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**Pilot Plant Studies Of The
Bioconversion Of Cellulose
And Production Of Ethanol**

Charles R. Wilke

September 30, 1977

Lawrence Berkeley Laboratory University of California/Berkeley

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PILOT PLANT STUDIES OF THE BIOCONVERSION OF CELLULOSE
AND PRODUCTION OF ETHANOL

under the auspices of the Department of Energy
Solar Energy Division--Fuels from Biomass Program

LAWRENCE BERKELEY LABORATORY

Charles R. Wilke, Principal Investigator

Report of Work Progress

September 30, 1977

Work performed under the auspices of the
U. S. Department of Energy.

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Appended Papers and Reports

Bazua, D.C. and C.R. Wilke, "Ethanol Effects on the Kinetics of a Continuous Fermentation and Saccharomyces cerevisiae. Biotechnol. and Bioeng., Symp. 7, 105-118 (1977).

Cysewski, Gerald R. and Charles R. Wilke, "Process Design and Economic Studies of Alternative Fermentation Methods to the Production of Ethanol, LBL-5963. (Submitted to Biotechnol. and Bioeng.).

This report summarizes briefly the progress over the period of July 1 to September 30, 1977, on those portions of the program primarily under Solar Energy Division sponsorship. Various reports have been issued or are in press which provide a full account of phases of the work which have been completed (see list appended). A listing is appended of the project personnel and topics presently under study in the entire program under funding by the Department of Energy, Solar Energy Division, Department of Energy, Division of Physical Research and National Science Foundation/RANN.

A. Analysis and Evaluation of Potential Raw Materials.

1. Chemical Analysis of the Kudzu Plant.

As promised in a previous progress report (1-30-77), the study on the Kudzu plant has been completed. Professor R.D. Tanner (Vanderbilt) has reported that immense quantities of this plant could be provided in the South.

Because of the small quantity available, the assay on this material was done indirectly, that is, the enzymatically hydrolyzed residue was assayed, and by material balance the assay of the original material was derived. Of course, this assay is not very accurate as it accounted for only 84% of the material. It is believed that the largest contributors to this discrepancy are the protein content and the azeotropic benzene/alcohol extractives which we were unable to obtain from the hydrolyzed plant residue. Therefore, the approximate derived composition was:

30.5 w% glucan	15.3% lignin
1.0 w% mannan	18.6% ash
6.7 w% xylan	
2.0 w% arabinan	
~1.9 w% unidentified carbohydrate	
~10% protein	

The enzyme hydrolyzate from a 5 w%, 2mm Wiley milled suspension hydrolyzed for 40 hours resulted in a solution containing 7.8 grams/liter of sugars with a composition of:

- 70.4 W% glucose
- 2.0 w% poly glucose (mainly cellobiose)
- 7.75w% xylose
- 3.8 w% arabinose
- ~9.6% unidentified sugar

The glucose obtained represents a 40.4% conversion of the glucan content of the plant. However the relatively low total sugar concentration obtained from the 5% suspension does not appear particularly encouraging for processing considerations.

2. Effect of NO_x Pretreatments on the Hydrolysis of Wheat Straw

Work has been continuing on the NO_x pretreatment of wheat straw. The process involves contacting the dry straw with NO + O₂ extraction of the treated material with water or caustic solutions and enzymatic hydrolysis of the solid residue.

All treatments have been conducted for 24 hours at 25°C and 1 atm pressure using 5 g of NO plus 7 g of O₂ per 100 g of wheat straw.

On a preliminary basis the most promising procedure thus far consists of the NO + O₂ contact followed by extraction with water for 2 hours at 100°C. In this case with subsequent enzymatic hydrolysis 50% of the original glucan is converted to glucose or cellobiose. In absence of the NO + O₂ pretreatment the corresponding conversion of glucan is only 22%. The results of the NO treatment are particularly encouraging in view of the poor response of wheat to chemical delignification with ethanol and with sodium chlorite obtained

in earlier experiments.

Work is continuing to determine optimum conditions for treatment as a basis for economic evaluation of the method. Also, the method will be assessed for effectiveness against other raw materials of current interest such as corn stover and tree biomass.

B. Utilization of Hemicellulose Sugars

Previous research concerning the fermentation of xylose to ethanol by Fusarium oxysporum showed growth ceasing before the sugar was completely consumed. This could be due to deficiencies of required substances or to the presence of an inhibitor that halts the growth of the organism.

Further investigations have been conducted by growing the fungus on our original medium and four additional media described in the literature. The salts from each medium were supplemented with 0.1% yeast extract, and 1% filter-sterile xylose was added separately to the autoclaved medium. As controls, additional media were made by adding only the yeast extract and only the xylose to the salts. Therefore, the organism was grown on a total of fifteen different media.

