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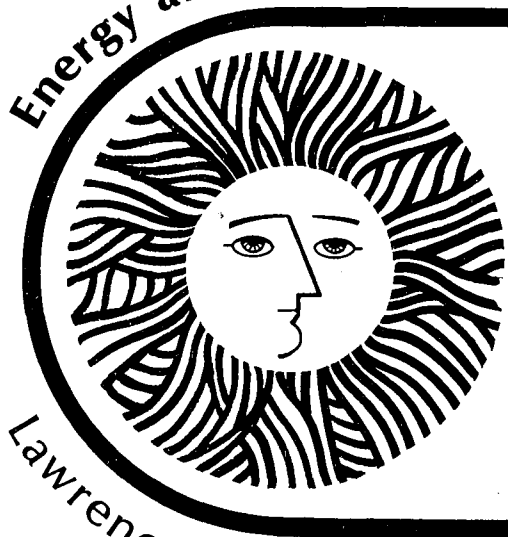
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Energy and Environment Division



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

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January 1977

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

under auspices of
U.S. Energy Research and Development Administration
Solar Energy Division--Fuels from Biomass Program

Lawrence Berkeley Laboratory
Charles R. Wilke, Principal Investigator

Report of Work Progress
January 31, 1977

This report summarizes briefly the progress in Fy 1977 to date on those portions of the program primarily under Solar Energy Division sponsorship. Various LBL 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 ERDA Solar Energy Division, ERDA Division of Physical Research and National Science Foundation/ RANN.

A. Collection of Potential Cellulosic Raw Materials

In cooperation with the Departments of Agronomy and Agricultural Engineering, University of California at Davis, and the U.S. Department of Agricultural Western Regional Laboratories, samples of the following materials were obtained:

1. Wheat Straw "Triticum Aestivum-L., em-Thell."
Grown in Yolo County, Ca., Fall of
1975 crop.
2. Barley Straw "Hordeum Vulgare-L.," from Yolo
County, Ca., Fall 1975.
3. Rice Straw "Oryza Sativa-L.," from Butte County,
Ca., Fall 1975.
4. Rice Hulls "Oryza Sativa-L.," from Butte County,
Ca., Fall 1974.
5. Sorghum "Bicolor Moench-L.," from U.C. Davis
Campus, Ca., Fall 1975.

6. Corn Stover "Zeamays-L.," from Sacramento County, Ca., Fall 1975.
7. Cotton Gin "Gossypium Hirsutum-L.,"
Trash Grown in Kern County, Ca.,
Summer 1974.

Samples of Douglas Fir and Redwood in original and steam treated forms were obtained from the Masonite Company. Newsprint (Wall Street Journal) was obtained as published and the ground wood from which the newsprint was made was obtained from Boise Cascade Corporation. Corn stalks in original and ball-milled form were supplied by General Motors Corporation.

B. Preparation and Analysis of Raw Materials

The various residues were air dried to less than a 12 percent moisture content to facilitate the milling operation, which was done with a Wiley mill equipped with a 2mm screen. The milled material was then air dried to an equilibrated moisture content of 7 to 12 percent. A sample of each was sieved to recover the 40-60 mesh fraction for compositional analysis. These results are shown in Table B.

Published methods of analysis on wood and grains were adapted or modified to suit these materials. Each material is extracted for six hours with the benzene/ethanol azeotrope. A sample of the dry extracted material is exhaustively hydrolyzed in $24.1N$ H_2SO_4 (72% w/w) and $0.8N$ H_2SO_4 (4% w/w). The sugars generated by this hydrolysis are measured by gas-liquid chromatography as their trimethylsilylated derivatives. The content of the hexoses such as glucose, mannose, galactose and the

Table B

COMPOSITION OF AGRICULTURAL AND WOOD RESIDUES

PERCENT COMPONENTS (BASED ON DRY WEIGHT)

