using pure simple substrates such as glucose and/or xylose. The metabolic pathways for glucose and xylose fermentation are well documented in the literature and hence, these substrates are continually used in many studies. Li and Fang (2007) have reported H_2 yields for a variety of substrates. These researchers report large yields are associated with simple sugars. While simple sugars are suitable for research work, they are not practical for large-scale H_2 production due to the enormous quantity of feedstock required. In order for biological H_2 production to be used to satisfy future energy demands, research focus needs to be shifted to using renewable biomass (Hawkes *et al.,* 2002).

Woody (forests) and non-woody (cropland) residues can provide an abundant supply of biomass that could be used to produce H_2. The most suitable area for harvesting materials for biomass is cropland (United States Office for Technological Advancement, 2008). The Canadian biomass harvest has an energy content of approximately 5.1 EJ, which is equivalent to 62% of the annual fossil fuel demand, with cropland accounting for approximately 1.96 EJ of the total (Wood and Layzell, 2003). The inventory of

lignocellulosic waste and residues includes corn stalk, corn straw, corn cob, wheat straw and switchgrass.

Lignocellulosic biomass is a complex sugar substrate that cannot be directly consumed by anaerobic bacteria for H_2 production (Fan and Zhang, 2006). However, methods for treating lignocellulosic biomass deriving it into biodegradable substrates are documented in literature (Fan *et al.,* 2008). In addition to biodegradable substrates, pretreating lignocellulosics has been shown to produce compounds that are inhibitory to H_2 production (Cao *et al.,* 2010). Furfural and hydroxyl methyl furfural are known as furans derivatives and are toxic to microorganisms. Methods to remove inhibitory chemicals must be considered in order to develop practical feedstocks from lignocellulosic materials.

1.5 Objectives

One objective of this study is to optimize an ion-exchange separation process to remove furans and furan derivatives using liquor derived from pretreating lignocellulosic materials. The second objective is to assess fermentative H_2 production using liquor derived from the steam explosion of a lignocellulosic material that is untreated and treated using an ion-exchange resin.

The sub-objectives of this study are as follows:

1. Determine the optimum temperature and pH for furfural and HMF removal using an ion-exchange resin.
2. Determine the range of pH and temperature for removing furfural and HMF.
3. Regenerate the XAD-4 resin and use response surface modeling and compare the performance of the regenerated resin and unused resin.

4. Demonstrate that furan derivatives can be removed simultaneously from a steam exploded switchgrass liquor using the XAD-4.
5. Compare H_2 production using glucose and the ion exchange treated and untreated liquor derived from the thermal treatment of a lignocellulosic material.

Chapter 2: Literature Review

Depleting fossil fuels have created global concerns such as climate change, pollution, ecology damage and human diseases. These problems can be alleviated by developing renewable energy supplies from biomass, wind and solar power. Many countries have developed policies to encourage industries and the public to use these new energy resources. The growth of this change can be rapidly enhanced by increasing resources to accelerate further research and development of alternative energy processes. Many biological processes are under development to increase renewable energy inventories such as hydrogen (H_2) and ethanol. Hydrogen together with other fuels such a bioethanol and biodiesel could potentially supplement a large fraction of fossil fuel demand in the near future. Biological H_2 production processes use less energy, utilizes low value renewable residues and they are more environmentally friendly when compared to fossil fuel based methods.

2.1 Anaerobic Degradation

During anaerobic digestion complex organic compounds are converted into a methane rich biogas by mixed microbial communities. The conversion of substrate into biogas products involves a series of complex biochemical reactions. Various fermentative pathways are possible throughout the process and are dependent on specific microorganisms. Under optimal operating conditions substrates are converted into methane, carbon dioxide and biomass. The four stages are of anaerobic degradation of organic compounds are as follows:

1. Hydrolysis,
2. Acidogenesis,

3. Acetogenesis and
4. Methanogenesis.

2.1.1 Hydrolysis

The first stage in the process involves the transformation of insoluble substrates into soluble monomers and polymers (simple sugars). Hydrolysis is the rate limiting step of the overall degradation process and it is dependent on pH, temperature and type of hydrolytic enzyme (Jordan and Mullen, 2007). The hydrolysis of a complex waste into a simple hexose sugar monomer is shown in equation 1 (Ostrem, 2004).

$(C_6H_{10}O_5)_{2n} + 2nH_2O \rightarrow 2nC_6H_{12}O_6$ where n = 1, 2, 3 … (2.1)

2.1.2 Acidogenesis

Acidogenesis involves the conversion of sugars into volatile fatty acids (VFAs) H_2 and carbon dioxide. The major VFA byproducts include acetic, propionic, butyric, lactic and formic acid. Acidogens have larger growth rates and they are resistant to inhibition by chemicals such as VFAs (Joubert and Britz, 1987). Several typical acidogenic reactions are depicted in equations 2.2-2.5.

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H_2$ (2.2)

$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$ (2.3)

$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COO- + 2H^+$ (2.4)

$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COO- + 2CO_2 + 2H_2 + H^+$ (2.5)

2.1.3 Acetogenesis

Acetogenic bacteria convert VFAs into H_2, carbon dioxide plus acetic acid (equations 2.6 and 2.7) (Ostrem, 2004). These reactions require a low H_2 partial pressure (less than 90 Pa) and they are pH sensitive (Joubert and Britz, 1987). Ethanol and other alcohols

are produced under low pH conditions (below 4.3) (Bahl *et. al.,* 1982). Between pH 4 and 5.5, solventogenesis predominates as a means to reduce the H_2 partial pressure remove VFAs and increase the pH (Monot *et. al.,* 1994).

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (2.6)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 1H^+ + 2H_2 \quad (2.7)$$

2.1.4 Methanogenesis

The terminal stage in the series of reactions is methanogenesis. Hydrogenotrophic methanogens produce methane by carbon dioxide reduction while acetoclastic methanogens split acetate into carbon dioxide plus methane (equations 2.8 and 2.9) (Gujer and Zehnder, 1983). Methanogens are slow growers and susceptible to changes in environmental conditions such as pH. These microorganisms adapt and grow well from pH 6.8 to 7.2; however, they are inhibited when the pH falls below 6.2 (Hassan *et. al.,* 2010). The H_2 yield is expected to increase because H_2 consumers are inhibited under low pH conditions.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.8)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.9)$$

2.2 Anaerobic Hydrogen Production

Mixed anaerobic microbial communities can be manipulated to produce H_2 instead of methane by uncoupling the syntrophic interaction between H_2-consumers and H_2-producers (Nandi and Sengupta, 1998). During the oxidation of organic substrates, electrons are used to reduce cofactors such as NAD^+ to NADH. These cofactors are reoxidized by the loss of electrons to electron acceptors such as carbon dioxide. Fermentative H_2 production is usually driven by the degradation of pyruvate, a three

carbon molecule produced from the breakdown of sugars (Figure 2.1). Pyruvate is degraded into acetyl-CoA with H_2 production catalyzed by pyruvate ferrodoxin oxidoreductase (Hallenbeck and Benemann, 2002). Acetyl-CoA is converted into acetyl-phosphate and acetyl-phosphate is oxidized into acetate plus ADP (Nath and Das, 2004). ADP is subsequently reduced to ATP. The complete reaction scheme is shown below.

$$\text{Pyruvate} + 2\ \text{Fd(ox)} + \text{CoA} \leftrightarrow \text{Acetyl-CoA} + CO_2 + 2\ \text{Fd(red)} \quad (2.10)$$

$$2\ \text{Fd(red)} \leftrightarrow 2\ \text{Fd(ox)} + H_2 \quad (2.11)$$

$$\text{Acetyl-CoA} \leftrightarrow \text{Acetyl-Phosphate} \quad (2.12)$$

$$\text{Acetyl-Phosphate} + \text{ADP} \leftrightarrow \text{Acetate} + \text{ATP} \quad (2.13)$$

A parallel reaction sequence involves pyruvate formate lyase (PFR) conversion of pyruvate to formate. Formate is then degraded to H_2 (Gottschalk and Andreeson, 1979).

$$\text{Pyruvate} + \text{CoA} \leftrightarrow \text{Acetyl-CoA} + \text{Formate} \quad (2.14)$$

$$\text{Formate} + H^+ \leftrightarrow H_2 + CO_2 \quad (2.15)$$

Hydrogen can also be produced via the nicotinamide adenine dinucleotide (NADH) pathway. NADH oxidation and NAD+ reduction is catalyzed by NADH ferredoxin oxidoreductase (equation 16). Proton reduction then leads to H_2 according to Tanisho *et al.,* 1998).

$$\text{NADH} + H^+ \leftrightarrow H_2 + NAD^+ \quad (2.16)$$

Not all metabolic reactions lead to H_2 production (Figure 2.1). The dominant reaction route depends on the culture, environmental conditions, engineering design factors, bacteria species and substrate.

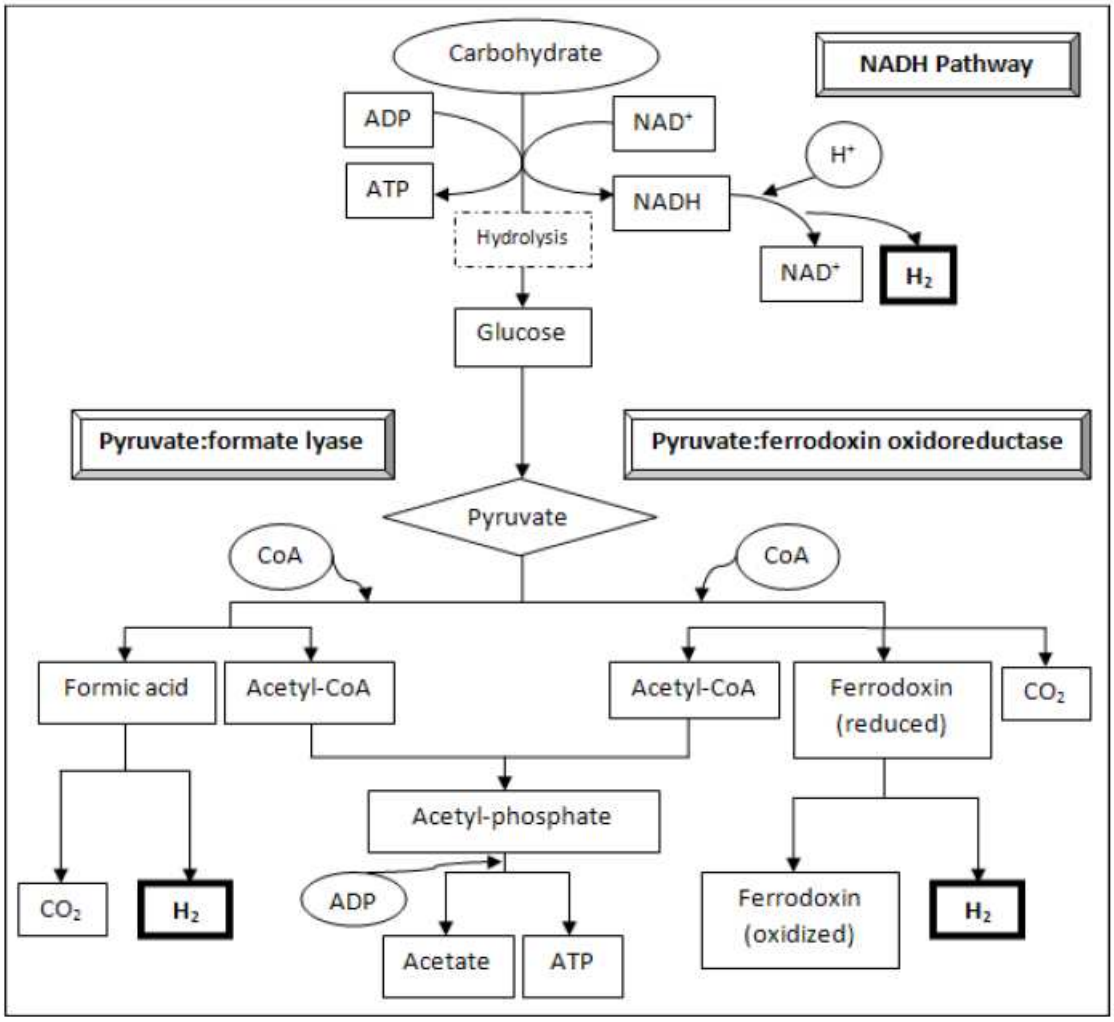

Figure 2.1: Hydrogen production pathways via pyruvate fermentation (Philpot, 2011; Nath and Das, 2004)

2.3 Microbial Thermodynamics

Biochemical reactions are affected by the quantity of free energy available to drive the reaction in the forward direction. In all reactions involving a microbial catalyst, a fraction of the energy from the substrate is converted into cellular mass and the remaining fraction is converted into heat, ATP plus byproducts. The energy available for work is defined as Gibbs free energy and is represented by the following equation:

$\Delta G = -n*F*\Delta E$

Where: ΔG = change in Gibbs free energy (J)

n = number of electrons transferred (mol)

F = number of Coulombs / Faraday (96485 C/mol)

ΔE = potential difference (V)

A reaction with a negative ΔG proceeds spontaneously. In comparison, a reaction with a positive ΔG requires energy and thus, is usually coupled with a spontaneous reaction that releases energy. The reaction rate is determined by the activation energy, concentration and temperature. Activation energy refers to the initial energy barrier that must be overcome for a reaction to proceed in the forward direction. Catalysts can be used to lower activation energy (the overall free energy of the reaction remains constant). Figure 2.2 shows the effect of lowering activation energy on the energy profile for a typical reaction. Many critical microbial reactions are mediated with enzymes (Mara and Horan, 2003). These reactions include those responsible for biological H_2 production.

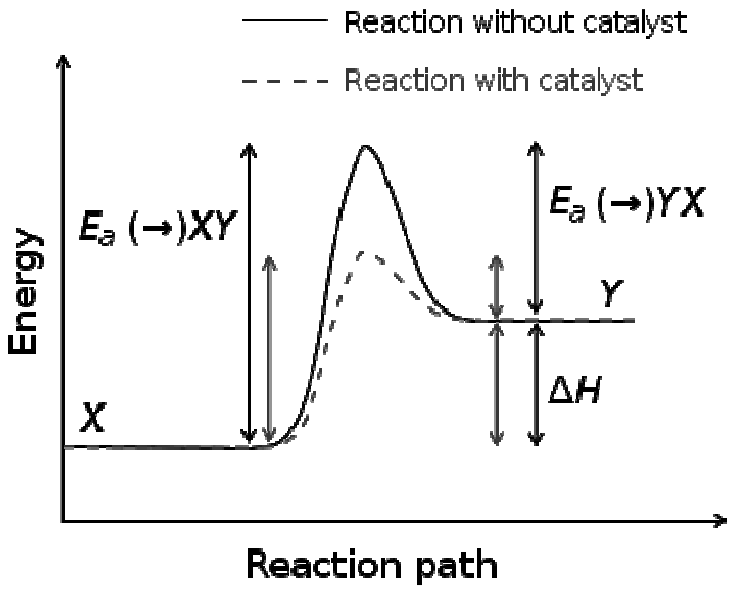

Figure 2.2: Activation energy of a catalyzed reaction

Oxidation and reduction half-reactions are combined together for an overall microbial reaction. In these reactions, electrons are transferred from an electron donor to an electron acceptor. Typically, electron donors such as carbohydrate, lipids and proteins are oxidized and electron acceptors are reduced. Oxygen and nitrate are the preferred electron acceptors, followed by sulfate and carbon dioxide (based on ΔG of associated reactions). Typical microbial half reactions are shown in Table 2.1. In anaerobic microbial communities, approximately 10% of the energy generated is used for cell synthesis and the remaining energy is released as heat and in byproducts (Mara and Horan, 2003).

Table 2.1: Selected microbial half-reactions (Yang and Okos, 2007)

Half Reaction	**$\Delta G^{o'}$ (aq) (kJ/electron equivalence)**
Electron Donor (Oxidation)	
Carbohydrates $1/4CH_2O + 1/4H_2O \rightarrow 1/4CO_2 + H^+ + e^-$	-41.8
Fats and Oils $1/46C_8H_{16}O + 15/46H_2O \rightarrow 4/23CO_2 + H^+ + e^-$	-27.6
Protein $1/66C_{16}H_{24}O_5N_4 + 27/66H_2O \rightarrow 8/33CO_2 + 2/23NH_4^+ + 31/33H^+ + e^-$	-32.2
Acetate $1/8CH_3COO^- + 3/8H_2O \rightarrow 1/8CO_2 + 1/8HCO_3^- + H^+ + e^-$	-27.6
Ethanol $1/12CH_3CH_2OH + ¼H_2O \rightarrow 1/6CO_2 + H^+ + e^-$	-31.8
Electron Acceptor (Reduction)	
Oxygen $1/4O_2 + H^+ + e^- \rightarrow 1/2H_2O$	-78.2
Nitrate $1/5NO_3 + H^+ + e^- \rightarrow 1/10N_2 + 3/5H_2O$	-71.6
Carbon Dioxide $1/8CO_2 + H^+ + e^- \rightarrow 1/8CH_4 + 1/4H_2O$	+24.3
Sulphate $1/8SO_4^{2-} + 19/16H^+ + e^- \rightarrow 1/16\ H_2S + 1/16HS^- + 1/2H_2O$	+21.3
Cell Mass $1/20NH_4^+ + 1/20HCO_3^- + 1/5CO_2 + H^+ + e^- \rightarrow 1/20C_5H_7O_2N + 9/20H_2O$	+31.4

2.4 Factors Affecting Biological Hydrogen Production

2.4.1 Effect of Nutrients

Nutrients such as nitrogen, phosphorus and trace metals are required for achieving optimal microbial growth. Nitrogen is used as a macronutrient for protein and DNA synthesis while phosphorus is used in DNA synthesis, energy storage and buffering capacity (Lin and Lay, 2005). Micronutrients include selected trace metals such as magnesium, iron, cobalt and nickel. They are cofactors needed for enzyme function. At elevated levels, metals can be toxic to bacteria and nitrogen and phosphorus can cause overgrowth of organisms such as algae (Li and Fang, 2007).

2.4.2 Effect of Temperature

Most studies have shown an increase in H_2 production with an increase in temperature. Typically, reaction rates increase by a factor of 2 for every 10^oC rise in temperature. However, in some cases, increasing the temperature has minimal effects on the H_2 yield. For example, Van Ginkel et al. (2001) noted that carbohydrate substrates have comparable yields for mesophilic (30-40°C) and thermophilic (50-65° C) microorganisms with maximum yields of approximately 330 mL H_2/g hexose. High H_2 yields using wastewater substrates have been reported at 60°C (Ueno *et al.,* 1996) and with solid waste at 55°C (Valdes-Vazquez *et al.,* 2005). Based on these reports, the substrate type and reaction and temperature will affect the maximum H_2 yield. When using carbohydrates, 35-40° C is the most common temperature range for achieving the highest H_2 yield.

When operating at temperatures above the ambient range, the benefits of increasing the H_2 yield must be weighed against the costs of operating at higher temperatures. At

higher temperature, elevated reaction rates allow for higher substrate loadings (Zoetemeyer *et al.,* 1982). Elevated H_2 partial pressure is not thermodynamically optimal for achieving a high H_2 yield. This problem can be alleviated by sparging the reactor contents and by operating at elevated temperatures. Hydrogen solubility decreases with increasing temperatures, which corresponds to a lower H_2 partial pressure. The diffusion of compounds into and out of the cell becomes more thermodynamically favorable at higher temperatures (Cirne *et al.,* 2007). Hence, operating at elevated temperatures is advantageous for microbial H_2 production.

2.4.3 Effect of pH

pH is an important factor that affects the performance of many microbial catalysts. Enzymatic activity and microbial processes such as nutrient transport are affected by pH. For example, hydrogenase activity is affected by pH. Methanogenic activity decreases substantially at pH values above 7.8 and below 6.3 (Chen *et al.,* 2002). Maximum H_2 yields have been reported between pH 5-7. Fang and Lui (2002) reported a yield of 286 mL H_2/mg hexose at an optimal pH of 5.5. In comparison, Li and Fang (2007) reported an optimal pH of 6.0 for H_2 production from carbohydrates. Notice a similar impact of pH and temperature on the H_2 yield. In both cases, the optimum H_2 yield is detected within a narrow range of pH and temperature.

The type of substrate can affect the optimum pH for H_2 production (Noike, 2002; Lee *et al.,* 2002). pH is shown to affect the metabolic routes linked to VFAs, alcohols, methane and H_2 production (Monot *et al.,* 1984; Bahl *et al.,* 1982). Butyrate production is favorable at lower pH values while propionate is selected at pH levels above 7 (Lay,

2000). Note many reported optimal pH values are inaccurate due to a lack of data clarifying if the pH was maintained over the duration of the studies.

2.4.4 Effect of Hydrogen Partial Pressure

Elevated H_2 levels can thermodynamically inhibit its production. At elevated H_2 levels, the degradation of VFAs becomes thermodynamically unfavourable. The free energy value for many acetogenic reactions is positive under standard conditions. However, these free energies can become negative by controlling the H_2 partial pressure. According to Ahring and Westermann (2008) for butyrate consumption to occur, the H_2 partial pressure must be below approximately 2 Pa. The maintenance of low H_2 levels allow for the reaction to proceed in the direction resulting in H_2 production. Thermodynamically H_2 consumption is more favourable than H_2 production. Notice methane production is favourable (ΔG of -131.0 kJ/mol) while acetate degradation to H_2 is unfavourable (ΔG of +94.9 kJ/mol) (Table 2.2). During H_2 production and consumption, the reaction free energy changes with variation in the H_2 partial pressure. Figure 2.3 shows that butyrate degradation is more thermodynamically favourable than methane production. Overcoming the thermodynamics limitations is important when maximizing the H_2 yields.

Table 2.2: Hydrogen production and consumption reactions (Schink, 1997)

Reaction	$\Delta G^{\circ'}$ (kJ/mol)
H_2-producing reactions	
$CH_3CH_2CH_2COO^- + 2H_2O \leftrightarrow 2CH_3COO^- + H^+ + 2H_2$	+43.6
$CH_3CH_2COO^- + 2H_2O \leftrightarrow CH_3COO^- + CO_2 + 3H_2$	+73.6
$CH_3COO^- + H^+ + 2H_2O \leftrightarrow 2CO_2 + 4H_2$	+94.9
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \leftrightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.5
$CH_3CH_2OH + H_2O \leftrightarrow CH_3COO^- + H^+ + 2H_2$	+1.9
H_2-consuming reactions	
$4H_2 + 2CO_2 \leftrightarrow CH_3COO^- + H^+ + 2H_2O$	-94.9
$4H_2 + CO_2 \leftrightarrow CH_4 + 2H_2O$	-131.0
$H_2 + HCO_3^- \leftrightarrow HCOO^- + H_2O$	-1.3
$H_2 + S \leftrightarrow H_2S$	-33.9
$4H_2 + SO_4^{2-} + H^+ \leftrightarrow HS^- + 4H_2O$	-151.0
$H_2C(NH_3^+)COO^- + H_2 \leftrightarrow CH_3COO^- + NH_4^+$	-78.0
Fumarate + H_2 ↔ succinate	-86.0

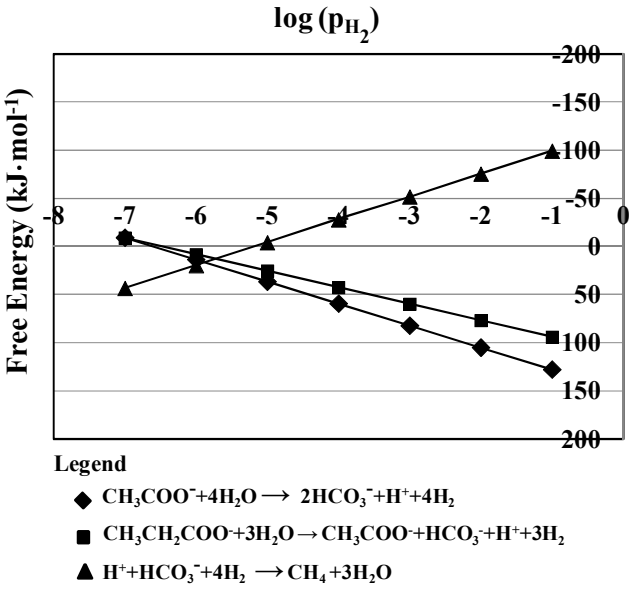

Figure 2.3: Free energy values for hydrogen producing and consuming reactions as a result of hydrogen production

Researchers have implemented several methods with varying degrees of success to reduce H_2 partial pressure. Sparging with nitrogen has been shown to increase the H_2 yield from 172 to 254 mL H_2/mg hexose (Hussey *et al.,* 2003). According to Lay (2000),

the simplest and most commonly used method to increase the H_2 yield is to increase mixing within the reactor.

2.4.5 Effect of Microbial Source

The bacterial culture can influence the variety and quantity of end products formed during the degradation of organic substrates. The bacteria species has a direct effect on the H_2 yield.

2.4.5.1 Pure Cultures

Researchers have used *Enterobacter* and *Clostridium* pure cultures for H_2 production studies (Li and Fang, 2007). In general, pure cultures produce higher H_2 yields because they consist solely of H_2 producing bacteria. Pure cultures require constant maintenance and are extremely sensitive to environmental condition changes making them impractical for large scale hydrogen production usage. Feedstock contamination will result in converting pure cultures into mixed cultures and a subsequent reduction in the H_2 yield.

2.4.5.2 Mixed Cultures

Mixed cultures contain a variety of species that exist in natural communities stemming from landfills, wastewater facilities, compost and soil. While they are much easier to maintain and the risk of contamination is much lower, the presence of H_2 consuming bacteria is a major problem because they are associated with lower H_2 yields.

2.4.6 Effect of Hydraulic Retention Time

Hydraulic retention time (HRT) is the average time for a volume element to enter and leave a reactor. Continuous stirred tank reactors (CSTRs) or semi-continuous are operated under a variety of HRTs. Batch reactors have no in and out flow and hence, they do not operate at a HRT. Batch reactors are generally easier to operate and control;

however, they are not applicable in cases when product demand is large for products such as bioethanol, biodiesel and biohydrogen.

In continuous flow reactors, shortened HRTs cause microorganism wash-out and this resulting in increased H_2 yields. In comparison, longer HRTs lead to methane production because the substrates and microorganisms are retained in the bioreactor. Short HRTs can be used to wash-out methanogens to eliminate the amount of methane producing microorganisms. Li and Fang (2007) reported that optimal HRT values for degrading simple carbohydrates can vary from 3 to 8 hours. Fang and Lui (2004) reported an optimal value of 13.7 hours while Chang et al. (2002) reported an HRT of 1 hour.

2.5 Substrates for Biological Production of Hydrogen

Simple sugars, cellulose and starch all contain an abundance of electron donors that can be used by mixed anaerobic cultures for H_2 production. The main advantage of these substances is their easy of degradability by mixed and pure anaerobic cultures. Proper operational efficiency requires a food to microorganism ratio (F/M) when utilizing CSTRs, both over and under feeding can result in reducing the H_2 yield (Lay, 2001; Van Ginkel *et al.,* 2001). Data from Li and Fang (2007) comparing H_2 yields from a variety of substrates has shown large H_2 yields based on simple sugars such as glucose.

Pure substrates are expensive and their use for large scale H_2 production is not be economically viable. Hence, greater research efforts are required to develop sustainable feedstocks using low value agriculture residues and wastes (Hawkes et al., 2002).

2.6 Biomass Feedstock

The estimated annual global primary production of biomass is equivalent to the 4,500 EJ (or 700 billion bbl oil) of solar energy captured each year (Ladanai *et. al.,* 2009).

Forests, cropland and wetlands provide an abundant supply of biomass that could be used to eventually satisfy global energy demands. The most suitable land for harvesting plants for biomass is cropland. The best croplands are only used for food production because of their value. However, researchers have realized that utilization of biomass waste (corn cob, corn stover, and wheat straw) generated from food production processes can add-value to these low-value products and subsequently reduce the cost for producing biofuels (United States Office for Technological Advancement, 2008). Underutilized hayland and pastureland can be easily converted to cropland for biomass production. Natural wetlands can be used for cultivating aquatic plants.

2.6.1 Agricultural Waste

Wastes generated from food processing, animal farming and crops conversion into food products can be used to produce fuels such as H_2 and ethanol. Three important crops grown in North America from which waste lignocellulosic feedstocks can be produced include corn, wheat and sugar. Currently, the most direct method of converting these feedstocks into bioenergy is via liquid fuel production (United States Office for Technological Advancement, 2008). Based on the lignocellulosic feedstocks utilization, ethanol yields are now economically variable. The United States Department of Energy (USDOE) estimated more than 1.3 billion tonnes of dry biomass can be produced in the United States (U.S.). The U.S. Department of Agriculture (USDA) further estimated that of the total 1.3 billion tonnes, 998 million tons is produced from agricultural lands (Perlack *et. al.,* 2005).

2.6.2 Lignocellulosic Biomass

Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin. Cellulose and hemicelluloses are bound tightly to the lignin component and separation of these components presents a major problem to researchers. Lignocellulosics are present in a variety of crops including corn stalk, wheat straw and switchgrass. Anaerobic bacteria are unable to produce H_2 from these complex solid lignocellulosic feedstocks (Fan and Zhang, 2006). Evidence showing that sugars derived from these low value wastes can be converted into H_2 by anaerobic microbial communities has been reported by Sankar (2011).

Low value biomass consists of approximately 30-50% cellulose and 20-40% hemicellulose on a dry mass basis (Lee et. al., 2007). Hence, the high sugar content is a major driver for developing fuels from these low value feedstocks. Key advantages for utilizing lignocellulosic biomass for biofuels are as follows (Verenium, 2008): use of non-food crops; relative low cost; and some lignocellulosics such as switch grass can be produced on marginal lands.

Cellulose is difficult to degrade into monomers because of stable glycosidic linkages. Hence, specific enzymes are to hydrolyze glycosidic the bonds. Hemi-cellulose (20-40% dray mass) is more easily degradable due to its branched and amorphous nature (Lee *et al.,* 2007). Hemi-cellulose is composed of many sugars and it is degraded enzymatically into xylose, mannose, galactose, rhamnose, and arabinose. Recent studies by Reaume (2009) have shown that xylose can produce H_2 with yields similar to that derived from glucose.

2.6.2.1 Lignocellulosic Biomass Pretreatment

Several pre-treatment methods are available for degrading lignocellulosic biomass into fermentable sugar. Using a 12% NaOH (w/w) solution at 70^{o}C was reported by Vrije et al. (2002) for pretreating corn cob. Steam explosion has been reported as a useful method for pretreating corn leaves under harsh temperature and pressure conditions Li and Chen (2007). Cao *et al.,* 2009 reported a H_2 yield of 2.24 mol/mol glucose using aliquor produced from dilute acid hydrolysis of corn cob with 1.7% sulphuric acid.

