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UTILIZATION OF CELLULOSIC MATERIALS THROUGH ENZYMATIC HYDROLYSIS I. FERMENTATION OF HYDROLYSATE TO ETHANOL AND SINGLE CELL PROTEIN

Gerald R. Cysewski and Charles R. Wilke

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UTILIZATION OF CELLULOSIC MATERIALS THROUGH ENZYMATIC HYDROLYSIS

I - FERMENTATION OF HYDROLYSATE TO ETHANOL AND SINGLE CELL PROTEIN *

by

Gerald R. Cysewski
and
Charles R. Wilke

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

ABSTRACT

Ethanol fermentation studies were conducted with Saccharomyces cerevisiae ATCC#4126, to determine the optimal conditions of oxygen tension and feed sugar concentration. In long term continuous culture maximum ethanol production was found to occur at 0.07 mmHg oxygen tension and 10% glucose feed concentration. Preliminary process design and cost studies are developed for industrial scale fermentations to produce ethanol and Torula yeast from sugars obtained by enzymatic hydrolysis of newsprint.

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*This paper was prepared for presentation at the First Chemical Congress of the North American Continent, Mexico City, Mexico, December 1-5, 1976

INTRODUCTION

This paper, the first of a two part series, presents new laboratory results for the alcohol fermentation and preliminary process designs for the manufacture of ethanol and Torula yeast from sugars produced by enzymatic hydrolysis of cellulose. These results are employed in the second paper to develop overall material and energy balances and cost estimates for processing of a typical cellulosic waste to produce ethanol plus by-product yeast and electricity.

Although the alcoholic fermentation has been classically thought of as totally anaerobic, numerous workers have shown that trace amounts of oxygen stimulate fermentation rates and that some strains of the yeast, Saccharomyces cerevisiae actually require oxygen as a growth factor (1)(2)(3). The stimulating effect of trace amounts of oxygen is of an anabolic nature rather than catabolic, enabling the cells to synthesize unsaturated lipids necessary for cellular membranes (4)(5). Because of catabolic repression, when the glucose concentration is above 100-200 mg/l, the catabolic metabolism of Saccharomyces becomes primarily anaerobic rather than aerobic even at high oxygen tensions (2)(3). However, at lower glucose concentration, the metabolism switches from anaerobic to aerobic and the Pasteur effect is observed. That is to say, because the energy available for growth per molecule of glucose consumed increases, the specific consumption of glucose decreases (6). Further, it has been shown that very high oxygen tensions (above 300 mmHg) inhibit both cell growth and alcohol production (6)(7).

This work was then undertaken to quantify the effect of oxygen and feed glucose concentrations on ethanol production in continuous culture. From the optimal laboratory fermentation conditions, an industrial scale continuous fermentation plant was designed and an economic evaluation conducted.

EXPERIMENTAL PROCEDURES

Saccharomyces cerevisiae, ATCC #4126 was used in the continuous alcohol fermentation studies in a 5 liter "Micro Ferm" fermenter (Fermentation Design, Inc.). The pH was controlled with a Fermentation Design pH-RT recorder-controller module used in conjunction with an Ingold 761-351B combination pH electrode. The pH was held at 4.0 by automatic addition of either 6M H_2SO_4 or 6M NaOH. A continuous and constant flow of medium was pumped to the fermenter with a kinetic clamp pump (Sigmamotor model TM-2.0-2) from a 20 liter reservoir. A fermenter working volume of 2 liters was maintained by an overflow port in the side of the fermenter jar. Air, sterilized by filtration was sparged through the fermenter and the oxygen tension measured with a New Brunswick model M1016-0202 oxygen probe connected to a Leeds and Northrup Speedomax Type G recorder. The oxygen tension was controlled by changing the RPM of the impeller and/or the air rate. A temperature of 35.0°C was used for all the fermentation experiments.

The fermenter was filled with two liters of fermentation broth, shown in Table 1 and autoclaved. (When the glucose concentration of the medium was changed, all other components were changed by the same ratio.) The fermenter was inoculated with a 2% inoculum and at the end of batch growth (usually 12 to 16 hours after inoculation) the medium feed pump was turned on. After three fermenter volumes (i.e. 6 liters) of broth passed through the fermenter, 10 ml samples of effluent were collected every 4 hours and the cell mass concentration measured. When the cell mass remained constant over a 12 hour period, steady state was assumed and a 20 ml sample was aseptically withdrawn from the fermenter for analysis. Experimental conditions were then changed and a new steady state established.

ASSAY PROCEDURES

Ethanol Concentrations. Ethanol was measured by gas chromatography using an Aerogrpah 1520 G-L Chromatograph. A 6 foot 1/4 inch column packed with Chromosorb-W acid wash type 60-80 mesh was used with a flame ionization detector. The injector and detector temperatures were 175°C and the column oven operated isothermally at 105°C.

Cell Mass. The cell mass concentrations were measured optically using a Fischer Electrophotometer with a 650 mμ filter. A calibration curve of cell mass versus absorbance was prepared by measuring the absorbance of samples of varying cell concentrations. The cell mass (g dry wt./liter) of these samples was determined by centrifuging the cells, washing twice with distilled water and drying the cells at 105°C until no further weight change occurred.

TABLE 1

BASE FERMENTATION MEDIUM

COMPONENT*	PER LITER
GLUCOSE (ANHYDROUS)	100 G
YEAST EXTRACT (DIFCO)	8.5 G
NH_4CL	1.32 G
$MGSO_4 \cdot 7H_2O$	0.11 G
$CACL_2$	0.06 G
ANTI-FOAM (GENERAL ELECTRIC AF60)	0.2 ML
TAP WATER TO	1 LITER

*ALL SALTS AND GLUCOSE REAGENT GRADE.

Glucose concentration. Glucose was determined by the dinitrosalicylic acid (DNS) and method (8).

RESULTS AND DISCUSSION

Initially the yeast showed a definite optimum for both ethanol production and cell mass production at 0.7 mm Hg oxygen tension. Above this oxygen tension ethanol and cell mass productivities declined. These findings are in direct agreement with the results reported by Cowland (2). However, Cowland was able to adapt the yeast to high oxygen tensions and eliminate the inhibitory effect of oxygen on cell production, but no gain in ethanol production was reported. The adaption process was also observed in this work. After approximately three weeks of continuous operation, it was observed that cultures developed the ability to sustain high cell mass production rates at high oxygen tension. Furthermore, the optimal ethanol productivity of the "adapted" culture was 42% greater than "unadapted" yeast at conditions of complete substrate utilization.