Material	Hexosans	Pentosans	Lignin	Ash	Protein
Barley Straw	40.	19.	13.5	10.8	
Corn Stover	36.	16.	15.1	4.3	4.0
Cotton Gin Trash	20.	6.0	17.6	14.8	
Rice Hulls	36.	15.	19.4	20.1	
Rice Straw	39.	17.	9.9	12.4	
Sorghum Straw	33.	18.	14.5	10.1	
Wheat Straw	36.	17.	12.5	9.6	3.0
Newsprint (Wall Street)	73.	4.5	21.0	0.35	-0-
Ground Wood*	54.	3.8	30.0	0.10	
Douglas Fir	57.	4.0	27.3	0.20	
Masonite Steam Treated Douglas Fir	59.	3.9	24.3	0.30	
Redwood	44.	5.7	33.9	0.40	
Masonite Steam Treated Redwood	42.5	3.6	26.5	0.50	
Corn Stalks (a)	35.	15.	18.5	5.4	4.0
Corn Stalks (a) (GM Ball Milled)	22.5	13.	16.1	5.6	-0-

* Boise Cascade Ground Wood bleached with hypochlorite for Wall Street Newsprint, a mixture of 60% Western Hemlock, 25% White Fir, 15% Western Spruce.

(a) Gathered from a field by personnel of the General Motors Technical Center in Warren, Michigan. The stalk was on the field for 1½ years.

pentose such as xylose and arabinose can be measured by this method. After correcting for the loss of sugar during hydrolysis, the content of each sugar in its polymerized form is reported as a percentage of the original dry weight.

The lignin content is measured on extracted samples that have been thoroughly washed with hot H₂O to remove substances that would erroneously be measured as lignin. This water washed material is dried and then exhaustively hydrolyzed by the method described previously. The residue remaining is collected, dried and weighed. The residue is then ashed at 600°C. The change in weight after ashing is taken as the lignin content. It is reported as a percentage of the original dry weight.

Ash is determined as the residue of the original dry material after ignition at 600°C. It should be clearly noted that the ash is composed of metal oxides and dehydrated compounds such as metal silicates which are of lower molecular weight than the original inorganic-organic compounds. Thus this error gives rise, in the case of straws, to a nontrivial unidentified fraction in the composition of the material along with the ashing error in the lignin assay and in the lignin readable substances removed by the hot H₂O wash.

C. Acid Extraction and Enzymatic Hydrolysis of Raw Materials

Enzymatic hydrolysis was performed on each Wiley milled original material in a 5 w/tw% suspension in cellulase enzyme solution (FPA¹ ≈ 3.5) at 45°C and stirred at ~200 rpm for 48 hours. The mixture was sampled at 20, 40 and 48 hour intervals, and the relative sugar production was tracked colorimetrically. After 48 hours, the hydrolysis was stopped and the solids were

¹ Filter Paper Activity

separated by filtration or centrifugation where necessary, as in the case of cotton gin trash. The washed solid residue was freeze dried and weight loss determined for material balance. A portion of a sample of the enzyme-sugar solution was subjected to GLC, the second portion was subjected to exhaustive hydrolysis and run again on GLC to determine the polymeric sugars present. The results are expressed as the sugars in Table C-1. Comparing 100 pounds of barley straw shown in Table B, for example, the 40 pounds of hexosan contains 37 pounds of glucan component, equivalent to 41.1 of glucose, resulting on enzymatic hydrolysis in 7 pounds of glucose in solution. This is a 17 percent conversion to glucose monomer, and an additional two percent conversion to polymeric glucose which is mainly cellobiose. Continuing, the original 19 pounds of pentosans contains 15 of xylan, equivalent to 17.05 pounds of xylose, resulting on hydrolysis as 3.05 pounds of xylose, a 17.9 percent conversion. Also, it should be noted that the washed residue of enzymatic hydrolysis could be recycled, or burned for power.

The acid pretreatment of these residues was derived from common acid hydrolysis techniques, only considerably less in acid concentration. The milled residue was reacted with 0.09M sulfuric acid in a solids to liquid ratio, which varied from 6 to 8.5:100 by weight. The stirred mixture was boiled for 5.5 hours. The relative sugar production was tracked colorimetrically by sampling the reaction mixture at generally one-hour intervals. The mixture was then cooled and the solid separated by filtration or centrifugation where necessary.

TABLE C-1

ENZYME HYDROLYSIS OF ORIGINAL MATERIAL

BASIS: 100 lb. OF ORIGINAL MATERIAL WILEY MILLED

Material	Glucose	Polymeric Glucose	Xylose*	Arabinose*	Other
Barley Straw	7.0	0.8	3.05	0.81	
Corn Stover	11.2	0.5	3.0	0.62	
Cotton Gin Trash	5.3	0.5	0.03	0.01	
Rice Hulls	5.3	0.3	0.33	0.08	
Rice Straw	17.5	<0.01	2.55	0.84	
Sorghum Straw	10.5	0.5	1.08	0.51	
Wheat Straw	8.9	<0.01	2.45	0.56	
Newsprint (Wall Street)	20.8	4.0	1.8	0.16	3.8 Mannose
Ground Wood (Boise Cascade)	8.0	0.8	0.44	0.1	2.3 Mannose
Corn Stalks (a)	6.8	0.3	1.6	0.45	0.15 Mannose
Corn Stalks (a) (GM Ball Milled)	22.9	0.1	10.5	--	

*Includes small amount (<0.05 polymeric pentose).

