

# Bioethanol production: from wood to fuel

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## Abstract

Increasing oil prices along with the climate change threat have forced governments, society and the transportation sector to consider alternative fuels. Biofuels presents itself as a suitable replacement and has received much attention over recent years fuelling various vehicles worldwide. Today, bioethanol (a liquid fuel replacement for petrol) is primarily produced using first-generation technologies which convert starch or sugar to ethanol. The feedstock for this technology is predominantly sugar-cane and maize, which competes directly with food security and has fuelled many political debates. Recent breakthroughs in the biological sciences have revealed a possible alternative whereby woody (lignocellulosic) materials could be used as feedstock. This second-generation technology is rapidly approaching commercialisation readiness and requires the addressing of key questions. One such question is whether a significant gain in ethanol yield could be attained by means of substrate and organism recycling. This paper uses a model for simultaneous saccharification and fermentation of lignocellulosic material in combination with continuous stirred tank reactor and recycling schemes to investigate the theoretical ethanol yields improvements possible. Final results indicate that under ideal conditions a possible 33 % increase in ethanol yield could be achieved through substrate and cell recycling under a dilution rate of 0.02 h<sup>-1</sup>.

Keywords: Hydrolysis, *Saccharomyces cerevisiae*, *Trichoderma reesei*, SSF, Enzyme

## 1 Introduction

Increasing oil prices are approaching 80 \$/bbl and political, social and environmental pressure is rising to reduce global greenhouse gas emissions. These factors place significant strain on the transportation sector, with new standards continuously forcing vehicle manufacturers to find ecologically (eco) friendly alternatives to the traditional “dirty” fossil fuels in use today. It is estimated that approximately 1 billion vehicles are utilised worldwide today (World Bank, 2008). If all these vehicles were replaced with environmental friendly alternatives, the reduction in greenhouse gas emissions would contribute significantly to minimising the effects of the current global climate change phenomenon.

There are various alternative options which the transportation sector can consider, with the most popular alternatives including; electric vehicles, hydrogen fuel cells and biofuels. At present the most promising medium-term solution for the transportation sector is the use of biofuels. These fuels are produced in different forms, including; ethanol, butanol, biodiesel and green diesels (hydrogenated sugar derivatives). Each derived from various feedstock and produced by specialised processes. Biofuels are considered less harmful to the environment as the CO<sub>2</sub> released from the production and combustion of these fuels are reabsorbed by the next generation feedstock grown, essentially closing the carbon cycle making the fuels essentially CO<sub>2</sub> neutral.

Bioethanol is a liquid fuel replacement for petrol, which has been mass-produced successfully in countries such as Brazil and the United States (Berlin et al., 2006). Current technology known as the 1<sup>st</sup>-generation bioethanol technology utilises starch and sugar, from maize and sugar-cane feedstock respectively. These sources have received much criticism from the food versus fuel debate and have led to the development of 2<sup>nd</sup>-generation bioethanol technology. This 2<sup>nd</sup>-generation technology utilises lignocellulosic (woody) material, overcoming the food versus fuel debate and allowing a much larger selection of feedstock, which is in abundance worldwide.

## 1.1 1<sup>st</sup>-Generation bioethanol technology

The primary feedstock for bioethanol production utilised worldwide is starch from maize in North America and from wheat and barley in Europe (Linde, 2008), while Brazil uses sugar from sugar-cane. Production of ethanol from starch (Figure 1) begins with either the dry or wet milling of the kernels. Dry milling refers to the crushing of the kernels to form a fine powder that is mixed with water to form a slurry, while wet milling uses dilute sulphuric acid to break the kernel structure. After milling, enzymes are added to the slurry to hydrolyse the starch to sugar. Hydrolysis refers to the breaking of the covalent bonds that bind glucose strings in starch using water with enzymes as catalyst. The glucose released through hydrolysis is fermented by a microorganism (usually yeast) producing ethanol. The ethanol containing mixture is distilled and the extracted ethanol dried to 99.9 % purity, which is ready for use in automobiles as a pure or petrol blended fuel.

