ACCELERATING ENZYMATIC HYDROLYSIS OF CORNSTARCH AND CELLULOSE USING CATIONIC POLYMERS

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ACCELERATING ENZYMATIC HYDROLYSIS OF CORNSTARCH AND CELLULOSE USING CATIONIC POLYMERS

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To my loving mother Sujatha Mora

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SUMMARY

The effect of cationic polymers on the rate of hydrolysis of cornstarch and cellulosic feedstocks was investigated. Poly(diallyldimethylammonium chloride) (p-DADMAC) and cationic polyacrylamides (c-PAMs) were used in the study. Experiments were performed to analyze the effect of both p-DADMAC and c-PAM on cornstarch liquefaction. Measurements were also made on the hydrolysis rates of bleached softwood to determine the mechanism through which cationic polymers accelerate cellulose hydrolysis. Additional experiments were performed to study the effect of cationic polymers on different lignocellulosic feedstocks such as sludge, wheat straw and brown pulp.

Studies on cornstarch hydrolysis showed that p-DADMAC increases the rate of α-amylase-induced cornstarch liquefaction, thereby reducing the enzyme dose necessary for optimal hydrolysis. A similar increase was also observed by c-PAMs, but p-DADMAC offers the added industrial benefits of lower cost, a lower catalytic concentration, and a much lower residual in distillers dry grain. p-DADMAC also increases enzyme–substrate binding, lowers the onset temperature of gelatinization, and reduces the viscosity of the gelatinized cornstarch. It is proposed that most of these benefits result from the higher charge density of p-DADMAC, which neutralizes the negative charge on the cornstarch at a lower polymer dose. The higher charge also inhibits the agglomeration of starch through charge reversal. These attributes of p-DADMAC offer cost and performance benefits that can be realized in existing corn ethanol facilities.

Studies on bleached softwood showed that cationic polyelectrolytes increase the cellulase-induced hydrolysis rates of bleached wood fiber. It was shown that the polymer associates mainly with the amorphous region of fiber and acts principally on endoglucanase. Fiber:water partitioning of the enzyme follows a Langmuir isotherm for the untreated fiber but a Freundlich isotherm is obeyed for the polymer-treated fiber. Both c-PAM and p-DADMAC increase the fiber:water partition coefficient of the enzyme.

The feasibility of converting fibrous sludge into glucose was demonstrated through an economic analysis. A kinetic model that accounts for product inhibition was used to estimate the cost:benefits of the process. In the proposed scheme, the sludge was enzymatically hydrolyzed in a sequence of CSTRs, the ash separated, and the product glucose concentrated through reverse osmosis. The water recovered was mostly recycled. The most important economic variable was the value of the glucose. However, even if the glucose was assumed to be of no value the avoided cost of sludge disposal approximately offsets the process costs.

Studies were performed on brown fiber from softwood and hardwood pulps. Both c-PAM and p-DADMAC increased the glucose production of brown pulp at lower kappa numbers. The effect of cationic polymer was negligible at high kappa number.

Overall, cationic polymers enhanced the production of glucose from cornstarch and different cellulosic feedstocks. The polymer can reduce the enzyme dosage depending on the conditions and feedstocks used.

CHAPTER 1: INTRODUCTION

To meet the growing demand for energy, the production of biofuels is expected to increase significantly over the coming decades. In a recent study, it was shown that global production of conventional fuel is approaching its peak and only one new barrel of oil is found for every four that the world consumes [1]. Hence, biofuels are considered as an immediate alternative to fossil fuels that have the ability to increase energy security, to limit greenhouse gas emissions, and to improve air quality. Ethanol is considered to be an important biofuel. It can be produced from cornstarch or cellulosic biomass. Enzymatic hydrolysis of cornstarch and cellulosic biomass is currently one of the principal routes towards the production of biofuels from these feedstocks [2]. Cornstarch and cellulose undergo enzymatic hydrolysis to produce glucose, which can be readily converted into ethanol by fermentation.

Corn is the most widely used feedstock to produce ethanol in the U.S. With the annual production of corn topping 12.1 billion bushels in 2010, the U.S. ranks as the world's largest corn producer. More than 50% of the gasoline sold in the U.S. is blended with ethanol. The U.S. Energy Policy Act of 2005 (EPAct) requires that renewable fuels make up four billion gallons of the nation's gasoline market starting in 2006, and 15 billion gallons by 2015 [2]. To increase ethanol production it is very important to increase the efficiency of enzymatic hydrolysis. Further reduction in costs is required for biofuels to be able to compete effectively with gasoline in the absence of government subsidies. The cost of enzyme remains a major component of the process economics for both cornstarch and cellulose hydrolysis [3-5].

Lignocellulosic feedstocks can be used for the production of biofuels. These feedstocks would be ideal because they are abundant and of low cost. They also do not compete with food crops. Various agricultural biomass sources such as unbleached pulp, wheat straw, corn stover, bagasse, and rice straw are considered lignocellulosic biomass. They mainly contain cellulose, hemicellulose and lignin. It is essential to disrupt the integrated cellulose-hemicellulose-lignin structure to improve enzyme accessibility. Several pretreatment methods, both physical and chemical, can be employed to partially or completely remove lignin (and sometimes hemicelluloses) [4, 6].

Industrial waste such as sludge from pulp and paper mills would be an ideal feedstock for producing bioethanol. Sludge is associated with costs for landfill disposal or incineration (current industrial practice) [7]. Producing ethanol from sludge not only makes a commodity chemical but also decreases the amount of waste that has to be treated. About five million tons of sludge is generated from pulp and paper mills in the U.S. [8]. A typical 1000 tons per day mill generates about 50 tons of sludge daily. Sludge from a kraft mill essentially contains fibers, fillers, lime mud, boiler ash etc. Fibers can be enzymatically converted into glucose, which can be further fermented to produce ethanol. Sludge from a recycle mill contains more ash, which is a dead load during enzymatic hydrolysis.

There is strong potential for using recovered fiber from pulp mills or repurposing non-competitive kraft mills to produce bioethanol. The value of bleached pulp is more than that of bioethanol, however repurposing an existing mill for bioethanol production

shows good potential. Several pulp and paper mills have been closed in the U.S., due to decreasing paper consumption. It is estimated that approximately 7 million tons of production was closed in the U.S. [9]. A closed mill will have all the equipment necessary for wood pretreatment and also will have access to a skilled workforce. It is not necessary to cook and completely bleach the wood. Partial bleaching or kraft cooking might be enough to produce high yield glucose [5].

In the current project, different feedstocks such as cornstarch, bleached fiber, partially bleached and unbleached fiber, wheat straw, and sludge were used to study the effect of cationic polymers on enzymatic hydrolysis for the production of glucose. Cationic polyelectrolytes (c-PAM) can increase the hydrolysis rates of both bleached wood fiber and cornstarch by more than 50% [10]. While modest by catalytic standards, the increase is, nonetheless, commercially significant. It was proposed that the polyelectrolyte attaches to and neutralizes the negatively charged substrates thereby reducing the repulsion experienced by the negatively charged enzyme and increasing the binding of the enzyme to the substrate [11]. Charge neutralization of a fiber by a cationic polymer is called “patching”, a process well established for fiber agglomeration [12]. There are several enzymes involved in the enzymatic conversion of fiber to glucose. Endoglucanases first break up the cellulose fiber into fragments. Exoglucanases then processively convert the fragments into cellobiose, which is hydrolyzed to glucose by cellobiase. The last step is comparatively rapid [13] and is not rate-controlling for fiber hydrolysis.

Dr. Kendra Maxwell performed several experiments to study the effect of c-PAM on cornstarch liquefaction [14]. Overall, the c-PAMs can reduce the optimal enzyme dose required for cornstarch hydrolysis by about 60%, which is economically very significant. A negative attribute is that it can increase viscosity by agglomerating starch components. Viscosity spikes can be difficult to accommodate in industrial operations where interruptions in mixing and pumping intensity are detrimental [15-17].

Several c-PAMs varying in charge, molecular weight and degree of branching are capable of increasing the hydrolysis rate [11], which suggests that they act through a non-specific mechanism. Hence, it seemed likely that other cationic polymers could also have a similar rate-enhancing effect, perhaps without the viscosity penalty of c-PAMs. This thesis describes the effect of polydiallyl dimethyl ammonium chloride (p-DADMAC), a commonly used retention aid in the paper industry [12, 18], on cornstarch hydrolysis.

The objectives of the current study are:

1. Compare the behavior of pDADMAC and c-PAM for cornstarch hydrolysis
2. Understand the mechanism through which the polymers act on the enzymatic hydrolysis of cornstarch and cellulose
3. Study the effect of polymers on different cellulosic feedstocks

Objective 1: Studies were performed to analyze the effect of p-DADMAC, which is cheaper than c-PAM, on cornstarch hydrolysis. Chapter 4 reports results and discusses the effects of c-PAM and p-DADMAC on cornstarch liquefaction.

Objective 2: Several binding studies were performed to analyze the effects of p-DADMAC and c-PAM on cornstarch and cellulose. Chapter 4 reports results on cornstarch liquefaction and Chapter 5 reports results from experiments performed on bleached fiber.

Objective 3: Experiments were performed to study the effect of cationic polymers on different lignocellulosic feedstocks. Chapter 6 reports results of an economic analysis on sludge saccharification. Chapters 7 and 8 report the effect of cationic polymers on wheat straw and brown pulp hydrolysis, respectively.

The current study has further explored the potential use of cationic polymers in the bioethanol industry. The results provided in the study provide insight into the mechanism through which cationic polymers act on cellulose and cornstarch during hydrolysis. In addition, the study also shows the effect of cationic polymers on different lignocellulosic feedstocks. The findings of this study will be helpful in reducing the amount of enzyme used for glucose production from different feedstocks. As the cost of enzyme remains a major component of the process economics, use of a cationic polymer will have a significant economic impact on industrial production of bioethanol.

CHAPTER 2: LITERATURE REVIEW

2.1 Biofuels

Global production of biofuels reached 79 billion liters in 2008, representing about 1% of total road-transport fuel energy consumption [19]. Brazil and the U.S. together accounted for almost 80% of global supply of biofuels. The increased use of biofuels offers many benefits, such as decreased reliance on petroleum, reduced environmental impacts, and developing energy security of importing countries. In spite of these many advantages, biofuels present some critical challenges. Among the challenges receiving major attention are the effects of biofuel development on food crop prices, availability of land resources, and, most importantly, high biofuel costs compared to petroleum fuels. Further reduction in costs is required for biofuels to be able to compete effectively with gasoline in the absence of government subsidies. Intensive research is being done to improve biofuel production by developing strategies to meet the concerns for cost effectiveness, food competition and ecosystem protection. Produced in a sustainable way, biofuels can be a permanent solution for fuel problems, leading to increased reliance on domestic fuel sources [20].

2.2 Cornstarch

Due to government regulations, ethanol production has been increased in recent years and about 25% [21]of corn produced in the U.S. is used in the production of ethanol. Due to legislations such as Energy Independence and Security Act of 2007, the production of ethanol from cornstarch has led to an aggressive expansion in the U.S. About 13.9 billion gallons of starch based bioethanol was produced in the U.S. in 2011[22]. Around 70-72% of corn is starch, which can readily be converted into glucose and thus to ethanol [23].

2.2.1 Properties of Starch

Starch is a highly reactive carbohydrate, which can be used in several ways such as in food products, coating for paper, adhesives, and as stated above, for ethanol production through physical, chemical or enzymatic modification. Starch consists of two major polymers: amylose and amylopectin (Figure 1 and Figure 2). Amylose is a linear polymer made up of about 6,000 repeating glucose units connected by α-1-4-glycosidic bonds. Amylopectin is one of the largest naturally occurring biopolymers with about 200,000 glucose units and with molecular weight more than 10^8 Da. Unlike amylose, amylopectin is a nonlinear polymer containing both α-1-4-glycosidic bonds and 4-6% α-1-6 branched side chains. Amylose is not soluble in cold water whereas amylopectin is. Cornstarch granules are generally round with a diameter ranging between 2-15 μm. Cornstarch typically contains 27% amylose with the remainder being amylopectin [24].

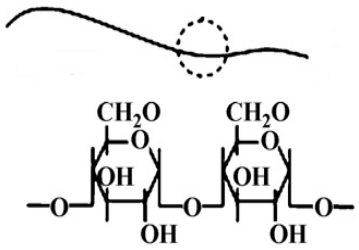


Figure 1: Amylose structure [14]

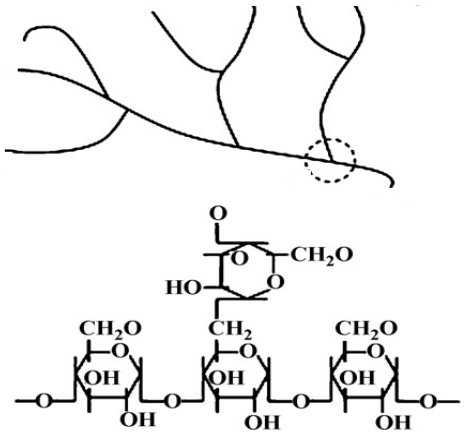


Figure 2: Amylopectin structure [14]

2.2.2 Starch Gelatinization

Amylose and amylopectin hydrate when heated in water. As the granules continue to expand, immobilizing much of the water present, the viscosity of the stock increases rapidly. This results in formation of a paste or gel and the process is termed gelatinization (Figure 3). Gelatinization is normally used to describe the collapse of starch granule, whereas pasting is used to describe changes in viscosity after the disruption of starch granule due to dissolution of starch [25, 26]. The temperature at which gelatinization starts is referred to as the gelatinization temperature, this is the temperature where the viscosity of the solution begins to increase. The gelatinization temperature of cornstarch is between 62-72°C and varies with factors such as starch concentration, pH of the stock,

rate of heating, and presence of specific polymers or salts [24, 27]. One of the important factors that affect gelatinization is the amylose content of cornstarch. Amyloses are linear polymers that are aligned in parallel and are attracted to each other through their hydroxyl groups. Due to these reasons, the gelatinization of starch with high amylose concentration requires more energy to disintegrate the firmly bonded crystalline structure. When cooled after gelatinization, they form firm gels. On the other hand, a starch solution with higher amylopectin is easy to gelatinize and retrograde to form weak gels.

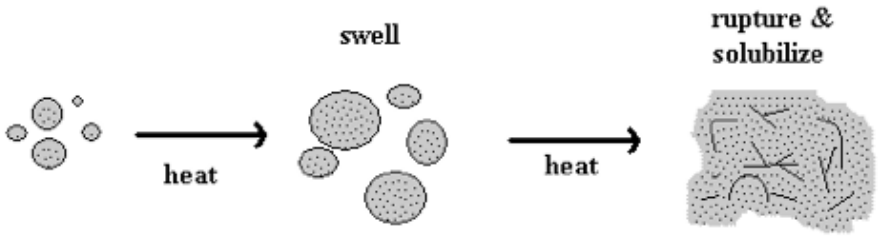


Figure 3: Gelatinization of cornstarch [14]

2.2.3 Effect of Different Additives on Starch Gelatinization

Polysaccharides are often used with starch to modify or control product quality, lower the production costs, maintain or obtain certain textures, control moisture etc[28, 29]. Many studies were performed to analyze the effect of hydrocolloids in cornstarch gelatinization. Hydrocolloids such as xanthan, alignate, carboxymethylcellulose, and guar gum has lowered the onset of gelatinization temperature of cornstarch. This was attributed to a measurable viscosity increase at a lower temperature compared to starch gelatinized without adding any hydrocolloids [29].

Xiaohong, *et al*. obtained pasting curves of cornstarch with and without hydrocolloids. Low cornstarch concentrations were used (3.6% starch). Higher viscosity was observed for cornstarch in the presence of caboxymethylcellulose, gellan, xanthan, guar gum, and sodium alginate. Figure 4 shows that the onset of gelatinization temperature was lower when gellan was used with cornstarch compared to cornstarch without gellan. The onset of viscosity increase in the presence of gellan began at a temperature 15°C lower than that of the control [29].

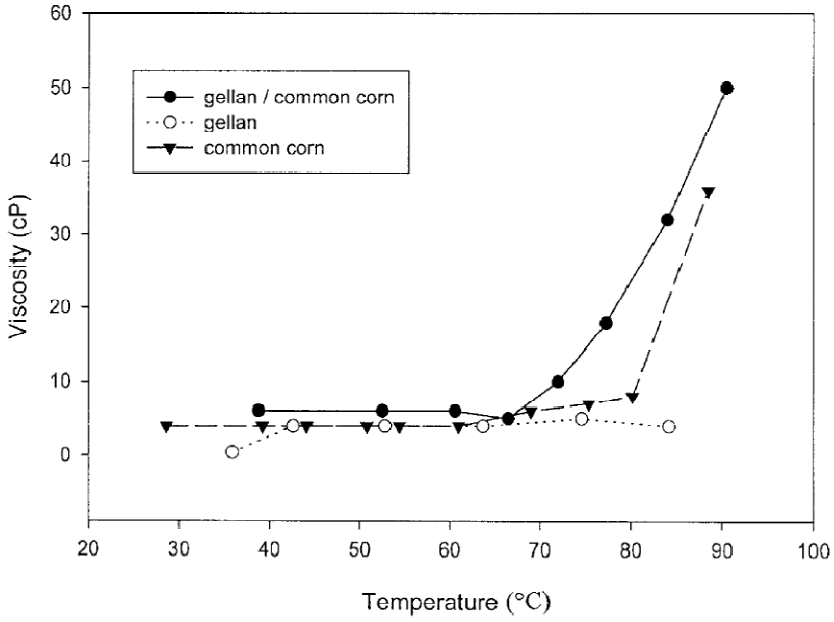


Figure 4: Change in onset of gelatinization temperature with polysaccharide [29]

Zhiting, *et al*. studied three different charged polysaccharides, Konjac glucomannan which is neutral, negatively charged carboxymethylcellulose, and positively charged chitosan. The viscosities of cornstarch dispersions were affected differently depending on the polysaccharide used, which suggest that all polysaccharides do not have similar effect during cornstarch gelatinization and pasting [30].

Addition of Konjac glucomannan increased the viscosity peaks as compared to control. Figure 5 shows that 1-3% KG has higher viscosity peaks, however the peak temperature is constant for all the cases. The figure also reports changes during retrogradation of cornstarch (decrease in temperature). Retrogradation, which is a very important factor to be considered for food and processing industries, is of little concern to the biofuel industry and is not discussed further [30].

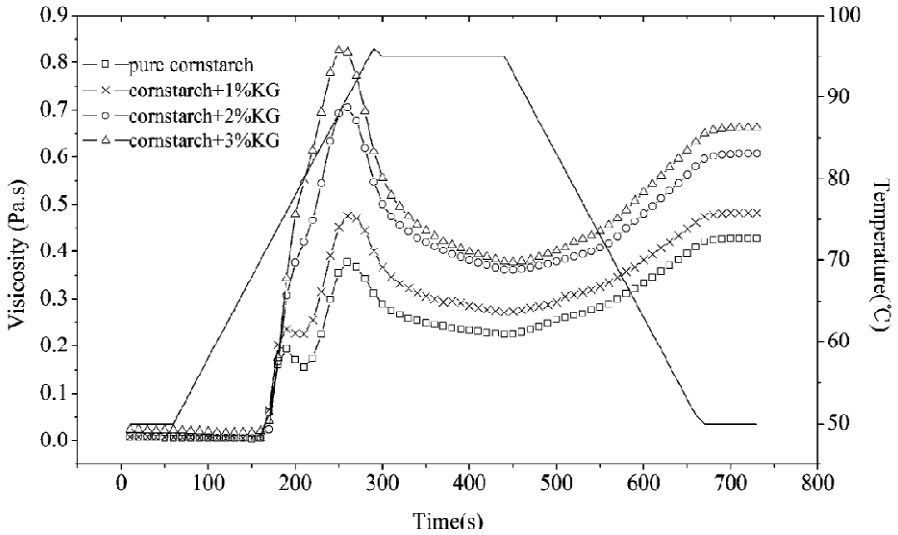


Figure 5: Pasting curves with and without polysaccharides [30]

The mechanism through which hydrocolloids interact with starch during gelatinization is not yet understood to an extent that it can be modeled or predicted. Several researchers have come up with different mechanisms. Xiaohong*, et al*. predicted that the reason polysaccharides decrease the onset of gelatinization or increase viscosity levels is due to intermolecular interactions between dissolved amylose chains and polysaccharides. Zhou*, et al.* suggested that polysaccharides reduce the chain mobility of starch in water by interacting with starch polymers, and increase gelatinization

parameters [31]. Some researchers suggested that certain hydrocolloids enhances granule swelling, which increases the leaching of amylose chains into water, where as some hydrocolloids such as xanthan restrict granule swelling by attaching to the granule surface and restrict the amount of water available to the granules [28-31].

BeMiller*, et al*. in his review summarized several mechanisms that have been reported in different research papers [28]. It was concluded that due to the complex nature of the starch-polymer system, which change significantly depending on temperature, pH shear rate, concentration and type of hydrocolloid used, several different mechanisms are probably operating and the contribution of each mechanism will vary with each individual system [28, 29, 32].

2.2.4 Enzymatic Hydrolysis of Cornstarch

Cornstarch can be converted into ethanol using enzymes by either dry-grind or wet-grind processes. The main product of the dry-grind process is ethanol whereas the wet-grind process produces additional high value products with ethanol. Fuel ethanol is mostly generated by using the dry-grind process in the U.S. As the current work is focused on ethanol production, the dry-grind process is considered in the current thesis. There are several other methods that use acids to break starch molecules into glucose. However, these processes are performed at temperatures greater than 140°C. In contrast, enzyme hydrolysis is performed at relatively mild conditions and enzymes have a higher specificity [33].

Cornstarch is converted into ethanol in two steps: hydrolysis of starch molecules into single glucose units, and fermentation of glucose to ethanol. As the current research

focuses on the hydrolysis of starch molecules into glucose, fermentation is not discussed. Hydrolysis is, in turn, achieved in two steps: liquefaction and saccharification (Figure 6), which are described in the following sections [34].

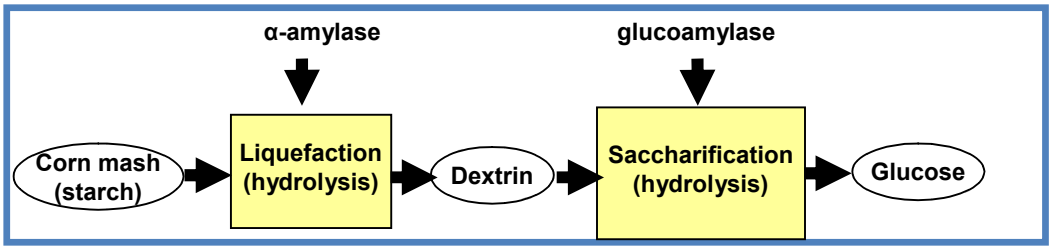


Figure 6: Hydrolysis of cornstarch to glucose

2.2.5 Liquefaction of Cornstarch

Starch solutions with 30 to 32% dry solids are gelatinized at elevated temperatures. The gelatinized starch is then treated with α-amylase to break down the starch molecules into shorter-chain-length dextrins. The1-4 glycosidic bonds of starch molecules are broken by α-amylase into smaller units of glucose, maltose, maltotriose, maltopentose, maltotetraose, and maltohexaose. After gelatinization, the stock is at 90-100°C so the enzyme must be thermally stable. The pH of the stock is generally adjusted to 6, as it is optimum for α-amylase. It is advisable to opt for an enzyme that is stable at lower pH as the later saccharification step is performed at pH 4.2-4.5 (the optimum pH for glucoamylase). Recent studies have shown that enzymes from *B. stearothermophilus* and *B. licheniformis* can be used at lower pH and higher temperature and are, therefore, best suited for the liquefaction of cornstarch [24, 27, 33].

2.2.6 Saccharification of Cornstarch

Glucoamylase is added after liquefaction by decreasing the temperature of the stock to 60°C and adjusting the pH to 4.5. Glucoamylase sequentially removes glucose units from the non-reducing ends until all the oligosaccharides are degraded to glucose units. Fungi strains *Aspergillus niger* or *Rhizopus sp.* are a source of industrial glucoamylase [24].

2.2.7 Distiller Dried Grains (DDG)

The residual that is left over after fermentation of cornstarch in the dry-milling process is termed DDG. Around 17-18 lbs of DDG is produced for every bushel of corn processed [35]. The production and content of DDG mainly depends on the hydrolysis and fermentation process. DDG contains high amounts of protein, and amino acids, which can be fed to livestock. Eighty percent of DDG produced in the United States is fed to ruminants; the other 20% is used by the poultry and swine industries [36].

2.3 New Technologies to Boost Starch Hydrolysis

Improving starch hydrolysis has been a priority since the advent of using starch for bioethanol production. Major companies such as DuPont and Monsanto developed hybrid corn seeds and advanced engineered enzymes to increase ethanol yields from cornstarch.

2.3.1 Highly Fermentable Corn Hybrids

Ethanol yield from cornstarch depends on several factors. One of the most important factors is the type of corn hybrid. It is very difficult to identify an ideal corn hybrid, which can produce higher ethanol yields; sometimes the same hybrid may not give high starch yields [37].

DuPont Pioneer's extensive research has generated several hybrids, which can give high ethanol yields. These hybrids are characterized as high total fermentable (HTF) corn. It was shown that around 2-4% increase in yield can be achieved with any of HTF corn hybrids [38]. Pioneer has more than 180 HTF ethanol hybrids[38]; hybrids are selected by farmers depending on irrigation practices, location, and soil conditions. Pioneer developed near infrared calibration (NIR), which can estimate the weighted ethanol yield average of corn delivered to the plant. This information can be used to predict the value of corn, which can be further used by formers to choose better HTF hybrid for the next crop. Monsanto, another major seed company, has also developed similar hybrids for higher ethanol yield, which are characterized by 90 processor preferred fermentable corn hybrids. Monsanto also provide NIR for better selection of hybrids. [37-39].

2.3.2 Enzyme Engineering and Immobilization

Liquefaction is performed at elevated temperatures (70-90^{o}C) that require enzymes, which are more thermostable. *Bacillus amyloliquefaciens* was replaced by *Bacillus stearothermophilus* and *Bacillus licheniformis* for their higher thermostability. However, these enzymes can only perform with higher activity at pH levels above 5.9 and fail to give optimum conversions at low pH levels [33, 40, 41].

The limitation stated above and many other industrial requirements have led to the development of new enzymes using enzyme engineering. Enzyme engineering can integrate desired properties such as higher thermostability, higher activity at low pH, ability to degrade highly concentrated starch, increased activity, resistance to product inhibition etc [40]. Different mutations were performed on *Bacillus licheniformis* some of which have shown improvements in multiple desired properties. For example, mutating Asn 172, His 156, and Ala 181 to Arg, Tyr, and Thr respectively has increased the thermostability of the enzyme [40, 41].

One of the most widely used technologies to improve enzyme activity is immobilizing enzyme during the process. The catalytic activity of enzyme is very complex and depends on several factors, including available active sites, substrate binding energy, co-factors involved, and chain structure and conformation. During reaction several of these factors remain stable; however structure and confirmation of the enzyme can be physically or chemically damaged from its natural state, which lowers the activity and performance of α-amylase. Immobilizing an enzyme stabilizes it and protects

it from various physical and chemical denaturing agents. There are several prominent methods for immobilizing α-amylase, which include using agents such as copolymers of butyl acrylate, poly(ethylene glycol dimethacrylate-*n*-vinyl), poly(hydroxyethyl methacrylate-co-glycidyl methacrylate), calcium alginate beads, silica particles etc. Around 70-90% retained activity for several cycles of operation was reported after immobilizing enzyme [40, 42, 43].

