DOE/EE/15958--T2

ERIP/DOE Quarterly Report #3 & 4

April 1, 1998 through Sept. 30, 1998

A Pilot Plant Scale Reactor/Separator for Ethanol from Cellulosics

ERIP DOE Project DE-FG01-97EE15958

Bio-Process Innovation
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Project Aim

The objective of this project is to develop and demonstrate a continuous, low energy process for the conversion of cellulosics to ethanol. This process involves a pretreatment step followed by enzymatic release of sugars and the consecutive simultaneous saccharification/fermentation (SSF) of cellulose (glucans) followed by hemi-cellulose (pentosans) in a multi-stage continuous stirred reactor separator (CSRS).

During quarters 3 and 4, we have completed a literature survey on cellulase production, activated one strain of *Trichoderma reesei*. We continued developing our proprietary Steep Delignification (SD) process for biomass pretreatement. Some problems with fermentations were traced to bad cellulase enzyme. Using commercial cellulase enzymes from Solvay & Genencor, SSF experiments with wheat straw showed 41 g/L ethanol and free xylose of 20 g/L after completion of the fermentation. From corn stover, we noted 36 g/L ethanol production from the cellulose fraction of the biomass, and 4 g/L free xylose at the completion of the SSF. We also began some work with paper mill sludge as a cellulose source, and in some preliminary experiments obtained 23 g/L ethanol during SSF of the sludge.

During year 2, a 130 L process scale unit will be operated to demonstrate the process using straw or cornstalks. Co-sponsors of this project include the Indiana Biomass Grants Program, Bio-Process Innovation.

DISCLAIMER

Project Objectives

There are three basic objectives to this ERIP project, 1) some basic research on co-production and/or recycling of cellulosic enzymes and development of pretreatment processing which allows enzymatic breakdown of the biomass 2) a lab scale development phase where the process is operated on a small scale, and process modeling, design, and economics, and finally, 3) a demonstration scale CSRS being built and operated.

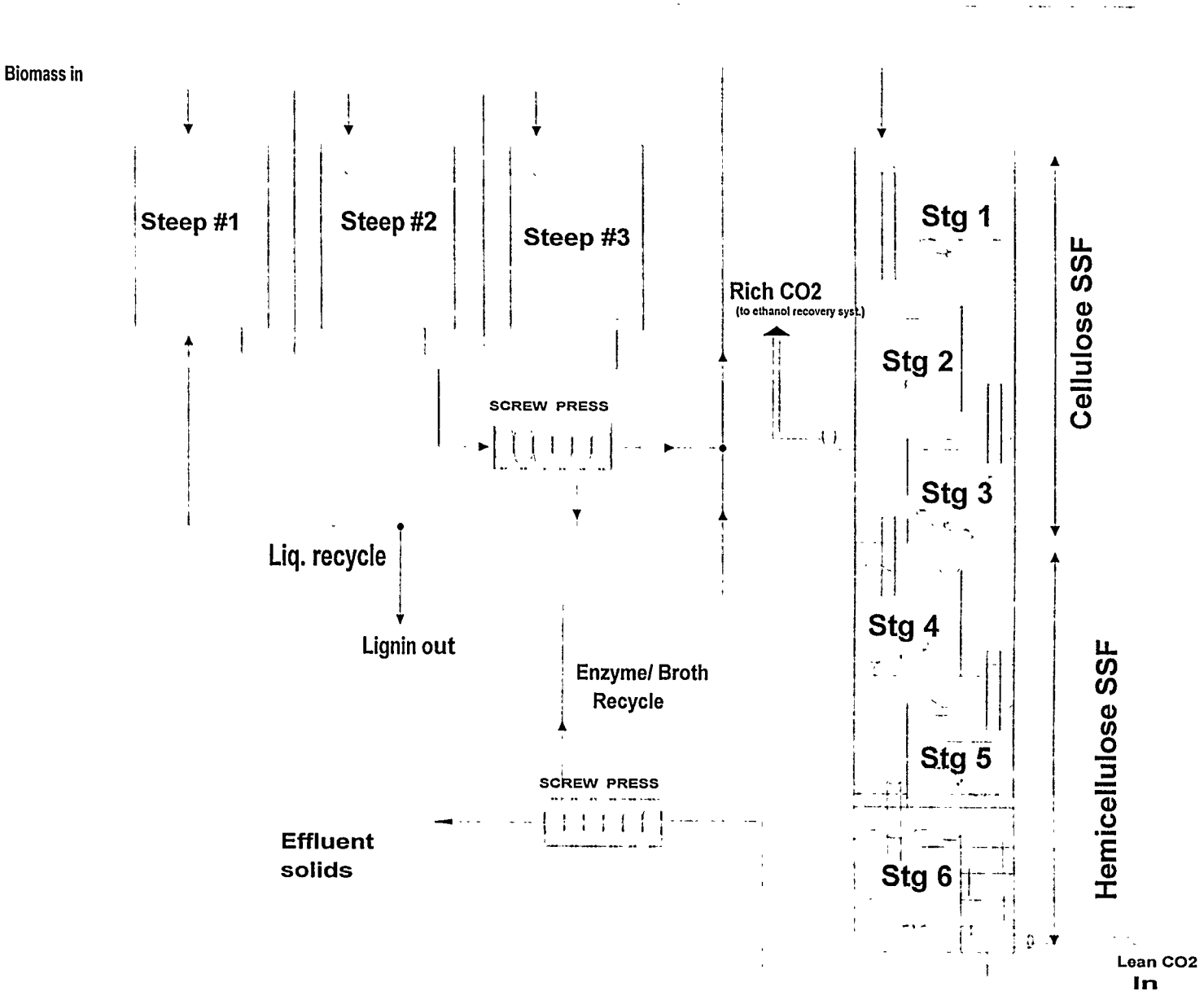
General Approach

Cellulosics, or biomass sources, are a great internal renewable resource for fuels and chemicals for the USA. In this project, we will be using corn stover and straw as biomass sources to produce ethanol. Ethanol production from cellulose is currently hampered by several major difficulties, most notably 1) the difficulty in reducing biomass to fermentable sugars economically, and 2) the difficulty in fermenting xylans, or five carbon sugars which are the breakdown monomer of hemicellulose, which constitutes between 15 and 25% of most cellulosics. There are two basic methodolgies for breaking down cellulosics to constituent sugars, acid and enzymatic (cellulase) processes. In our work, we will be focussing on an enzymatic process which should require much milder pH's and reduced capital costs. However, if enzymes are purchased, the enzyme cost alone can cause the biomass based ethanol to cost more than the current market price for ethanol.

