

**A Pilot Plant Scale Reactor/Separator
for the Reduced Energy
Production of Ethanol from Cellulosic Materials.**

A Proposal to the Energy Related Inventions Program

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Executive Summary

A Pilot Plant Continuous Stirred Reactor Separator for the Production of Ethanol from Cellulosic Materials is a proposal in which BPI follows up our current project with the NIST-DOE Energy Related Inventions Program (consisting of converting starch and sugars to ethanol in a Continuous Stirred Reactor Separator (CSRS) on the lab and pilot scale) with the application of the CSRS reactor technology to pretreated cellulosics. Lab work on process development for cellulose conversion is being done under the current project, and these efforts will be continued during the first 12 months of this new project, and then followed by a demonstration scale test of ethanol production from biomass/cellulosics using a 15,000 L. CSRS. This CSRS will be able to utilize the low energy SAED (solvent absorption-extractive distillation) ethanol recovery system designed and built as a part of the current project.

A high pressure-high temperature extrusion pretreatment (the Xylan Delignification Process) of biomass will be followed by a two stage enzymatic hydrolysis and fermentation process in the CSRS. A hot rinsing of the pretreated biomass will give a 4 to 6% hemicellulose /lignin stream. The first portion of the fermentation process is a simultaneous saccharification and fermentation (SSF) of this hemicellulose-xylose stream which is solubilized by the Xylan pretreatment. A strain of *Pichia stipitus* (which has been found to give very good conversion efficiencies of xylose to ethanol in our current studies) will be used to ferment the xylose as it is released. Following this simultaneous saccharification and fermentation (SSF) of hemicellulose in stages 1 and 2, the cellulose fibers are added to the broth for the second portion of the fermentation, the SSF of cellulose-glucose which occurs in stages 3, 4 and 5. These processes are combined with ethanol separation in the CSRS to allow high speed fermentation of the released xylose and glucose. Process kinetics and small scale batch and continuous tests will be performed at BPI's laboratory during the first 12 months of the project, with some tests performed on enzyme development and comparisons of commercial cellulases and hemi-cellulases at Purdue University as a sub-contract. A process model/design will be developed to allow optimization of the process conditions and to give cost estimates for biomass to ethanol facilities as a function of scale.

Total costs for the project are estimated at \$347,750 over a 24 month period. In this proposal we request \$92,500 from NIST-ERIP , with \$255,250 being provided by other project co-sponsors:.

Section 1. Technical Description

Introduction

The conversion of biomass (through enzymatic techniques) to ethanol consists of four basic steps, 1- pretreatment of the biomass to allow enzymatic attack of the bio-polymers, 2- conversion and fermentation of the hemicellulose/xylose fraction, 3- conversion and fermentation of the cellulose/glucose fraction, and 4- separation and concentration of the ethanol. There are many different technologies currently under development to convert biomass to ethanol, but despite some rather expensive development efforts, there are no successfully operating biomass to ethanol plants running presently in the US to our knowledge. In this proposal, we are focusing on an enzymatic, two stage conversion process of biomass to ethanol, using the CSRS reactor technology to speed fermentation rates. We feel as though this technology may have substantial advantages over acid based or competing enzymatic based processes which are under development by various organizations.

Biomass is a mix of three basic components, lignin, cellulose and hemicellulose. Lignin serves as a sort of 'glue' giving the biomass fibers its structural strength, while hemicellulose and cellulose polymers are the basic building blocks of the fibers. In order to break down the hemicellulose and cellulose to sugars, the basic structure of the biomass must be attacked. Once the structure of the biomass is disrupted, the hemicellulose and cellulose can be converted to sugars enzymatically.

Pre-treatment

In this project we will utilize the peroxide extrusion process and cooperate with Xylan Inc who have developed this technology. Xylan Inc has agreed to both provide samples of different cellulosic materials pretreated using their process and to provide one truck load (60,000 pounds) of pretreated corn stalks for our pilot scale test. The Xylan-Delignification-Process (XDP) utilizes extrusion technology in conjunction with alkaline hydrogen peroxide. Carr and Doane (1984) reported on the use of a similar process for straw pretreatment, and Chen and Wayman (1989) also utilize this sort of treatment for Aspen pretreatment. The Xylan method continuously treats lignocellulosic biomass by reacting the biomass with a reaction medium containing an aqueous solution of alkali agent (pH 11.5) which softens the lignin and allows water to enter the biomass. The cellulosic biomass is then fed into a pressurized extruder/ reactor in an oxygen atmosphere at a temperature between 235-275 F. and pressures up to 400 psi. These high temperatures and pressures allow minimization of chemicals as compared to other technologies for cellulose pretreatment (low acid-high temp, or high acid-low temp). The mechanical extrusion system mixes, grinds, sterilizes, and disrupts the cellulosic biomass cell walls. Exiting the reactor barrel is a liquid/solid mixture stream containing lignin and hemicellulose sugars,

and cellulose fibers, suitable for paper, cattle feed, or enzymatic hydrolysis to glucose. Exit temperatures from the extruder are in the range of 115 to 125 C.