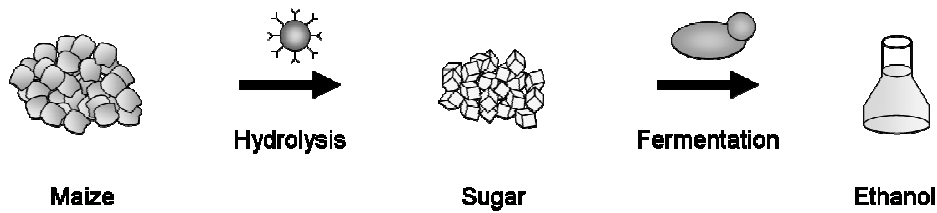


Figure 1: Starch conversion to bioethanol

## 1.2 2<sup>nd</sup>-Generation bioethanol technology

Lignocellulosic ethanol is the future for bioethanol and is produced from the most abundant raw material in nature, namely; cellulosic biomass (Lee, 1997). This feedstock includes hardwood, softwood, grasses and agricultural residues, with additional materials of potential interest such as old newspapers, office paper and municipal waste (Lee, 1997). Lignocellulosic materials consist primarily of three major components, namely: cellulose (40 % - 60 %), hemicellulose (20 % - 40 %) and lignin (10 % - 25 %) (Hamelinck et al., 2005). Lignin is an extremely resistant aromatic structure that protects the cellulose bundles and hemicellulose chains from chemical or enzymatic degradation (Figure 2).

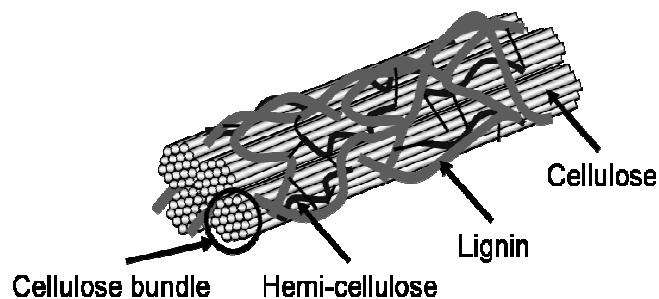
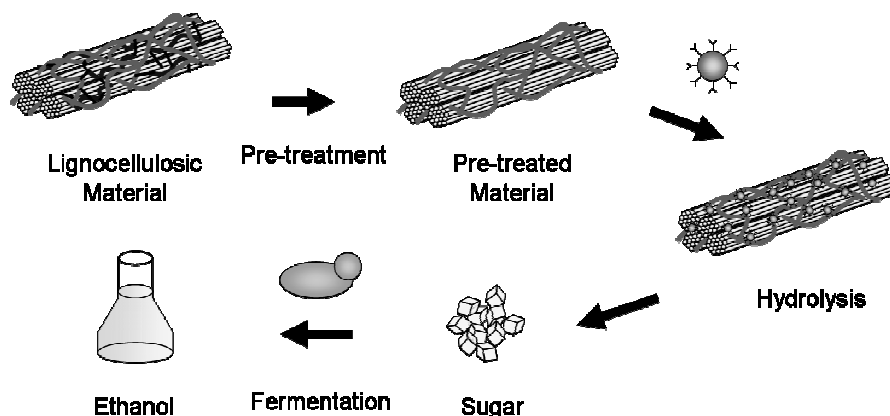


Figure 2: The lignocellulosic structure with cellulose bundles encapsulated by hemicellulose and lignin

Bioethanol production from lignocellulosic materials (Figure 3) requires a pretreatment process to reduce the recalcitrance of the feedstock by breaking the hydrogen bonds that attach the lignin, cellulose and hemicellulose together, exposing the cellulosic structures for chemical or enzymatic conversion (Berlin et al., 2006). The pretreatment method utilised depends greatly on the feedstock and may include milling, dilute / strong acid treatment or steam explosion. Chemical or enzymatic hydrolysis of the exposed cellulose components to simple sugars allows

microorganisms (bacteria or yeast) to ferment the sugars to ethanol (Berlin et al., 2006).



**Figure 3: Lignocellulosic conversion to bioethanol**

The major advantage of this technology is the large range of feedstock available for ethanol production and improved efficiency of current agricultural land utilisation over the first generation technology by including cellulosic sources (Linde, 2008). This permits the food and fuel industry to co-exist, both receiving feedstock from the same agricultural land increasing the options and subsequent financial income of the commercial farmer, while creating new job opportunities.

