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PROCESS DEVELOPMENT STUDIES ON THE BIOCONVERSION
OF CELLULOSE AND PRODUCTION OF ETHANOL

Charles R. Wilke and Harvey W. Blanch

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PROCESS DEVELOPMENT STUDIES ON THE BIOCONVERSION OF CELLULOSE
AND PRODUCTION OF ETHANOL

Charles R. Wilke and Harvey W. Blanch
Principal Investigators

Department of Chemical Engineering
and
The Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

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I. RAW MATERIALS AND PROCESS EVALUATION

A. High Pressure HCl Conversion of Wood

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This section (Pages 3 to 7 inclusive) deleted because of matter subject to patent not released by DOE.

B. Preliminary Studies on Moderate Pressure Sulfur Dioxide on Wood

Because of the low solubility of sulfur dioxide (at 40°C) of 54.1 mg (0.845 m.mols) per gram of water versus 633 mg (17.37 m.mols) hydrogen chloride per gram of water, it was decided to try to react SO₂ with samples of 2 mM Wiley milled Populus analogous to the HCl process. However, due to the low pressure of only 38 psi at 21°C, the sulfur dioxide produced a minimal carbohydrate conversion of less than 10% upon secondary hydrolysis.

Another trial at a higher temperature will be tried to see if SO₂ can be inserted into the chemical structure of this lignocellulosic material such as has been reported for HCl (2). It is expected that, at a temperature of about 42°C, the insertion of SO₂ into lignin polymer should occur before appreciable amounts of cellulose degradation occurs. Again, upon secondary hydrolysis some of the lignin will dissolve as lignosulfonic acids.

II. ENZYME FERMENTATION STUDIES

A. Cellulase Production

1. Batch Fermentation

In the present studies, evaluations were made of Trichoderma viride strain L-5, comparing cellulase productivity with strain Rut-C-30, upon which most of our previous process development have been used.

Fermentation operations were conducted in 5-liter New Brunswick fermentors. The medium as devised by Mandels was used for all experiments. Ball-milled solka floc (BW 200) and Tween 80 at 1.0% and 0.02% concentration, respectively, was used for all runs. Four-day old, 10% mycelium inoculum

was used for all fermentations. The pH was adjusted with $2N$ NaOH. and $2N$ H_2SO_4 .

Table 1 shows the effect of operating conditions on state variables.

Run #2 and #5 show that controlling the temperature of $28^\circ C$ throughout the fermentation decreases the β -glucosidase activity and soluble protein, while C_1 , C_x and FPA activities remain the same. The higher level of β -glucosidase in run #5 would permit more rapid conversion of cellobiose to glucose. This then relieves the cellobiose inhibition of the C_1 enzyme and hence increases the rate of depolymerization of crystalline cellulose.

Table 2 shows the comparison of Rut L-5 strain with Rut-C-30. Using 1% substrate as the carbon source (run #3), FPA, C_1 and β -glucosidase are higher with Rut-C-30.

2 Continuous Cellulase Production Rut-C-30

Studies were carried out in order to optimize individually the 1st and 2nd stage of the two-stage continuous system for cellulase activity manipulating pH, temperature, Tween-80 level, substrate concentration and dilution rates. Part of the results are shown in Table 3.

Run #1 and #2 show that decreasing Tween 80 level by half has no effect on filter paper activity. Increasing substrate concentration in the feed from 1% to 2.5% increases the filter paper activity from 1.03 and 1.6 to 2.48 and 3.6, respectively, in the first and second stage of the two-stage continuous system. Increasing dilution rate from 0.02 hr^{-1} to 0.04 hr^{-1} in the second stage results in the increase of productivity from 0.072 to 0.137.

B. Studies on Composition of Cellulase Enzyme

Cellulase is composed of three distinct types of activities, characterized by their action on crystalline cellulose, amorphous cellulose, or cellobiose. These components have been separated.