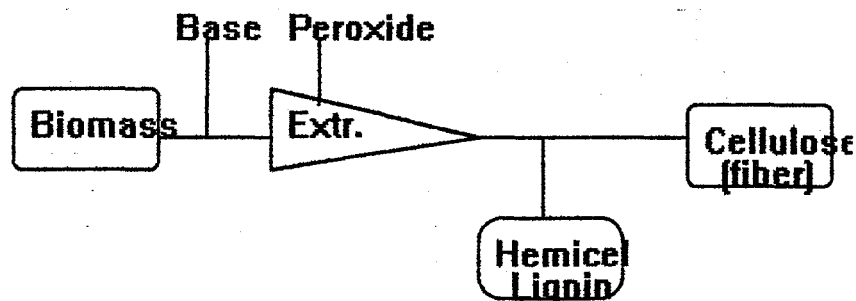


Figure 1. Xylan Delignification Process

Tyson (1993) suggests that temperatures of 138 C and a pressure of 340 psi seemed best during tests with hardwoods. Hydrogen peroxide is added to the barrel of the extruder to help catalyze the breakdown of the fibrous biomass structure. The wet /fibrous product from the extrusion process consists of a lignin /soluble hemicellulose stream and a fibrous cellulose. Squeezing and washing the cellulose gives two streams, the lignin/soluble hemicellulose, and the solid fibrous cellulose. The cellulose stream could be used for ethanol production, paper production, or cattle feed. In this project, we will be concentrating on ethanol production from this fibrous solid material.

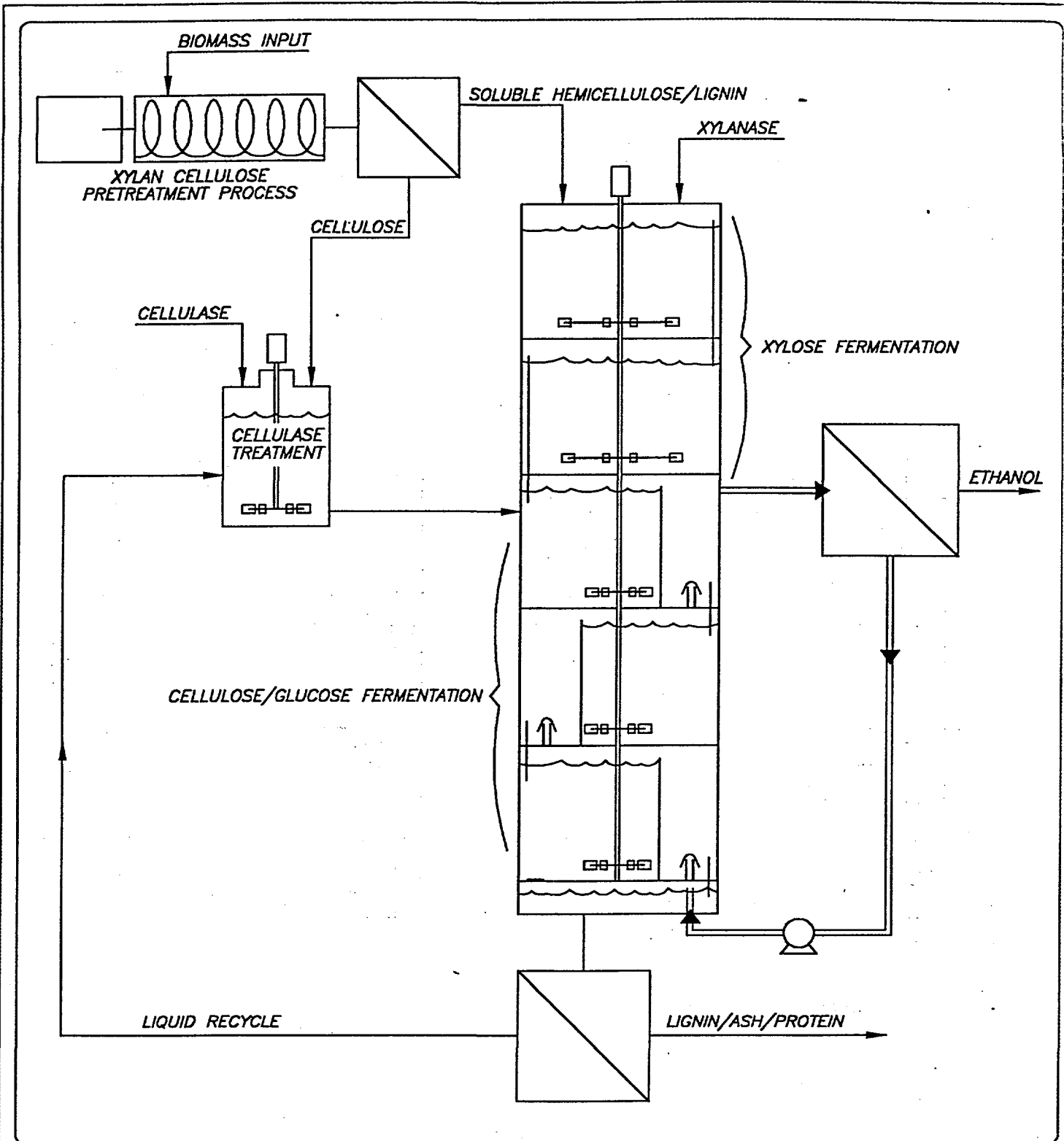
The liquid lignin/soluble hemicellulose stream is easily converted to xylose enzymatically, after which it can be converted to ethanol using various fermentation strategies. In previous lab tests, when treating this stream (from a pre-treated wood chip process) with hemicellulase, the conversion of the hemicellulose to xylose was noted, with 20 g/l xylose being determined after hydrolysis along with 2.2 g/l of glucose (Tyson, 1993). The cellulosic fibers stream can then be broken down to glucose via a similar enzymatic treatment. The cellulosic fiber stream, when added to water and hydrolyzed with cellulase gave a 10.7 g/l ethanol product during a Simultaneous Saccharification Fermentation (SSF) with a 31.9% yield of ethanol from dry matter yield (Tyson, 1993). Co-products of the fermentation included lactic acid and acetic acid at 3.3 and 1.6 g/l final concentrations respectively with 4.2 g/l glucose also remaining unfermented (Tyson, 1993). SSF of corn stalks pretreated by the Xylan process as run in a batch test are shown in Figure 2 (Tyson, Dale, Zhou and Lei, 1995)