### 1.3 Modelling

Modelling the production of ethanol from cellulosic materials presents a challenge for engineers and biologists. These systems involve a vast number of parameters which affect the reaction kinetics of hydrolysis and fermentation of cellulose. Mixing conditions in these reactors are primarily affected by the fluid viscosity and agitation speed, with the viscosity highly dependent on the cellulose particle properties and soluble constituent concentrations. Optimal reaction rates and product yields are attained under fully suspended mixing conditions and are highly sensitive to temperature, acidity, soluble constituent concentrations, available substrate surface and the organism characteristics and enzyme properties.

Many models have been proposed to predict the complex enzyme kinetics responsible for the hydrolysis of cellulose to sugar (Converse et al. 1988, Gusakov and Sinitsyn 1985, Scheiding et al. 1984, Caminal et al. 1985, Converse and Optekar 1993). These models used either Langmuir isotherms or Michaelis-Menten type equations to model enzyme adsorption to cellulose (Zhang and Lynd, 2004). A complete model for simultaneous saccharification and fermentation of dilute acid pretreated poplar feedstock was developed by South et al. (1995) based on work from Phillippidis et al. (1992) and later modified by Shao et al. (2008) and Zhang et al. (2009) for use with paper-sludge.

Simultaneous saccharification and fermentation involves the simultaneous hydrolysis of cellulose to sugars by enzymes and the fermentation of these sugars by the organism to form ethanol. This differs from separate saccharification and fermentation where each process is performed separately, first by hydrolysing the cellulose under conditions ideal for the enzymes and then fermenting the sugars under favourable conditions for the organism. Separate saccharification and fermentation however suffers a significant drawback as separate hydrolysis of cellulose results in significant concentrations of sugars which inhibit the conversion of cellobiose (short chain glucose polymers), which subsequently inhibits the hydrolysis of the cellulose by the enzymes. Simultaneous saccharification and fermentation thus provides a significant improvement in product yields as the organism consumes the sugars as they are produced, preventing product inhibition and subsequently achieving higher cellulose conversion (Linde et al., 2008).

## **1.4 Objectives**

The objective of this study was to investigate the possibility of increased ethanol yields from continuously stirred tank reactors employing cell and substrate recycling schemes. The study involved evaluating the effects of lignin presence on the hydrolysis rate of cellulose, the investigation of increased cellulose conversion with higher cellulase loadings and the benefit of cellulose recycling.

## **2 Conceptual method**

It is proposed that recycling yeast cells and substrate back to the reactor will produce higher ethanol yields as the substrate and yeast concentrations will remain high allowing for maximum conversion conditions to exist. Implementing a feedback scheme in combination with a continuous stirred tank reactor (CSTR) configuration has the added benefit of removing excess ethanol from the reactor. This is especially useful as ethanol inhibits the growth and performance of the organism, thus maintaining low concentrations of this potentially toxic inhibitor is favourable. Substrate recycling bears a further advantage as it returns adsorbed enzymes to the reactor, always ensuring a higher enzyme loading, reducing the excess cost of continuously adding large volumes of expensive enzymes.

## **3 Research methodology**

### **3.1 Numerical Model**

The reaction kinetics model proposed by South et al. (1995) for pretreated poplar was modified using concepts from Shao et al. (2008) to improve numerical stability and coded into the Matlab R2007a Student Edition (MathWorks, Inc, USA) environment. The model was verified by comparing the results with South et al. (1995). The reactors investigated were assumed to operate under ideal mixing conditions resulting in a homogeneous solution and that the pretreated lignocellulose material contained no hemicellulose to avoid by-products such as furfural and hydroxymethyl furfural forming during hydrolysis.

A reactor substrate concentration of 125 g/L was selected for this study allowing the substrate to be concentrated to 250 g/L and fed back to the reactor without causing blockage within the system. The lignocellulosic substrate was assumed as softwood with a composition of 38.6 % lignin and 61.4 % fermentable cellulose (Hamelinck et al.,2005). Complete conversion of cellulose was assumed, with glucose, ethanol and carbon dioxide as products.

