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**INTER-AMERICAN INSTITUTE FOR COOPERATION
ON AGRICULTURE
TECHNOLOGY GENERATION AND TRANSFER PROGRAM**

**INDUSTRIAL BIOTECHNOLOGICAL PROCESSES AND
PRODUCTS FOR AGROINDUSTRIAL WASTE TREATMENT**

FINAL REPORT

Submitted by

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To the

**Canadian Coordinator
IICA-AGRICULTURE CANADA-CIDA Project
Ottawa**

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PRODUCTS FOR AGROINDUSTRIAL WASTE TREATMENT**

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EXECUTIVE SUMMARY

IICA initiated a project to evaluate Canadian industrial biotechnological processes and products for agroindustrial waste treatment. The project's objectives are listed below:

- Identify the most important problems and needs of [solid] agroindustrial waste treatment (coffee, sugar, vegetable oil, pulp and paper, bananas) in Latin American and the Caribbean (LAC) countries.
- Describe Canadian biotechnological processes or products for the treatment [upgrading] of agroindustrial wastes relevant to LAC problems.
- Identify conditions and opportunities for technology transfer: companies and status of technology ownership and interest to sell/share/exploit jointly these technologies.
- Develop recommendations and strategies for access to/transfer of technologies.

This short introductory assessment of Canadian biotechnology processes and products to upgrade **solid** waste materials showed that some fundamental research has been, and is still being, undertaken in this field. The major emphasis in Canada, however, is on **effluent** treatment to meet increasingly stringent environmental regulations. This is the underlying reason why two major research institutes, the National Research Council's Biotechnology Research Institute in Montreal and the Pulp and Paper Research Institute (PAPRICAN) in Pointe Claire and British Columbia are not conducting experiments in solid waste treatment. Both, however, indicated a future interest in solid waste research.

The two major products researched in Canada (as well as in other



countries) include Single Cell Protein (SCP) also known as microbial biomass product (MBP), and ethanol. Additional products include several other chemicals, as well as plastics, pectin and other products. The substrates include primarily lignocellulosic materials of wood origin including bagasse. Research on the upgrading of tropic by-products is being undertaken in other countries.

Fundamental research however has not led to **applied** research. Furthermore, commercialization of some of the technologies has lacked.

In Canada, only four companies are actually offering proven commercial processes and products for the upgrading of wastes. StakeTech offers a proven steam explosion process to produce either SCP, several chemicals and/or fodder. Thermo Tech Technologies Inc. offers a bacterial fermentation process to produce SCP and animal feed. Tigney Technology Inc. has an organic refinery process of hemicellulosics (offered under the auspices of the Biofocus Foundation). IOGEN Corporation sells Cellulase-100, an enzyme to break down lignocellulosic bonds. Several universities and research institutes also offer technologies on a laboratory or pre-pilot scale. They include the University of Waterloo, the University of Sherbrooke, McGill and Ortech.

The general conclusions derived from these assessments are that some of the technologies are not yet ready for commercialization or, depending on cost/benefits, not yet economical. These are not only our conclusions but also those of several researchers quoted in the last part of this report.

However, the combination of hugh quantities and relatively easy accessibility of lignocellulosic waste in many parts of the world, environmental problems and economic needs particularly in developing countries, would make it imperative concentrate on applied research and development of the commercialization of technologies in order to achieve long term economic benefits.



Our recommended strategy represents an implementable long-term action plan and program.

First, environmental problems with regard to specific waste disposal problems and economic needs as well as costs for imported products such as protein, fuel, chemicals etc. should be prioritized for LAC countries.

With these priorities in hand, we recommend that IICA staff and interested LAC company representatives come to Canada to meet companies with commercial biotechnology processes and products to discuss details, tech transfer, joint ventures and other relevant aspects.

These discussions would subsequently lead to the initiation and preparation of a comprehensive commercial feasibility evaluation by LAC companies, the Canadian counterpart and IICA staff. This evaluation would include a technical, market, business and financial assessment of one or more technologies applicable to a specific need by one or more LAC companies.

This feasibility evaluation would then form the basis for implementing one or more initial commercial operations with local, Canadian and international participation and financing. This nucleus would be expected to lead to additional ventures either with the same or other technologies for the long-term benefit of LAC economies.



I PROBLEMS AND NEEDS OF SOLID AGROINDUSTRIAL WASTE PRODUCTS DISPOSITION

Efforts were undertaken to identify the most important problems and needs relating to the disposition of solid agroindustrial waste products from processing operations in LAC countries. The products listed in the Terms of Reference included coffee, sugarcane, vegetable or palm oil, banana and pulp and paper.

Six leading international research institutes were identified as research centres working with one or more of the five products. They included the following:

- Instituto Centroamericano de Investigacion y Technologica Industrial (ICAITI), Guatemala City, Guatemala: Dr. Carlos Rolz, Head, Applied Research Division.
- Caribbean Industrial Research Institute, Trinidad and Tobago: Dr. Ali Desmond, Director.
- Honduran Coffee Institute, Tegucigalpa, DC, Honduras: Mr. Ramiro Rodriguez Lanza, General Manager.
- Sugar Industry Research Institute (Agricultural Division), Manchester, Jamaica: Dr. Michael Shaw, Director.
- Centro de Tecnologia Copersucar, c/o Cooperativa Central des Produtores de Acucar et Alcool do Estado de Sao Paulo, Sao Paulo, Brazil: Dr. Manoel Sobral, Manager.
- Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia: Dr. Jaji Abdul Halim Haji Hassan, Director General, (we were subsequently informed that the new Director General was Dr. Yusof Basiron).

A cover letter or fax with the enclosed memorandum was sent to each



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MEMORANDUM

BIOTECHNOLOGICAL PROCESSES OR PRODUCTS FOR AGROINDUSTRIAL WASTE TREATMENT

INMARINT Ltd., an international marketing, technology and business development consulting company, located in Guelph, Ontario, was awarded a small contract by the Inter-American Institute for Cooperation on Agriculture (IICA) and the Canadian International Development Agency (CIDA) to research the Subject Topic.

The contract's objectives are to:

- a) Identify the most important problems and needs relating to the disposition of solid agroindustrial waste products from processing operations in Latin America and the Caribbean (LAC). The processing operations include coffee, sugar cane, vegetable (palm) oil, bananas and pulp and paper. The wastes may be in the form of either solids, particles, fibres, paste or sludge. Effluents are excluded.
- b) Identify Canadian biotechnological processes or products to convert, upgrade or improve the identified wastes to beneficial products such as feed and food products (eg. single-cell protein etc.), fertilizer, chemicals etc.
- c) Identify opportunities and conditions for technology transfer from Can. research institutes and companies to LAC processing companies, including joint ventures and other forms of collaboration.
- d) Develop relevant recommendations and strategies.

Our specific enquiry concerns the first objective of identifying and describing the most important problems and needs of particular solid agro-industrial waste products relating to one or more of the identified product groups that may be studied and evaluated in your Institute's applied research and development activities.

Could you please provide a description and details of the material, physical and, where possible, chemical characteristics of the particular wastes and by-products such as consistency, dry matter content, pH, handling problems etc.

These and any other details you may wish to provide, would be most helpful in linking biotechnological processes and products to particular wastes for their upgrading and improvement.

We would also welcome any other suggestions and comments you may have.



of the Directors. Replies were received in time from the Instituto Centroamericano de Investigacion y Technologica Industrial (Dr. Carlos Rolz), the Caribbean Industrial Research Institute (Ramnarine Ramnagibsingh, Mgr., Business Development, Planning & Coordination), the Centro de Tecnologia Copersucar (Dr. Carlos Eduardo Vaz Rossell, Head of Process Division) and the Sugar Industry Research Institute (Dr. Michael G. Hylton, Dir. of Research). The letter to the Honduran Coffee Institute was returned by the postal service.

The information received from these four institutes, plus information from other sources, are summarized in this Section.

Recommended methods for the characterization of agricultural residues and feed products derived through bioconversion have been established by the Commission on Biotechnology of the Applied Chemistry Division of the International Union of Pure and Applied Chemistry established (Ringfeil, Moo-Young, M., 1987). This updated set of guidelines has been developed with the objective of providing a frame of reference which could be used to evaluate microbial biomass products grown on agroindustrial raw materials. The guidelines make reference to important agroindustrial materials like lignocellulosic crop residues, and other wastes. It would be helpful for future fundamental as well as applied research and biomass upgrading, to have lignocellulosic materials defined by these detailed guidelines.

A. PULP AND PAPER WASTE

The composition of solid pulp and paper waste was derived from North American sources. The proportion of cellulose, hemicellulose and lignin depends on the type of trees such as coniferous (evergreen needled-bearing) or deciduous (annual leaf-bearing) types and their varieties as well as on



growing conditions such as soil, climate, temperature and other related factors.

The composition of the major constituents of a typical deciduous tree (birch) and a typical coniferous tree (spruce) is shown in Table 1 (Cowling, 1975):

Table 1: Composition of Birch and Spruce Fibers

Constituent	Birch (% by wt)	Spruce (% by wt)
Holocellulose	77.6	70.7
cellulose	44.9	46.1
noncellulosic polysaccharides	32.7	24.6
Lignin	19.3	26.3
Protein (N x 6.25)	0.5	0.2
Extractable extraneous materials	2.3	2.5
Ash	0.3	0.3

An interesting observation was made by Dr. Esdale of the StakeTech Company with regard to digestibility of deciduous and coniferous lignocellulosic composites. Due to different bonding structures of the cellulose microfibrils with the lignin and hemicellulose components, the digestion for deciduous lignocellulosics was about 60% and for coniferous lignocellulosics only about 15%. This observation, in our opinion, may be crucial for the future industrial/commercial upgrading of lignocellulosic base materials in LAC countries.

Dr. Esdale also mentioned that Dr. Tosi of the Tropical Science Centre in San Jose, Costa Rica identified some 300 tropical trees. It would be



interesting to also know, in addition to their physical composition, the distribution between coniferous and deciduous forests in LAC countries. This would greatly facilitate the identification and the determination of specific biotechnological upgrading processes of the various lignocellulosic materials in specific countries.

B. SUGARCANE BAGASSE

Sugarcane bagasse consists essentially of cellulose, hemicellulose and lignin. The percentages differ greatly depending on the culture and the regions where the sugarcane is grown. Rolz (1984) indicated that one type of bagasse contains 44% cellulose, 40% hemicellulose and 15% lignin. The *in-vitro* dry matter digestibility (IVDMD) of such products is relatively low due to the lignin content of bagasse and its structural characteristics. One sample, tested by the ICAITI's EX-FERM method, showed an IVDMD of 18.8% although the values quoted in the literature could range from 9.2 to 31.6%.

It should be pointed out that in Section III the digestibility of sugarcane bagasse with the StakeTech process was indicated at about 60%.

Villacencio (1974) indicated the following composition of sugarcane bagasse for paper making in Table 2 on the following page:



Table 2: Composition of Bagasse

	Composition (% dry basis)				
	Cellulose	α -Cellulose	Hemicellulose (pentosans: araban, gal- stan, xylin etc.)	Lignin	Ash
Louisiana					
Whole	58.4	36.8	29.4	21.3	2.9
Fiber	61.4	38.7	30.0	20.7	2.0
Pith	54.6	32.3	29.9	21.3	4.6
Philippines					
Whole	56.8	34.9	31.8	22.3	2.3
Fiber	62.9	41.2	31.2	21.8	1.2
Pith	55.4	34.9	33.2	22.5	2.6

The fibre dimension and length/width ratio of bagasse is also noteworthy as shown in Table 3 below:

Table 3: Fiber Dimension of Bagasse Fiber and Pith (Hawaii)

Sample	Length (mm)	Length/width ratio
1		
Fiber	1.34	70.5
Pith	0.37	4.6
2		
Fiber	1.82	29.1
Pith	0.26	3.3
3		
Fiber	1.24	59.0
Pith	0.36	6.3



It should be noted that according to this reference, a major portion of bagasse is used for paper making with paper mills in operation in 19 countries with several countries in Central and South America.

The Centro de Tecnologia Copersucar reported that the solid wastes and by-products available after the processing of sugarcane to sucrose and ethanol are:

- cane leaves and trashes
- bagasse
- filter mud
- yeast sludge

The cane leaves and trashes are used as a fuel for boilers, feedstock for pulp and paper, panelboards, feedlots and as a source material for wood saccharification processes. They contain 20.1% total reducing sugars, 41.7% cellulose and 27.7% hemicellulose.

Bagasse is the fibrous by-product after sugarcane processing for juice extraction. It contains 43.2% cellulose, 32.3% hemicellulose and 21.0% lignin. The main use for bagasse at the Sugar and Ethanol industry is as a fuel for boilers. An efficient mill can generate in excess of 5-6% of total bagasse that can be used as fuel, raw material for pulp and paper, panelboards,, feedlots for cattle, furfural and raw materials for saccharification process (in development).

Filter mud is the waste solid obtained after the process of clarifying cane juice. It is separated by rotary vacuum filters. It is a sticky, dark brown colored material, that smells like molasses. It has a high moisture content (65-80%). It is used as a fertilizer at the cane yards because of its high phosphate content. It can also be used as a feed for cattle considering protein and sugar content. Waxes can be extracted and refined. Additional details re complete composition materials etc. have been supplied and are available.



In Jamaica, bagasse and filter press mud are the two main solid by-products of the sugar industry. Bagasse is largely burnt as fuel for sugarcane processing and presently there is very little excess. Its typical composition is: 45% Fiber, 2-3% Insoluble Solids, 2-3% Soluble Solids, and 50% Water.

C. COFFEE WASTES

The wet processing of coffee berries to produce the beans yields several by-products and wastes. Among the most important ones are the pulp (skin and mesocarp of the fruit) and the waste waters from the washing of beans (fermented mucilage).

With a wet processing operation, 100 kg of fresh coffee berries yield 36.6 kg of green coffee beans, 40 kg of pulp, 20 kg of mucilage and 3.4 kg of dry parchment (Calzada, J., F.J. Rolz, C., 1983).

The parchment is widely used as a fuel to provide the heat for the drying operations but the pulp and waste waters can not be burned directly because of their high water content. Pulp has 85% moisture.

Fruit pulp has been used as feed for fish, ruminants, chickens and pigs.

In other research, the by-product yields were indicated as 44.8 kg of pulp from 100 kg of coffee fruit or 26.9 kg of pressed pulp (Garcia, R., 1990).

D. PALM OIL WASTE

Information on palm oil waste was received from Dr. J.M. deMan, Professor Emeritus of the Department of Food Science, Ontario Agricultural College, Guelph University. Dr. deMan chairs the Technical Subcommittee of the Palm Oil Research Institute Advisory Committee.

The solid by-products of the palm oil processes include

- mesocarp fiber (press cake fibre)



- empty food bunches (EFB)
- shell (produced when the kernels are extracted from the seeds)

The fiber and shell are currently the main sources of energy in palm oil mills. When burnt, they produce more than sufficient energy to meet the mills energy requirements. EFB's are now largely used as mulch since burning is discouraged.

Details as to fiber content and other characteristics were not contained in this reference (Palm Oil Developments No. 17, PORIM).

E. BANANA WASTE PRODUCTS

Processed products made from bananas include banana figs (or fingers, consisting of whole, peeled, dried fruit), powder, puree, slices, chips, jams and berry juice.

The by-product is the banana peel or peelings. According to estimates derived from a feasibility study of canned banana puree production in Ecuador, the banana peel is about 53% of the entire banana. This percentage may vary depending on the cultivar, type of banana and degree of ripeness and is generally considered to be approximately 50%. Details as to the composition, fiber content and other physical/chemical characteristics were not yet obtained.



II IDENTIFICATION OF RELEVANT INDUSTRIAL BIOTECHNOLOGY PROCESSES AND PRODUCTS

A INTRODUCTION

In the course of this project, several hundred reference sources were scanned from the data bases of the University of Guelph and the Centre of Industrial, Scientific and Technical Information (CISTI) of the National Research Council of Canada. The data bases included AGRICOLA Commonwealth Agricultural Bureau (CAB), Derwent Biotechnology Abstracts, and Food Science and Technology Abstracts.

Approximately 125 scientific papers and abstracts were reviewed in more detail of which about 35 have been covered in this report.

Three salient characteristics emerged from this review as follows:

- With the exception of two technologies, namely single cell protein (SCP) and ethanol production, there was no significant research conducted on other solid waste upgrading technologies in Canada.
- There is a considerable amount of **fundamental** R&D undertaken; we were not able to identify **applied** R&D projects.
- As anticipated, Canadian R&D is concentrating on pulp and paper waste and "structurally related" bagasse treatment rather than coffee pulp, banana peels, and palm oil kernels. Several references relating to the latter waste products were found being researched outside of Canada, notably in Guatemala, Mexico, Brazil, Malaysia and India as well as in the U.S., the U.K., South Africa and Egypt.

Composting was not included in this survey since it does not involve a biotechnological process; the biomass is upgraded only slightly.

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B. LIMITED CANADIAN RESEARCH IN SOLID INDUSTRIAL WASTE TREATMENT

In the course of our search for biotechnological processes and products to upgrade ~~solid~~ agroindustrial waste, we found that most Canadian efforts, primarily by many of the larger paper mills, are directed towards effluents. The treatment of solid wastes either does not appear to be of urgency nor of interest at this time. There are a number of reasons for this situation:

- The major emphasis by institutions such as the Pulp and Paper Research Institute (PAPRICAN) and the Biotechnology Research Institute of the National Research Council in Montreal, as well as major pulp and paper companies such as Domtar, McMillan, Noranda, Abitibi, Cascades and others, is on effluents primarily in order to meet increasingly stringent environmental pollution regulations.
- A protein deficiency does not exist in Canada as there is sufficient soybean and other protein available at moderate and competitive costs.
- In many paper mills, medium and fine cellulose fibres are retrieved through filters and put back into the pulping process; new pulp and paper processes continuously reduce the output of medium and fine cellulose fibres.
- The above process improvements in many mills leave only very small and colloidal fibres and solids which are hard to retrieve for conversion into feed, food or fodder products (e.g. SCP, ethanol and other chemicals). Other sources, however, indicate that a disposal of solid pulp and paper waste would still represent a problem.



- Despite the substantial research carried out in Canada, the U.S. and overseas, the economic viability of some of the solid waste treatment processes established in the laboratories has not yet been demonstrated.

C. SEPARATION OF LIGNOCELLULOSIC WASTE COMPOUNDS

Practically all R&D projects start with clean **agricultural** substrates such as separated or "pure" lignocellulosics or cellulosic waste products such as bagasse, palm oil kernels, corn stover and others. These substrates not only facilitate but also "guarantee" good and measurable results of bacterial/fungi fermentation allowing the microorganisms to grow in an optimum environment including temperature, pH, aerobic/anaerobic systems etc. unencumbered by "foreign" contaminants in substrates which could reduce fermentation and output.

Industrial waste streams especially from pulp and paper mills, however, contain a mixture of waste products in addition to lignocellulosic material. They may include fillers, chemicals, inks, colours, solvents and other contaminants (Dr. Lo; 1993). This presents a serious problem for biotechnological treatment and upgrading of solid waste products.

This problem, in our opinion, may possibly be solved by one of two alternatives: Either separate the various components in order to arrive at a relatively pure lignocellulosic product as a substrate for proper fermentation, or undertake the fermentation of a heterogeneous waste product with a group of different microorganisms (if feasible) possibly resulting in a decreased efficiency and/or reduced yield (of protein).

In our literature search we have not come across this problem mainly because the vast majority of lignocellulosic waste is of agricultural origin. This



problem, however, should be further assessed in subsequent more detailed research and evaluations, as, in our opinion, it may have a considerable bearing on the practicability and efficiency of the treatment and upgrading of lignocellulosic waste from pulp and paper mills.

D. PRE-TREATMENT OF LIGNOCELLULOSIC WASTE

Lignin is interspersed with hemicellulose and forms protective seals of cellulose microfibrils which prevent access of degradative enzymes to the cellulose and hemicellulose in woody plant tissues (Sidwick, 1987).

Hydrolysis or degradation of cellulose, i.e., its conversion to soluble simple sugars, requires that the hydrolytic reagents (chemicals, microbial enzymes) come in direct physical contact with molecules of cellulose. However, in most natural lignocellulosics the cellulose lies protected by lignin and hemicellulose which hinder degradation by restricting the access of the hydrolytic reagents to cellulose fibres. Hence, for effective cellulose degradation, the lignin and hemicellulose must first be removed from the lignocellulosic composites such as wood and corn stover. Various types of chemical and physical pretreatments (e.g., steam explosion, alkaline hydrolysis of lignin and hemicellulose, irradiation, etc.) are used to achieve this objective.