Fermentation

The major goal of this project is operation of a demonstration scale CSRS unit running on biomass materials. We plan to build a 15,000 liter pilot plant to demonstrate the cellulose to ethanol technology shown in Figure 3. The economics of biomass conversion to ethanol are greatly improved if both the hemicellulose and the cellulose are converted to ethanol (Ladisich and Swartzkopf, 1993). This Xylan Delignification Process (XDP) causes a breakdown of the lignin and hemicellulose which can then be separated from the cellulose by rinsing. Fermentation of the xylose can then occur prior to cellulose fermentation. This pre-saccharification of xylose eliminates the problem of trying to ferment xylose in the presence of glucose. Grooten et al (1991) suggests a similar strategy following the poor results noted in his co-fermentation experiments. The conversion of xylose to ethanol is recently been improved with a variety of strains found to be able to efficiently convert xylose to ethanol. These strains include *Pichia stipitis*, *Pachysolen tannophilus*, *Candida shehatae*, and genetically modified yeast and bacteria. Thermophilic anaerobic conversion of cellulose to ethanol has also been demonstrated by several researchers (Zertuche and Zall, 1982; Shahbazi et al, 1991) but this organism is only able to produce 5 to 7 g/l or less of ethanol, and generally produces a number of side products (organic acids).

Genetically engineered *Saccharomyces cerevisiae* able to ferment xylose have been reported by Tantirungkij et al (1993) who were not able to actually generate much ethanol, and Ho et al (1994) who seems to have successfully completed a fermentation of both glucose and xylose with a transformed yeast. A recombinant *E. coli* has been shown to be able to ferment xylose well, with optimal pH of 6 to 7 (Bealle et al, 1991). Actual fermentation of biomass hydrolysate has not been able to duplicate these results, with ongoing experiments trying to determine the components poisoning the fermentation (Bothast, 1994).

Work performed during the current phase of this project led to the selection of one strain of *Pichia stipitus* which showed a conversion efficiency of 0.46 g ethanol per g xylose (Zhao and Dale, 1994- Appendix 2). These high conversion efficiencies however, were accomplished at a much lower specific ethanol production rate (gram ethanol per gram cell per hour) than is generally accomplished with *S. cerevisiae*. Thus, high cell densities will need to be maintained to keep the residence time under 24 hours on the two xylose fermentation stages. These xylose fermenting strains are much more strongly inhibited by ethanol as well, with a maximum ethanol tolerance of about 3%. We will continue to monitor the literature for organisms which might have performance advantages as compared to the strain we currently plan to utilize. Work during this project will include lab scale testing of cellulose to ethanol process design and testing followed by pilot scale implementation of the process. During the final 3 months of the project, the 15,000 liter unit will be operated on pretreated corn stalks.



Drawing Title:
Figure 3 BIOMASS PROCESS

Drawing No.
 SCHEM6

Scale: NONE Date: 3/13/95

Issue: Drawn By: BLK

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The Continuous Stirred Reactor Separator (CSRS) consists of stirred reactors matched with gas-liquid contractor stages. Removal of the ethanol during fermentation allows the fermentation rate to increase. Phase 1 work with the assistance of the NIST-DOE Energy Related Inventions Program led to the construction, testing and operation of a 24,000 liter pilot scale CSRS unit during 1994 with operation shown on starch and sugar streams. This CSRS is a new type reactor which allows simultaneous saccharification, fermentation, and ethanol separation in a combined process. Combining these reactions allows significant improvements in each operation. To operate the CSRS on biomass rather than starch, two cultures will be maintained within the reactor, a xylose fermenting yeast strain on stages one and two, and glucose fermenting yeasts on stages three, four and five.