(a) Gathered from a field by personnel of the General Motors Technical Center in Warren, Michigan. The stalk was on the field for 1½ years.

9 7 2 1 0 6 4 0 0 0

The solid was washed and freeze dried to 100 percent dryness and a sample was removed for composition analysis and material balance as described previously. The residual acid concentration was determined in each liquor. Sorghum straw and cotton gin trash consumed 7.4 and 18.2 percent of the acid respectively. Samples of the liquor were analyzed for composition as described previously, and the results are shown in Table C-2. As can be noted, approximately one third of the pentosans are extracted from the straw and over one half of the mannan from newsprint and ground wood. More of these sugars can be extracted at the expense of increased glucose concentration which was not desired. Moderate amounts of lignin appeared in some of the acid straw liquors which is desirable, since it represents a moderate delignification of the substrate and is acid soluble and thus does not represent a perturbation on subsequent processes with these liquors. A portion of each acid treated substrate was subjected to enzymatic hydrolysis and the hydrolyzates were analyzed as described previously. The results are shown in Table C-2A. Improved glucose yields are observed in most cases except cotton gin trash and rice hulls. It would appear, except for cotton gin trash and rice hulls, that technically the acid extraction on the straw, newsprint, and ground wood is warranted since acid is not consumed, thus recycleable and improved yields of glucose are obtained.

The concept of alkali treatment on wood is well known, and has been applied here to effect delignification on agricultural residues. However, it has been neglected in most published reports, to note the considerable consumption of the

Table C-2

ACID EXTRACTION OF ORIGINAL MATERIAL

BASIS: 100 lb. OF ORIGINAL MATERIAL

Material	Glucose	Polymeric Glucose	Xylose	Arabinose	Other
Barley Straw	1.2	2.0	5.9	2.4	
Corn Stover	1.9	1.0	12.2	2.4	3.3 Lignin
Cotton Gin Trash	0.09	0.35	0.32	0.05	
Rice Hulls	2.7	1.3	8.1	1.6	2.8 Lignin
Rice Straw	1.7	2.1	11.2	2.5	2.2 Lignin
Sorghum Straw	0.31	2.3	8.4	1.5	3.0 Lignin
Wheat Straw	2.5	1.2	10.9	2.0	
Newsprint	3.1	<0.1	2.2	0.1	9.8 Mannose
Ground Wood (Boise Cascade)	1.6	<0.1	2.7	<0.1	8.2 Mannose

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Table C-2A

ENZYME HYDROLYSIS OF ACID TREATED SOLID

BASIS: 100 lb. ORIGINAL MATERIAL

Material	Glucose	Polymeric Glucose	Xylose*	Arabinose*	Other
Barley	13.0	0.5	0.35	0.05	
Corn Stover	15.7	1.1	0.81	0.11	
Cotton Gin Trash	5.7	0.84	0.57	0.06	
Rice Hulls	4.0	0.8	0.04	0.03	
Rice Straw	18.6	2.6	0.95	0.10	
Sorghum Straw	14.7	0.3	0.82	0.13	
Wheat Straw	12.5	1.2	2.36	0.10	
Newsprint	9.8	0.8	0.7	0.08	2.0 Mannose
Ground Wood (Boise Cascade)	10.3	1.0	0.23	--	1.3 Mannose

*Contains small amount (<0.05) polymeric (pentose).