Pretreating lignocellulosics has a major disadvantage because of the production of furans, which are potent microbial inhibitors (Cao *et al.,* 2009). The levels of sugars and furans produced are dependent upon the severity of the pretreatment conditions. Under harsh conditions, pentose sugars are converted into furfural while hexose sugars are converted into hydroxyl methyl furfural (HMF). A major objective for many researchers is to develop pretreatment methods that are able to release the largest amount of sugars while minimizing the formation of furans.

2.7 Hydrogen Production Using Pretreated Liginocelluosic Biomass

A significant amount of research has been conducted on using liquor derived from pretreating lignocellulosic biomass for H_2 production. Quéméneur *et* al. (2012) reported the inhibition of H_2 was more from a liquor containing furan derivatives (0.40-0.51 mol H_2/mol xylose) when compared to yield for a feed containing phenolic compounds (1.28-1.39 mol H_2/mol xylose). Under harsh pretreatment conditions, hemicellulose sugars are converted into furan derivatives and hence, optimizing sugar concentrations while minimizing the furan levels is a priority research priority during process development. Clark and Mackie (1987) found that impregnating wood with SO_2 or H_2SO_4 prior to steam

explosion greatly improves hemicellulose derived sugar recovery in the final liquor. *S. cerevisiae* has been shown to reduce furfural into furfuryl alcohol and furoic acid under aerobic and anaerobic conditions during fermentation (Taherzadeh *et al.,* 1998; Villa, 1992). According to Palmqvist and Hahn-Hägerda (2000), furfuryl alcohol and furoic acid have a slight inhibitory affect on cell growth under anaerobic conditions. In comparison, both furfural and HMF (at 1 $g \cdot L^{-1}$) have been reported to stimulate the growth of *Clostridium beijerinckii* BA101 as well as the production of acetone-butanol-ethanol via non-H_2-producing pathways (Ezeji *et. al.,* 2007). Other studies have demonstrated that a decrease in H_2 yield in the presence of furans is associated with a decrease in several clostridia species. These studies concluded that *Clostridum beijerinkii* was more resistant to inhibitors, making it ideal for H_2 production from lignocellulosic biomass hydroslate (Quéméneur *et. al.,* 2012).

Chapter 3: Materials and Methods

3.1 Experimental Plan

Experiments were divided into three stages in order to execute research objectives (Figure 3.1). In the first stage, the capacity of the resin to remove each furan derivative was examined as a function of pH, temperature and the initial furan concentration. In addition, regeneration and reuse of the resin was assessed using furfural and HMF. The second stage of the study examined the ability of the resin to remove furfural and HMF simultaneously in mixtures. The final and third stage of the experiments was to examine the benefits of treating steam exploded switchgrass liquor with XAD-4 resin before using it as a substrate for fermentative H_2 production.

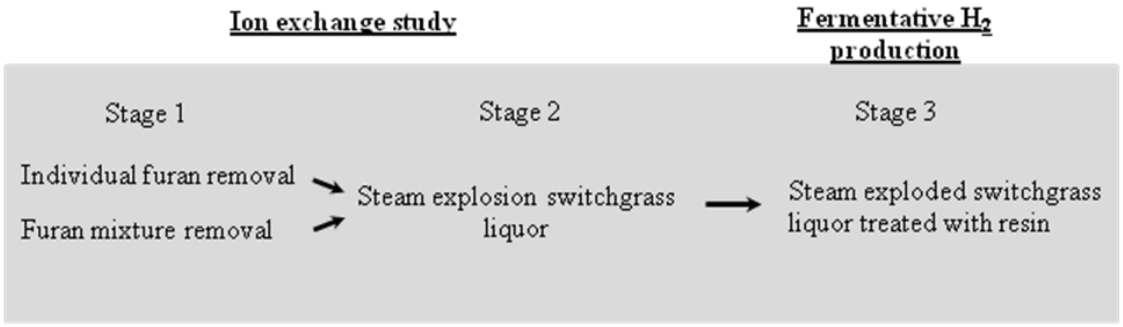

Figure 3.1: Experimental design process

The experimental approach to accomplish stage 1 involved a three level, three factor Box-Benkhen experimental design (BBD). Two separate BBDs were used to examine furfural and HMF removal (Tables 3.1 and 3.2). The experimental plan for a three factor, three level BBD is shown in Table 3.3.

Table 3.1: BBD factors and levels for furfural removal

	Levels		
Factors	**-1**	**0**	**1**
pH	5	6	7
Temp (°C)	24	37	50
Furfural (g/L)	1	3	5

Table 3.2: BBD factors and levels for HMF removal

	Levels		
Factors	**-1**	**0**	**1**
pH	5	6	7
Temp (°C)	24	37	50
HMF(g/L)	0.25	0.50	0.75

Table 3.3: Three factor, three level BBD

Experiment Number	pH	Temp	Initial Concentration
1	-1	-1	0
2	1	-1	0
3	-1	1	0
4	1	1	0
5	-1	0	-1
6	1	0	-1
7	-1	0	1
8	1	0	1
9	0	-1	-1
10	0	1	-1
11	0	-1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

A three level, three factor BBD is composed of 13 experiments instead of the 27 required for a full factorial design. Two additional centre point experiments were conducted in order to quantify among-replicate variability (errors) generated from the experiments.

The results of the BBD were used to develop a quadratic equation composed of 10 coefficients. The model equation was used to predict the final inhibitor concentration based on given initial conditions. The BBD for furfural was repeated using regenerated resin.

The experimental plan for stage 2 consisted of simultaneously removing both furfural and HMF. Stage 2 experiments were used to verify data generated in stage 1 and the conditions established for removing furfural and HMF can be useful in establishing parameters for optimum removal of furans from switchgrass. The experimental plan for stage two involved varying the pH between 5, 6 and 7, while maintaining a constant temperature of 37°C with furfural and HMF removal occurring simultaneously. The initial furfural and HMF concentrations were equivalent to the concentrations in the steam exploded switchgrass liquor. Unused and regenerated resins were used to remove furfural and HMF in water and in switchgrass liquor. Both stage 1 and stage 2 experimental plans examined the removal of 2g XAD-4 resin/15ml liquid volume.

Conditions for the third set of experiments are summarized in Table 3.4. Experiments in stage 3 were conducted in 160 mL serum bottles with 50 mL liquid volume. Furans were added to determine their effects on H_2 producing microbial cultures. All cultures were fed linoleic acid (LA), an LCFA that inhibits methanogens (Philpot, 2011). The pH was adjusted to 5.5 to inhibit methanogenesis (Chen *et al.,* 2002). Sugar (5000 mg/L) was added from a glucose stock solution (100000 mg/L) and also from switchgrass liquor. The sugar content of the switchgrass liquor was 30,000 mg/L (glucose and xylose). The volatile suspended solids (VSS) in the microbial batch reactors (150 mL) was adjusted to 2000 mg/L. Liquid and gas samples were removed at regular time

intervals for gas, VFAs, alcohols and sugar analysis. All experiments were conducted in triplicate.

Table 3.4: Stage 3 experimental summary design conditions

Bottles	Substrate	HMF Added (g/L)	Furfural Added (g/L)
1,2,3	Glucose	0	0
4,5,6	Glucose	0	2.0
7,8,9	Glucose	0.25	0
10,11,12	Glucose	0.25	2.0
13,14,15	Untreated Switchgrass Liquor	0	0
16,17,18	Treated Switchgrass Liquor	0	0

3.2 Inoculum Source and Culture Maintenance

The inoculum source for the microbial experiment was procured from a facility treating effluent from a brewery located in Guelph, Ontario. The culture (approximately 10000 mg/L VSS) was maintained at 37°C in a 5-L batch reactor covered in aluminum foil to prevent photosynthetic growth. The reactor working volume was set at 4 L. The reactor was fed glucose (5000 mg/L) every 5 to 7 days. At the end of a feeding period, mixing was terminated for 3-4 hours to settle the solids in the bioreactor. The top liquid layer (approximately 2 L) was decanted and fresh basal media (2 L) was added to the reactor. The composition of the basal media was prepared in accordance with the procedure described by Ray *et al.* (2009). The pH of the reactor was determined to insure the culture was operating within a range of 6 to 7.6.

3.3 Basal Media Characteristics

Basal media fed to the 5-L and 160-mL batch reactors were prepared according to Table 3.5 (Ray *et al.,* 2009).

Table 3.5: Basal Media Constituents

Chemical	Concentration (mg/L)
$NaHCO_3$	6000
NH_4HCO_3	70
K_2HPO_4	14
$(NH_4)_2SO_4$	10
Yeast Extract	10
Resazurin	1.0
$CuCl_2$-$2H_2O$	0.03
Na_2SeO_3	0.1
$CoCl_2$-$4H_2O$	0.15
$MnCl_2$-$6H_2O$	0.5
$NiCl_2$-$6H_2O$	0.05
H_3BO_3	0.05
KCl	25
$ZnCl_2$	0.05
$MgCl_2$-$4H_2O$	9
EDTA	1.0
$(NH_4)_6MoO_7$-$4H_2O$	0.09
$FeCl_2$-$4H_2O$	2.0
Na_2S	3.0

3.4 Experimental Details and Preparation

3.4.1 Furan Removal Studies

All experiments for stage 1 and 2 were conducted using 20-mL vials that were sealed using Teflon®-lined silicon rubber septas and capped with aluminum crimp caps. First, 2g of XAD-4 resin was added to the vials. Next, approximately 10 mL of milli-Q (MQ) water was added followed by the required volume (Tables 3.1 and 3.2) of furan stock solution (50000 mg/L). MQ water was added to a total liquid volume of 15 mL. The pH of the solution was adjusted with 1N hydrochloric acid or 1N sodium hydroxide. The vials were sealed, capped shaken at 200 rpm. Samples were removed to monitor the furans levels in the aqueous phase at periodic intervals.

3.4.2 Fermentative Hydrogen Production Studies

Preparation details of the batch reactors for H_2 fermentative studies in stage 3 are described by Ray et al. (2009). A summary of preparing the reactors is described in this section. All of the experiments in stage three were conducted using 160 mL batch reactors wrapped in aluminum foil and maintained at 37^{o}C. The bottles were prepared in a Coy® anaerobic chamber (Figure 3.1). Each bottle was injected with the required amount of culture and basal media to a final VSS of 2000 mg/L. The total liquid volume was set at 50 mL. The pH was adjusted to 5.5 with 1N hydrochloric acid or 1N NaOH. Before adding the substrates, the solids were settled and a volume of liquid was removed equivalent to the volume of stock solution added was removed.

All substrates were added to the batch reactors in the glove box. Reactors fed 2000 mg/l of LA was allowed to mix for 24 hours before adding glucose or the resin treated steam exploded switchgrass liquor (Table 3.4). Furfural (2000 mg/l) the reactors were mixed for 24 hours before adding 5000 mg/l of the sugar substrate (glucose or resin treated steam exploded switchgrass liquor). After the substrate was injected at time = 0 hr liquid and gas samples were removed for analysis.

After injecting all the substrates, the bottles were sealed using Teflon®-lined silicon rubber septa and capped with aluminum crimp caps. To avoid a negative pressure from developing during headspace sampling, 20 mL of the anaerobic chamber gas mixture (80%N_2/20%CO_2) was injected into the headspace of the 160-mL batch reactors. The bottles were removed from the chamber and placed in a Lab Line orbital shaker (Max 4000, Barnstead) set at 200 rpm and maintained at 37°C.

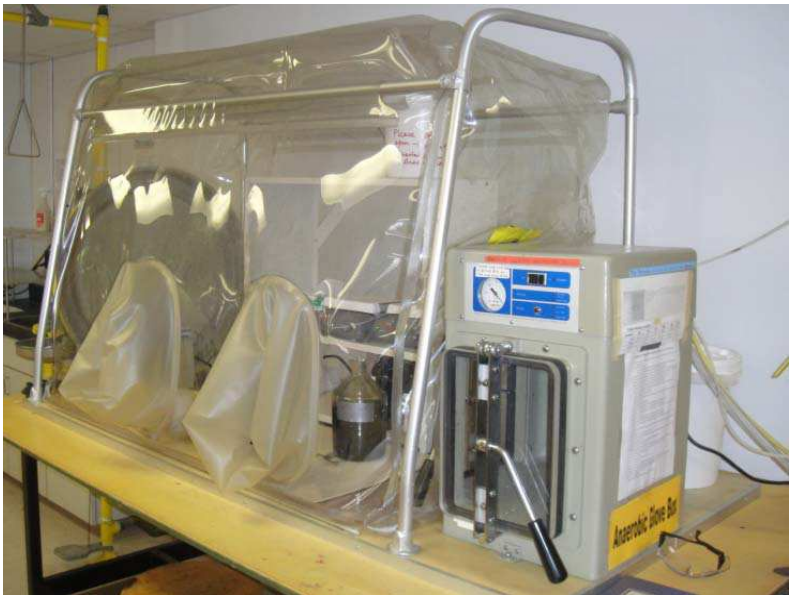

Figure 3.2: Coy® anaerobic chamber

3.5 LCFA Delivery Method

The long chain fatty acid (LCFA), linoleic acid (LA), was 18 carbons long and slightly soluble in water (Raston and Hoerr, 1942). Dispersing LA in basal media was required to enhance its mass transfer to microorganisms. A LA stock solution (50,000 mg/L) was prepared *au bain-marie* by mixing LA and NAOH pellets at 50°C with vigorous stirring (Angelidaki and Ahring, 1992). For LA, 0.142 g of NaOH was used per gram of LCFA.

3.6 VSS/TSS Measurements

The volatile suspended solids (VSS) and total suspended solids (TSS) concentration were measured in accordance with Standard Method of Analysis (APHA, AWWA, WEF, 1992). VSS/TSS measurements were conducted periodically to ensure the VSS concentration was maintained in the 5L batch reactor.

3.7 pH Measurements

An important characteristic for batch reactor (5L) maintenance, batch reactor (160 mL) preparation and furan removal was pH adjustment and monitoring. Measurements were conducted using a VWR SR40C Symphony pH meter (Orion). The instrument was calibrated with pH 4 and pH 7 standard buffer solutions.

3.8 Analytical Methods

3.8.1 Gas Sample Analysis

Headspace samples from the 160 mL batch reactors were injected into a Varian 3800 gas chromatograph (GC). The GC was configured with a thermal conductivity detector (TCD) and a 2-m x 1.0-mm diameter (ID) (OD = 1.6 mm) packed Shincarbon ST (Restek, USA) column. The injector was set at 100°C while the oven and detector were set at 200°C. Nitrogen (99.99%, Praxair, ON) was the gas carrier with a flowing at 20 mL/min. Calibration curves are shown in Appendix I. The detection limits for H_2 and methane were 0.25 mL/160 mL bottle.

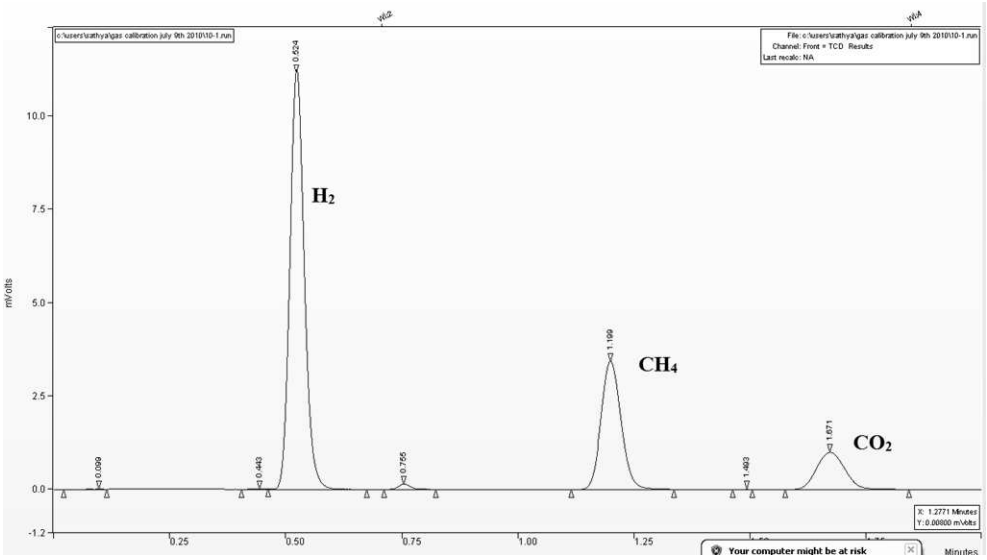

Figure 3.3: Chromatogram demonstrating the peaks of H_2, CH_4 and CO_2

3.8.2 Liquid Samples

3.8.2.1 Furan Derivatives

Furfural and HMF were analyzed for using high pressure liquid chromatography (HPLC) (Dionex Ultimate 3000, Sunnyvale, CA). The instrument was configured with a UV-visible photodiode detector set at 215 nm and an Acclaim C18-3 um (2.1 mm I.D. and 100 mm long) column. The analysis was conducted isothermally with the oven temperature set to 50°C and an eluent flowing at 0.2 mL/min. The eluent was a MQ water mixture containing methanol (20%) and phosphoric acid (0.1%). The calibration curves are shown in Appendix II. The detection limits for furfural and HMF were 1.0 mg/L and 0.5 mg/L, respectively.

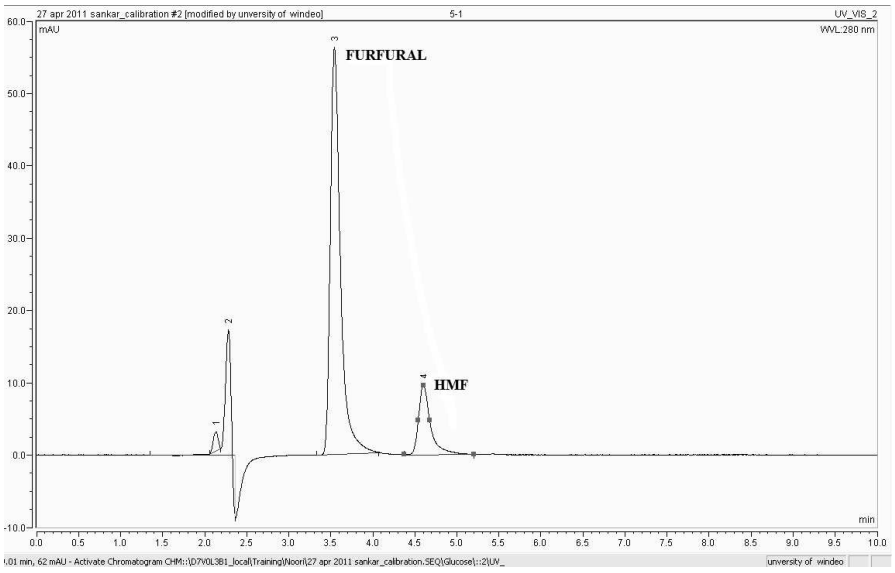

Figure 3.4: Chromatograph demonstrating furfural and HMF peaks

3.9.2.2 VFAs

Acetic, propionic, butyric, formic and lactic acid were analyzed using an HPLC (Dionex Ultimate 3000, Sunnyvale, CA). The instrument was configured with a UV-visible photodiode detector set at 215nm and an Acclaim C18-3 um (2.1 mm I.D. and 100

mm long) column. The analysis was conducted isothermally with the oven temperature set to 55°C and an eluent flow set at 0.3 mL/min. The eluent was a mixture of methanol (10%) and phosphoric acid (90%) at pH 3. The detection limits for the VFAs were 5 mg/L. The calibration curves are shown in Appendix III.

3.8.2.3 Alcohols and Sugars

Glucose, xylose, ethanol, propanol, iso-propanol, butanol and iso-butanol were measured using a DX-600 Ion Chromatograph (IC) (Dionex, Sunnyvale, CA) equipped with an automated sampler (AS40), a gradient pump (GP50), a liquid chromatography oven (LC10) and a electrochemical detector (ED50). The IC was configured with a 3 mm i.d. x 100 mm long CarboPac^{TM} PA20 (Dionex) analytical column (Dionex) and a 3 mm I.D. x 30 mm long PA20 (Dionex) guard column with a 25 ul sample loop. The 480 mmol NaOH eluent flow was set at 0.2 mL/min. The detection limit was 1 mg/L for glucose, xylose, ethanol, propanol, iso-propanol, butanol and iso-butanol. The calibration curves are shown in Appendix IV.

3.9 Experimental Sampling Plans

3.9.1 Furan Removal Optimization Sampling Plan

For stage 1 and 2 experiments, liquid samples were removed at regular time intervals (20, 40, 60, 90, 120, 180, 240, 300 and 360 min). The sampling plan for stage 3 experiments is shown below in Table 3.6.

Table 3.6: Stage 3 experimental sampling plan

Time (hr)	0	2	4	6	8	12	16	24	48	72	96
Glucose	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
VFAs/Alcohols	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Gas	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes

3.10 Sample Treatment

Liquid samples for IC and HPLC analysis were diluted with MQ water and filtered to remove suspended solids and heavy metals. The first filtering process used a 25-mm diameter 0.45 μm polypropylene membrane to remove suspended solids. In the second filter, a 1-mL polypropylene cartridge fitted with a 20 μm PE frit and filled with Chelex® 100 to 200 mesh resin was used to remove heavy metals.

3.11 Furan Removal Using the Amberlite XAD-4 Resin

Furfural and HMF were removed from a liquid using the XAD-4 resin (Octochem Inc., IL) via ion exchange. Based on the manufacturer's data, XAD-4 resin is functional at temperature up to 300 °C, at any pH and with a mean surface area of 725 m^2/g. The chemical structure of the XAD-4 resin is shown in Figure 3.4. The resin is a polymeric adsorbent consisting of white insoluble beads. The manufacturer recommends it is used to adsorb low molecular weight organic substances. Before using the resin, it was washed with water to remove salts (ROHM and HAAS, 2006).

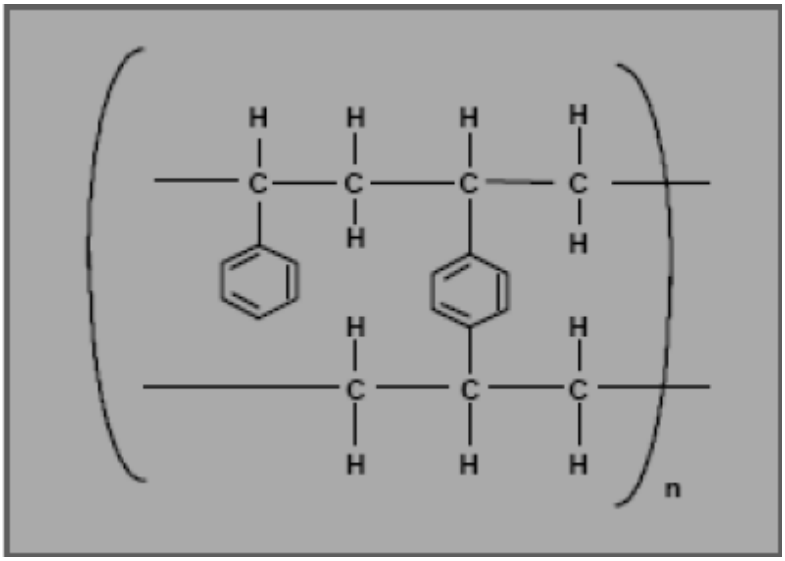

Figure 3.5: Chemical structure of XAD-4 resin

3.11.1 XAD-4 Resin Regeneration

Used resin was washed with MQ grade water (1 g resin/4 mL water). Next, the resin was soaked in a 50% H_2O_2 solution for 24 hours and washed again with MQ water again before soaking in a 1.5% NaOH solution for 24 hours. The resin was washed a third time with MQ before soaking in a 1.5% HCl solution for 24 hours. Next, the acid solution was decanted and the resin was mixed for 2 hours with 100 mL MQ water. The liquid phase was decanted and the resin was ready for reuse.

3.12 Switchgrass Pretreatment

In order for switchgrass to be used as a substrate for fermentative H_2 production, it was treated using steam explosion under elevated temperature conditions to release the cellulose and hemicelluloses components into the liquid phase. First, the switchgrass was dried at 100°C. Samples were weighed before and after drying. The leaves were shredded and mixed with water in a 1:10 ratio before steam explosion. The samples were samples steam exploded at 190°C for 8 min. The liquor produced from the steam

explosion was filtered using 25 mm diameter 45 um filter paper (VMR Inc.) to remove solids. The filtered liquor was acid treated using 2% sulphuric acid to convert complex sugars into simple sugar monomers. The liquor was autoclaved for 15 min at 100°C. The pH was adjusted to 5.5 using potassium hydroxide pellets. The liquor was filtered again and then stored at 4°C.

3.13 Switchgrass Liquor Characterization

The sugar, furfural and HMF content in the switchgrass liquor was determined in accordance with methods reported by Tappi (2009) and Goering and Soest (1972).

3.14 Statistical Analysis and Response Surface Methodology (RSM)

A Full-Factorial design (FFD) is often considered impractical due to a large number of experiments. Instead, more commonly selected experimental design procedures that are based upon the desirable feature of accurate prediction throughout the factor space were used Central Composite design (CCD) and Box–Benkhen design (BBD) (Myer and Montogomery, 2002; Box et. al., 1978). However, for a quadratic response surface model with three or more factors, the BBD procedure is much more advantageous than the CCD (Myer and Montogomery, 2002 Box et. al., 1978).

A full quadratic (second order) response surface was analyzed for the BBD. The experimental response (mg/L furan) was analyzed statistically using Minitab 16 (Minitab Inc., State College, PA). Three experiments were conducted at the central points to estimate the magnitude of error or "noise" in the experimental analysis. Responses from process factors other than those selected for the experimental design are considered as errors for the experimental design under consideration.

A multiple regression analysis (method of least square) was performed to determine the coefficient values for the model using experimental data (Box and Draper, 1986).

The residual furan concentrations (response) recorded at each design point of the BBD (see Tables 4.1) were used as experimental data. An analysis of variance (ANOVA) was conducted using the experimental response to evaluate a full quadratic approximation of the response surface model of the BBD. The order of the response model was used to determine the degree of curvature of the response surface model (Box et. al., 1978). The model was verified using an analysis of residuals. The residual is the difference between the model prediction and the experimental outcome at identical factor levels within the design space under consideration (Myer and Montogomery, 2002). The residuals are expected to follow a normal distribution (occurrences are random) for a model with good predictability characteristics (Box and Draper, 1986).

The Anderson-Darling test is a statistical tool used to quantify the deviation of residuals from a normal distribution. The validity of residuals distribution in Anderson-Darling test at a 5% level of significance confirms the accuracy of the model (Stephens, 1974). The Anderson-Darling test was conducted using the residuals that were determined for the response surface model.

Chapter 4: Optimizing Furfural and HMF Removal Using XAD-4 Resin

Furfural and HMF removal using XAD-4 resin is dependent on a variety of factors. These experiments examined the effects of pH and temperature as well as the furan initial concentration on removing furfural and HMF from the liquid phase. All experiments were conducted in triplicate. A response surface modeling (RSM) was used to determine optimal temperature, pH conditions and initial furan concentration within the experimental range under consideration. Furfural and HMF have been reported to inhibit different populations in H_2 producing mixed microbial cultures at concentrations greater than 500 mg/L (Cao *et al.,* 2009).

Experiments were conducted using a BBD for three factors (pH, temperature and initial concentration) at three levels. This allowed for only 15 experiments to be run instead of 27 experiments based on a full-factorial design. The outputs of these experiments were converted into 2 variable contour plots to demonstrate the impact of 2 variables on furan removal. An analysis of variance (ANOVA) was conducted on the data generated from all three experiments to determine the significance of the factors as linear, squared and interacting factors. MINITAB 16 (Minitab Inc., State College PA) was used to determine the linear, square and interaction coefficients for a quadratic equation.

4.1 Furan Removal Plots

All experiments were conducted for a period 360 min. However, note in some cases the expected time for attaining adequate removal was less than 360 min (furan concentrations less than 500 mg/L). The percent removal efficiencies for all the BBD experiments is summarized in Table 4.1 Furfural and HMF removal under different

conditions is shown in Figures 4.1 through 4.13. At initial concentrations of 3000 mg/L furfural and 500 mg/L HMF, the quantity of furfural and HMF removed after 360 min using resin that was not regenerated was 95.2 ± 0.4% and 94.3 ± 1.0%, respectively, at a pH set at 6 and 37°C. In comparison, for the regenerated resin, the percent furfural removed reached 95.6 ± 0.4%.

Table 4.1: Furan Removal Efficiencies for all BBD Experiments

Expt #	pH	Temp (°C) ±1°C	Percent Removed (%)		
			Furfural with Unused Resin	HMF with Unused Resin	Furfural with Regenerated Resin
1	5	24	98.±0.3	93.5±1.3	96.6±0.2
2	7	24	97.8±0.3	96.9±0.9	97.2±0.3
3	5	50	98.4±0.4	91.2±1.4	97.3±0.4
4	7	50	98.3±0.4	95.7±1.6	97.2±0.5
5	5	37	99.6±0.2	94.8±1.0	97.7±0.4
6	7	37	95.5±0.5	95.9±0.8	95.5±1.1
7	5	37	97.7±0.3	95.3±1.1	96.0±0.4
8	7	37	96.8±0.2	95.0±0.4	94.7±0.4
9	6	24	97.4±0.3	93.8±1.4	96.1±0.4
10	6	50	99.2±0.2	99.2±0.4	97.0±0.6
11	6	24	95.5±0.6	92.3±0.9	95.0±0.8
12	6	50	95.7±0.8	91.0±0.7	94.6±1.3
13	6	37	97.9±0.3	94.3±1.0	97.0±0.4
14	6	37	97.3±0.2	96.6±0.4	96.6±0.4
15	6	37	97.2±0.4	96.4±0.6	96.6±0.5

The minimum furan removal scenario was observed with initial concentration of 5000 mg/L furfural and 750 mg/L HMF at a pH of 6 and 50°C. For furfural and HMF removal utilizing resin that was not regenerated, the removal efficiencies were 95.7 ± 0.8% and 91.0 ± 0.7%, respectively. For furfural, the removal efficiency using regenerated resin was 94.6 ± 1.3%. The removal efficiencies at the lowest initial concentration for furfural

(1000 mg/L) and HMF (250 mg/L), with environmental conditions set at pH 5 and 37°C, were 99.6 ± 0.2% and 94.8 ± 1%, respectively. For regenerated resin the furfural removal efficiency was 97.7 ± 0.4% under the same conditions.