The separation scheme is shown in figure 3. Raw culture filtrate is

Table 1
Effect of Operating Conditions on State Variables

OPERATING CONDITIONS						STATE VARIABLES				
Run #		Temp. (°C)	S ₀ %	T-80%	C/N	FPA	β-Gluc.	C ₁	C _x	Soluble Protein
1	Controlled not to go below 3.3	28	1.0	0.02	8.4	2.02	0.84	0.21	112	5.2
2	Controlled not to go below 3.3	28	1.0	0.02	8.4	2.1	2.1	0.176	119	4.4
3.	Controlled at 4.0	28	1.0	0.02	8.4	1.75	1.15	1.17	90	6.1
4	Controlled at 5.5	28	1.0	0.02	8.4	1.22	1.43	0.11	60	6.5
5	Controlled not to go below 5.0	31 (0-6 hr) 28 (RT)*	1.0	0.02	8.4	2.3	2.5	0.177	120	6.1
6	Controlled not to go below 5.0	31° (0-12hr) 28° (RT)*	1.0	0.02	8.4	1.9	1.55	0.12	101	6.25

* (RT)= remainder of time

Table 2

Comparison of Rut-L-5 with Rut-C-30

Run #	S ₀ (%)	Strain	FPA U/ml	β -Glucosidase U/ml	C ₁ U/ml	C _x U/ml	Soluble Protein mg.ml ⁻¹	Remarks
1	1	Rut-L-5	2.3	2.5	0.177	120	3.1	pH controlled not to go below 5.0. 31°C for 0-6 hrs. 28°C for remainder of fermentation time (RT).
2	1	Rut-C-30	3.1	3.3	0.195	84	3.3	pH controlled not to go below 5.0 Temp. 25°C.
3	1	Rut-C-30	3.0	4.3	0.26	105	3.3	pH controlled not to go below 5.0 28°C for 0-9 hrs. 25°C for RT

Table 3

Two-State Continuous Production of Cellulase

Run #	CONTROLLED VARIABLES								RESULTS*			
	Inlet Sub ^{**} Conc. (g/l)	Tween 80 level (%)	Temperature (°C)		pH		Dilution Rate (hr ⁻¹)		Productivity		FPA	
			F ₁	F ₂	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂
1	10	0.02	25	25	5.0	5.0	0.02	0.02	0.021	0.032	1.03	1.6
2	10	0.01	25	25	5.0	5.0	0.02	0.02	0.020	0.030	0.98	1.49
3	25	0.02	25	25	5.0	5.0	0.02	0.02	0.050	0.072	2.48	3.6
4	25	0.02	25	25	5.0	5.0	0.02	0.04	0.052	0.137	2.6	3.42

* for non-recycled system

** ball milled solka floc (200 mesh)

F₁ = first stage; F₂ = 2nd stage

Figure 3. Scheme for Separation of Cellulase Components

concentrated by ultrafiltration with an Amicon UM2 membrane, removing material of molecular weight less than 1,000. The concentrate is subjected to gel-permeation chromatography (GPC), yielding two fractions. the low molecular weight fraction contains non-essential material and is discarded. The high molecular weight fraction, containing enzyme, is concentrated by lyophilization and resuspended in citrate buffer.

The final resolution is accomplished by ion-exchange chromatography. Using a 91 x 1.5 cm-column packed with DEAE-Sephadex, elution with an ionic gradient constructed of 5 mM citrate and 50 mM citrate at pH 5.5, washes out β -glucosidase, followed by two components of glucanohydrolase (C_x) and a single cellobiohydrolase (C_1). The component activities are determined by chemical assay and shown in figure 4.

Polyacrylamide gel electrophoresis is used for the detection and analysis of the enzymes at each stage in the separation. This technique gives the molecular weights of the four resolved components, shown in Table 4. Xylanase and protease activities have not been localized in single fractions.

C. Economic Optimization of Processes for Hydrolysis of Corn Stover

During the fall, experiments were conducted to study the performance of enzyme Rut-C-30 in the hydrolysis of acid-pretreated corn stover to produce glucose. This enzyme is being developed as one of the possible substitutes for enzyme QM-9414 currently in use.

A series of batch experiments were carried out to study the kinetics of the hydrolysis reaction at various enzyme filter paper activities. All experiments were carried out with 5% by weight solids and a total volume of 100 ml. The temperature was maintained at 45°C. The enzyme concentration was 14, 7, 3.5 FPA, respectively, for the three batches conducted. Samples obtained at various intervals were analyzed for sugar concentration by means

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Figure 4. Components of Cellulase Enzyme.

Table 4

COMPONENTS OF CELLULASE

<u>COMPONENT</u>	<u>MOLECULAR WEIGHT</u>
β -Glucosidase	73,000
C_x-1	32,500
C_x-2	17,000
C_1	87,000

of a DNS sugar assay. Material balance on the solids was done by measuring the weight upon drying.