Figure 1 plots various productivities obtained with the adapted yeast against oxygen tension at a dilution rate of 0.22 hr^{-1}. As shown, the "adapted" yeast required an oxygen tension of only 0.07 mm Hg for optimal ethanol production. This is an order of magnitude less than that required by the "unadapted" yeast. Also, as is evident from Figure 2, above an oxygen tension of 1.7 mm Hg the cell mass productivity drastically increased with a corresponding decrease in ethanol productivities. An additional point to be made from Figure 1 is the low fermentor productivities obtained at zero

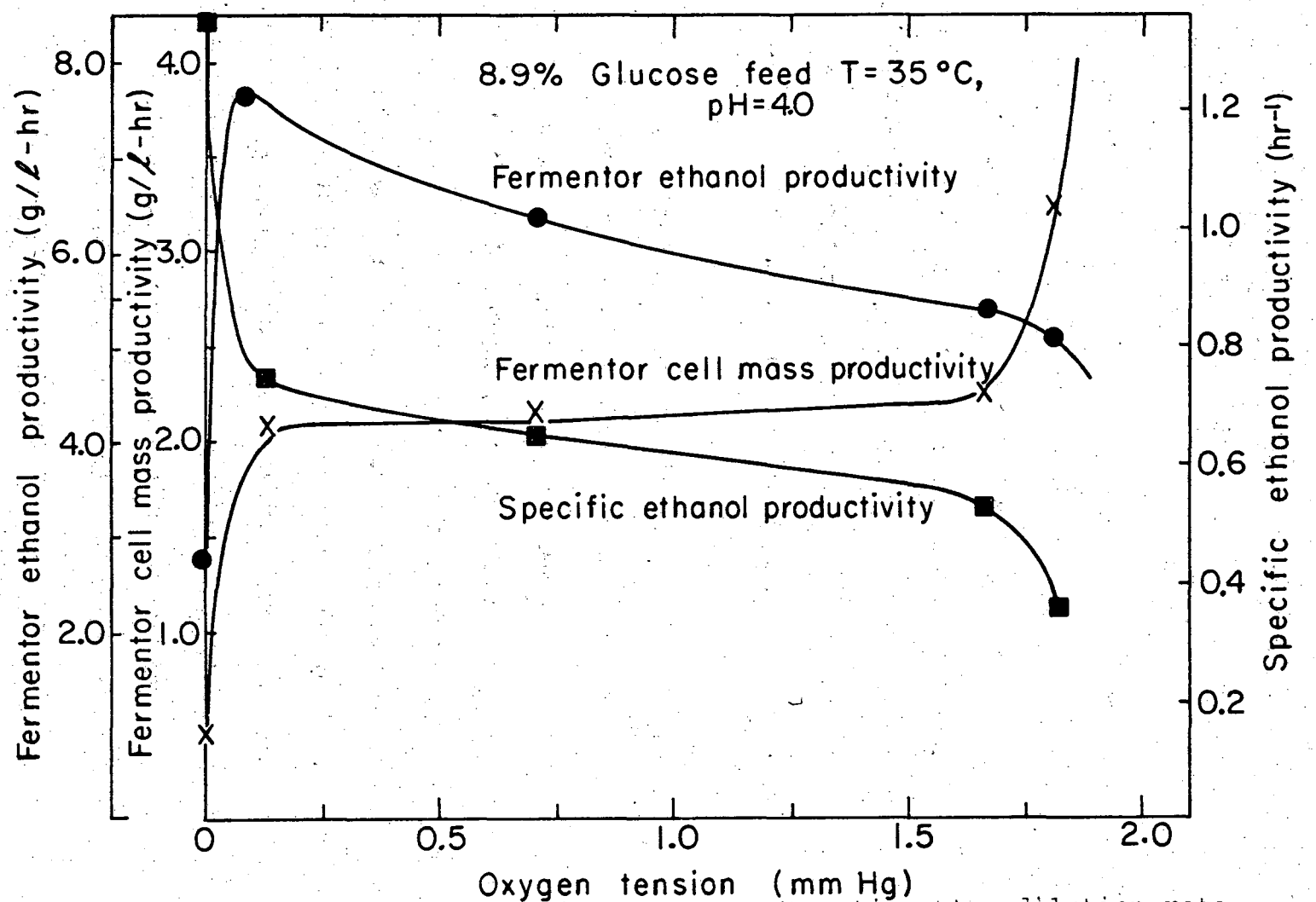

Fig. 1 Productivities as a function of oxygen tenstion at a dilution rate of 0.22 hr^{-1}.

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oxygen tension. These data at zero tension were obtained using an air saturated feed and not sparging air through the fermentor and, hence, represent the operations of a continuous ethanol fermentation naively operated in an anaerobic mode. (Similar low fermentor productivities were obtained with "unadapted" yeast at zero oxygen tension).

Once the culture had been adapted to high oxygen tensions it could be deadapted by running the fermentor at a low dilution rate (0.05 hr^{-1}) and stopping the air flow to the fermentor. The adaption process could then be reinitiated by maintaining an oxygen tension of 1.8 mm Hg for a period of 3 to 5 days. Because of the aforementioned increase in ethanol productivity and reduced oxygen requirement the remaining experimental studies were conducted with "adapted" yeast.

The results of a typical continuous fermentation with an adapted culture are shown in Figure 2. The fermentor ethanol productivity, ethanol, glucose and cell mass concentration are plotted against dilution rate. Ethanol productivity is highest at high dilution rates, however, a large percent of the glucose is unfermented at these conditions. As a result, the fermentor must be operated at a low dilution rate (0.17 hr^{-1}) to obtain complete utilization of the glucose. Although not clearly shown in Figure 2, the glucose concentration was independent of dilution rate below 0.19 hr^{-1} and could not be reduced below 0.3 wt%. This rather high residual glucose level may be explained by the Pasteur effect. Since air was continually sparged through the fermentor to maintain the optimum oxygen tension, the yeast metabolism became more aerobic as the glucose level approached 0.3% and catabolic