The goals of this project are thus to make progress towards reducing three basic barriers to commercialization of biomass to ethanol via enzymatic hydrolysis, 1) co-production and/or recycling of cellulase (and hemicellulases) will be examined closely, 2) a low temperature/low pressure chemical/ chemical recycle process for biomass pretreatement, and 3) successive fermentation of cellulose/glucose followed by hemicellulose/xylose in a new bioreactor, the continuous stirred reactor/ separator (CSRS).

Ethanol production from cellulosics can be improved by various means:

1) Develop a high density of cells within the reactor so as to convert sugars to ethanol quickly
2) Combine enzymatic conversion of cellulose and hemi-cellulose polymers with fermentation so as to keep sugar levels low, improving enzymatic conversion rates
3) Convert both xylose and glucose to ethanol
4) Either co-produce crude cellulase enzyme or recycle the enzymes so as to reduce enzyme costs.
5) Separate ethanol from the reactor broth so as to keep reaction rates high



Notes

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Title: Architectural Drawing Board

Date: 4/98

Scale:

Sheet: ANSI D Size (22 in x 34 in)

A central effort of this project is to develop and demonstrate a continuous stirred reactor separator for the successive fermentation of xylans and glucans which will incorporate all the above design parameters.. Biomass generally consists of about 25-30% lignin, 25-30% hemicellulose, and 30-45% cellulose. The ideal process for biomass conversion to useful products must utilize each of these components. We are developing a delignification/pretreatment, followed by enzymatic release of xylans and glucans from the hemicellulose and cellulose respectively. Combining reaction (fermentation) with enzymatic release of sugars (Simultaneous Saccharification and Fermentation or SSF) improves the enzyme kinetics due to reduction of product inhibition. The basic process flows of the CSRS process for cellulose as we currently envisage it are shown in Figure 1.

Progress Report

This project is divided into three phases, 1) a basic phase consisting of laboratory studies on enzyme production, enzyme performance and enzyme recycling in cellulose breakdown, and pretreatment effectiveness, 2) a lab development stage consisting of operating batch bench scale saccharification fermentations (SSF) trials and 3) an applied or demonstration phase focusing on construction and operation of a 130 L or larger CSRS for the production of ethanol. During quarters 3 and 4, we have continued development of our non-acid pretreatment process, testing performance on several different biomass substrates, then completed SSF experiments of straw, paper mill sludge, and corn stover. Simultaneous saccharification/ fermentation (SSF) batch tests and continued the design of the 130 L pilot CSRS unit.

During our second two quarters, we have been focussing on

1) pretreatment studies (Task 1. b),

2) simultaneous saccharification/fermentation of pretreated biomass,

3) beginning some work with paper mill sludge as a cellulose source, and

4) beginning work on cellulase production, completing a literature survey and activiating one strain of cellulase producing fungi, *Trichoderma reesei* which we had in our culture library.

1. **Basic Research**-The basic research is being performed at BPI's laboratory in W. Lafayette, IN.

Task 1. a) Cellulase/hemicellulase production and performance comparison- The enzymatic conversion of cellullose is often the rate limiting step during the simultaneous saccharification and fermentation. During quarters 1 and 2, we demonstrated that recycled fermentation broth retained a high degree of

enzymatic ability. Thus, our prelimary focus is now on recycling enzymes rather than the co-production in our upcoming pilot plant efforts. In the CSRS, temperatures stay low during the ethanol separation, so there is no destruction of the enzymes as would occur during a standard distillation process.

Co-production of enzymes does however, would have a major beneficial effect on economics, so we will continue to pursue this line of research.

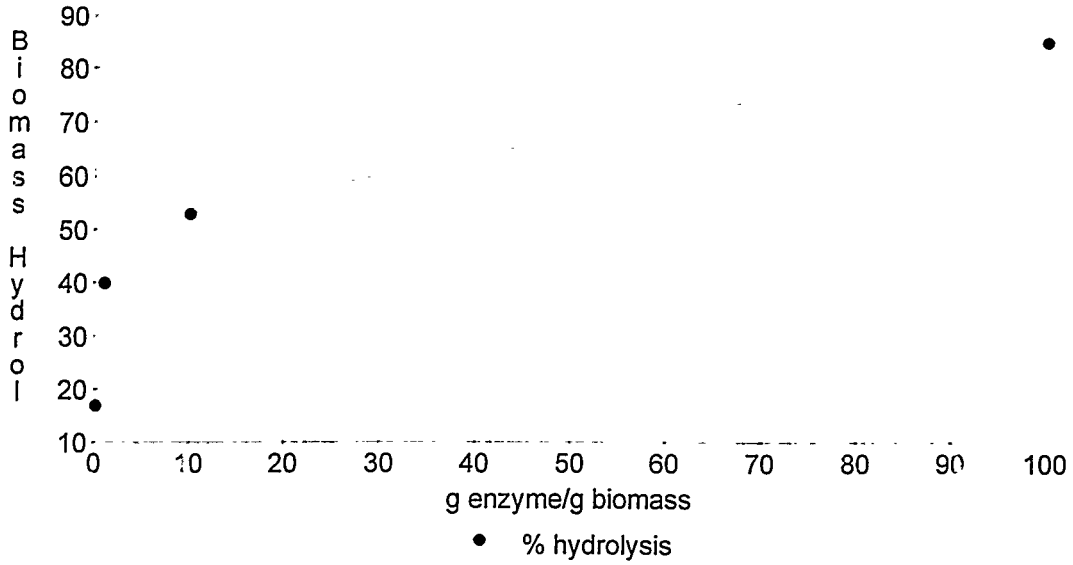
During Q4, we have activated the one strain of *Trichoderma reesei* that we have in our own culture library. We have grown-up several batches of this Trichoderma reesei on glucose, and checked for production of cellulase. During our next quarter we will obtain some of the hyperproducing strains described in the literature (Appendix 1).