2.3.3 Effect of Metal Ions on α-Amylase Activity

Several studies have stated that metal ions influence the activity of α-amylase [40, 42, 44]. Some metal ions such as Ca^{2+}, K^{+}, Co^{2+}, Mg^{2+}, Ba^{2+}, Ni^{2+}, Al^{3+} and Fe^{3+} reported increase in enzyme activity, where as metal ions such as Na^{+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+} and Hg^{+} have promoted or inhibited activity of α-amylase depending on the organism [40]. Several α-amylases are naturally metal ion-dependent, and these metals stabilize enzyme structure (figure 7) [44].

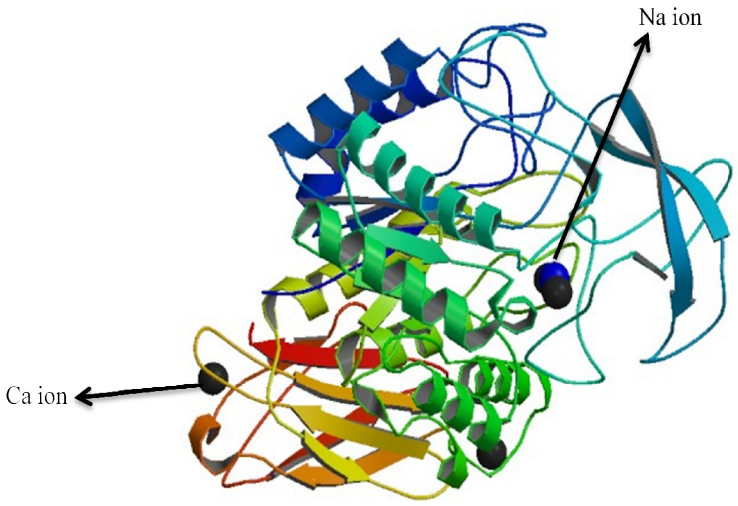


Figure 7: Structure of *Bacillus amyloliquefaciens* [44]

2.4 Conversion of Lignocellulosic Biomass to Ethanol

Lignocellulosic biomass is plentiful and is the most abundant resources in nature. Lignocellulosic biomass include both non-woody (herbaceous) and woody crops. Woody crops consist of hardwood (maple, oak etc.) and softwood (pine, redwood, Douglas fir etc.) trees. Non-woody crops include wheat straw, corn straw, switch grass etc. Currently, biofuel is mostly produced from starch based feedstocks. Utilizing lower-value feedstocks such as wheat straw, corn stover, switch grass, and cane bagasse, which are agricultural by-products, can significantly reduce the cost of ethanol production [45, 46].

Unlike starch, lignocelluloses are a complex structure of cellulose, hemicellulose, and lignin integrated together. The composition and structure of these elements vary depending on the source of feedstock.

2.4.1 Softwood and Hardwood

Softwood consist ~44% cellulose, which is similar to that of hardwood. The composition of lignin and extractive are higher in softwood, compared to hardwood. Due to high lignin content, pulping softwood is more challenging than hardwood. Figure 8 shows average compositions of softwood and hardwood. The total carbohydrate content in the wood is termed as holocellulose [46, 47].

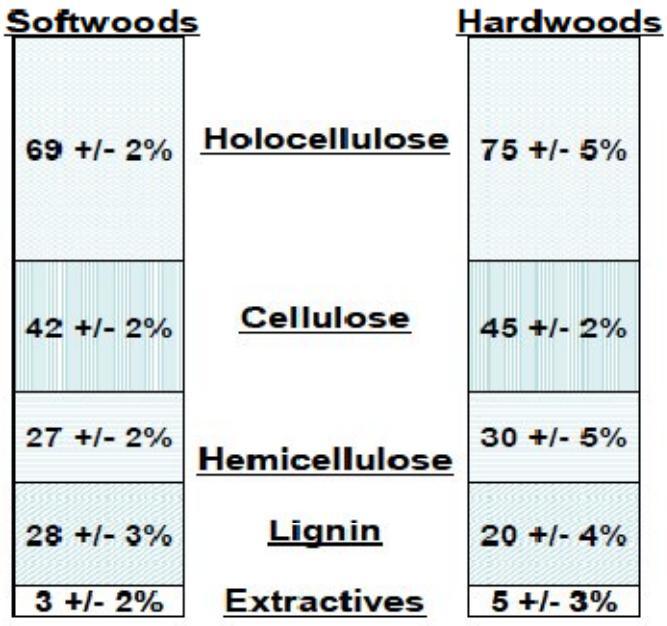


Figure 8: Composition of softwood and hardwood [46]

2.4.2 Non-Woody Crops

Non-woody crops, which include corn stover, wheat straw, rice straw, bagasse fiber etc. contain lower amount of lignin compared to woody crops. The composition of these crops depends on several factors such as crop variety, region, soil type, type of harvesting practice, and weather. Compositions of some lignocellulosic biomass are listed in Table 1 (based on dry weight) [45].

Table 1: Lignin and carbohydrates composition of various non-woody crops [45]

	Corn stover	Wheat straw	Rice straw	Bagasse fiber
Carbohydrates	58.1%	61.4%	62.5%	65%
Lignin	15.1%	14.5%	9.9%	18.4%

2.4.3 Cellulose

Cellulose is a long chain polymer with repeating units of D-glucose linked via beta-1,4 glycosidic bonds (Figure 9) with a degree of polymerization between 3500 to 10,000. These long chained polymers are referred to as elemental fibrils. The beta linkage makes cellulose chemically different from starch and due to a lack of enzymes that can break this linkage in the human body, humans cannot digest cellulose. Microfibrils, which consist of approximately 250 glucose units, are made up of elemental fibrils. These microfibrils form cellulose fibers, which are oriented in both amorphous and crystalline forms. Around 70% of the cellulose found in nature is crystalline. It is very hard to break cellulose apart as single unit glucoses because of the strong hydrogen bonds and van der Waal forces [47, 48]. Crystalline cellulose is harder to degrade as enzymes are too large to approach the linkage between cellulosic chains for degrading them. Amorphous regions are more susceptible to hydrolysis. Pulped fiber characteristics depend on the wood source. Softwood has higher average fiber length and diameter, which gives stronger paper when pulped compared to hardwood. The average fiber length of softwood is around 3.5 mm, whereas hardwood fiber length is much shorter at around 1.4 mm. The Table below shows fiber length and diameter of different wood species [46].

Table 2: Fiber length and diameter of different softwood and hardwood pulps [46]

	Softwood			Hardwood		
	Black Spruce	Douglas fir	Redwood	Aspen	Oak	Birch
Fiber Length (mm)	3.5	3.9	6.1	1.04	1.4	1.85
Fiber Diameter (μm)	25-30	35-45	50-65	10-27	14-22	20-36

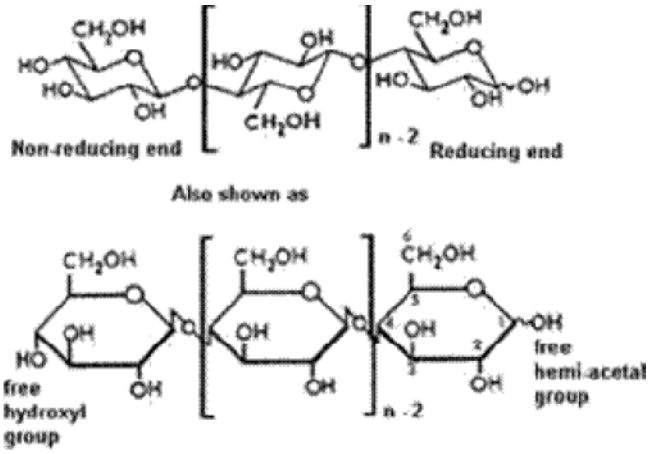


Figure 9: Cellulose structures [47]

2.4.4 Hemicellulose

Hemicelluloses provide bonding between cellulose through hydrogen bonds and direct linkage by improving the structural property of wood. They are polysaccharides of different sugar monomers with a DP of 100-200 and is completely amorphous unlike cellulose [49]. There are two principal types of hemicellulose: xylan (Figure 10) and glucomannans (Figure 11). Xylan is mostly found between lignin and cellulose, integrating them. Xylans are present in higher concentration in hardwood (15-30%) compared to softwood (7-10%). The hardwood DP ranging from 150 to 200 is higher

than softwood DP ranging from 70 to 130. 1,4-Linked β-D-xylopyranosyl units form the backbone of xylan and the side groups are species dependent. The side chains mainly include 4-O-methyl-D-glucuronopyranosyl, acetyl, feruloyl. In hardwood xylan exists as o-acety-l-4-methylglucuronoxylan and in softwood it exists as arabino-4-omethulgucuronoxlan (Figure 10) [50, 51]. Glucomannans contains straight chain backbone with mannose units. Compared to xylan, glucomannans are easily degraded by chemicals.

Figure 10: Structure of xylan [51]

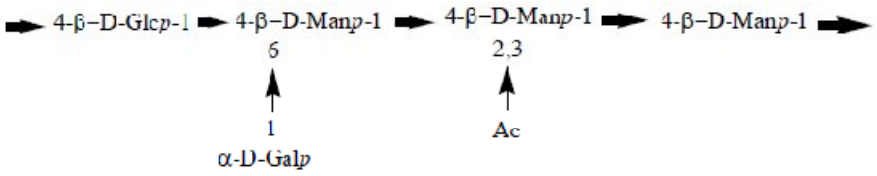


Figure 11: Structure of glucomannan [51]

The distribution of xylan and glucomannan varies depending on wood species (Table 3). Hemicelluloses distribution in wood fibers also depends on species and growing conditions. In softwood, most of the xylan is concentrated on the outer layer of secondary wall (S2), and the innermost fiber wall layer and in hardwood xylan is mostly found only in the outermost layers of secondary fiber wall.

Table 3: Hemicellulose in softwood and hardwood [51]

Component	Pine (% of dry Wt)	Birch (% of dry Wt)
Xylan	5-11	22-30
Glucomannan	14-20	1-4

2.4.5 Lignin

Lignin is an amorphous complex polymer essential to plant life. It provides mechanical support to the tree and also thermoplasticity, which is very important for bonding. It also imparts rigidity and stiffness to cell walls and gives hydrophobicity to tree, which protects trees from water penetration. Three types of lignin have been identified in wood based on their building blocks. They are guaiacyl (one methoxyl group), syringyl (two methoxyl groups), and p-hydroxygphenyl (no methoxyl groups). Guaiacyl is present in softwood, hardwood, and most non-woody plants. Syringyl is absent in softwood and resides in hardwood and non-woody plants. P-hydroxyphenyl resides mostly in non-woody plants. Figure 12 below shows the building blocks of lignin [46, 52].

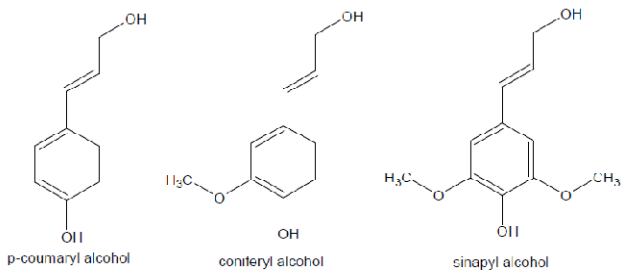


Figure 12: Building blocks of lignin

Lignin in wood can be measured by kappa number or Klason lignin number. Sulfuric acid is used to determine Klason number, lignin is determined as a residue in this method. Potassium permanganate is used to determine kappa number. This is most widely used method to determine leftover lignin after kraft pulping and subsequent bleaching stages. Kappa number is accurate for softwood pulps, whereas kappa numbers measured for hardwood are not precise due to presence of hexenuronic acids.

2.4.6 Kraft Pulping

Kraft pulping is a widely used pulping process in the world. In this process wood is chemically converted into pulp. High strength pulp is obtained in this process, however lower yields (47%) are attained due to non-selectivity of chemicals used. Highly colored pulp is obtained after kraft pulping process. In a typical batch pulping, the digester is filled with chips and white liquor (NaOH and NaSH) is added at an elevated temperature (170^{o}C). Chips are cooked at this temperature between 0.5 to 2 hours depending on degree of lignin that has to be removed. 4:1 wood to water ratio is maintained in the reactor [46, 52].

NaOH swells the wood chips, which allows good distribution of chemicals into chips. NaOH reacts with lignin by converting phenols into salts. NaOH is not selective towards lignin and reacts with carbohydrates by peeling and secession reaction, thus reducing the overall yield. NaSH is very selective and reacts only with lignin [46].

2.4.7 Hemicellulose and Lignin after Kraft Pulping

Due to the crystallinity and linearity of wood cellulose, they show resistant towards the harsh conditions of cooking. Unlike cellulose, hemicellulose components of the wood are modified heavily during conventional cooking. Xylan is partially soluble in the alkaline cooking liquor. The side groups and acetic acid residues of xylan are chemically altered by cooking liquor [51]. The methylglucoronic acid side groups in both residual and solubilized xylan are converted into hexenuronic acids. It has been shown that hexenuronic acids lead to higher consumption of bleaching chemicals thus reducing the effect of bleaching and increasing the amount of chemical requirement to achieve similar brightness [53, 54].

Xylan and lignin that are solubilized during the cooking process tend to readsorb on to the surface of the cellulose microfibrils. Lignin and xylan are believed to form covalent bonds leading to lignin carbohydrate complexes (LCC's) and are relocated to the surface of the fiber, resulting in increased lignin concentration on the fiber surface [55]. As the hydroxide is consumed toward the end of the pulping, the solubilized xylan and lignin tend to relocate on to the surface of the fiber. This phenomenon is significant in batch cooking process where there is no flux of the cooking liquor during pulping, compared to the continuous digester where the cooking liqueur is removed and replaced [56].

After kraft cooking most of the xylan is assumed to be accumulated on the outer surface layer for both softwood and hardwood pulps. Even though most of lignin is

removed in kraft pulping process, the presence of remaining lignin on the fiber surface might act as a rate limiting step for enzymatic hydrolysis of kraft pulp for glucose production. A model detailing the proposed structure of unbleached softwood pulp through kraft pulping is show in Figure 13.

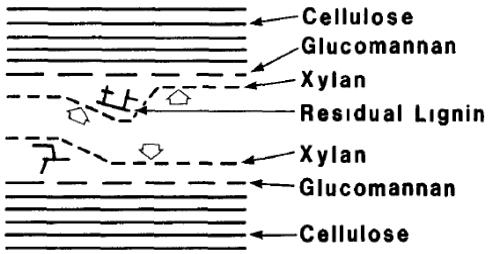


Figure 13: Proposed structure of secondary wall of unbleached softwood kraft pulp [49]

2.4.8 Enzymatic Conversion of Cellulose to Ethanol

Enzymatic conversion of lignocellulosic feedstock typically starts with a pretreatment stage where lignin and hemicellulose are partially or completely removed by chemical or mechanical pretreatments. This step is followed by enzymatic hydrolysis, where cellulases are added to break cellulosic chains into glucose units. The sugars formed are further fermented in the final stage by adding yeast. Figure 14 shows the hydrolysis and fermentation process of converting lignocelluloses into ethanol [6].

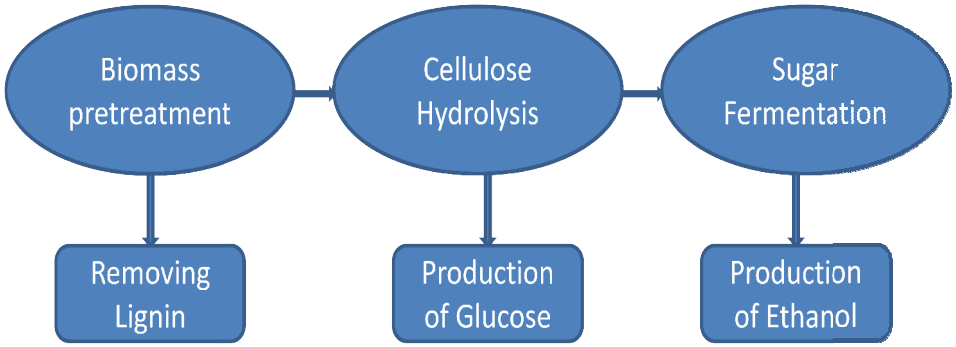


Figure 14: Conversion of lignocelluloses to ethanol [6]

2.4.9 Feedstock Pretreatment

The main components (lignin, cellulose and hemicellulose) of lignocellulosic feedstocks in their natural state are tightly packed. The main objective of pretreatment is to disrupt the integrated structure and expose the cellulosic surface area (Figure 14). This would allow easy access to enzyme during hydrolysis and give high conversions [57].

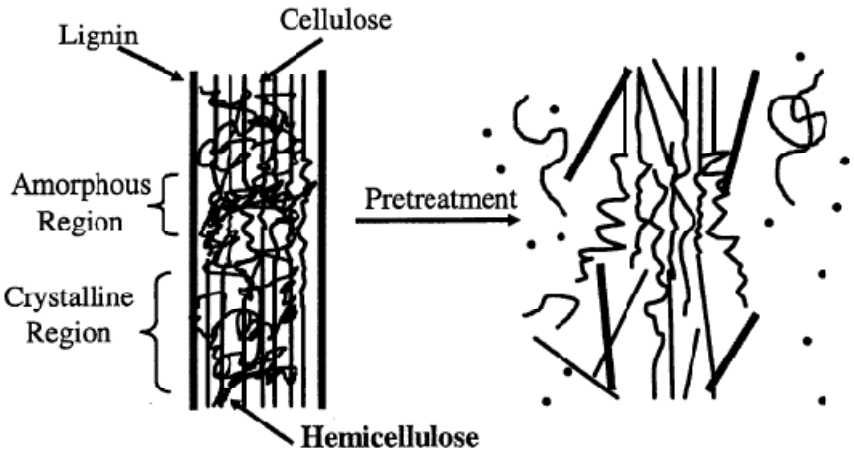


Figure 15: Pretreatment effect on lignocellulose [58]

Pretreatment generally include the use of a combination of wet milling, dry grinding, or mechanical refining to increase the surface area for future chemical or

enzyme treatments. Several chemical pretreatments have been stated in the literature to increase the accessibility of cellulose to enzymatic hydrolysis. These pretreatments are listed in Table 4, with typical conditions stated. Dilute sulfuric acid pretreatment can effectively remove lignin and hemicellulose. All these methods were effective in altering lignin structure and disrupting the integrated structure [47, 59].

Table 4: Different technologies for pretreatment of lignocellulose [47, 59]

Pretreatment Technology	Chemicals used	Temp. (^{o}C)	Pressure (atm)	Reaction time (min)	Solids (wt.%)	Increase accessible surface area	Removes hemi-cellulose	Removes lignin / alter structure
Dilute sulfuric acid	0.5%-3.0% sulfuric acid	130-200	3-15	2-30	10-40	M	M	m / M
Ammonia fiber explosion (AFEX)	100% (1:1) anhydrous ammonia	70-90	15-20	<5	60-90	M	m	m / M
Ammonia Recycle Percolatin (ARP)	10-15 wt. % ammonia	150-170	9-17	10-20	15-30	M	m	m / M
Lime	0.05-0.15 g Ca(OH)2 / g biomass	70-130	1-6	1-6 h	5-20	M	m	M / M
Lime + air	0.05-0.15 g Ca(OH)2 / g biomass	25-60	1	1 week – 2 months	10-20	M	m	M / M
Major effect: M, Minor effect: m, Not determined: ND								

2.4.10 Enzymatic Hydrolysis of Cellulose

Cellulases are a group of enzymes comprising endogluconases, exoglucanases and beta-glucosidases. Cellulase degrades cellulose into glucose. This class of enzyme is most commonly extracted from the fungal system *Phanerochaete chrysosporium* and *Trichoderma species*. Recent studies have shown that enzymes produced by *T. reesei*

give a higher glucose output and have higher stability during conversion of cellulose to glucose.

Endoglucanase disrupts the crystalline structure of cellulose by breaking the 1,4-beta-D glycosidic linkages in cellulose. Exoglucanases consists two main types Cellobiohydrolase I (CBHI), which acts at reducing ends and cellobiohydrolase II (CBHII), which acts at non-reducing ends. Beta-glucosidase (cellobiase) creates glucose units by attacking cellobiose disaccharide (Figure 16) [47].

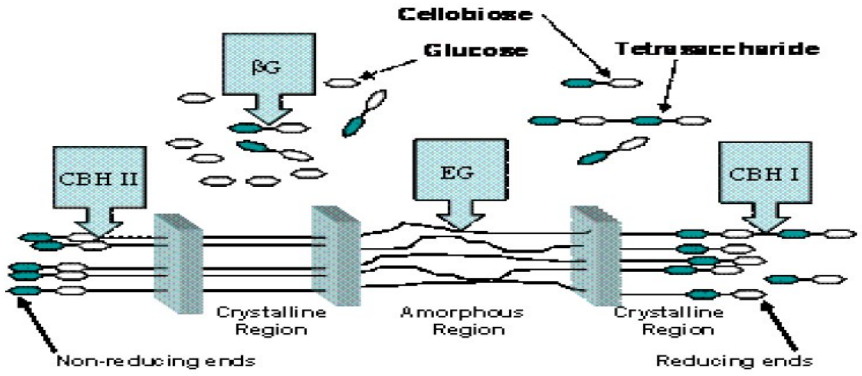


Figure 16: Cellulose degradation into glucose [47].

2.5 Previous Studies on Effect of Cationic Polymers on Cellulose and Cornstarch Hydrolysis

Cationic polyacrylamides (c-PAMs) which are mainly used as flocculants in industries have been found to enhance the enzymatic hydrolysis of cellulose and cornstarch [10]. Studies have shown that c-PAM increases the binding of enzyme to substrate significantly, thus enhancing the enzymatic hydrolysis and increasing glucose yields [10]. Several c-PAMs with varying charge, molecular weight, and degree of branching are capable of increasing the hydrolysis rate. Due to the fact that c-PAM can boost both cornstarch and cellulose hydrolysis, which are two different systems, it was suggested that the polymer was acting through a non-specific mechanism [11]. Considerable work has been done to study the effect of c-PAM on both cornstarch and cellulose hydrolysis. Little work has been done so far on studying the effect of different cationic polymers such as polydially dimethyl ammonium chloride (p-DADMAC) that are less expensive than c-PAM on enzymatic hydrolysis of cornstarch and cellulose. These cationic polymers, which are readily available, can reduce the enzyme dose required for a given yield, thus reducing overall process cost.

2.5.1 Cellulose Hydrolysis

A cellulose sheet was soaked into a c-PAM solution and was exposed to cellulases. Total organic carbon (TOC) was measured for both c-PAM mediated hydrolysis and control (without c-PAM). Figure 18 shows that addition of c-PAM increases TOC levels significantly compared to the control [10].

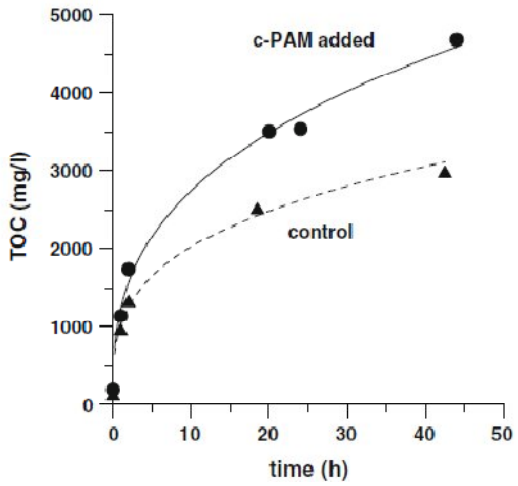


Figure 17: Effect of c-PAM on cellulose hydrolysis[10]

When a fiber suspension was shaken in a water bath instead of more vigorous agitation, no improvement in hydrolysis was observed with c-PAM. It was suggested that c-PAM flocculates the fibers, reducing the surface area available to the enzyme. To study the effect of agitation on c-PAM meditated hydrolysis, several experiments were performed by Reye*, et al*. (2011) at different Reynolds numbers (figure 19) [47]. Glucose production increased with increasing Reynolds number, because high agitation obstructs fiber flocculation. The glucose levels decrease at very high Reynolds number because high shear damages the enzyme and lowers its activity [47].

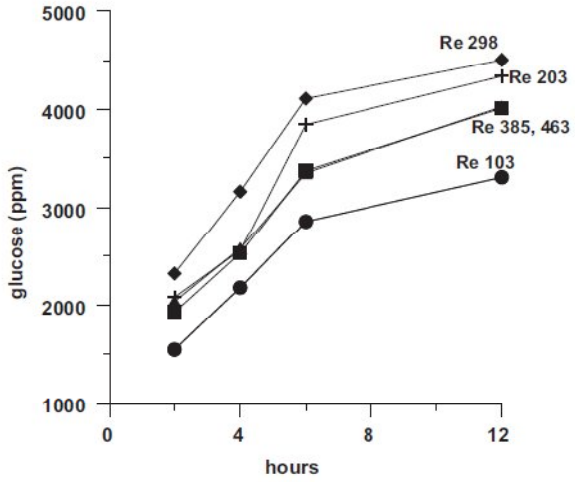


Figure 18: Effect of Reynolds number on c-PAM mediated cellulose hydrolysis [47]

Cellulases as explained in section 2.4.10 contain three enzyme groups. Endoglucanases attack cellulose chains randomly and break them [60]. It was suggested that the kinked regions in fiber are highly susceptible to endoglucanases [61]. An attack on a kink would decrease fiber length and kink angle [62]. Exoglucanases attaches to a cellulose chain and processiveley cleaves out celloboise units. The reaction with endoglucanases is typically considered to be rate limiting; however some researchers have suggested that both endoglucanase and theexoglucanase can be rate limiting [63]. Cellobiase rapidly converts cellobiose to glucose.

In the Figure below, it can be seen that c-PAM decreases the kink angle faster over and above that of the control. This suggests that c-PAM enhances the activity of endoglucanases as compared to exoglucanases and celloboise. Exoglucanase attaches to an end of a cellulose chain and processiveley cleaves out celloboise units. The activity of exoglucanases depends on the availability of fiber ends. This suggests that c-PAM indirectly enhances exoglucanases rate by increasing fiber fragments [62].

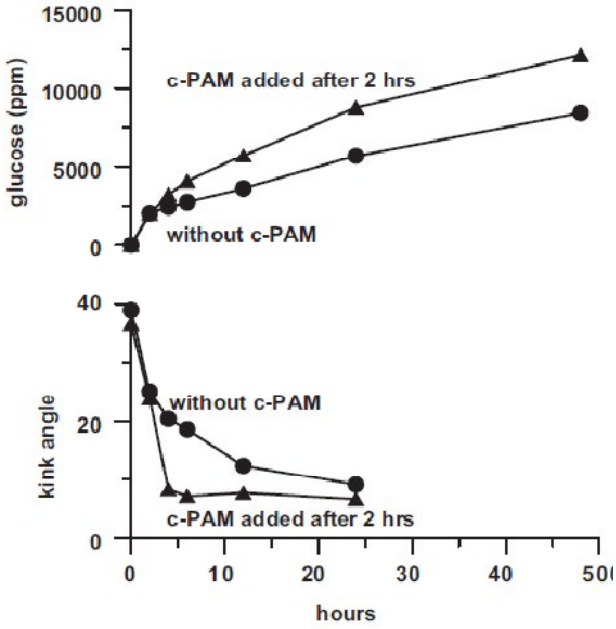


Figure 19: Effect of c-PAM on cellulose hydrolysis [62]

Binding studies were performed by Reye*, et al*. (2009) to analyze the effect of c-PAM on binding between enzyme and cellulose. The Figure below shows that c-PAM enhanced the binding of enzyme to substrate. It was suggested that the increased binding led to higher glucose levels [10].