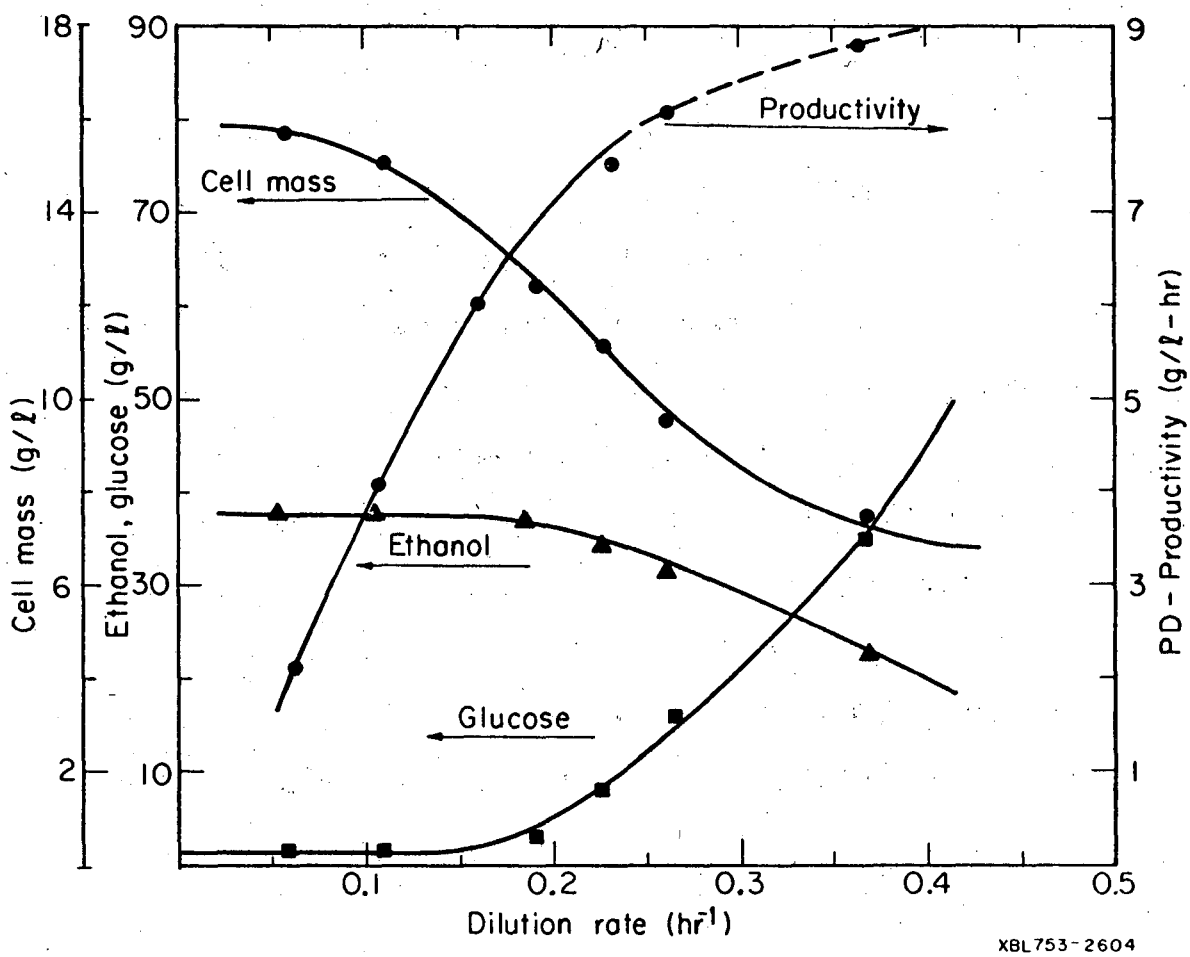

Fig. 2. Results of Continuous Fermentation with Adapted Yeast. 8.9% Glucose Feed, Oxygen Tension 0.07 mm Hg.

repression lifted, resulting in a lower glucose consumption rate. Thus, to overcome this problem a second stage anaerobic fermentation is needed. Experiments using a 1 liter "mini-ferm" (Fermentation Design, Inc.) as the second anaerobic stage did indeed lower the residual glucose concentration to 0.02% at a total system dilution rate of 0.13 hr^{-1}. (Total system dilution rate = medium flow/(sum of volumes of both stages).

Figures 3 - 5 illustrate the effect of feed sugar concentration on continuous alcohol production. In these experiments the oxygen tension was held at 0.07 mmHg. Figure 3 plots the effluent ethanol concentration against dilution rate for various sugar feed concentrations. As the dilution rate increases the ethanol concentration drops due to incomplete utilization of the glucose. At high glucose concentrations the ethanol drops more rapidly with dilution rate because alcohol inhibition becomes an important factor as shown by Bazua and Wilke (9). An explanation of the continued steep decrease in alcohol concentration at high dilution rates for the 12.1% feed is not clear but may be due to a combination of ethanol and glucose inhibition.