We have completed a literature survey of cellulase production, and mapped out a strategy for production of enzymes. This is included as Appendix 1. We will be working on this phase of the project during quarter 5. We have activated the 1 strain of *Trichoderma reesei*, and are in the process of obtaining 2 or 3 other strains from the NRRL culture collection which are decribed in the literature as being highly productive of cellulase. As described in the literature survey, use of biomass extract as a culture medium has beneficial effects besides being a low cost substrate.

The performance of a liquid enzyme (used as NREL standard) from Env. Biotechnology of Menlo Park, CA is shown below on SD process pretreated wheat straw after 96 hours and 42C.

Figure 2.

Enzyme Performance



As per this figure, between 2 and 10 g enzyme was required to achieve hydrolysis. SSF improves hydrolysis by removing inhibitory cellobiose and glucose products of the enzyme reaction.

Task 1. b) Development/ Optimization of a delignification pretreatment process for straw and/or corn stalks .

In this portion of the project, we have beem developing an environmentally benign, non- sulfite, non-acid, method for ptreteating the biomass with the intent of allowing the quick and complete enzymatic attack of the xylan (hemicellulose) and glucan (cellulose) polymers. We have completed a fair amount of work on a combined chemical and physical process which was first proposed by G. Tyson of Xylan Inc. This process, the Xylan Delignification Process (XDP) consists of extrusion in the presence of peroxide and base. We described this work in our Q1/Q2 report

During the Q3 and Q4, we have used our own proprietary low temperature steeping delignificaiton/ extraction pretreatment for biomass to solubilize lignin. All SSF experiments completed during Q3 and Q4 were based on the BPI Steep Delignification (SD) process. Some of our results are summarized in Table 1.

Table 1. BPI's Steep Delignification Biomass Pretreatment Process

Corn Stover: 49% solubilization of lignin/ ash

Lignin characteristic: acid soluble to acid insoluble ratio 7:1

free xylose < 1 g/L

Composition :	pentosans	15%	
	hexans	35%	
	lignin	15%	
			(ref)

SSF Utilization of enzyme saccharified pentosans/hexans 77%

Wheat Straw: 43% solubilization of lignin/ ash

Composition:	pentosans	30.1	19.1	20	19
	hexans	35.2	39.5	51	86
	lignin	15.4	24	n.m.	
	ash	7.5	11	n.m.	
		Carr	Zhao	this study	

SSF Utilization of enzyme shaccharified pentosans/hexans 85%

2. Lab. Development : There will be two parts to our laboratory process development phase:1) Bench scale testing of the saccharification/fermetnation process, and 2) Process modeling and economic optimization/costing. During Q3 adn Q4, we have performed a number of fermentations of pretreated biomass.

2.1) Bench Scale SSF of pretreated (BPI's SP Process) biomass

During Q3, we performed a number of experiments using an enzyme obtained from Environmental Bio Tech, Menlo Park, Calif. which is an enzyme suggested by NREL for standard studies on SSF of cellulose. However, in repeated studies we were able to show reasonably good saccharification of biomass to free sugars, but were unable to ferment these sugars. Contamination of the fermentations by bacteria was noted, and an examination of the enzyme seemed to show bacteria. We then sterilized the enzyme by two different techniques, 1) filter (0.2 u) sterilization, and 2) addition of hydrogen peroxide to the enzyme. Even after these processes, the yeast did not perform the desired SSF. We added antibiotics to the fermentation broth and again noted no fermentation. We eventually concluded that there was some sort of inhibitory compound in the enzyme which was stopping the yeast fermentation. An unidentified peak was noted in our chromatographs as per Figure xx.

We then used 3 different enzymes:

1) TRL Cellulase (a liquid concentrate) from Solvay, who were aquired by Genencor
2) Cellulase 4000 (a solid granular concentrate) also from Solvay

and recently, we obtained two cellulases from Genencor

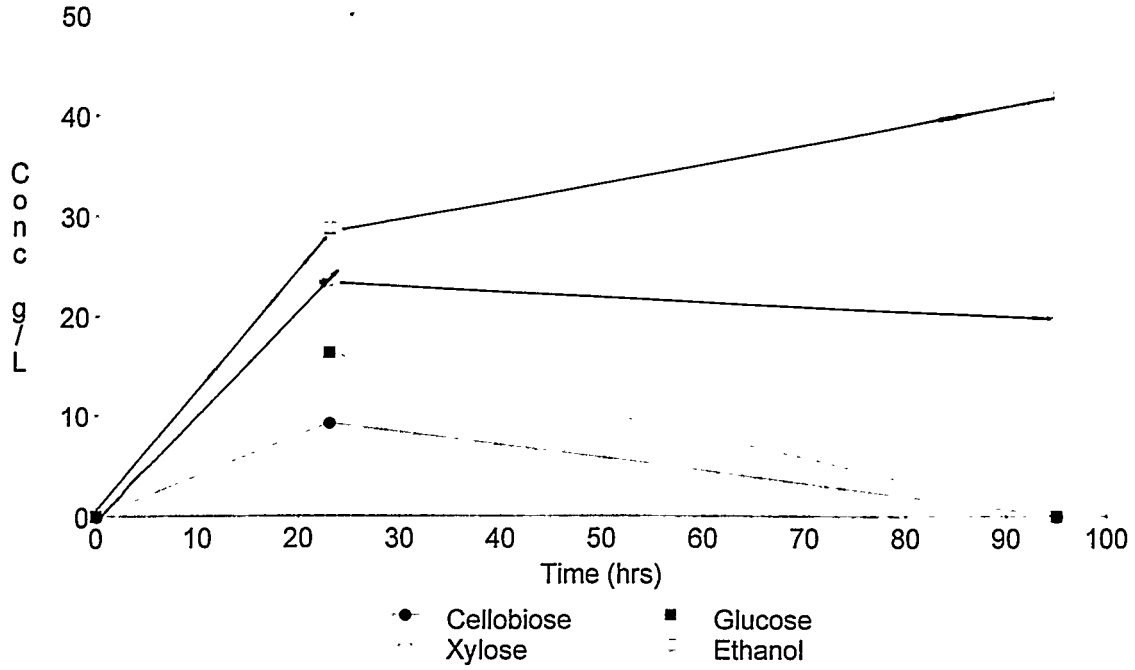
3) Specyzme CP (a liquid concentrate)
4) Spezyme CE (a liquid concentrate)

Product descriptions of these enzymes are included with Appendix 1. Successful fermentations were obtained with all of these enzymes, and we will by using the Spezyme enzymes in our future work due to the commercial availability of this enzyme.