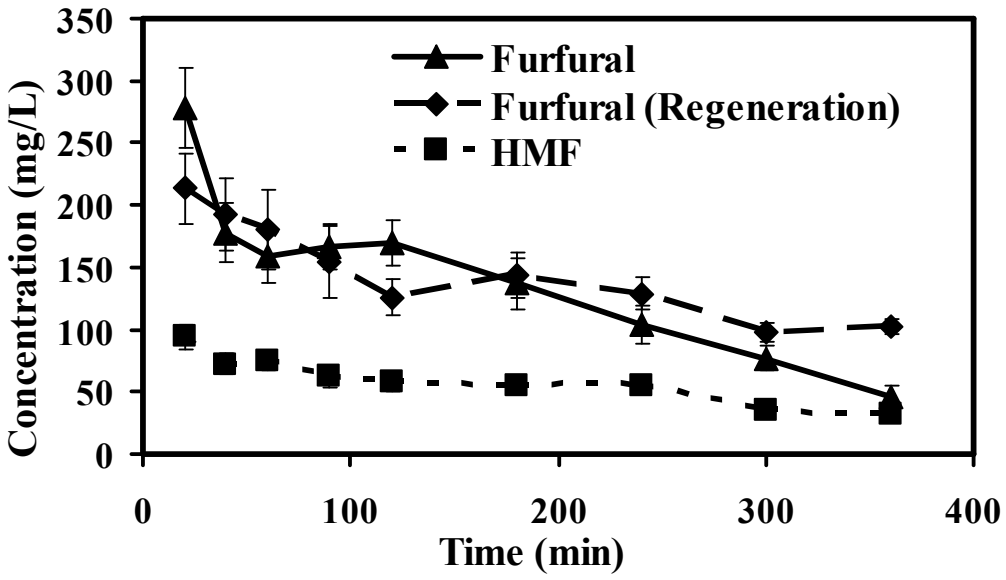

Figure 4.1 Furan removal by resin with initial concentrations of 3000 mg/L furfural and 500 mg/L HMF at pH 5 and 24 °C

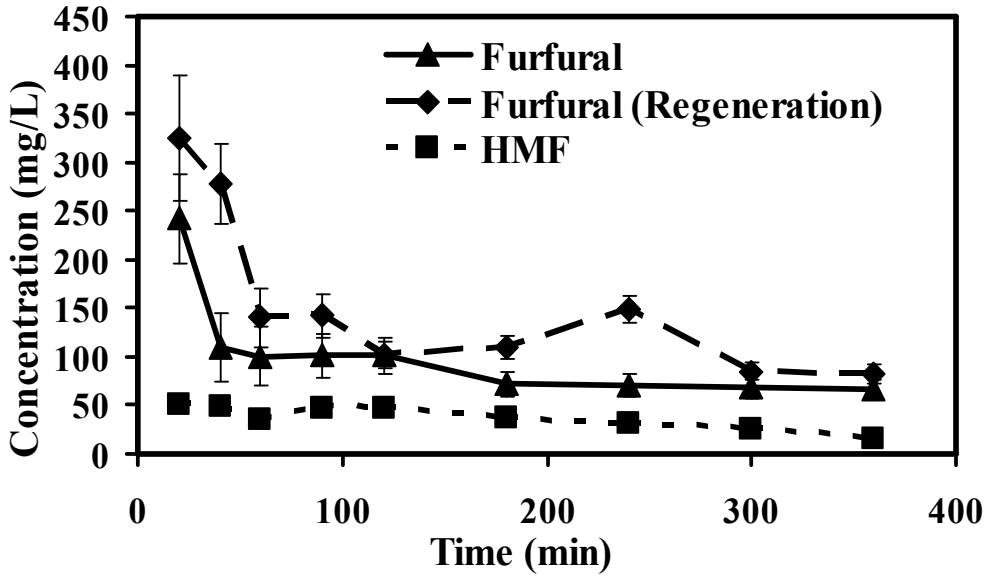

Figure 4.2: Furan removal by resin with initial concentrations of 3000 mg/L furfural and 500 mg/L HMF at pH 7 and 24 °C

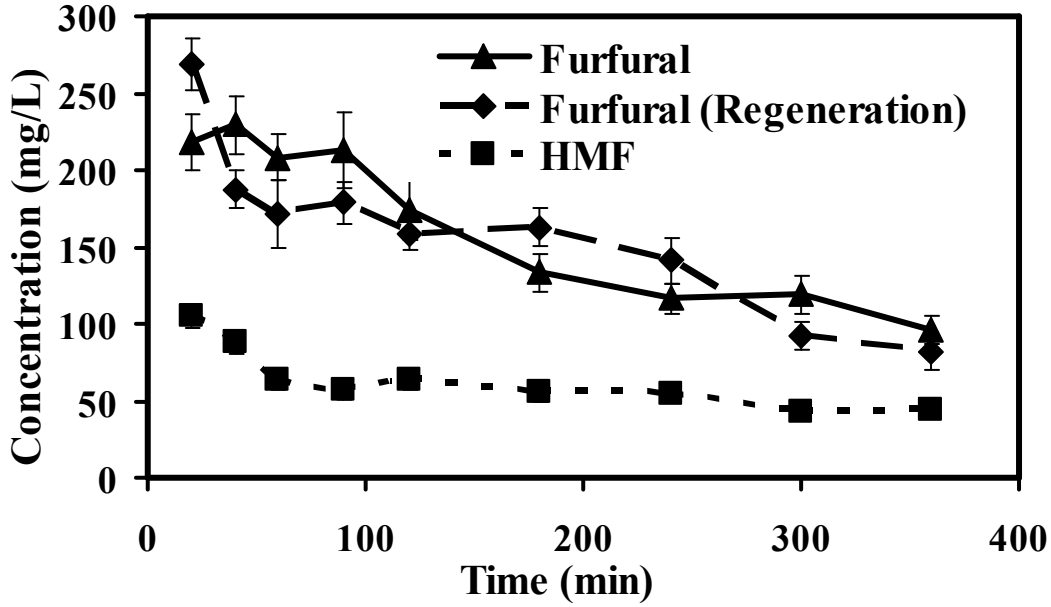

Figure 4.3 Furan removal by resin with initial concentrations of 3000 mg/L furfural and 500 mg/L HMF at pH 5 and 50 °C

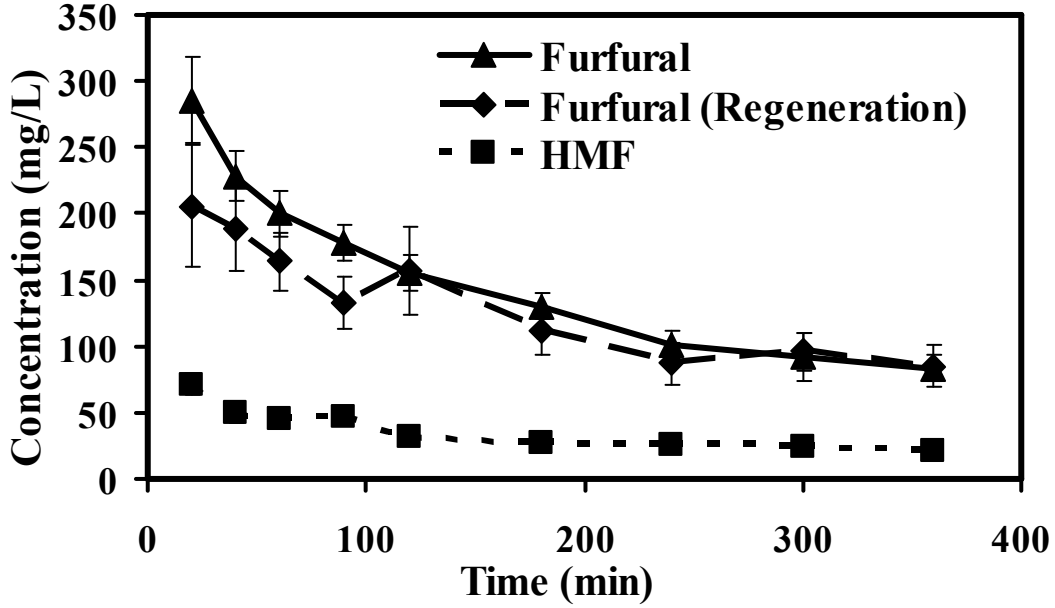

Figure 4.4 Furan removal by resin with initial concentrations of 3000 mg/L furfural and 500 mg/L HMF at pH 7 and 50 °C

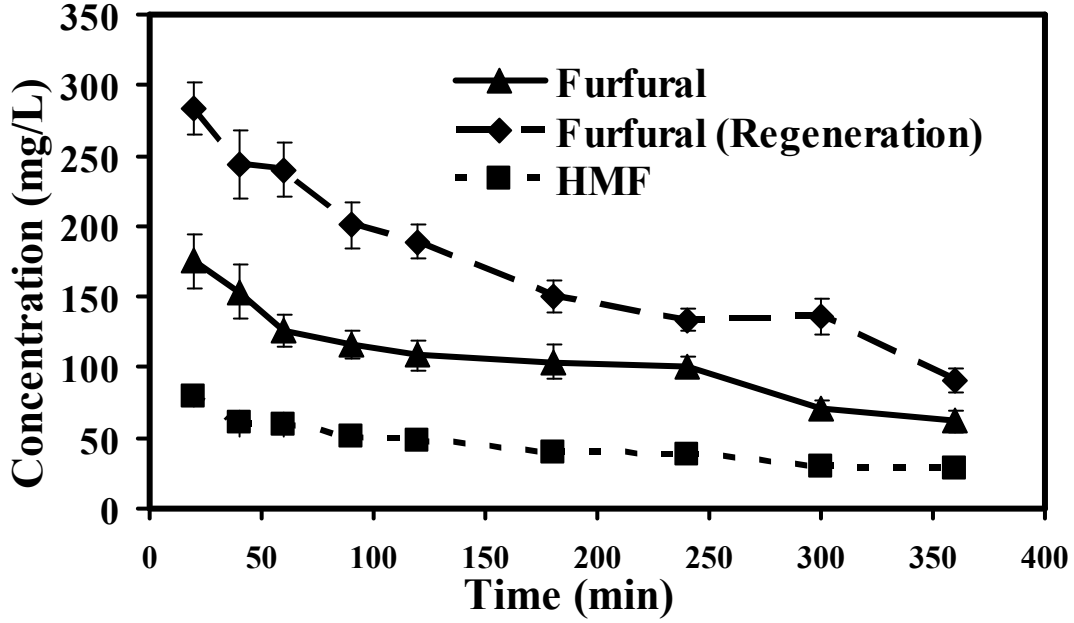

Figure 4.5 Furan removal by resin with initial concentrations of 3000 mg/L furfural and 500 mg/L HMF at pH 6 and 37 °C

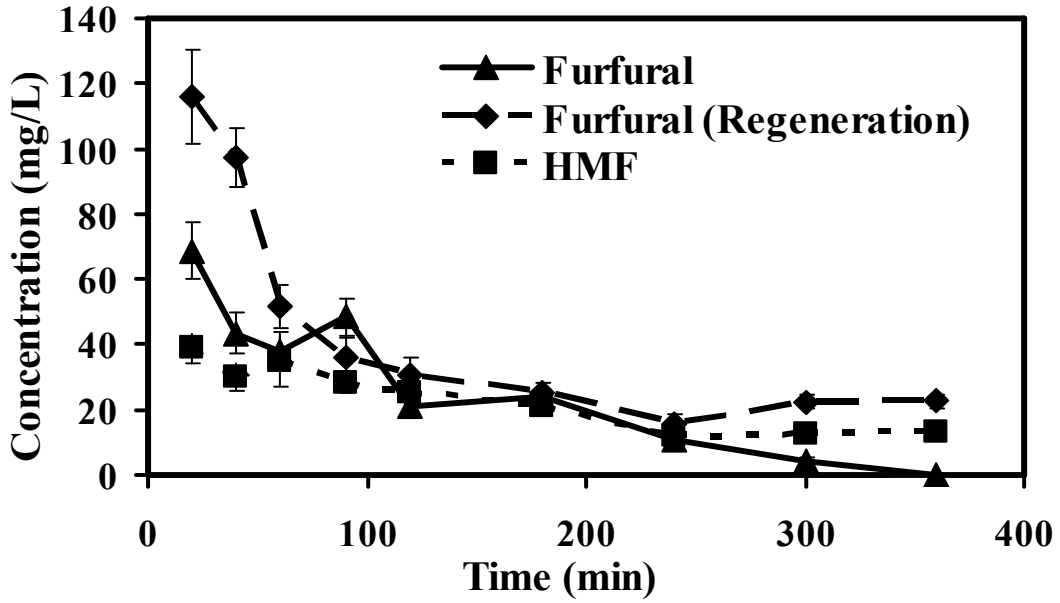

Figure 4.6: Furan removal by resin with initial concentrations of 1000 mg/L furfural and 250 mg/L HMF at pH 5 and 37 °C

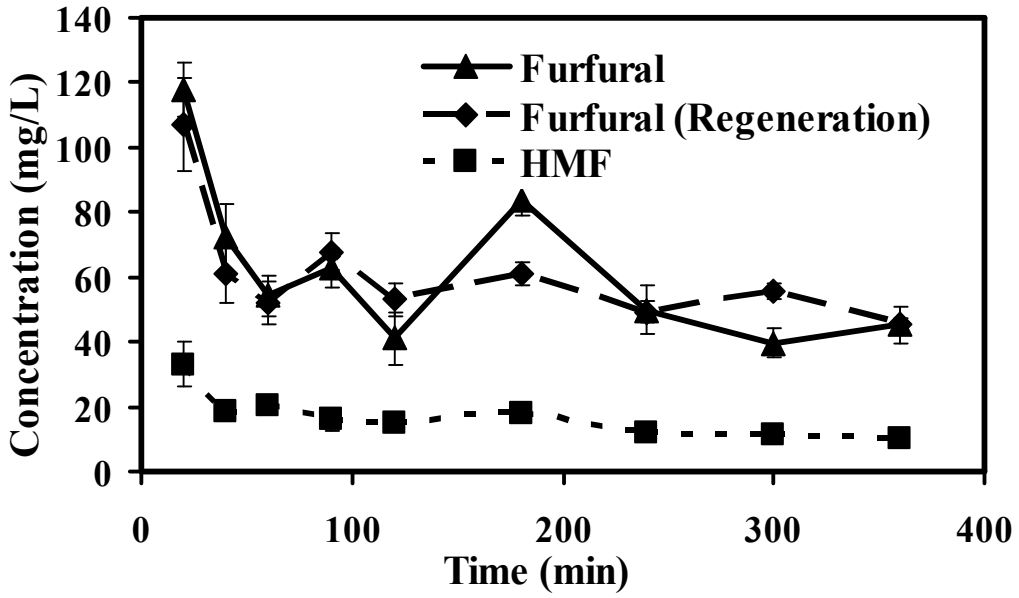

Figure 4.7: Furan removal by resin with initial concentrations of 1000 mg/L furfural and 250 mg/L HMF at pH 7 and 37 °C

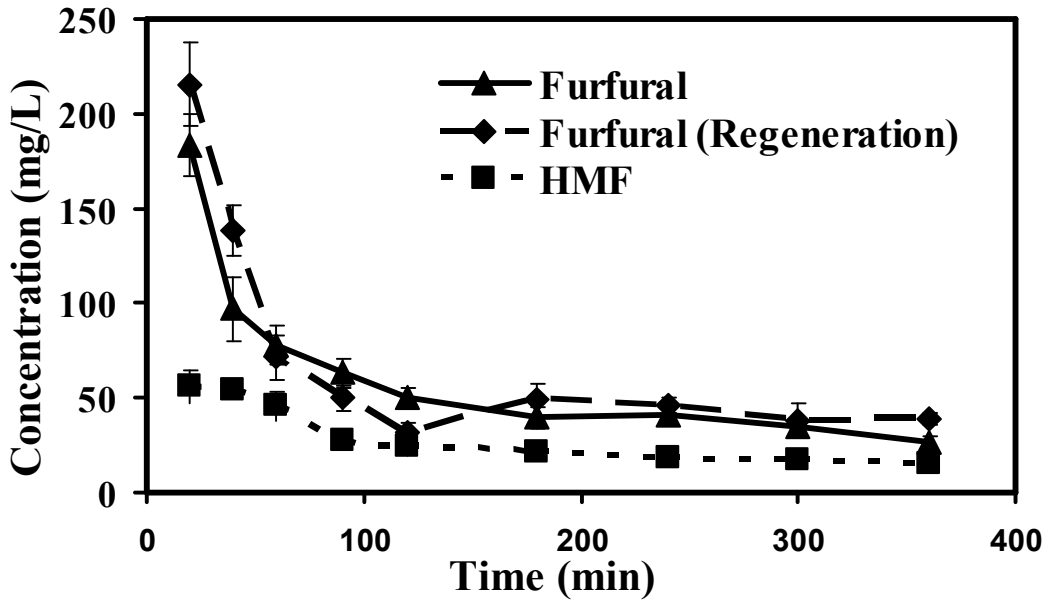

Figure 4.8: Furan removal by resin with initial concentrations of 1000 mg/L furfural and 250 mg/L HMF at pH 6 and 24 °C

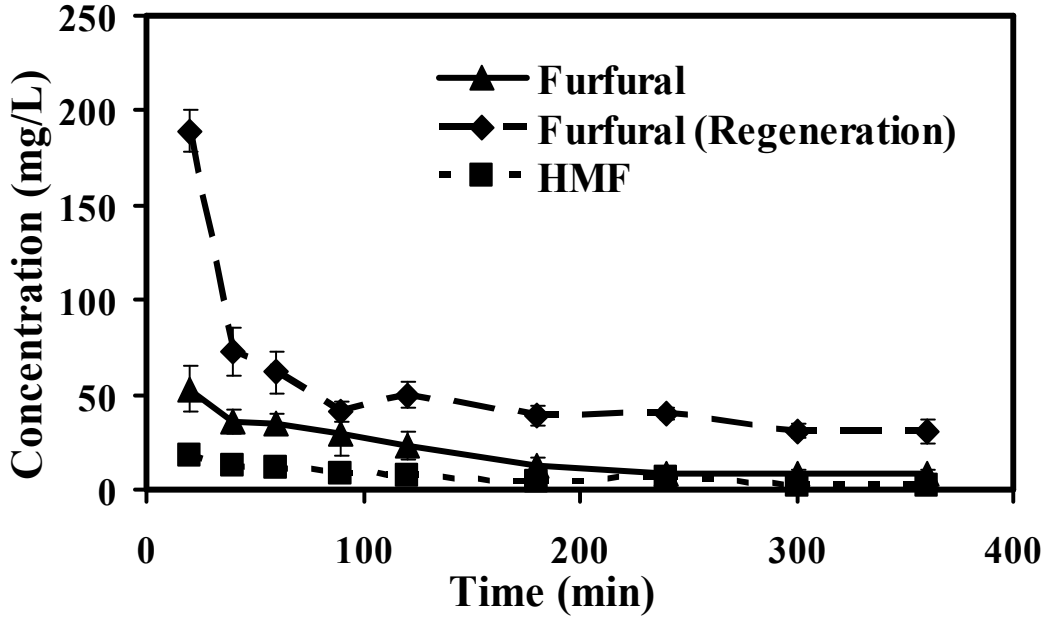

Figure 4.9: Furan removal by resin with initial concentrations of 1000 mg/L furfural and 250 mg/L HMF at pH 6 and 50 °C

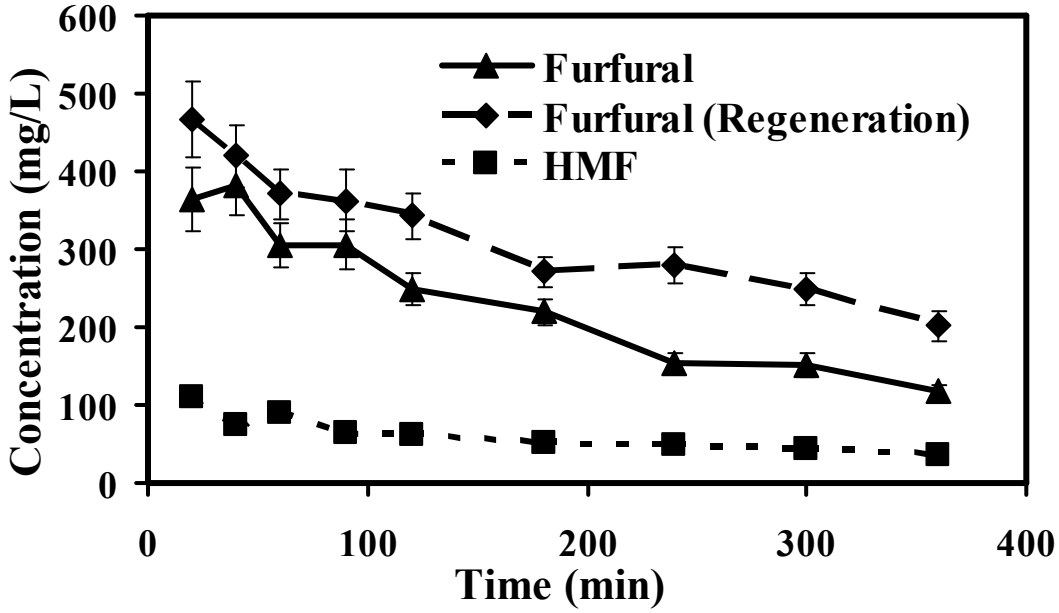

Figure 4.10: Furan removal by resin with initial concentrations of 5000 mg/L for furfural and 750 mg/L for HMF at pH 5 and 37°C

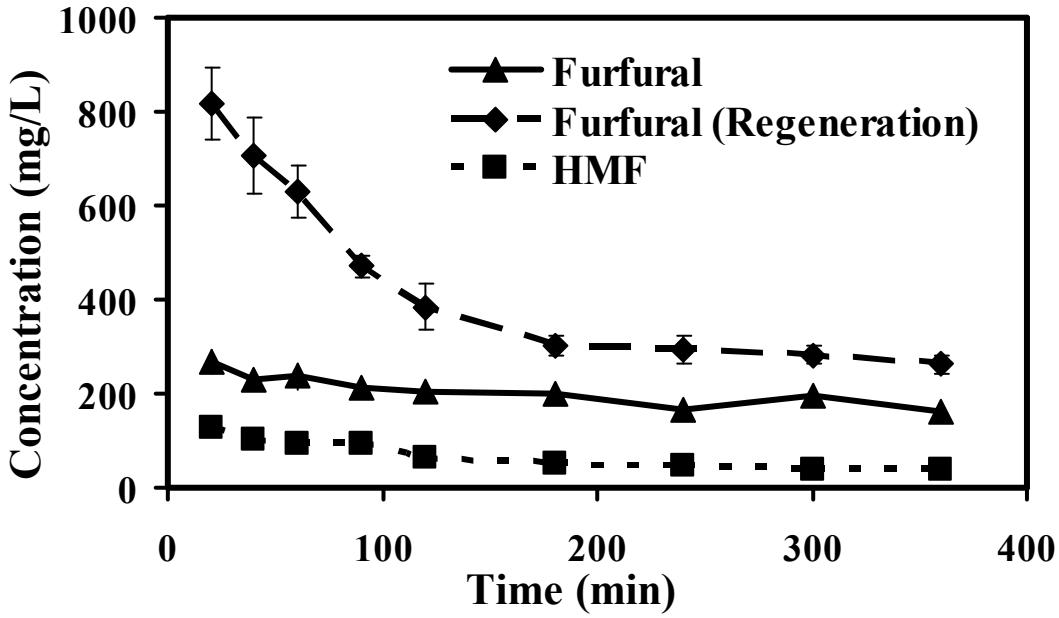

Figure 4.11: Furan removal by unused resin with initial concentrations of 5000 mg/L for furfural and 750 mg/L for HMF at pH 7 and 37°C

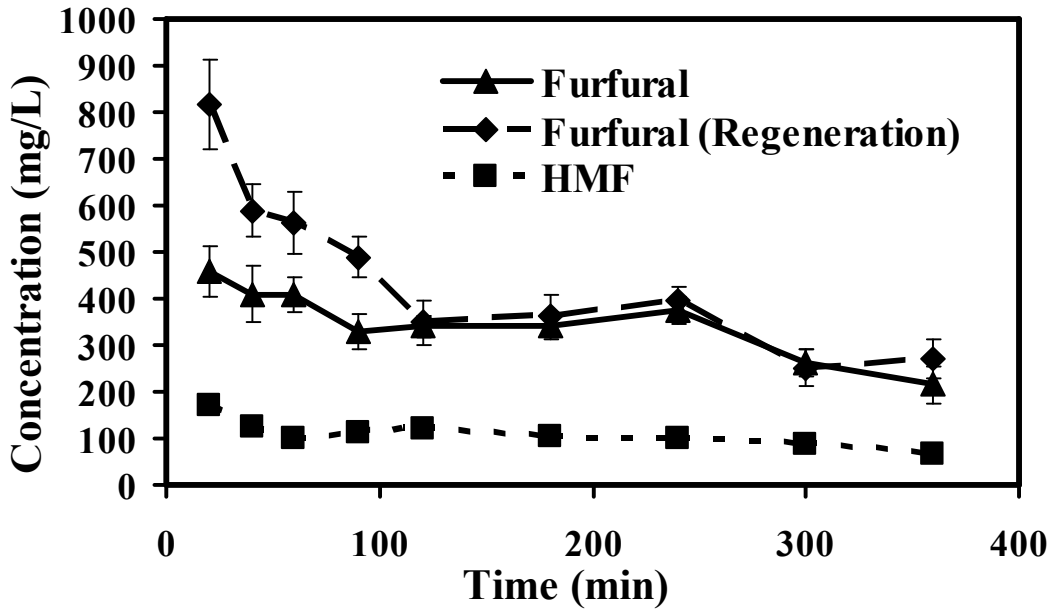

Figure 4.12: Furan removal by resin with initial concentrations of 5000 mg/L for furfural and 750 mg/L for HMF at pH 6 and 24°C

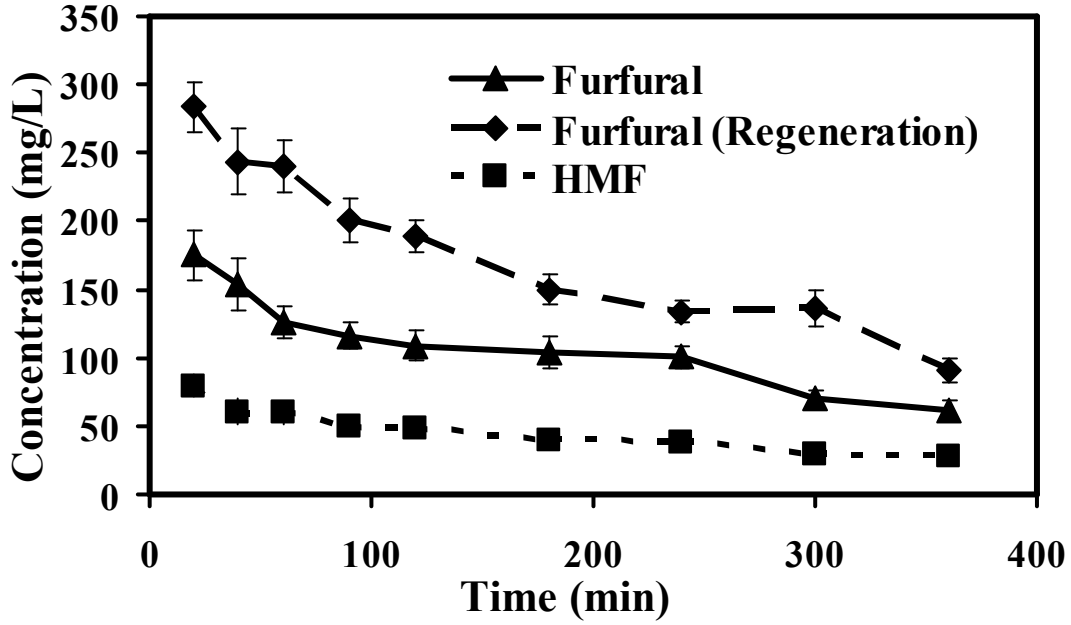

Figure 4.13: Furan removal by resin with initial concentrations of 5000 mg/L for furfural and 750 mg/L for HMF at pH 6 and 50°C

4.2 Response Model for Furfural Removal Using Unused Resin

Contour plots based on two factors were developed using MINITAB 16. Two factors were varied across the experimental range while a third factor was kept constant. Contours for temperature versus pH (initial furfural concentration constant at 3000 mg/L), pH versus initial concentration (temperature constant at 37°C) and temperature versus initial concentration (pH constant at 6) are shown in Figures 4.14 to 4.16. The plots are all based on the furfural BBD which was shown in Table 4.1. Sections on the plots where the contour lines are close together indicate better furfural removal over a small change in a variable.

The residual furfural concentrations were all below 500 mg/L (Table 4.2) even when the initial concentration was increased to 5000 mg/L. At a fixed furfural concentration (3000 mg/L) and for any fixed temperature condition from 30^{o}C to 50^{o}C, reducing the pH from 6.5 to 5.0 resulted in increasing the percent furfural removed (Figure 4.14). At 37^{o}C and for a fixed pH value from 5.0 to 7.0, the percent furfural removed increased with decreasing initial concentrations (Figure 4.15). At pH 6 and a temperature condition from 25^{o}C to 50^{o}C, the percent furfural removed increases with decreasing initial furfural concentrations (Figure 4.16).

Notice a larger fraction of furfural was removed under low pH conditions from 5.0 to 5.3 (Figure 4.14). However, varying the pH from 5.5 to 6.5 caused a change in response by only 10 mg/L.