The following observations were made:

1. Initial rates of Rut-C-30 at all FPA's are faster than those obtained with enzyme QM-9414 currently in use.
2. Conversion to sugar at 40 hr is 65% of theoretical for FPA 14 and 58% for FPA 3.5, significantly higher than the 44% obtained with 9414 at FPA 3.5.
3. No difference is observed in rate of conversion for experiments conducted with C-30 at FPA 14 and FPA 7

The preliminary results indicate a potentially significant reduction in the cost of producing sugar from corn stover. The two factors upon which this conclusion relies are the ability to produce enzyme at between 14 and 7 FPA continuously and the ability to duplicate the hydrolysis data in a continuous operation.

During the winter quarter attempts will be made to conduct the hydrolysis reaction continuously. The data obtained from the experiments will be useful for subsequent process economic studies.

III. ETHANOL FERMENTATION STUDIES

A. Media Development

Development of a optimum medium for continuous ethanol fermentation has been continued this past quarter. The emphasis has been on determining the important growth factors for yeast growth and ethanol production. The procedure consisted of pulse injections of a series of nutrients and then step changes in the feed concentrations of the limiting nutrients to a continuous culture (1a).

For 10 g/l glucose concentration, the results are summarized in Table 5. With a minimal level of yeast extract of 0.1 g/l, the cell yield was 0.33 grams dry wt/l (which is approximately equal to the optical density). Increasing the yeast extract to 0.35 g/l resulted in total sugar utilization and about 1.22 g/l dry weight, indicating the growth limiting factors were in yeast extract.

It was then shown that the first growth factor deficiency which has to be satisfied before the effects of the other vitamin deficiencies could be discerned is that for biotin. Increasing the concentrations of the other vitamins had no effect when biotin was not added. With biotin added, increasing the pantothenic acid and then the pyridoxine concentrations each increased the cell yield.

With these three vitamins in excess, further addition of other vitamins had no significant effect on cell yield but may have increased the ethanol yield. In fact, with all the vitamins in excess it appeared that the sugar was totally utilized but with more ethanol and less cell mass than with high yeast extract concentration.

The next experiment was to see the effect of going from 10 g/l to 100 g/l glucose with mainly synthetic growth factors to determine if the medium could be scaled up for running the vacu-ferm system. Linearly increasing all the medium components in relationship to the glucose resulted in a cell yield of only about 3 g/l dry weight and 18 g/l ethanol. The low yields with the high concentration medium may have been due to a combination of ethanol and substrate inhibitions. However, most of the existing models for ethanol inhibition indicate 18 g/l ethanol should not have a great effect. Therefore, the problem is most likely that of substrate inhibition due to the glucose, minerals, and/or vitamins. Possibly because of the mechanism of transport of these substrates to the cell, it may not be proper to scale up the

Table 5
Effect of Growth Factors on Continuous Ethanol Fermentation
for 10 g/l Glucose

<u>Growth Factors</u>	<u>Optical Density</u> <u>dry wtg (g/l)</u>	<u>Ethanol</u> <u>(g/l)</u>
Yeast Extract (0.1 g/l)	0.33	1.3
" " (0.35 g/l)	1.22	3.5
" " (0.1 g/l) + Biotin (4 μ g/l)	0.43-0.53	2.0-3.6
" " (0.1 g/l) + Biotin (4 μ g/l) + Pantothenic Acid 1.25 mg/l)	0.733	
" " " " " " + Pyriodoxine (1.25 mg/l)	0.91	
Above + All Other Vitamins	0.95	3.9

minerals and vitamins in proportion to the glucose. Also the cells may require more time to adapt to the higher substrate concentrations.

In the next experiments the mineral and vitamin requirements for 100 g/l glucose will be determined experimentally rather than by scaling up the 10 g/l case. The effect of yeast extract at the higher glucose level will also be determined. Mass balances will also be obtained for the minerals by analyzing the yeast and medium. Cell growth will be more closely followed by measuring dry weight and cell numbers as well as optical density.

B. Process Development Studies of Ethanol Production

Last quarter, a detailed engineering and economic analysis of the novel flash-ferm process was presented (1b,3). Several advantages over the earlier vacu-ferm process and conventional batch processes were shown.

In the flash-ferm process, fermenting beer is rapidly cycled from an atmospheric pressure fermentor through a low-pressure flash vessel. Ethanol is boiled away and beer ethanol concentration is thus maintained below 3.5 wt%. End product inhibition is eliminated and super high continuous ethanol productivities should be possible.