The SSF of wheat straw is shown in Figure 3. For this fermentation we used TRL Cellulase from Solvay, and an initial biomass (pretreated) of 111 g/L.

Figure 3.

SSF of SD Treated Wheat Straw



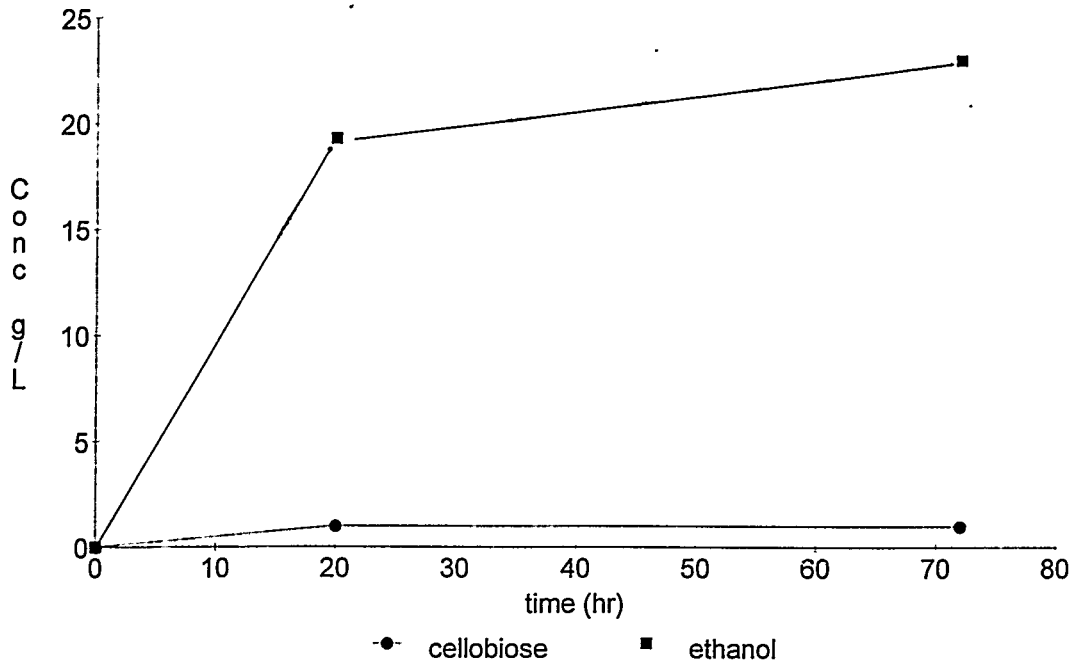
As per Figure 3, 41 g/L ethanol was obtained in the SSF of the wheat straw. This fermentation was run at 43 C., using Solvay Cellulase 4000 Aspergillus based cellulase.

Paper sludge was obtained from a Wisconsin paper mill. This stream is currently being land applied as a waste stream. We completed several experiments with this product. The basic make-up was 31% solids, few fibers, no spoilage problems with bacteria, a pH of 8.3-9.0, with a fairly high buffering capacity. 1 gram of concentrated H3PO4 was required to drop the pH of a solution with 7.1 grams (dry basis) of PMS.

A SSF using enzyme and high temperature tolerant yeast was performed using enzyme from Env. Biotechnology of Menlo Park, CA . Approximately 55% of the sludge was solubilized/hydrolysed, but only 6.3 g/L ethanol was measured. Repeating this experiment at 43 C. with Cellulase 4000 from Solvay, 23 g/L was obtained in a 72 hour SSF as shown in Figure 4. No glucose or xylose was noted in the fermentation broth indicating good conversion of sugars to ethanol, and the lack of hemicellulose in the sludge.

Figure 4.

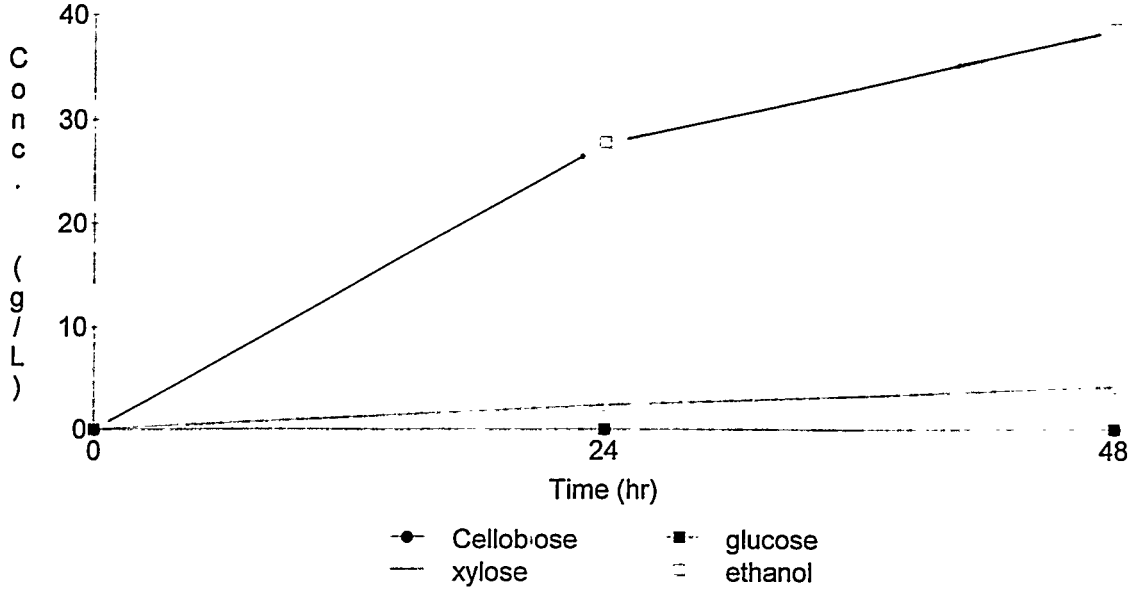
SSF of Paper Mill Sludge



An example of one of our corn stover SSF is shown in Figure 5. In this fermentation, we used a temperature tolerant yeast strain developed by our lab, BPI ADPY 15 which is an adapted flocculating K. marxianus type of yeast.