Lignin and hemicellulose are polymeric substances which bind or glue the cellulose microfibrils into a composite which gives structural strength to plants. Hemicellulose is made up of sugar molecules linked into a highly branched structure. However, unlike in cellulose, the bonds between the sugar molecules in hemicellulose are relatively easily broken (hydrolysed) in various types of pretreatments (e.g., alkaline hydrolysis). Lignin is similarly easily hydrolysed. Unlike cellulose and hemicellulose, lignin is not made up of sugars; instead, it is a highly branched polymer of phenolic substances. Lignin

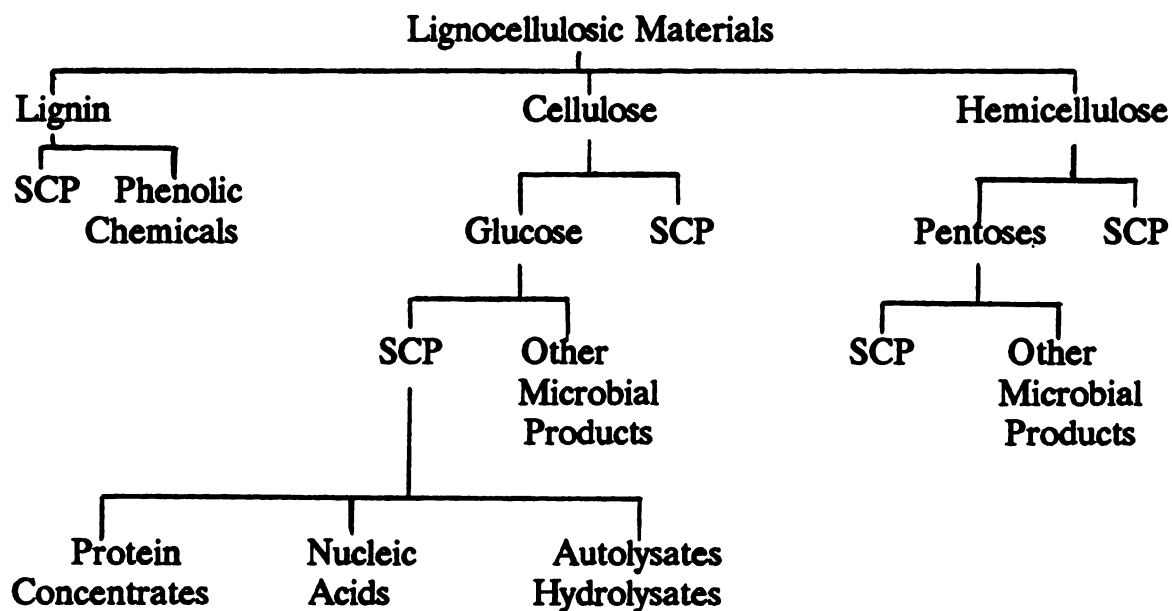


and hemicellulose usually constitute only a few percent of the lignocellulosic material.

E. BIOTECHNOLOGICAL PROCESSES TO UPGRADE AGROINDUSTRIAL WASTES

A number of products can be derived from cellulose and hemicellulose as illustrated by Litchfield (1985):

Figure 1: Products of microbiological conversion of Lignocellulosic Materials.



Cellulose can be assimilated directly by cellulolytic microorganisms or, after conversion to glucose, can be used as a substrate for producing SCP or other microbial products of interest as food additives.



Hemicellulose can be utilized directly for SCP production by microorganisms that can hydrolyse the substrate or after conversion to pentoses, for the production of SCP or other microbial products.

Some of the desirable characteristics of substrates for microbial production of foods or food additives include the following (Litchfield, 1985):

- availability - the substrate must be readily available in a uniform supply over the entire year.
- composition and physical characteristics - the substrate should have low concentrations of furfural, hydroxymethylfurfural, ash and heavy metals. Also, the substrate should be readily handled by commercial bulk handling equipment.
- performance - the substrate should give commercially attractive growth rates, and yields with the microorganism of choice.
- cost - there is a trade-off among the cost of substrate, yield of product, and the selling price of the product that must be considered in selecting the substrate for a particular process.

1. Processes of Pre-Treatment of Lignocellulosic Waste

As indicated in the previous section, fibrous lignocellulosic residues should be pretreated or hydrolyzed for the efficient fermentation by microorganisms. The exception is the fungus *Chaetomium cellulolyticum* which is capable of rapidly converting cellulosic substances directly to protein without the usual hydrolysis pre-treatments; McDonald (1984).

In a hypothesis, Rolz (1984) indicated that in order to increase the rates of enzymatic reactions to separate the micro components of lignocellulosic materials for their further biotransformations, pre-treatment operations must



be an integral part of any process scheme. There is no optimal or best treatment scheme as it will depend on the process, the raw material, the product and microorganisms used.

In physical and chemical pre-treatment experiments using lignocellulosic sugarcane residue, Rolz (1987) established its composition and enzymatic digestibility. After the fermentation of the sugarcane chips with yeast according to the EX-FERM process, pretreatments were carried out with sodium hydroxide; sodium carbonate less calcium hydroxide; gaseous ammonia; SO₂; steam explosion; alkaline organosolv; aqueous phenol; and a full sodacook.

The various pre-treatment results indicated the different effects upon chemical composition, in vitro enzymatic digestibility and susceptibility to enzymatic hydrolysis of the EX-FERmented sugarcane chips.

All treatments were effective and increased the in vitro dry material digestibility (IVDMED), the initial rate of holocellulose hydrolysis and the saccharification extent. The best pre-treatments were the organosolv variant, the steam explosion, the sodium hydroxide and the aqueous phenol. Ligno and hemicellulose contents decreased in all samples. Delignification was higher for the organosolv, aqueous phenol and steam exploded samples. Hemicellulose was fully hydrolysed in the aqueous phenol and steam exploded samples.

In another experiment, Rodriguez-Vazquez (1992) examined the results of a dry vs. wet pre-treatment of Mexican sugarcane bagasse pith for SCP production. Bagasse was sprayed with a 0.1 g of sodium hydroxide per g pith and 0.2 g per g/pith. In vitro - digestibility and microbial growth were obtained with a mixed culture of *Cellulomonas flavigena* and *Xantomomas sp.* The results indicated the advantages of the dry pre-treatment with a 76% maximum digestibility for the dry vs. 71% digestibility for wet pretreatment.



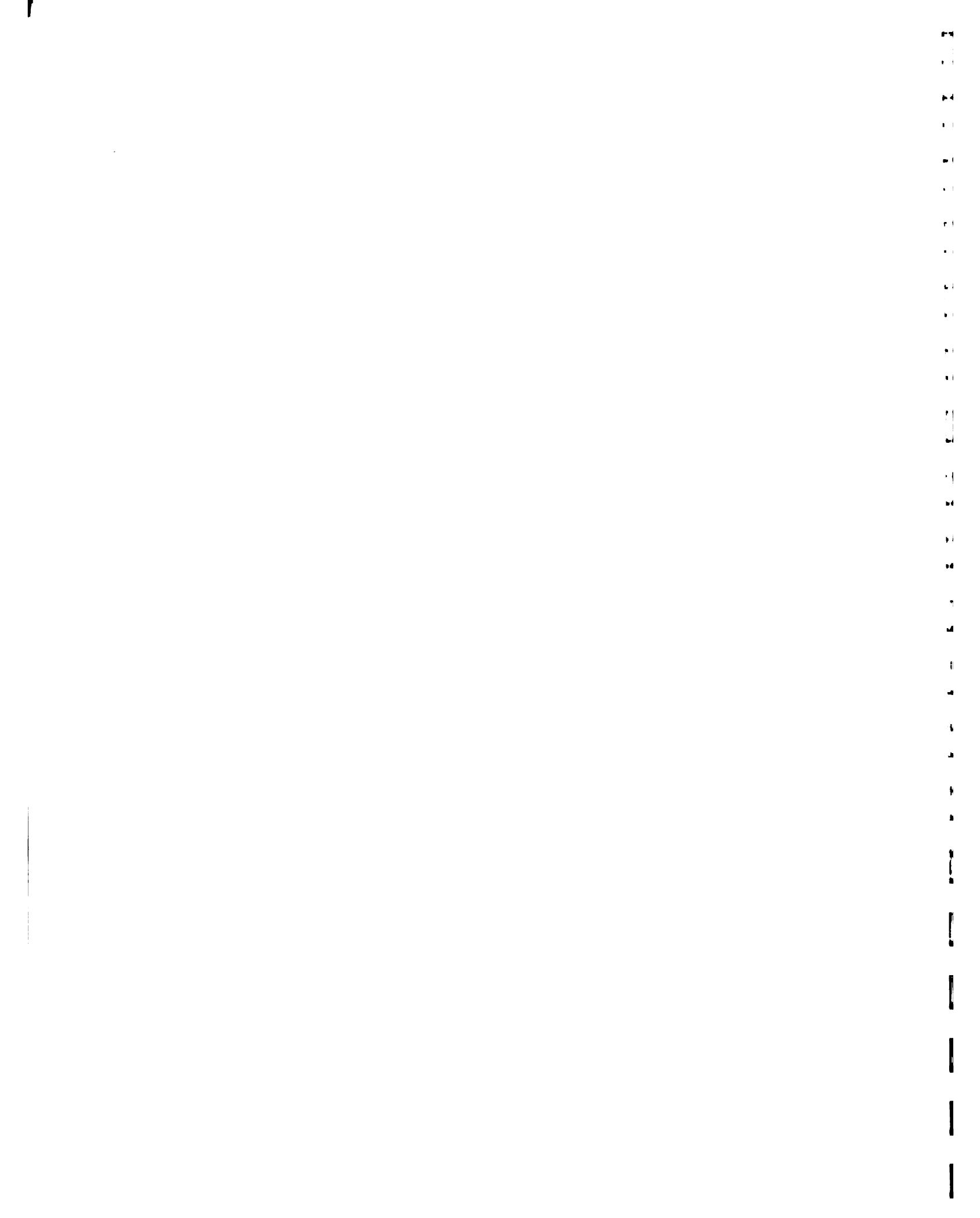
Furthermore, the dry process required less equipment and reduced costs and waste products.

Since the complex polymeric lignocellulosics are recalcitrant to biodegradation, research to develop an economical process for enzyme production was undertaken by Oguntimein et al. (1992). Cellulases and beta-glucosidases were produced by *Neurospora sitophila* in sugarcane bagasses as carbon source. The effects of several parameters were researched. Crude protein production increased to 1.89 g/l in the first 40 hours and declined slightly to about 1.1 g/l after 120 hours. The partial enzymatic activity is shown below in Table 4.

Table 4. Enzyme activity and productivity with different carbon sources

	Enzyme activity (U/ml)		
	Filter Paper Activity	Carboxy methyl cellulose	Beta-glucosidase
Chinese bagasse	0.142	1.48	0.041
Mexican bagasse	0.073	1.06	0.048

Fungal growth pretreatment with white-rot fungi on sugarcane lignocellulosic residue was researched by Rolz (1987). Twelve white-rot fungi were grown in a solid state culture on sugarcane chips previously fermented by yeast employing ICAITI's EX-FERM process. After five to six weeks at 20°C all fungi (with the exception of one) produced a solid residue which had a lower in-vitro dry matter enzymatic digestibility than the original bagasse. Lignin losses were similar for all fungi tested. Similar amounts of hemicellulose were degraded. Most fungi showed a preference for hemicellulose hydrolysis over cellulose degradation.



Pretreatment experiments carried out at ICAITI included the pulping of sugarcane bagasse with a mixture of ethanol-water solution in the presence of sodium hydroxide and anthraquinone (Valladares, Rolz et al. 1984). The effects of sodium hydroxide and anthraquinone were researched. The effect of the sodium hydroxide can be ascribed to a further cleavage of the products of the ethanolysis reaction and to anthraquinone for the conversion of these by-products to their more soluble sodium derivatives.

In the late 1970's, the StakeTech company in Norval, Ontario invented a steam explosion process to break the natural chemical bonding between lignin/cellulose/hemicellulose. This versatile process (described in the following section) can be used for different applications such as pretreatment, pulp production and numerous end products such as feed, chemicals, binders, SCP, adhesives, glucose etc.

An application of the STAKE II process was used at the University of Sherbrooke by Heitz M., Chornet et al. (1991). The aim was to find operational zones at which the recoveries of hemicelluloses (pentosans), lignin and cellulose were maximized. The objective of this test was to demonstrate the degree of opening of the structure of the treated material.

Fractionation of a poplar prototype hardwood, *Populus tremuloides* was optimized in a 4 ton/hr. pilot plant operation at the University of Sherbrooke, Quebec. A severity scale combining time and temperature parameters to express the severity of given pretreatment was developed.

The author reported experiments carried out with STAKE II continuous reactors at 2 t/hr. demonstrated that with temperature, pressure and feed rate controls, stable operating conditions can easily be maintained. The data on the yields and properties of the polymer fractions obtained at the semi-industrial scale separations showed a good agreement with laboratory experiences at

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similar severity levels.

Stake Technology's improved steam explosion process for lignocellulosic materials was tested in three independent trials at the University of Quebec at Trois Rivieres (UQTR), Hymac Ltd. and StakeTech (Yu E.K.C., Taylor J.D., 1992). The experimental protocol was designed jointly by StakeTech PAPRICAN, Hymac and the UQTR.

The objectives of the tests were to establish the energy requirements and quality of steam explosion pulp (S-pulp) of aspen (*Populus tremnloides*). The correlated results showed that:

- the steam explosion (StakeTech) process produced a good quality high-yield pulp.
- the S-pulp process required about half of the energy than corresponding processes (conventional chemimechanical pulps; CMP or chemithermo mechanical pulps; CTMP).
- The physical strength of S-pulp was consistently higher than conventional pulps
- S-pulps could be produced consistently at about 90% yield
- the S-pulp's process requires only about 50% chemical charge compared with the CMP process.

2. Biological Processes to Produce Food and Feed Substances

Considerable research has been undertaken for the production of single cell protein (SCP) with numerous microorganisms. The term 'single cell protein' was established at MIT in 1966 by Prof. Wilson (Goldberg, 1985). SCP are dried cells of microorganisms such as bacteria, yeasts, molds/fungi grown in large scale culture systems for use as protein sources in human foods or animal feeds. Soon it was recognized that 'single cell protein' terminology



was not accurate - many of the SCP microorganisms being multicellular.

Hence, at a bioconversion conference in Guatemala in 1978, a new name was established for protein products derived from microbial processes. The name microbial biomass product (MBP) was coined. Since then, both definitions of SCP and MBP have been used synonymously.

A list of desirable physiological characteristics in microorganisms to be used for the production of SCP has been published by Goldberg (1985) and is shown in Table 5:

Table 5: Desirable physiological characteristics in microorganisms to be used in SCP production

- High growth rate
- Growth on simple media - no requirement for expensive growth factors
- High yield coefficient on a carbon substrate
- High affinity for the carbon substrate
- Ability to utilize complex substrate and/or mixture of carbon substrates when required
- Ability to grow at high cell densities
- Resistance to substrate and/or product toxicity
- Stable growth in continuous culture
- High optimum growth temperature
- Stable fermentation - resistance to contamination
- Capability of genetic modification
- Capability of using ammonia as nitrogen source
- pH tolerant

Other requirements:

- Protein, fat and carbohydrate content of high quality
- Low nucleic acid content
- High nutrient content
- High digestibility
- Absence of toxicity
- Good taste
- Ease of recovery
- Amenable to further processing, e.g. drying, without changes of color, texture, smell, etc.

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Microorganisms suitable for SCP production are divided into four main categories namely bacteria, yeasts, fungi and algae. They are summarized below (Goldberg, 1985):

a. Bacteria

The most important characteristic is the efficiency of conversion of substrate carbon to cell carbon. A high conversion efficiency results in a high overall cell yield and productivity. Bacteria as process organisms offer some attractive advantages. The growth rate and cellular yield from carbon sources are higher for bacteria than for other organisms. They generally have higher protein contents than other organisms, and the quality of the protein is better in bacteria compared to other microorganisms. Disadvantages include the small size of bacteria which causes difficulties during the separation stage. Furthermore there is only a limited number of bacterial species that have been grown specifically for food purposes. Also, the relatively high content of nucleic acids renders their use unsuitable for human food as excessive intake of nucleic acids is implicated with certain metabolic disorders such as gout.

b. Yeasts

Considerable experience has been obtained in the manufacture and use of microbial foods with certain yeasts for human consumption primarily in Germany due to World Wars I and II as a meat substitute or meat extender. An example is the well known fodder yeast, the dried yeast obtained as a brewing industry by-product used as a feeding supplement for animals. Yeasts are a good source of the B group vitamins and also provide small amounts of vitamin E and vitamin D. However, not all yeasts are suitable for food or feeds.

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c. Fungi

The idea of mass cultivation of fungi as a protein source is relatively new. Recent research on fungal based SCP has shown that

- the growth rates of fungi are usually slower than those of yeast and bacteria
- their protein content is usually lower than that of bacteria and yeasts
- fungal protein is often deficient in cell and amino acids
- the problem of digestibility of the cell wall is often a serious consideration
- fungi are more of an unknown entity with regard to nutrition and toxicology

On the other hand fungi have some important advantages namely:

- most fungi produce a range of carbohydrolyzing enzymes and thus are able to grow on complex raw materials such as cellulose, hemicellulose and starch
- they can usually be recovered by simple filtration which offers a significant reduction in capital and processing costs
- they give rise to a fibrous mass and are easy to process into textured products such as meat substitutes.

d. Algae

Algae are often thought to be an effective source of SCP owing to their photosynthetic abilities. Algae protein production can be carried out in large open pools, natural lakes or ponds. Their growth rates are lower than both bacteria and yeasts although the separation procedure is easier. The raw materials suggested for SCP production and the organisms used are shown in the Table 6 below by Goldberg (1985):

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Table 6: Raw materials suggested for SCP production and organisms used

Raw Material	Organism(s) Used
Lignin	<i>Trichosporon penicillatum</i> 3G
Cellulosic wastes	<i>Candida utilis</i> and <i>Saccharomyces cerevisiae</i> <i>Chaetomium cellulolyticum</i> * <i>Cellulomonas</i> sp.
Bagasse substrate	<i>Aspergillus terreus</i> GN1
Sugarcane bagasse	<i>Cellulomonas</i> strain II bc <i>Trichoderma longibrachiatrum</i> Rifai <i>Candida utilis</i> and <i>Paecilomyces variotti</i>
Coffee pulp juice	<i>Aspergillus oryzae</i> , <i>Trichoderma harzianum</i> , <i>Penicillium crustosum</i> , <i>Glucocladium deliquescens</i>
Banane waste	<i>Pichia spartinae</i>
Skin of green plantain (mostly starchy)	<i>Pichia spartinae</i>
Palm oil (triglycerides and saturated fatty acids) Malaysia Japan Japan	<i>Candida tropicalis</i> , <i>Candida lipolytica</i> <i>Torulopsis candida</i> TAMY128 <i>Candida blankii</i> CBS 1898
Coffee wastes	<i>Trichoderma harzianum</i> Rifai
Palm oil and rape seed oil (France)	<i>Candida curvata</i> , <i>Candida rugosa</i> , <i>Candida deformans</i> , <i>Candida lipolytica</i> , <i>Candida parapsilosis</i> , <i>Cryptococcus uniguttulatus</i> , <i>Geotrichum candidum</i> , <i>Trichosporum cutaneum</i> , <i>Rhodotorula pilimance</i>

*It should be noted that since the publication of this information in 1985, the *Chaetomium cellulolyticum* has been determined to be unsuitable as SCP.

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It is worthwhile noting that certain micro- and macro-algae have been used as human food for centuries in parts of Africa, Japan and China. Similarly, the bakers' and brewers' yeasts and several fungi as human food has a long history. Also, some macrofungi (mushrooms) are a very successful human food utilising many agricultural solid wastes (manures, lignocellulosics, etc.)

In addition to the Waterloo University, several companies have also conducted research with SCP production from several raw materials. Details and characteristics of selected SCP processes are shown in Table 6 (Goldberg, 1985) on the following page.

Substantial research to convert cellulosic waste to protein-rich substances has been undertaken at the University of Waterloo by Moo-Young. In earlier experiments, the fungus *Chaetomium cellulolyticum* was used to convert lignocellulosic materials such as crop residues, bagasse, sawdust, pulp mill lignocellulosic wastes and others. The process uses a three stage operation as follows:

- thermal and/or chemical pre-treatment of cellulosic material
- aerobic fermentation of the pretreated material with nutrient supplements
- recovery of the SCP product.

The cellulosic material is pre-treated with hot water or dilute alkali and continuously fed into a 1000 litre conventional aerated fermenter operating at pH 4.5 and at 37°C. The recovery of the fungal mycelia is accomplished by filtration; the dried SCP contains about 45% (of dry weight) crude protein.