The reactor's separation stages between stages 3, 4, 5 and exit must be designed so as to not clog with cellulose fibers. We tentatively plan to use a sieve plate design with slightly reduced plate area between holes to keep solids from settling. Downcomers will also be designed to be clog resistant. The designs will be tested on a 6 liter glass lab-scale unit during months 1-12 of the project.

Enzymes

Cellulase and/or xylanase will be added to the stages to release the sugars from the biomass. If the enzymes must be purchased, and are not able to be recycled, enzyme costs become an expensive part of the fermentation process. Enzyme costs for biomass hydrolysis were estimated by Lynd et al, (1991) as falling from \$1.00/gal for a 24 hour hydrolysis, to \$0.20/gal for a 5 day hydrolysis. Fermentation costs were estimated to increase from \$0.20 to \$0.38/gal as fermentation time increased. These sorts of high enzyme costs are a significant barrier to commercial implementation of enzyme based biomass to ethanol processes. In informal verbal conversations with H. Pesonen of ALKO LTD. (a Finnish company making and selling grain to ethanol fermentation systems), Mr. Pesonen stated that in their biomass to ethanol efforts, they found it necessary to generate their own enzyme on site to allow economical enzyme conversion of biomass to ethanol.

In this project, we will prepare some of our own cellulase/hemicellulase from three to four different organisms. Preliminarily we plan to grow strains of *Trichoderma reesei*, *Thielavia terrestris*, and *Aspergillus niger* as these cellulase producing fungi have temperature and pH optimums for growth and productivity near to yeasts. Co-culture and pre-culturing media to develop cellulases will then be examined. Fed batch and induction systems as proposed by Hendy et al (1984), Mandels and Weber (1969) and McLean and Abear (1986) will be evaluated to maximize enzyme production. We also plan to preliminarily evaluate a thermophilic bacterium, *Acidothermus cellulolyticus* which has been shown to excrete thermotolerant cellulases and is being developed by NREL researchers. A recent patent (Tucker et al, 1992) describes the use of this organism to

produce thermotolerant cellulase enzymes. The enzymatic breakdown of cellulose/hemicellulose requires three basic types of cellulase, endoglucanase which breaks down the cellulose polymer from within the chain, exoglucanase which works from the ends of the cellulose polymer, and cellobiase. Preliminary process design calls for the continuous production of cellulase in the hydrolysate tank (Figure 3) by culturing the cellulase organisms along with the pretreated cellulose. The broth from the CRS containing the cellulase enzymes will be recirculated to the hydrolysate tank, allowing the enzyme levels to build-up over time. The cellulolytic organism, and optimal conditions in the enzyme generation stages will be designed during phase 1 and 2 based on experimental findings and performances on small scale tests. The possibility of separate production and or concentration of the cellulase to the hydrolysis tank will also be studied and economically compared to the purchase of commercial cellulase. If recycling or immobilization of the enzymes allows economical use of commercial enzymes then this option will be tested on the pilot unit. The possibility of co-culturing cellulolytic molds with the fermentation media will be evaluated as will some preliminary trials with immobilized enzymes as per Chen and Wayman (1989).

2. Technical Advantages

Bio-Process Innovations Inc. has designed, modeled and patented the CSRS for the production of ethanol from various substrates. This novel reactor/separator is coupled with a solvent ethanol recovery system to give a low energy continuous process for the production of ethanol from starch or biomass. Combining reaction with separation allows the fermentation of concentrated streams even if the fermenting microbe is strongly inhibited by ethanol. Simultaneous saccharification and fermentation of polysaccharides such as hemicellulose and cellulose can be quickly completed in this bio-reactor/separator, with the fermentation coupled with ethanol removal. Combining these reactions within the reactor vessel allows significant improvements in each operation as both enzymatic and ethanol reactions are product inhibited.

The reactor-separator concept of this invention consists of stirred tank type reactors operated in series, with the liquid streams moving from tank to tank contacted with a stripping gas to remove the ethanol product. Saccharification (of both polymers such as starch and cellulose) is sped by the reduction of sugar concentration as the sugar is fermented to ethanol. Fermentation is sped by the removal of the toxic ethanol product, and ethanol purification and concentration costs are reduced by the enrichment of the ethanol in the vapor phase. The gas stream is co-current to the tank to tank liquid flow in the enriching section, and counter-current in the stripping section. The final effluent from the CSRS is ideally characterized by complete saccharification of all polysaccharides (hemicellulose and cellulose polymers), complete fermentation of sugars to ethanol and complete removal or separation of the ethanol into the gas phase.

A schematic of the CSRS process is shown in Figure 3 for a system for biomass conversion to ethanol as suggested in this proposal.