### **3.2 Effects of lignin**

Cellulases adsorb onto both cellulose and lignin surfaces. Enzymes adsorbed to cellulose hydrolyse the substrate with the surrounding water and detach from the surface once completing a cellulose chain. Enzymes that adsorb to the lignin surface however are unable to perform any type of reaction and do not detach. This effectively reduces the concentration of available free enzymes in the reactor broth lower the hydrolysis rate of the reaction.

The effects of lignin on the hydrolysis of cellulose were evaluated with a batch type reactor configuration. The control case assumed an initial cellulose concentration of 125 g/L and yeast cell loading of 2.5 g/L with a cellulase loading of 10 filter paper units (FPU)/g cellulose and a  $\beta$ -glucosidase loading of 50 international units (IU)/g cellulose. The simulation was repeated with lignin present at 78.75 g/L, constituting 38.6 % of the substrate composition.

### **3.3 Enzyme loading**

Enzyme loading generally increases the hydrolysis rate of cellulose as the greater the enzyme concentration the higher the probability of an enzyme protein adsorbing to an available bonding site on the cellulose surface and hydrolysing the substrate. The effect of increased enzyme loading is however limited by the available substrate surface. If all available bonding sites are actively occupied by enzymes, adding additional enzymes would have negligible effect.

The effects of increased enzyme loading were investigated using a continuous stirred tank reactor configuration. Steady-state conditions were simulated for a substrate feed concentration of 125 g/L cellulose and  $\beta$ -glucosidase loading of 50 IU/g cellulose at various dilution rates to determine the converted substrate concentrations. Three cellulase feed concentrations of 10 FPU/g cellulose, 20 FPU/g cellulose and 30 FPU/g cellulose were evaluated.

### 3.4 Cell and substrate recycling

Recycling of microorganism cells and unconverted lignocellulosic substrate back to the reactor increases the average residence time the cellulose remains in the reactor improving the total substrate conversion. Adsorbed enzymes are also transported with the substrate back to the reactor, collectively raising the reactor enzyme concentration and increasing hydrolysis rates which improve efficiency.

The effects of cell and substrate recycling were investigated using a continuous stirred tank reactor configuration with a concentrated feedback loop. Steady-state conditions were simulated for a substrate feed concentration of 125 g/L cellulose, cellulase feed concentration of 10 FPU/g cellulose and  $\beta$ -glucosidase feed concentration of 50 IU/g cellulose at dilution rates between  $0.01 \text{ h}^{-1}$  and  $0.1 \text{ h}^{-1}$  to determine the converted substrate concentrations. Cell and substrate recycle benefits were investigated at three recycling percentages of 30 %, 60 % and 90 % of the exiting substrate concentration.

## 4 Results

### 4.1 Effects of Lignin

The presence of lignin during hydrolysis caused a lower cellulosic conversion rate due to the decrease in free enzymes concentrations in the broth. The ethanol concentration throughout the batch reaction decreased with less than 5 % when lignin was present (Figure 4).

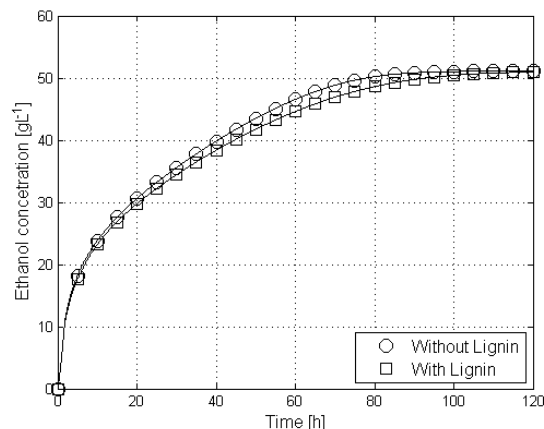


Figure 4: Batch ethanol concentrations with and without lignin

Cellulases have a higher affinity for cellulose over lignin, causing the majority of the cellulases to bond to the cellulose. As the substrate is hydrolysed decreasing its concentration, the excess free enzymes adsorb to the lignin (Figure 5).