For the analysis of the flash-ferm process, it was assumed that the high (80 g/l.hr) ethanol productivity demonstrated by Cysewski (4) for the vacu-ferm process could be achieved. A cautionary note was made, however, that the effect of repeated cycling of yeast through a large pressure differential (entering and leaving the flash vessel) had not been fully studied, and some inhibition might occur.

During this quarter, a laboratory flash-ferm apparatus (3 liter fermentor capacity) has been assembled to allow testing of the assumptions of the earlier economic analysis and provide further data for design optimization. A detailed description of the apparatus is provided here to facilitate interpretation of

experimental results to be presented in future quarters.

1. Flash-Ferm Apparatus Overview

The flash-ferm apparatus is shown schematically in figure 5 .

Continuous fermentation of sugar solutions to ethanol and carbon dioxide is carried out in a five liter fermentor (a) (three liters working volume). Carbon dioxide product and sparged air are directly vented through an overhead condenser (b) which prevents ethanol vapor loss.

Fermentation beer is drawn by vacuum into an auxiliary isothermal vacuum flash vessel (c) where it boils. Ethanol and water vapors are taken as flash vessel overhead products, condensed in a condenser train (d) and collected in a dry ice cooled collection vessel (e). The ratio of ethanol to water in this final product is fixed by the temperature and pressure of flash vessel operation. Ethanol depleted beer is pumped (f) back to the fermentor from the flash vessel. Fermentor ethanol concentration is thus maintained at a low level.

A bleed stream (g) of fermentation beer may be taken directly from the fermentor to prevent buildup of nonvolatile feed components and fermentation products. A settler (h) in the bleed line allows recycle of concentrated yeast cells to the fermentor. Cell loss is minimized to allow very high cell density continuous operation.

Makeup feed is provided in response to ethanol product and other fermentor beer loss. As ethanol and water products are removed in the flash vessel, and as beer drains directly from the fermentor in the bleed, the liquid level in the system drops. This liquid level drop is detected by a probe in the fermentor, and a feed pump (i) is triggered to pump additional media from the feed reservoir (j) into the fermentor.

Temperature (T), pH (pH) and pressure (P) are continuously monitored and controlled throughout the system. Liquid phase oxygen tension and all

FLASH - FERM APPARATUS OVERVIEW

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Figure 5.

major liquid and gas flow rates are accurately measured.

Samples (S) may be taken of feed and product streams as well as of all major internal flows and from the two vessels.

2. The Fermentor

The fermentor is detailed in figure 6. A modified 5-liter "Micro Ferm" fermentor (Fermentation Design Model MA501) - 16 cm diameter, 30 cm deep, is used. Agitation is with an 8 cm diameter, 6 blade turbine impeller, mounted 11 cm above the fermentor bottom.

Fermentor pH is controlled with a Fermentation design Ph-RT recorder-controller module used in conjunction with an Ingold 761-351B combination pH electrode. pH is held at the set point by automatic addition of either 5 N H_2SO_4 or 5N NaOH.

Temperature, agitation, and air (or oxygen) flow are controlled by the Fermentation Design "Micro Ferm" controller. Temperature is held constant by passing heated or cooled water through a single loop 9 mm diameter exchanger in the fermentor.

Air (or oxygen) is filtered through a 60 cm long, 5 cm diameter glasswool packed prefilter before the flow rate controller and then through a sterile 15 cm long 2.5 cm diameter glasswool final filter mounted at the fermentor sparge inlet port. Sparging is through a single 3 mm diameter orifice located just above the bottom magnetic coupler and offset 2 cm from vessel center. The fermentor head space can also be air blanketed through a similar filter arrangement to provide a rapid gas outflow when the inoculation port is opened.

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Figure 6.

The fermenter is vented through a Ross, model SSCF stainless steel shell in tube heat exchanger with fermentor gases passed through the tube side, and 0°C water/ethylene glycol solution passed through the shell side. A final 15 cm long, 2.5 cm diameter sterile glass wool packed filter prevents back contamination, and exit gas flow rate is measured with a Precision Company wet test meter.

A 25 mm inoculation port and ports for substrate feed, flash vessel beer return, yeast cell recycle return, acid-base addition, and antifoam addition are provided through the fermentor head. An additional port for the flash vessel beer feed and bleed stream is provided through the glass fermentor wall, 3.5 cm above the fermentor bottom. Fermentor liquid and headspace thermo-wells and the fermentor liquid level probe also enter through the fermentor head plate.