Table 6: Characteristics of selected single cell protein processes

Table 6: Characteristics of selected single cell protein processes

Name of SCP product Item	"Pruteen" bacterial protein	"Pekilo" mold protein	Algal protein	"Mycoprotein" fungal protein	Yeast protein	"Waterloo" fungal protein
Organism	<i>Methylophilus methylotrophus</i>	<i>Puccinomyces variotii</i>	<i>Microcystidium</i> , <i>Euglena</i> , <i>Chlorella</i> , <i>Scenedesmus</i>	<i>Fusarium graminearum</i>	<i>Endomycopsis (Candida) lipolytica</i> FH-H-5027	<i>Chaetomium cellulolyticum</i> *
Raw material	Methanol	Sulfite liquor	CO ₂ , domestic sewage	Glucose	n-paraffins	Cellulosis wastes
Company or institute	ICI	United Paper Mills	Technion	RHM	Hoechst-Uhde Gelsenberg	University of Waterloo
Location	Billingham, England	Jamsankoski, Finland	Haifa, Israel	High Wycombe, England	Frankfurt, West Germany	Waterloo, Ontario, Canada
Type of process	Continuous	Continuous	Continuous	Continuous	Continuous	Continuous
Fermentor size	1500-m ³	2 × 360-m ³	Pond area: 120 m ² , 150 m ² , 2 × 1000 m ²	2 × 1.3-m ³	4-m ²	1-m ³
Type of fermentor	Air lift	Impeller-agitated	Aerated pond (HIRAP)	Impeller-agitated	Air lift	Agitated
Sterility	Aseptic	Aseptic	Nonaseptic	Aseptic	Aseptic	Aseptic
pH	6.5-6.9	4.5-4.7	-	6.0	3.5	5.5
Temperature (°C)	34-37	38-39	Ambient	30	30	37
Specific growth rate (μ) or dilution rate (D) in h ⁻¹	0.16-0.19	0.2	2.5-4*	0.18	0.11	0.24
Concentration of cells (g/liter; dry wt)	30	17	0.1-0.2	15-20	15-20	-
Y _p , g cell dry wt/g substrate	0.5	0.47	-	0.48-0.5	0.95	-
Productivity (g cell dry wt/liter · h)	4.8-5.7	2.7-2.8	7 kg/m ² · year	3-4	2	-
Total production (ton/year)	50,000-70,000	10,000	14	50-1000	20	-
Product recovery	Agglomeration and centrifugation	Filtration	Coagulation, flotation, centrifugation	Filtration	Nozzle-type separator, decantor	Filtration
Crude protein (% of dry wt)	83-85	57-63	57	56	58	45
True protein (% of dry wt)	59-64	-	-	50	55	-
Intended for use as:	Animal feed	Animal feed	Animal feed	Human food	Animal feed	Animal feed
Other products formed	CO ₂	Water of low BOD	Water of low BOD	-	-	CH ₄ , CO ₂ and water of low BOD
References No.	[7, 8, 55]	[380]	[623]	[8, 45, 624]	[65, 66]	[625]

* Retention time, days

*It should be noted that the new Waterloo process has replaced *C. cellulolyticum* with *N. sitophila* while process details are essentially unchanged.

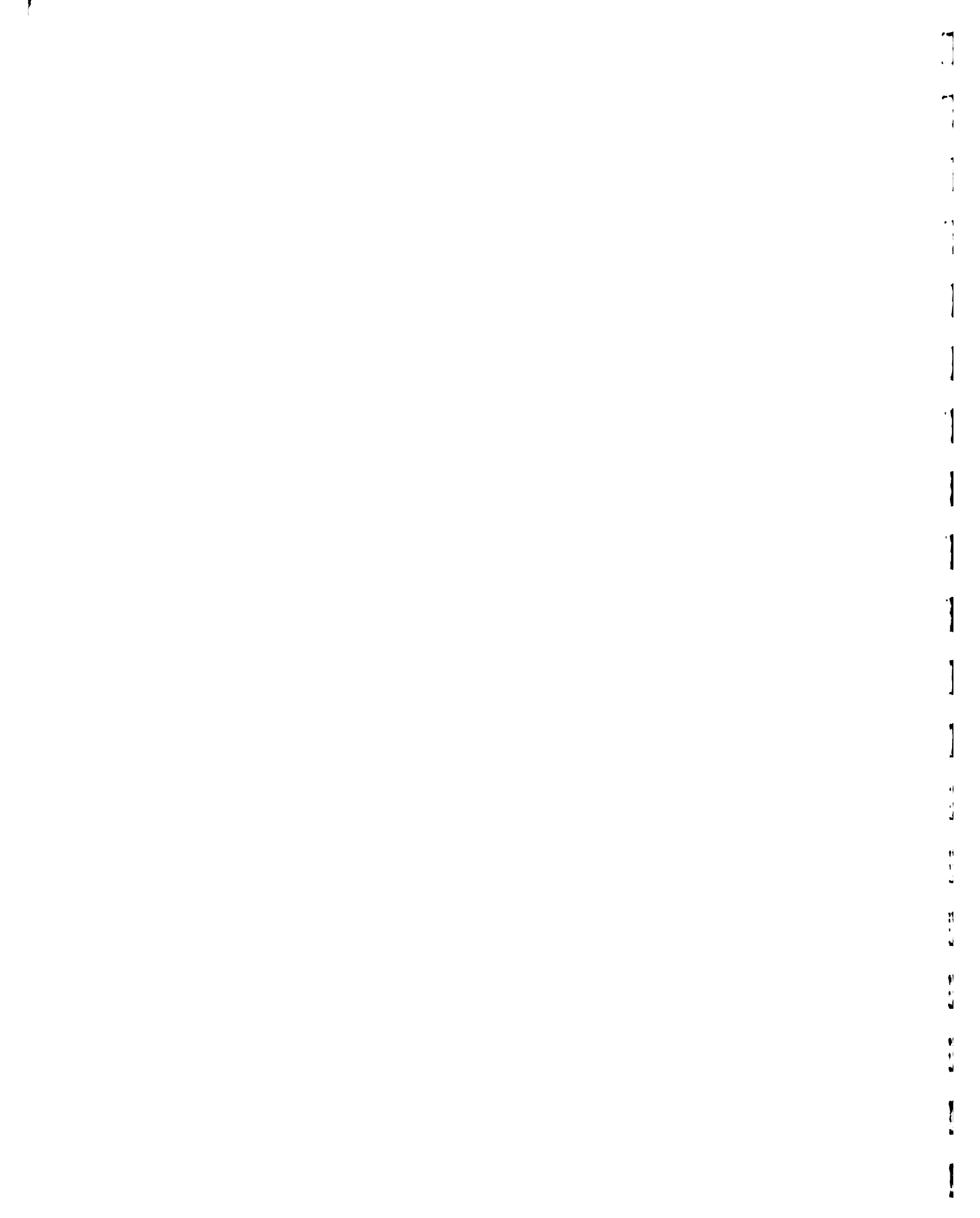
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The "Waterloo" process has several unique characteristics which differ substantially from other processes. The characteristics are as follows:

- the process costs are minimized by using relatively inexpensive raw materials such as waste residues from pulp and paper, bagasse and others
- the versatile equipment can handle different raw materials
- the continuous process employs low technology operations
- the economy of the process is further improved by replacing the fertilizer with aerobically pre-digested animal manure as the source of nitrogen, phosphorus, potassium and various essential minerals.
- the process employs simple methods for the separation and recovery of biomass using agglomeration, coagulation and flotation techniques.

The above characteristics result in relatively low capital investment and operational expenditures for the manufacture of the fungal SCP product in comparison to those of other SCP processes using expensive feed stocks and high technology operations. This would allow for economic operation of relatively small size plants in medium sized villages in developing countries.

Commercialization of the Waterloo SCP bioconversion process was initiated by McDonald (1987) of Envirocon Canada Inc. in Vancouver. Envirocon obtained the licence for the fermentation process and constructed a pilot plant in Vancouver to conduct process development activities for 12 months. The total cost of the development of the process approached \$5 million of which Envirocon contributed in excess of \$2 million with the remainder provided by various public sectors funding programs. The process used a three staged operation namely: thermal and/or chemical pretreatment



of the cellulosic based material; aerobic fermentation of the pretreated material with nutrient supplements; and separation of the suspended solids from the fermentation brew.

Envirocon's biomass conversion technology was founded on the approach of marketing the technology itself rather than the products. The fungus *Chaetomiium cellulolyticum* was selected because of its ability to rapidly convert cellulosic substrates directly to protein without the usual hydrolysis-type pretreatments. The first serious evaluation of the market potential took place during the development phase. The global market for proteins had been thoroughly analysed from widely published information forecasting the long term growth of the demand for protein. Companies such as B.P., ICI, Phillips Petroleum and others recognized this trend and also developed SCP technology at that time.

Analysis of typical applications indicated that a commercial plant would have a processing capacity of approximately 10 tons per day of waste biomass representing a fermentation volume of about 100 cubic metres.

The construction of the pilot plant was completed in the Fall of 1982 and operated for 12 months until September 1983.

Due to a major technical obstacle of possible product toxicity, the process and development activities were stopped in September 1983. While there was an aware of a toxigenic potential of *Chaetomium* species, it was not perceived to be a serious problem until the results of the preliminary feeding trials with chicks resulted in a significant mortality rate among those fed a diet containing SCP. Since a number of metabolites of *Chaetomium* were possible toxins, concentrations were found to far exceed the acceptable levels. Due to possible funding limitations, Envirocon was unable to carry on research into the toxicity and had to cease operations.

In subsequent research to avoid possible contamination by toxins with SCP production, Moo-Young et al. (1992) used a food grade fungus *Neurospora sitophila*. The fungus has been used in the Orient as a food for a long time. It also has the advantage of being one of the faster growing microfungi.

Conceptually, *N. sitophila* mycroprotein production process consists of the following steps:

- Size reduction of the dry cellulosic residue by milling or grinding;
- Treatment of the residue with alkali, acid and/or steam to increase the accessibility of the cellulose in the particles;
- Fermentation of the residue with *N. sitophila* either in submerged or surface culture;
- Solid-liquid separation and dehydration of the product for direct use as fodder;
- Blending, possible nucleic acid reduction, texturizing and flavouring operations for human food applications.

Typically, dry lignocellulosic material (bagasse, corn stover) is ground to < 1 mm particle size, sodium hydroxide (0.15 kg NaOH/kg solids) is added and the material is autoclaved at 121°C for 30 minutes. Sterile nutrients (mainly nitrates and phosphates) are added and the slurry is made up to 10 kg/m³ lignocellulosic solids in water. The pH is adjusted to around 7.0 and the temperature is kept in the 25-35°C range. A 5% (by volume) inoculum of *N. sitophila* is added. The fermentation is carried out in stirred tank or stirred-airlift hybrid bioreactors for up to four days. Throughout, the fermenter is aerated to keep the dissolved oxygen level above 20% of air saturation. After fermentation, the biomass is harvested (filtration or centrifugation) and dried. The dry material may be ground to give a crude animal-feed protein

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supplement. Depending on the lignocellulosic substrate used, up to 90% of the initial cellulose present is converted into fungal biomass. The crude protein concentration in the final product may reach up to 50%.

Blending, texturizing, colouring and flavouring operations may be used to enhance the palatability and the organoleptic properties of the product. These operations are in common use in the food processing industry, and have been developed also for the fungal protein food "Quorn" being marketed in the United Kingdom. Nucleic acid reduction is also used in Quorn manufacture. The reduction of nucleic acids may be required if the product is used for human food. The dietary level of RNA should not exceed 2 g per day: breakdown of RNA in the human body leads to elevated levels of uric acid which may cause such metabolic disorders as kidney stones. In animals uric acid is readily excreted by conversion to allantoin, and RNA reduction in feeds is not necessary.

The *N. sitophila* raw protein has a pleasant almond smell; it has potential economic applications in feed and food preparations.

SCP experiments with Mexican sugarcane bagasse, cassava as well as methanol and molasses were conducted by De la Torre-Louis and Cotera (1986). The pre-treated sugarcane bagasse was fermented with a mixed bacterial culture *Cellulomonas flavigena* and *Xanthomonas sp.* The initial research included several batches and was subsequently changed to a continuous counter-current treatment unit in order to estimate capital costs. Residual substrate was separated from the bacteria cell cream on a rotary vacuum filter dried and stored.

The two products obtained contained 12-15% protein and a microbial biomass with 60-65% protein. The forage is similar to dry alfa-alfa with a protein content and digestibility of 60%.

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The authors conclude that the economics depend strongly on the carbon source costs with the availability and price of substrates depending on raw materials at each location.

Using several references, the authors established an investment estimate for a 50,000 ton production capacity per year, operating 300 days per year, of U.S. \$ 78.2 million. The projected SCP production costs were U.S. \$1,098/ton, the raw materials and utilities U.S. \$465/ton and the protein cost U.S. \$1,830/ton. The protein content is 60%. They projected that a reduction of the alkali consumption to 60% of the actual value and a reduction of capital investment by 10% would bring the SCP production costs below U.S. \$1,000 per ton. Furthermore, by recovering solubilized hemicelluloses and lignocellulosics, the overall economy of the process could be improved. They felt that with a shortage of proteins for human and animal nutrition in Mexico, and soybean imports averaging 800,000 tons per year (in the mid 1980s), the production of single cell protein would be of substantial benefit to Mexico.

3. Biotechnical Processes to Produce Ethanol

Ethanol or ethyl alcohol is a product primarily used for the blending of gasoline to increase the octane rating and allow a cleaner combustion process to reduce emission of some polluting compounds.

Ethanol is being produced from sugarcane, spent sulphite liquor, corn and wheat.

Brazil has been a leader in the production of ethanol. The program represents an independence from the world petroleum market. With the adoption of Proalcohol, the production of alcohol from sugarcane juice increased to 12 billion litres of alcohol per year in the late 1990s. Following the extraction of the juice, the remaining bagasse, however, represents a

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disposal problem and at the same time a challenge as inexpensive abundant source of carbohydrates with the potential of fermentation to ethanol.

Recent interest has been concentrating on the production of ethanol with biotechnological processes from sugarcane bagasse and other lignocellulosic wastes such as pulp and paper residues.

Rolz (1984) developed a hypothesis dealing with existing alternatives for employing lignocellulosic residues by extraction/fermentation of sucrose present in sugarcane with the action of microorganisms using ICAITI's EX-FERM process. As indicated earlier, a pre-treated hydrocellulosic material is more susceptible to biotransformation to ethanol and other chemicals than untreated material.

Biotransformation can be classified into three groups: indirect, direct and "intermediate". In the indirect, the basic objective is to hydrolyse the hollocellulose to monomeric units with chemicals or enzymes for the transformation into ethanol. In the direct process, the conversion of hollocellulose into ethanol is done in one step avoiding the production and accumulation of monomers. In the new "intermediate - acid" alternative, studied by ICAITI scientists, the hollocellulose is transformed into volatile organic acids which subsequently will be biotransformed to ethanol.

Roberto (1991) researched the production of ethanol from sugarcane bagasse hemicellulosic hydrolysate with a xylose-fermenting yeast *Pichia stiptis* CBS 5773 and three other yeasts in semi-synthetic xylose-containing media and in sugarcane bagasse hemicellulosic hydrolysate. An effective hydrolysate treatment employing neutralization with NaOH and activated charcoal was established. Materials and methods were described and results of research reviewed. The results showed that the fermentation of ethanol with *Pichia stiptis* was far superior compared to the other three yeasts. The inhibition of

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fermentation parameters was also observed and evaluated. An observation of interest was that the fermentation was in fact consistent and high yielding despite there being a low amount of cell replication in the second and third fermentations and despite there being a decline in cell numbers during the fourth fermentation. It was also acknowledged that a large scale process would not employ an activated charcoal NaOH treatment as described in this research. However, the ability of *Pichia stiptis* to readily ferment hydrolysate treated in the manner, demonstrated the potential of using appropriately-treated hydrolysate fermentation substrate. Furthermore, the identification and removal of inhibitors from crude substrates was projected to be a vital component.

The production of ethyl alcohol from banana peels in India was researched by Tewari (1985). The peels were saccharified with H_2SO_4 , cellulase and steam, and fermented with *Saccharomyces ceravisiae* var. *ellipsoideus*. Depending on the concentration, pressure and time, different rates of saccharification were achieved. The process was further accelerated by steaming and autoclaving. With the cellulase, an ethanol yield of 1.38%, and with H_2SO_4 treatments, an ethanol yield of 0.78% was obtained. Further details could not be obtained since the Journal of Research of the Punjab Agricultural University was not available in Canada.

An Ontario company, Commercial Alcohols Inc. of Tirerton, started ethanol production from corn last year for UCO Petroleum Inc., the distributor of ethanol blended gasolines. The company is jointly owned by United Cooperatives of Ontario (UCO) and Sunoco. UCO Petroleum distributes the blend in 21 Ontario gasoline stations.

In Western Canada, Mohawk Oil Co. Ltd. produces ethanol blends in its Minnedosa, Manitoba plant (12 million litres) and Poundmaker Lanigan

Saskatchewan plant (13 million litres). It distributes two ethanol blends in 289 retail outlets.

The production and consumption of ethanol appears to be increasing rapidly in some states in the U.S. In the state of Minnesota, for example, ethanol consumption has increased four fold since 1988 to a projected 60 million gallons per year in 1992. It accounts for 30% of all gasoline sold in the State. That share was expected to increase sharply in November of 1992 under the Oxygenated Fuel Requirements and of the Federal Clean Air Act (Ye, 1992).

In the past, Minnesota converted only about 1% of its corn into ethanol. As a comparison, Illinois already processes 19% and Iowa 8% of its corn production to ethanol.

Efforts are now underway to increase the ethanol production in Minnesota which, in 1992, was only 20 million gallons per year or 1/3 of the consumption. It is made from corn and other grains produced domestically. The program reduces the State's dependency on oil imports, provides fuel self-sufficiency, improves balance of payments, and reduces carbon monoxide emissions as well as reducing farm surplus and farm program payments.

Plans are being developed for a 200 million gallon a year production which would provide a 100% marketshare of a 10% blended ethanol and gasoline mixture sold at filling stations in Minnesota.

Although the ethanol production from spent sulphite liquor or an effluent is beyond the objectives of this evaluation, it might be of interest to some LAC companies that we identified two companies producing ethanol from spent sulphite liquor.

Temeco Enterprises Inc. in Temiscaming in the Province of Quebec is a small company producing a pharmaceutical purity grade of ethanol from

spent sulphite liquor. For further details refer to this company in the next section.

Another company is the Quebec Ontario Paper Company who produced ethanol at its pulp and paper plant in Thorold, Ontario. We were informed, however, that the company ceased ethanol production in 1988; no reasons were given. In one of the references, it was indicated that in order to be economically viable, the ethanol production from spent sulfite liquor should be incorporated in the pulp and paper making process from the beginning and not be added afterwards.

4. Biotechnological Processes to Produce Other Products

Through our literature search and personal contacts we identified six products resulting from the fermentation of agro-industrial waste products: biogas, biodegradable plastics, irradiated livestock feed from empty palm fruit bunches, pectin, organically refined fibers and chemicals and a list of numerous derivatives from lignocellulosic components.

Coffee pulp represents a pollution threat to the coffee industry. It causes odour and nuisance in coffee estates, creating numerous problems for its disposal. Thus, research attempts have been undertaken to treat the coffee pulp with biotechnological processes. The literature search did not provide any references relating to Canadian research in this field.

Research in the U.K. and the U.S. has been undertaken by Boopathy (1984, 1987 and 1988). These experiments included the production of methane gas from coffee pulp. Methane-forming bacteria are present in most manure handling systems, but take a long time to multiply to an efficient methane producing population; up to six weeks are required to achieve maximum gas production. In order to reduce the time for this material to become

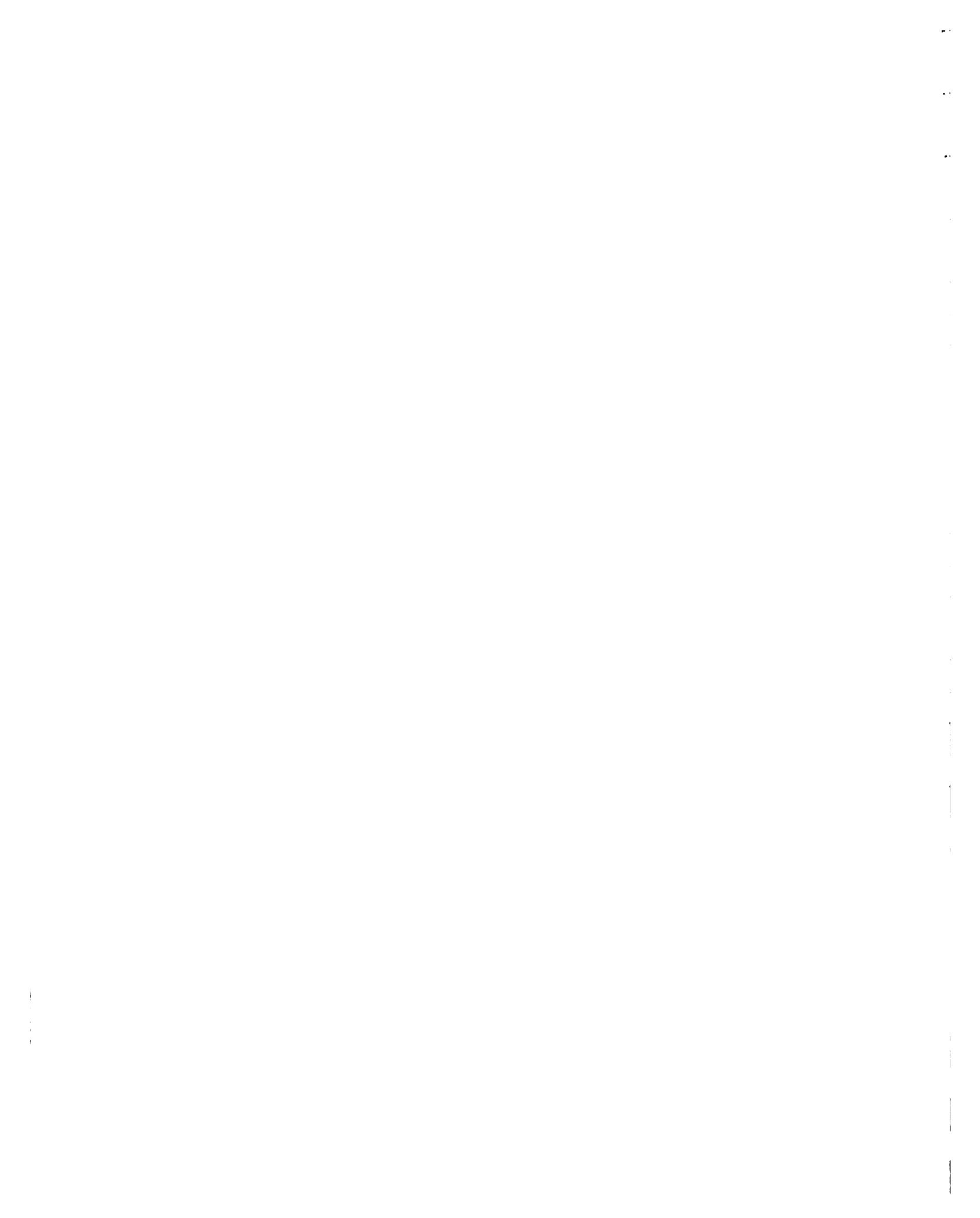
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established in a new digester, it was suggested that actively-digested material or inoculants be added to aid the start up.

