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ORNL
FOREIGN TRIP REPORT
ORNL/FTR-3803

DATE: October 25, 1990

SUBJECT: Report of Foreign Travel of Jonathan Woodward,
Development Staff Member II, Chemical Technology Division

TO: Alvin W. Trivelpiece

FROM: Jonathan Woodward

PURPOSE: To participate in the International Energy Agency (IEA) Network planning meeting for the coordination of ongoing national programs in "Biotechnology for the Conversion of Lignocellulosics" at the Institut Français du Pétrole (IFP), Rueil-Malmaison, France, and to discuss a proposed collaboration of research between the traveler and Dr. Martin Beevers of Aston University, Birmingham, U.K.

SITES VISITED:	9/11-12/90	Institut Français du Pétrole Rueil-Malmaison, France	J. Saddler
	9/16/90	Aston University Birmingham, U.K.	M. Beevers

ABSTRACT: The purpose of this trip was twofold -- firstly, to participate in the International Energy Agency Network planning meeting, held at the Institut Français du Pétrole (IFP), Rueil-Malmaison, France, for the coordination of national programs on "Biotechnology for the Conversion of Lignocellulosics" and, secondly, to visit the laboratory of Dr. Martin Beevers at Aston University, Birmingham, U.K., for discussions on our proposed research collaboration entitled "Structural Studies on Native and Modified Forms of Cellobiohydrolase I from *Trichoderma reesei*." The IEA meeting consisted of 18 participants from 12 countries and focused on the organization of "round-robin" comparative testing in specific areas associated with the bioconversion of lignocellulosics. The discussions at Aston University focused on the mechanism whereby the proposed collaborative research project could be achieved if funding by the National Science Foundation, through an interagency agreement with the U.S. Department of Energy, was achieved.

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REPORT OF TRAVEL TO FRANCE AND THE UNITED KINGDOM

October 9-18, 1990

Jonathan Woodward

INTRODUCTION

This report summarizes the results of the traveler's participation in the International Energy Agency (IEA) Network planning meeting for "Biotechnology for the Conversion of Lignocellulosics," held at the Institut Français du Pétrole (IFP), Rueil-Malmaison, France. It also summarizes the results of discussions held at Aston University, Birmingham, U.K., with Dr. Martin Beevers with whom the traveler is attempting to initiate a collaborative research project that will be beneficial to ongoing research programs at Oak Ridge National Laboratory (ORNL). The itinerary for the trip is given in Appendix A; the names of the people contacted are listed in Appendix B. Also, pertinent information about the Institut Français du Pétrole is attached (Appendix C).

IEA NETWORK PLANNING MEETING FOR
"BIOTECHNOLOGY FOR THE CONVERSION OF LIGNOCELLULOSICS"
OCTOBER 11-12, 1990

The IEA was established in 1974 arising from an intergovernmental conference on energy. Its aims are to improve the world's supply of energy through the development of alternative energy sources that would offset any disruptions to the supply of oil. There are 21 member states, with representatives of each state on the board of governors. The primary activity of the IEA is cooperation among member states to reduce excessive oil dependence through energy conservation and energy development research in general. The U.S.A. is a member state.

One subgroup of the IEA, known as Task IV, deals with the Bioconversion of Lignocellulosics and was initiated in 1987 after the amalgamation of two other subgroups: (1) pretreatment of lignocellulosics and (2) conversion of pentoses to ethanol. This subgroup consists of representatives from various laboratories representing the member states, and the initial planning meeting in 1985, consisting of the participating laboratories shown in Table 1, agreed on a program of cooperative research whose ultimate goals were to coordinate ongoing national programs on biotechnology leading to improved lignocellulosic conversion processes for energy production and conservation.

The proposed program consisted of (1) characterization and quantification of untreated and pretreated lignocellulosic substrates; (2) assay, production, adsorption, and recycle of cellulase; and (3) process economics and equipment. So far, two projects have been completed: (1) a comparative

Table 1. Laboratories participating in the initial planning meeting of the IEA Task IV

Laboratory	Country
Forest Research Institute, Rotorua	New Zealand
University of Graz, Graz	Austria
Solar Energy Research Institute, Golden	U.S.A.
BFH Inst. Wood Chemistry, Hamburg	Germany
Swedish Pulp and Paper Res. Inst., Stockholm	Sweden
University of Lund, Lund	Sweden
Forintek Canada Corp., Ottawa	Canada
Ruakua Agricultural Research Centre, Hamilton	New Zealand
Inst. Français du Pétrole, Rueil-Malmaison	France
CSIRO, Clayton	Australia
University of Innsbruck, Innsbruck	Austria
Mississippi State University	U.S.A.

analysis of a softwood, two hardwoods, and wheat straw and (2) a comparison of the hydrolysis of pure cellulose and pretreated spruce and aspen wood using *Trichoderma reesei* cellulase. Several laboratories participated in these worldwide, round-robin projects, the objective being to standardize data on the bioconversion of lignocellulosics for general use.