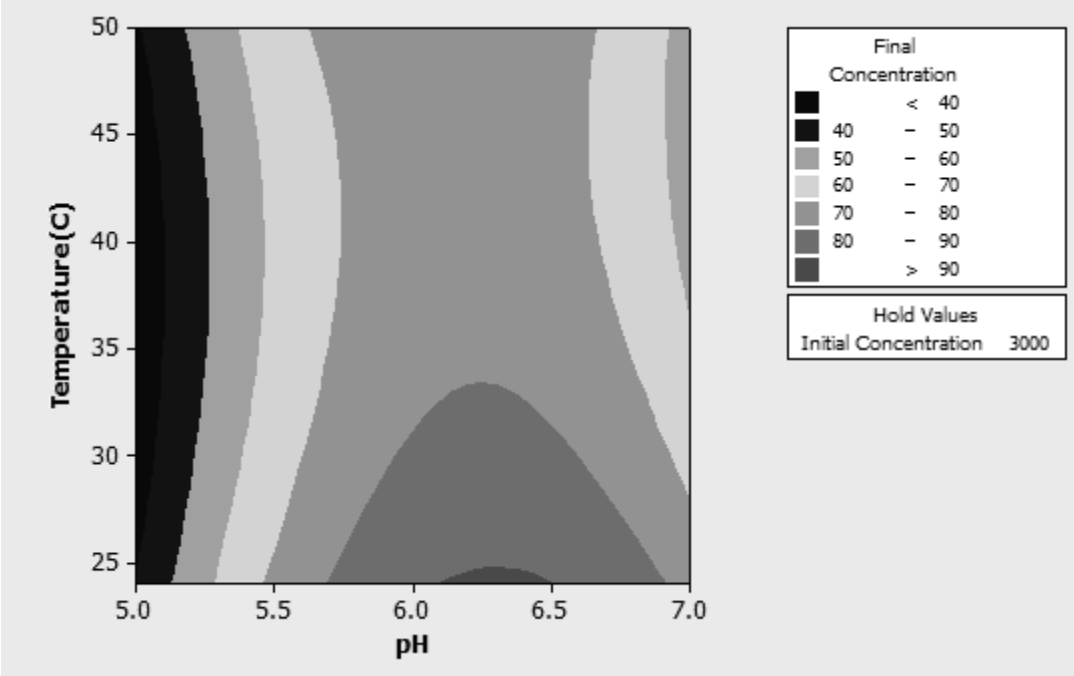

Figure 4.14: Response (Residual furfural concentrations (mg/L)) plot for pH and temperature using unused resin at a constant initial concentration of 3000 mg/L

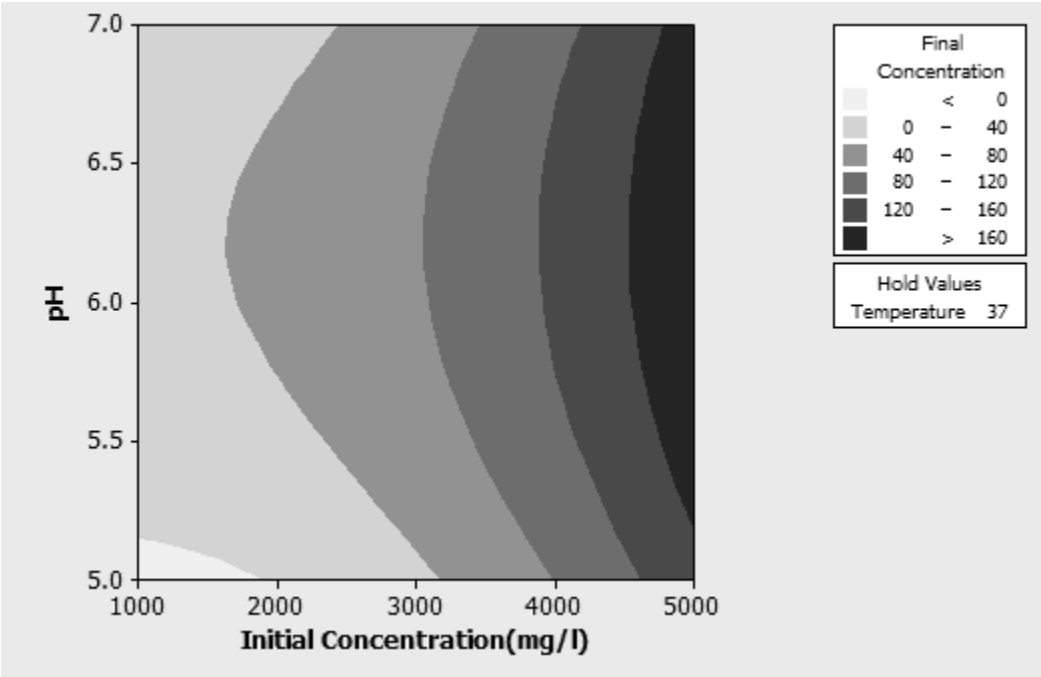

Figure 4.15: Response (Residual furfural concentrations (mg/L)) plot for initial furfural concentration and pH using unused resin at a constant temperature of 37°C

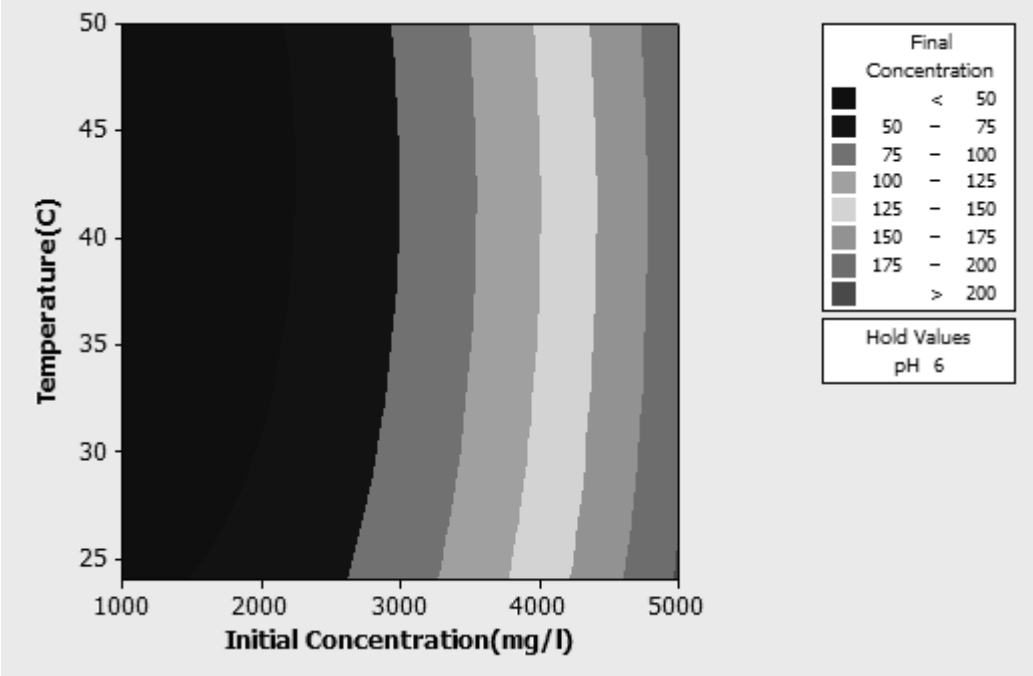

Figure 4.16: Response (Residual furfural concentration (mg/L)) plot for initial furfural concentration and temperature using unused resin at a constant pH of 6

Table 4.2: BBD and response (Residual Furfural concentrations) for unused resin

Expt. #	pH	Temp. (^{o}C) ±1^{o}C	Initial Furfural Concentration (mg/L)	Residual Furfural Concentration (mg/L)	Percent Removed
1	5	24	3000	45±10	98.±0.3
2	7	24	3000	66±9	97.8±0.3
3	5	50	3000	48±12	98.4±0.4
4	7	50	3000	50±13	98.3±0.4
5	5	37	1000	4±2	99.6±0.2
6	7	37	1000	45±5	95.5±0.5
7	5	37	5000	117±15	97.7±0.3
8	7	37	5000	161±11	96.8±0.2
9	6	24	1000	26±3	97.4±0.3
10	6	50	1000	8±2	99.2±0.2
11	6	24	5000	226±30	95.5±0.6
12	6	50	5000	214±39	95.7±0.8
13	6	37	3000	61±8	97.9±0.3
14	6	37	3000	81±7	97.3±0.2
15	6	37	3000	85±12	97.2±0.4

A RSM was developed using MINITAB 16. The ANOVA data was used to evaluate the significance of various terms (Table 4.3).

Table 4.3: ANOVA for residual furfural remaining after using unused resin

Source		Degrees of Freedom	Sequential Sum of Squares	F	P
Regression		9	61461.3	7.15	0.022
	Linear	3	52351.2	18.26	0.004
	pH	1	1458.8	1.53	0.272
	Temperature	1	244.5	0.26	0.634
	Initial Concentration	1	50647.9	53	0.0001
	Square	3	9007.1	3.14	0.125
	pH*pH	1	4130.8	3.52	0.119
	Temperature* Temperature	1	61.3	0.18	0.688
	Initial Concentration*Initial Concentration	1	4815.1	5.04	0.075
	Interaction	3	102.9	0.04	0.99
	pH*Temperature	1	89.4	0.09	0.772
	pH*Initial Concentration	1	2.8	0.00	0.959
	Temperature*Initial Concentration	1	10.8	0.01	0.92
Residual Error		5	4777.9		
	Lack-of-Fit	3	4468.4	9.62	0.096
	Pure Error	2	309.5		
Total		14	66239.2		

Small p-values (0.05) are indicative of a statistically significant variable. Overall, linear factors have a p-value of 0.004 hence; they are more significant, followed by squared factors with a p-value at 0.125 and interaction factors with value of 0.990. Notice the most significant factor is the initial concentration with a p-value of 0.001. All of the squared and interaction terms are not statistically significant because they associated with large p-values.

The larger the F-value, the more likely the factor is considered to be statistically significant. An F-value is an indication of the statistical significance as the p-values. The linear factors are the most significant, with initial concentration as the most significant individual factor. The interaction terms all had an individual F-value of close to zero, making them non-significant.

The general quadratic equation for the RSM is shown as equation 4.1. The model with the coefficients is shown as equation 4.2. In equation 4.2, all the coefficients are included because the complete equation without neglecting any terms resulted in the best fit with the experimental data. If the temperature term or any other terms were neglected, the modified equation results in an equation that cannot predict the experimental value with a good degree of accuracy.

$$\begin{aligned}\text{Residual furural concentration} &= a_0 + a_1 \times (\textit{Furfural Conc.}) \\ &+ a_2 \times (pH) + a_3 \times (\textit{Temp.}) + a_4 \times (\textit{Furfural Conc.})^2 \\ &+ a_5 \times (pH)^2 + a_6 \times (\textit{Temp.})^2 + a_7 \times (\textit{Fufural Conc.}) \times (pH) \\ &+ a_8 \times (\textit{Furfural Conc.}) \times (\text{Temp.}) + a_9 \times (pH) \times (\text{Temp.}) + \varepsilon \qquad (4.1)\end{aligned}$$

$$\begin{aligned}\text{Residual furural concentration} &= -1125.2 - 0.019208 \times (\textit{Furfural Conc.}) \\ &+ 388.059 \times (pH) - 1.42744 \times (\textit{Temp.}) + 9.03 \times 10^{-6} \times (\textit{Furfural Conc.})^2 \\ &- 30.1954 \times (pH)^2 + 0.0404709 \times (\textit{Temp.})^2 + 0.0000415 \times (\textit{Fufural Conc.}) \times (pH) \\ &+ 6.31 \times 10^{-5} \times (\textit{Furfural Conc.}) \times (\text{Temp.}) - 0.363654 \times (pH) \times (\text{Temp.}) \qquad (4.2)\end{aligned}$$

The regression coefficients for residual furfural (response) model based on using unused resin are shown in Table 4.4. Equation 4.2 is the model that can be used to predict the residual furfural concentration for unused resin. Equation 4.2 applies only for the

experimental boundaries used in BBD for all three factors. The fit of the model was checked by the coefficient of determination R^2, which was calculated to be 0.9308, indicating that approximately 93.08% of the variability in the response could be explained by the model (Figure 4.17). The model also showed statistically insignificant lack of fit ($p = 0.096$), implying that there was a 9.6% chance that the lack of fit F-value could occur due to noise. This result suggests a reasonable good fit between the experimental data and the model.

The accuracy of the model was tested using an analysis of residuals (difference between experimental and model values). The Anderson-Darling (AD) statistic was used to confirm normal distribution of the residuals (Figure 4.18).

Table 4.4: Regression coefficients for residual furfural (response) model based on using unused resin

Term	Regression Coefficient	Units
Constant	-1125.17	mg/L
pH	388.059	mg/L
Temperature	-1.42744	mg/(l*°C)
Initial Concentration	-0.0192083	
pH*pH	-30.1954	mg/L
Temperature*Temperature	0.0404709	mg(l*°C*°C)
Initial Concentration*Initial Concentration	9.02802E-06	l/mg
pH*Temperature	-0.363654	mg/(l*°C)
pH*Initial Concentration	0.0000415	l/mg
Temperature*Initial Concentration	6.30769E-05	(l*°C)/mg

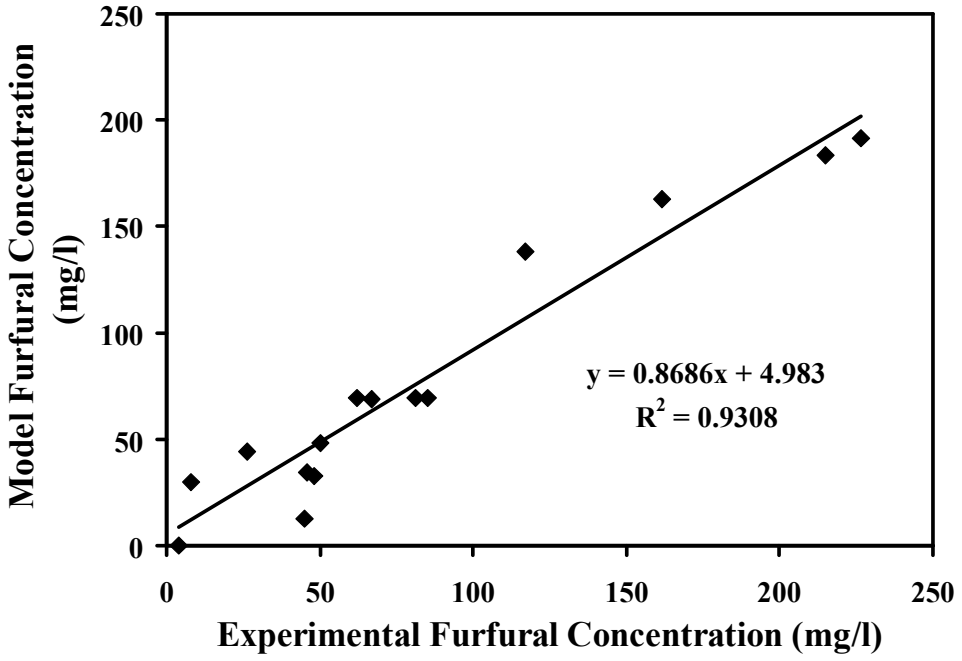

Figure 4.17: Plot model of predicted furfural removal against experimental furfural removal

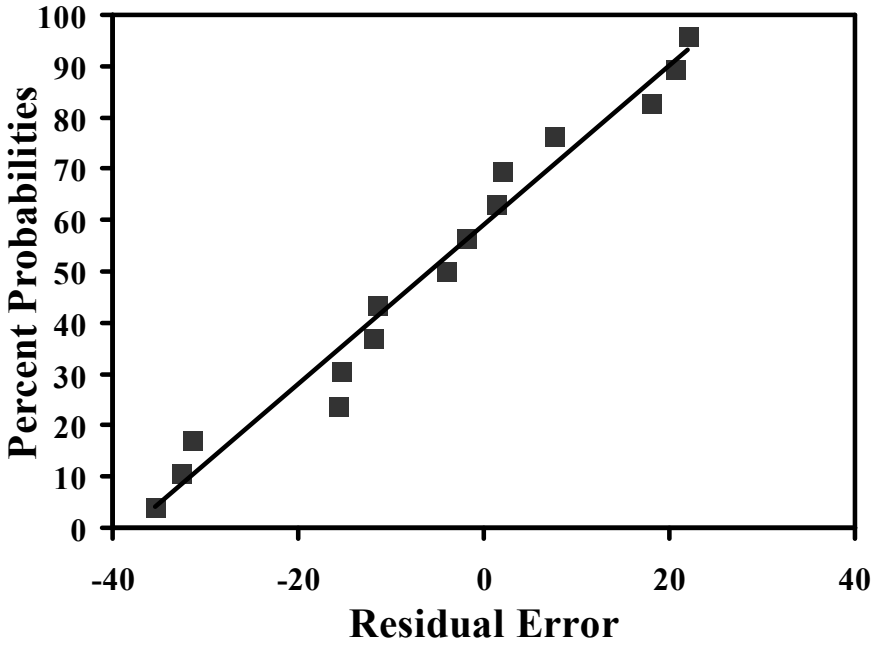

Figure 4.18: Anderson-Darling normality plot of residuals

The mean and standard deviation of the residuals were -5.914 and 18.626, respectively. The calculated AD test statistic was 0.2611, which is less than the critical value of 0.752 for a sample size of 15 and the associated p-value (0.655) of the AD statistic was significant at a 5% level. The computed AD statistic was less than the critical value and this confirms a normal-fit of the probability distribution of the residuals.

4.3 Response Model for Furfural Removal Using Regenerated Resin

Contour plots were developed to model furfural removal using the regenerated resin. Conditions used to develop the contour plots (Figures 4.19 - 4.21) for the regenerated resin are the same as those for studies conducted with the unused or new resin.

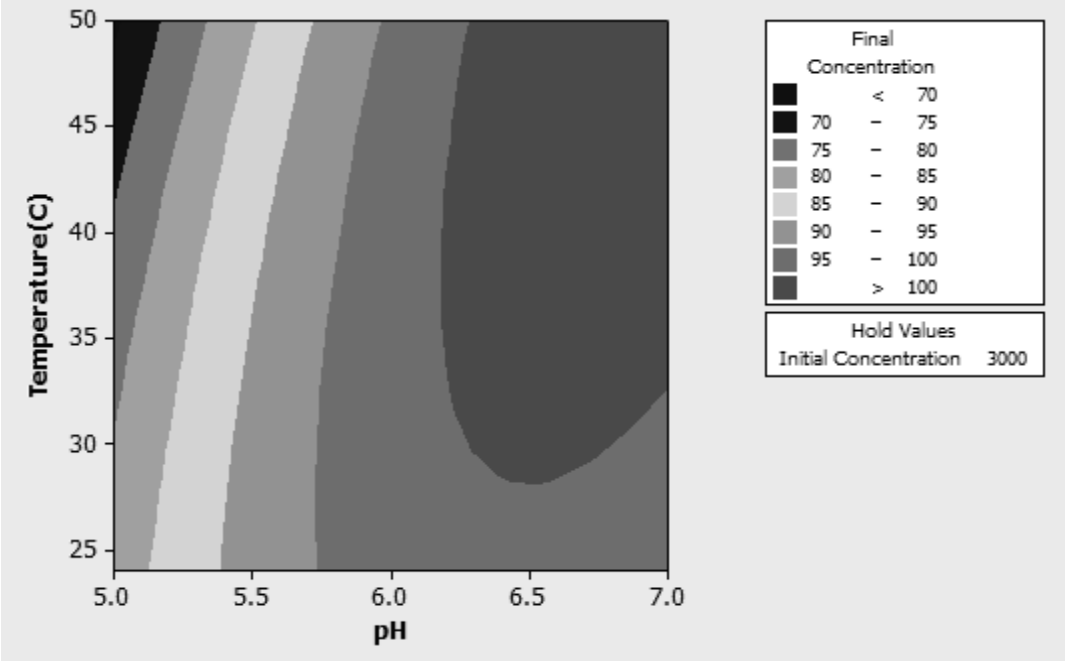

Figure 4.19: Response (Residual furfural concentrations (mg/L)) plot for pH and temperature using regenerated resin at a constant initial concentration of 3000 mg/L

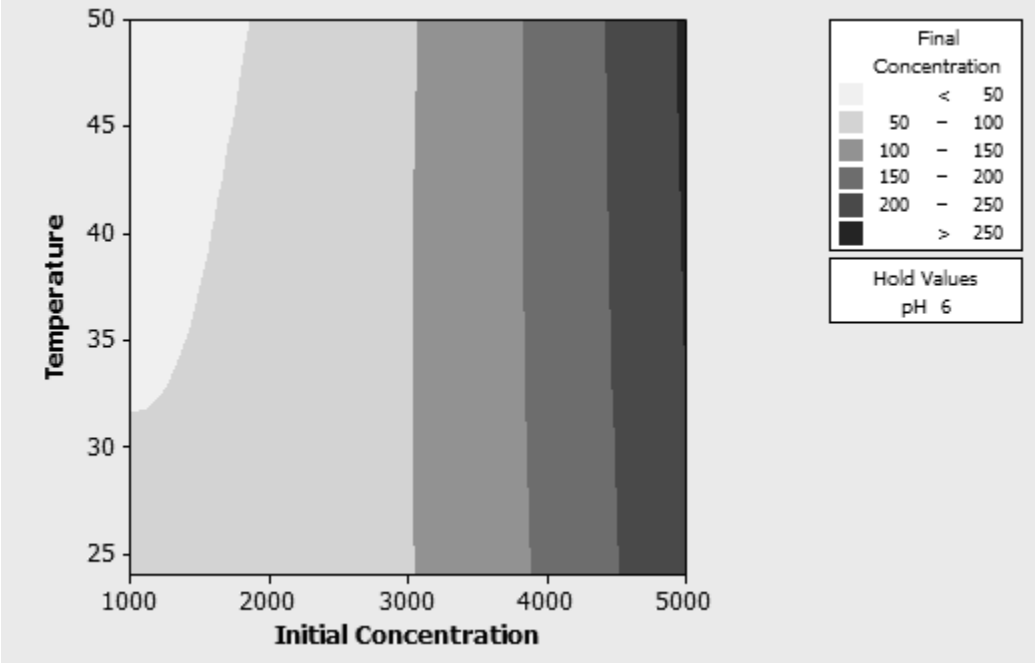

Figure 4.20: Response (Residual furfural concentrations (mg/L)) plot for temperature and initial concentration using regenerated resin at a constant pH of 6

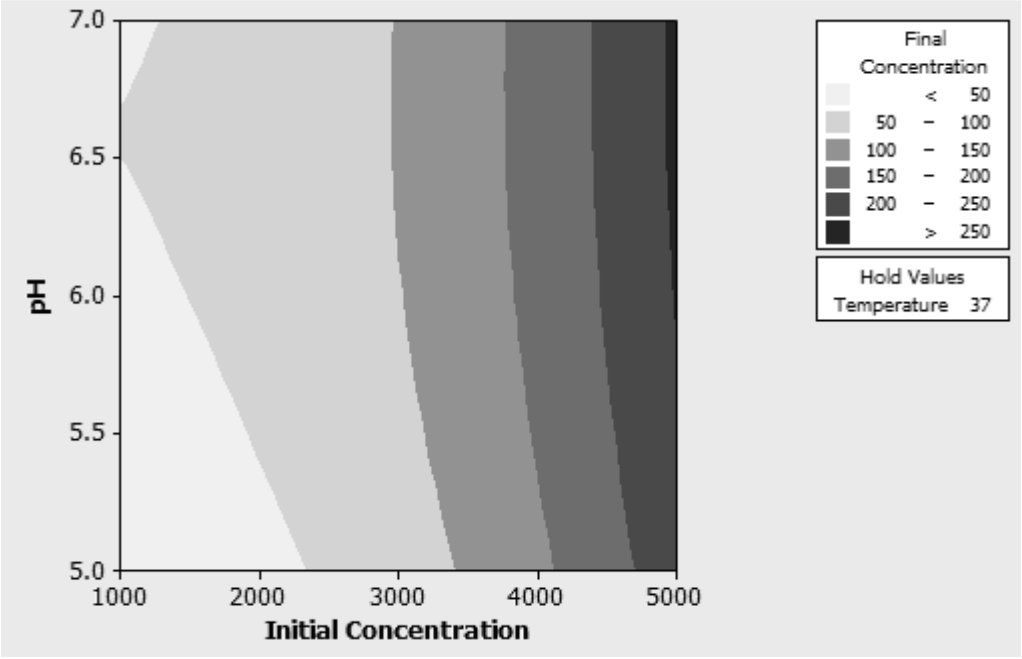

Figure 4.21: Final furfural concentrations (mg/L) contour plot using regenerated resin based on pH and initial concentration values with a temperature of 37 °C

The contours plots for the unused resin are similar to those for the regenerated resin (Figures 4.19, 4.20 and 4.21). However, note the regenerated resin performance is slightly poorer or equal in removing furfural over the range of initial concentrations under consideration (Figures 4.16 and 4.20). For example, at 37°C and at a pH set a 6 and an initial concentration of 2000 mg/L, the response is 40 to 80 mg/L residual furfural (Figure 4.15). In comparison, the response is 50 to 100 mg/L residual furfural for the same conditions (Figure 4.20). At pH 6 and initial furfural levels from 2000 to 5000 mg/L, temperature has no effect on the residual furfural level (Figure 4.20). The plots shown in Figures, 4.19, 4.20 and 4.21 are based on the residual concentrations shown in Table 4.5.

Table 4.5: BBD and response (Residual furfural conscentrations) for regenerated resin

Experiment Number	pH	Temp. (^{o}C) $\pm1^{o}$C	Initial Furfural Concentration (mg/L)	Residual Furfural Concentration (mg/L)	Percent Removal
1	5	24	3000	101±7	96.6±0.2
2	7	24	3000	82±10	97.2±0.3
3	5	50	3000	81±11	97.3±0.4
4	7	50	3000	85±16	97.2±0.5
5	5	37	1000	22±4	97.7±0.4
6	7	37	1000	45±10	95.5±1.0
7	5	37	5000	201±19	96.0±0.4
8	7	37	5000	262±19	94.7±0.4
9	6	24	1000	38±4	96.1±0.4
10	6	50	1000	30±6	97.0±0.6
11	6	24	5000	251±40	95.0±0.8
12	6	50	5000	271±62	94.6±1.3
13	6	37	3000	90±11	97.0±0.4
14	6	37	3000	102±12	96.6±0.4
15	6	37	3000	100±15	96.6±0.5

All of the residual concentrations in Table 4.5 are well below 500 mg/L. In general, the residual concentrations in Table 4.5 are all greater than the residual furfural concentrations from studies conducted with the unused resin. This difference is based on a comparison of the residual levels in Tables 4.1 and 4.5 which show values varying from a less than 1% difference to 475%. Note although the greatest percent difference is 475%, the residual furfural concentrations for the unused and regenerated resin were 4 mg/L and 23 mg/L, respectively (experiment number 5).

ANOVA was conducted in order determine the significance of single and interacting factors on the residual concentrations (Table 4.6). The most significant individual factor is the initial concentration with an F value of 129.48 and a p value of 0.000. The squared initial concentration term is also significant with an F value of 15.01 and a p value of 0.051. None of the other factors (linear, squared and interaction) were statistically significant. However, note neglecting any of the terms resulted in a model that was unable to predict the experimental residual concentration. Hence, none of the terms were neglected from the model equation. Notice the contours plots for the furfural data with regenerated resin are similar the contours for studies conducted with furfural removal with unused resin.

Table 4.6: ANOVA for residual furfural remaining after using regenerated resin

Source		Degrees of Freedom	Sequential Sum of Squares	F	P
Regression		9	95733.2	16.44	0.003
	Linear				
	pH	1	1215.7	1.88	0.229
	Temperature	1	6	0.01	0.927
	Initial Concentration	1	83789.9	129.48	0.000
	Square	3	10377.5	5.35	0.051
	pH*pH	1	553.1	0.42	0.544
	Temperature* Temperature	1	112.6	0.01	0.911
	Initial Concentration* Initial Concentration	1	9712.7	15.01	0.012
	Interaction	3	344.1	0.18	0.907
	pH*Temperature	1	125.2	0.19	0.678
	pH*Initial Concentration	1	16.2	0.03	0.880
	Temperature*Initial Concentration	1	202.6	0.31	0.600
Residual Error		5	3235.7		
	Lack-of-Fit	3	3155.5	26.23	0.037
	Pure Error	2	80.2		
Total		14	98978.8		

The regression coefficients for the quadratic equation are shown in Table 4.7.

Table 4.7: Regression coefficients for residual furfural (response) model based on using regenerated resin

Term	Regression Coefficient	Units
Constant	-190.067	mg/L
pH	96.6858	mg/L
Temperature	-2.78675	mg/(l*°C)
Initial Concentration	-0.0419363	
pH*pH	-6.0875	mg/L
Temperature*Temperature	-0.00923817	mg(l*°C*°C)
Initial Concentration*Initial Concentration	0.000012822	l/mg
pH*Temperature	0.430385	mg/(l*°C)
pH*Initial Concentration	0.0010075	l/mg
Temperature*Initial Concentration	0.00027375	(l*°C)/mg

Equation 4.3 is the model that can be used to predict the residual furfural concentration for the regenerated resin:

$$\begin{aligned}\text{Residual fururalconcentration} &= -190.067 - 0.0419363\times(Furfural\ \ Conc.)\\ &+ 96.6958\times(pH) - 2.78675\times(Temp.) + 0.0000128225\times(Furfural\ Conc.)^2\\ &- 6.0875\times(pH)^2 - 0.00923817\times(Temp.)^2 + 0.0010075\times(Fufural\ \ Conc.)\times(pH)\\ &+ 0.00027375\times(Furfural\ \ Conc.)\times(\text{T}emp.) + 0.430385\times(pH)\times(\text{T}emp.)\end{aligned} \quad (4.3)$$

This equation is applicable for the experimental boundaries used in BBD for all three factors considered for the regenerated resin. Residuals (difference between model outputs and experimental results) were plotted (Figure 4.22) to verify a linear relationship between the predicted values and those obtained experimentally. The plot is linear with a regression coefficient of 0.945 (R^2 value 0.8931). This indicates a reasonable linear relationship between the experimental values and the output values from the model. The

Anderson-Darling normality plot (Figure 4.23) demonstrates a normal distribution of the residuals.