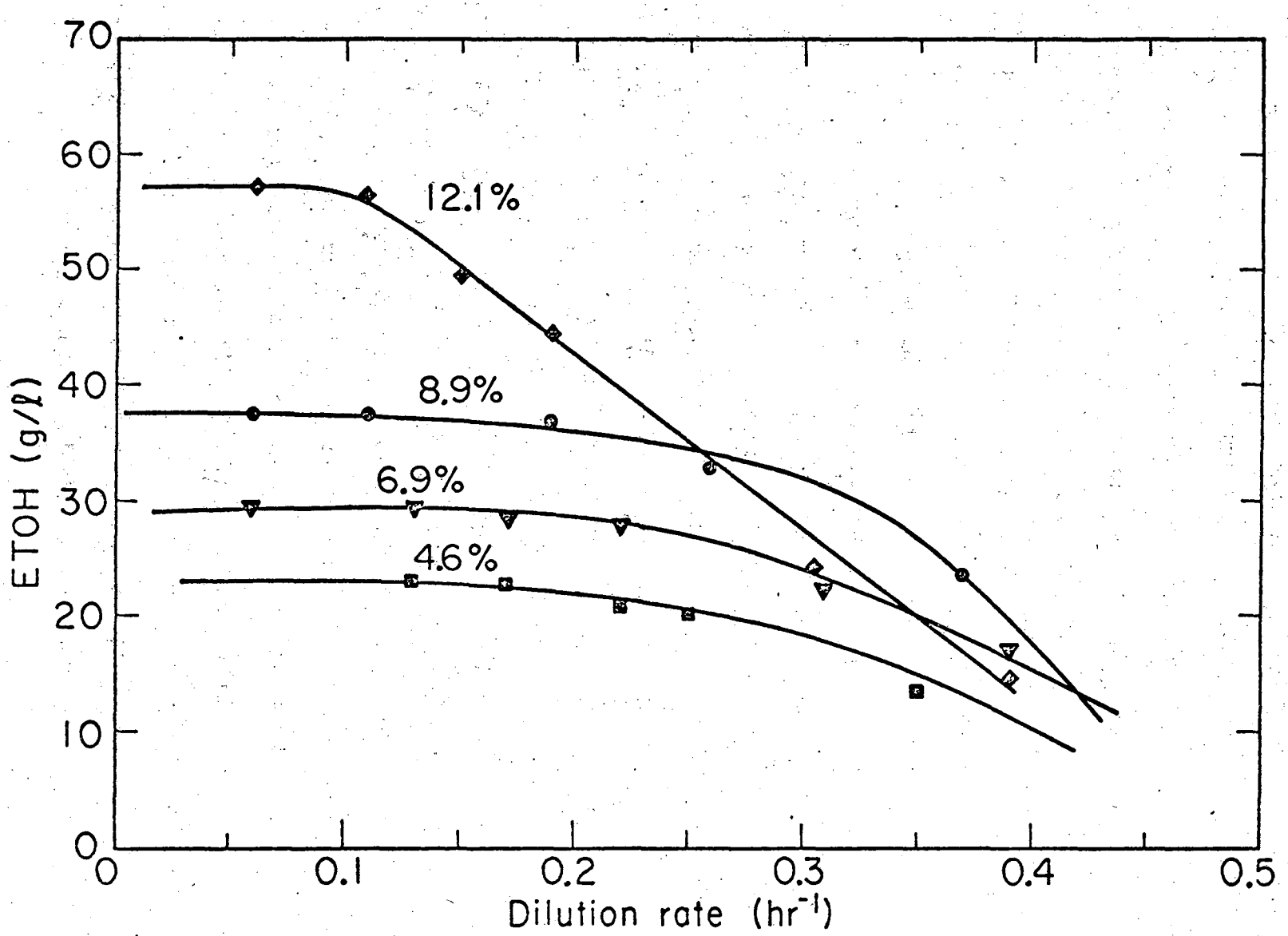

Figure 3. Effluent Ethanol Concentration as a Function of Dilution Rate for Various Feed Sugar Concentrations (adapted culture).

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The dilution rate required to ferment essentially all the glucose in the feed is shown as a function of feed sugar concentration in Figure 4. The required dilution rate has a linear relation to feed glucose concentration at low concentrations, but as the feed concentration increases the curve bends toward lower dilution rates. This is due to alcohol inhibition, since higher sugar concentrations imply higher ethanol concentrations when the sugar is totally fermented. For the 16% sugar feed, 7.6% ethanol was present in the fermented broth and the specific cell ethanol productivity was reduced by 74% compared to conditions of negligible alcohol inhibition. This agrees with the work of Bazua (9) who used ethanol enriched feeds rather than producing the ethanol by fermentation of concentrated sugar solutions.

Figure 5 is a combination of data presented in Figure 3 and 4. Defining "complete" substrate utilization as only 0.3 wt.% sugar unfermented, Figure 5 relates specific cell productivity and fermenter productivity to initial feed sugar concentrations. It is interesting to note that an optimum fermenter productivity exists at a 10% sugar feed. Above this value the specific cell productivity decreases due to ethanol inhibition, while if the sugar concentration decreases the cell mass drops, resulting in a lower fermenter productivity even though the specific productivity increases. It should be pointed out that the productivities shown in Figure 5 are those at complete substrate utilization and not the maximum productivities obtainable for a given substrate concentration.