The traveler was invited to participate in the IEA Network planning meeting for "Biotechnology for the Conversion of Lignocellulosics," October 11-12, 1990, held at the Institut Français du Pétrole, Rueil-Malmaison, France, because of his recognized expertise in this area.

The Institut Français du Pétrole (IFP) was an appropriate location for this meeting because the development of biotechnological processes for lignocellulosic conversion is a major topic of research and development. Established in 1944, IFP is a not-for-profit organization under government control that carries out research, trains specialists, and disseminates information in the field of energy and hydrocarbons. The opening address was given by Monsieur Philippe Renault, director of biotechnology research, who introduced a short film about IFP. The information in this film is summarized in the attached article entitled "Institut Français du Pétrole-Preparing for tomorrow" (see Appendix C). Monsieur Jean-Paul Vandecasteele of the division of biotechnology and environmental studies then presented details about the experimental program at IFP at its biotechnology facilities

at Soustons (south of Bordeaux). A summary of the lignocellulosic bioconversion research and development at IFP is attached (Appendix C).

After this introduction, the participants introduced themselves and briefly described their research activities. There were 18 participants representing 12 countries (see Appendix B). Of particular interest was the report by Marianne Hayn, Institute of Biochemistry, University of Graz, Austria, that described the results of the IEA activity, in which several laboratories had participated, concerning the enzymatic hydrolysis of a standard cellulose, pretreated spruce and aspen wood, using a standard cellulase enzyme. Basically, each laboratory (11 in all), incubated the substrate (2% w/v) with 200 filter paper units of cellulase (*T. reesei* celluclast from NOVO enzymes) and determined the amount of glucose liberated at various time intervals up to 72 h. The maximum digestibility of cellulose as calculated by the 11 participating laboratories was 64.3 +/- 3.7% (mean +/- standard deviation). Similar reproducibility was obtained with pretreated aspen and spruce wood. It was concluded, therefore, that standardized conditions may be formulated and used worldwide for the bioconversion of lignocellulosic materials.

The remainder of the meeting was dedicated to the future planning of activities that could be reported at the IEA network symposium on "Biotechnology for the Conversion of Lignocellulosics" to be held May 3-6, 1991, in Colorado Springs, Colorado.

The traveler was requested to coordinate a round-robin activity in the area of cellulase assay and application by the leader of this IEA subgroup, Dr. J. Saddler of The University of British Columbia, Canada. In this regard, it was decided that the next effort should be a comparison of the hydrolysis of steam-exploded wood (provided by IFP) by a commercial cellulase/ β -glucosidase mixture or a purified cellobiohydrolase I/ β -glucosidase mixture. These studies will be performed in order to determine whether efficient hydrolysis of lignocellulosics can be achieved with only two cellulase components. Several laboratories agreed to participate in this round-robin study. The cellulase and β -glucosidase enzymes will be provided by N. K. Lange of NOVO Nordisk in Denmark, and M. Claeysens and the traveler will be responsible for providing the cellobiohydrolase enzyme. The results of this study will be reported at the IEA network symposium in Colorado Springs during 1991.

Other round-robin studies that will be planned, organized, and executed by various participants in this meeting are listed below.

<u>Activity</u>	<u>Coordinator</u>
Xylanase/hemicellulase assay and application	K. Poutanen
Protein assay	K. Grohmann
Substrate analysis	J. Puls
Pentose fermentation	B. Hahn-Hagerdal/ M. J. Beck
Modeling	G. Zacchi

The traveler suggested that one of the ultimate aims of this subgroup of the IEA should be the production of a handbook that could be referred to and used as a guide for those researchers/companies who wish to utilize biomass as a means of providing fuels and chemicals. Such a handbook would result from the round-robin studies currently under way and would probably not be available until sometime in the late 90s.