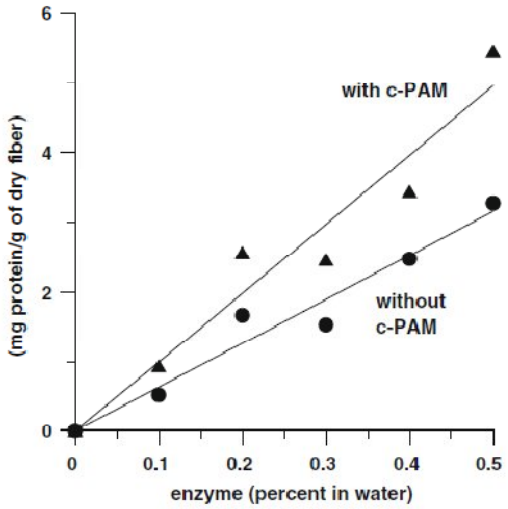


Figure 20: Effect of c-PAM on enzyme bound to cellulose [10]

2.5.2 Proposed Mechanism

Previous studies have shown that c-PAM neutralize the charge on a fiber surface and enhance agglomeration [12]. This mechanism is called the patch mechanism. Due to high electrostatic attraction, polymers attach themselves onto the surface of the substrate in a flat conformation. Because of strong electrostatic attractions, charge density plays an important role in this mechanism [12].

Both the enzyme and fiber used in cellulose hydrolysis are negatively charged. It was proposed that c-PAM neutralizes certain parts of fiber surface, thus reducing the repulsive barrier between enzyme and fiber (Figure 21). This boosts the binding between enzyme and substrate and enhances the rate of hydrolysis [11].

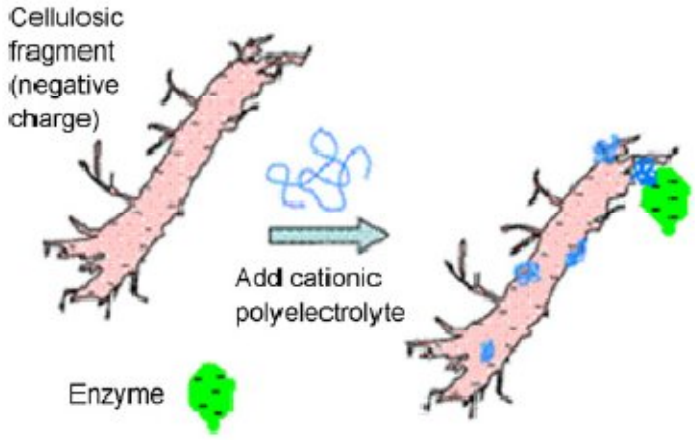


Figure 21: Proposed patch mechanism [11]

2.5.3 Cornstarch Hydrolysis

Maxwell*, et al.* has performed extensive research to study the effect of c-PAM on cornstarch hydrolysis [14, 23]. Unlike cellulose, cornstarch undergoes swelling and subsequent gelatinization at an elevated temperature during hydrolysis. Initial experiments performed at 1% corn starch and 0.004% enzyme at 50^{o}C by Maxwell*, et al.* has shown that c-PAM enhanced glucose production. In Figure 23, it can be observed that higher glucose levels were obtained when c-PAM was added [23].

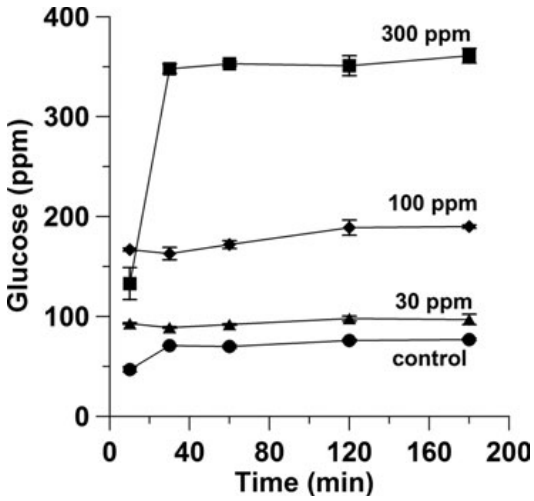


Figure 22: Effect of different c-PAM concentrations on cornstarch hydrolysis [23]

Figure 24 below demonstrates that the amount of enzyme used can be lowered by adding c-PAM. Experiments were performed by Maxwell*, et al.* at 30% cornstarch concentration, and different enzyme doses at 70^{o}C. Brix levels were reported instead of glucose levels. Brix is a refractive index based measurement of sucrose-equivalent sugar. α-Amylase randomly cleaves out α-1-4 glycosidic bonds and produces short oligosaccharide. Through interpolation of the Figure below, it was shown that enzyme dose can be reduced by about 62% when c-PAM (100 ppm) is used [14, 23].

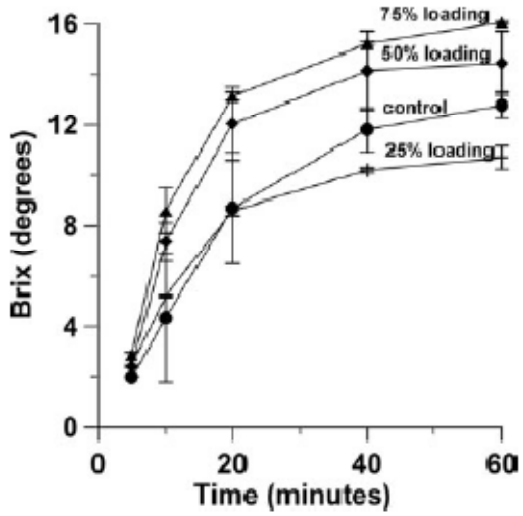


Figure 23: Effect of reducing α-amylase dosage with c-PAM [23]

Binding experiments were performed by Maxwell*, et al.* to study the effect of c-PAM on enzyme bound to cornstarch, and the affinity of c-PAM towards cornstarch [23]. Figure 25 shows the distribution of c-PAM between water and starch. The partition coefficients are very high, which suggest that c-PAM has a high affinity towards starch.

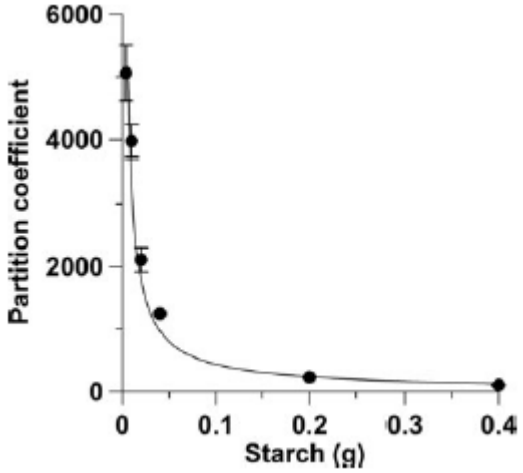


Figure 24: c-PAM partition between cornstarch and water [23]

The following figure demonstrates that the enzyme affinity towards cornstarch increases significantly in presence of c-PAM. c-PAM binds with cornstarch and increases the affinity of enzyme towards starch. The combined effect leads to higher Brix levels during hydrolysis.

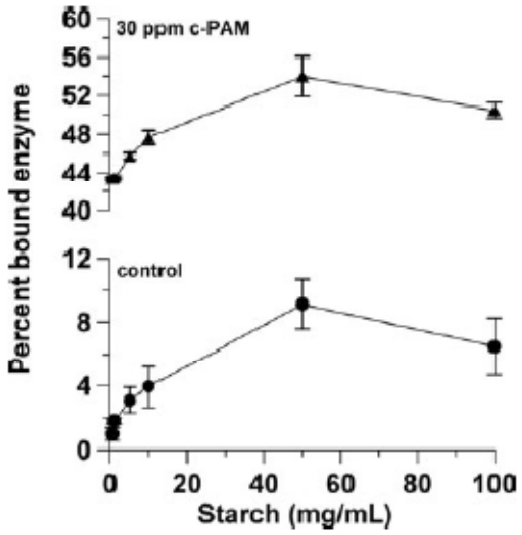


Figure 25: c-PAM effect on enzyme bound to cornstarch [23]

Viscosity studies showed that c-PAM did not have any effect on swelling of cornstarch granules. However, c-PAM increased cornstarch solubilization, which was determined by measuring dissolved organic carbon (DOC). It was reported that c-PAM decreased the onset of gelatinization temperature by 8^{o}C [23]. This is very important as it allows hydrolysis to begin at lower temperature. The Figure below shows the effect of c-PAM on cornstarch gelatinization.

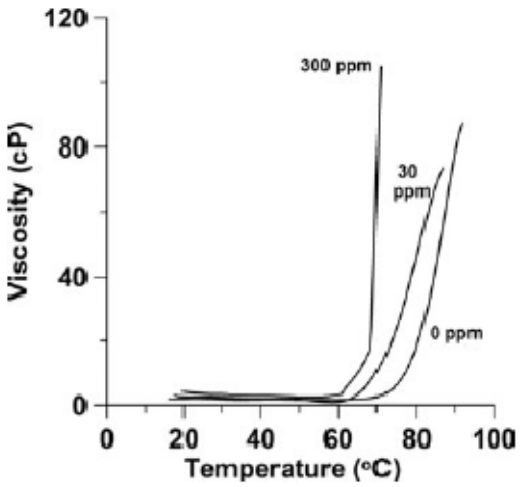


Figure 26: c-PAM effect on cornstarch onset of gelatinization temperature [23]

As with cellulose hydrolysis, it was reported that c-PAM neutralizes negatively charged cornstarch and enhances the binding of enzyme to substrate, thus increasing the rate of hydrolysis. However, unlike cellulose hydrolysis, c-PAM has a synergistic effect on cornstarch hydrolysis, by influencing both gelatinization and hydrolysis [23]. Due to the fact that c-PAM can boost both cornstarch and cellulose hydrolysis, which are two different systems, it was suggested that the polymer acts through a non-specific mechanism.

CHAPTER 3: MATERIALS AND METHODS

3.1 Cornstarch Liquefaction

Several studies were performed to analyze the effect of cationic polymer on gelatinization and liquefaction of cornstarch. This section discusses the materials and methods used in these studies. Sections 3.1.1, 3.1.2, and 3.1.3 explain the materials used and sections 3.1.4, 3.1.5, 3.1.6, and 3.1.7 discusses the methods used. All experiments were performed at pH 6.0.

3.1.1 Starch

Unmodified regular cornstarch with approximately 73% amylopectin and 27% amylose was purchased from Sigma-Aldrich. The moisture content of the starch as specified by the supplier was 9.8% ± 0.8%.

3.1.2 Enzymes

α-Amylase: BAN 480 L bacterial amylase solution, a gift from Novozymes, (Franklinton, NC) was used in the current study. The enzyme is produced from *Bacillus amyloliquefaciens* through submerged fermentation. The protein concentration of the solution was 38 ± 0.2 mg/ml, which was determined by using a BCA (bicinchoninic acid) protein assay kit. The activity was 3.5 ± 0.6 U/ml, where 1 U is the amount of enzyme that releases 1 μmol/min of glucose from raw cornstarch at pH 6 and 50^{o}C.

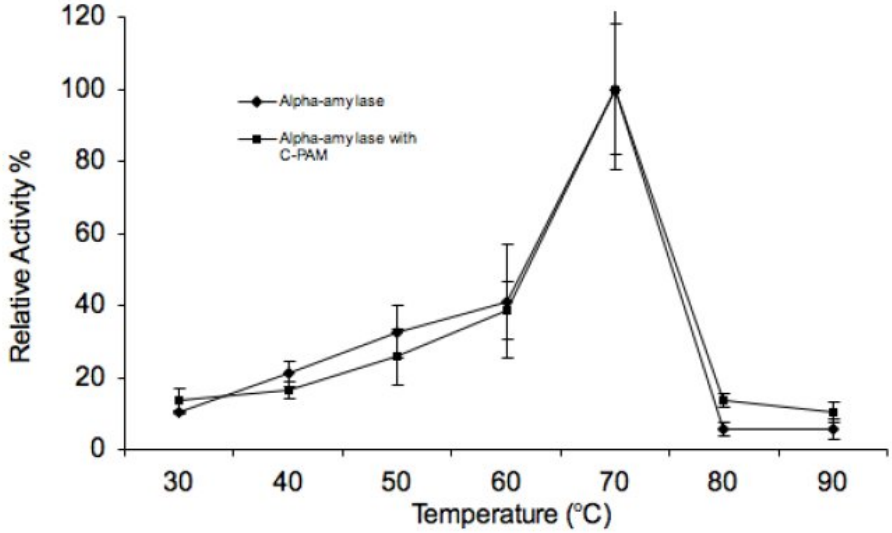


Figure 27: α-Amylase activity at different temperatures

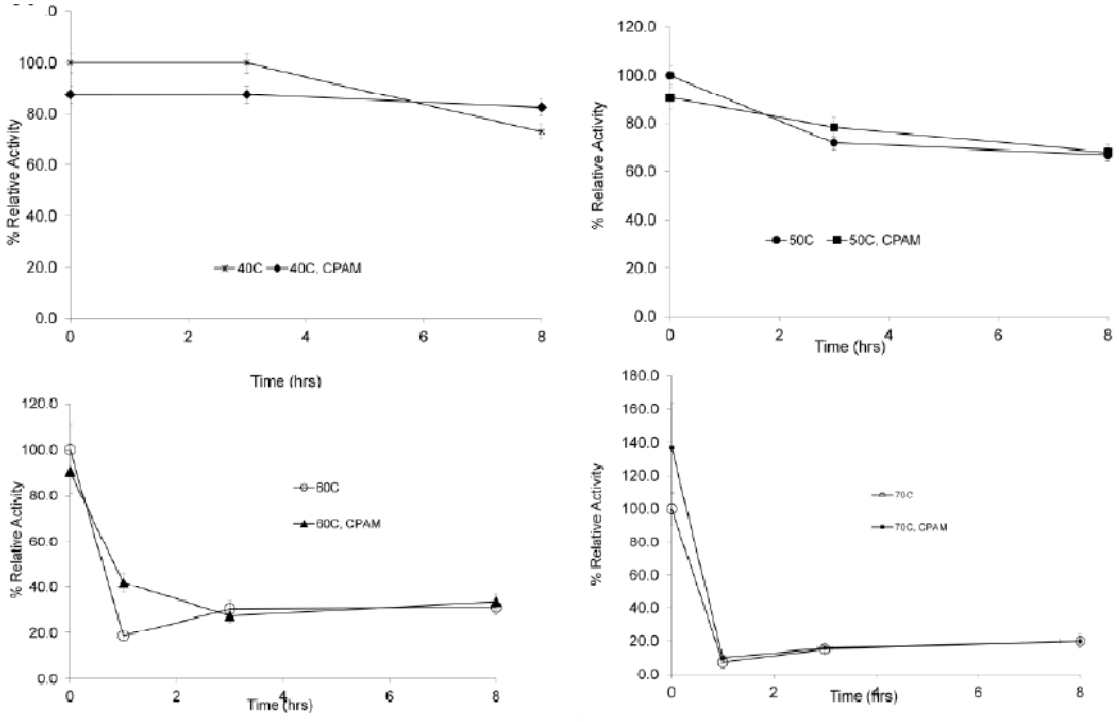


Figure 28: α-Amylase stability at different temperatures

α-Amylase activity with and without c-PAM at different temperatures was measured and expressed as percent relative activity (experimental work performed by Dr. Kendra Maxwell). Figure 28, shows that the activity of α-amylase increases with temperature and reaches its peak at 70^{o}C. It can be observed in Figure 29, that the stability of enzyme decreases with increasing temperature. At 40^{o}C and 50^{o}C, the enzyme is stable over a period of time, whereas at 60^{o}C and 70^{o}C it loses activity within the first hour. The enzyme is very active at 70^{o}C (Figure 28). c-PAM had minimal to no effect on enzyme activity and stability.

Glucoamylase: Starch is decomposed into glucose by glucoamylase, which removes glucose units from the non-reducing end of the polysaccharide chain. The enzyme is produced from *Asp. niger.* through submerged fermentation. Glucoamylase (NS 22032) was obtained from Novozymes (Franklinton, NC). The protein content of the enzyme was 254.4 ± 0.1 mg/ml. The protein concentration was measured using the procedure stated above.

3.1.3 Polymers

The c-PAMs used (XP10023, XP10025 and XP10033) were provided by Eka Chemicals, Marietta, GA. Their cationicities were 10, 40 and 80%, respectively; their molecular weights were in the 5-6 MDa range. p-DADMACs (XP10030 and XP10020 of molecular weight 0.76 and 1.8 MDa, respectively) were also provided by Eka at 40% and 20% solids, respectively. The charge density of the active ingredient was 6.2 meq/g. c-PAMs were provided as dry white granules. They were dissolved in deionized water and stirred for one hour to allow the granules to completely solubilize in water. p-DADMACs

were provided as concentrated solutions, which were diluted depending on experimental requirements.

The hydrolytic stability of the c-PAMs was analyzed by measuring their zeta potentials over 24 hours. The zeta potential of c-PAM (XP10025) at 100 ppm was determined with a Malvern Zetasizer 3000 instrument. Experiments were performed at 50^{o}C and 70^{o}C. Figure 30 shows that the polymer retained most of its charge during the first two hours (experimental work performed by Dr. Kendra Maxwell). This shows that polymer charge is relatively constant during cornstarch hydrolysis, which is typically performed for an hour.

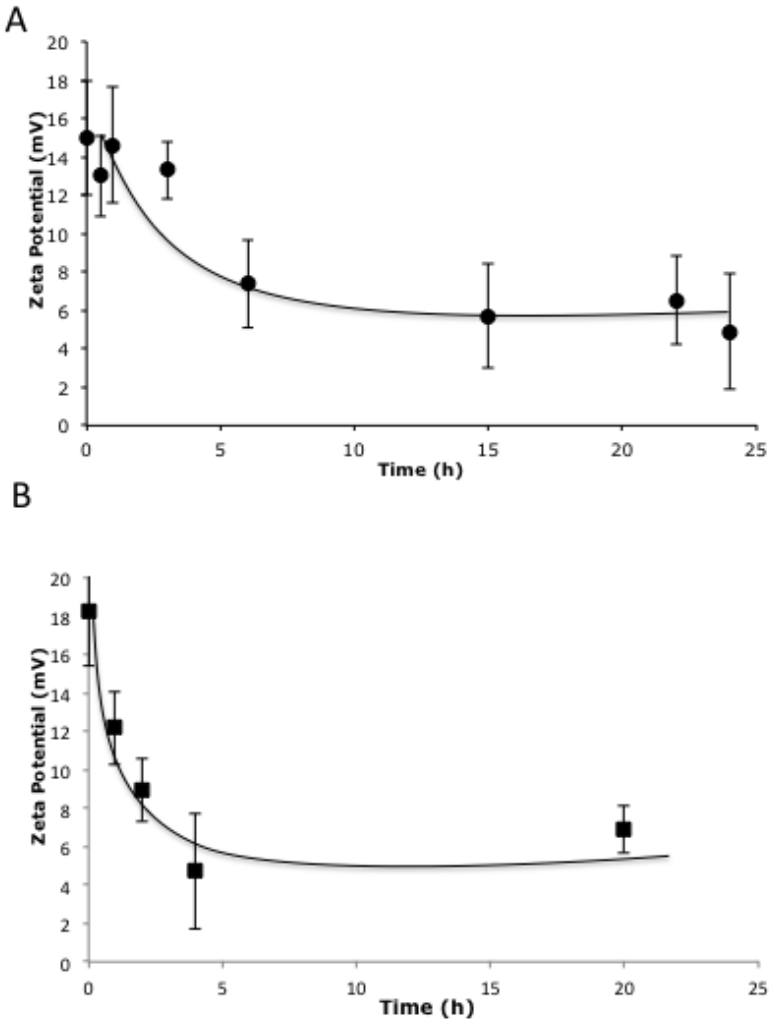


Figure 29: Hydrolytic stability of c-PAM (A) at 50^{o}C and (B) at 70^{o}C

The hydrolytic stability of p-DADMAC was determined by performing standard PVSK (potassium polyvinyl sulfate) charge titration. p-DADMAC solution was heated in a water bath at 90^{o}C and samples were taken periodically. Active polymer weight was calculated from the results obtained. The p-DADMAC (XP10030) lost 49% of its charge over 24 hours at pH 4.1. This result is broadly consistent with the results of Wilson*, et al.* who found that the molecular weight of a p-DADMAC in solution began decreasing at 80^{o}C [64]. Figure 31 shows that the polymer retained most of its charge during the first

three hours. It can be concluded that both c-PAM and p-DADMAC charge is relatively unchanged during cornstarch hydrolysis.

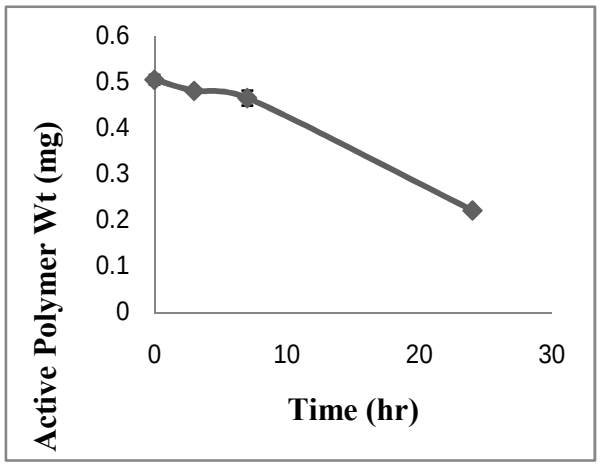


Figure 30: Hydrolytic stability of p-DADMAC

3.1.4 Enzyme Binding Studies

Enzyme binding to cornstarch was measured by adding α-amylase (0.6% by volume) to cornstarch (0.05% to 10%) in a cold room at 4^{o}C and shaking the mixtures for 30 minutes. The suspensions were centrifuged at 4^{o}C at 13,400 RCF for 10 minutes and the protein content of the supernatant determined. A low temperature environment was used to minimize activity of enzyme towards cornstarch hydrolysis. BCA protein assay was used to determine the protein content. For p-DADMAC measurements, experiments were performed with a similar procedure by adding different p-DADMAC concentrations in cornstarch solution. The concentrations reported for all the polymers are based on active ingredient and are expressed in terms of the total weight of cornstarch and water present. For example, 100 ppm p-DADMAC in a 30% cornstarch suspension represents 100 mg of p-DADMAC in a suspension of 300 g of cornstarch in 700 g of water. The

enzyme concentrations in the system are either expressed on a total mass basis or on a percent volume basis. All experiments were done in two replicates; the measurement uncertainty was about 1%.

Dissociation constants were measured using a method proposed by Warren*, et al*.[65]. The amount of enzyme bound to the substrate was calculated by using the equations below.

$$[?]_{?????} + [?]_{??????????} = [?]_{?? ???}? \quad (1)$$

$[?]_{?????}$ is the amount of enzyme bound to the substrate, $[?]_{??????????}$ is amount of enzyme unbound to cornstarch, and $[?]_{?? ????}?$ is the amount of enzyme added to the cornstarch solution initially. A one-site binding model was used to determine dissociation constants.

$$[?]_{?????} = \frac{?_? ??[?]}{?_?+[?]} \quad (2)$$

$[?]$ is the substrate concentration, $?_? ??$ represents the maximum amount of enzyme bound to cornstarch, $?_?$ represents dissociation constant. The above equation can be represented as

$$\frac{[?]_{?????}}{[?]} = \frac{?_? ??}{?_?} - \frac{[?]_{?????}}{?_?} \quad (3)$$

A plot of $\frac{[?]_{?????}}{[?]}$ vs. $[?]_{?????}$ gave a straight line from which $?_{?\,??}$ and $?_{?}$ were calculated (Figure 32).

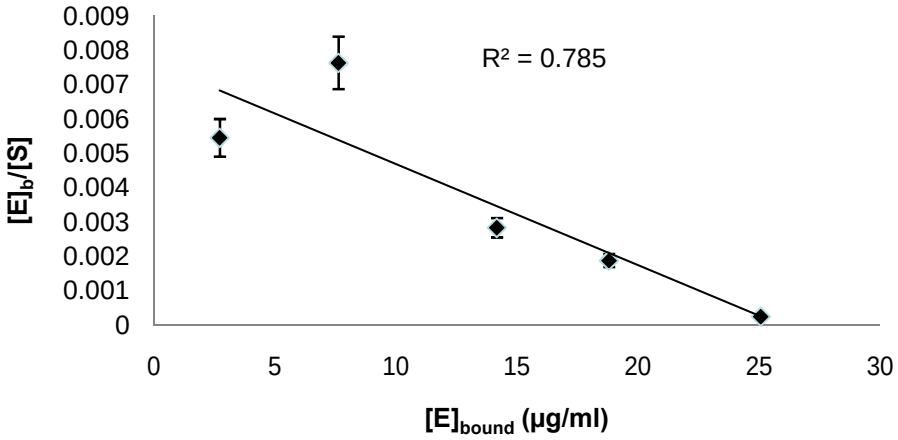


Figure 31: Graph obtained for dissociation constant calculations

3.1.5 Polymer Adsorption to Cornstarch Studies

Charge titration was used to determine the amount of polymer adsorbed to cornstarch. Charge titration is traditionally used to measure the charge of fibers, fillers, and anionic and cationic trash levels in water. Both the anionic and cationic charge of solids can be estimated by direct or back titrations. p-DADMAC and PVSK are commonly used titrates. For the current study, titrates were obtained from BTF Americas Inc., Norcross GA as 0.001 N standard solutions. By performing back titrations, p-DADMAC can be used to measure the charge of negatively charged solids whereas PVSK can be used to determine the charge of cationic polymers.

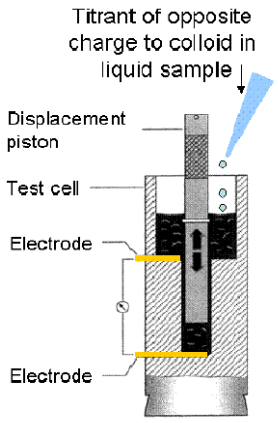


Figure 32: Mutek particle charge detector

A Mutek Particle Charge Detector (PCD) was used to perform charge titration studies (Figure 32). The PCD contains a cylindrical test cell with a displacement piston. The test cell was filled with the sample. The piston oscillated at a rate of 4 times per second, which in turn displaced the sample. The streaming current of the charged ions present in the sample was measured in mV. After the streaming current reading stabilized, an automatic titrator was used to introduce PVSK into the test cell until the charge of the sample was neutralized and the measured current dropped to zero mV. The total charge (Q) can be calculated by using the following formula.

$$? = \frac{? . ?}{?} . ?$$

V represents the total volume of titrant used to neutralize the sample, c is the concentration of the titrant in mole per liter, m is the quantity of active substance present in the sample used (grams), and F is Faraday' constant.

p-DADMAC binding to cornstarch was measured by the solution depletion method [66, 67]. Starch (1-15%) was shaken with a p-DADMAC solution (50 ppm) at 25^{o}C for 30 min and centrifuged at 3,000 RCF for 15 min. The supernatant was charge-titrated against PVSK using PCD. Instead of calculating total charge Q, a plot was obtained between active p-DADMAC loading in the solution at different concentrations vs. amount of PVSK utilized to neutralize the charge (Figure 33). The standardized graph was then used to determine the active p-DADMAC content in the supernatant based on the total PVSK needed to neutralize the sample. The amount of p-DADMAC adsorbed to the starch was calculated from the difference in charge in the supernatant between p-DADMAC alone and that from the starch/p-DADMAC samples. The analytical uncertainty was about 6%.