alkali in these processes. Since boiling alkali solutions absorb carbon dioxide readily from the atmosphere, a simple closed system was devised. The acid treated substrate was added to the stirred and boiling 0.25M sodium hydroxide (1 w/w%) solution and heating was continued for three hours. The mixture was sampled in 30-minute intervals to track the relative sugar production in the reaction liquor. It was found to be trivial in all cases. The solids to liquor ratio, by weight, varied from 6.2 to 8.4:100. After three hours the reaction mixture, best described as slimes, were cooled and neutralized quantitatively, and thus the alkali consumption determined. This also facilitated the separation of the slime from the liquor. The substrate was washed, freeze dried and the composition and material balance was determined. Partial delignification was observed with the straws except in the case of cotton gin trash, rice hulls, and wheat. Barley straw lignin decreased to 8.8 percent, corn stover to 4.3 percent, cotton gin trash increased to 27.9 percent, rice straw decreased to 6.6 percent, and sorghum to 6.2 percent. The liquors were analyzed as previously and the results are included in Table C-3 for comparison. By inspection it is obvious that the alkali treatment, for yields of sugars and coupled with alkali consumption, is unwarranted.

In progress is a study on kudzu plant which we have been informed, by R.D. Tanner of Vanderbilt University at Nashville, Tennessee, that has taken over many forests and farms in the southern states and there is a great interest in finding a use

Table C-3

TOTAL YIELD SUMMARY

BASIS: 100 lb. ORIGINAL MATERIAL

Material	Acid Treatment & Enzyme Hydrolysis			Acid-base Treatment & Enzyme Hydrolysis		
	Glucose	Poly Glucose	Pentoses	Glucose	Poly Glucose	Pentoses
Barley Straw	14.2	2.5	8.7	17.2	4.9	8.8
Corn Stover	17.6	2.1	15.5	15.7	9.2	16.4
Cotton Gin Trash	5.8	1.2	1.0	7.0	2.2	0.98
Rice Hulls	6.7	2.1	9.7	10.2	2.6	10.1
Rice Straw	20.3	4.7	14.8	17.4	10.5	14.6
Sorghum Straw	15.0	2.6	10.9	14.2	12.0	12.4
Wheat Straw	15.0	2.4	15.4	17.1	4.8	15.5
Newsprint	12.9 & 11.8 Mannose	0.86	3.1			
Ground Wood	11.9 & 9.5 Mannose	1.0	3.0			

for it. The reported composition of approximately 60 percent carbohydrate (and a questionable 18 percent protein content) offers some promise. Enzymatic hydrolysis, of only a 10 gram sample we received, showed ~31 percent conversion of available carbohydrate (comparable to newsprint). A study will begin shortly on corn cob pellet residues from the Anderson Co. of Maumee, Ohio.

D. Utilization of Hemicellulose sugars

Because of the substantial xylose fraction in most cellulosic materials of potential interest recovery and utilization of this sugar is being studied in addition to the work on glucose. The present project is concentrating on the production of ethanol as a probable major end product, both for glucose and xylose.

Xylose fermentation studies are in progress employing the fungus Fusarium oxysporum. The pH optimum has been determined to be 5.0 with a maximum specific growth rate of 0.018 hr^{-1} . Ammonium nitrate was found to be a preferred nitrogen source. Yield factors were found to be 0.1 for cell mass and 0.35 for ethanol (gm/gm xylose consumed).

A preliminary experiment was made in continuous culture employing a settler to obtain cell recycle. The cell mass was increased to 5 times normal over a 30 day run. This method will be explored more fully at a later time. Presently, experiments are in progress to determine the extent of product inhibition and the optimum xylose content of the feed for continuous culture.

E. High Pressure Steam Treatment

Samples of Douglas Fir and Redwood obtained from the Masonite high pressure steam treatment process were evaluated in the standard analytical and treatment scheme described in Section A, B and C above. The major results are given in Table E.

Comparison with results obtained on the treated and untreated materials indicate that little benefit to subsequent enzymatic hydrolysis resulted from the high pressure steam. Therefore, this study has been discontinued.

F. Process Design and Economic Studies

Relatively little effort has gone into this phase of the project pending the outcome of laboratory work which might lead to revision of the processing schemes that have been assessed previously. A report has been issued (LBL-5267) which gives a detailed review of the research results underlying the previous process design studies and discusses the validity and limitations of the various assumptions. A theoretical analysis of residence time distribution effects in multi-stage continuous enzyme induction fermentors was completed to provide a basis for estimation of the effectiveness of various potential design configurations (LBL-5270).