The final part of the planning meeting concerned the IEA network symposium to be held in Colorado Springs in May 1991. The emphasis of this meeting will be an evaluation of the available technology for lignocellulosic bioconversion and areas in need of improvement. Results of the round-robin studies currently under way will also be reported at that time.

VISIT TO ASTON UNIVERSITY, BIRMINGHAM, U.K. OCTOBER 16, 1990

The traveler visited Dr. Martin Beevers, a faculty member of the Department of Chemical Engineering and Applied Chemistry, at Aston University for discussions on their proposed cooperative research project entitled "Structural Studies on Native and Modified Forms of Cellobiohydrolase I from *Trichoderma reesei*." The traveler and Dr. Beevers have collaborated to compile a cooperative research proposal that has now been submitted to the Division of International Programs, National Science Foundation, with whom the U.S. Department of Energy has an Interagency Agreement.

A summary of the discussions between the traveler and Dr. Beevers is given below.

1. Initial studies will compare the conformation of native cellobiohydrolase I (CBH I) to that of "core" CBH I (i.e., the native enzyme minus its C-terminal or cellulose-binding moiety).
2. Approximately 50 mg of each enzyme will be required to get proton spectra.

3. Electro-optical Kerr effects for the native and "core" enzyme will be measured. Such effects are dependent on molecular configuration.
4. Initial studies on the electro-optical Kerr effects will be carried out by Dr. Beevers at Aston using lysozyme as a model enzyme.
5. Dr. Beevers will apply to the Science and Engineering Research Council of Great Britain for a Ph.D. studentship that will bring in a student whose research project will be based upon the collaboration.
6. If the National Science Foundation funds the collaboration through its interagency agreement with the U.S. Department of Energy, the traveler will supply the purified native and modified CBH I enzymes to Dr. Beevers and also gain new expertise in their structural determination.

APPENDIX A

ITINERARY

10/9-10/90	Travel from Oak Ridge to Paris, France
10/11-12/90	IEA Meeting on "Biotechnology for the Conversion of Lignocellulosics," Institut Français du Pétrole, Rueil-Malmaison, France
10/13-15/90	Weekend and travel to Birmingham, U.K.
10/16/90	Visit to Aston University, Birmingham, U.K.
10/17-18/90	Travel from Birmingham, U.K., to Oak Ridge

APPENDIX B

PERSONS CONTACTED

Institut Français du Pétrole, Rueil-Malmaison, France

M. J. Beck	Tennessee Valley Authority Muscle Shoals, Alabama, U.S.A.
C. Breuil	Forintek Ottawa, Canada
M. Claeysens	State University Ghent, Belgium
K. Grohmann	Solar Energy Research Institute Golden, Colorado, U.S.A.
M. Hayn	Institute für Biochemie Graz, Austria
N. K. Lange	Novo Nordisk, Enzyme Process Division R&D Bagsvaerd, Denmark
A. Marzetti	Stazione Sperimentale per la Cellulosa Milano, Italy
J. Pourquie	Institut Français du Pétrole Rueil-Malmaison, France
K. Poutanen	VTT, Technical Research Centre of Finland Espoo, Finland
J. Puls	Wood Research Institute Hamburg, Germany
P. Renault	Institut Français du Pétrole Rueil-Malmaison, France
J. Saddler	University of British Columbia, Dept. of Harvesting and Wood Science Vancouver, B.C., Canada
I. Storro	SINTEF Trondheim, Norway
J. P. Vandecasteele	Institut Français du Pétrole Rueil-Malmaison, France

PERSONS CONTACTED - continued

L. Viikari	VTT, Technical Research Centre of Finland Espoo, Finland
G. Zacchi	University of Lund, Chemical Centre, Chemical Engineering Lund, Sweden
W. Zimmermann	Swiss Federal Institute of Technology Zurich, Switzerland

Aston University

M. Beevers	Aston University Birmingham, United Kingdom
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APPENDIX C
INFORMATION ON ACTIVITIES AT IFP

Created in 1944 in the form of a not-for-profit professional establishment, the Institut Français du Pétrole is a research, training and information center under the control of the Ministry for Industry and the Ministry for Economy and Finance.

Facilities

To carry out its threefold mission, IFP's budget for 1990 is about MFF 1300. IFP has a staff of 1810, two-thirds of whom are engineers and technicians, in all disciplines.