3. The Flash Vessel

Figure 7 depicts the flash vessel. The pyrex flash vessel--16 cm diameter, 59 cm tall, was made by special order. Beer enters through a sidewall port 15 cm above the vessel bottom, and leaves through a similar port 5 cm above the bottom and rotated 120° from the first. An additional sidewall port is located 22 cm from the vessel bottom and directly above the beer return port. The final sideport is used for return pump leg pressure equalization, as described under "Beer Cycling System." A liquid level probe mounted through the fermentor head plate is used to maintain the liquid depth at approximately 8 cm. The large vessel headspace is provided to insure foam disentrainment before vapors enter the overhead condensers.

Excessive foaming is detected by a Versaford Electronic Liquid Level Controller (Cole Palmer model 7186) with the foam probe mounted through the vessel headplate. The foam probe was made by special order and is assembled from a 5 mm diameter stainless steel rod forced through a tightly fitting 12 mm

LABORATORY FLASH VESSEL

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Figure 7

O.D. teflon tube. The exposed tip is sharpened to a 90° point. The teflon probe covering is used because of its hydrophobic surface properties which prevent a condensate film from coating the probe. A liquid film would short-circuit the probe and cause the sensor to register a false high foam level.

The temperature is controlled by cycling 2 liters/min of heated water through a seventeen turn 14 cm diameter coil of 6 mm stainless steel heat exchange tubing in the flash vessel. In operation, entering beer splashes onto the coil and the vapors flash away. Beer then drips down the coil into the stirred boiling liquid reservoir at the flash vessel bottom and is returned to the fermentor. Exchanger heating water temperature is controlled by a Hallikainen Instruments "Thermotrol" proportional temperature controller. A resistance thermometer monitors the hot water reservoir and additional heating is provided by energizing a 1500 watt heater in the water return line. The heater is constructed of a four turn, 25 cm diameter coil of 12 mm diameter copper tubing wrapped with electrical heating tape and insulated with asbestos cloth. The flash vessel is lagged with a 1 cm thick high density foam blanket except for a 3 cm wide viewing strip up the length of the vessel.

A headplate port, fitted with a 15 cm long 2.5 cm diameter sterile filter interfaces to the flash vessel pressure control system. Beer liquid and headspace thermo-wells are provided.

Product vapors exit the flash vessel through a 25 mm port in the head plate. A 60 cm long insulated pipe leads up to the condenser train. Two, American Standard No. 87M200-8A2 stainless steel shell in tube heat exchangers are used. Flash-vessel vapors are condensed on the shell side of the exchangers by a 0°C ethylene glycol/water solution chilled by a Blue M Model PCC-12A-3 portable cooler. Condenser product drains into a 20 l glass carboy packed in dry ice. Flow to the carboy can be closed off with a valve in the

product line to allow carboy replacement. The carboy is connected to the vacuum pumping system.

A valved side stem branches from the product line. A sample tube can be sealed to the side stem below the valve and evacuated. Opening the valve allows collection of small product samples.

4. Beer Cycling System

The beer cycling system is detailed in figure 8. Beer is sucked by vacuum from the fermentor into the flash vessel. When the flash vessel liquid level probe (similar to that used for foam detection) senses a low liquid level in the flash vessel, a solenoid valve is activated. Beer is sucked from the bottom sidewall port of the fermentor past the bleed stream side tap, through a flow rate controlling needle valve, through the solenoid then into the flash vessel.

Beer recycle back to the fermentor is more difficult. A 120 liter per hour capacity, adjustable flow rate, diaphragm pump (AMF-CUNO Model H3971-121) is used. The diaphragm pump can work against a high vacuum and applies very little destructive shear to the cycling yeast. However, the fermentor beer is at its flash point. If fed directly to the pump, the beer flashes on the intake stroke filling the pump head with vapor and preventing pumping. Two measures have been taken to assure against this problem. Flash vessel beer passes through a 45 cm long 6 mm single tube heat exchanger (angled downward 5° from horizontal) to cool it below the flash point. The pump is placed one meter below the flash vessel so that a 70 mm Hg liquid head pressure is exerted in addition to the flash vessel pressure. The pump head pressure leg is a 20 mm diameter tube to allow easy bubble rise and prevent vapor locking of the line. The flash vessel beer exchanger is connected to a "T" in the head pressure leg. Bubbles rise past this "T", disentrain from the liquid and the vapor is then returned through a vapor line to the flash vessel headspace.

Figure 8.

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After passing through the pump, beer flows past a sidestem sampling valve and back to the fermentor.