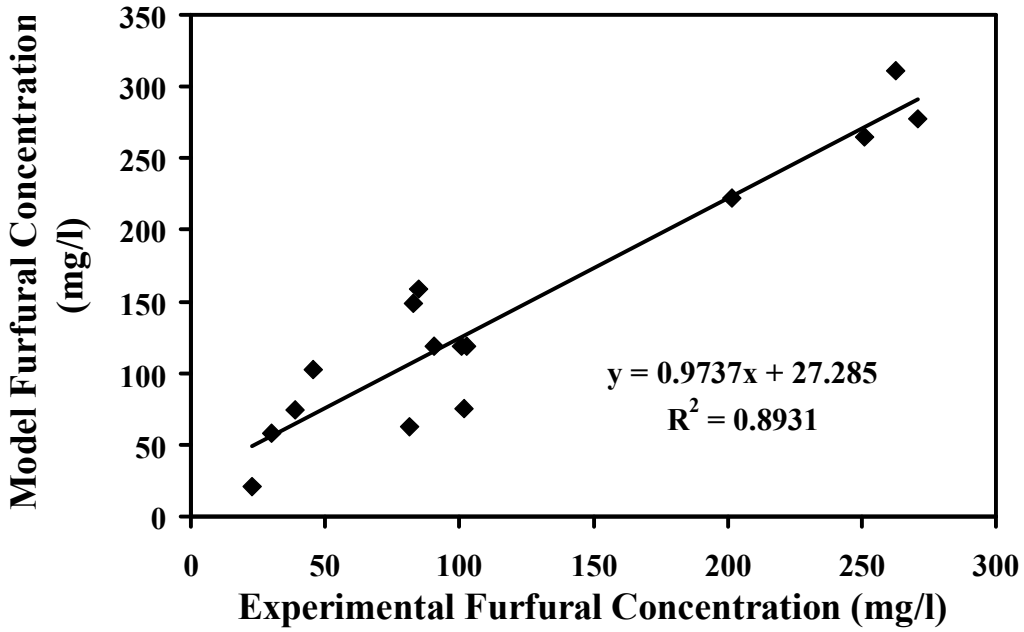

Figure 4.22: Plot model of predicted furfural removal against experimental removal

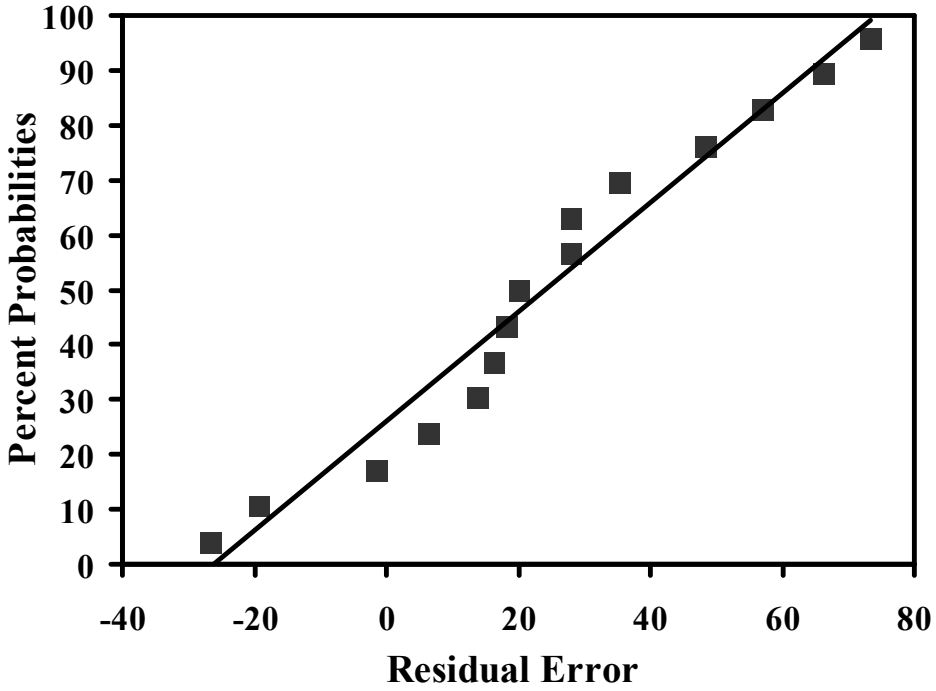

Figure 4.23: Anderson-Darling normality plot of residuals

The mean and standard deviation of the residuals were 24.18 and 28.92, respectively. The AD test statistic was 0.181, which is less than the critical value of 0.752 for a sample size of 15 and the associated p-value (0.655) of the AD statistic was significant at a 5% level. The computed AD statistic was lower than the critical value and this confirmed a normal-fit of the probability distribution of the residuals.

4.4 Response surface model for HMF removal

Contour plots were also developed to model HMF removal using the XAD-4 resin. The contour plots resulting from the BBD experimental design for HMF removal is shown in Figures 4.24 through 4.26. The factors were the same as those used in the furfural design, except the initial concentrations were 250, 500 and 750 mg/L instead of 1000, 3000 and 5000 mg/L.

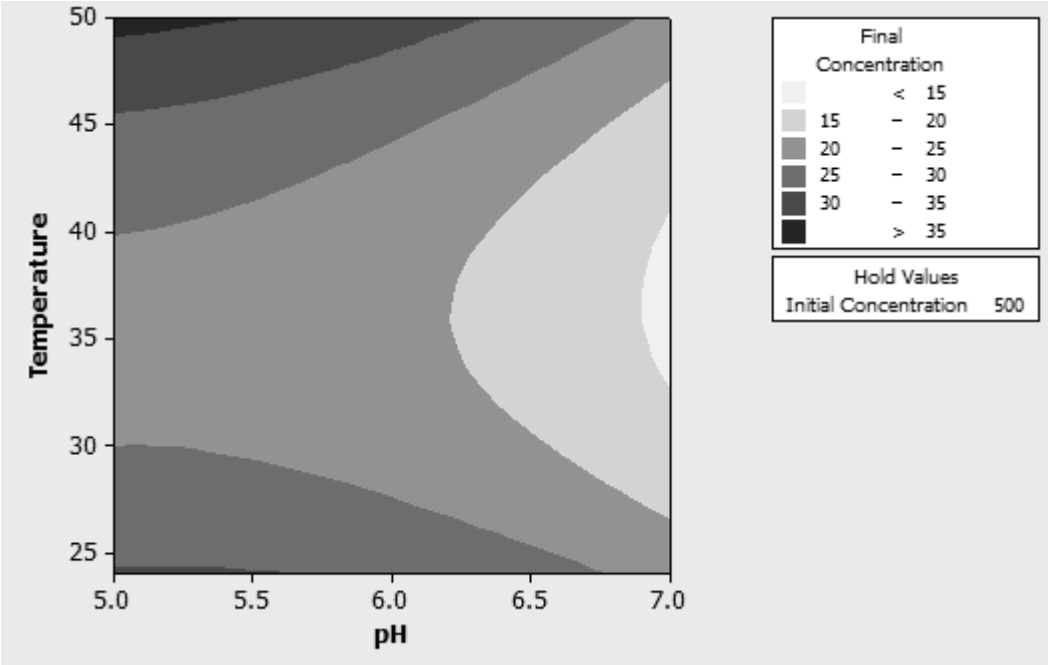

Figure 4.24: Response (Residual HMF concentrations (mg/L)) plot for pH and temperature using unused resin at a constant initial concentration of 500 mg/L

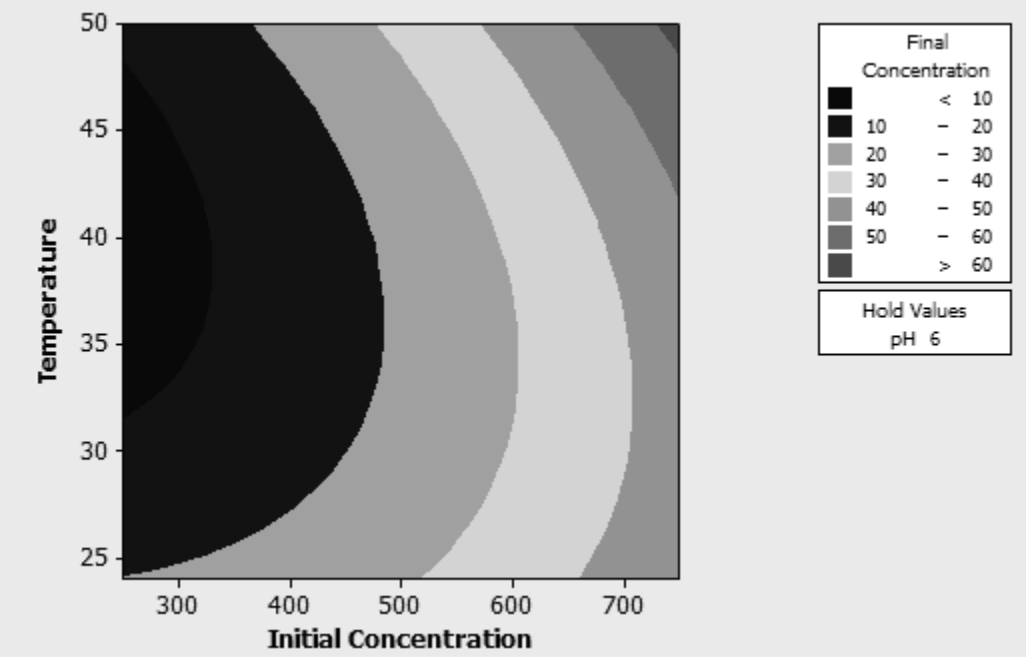

Figure 4.25: Response (Residual HMF concentrations (mg/L)) plot for initial concentration and temperature using unused resin at a constant pH of

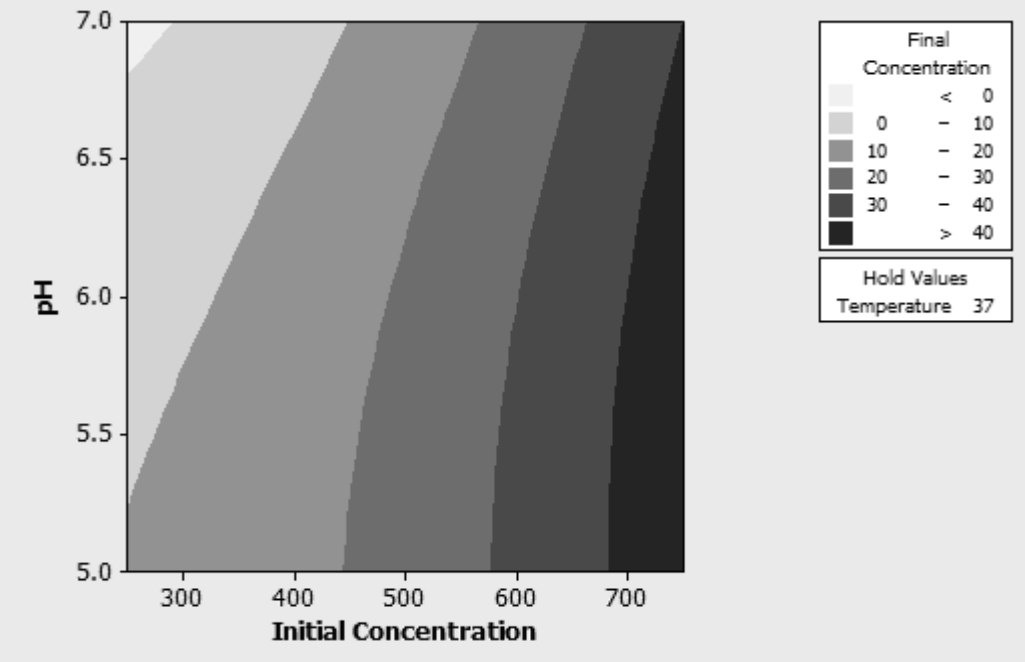

Figure 4.26: Response (Residual HMF concentrations (mg/L)) plot for varying initial concentration and pH using unused resin at 37 °C

The BBD design and HMF residual concentration are shown in Table 4.8. Data from Table 4.8 was used to create the contour plots and quadratic model equation. HMF

removal using the unused resin increased when the pH was changed from 5 to 7 (Figures 4.24 and 4.26). The optimal temperature was approximately 37°C with the final concentration decreasing as the temperature approached 37°C from both sides (Figures 4.24 and 4.25). Note a reduction in the residual concentration as the initial concentration decreases (Figure 4.25 and 4.26).

Table 4.8: BBD and response (Residual HMF concentration) for unused resin

Expt #	pH	Temp (^{o}C) $\pm1^{o}$C	Initial HMF Concentration (mg/L)	Final HMF Concentration (mg/L)	Percent Removal
1	5	24	500	32±6	93.5±1.3
2	7	24	500	15±4	96.9±0.9
3	5	50	500	43±7	91.2±1.4
4	7	50	500	21±8	95.7±1.6
5	5	37	250	12±3	94.8±1.0
6	7	37	250	10±4	95.9±0.8
7	5	37	750	35±8	95.3±1.1
8	7	37	750	37±3	95.0±0.4
9	6	24	250	15±4	93.8±1.4
10	6	50	250	2±1	99.2±0.4
11	6	24	750	57±5	92.3±0.9
12	6	50	750	67±7	91.0±0.7
13	6	37	500	28±5	94.3±1.0
14	6	37	500	16±2	96.6±0.4
15	6	37	500	18±3	96.4±0.6

All the residual concentration values were below 100 mg/L. This is likely attributed to the lower starting concentration. The lower initial concentration was selected based on the HMF concentration detected in steam exploded liquors produced from corn cobs and switchgrass.

ANOVA was conducted in order to determine the significance of single and interacting factors on the residual concentration (Table 4.9).

Table 4.9: ANOVA for residual HMF remaining after using unused resin

Source		Degrees of Freedom	Sequential Sum of Squares	F	P
Regression		9	3891.76	2.92	0.125
	Linear	3	3304.25	7.43	0.027
	pH	1	191.69	1.29	0.307
	Temperature	1	21.62	0.15	0.718
	Initial Concentration	1	3090.95	20.86	0.006
	Square	3	438.16	0.99	0.47
	pH*pH	1	39.17	0.12	0.742
	Temperature*Temperature	1	306.02	0.63	0.464
	Initial Concentration*Initial Concentration	1	92.97	2.24	0.195
	Interaction	3	149.35	0.34	0.801
	pH*Temperature	1	7.56	0.05	0.83
	pH*Initial Concentration	1	7.34	0.05	0.833
	Temperature*Initial Concentration	1	134.44	0.91	0.385
Residual Error		5	740.96		
	Lack-of-Fit	3	662.35	5.62	0.155
	Pure Error	2	78.61		
Total		14	4632.72		

The ANOVA indicate the most statistically significant terms are linear in pH and initial concentration. However, neglecting any of the terms resulted in a model that was unable to predict the experimental residual concentration. Hence, none of the terms were neglected from the model equation. Notice the contours plots for the HMF data are similar the contours for studies conducted with HMF.

Table 4.10 Regression coefficients for HMF response model based on using unused resin

Term	Regression Coefficient	Units
Constant	49.6881	mg/L
pH	22.7635	mg/L
Temperature	-4.27439	mg/(l*C)
Initial Concentration	-0.100184	
pH*pH	-2.20458	mg/L
Temperature*Temperature	0.0559936	mg(l*C*C)
Initial Concentration*Initial Concentration	8.02867E-05	l/mg
pH*Temperature	-0.105769	mg/(l*C)
pH*Initial Concentration	0.00542	l/mg
Temperature*Initial Concentration	0.00178385	(l*C)/mg

Equation 4.4 can be used to predict the residual HMF concentration.

$$\begin{aligned}\text{The model Residual furural concentration} &= 49.68817 - 0.100184\times(HMF\ Conc.)\\ &+ 22.7635\times(pH) - 4.27439\times(Temp.) + 0.0000802867\times(HMF\,Conc.)^2\\ &- 2.20458\times(pH)^2 + 0.0559936\times(Temp.)^2 + 0.00542\times(HMF\,Conc.)\times(pH)\\ &+ 0.00178385\times(HMF\ Conc.)\times(\text{T}emp.) - 0.105769\times(pH)\times(\text{T}emp.) \qquad (4.4)\end{aligned}$$

Equation 4.4 is applicable only for the experimental boundaries used in the BBD for all three factors. Verification of linearity between the relationship between the predicted and experimental residual concentration is shown in Figure 4.27. The fit of the model was checked by the coefficient of determination R^2, which was calculated to be 0.8497, indicating that approximately 85% of the variability in the response could be explained by the model. The model also showed statistically insignificant lack of fit ($p = 0.115$), implying that there was a 11.5% chance that the lack of fit F-value could occur due to noise. This result suggests a reasonable good fit between the experimental data and the model.

The Anderson-Darling normality plot (Figure 4.28) demonstrates a normal distribution of the residuals.

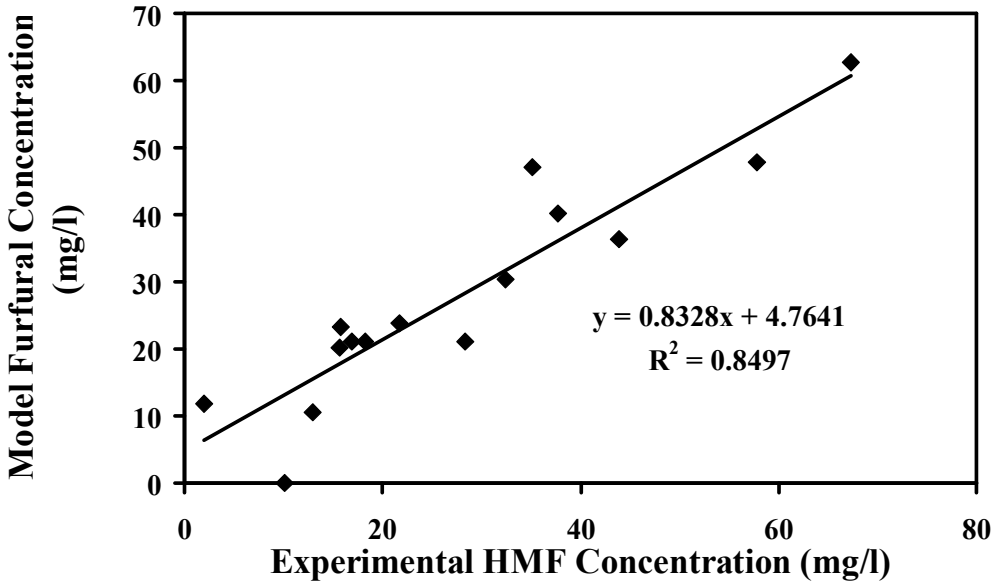

Figure 4.27: Predicted versus experimental residual HMF concentration for unused resin

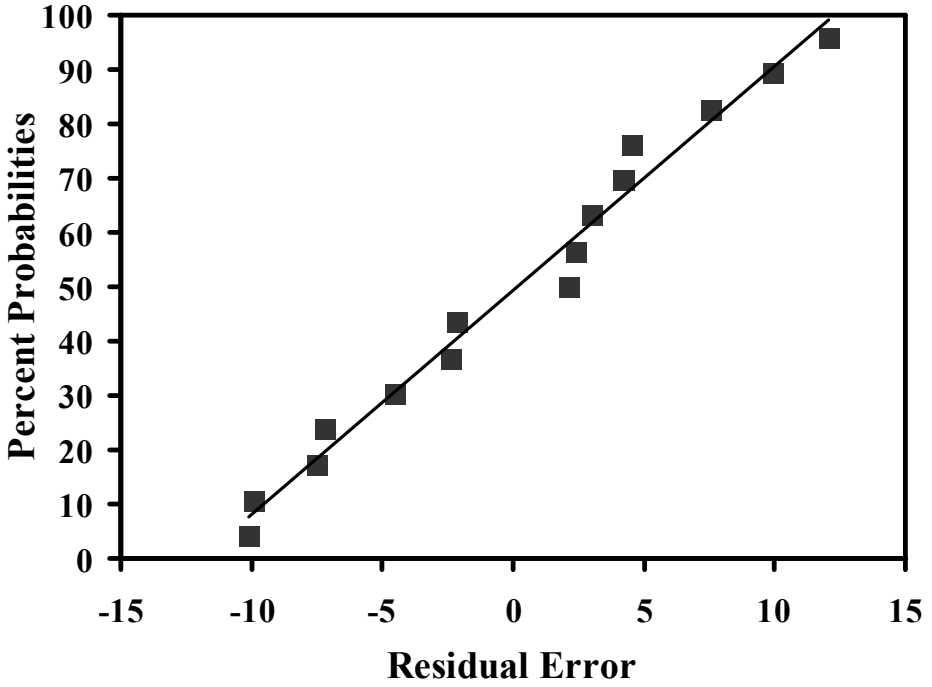

Figure 4.28: Anderson-Darling normality plot of residuals for HMF treated using unused resin

The mean and standard deviation of the residuals are 0.129 and 7.061, respectively. The calculated AD test statistic was 0.257, which is less than the critical value of 0.752 for a sample size of 15 and the associated p-value (0.669) of the AD statistic was significant at a 5% level. The lower computed AD statistic when compared to the critical value confirms a normal-fit of the probability distribution of the residuals.

4.5 Initial Furan Removal Rates

Table 4.11 shows the initial furan removal rates for all of the experiments with furans. The rates are larger for the unused resin than for regenerated resin for the majority of the experiments. The rates were largely depended on the initial concentration of the furan for both furfural and HMF. A higher initial furan concentration resulted in a higher initial removal rate. Little significance could be attributed to the pH/temperature conditions on the initial removal rates. Regeneration of the resin had no effect on the initial removal rate for furfural. The initial furfural removal rates were greater than the initial HMF removal rates. This suggests the resin selectively adsorbs furfural compared to HMF.

Table 4.11: Initial furan removal rates for BBD

Exp. #	pH	Temp (^{o}C)	Initial Concentration Level[1]	Initial Furan Removal Rate (mg furan/mg resin·min)		
				Furfural (with unused resin)	Furfural (with regenerated resin)	HMF (with unused resin)
1	5	24	0	26.9±0.7	27.0±0.4	4.0±0.1
2	7	24	0	27.1±0.3	26.6±0.6	4.5±0.0
3	5	50	0	27.9±0.3	27.8±0.3	3.9±0.1
4	7	50	0	27.1±0.6	25.8±0.5	4.2±0.2
5	5	37	-1	9.2±0.1	8.8±0.1	2.1±0.1
6	7	37	-1	8.7±0.1	8.0±0.0	2.1±0.0
7	5	37	1	46.4±1.1	44.2±1.3	6.3±0.3
8	7	37	1	47.1±0.3	41.4±1.1	6.1±0.2
9	6	24	-1	7.9±0.1	7.6±0.1	1.9±0.1
10	6	50	-1	9.2±0.3	7.7±0.2	2.3±0.1
11	6	24	1	43.8±.8	42.1±0.5	5.6±0.2
12	6	50	1	45.3±0.7	41.1±0.8	5.6±0.1
13	6	37	0	28.2±0.2	27.0±0.4	4.1±0.3
14	6	37	0	27.9±0.3	26.9±0.5	4.5±0.3
15	6	37	0	28.0±0.1	27.0±0.4	4.3±0.1

Note 1: Initial Concentration levels -1, 0, 1 are 1000, 3000 and 5000 mg/L for furfural and 250, 500 and 750 mg/L for HMF respectively

4.6 Discussion

Both unused and regenerated resin removed furfural to levels below 300 mg/L in all experiments, which is significantly less than levels that have been reported to inhibit anaerobic cultures (Cao *et. al.,* 2010). This is important because greater than 500 mg/L furfural can affect H_2 yields in mixed anaerobic communities. Pretreating lignocellulosic biomass such as switchgrass using steam explosion results in furfural concentrations of approximately 1700 to 2300 mg/L (Cao et. al., 2010). In comparison to data reported by Cao *et. al.,* (2010), at an initial concentration of 5000 mg/L, the furfural levels in solution

remained below 300 mg/L for various combinations of pH and temperature. This indicates that the resin can be used to remove furfural from switchgrass liquor under the pH and temperature ranges based on the BBD.

The HMF levels detected were below 70 mg/L for all the conditions examined. Data by Cao *et al.* (2010) demonstrated that inhibitors have a synergistic inhibition on H_2 yields. The combined maximum levels of furfural and HMF detected were below the 500 mg/L threshold. HMF concentrations generated from pretreating switchgrass are normally within the 200 mg/L to 350 mg/L range (Cao *et. al.,* 2010). With the XAD-4 resin treating a 750 mg/L solution to below 70 mg/L, this indicates that the resin was able to remove both furfural and HMF to levels that will not inhibit H_2 production in cultures fed a switchgrass liquor.

The resin was able to remove the furans rapidly at the beginning of each experiment; however, the removal rate decreased with time. In all cases where the initial HMF concentration was 750 mg/L, the concentration decreased to below 500 mg/L within the first 20 minutes. The only condition when the residual concentrations exceeded 500 mg/L furfural after 20 minutes with unused resin was when the initial concentration was at 5000 mg/L at a pH of 6 and 24^{o}C. In all experiments where the initial concentrations were 5000 mg/L, the furfural concentrations were depleted to 400 mg/L or less within 60 minutes. When using regenerated resin for furfural removal, all of the experiments showed residual concentrations reaching less than 500 mg/L after 20 minutes except for four experiments where the initial concentration was 5000 mg/L. Under these experimental conditions, the concentrations decreased to less than 400 mg/L within 120 minutes. If the initial furfural and HMF concentrations are less than 3000 mg/L and 500

mg/L, respectively, in the switchgrass liquor, based on data from these studies, it can be concluded that 2 g resin/15 mL liquor resin concentration would be able to remove HMF and furfural to acceptable levels in less than 60 minutes. The ANOVA data indicate that the only factors that are statistically significant are the initial furan concentration and pH. However, developing the model based on pH and initial concentration was insufficient to predict the residual furan level. Hence, all the models developed for unused and regenerated resin included all the terms. Including all the terms accounted for a model that was able to predict the residual furan concentration. The response models prediction for the conditions examined correlated reasonably well with the experimental data. The R^2 value for the 3 regression equation ranged from 0.849 to 0.931. All three models had residuals that follow a normal pattern as per the Anderson-Darling statistic. Hence, the models can be used to predict the residual concentrations within the range of conditions for the three factors.

The residual concentrations for the unused and regenerated resin indicate that the XAD-4 resin can successfully be regenerated. The percent removed for the regenerated resin were within 2% of the percent removed using the unused.

The contour plots can be used to infer temperatures, pH and initial furan concentration that can lead to reduced residual concentrations. The ANOVA analysis suggested the statistical important variables were pH and initial concentration. However, the modified model could not predict the residual furan levels. Hence, none of the terms were removed from the quadratic equation. The model was able to predict the residual furan concentration under the conditions examined. Under all the conditions examined, 91% to 99% furan was removed from the aqueous phase. This high amount of removal was

likely attributed to the quantity of resin added to the aqueous phase. The response is expected to be affected by pH, temperature and initial furan concentration. However, in this study, the quantity of resin added was large and it did not significantly affect the quantity of furan removed. Notice greater than 95% of the furan was removed irrespective of the pH, temperature and initial furan concentration. Future work should assess the impact of varying the quantity of resin on the percent of furan removed. Data from this study demonstrated that within a pH 5 to 7, 24^{o}C to 50^{o}C and 1000 mg/L to 5000 mg/L furfural or 250 mg/L to 750 mg/L HMF the resin is effective in reducing the furans to levels that are not inhibitory to anaerobic microorganisms. Nilvebrant (2001) reported 8g anion resin/50 mL hydroslate removed 65% from 5.10 g/l of HMF and 68% from 0.82 g/l of furfural after 1 hour of incubation. Note the anion resin also removed 75% of the glucose present in the pretreatment liquor. Nilvebrant (2001) also reported that XAD-8 resin removed 42% of the HMF and 65% of the furfural without removing any glucose after 1 hour of incubation.

Chapter 5: Furan Removal from Milli-Q Water and Switchgrass Liquor

Studies with individual furans have established that the XAD-4 resin was able to remove both furfural and HMF from an aqueous solution under varying pH, temperature and initial furan concentration. In stage 2, the experiments are designed to establish if the XAD-4 resin can remove mixtures of furfural and HMF to satisfactory levels. In the first series of experiments, removing both furfural and HMF from milli-Q water was examined under conditions established from previous studies. In the subsequent experiments, furan removal was examined using steam exploded switchgrass liquor.

5.1 Switchgrass Liquor Composition

Steam exploded switchgrass liquor was analyzed for sugar content, acetic acid and furan derivatives (Table 5.1)

Table 5.1: Sugar and inhibitor concentration in pretreated switchgrass liquor

Component		Concentration (mg/L)
Total Sugar[1]		32400 ± 1250
	Glucose	20100 ± 1050
	Xylose	12100 ± 590
Total Furans		2166 ± 54
	Furfural	1942 ± 53
	HMF	224 ± 8
Acetate		4200 ± 330

Note 1: Arabinose, mannose and galactose were present in concentrations less than 100 mg/l.

The furan concentrations from pretreated switchgrass are used when simulating experiments requiring furfural and HMF concentrations that match pretreated switchgrass liquor. Sugar composition is used for designing hydrogen fermentation experiments comparing switchgrass liquor yields to pure glucose yields.

5.2 Simultaneous Removal of Furfural and HMF Using Unused and Regenerated Resin

Experiments were conducted using unused and regenerated resin to remove HMF and furfural for 360 min at pH 5, 6 and 7 and 21^{o}C (Figures 5.1 and 5.2). At an initial furfural concentration of 2000 mg/L, the residual furfural levels were approximately the same for pH 5, 6 and 7. Similarly, the residual HMF concentration was also approximately the same at pH 5, 6 and 7 when the initial HMF level was set at 250 mg/L. In case of furfural and HMF, the percent removed were 99.1±0.3% and 96.4±0.9%, respectively, under the various pH conditions.

When regenerated resin was used, at an initial concentration of 2000 mg/L, the residual furfural concentration were 18±2.7 mg/L and 27±3.9 mg/L at pH 5 and 6, respectively. Under these conditions, the percent furfural removed ranged from 98.7±0.5% to 99.1±0.2%. With an initial concentration of 250 mg/L of HMF, the residual concentrations reached 13±2.3 mg/L and 19±2.6 mg/L at a pH of 5 and 6, respectively. The percent HMF removed ranged from 92.3±1.3% to 94.7±1.1%.