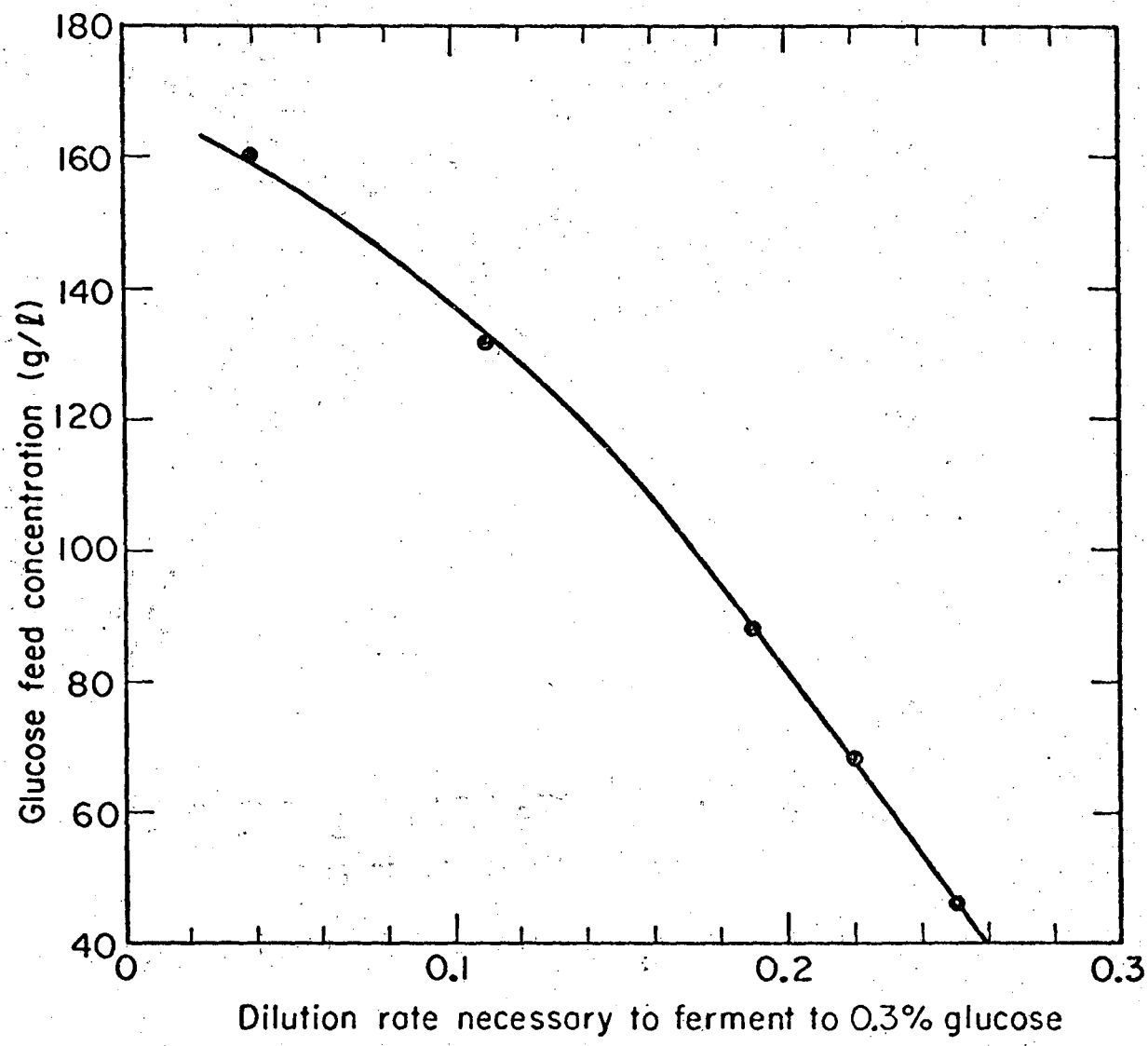

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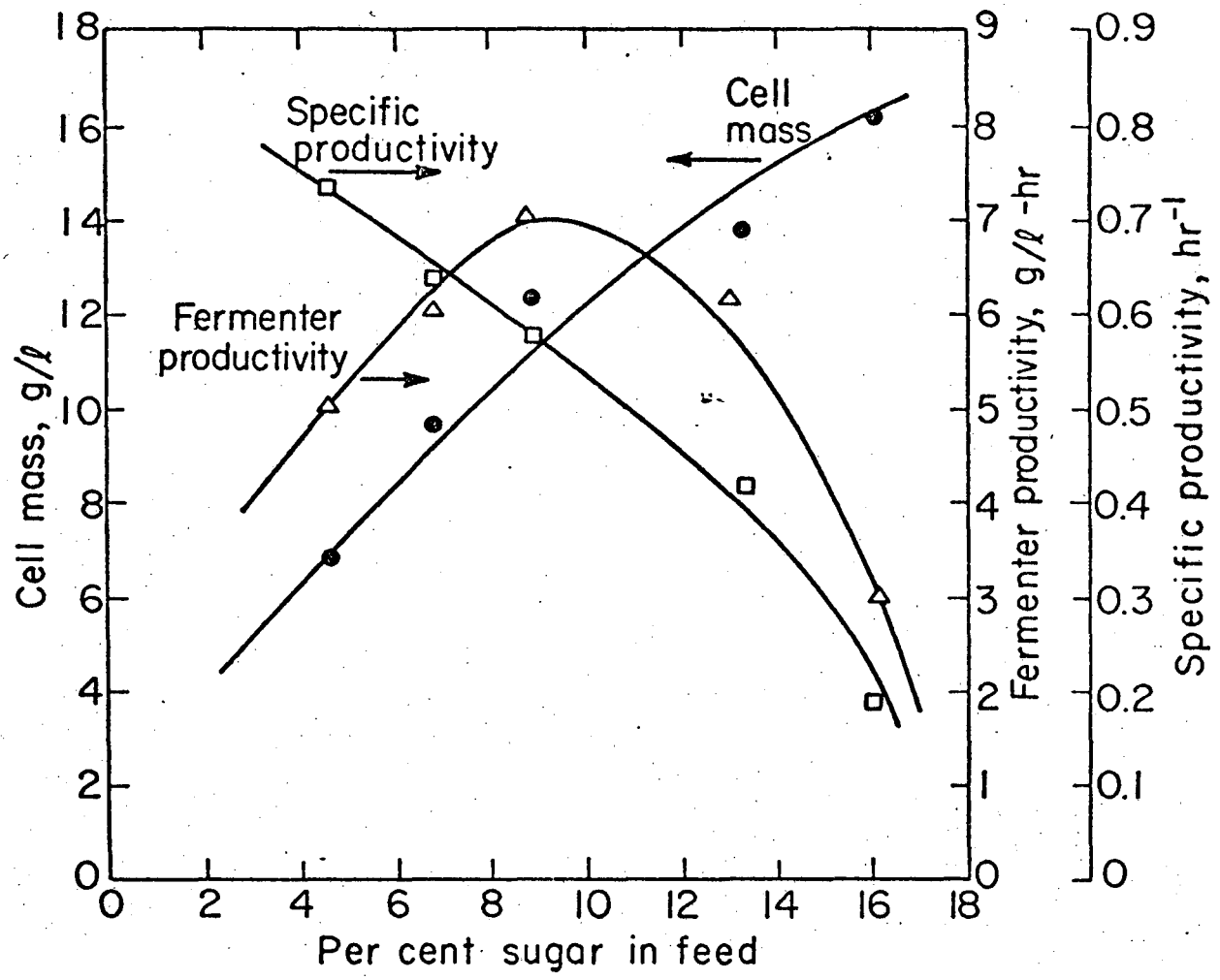
Figure 4. Dilution Rate Necessary to Ferment to 0.3% glucose as a Function of Glucose Feed Concentration (adapted culture).



Conditions at "Complete" Substrate Utilization

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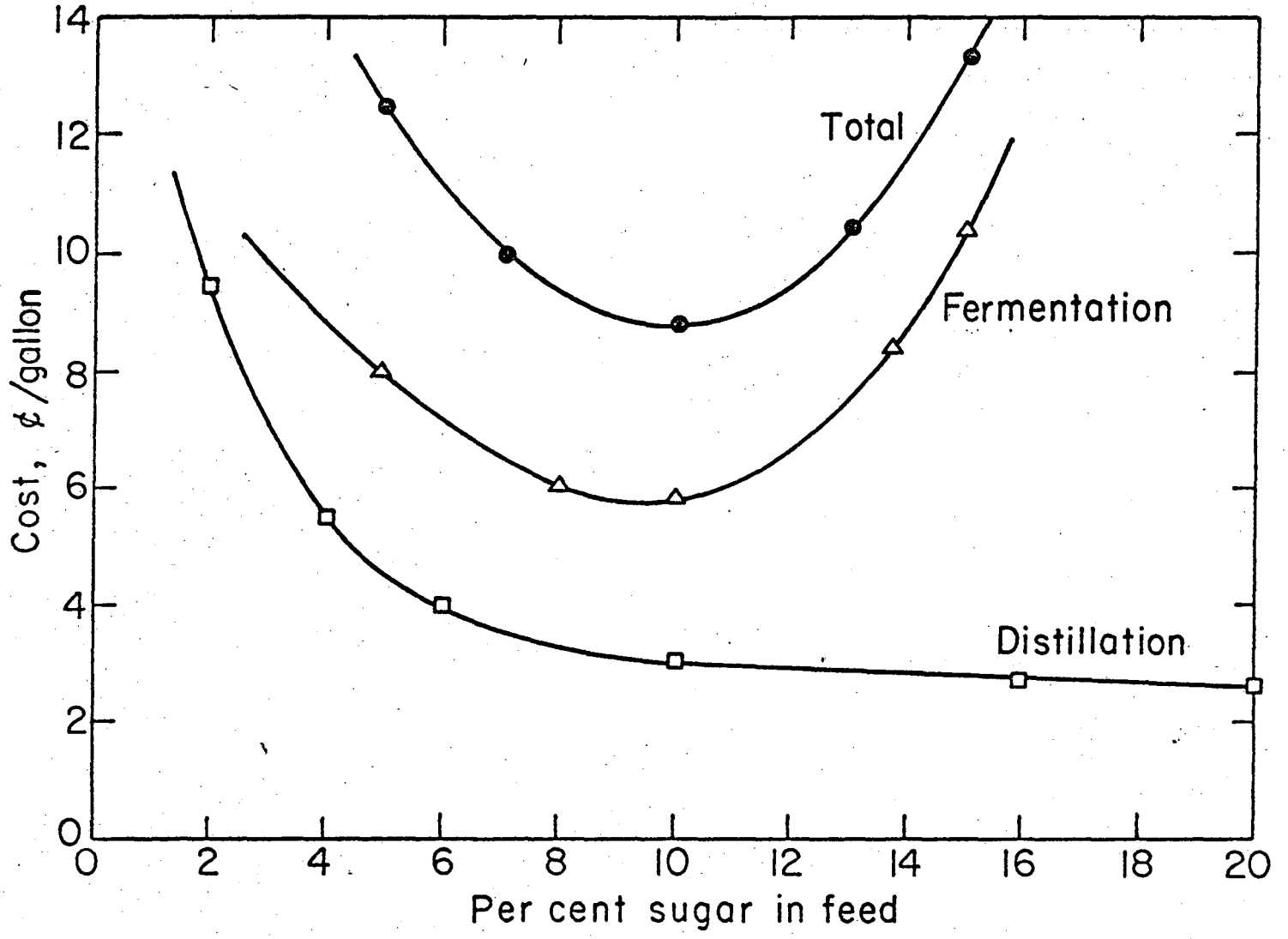
Figure 5. Ethanol Productivities and Cell Mass Concentration as a Function of Feed Sugar Concentration (adapted culture).