The current project has included process development and demonstration of a low energy Solvent Absorption/Extractive Distillation (SAED) system for the recovery of anhydrous ethanol from ethanol vapors leaving the reactor/separator. A diagram of the SAED system is shown in Figure 4. As part of the current project, non-toxic, non-volatile solvents have been screened and tested. We have determined a solvent mixture which will allow good selectivity for ethanol over water. Our calculations based on the properties of this chosen solvent mix indicate that anhydrous ethanol may be obtained from concentrated sugar feed streams with an energy consumption ranging from 22,000 btu/gal at 5% sugar feed to 5,500 BTU/gal at 30% sugar feed by using a temperature swing between the absorber and the reactor as shown in Figure 5. This corresponds to a savings of about 50% over conventional processing using the current industry standard of distillation and molecular sieve dehydration as also shown in Figure 5.

There are several basic methods for cellulose breakdown, strong acid, dilute acid, ammonia explosion, steam explosion, and peroxide extrusion. Acid (sulfuric or hydrochloric) can serve both for disruption and hydrolysis of the cellulosic polymers. Strong acid allows complete breakdown of the components in the biomass to sugars, but also requires large volumes of concentrated sulfuric acid and can result in the production of furfural, an inhibitory byproduct (Goldstein and Easter, 1992; Ladisch and Swartzkopf, 1991). Dilute acid allows reduced acid concentrations, but requires higher temperatures, and again gives furfural. Ammonia explosion is a process being promoted by AFEX (B. Dale et al, 1985; AARC Bulletin, 1994)) which uses a quick pressure reduction after soaking the biomass with liquid ammonia solution. This system does not directly liberate any sugars, but allows the polymers (hemicellulose and cellulose) to be attacked enzymatically and reduced to sugars. Steam explosion and peroxide extrusion are similar processes. Steam explosion is being developed by Stake Technology which involves extrusion of the biomass at a high temperature and pressure, while peroxide extrusion uses a chemical pretreatment along with extrusion to accomplish the same goal of breaking down the internal structure of the biomass fibers. Steam explosion and peroxide extrusion allow enzymatic hydrolysis of the cellulosic polymers.

The Xylan process, by eliminating acid hydrolysis eliminates the production of toxic byproducts or carbonization (acid burning) associated with conventional sulfuric or hydrochloric treatments. The Xylan Delignification Process (XDP) seems also to be simpler and lower cost than the ammonia explosion (AFEX process, Dale et al., 1985) in which a high pressure explosion chambers must be developed, tested and scaled-up. We will follow the XDP process with an enzymatic hydrolysis of the biomass polymers. Enzymatic hydrolysis is slower than

acid or caustic chemical hydrolysis, but does not require the large volumes of chemicals, chemical recovery or neutralization, or the plant to be constructed of special acid/caustic compatible materials. We feel that the mild conditions and low chemical costs associated with enzymatic hydrolysis will outweigh the advantages of chemical (acid or caustic) hydrolysis. Both concentrated and dilute acid processes for biomass conversion are being pilot tested by TVA and NREL researchers (Barrier et al, 1986).

3. Energy Impact

This project will have two basic energy impacts. First, the goal is to replace fossil/imported oil energy sources with renewable, domestic liquid fuel, i.e. ethanol as a gasoline extender/replacer. Secondly, the goal is to produce ethanol economically in an environmentally friendly and energy efficient manner from domestic cellulosic biomass sources. Currently, almost all ethanol in the US is made from corn/grain starch sources. There is currently excess grain producing capacity in the US which allows the use of this grain for ethanol production, but in the long run, as export markets improve, it may be more beneficial to use the grain for human consumption. Biomass resources in the US are enormous, and include paper wastes, urban lawn wastes, wood pulp, straw, corn stalks, saw dust, sugar cane bagasse, etc. There is also a fair amount of current research on production of biomass crops such as switch grass and alfalfa for biomass to ethanol processes with a recent issue of Biomass & Bioenergy devoted to research papers on dedicated biomass feedstock supply systems in the US (B&B 6:3 1994).

Our efforts in this project focus on energy and capital savings for a process involved with the conversion process of cellulose to sugars and ethanol. Energy savings are attained by combining the CSRS reactor concept with solvent absorption of the ethanol from the gas stream exiting the CSRS. The enrichment of ethanol in the vapor stream (with a concentration of 5 to 8 times higher than liquid) combined with a further enrichment of ethanol in the solvent absorber (limited water solubility in the solvent) allows highly concentrated ethanol to be easily recovered from the solvent in a solvent stripping column. An anhydrous ethanol product may be recovered from the solvent using a simple extractive distillation procedure using the same solvent used for the ethanol absorption. As the ethanol concentration in the stage from which the gas exits is allowed to increase, energy costs for ethanol recovery drop. A part of this years project involved solvent testing and selection, combined with design and installation of the SAED system.