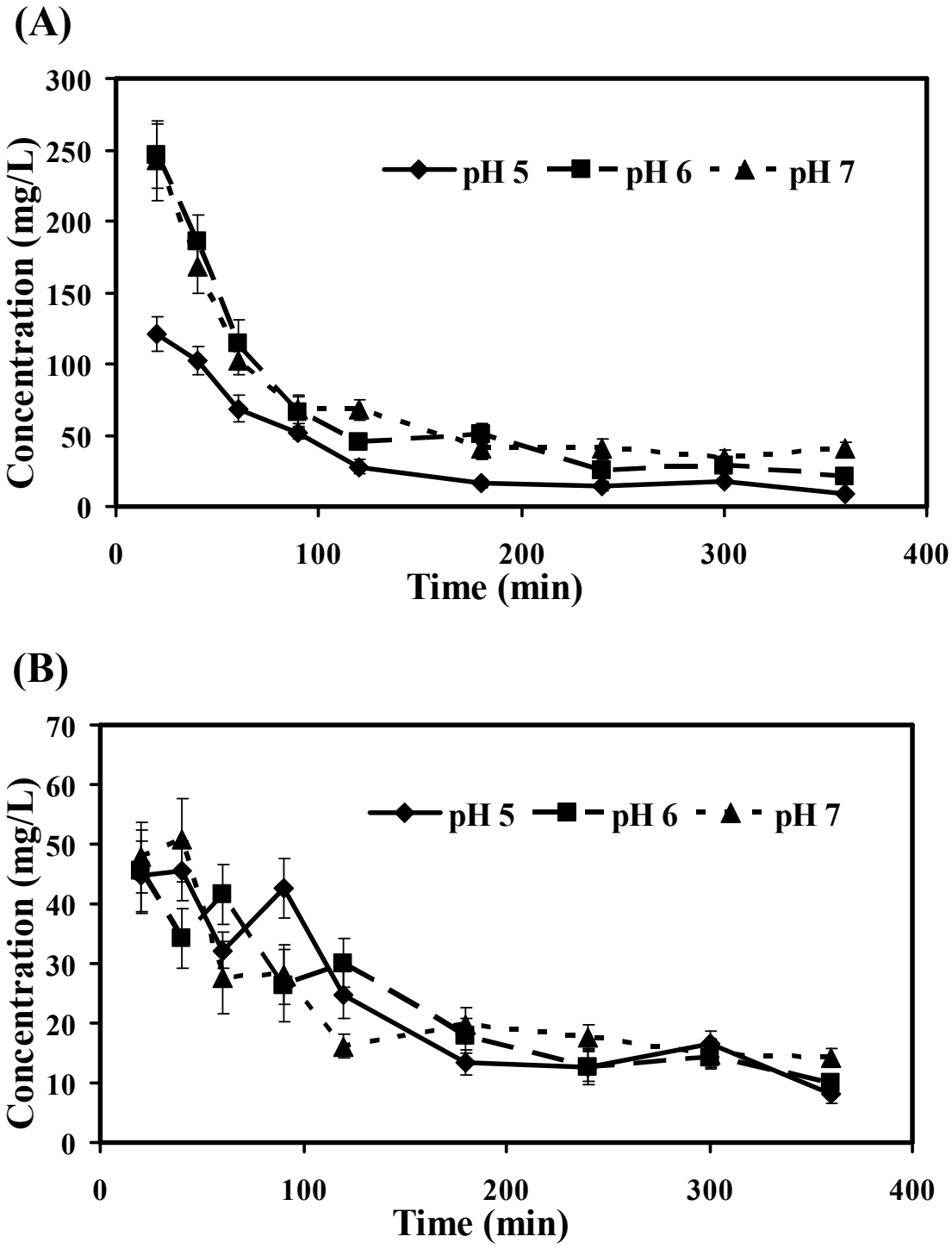

Figure 5.1: Removal for both furans added to a water solution (A) Furfural (B) HMF

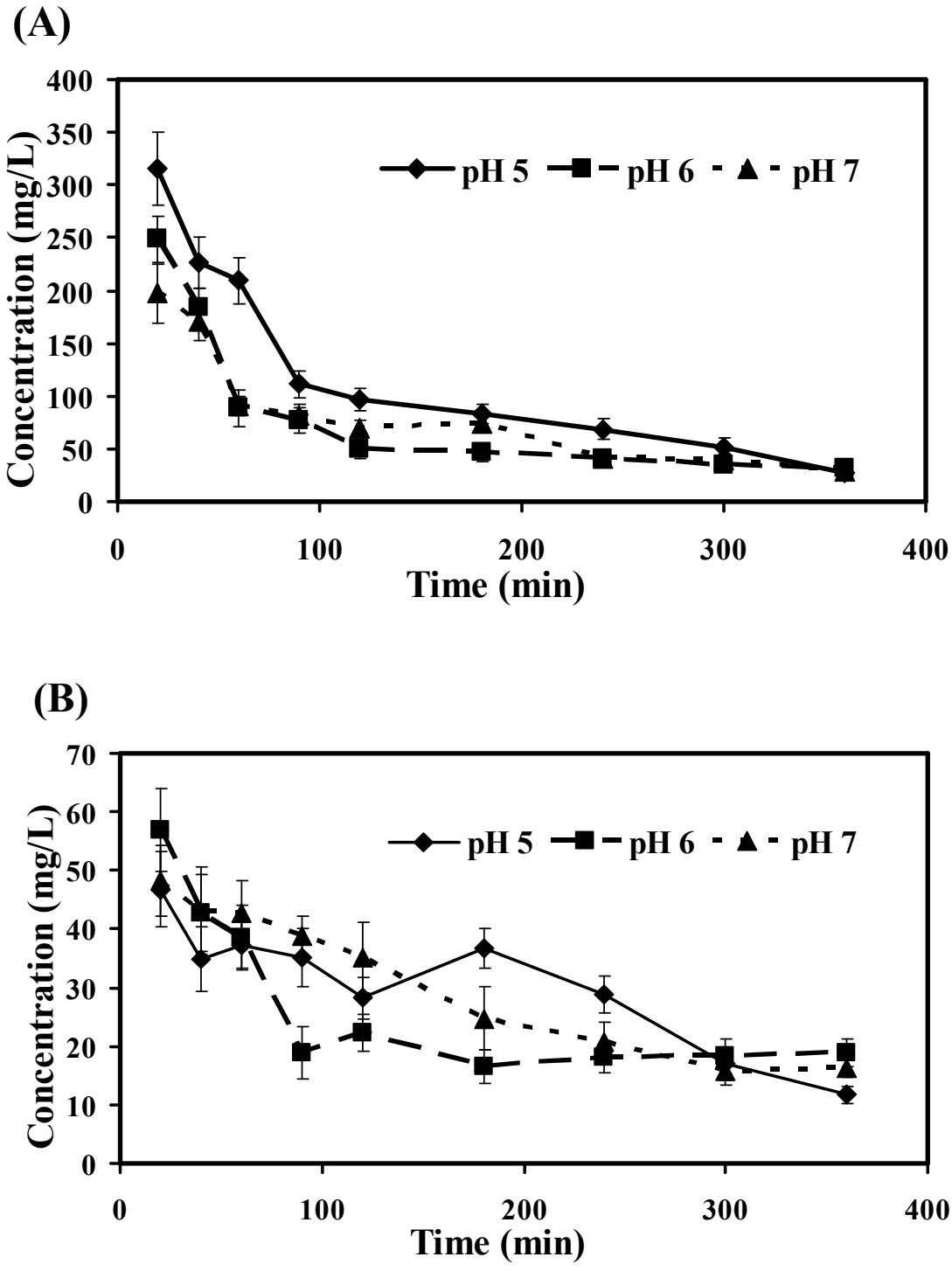

Figure 5.2: Furan removal with regenerated resin from solutions containing both furans (A) Furfural (B) HMF

Table 5.2 shows the initial furan removal rates for the above experiments. For unused resin, a pH of 5 has the highest initial removal rate for furfural, where as the values are all relatively the same for HMF. For unused resin furfural and HMF initial removal rates are slightly higher at a pH of 5 compared to pH 6 and pH 7. In general, the initial removal rates for furfural were greater than those for HMF.

Table 5.2: Initial furan removal rates when both furans are present in solution

Furan Initial Removal Rate (mg furan/mg resin·min)				
	Unused Resin		**Regenerated Resin**	
pH	**Furfural**	**HMF**	**Furfural**	**HMF**
5	18.7±2.2	2.1±0.4	17.5±1.6	2.0±0.2
6	17.3±1.8	2.0±0.4	17.4±1.1	1.9±0.3
7	17.3±1.7	2.0±0.2	17.3±1.3	1.9±0.2

5.3 Furan Removal from Switchgrass Liquor Using Unused and Regenerated Resin

The initial furfural and HMF concentration in the switchgrass liquor was 1942±53 mg/L and 224±8 mg/L, respectively. Under the experimental conditions the residual furfural and HMF concentrations of 23±3.5 mg/L and 10 ±0 .8 mg/L correspond to percent removals of 98.8±0.5% and 95.5±0.4%, respectively (Figure 5.3).

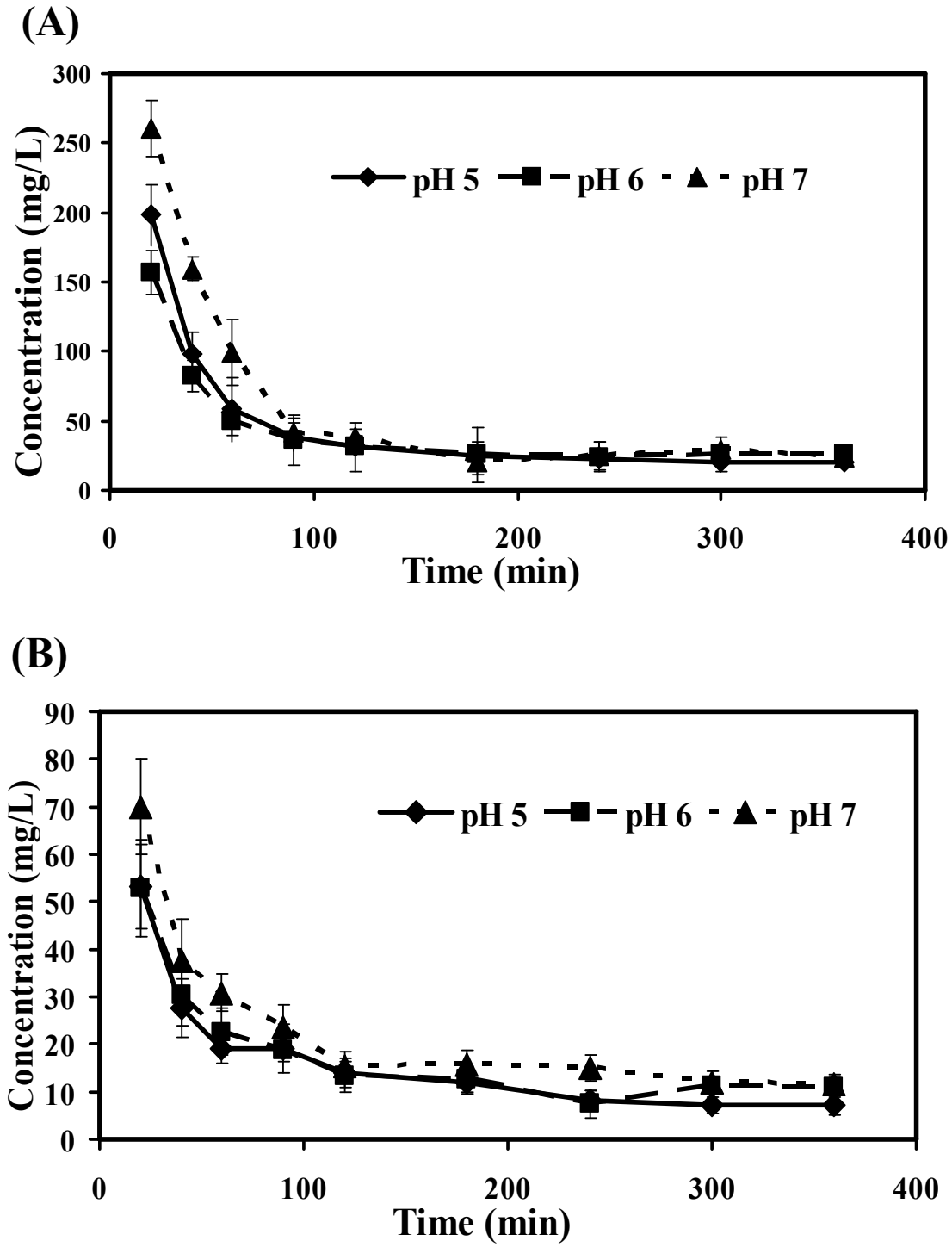

Figure 5.3: Furan removal from switchgrass liquor (A) Furfural (B) HMF

When the regenerated resin was used, the average residual furfural and HMF concentrations were 21±2.2 mg/L and 15±1.8 mg/L, respectively, for all the different pH

conditions (Figure 5.4). The average residual furfural and HMF concentrations correspond to 98.9±0.5% and 94.0±1.1% removals, respectively.

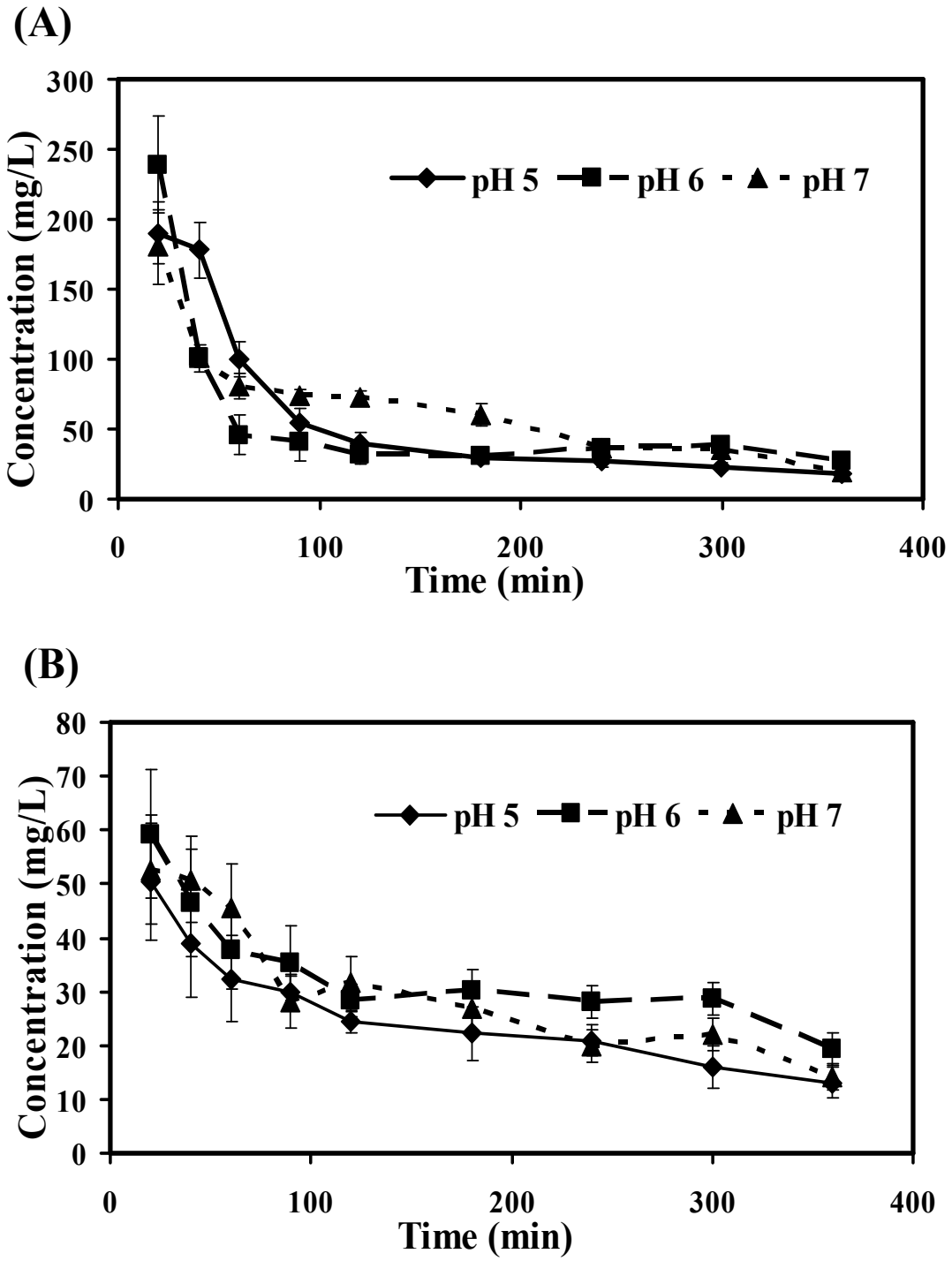

Figure 5.4: Furan removal from switchgrass liquor using regenerated resin (A) Furfural removal (B) HMF removal

Initial furan removal rates are tabulated in Table 5.3. Statistically the unused resin had the same initial removal rate as regenerated resin for both furans (Tukey's test was used). The initial removal rate was high for furfural than it was for HMF.

Table 5.3: Initial furan removal rates from switchgrass liquour

	Furan Removal Rate (mg furan/mg resin·min)			
	Unused Resin		Regenerated Resin	
pH	**Furfural**	**HMF**	**Furfural**	**HMF**
5	17.6±1.8	1.7±0.2	15.7±2.4	1.9±0.3
6	17.3±1.4	1.7±0.1	15.0±1.9	1.8±0.3
7	16.5±1.4	1.5±0.4	14.9±1.3	1.8±0.1

5.4 Discussion

In a mixture of only 2 components, the percent furfural and HMF removed when comparing the unused and regenerated resin ranged from 92±1.4% to 99±0.2%. The unused and regenerated resin was able to remove furfural and HMF from a mixture to levels which would be considered non-inhibitory to anaerobic microorganisms. In the switchgrass liquor, furfural and HMF were removed to levels that are likely non-inhibitory to microorganisms. The percent furfural and HMF removed was 98±0.4% and 94 ± 0.8%, respectively.

In the unused resin, the furfural and HMF initial removal rates were different when compared to the rates for the regenerated resin. However, notice for most of the conditions examined, the removal rates (over 360 min) were statistically the same for furfural or HMF. This different initial removal rates between the two chemicals are likely due to changes in adsorption activity, which are associated with selectivity of the resin for furfural when compared to HMF. In experiments conducting with switchgrass,

the furfural concentration reached 50 mg/L for the different pH conditions within 90 minutes whereas in case of the regenerated resin, the same residual level was attained within180 min.

In the case of unused resin, removing HMF to below 20 mg/L took place within 120 minutes; however, reaching the same concentration with the regenerated resin was attained within 360 min. In general, the pH had a small impact on the residual concentration after 360 minutes; however, it did affect how rapidly they removed were removed from solution The initial removal rates trend as function of pH was statistically the same at 37°C. An operating temperature of 37^{o}C was selected anaerobic microbial cultures are maintained under this condition.

A possible reason for differences in the removal times for the 2 compounds to the similar residual levels when using unused and regenerated resin can be attributed to different active sites. Notice the initial removal took place on sites which are highly selective to both compounds; however, with increasing time, furfural and HMF removal could be attributed to sites which were less active on the regenerated resin.

Chapter 6: Hydrogen Production from XAD-4 Treated Switchgrass Liquor

Stage 3 experiments were designed to demonstrate the impact of not treating and treating switchgrass liquor on fermentative H_2 production. The experimental design plan is summarized in Table 6.1. Throughout this chapter H_2 yields for switchgrass liquor are converted from mol H_2/mol substrate to mol H_2/mol glucose in order for direct comparison to experiments using pure glucose as a substrate.

Table 6.1: Experimental design[1,2]

Bottles	Substrate	HMF Added	Furfural Added
1,2,3	Glucose	No	No
4,5,6	Glucose	No	Yes
7,8,9	Glucose	Yes	No
10,11,12	Glucose	Yes	Yes
13,14,15	Untreated Switchgrass Liquor	No	No
16,17,18	Treated Switchgrass Liquor	No	No

Notes
1. Culture maintained at 37^{o}C and pH set at 5.5
2. 2000 mg/L linoleic acid injected to prevent methanogenesis

6.1 Hydrogen Production and Removal – Furans and switchgrass liquor

Hydrogen production was observed in controls and cultures fed furans (Figure 6.1). Hydrogen production was inhibited in cultures fed 2000 mg/L furfural when compared to the controls. A 12 hour lag phase in H_2 production was observed in cultures fed furfural. No lag phase in H_2 production was detected in cultures fed 300 mg/L HMF and these cultures produced lower amounts of H_2 when compared to the controls. In the presence of both HMF and furfural, the cultures produced H_2 only after a 12 hour lag phase.

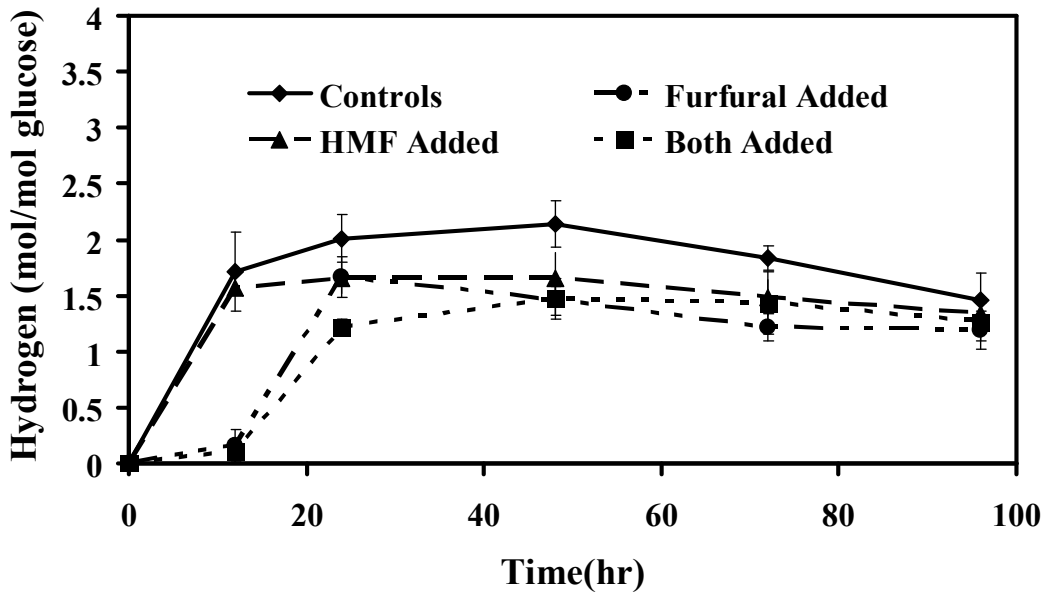

Figure 6.1 Effect of furans on H_2 production

Cultures containing untreated switchgrass showed a 12 hour lag phase before producing H_2 (Figure 6.2). No lag in H_2 production was observed in cultures fed treated switchgrass liquor. In the controls, the H_2 yield was 2.14±0.21 mol/mol glucose. The maximum H_2 yield for cultures fed untreated and treated switchgrass liquor were 1.8±0.11 mol/mol glucose and 2.26±0.14 mol/mol glucose, respectively.

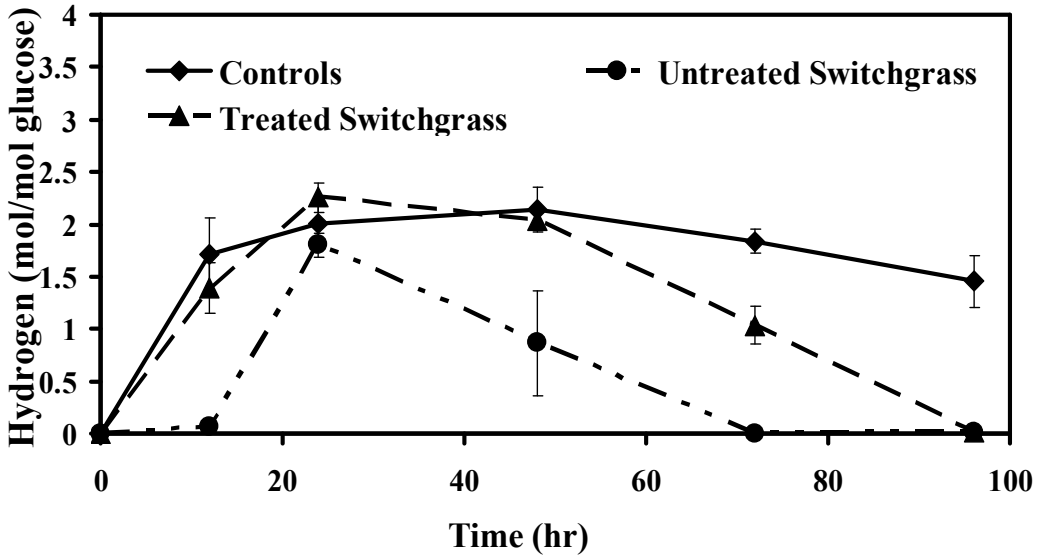

Figure 6.2 Hydrogen production using treated and untreated switchgrass liquor

The maximum H_2 yields are shown in Table 6.2. A maximum yield of 2.26±0.14 mol H_2/mol glucose was observed in cultures fed treated switchgrass. The yield observed in cultures fed treated switchgrass was statistically the same as the controls with a maximum H_2 yield of 2.14±0.21 mol H_2/mol glucose. The H_2 yield for cultures fed furfural (1.66±0.19 mol/mol glucose) or HMF (1.66 ± 0.13 mol/mol glucose) were less than that for the control cultures. Relative to the maximum H_2 yield, the yield (1.47±0.19 mol H_2/mol glucose) was less in cultures fed HMF plus furfural. The H_2 yield for untreated switchgrass was 1.80±0.11 mol H_2/mol glucose. The Tukey's Test was conducted to confirm the significance between the 6 mean maximum hydrogen yields. No statistical significant differences were observed between the yield in the control and cultures with treated switchgrass (labeled a). Likewise there was no statistical difference between cultures when comparing cultures fed furfural, HMF, both furans and untreated switchgrass (labeled b). Yields labeled with subscript 'a' was statistically different than

thos labeled with 'b'. The Tukey's test was based on a critical value of 4.49 stemming from 95% confidence, 6 means and 18 degrees of freedom which led to a test value of 0.429745.

Table 6.2: Maximum H_2 yields for cultures fed different substrates

Experiment	Maximum Yield (mol H_2/mol glucose)
Control	2.14 ± 0.21^a
Furfural Added	1.66 ± 0.19^b
HMFAdded	1.66 ± 0.13^b
Both Added	1.47 ± 0.19^b
Untreated Switchgrass	1.80 ± 0.11^b
Treated Switchgrass	2.26 ± 0.14^a

Note;
1. Yields labeled with the same superscript letter are statistically the same.

Relative to the controls, significant quantities of H_2 was consumed in cultures fed untreated switchgrass and treated switchgrass (Table 6.3). The H_2 consumption rate for all the conditions are shown in Figure 6.4. Maximum H_2 consumption was observed in cultures fed untreated and treated switchgrass liquor (1.79 To 2.25 mol H_2/mol glucose). In comparison, the minimum H_2 consumption (0.21 To 0.47 mol/mol glucose) was observed in cultures fed furfural, HMF and furfural plus HMF. The Tukey's test showed that the consumption in the control culture and cultures fed furans were statistically the same (Table 6.3, labeled as 'a'). Statistically the same H_2 consumption (Table 6.3, labeled as 'a' or 'b') was observed in cultures fed treated and untreated switchgrass liquor. The cultures labeled as 'a' had statistically different H_2 consumption compared to cultures labeled with 'b'.

Table 6.3 Hydrogen Consumption for different substrates

Experiment	H_2 Consumption (mol H_2/mol glucose)
Control	0.69 ± 0.24^a
Furfural Added	0.47 ± 0.25^a
HMFAdded	0.32 ± 0.22^a
Both Added	0.21 ± 0.25^a
Untreated Switchgrass	1.79 ± 0.11^b
Treated Switchgrass	2.25 ± 0.14^b

Note;
1. Yields labeled with the same superscript letter are statistically the same.

6.2 Methane Production

Low levels of methane were detected in cultures fed with any of the substrates (Figures 6.3). The largest methane yield was observed in the controls fed glucose (0.0164 mol/mol glucose). The methane yield for the treated switchgrass was 0.0123 mol/mol glucose. The lowest methane yield was observed in cultures fed furfural plus HMF (0.0071 mol/mol glucose).

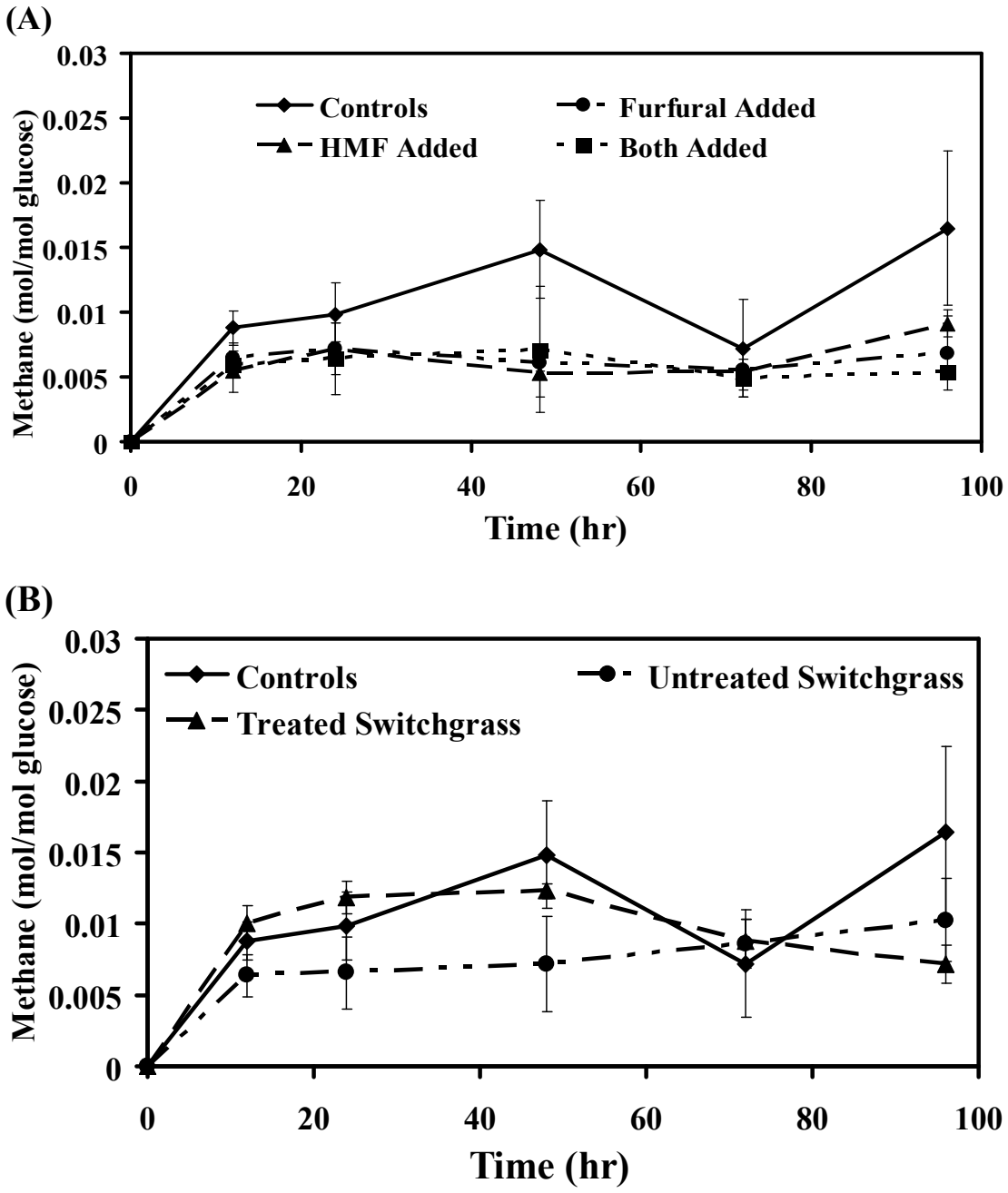

Figure 6.3: Methane production (A) With furans added (B) Treated and untreated switchgrass liquor

6.3 Substrate Degradation

Sugars were degraded from 5000 mg/L to levels reaching approximately 220 mg/L within 24 hours in the controls (Figure 6.4). A large fraction (99%) of glucose was consumed within 16 hours in the controls and cultures fed HMF. In comparison, greater

than 95% sugar removal in cultures fed furfural, furfural plus HMF and untreated switchgrass liquor was attained only until after approximately 24 hours (Figure 6.4). The initial sugar degradation rates for the various conditions examined are shown in Table 6.2. Cultures fed furans had a lower initial degradation rate than the controls. Culture fed both inhibitors and culture fed furfural were both lower than culture fed HMF. Likewise treated switchgrass had a higher initial degradation rate than untreated switchgrass. The Tukey's test showed that the controls, cultures fed HMF and those receiving untreated switchgrass liquor all had statistically different initial degradation rate when compared to the other cultures.