The experiments were carried out in 25 mm x 150 mm serum capped test tubes and incubated at 30°C. The cultures were placed on a rocking platform to keep the fungus homogeneously dispersed. Optical density readings were plotted as a function of time to determine the growth kinetics in the different media. At regular intervals, CO₂ was bled from the cultures.

The growth curves showed that growth on the media containing both yeast extract and xylose exceeded the sum of the control curves. This indicates that the yeast extract is providing one or more necessary growth factors in all cases.

After growth ceased, the cultures were filtered to obtain the dry cell mass. The supernate was assayed for xylose and ethanol.

In all cases the addition of .1% yeast extract gave more complete consumption of xylose and greater production of cell mass. Complete consumption of xylose was observed in the original medium, contrary to earlier findings. One of the additional media also showed complete substrate consumption and furthermore produced more cell mass than the original medium.

Experiments were conducted with standard solutions of ethanol which demonstrated that considerable amounts of ethanol can be lost during culture filtration for collection of the cell-free supernate. Therefore, in future experiments centrifuged samples will be used in determining ethanol concentrations.

Autoclaving xylose in the medium may produce an inhibitor that halted growth in earlier investigations. Experiments are underway to compare the results when filter sterile xylose is added separately to the autoclaved media with the results when the xylose is autoclaved in the media. Investigations are now being restricted to the best growth medium, our original medium, and a third complete mineral medium.

C. Process Design and Economic Studies

1. Hydrolysis Process

A study is in progress to re-evaluate certain assumptions and to update and optimize the previous process design for hydrolysis of newsprint. A tentative flow sheet and material balance have been prepared for processing of corn stover incorporating a dilute acid pretreatment for xylose recovery and enzymatic hydrolysis of the residual solids. Batelle is preparing an economic analysis based on these process specifications.

2. Ethanol Fermentation

A revised economic analysis of the vacuum and cell recycle fermentation schemes for production of ethanol from molasses has been computed (LBL-5963) and submitted to Biotechnology and Bioengineering for publication.

The results indicated that employment of vacuum plus cell recycle can reduce the fermentation cost (exclusive of raw materials) from 27.5¢/gallon (batch) to 12.7¢ per gallon. Total production cost with sugar at 5¢ per pound (exclusive of yeast credit) is 87¢ per gallon for the vacuum plus recycle system. While absolute cost levels may vary with various cost assumptions required for specific situations the relative comparison of the various processing schemes should be valid.

D. Pilot Plant Process Development and Design Studies

1. Enhanced Cellulase Production

Most of the work completed during this period was described in the previous report. Further work will focus on dividing the enzyme production step into two stages, the first concerned with maximizing cell-growth and the second concerned with maximizing enzyme productivity. Temperature and pH will be the variables studied in these stages.

In addition, a number of physical modifications will be made in the fermentation equipment to overcome previous difficulties. These will include:

- a) Continuous removal of foam from the fermenter and continuously feeding it back in liquid form by using a "vacuum-nozzle" method.
- b) Continuous addition and removal of substrate and enzyme respectively by using the modified "T" circulation method.
- c) Continuous separation of enzyme from cells during fermentation and recycling the cells into the fermenter using the "flow settling" process.
- d) Use of a disc turbine in the bottom of the impeller shaft to provide

good gas transfer and use of a marine impeller mounted above it to provide good circulation.

e) Modification of the sampling process to include a steam sterilization step.

2. Continuous Hydrolysis

The continuous enzymatic hydrolysis of cellulose has been successfully carried out in multi-stage constant stirring tank reactors. At 30 hours mean residence time, the glucose concentration ratio of CSTR v.s. batch is 90 percent for two stages and 95% for three stages. These results are in reasonable agreement with values based on residence time distribution theory for well-stirred vessels. To confirm this, a residence time distribution of the fluid in CSTR has been tested. The age distribution E curve was plotted and compared with the theoretical E curve. Both are in close agreement with each other within experimental accuracy.

The glucose inhibition effects in the batch and the continuous hydrolysis have been analyzed. The same source of enzyme solution was used with different glucose concentrations. Then the batch and the continuous hydrolysis processes were conducted as usual. The results showed that as the glucose concentrations in the enzyme solution increased, the resulting glucose production decreased and the cellobiose concentrations increased. For a two-stage continuous hydrolysis and 20 hours mean residence time, 10 g/l, 20 g/l and 40 g/l glucose concentrations premixed with enzyme solution resulted in 33 percent, 47 percent and 70 percent less glucose production than without glucose addition. Since the cellobiose concentrations increased as the premixed glucose increased, the effect of glucose inhibition on total reducing sugar production may be due in part to cellobiose inhibition. These results support the companion effort in studying the use of a separate β -glucosidase treatment in the hydrolysis process

to reduce cellobiose levels.