Figure 5.

SSF of SD treated Corn Stover



2.1a. Enzyme Recyling Results described in our Q1/Q2 report indicated that a high degree of enzymatic activity could be maintained by recycling the fermentation broth as per our process design of Figure 1.

2.1b. Xylose Fermentation BPI is developing a flocclulent S. cerevisae strain, (Dale, 1995), and as a part of this project, a flocculent strain of *Pichia stipitus* was developed for the xylose fermentation during 1997. Our work with this strain during Q4 indicated little ethanol generation, similar to the results reported in our Q1/Q2 report. Thus, we will have to re-order our parent strain, and run our flocculation adaptation reactor. We intend to do this during Q5.

2. b) Process modeling/economics- This modeling effort will follow gathering of data from pretreatment, enzyme generation, and fermentation trials. This will be completed by Q6.

3. Process Scale Operations:

Our demonstration scale research consists of building and operating a process scale CSRS for operation on cellulosics. We will operate the 130 L reactor during Q7 and Q8. A 130 liter pilot scale CSRS (12" diameter column) was constructed during 1997. An auxiliary absorption column and gas circulation need to be fabricated for ethanol removal from the recirculating CO2 stream, and the pretreatment vessels and pretreatment chemical recycle system designed (BPI's Steep Delignification process).

Appendix 1. Cellulase Production by Microbial Fermentation: A Literature Summary

Cellulosic biomass sources can be converted to sugars either by acid hydrolysis, or by enzymatic hydrolysis. Strong or weak acid hydrolysis requires use of an acid solution, and process use of acid resistant piping/ tankage. Large amounts of acid are added the biomass to 1) delignify, and 2) release the sugars. With strong acid, hydrolysis can be attained at low temperatures, and with weak acid (1-3%) high pressures and temperatures allows hydrolysis. The acid is then neutralized with lime, and calcium sulfate precipitated. Thus the 'traditional' acid process uses large amounts of acid, and generates a number of waste streams. There has been an effort in the research community to work on methods for recycling the acid. Work at Purdue (weak acid process) and TVA has focussed on sulfuric acid recovery by chromatographic means, while Golstein & Easter (1992) suggested hydrochloric acid recycling by evaporation.

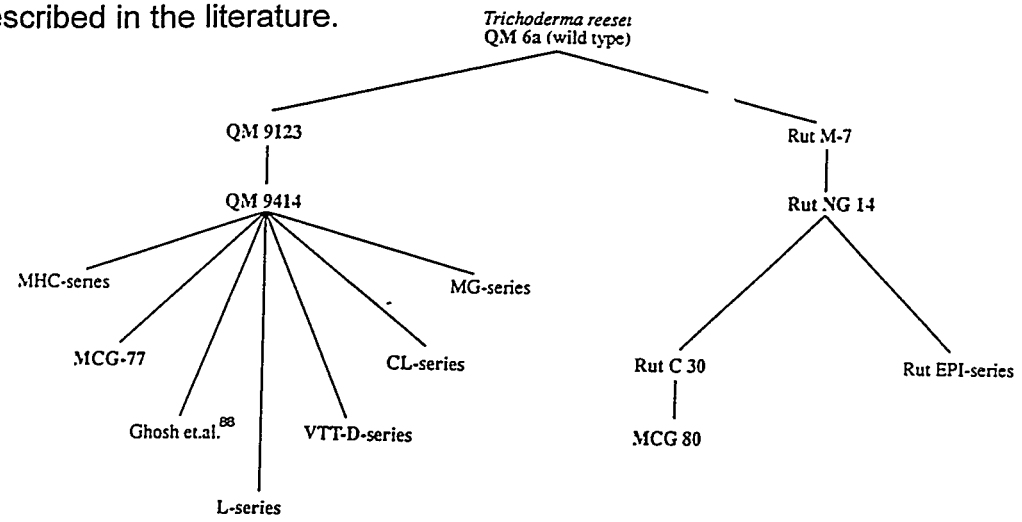
Enzymatic hydrolysis allows low temperature and mild conditions for release of the sugars. Some sort of pretreatment is required to break-up the structure of the biomass, and to release or remove the lignin. After this pre-treatment, enzyme is added to the biomass and sugars released. This method of releasing sugars is widely seen as the future for ethanol production from cellulose. However, the current cost of commercial cellulase enzyme concentrate, $9. to $20. per liter, and the amount of cellulase volume needed to hydrolyse the sugars in the cellulosic biomass, up to 200 ml enzyme per kilogram of dry biomass solids can make enzyme costs prohibitive for ethanol production. Enzyme costs alone could run over $9. per gallon under a single use process design if lab scale levels of enzyme were used. An analysis by Wright (1988) indicated an enzyme cost of $0.61/ gallon for a large scale celllulose to ethanol facility, and total ethanol production costs of $2.66/ gallon. Thus, the research goals of this project are focused on reducing this cost by enzyme recycling and/or enzyme co-production.

Pretreatment of the biomass can be accomplished by a variety of methods. 1) Physical, 2) Thermo/physical, 3) Chemical, and 4) Thermo/physical/chemical. Physical pre-treatment consists largely of grinding the biomass. Thermophysical pretreatment refers to extrusion or 'steam explosion' type pretreatments. Chemical pretreatments include acids, bases, and organic/ inorganic solvents, with the most common being acid hydrolysis as mentioned above. Finally, thermo/physical/chemical refers to some combination of temperatures, chemicals and physical treatment of the biomass. Examples of this 'combination' include ammonia explosion (AFEX) as developed at Texas A&M, the Xylan Delignification Process (XDP) using ammonia, peroxide and an extrusion, and others. With all of these processes, there are four major costs to evaluate: 1) the cost of energy to operate the pumps/ extruders/ grinders ect., 2)

the cost of the chemicals, 3) the cost of recovering or neutralizing the chemicals, and 4) the capital costs for the apparatus used.