Most of its facilities are located in Rueil-Malmaison (near Paris) and at the industrial research and development center at Solaize (near Lyon).

Information and Documentation

IFP has been given the mission of "informing public authorities, industry and technicians about scientific know-how and industrial technology"

To do this, IFP has developed a policy of data acquisition and synthesis based on:

- a Documentation Center for technology and economics, open to the general public, where more than 220,000 books, maps and miscellaneous documents may be consulted, along with 3,330 different periodicals, and where online searching of international databanks and databases is also possible;
- a specialized subsidiary, Editions Technip, that ensures the publication of books written by scientists, technicians and economists specializing in energy issues.

Training

The Ecole Nationale Supérieure du Pétrole et des Moteurs (ENSPM) is an integral part of IFP. This specialized graduate school accepts graduates of the leading French engineering schools (Grandes Ecoles) or university graduates of the same level (167 in 1989/1990). Its courses are held in five specialized graduate centers for:

- exploration
- drilling and production
- refining and engineering
- combustion, engines and applications of energy technology
- economics and management.

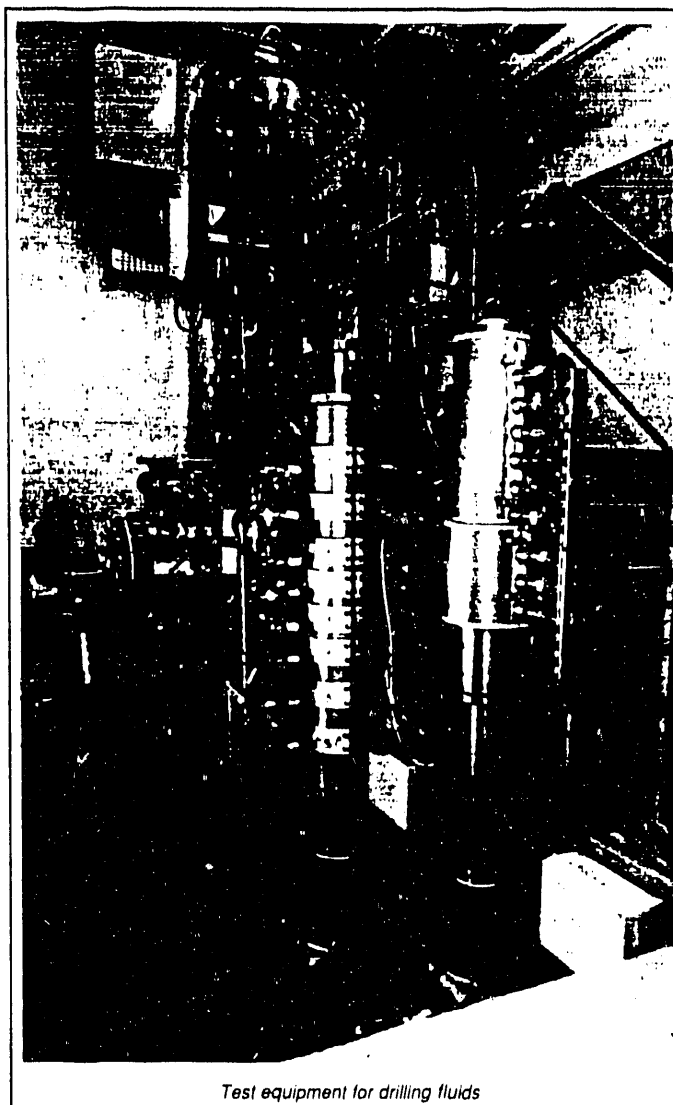
The number of students in each center is determined annually according to the needs of industry. One third to one fourth of them are foreign students. ENSPM has concluded many cooperation agreements with foreign universities concerning exchanges of teachers and students.

Within the framework of training by doing research, ENSPM, in association with universities, provides courses toward DEA degrees (Diplôme d'Etudes Approfondies) for 36 students in Petroleum Science and Energy Economics, and supervises and organizes the laboratory work and research of 125 doctoral candidates (Science or Economics).

In addition to this training, a great effort is being made in continuing education. More than 1000 engineers and 3000 executives and technicians attend specialized refresher courses every year.

Research and Development

This is the essential part of IFP's activities, taking up more than 70 percent of its budget in 1990. For this mission, IFP acts as a technical research center



Test equipment for drilling fluids

operating in sectors such as the petroleum, chemical, mechanical construction and automotive industries and also in the oil service sector. The following research topics are covered.