Cultures fed both furans, furfural or those receiving treated switchgrass all had statistically the same initial degradation rate (Table 6.4). Note that in the switchgrass liquor, approximately 62% glucose and 38% xylose were removed over 24 hours which could account for the higher initial degradation rates when pure glucose was used compared to switchgrass liquor.

The lowest substrate degradation rate was observed in cultures fed untreated switchgrass liquor. Although greater than 95% of the sugars in were removed in the treated and untreated switchgrass liquor, a slower initial rate was observed in cultures fed with the untreated switchgrass liquor.

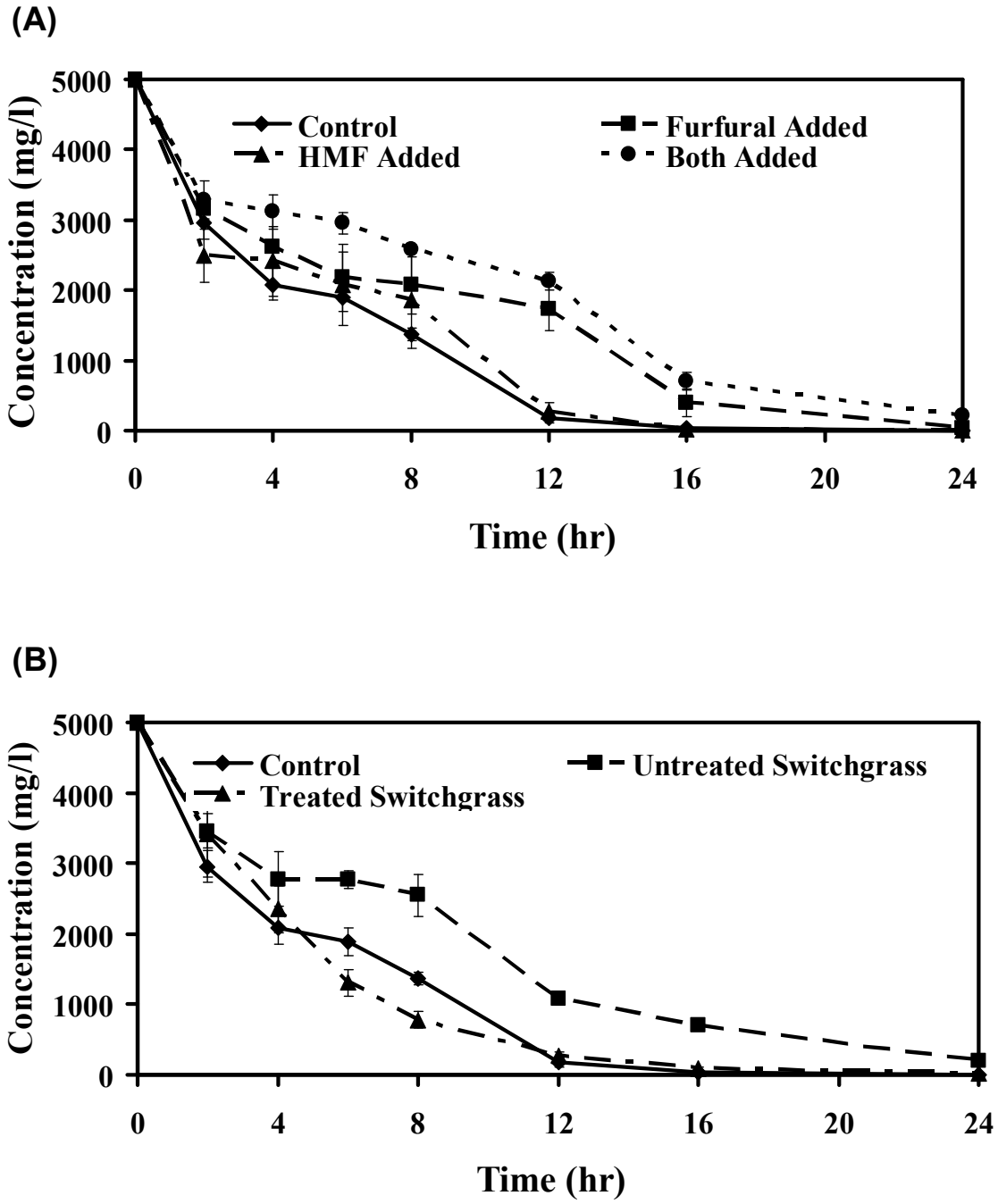

Figure 6.4: Substrate degradation (A) Glucose with furans added (B) With treated and untreated switchgrass

Table 6.4: Initial substrate degradation

Experiment	Glucose Removal Rate ((µg/mg VSS)/min)	Tukey Significance
Controls	17.3 ± 1.2^{a}	A
Furfural Added	10.5 ± 0.7^{b}	B
HMF Added	13.8 ± 0.8^{c}	C
Both Added	10.3 ± 0.3^{b}	B
Untreated Switchgrass	8.2 ± 0.5^{d}	D
Treated Switchgrass	11.4 ± 0.7^{b}	B

Note;

1. Yields labeled with the same superscript letter is statistically the same.

6.4 VFA Production

The VFA's by-products detected included acetic, propionic, butyric and lactic acids (Figures 6.5 to 6.8). The acetic acid level reached a maximum of approximately 900 mg/L in the control cultures after 360 min. In cultures fed untreated switchgrass liquor, the maximum acetate level was approximately 3400 mg/L. In comparison, in the treated switchgrass liquor, the maximum acetate level attained approximately 1480 mg/L. In cultures fed furfural, HMF, furfural plus HMF or untreated switchgrass liquor, the acetate levels reached maximum levels ranging from 500 to 900 mg/L.

The propionic acid levels reached a maximum at approximately 1200 mg/L in the controls after 360 min. In comparison, propionic acid levels in the treated switchgrass liquor reached a maximum level of approximately 1090 mg/L, compared to a maximum level of approximately 790 mg/L in the untreated switchgrass liquor. Cultures receiving inhibitors all had lower maximums than the control, with cultures receiving both inhibitors having the lowest maximum at approximately 560 mg/L.

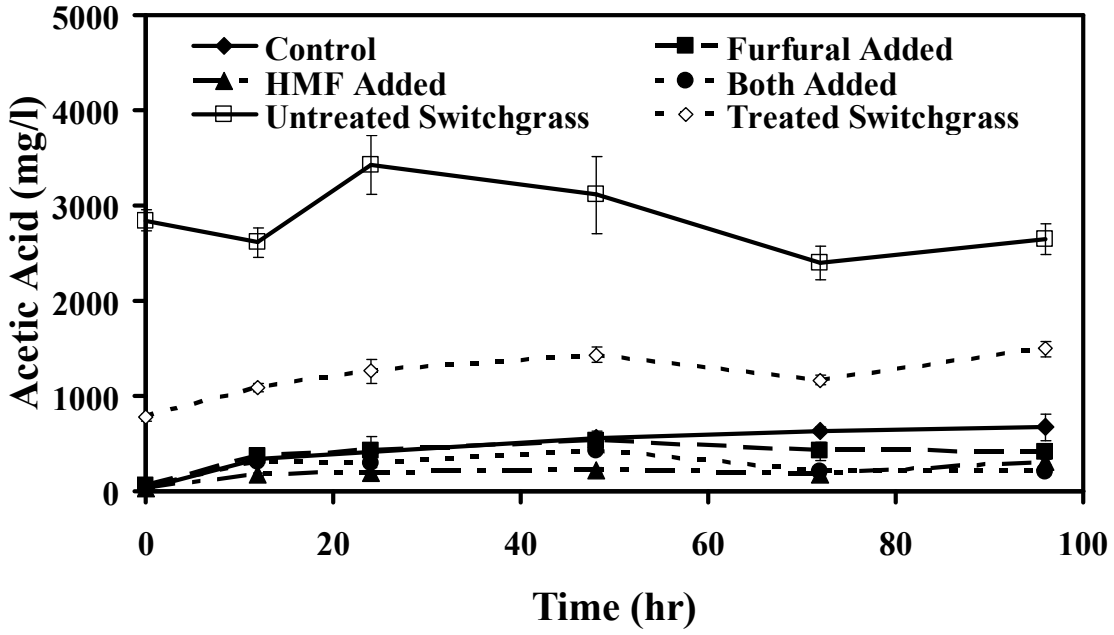

Figure 6.5: Acetic acid production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

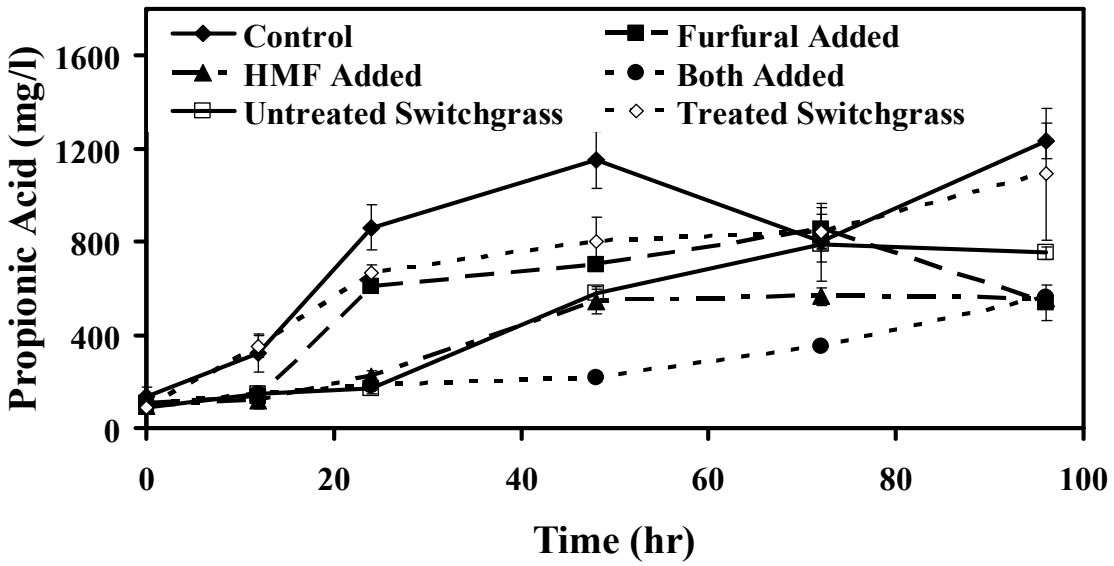

Figure 6.6: Propionic acid production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

The butyric acid concentration was less than 100 mg/L under all the experimental conditions under consideration. A maximum level of approximately 100 mg/L was

detected in cultures fed furfural. The lactic and butyric acid levels (< 100 mg/L) were very low in comparison to acetic and propionic acids.

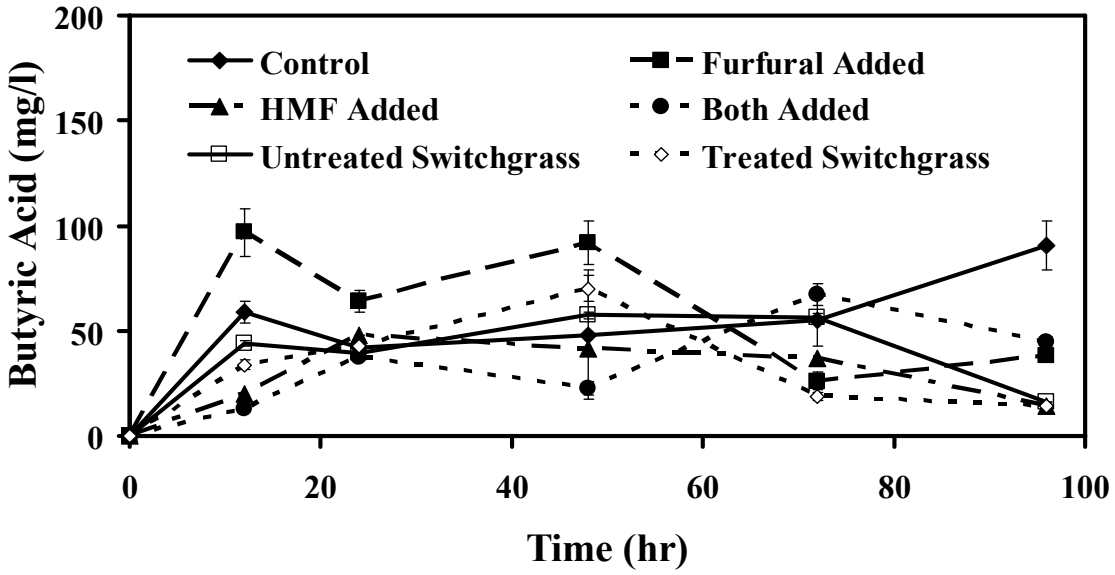

Figure 6.7: Butyric acid production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

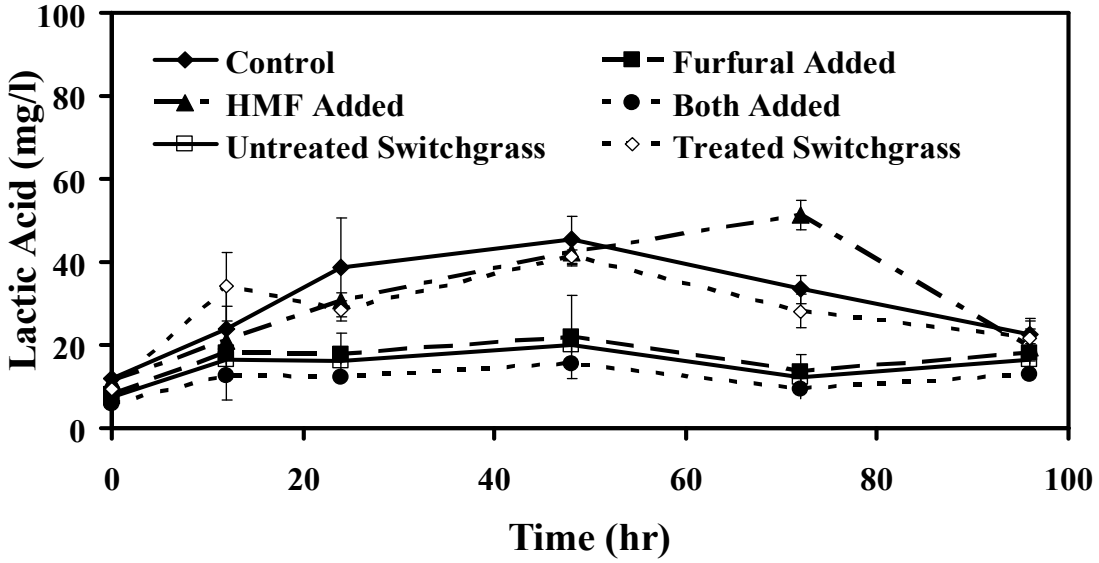

Figure 6.8: Lactic acid production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

6.5 Alcohol production

The alcohols produced at elevated levels were ethanol and iso-propanol (Figures 6.9 and 6.10). Approximately 140 to 500 mg/L ethanol was detected after 48 hours of incubation; however, the levels gradually decreased to less than approximately 160 mg/L after 96 hours. The ethanol levels in cultures fed treated switchgrass liquor were greater than the levels in cultures fed untreated switchgrass. Except for cultures fed furfural, the iso-propanol levels on average ranged from 200 mg/L to 700 mg/L at incubation times from t = 24 hours to t = 96 hours (Figure 6.10). In comparison, n-propanol and iso-butanol were also produced but at lower levels (Figures 6.11 and 6.12). Except for an increase of approximately 350 mg/L in the controls from 72 hours to 96 hours, the n-propanol levels were on average 75 mg/L in the remaining cultures. The iso-butanol levels over the duration of the study were less than approximately 130 mg/L in the controls and cultures fed furfural and HMF. Iso-butanol was not produced in cultured

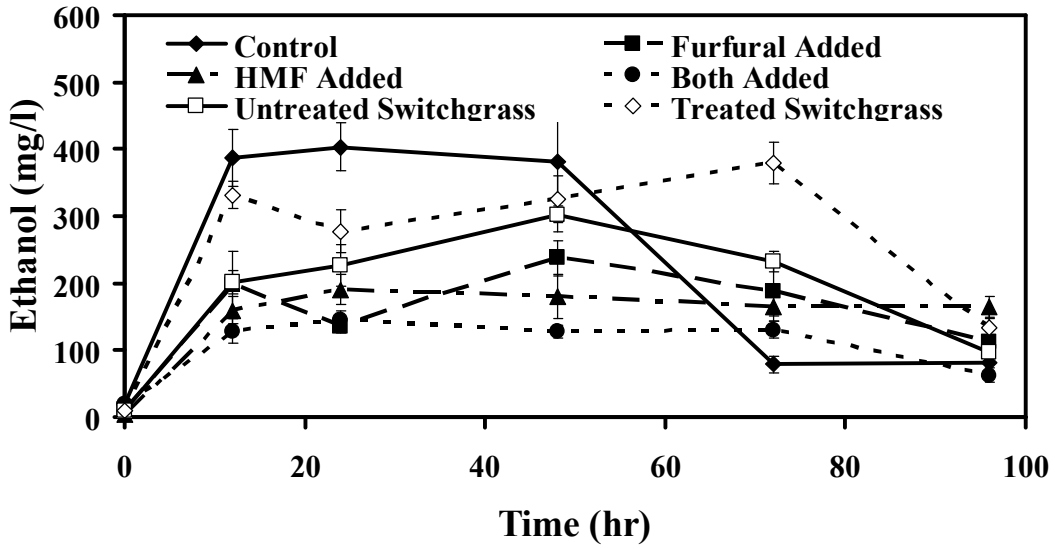

Figure 6.9: Ethanol production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

fed untreated and treated switchgrass liquor. n-Butanol was not produced in any of the cultures.

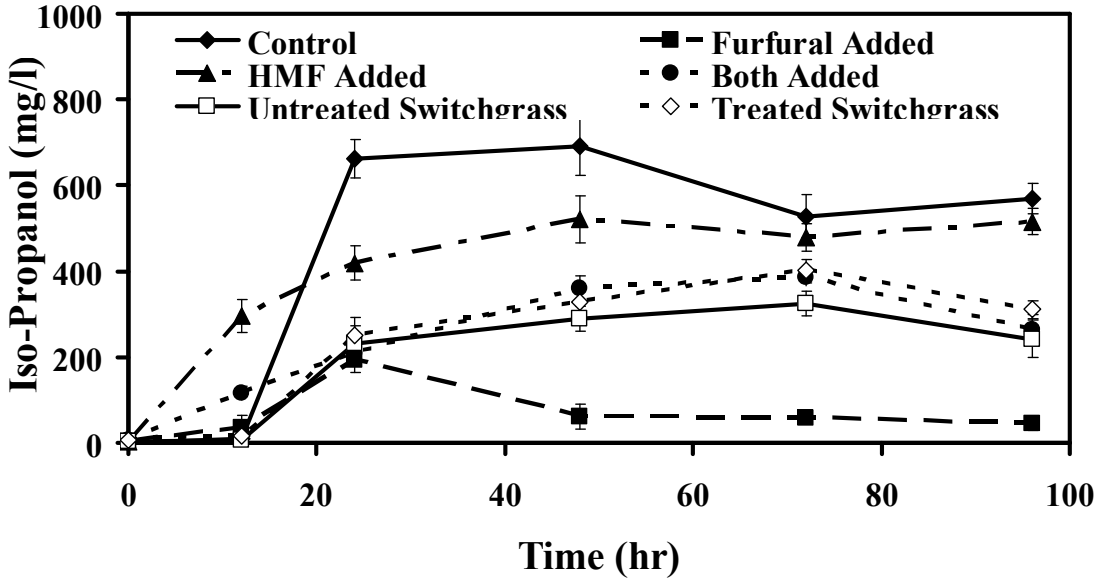

Figure 6.10: Iso-propanol production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

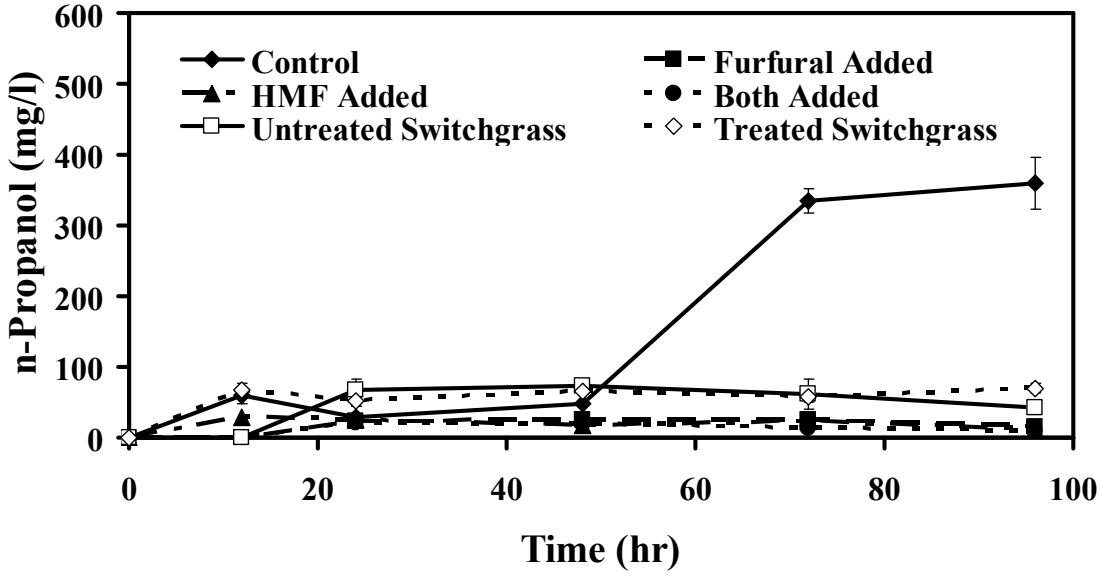

Figure 6.11: n-Propanol production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

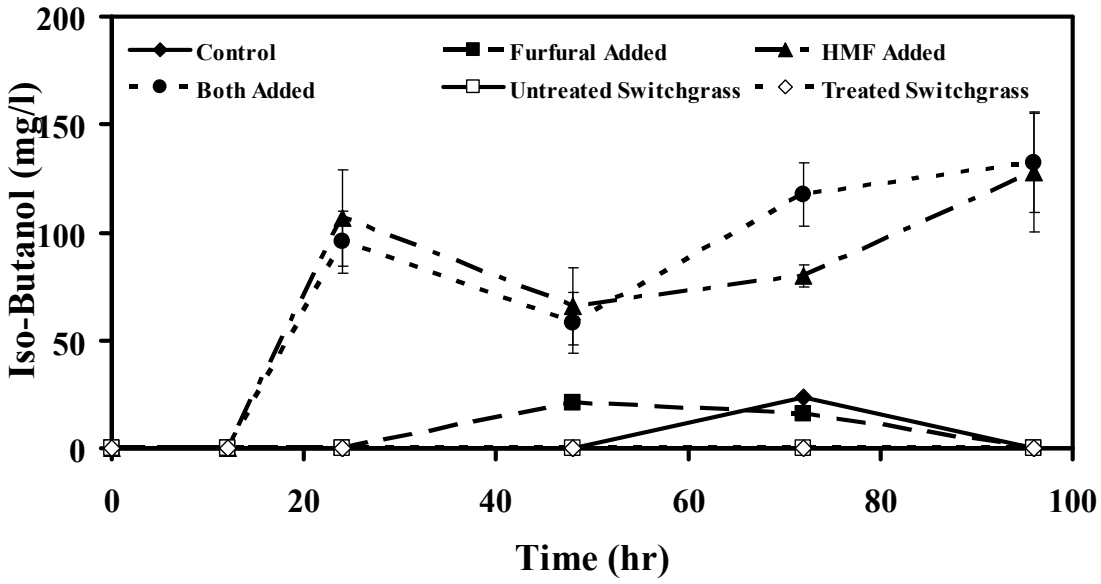

Figure 6.12: Iso-butanol production in cultures fed furfural, HMF, furfural plus HMF, untreated switchgrass liquor or treated switchgrass liquor.

6.6: Discussion

The quantity of HMF produced in the steam exploded switchgrass liquor was below the level that affects fermentative H_2 production (Cao *et. al.,* 2010). In comparison, the quantity of furfural generated was approximately four times the amount that can impair H_2 production (Cao *et. al.,* 2010). HMF and furfural have been reported to synergistically affect H_2 production. The level of HMF used in this study was similar to the levels reported by Quéméneur *et al.* (2012) for liquor produced from the steam explosion process for low value biomass. An HMF concentration of 250 mg/L did not show a lag in H_2 production when compared to the controls. However, a furfural concentration of 2000 mg/L caused a lag in H_2 production by 12 hours when compared to the control. The lag phase in H_2 production is possibly due to the synergistic inhibition action of LA plus fufural. This effect is especially noticeably in cultures fed fufural, fufural plus HMF and untreated switchgrass liquor. Hydrogen producers such as

acidogens and acetogens were likely inhibited but subsequently, these organisms produced H_2 by relieving the inhibitory stress.

The effect of furfural on the maximum H_2 yield was greater compared to HMF. The H_2 yields attained in the presence of furfural and HMF were 1.66±0.19 mol/mol glucose and 1.66±0.13 mol/mol glucose, respectively. Note the H_2 removal rate in the presence of furfural and HMF were 0.47 mol/mol glucose and 0.32 mol/mol glucose, respectively. Hydrogen removal is mediated by the presence of H_2-consumers such as homoacetogens, hydrogenotrophic methanogens and sulfate reducing bacteria (SRB). Reaction 6.1, 6.2 and 6.3 show H_2 consumption by homoacetogens, hydrogenotrophic methanogens and SRBs.

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (6.1)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (6.2)$$

$$4H_2 + SO_4^{-2} + H^+ \rightarrow HS^- + 4H_2O \quad (6.3)$$

The H_2 removal rate for the controls (containing LA) is similar to cultures fed furans (furfural, HMF and furfural plus HMF) and LA. This suggests the inhibitory effect due to LA was dominant over that caused by furfural, HMF and furfural plus HMF. Note in cultures fed switchgrass liquor (untreated and treated) plus LA, the H_2 removal rate was greater when compared to the controls. The increased H_2 removal rates for cultures fed untreated and treated switchgrass liquor is unexpected because LA is able to inhibit H_2 consumers to the same degree when compared to the controls. Reasons for the increased H_2 removal rates in cultures fed the switchgrass liquor plus LA are unknown. Adding LA at threshold levels and adjusting the pH to 5.5 are effective methods to inhibit methanogens (Philpot, 2011**;** Reaume, 2009). In all the conditions examined, inhibition

of methanogens was the cause for the relatively low methane yield. Hydrogen consumption did not correlate with increasing methane yield. Utilization of H_2 to produce acetic acid was likely mediated by homoacetogens.

Acetic acid is produced as a byproduct from the steam explosion of lignocellulosics (Gravitis *et. al.,* 2004). The elevated acetic acid level in the untreated and treated liquor is a result of pretreating switchgrass using steam explosion. In studies conducted with the switchgrass liquor, acetic acid remained elevated over the duration of the study because aceticlastic methanogens were inhibited by LA. Under low pH conditions, acetic acid is inhibitory to a variety of microorganisms (Sundberg and Jonsson, 2004) and as a result, the H_2 yield could be affected in mixed anaerobic H_2 producing cultures.

Over the duration of the study, propionic acid was not degraded and hence, the levels remained relatively constant. The elevated ethanol and iso-propanol levels suggest solventogenesis was the major route. Instead of acetate production, the pathway shifted to producing ethanol. The low lactate and elevated propionate levels indicate the propionate production pathway proceed easily even under elevated high H_2 partial pressures.

Control cultures consumed glucose faster in comparison to cultures fed glucose plus furans. The larger glucose initial degradation rates (Table 6.5) in the controls when compared to cultures fed LA and furans strongly suggest inhibition of H_2 producing microorganisms. Inhibition of H_2 producers did not impair the production of H_2 and the higher H_2 yields were associated with fast glucose removal times.

Chapter 7: Conclusions

The objectives of this study were twofold. Optimal removal of furfural and HMF using XAD-4 resin based on temperature, pH and initial furfural concentration was examined, along with the benefits of using XAD-4 resin to treat switchgrass liquor before it was used as a substrate for fermentative H_2 production. Data from this study could be used in laboratory scale studies to assess the pretreatment of liquor generated from the steam explosion of low value biomass. Data from these studies can also be used in continuous flow bioreactors studies to develop a microbial H_2 production process.

Using pure cultures on a large scale is impractical due to excessive costs, maintenance and contamination. In comparison, mixed cultures contain a variety of bacteria species that can convert complex organic molecules into H_2 plus carbon metabolites. Many methods have been developed to eliminate or inhibit H_2 consumers while retaining a 'healthy' H_2 producing microbial population.

Using pure substrates as feedstocks for full-scale applications is not sustainable and economically feasible. Pretreatment processes such as steam explosion have been developed to convert low-value agriculture wastes into mixtures of compound containing biodegradable substrates. However, a major issue during pretreating is the generation of microbial inhibitors such as furfural and HMF. In order for agriculture wastes to be a viable feedstock, conversion and separation processes have to be developed which are efficient and economical.

The main conclusions of this study are as follows:

1. The XAD-4 resin was able to remove furfural and HMF to acceptable levels that were not inhibitory to H_2 fermentative microorganisms. Furan removal efficiencies were greater than 90% under all the conditions examined.
2. Furan removal by the XAD-4 resin was not significantly affected by pH and temperature over the range of conditions examined
3. The XAD-4 was effective in removing furans from steam exploded switch grass liquor to levels that are not inhibitory to H_2 fermentative microorganisms.
4. The XAD-4 resin was successfully regenerated for reuse in removing furans.
5. Without inhibitors, the maximum H_2 yield was 2.14±0.21 mol H_2/mol glucose. In comparison, the H_2 yield was 1.66±0.13 and 1.47±0.19 mol H_2/mol glucose for cultures fed furfural and HMF, respectively. For cultures fed furfural plus HMF the yield was reduced to 1.47±0.9 mol H_2/mol glucose.
6. The H_2 yields for untreated and treated switchgrass liquor were 2.26±0.14 and 1.80±0.14 mol H_2/mol glucose, respectively.