A tentative processing scheme was evaluated for processing of wheat straw including production of xylose by dilute acid extraction followed by enzymatic hydrolysis of the residual solids (LBL-5275). For a process handling 1439 tons wheat

TABLE E

Enzymatic Hydrolysis of Wood and Steam Treated Wood (Masonite Process) (a)

Material	Glucose	Polymeric Glucose	Xylose*	Arabinose*	Other
Douglas Fir Chips	1.4	0.02	0.08	0.02	0.5 Mannose
Steam Treated	2.4	0.1	1.24	0.4	1.8 Mannose
Redwood Chips	1.3	0.02	0.11	0.02	0.4 Mannose
Steam Treated	2.6	0.1	2.7	0.6	2.7 Mannose

(a) Basis is 100 lb. in all cases (Steam treated material balance is unknown)

* Includes small amount (<.05 polymeric pentose)

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straw per day, it was estimated that there might be produced 170 tons of xylose at \$.03 per lb and 174 ton of glucose at \$0.10 per lb, exclusive of any raw material cost. These results are very tentative, serving primarily to indicate that processing of such materials shows promise of ultimate economic feasibility with further research.

H. Pilot Plant Process Development and Design Studies

The current laboratory work is leading toward the construction and operation of a mini-plant assembly to evaluate an integrated continuous operation of the process steps.

The initial phase of the mini-plant study will include enzyme production, hydrolysis and enzyme recovery from the hydrolyzate. This phase will assess problems associated with the effects of recycle streams in altering the enzyme composition and in maintenance of sterility under sustained fermentation and hydrolysis operation. Also various potential raw materials will be examined for their processing characteristics and carbohydrate yield with and without various pretreatments.

A second phase of the mini-plant study will incorporate enzyme recovery from the hydrolyzed solids. This step is farther from final definition than those of the first phase pending the outcome of work in progress on enzyme desorption.

A third mini-plant phase will study the conversion of the sugars produced from the various raw materials to ethanol. Basic studies of the fermentation of glucose and xylose to ethanol employing vacuum and/or cell recycle will be incorporated into a model unit to assess efforts of recycle streams, energy recovery techniques, nutrient requirements and product recovery problems

under sustained continuous operation. Feed stocks for this unit will be sugars produced in the hydrolysis mini-plant, although it is not anticipated that the two operations will be directly interconnected. Considerable basic work remains to be completed before the exact configuration of the alcohol mini-plant can be set, probably in FY 1979.

During the present year to date laboratory work on the separate process steps has been continuing in an effort to reach as firm a definition of the process as practically possible before commencing the mini-plant assembly. New information developed in basic studies of the enzyme production and hydrolysis steps indicate that sufficient revision of the original processing concept may be forthcoming to warrant deferment of the mini-plant construction as originally designed for another six months or so. Meanwhile, laboratory work and engineering analysis are proceeding to provide the basis for a new process design and corresponding mini-plant study. Recent developments in those areas most closely related to mini-plant application will be reviewed briefly below.

(1) Cell Recycle System for Cellulase Production

Early studies of this method employed a single stage with settler attached and with a centrifugal separator attached to permit cell recycle. Results have been summarized in LBL-5267 covering the work through June 1976.

A marked increase in both cell density and enzyme productivity was achieved relative to earlier results with no recycling. In the course of this investigation, it was frequently observed that rapid cellulase production was obtained when continuous operation was interrupted and the cells were forced to utilize the remaining limited amount of cellulose. Also, in previous investigations of both batch and semicontinuous recycle operations, higher levels of cellulase were excreted when the culture was starved for cellulose. These observations suggested an investigation of a multistage induction system in which the first stage would be used for cell growth on cellulose and subsequent stages would be used for enzyme induction.

Three fermentors in series were employed, operating at working volumes of 4.5, 4.5 and 6 liters in the first, second and third stages, respectively. One percent Solka Floc medium was supplied to the first stage, and enzyme solution was continuously pumped out from a settler mounted in the third stage. A portion of the suspension of cells in the third stage was pumped back intermittently to the first stage to prevent cell washout. Temperature was maintained at $30 \pm 0.4^\circ\text{C}$ and pH at 4.8 ± 0.3 for all three stages.

Steady state was achieved in about a week. An average effluent enzyme concentration of 3.8 FPA at an overall dilution rate, excluding the cell blending stream, of 0.22 hr^{-1} was achieved. Average cell densities were maintained

at 3.9, 4.2 and 5.4 gm/liter in the first, second and third stages, respectively. These results give a specific productivity of 0.143 mg of soluble protein per mg of biomass per day. The productivity is compared with that of previous experiments in the table below. The three-stage process shows a marked increase in specific productivity over a continuous single-stage recycle system and is somewhat better than a semicontinuous operation.