4. Cost and Economic Considerations

Ethanol production in the USA offers a renewable source of liquid fuel produced within the borders of our own nation as well as offering a market for excess grain/biomass crop production capacity of the midwestern states. However, in order for the ethanol fuel industry to be able to expand without governmental subsidies, ethanol production costs must be reduced closer to the level of refined unleaded gasoline (\$0.55-\$0.75 per gallon). Ethanol production costs can be reduced via:

- 1) reducing costs of substrate
- 2) increasing the efficiency of substrate conversion to ethanol
- 3) reducing the energy costs for purifying and dehydrating the ethanol
- 4) reducing the amount of bottoms waters which must be treated
- 5) reducing the capital costs for the ethanol processing plant
- 6) reducing the labor for operating the ethanol plant

The reactor/separator technology as being developed in the current project can meet several of these goals, namely increased conversion efficiency, reduced effluents, reduced capital, and reduced labor. In this proposal, we are trying to meet the first goal: low substrate cost through the utilization of biomass/paper wastes. In this proposal we suggest the testing of the lab and a 15,000 liter pilot unit on cellulosic materials (corn stalks). We would hope that this project will lead directly to the implementation of cellulose to ethanol on full scale plant size using waste cellulose such as waste paper, separated paper/cellulose from municipal solid waste plants, field crops such as switch grass and corn stalks, and saw dust.

As our nations oil supplies are depleted, and clean air requirements are stiffened, the need for ethanol fuels is becoming a national priority. If, however, current high energy technology (utilizing coal fired boilers) uses 40 to 120,000 BTU's of coal energy to produce 84,000 BTU's of liquid fuel energy per gallon of ethanol, the net effect is to produce a lot of coal fired boiler stack gas emissions to reduce car exhaust emissions. The overall effect on the US environment of this trade-off is open to debate. Current batch fermentation technology for ethanol production from corn requires large scale operations (12-50 million gal/yr. of ethanol), a large capital investment (\$2-4.00/ annual gallon), and is energy intensive. About half of this energy is associated with drying and evaporating the stillage, and half with fermentation and distillation. Presently, ethanol production level in the USA is at about 1,100 million gallons/yr. The total market for ethanol as a 10% blend in gasoline would be 12 billion gallons. As more and more ethanol is produced, it is important to our net energy position that ethanol be produced domestically in an energy efficient fashion. It is also important to develop processing for alternative feed stocks, such as biomass crops, rather than corn. Ethanol fuel production can help utilize excess corn capacity

(perhaps 20% of the nations crop) but further ethanol production must use other feedstocks if corn and grain prices are to be kept within 30 to 40% of the current levels.

A preliminary cost analysis for the Xylan/BPI biomass ethanol process was prepared based on cost and power estimates on extruders from Insta-Pro Intl. (a manufacturer), and cost estimates for the CSRS based on model size estimates and design costs equations as presented by Wood (1980, Engineering Economics in the Chemical Processing Industry). This analysis is presented in Table 1 for four basic scales, 1 million gal/yr, 5 million, 10 million, and 50 million gallons of ethanol per year. Assumptions were based on biomass composition of 35% cellulose, 28% hemicellulose, 30% lignin, and 8% ash. Only the costs of pretreatment and fermentation were determined. We did not estimate the costs of other necessary auxiliaries associated with a biomass to ethanol facility (raw material, labor, storage facilities), as these costs would be site specific (based on the raw material). As per Table 1, a net cost of between \$0.28 and \$0.35/gallon was determined for the process due to required fermentation nutrients, pretreatment chemicals, and energy costs to distill anhydrous ethanol. Pretreatment chemicals were determined to cost about \$0.120/gal. During our year 1 investigations, the possibility of substituting lower cost chemicals (specifically ammonia for sodium hydroxide, and lower amounts of hydrogen peroxide) will be studied with the intent of bringing this cost down to about \$0.05/gal. Labor, raw material, and waste treatment costs were not determined as mentioned previously. However, even at the smaller scales evaluated, 1 and 5 million gal/yr, a net cost per gallon (for pretreatment, fermentation, and ethanol recovery) of \$0.302 to \$0.355/gallon of anhydrous ethanol was determined. If the ethanol is sold at \$1.05/gallon, a ROI of 61% can be determined at the 1 million gallon scale, and 140% at the 5 million gal/yr scale suggesting that the process should in fact be quite profitable, even when added costs for labor, materials handling/storage, and raw product are added. By-product sales of yeast, CO₂ and lignin could also help improve the bottom line of the bio-refinery. Dried yeast currently sell at about \$.30/# for feed purposes, while lignin (sodium lignate sulfanate) has a selling price of around \$0.16/# (Chem. Marketing Rep, 1994).