PROCESS DESIGN AND ECONOMICS

Using the optimal laboratory fermentation conditions described above for adapted cultures, an industrial size fermentation plant producing 24,000 gal/day of 95% ethanol was designed. The fermentation substrate was taken to be sugars produced from the enzymatic hydrolysis of cellulosic waste as proposed in the process design studies of Wilke et al. (10). The design basis is shown in Table 2. The hydrolysis product was found to be 70% fermentable by Saccharomyces, thus requiring a 14.3% solution of hydrolysate sugars to obtain the optimum feed of 10% fermentable sugars. Preliminary cost analysis showed it economically favorable to concentrate the sugar to 14.3%. The concentration costs of 2.7¢/gal. of ethanol produced (see below) being more than offset by the savings in fermentation and distillation costs as shown in Figure 6. A computer process model was used to design and optimize the ethanol fermentation plant and a single cell protein process which consumes the residual sugars left after the alcohol fermentation.



Ethanol Production Costs
(2.37×10^4 gal/day of 95% Ethanol)

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Figure 6. Ethanol Production Costs Excluding Raw Materials as a Function of Feed Sugar Concentration.

Figure 7 shows a schematic flow diagram of the ethanol fermentation process. The principal items of equipment corresponding to the flow sheet are listed in Table 3. The evaporator which concentrates the hydrolysate sugar solution is not shown although it has been included in the process cost analysis.

After the hydrolysate sugars have been evaporatively concentrated from 4.0% to a 14.3% solution, protein and mineral supplements, shown in Table 4, are mixed with the sugars. Sterilized by steam injection, the fermentation broth is distributed to five continuous fermenters, each operating at a dilution rate of 0.17 hr^{-1}. A low flow of air (8.0×10^{-4} VVM) is sparged through the fermenters to maintain the oxygen tension at the optimum level of 0.07 mmHg. The fermented beer then passes to two continuous centrifuges and the yeast is removed. The yeast is subsequently dried and stored for sale as a protein feed supplement. The clarified beer from the centrifuges is next distilled to concentrate the ethanol to 95 wt%. An absorber using the distillate bottoms as the absorbing liquid, is employed to recover ethanol lost in the exit gases (air and CO_2) from the fermenters. The ethanol rich stream from the absorber is also fed to the main distillation unit for final ethanol recovery.

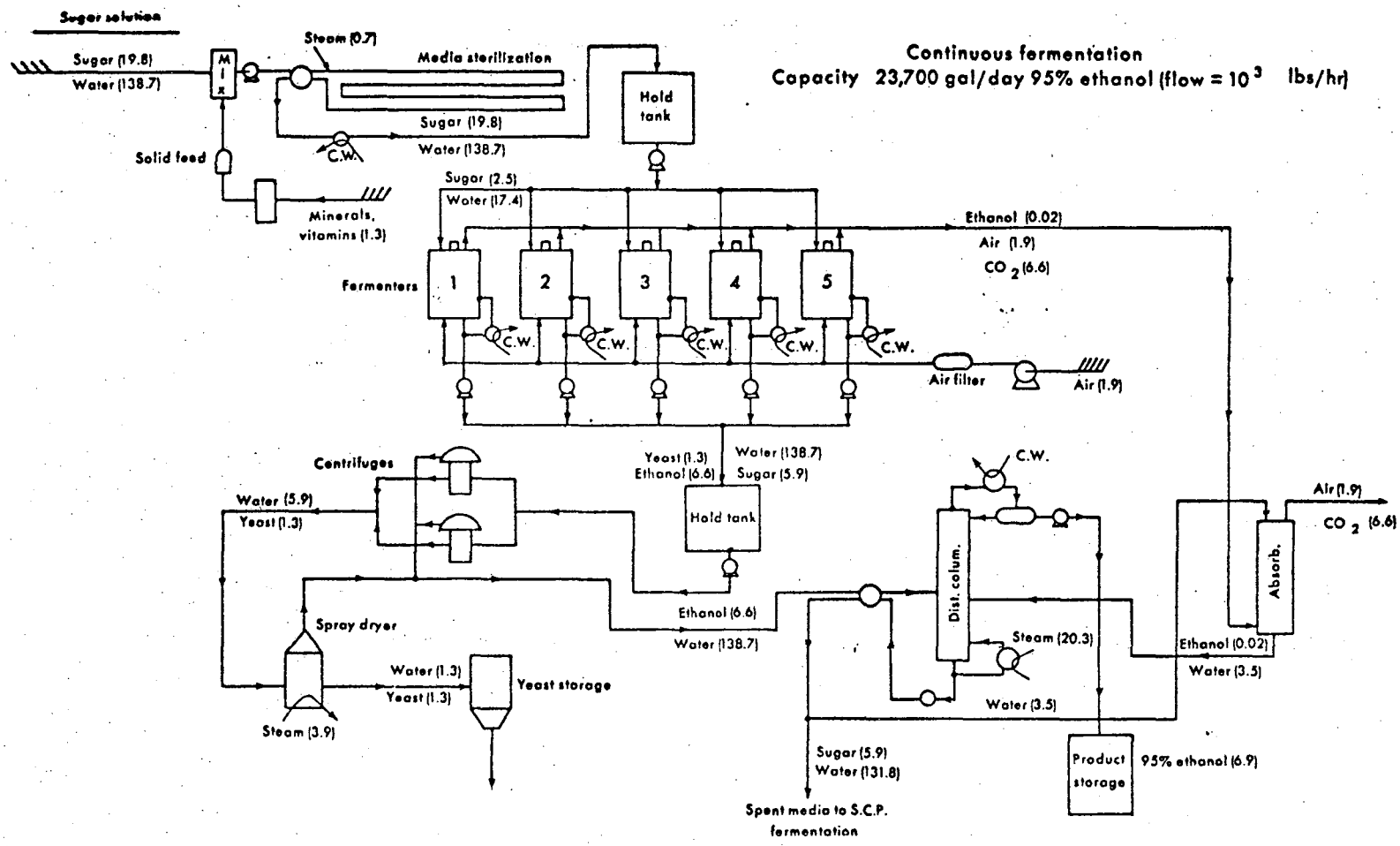

Figure 7. Flow Diagram for Ethanol-Torula Yeast Production.