Exploration/Reservoir Engineering

Involves the exploration of new areas, the petroleum assessment of sedimentary basins by geological and geochemical modeling, the development of equipment and techniques for geophysical data acquisition, the surveying of complex geologic structures, the characterizing of reservoirs and of the dynamics of these reservoirs, new processes for production and the stimulation of production (horizontal drain holes, fracturing), and improving the recovery rate of oil in place.

Industrial Production Systems include directional drilling, the automation and control of operations, borehole equipment, multiphase and subsea production, the design and engineering of offshore structures, and the development of flexible flowlines for the gathering and pipelining of oil and gas.

Refining and Petroleum Products: IFP has a complete range of refining processes and the principal petrochemical plants. A concerted research effort attempts to constantly improve the performances of processes, to maintain their competitiveness and to facilitate their adaptation to new market conditions, particularly concerning the conversion of heavy cuts and residues, and the manufacturing of base stocks for fuels and for the production of olefins or aromatics. For this, special efforts are devoted to:

- the upgrading of natural gas;
- research on high added-value products such as additives for fuels and lubricants, special polymers and composite materials;
- the development of ways to control pollution by hydrocarbons;
- the exploration of new routes in the field of biotechnology.

Energy involves advanced research on engines, the optimizing of the formulation of fuels, engineering and construction of probative engines; the processing of exhaust gas and the prevention of air, thermal, industrial and home pollution, and lubrication.

Promotion of Research Achievements

IFP is independent of oil companies, of the petroleum equipment and service sector, and of the automotive industry, but, in close concert with them, implements a dynamic policy of exploiting its achievements in a context of competition as well as international cooperation. This policy takes on the form of:

- cooperation agreements, either directly or via specialized firms in the IFP/ISIS group, for training, technical assistance and joint research;
- the granting of licenses to use IFP processes, which is the case in particular of more than 765 refining and petrochemical plants using IFP technology;
- the creation of consultancy firms (BEICIP, Franlab, Horwell, Géodia, etc.), engineering firms (Technip, Technip Géoproduction) or manufacturers of new products and equipment (Procatalse, Eurecat, Zéocat, Collexip, SMFI, Bent-O-Matic, etc.), all of which are grouped within the holding company ISIS (Internationale de Services Industriels et Scientifiques).

THE EXPERIMENTAL
BIOTECHNOLOGY FACILITIES AT
SOUSTONS

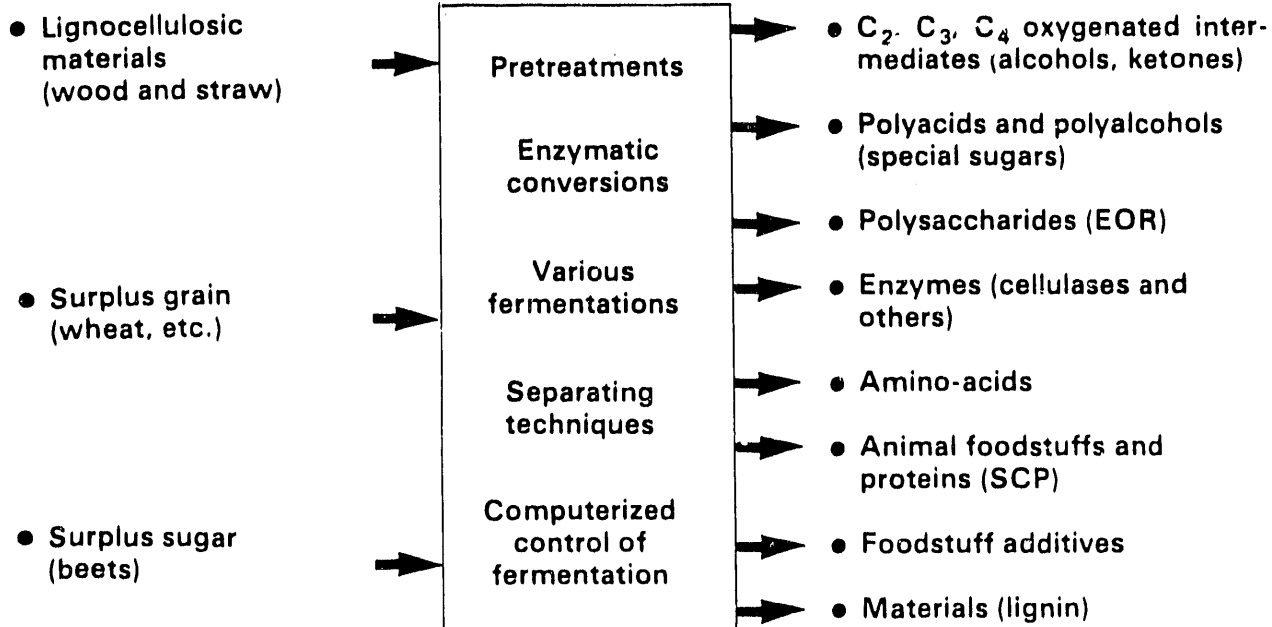
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BIOTECHNOLOGY APPLICATIONS