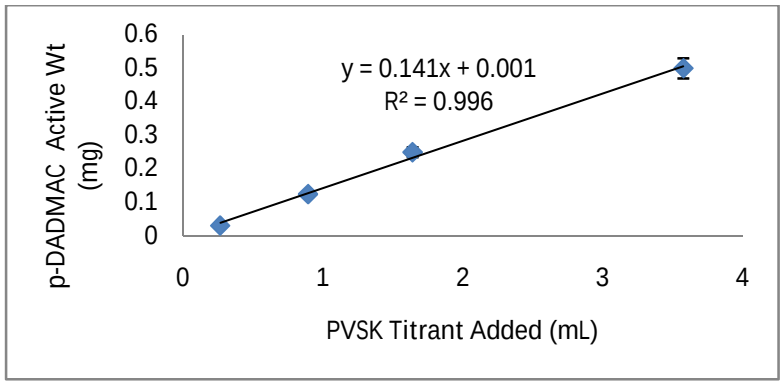


Figure 33: Polymer active content vs. titrant added

3.1.6 Viscosity Studies

Viscosity was determined with a Grace Instruments M3500 viscometer. The instrument has the following measurement range: temperature between -12^{o}C to 100^{o}C, speed between 0.01-600 rpm, viscosity between 0.5 to 5000000 Cp, along with an accuracy of ± 5%. Aluminum foil was used to insulate the reactor surface. Four sets of experiments were performed to determine the effect of cationic polymers on cornstarch gelatinization and enzymatic hydrolysis.

The first set of experiments was performed to determine effect of cationic polymers on gelatinization temperature. An 8% starch suspension was heated from room temperature to 90^{o}C at 2.5^{o}C/min at a shear rate of 400 s^{-1}. The experiment was continued until a sharp rise in viscosity was observed. The temperature at which the viscosity rise occurred was noted. Three sets of temperature readings were taken during the viscosity rise. The results were averaged to determine the gelatinization temperature.

A second set of experiments were performed to determine the effect of cationic polymers on viscosity rise and hydrolysis of cornstarch. An 8% starch suspension was heated from room temperature to 90^{o}C at 2.5^{o}C/min at a shear rate of 400 s^{-1} and held at those conditions for 40 min. α-Amylase (0.036%) was added after about 42 minutes to initiate hydrolysis. This is a departure from industry practice where the α-amylase is added during the preparation of the slurry [27]. The delay in α-amylase addition was done to highlight changes in viscosity caused by the cationic polymers.

A third set of experiments was performed with a 30% cornstarch suspension under similar conditions of temperature and shear rate mentioned above. α-Amylase (0.036%) was added initially for this experiment to be consistent with industry practice.

A fourth set of experiments were performed to study the effect of the polymer addition point in the gelatinization of cornstarch. A 300 ppm p-DADMAC solution was added at three different time intervals. The temperature, shear rate, enzyme loading, and enzyme addition time were similar to those mentioned above.

All four experiment sets were performed with and without the addition of cationic polymers. Both c-PAMs and p-DADMACs were used to study the effect of different cationic polymer on the viscosity of cornstarch.

3.1.7 Liquefaction Studies

Experiments were performed to study the effect of p-DADMAC on cornstarch liquefaction. They were conducted at various polymer, enzyme, and cornstarch concentrations. Samples were taken periodically and were analyzed for Brix and glucose levels. Brix is a refractive index based measurement of sucrose-equivalent sugar.

Polymer Loading: Different polymer loadings were used to study the effect of polymer loading in cornstarch hydrolysis. The polymer loadings were generally 100 ppm, 150 ppm, 200 ppm, 300 ppm, or 500 ppm. The potential interference of polymers in the absorbance measurement of glucose or sugars was measured. It was determined that the interference was negligible at the polymer loadings used.

Temperature: Experiments were conducted at different temperatures for various reasons, e.g. 80^{o}C and 85^{o}C were used to mimic industrial conditions as starch is hydrolyzed at elevated temperature in industrial process. As α-amylase used in this study is very stable at 70^{o}C, (see section 3.2.1) this temperature was used in certain experiments.

Enzyme Loading: α-amylase loading was varied from 1% to 0.2% based on the volume of the reactor. Several experiments were performed at 0.2% based on cornstarch weight to mimic industrial process conditions.

Starch Concentration: 1% cornstarch (w/v) was used in preliminary studies to demonstrate the effect of polymer on cornstarch hydrolysis (as it is easy to measure glucose at lower cornstarch concentration). Hydrolysis at 8% cornstarch (w/v) was performed in conjunction with viscosity studies as explained in the above section 3.3.3. Cornstarch at 30% (w/v) was used to mimic industrial process conditions.

1% Cornstarch Hydrolysis: Three experiments are reported in the current thesis; all were carried out at 1% cornstarch (0.5 grams/50ml), 85^{o} C, 120 rpm (shaking), and 0.2% α-amylase (v/v). The first experiment was performed by adding enzyme before gelatinizing the starch at time zero. The second experiment was performed by gelatinizing the cornstarch for five minutes before adding α-amylase. The third experiment was performed by adding 0.2% α-amylase (v/v) after 15 minutes of

gelatinization and the experiment was continued by decreasing the temperature to 50°C and adding 0.01% (v/v) glucoamylase after 60 minutes of hydrolysis.

8% Cornstarch Hydrolysis: Experiments were performed at an 8% cornstarch (8 grams/100 ml), 0.2% α-amylase (v/v), 70°C, and 165 rpm (shaking). Enzyme was added after 15 minutes of cornstarch gelatinization.

30% Cornstarch Hydrolysis: Several sets of experiments were performed at 30% cornstarch concentration, which were done at 30% cornstarch (60 grams/200ml), 0.2% (based on cornstarch weight) enzyme, 70°C, and 230 rpm (stirring). Experiments were performed by varying p-DADMAC concentration and enzyme loading to determine optimum polymer dosage and to estimate amount of enzyme that can be saved by using polymer.

For all the experiments, samples were taken periodically and filtered. Glucose was determined with a GOPOD format d-glucose assay kit from Megazyme International, Wicklow, Ireland, adapted to a DA3500 Discrete Analyzer from OI Corp., College Station, TX. The average error from duplicate measurements was 4.5%. For 8% and 30% cornstarch hydrolysis, Brix was measured with a Sper Scientific 300034 digital refractometer. The uncertainty was 0.03 °.

3.2 Bleached Fiber Saccharification

3.2.1 Rate Measurements

A 2% (w/v) suspension of bleached softwood pulp (from Weyerhaeuser's Grande Prairie mill in Alberta, Canada) was stirred with a 0.1% (v/v) commercial cellulase preparation (Ctec Cellic 2, 119 FPU/ml, from Novozymes) at 220 rpm and at 50^{o}C. c-PAM from Eka Chemicals (XP10035, 35% charge) was added after 2 hours at 250 mg polymer/l. Lu*, et al.* have shown that the c-PAM is most effective when added after about 2 hours when the fibers have shortened somewhat. Prior to that the c-PAM tends to agglomerate the fibers and reduces their surface area [68]. Samples were collected periodically. They were filtered and analyzed for glucose.

3.2.2 Measurement of Partition Coefficients

Bleached softwood pulp (from Weyerhaeuser's Grande Prairie mill in Alberta, Canada) was screened through a 28-mesh (0.6 mm) screen to remove fines so as to generate a more uniform furnish. The fines content of the screened material was 3.9%. Fines are classified as fiber fragments shorter than 0.1 mm. The fiber length (length-weighted) was 2.64 mm. Both measurements were made with a Fiber Quality Analyzer from Optest, Hawkesbury, Ontario, Canada. Thus, the fiber was a polycomponent mixture distributed about an average fiber length of 2.6 mm.

The fiber (1 g) was suspended in 100 ml water containing 1,000 mg c-PAM/liter or 1,000 mg p-DADMAC/liter. It was then filtered out and resuspended in 25 ml of water. Cellulase was prepared at various concentrations in 25 ml of water and stored at 4^{o}C. The fiber and enzyme preparations were mixed and incubated at 4^{o}C for 1 hour with

continuous shaking. Previous work has shown that equilibrium is reached within 1 hour[62]. The samples were filtered through a 0.2 μm GHP (low protein binding) syringe filter and the protein remaining in solution was assayed with a BCA Protein Assay Kit from Pierce Protein Research Products. The protein associated with the fiber was obtained by difference. The dimensionless fiber:water partition coefficient ($K_{f/w}$) of the protein was calculated as the ratio of the amount of protein associated with fiber to that of an equal weight of water. The average deviation from duplicate measurements was less than 4%.

3.2.3 Measurement of Specific Resistance to Filtration

Specific resistance to filtration can be determined with the Buchner funnel test method. This method is widely adopted to evaluate sludge filtration or dewatering solid suspensions. The experimental setup consists of a Buchner funnel (9 cm), graduated glass cylinder, vacuum pump, and a timer (Figure 35). An optimum dose of conditioner (polymer) can be determined by plotting polymer dosage vs. specific resistance. Specific resistance can be calculated by using the following equation [69].

$$? = \frac{2? ? ^2 ?}{\mu ?} \quad (12)$$

R is specific resistance (m/Kg), A is area of the filter (m^2), P is test pressure (N/ m^2), μ is viscosity (N.s/ m^2), C is mass of dry solids per unit volume of filtrate (kg/ m^3), and b is slope of t/V vs. V (s/m^6) where t is time from start (s) and V is total volume (m^3).

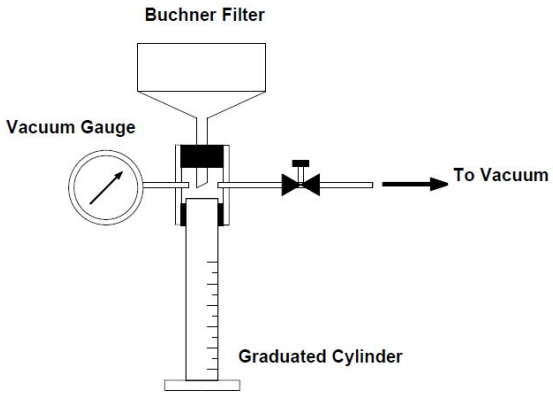


Figure 34: Experimental setup to measure specific resistance [69]

A 1% (w/v) bleached softwood pulp (from Weyerhaeuser's Grande Prairie mill in Alberta, Canada) was prepared with different p-DADMAC concentrations (0 ppm, 100 ppm, 500 ppm, and 1000 ppm). The Buchner funnel filter test was used to determine the specific resistance of softwood fiber. A vacuum pump connected through a pressure gauge was used to filter the solution. Whatman 4 filter paper was used to filter the fiber slurry. The volume of solution collected through filtration was measured at different time intervals and a time/volume vs. volume plot was made (Figure 36).The test pressure was noted. The slope of the time/volume vs. volume plot was determined and used to estimate the specific resistance of softwood fiber at different polymer loadings. The experiments were duplicated.

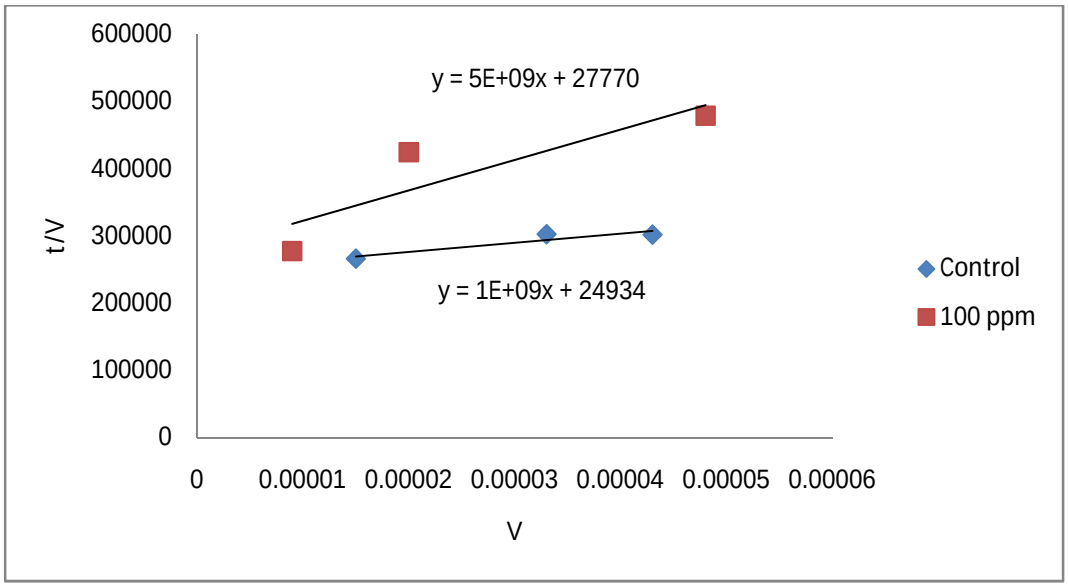


Figure 35: Time/volume vs. volume plot

3.3 Economic Analysis of Sludge Hydrolysis

Sludge from a paper mill that produces a bleached product is essentially free of lignin. Some of the hemicelluloses are dissolved out during pulping and papermaking so the fibrous fraction of the sludge is enriched in cellulose. The fiber length of sludge is relatively short as it includes some of the fines produced during papermaking. Fines are especially susceptible to enzymatic hydrolysis due to their high surface area. The kinetic model used in the current study was developed by Zheng*, et al.* [13]. The model developed by Zheng*, et al.* was for enzymatic hydrolysis of ryegrass, which was modified accordingly to incorporate cellulose hydrolysis. The model was implemented in MATLAB.

Cellulase is assumed to sorb to substrate through a Langmuir isotherm. Equations governing sorption to ryegrass lignin were excluded in the current study because sludge is mostly lignin-free. The model incorporates first order cellulose-to-cellobiose, cellobiose-to-glucose and cellulose-to-glucose conversions as shown in Figure 37. All three processes can be inhibited by glucose or cellobiose. The cellobiose-to-glucose conversion should be independent of the nature of the cellulosic substrate. Importantly, competitive inhibition of cellulase by glucose and celloboise are accounted for. Equations that describe the model have been described by Zheng*, et al.* (microfluid model). Some of the important equations used in the model are stated below.

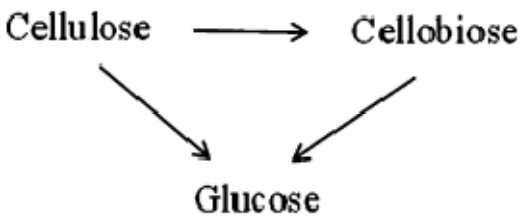


Figure 36: Schematic of cellulose hydrolysis

Mass balance equation for cellulose:

$$?_{?-1} - ?_{?} - ?(?_1 + ?_2) = 0 \quad (4)$$

C_o is the initial cellulose concentration (g/L), C_i is the cellulose concentration at any given time (g/L), τ is residence time of reactor (h), r_1 is cellulose to celloboise reaction rate (g/L/h) and r_2 is cellulose to glucose reaction rate (g/L/h).

Mass balance equation for cellobiose:

$$?_{2?-1} - ?_{2?} + ?(1.056?_1 - ?_3) = 0 \quad (5)$$

G_2 is the cellobiose concentration at any given time (g/L), r_3 is cellobiose to glucose reaction rate (g/L/h).

Mass balance equation for glucose:

$$?_{?-1} - ?_{?} + ?(1.1116?_2 + 1.053?_3) = 0 \quad (6)$$

G is the glucose concentration in the reaction at any given time.

Unless noted otherwise, the variables used were model defaults. A minor difference is that the model was implemented at room temperature (25^{o}C), whereas Zheng used a higher temperature of 50^{o}C. It has been shown that sludge hydrolysis is relatively insensitive to temperature because enzyme binding is inversely temperature dependent, whereas the hydrolytic step increases with temperature. The two effects tend to offset each other [68].

In the work done by Lu*, et al.* it was found that a 1 % suspension of bleached fiber hydrolyzed to 2,300 ppm glucose when treated with a 1 % commercial cellulase mixture for 6 h (reference). The Zheng model provides a higher glucose estimate of 3,170 ppm. The correspondence is considered to be good given the difference in the type of furnish (ryegrass vs. bleached pulp fiber) and in light of the several assumptions made.

A small point of difference is that Zheng used an enzyme/substrate binding constant of 0.6 l/g. The measured value for bleached kraft fiber is 4.2 l/g for bleached pulp fiber. Use of the higher value leads to a marginally higher estimate of 3,300 ppm (vs. 3,170 ppm) glucose because the enzyme is substantially bound to the fiber in both

cases. The rate is, therefore, insensitive to the binding constant over this range and the original 0.6 l/g value was retained in the interest of continuity.

Because of the progress in enzyme development, new commercial cellulases are at least twice as efficient as the one used in the rate work discussed above. Of the three processes in Figure, the cellulose-to-cellobiose conversion predominates for glucose conversion. A simple doubling of this rate constant in the model does not double the hydrolysis rate because of product inhibition. The rate constant had to be increased by a factor of ten to achieve the measured doubling in rate. Increasing the other two rates in Figure had only a minor effect. The slower cellulase used in the Zheng model is designated as ‘‘default’’ and the new cellulase is called ‘‘advanced’’.

3.4 Brown Pulp Saccharification

3.4.1 Pretreatment of Biomass (Hardwood)

Two sets of hardwood biomass were obtained from different sources. The kappa numbers of the pulps were measured. The first hardwood pulp (HW24) kappa number was 24. Mixed chips from oak, aspen and birch were cooked by Blair Carter at Institute of Paper Science and Technology, Atlanta GA. The kappa number of the second hardwood (aspen) pulp (HW14) obtained from NewPage was 14.3. The HW14 pulp was further delignified to reduce the amount of lignin by oxygen delignification at 12% pulp consistency. The conditions used were 1.7% NaOH (based on OD pulp), temperature 90^{o}C, gaseous oxygen inlet pressure 80 psi, and time 90 minute. All these condition are within the range of typical industrial practice [46].

The kappa number of HW14 after oxygen delignification was 8.77 (HW8). HW8 was further delignified by chlorine dioxide delignification and NaOH extraction. The kappa number of the pulp obtained after chlorine dioxide delignification was less than 2. Chlorine dioxide was applied at 5% pulp consistency, 1% ClO_2 (based on OD pulp), temperature 50^{o}C, and time 60 minutes. All these condition are within the range of typical industrial practice [46]. NaOH extraction was performed at 10% pulp consistency, 3% NaOH (based on OD pulp), 60 minutes, and temperature 50^{o}C.

3.4.2 Hardwood Saccharification

Hydrolysis was performed to three pulps obtained from above pretreatment methods. They are HW24 (kappa number 24), HW14 (kappa number 14) and HW2 (kappa number less than 2). Hydrolysis was performed with and without cationic polymers. c-PAM (XP10025, 40% cationicity) and p- DADMAC (XP10030, 100% cationicity) were used at different concentrations. Saccharification conditions were maintained at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 400 rpm, and 0.1% (v/v) cellulase (31.8 FPU/ml). Samples were taken periodically and glucose was determined. Fiber quality analysis was performed during hydrolysis for HW 24 and HW14 to determine the changes in fiber length, and fines content. Fiber length and percent fines were analyzed using fiber quality analysis and are reported in Table 5. BCA protein analysis was performed at 1% cellulose (w/v) and 0.1 % cellulase (v/v) to determine the enzyme absorbed to cellulose for HW24, HW14, HW8 and HW2.

Table 5: Fiber properties

Pulp	Kappa Number	Weight Weighted (Mean Length) mm	Fines (%)
Hardwood	24	1.21	11.6
	14	1.03	12.3
	bleached	0.92	8.3
Softwood	76	2.88	20
	40	2.93	17.6
	25	3.34	23.6

3.4.3 Softwood Saccharification

Softwood pulp was provided by Dr. Ved Naithani of North Carolina State University (MeadWestvaco, Evadale, TX). Softwood chips were cooked at different levels to obtain pulps of three different kappa numbers. The kappa numbers were 76, 40 and 25; they are referred as SW76, SW40 and SW25 respectively. Fiber length and percent fines were analyzed using fiber quality analysis and are reported in Table 5. Hydrolysis was performed with and without cationic polymers. c-PAM (XP10025, 40% cationicity) and p- DADMAC (XP10030, 100% cationicity) were used at different concentrations. The hydrolysis conditions were similar to those of hardwood saccharification explained in the above section.

3.5 Wheat Straw Saccharification

3.5.1 Pretreatment of Biomass (Wheat Straw)

Wheat straw was obtained from Eka chemicals. Two pretreatment methods were used. Acid pretreatment: wheat straw (particle size ≤ 3.36 mm) was treated with 0.2% H_2SO_4 for 15 minutes and was washed with water subsequently. Chlorine dioxide pretreatment: wheat straw (particle size ≤ 3.36 mm) was pretreated using chlorine dioxide (procedure explained in section 4.4.1).

3.5.2 Saccharification of Biomass (Wheat Straw)

Three sets of experiments were performed to study the effect of cationic polymers on wheat straw hydrolysis.

Untreated Wheat Straw: Saccharification conditions were maintained at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 250 rpm, 1% wheat straw (92.69% OD w/v) and 0.1% (v/v) cellulase (31.8 FPU/ml). c-PAM (XP10025, 40% cationicity) was used for polymer mediated hydrolysis.

Acid Pretreated Wheat Straw: Saccharification conditions were maintained at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 400 rpm, 1% wheat straw (92.69% OD w/v) and 0.1% (v/v) cellulase (31.8 FPU/ml). c-PAM (XP10025, 40% cationicity) was used for polymer mediated hydrolysis.

Chlorine Dioxide Pretreated Wheat Straw: Saccharification conditions were maintained at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 400 rpm, 3% wheat straw (89%

OD w/v) and 0.2% (v/v) cellulase (31.8 FPU/ml). c-PAM (XP10025, 40% cationicity) was used for polymer mediated hydrolysis.

Samples were taken periodically and were analyzed for glucose for all the experiments stated above.

3.6 Assays Used

3.6.1 Glucose Assay

Megazyme D-Glucose (glucose oxidase/peroxidase GOPOD) assay kits were used to measure glucose levels. Highly purified glucose oxidase and peroxidase made this assay ideal for analyzing glucose levels from plant sources. Due to the presence of sugars such as sucrose in the solution, it was important that the assay used did not have any sucrose degrading enzymes.

In the GOPOD assay, glucose oxidase enzyme reacts with D-glucose in the presence of oxygen and water to produce D-gluconate and hydrogen peroxide. The hydrogen peroxide resulting from the reaction was further reacted in the presence of peroxidase to produce quinonemine dye (Figure 38). The concentration of quinonemine dye produced can be measured by UV absorbance at 510 nm. This can be converted into the amount of glucose present in the sample.

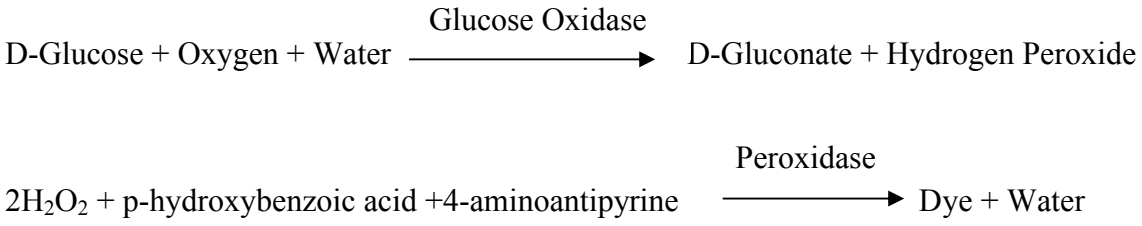


Figure 37: Reactions involved in glucose assay

The GOPOD assay was automated by using a DA3500 Discrete Analyzer from OI Corporation, College Station, TX.

3.6.2 Protein Assay

The BCA protein assay was used to determine the protein content of enzyme. This method uses the biuret reaction (reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium). The formation of Cu^{+1} can be detected by reagent bicinchoninic acid. The product of the reaction is in purple color, which exhibits absorbance at 562 nm. This absorbance was linear with protein concentrations. Bovine serum albumin (BSA) was used as a standard protein, which was used as a reference. Several dilutions were made with BSA and corresponding UV measurements were noted. A standardized graph was obtained for BSA protein content vs. UV adsorption measurements. This graph was used to determine protein concentration.

Different temperature protocols can be used to determine protein content by BCA protein assay kit. In the current study, the room temperature protocol was adopted and enzyme was diluted accordingly to ensure that the protein content measured was within the range of assay.

CHAPTER 4: CORNSTARCH LIQUEFACTION

4.1 Binding Studies

4.1.1 Polymer Adsorption to Cornstarch Studies

The distribution of p-DADMAC (XP 10030) between starch and water is illustrated in Figure 38. The binding of p-DADMAC to starch was measured by the solution depletion method by adding different concentrations of starch to 50 ppm of a p-DADMAC solution. The binding of c-PAM to starch was measured in a similar way by adding different concentrations of starch to 20 ppm of a c-PAM solution (experimental work performed by Dr. Kendra Maxwell).

As noted earlier in analogous measurements of c-PAM binding to starch (Figure 39) [23] the partition coefficient (the ratio of p-DADMAC bound to starch to that present in an equal weight of water) is high when the starch content in the suspension is low. It then levels off at a value of about 0.3 at higher starch levels (0.2 grams). Similar behavior is observed with c-PAM where the partition coefficient levels off at 0.2 grams of starch. It is likely that starch-derived colloidal material solubilizes p-DADMAC in water and decreases the partition coefficient at high solids. This type of behavior is well known in the literature on sorption to soils, where dissolved organic matter from soil solubilizes the sorbate and reduces the partition coefficient [70-72].

The situation is more complex here because the p-DADMAC can also bind the colloidal material to solids. However, for the current study, the focus is to show that p-DADMAC binds to cornstarch solids and the mechanism of binding is not further

explored. The asymptotic value of approximately 0.3 in Figure 38 is two orders of magnitude lower than the corresponding value for c-PAM (the partition coefficient levels off at about 107 as shown in Figure 39). The likely reason is charge reversal. Because p-DADMAC is more highly charged than c-PAM, it will reverse the charge on the cornstarch surface at lower dosage and will inhibit the binding of additional polymer.

It can be observed from Figures 38 and 39 that both c-PAM and p-DADMAC partition coefficients level off at approximately 0.2 grams cornstarch, suggesting a similar mechanism of binding to cornstarch for both polymers. However, as stated above, due to charge reversal, p-DADMAC binding to cornstarch is significantly lower than that of c-PAM. For studies performed on c-PAM and p-DADMAC binding to cellulose, a similar trend was observed [73] (see chapter 5). Again, the partition coefficient obtained with c-PAM was significantly higher as compared to p-DADMAC.