5. Fermentor Beer Bleed System

The fermentor bleed is taken as a side tap from the flash vessel feed stream. Flow rate is controlled with a calibrated Signamotor Model AL-4E kinetic clamp pump. The product is collected in a 20 liter carboy.

Several alternative methods for yeast concentration and recycle are still under consideration. These will be tested under actual operation. The method chosen will be reported on later.

6. Substrate Feed System

Substrate is prepared by premixing ingredients in a 220 liter agitated, heated, polyethylene mixing tank. The A Gearchem Model G4-ACKKAV-B high pressure pump forces feed through a Pall cartridge prefilter (model MCY1001BA) and a sterile 0.2 μ M microbe removable filter (model SDL1AR16P).

A 220 liter sterile polyethylene drum, vented through a 15 cm long 2.5 cm diameter glasswool filter serves as the feed reservoir. A liquid level probe in the fermentor (similar to the flash vessel foam level detector) senses a low liquid level and activates the Manostat Veristaltic kinetic clamp feed pump. Feed flow rate and total accumulated feed volume are accurately measured ($\pm 0.5\%$) by a Fluidyne model 213-200 positive displacement flow meter and Fluidyne Model 1227 microprocessor.

7. Vacuum System

The flash-vessel is connected to the vacuum system through the product collection vessel. The vacuum is obtained with a 8 liter per minute displacement capacity Kinney Model K2-8 vacuum pump running continuously. The product

collection vessel is connected to a 40 liter pressure surge tank through a liquid nitrogen cooled final vapor condenser and to the pump. Pressure is controlled by a Manowatch Model MW-1 controller connected through a sterile filter to the flash vessel head space. The controller activates a solenoid valve to bleed filtered air into the vacuum line just before the vacuum pump whenever the pressure goes below the set point. Arranged in this way, the flash-vessel pressure cycles by 1-2 mmHg about its set point, taking 45 to 60 seconds per cycle. As the pressure drops below the set point, foaming becomes excessive. If the foam rises too high in the flash vessel, the foam probe is triggered and antifoam agent is pumped into the fermentor. When the pressure reaches about 1 mmHg below the set point, the solenoid is activated and air bleeds rapidly into the system. Boiling and foaming subside and an accurate measure of vessel liquid level can be made. If the liquid level is too low, the liquid level probe activates the solenoid to bring more beer from the fermentor.

8. Materials of Construction

All beer wetted parts are of pyrex glass, teflon, silicone or stainless steel.

9. Conclusion

Completion of this apparatus makes available a highly versatile new system for product inhibition free fermentation. Operation over the next four quarters will allow full optimization of the new flash-ferm process.

IV. UTILIZATION OF HEMICELLULOSE SUGARS

A. Xylose Fermentation

During this past quarter, work continued with Bacillus macerans grown on a 2% xylose solution in a continuous culture. In the first runs, nitrogen was sparged through the fermentor liquid at a rate of 0.05 VVM. During this quarter, sparging rates of 0 and 0.1 VVM were tried. The higher rate produced little change in growth patterns from those observed at 0.05 VVM. No sparging produced large changes. The cell density was less than half that at 0.05 VVM with a concomitant poor consumption of substrate, as is shown in Table 6. Considering the results, future work will probably be done at a sparging rate of 0.05 VVM.

Runs at 0.05 VVM were made with a feed enriched with ethanol so that the final ethanol concentration was about 0.7% (about double the concentration that the bacteria produced). The effect of this, is lower cell densities and poorer consumption of substrate. More runs like this will be made, but the alcohol concentration will be higher.

B. Xylanase Production

In shake flask, Chaetomium trilaterate No. 2264 grows in pellet form. The size of pellets varies from flask to flask, some being very fine and the largest is about 5 mm in diameter. As the pellets grow, the color of the broth changes from milky white to pink.

The results of batch growth studies are shown in Table 7. The enzyme production rate in shake flask and 5-liter fermentor showed a marked difference because of pH control in the fermenter.