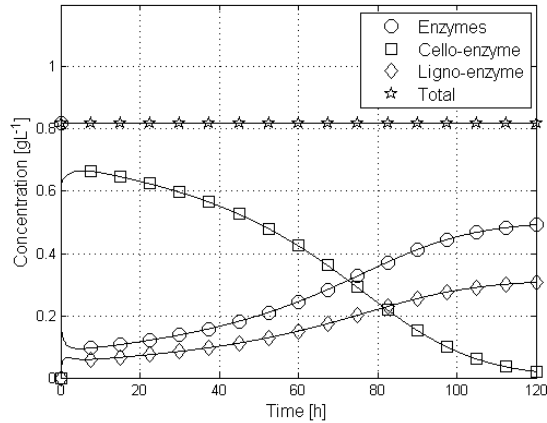


Figure 5: Enzyme concentration to illustrate enzyme affinity

## 4.2 Enzyme loading

Increasing the feed cellulase concentration in a CSTR configuration caused higher hydrolysis rates and improved conversion of the cellulose substrate (Figure 6). Residence time in the reactor affects the total substrate conversion, with dilution rates of  $D = 0.1 \text{ h}^{-1}$  and less producing significantly higher total substrate conversion. It is important to remember that a dilution rate of  $D = 0 \text{ h}^{-1}$  is essentially a batch tank reactor, which produces the maximum substrate conversion.

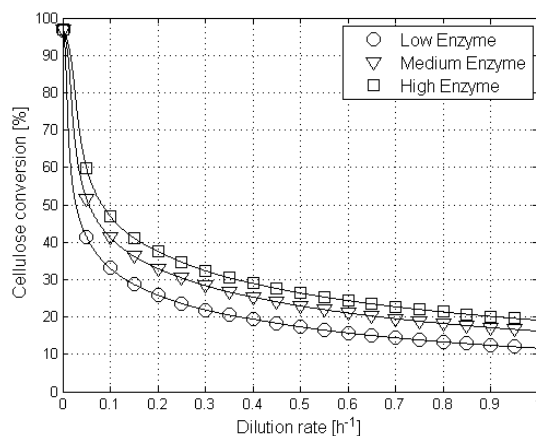


Figure 6: Total conversion for various enzyme loadings

A second important limitation on CSTR configurations is the maximum growth rate of the organism. Operating a CSTR at dilution rates greater than the maximum growth rate essentially flushes the organism from the system preventing fermentation. The *Saccharomyces cerevisiae* organism used in this study has a maximum growth rate of  $\mu = 0.4 \text{ h}^{-1}$  (Figure 7).

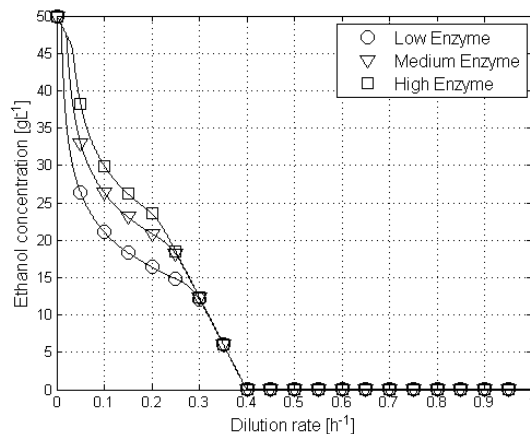


Figure 7: Enzyme loading effects on ethanol concentrations

### 4.3 Recycled cells and substrate

One primary concern when implementing a feedback loop in a CSTR system is the overflowing and subsequent blockage or damage of the reactors. Setting a reactor limitation of 125 g/L substrate and simulating cell and substrate recycling schemes revealed that dilution rates below  $D = 0.09 \text{ h}^{-1}$  have essentially no risk of overflowing the reactors (Figure 8).

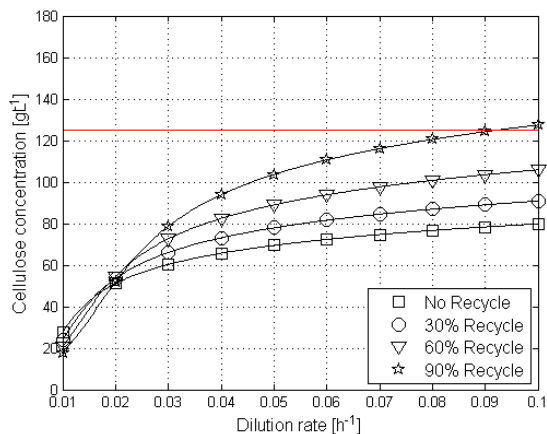


Figure 8: Reactor cellulose concentrations

Evaluation of the ethanol concentrations that eventually exit the reactors indicated that higher recycling percentages increase final ethanol concentrations. A maximum concentration was observed at a dilution rate of  $D = 0.02 \text{ h}^{-1}$  with a 33 % high concentration when compared to a standard CSTR operating under similar conditions (Figure 9).