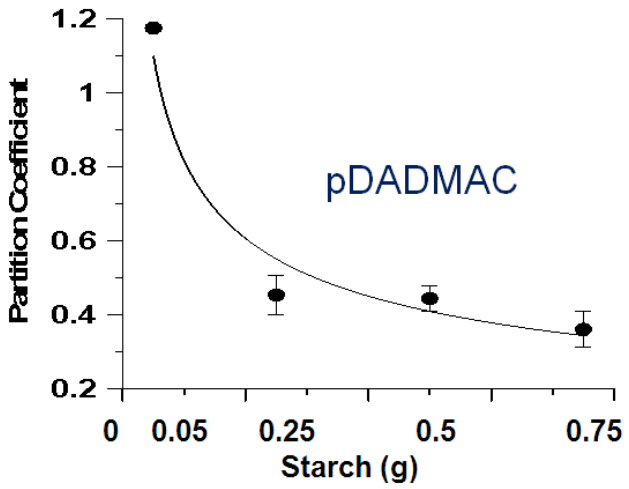


Figure 38: Partition coefficient of p-DADMAC (XP10030) between cornstarch and water

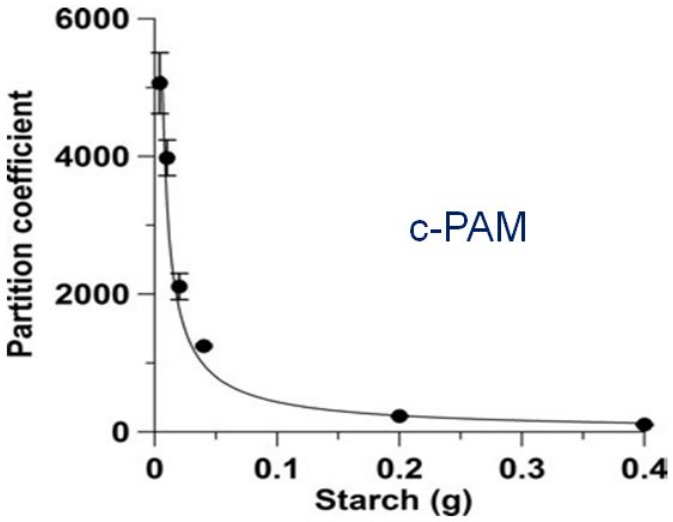


Figure 39: Partition coefficient of c-PAM (XP10025) between cornstarch and water [23]

4.1.2 Enzyme Binding Studies

The effect of p-DADMAC on the binding of α-amylase to cornstarch is illustrated in Figure 40 and Table 6. Studies were performed at different p-DADMAC (XP 10030) concentrations of 100 ppm, 300 ppm and 500 ppm. Figure 40 shows the percent of enzyme bound to cornstarch and Table 6 shows the estimated dissociation constants. The binding constants obtained for control (without polymer) compare well to those estimated by Warren*, et al.* [65].

It can be seen from Figure 40 and Table 6 that p-DADMAC significantly enhances the binding of enzyme to substrate. For the control, the percent of enzyme bound to cornstarch was less than 10%, whereas when p-DADMAC was used, the percent of enzyme bound to cornstarch was around 50% (Figure 40). Binding is similar for both the p-DADMAC concentrations used. From Table 6, it can be observed that the dissociation constant measured for the control is significantly higher than those for the p-DADMACs. A similar approach was used by several researchers to identify promising enzymes by comparing dissociation or binding constants [74].

The binding of p-DADMAC is very similar to that measured for c-PAM [23] even though p-DADMAC binds to cornstarch to a much lower degree than does c-PAM. The higher charge of p-DADMAC reduces its binding to cornstarch as discussed above, but it also attracts more of the negatively charged enzyme to the cornstarch. The two effects apparently offset each other so that both polymers increase the binding of enzyme to cornstarch by comparable amounts. The overall conclusion from these binding

measurements is that both p-DADMAC and c-PAM increase enzyme-substrate binding to the same extent, even though the binding constants of the polymers themselves are different.

As with the c-PAMs, p-DADMAC increases both enzyme-substrate binding and (as described below) the rate of cornstarch liquefaction, which suggests a cause-and-effect relationship. However, the enzyme is appreciably bound to cornstarch even without the p-DADMAC, so the relationship between binding and hydrolysis is complex.

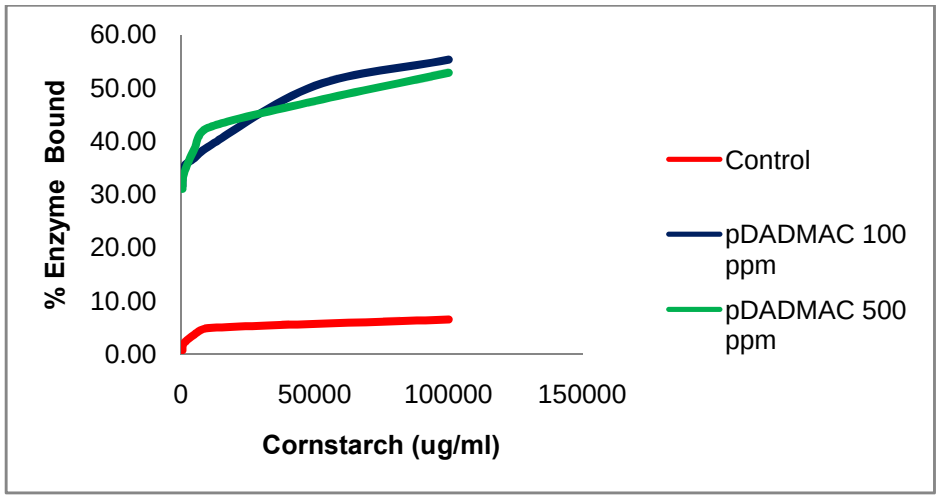


Figure 40: Effect of p-DADMAC (XP 10030) on the binding of α-amylase to cornstarch

Table 6: Dissociation constants of α-amylase bound to cornstarch

Polymer/Concentration	**K_d (ug/ml)**
Control	3401
p-DADMAC, 100 ppm	505
p-DADMAC, 300 ppm	709
p-DADMAC, 500 ppm	394

4.2 Viscosity Studies

4.2.1 Gelatinization Temperature

Experiments were performed to estimate the average temperature for the onset of gelatinization. The average temperature for the control (without polymer) was 72.7 ± 1.3^{o}C. The onset of gelatinization temperature varies with factors such as starch concentration, pH, and the rate of heating. The value obtained in the experiments is on par with literature values [25, 26].

The onset of gelatinization temperature for both XP 10030 and XP 10020 used in the current study was in the range of 65.6 ± 1.3^{o}C (Figure 41). This shows that the addition of p-DADMAC has significantly decreased the onset of gelatinization temperature. Similar behavior was observed in the studies conducted on effects of c-PAMs by Dr. Kendra Maxwell. The onset of gelatinization temperature when different c-PAMs were used was 62 ± 2^{o}C [14]. The phenomenon through which cationic polymers lowers the onset of gelatinization temperature is attributed to a measurable viscosity increase at a lower temperature compared to that of starch gelatinized without adding any cationic polymer. This is of practical importance, as the hydrolysis can start faster at milder conditions when cationic polymers are used.

Several studies have reported changes in cornstarch gelatinization behavior by adding different additives such as polysaccharides and salts. Xiaohong*, et al.* obtained pasting curves of cornstarch with and without hydrocolloids [29]. It was observed that higher viscosity was obtained when gellan, xanthan, guar gum or sodium alginate was

added in the cornstarch. For gellan induced cornstarch gelatinization, the onset temperature was 15^{o}C lower than the control [29].

The mechanism through which hydrocolloids interact with cornstarch during gelatinization is not uniform, and different assumptions have been stated in the literature [28, 29]. It is possible that cationic polymers which are good agglomerants induce friction between particles, which could lead to early rupture [23].

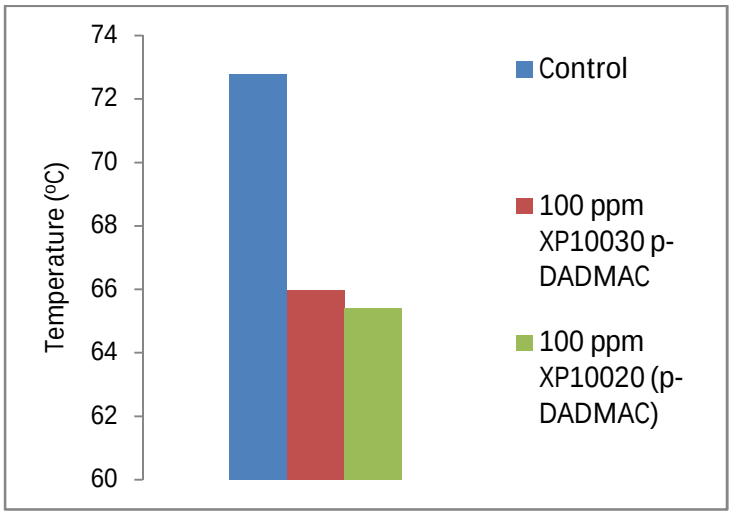


Figure 41: Effect of p-DADMAC on gelatinization temperature (average standard deviation is 1.3^{o}C)

4.2.2 Effect of Cationic Polymers on Pasting Curves

Experiments were performed at 8% (w/v) cornstarch concentration. A lower cornstarch concentration was used in this study, as it is hard to stir higher concentration cornstarch slurry without adding enzyme during gelatinization. Enzyme was added after 42 minutes of gelatinization, when the viscosity of the solution was constant with time. For all the experiments performed, temperature was raised from room temperature to around 90^{o}C at a uniform rate and was kept constant. The approximate temperatures at 10 and 20 minutes were 43 and 72^{o}C, respectively. The temperature plateaued at about 90^{o}C after about 28 minutes.

The viscosity of cornstarch spikes during gelatinization. It can be observed in Figure 42 and Figure 43 that as the temperature increased, after about 15 minutes the viscosity of cornstarch slurry started going up at a steady rate. Around 70^{o}C, there was a sharp rise in viscosity. After about 25 minutes, the viscosity reached its peak and was stable. It can be seen that the viscosity of the solution decreased rapidly after the addition of enzyme. Figures 42 and 43 show the changes in viscosity of cornstarch with time and temperature for both p-DADMAC and c-PAM induced gelatinization. Figure 44 shows a better comparison between c-PAM and p-DADMAC behavior on cornstarch gelatinization.

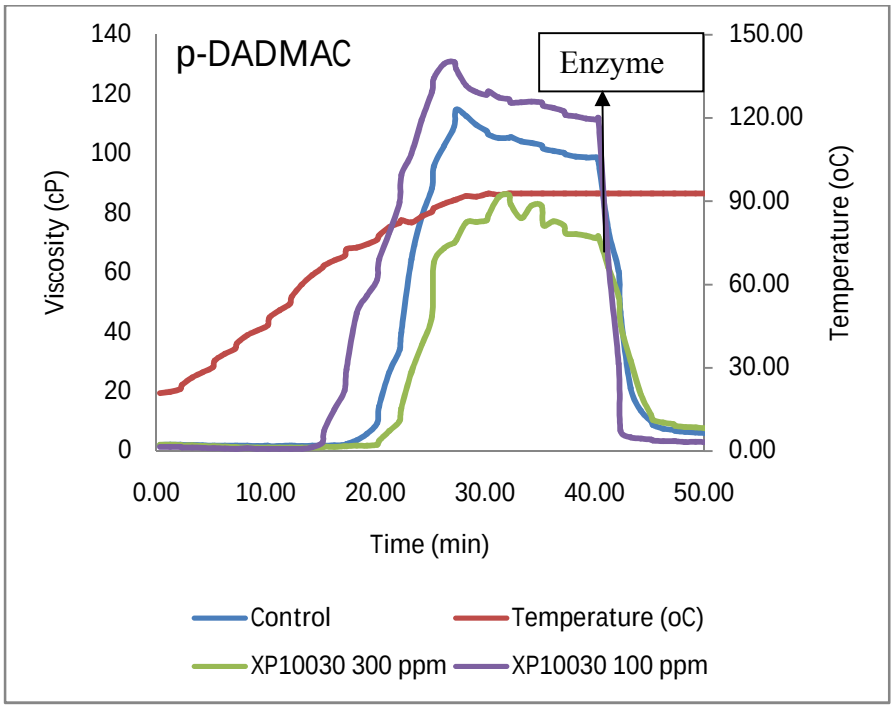


Figure 42: Effect of p-DADMAC on pasting curves of cornstarch

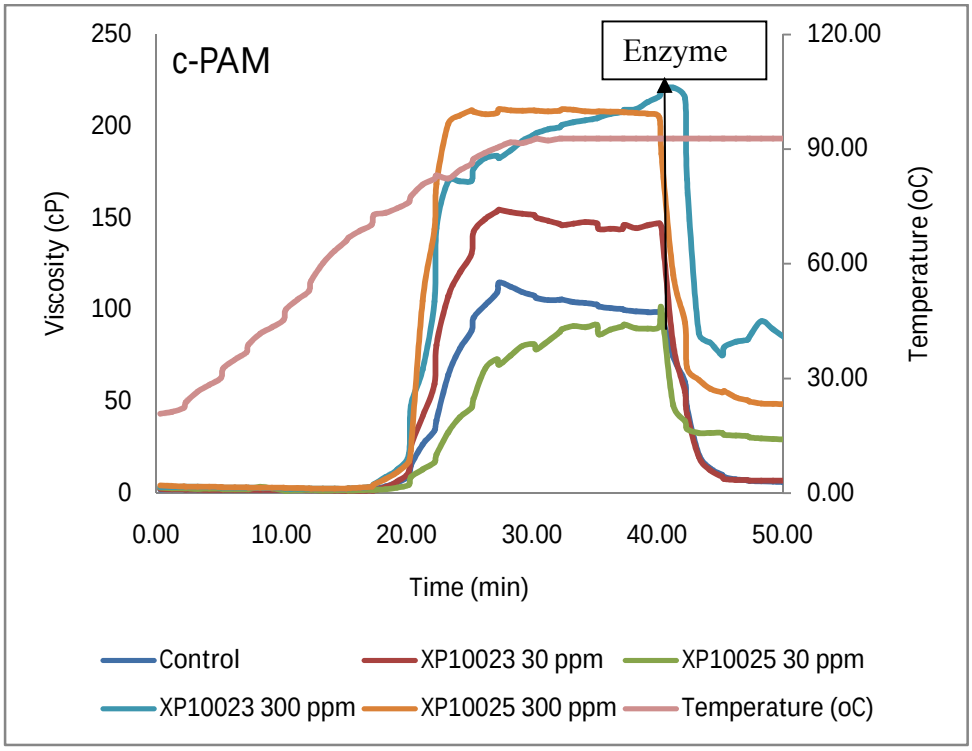


Figure 43: Effect of c-PAM on pasting curves of cornstarch

Through a study of c-PAMs of differing cationicities, it was shown that there is an inverse relationship between viscosity and glucose production [23]. The higher viscosity lowers the rate of hydrolysis, probably by slowing down diffusion. The effects of c-PAM and p-DADMAC on viscosity are compared in Figure 44. Data for only two c-PAMs are presented to preserve clarity (Figure 44); the same trend was observed for XP10033. It is apparent that the c-PAMs mostly increase both peak and final viscosities. p-DADMAC changes viscosity to a much smaller extent. Little or no change in viscosity over that of the control was seen at p-DADMAC concentrations below 100 ppm. The profile for XP10020 was almost identical to that of XP10030 when compared at a concentration of 100 ppm.

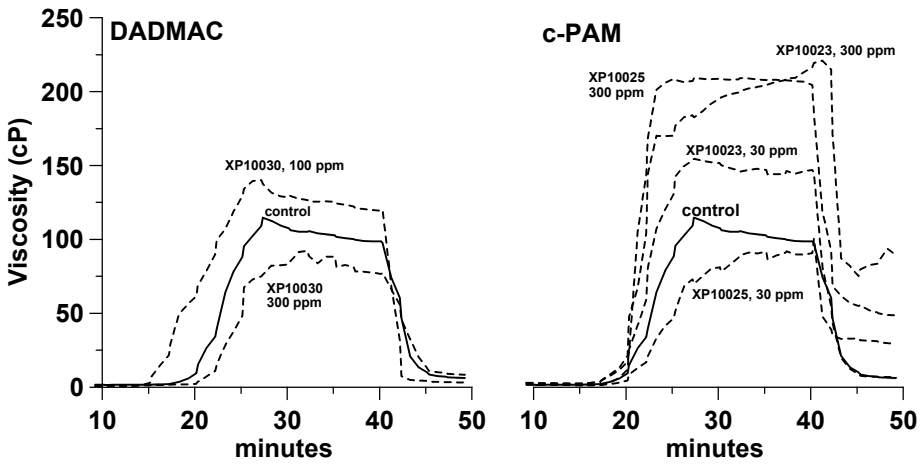


Figure 44: Effect of p-DADMAC and c-PAM on cornstarch viscosity

The principal purpose of the polymers is to increase the rate of hydrolysis of either cornstarch [23] or cellulosic fiber [73]. Because the gelatinization of cornstarch is rapid, an appreciable amount of colloidal material is formed, and the agglomeration of these compounds can be enhanced by the polymer, leading to an increase in viscosity.

This situation does not arise for cellulosic fiber because there is no significant accumulation of colloidal material. c-PAMs agglomerate the colloids generated from cornstarch as reflected by the increase in viscosity. The p-DADMAC does not because once bound to the colloidal material, its much higher charge density [75, 76] reverses the charge of the colloids from negative to positive and the polymer stabilizes, rather than agglomerates, the suspension. This type of behavior is well known in the literature on sludge dewatering where overdosing by a cationic polymer leads to deflocculation [23, 77].

Although the charge difference between p-DADMAC and c-PAM explains their different effects on viscosity, other structural factors between the two polymers may come into play. p-DADMAC is of much lower molecular weight, which makes it unable to flocculate particles through bridging. It must, therefore, act principally through patching as discussed above. The c-PAM is capable of both bridging and patching and leads to greater flocculation than does p-DADMAC. In principle, bridging alone could account for the higher viscosity that arises from the use of c-PAM. However, p-DADMAC decreases the final viscosity, as shown in Figure 41, to a level even lower than that of the control, which implicates charge reversal as the root cause.

It can be seen in Figures 41 and 42 that both c-PAM and p-DADMAC reduce the temperature for the onset of gelatinization as signaled by the sharp rise in viscosity at about 20 minutes. This drop is of practical importance because it allows hydrolysis to begin at a lower temperature.

It can be seen in Figures 41 and 42 that the viscosity of the cornstarch slurry dropped rapidly after enzyme addition. Enzyme cleaves α-1-4 glycosidic bonds of amylose and amylopectin chains in the solutions. The DP of the chains drops, in turn decreasing the viscosity of the solution.

4.2.3 Effect of p-DADMAC on the Viscosity of Cornstarch during Liquefaction

Experiments were performed to study the effect of p-DADMAC on the liquefaction of cornstarch. Starch solutions with 30% dry solids were used, which is similar to industrial practice [27]. Enzyme was added at time zero, before starting liquefaction. For all the experiments performed, the temperature was raised from room temperature to around 90^{o}C at a uniform rate and was kept constant. The temperature plateaued at about 90^{o}C after about 28 minutes.

In Figure 45, lower viscosities can be observed for the control initially, but as the temperature increased, the viscosity of the slurry increases, reaching a peak at about 1000 seconds. Due to continued liquefaction by enzyme, the viscosity decreases at the end of the reaction. Similar studies were performed by Słomińska*, et al.* on cornstarch liquefaction. The cornstarch viscosity curve for control presented here is generally consistent with that of Słomińska*,, et al.* who performed cornstarch liquefaction at 20% solids concentration [78]. It can be observed that with pDADMAC, the viscosity peak of cornstarch remains similar to that of control (without polymer), but occurs faster and at a lower temperature.

The final viscosity of cornstarch/pDADMAC is 30-40% lower than that of the control at the end of hydrolysis, indicating that pDADMAC induced more complete hydrolysis. This conclusion is consistent with hydrolysis experiments discussed in Section 4.3. This phenomenon is industrially significant, as reduced viscosity would enhance heat exchanger efficiency, need less power for mixing, and would make the subsequent saccharification stage more effective. A similar approach was used by Yuguo, *et al.* to compare different bacterial sources of enzyme on cornstarch liquefaction [79]. By comparing viscosities with different enzyme isoforms, they were able to identify an ideal mix of enzymes for cornstarch liquefaction [79].

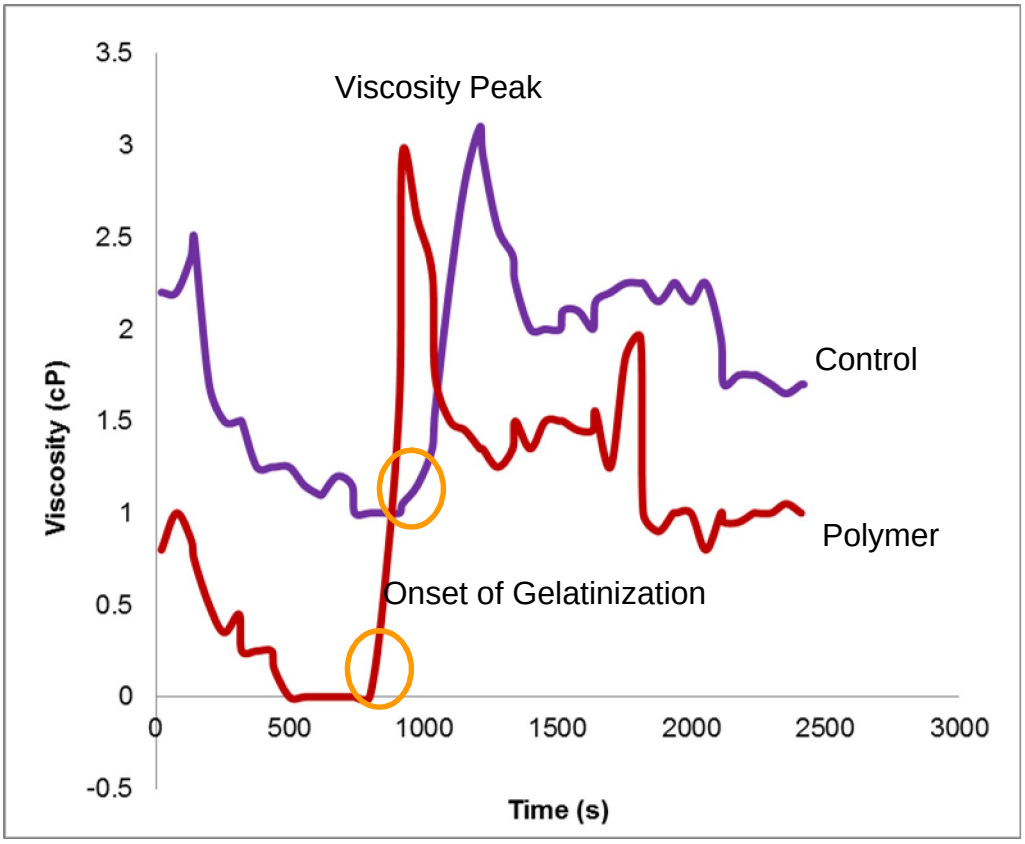


Figure 45: p-DADMAC effect on liquefaction of cornstarch

4.2.4 Effect of p-DADMAC Addition Point on the Viscosity of Cornstarch

Experiments were performed at 8% (w/v) cornstarch concentration. A lower cornstarch concentration was used in the study, as it is hard to stir any higher cornstarch concentration without adding enzyme during gelatinization. For all the experiments performed, the temperature was raised from room temperature to around 90^{o}C at a uniform rate and was then kept constant. The temperature plateaued at about 90^{o}C after about 28 minutes.

In the first experiment, 40 grams of 1000 ppm p-DADMAC solution was added to a 400 gram cornstarch slurry after 40 minutes, which would make the concentration of p-DADMAC in solution 100 ppm. Enzyme at 0.12 (v/weight of cornstarch) was added after 42 minutes. Water (40 grams) was added to the control to maintain the volume of the reactor equal to that of polymer-treated suspension.

It can be observed that both the control and p-DADMAC viscosities dramatically increase after water and p-DADMAC solution were added, respectively. It is a known fact that the viscosity of the slurry increases via the addition of water; corn granules absorb water available to them at higher temperatures, subsequently swelling and releasing amylose and amylopectic chains, and leading to higher viscosities [27]. However, when enzyme was added after 42 minutes, it can be observed that the viscosity of the slurry decreased significantly. Enzymes cleave α-1-4 glycosidic bonds of amylose and amylopectin chains. The DP of the chains decreases, thereby decreasing the viscosity of the solution.

It can be observed in Figure 46 that the viscosity drop with p-DADMAC is more pronounced compared to the control. This is consistent with the experiments discussed in Sections 4.2.3 and 4.2.4. Whether polymer was added before or after gelatinization did not make any difference to the pasting curves.

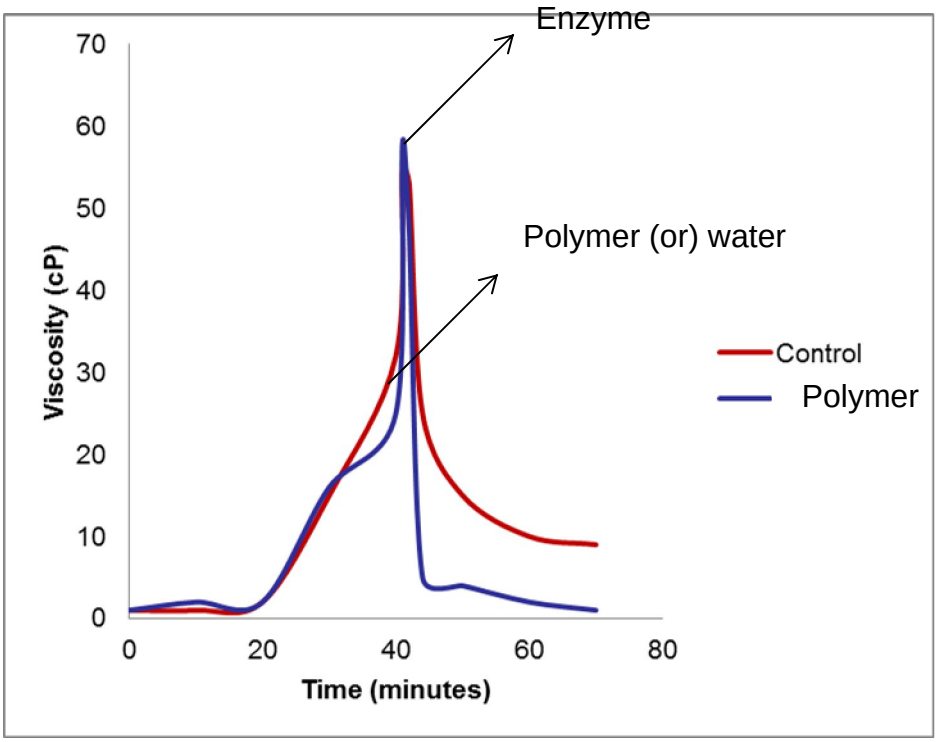


Figure 46: Effect of p-DADMAC addition point on liquefaction of cornstarch (I)

The second set of experiment was performed by adding polymer at three different stages. Concentrated polymer solution was added in order to maintain the volume of the reactor. p-DADMAC (100 ppm) was added at 15, 25 and 55 minutes during cornstarch gelatinization. The viscosity spike at time 40 minutes and the viscosity at the end of liquefaction are similar to that of the experiment performed above.