An experiment was conducted to find out the correct or best inoculant material for the startup of an anaerobic digestion for coffee pulp as a substrate. Three different inoculants were tested for their efficiency in producing maximum methanogenic activity, namely, slurry from cow dung biogas plant, sewage sludge, and rumen fluid. The slurry from the cow dung digester was found to be the best seed or inoculant material for methanogenic activities, as expressed by the maximum gas yield, substrate utilization, and microbial activities in the treatment.

While earlier R&D was undertaken by Boopathy at the Department of Chemical Engineering of the University of Strathclyde in Glasgow, subsequent research was done at the College of Agriculture, Department of Agricultural Engineering, University of Columbia. The objective of the research was to determine the best solids concentration ratio between the coffee pulp and cow dung. An experiment was undertaken in a 2.5 litre lab fermenter and monitored regularly with special reference to physical and chemical characteristics of the system. Solid concentrations varied from 4.3, 10, 20, 25, 30, 35, 40 and 46.15%. Observations were made over a time span of 10 weeks. Results indicated that methane gas was obtained from all solids concentrations tested but the maximum gas production was found in the fermenter with a 25% total solids concentration.

These research results would indicate that the fermentation to obtain methane gas would represent a viable solution for the coffee pulp problem. The possibility should be examined for a LAC research institute to follow up the lab experiment with a pilot plant operation to establish parameters on a larger scale. Alternatively, subcontract research could be undertaken either in



LAC countries or in Canada.

In an experiment to convert banana waste, Mishbah-ul-Hasan (1989) was conducting research in the production of biogas from dried, ripe banana peelings. The peelings were aerobically fermented for 48 hours before being placed in a series of digester bottles. In trials, enough biogas (comprising 70% methane) was produced from the peelings to make the process (according to Misbah-ul-Hasan) economically feasible. The biogas yields increased when the banana peelings were mixed with cow dung.

The composition of substrates and resulting generation of biogas are shown in Table 7:

Table 7: Composition of Substrates and Related Biogas Generation

Experiment	Composition of Substrate	Amount of Biogas Generation (ml)
1	240 g banana peelings	3,090
3	225 g banana peelings 2 g ammonium phosphate	3,230
4	240 g banana peelings 15 g cow dung	3,910

A completely new method of conversion concerning the fermentation of waste agricultural material into biodegradable plastics was discussed with Bruce A. Ramsay and Shirley Ann Ramsay (1990, 1992). Their Polyferm Company is a small Montreal-based company with expertise in fermentation and separation processes for poly- β -hydroxyalkanoate (PHA) production. PHAs are a family of biodegradable plastics produced from renewable resources. One of Polyferm's mandates is to further develop and subsequently

commercialize PHA technology developed at Ecole Polytechnique de Montreal, Canada's largest engineering school.

Polyferm has effective control of one patent (through Ecole Polytechnique) relating to the chemical oxidation of bacterial biomass to release PHA granules. It is in the process of obtaining another patent relating to fermentation process design and another relating to the use of agro-industrial wastes as substrates for PHA production. Of particular note is Polyferm's experience in the conversion of biomass hydrolyzates (especially those containing pentose sugars) into biodegradable plastics.

Research to upgrade empty food bunches (EFB), a major cellulosic waste product from the palm oil industry, for animal feed by radiation/fermentation treatment was done by Kume (1991). The method includes irradiation, to decontaminate microorganisms in fermentation media, inoculation with useful microorganisms, microbial digestion of cellulose, and production of protein and edible mushrooms. The contents of alpha-cellulose, hemicellulose and lignin in EFB substrate was partly sterilized by irradiation at 10 kGy and inoculated with several microorganism. *Coprimus cinereus* was selected as the most suitable inoculant. The fermentation of EFB with *C. cinereus* was thought as appropriate for animal feed production in Malaysia.

Another form of upgrading wastes includes the extraction and purification of pectin from coffee pulp and coffee pulp juice as researched by Garcia et al. (1991). Pectin from three coffee varieties was extracted and purified and its physical-chemical characteristics determined.

Out of 100 kg coffee crude, 44.84 kg of pulp are the residue. After pressing, 26.9 kg of pressed pulp and 17.94 of juice are separated. After several filtering, washing and drying processes, 109.1 g of pectin are extracted from the pulp and 49.28 g from the juice.

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An organic refinery process to produce fibers and chemicals from lignocellulosic materials has been developed by Tisney Inc. in Alberta. Over the past fifteen years, the company has developed an organic material refinery process which is a highly flexible continuum of processes to dissociate the lignoxylan complex which binds cellulose into a matrix in wood, straw, bagasse and other woody materials. By controlling the level of thermomechanical energy imparted to the matrix, a range of fibres can be produced, from a 97 to 99 percent pure, structurally weak, low DP cellulose fibre, to a sixty percent pure high yield cellulose fibre which is comparable in strength and molecular weight with CTMP.

Between these two extremes is a continuum of fibres having progressively lower strength and yield but with corresponding increased yields in related co-products. There are several major advantages which the Tigney Process enjoys over conventional pulp processes to produce cellulose fibres. These are mostly derived from the fact that no chemicals are involved in the dissociation (cracking) stage of the process and the contact time at high temperature is less than a minute. Several downstream advantages result from these major front end differences between conventional processes and the Tigney processes. The first is that the xylan is not sulphonated, thus the neutral sugars are pure and water soluble. The lignin is carbohydrate free and highly reactive. It is not water soluble. The sugars are easily separable from the lignin by an extraction with water. The lignin can then be extracted by a mild (0.5%) caustic solution. Xylan (a carbohydrate polymer) and lignin (a phenolic aromatic polymer) are nature's second and third most abundant organic materials. There is a wide range of undeveloped but potentially valuable applications for both of these materials.

The Tigney process extracts a third major fraction of woody materials

which consists of oils, fatty acids, lipids, plant steroids etc. This fraction is useful and has market value if cleanly extracted. By extracting this fraction prior to the bleach stage, Tigney is able to produce useful marketable co-products avoiding the toxicity, maintenance problems and end product quality difficulties which occur in conventional processes. Unsulphonated components have a positive market value.

The residue from the above extractions consists of cellulose fibres as they were produced in the dissociation stage of the process. They can be controlled to be high molecular weight relatively intact fibres. At the other end of the process spectrum, they can also be low molecular weight, remarkably pure cellulose suitable for bleaching to a dissolving grade cellulose pulp. Since all of the components of the starting material end up as product, the system reportedly is environmentally benign.

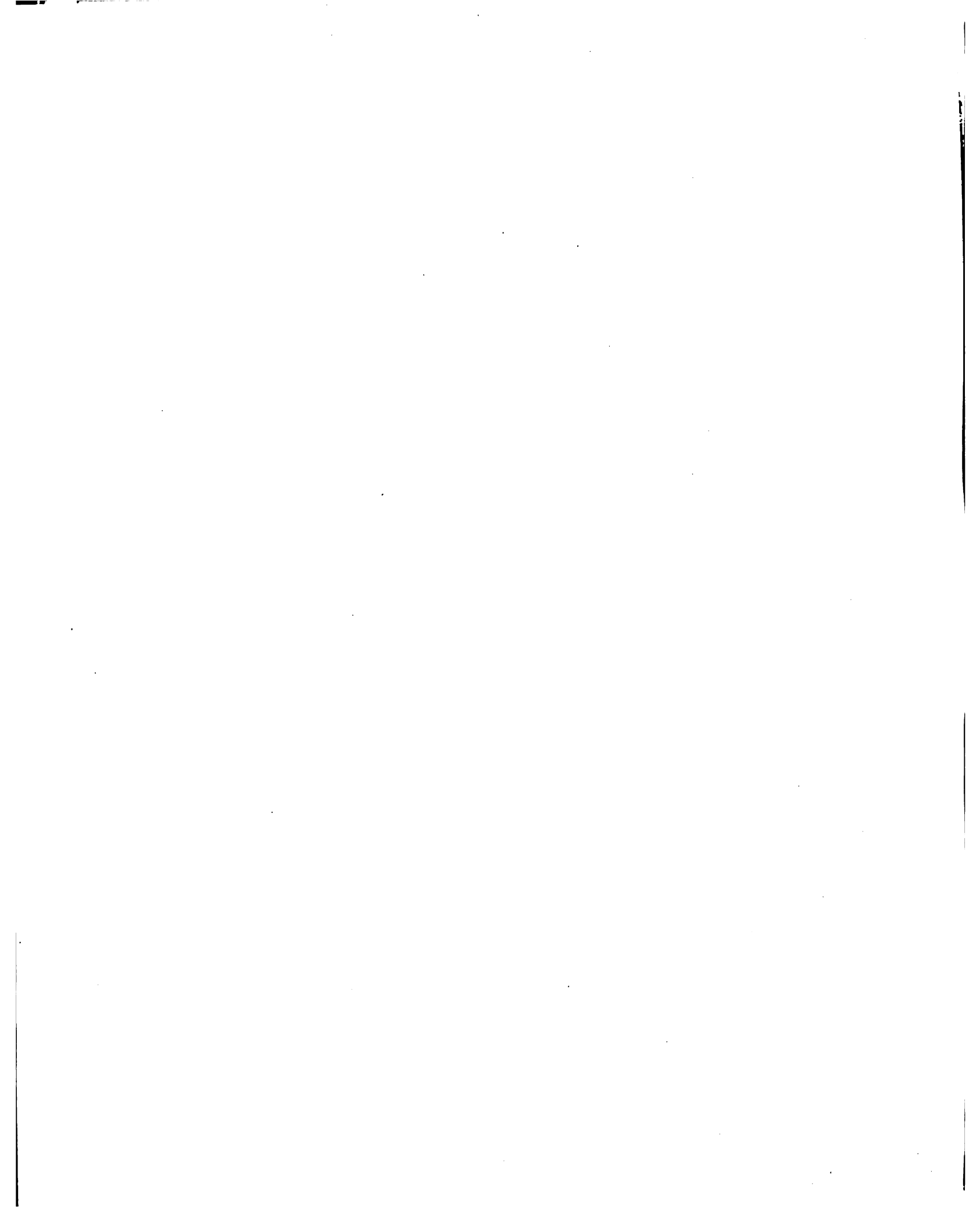
The steam explosion or StakeTech Biomass Conversion (SBC) process developed by the StakeTechnology company described in the next section is also used to produce a large variety of chemicals as shown on the enclosure of the next page.

5. Biotechnical Products

Two biotechnical products were identified. The first, an Iogen cellulase, is a food grade enzyme preparation made by submerged culture fermentation of a selected strain of the fungus *Trichoderma reesei*. The product is a combination of several enzymes that have the ability to partially or completely break down soluble and insoluble cellulosic and hemicellulosics. Further details are described in the next section by its manufacturer, Iogen Corporation.

The second product is a catalytic nutrient supplied by the Atara







IOGEN CELLULASE-100

Description

logen Cellulase-100 is a food grade enzyme preparation made by submerged culture fermentation of a selected strain of the fungus *Trichoderma-reesei*. Cellulase-100 contains a combination of several enzymes that have the ability to degrade the complex carbohydrates that make up plant cell walls. The enzymes can completely or partially break down soluble and insoluble cellulosic and hemicellulosic material found in fruit, vegetables, cereal grains, and wood products. They can consequently reduce viscosities, liberate or solublize insoluble components, or digest oligosaccharides in mixtures containing materials of plant origin.

All the activities required to hydrolyze cellulose are found in Cellulase-100. The preparation contains a balanced mixture of cellobiohydrolase, endoglucanase, and β -glucosidase. These components are highly effective on the β -D-1,4 glucosidic linkage of cellulose, its oligomers, and derivatives. They also have significant activity on β -glucan linkages found in cereal grains, and xylosidic linkages found in plant hemi-cellulose.

Product Specification

Activity:	140 FPU/ml
Form:	Amber liquid
Solubility:	Miscible in H ₂ O
pH:	Typical 4.5
S.G.:	Typical 1.17

logen Cellulase-100 complies with FAO/WHO and FCC recommended specification for food grade enzymes, supplemented with maximum limits of 5×10^4 /g for total viable count, and 10^2 /g for molds. A detailed specification sheet is available on request.

Activity Determination

One FPU of activity is equal to the amount of enzyme required for producing 1 micromole/minute reducing carbohydrate under standard conditions. logen uses the IEA standard analytical method (description available on request.)

Applications

logen Cellulase-100 can be used in a wide range of applications where it is desirable to reduce viscosities, increase extraction yields, liberate or solublize components, or digest oligosaccharides. Cellulases have shown to be of benefit in:

- Animal feed
- Baked goods
- Brewing
- Corn milling
- Fruit processing
- Paper production
- Starch processing
- Textile processing
- Waste treatment
- Wine production

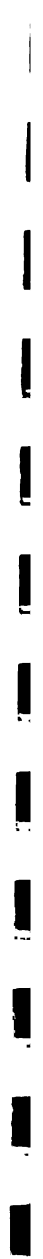
Dosage requirements are highly dependent on the specific substrate, the desired reaction and the processing conditions. Typically, applications requiring soluble gum digestion use as little as 0.02% enzyme (w/w), while complete hydrolysis of insoluble fiber can require as much as 2.0% enzyme (w/w). For initial evaluations, levels of 0.1% to 1.0% on a dry weight basis are recommended. Once the desired effect is established, it may be possible to reduce the dosage substantially.

Handling Precautions

The product is non-flammable and completely miscible with water. As the enzyme can be a mild irritant to skin and eyes, unnecessary contact with the product should be avoided. It is recommended to use protective spectacles and gloves during handling. In case of spillage or accident, rinse by flushing with water. Consult the Material Safety Data Sheet for further information.

Storage

Refrigerated storage is not required. logen Cellulase-100 can be stored and handled at temperatures up to 25°C without detrimental effects. The preparation can handle temperatures up to 50°C temporarily without activity loss. Cooler storage temperatures extend shelf life. Freezing does not harm the product, although if frozen, the enzyme should be fully thawed and mixed before use. Consult logen for further information on product storage in specific conditions and applications.



Corporation. The catalytic nutrient will enhance the reaction that takes place in biological process vessels for the treatment of wastes. From empirical tests to date it is very effective with agricultural, pulp and paper and domestic wastes. Atara are in the process of arranging tests with two or three well known scientific institutions, e.g. the Biological Research Institute of Canada, MacDonald College, Zenon Industries, to confirm the findings. The company is confident that this product will significantly assist in the treatment of agricultural and other organic wastes. In particular, it will also be very effective in conjunction with Atara's process tanks.

III CANADIAN INSTITUTIONS AND COMPANIES WITH BIOTECHNOLOGICAL PROCESSES AND PRODUCTS

A. GOVERNMENT DEPARTMENTS AND RESEARCH ORGANIZATIONS

Our initial efforts concentrated on contacting government departments and national research organizations engaged primarily in the treatment and upgrading of agroindustrial waste. We discovered that their principal efforts were directed towards **effluents or liquids** rather than **solid** waste treatment as explained in the previous section. Many companies have to modify their operations and waste utilization systems in order to meet increasingly stringent effluent environmental regulations.

The following departments and research organizations were contacted:

Industry Science and Technology Canada,
Biotechnology Directorate
Chemicals and Bio-industries Branch
235 Queen Street
Ottawa, Ontario
613-954-1035

Carol Cheffins, Director
John F. Jaworski, Senior Industry Development Officer
Moe Suleiman, Industry Development Officer

This is a new Directorate established after the World Environmental Congress in Rio de Janeiro. Its overall objective is to contribute towards sustainable development, encourage Canadian companies to increase value-added activities through advanced technology, and to encourage technology transfer and joint venture opportunities of mutual interest to licensor and licensees.

The Directorate funded a project of bio-tech transfer opportunities in

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Mexico, Venezuela, Brazil, Argentina and Chile undertaken by Chris Ostrowski formerly of the University of Guelph, Tech Transfer Office and now with TPI Incorporated in Toronto. The study has just been completed. As a project collaborator, Ernani Fiori of the ICA Ottawa office is aware of the project.

A strong interest was expressed by John Jaworski in our project. In the course of a meeting in Ottawa, he allowed us to peruse the 1993 Canadian Biotechnology Companies Directory to identify companies with biotechnology activities relevant to our project.

Agriculture Canada
Research Branch
Sir John Carling Building
930 Carling Avenue
Ottawa, Ontario
613-995-7084

Mark Stumborg, Special Advisor (Ethanol)

Following a lead regarding an ethanol program by Agriculture Canada, I met with Mark Stumborg. Stumborg is part of the overall Energy Mines and Resources' Alternative Energy Program (see EMR details below). His function is to identify and assess cost/benefits of agricultural/agroindustrial waste products as substrates for ethanol production. Since he has just started this activity he has not yet had time to identify and evaluate sources of raw materials.

Energy, Mines and Resources Canada
Alternative Energy Division
CANMET
580 Booth Street - 7th floor
Ottawa, Ontario
613-996-6195

Joe Robert, P.Eng., A/Chief, Bioenergy Group

At the suggestion of Mark Stumborg of Agriculture Canada I met with Joe Robert. As it turned out this \$7 million Alternative Energy Program is not concerned with cellulosic conversion/upgrading but rather with the assessment of alternative energy research and improvement of wood-based resource materials. It is therefore of no interest to this project at this time.

Biotechnological Research Institute (BRI)
National Research Council of Canada
6100 Royalmount Ave.
Montreal, Quebec
H4P 2R2
514-496-6100; 514-496-6181

Dr. B. Guiot, Research Officer

Dr. Guiot was an early contact to inquire about the activities of the Biochemical Engineering Section of the Bio-Engineering Group of the BRI. Although very helpful, he was not in a position to assist us because the emphasis of this group is almost exclusively on effluent treatment and not on solid waste upgrading. He provided us with some additional contacts and also indicated that his group was planning to undertake a joint project with ATARA Consulting Engineering in Montreal on the fermentation technology of solid waste products. Further details of that are described in the section on ATARA.

Institute for Environmental Chemistry
National Research Council of Canada
Montreal Road
Ottawa, Ontario
613-990-6618

Terry Kimmel

Among other activities, this group researches waste production and treatment. In a phone call, we were informed that this activity is primarily deals with pyrolysis.