Section 5. Commercial Potential and Market Considerations

Biomass is a generic term for plant substances which can be harvested from crop and timberlands and used as a renewable source of organic chemicals. A major goal within the Dept. of Energy and US Dept. of Agriculture is the development of an economic and environmentally responsible bio-fuels program to replace or supplement the use of petroleum as a liquid transportation fuel. Each gallon of ethanol produced in the USA displaces about 0.7 gallons of petroleum on an energy value basis. Our nation is currently importing about 50% of our petroleum needs (Gov. Et. Coal., 1994) with 110 billion gallons of gasoline con-

sumed in 1992 (Biofuels Update, 1994). Non-food crops such as switch grass and poplars grown on ground not used for food crops could produce 190 to 270 million tons of cellulosic biomass, which could then be turned into 30-50 billion gallons of ethanol (Brower, 1994). The major goal of this project is to help develop cost effective technology for the conversion of cellulose to ethanol, to allow the substitution of a renewable resource, biomass, for a non-renewable resource, oil. There is also a national security issue, in that our nation is dependent on foreign oil for 50% of our oil needs. Renewable resources, within the bounds of our own nation, could replace these imports with ethanol, and provide many jobs in production, collection and processing of the biomass.

Ethanol production in the USA offers a renewable source of liquid fuel produced within the borders of our own nation as well as offering a market for excess grain/biomass crop production capacity of the midwestern states. However, in order for the ethanol fuel industry to be able to expand without governmental subsidies, ethanol production costs must be reduced closer to the level of refined unleaded gasoline (\$0.55-\$0.75 per gallon). We hope that this project will lead to the implementation of cellulose to ethanol processes on full scale plant size using waste cellulose such as waste paper, separated paper/cellulose from municipal solid waste plants, field crops such as switch grass and corn stalks, and saw dust.

In a recent RFP from the Great Lakes Biomass Council (1995) it is reported that the EPA mandates on reformulated gasoline should increase the demand for ethanol by over 600 million gal/yr. Also reported are the following benefits of ethanol production in the USA:

- 1) the GAO estimates that increasing ethanol production from the current 1 billion gal/yr to 2-5 billion gal/yr would increase net farm income by \$415 million over an 8 year period.
- 2) The Federal government would save approximately \$500 to 600 million/yr even if tax subsidies to ethanol are continued at current levels.
- 3) Utilization of agricultural wastes as a feed stock will reduce price pressures on higher value feedstocks (grains).

It is our hope that current large scale ethanol producers will be able to expand production using this technology- perhaps continuing using current facilities for corn to ethanol while adding on biomass to ethanol capabilities. We also plan to market the process to farm co-ops in rural areas where biomass production could supplement or replace traditional grain crops. Finally, we see a market in the Municipal Solid Waste (MSW) market where paper/cellulose are being picked up and recycled. Goodman and Walters (1991) show that about 66 million tons/yr. of paper and paperboard are estimated to be discarded into MSW in the year 2000 along with 19 million tons of yard wastes. If 30% of this stream were converted to ethanol at a conversion rate of .3# ethanol/1 pound biomass, these streams alone could give 2.3 billion gallons/yr. of ethanol. Agricultural

crop residues such as corn stalks, wheat straw, or cotton stems have been estimated to be about 812 million tons/yr. which if 30% utilized could give 22 billion gallons of ethanol. The possibility of biomass crops such as hemp, kenaf, or other highly productive crops grown on marginal lands could expand this biomass availability even more. We feel that this development and demonstration project will allow the demonstration of several symbiotic technologies. The Xylan pretreatment system allows simultaneous saccharification and fermentation, while the CSRS further improves the kinetics of fermentation by keeping the ethanol levels low, which is particularly important during the first xylose-fermentation stages. Xylose fermenting yeast show fairly complete inhibition at 30 g/l ethanol. We hope that this technology would be applied to rural small scale ethanol production from biomass crops in the mid-west states, ethanol from cane bagasse in southern states, ethanol production from paper/cellulose separated from municipal solid waste (MSW) streams and recycled paper streams, saw dust from saw mill operations, and similar projects. It is a major goal of BPI to develop cost effective, low labor, efficient ethanol production processes and to market-license this technology as quickly as possible.

There are several competing technologies/processes for both biomass pretreatment and biomass fermentation. Which of these biomass systems is eventually most widely used is purely a response of the market to the profitability of the process. The Xylan process, by eliminating acid hydrolysis eliminates the production of toxic by-products or carbonization (acid burning) associated with conventional sulfuric or hydrochloric treatments. The Xylan process seems also to be simpler and lower cost than the ammonia explosion (AFEX process, Dale et al., 1985) in which a high pressure explosion chambers must be developed, tested and scaled-up. We will follow the XPD process with an enzymatic hydrolysis of the biomass polymers. Enzymatic hydrolysis is slower than acid or caustic chemical hydrolysis, but does not require the large volumes of chemicals, chemical recovery or neutralization, or the plant to be constructed of special acid/caustic compatible materials. We feel that the mild conditions and low chemical costs associated with enzymatic hydrolysis will outweigh the advantages of chemical (acid or caustic) hydrolysis. The expense of purchased enzyme is one concern associated with this process, hence the possibility of co-producing enzymes at the plant rather than purchasing the enzyme will be a part of our research/economic investigation.