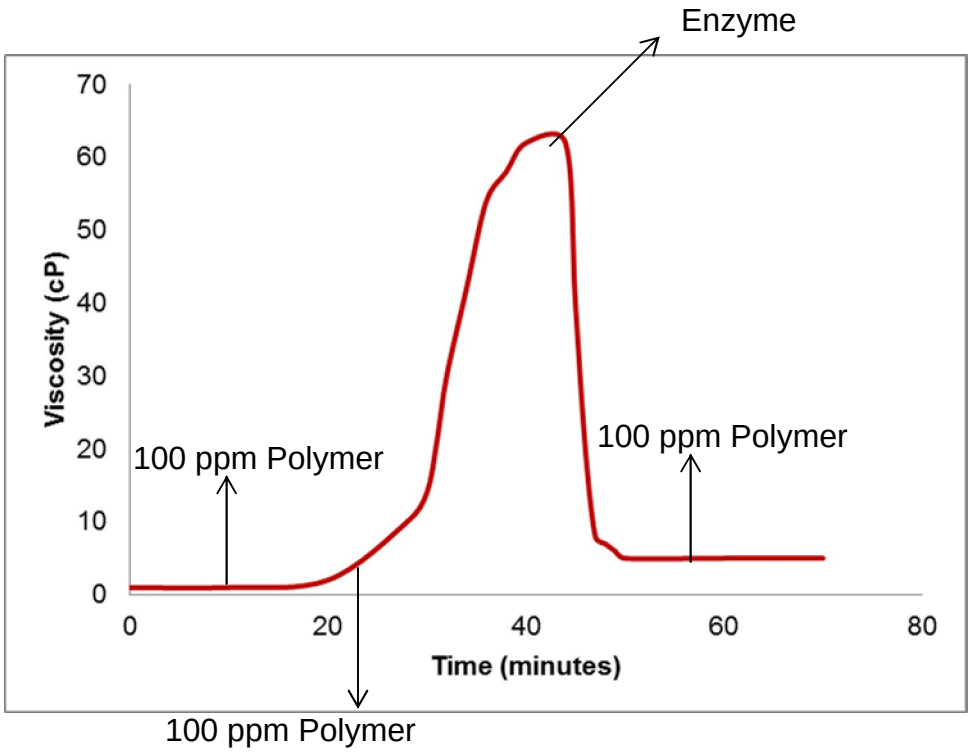


Figure 47: Effect of p-DADMAC addition point on liquefaction of cornstarch (II)

The time of addition of cationic polymers plays a significant role in the process of enzymatic hydrolysis of cellulose. Lu, *et al.* have suggested that for softwood saccharification it is better to add cationic polymer after two hours of initial hydrolysis [68]. Cationic polymers tend to agglomerate long fibers, decreasing the surface area available for the enzyme to act, whereas after two hours, these long fibers are shortened, and addition of cationic polymers at this point leads to increased glucose production (patch mechanism).

Cationic polymers act via two different mechanisms in cornstarch liquefaction. (1) The first effect is the ability of cationic polymers to enhance cornstarch solubilization by inducing disintegration of granules, and thus reducing the onset of gelatinization

temperature. (2) The second effect is the ability of cationic polymers to increase the binding of enzyme to substrate through a patch mechanism, as described in chapter 2. These combined effects lead to viscosity reduction at the end of liquefaction (thus increasing efficiency of heat exchange), and produce higher yields. p-DADMAC is very stable at the standard conditions maintained during cornstarch liquefaction. From the above explanation, it can be concluded that, unlike softwood saccharification, addition of polymer initially before gelatinization would be more beneficial for cornstarch liquefaction.

4.3 Liquefaction Studies

Liquefaction rates were measured at 1% cornstarch loading at 70°C. Two sets of experiments were performed by adding enzyme initially at time zero (Figure 48) and after 5 minutes of gelatinization (Figure 49). Enzyme is also added at time zero and after gelatinization begins in industrial practice [27]. It can be observed from Figure 48 that the addition of polymer increases glucose production by 42% compared to control after 30 minutes of hydrolysis.

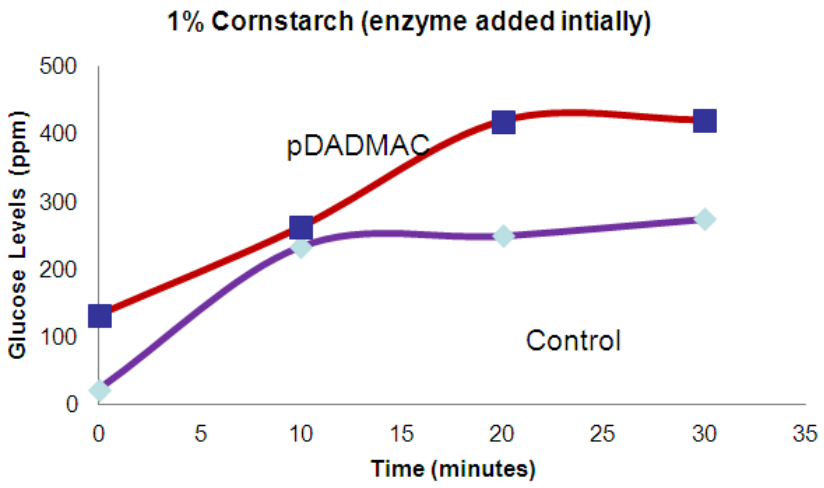


Figure 48: p-DADMAC effect on liquefaction of cornstarch (enzyme added at time zero)

Figure 49 shows the effect of adding enzyme after gelatinization. Glucose production is higher (for runs both with and without polymer) as compared to the results in Figure 48. These results are attributed to the fact that addition of enzyme during gelatinization may lead to partial enzyme deactivation. Studies done on α-amylase stability at elevated temperature have shown that there is a decrease in enzyme activity over time at 70°C (the operating temperature for the current experiments).

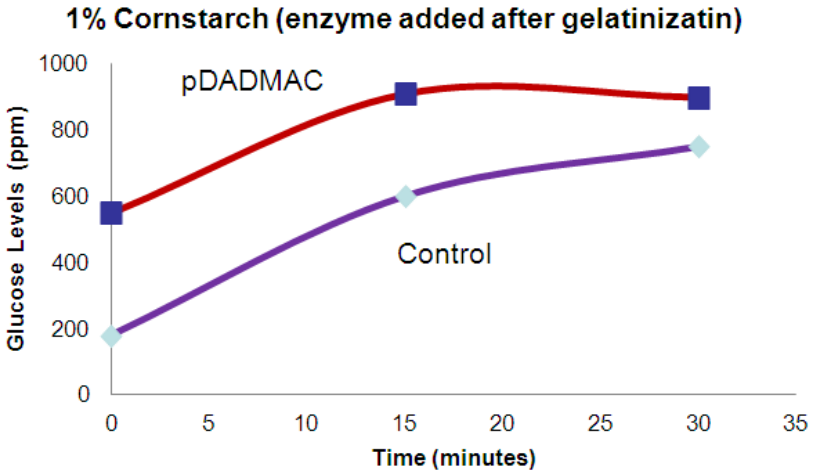


Figure 49: p-DADMAC effect on liquefaction of cornstarch (enzyme added after gelatinization)

It can be observed in both Figure 48 and Figure 49 that p-DADMAC increased glucose production during liquefaction. Maxwell*, et al.* [23] and Reye*, et al.* [62] have proposed a mechanism for cationic polymer behavior during enzymatic hydrolysis. Both the enzyme and cornstarch used in liquefaction are negatively charged. It was proposed that cationic polymer neutralizes certain parts of the starch surface, thus reducing the repulsive barrier between enzyme and substrate. This boosts the binding between enzyme and substrate, and enhances the rate of hydrolysis.

Experiments were performed to study the effect of glucoamylase on p-DADMAC mediated saccharification (Table 7). The overall glucose production after addition of glucoamylase is already very high, which made it difficult to conclude if polymer influences saccharification under the conditions chosen.

Table 7: Glucose Levels (in ppm) at 1% Cornstarch (α-amylase added after gelatinization)

	Time (minutes)	Control	XP-10030 pDADMAC 100 ppm	XP-10030 pDADMAC 300 ppm
No Enzyme		180	200	550
α-amylase added	0	600	610	910
	30	750	620	900
glucoamylase added	60	5090	4950	5270
	90	8640	6260	8130
	120	8250	9650	9670
	360	8440	7930*	9680
	570	9430	9570	8490*
*possibly erroneous as it represents a drop from the preceding value				

Several studies have been reported in the literature to identify optimum enzyme, pH and temperature for liquefaction [78, 79] and many of them have assumed that when the rate of liquefaction was enhanced, this will naturally lead to higher glucose levels after saccharification, and thus produce high ethanol yields. For the reasons stated above, the effect of glucoamylase was not further explored.

Liquefaction rates were measured at 8% and 30% cornstarch loading. The effect of 100 ppm p-DADMAC (XP 10030) on the liquefaction rate of 8% and 30% cornstarch is illustrated in Figures 50 and 51, respectively. Higher p-DDMAC levels of up to 500 ppm gave similar increases as did use of the other p-DADMAC, XP 10020. Brix is the equivalent of 1 g of sucrose in 100 g of solution and is commonly used in the industry as a measure of cornstarch liquefaction. Rate measurements were begun immediately after

addition of the amylase; the high initial conversion reflects rapid liquefaction, which is the expected outcome. It is clear that the p-DADMAC increases the degree of conversion over the measurement period. The increase in Brix induced by p-DADMAC is very similar to that measured for various c-PAMs.

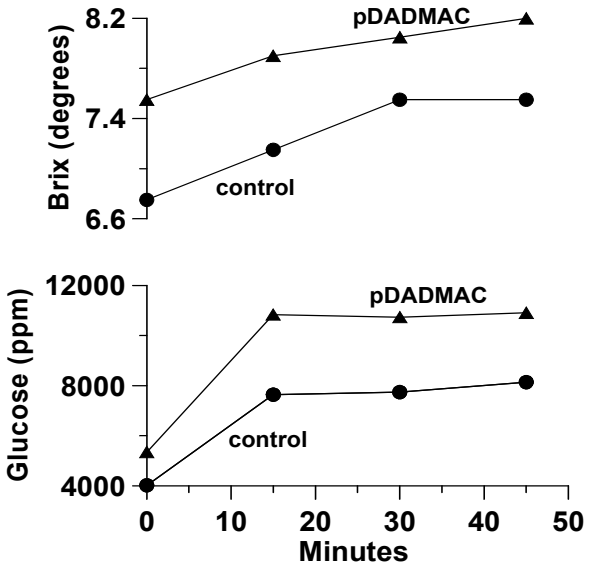


Figure 50: Effect of 100 ppm p-DADMAC (XP 10030) on the liquefaction rate of 8% cornstarch

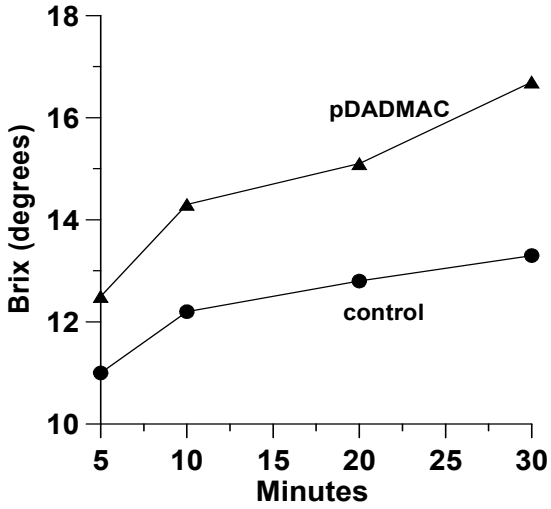


Figure 51: Effect of 100 ppm p-DADMAC (XP 10030) on the liquefaction rate of 30% cornstarch

The increase in Brix induced by different doses of p-DADMAC is illustrated in Figure 52. The values were taken at 55 minutes, where Brix reached its peak value. A control with no polymer was run along with each measurement to factor out the effect of small changes in temperature. It is clear that the p-DADMAC increases Brix, and that the benefit levels off at a p-DADMAC value of 25 ppm. These results suggest that p-DADMAC should be able to reduce the enzyme dose required for optimal hydrolysis.

The degree to which this occurs is illustrated in Figure 53 where liquefaction was conducted at two enzyme levels with and without 50 ppm p-DADMAC. The points were taken at 55 minutes into the hydrolysis where the Brix values were at a maximum. p-DADMAC increases Brix at both enzyme levels. The Brix level for the p-DADMAC run at 0.015% enzyme is higher than that obtained for the control run at 0.03%, which means that the p-DADMAC can reduce the enzyme dose by over a factor of two.

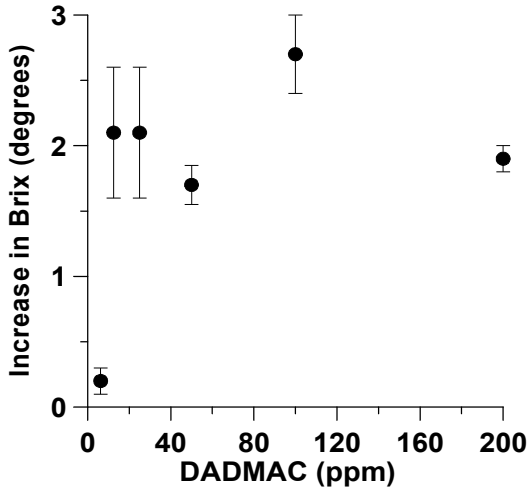


Figure 52: Effect of p-DADMAC (XP 10030) dose on Brix

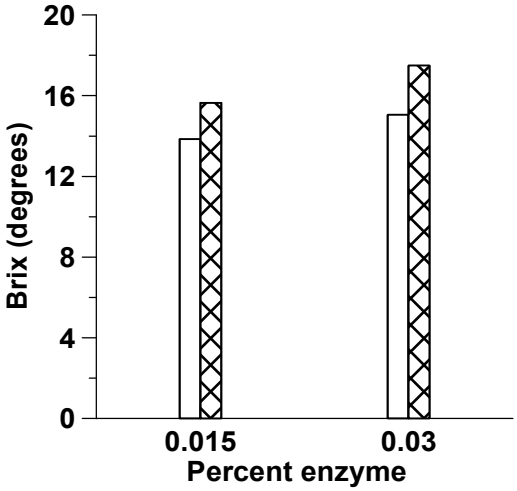


Figure 53: Brix increase with p-DADMAC (cross-hatched and open bars represent runs with (50 ppm) and without p-DADMAC, respectively)

The major benefit of including a cationic polymer in cornstarch liquefaction is that it reduces the dosage (and the cost) of the α-amylase required for the conversion. The p-DADMACs and c-PAMs behave similarly insofar as enzyme binding and the hydrolysis rate is concerned. The mechanism appears to be quite general because several polymers are able to increase the hydrolysis rate. Although only one source of α-amylase

was used, the approach is likely to apply to others that are commercially available or are under development [40].

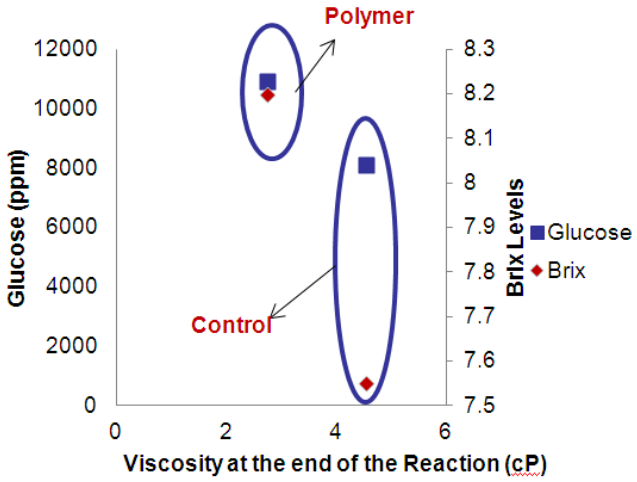


Figure 54: p-DADMAC effect on viscosity and liquefaction of cornstarch

Figure 54 shows glucose, Brix and viscosity levels at the end of liquefaction for a 8% cornstarch concentration. The figure clearly represents the relation between viscosity and glucose/Brix levels. At a lower viscosity for p-DADMAC (2.2 cP) mediated hydrolysis, the Brix/glucose levels are higher.

The higher viscosity that results from c-PAM is absent for p-DADMAC and the viscosity of the liquefied product is lower. Operationally, these are major advantages. As discussed earlier, the higher charge of the p-DADMAC leads to charge reversal of the solubilized components and inhibits agglomeration. Ideally, a polymer should be able to increase productive binding of enzyme to substrate, without agglomerating the substrate. The c-PAMs need more careful handling in this regard. p-DADMAC is superior in that it

retains the rate-enhancing benefit of c-PAMs without incurring the viscosity penalty. It is also cheaper. Finally, the lower cornstarch:water partition coefficient of the p-DADMAC as compared to that for c-PAM means that much less of it will associate with distiller's dry grain (DDG), a residual of the process that is used as animal feed. While feed safety regulations for these polymers have not been established for this application, a lower chemical concentration in the feed is always preferable [80].

4.4 Starch Hydrolysis Kinetics

The reaction kinetics of enzymatic hydrolysis of cornstarch was determined for both control, and p-DADMAC mediated cornstarch hydrolysis. Experiments performed for Brix analysis both for control and p-DADMAC were used to estimate kinetic parameters. Kinetics for starch hydrolysis was studied by many researchers. Pasri*, et al.* Yankov*, et al.* and others have used Michaelis-Menten's approach to study kinetics [81-83].

Brix levels (Section 4.3) were converted into an amount of substrate consumed by reaction (Equation 7). Conversion (X) was calculated by dividing the measured Brix levels by 24 (the highest Brix that can be obtained from 30% cornstarch liquefaction). Figure 55 shows substrate concentration vs. time for both the control and p-DADMAC. A plot between initial rates of reaction vs. concentration of substrate is presented in Figure 56.

$$? = ?_? (1 - ?) \quad (7)$$

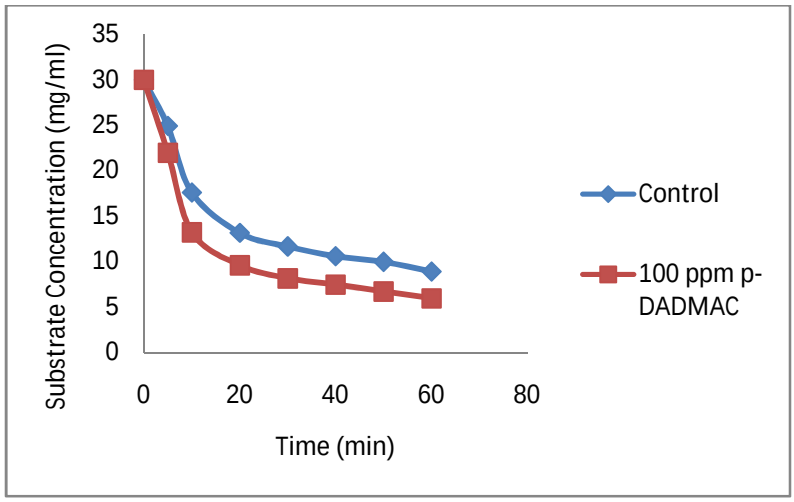


Figure 55: Substrate vs. time plot

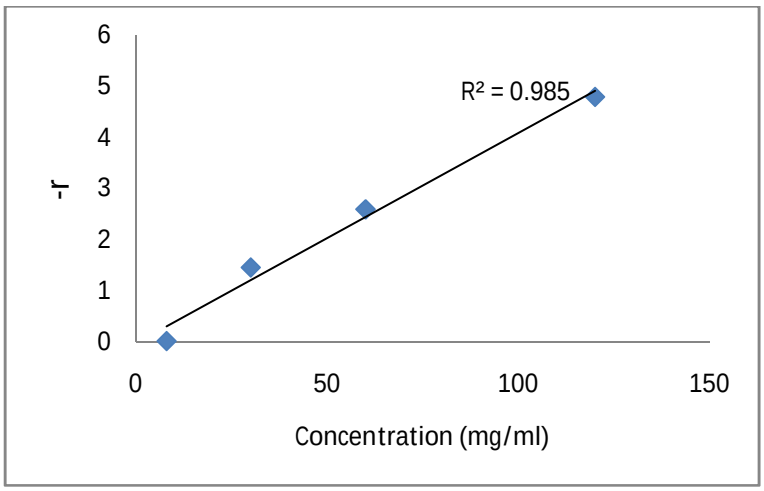


Figure 56: Initial reaction rates vs. concentration plot for control (without polymer)

It can be observed from Figure 56 that the rate of the reaction is directly proportional to concentration, indicating first order kinetics.

The mass balance of a reaction with first order reaction kinetics can be written as:

$$-\frac{??}{??} = ?? \quad (8)$$

The above equation can be integrated, and by assuming condition t=0, $S = S_o$, the equation can further be stated as:

$$\frac{\ln ?_0}{?} = ?? \quad (9)$$

A plot between $\ln S_o/S$ vs. t will give the value of K. Figure 57 and Figure 58 show the plots for control and p-DADMAC respectively. The reaction rate constant for control was 0.0326 min^{-1} and the value is similar to that estimated by Komolprasert*, et al.* [84]. The rate constant estimated by Komolprasert, who also assumed first order kinetics was 0.033min^{-1}. The reaction rate constant for p-DADMAC mediated hydrolysis was 0.044 min^{-1}.

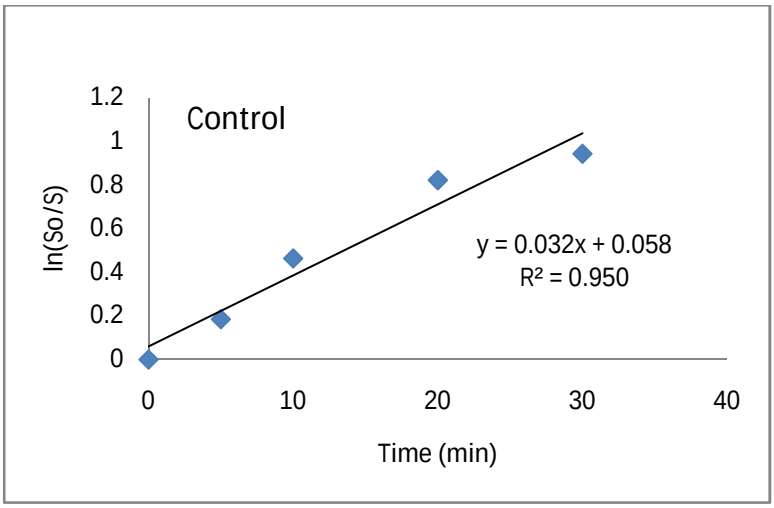


Figure 57: lnS_o/S vs. t for control

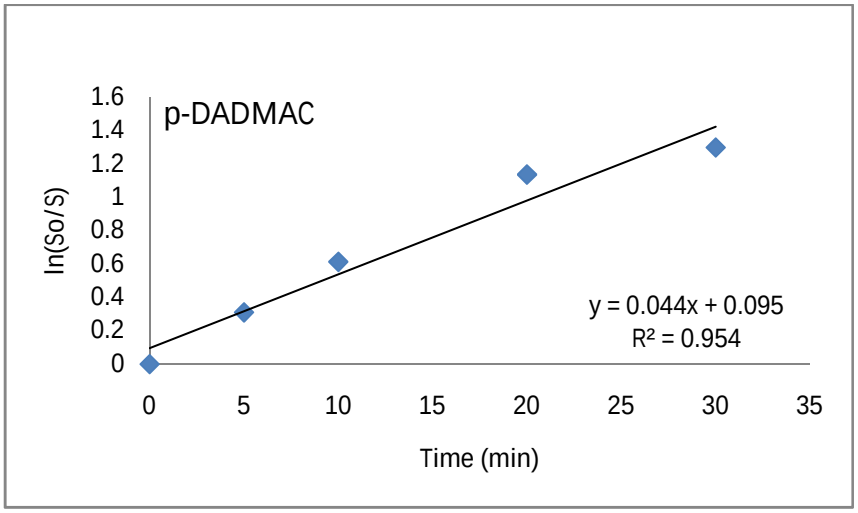


Figure 58: lnS_o/S vs. t for p-DADMAC

For Figures 57 and 58, data points were considered only for the first 30 minutes because the enzyme loses its activity rapidly at 70°C. Product inhibition is also a factor. The rate constant was higher for the polymer-mediated hydrolysis (0.044 min^{-1}) than for the control (0.0326 min^{-1}). This is consistent with other experiments performed to estimate binding constants and sugar levels with and without p-DADMAC. The polymer enhances both binding and Brix levels, which can be represented by a higher reaction rate

constant. Similar studies were performed to deduce cationic polymer effects on cellulose hydrolysis, whereby it was predicted that the effect of polymers on hydrolysis can be incorporated by increasing the rate constant.

CHAPTER 5: BLEACHED FIBER SACCHARIFICATION

5.1 Rate Measurements

Figure 59 illustrates the changes induced by the polymer on fiber properties such as fiber length, the kink angle and on the generation of glucose and fines. These fiber parameters are commonly used in the paper industry and are easily obtained with a Fiber Quality Analyzer but they do not appear to have been utilized by the biomass community. In order to highlight the changes induced by the polymer, a difference plot is presented in Figure 60. The changes in Figure 59 are absolute values; the kink angle and fiber length initially decrease with time, whereas the fines and glucose levels increase. The fines concentration rises initially because the fiber is progressively shortened by the endoglucanase. The polymer clearly increases the rate of this process and it must, therefore, have a greater effect on endoglucanase than on exoglucanase. Were it otherwise, the fines level would have fallen initially because the rate of consumption of the fines would have exceeded its rate of production. The difference in all the fiber properties reaches a maximum after 6 h (Figure 60). The glucose concentration at 6 h is about 4,000 mg/l for the runs that included c-PAM (Figure 59). This reflects about 20% conversion of cellulose, which is also the approximate amorphous content of a bleached softwood fiber used earlier [8]. Hence, the likely reason that the polymer preferentially increases the rate of endoglucanase hydrolysis is that it associates more with the amorphous regions of fiber that are particularly susceptible to endoglucanases attack [85, 86]. Further evidence for this preferential association comes from the partitioning measurements described below.