Pulp and Paper Research Institute of Canada (PAPRICAN)
570 St. John's Boulevard
Pointe Claire, Quebec
H9R 3J9
514-630-4100

Dr. Lubo Jurasek
514-398-6276

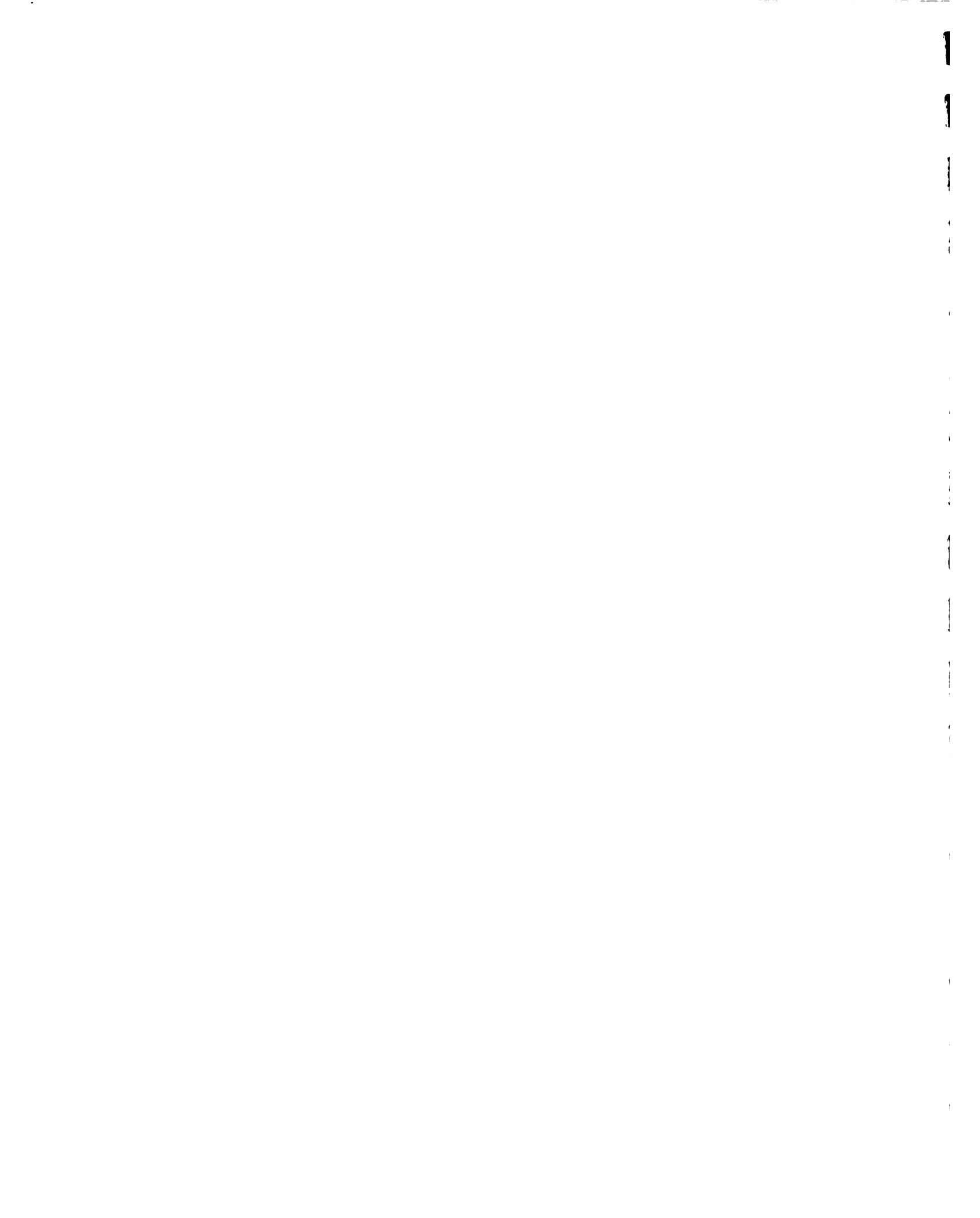
In a telephone conversation, Dr. Jurasek who is probably recognized as an authority in the pulp and paper industry, indicated that their current biotechnology program does not deal with the treatment of solid waste.

For over 60 years, PAPRICAN at Pointe Claire, McGill and Vancouver has been a principal research and development organization of the Canadian pulp and paper industries.

Research within the biotechnology group covers a broad spectrum of applied projects of the modification and degradation of wood polysaccharides and lignins and related activities. Research and development of **solid** waste treatments and systems reported is of increasing interest.

Current research interests include:

- Biological bleaching of kraft pulps with fungus Trametes versicolor.



- Bleaching pre-treatment with xylanase.
- CTMP and kraft bleachery effluent treatments.

Jurasek provided names and leads which were followed up.

Pulp and Paper Research Institute (PAPRICAN)/University of British
Columbia
Vancouver
604-222-3200

Dr. Jack Saddler, Chair for Forest Biotechnology

In a telephone conversation (suggested by Jurasek), Dr. Sadler also indicated that the western section of PAPRICAN does not conduct research in lignocellulosic waste products. He provided additional names and leads which were followed up.

B. BIOTECHNOLOGY INSTITUTIONS AND COMPANIES

1. Institutions and Companies with Relevant Biotechnology Activities

University of Waterloo
Department of Chemical Engineering
Waterloo, Ontario
N2L 3G1
519-888-4006

Prof. M. Moo-Young, PhD, P.Eng., Professor
NSERC Industrial Biotechnology Chair
Yusuf Chisti, PhD, Research Engineer and Adjunct Professor

Technology: The Industrial Biotechnology Chair is one of several industrial research chairs established by the Natural Sciences and Engineering Research Council (NSERC) in co-sponsorship with the university and nine industrial corporations. Further details as to personnel, facilities, expertise and



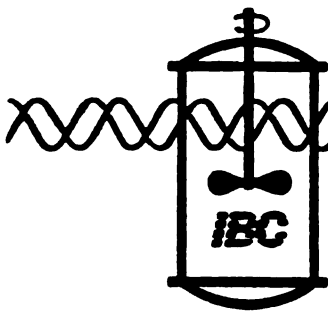
Prof. M. Moo-Young, PhD, PEng
Dept. of Chemical Engineering
University of Waterloo
Waterloo, Ontario
Canada N2L 3G1

Phone (519) 888-4006 Telex 069-55259

Fax (519) 746-4979

- Focus** Biochemical Engineering and Fermentation Technology
- Structure** The Chair is one of several Industrial Research Chairs established at Canadian universities by NSERC (Natural Sciences and Engineering Research Council) in co-sponsorship with industry. This Chair also operates an international UN-sponsored MIRCEN (Microbiological Resource Centre).
- Funding** By NSERC with supplementary support from the University and nine industrial corporations (Alfa-Laval, Apotex, Esso Chemical, Linde/Union Carbide, Pharmacia, Rohm & Haas, Shell, SNC, Vetrepharm). In addition, the Chair receives grants, and fees from consultancies, contract research and workshops.
- Personnel** The infrastructure personnel: the Chair professor, an assistant professor, a research engineer and a technician, supported by a group of about twenty co-workers consisting of research associates, post-doctoral fellows and graduate students. Collaborations with other faculty members significantly increase the overall research activities of the Chair.
- Facilities** Equipment for enzyme biotransformations and for culturing microbial, plant and animal cells (including recombinants and hybridomas). Facilities include a 1,000L flexible fermentation pilot plant and a large-scale chromatography bioseparations unit.
- Expertise** Analysis, design, optimization and scale-up of bioreactor systems and associated downstream processing. Techno-economic evaluations. Special expertise on mass transfer, mixing, shear-sensitive materials, non-Newtonian flows, immobilized biocatalysts, complex biokinetics, process integration.
- Opportunities** Innovations for the healthcare, food, chemical, energy, and waste industries. Examples include: recombinant proteins, agro-chemicals, monoclonal antibodies, morphinan alkaloids, antibiotics, vaccines, single cell proteins, amino acids, ethanol, organic acids, biogas; equipment hardware and software; bioremediation. Inventions, some patented, are available for licensing to commercial enterprises.
- Consultancies** To industrial and government corporations. Previous clients include: Cetus, Kelco, Robinhood, ICI, Unilever, Sulzer, Rhône Poulenc, DuPont, UNIDO, FAO.
- Publications** Books produced by the Chair include: *Comprehensive Biotechnology*, Pergamon (1985); *Waste Treatment*, Pergamon (1982); *Biotechnology and Renewable Energy*, Elsevier (1986); *Microbial Biomass Proteins*, Elsevier (1986); *Bioreactor Immobilized Enzymes and Cells*, Elsevier (1988). The Chair professor is also the executive editor of *Biotechnology Advances*, a quarterly review journal, Pergamon (1981-).

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Prof. M. Moo-Young, PhD, PEng
Dept. of Chemical Engineering
University of Waterloo
Waterloo, Ontario
Canada N2L 3G1

Phone (519) 888-4008 Telex 089-55259

Fax (519) 746-4979

Current Research Activities (W/92)

Healthcare/Medical

- Antibiotics from Fungal Fermentation Processes (Patent Held)
- Monoclonal Antibodies from Immobilized Hybridoma Cell Bioreactors (Patent Pending)
- Morphinan Intermediates (L-DOPA) and Alkaloids by Biomimetic Enzyme Technology
- Extracellular Bioprotein Products from Packed-Bed Yeast Fermentations
- Production of Hormones for the Livestock Industry

Food/Fine Chemicals

- Upgrading Cellulosic Materials into Protein-Rich, Zero-Cholesterol, SCP Products (Patents Held)
- Process Control of Production of Amino Acids
- Natural Flavours and Fragrances by Biotransformation Systems

Bulk Chemicals/Fuels

- Production of Ethanol by Immobilized-Cell Packed-Bed Yeast Bioreactor
- Production of Methane by Two-Stage Anaerobic Digestion
- Methodologies for Production of Industrial Enzymes (Patent Held)
- Upgrading Bitumen and Heavy Oils by Microbial Desulphurization
- (Fermentation Production of Microbial Inoculants)

Environmental/Pollution Control

- Biodegradation of Toxic Chemicals in Contaminated Soils
- Bioconversion of Agricultural Residues into Proteinaceous Animal Feed Ingredients (Patent Held)
- Enhanced BOD Reduction by Activated Sludge Techniques
- Bioleaching and Biosorption of Mine Tailings

Equipment/Instrumentation

- Handling of Viscous Non-Newtonian Fermentation Media (Patents Held)
- Low-Shear Bioreactor Devices for Multiphase Contacting (Patent Held)
- Tubular Bioreactor Devices for Solid-State and Plug-Flow Fermentations (Patent Held)
- Mathematical Modelling and Artificial Intelligence for Bioprocess Control
- Nylon-Based Membrane Systems for Bioseparations
- Scaling-Up Chromatographic Fractionations in Downstream Processing
- Cell Disruption in Novel Annular Bead Mill
- Process Software for Competitive ELISA Technique

opportunities as well as current research activities are enclosed.

According to our research, Dr. Moo-Young and his staff are at the leading edge of SCP research and technology and are internationally recognized for their research and results. Since their research has already been described in the previous section, there is no need to repeat their activities.

Technology Transfer: The enclosed summary from the University of Waterloo Technology Transfer Licensing Office provides further details. In a meeting with E.B. Cross, Associate Director, TTLO, the following tech transfer conditions and agreements pertain to this university:

- Initial discussion and negotiations are usually conducted with the inventor(s) at the various technology centers.
- Subsequent negotiations are held with the TTLO and the inventor.
- TTLO's preferred way would be to licence a technology to an experienced international engineering company rather than to the end-user. The engineering company would have the technical/operational and marketing experience to conduct a feasibility evaluation for a successful commercialization.

Further details are described in the attached brochure.

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PROTEINACEOUS FOOD FROM CELLULOSIC WASTES

DESCRIPTION AND APPLICATION:

Cellulosic materials occur abundantly as agricultural and industrial residues or surplus by-products (straw, stovers, bagasse, cereal brans, wood sawdust, clarifier sludge). A novel aerobic slurry fermentation process has been developed which eliminates the pollution potential by directly bioconverting the solid waste materials (no need for liquefaction pretreatment) into proteinaceous food products suitable for animal nutrition in the livestock industries (cattle, swine, poultry). Product recovery is by simple filtration. Depending on the application requirement, the product consists of about 50-95% DM (dry matter basis) of a known food-grade filamentous microbial organism (*Neurospora* species), the remaining component being unconverted lignocellulose which is left as dietary fibre. The proximate composition of the so-called SCP (single-cell protein) product indicates a nutritious mixture of protein (45%), fats (5%), carbohydrates (45%), rich in vitamin B-complex, zero cholesterol and only 2% nucleic acids. The conversion efficiency is about 50% cellulosic material to SCP product in 5-10 hours depending on feedstock source. The essential amino-acids profile compares favourably with soymeal.

STAGE OF DEVELOPMENT:

The fermentation process has been tested successfully on several cellulosic residues (including cornstover, sugarcane bagasse, kraft paper sludge and wheat bran) in a 75-litre fermentor in batch and continuous operations. Further scale-up testing has been done batchwise in a 1000-litre fermentation pilot plant. Intellectual property includes a low-shear fermentor design, media additives and the generic bioconversion technology.

STATUS OF LICENSE:

Two U.S. patents have been granted and another is pending for the related inventions. At present, a Mexican consortium has an option on licensing for local uses of the technology in the upgrading of sugarcane bagasse to animal feed ingredients. Other countries expressing interest include: India, Phillipines, China, Cuba, France. Certain rights to the commercial use and/or distribution of the technologies are available to investors and manufacturers.

CONTACT:

Mr. E.B. Cross, Associate Director, TTLO





What is the Technology Transfer and Licensing Office?

Formed in 1990, the Technology Transfer and Licensing Office (TTLO) replaced the Waterloo Centre for Process Development and the Commercial Development Office. The mission of the TTLO is:

to facilitate the development and transfer of technology to industry and to generate income for reinvestment in the university research community.

• The University of Waterloo encourages the use of its resources to create intellectual property and to integrate this with scholarly activity. Intellectual property is defined as the material benefits of research and the rights of ownership of such property are vested in the creators unless those rights are qualified by written agreements to the contrary.

• When the university enters agreements where it is expected to license or convey the rights to intellectual property resulting from research activity, such agreements are negotiated with the full understanding and support of the principal investigator.

Technology Transfer and Licensing Office



University of Waterloo
Office of Research

Services provided by the TTLO

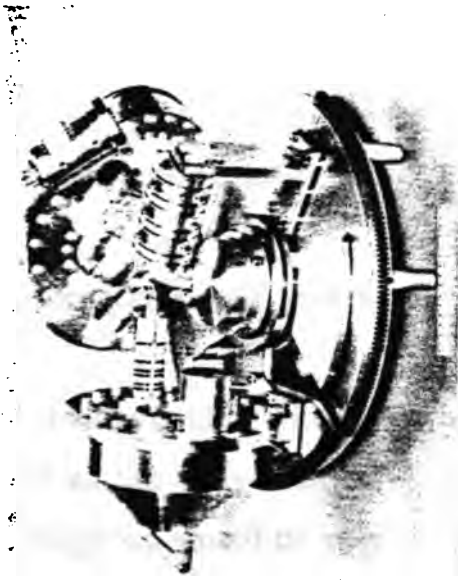
Advice and counsel to faculty members regarding appropriate clauses to be considered to protect the inventor's rights in any intellectual property developed.

Expertise to bring inventions (developed as a result of university research activities) forward for public use and benefit.

Help in identifying potential private or public sector partners for technology transfer.

Funds and guidance to protect and develop intellectual property so that organizations equipped with the necessary resources can bring the technology to its full commercial potential. Including:

- developing an action plan in co-operation with the principal researchers to perform: preliminary technology assessment/feasibility; patent search; market survey; competitive analysis; economic analysis and overall commercial feasibility.
- identifying complementary areas of research or technologies where joint development projects may accelerate the commercialization process.
- conducting market studies (which can be in depth studies by outside consultants when appropriate) to evaluate the best route to achieve successful technology transfer.
- facilitating the production of a proposal to enable the inventor to obtain funding for the development of additional feasibility models/prototypes and commercialization plans.



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University of Sherbrooke
The Research Group Conversion Technologies
Department of Chemical Engineering
Faculty of Applied Sciences
Sherbrooke, Quebec
J1K 2R1
819-821-7170

Dr. Estaban Chornet, Professor of Chemical Engineering

Technology: This group has an ongoing major program in biomass conversion. Two directions are followed:

- pretreatment/fractionation via steam/aqueous and organosolv technologies;
- gasification of residual biomass aiming at clean syngas to be used as fuel for turbine/engines or as synthesis gas.

The group has ongoing collaborative research programs with several other universities.

In a discussion, Dr. Chornet indicated research activities dealing with the production of paper, adhesives and chemicals as well as the conversion of hemicellulosics, lignins and cellulosic fibres. He and his group would be very interested to collaborate with interested companies in the upgrading of lignocellulosic wastes.

His group has a demonstration unit in Spain and is planning to build a pilot plant in Brazil where the go ahead will depend on the political situation. Incidentally, Dr. Chornet's lab has purchased and is using a steam explosion unit from StakeTech in Norval (see StakeTech below). Further details about the Group's activities and research are shown in the enclosed summaries.



THE RESEARCH GROUP ON CONVERSION TECHNOLOGIES
 DEPARTMENT OF CHEMICAL ENGINEERING
 FACULTY OF APPLIED SCIENCES
 UNIVERSITÉ DE SHERBROOKE (UdeS)
 SHERBROOKE, QUÉBEC, CANADA, J1K 2R1
 Tél.: (819) 821-7170; Fax: (819) 821-7955

**Group
Profile:**

The group is recognized, within the UdeS, as a unit devoted to innovative research and development as well as training of graduate students in the area of conversion technologies and processes applied to energy and chemicals from bioresources and wastes.

The group's composition is as follows:

- * senior researcher: E. Chornet, professor of Chemical Engineering
- * assistant professors: P.F. Vidal and M. Heitz
- * adjunct professors: R.P. Overend and N. Abatzoglou
- * research coordinator: P. Jollez
- * head, process laboratory: J.P. Lemonnier
- * head, analytical laboratory: M. Trotter
- * additional staff: Five professionals, four technicians and one secretary
- * graduate students: eight to ten students conduct their graduate thesis within the group
- * postdoctoral fellows: the group hosts, regularly, one PDF or invited researcher

The group has ongoing collaborative research programs with the Universitat Rovira i Virgili (Tarragona, Spain); Universitat Politècnica de Catalunya (Barcelona, Spain); Institut National Polytechnique (Toulouse, France); and National Technical University (Athens, Greece). Within the UdeS the group has on-going collaborative programs with researchers in biochemistry and microbiology.

The group has been named, in 1992, the "Thermochemical Conversion Module" of the Centre québécois de valorisation de la biomasse (C.Q.V.B), a state-owned corporation created to stimulate new biomass-based joint ventures with the industrial sector in the province of Quebec.

Research

Orientations: The group has an ongoing major program in biomass conversion. Two main directions are followed:

- pretreatment/fractionation via steam/aqueous and organosolv technologies;
- gasification of residual biomass aiming at clean syngas to be used as fuel for turbines/engines or as synthesis gas.

Scientifically, the group has based its strategy on a comprehensive approach involving thermo-mechano-chemistry, kinetics of non-homogeneous systems, acid-base catalysis and reaction engineering of two and three phase systems.

Technologically, the group has developed an expertise in high shear reactors (jet reactors), rapid mixing regimes, thermomechanical processes (which include the family of steam explosion related technologies) and fluidized bed reactors (which include gas filtration and reforming).

The product-targetted strategies include a variety of projects on: (a) the conversion of hemicelluloses into hydroxyacids, diacids and ester derivatives as well as polyols; (b) the conversion of lignins into oxyaromatics (aldehydes, acids and ethers); (c) cellulosic fibers and special celluloses; (d) chitosan polymers; (e) peat-derived products; (f) conversion of complex polysaccharides (including cellulose) into fermentable sugars.

Recently, the group is applying its know-how in reaction and reactor technology as well as in separation processes to environmentally-driven projects: (i) the destruction of toxicity in industrial wastewaters via a novel rapid wet oxidation approach, the Oxyjet process; (ii) the conversion of hazardous wastes into clean gas via gasification/reforming; (iii) the upgrading of heavy oil residues.

Facilities: The group has a wide spectrum of facilities including both laboratory-type equipment and specially designed units developed as a consequence of the group's technological thrust.

The process laboratory is equipped with:

- standard autoclaves (magnetically-driven)
- rapidly heated bubbling microautoclaves
- cascade reactors
- catalytic flow reactor (fixed bed)
- process development units:
 - mini-vapour-cracker (an improved configuration of the classical "steam explosion gun" capable of treating between 300 and 600 g of chips, shavings or finely divided material);
 - tubular reactor unit capable of treating medium consistency suspensions up to 0.5 L/min. The unit is equipped with homogenizing valves and rapid heat injectors. This unit can operate in plug flow regimes;
 - two jet reactor units for specific two-phase (G/L) reacting systems.
- pilot units:
 - a 50 kg/h fluidized bed atmospheric gasification reactor including ancillary equipment: feeding system; gas filters and isokinetic sampling. A newly designed reformer will be added during the Fall 1992.
 - a 4 tonne/h STAKE II feeder coupled with a digester. The ensemble is used as a demonstration unit for steam explosion projects.

The analytical laboratory is equipped with state-of-the-art instrumentation including GC, GC/MS, HPLC, GPC, FTIR, TGA/DTA/DSC, viscosimetry, VPO, microtox, and elemental analyzer. Access to specialized equipment such as OM, SEM, TEM, XRD, XRES, and ^{13}C nmr, is warranted through internal arrangements within the university.

Funding Profile:

Projects of fundamental nature, which normally include the graduate thesis, are sponsored by federal (NSERC), provincial (FCAR) and internal university grants.

Projects having an applied nature, either precompetitive or of immediate interest of industry are carried out contractually. The group has a long standing solid record of performance in contract research.



One third of the budget of the group is covered by grants and the remaining two thirds are derived from external contracts.

Publications: The group publishes regularly in internationally recognized peer-review journals. As well, members of the group participate to major conferences and seminars where the diversified spectrum of activities of the group needs to be known.

Proprietary know-how: The group has a policy of protection of know-how under the umbrella of the office of research of the university. Some of this know-how is protected by patents. In any contract the ownership of results and access to possible prior know-how of the group is negotiated between the parties involved and the university.