Fermentation processes for biomass hydrolysate are being developed by Quadrex, Inc. who suggest a capital cost of \$2.00 per gallon at the 10 million gallon scale for their process (Co. Lit. 1992). Our calculations indicate a capital cost of under \$1.00 per gallon at this scale for our XDP/CSRS process. Quadrex have a genetically engineered bacteria which is able to metabolize both glucose and xylose well (Bealle et al, 1991) but actual performance of the organism in biomass hydrolysate has not been good due to poisoning (Bothast, 1994). Ho et al. (1994) at Purdue are currently developing a yeast which can apparently fer-

ment both xylose and glucose if nutritional conditions are rich. We feel our yeast strain may be the best currently available, but we will certainly be watching for better and more robust fermentation organisms which could be used in our reactor system.

Section 6. Needed Support/Budget

This project holds the prospect of making a significant impact on the energy use during production of liquid fuels in the US. BPI is a very small company with little or no financial resources to develop some of the ideas discussed in this project proposal. Thus governmental assistance during this development/demonstration phase of the invention process is critical to the timely development of the technology. We feel fairly confident that the input of governmental funds at this stage of our project will eventually return many times this investment in infrastructure, jobs, and taxes paid to the US government.

Work Plan

This project is divided into two phases, 1) the basic phase consisting of laboratory studies and process design, and 2) an applied or demonstration phase focusing on operation of the 15,000 liter CSRS for the production of ethanol at Permeate Refining Inc, in eastern Iowa.

Basic Research-The basic research will be performed at BPI's laboratory in W. Lafayette, IN. Dr. Dale will directly oversee the development and testing of the cellulosics fermentations, while the actual experimentation will be performed by Mr. Zhao, a biochemical engineering researcher. Mr. Zhao will be working on this project on a 75% time basis developing enzymes, time/temperature extraction data, and improving conversion efficiencies for biomass to ethanol. During the current ERIP study, xylose conversion kinetics for several strains of yeast were determined and one strain singled out for our further use. Kinetic data obtained in the lab will be incorporated into a process model of the cellulose conversion/fermentation/ethanol separation in the CSRS fermenter by Dr. Dale of BPI who has extensive experience in modeling of fermentation and separation systems (Dale et al, 1990). There will be three parts to our basic process development research:

- 1) Cellulase/hemicellulase production and performance comparison- The enzymatic conversion of the hemicellulose and cellulose tends to be the rate limiting step during the simultaneous saccharification and fermentation of these polymers. Strains of cellulase producing enzymes, (*T. reesei*, *A. niger*, *Thielavia terrestris*, and *A. thermocellum*) will be compared in lab studies for cellulase and hemicellulase activity. Based on these studies, the continuous cultivation of cellulase by one of these organisms will be implemented on the pilot study. Commercial enzymes will be compared for cost and effectiveness in hemi- and

cellulose hydrolysis. These will be obtained from three major enzyme companies 1) Solvay, 2) Genencor, and 3) Novo.

2) Bench scale testing of proposed biomass fermentation process-

A 4 liter CSRS has been constructed and is currently being operated to identify possible process improvement which would expand the application of the CSRS technology. This unit may have to be rebuilt to handle the biomass as some clogging problems were noted with starch, and the biomass, due to its fibrous nature will show an even stronger tendency to clog a bubble plate type contactor. We plan to build a glass CSRS lab scale unit with sieve type gas-liquid plates. We will implement the best enzyme/temperature program as determined from our batch scale tests along with the two stage (hemi/xylose SSF followed by cellulose/glucose SSF) process as shown in Figure 3. This reactor system will be operated from months 6 through 12. Experiments designed to improve the performance of the entire process will be continued during the entire course of the project. In this phase of the research we will also be testing the use of liquid ammonium hydroxide to replace sodium hydroxide as a pre-softening agent. If successful, this will reduce the cost of pretreatment chemicals substantially, as well as reducing the salts in the stream, as the ammonia will act as a nutrient during the fermentation process.

3) Process modeling/economics- A detailed process model of the process will be constructed including kinetics of enzyme hydrolysis, yeast conversion kinetics, and reactor size and ethanol separation characteristics. This model will be used to help optimize operational parameters for both lab and pilot scale tests, and to design and determine economics of full scale biomass to ethanol plants on various substrates at various scales of operation.

Applied Research- A pilot scale 15,000 liter 5 stage CSRS will be built during months 12-16, and tested on cellulosic feed stocks at the ethanol plant in Hopkinton during months 17-24 of the project. Xylan Inc will provide 60,000 pounds (one semi truck load) of pretreated corn stalks. Dr. Dale of BPI will develop the detailed designs for the 15,000 liter, 5 stage CSRS system by month 12, using experience gained from the glass lab scale unit. The unit will be built during months 12-15 by Merrill I&S and installed by month 16. The CSRS will then be run on pretreated corn stalks, with 60,000 # of stalks provided by Xylan Inc. over a period of 3 to 6 months

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