Cellulase is produced by a number of microorganisms, and in nature, is responsible for the degradation and decay of all lignocellulosic plant materials generated each year on the planet. Investigations focusing on which organisms seem to produce the most cellulase have focused on Aspergillus, Trichoderma , and white rot fungi. Other species important in cellulase production include Pennicillium, and the bacterium Acidothermus celluloyticus (Shiang et. al, 1991). There have been two reviews on cellulase, 1) by Persson et al,(1991) who review the literature on the production of cellulase from fungal sources, and 2) by Coughlan (1992) who review literature on the use of enzymes to hydrolyse cellulosis. Most work to date focuses on Trichoderma reesei as a highly productive source of enzyme.

Cellulase is a mix of three basic enzymes, 1) endo-beta-glucanase, which cleaves the linear cellulose polymer into cellobiose, 2) exo-beta-glucanase, which works on the branched cellulose polymer, breaking off linear glucan polymers, and 3) beta-glucosidase which breaks cellobiose (2 glucan diose sugar) down to 2 glucose molecules. Chahal et al (1996,a, 1996 b) suggests that a ratio of beta-glucosidase to glucanses of 1.0 is optimal for complete hydrolysis of cellulose. Release of the enzymes by Trichoderma reesei during fermentation is only at low levels unless production is stimulated by 1) induction, and/or 2) screening for hyper-productive mutants. Mutations have been induced using UV light with some mutagenic chemicals. Persson et al (1991), Figure 2, shows the parentage of the mutated Trichoderma reesei strains which are described in the literature.



Enzyme generation by Trichoderma reesei can either be on soluble or non-soluble substrates. Generation on soluble substrates has the advantage of generation of the free enzyme, while when a solid substrate (cellulose, or crude biomass) is used a substantial fraction of the enzyme may adsorb to the the cellulose. (Adsorption of the enzyme to the solid substrate is the first step in the hydrolysis process.) Glucose, xylose and lactose are generally used as soluble substrates. Final enzyme concentrations reported range from 0.25 to 6.4

FPU/ml (Mandels et al, 1976, Mishra et al, 1982, and Chaudhuri et al., 1993) Addition of a small amount of cellulose to the fermentation broth has been shown by several researchers to boost cellulase to 2.6 to 10.5 FPU/ml (Persson et al, 1991). Warzywoda et al, (1992) show up to 21.5 FPU/ml on a lactose/glucose medium, and also demonstrate a boost in xylanase activty if pentose extract from pretreated biomass is used as a substrate for enzyme production.

Enzyme production on non-soluble substrates, cellulose and pretreated ligno-cellulosics was reported by Viesturs et al, 1996. Promotion of continued enzyme production during batch fermentations was promoted by 1) addition of acetate and ethanol, and 2) ligno-cellulosics. A enzyme final broth concentration of 4.0 FPU/ ml was obtained. A solid state 'pan' reactor is decribed by Chahal et al (1996a, 1996b) in which 6 to 9.25 FPA/ml were obtained. Persson et al (1991) conclude that higher levels of cellulase can be obtained through the addition of a non-soluble substrate to the medium although soluble substrates would be the major carbon source for cell growth.

The action of cellulase is generally proposed as a two stage sort of mechanism, adsorption of the enzyme to the cyrstalline solid cellulose polymer, followed by the successive cleaving off of cellobiose sugars. The cellobiose is then cleaved to gluocose by beta-glucosidase. Cellulase activity is inhibited by cellobiose, glucose, and ethanol. Holtzapple et al (1990) reviewed the literature on cellulase inhibition by substrate and product, and concluded that glucose, cellobiose and ethanol are non-competetive inhibitors. The highest inhibition constant was found to be associated with cellobiose. Conversion of cellobiose to 1) glucose and 2) then to ethanol in a SSF type fermentation reduces product binding inhibition constant in the enzyme catalyzed hydrolysis by a factor of 16 time for Trichoderma reesei. Thus, the SSF has definite advantages for the enzyme process. Ethanol removal, as per the CCRS proposed in this project, will further remove ethanol as an inhibitor, although, the enzyme inhibition due to ethanol is not large. SSF increased hydrolysis from 55% to 65% using the same enzyme level after 4 days in a set of data presented by Hogan et al (1990) using 10 FPU/ml.

The minimum amount of enzyme needed to complete hydrolysis of biomass is generally determined to be about 5 to10 FPU per g of biomass . The cellulase adsorbes to the biomass and begins the successive release of cellobiose. Adsorption rates can be used to determine the minimum amount of cellulase needed to complete a hydrolysis. Using 10 FPU/ml(Hogan et al, 1990) who also show that hydrolysis rates can be aided through SSF removal of inhibitory cellobiose product.

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SPEZYME® CP

Cellulase

Delivering Innovation Through Biotechnology®

Product Information

■ INTRODUCTION

SPEZYME CP cellulase is an enzyme preparation intended for the starch and alcohol industries. This product is capable of reducing viscosity and improving separation of different grain fractions. SPEZYME CP contains a combination of enzymes which effectively modify and digest non-starch carbohydrates, the structural material of plant cells. This plant material is composed mainly of cellulose, hemicellulose, and β-glucans which are cross-linked with each other and also with lignin, pectins, proteins, starch, and lipids. SPEZYME CP enzyme is produced by controlled fermentation of *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*).

■ UNIT DEFINITION

The activity of SPEZYME CP enzyme is expressed in **G**enencor **C**ellulase **U**nits (**GCU**) per milliliter. The assay measures the amount of glucose released during the incubation of enzyme solution with a specified type of filter paper at 50° C in 60 minutes and is compared against an internal standard. The assay method is available upon request.

■ CHARACTERISTICS

SPEZYME CP enzyme is effective especially on cellulose, hemicellulose, and β-glucans. As a cellulase enzyme complex, the product contains multiple enzyme activities but is standardized on the basis of its activity on filter paper. The performance of this enzyme preparation is a result of the synergistic effect of all the main and side activities, and cannot be evaluated only on the basis of the declared activity.

Declared activity:	90 GCU/ml (minimum)
Appearance:	Amber liquid
Solubility:	Completely miscible in water

SPEZYME CP enzyme complies with current FAO/WHO and FCC recommended specifications for food-grade enzymes.

■ APPLICATIONS

In the **corn wet milling** industry, SPEZYME CP enzyme can be used to improve the separation of starch, gluten, and fiber fractions and to make the filtration of individual product streams easier.