Technology Transfer: The technology transfer from the university research and its patents is handled by the Centre québécois de valorisation de la biomasse (C.Q.V.B.). This is a state owned corporation created to stimulate new biomass based joint ventures with the industrial section in the Province of Quebec. Details on tech transfer and potential joint ventures with companies outside of the province have not been identified at this point.

StakeTech Technologies Ltd.
2836 Highway 7
Norval, Ontario
L0P 1K0
416-455-1990

W.J. (Bill) Esdale, Ph.D.
Executive Vice-President

Technology: This company has been in existence since the late 1970's. It initiated the steam explosion process with the primary purpose of breaking down the lignocellulosic waste products of coniferous and deciduous cellulose to cattle feed. For a number of reasons several feed lots which used the patented StakeTech Biomass Conversion (SBC) process have discontinued the operations due to economic and climatic reasons beyond the control of StakeTech. However, the company has expanded its activities to the production of numerous chemicals from lignocellulosics as described in the previous section.

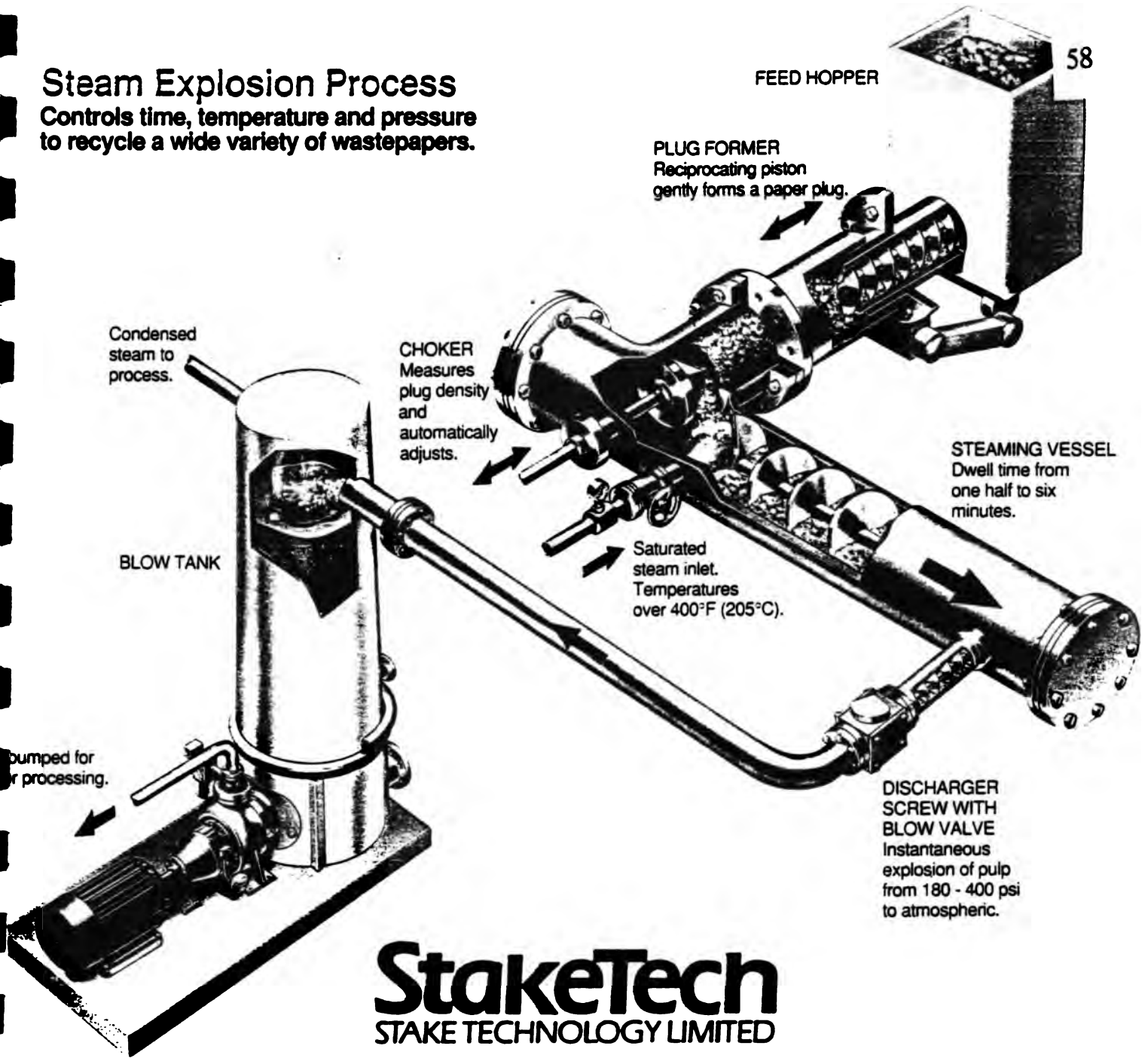
Using its technology, the company is currently involved in the following projects:

- a paper recycling project in California



Steam Explosion Process

Controls time, temperature and pressure to recycle a wide variety of wastepapers.



StakeTech

STAKE TECHNOLOGY LIMITED

Performance Capabilities

- Continuous operation reduces downtime, increases production, lowers operating cost.
- Proven Steam Explosion Technology makes use of previously unacceptable furnishes.
- Recycles a wide range of waste papers and mixes; disperses wax, latex and hard to handle inks and varnishes.
- Environmentally friendly, environmentally sound.
- Treats plastic, hot melts, poly coatings, wet strength materials.

User Benefits

- Produces high quality contaminant-free pulp at the lowest cost.
- Maximum system flexibility.
- Reduces consumption of chemicals, resulting in lower wastewater treatment costs.
- Allows paper mills to use more recycled pulp in their production processes.
- Lower energy, maintenance requirements.



Ontario

Ministry
of
Energy

Project Summary

Fuel ethanol from waste paper shows promise

Every year, Ontario's municipal waste stream receives an estimated 3.2 million tonnes of mixed waste paper, including office and ledger paper, paperboard and magazines. Over the same one-year period, Ontario drivers consume some 12.7 billion litres of gasoline. These two apparently unrelated statistics recently became the focus for an investigation by two enterprising Ontario companies that decided to examine the potential for turning them into profits.

Stake Technology Ltd. of Norval and Bio-hol Developments of Downsview joined forces to investigate the technical and economical feasibility of producing fuel ethanol, a gasoline additive, using some of the millions of tonnes of waste paper Ontarians

discard every year. Each company received a grant of about \$98,000 from the Ontario Ministry of Energy's EnerSearch Program to assist them to carry out a coordinated research project.

Each company took a different technical route to meeting the challenges posed by the project, using two different proven technologies for producing ethanol. But the two firms cooperated closely on each phase of the project.

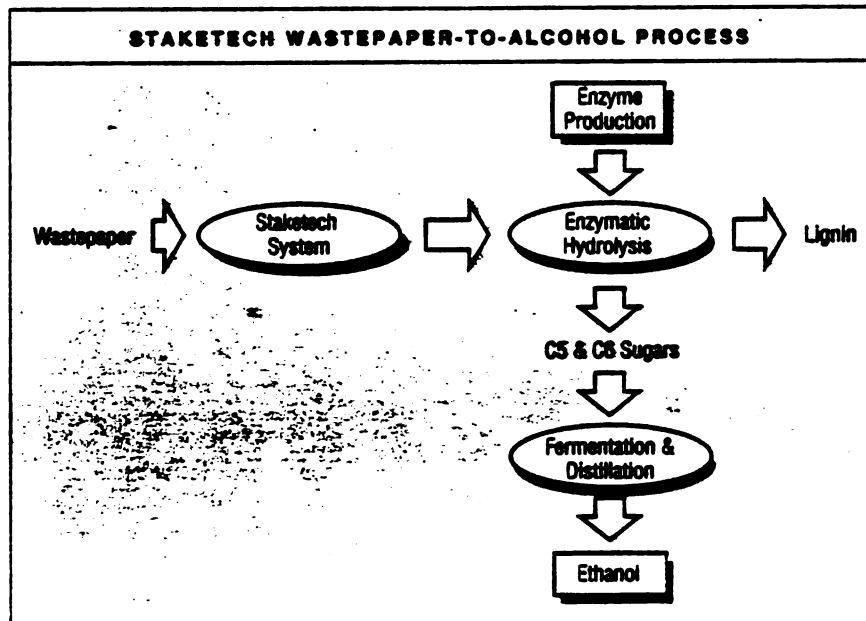
The results of the project demonstrated that converting waste paper to ethanol – using the technology developed for converting wood waste to ethanol – has the potential to become commercially viable. What's more, the project proved that large quantities of fuel

ethanol could be produced from waste paper and waste paper products.

The project's success has led to the formation of a consortium of private companies that intends to exploit the technology. The consortium is currently studying the feasibility of building a 50-million-litre-per-year ethanol plant near Kincardine, Ontario, that would be the first plant in the world to use paperboard waste as the raw material for ethanol.

HIGHLIGHTS

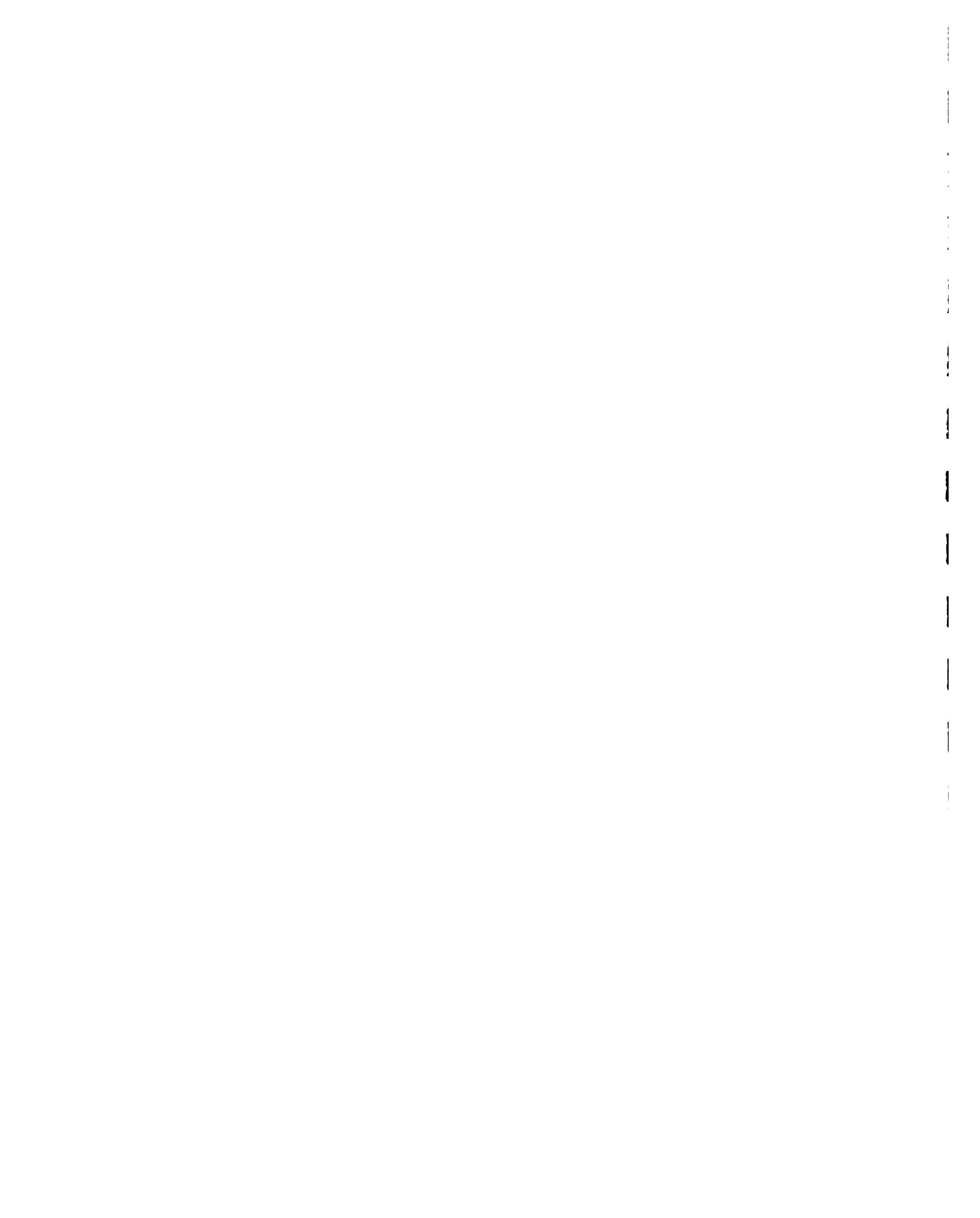
- ↳ New process yields 350 litres of ethanol per tonne of mixed paper.
- ↳ Project demonstrates that converting waste paper to fuel ethanol can be commercially viable, depending on the price of waste paper.
- ↳ Process can also address important environmental and energy concerns.
- ↳ As an additive in gasoline, fuel ethanol made from waste paper offers a clean-burning, renewable, indigenous source of energy that could displace up to 1.2 billion litres of gasoline used in Ontario each year.



- a joint recycling project with Black Clawson to steam explode recycling paper which shatters the ink particles and thus saves further processing, time and costs.
- a plant in Medicine Hat, Alberta, for tissue production
- a plant in Finland to steam explode birch chips for the production of xylol and cattle feed
- a plant in Soustons, France to produce gasohol
- a project at the Polytechnic University in Virginia
- a plant for ENEA in Italy (Italy's largest chemical concern)
- collaboration at the University of Sherbrooke for the development and research of new products
- Feasibility project to produce fuel ethanol from waste paper at the Bruce Energy Park near Kincardine, Ontario. A consortium of several companies and ministries have been established. A feasibility study is currently underway for Phase I. Dr. Esdale indicated that Phase II would include the evaluation of ethanol from bagasse, and Phase III would evaluate the production of ethanol from **lignocellulosic waste** products.

Details of the steam explosion process are enclosed. It should be noted that the operating temperature is 450°F with pressures up to 400 psi. The process time varies between 1-6 minutes.

Technology Transfer: Dr. Esdale indicated that StakeTech already has licensing agreements and joint ventures with other companies and is very interested to look at any opportunities to expand the tech transfer with new companies in the LAC region. The company does not have a standard tech



transfer contract. Conditions depend on the potential partner, technology, extent of interest for joint venture, joint capital and development activities etc.

Atara Corporation
9700 Henri Bourassa W.
Ville St. Laurent, Quebec
H4S 1R5
514-331-8332

Michael Ogilvie, President
Declan Murphy, Vice-President & General Manager
Doug Galbraith, Vice-President Marketing & Product Div.

Technology: Atara is a leading consulting engineering company with substantial experience in biodegradation and fermentation processes.

The company has developed a new bioreactor with significant improvements for the degradation of organic wastes in aerobic and anaerobic fermentation/digestion processes.

The design of these vessels is in the process of patent application to be filed before the end of March 1993. They will be very efficient not only in their treatment capabilities but also as separators of liquids and solids. They will enable high rate processes to be achieved without the secondary problems that are normally encountered in the operation of such processes.

The company is also establishing a joint venture with a Californian supplier of a catalytic nutrient which will greatly enhance the fermentation for waste processes. Details have been described in the previous section.

According to Dr. S. Guiot, the Biochemical Engineering Section of the Biotechnological Research Institute in Montreal plans to establish a joint research project with Atara co-funded by the National Research Council.



Technology Transfer: Mr. Ogilvie indicated that in view of the development and research work for both the new bioreactor and the supply of the catalytic nutrient, it would be premature to discuss details of tech transfer, commercialization and joint venture. He indicated, however, that Atara would, at the proper time, be interested in pursuing joint venture or licensing discussions with responsible companies in Latin America. He feels that it will not be feasible for Atara to develop all markets themselves and they will be seeking compatible associates or partners with significant experience in the field.

Domtar Research Centre
P.O. Box 300
Senneville Road
Senneville, Quebec
H3R 1L6

Robert Eamer, Ph.D., Director of Research
Amy Lo, Ph.D., Researcher

Technology: Domtar is one of Canada's largest pulp and paper companies with several mills in Canada.

Dr. Eamer indicated that the current R&D is primarily focused on applications of effluent treatment to meet environmental regulations. More recent R&D involves the conversion of waste to agricultural materials as well as to fuels (anaerobic processes).

Dr. Eamer is open to discuss any direct and applied R&D for the upgrading of solid cellulosic waste materials which might be of mutual benefit to interested third party companies in the LAC region and Domtar.

In a subsequent telephone discussion suggested by Dr. Eamer, Dr. Amy Lo further confirmed Domtar Research Centre's plans to look into biosolids



and waste fibre utilization and upgrading.

Discussing various aspects of SCP, Dr. Lo indicated that researchers generally use pure or sterile substrates of agricultural cellulosic waste materials. Industrial waste whether effluent, sludge or solid, is more like garbage including fillers, plastics, inking, solids, etc. To her knowledge, no research has been undertaken to date to establish if mixed colonies of microorganisms are able to convert a mixture of lignocellulosic garbage waste to SCP and other products or if such waste should first be separated for subsequent biotechnological upgrading of selected components.

Abitibi Price Inc.
Sheridan Research Park
Mississauga, Ontario
416-822-4770

Dr. Phil Whiting

Technologies: Dr. Whiting indicated that solids will become less available because Abitibi Price is working on a new process to retrieve and return "fines" (or fine fibres) to the paper making process. He feels that hydrolysis of lignocellulosics is too expensive for conversion.

Abitibi is working on a new technology with results expected in about six months. He did not go into details.

Technology Transfer: He mentioned that it was premature at this time to discuss tech transfer.



Ortech International
2395 Speakman Drive
Mississauga, Ontario
L5K 1B3
416-822-4111

John Christison, Ph.D.,
Food, Pharmaceutical and Consumer Products

Technology: Ortech developed on a pilot scale the Hyperferm process, an integrated fermented/purifier based on the simultaneous removal of volatile product by vapour phase stripping to maintain a continuous fermentation to produce ethanol with a product concentrate containing 14.4% (w/v) productivity. The process has been maintained at a relatively low 41°C. for extended periods. The substrates are corn syrup, glucose, or maltose. It should be noted however, according to Christison the major goal is to produce these alcohols directly from **lignocellulosic materials** and he feels that this process could be easily developed for cellulose conversion technology.

Consolidated Thermotech International Inc.
#203-1120 Austin Avenue
Coquitlam, B.C.
V3K 3P5
604-937-5274

Seann Maxwell, Vice-President
206-868-2549 (Seattle)

Technology: The company utilizes a patented thermophilic bacterial fermentation process for the digestion and pasteurization of biodegradable wastes. The Thermo Master digester is an engineered and carefully controlled environment in which the bacteria thrive and efficiently and cost effectively digest organic waste turning it into products such as SCP and animal feed.



The technology could readily convert sugar cane bagasse, coffee pulp and banana peels to high grade animal feeds. It could probably digest palm oil and perhaps the pulp and paper wastes. In these latter two cases, the company would be pleased to conduct digestion trials on these materials if they could be supplied to them. Details are shown on the attached sheet.

The company has been very active as demonstrated by the following activities:

License Agreements:

- Delta Bio Recycling, Delta, B.C.
- Calgary Bio Recycling
- Alberta Bio Recycling, Edmonton
- Edmonton Northlands, Edmonton (negotiation)

Joint Ventures:

- Burlington Bio Recycling Corp.
- Several other negotiations underway.

The company also has contractual relationships with other biotechnology and consulting engineering companies.

Technology Transfer: Thermo Tech is definitely willing to enter a technology transfer arrangement whereby the company would supply the technology, a turn-key plant, plant commissioning and start-up, operational and management training and even, take back a contract to market the end products. Depending on the country, the company would be prepared to consider an investment in the plant and will wherever possible assist in raising financing. The company would also provide management supervision to ensure proper plant operations and product quality.