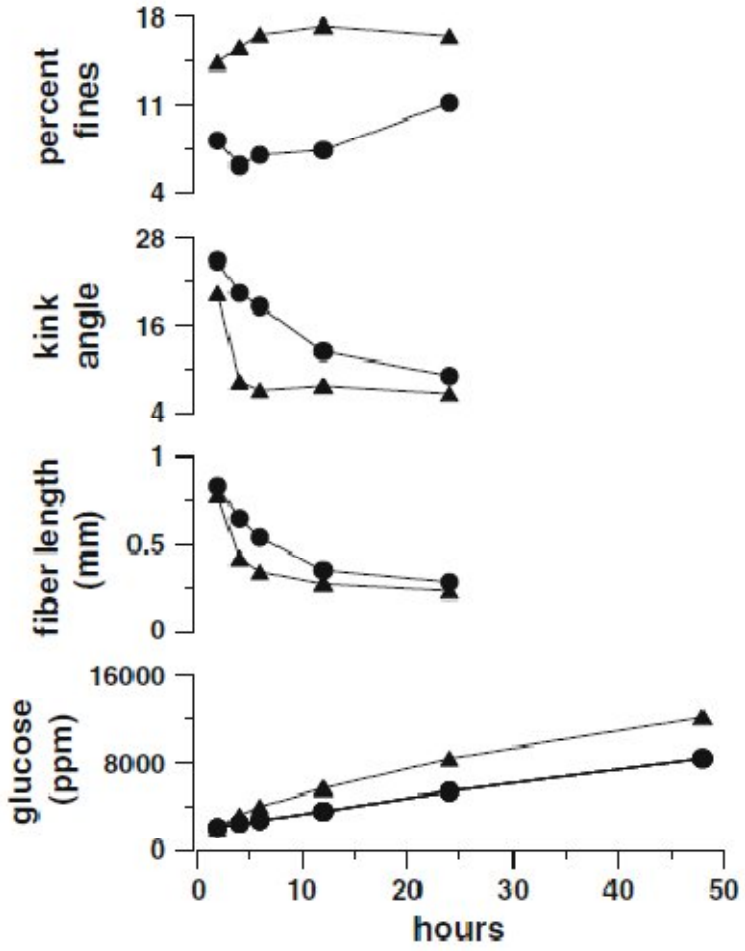


Figure 59: Effect of c-PAM (XP10035, 250 mg/l) on the hydrolysis of bleached softwood fiber by 0.1% (by volume) Ctec Cellic 2. The circles represent measurements on untreated fiber; the triangles are for fiber treated with c-PAM

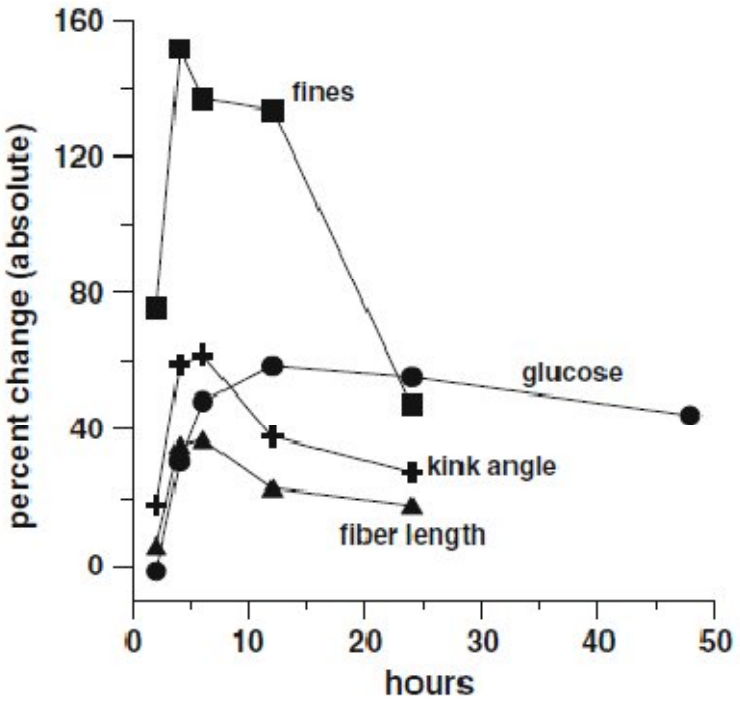


Figure 60: Difference plots for the results in Figure 59

5.2 Partitioning of Enzyme between Fiber and Water

The distribution of the enzyme between fiber and water, both with and without polymer treatment is illustrated in Figure 61 and 62. The results are expressed as dimensionless partition coefficients ($K_{f/w}$) in Figure 61, which reflect the propensity of the enzyme to associate with fiber as opposed to remaining in solution. A more conventional sorption format is followed in Figure 62. The lower trace in Figure 61 shows the distribution without the polymer where $K_{f/w}$ remains constant across various enzyme concentrations, i.e. a Langmuir isotherm is followed. Others have used Langmuir isotherms to model cellulase sorption to cellulose [13, 87, 88]. In the presence of c-PAM, a Freundlich isotherm ($n = 1.7$, $r^2 = 0.992$) is obtained for the upper trace in Figure 61. If the c-PAM were to coat the fiber uniformly a Langmuir isotherm would have likely resulted. Given that the Figure 60 results indicate that the c-PAM promotes attack on the amorphous regions, it follows that c-PAM principally binds to these amorphous regions. While only a single c-PAM was used for the binding measurements, a wide variety of c-PAMs have been shown to catalyze the cellulase-mediated hydrolysis of cellulosic fiber and it is likely that the same mechanism of binding also applies to the other polymers. When p-DADMAC was used for the binding measurements, high $K_{f/w}$ values were obtained compared to control. p-DADMAC has lower $K_{f/w}$ values, compared to c-PAM. High charge density of p-DADMAC reverses the charge of the colloids from negative to positive. This reduces the amount of p-DADMAC attached to fiber and also enzyme bound to the fiber.

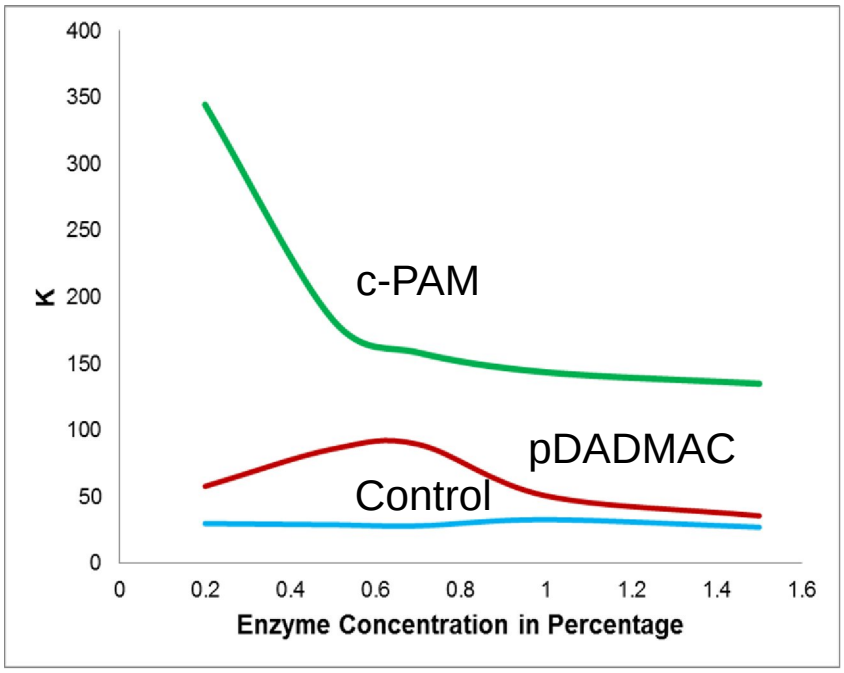


Figure 61: Fiber:water partitioning of cellulase (on 2% softwood fiber with and without cationic polymers)

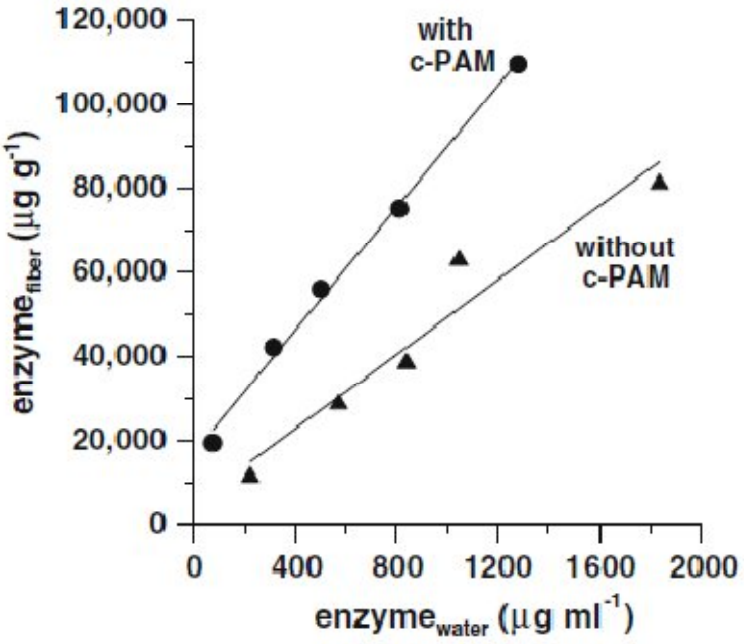


Figure 62: Sorption isotherms for cellulase with and without c-PAM

5.3 Specific Resistance to Filtration

A conventional measure of flocculation used in the wastewater industry is the specific resistance to filtration (SRF) [69]. The drainage rate of a fiber suspension is measured under vacuum with and without added polymer and then converted to SRF through a relationship that includes the pressure drop, the viscosity of the filtrate and the surface area of the filter paper. The SRF value is an index of filterability; as the fibers agglomerate, channels for water drainage open up and the cake dewaters more easily.

SRF values were measured to obtain the optimum dosage of p-DADMAC. The optimum p-DADMAC concentration according to Figure 63 is 100 ppm. As the polymer concentration increases from 100 ppm, the SRF rises, reaching its maximum at 1000 ppm. The increase is expected; overloading the fiber surface with the polymer reverses the charge and makes the surface positive. The resulting charge repulsion inhibits flocculation. Importantly, the optimal p-DADMAC concentration is of the same magnitude used for the enzyme applications. This similarity supports the position that the p-DADMAC increases the rate of glucose generation by reducing the zeta potential of the surface of the fiber.

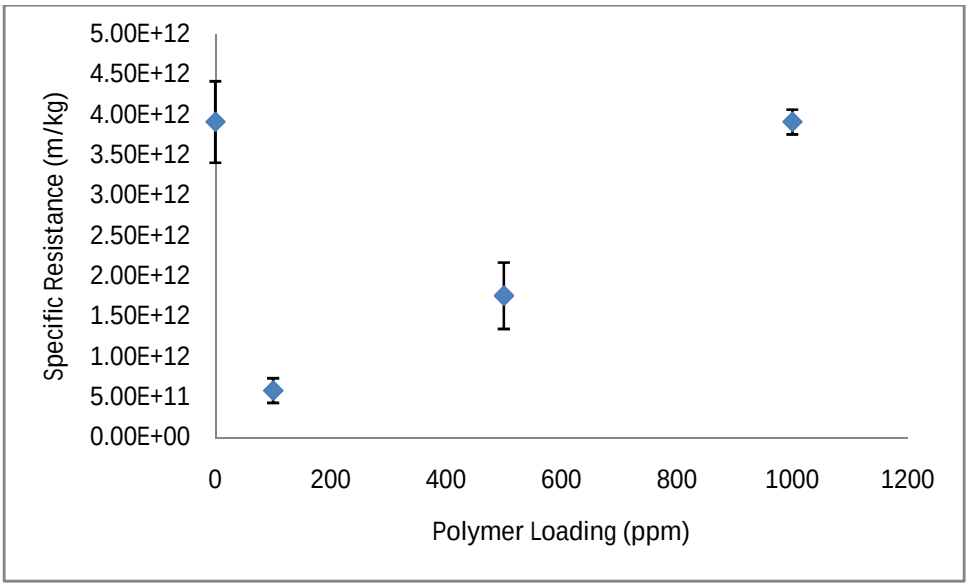


Figure 63: Specific resistance to filtration of p-DADMAC

CHAPTER 6: ECONOMIC ANALYSIS OF SLUDGE HYDROLYSIS

6.1 Economic Analysis

The previous chapters have addressed the enzymatic hydrolysis of fiber. An economically viable source of fiber is cellulosic sludge because it has a negative value. An economic analysis of sludge hydrolysis was conducted based on the schematic in Figure 64. The reactions considered during hydrolysis are shown in Figure 65. The hydrolysis is conducted in a series of five CSTRs of equal volume with the inert solids (ash) being removed either in a centrifugal cleaner (as shown) or in a settling tank. The glucose is concentrated through membrane filtration and the water recycled. The economics are conservatively based on a reverse osmosis membrane, although it is possible that less expensive ultrafiltration will suffice. Water is recycled to minimize the volume that would otherwise require disposal. The principal cost elements are capital, enzyme, and water treatment costs. The principal benefits are the value of the glucose produced and the avoided cost of sludge disposal. The power cost for the membrane operation is included, but that for the impellers for the reactors and for the centrifugal cleaners are neglected. The capital cost of the cleaners is also neglected because it is a minor component of the overall capital.

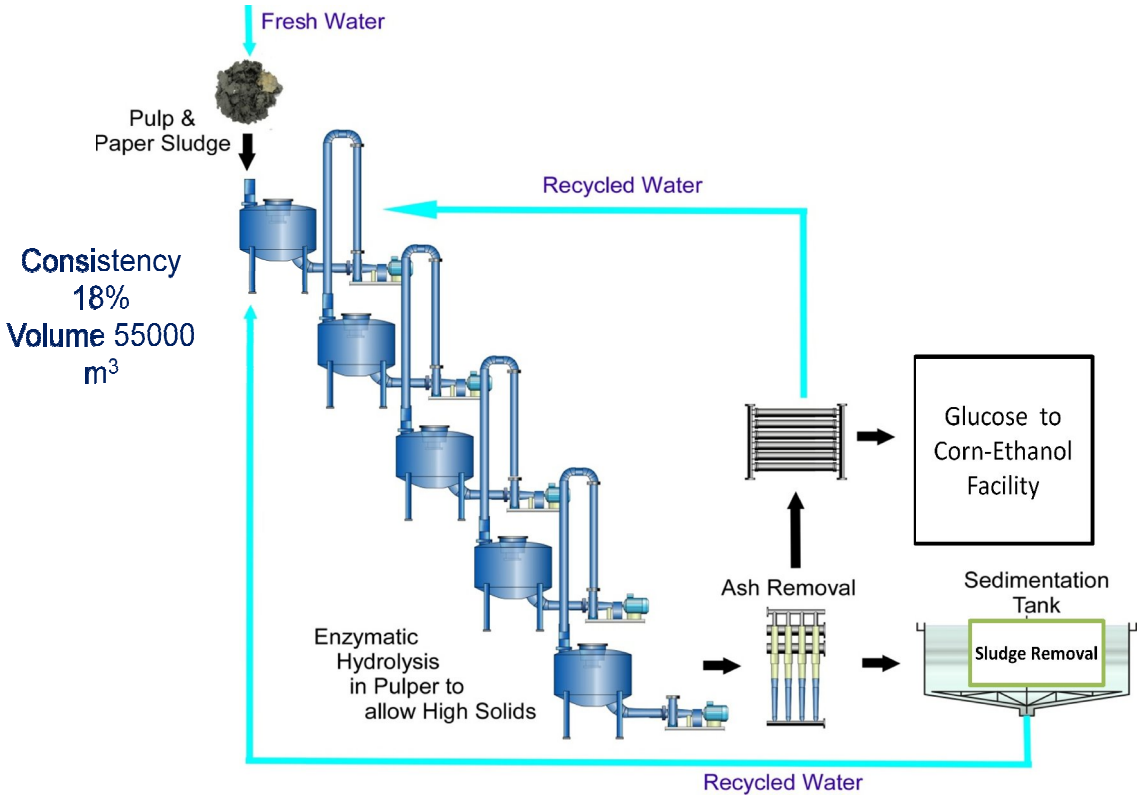


Figure 64: Schematic for the conversion of the cellulosic component of sludge to glucose

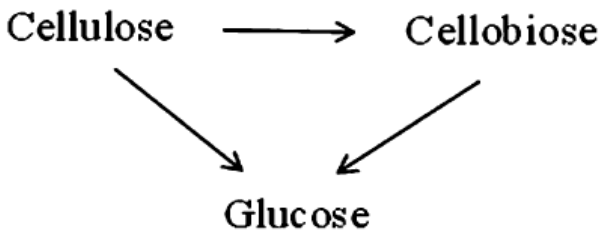


Figure 65: Schematic of cellulose hydrolysis. Each step is assumed to be first-order with potential inhibition by glucose and cellobiose

The calculations are based on 50 tonnes per day of the cellulosic component of sludge. The actual sludge mass may be higher because of the presence of inerts. The solids content (consistency) of the feed is 18%, which is near the maximum consistency that can be accommodated in a conventional batch pulper, which is a CSTR used in the paper industry to disintegrate recycled fiber. The volume of each CSTR was taken as 55 m^3, which is typical for a large repulper used in the recycle paper industry. Under these conditions the residence time in each reactor (CSTR) is 4.75 hrs and the volumetric flow rate is 11.6 m^3 per hour. The cumulative conversions by using the MATLAB program after exposure to each of the five reactors are illustrated in Figure 66. The various inhibitions by glucose and cellobiose built into the model are important; without them the conversions in Figure 66 would have been much higher and would have led to artificially favorable economics.

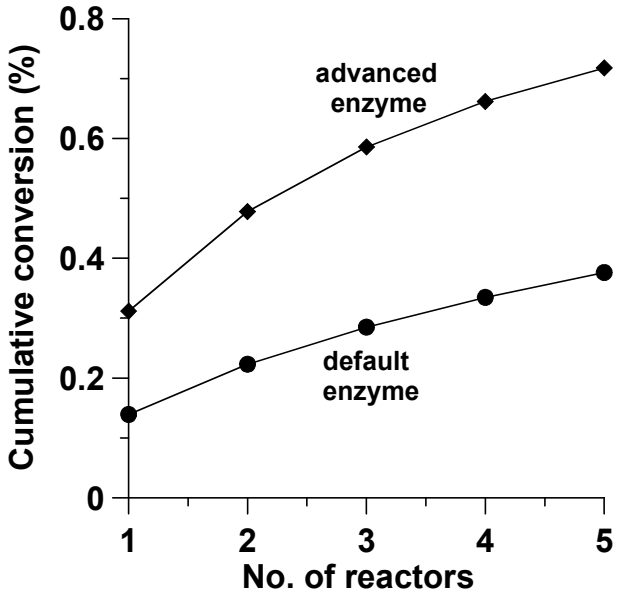


Figure 66: Fiber hydrolysis in a series of reactors

The cost elements used for the cost:benefit analysis to follow are provided in Table 8. The cost of CSTRs can vary depending on the manufacturer and a conservative value of $2 million for the set of five. Only the first reactor need be of higher power, because the fiber shortens rapidly when exposed to the enzyme and leads to a reduction in viscosity, which makes mixing easier. Also, the solids will progressively dissolve and the mixing load on the reactors later in the train will be correspondingly lower. The capital cost is amortized at 6% over twenty years.

A reference value for the bulk enzyme cost is unavailable, because prices are usually negotiated. An estimate of $4/kg is used here. However, the enzyme cost does not swing the economics and small changes in its cost do not affect the overall conclusions. It is difficult to assign a price to the membrane-purified glucose because this material is not yet available. Glucose prices are volatile; the average 2011 price for raw sugar is $0.59/kg. A base value of $0.30/kg is used in order to be conservative. The avoided cost of sludge disposal was taken as $60/dry tonne, which is what incurred by a mill in South Georgia and is typical of the industry. The cost for membrane separation (including capital and power) is estimated at $0.5/$m^3$.

Table 8: Cost elements for fibrous sludge hydrolysis

CSTR (5):	$2 million
Enzyme:	$4/kg
Glucose:	$0.59/kg
Avoided cost of sludge disposal:	$60/dry tonne
Water treatment cost:	$ 0.5/m^3

Results for the base case (where the glucose is valued at $0.30/kg) are shown in Table 9. It is apparent that the value of glucose swings the economics and that reasonable changes in the other parameters have a relatively small effect. Enzyme costs have been falling and are likely to continue to do so near term. The value of glucose is volatile but the long term trend points upwards. Sludge disposal costs are also almost certain to rise. All these changes will increase the overall savings. It is difficult to predict future water treatment costs. Newer membrane technology will reduce the cost, but the cost of power will rise. In any case, this cost is comparatively low and will not significantly impact the economics.

Table 9: Cost:benefits (dollars per day) for sludge hydrolysis using a glucose value of $0.30/kg

	Conventional enzyme	Advanced enzyme
Enzyme	(1,110)	(1,110)
Glucose	5,640	10,800
Water treatment	(140)	(140)
Avoided sludge disposal cost	1,130	2,150
Disposal of residual sludge	(1,870)	(850)
Amortization	(480)	(480)
Overall savings	3,170	10,370

It follows from the results in Table 9 that the economics are principally dependent on the value of glucose. It is likely that the economics would be even more favorable if the number of reactors is increased because up to a point the value of the additional glucose would more than offset the cost of the additional capital. The dependence of the cost:benefits on the value of glucose is illustrated in Figure 67. For the advanced enzyme, break-even occurs even if no value is attributed to the glucose because the various costs incurred are offset by the avoided cost of sludge disposal. A small cost is incurred if the default enzyme is used without an allowance for glucose. For the base case of a $0.30/kg value for glucose, annual savings of $1 and $3.6 million are projected for the default and advanced enzymes, respectively. Of course, there is also the green benefit of the reuse of residuals. While the effect of polymer on reducing enzyme dose was not considered for

the current economic anaylysis, the savings can be increased by about 10% if the polymer were applied.

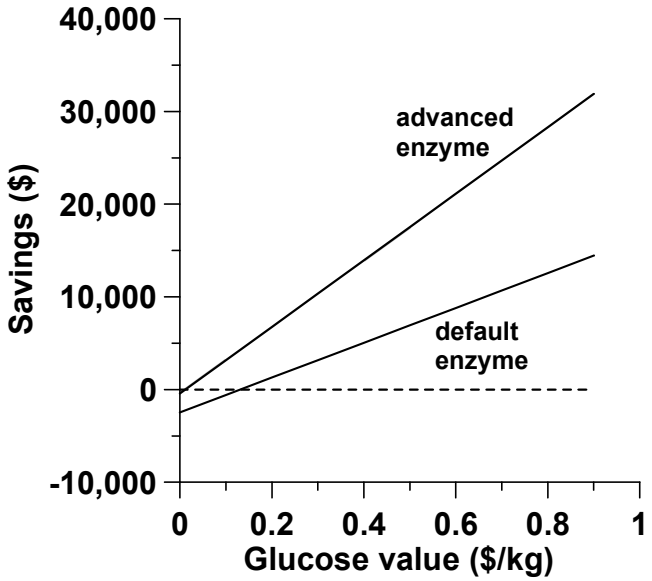


Figure 67: Dependence of the cost:benefits on the value of glucose

CHAPTER 7: WHEAT STRAW HYDROLYSIS

7.1 Effect of c-PAM on Physically Treated Wheat Straw

Experiments were conducted to study the effect of cationic polymers on wheat straw hydrolysis. Wheat straw typically contains approximately 35-45% cellulose, 20-30% hemicellulose, and 8-15% lignin. Enzymatic hydrolysis of wheat straw cannot be performed due to the crystalline structure of cellulose and the presence of lignin. To improve the accessibility of enzyme to cellulose, many pretreatments have been suggested in the literature [4, 6, 45]. For the current experiment, milled wheat straw was used, which was further screened through a standard 6 mesh (1.23 mm pore diameter). Milling improves the specific surface area available to the enzyme, and decreases DP [3]. Experiments were performed at 1% wheat straw (92.69% OD w/v) and 0.1% (v/v) cellulase with and without polymer (XP 10025).

Table 10: Effect of c-PAM on wheat straw

	Glucose (ppm)	
Time (hr)	Control	XP10025 c-PAM (150 ppm)
1	480	530
2	565	550
4	890	1100
20	1375	1330
25	1540	1610

It can be observed from Table 10 that c-PAM did not have any effect on hydrolysis. The glucose results obtained for both control and polymer-mediated hydrolysis were similar. The glucose levels for both control and polymer applications are low, as lignin and hemicellulose present in the untreated wheat straw hinder enzymatic hydrolysis.

7.2 Effect of c-PAM on Chemically Pretreated Wheat Straw

Several chemical pretreatments have been reported in the literature. These include alkaline, dilute acid, oxidative, carbon dioxide, and ammonia pretreatments. As each pretreatment can have a different effect on cellulose, hemicellulose and lignin it is hard to identify an ideal pretreatment method for wheat straw hydrolysis. The current study was performed to analyze the effect of c-PAM, and acid and base pretreatment.

Figure 68 shows glucose levels obtained for hydrolysis performed after alkaline pretreatment (0.5% NaOH, autoclaved for 60 minutes at 121°C,). Following pretreatment, experiments were performed with 3% (w/v) wheat straw, 0.2% (v/v) cellulose, at 50°C and pH 4.8, in 0.05M sodium citrate buffer. Chloramine T was added to inhibit bacterial growth during hydrolysis (experiments performed by Ikay Okafor).

There are several factors that inhibit enzymatic hydrolysis of wheat straw, such as the crystallinity of cellulose (influences initial hydrolysis rate), lignin content, surface area, and hemicellulose content [89, 90]. The goal of the pretreatment is to reduce these effects and to increase the accessibility of enzyme to cellulose. Removal of lignin with pretreatment is not always directly related to amount of glucose that can be produced during enzymatic hydrolysis [89].

Alkaline pretreatment causes swelling of biomass due to saponification. This causes partial decrystallization of cellulose. Alkaline treatment also disrupts the lignin structure and partially dissolves hemicellulose. Acid pretreatment, on the other hand,

mainly dissolves hemicellulose, and thereby increases enzyme accessibility to cellulose. Acid pretreatment also partially dissolves lignin [3, 4, 90].

Figure 68 shows that when alkaline pretreatment was used, glucose production increased compared to control (no chemical pretreatment). Many researchers produced similar results and reported more than 100 percent increase in glucose production after alkaline pretreatment [91, 92]. Alkaline pretreatment as mentioned above disrupts the cellulose-lignin-hemicellulose structural order, which enhances enzyme-substrate accessibility resulting in higher glucose production.

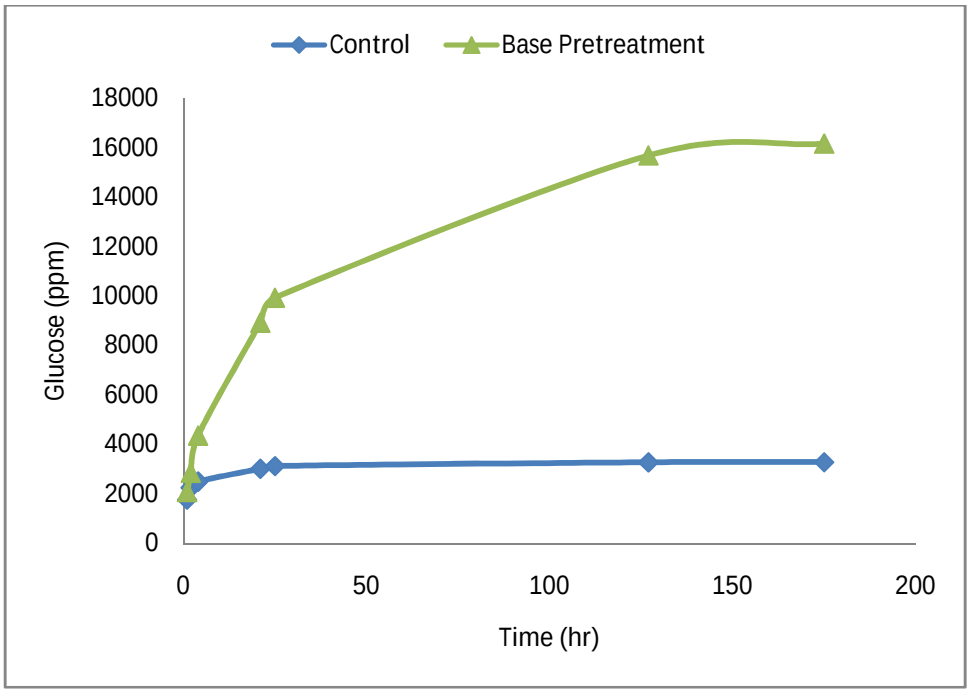


Figure 68: Effect of alkaline pretreatment on wheat straw saccharification

Experiments were performed to obtain optimum alkaline concentration for wheat straw hydrolysis. Figure 69 shows that the amount of glucose produced after 48 hours of enzymatic hydrolysis increases with increasing NaOH concentration. The results are consistent with those of Han, *et al.* who performed similar experiments [91]. Han, *et al.* showed that wheat straw enzymatic hydrolysis is constant with respect to NaOH concentration used for pretreatment above 1 percent NaOH concentration [91].