TABLE 2

SUGAR CONCENTRATION	14.3%, 70% FERMENTABLE
DILUTION RATE	0.17
TEMPERATURE	35°C
CELL YIELD FACTOR, Y(X/S)	0.1
ETHANOL YIELD FACTOR, Y(P/S)	0.465

ETHANOL FERMENTATION DESIGN BASIS

Table 3

Item	Unit Specification	No. of Units	Cost/Unit $ FOB
Sugar Evaporator	7 effect, 2.55×10^4 ft^2 Total area	1	$186,000
Ethanol Fermentation		Total	$763,000
Fermenter	Vol. 2.44×10^4 Gal. Stainless steel construction	5	51,400
Agitator	10.8 H.P. stainless steel construction	5	5,600
Air compressor	35.5 H.P. centrifuger type 30 psig.	1	19,900
Air Filter	0.4x0.3 meters glass fibers	5	200
Media sterilizer	4.2x0.6 meters insulated stainless steel pipe	1	2,900
Preheat exchanger coupled with sterilizer	2750 ft^2 stainless steel	1	49,200
Cooler exchanger coupled with sterilizer	1190 ft^2, stainless steel	1	28,800
Heat removal exchanger coupled with fermenter	245.3 ft^2, stainless steel	5	10,400
Solid feeds	Screw conveyor 4T/D	4	1,500
Nutrient mixing tank and agitator	Vol. 4.6×10^3 Gal., Stainless steel	1	10,300
Sugar solution storage tank	Vol. 4.6×10^4 Gal., Stainless steel	1	54,260
In plant beer storage tank	Vol. 4.6×10^4 Gal., Stainless steel	1	54,260

Item	Unit Specification	No. of Units	Cost/Unit $ FOB
Centrifuges	Nozzle type bowl, 40 HP	2	$ 44,000
Yeast spray dryer		1	22,800
Product alcohol storage	Vol. 770,000 Gal. Carbon steel	1	64,700
Pumps and drivers		10	2,400
Ethanol Recovery		Total	$ 124,700
Distillation column	5.2 ft dia. 76 sieve trays	1	49,300
Condenser	890 ft^2 carbon steel	1	23,700
Reboiler	630 ft^2 carbon steel	1	19,000
Preheat exchanger	990 ft^2 carbon steel	1	25,500
Ethanol absorber	4 ft dia. 23 ft high 1" Raschig rings	1	7,200
SCP Fermentation		Total	$ 659,000
Fermenter	Vol. 3.26×10^4 Gal. Stainless steel construction	2	45,200
Agitator	136.3 HP Stainless steel construction	2	23,200
Heat removal exchanger coupled with fermenter	5598 ft^2 stainless steel construction	2	77,400

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Item	Unit Specification	No. of Units	Cost/Unit $ FOB
Air compressor	455 HP centrifuger Type 30 psig	1	$126,900
Air filters	1.4x0.4 meters, glass fibers	2	2,500
Media Sterilizer	4.2x0.6 meters insulated stainless steel pipe	1	2,900
Preheat exchanger coupled with sterilizer	2750 ft^2 stainless steel	1	49,100
Cooler exchanger coupled with sterilizer	1190 ft^2 stainless steel	1	28,700
Solid feeders	screw conveyors	4	1,500
Nutrient mixing tank with agitator	Vol. 4.6×10^3 Gal. stainless steel	1	10,300
Yeast spray dryer		1	34,500
Centrifuge	Nozzle type bowl	2	44,000
Pumps and drives		5	3,200

TABLE 4

COMPONENT[1]	$/T	T/DAY	G/L
$(NH_4)_2SO_4$	90	6.06	3.65
$MgSO_4 \cdot 7H_2O$	110	0.26	0.16
CaCl	33	0.14	0.08
PROTEIN NUTRIENT[2]	960	4.76	2.86

MEDIUM RAW MATERIALS--ETHANOL FERMENTATION

1. TRACE ELEMENTS ARE ASSUMED SUPPLIED BY PROCESS WATER
2. AMBER BYF-300 AMBER LABORATORIES, JUNEAU, WISCONSIN

TABLE 5

TORULA YEAST

DILUTION RATE	0.32 HR^{-1}
TEMPERATURE	40°C
CELL YIELD FACTOR, Y(X/S)	0.46

SCP FERMENTATION DESIGN BASIS

Saccharomyces cerevisiae used in the ethanol fermentation will ferment only 70% of the reducing sugars in the hydrolysate. The remaining 30% of the sugars (xylose and cellobiose) are fed to an aerobic fermentation process to produce single cell protein from a Torula yeast. Although Torula yeasts will ferment the remaining sugars, it is a facultative aerobe and does not produce ethanol. Table 5 lists the design assumption for the SCP fermentation.

The SCP fermentation design was based on data presented by Maxon and Johnson (11) and Mandels and Weber (12). A Torula yeast was chosen since it has been shown to ferment all of the reducing sugars present in the hydrolysate. (12) This design is of course speculative and is included to indicate general processing costs. Further data on the cell mass production from the residual sugars left after the ethanol fermentation is needed.