COMPARISON OF ENZYME PRODUCTIVITIES

Dilution Rate hr ⁻¹	Filter Paper Activity	Productivity mg Sol. Protein ml-day	X _T Cell Density mg/ml	Specific Productivity mg Sol. Protein mg cell-day
Mitra-Single stage, no recycle				
0.021	1.35	0.153	4.4	0.036
Mitra--Two-stage, no recycle				
0.021	0.83	0.101	2.2	0.046
Single-stage with recycle, semicontinuous				
0.02	3.95	0.690	5.15	0.134
Single-stage with recycle, continuous				
0.022	3.65	0.583	6.85	0.085
Three-stage with recycle, continuous				
0.022	3.80	0.66	4.6	0.143

Further improvement in productivity will be sought by maintaining different environmental conditions in each of the multistages. Higher temperature in the first stage should lead to maximum cell growth, while lower temperature in the last stage should lead to maximum enzyme induction. Other parameters, such as pH and aeration will be also be varied to determine their effect.

An experiment currently in progress is employing a single growth stage operating on Solka Floc as the carbon source followed by a single induction stage to which the cell recycle is directed. Use of the Solka Floc for the growth stage appears feasible with the economic advantage of not requiring use of hydrolyzate sugars for growth. In practice it is anticipated that a suitably delignified portion of the plant feed would be provided instead of Solka Floc for the enzyme system. Methods for delignification are being investigated in other phases of this project.

(2) Continous Hydrolysis

This study is being made to assess the effect of short circuiting in well mixed hydrolysis reactors and to provide a basis for reactor design in the mini-plant and large scale processing.

Continuous enzymatic hydrolysis of newsprint has been carried out in a one-stage CSTR. Preweighed Wiley-milled newsprint was evenly distributed on a belt conveyor, and then continously carried to the reactor. The working volume in the reactor was kept constant by a liquid level controlling

device. This specific rate solid-feeding system has worked out satisfactorily.

The continuous hydrolysis was run at mean residence times of 20 and 30 hours. Samples were taken at two-hour intervals until glucose concentration in the reactor reached steady state. Sugar levels were analyzed by DNS test and high pressure liquid chromatography. The results showed that the glucose concentrations from a single stage CSTR were approximately 80 percent of those from the batch hydrolysis at the same residence time. Theoretical calculations based on the batch hydrolysis curve indicated that the glucose level for a one-stage CSTR should be 75 percent of that for batch hydrolysis and that five stages of CSTR would approach 95 percent of the batch value. Thus for a single-stage CSTR theory and experiment are in reasonably close agreement.

Construction is in progress to increase the number of stages.

(3) Hydrolysis with Consecutive Enzyme Additions

Hydrolysis studies of Wiley-milled newsprint were conducted in which fresh portions of enzyme were added to the hydrolysis mixture at 10-hour intervals. A four-stage treatment increased the percentage saccharification from 37 percent to 59 percent, an increase of more than 50 percent in total sugar yield during forty-hours hydrolysis. Analysis of the adsorption of the C_1 and C_x components suggested that in a single stage treatment strong adsorption of the C_1 complex essentially immobilizes it and inhibits the hydrolysis process. These studies

are described fully in LBL 5950 (in press).

These results suggest also that a countercurrent hydrolysis process may be considerably more effective than the cocurrent scheme visualized heretofore. Such a scheme might incorporate enzyme recovery from the hydrolyzate and the hydrolysis into a single operation without increasing the complexity and cost of the process equipment materially. A project has been initiated to explore this latter possibility

(4) Ethanol Fermentation Studies

In addition to the xylose fermentation (Section D above) work has continued on the vacuum and cell recycle fermentation system (primarily under Division of Physical Research support). No new phases of this work have reached completion. The work in progress in studying media component requirements to minimize costs and using a more effective settler to determine the feasible upper limit of cell density. Details of completed work including progress schemes and economic analyses are described in LBL-4480 and LBL-5261 (in press) and LBL-5963 (in press).

(5) Mini-plant Design and Construction

As noted earlier the mini-plant process study as designed previously has been deferred pending the outcome of the studies in progress described above. It is anticipated, however, that equipment used for these studies may be incorporated into the mini-plant assembly in due course.