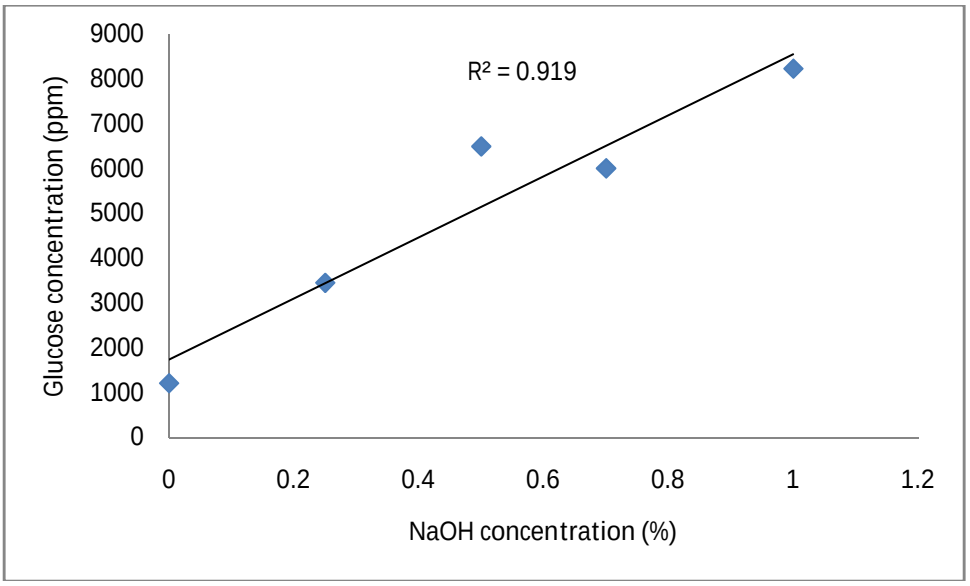


Figure 69: Glucose levels after 48 hours of hydrolysis vs. alkaline concentration

7.2.1 Effect of c-PAM on Alkaline Pretreated Wheat Straw

Experiments were performed to study the effect of c-PAM on alkaline pretreated wheat straw. The experimental conditions were maintained as mentioned above (experimental work performed by Ikay Okafor). XP10025 was used at 100 and 200 ppm. It can observed form Table 11 that c-PAM has no effect on hydrolysis and the results are very similar to those from the control.

Table 11: Glucose levels for c-PAM mediated alkaline pretreated wheat straw saccharification

Time (hr)	100ppm c-PAM	200ppm c-PAM	Control
1	2035	2129	2163
2	3017	3023	3147
7	4603	4554	4381
20	6774	6855	6852
24	7135	7590	6864
31.5	7341	7449	7139
79.5	8861	8276	8559

It can be observed from Tables 12 and 13 (experiments performed by Ikay Okafor) that c-PAM agglomerates the wheat straw. This would decrease the surface area available to enzyme and is presumed to decrease glucose production below control. However, glucose levels with and without c-PAM were similar (Table 11). This is attributed to the fact that c-PAM enhances the binding of enzyme to fiber. It is probable

that these two effects (agglomeration and increased binding of enzyme) offset each other and result in a similar rate of glucose production.

Table 12: Control and c-PAM mediated hydrolysis of wheat straw (Magnification: 1X)

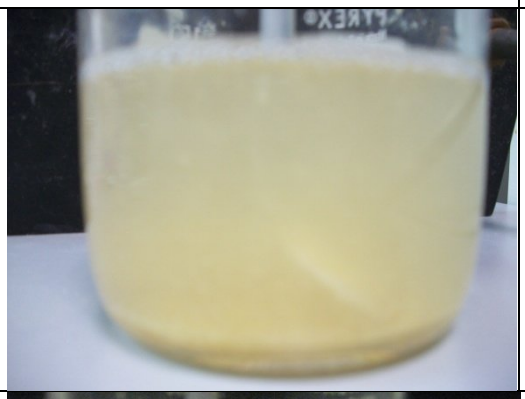
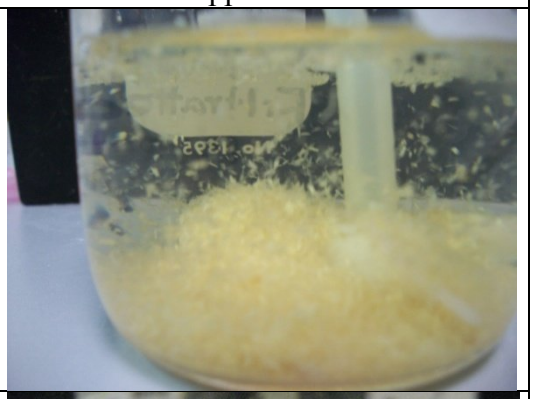
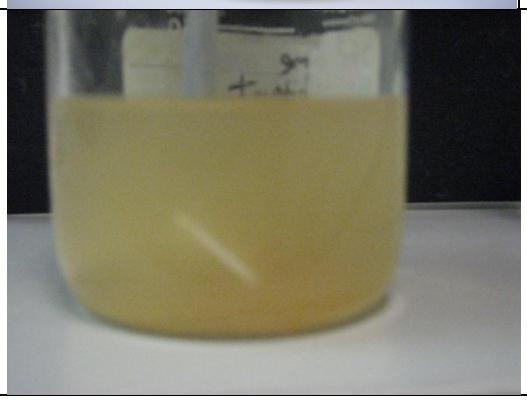
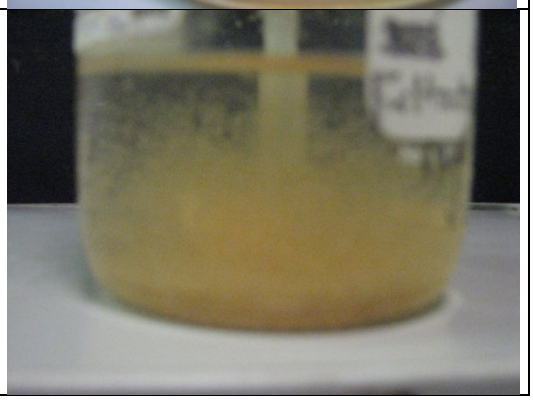
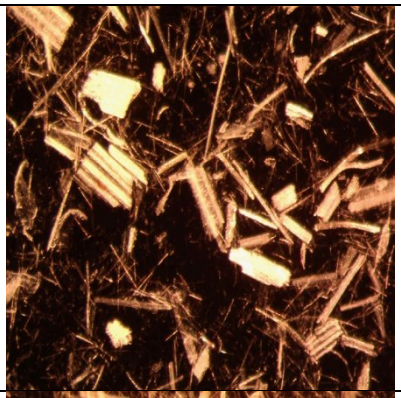
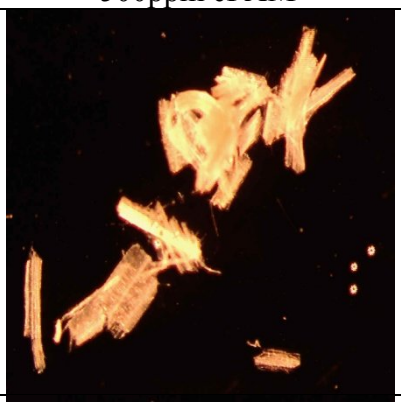
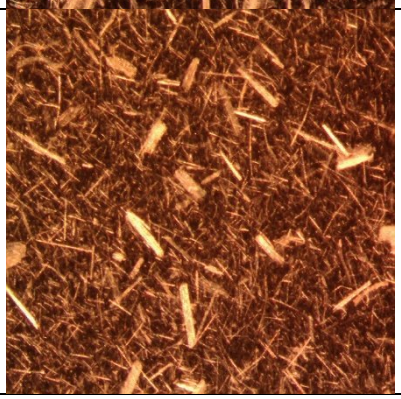
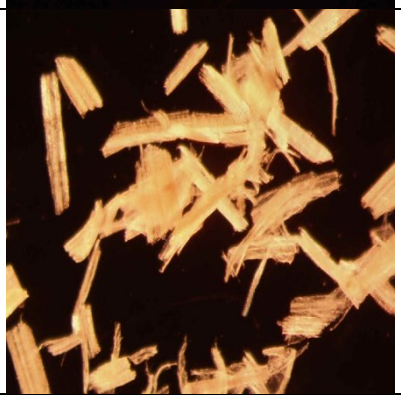
	No CPAM	500ppm cPAM
2 hours		
24 hours		

Table 13: Control and c-PAM mediated hydrolysis of wheat straw (Magnification: 7X)

	No CPAM	500ppm cPAM
1 hour		
2 hours		
24 hours		

7.2.2 Effect of c-PAM on Acid Pretreated Wheat Straw

Table 14 shows the effect of c-PAM on acid pretreated (0.2% H_2SO_4 for 15 minutes and then washed with water) wheat straw. Experiments were performed at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 400 rpm, 1% wheat straw (w/v) and 0.1% (v/v) cellulase. Many studies have shown that higher glucose levels can be obtained through acid pretreatment [93, 94]. It can be seen in Table 14 that glucose levels are higher for both control and c-PAM compared to Table 10. Acid pretreatment solubilizes hemicellulose, increasing the availability of cellulose to enzyme and leading to a higher reaction rate [90, 93, 95].

Table 14: Effect of c-PAM on acid pretreated wheat straw saccharification

	Glucose ppm	
Time	Control	XP10025 150 ppm
1	650	531
2	808	892
4	1300	1405
8	1621	1570
16	1970	1800

Table 15 shows the effect of c-PAM on oxygen delignified wheat straw. The conditions used for oxygen delignification were 1.7% NaOH (w/w based on OD pulp), temperature of 90^{o}C, gaseous oxygen inlet pressure of 80 psi, and a time of 90 minutes. Experiments were performed at 50^{o}C, pH 4.8, 0.05M sodium citrate buffer, 400 rpm, 1%

wheat straw (w/v) and 0.1% (v/v) cellulase. Three different c-PAMs XP10025 (130 ppm), XP10031 (300 ppm), and XP10023 (400 ppm) were used in the experiment. It can be seen from Table 15 that there is no difference in glucose levels with and without c-PAM mediating hydrolysis.

Table 15: Effect of c-PAM on oxygen delignified wheat straw saccharification

	Glucose ppm			
Time (hr)	Control	XP10023 (400 ppm)	XP10031(300 ppm)	XP10025 (130 ppm)
1	1250	1271	1302	1426
2	1524	1734	1511	1478
7	2312	2414	3357	2642
21	2987	2876	3138	2911

Acid and alkaline pretreatments have some advantages and disadvantages. Alkaline treatment effectively removes lignin and disrupts the crystallinity of cellulose. However, this treatment involves an expensive alkaline catalyst. Acid treatment solubilizes hemicellulose and produces more glucose. However, it also involves high cost acids (when concentrated acids are used) and has corrosion issues [95].

For both treatments, c-PAM did not produce an improvement in glucose production. This is probably due to the fact that both pretreatments employed in the study did not sufficiently disrupt the lignin-cellulose-hemicellulose structure of wheat straw,

and by the time wheat straw disintegrated, the enzyme was distributed well so that the effect of c-PAM was negligible. It can be seen from Tables 12 and 13 that c-PAM has an effect on wheat straw during hydrolysis, and if used in an appropriate way, c-PAM has the potential to enhance the enzymatic hydrolysis of lignocellulosic biomass (probably by different pretreatment methods). Ji, *et al.* recently showed that addition of c-PAM to a non-shear hybrid alkaline pretreatment of corn stover increased the production of glucose during enzymatic hydrolysis [96]. It was suggested that c-PAM altered lignin structure on the surface of corn stover in a way that increased the accessibility of enzyme to cellulose.

CHAPTER 8: BROWN FIBER SACCHARIFICATION

The presence of lignin impairs the enzymatic hydrolysis of wood pulp fiber to sugars. The lignin is believed to bind to the enzyme and/or prevent access to the surface of the cellulose. Removing all the lignin through pulping is not economically viable for biofuel production because a significant amount of cellulose would also be lost. Several studies have examined the role of lignin in fiber hydrolysis. In a cost:benefit model for the production of cellulosic ethanol, Phillips, *et al.* proposed using 100 kappa softwood and 70 kappa hardwood as feedstocks [5, 9]. Kappa number is roughly equal to the percent lignin remaining in pulp divided by 0.15 [97]. The relationship is influenced by the type of wood and the conditions used to pulp it. Monrroy, *et al.* found that kappa 43 eucalyptus pulp underwent 93% hydrolysis [98]. Chang, *et al.* [99] and Zhu, *et al.* [100] showed that for poplar the total sugar conversion began to decrease at lignin levels exceeding 10-15%. Clearly, lignin inhibits cellulose hydrolysis.

As shown earlier, cationic polymers can increase the rate of hydrolysis of both cornstarch and bleached cellulosic fiber. In order to extend this approach to mills that make unbleached paper products, the effect of cationic polymers was studied on fibers containing various amounts of lignin.

Binding constants were measured as described before [73]. Kinetic measurements were made in duplicate. Because the runs were not necessarily run simultaneously the solutions were sampled at different times on occasion. Where they were sampled simultaneously the uncertainty was less that 5%.

Binding constants for the association of cellulase to fiber are reported in Table 16. They are very similar across all the samples, indicating that lignin does not significantly affect the binding. This is not surprising because the lignin content for the pulps used is relatively low compared to the amount of cellulose present. Results from hydrolyzing hardwood fiber pulped to different levels with and without added c-PAM and p-DADMAC are illustrated in Figure 70. As expected, lignin retards both the rate and the degree of conversion over the period of measurement for the control (without polymer). The lignin probably shields the cellulose from enzymatic attack. However, the rate enhancing effect of the polymer is maintained, so that a benefit will be obtained with brown pulp.

This is especially important for the sludge application, because if brown sludge is to be hydrolyzed, then use of the polymer will provide a cost advantage. The rate and the conversion both increase by about the same extent for the two polymers. A similar situation exists for softwood pulp as shown in Figure 71, although glucose production is much slower. Hydrolysis of softwood is slower than that of hardwood. Yu, *et al.* [101] have noted a similar difference in rate, but with pulps containing much more (12%) lignin. Differences in crystallinity, pore size and other factors have been suggested as factors that can affect the hydrolysis rate [102].

Table 16: Partition Coefficients (Standard Deviation 5.8)

Pulp	Kappa Number	Partition Coefficient
Hardwood	24	68
	14	76
	Bleached	82
Softwood	76	77
	40	71
	25	82

A comparison of Figures 70 and 71 demonstrates that the rate of softwood hydrolysis is about half of that of hardwood. The presence of lignin does not explain the difference because the rate difference persists at comparable kappa numbers. The difference in fiber length between hardwood and softwood is unlikely to be responsible because the rate has been shown to be independent of fiber length [103]. Crystallinity is known to retard the rate of hydrolysis, but Agarwal, *et al.* [104] have shown that hardwood fibers are more crystalline, so crystallinity cannot be a factor here. Hence, the difference in rate between hardwood and softwood fibers cannot be traced to the fiber properties that have been measured. It is possible that transport across the cell walls is important. However, a difference in rate of a factor of two is thermodynamically quite small and it will be difficult to attribute this difference to fiber properties.

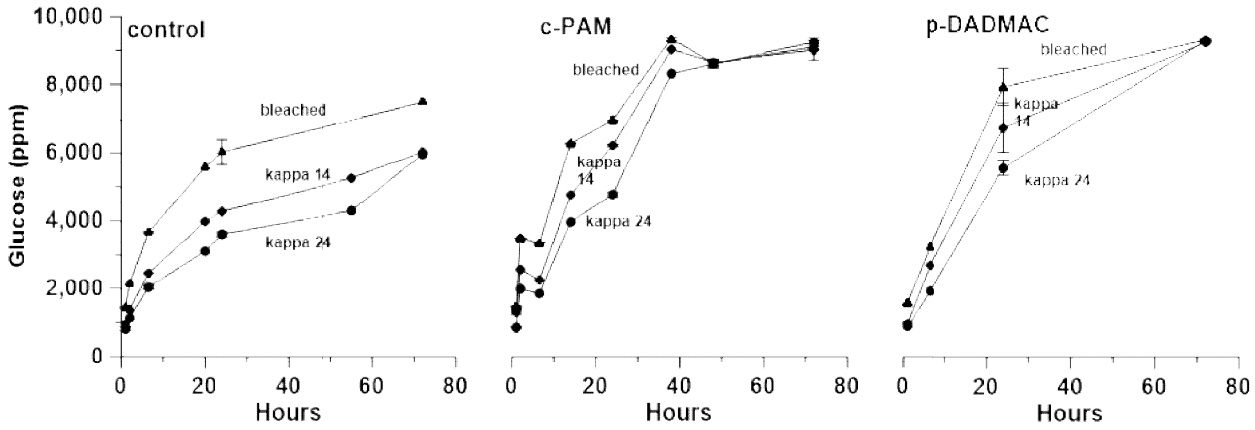


Figure 70: Effect of cationic polymers on the rate of glucose formation from hardwood pulps

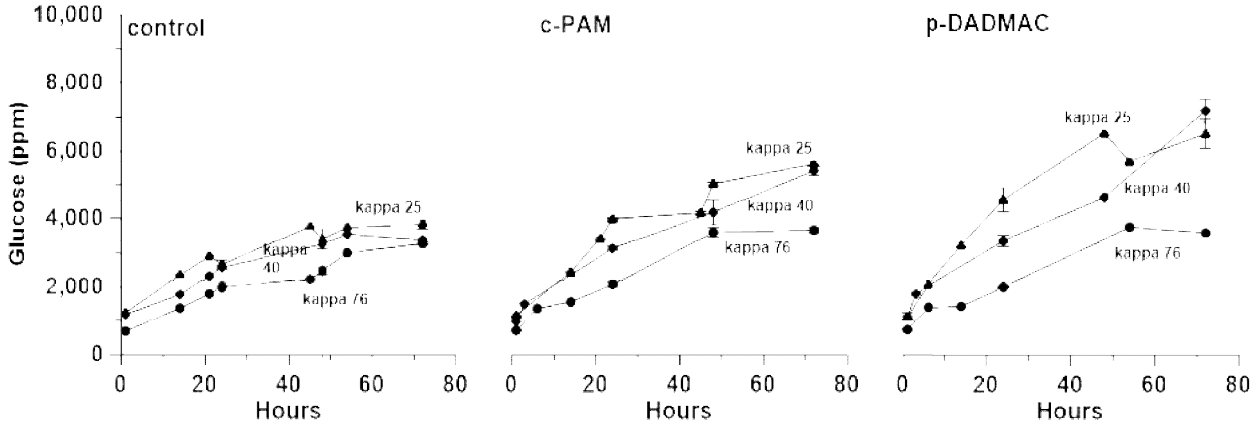


Figure 71: Effect of cationic polymers on the rate of glucose formation from softwood pulps

CHAPTER 9: CONCLUSIONS AND FUTURE WORK

9.1 Cornstarch Liquefaction

9.1.1 Binding Studies

Through charge titration studies it has been determined that p-DADMAC has an affinity towards cornstarch. Compared to c-PAM, the binding of p-DADMAC to cornstarch was significantly lower. This phenomenon occurs due to charge reversal. p-DADMAC is more highly charged than c-PAM, so that it will reverse the charge on the cornstarch surface at lower dosage and will inhibit the binding of additional polymer. A lower polymer dosage is preferred as it means there will be a low polymer concentration associated with distiller dry grain, which is used as animal feed.

Through BCA protein studies, it has been determined that p-DADMAC increases the binding of enzyme to cornstarch. The dissociation constant measured for the control was significantly higher than that when p-DADMACs were present. The association of cationic polymer to the enzyme is driven by electrostatic interactions. p-DADMAC, being a highly charged polymer, attracts negatively charged enzyme to the cornstarch.

9.1.2 Viscosity Studies

Both p-DADMAC and c-PAM decrease the temperature for the onset of gelatinization. The average onset temperature for the control (without polymer) was 72.7 ± 1.3°C; that for the p-DADMAC treated sample was 65.6 ± 1.3°C.

Through the effect of cationic polymers on pasting curve studies of cornstarch, it was determined that c-PAM (at certain concentrations and cationicity) increases peak and final viscosities. The higher viscosity lowers the rate of hydrolysis by slowing down diffusion. The peak and final viscosities for p-DADMAC-induced gelatinization were similar to those for the control.

It was shown that the viscosity peak for both p-DADMAC and control were similar during liquefaction. p-DADMAC reached its viscosity peak faster than control, suggesting faster gelatinization. The final viscosity level for p-DAMAC was lower than control, consistent with enhanced hydrolysis. As cationic polymers affect both gelatinization of cornstarch and the ability of the enzyme to bind to substrate, it was concluded that adding polymer initially before gelatinization is optimal.

9.1.3 Liquefaction

Experiments conducted at different concentrations of cornstarch and p-DADMAC produced higher glucose or Brix levels when polymer was used. The enzyme dose can be reduced by half by using 30 ppm of p-DADMAC during liquefaction. Kinetic studies showed that the rate constant obtained for p-DADMAC-mediated hydrolysis is higher than that of the control.

9.2 Bleached Fiber Saccharification

It was shown through difference plot that cationic polymer increases the rate of endoglucanase hydrolysis. The binding of the overall mixture of cellulase enzymes to fiber follows a Langmuir isotherm but switches to Freundlich in the presence of c-PAM. It was interpreted that the polymer associates more with the amorphous region of fiber than with the crystalline. While only a single c-PAM was used for the binding measurements, a wide variety of c-PAMs were shown to catalyze the cellulase-mediated hydrolysis of cellulosic fiber and it is likely that the same mechanism of binding also applies to the other polymers.

9.3 Economic Analysis of Sludge Hydrolysis

The economic feasibility of converting fibrous sludge from paper mills making a bleached product into glucose was demonstrated through an economic analysis. The cost:benefits are predominantly dictated by the value of glucose. The process costs incurred are roughly balanced by the avoided cost of sludge disposal. Annual savings of up to $3.6 million are projected for a typical mill. .

9.4 Brown Pulp Saccharification

Cationic polymers increase enzymatic hydrolysis of hardwood pulps for kappa numbers 24, 14, 8 and < 2. Polymers enhanced the enzymatic hydrolysis of softwood pulps at lower kappa numbers (40 and 25). Polymers had no effect on higher kappa number softwood pulps (i.e,, kappa number 76).

9.5 Overall Conclusions

9.5.1 Cornstarch Hydrolysis

- p-DADMAC accelerates the rate of amylase-catalyzed hydrolysis of cornstarch. A 9 percent increase in sugar yield is observed for hydrolysis experiments conducted at 1% (w/v) cornstarch concentration.
- A substantial amount of enzyme (50 %) can be saved by adding a small amount of cationic polymer, without changing any process conditions.
- Both c-PAM and p-DADMAC reduce the temperature of the onset of gelatinization. Cationic polymer-mediated hydrolysis leads to faster gelatinization and hydrolysis.
- p-DADMAC offers additional benefits compared to cPAM with respect to viscosity spikes, polymer-starch binding and cost.

9.5.3 Cellulose Saccharification

- During bleached fiber hydrolysis, cationic polymers primarily attack amorphous regions, promoting endoglucanase activity.
- Cationic polymers significantly increase the binding of enzyme to substrate.
- Cationic polymers show potential in sludge treatment (up to $3.6 million in savings estimated per for a mill releasing 50 tons of sludge per day).
- Cationic polymers enhance the production of glucose from brown hardwood and softwood pulps.

9.6 Future Work

Purified cornstarch was used for the work performed on cornstarch liquefaction in the current study to have a consistent starting substrate. However, a mashed corn mixture is typically used in industry. Even though several conditions used in the experiments performed were similar to those common to industrial practice, all the experiments were conducted at a lab scale. A scale up process and pilot plot runs have to be performed before using cationic polymers in real time. Ethanol is produced from different starch sources across the world. Experiments can be done to study the effect of cationic polymers on rice starch, wheat starch, and potato starch liquefaction.

Further work can be performed on different lignocellulosic biomass. In the current study, it was shown that cationic polymers enhance enzymatic hydrolysis of bleached fiber, sludge, and unbleached or partially bleached fiber. Other potential feedstocks such as switch grass, bagasse and corn stover can be explored.

APPENDIX

Equations used for economic analysis of sludge hydrolysis are stated below. Fsolve function in MATLAB was used to solve multiple non-linear equations.

$$SR = ? \frac{?}{?_?} \quad (1)$$

The below equation shows Langmuir isotherm for enzyme adsorption to substrate in presence of lignin.

$$\frac{?_{1?}}{?} = \frac{?_{1?}\ ?_?{?}_{1?}{?}_?{?}_{1?}}{1+?_{1?}{?}_?{?}_{1?}} \quad (2)$$

As enzyme has the ability to absorp on both cellulose and lignin. The equation below shows the adsorption of enzyme to lignin.

$$\frac{?_{??}}{?} = \frac{?_{??}\ ?_?{?}_{??}{?}_?{?}_{??}}{1+?_{??}{?}_?{?}_{??}} \quad (3)$$

For the current study lignin is considered to be negligible so E_{ibL} is assumed to be zero.

$$?_{1?} = ?_{1?} - ?_{1?}? \quad (4)$$

Cellulose to cellobiose reaction rate:

$$?_1 = \frac{?_{1?}{?}_{1?}\ SR\ ?}{1+?\frac{?_2}{?_{1?}}?+(\frac{?}{?_{1?}})} \quad (5)$$

Cellulose to glucose reaction rate:

$$?_2 = \frac{?_{2?}{?}_{1?}SR\ ?}{1+?\frac{?_2}{?_{2?}}?+(\frac{?}{?_{21?}})} \quad (6)$$

Cellobiose to glucose reaction rate:

$$?_3 = \frac{?_{3?}?_{2??}?_2}{?_{3?}\ ?1+?\frac{?}{?_{3??}}??+?_2} \quad (7)$$

Table 1: Kinetic parameters estimated by Zheng *et al.*

Kinetic Parameters measured by Zheng *et al.*	
k_{1r}	16.5 (mL/mgh)
K_{1IG2}	0.04 (mg/mL)
K_{1IG}	0.1 (mg/mL)
k_{2r}	7.1 (mL/mgh)
K_{2IG2}	132.5 (mg/mL)
K_{2IG}	0.01 (mg/mL)
k_{3r}	267.6 (h^{-1})
K_{3M}	25.5 (mg/mL)
K_{3IG}	2.1 (mg/mL)

C is cellulose concentration at any given time (mg/mL)

E_{ib} is enzyme concentration bound to substrate (cellulose and lignin) (mg protein/mL)

E_{1bc} is enzyme concentration bound to cellulose in substrate (mg protein/mL)

E_{1bL} is enzyme concentration bound to lignin in substrate (mg protein/mL)

E_{if} is concentration of free enzyme in solution (mg protein/mL)

E_{ifL} is concentration of free enzyme in solution in presence of lignin (mg protein/mL)

E_1max is maximum amount of enzyme that can be observed on substrate (mg protein/g substrate)

E_{imaxL} is maximum amount of enzyme that can be observed in a unit mass of lignin (mg protein/g lignin)

E_{iT} is total enzyme concentration (mg protein/mL)

G is glucose concentration (mg/mL)

G_2 is cellobiose concentration (mg/mL)

K_1ad is dissociation constant for enzyme reaction with substrate (mL/mg protein)

K_{iadL} is dissociation constant for enzyme reaction with lignin (mL/mg protein)

k_{ir} is reaction rate constants (i=1 for cellulose to cellobiose, i=2 for cellulose to glucose, i=3 cellobiose to glucose) (mL/mg.h)

K_{iIG} is glucose product inhibition constant (i=1,2,3 is same as defined for kir) (mg/mL)

K_{iIG2} is cellobiose product inhibition constant (i=1,2,3 is same as defined for kir) (mg/mL)

K_{3M} is cellobiose saturation constant (mg/mL)

L lignin content (g/mL)

r_i reaction rates (i=1,2,3 is same as defined for kir) (mg/mL/h)

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