The single cell protein fermentation, although not shown, resembles the alcohol fermentation process excluding the distillation and absorption columns. Of course the aeration and agitation rates are much higher for the production of cell mass. The principal items of equipment for the SCP fermentation are described in Table 3. From the distillation column the sugar solution is supplemented with minerals and protein components shown in Table 6. Two parallel continuous fermenters operating at a dilution rate of 0.318 hr^{-1} are employed in the SCP fermentation. In order to satisfy the oxygen demand of the yeast an

TABLE 6

COMPONENT[1]	$/T	T/DAY	G/L
$(NH_4)_2 SO_4$	90	11.95	7.9
$(MgSO_4) \cdot 7H_2O$	110	0.51	3.4
$CaCL_2$	33	0.28	0.19
PROTEIN NUTRIENT[2]	960	9.39	6.27

MEDIUM RAW MATERIALS--SCP FERMENTATION

1. TRACE ELEMENTS ARE ASSUMED SUPPLIED BY PROCESS WATER
2. AMBER BYF-300 AMBER LABORATORIES JUNEAU, WISCONSIN

an air rate of 0.3 VVM and a power per unit volume ratio of 1.38 HP/m^3 was necessary. Also, the fermenters were operated at a total pressure of 2.6 atm to enhance the oxygen transfer.* After the yeast has been removed from the broth by centrifugation, the yeast stream is spray dried and packaged for sale.

Cost Estimation. A preliminary cost estimate was made for the above mentioned ethanol and SCP fermentation processes to determine the required capital investment and cost per gallon of 95% ethanol.

The general cost estimation procedures were those recommended by Peters (13) and Gutherie (14).

The fixed capital cost is estimated as a multiple of the purchased cost of the principal items of equipment. In the present case a multiplier of 3.1 was used in conjunction with the FOB equipment costs listed in Table 3. The manufacturing cost is subdivided into investment related costs, labor costs, utility costs, raw material costs and sugar costs. The sugar cost was taken at a base cost of 5.2¢/lb as presented by Wilke, et al. (10). The steam and power costs were estimated assuming that they would be generated using spent solids from the hydrolysis process as fuel. A summary of utility costs are shown in Table 7. A base labor rate of $5.60/hr was assumed for the operation of the fermentation process.

The fixed capital costs for the overall process are shown in Table 8. A total fixed capital of $5.37 x 10^6 is required to produce 24,000 gal/day of 95% ethanol from the hydrolysate sugars.

*These conditions were found to be optimal for the SCP fermentation from a computer model of the fermentation process.

TABLE 7

	UNIT	UNIT COST	UNITS/HR. SUGAR CONC.	ETHANOL FERM.	DISTILLATION	SCP FERM.
POWER	KWH	1.0¢	5.8	146	8.8	762
STEAM	1000 LB	32.5¢	58	4.2	20.2	4.2
WATER COOLING	1000 GAL	12.8¢	-	24.5	21.5	114

UTILITIES COSTS

TABLE 8

	$\$10^6$	% OF TOTAL
SUGAR CONCENTRATION	0.58	10.8
ALCOHOL FERMENTATION	2.36	43.9
DISTILLATION	0.39	7.3
SCP FERMENTATION	2.04	3.80
TOTAL	5.37	100

CAPITAL INVESTMENT SUMMARY

A breakdown of ethanol production costs is shown in Tables 9 and 10. Of the $1.05/gal production cost 68.6% is related to the sugar cost of 5.2¢/lb.

This is also reflected in the SCP fermentation processing costs shown in Table 11. The sugar costs amounts to 49% of the yeast production cost of 30.0¢/lb. A somewhat heavy charge is made for nutrient supplements in the SCP process. The nutrient requirement was based on the yeast cell mass composition assuming no vitamin or protein components are in the hydrolyzate sugars. These media supplement costs would be reduced if agricultural or municipal wastes, which contain many vitamins and minerals, were hydrolyzed instead of the newsprint used in the base design case of Wilke et al. (10).

The above costs for ethanol and SCP should be considered within the context of the particular cellulose processing scheme, of which they would be a part. Such an analysis is presented in the following paper (15), in which alcohol is taken as the primary product resulting from enzymatic hydrolysis of newsprint, and cost credits are estimated for by-product yeast and electrical power.

TABLE 9

	¢/GAL 95% ETOH	PERCENT OF TOTAL
SUGAR CONCENTRATION	2.7	2.6
FERMENTATION	5.4	5.1
DISTILLATION	2.5	2.4
YEAST RECOVERY	1.0	1.0
RAW MATERIALS	21.4	18.3
SUGAR	72.2	68.6
TOTAL	105.2	100

PROCESSING COST DISTRIBUTION--ETHANOL PRODUCTION

TABLE 10

	¢/GAL 95% ETOH	PERCENT OF TOTAL
INVESTMENT RELATED	7.5	7.2
LABOR	1.6	1.5
UTILITIES	2.5	2.4
RAW MATERIALS	21.4	18.3
SUGAR	72.2	68.6
TOTAL	105.2	100

PROCESSING COST DISTRIBUTION--ETHANOL FERMENTATION
(INCLUDES DISTILLATION AND SUGAR CONCENTRATION)

TABLE 11

	¢/LB YEAST	PERCENT OF TOTAL
INVESTMENT RELATED	1.6	5.3
LABOR RELATED	0.3	1.0
UTILITIES	0.9	3.1
RAW MATERIALS	15.5	51.6
SUGAR COSTS	11.7	39.0
TOTAL	30.0	100

PROCESSING COST DISTRIBUTION--SCP FERMENTATION

CONCLUSIONS

Although careful control of oxygen tension and using optimum feed sugar concentrations in the alcohol fermentation may reduce capital expenditures by as much as 50%, sugar cost dominates the economics of ethanol production. Every ¢/lb of sugar adds 14¢/gal to the ethanol production cost, while the basic fermentation charges excluding sugar are only 20¢ to 30¢ per gallon. Thus, before ethanol fermentation process improvements and optimization become of equal economic importance to sugar cost, the base sugar cost must be reduced to 2¢ to 3¢ per pound.

However, the presented experimental work does demonstrate the practicality of continuous ethanol fermentation. Under the optimal fermentation conditions, continuous alcohol production in the laboratory fermenter has been maintained for 60 days without problems of contamination and strain degradation.

Acknowledgment

This work is part of a general program on utilization of cellulose as a chemical and energy resource conducted under the auspices of the Energy Research and Development Administration (ERDA).

Figure Captions

Figure 1 Productivities as a Function of Oxygen Tension at a Dilution Rate of 0.22 hr^{-1} (adapted yeast).

Figure 2 Results of Continuous Fermentation with Adapted Yeast. 8.9% Glucose Feed, Oxygen Tension 0.07 mm Hg.

Figure 3 Effluent Ethanol Concentration as a Function of Dilution Rate for Various Feed Sugar Concentrations (adapted culture).

Figure 4 Dilution Rate Necessary to Ferment to 0.3% Glucose as a Function of Glucose Feed Concentration (adapted culture).

Figure 5 Ethanol Productivities and Cell Mass Concentration as a Function of Feed Sugar Concentration (adapted culture).

Figure 6 Ethanol Production Costs Excluding Raw Materials as a Function of Feed Sugar Concentration

Figure 7 Flow Diagram for Ethanol-Torula Yeast Production.

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TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720