Published Papers

1. Cysewski, Gerald, and C. R. Wilke, "Utilization of Cellulosic Materials through Enzymatic Hydrolysis. I. Fermentation of Hydrolysate to Ethanol and Single Protein," *Biotechnol. and Bioeng.* 18, 1297-1313 (1976).
2. Wilke, C. R., G. R. Cysewski, R. D. Yang, and Urs von Stockar: "Utilization of Cellulosic Materials through Enzymatic Hydrolysis. II. Preliminary Assessment of an Integrated Processing Scheme." *Biotechnol. and Bioeng.* 18, 1315-1323
3. Wilke, C. R. and Gautam Mitra: PATENT: "Conversion of Cellulosic Materials to Sugar" U.S. Patent 3,972,775, August 1976.
4. Wilke, C.R., Ren Der Yang and Urs von Stockar: "Preliminary Cost Analysis for Enzymatic Hydrolysis of Newsprint," *Biotechnol. and Bioeng. Symp. No. 6*, 155-175 (1976).

Published Reports

1. Cysewski, Gerald and Charles R. Wilke: "Fermentation Kinetics and Process Economics for the Production of Ethanol." LBL-4480, (March 1976).
2. Carroad, Paul A., and Charles R. Wilke, "Studies of Lignin Degrading Fungi and enzymatic Delignification of Cellulosic Materials," LBL-4490 (May 1976).
3. Wilke, Charles R., Urs von Stockar, and Ren Der Yang: "Process Design Basis for Enzymatic Hydrolysis for Newsprint." LBL-5267 (July 1976). Presented at the AIChE Meeting, Los Angeles, Ca., November 16-20, 1975.

Published Reports

4. Carroad, P. A. and C. R. Wilke: "Cell Growth and Catcholase Production for Polyporus versicolor in Submerged Culture." LBL-5263, August 1976. (Accepted by Applied and Environmental Microbiology Journal).
5. Carroad P. A. and C. R. Wilke: "Exponential Growth Kinetics for Polyporus versicolor and Pleurotus ostreatus. LBL-5264, August 1976. (Accepted by Applied and Environmental Microbiol.)
6. von Stöckar, Urs, Ren Der Yang, and Charles R. Wilke: The Computation of the Fraction of Induced Cells in Enzyme Induction Systems," LBL-5270, September 1976. (Submitted to Biotechnol and Bioengineering).

Reports and papers to be published

1. Cysewski, Gerald and C. R. Wilke, "Rapid Ethanol Fermentations Using Vacuum and Cell Recycle," LBL-5261 (in press) (submitted to Biotechnol. and Bioeng., John Wiley & Sons.
2. Wilke, C.R., "Systems for Hydrolyzing Cellulose to Generate Fermentable Carbohydrates," paper presented at Symposium on Bioconversion of Solar Energy at Campinas, Brazil, 1976. LBL-5275 (in press).
3. Castanon, Maria, and C.R.Wilke, "Studies on the Enzymatic Hydrolysis of Newsprint," University of California and Lawrence Berkeley Laboratory, LBL-5950 (in press).
4. Carroad, Paul A. and Charles R. Wilke, "Enzymes and Microorganisms in Food Industry Waste Processing and Conversion to Useful Products," LBL-5962 (in press) (Submitted to Resource Recovery and Conservation).
5. Cysewski, Gerald R. and Charles R. Wilke; "Process Design and Economic Studies of Alternative Fermentation Methods of the Production of Ethanol." LBL-5963 (in press). Paper presented at the AIChE meeting, Chicago, Illinois, November 14-18, 1976.
6. Sciamanna, Aldo, Ray Freitas and C. R. Wilke: Composition and Utilization of Cellulose from Agricultural Residue for Chemicals," LBL-5966 (in press).
7. Freitas, Ray, C. R. Wilke and Bill Long: "Procedures for Analysis of Solids and Liquors from Cellulosic Sources," LBL-5967 (in press).

Reports and Papers to be Published

8. Yamanaka, Yoshio, Paul A. Carroad, Mohammad Riaz and Charles R. Wilke: "Decomposition of Lignin and Cellobiose in Relation to the Enzymatic Hydrolysis of Cellulose," LBL-5960 (in Press). Presented at the National Science Foundation Symposium on Research Applied to National Needs, Washington, D.C., Nov. 7-9, 1976.