Chapter 8: Engineering Significance and Future Recommendations

Sustainable fermentative H_2 production using mixed anaerobic communities is linked to developing inexpensive feedstock chemicals. Low yields coupled with expensive feedstock costs have prevented the development of full-scale industrial processes. Past studies have provided evidence that attaining yields to greater than 3 mol H_2/ mol substrate is possible by inhibiting H_2 consumers.

Utilizing pure feedstocks such as glucose are not economically feasible and sustainable for full-scale processes. Developing methods to reduce feedstock cost will eventually lead to an economical H_2 production process. Converting low-value lignocellulosic biomass into biodegradable substrates is possible using pretreatment methods such acid treatment and steam explosion. However, inhibitory byproduct chemicals (furfural and HMF) generated by the pretreatment process have to be removed before feeding substrates to microbial reactors.

This research work focused on optimizing the use of XAD-4 (an ion-exchange resin) to remove furfural and HMF from the steam exploded liquor. Treating the switchgrass liquor with a resin was able to increase the H_2 yield.

A list of recommendations for future research work is as follows:

1. Conduct continuous flow bioreactor studies using the treated switchgrass liquor. Factors that can be studied include reducing the pH, increasing HRT, organic loading and sparging the reactor liquid to increase the H_2 yield. .
2. Assess the effect of using different low value treated steam exploded liquor on the H_2 yield.

3. Examine the effects of inoculating the bioreactor with microbial cultures from different sources.

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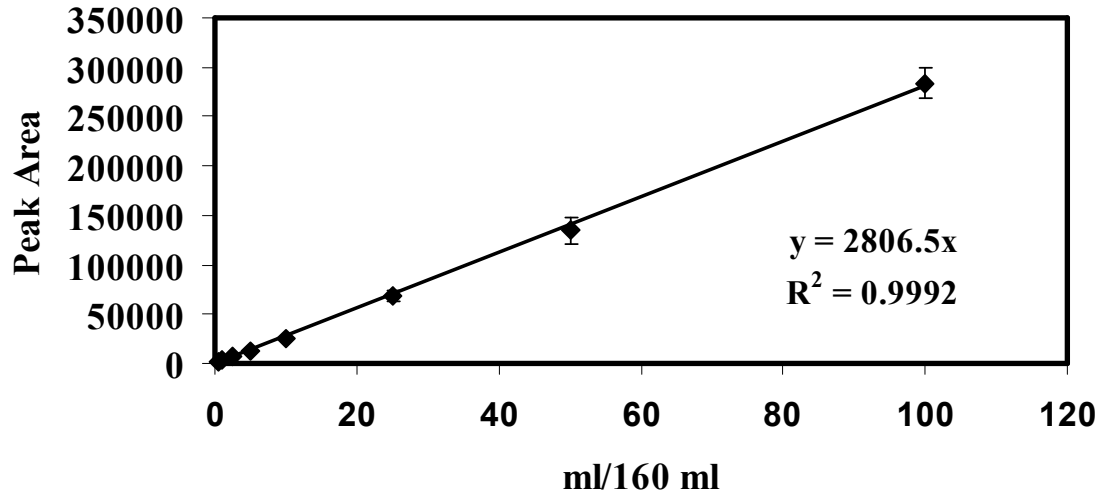
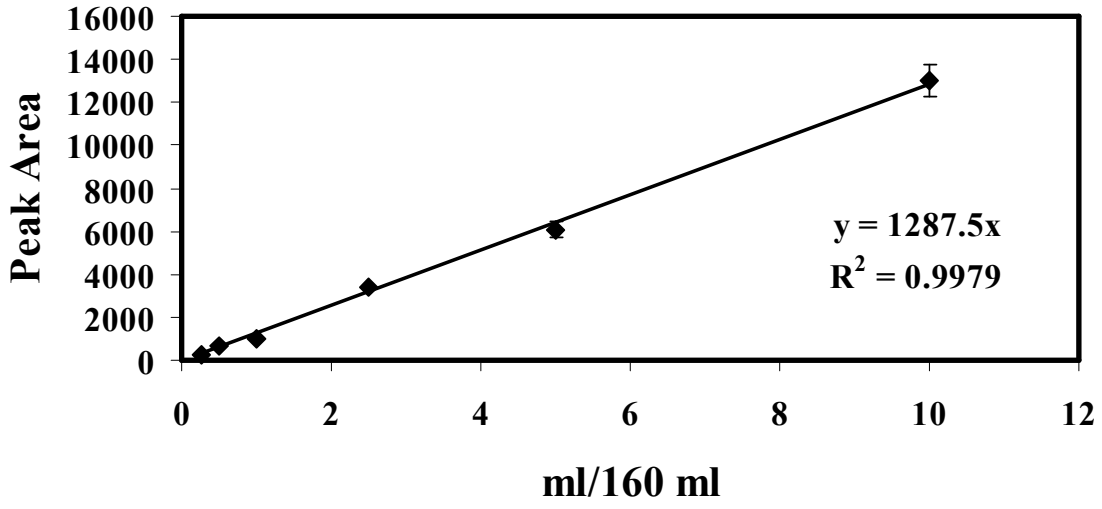
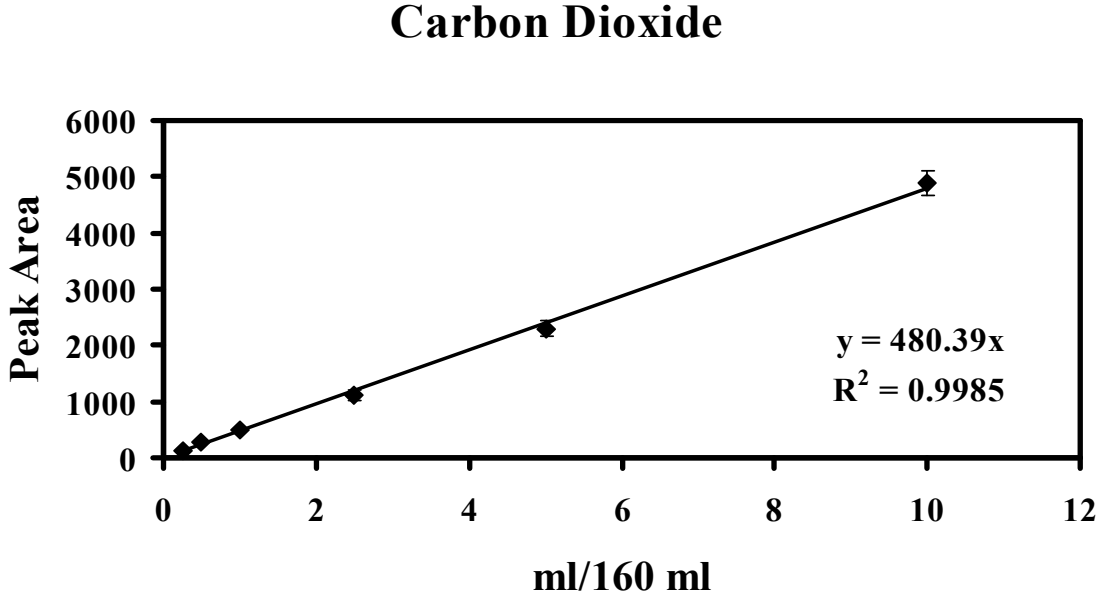
Appendix I: Gas Calibration Curves

Hydrogen



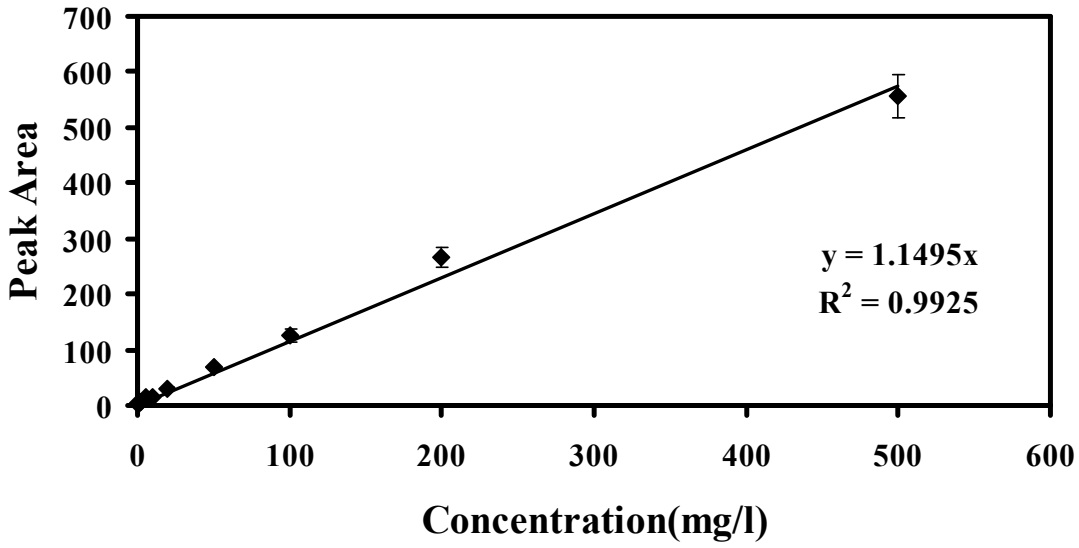
Methane



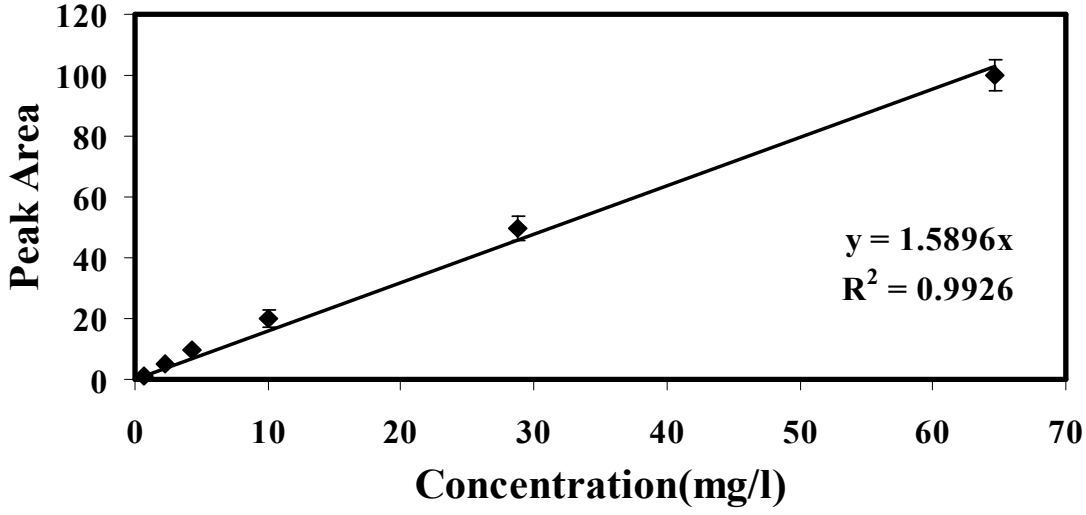

Carbon Dioxide
Peak Area
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
ml/160 ml
y = 480.39x
R2 = 0.9985

Appendix II: Furan Calibration Curves

Furfural

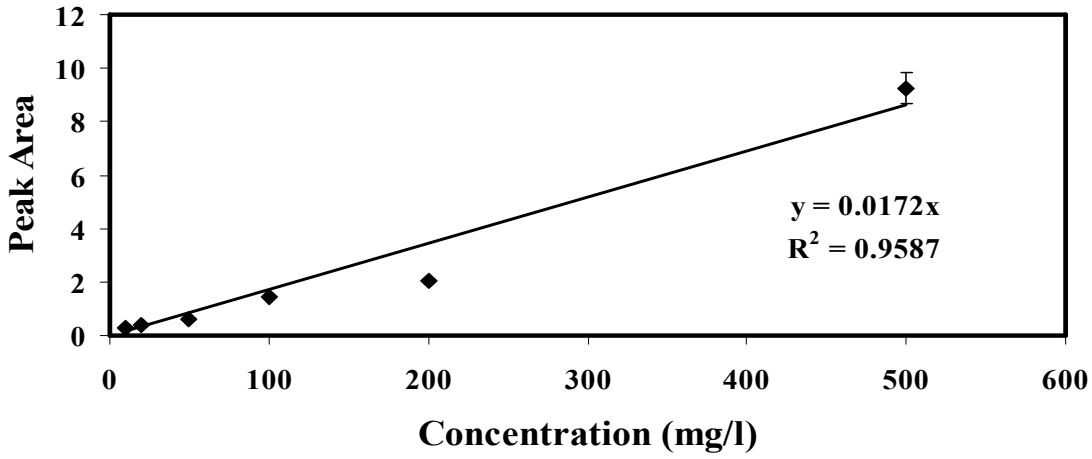


HMF

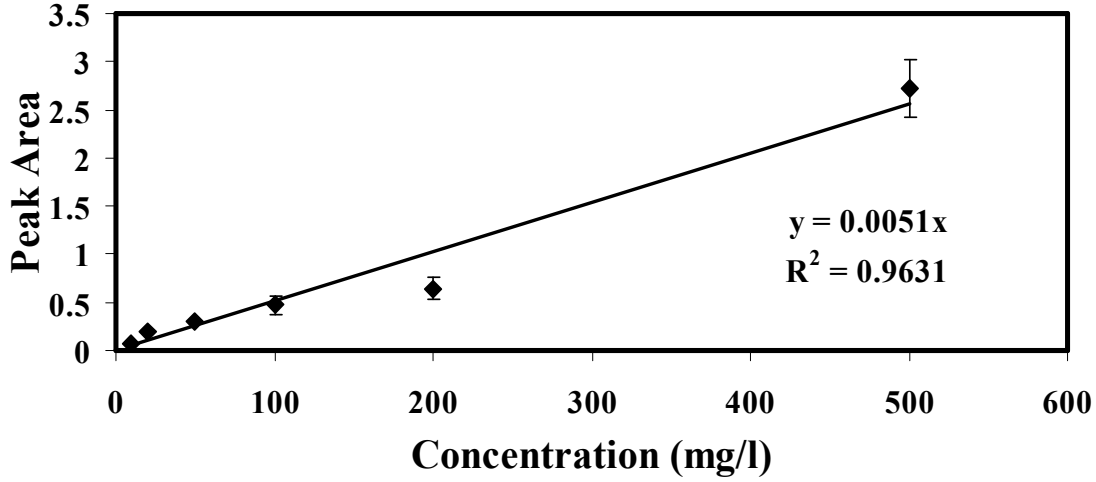


Appendix III: VFA Calibration Curves

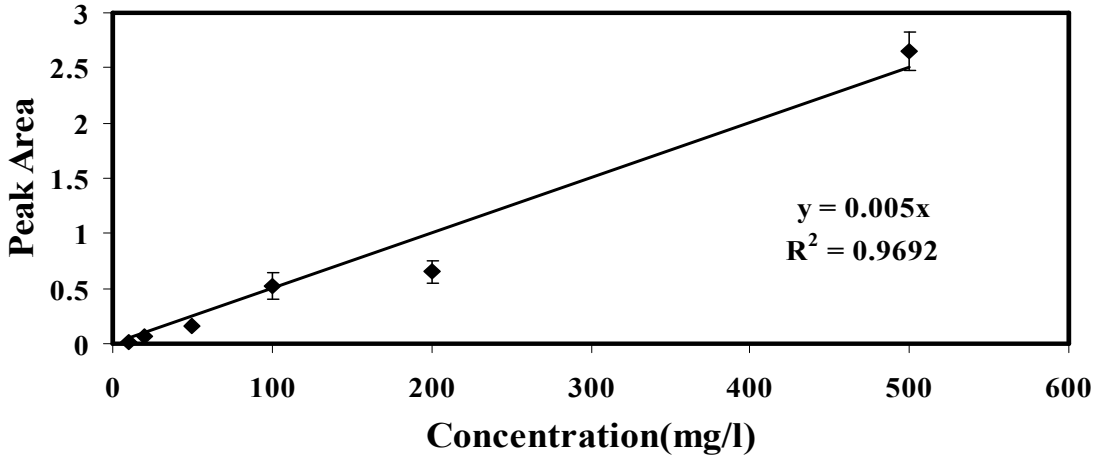
Acetic Acid



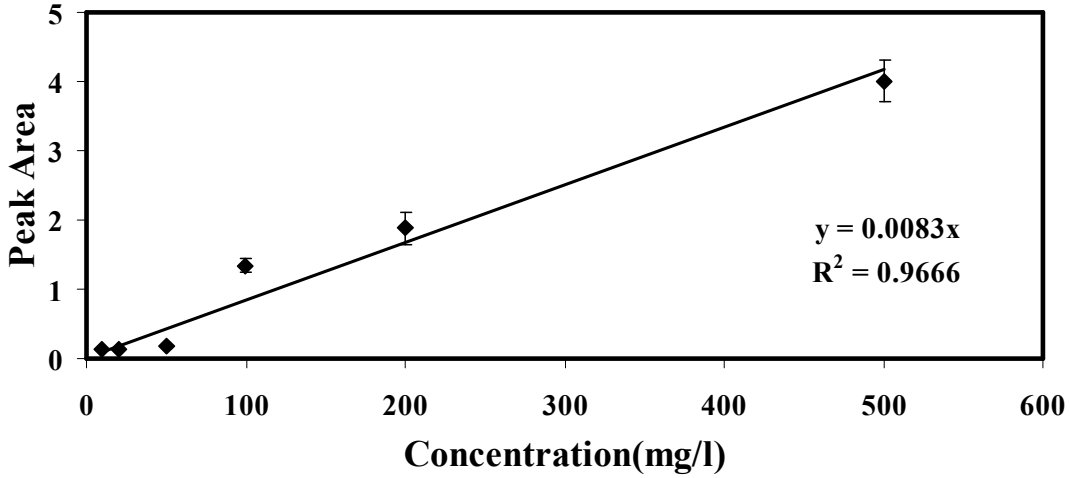
Propionic Acid



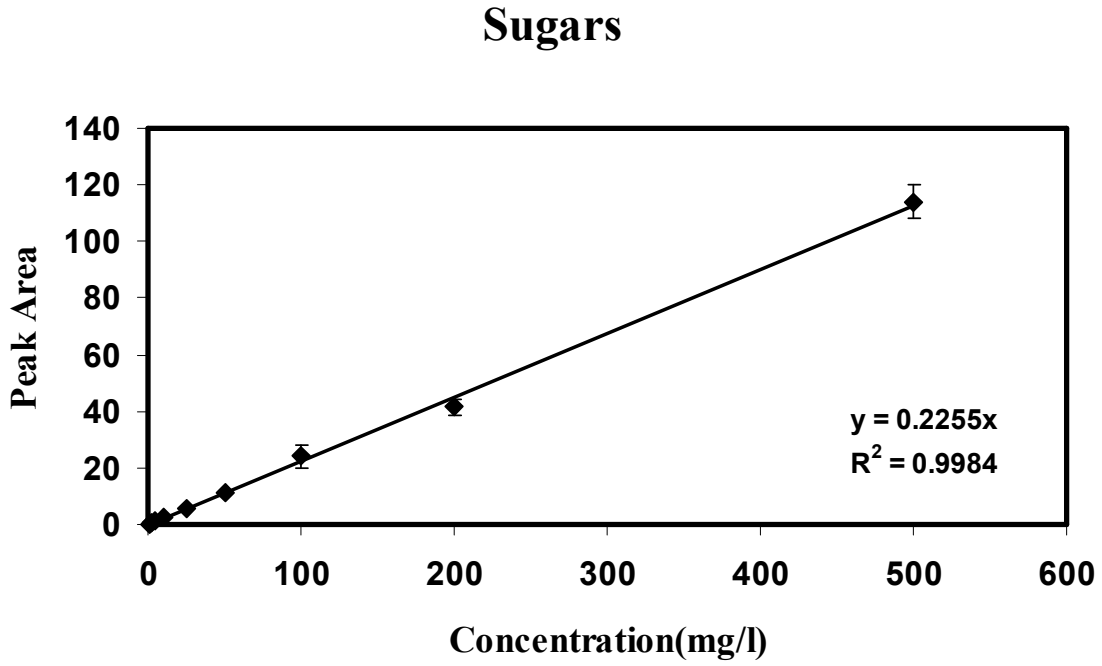
Butyric Acid



Lactic Acid

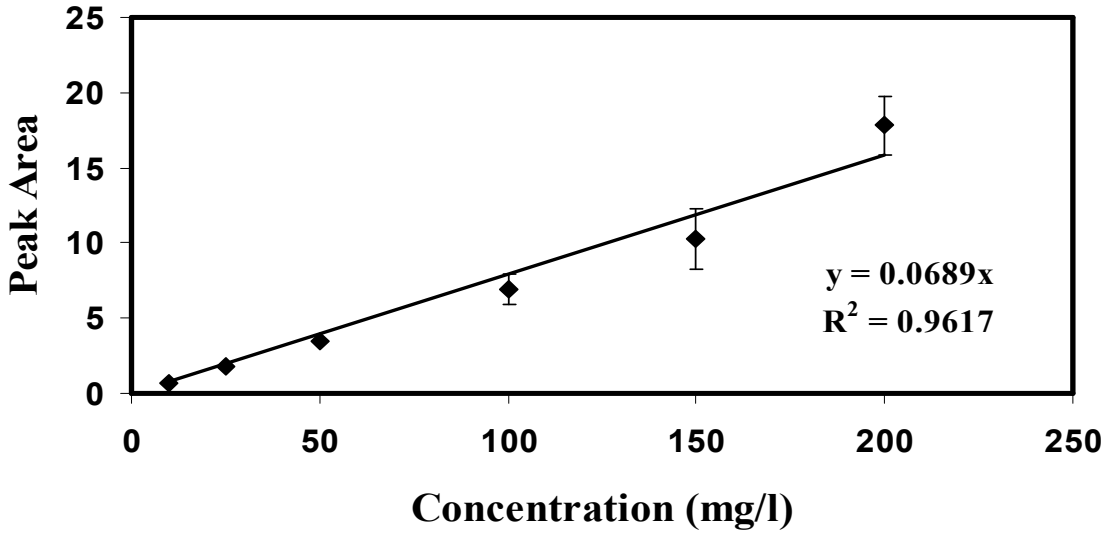


Appendix IV: Sugar Calibration Curves

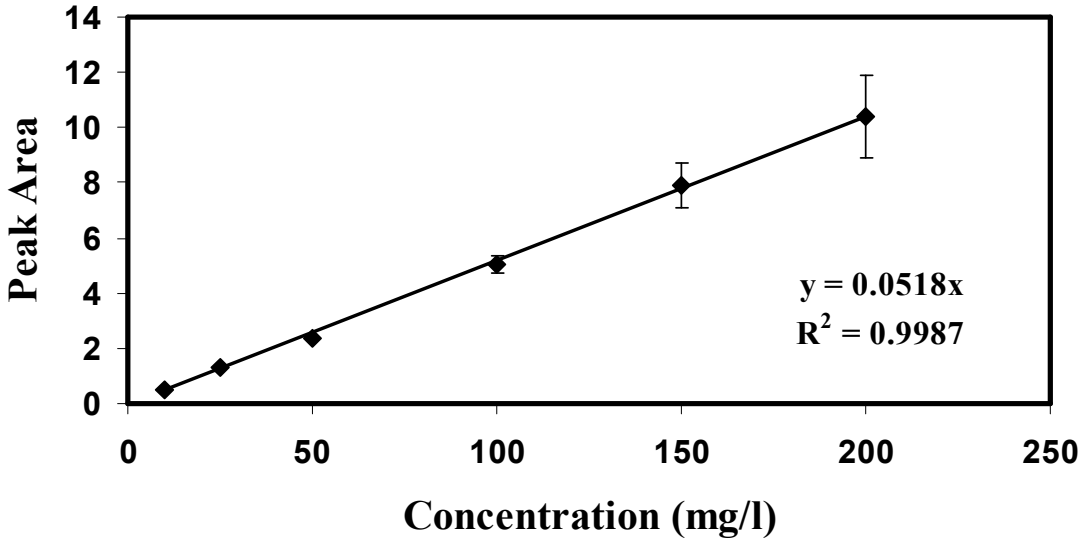


Appendix V: Alcohol Calibration Curves

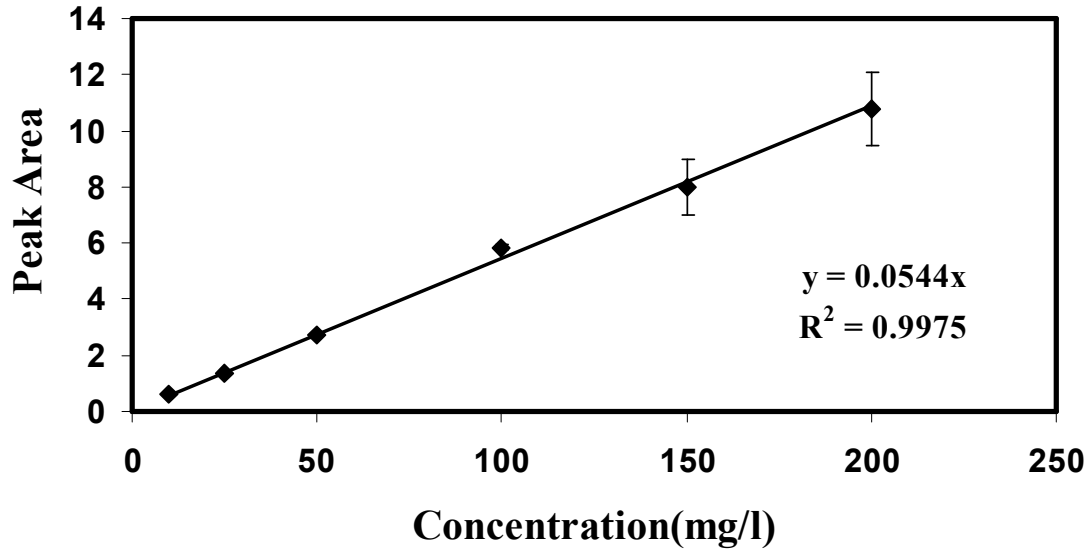
Ethanol



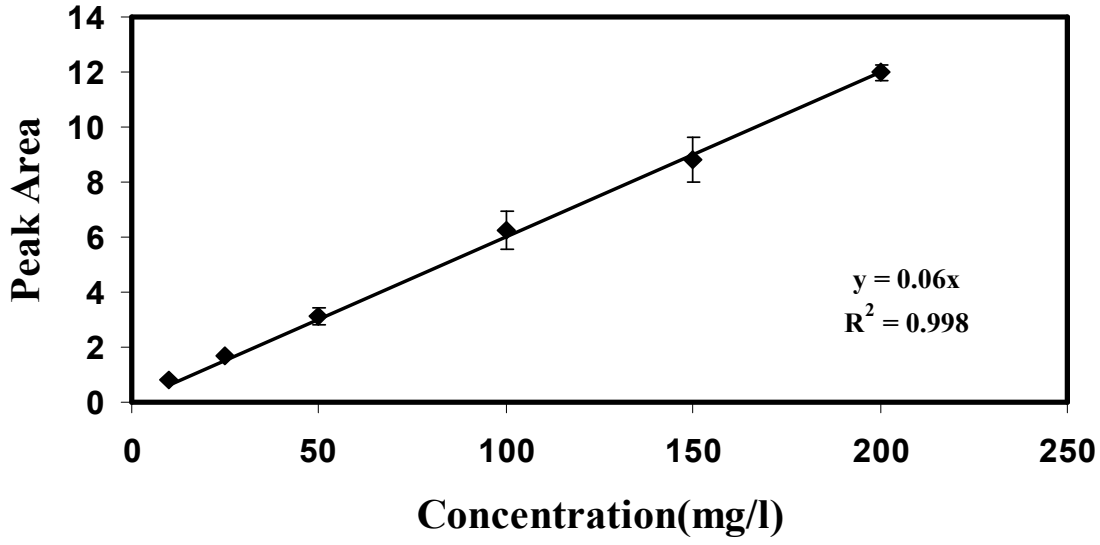
Iso-Propanol



n-Propanol



Iso-Butanol



Appendix VI: Sample Calculations

VSS/TSS Calculation:

TSS= [mass@105 C-empty mass]/Volume

VSS= [mass@550 C- mass@550 C]/Volume

empty mass= dry mass of the aluminum tin(g) + filter paper(g)

mass @ 105 C= mass of aluminum tin(g) + filter paper(g) + sample after 1 hour in the 105 C oven(g)

mass @ 550 C= of aluminum tin(g) + filter paper(g) + sample after 1 hour in the 550 C muffle furnace(g)

Volume= sample volume (mL)

Example:

Empty Mass(g)	mass@105C(g)	mass @550C(g)	Volume(mL)
0.9083	0.9443	0.9109	4.8

TSS=[0.9443-0.9083]/4.8 = 0.0075 mg/mL = 7500 mg/L

VSS=[0.9443-0.9109]/4.8 = 0.006958 mg/mL = 6958 mg/L

Yield Calculation:

5g/l of glucose was injected into each bottle with a liquid volume of 50mL. Assuming 0.002000 mol of H_2 has been calculated at a given time:

amount of glucose(g)= volume(l)*glucose(g/l)=0.05*5= 0.25

mol of glucose= amount of glucose(g)/molecular weight of glucose(g/mol)
=0.25/1860.16
= 0.001387655

hydrogen yield = mol hydrogen/mol of glucose=0.002000/0.001387655
=1.44 mol H_2/mol glucose

Glucose Initial Degradation Calculation:

Assuming 5000 mg/L initial glucose concentration and 2000 mg/L culture VSS

Using first three data points for the control culture degradation results in:

$C(mg/L) = 5000-2070.4t + 335.3t^2$
$-(dc/dt) = -2070.4 + 335.3t$

When t=0 dc/dt= 2070.4 (mg/L)/hr = 17.25 (μg/mg VSS)/min

Furfural Initial Degradation Calculation:

Assume the first three data points from a furfural degradation curve (t=0-40min) form an initial removal curve of:

$C(mg/L)= 3000 – 201.74t +3.277t^2$
$-(dc/dt)= -201.4 + 3.277t$

When t=0 dc/dt= 201.64 (mg/L)/min

Considering 2g resin/15 mL results in

dc/dt=201.64 (mg/L)/min X 2g resin/15mL
= 26.9 (mg furfural/mg resin)/min

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