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The benefits of a Thermo Master Plant

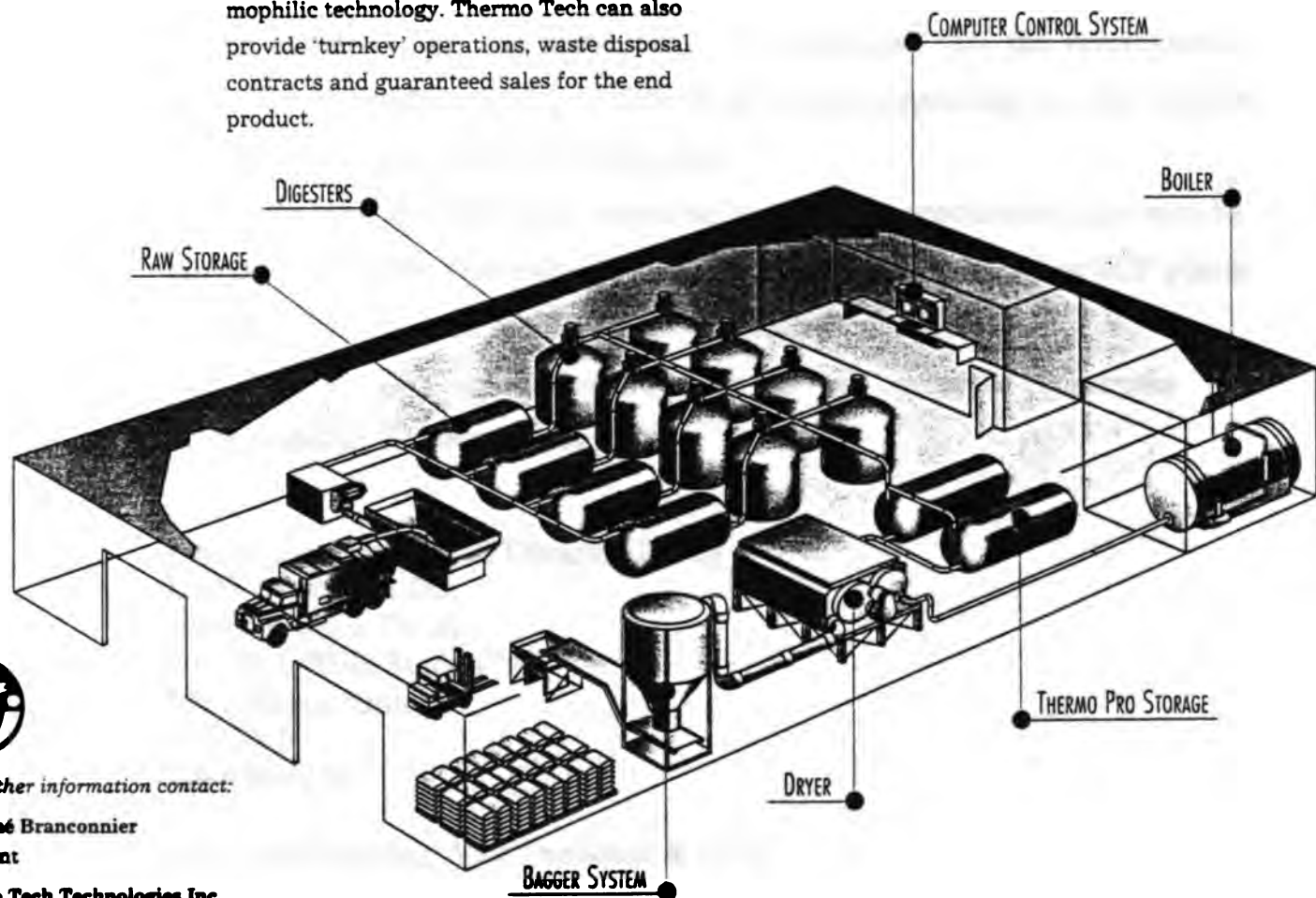
- A proven patented process
- One of the only viable alternatives to landfill disposal
- A proven and more economical solution for waste disposal
- Recycles misplaced resources into Thermo Pro products
- Revenue from two sources, substantial tipping fees and guaranteed end product sales
- Central plant has a guaranteed supply of material
- Low construction, operating and maintenance costs
- Plant is cost-effective with a short pay-back
- Low capital investment

The opportunity

Thermo Tech will enter into joint ventures through the licensing of the patented thermophilic technology. Thermo Tech can also provide 'turnkey' operations, waste disposal contracts and guaranteed sales for the end product.

Thermo Tech Technologies Inc. a Public Company, Trading Symbol "TTR.V" operating through its two subsidiaries: Thermo Tech Waste Systems Inc. and Thermo Tech Bio-Recycling Corp.

Pollution is the problem of the '90s. Recycling, reducing and reusing is the business of the '90s.



For further information contact:

René Branconnier
President

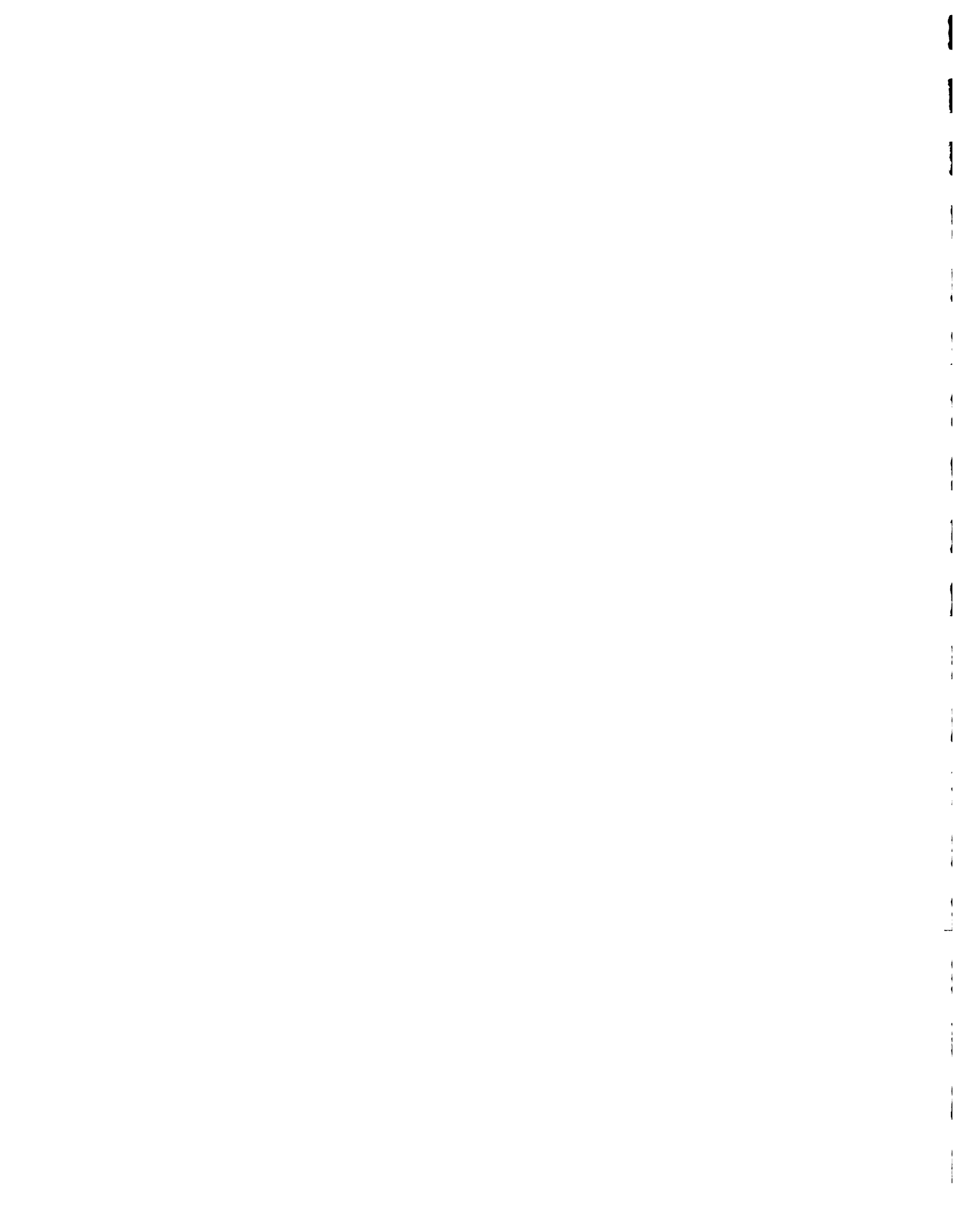
Thermo Tech Technologies Inc.

Suite 203, 1120 Austin Avenue
Vancouver, B.C. Canada.

V6K 3P5.

tel: (604) 937-3022

fax: (604) 937-3274



ICI Bio Products
Sheridan Park Research Centre
2101 Hadwen Road
Mississauga, Ontario
L5K 2L3
416-823-7160

David J. Gannon, Ph.D., Manager, North American Technical Centre

Technology: ICI is not doing much work on solid state fermentation or SCP technology.

He feels that enzymatic processes are less expensive than others for bleaching and dissolving lignocellulose lignin for lignocellulose products for xylane in hardwoods and softwoods. He mentioned that the fermentation process and product quality is difficult to control depending on the process characteristics and quality of substrates.

He feels that SCP from methanol due to high production costs may be uneconomic via the fermentation process. He also mentioned that SCP plants in Russia had to be closed due to serious water pollution.

ICI is also conducting research to use cellulose substrates for biodegradable film manufacture.

United Cooperatives of Ontario (UCO)
UCO Petroleum Inc.
5600 Cancross Court
P.O. Box 7030, Station "A"
Mississauga, Ontario
L5B 2N6
416-890-4250

Glen Perschbacher, Vice President & G.M.

Technology: UCO, the largest farm supply cooperative in Ontario, and Sunoco Inc. formed a joint venture company called UCO Petroleum Inc. to produce and market ethanol-blended gasolines in Ontario which started in May of 1992.

Ethanol is produced from corn by Commercial Alcohols Inc. in Tiverton near Kincardine. The project has the full support of the Ontario Corn Growers Association.

The ethanol is blended with gasoline from the Sunoco Sarnia refinery in Sarnia and then shipped to 21 Gas Bar outlets in southern Ontario by UCO Petroleum Inc.

Temeco Enterprises Inc.
P.O. Box 3000
Temiscaming, Quebec
J0Z 3R0

Mr. Gerry Grewe, Manager R&D

Technology: This company was selected since its profile indicated "conversion of cellulose waste to ethanol". The discussion with Gerry Grewe, who is in charge of managing, marketing and R&D activities, was challenging and frank.

The company operates a high purity ethanol plant started in 1990. It produces 18 M/l of 95% pharmaceutical grade ethanol by a process based on fermentation of spent sulfite liquor from adjoining pulp mills.

According to Mr. Grewe, the conversion of cellulosic waste into industrial or fuel grade ethanol would neither be economically viable nor environmentally acceptable. In laboratory experiments it appears to be feasible, but not in pilot plants or commercial operations. The lowest production costs of industrial or fuel grade ethanol is 45-50 cents per litre compared with the gasoline costs before taxes of 20 cents per litre. Even

using cellulosic waste in Central and South American countries as a raw material at almost no cost would not reduce the ethanol production cost significantly because environmental compliance costs may be high. Thus, a heavy subsidy would still be required.

Crewe indicated that Temeco has plans to look into the conversion of cellulose waste via the Pentos conversion process in the near future. The company plans to erect a pilot operation at \$1-2 million and if successful may follow-up with a commercial feasibility plant of \$5-6 million.

Technology Transfer: Since a production of high purity ethanol is beyond the scope of this project and since the plans of cellulose waste conversion are in the near future, it was premature to discuss tech transfer details.

McGill University
Department of Chemistry
Pulp and Paper Building
3420 University Street
Montreal, P.Q.
514-398-6276

R.H. Marchessault, NSERC-Xerox Professor

Dr. Marchessault was advised by Paprican of our project and was kind enough to send a paper on the production of biodegradable thermoplastic material via fermentation technology. This material can be made from carbohydrate waste from wood specifically from glucose or xylose. Further details of this process are described in the previous section. (See: Polyferm Inc. below).

Technology Transfer: Since it appears to us that this is a sophisticated technology and may required years of refinement we have not discussed tech transfer.

Polyferm Inc.
1044 Woodside Drive
Dollard-des-Ormeaux
Quebec H9B 1P9
514-340-4525

Juliana Akit Ramsay, Ph.D., President
Bruce A. Ramsay, Ph.D., Vice President

Technology: This husband and wife team developed the production of biodegradable plastics from lignocellulosic materials. Details are described in the previous section

Dr. Bruce Ramsay is also Director of Biopro Projects of the Ecole Polytechnique which is on the campus of the University of Montreal in Montreal.

Technology Transfer: We believe that in view of the sophistication and preliminary research stage of technology, it is premature to discuss tech transfer.

Mevex Corporation
P.O. Box 1778
108 Willowlea Road
West Carleton Industrial park
Stittsville, Ontario
K2S 1B4
613-831-2664

Mike de Wilton, Co-founder and Co-owner
Peter Brown, Co-founder and Co-owner

Technology: This company has been contacted in relation to the irradiation of empty fruit bunches (EMB) of the palm oil industry (see previous chapter).

MEVEX designs, builds, sells and services industrial electron linear accelerators. It also operates a pilot electron beam facility enabling potential uses of electron beam techniques to experiment and develop processes to suit their needs.

The MEVEX equipment model 10/20 has a 10 Mev electron beam energy at 20 kW of average beam power. These accelerators are cost effective, reliable, compact, safe and provide adjustable energy and power and either horizontal or vertical beam delivery. Since they use electricity (only when in operation) they are more cost effective than gamma irradiators.

Technology Transfer: This sophisticated and proprietary technology does not lend itself to licensing or technology transfer.

**Tigney Technology Incorporated
439 - 22560 Wye Road
Sherwood Park, Alberta
T0A 4T6
403-464-2960**

Ted De Long

This company was contacted at the suggestion of Prof. Marchessault of McGill University's Department of Chemistry.

Mr. De Long indicated that Tigney is committed to the Biofocus Corporation Ltd. for transfer of their technology to the Third World. This is a foundation sponsored by the World Academy of Art and Science (WAAS).

The objectives of the Biofocus Foundation are to:

- **Develop local resources and markets.**
- **Promote commercial ventures supported by scientific, technical, market and business management expertise.**
- **Identify target areas, local entrepreneurs and commercial business opportunities for biotechnology and genetic engineering.**
- **Assist in business plan preparation and presentation to potential investors.**

The address and contact person are:

**Biofocus Foundation
The Old Observatory
S-11360 Stockholm
Sweden
Tel: 468-30-49-30
Fax: 468-31-46-20
Dr. Carl-Goran Heden, V.P.
(immediate past President of WAAS)**

2. Suppliers of Products

IOGEN Corporation
400 Hunt Club Road
Ottawa, Ontario
K1G 3N3
613-733-9830

Kevin J. Foody, Cellulase Product Manager

IOGEN reportedly uses a steam explosion process to produce enzymes for sale.

One of the enzymes is Iogen Cellulase 100 which is a food grade enzyme preparation. Details are shown on the enclosed sheet.

The company holds patents and proprietary technology relating to thermal, chemical, and biochemical fibre processing and has become a leading manufacturer and marketer of biochemically derived catalysts used for fibre modification.

My offer to meet with Mr. Foody while in Ottawa was not accepted.

According to one trade source, IOGEN was quoted as having the cheapest and most effective enzymes.



Atara Corporation
9700 Henri Bourassa W.
Ville St. Laurent, Quebec
H4S 1R5
514-331-8332

Michael Ogilvie, President
Declan Murphy, Vice-President & General Manager
Doug Galbraith, Vice-President Marketing & Product Div.

Technology: Atara is a leading consulting engineering company with substantial experience in biodegradation and fermentation processes.

The company supplies a catalytic nutrient which enhances reactions in biological process vessels for the treatment of wastes. Further details are described in the previous section.

3. Companies With Support Systems

Three companies were identified to provide control process, support systems and system design as follows:

BioExpert Inc.
3075 Hamel Blvd., Suite #227
Quebec City, Quebec
G1P 4C6
418-877-5711

Mr. Alain Marchand, President

The company has experience in developing biotechnology control processing instrumentation. The company integrates computer science, electronic devices and biochemistry knowledge to design instrumentation.



Polydex Biologicals
415 Comstock Road
Scarborough, Ontario
M1L 2H4
416-755-2231

Mr. George Usher, President

The company provides chromatography support materials (HPLC) for the preparation, purification and analysis of biologically active molecules.

Stanley Industrial Consultants Ltd.
10160 112th Street
Edmonton, Alberta
T5K 2L6
403-423-4777

B.E. Novak, President

The company is part of the Stanley Technology Group, a Canadian group of 1100 employees. It provides multidisciplinary services and research in waste treatment for forest, food and other industries. The company does the system design work for Consolidated Thermotech International in Vancouver (see above).

4. Other Companies

Several other companies that were suggested by various people in the waste utilization field or whose profiles from the 1993 Biotechnology Directory indicated that they had technologies or products relating to the upgrading of solid waste, were contacted. As it turned out, neither their technologies nor their products had a direct relationship with the objectives of this project. The names of the companies and their activities are nevertheless added in case some spinoff needs might develop for which their technologies might be applicable.

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**Forintek Canada Corp.
800 Montreal Road
Ottawa, Ontario
K1G 3Z5
613- 744-0963**

Brenda McAfee

Forintek is a private, non-profit, wood products research organization engaged in R&D for private industry and governments.

In the past, Forintek was involved in lignocellulosic and steam explosion processes but it appears that their objective has been changed to biotechnology for wood preservation, biopesticides for wood products and development of new uses of wood products for biomass energy.

Current projects include an activity as curator of a fungi collection; a biocontrol project; and research on the control of staining fungi which degrade and discolour lumber.

**SNC Group Inc.
2 Place Felix-Martin
Montreal, Quebec
H2Z 1Z3
514-866-1000**

Bechera Safi

SNC, the largest consulting engineering company in Canada, was contacted at the suggestion of Mr. Jaworski of the ISTC Biotechnology Directorate.

Although the company offers technologies in anaerobic bioreactors for waste treatment and conversion, current research activities are concentrating on pyrolysis, sludge conversion from municipal waste water, and surfactants for asphalt.

ADI Group Inc.
P.O. Box 44, Station "A"
1133 Regent Street, Suite 407
Fredericton, New Brunswick
E3B 4Y2
506-452-9000

L.A. Pearson, V.P. International Affairs

ADI was contacted at the suggestion of Dr. Guiot of the Biotechnology Research Institute since they are specializing in anaerobic processes.

Information received, however, applies almost exclusively to waste water treatment in which ADI seems to have a considerable technology and has built numerous plants in Canada and other countries. The company has developed a patented ADI-BVX system which is the least costly of seven comparative anaerobic technologies developed by ADI.

Atlantic Microbiology Ltd.
Oak Point Research Centre
R.R. #1
Hampstead, N.B.
E0G 1Y0
506-425-2569

Lucy Wilson

Since the profile of this company indicated anaerobic treatment of cellulosic waste we contacted the company and were informed by Ms. Lucy Wilson that the cellulosic waste included bark and sawdust.



**Canola Council of Canada
Room 301 - 433 Main Street
Winnipeg, Manitoba
R3B 1B3
204-982-2100**

Francois Catellier

The Canola Council was contacted to find out about the Council's goals or processes concerning the treatment of waste products from the canola seeds.

Catellier mentioned that the hulls and residue of the canola seeds are transformed into a canola meal (or cake) which is a high quality livestock ration with 36% protein. Current research is concentrated on dehulling or reducing the cellulose of the canola seed hulls.



IV CONCLUSIONS AND RECOMMENDATIONS

A. CONCLUSIONS

1. Technical, Economic and Market Considerations

This relatively brief identification and assessment of industrial biotechnological processes to upgrade solid agroindustrial waste products, unfortunately, did not provide the anticipated answers. In many cases, proven industrial results and hoped-for economically-viable commercial applications of some of the biotechnological processes have not yet been achieved in Canada (and even in some overseas countries).

In some cases, even hypotheses as well as projections have been questioned by some researchers as shown below:

- An estimated or perceived world shortage of protein, especially in developing countries, has been questioned; this perception has reportedly reduced the emphasis on recent SCP research.
- The availability of "free" or low cost agroindustrial wastes such as lignocellulosic waste and its components in certain parts of the world has been questioned.
- Some results of enzymatic hydrolysis of some lignocellulosic residues (e.g. bagasse, pulp and paper waste etc.) have been contradicted.

Since we do not want to appear to take a negative position, we felt it best to quote the opinions and projections of some scientists and researchers below.

According to Goldberg (1987) several publications deal with the economic analyses of processes for the production of microbial biomass. However it is difficult to obtain a realistic economic basis for assessing the



relative merits of alternative SCP processes and the advantages of the production of industrial or SCP vs. agricultural protein. This is due to several factors:

- Cost estimates given in the literature are usually supplied by scientists working either in academia or for consulting firms not employed by biomass manufacturers.
- Analyses are made on laboratory scale or pilot scale and not on industrial scale processes.
- Comparison of alternative routes and technologies tend to be unreliable due to the fact that the effect of inflation on the cost quote is frequently unqualified and that some costing exercises refer to commercial technology while others only refer to technology at some state of development.
- Any review of economics is liable to be out-of-date rather quickly due to unpredictable changes in feed stock prices, energy costs, changes in plant construction and interest charges.
- Various high-technology SCP processes are capital intensive making it only viable for large companies with substantial government assistance.

It was hoped that low technology "village-type" processes would be substantially less capital intensive but more labour intensive and thus reduce costs substantially.

In an earlier publication dealing particularly with SCP, Goldberg (1985) indicated that there appears to have been an over-optimism on the SCP production. The estimated expenditure in SCP R&D were believed to be about \$1000 million with several large companies responsible for the major part (BP, ICI, Shell, Liquichimia, Dainippon, Mitsubishi, RHM and others) and

numerous research groups. It is only RMH which now produces fungal SCP for human food sold in U.K. supermarkets (McNaughton, 1989).

The world's protein shortage was estimated at about 10 million tons in 1980 and forecast to increase to 25 million tons in the year 2000. Yet, the current annual SCP production (presumably in 1983/84) was only about 2 million tons the majority of which was yeast from cane or beet molasses. Even this estimate was questioned by some authors. The USSR alone reportedly produced more than 1 million tons.