Table 6

Xylose Fermentation Environmental Conditions and Results

Dilution Rate (hr^{-1})	Cell Density ($\frac{\text{gm}}{\text{L}}$)	Substrate Remaining ($\frac{\text{gm}}{\text{L}}$)
2% Xylose, 0.05 VVM		
0.03	0.68	0.2
0.06	0.74	6.5
0.09	0.74	11.2
2% Xylose, -0- VVM		
0.03	0.30	15.4
0.06	0.25	16.5
0.09	0.30	16.6
2% Xylose, 0.10 VVM		
0.03	0.65	-0-
0.06	0.65	7.0
2% Xylose, 0.05 VVM 7 $\frac{\text{gm}}{\text{L}}$ EtOH in the Fermentor		
0.03	0.65	2.6
0.06	0.53	9.8

Table 7
Xylanase Production

Mode of Operation	Type of Substrate	Results			Remarks		
Final Enzyme Activity							
Shake Flask (200 ml medium in 500 ml flask)	larchwood	12.7 ^a	23.4 ^b	4.69 ^c	a,b, : with 1%, 2% substrate (0.1% Yeast) c , 2% Substrate, (0.05% Yeast)		
	wheat bran	32.6 ^d	32.5 ^e		d,e: with 3.5%, 7.0% substrate, (0.1%, 0.2% yeast extract for 3.5% and 7.0% substrate conc.)		
	larchwood	6.79 ^f	4.45 ^g	14.64 ^h	Activities with 2,4 and 6 day inoculum age (f,g,h).		
Submerged fermentation	larchwood	0.54*	(0.65) ⁺		pH	S _o (%)	Yeast Ext. (%)
		6.60	(0.56)		6.0	1	0.1
		1.95	(0.50)		7.0	1	0.1
Fer. vol: 5 l		6.03	(1.09)		6.5	1	0.1
		1.95	(0.71)		7.5	1	0.1
Liq. vol: 3 l.		6.60	(1.28)		8.0	1	0.1
		6.25	(0.80)		7.0	1	0.3
					7.0	1	0.2

*= Enzyme Activity(I.U./ml)

+ = Soluble Protein (mg/ml.)

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EXECUTIVE SUMMARY

SOLAR PROGRESS QUARTER REPORT

DECEMBER 1979

C.R. WILKE AND H.W. BLANCH

PRINCIPAL INVESTIGATORS

Department of Chemical Engineering
and
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

I. Raw Materials and Process Evaluation

A. High Pressure Hydrogen Chloride Process

This paragraph deleted because of matter subject to patent not released by DOE.

B. Moderate Pressure Sulfur Dioxide Hydrolysis on Wood

Preliminary studies show minimal carbohydrate conversion of wood and sulfur dioxide at pressures of 38 psi at room temperature. A further study of higher pressure sulfur dioxide, but below appreciable carbohydrate degradation temperature, is indicated.

II. Enzyme Fermentation Studies

A. Cellulase Production

1. Batch Fermentation

Evaluation studies on Trichoderma viride strain Rut-L-5 and comparison studies were performed with Rut-C-30. The Mandels medium with ball milled solka floc and Tween 80 was used for all the experiments. Under comparable conditions of

production, Rut-C-30 appears to be somewhat superior in FPA, β -G, C_1 and soluble protein. Rut-L-5 appears to be slightly higher in C_x activity.

2. Continuous Production of Rut-C-30

Studies were performed to optimize individually the 1st and 2nd stage of the two stages continuous production system, by manipulating the pH, temp., Tween-80 level, substrate concentration and the dilution rate. Decreasing the Tween 80 level in half did not affect the resultant FPA. Increasing the substrate concentration by about 2-5 increased the FPA by about the same factor in both stages. Increasing the dilution rate by a factor of two in the second stage, doubles the productivity.

B. Studies on Composition of Cellulase Enzyme

The known major components of cellulase from Tv QM-9414, characterized by activities on crystalline and amorphous cellulose, have been separated. The scheme was performed by concentration by ultrafiltration (with Amicon UM-2 membrane), then gel permeation chromatography (with Sephadex G-75), then lyophilization followed by ion exchange chromatography (with DEAE Sephadex and ion gradient elution). The component activities FPA, C_1 , C_x , β -G, and S.P. were determined on each fraction. B-glucosidase elutes first followed by two components of glucanhydrolase (C_x) and a single cellobiohydrolase (C_1). Polyacrylamide gel electrophoresis was used for detection and analysis at each stage of the separation showing the four resolved components and their 17 K to 87 K molecular weights.