SUBSTRATES

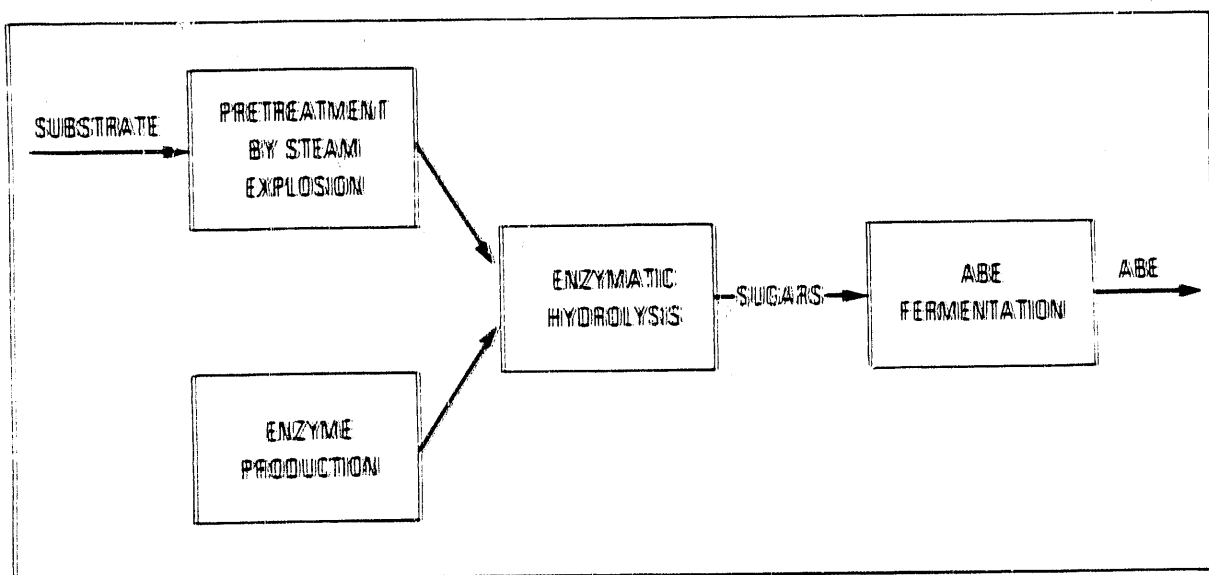
TECHNIQUES

PRODUCTS



LIGNOCELLULOSIC BIOMASS CONVERSION INTO BUTANOL ACETONE

The experimental program at the Soustons facilities includes the steps shown in the flowchart below.



- Steam explosion pretreatment makes the cellulose fraction digestible by cellulolytic enzyme action. Depending on the conditions implemented, the solubilization and hydrolysis of hemicellulose can be made more or less complete, and the lignin more or less easy to extract.
- Enzymes are produced on site by an original fermentation process using a selected strain of the fungus *Trichoderma reesei*.
- Enzymatic hydrolysis is carried out under mild conditions (pH 4.8; 50 °C). It induces the conversion of cellulose and hemicelluloses into their constituent monomers, glucose, xylose, mannose, etc.
- ABE fermentation under anaerobic conditions converts C₅ and C₆ sugars into a mixture of 2/3 butanol and 1/3 acetone through the action of *Clostridium acetobutylicum*.
- The overall yield anticipated for conversion is 1 metric ton of butanol acetone for 5-6 tons of lignocellulosic matter processed.
- This process is being tested on grain straw, corn stover, deciduous and coniferous wood, etc.

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