In the **wheat starch** industry, SPEZYME CP enzyme can be used to improve the separation of starch and gluten of poor- and medium-quality wheat crops. It will also reduce viscosity of effluent streams. Additional application information is available upon request.

In the **fuel alcohol** industry, SPEZYME CP enzyme can be used when milled grain is used as raw material. The advantages of using SPEZYME CP enzyme are improved starch utilization and the hydrolysis of non-starch carbohydrates which otherwise increase the mass viscosity or cause fouling problems in distilling equipment.

■ DOSAGE GUIDELINES

The optimum dosage levels of SPEZYME CP enzyme will vary with different substrates and operating conditions such as pH, temperature, and reaction time. Small-scale experiments are advised in order to determine the proper level of enzyme addition. With no previous use information for a specific application, SPEZYME CP enzyme should be initially tested at 0.4 - 0.5 liters/metric ton dry substance (**DS**), after which the dosage can be lowered gradually to determine the level where the enzyme still has the required effect.

■ EFFECT OF TEMPERATURE AND pH

SPEZYME CP enzyme has the best operational stability in the following temperature and pH ranges:

Temperature	*pH*
70° C (158° F)	4.5 - 5.0
50 - 65° C (122 - 149° F)	4.0 - 5.5
<50° C (<122° F)	3.5 - 6.5

SPEZYME CP enzyme is easily inactivated at temperatures above 70° C (158° F) or at pH levels above 7.0.

■ PACKAGING

SPEZYME CP enzyme is available in 25-liter polyethylene pails, 200-liter polyethylene drums, and 1000-liter containers.

■ STORAGE

This product will meet the declared activity upon arrival at the customer's plant. During storage at 4° C (40° F), the activity will remain constant for at least 18 months, and at 25° C (77° F) for at least 12 months. It is advisable to store SPEZYME CP enzyme under refrigerated conditions. Storage above 25° C (77° F) should be avoided.

GENENCOR INTERNATIONAL

SPEZYME® CE

Genencor® Cellulase Enzyme

Delivering Innovation Through Biotechnology®

Product Information

INTRODUCTION

SPEZYME CE cellulase is an enzyme preparation intended for the starch and alcohol industries. This product is capable of reducing viscosity and improving separation of different grain fractions. SPEZYME CE contains a combination of enzymes which effectively modify and digest non-starch carbohydrates, the structural material of plant cells. This plant material is composed mainly of cellulose, hemicellulose, and β-glucans which are cross-linked with each other and also with lignin, pectins, proteins, starch, and lipids. SPEZYME CE enzyme is produced by controlled fermentation of *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*).

CHARACTERISTICS

SPEZYME CE enzyme is effective especially on cellulose, hemicellulose, and β-glucans. As a cellulase enzyme complex, the product contains multiple enzyme activities but is standardized on the basis of its activity on carboxymethylcellulose (**CMC**). The performance of this enzyme preparation is a result of the synergistic effect of all the main and side activities, and cannot be evaluated only on the basis of the declared activity.

Declared activity:	CMC 2500 IU/ml
Appearance:	Amber liquid
Solubility:	Completely miscible in water

SPEZYME CE enzyme complies with current FAO/WHO and FCC recommended specifications for food-grade enzymes.

UNIT DEFINITION

One CMC unit of activity liberates 1 mmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions (50° C [122° F], pH 4.8). The detailed assay procedure is available upon request.

APPLICATIONS

In the **corn wet milling** industry, SPEZYME CE enzyme can be used to improve the separation of starch, gluten, and fiber fractions and to make the filtration of individual product streams easier.

In the **wheat starch** industry, SPEZYME CE enzyme can be used to improve the separation of starch and gluten of poor- and medium-quality wheat crops. It will also reduce viscosity of effluent streams. Additional application information is available upon request.

In the **fuel alcohol** industry, SPEZYME CE enzyme can be used when milled grain is used as raw material. The advantages of using SPEZYME CE enzyme are improved starch utilization and the hydrolysis of non-starch carbohydrates which otherwise increase the mass viscosity or cause fouling problems in distilling equipment.

DOSAGE GUIDELINES

The optimum dosage levels of SPEZYME CE enzyme will vary with different substrates and operating conditions such as pH, temperature, and reaction time. Small-scale experiments are advised in order to determine the proper level of enzyme addition. With no previous use information for a specific application, SPEZYME CE enzyme should be initially tested at 0.4 - 0.5 liters/metric ton dry substance (**DS**), after which the dosage can be lowered gradually to determine the level where the enzyme still has the required effect.

EFFECT OF TEMPERATURE AND pH

SPEZYME CE enzyme has the best operational stability in the following temperature and pH ranges:

Temperature	*pH*
70° C (158° F)	4.5 - 5.0
50 - 65° C (122 - 149° F)	4.0 - 5.5
<50° C (<122° F)	3.5 - 6.5

SPEZYME CE enzyme is easily inactivated at temperatures above 70° C (158° F) or at pH levels above 7.0.

PACKAGING

SPEZYME CE enzyme is available in 25-liter polyethylene pails or 200-liter polyethylene drums. Bulk deliveries are also available.

GENENCOR INTERNATIONAL

ISO 9002

SPEZYME® CP

Genencor® Cellulase Enzyme

Delivering Innovation Through Biotechnology®

Product Information

■ INTRODUCTION

SPEZYME CP cellulase is an enzyme preparation intended for the starch and alcohol industries. This product is capable of reducing viscosity and improving separation of different grain fractions. SPEZYME CP contains a combination of enzymes which effectively modify and digest non-starch carbohydrates, the structural material of plant cells. This plant material is composed mainly of cellulose, hemicellulose, and β-glucans which are cross-linked with each other and also with lignin, pectins, proteins, starch, and lipids. SPEZYME CP enzyme is produced by controlled fermentation of *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*).

■ UNIT DEFINITION

The activity of SPEZYME CP enzyme is expressed in **G**enencor **C**ellulase **U**nits (**GCU**) per milliliter. The assay measures the amount of glucose released during the incubation of enzyme solution with a specified type of filter paper at 50° C in 60 minutes and is compared against an internal standard. The assay method is available upon request.