Invited Lectures

1. "Systems for Hydrolyzing Cellulose to Generate Fermentable Carbohydrates," Energy and Bioconversion Symposium, Sao Paulo (Campinas) Brazil, July 7-8, 1976.
2. "Cellulose, Food and Energy, International Congress on Engineering and Food," August 9-13, 1976.
3. Panel Discussion: "Fuels for Sugar Crops," ERDA Conference Battelle Institute, Columbus, Ohio, October 15, 1976.
4. "Decomposition of Lignin and Cellobiose in Relation to the Enzymatic Hydrolysis of Cellulose," Presented at the National Science Foundation Symposium on Research Applied to National Needs, Washington, D.C., Nov. 7-9, 1976.
5. "Process Design and Economic Studies of Alternative Fermentation Methods of the Production of Ethanol," AIChE Meeting, Chicago, Illinois, Nov. 14-18, 1976.

Appendix

CELLULOSE BIOCONVERSION AND PILOT PLANT STUDIES

Lawrence Berkeley Laboratory

Project StaffFaculty Investigator

Charles R. Wilke, Professor of Chemical Engineering

Postdoctoral InvestigatorsTakeshi Harima, Biochemical Engineering, Univ. of Pennsylvania,
Ph.D. 1977.Mohammad Riaz, Microbial Biochemistry, University of Wales, U.K.
Ph.D. 1969.Steven Rosenberg, Bacteriology, University of California, Berkeley
Ph.D. 1970.Ren-Der Yang, Chemical Engineering and Biochemical Engineering,
University of Pennsylvania, Ph.D. 1974.Maria Castanon (to 10/76), Faculty of Pharmacy, University of
Madrid, Ph.D. 1971Urs von Stockar (to 12/76) Chemistry and Chemical Engineering,
Swiss Federal Institute of Technology,
Zurich, Ph.D. 1972.Graduate StudentsTom Batter, Food Science and Nutrition, University of California,
Berkeley, B.S. 1974. M.S. CandidateRon Borrevik, Chemical Engineering, University of Washington,
B.S. 1976. M.S. CandidateMary Jane Howell, Chemical Engineering, Cornell University
B.S. 1976. M.S. CandidateSteven Isaacs, Chemical Engineering, Massachusetts Institute of
Technology, B.S. 1976. M.S. CandidateRichard Lindsey, Chemical Engineering, Illinois Institute of
Technology, B.S. 1976. M.S. CandidateShu-yin Wei, Biostatistics, Cornell University, B.S. 1974.
M.S. Candidate.Harry Wong, Chemical Engineering, Massachusetts Institute of
Technology, M.S. 1973. Ph.D. Candidate

Support Staff

Al Sciamanna, Chemist,

M.S. in Chemistry, Univ. of San
Francisco, 1952.

Ray Freitas, Biochemist

B.A. Biochemistry, 1974, B.S.
Chemistry, 1975.

Bill Long, Technician

Current Projects and Assignments

- (DPR and Solar)* Cellulase Production with Cell Recycle
Ren-Der Yang, and Bill Long
- (DPR) Enzymatic Hydrolysis Kinetics
Ren-Der Yang and Jessie Wei
- (DPR) Enzyme Recovery (Adsorption-Desorption)
Mohammad Riaz and Richard Lindsay
- (Solar) New Raw Material Analyses and Carbohydrate Yields
Aldo Sciamanna, Ray Freitas and Bill Long
- (Solar) Hemicellulose Extraction and Hydrolysis
Aldo Sciamanna and Ray Freitas
- (DPR and Solar) Carbohydrate Fermentations--Glucose, Xylose
Takeshi Harima, Harry Wong and Tom Batter
- (DPR and NSF) Mixed Enzyme Development--C₂, β -Glucosidase
Mohammad Riaz, Mary Jane Howell and Steven Isaacs
- (DPR) Chemical Lignin Removal
Aldo Sciamanna and Ronald Borrevik
- (DPR, Solar and NSF) Chromatographic Separations and Analysis
Ray Freitas, Takeshi Harima, Mohammad Riaz and Bill Long

(DPR) Fermentation Control via CO₂, O₂ Monitoring

Takeshi Harima

(NSF) Enzymatic Lignin Decomposition

Steven Rosenberg, Ray Freitas

(Solar) Pilot Plant Planning

Ren-Der Yang

* Items in parenthesis designate primary sponsorship

DPR = ERDA, Division of Physical Research

Solar = ERDA, Solar Energy--Fuels from Biomass Division

NSF NSF/RANN

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