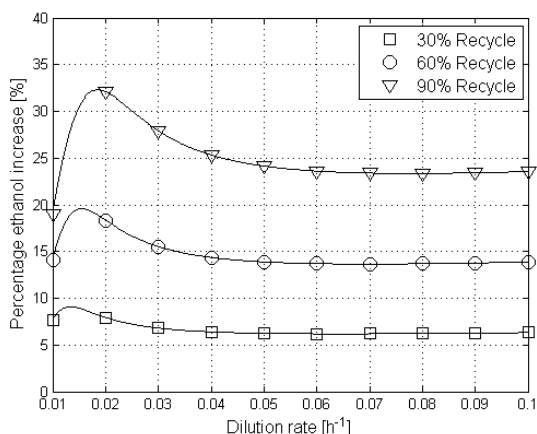


Figure 9: Percentage ethanol concentration increase caused by cellulose and yeast recycling

## 5 Conclusions and recommendations

### 5.1 Effects of lignin

Conditions with high cellulose concentrations are minimally affected by the presence of lignin as cellulases have a greater affinity for cellulose than lignin. It was observed that when cellulose concentrations decrease significantly, more cellulases adsorb to the non-reactive lignin reducing the number of free enzymes available, inhibiting hydrolysis.

Substrate recycle schemes are significantly affected by the presence of lignin. This is due to the effective concentrating of the lignin in the reactor with each cycle, as lignin is insoluble and non-reactive. The presence of lignin thus results in the reactor rapidly filling and becoming block or damaged. Furthermore, the concentration of lignin would soon exceed that of the cellulose substrate resulting in highly inefficient hydrolysis and thus ethanol production. Thus pretreatment should include methods of removing excess lignin from lignocellulosic substrates before hydrolysis and fermentation.

## 5.2 Enzyme loading

Cellulase loadings of 10 FPU/g cellulose, 20 FPU/g cellulose and 30 FPU/g cellulose were investigated. Observations indicated that dilution rates of  $D < 0.04 \text{ h}^{-1}$  resulted in high cellulose conversion in excess of 70 % and that higher cellulase loadings significantly improve the hydrolysis rate. Cellulases are however extremely expensive to produce or purchase, thus negatively affecting the attractiveness of increased enzyme loading.

Cellulase loading in excess of 30 FPU/g cellulose have been shown to have little benefit as the enzyme capacity of the cellulose substrate becomes limiting. This is caused by the limited bonding sites available on the substrate surface and once saturated is unable to accommodate additional enzymes.

## 5.3 Cell and substrate recycling

Organism and cellulose substrate recycling simulations indicated increased cellulose conversion producing higher ethanol concentrations when compared to a standard CSTR configuration. Simulations indicated a possible ethanol concentration increase of 33 % at a dilution rate of  $D = 0.02 \text{ h}^{-1}$  with 90 % substrate recycle.

A dilution rate of  $D = 0.02 \text{ h}^{-1}$  equates to an average residence time of 50 h. Cellulases adsorbed to the residual substrate are continuously returned to the reactor where fresh substrate is available. This effectively increases the enzyme loading of the reactor, improving hydrolysis rates as enzymes hydrolyse fresh substrate significantly more efficient (Tu et al., 2007). Cellulases have been shown to remain active for periods exceeding 48 h, though the industrial environment would rapidly damage or destroy these proteins during longer operation. Recycle reactors thus require a continuous supply of new enzymes.

Operating recycle reactors at much higher dilution rates are impractical as such systems would have low substrate conversion rates, thus rapidly overfilling the reactors, blocking or even damaging the reactors.

## 6 Acknowledgements

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## 7 References

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