■ CHARACTERISTICS

SPEZYME CP enzyme is effective especially on cellulose, hemicellulose, and β-glucans. As a cellulase enzyme complex, the product contains multiple enzyme activities but is standardized on the basis of its activity on filter paper. The performance of this enzyme preparation is a result of the synergistic effect of all the main and side activities, and cannot be evaluated only on the basis of the declared activity.

Declared activity:	90 GCU/ml (minimum)
Appearance:	Amber liquid
Solubility:	Completely miscible in water

SPEZYME CP enzyme complies with current FAO/WHO and FCC recommended specifications for food-grade enzymes.

■ APPLICATIONS

In the **corn wet milling** industry, SPEZYME CP enzyme can be used to improve the separation of starch, gluten, and fiber fractions and to make the filtration of individual product streams easier.

In the **wheat starch** industry, SPEZYME CP enzyme can be used to improve the separation of starch and gluten of poor- and medium-quality wheat crops. It will also reduce viscosity of effluent streams. Additional application information is available upon request.

In the **fuel alcohol** industry, SPEZYME CP enzyme can be used when milled grain is used as raw material. The advantages of using SPEZYME CP enzyme are improved starch utilization and the hydrolysis of non-starch carbohydrates which otherwise increase the mass viscosity or cause fouling problems in distilling equipment.

■ DOSAGE GUIDELINES

The optimum dosage levels of SPEZYME CP enzyme will vary with different substrates and operating conditions such as pH, temperature, and reaction time. Small-scale experiments are advised in order to determine the proper level of enzyme addition. With no previous use information for a specific application, SPEZYME CP enzyme should be initially tested at 0.4 - 0.5 liters/metric ton dry substance (**DS**), after which the dosage can be lowered gradually to determine the level where the enzyme still has the required effect.

■ EFFECT OF TEMPERATURE AND pH

SPEZYME CP enzyme has the best operational stability in the following temperature and pH ranges:

Temperature	***pH***
70° C (158° F)	4.5 - 5.0
50 - 65° C (122 149° F)	4.0 - 5.5
<50° C (<122° F)	3.5 - 6.5

SPEZYME CP enzyme is easily inactivated at temperatures above 70° C (158° F) or at pH levels above 7.0.

■ PACKAGING

SPEZYME CP enzyme is available in 25-liter polyethylene pails, 200-liter polyethylene drums, and 1000-liter containers.

■ STORAGE

This product will meet the declared activity upon arrival at the customer's plant. During storage at 4° C (40° F), the activity will remain constant for at least 18 months, and at 25° C (77° F) for at least 12 months. It is advisable to store SPEZYME CP enzyme under refrigerated conditions. Storage above 25° C (77° F) should be avoided.

GENENCOR INTERNATIONAL ISO 9002

SOLVAY ENZYMES

CELLULASE TRL

Liquid Trichoderma Reesei For Cellulose Hydrolysis

■ INTRODUCTION

Multi-enzyme cellulase complexes capable of converting cellulose to glucose contain at least three distinct enzyme components which degrade native cellulose.

The action of the C_1 component on cellulose is not well defined. There is no evidence that the component hydrolyzes glucosidic bonds, but rather it appears that the C_1 - component disrupts the structure of native cellulose by weakening the hydrogen bonds. The action is required before hydrolysis of highly structure forms of cellulose (cotton, crystalline cellulose wood, etc.) can occur.

The C_x - component consists of B-1, 4-glucanases. Exo-B-1, 4-glucanase successivley removes single glucose units from the nonreducing end of the cellulose chain, while endo-B-1, 4-glucanases randomly hydrolyze the interior glucosidic bonds of cellulose liberating oligomers of lower molecular weight.

The B-glucosidase, including cellobiase, are primarily active on the dimers and oligomers of cellulose.

■ DESCRIPTION

Cellulase TRL is a cellulase complex obtained by a controlled fermentation of Trichoderma reesei. The enzyme specifically hydrolyzes the B-D-1, 4-glucosidic bonds of cellulase, its oligomers, and derivatives.

The cellulase complex consist of both exo--glucanases and endo-glucanases which directly attack soluble cellulose derivatives and native cellulose. Cellulase TRL is characterized by its significant saccharifying (glucose liberating) actions on cellulose. Prolonged cellulose hydrolysis results in the formation of substantial levels of glucose.

Cellulase TRL is available as a liquid with a activity NLT 10,000 Viscometric Cellulase Units per gram.

■ PROPERTIES

Activity	NLT 10,000 CU/g NLT 140 FPU/ml
Form and Color	Amber liquid
Odor	Free of offensive odor
Taste	Free of offensive taste
Specific Gravity	1.05 to 1.15

Solvay Enzymes, Inc. PO Box 4226, Elkhart, IN 46514-0226
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SOLVAY ENZYMES

CELLULASE 4,000

A Multi-Enzyme Cellulase Complex Of A. Niger

INTRODUCTION

Multi-enzyme cellulase complexes capable of converting cellulose to glucose contain at least three distinct enzyme components which degrade native cellulose.

The action of the C_1–component on cellulose is not well defined. There is no evidence that the component hydrolyzes glucosidic bonds, but rather it appears that the C_1–component disrupts the structure of native cellulose by weakening the hydrogen bonds. The action is required before hydrolysis of highly structured forms of cellulose (cotton, crystalline cellulose, wood, etc.) can occur.

The Cx–component consist of ß-1,4-glucanases. Exo-ß-1,4-glucanase successively removes single glucose units from the nonreducing end of the cellulose chain, while endo-ß-1,4-glucanases randomly hydrolyze the interior glucosidic bonds of cellulose liberating oligomers of lower molecular weight.

The ß-glucosidases, including cellobiase, are primarily active on the smaller molecular weight cellulose hydrolysates. During cellulose breakdown they are active on the dimers and oligomers of cellulose.

DESCRIPTION

CELLULASE 4000 is a food grade cellulase complex obtained by a controlled fermentation of *Aspergillus niger*. The enzyme specifically hydrolyzes the ß-D-1,4-glucosidic bond of cellulose, its oligomers, and derivatives.

Product	Activity (CU/g)	Comment
Cellulase 4,000	4,000	Standardized with lactose

PROPERTIES

Form	Amorphous dry powder
Color	Light tan to white
Odor	Free of offensive odor
Taste	Free of offensive taste

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