C. Economic Optimization of Hydrolysis of Corn Stover

As a substitute for enzyme from Tv QM-9414, enzyme solutions of various concentrations derived from Rut-C-30 were used to hydrolyze corn stover. The results show that the initial rates of hydrolysis with Rut-C-30 at all initial

FPA's are somewhat faster than those obtained with QM-9414. Carbohydrate conversion to sugar at 40 hours hydrolysis is 65% of the theoretical for FPA 14 and 58% for FPA 3.5. This is significantly higher than the 44% obtained with QM-9414 at FPA 3.5. An interesting observation is the negligible difference in the rates of conversion conducted with Rut-C-30 at FPA 14 and 7. The results indicate a potentially significant reduction in the cost of producing sugar from corn stover. Studies of this enzyme for recovery and use in continuous hydrolysis is now desirable.

III. Ethanol Fermentation Studies

A. Media Development

Development of optimum medium for continuous ethanol fermentation is being continued. Emphasis has been on determining the important growth factors for yeast and ethanol production. The experiments show that some of growth limiting factors were in the yeast extract. Biotin appears to be the first growth factor deficiency which has to be satisfied. With biotin present the other vitamin deficiencies were discerned. With excess biotin, pantothenic acid and then pyridoxine becomes limiting. Further additions of the other vitamins had no significant effect on cell yield but increased the ethanol concentration.

The glucose concentration was increased to 10% in a medium containing mainly synthetic growth factors to determine if the medium could be scaled up for running the vacu-ferm system. Linear increase of all the medium components in relationship to the glucose resulted in cell yields of only about 3 g/l dry weight and about 18 g/l ethanol. The problem appears most likely to be due to ethanol inhibition or growth factor deficiencies. Future experiments on mineral and vitamin requirements for 10% glucose solution will be determined.

B. Process Development Studies of Ethanol Production

A detailed engineering and economic analysis of the flash ferm process was presented in the previous progress report. The advantages over the earlier vacu-ferm process and conventional batch process were shown.

A laboratory flash-ferm apparatus of about 3 liter capacity has been assembled to allow testing of the assumptions of the earlier economic analysis and provide further data for design optimumization. A detailed description is provided in the present progress report to facilitate interpretation of results of present and forthcoming experiments.

IV. Utilization of Hemicellulose Sugars

A. Xylose Fermentation

Studies were continued with Bacillus macerans grown on xylose solutions in continuous cultures. It appears that nitrogen sparged fermentation at 0.05 VVM is satisfactory. Higher sparging rates produce little change in growth patterns and less or no sparging produced large and unsatisfactory changes. Experiments with alcohol added (about double the concentration of that produced by the bacteria) resulted in lowered cell densities and poorer consumption of the substrate.

B. Xylanase Production

In shake flasks, Chaetomium trilaterate -2264 grows in pellet form. The size varies and as growth proceeds the broth changes from milk white to pink. The results of batch growth studies in shake flasks and 5 liter fermentor show marked differences and apparently are due to pH control used in the fermentor.

APPENDIX

PROCESS DEVELOPMENT STUDIES ON THE
BIOCONVERSION OF CELLULOSE AND PRODUCTION OF ETHANOL

LAWRENCE BERKELEY LABORATORY

Project Staff

Faculty Investigators

Charles R. Wilke, Professor of Chemical Engineering
Harvey W. Blanch, Associate Professor Chemical Engineering

Postdoctoral Investigators

Steven L. Rosenberg, Bacteriology, UCB, Berkeley, Ph.D. 1970
S. Kishen Tangu, Chemical Engineering, Indian Inst. of Tech., Delhi, India Ph.D. 1976

Graduate Students

Thomas Delfino, Chemical Engineering, UCB, B.S. ChE 1978, M.S. Candidate
George Dove, Chemical Engineering, UCB, B.S. 1978, MS Candidate
Ray Freitas, Biochemistry, BA 1974 and Chemistry, B.S. 1975, MS Candidate
Brian Maiorella, Chemical Engineering, M.I.T., B.S. 1978 Ph.D. Candidate
Kevin Murphy, Chemical Engineering, Mich. State U., BS, 1978, Ph.D. Candidate
Javier Perez, Chemical Engineering, Northwestern U., BS 1978, MS. Candidate
Bruce Vick Roy, Chemical Engineering, Univ. of Delaware, BS 1979, Ph.D. Candidate
Steven Wald, Chemical Engineering, Cornell, U. B.S. 1979, MS Candidate
Dale Wiley, Chemical Engineering, UCSB, MS 1977, Ph.D. Candidate
Harry Wong, Chemical Engineering, M.I.T., M.S. 1973, Ph.D. Candidate

Support Staff

Aldo Sciamanna, Chemist M.S. in Chemistry, Univ of San Francisco, 1952
Bill Long, Technician

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