3. Countercurrent Hydrolysis

Previous efforts to construct a small-scale countercurrent cellulose contacting system have been unsuccessful. For this reason feasibility studies are now underway to determine handling characteristics of the cellulose-enzyme system. Batch settling tests have provided the information necessary to design a gravitational settling system; and that design is expected to be completed soon. Future plans include:

1. Running a Weber extraction to simulate countercurrent contacting,
2. Testing the feasibility of using a tower design based on gravitational settling; and
3. Investigation of hydroclones or filtration systems for designing a countercurrent contacting system.

Published Papers

Bazua, D.C. and C.R. Wilke, "Ethanol Effects on the Kinetics of a Continuous Fermentation and Saccharomyces cerevisiae, Biotechnol. and Bioeng. Symp. 7, 105-118 (1977).

Published Reports

Cysewski, Gerald R. and Charles R. Wilke, "Process Design and Economic Studies of Alternative Fermentation Methods to the Production of Ethanol," LBL-5963. Submitted to Biotechnol. and Bioeng.).

Reports to be Published

Sciamanna, Aldo, Ray Freitas and C.R. Wilke, "Composition and Utilization of Cellulose from Agricultural Residue for Chemicals," LBL-5966 (in press).

Wilke, C.R., "Cellulose, Food and Energy," LBL (in press). To be published in the Proceedings of International Congress on Engineering and Food, Boston, August 9-10, 1976. LBL-5275.

Freitas, Ray, C.R. Wilke and Bill Long, "Procedures for Analysis of Solids and Liquors from Cellulosic Sources," LBL-5967 (in press).

Batter, Thomas R. and C.R. Wilke, "A Study of Xylose to Ethanol by Fusarium oxysporum," LBL-6351 (in press).

Wilke, C.R., "Production of Sugars and Ethanol Based on the Enzymatic Hydrolysis of Cellulose." LBL-6814 (in press). To be published in Proceedings of ERDA Conference, Urbana, Illinois, April 1977.

Harima, Takeshi, and C.R. Wilke, "Comparison of Batch and Continuous Cellulose Hydrolysis with and without Ethanol Fermentation." Report in Preparation.

Harima Takeshi, and C.R. Wilke, "Enhancement of Enzymatic Hydrolysis of Cellulose by Coupling with Ethanol Fermentation." Report in preparation.

APPENDIX

CELLULOSE BIOCONVERSION AND PILOT PLANT STUDIES

LAWRENCE BERKELEY LABORATORY

Project StaffFaculty Investigator

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Mohammad Riaz, Microbial Biochemistry, University of Wales, U.K., Ph.D. 1969.

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Invited Lectures

Lignin Degradation by Fungi and the Biological Production of Fuels from Plant Biomass, California Mushroom and Compost Science Society, Wednesday, October 5, 1977. Steven Rosenberg.

Participated in Energy Fair, Anaheim, California, November 3,4,5, and 6. Exhibit on Cellulose Conversion. Richard Lindsey.

CELLULOSE BIOCONVERSION TO SUGARS AND ETHANOL

BERKELEY PROGRAM -- SEPTEMBER, 1977

Evaluation of Potential Raw Materials (Agricultural Residues)

- (a) Analysis of composition--carbohydrates, lignin, etc.
- (b) Hemicellulose extraction--dilute acid pretreatment
- (c) Alkali pretreatment
- (d) Enzymatic hydrolysis of original and pretreated materials

Cellulase Production

- (a) Multistage fermentation with cell recycle
- (b) T. viride growth model via CO₂ utilization

Hydrolysis Kinetics (T. viride cellulase)

- (a) CSTR--short circuiting effects
- (b) Countercurrent hydrolysis
- (c) Product inhibition

Enzyme Adsorption-Desorption

- (a) Adsorption of C₁, C_x and β -glucosidase activities on cellulose
- (b) Enzyme Fractionation by adsorption on cellulose
- (c) Enzyme Desorption--effect of additives

Mixed or Supplementary Enzyme System Development

- (a) β -glucosidase (NSF project)
- (b) C₂ complex from T. koningii

Chemical Delignification Studies

- (a) Glycol treatment
- (b) Ethanol treatment
- (c) Assessment of other potential methods

Enzymatic and Microbial Delignification

- (a) Study of Phanerochaete chrysosporium (NSF Project)

Cellulose and Glucose Fermentation to Ethanol

- (a) Vacuum and cell recycle system development
- (b) Media optimization
- (c) Simultaneous enzymatic hydrolysis and fermentation

Xylose Fermentation to Ethanol

- (a) Fusarium oxysporum (f. sp. lini)

This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.