SCP has not proved to be the solution to hunger in developing countries nor has it generated good economic returns. The reasons for failure to reach high production rates and positive financial results were quoted as:

- increased substrate costs
- decreased relative cost of alternative proteins (e.g. soybean meal)
- soybean protein costs not having risen in the same way as SCP production costs
- highly increased energy costs
- increased plant production costs
- higher interest rates in western countries
- political directives
- psychological barriers of public opinion of accepting the new product primarily the realization of SCP as human foods
- difficulty in providing a tasty product with no digestion problems
- lack of experienced manpower in this field

The current conditions (in the mid-1980s) showed that the costs of the state-of-the-art SCP production could not compete with the prices of conventional protein sources, especially that of soybean meal (Goldberg, 1985). At that time, almost no SCP production plant was operating economically, even



though the Soviet Union and Eastern European countries have developed massive SCP production plants. The construction of these plants was the result of political and economic directives under the communist regimes which wanted to remain independent of excessive reliance of imported animal feed. According to ICI comments, many of these plants had to be shutdown due to severe pollution.

Buckle (1989) indicated that it would be unlikely that food waste containing cellulose, hemicellulose or starch would be available in sufficient quantities to warrant SCP production on a reasonable scale except in special circumstances e.g. Syba process developed by the Swedish Sugar Co. He also indicated that the realization in recent years that there is no substantial world shortage of protein has reduced the interest and emphasis on SCP research.

Rolz (1987) indicated that the major parameters influencing the extent and rate of further enzymatic hydrolysis of solid residues obtained after the solid substrate growth of basidiomycetes are not yet completely determined and that in fact contradictory results have been published in the past as substantiated by ICAITI's research. He also quoted other research sources indicating that some new experimental approaches would need to be developed to better understand how white-rot enzymes interact with surface components.

Anderson (1987) stated that the production of chemicals, fuels and single cell proteins from lignocellulose residues and wastes can not yet compete economically with that of feed stocks derived from oil. However, the tonnage and general availability of lignocellulose in certain parts of the world remains attractive enough for research to continue. If significant advances can be made in conversion rate, recovery of immediate products and digester or fermenter designs to accommodate mixed species, the economics could be improved.

Litchfield (1986) touched on the technical, economic and market parameters with relative comparisons of selling prices for microbial protein products, plant protein products and animal protein products. Since the market conditions changed substantially since then, these figures should only be used as a general indication. They would have to be updated taking into account new costs and prices.

2. Commercialization Considerations

In view of the above observations, the commercialization of specific biotechnological processes may not be economically feasible until more realistic technical and economic results have been achieved.

However, the combination of high quantities and relatively easy accessibility of lignocellulosic waste in many parts of the world, environmental problems and economic needs particularly in developing countries, would make it imperative to concentrate applied research and development of the commercialization of technologies in order to achieve long term economic benefits.

Valladares (1984) indicated that in spite of the huge experimental work carried out regarding organic solvent delignification of lignocellulosic materials, neither a definite process nor industrial plant has been developed or designed. He quoted two authors who had proposed a preliminary layout for an industrial plant using organosolv processes that could be commercially and economically feasible.

On the conversion of corn to ethanol, Morris (1992) indicated that the total energy used in growing corn and processing it into ethanol and other products is 75,811 BTUs per gallon of ethanol. Ethanol contains 76,000 BTUs a gallon and the replacement energy value for other co-products is 24,950 BTUs. Thus the total energy output of 100,950 BTUs provides a net energy



gain of 25,139 BTUs or an energy output-input ratio of 1.33:1.

Assuming the best existing operations, most energy efficient farms and state-of-the-art conversion, the net energy ratio could be as much as 2.21:1. It is interesting to note that Morris also stated that the **net energy ratio for cellulosic crops based on current data could be 2.45:1 [in the U.S.]**. He projected that if ethanol should become a significant transportation fuel and increasing its marketshare, the raw material will probably be cellulosic crops which have a much better than 2:1 net energy conversion. Morris did not provide cost/benefit comparisons.

A commercialization of any process, of course, depends on a number of critical factors derived from a feasibility evaluation including costs of raw materials, energy, process, operation, facility, wages, overhead etc. vs. the competitive market value of the products manufactured.

B. RECOMMENDATIONS

The following recommendations have been developed as a systematic follow-up of this preliminary assessment of biotechnological processes and products in order to lead to the commercialization of one or more processes with companies in the LAC region. A commercialization would provide valuable new products and, at the same time, alleviate serious waste disposal problems.

Some initial actions to identify specific problems and needs would have to be undertaken by IICA staff members with the collaboration of representatives of interested LAC companies. Subsequently, the Canadian counterpart would initiate a feasibility study of one or more projects with collaboration of IICA staff and representatives of LAC companies. The assessment would be anticipated to lead to one or more beneficial and



successful manufacturing operations.

The recommended action plan is outlined below:

1. Prioritization of Environmental Problems

(IICA Staff and LAC Company Representatives)

- Identify environmental regulations with regards to disposal of specific waste products in selected countries.
- Identify disposition problems of waste products particularly in terms of urgency for companies to comply with the regulations.
- Determine annual quantities and collection/transportation logistics and disposition of waste products in specific countries, industries and plants.
- Prioritize problems and needs.

2. Prioritization of Economic Needs

(IICA Staff and LAC Company Representatives)

- Identify and project total protein needs in LAC countries.
- Determine cost of landed protein products (for food or feed purposes) in LAC countries.
- Identify fuel requirements (for ethanol mixture) and determine landed costs of gasoline.
- Identify needs of other chemicals and products which could be produced by biotechnological processes.
- Prioritize needs.

3. Selection of Most Urgent Environmental/Economic Needs

(IICA Staff and LAC Company Representatives)



The above activities will result in a prioritized list of actions with regard to combined environmental/economic needs for relevant solutions to upgrade specific agroindustrial wastes to either protein, ethanol, chemicals, fodder and/or other products. The most urgent and/or beneficial needs would be selected for commercial follow-ups.

4. Visit of IICA and LAC Company Representatives to Canada

(IICA and LAC Company Representatives with Canadian Counterpart)

In order to obtain a good knowledge and understanding of the Canadian technologies and companies, we strongly recommend that representatives of IICA and some collaborating companies come to Canada, meet with the Canadian counterpart, and with representatives of the respective biotechnology companies and research institutes, to discuss all pertinent aspects of the research technology(ies), tech transfer, commercialization, joint ventures. These personal contacts would be very beneficial in the long run for technical and commercial relations.

If, for instance, the SCP technology would be a priority, we would recommend to meet with representatives of the following companies and institutes:

- StakeTech Company
- Thermotech Inc.
- Waterloo University
- Sherbrooke University

If, for instance, ethanol from lignocellulosic waste would be a priority, we would recommend to meet with representatives of the following companies:

- StakeTech Company
- Commercial Alcohols



- ORTECH
- Tigney
- Mohawk

It might be possible that some costs could be covered by the Canadian Program for Export Market Development (PEMD) of External Affairs and International Trade Canada or the Export Expansion Fund of Agriculture Canada. Details of these programs are shown in Appendices B and C.

5. Feasibility Evaluation(s) of Selected Biotechnological Application(s)
(Canadian Counterpart, IICA Staff and LAC Company Representatives)

Subsequently, a comprehensive economic feasibility evaluation would be initiated by the Canadian counterpart with the collaboration of IICA and LAC Company representatives for one or more agroindustrial waste products for upgrading with an appropriate biotechnological process as follows:

- Identify the specific needs of the waste product, its composition physical and chemical characteristics.
- Select the most appropriate biotechnological process, preferably a proven commercialized Canadian application by a company or research institution.

If the project looks feasible and additional applied research would still be required, the possibility of partial funding through the National Research Council's Industrial Research Assistance Program (IRAP) should be considered. Details are shown in Appendix D. Depending on the stage or readiness of commercialization of a technology, the construction of a pilot plant or semi-commercial operation should be considered.

- Select, if necessary, an international consulting engineering company to develop an integrated system of production.

一、二、三、四、五、六、七、八、九、十、十一、十二、十三、十四、十五、十六、十七、十八、十九、二十、二十一、二十二、二十三、二十四、二十五、二十六、二十七、二十八、二十九、三十、三十一、三十二、三十三、三十四、三十五、三十六、三十七、三十八、三十九、四十、四十一、四十二、四十三、四十四、四十五、四十六、四十七、四十八、四十九、五十、五十一、五十二、五十三、五十四、五十五、五十六、五十七、五十八、五十九、六十、六十一、六十二、六十三、六十四、六十五、六十六、六十七、六十八、六十九、七十、七十一、七十二、七十三、七十四、七十五、七十六、七十七、七十八、七十九、八十、八十一、八十二、八十三、八十四、八十五、八十六、八十七、八十八、八十九、九十、九十一、九十二、九十三、九十四、九十五、九十六、九十七、九十八、九十九、一百

- Identify and quantify all input costs: raw material, quantities and costs, handling, pretreatment, processing, operation, wages etc.
- Project capital investment costs for a plant including land, buildings, infrastructure etc.
- Identify markets for derived product(s), volumes, prices for local sales.
- Identify and quantify, if necessary, competitive products, availability and prices.
- Prepare cost/benefit projections, forecasts, cash flow, internal rate of return etc.

6. Project Commercialization

(LAC Company Representative, IICA Staff and Canadian Counterpart)

The final activity would be the implementation of a first commercial project jointly initiated by a LAC Company, IICA and the Canadian counterpart. This would involve, in short, a finalization of financial projections, identification of and negotiations with lending sources (local, regional, Canadian and World Bank financing), technology transfer arrangements, acquisition of land, construction of plant, staffing and startup of operation.



APPENDIX A

REFERENCES

- Alfani, F., Canterella, M. (1987) Lignin and cellulose biodegradation. *Biotechnology of Waste Treatment and Exploitation*. Editor: Sidwick J.M. et al. Ellis Horwood Series in Water and Wastewater Technology.
- Boopathy, R. (1988) Dry anaerobic methane fermentation of coffee pulp. *Journal of Coffee Research*, 18 (2), p. 59-72.
- Boopathy, R. (1987) Inoculum source for anaerobic fermentation of coffee pulp. *Applied Microbiology and Biotechnology*, 26 (9), p. 588-594.
- Buckle, K.A. (1989) Biotechnology opportunities in waste treatment and utilization for the Food Industry. *Biotechnology and Food Industry*. Editor: Rogers et al. p. 261-276. Gordon and Breach Science Publishers, New York.
- Calzada, J.F., Rolz, C. (1983) Anerobic digestion in the integrated utilization of coffee wastes. *Proceedings of Third International Symposium on Anaerobic Digestion*, Boston.
- Cowling, E.B. (1975) Physical and chemical constraints in the hydrolysis of cellulose and lignocellulosic materials. *Cellulose as a Chemical and Energy Resource*. *Biotechnology and Bioengineering Symposium No. 5*. Editor: Wilkie, C.R. John Wiley & Sons.
- De la Torre-Louis, M., Cotera, L.B.F. (1986) Technoeconomic evaluations of various substrates for SCP production: A case study for MENTCO. *Microbial Biomass Proteins*. Editor: Moo-Young. Elsevier Appl. Sc., London, New York.
- Ferranti, M.P. (1987) Environmental biotechnology in Europe: Community activities. *Biotechnology of Waste Treatment and Exploitation*. Editor: Sidwick, J.M. et al. Ellis Horwood Series in Water and Wastewater Technology.
- Garcia, R. et al. (1991) Characterization of Coffee Pectin. *Lebensm. Wiss. u. Technol.*, 24, p. 125-129.



- Goldberg, I. (1985) Single cell protein. *Biotechnology Monographs*. Springer Verlag Berlin, Heidelberg, New York, p. 3, p. 11-13, p. 114-115, p. 124, p. 153-156.
- Heitz, M., Chornet, E., et al. (1991) Fractionation of *Populus tremuloides* at the pilot plant scale: Optimization of steam pretreatment conditions using STAKE II Technology. *Bioresource Technology*, 35, p. 23-32.
- Kay, D.E. (1967) Banana Products, Part I, Tropical Products Institute Report, G32 UK Min. of Overseas Development. p. 30.
- Kume, T. et al. (1991) Upgrading of empty fruit bunch of oil palm to animal feeds by radiation-fermentation treatment - waste disposal and feedstuff preparation by solid-state fermentation using *Coprinus cinereus*. *Tree Cult. Prot. Util.* (114) CODEN 9999Y.
- Litchfield, J.H. (1986) Technical, Economic and Market Strategies for Microbial Biomass Proteins. *Microbial Biomass Proteins*. Editor: Moo-Young et al. Elsevier Applied Science, London and New York.
- Lo, Amy C., Domtar Research Centre, Senneville, P.Q. Personal Communication 1993.
- MacDonald, R.G. (1987) Industrial experience in commercialization of a biomass conversion process. *Biomass Conversion Technology, Principles and Practice*, PerGamon Press (Edited by Murray Moo-Young et al.)
- Misban-ul-Hasan et al. (1989) Production of biogas from dried banana peelings. *Pakistan Journal of Scientific and Industrial Research*, 32 (3), p. 212-215.
- Moo-Young, M., Chisti, Y., Vlach, D. (1992) Fermentative conversion of cellulosic substrates to microbial protein by *Neurospora sitophila*. *Biotechnology Letters*, Vol. 14, No. 9, p. 863-868.
- Morris, D., Ahemd I. (1992) How much energy does it take to make a gallon of Ethanol? Institute of Local Self Reliance, Minneapolis and Washington, D.C., Dec. 92.
- Oguntimein, G., Vlach, D., Moo-Young, M. (1992) Production of cellulolytic enzymes by *Neurospora sitophila* grown on cellulosic materials. *Bioresource Technology*, 39, p. 277-283.



- Ringpheil, M., Moo-Young, M., Rolz, C. et al. (1987) Recommended methods for characterization of agricultural residues and feed products derived through Bioconversion. Comm. on Biotechnology, Appl. Chem. Dir., Intl. Union of Pure and Applied Chemistry. Pure & Appl. Chem. Vol 59, No. 5, p. 723-730.
- Ramsay, B. (1992) Fermentation of waste agricultural material for conversion into biodegradable plastic. Conf. on Chemistry and Sustainable Dev., CHEMRAN VIII Moscow, Sept. 1992.
- Ramsay, J.A. (1990) Poly-beta-hydroxyalcanoic acids (PHA's): unique, microbially produced thermoplastics. Applied Phycology Forum. Vol. 7, No. 3, p. 1-6.
- Roberto, I.C. et al. (1991) Utilization of sugarcane bagasse hemicellulosic hydrolysate by *Pichia stipitis* for the production of ethanol. Process Biochemistry 26 (1991), 15-21.
- Rodriguez-Vazquez, R. et al. (1992) Sugarcane bagasse pith dry pretreatment for single cell protein production. Bioresource Technology, 39, p. 17-22.
- Rolz, C., de Leon R. (1986) Summary of current research activities in the Central American Biotechnology MIRCEN. Mircen Journal 2, p. 221-230.
- Rolz, C. (1984) Bioconversion of Lignocellulose. Biotechnology in the Americas: Prospects for Developing Countries, Symposium San José, Costa Rica 1983. Intercercia Assn. Washington, D.C., 1984.
- Rolz, C. et al. (1978) Effects of some physical and chemical pretreatments on the composition, enzymatic hydrolysis and digestibility of lignocellulosic sugarcane residue. Process Biochemistry, 22 (1), p. 17-23.
- Rolz, C. et al. (1987) White-rot fungal growth on sugarcane lignocellulosic residue. Appl. Microbiol. Biotechnology. 25, p. 535-541.
- Tewari, H.K. et al. (1985) Production of ethyl alcohol from banana peels. Journal of Research, Punjab Agric. Univ., 22 (4), p. 703-711.
- The Oil Palm Industry: Its Impact on the Environment (1992). PORIM Publ. Comm. Palm Oil Developments. No. 17, Palm Oil Res. Inst. of Malaysia.



Valladares, J., Rolz, C. et al (1984) Pulping of sugarcane bagasse with a mixture of ethanol-water solution in presence of sodium hydroxide and anthraquinone. Nonwood Plant Fiber Pulping Process Rep. No. 15, TAPPI Press, p. 23-28.

Villacicencio (1974) ISSCT, p. 1783. Title and other details were not provided by the research institute.

Ye, S. (1992) Economic Impact of the Ethanol Industry in Minnesota: Present Situations and Future Opportunities. Prel. Report, Marketing Div., Minnesota Dept. of Agriculture.

Yu, E.K.C. (1992) Industrial scale testing of continuous high yield explosion pulping of aspen - a review. TAPPI Proceedings. 1992 Pulping Conf. p. 1259-1265.



Program for Export Market Development

APPENDIX B

GENERAL INFORMATION

Program Objective

PEMD's objective is to increase export sales of Canadian goods and services. The program accomplishes this by sharing the costs of activities that companies normally could not, or would not, undertake alone, thereby reducing the risks involved in entering a foreign market. PEMD encourages Canadian companies not previously involved in exporting to become exporters, and encourages Canadian exporters to enter new geographic markets and to introduce new products to existing markets.

Types of Assistance Available

The program offers Canadian businesses limited financial assistance to share in the undertaking of various types of trade promotion activities. All activities must be commercial in nature; that is, they must focus on generating export sales.

Activities include:

- participation in recognized trade fairs outside Canada;
- visits outside Canada to identify markets;
- visits by foreign buyers or foreign sales agents to Canada, or to another approved location;
- marketing agreements (marketing campaigns consisting of a number of trade fairs and visits directed toward a single target market) for medium-sized companies experienced in exporting;
- capital project bidding for major capital projects outside Canada involving international competition/formal bidding procedures;
- special activities undertaken by sector-specific, non-sales trade associations for the benefit of their members.

General Eligibility

Eligibility for PEMD assistance requires that organizations come under one of the following categories:

- an incorporated business;
- an independent firm of professionals, such as architects or engineers;
- a sector-specific, non-sales trade association (for the Special Activities element only).



Program participants must also be export-ready.

They must:

- ▶ be established and operating in Canada and have current sales performance data;
- ▶ have satisfactory marketing and managerial capabilities;
- ▶ be financially able to successfully complete the project;
- ▶ have an exportable product or service that meets Canadian content criteria of 60 per cent;
- ▶ have fulfilled reporting and repayment requirements on any and all previous PEMD assistance;
- ▶ be registered (or in the process of registering) with EAITC's WIN Exports, or ISTC's Business Opportunities Sourcing System (BOSS).

Ineligible Applicants

PEMD assistance is not available to:

- ▶ publicly funded institutions, Crown corporations, Crown-owned companies (50% or more), and their affiliates and subsidiaries;
- ▶ applicants who have been consistently unsuccessful in generating export sales as a result of previous PEMD assistance.





EXPORT EXPANSION FUND

1. WHAT IS THE EXPORT EXPANSION FUND (EEF)

The Export Expansion Fund (EEF) was established in 1984 following approval by Treasury Board, with the objective of funding activities aimed at resolving technical constraints to the expansion of the export of Canadian agri-food products.

The program assists the Canadian agri-food sector in overcoming a wide range of constraints which could be as simple as ignorance on the part of potential foreign buyers of the quality and diversity of Canadian agri-food products to impediments to our trade as a result of differing regulations and standards associated with animal and plant health requirements. The products encompass the whole range from livestock, livestock genetics, meat, horticulture products, seeds for sowing, grains and oilseeds, feed and feed components, special crops to processed food and related services.

EEF is not a contribution program under which the private sector can apply directly for assistance. For the most part, it is a program that industry, through their associations, use to assist in their market development activities in the context of the Agri-food Industry Market Strategy (AIMS) initiative. It is expected that AIMS will be the route for most proposals to be funded by EEF. However, EEF may also fund other proposals which are departmental commitments such as bilateral agreements between Canada and various countries. These agreements anticipate the transfer of Canadian agri-food technology and in many cases this will assist the Canadian Agri-food industry in its export market development activities.

2. TYPICAL ACTIVITIES FUNDED BY EEF**Exploratory Technical Outgoing Missions**

EEF funds visits to identify possible technical barriers to export trade; to discuss and resolve specific technical/scientific issues identified as barriers to trade; or to create an environment conducive to expanding exports of agri-food products;

Incoming Missions

EEF also funds visits to Canada of representatives from foreign governments and agencies and/or representatives from foreign industry or industry associations on technical agri-food trade issues;

Promotional Material

EEF funds the production and distribution of brochures, posters, videos and other material of a technical/scientific nature which describe Canadian products, production techniques, marketing practices, product quality and performance grading and quality assurance as well as related transportation and handling systems.



Reports

EEF funds, under contract, market reports or reports on technical subjects related to specific agri-food products or exports markets.

3. OBLIGATIONS OF INDUSTRY ASSOCIATIONS ON PROJECTS FUNDED UNDER THE AIMS INITIATIVE

EEF funding of a project under the AIMS initiative imposes an obligation on the Industry Associations to report on the results of the activity to the industry at large. The Department agrees to contribute a portion of the funds to cover the costs of a project and the Association must submit a Final Report which will explain what this activity accomplished to respond to industry needs. The Final Report serves as the "deliverable" in contractual terms to satisfy the requirement that government funds were appropriately used in accordance with the terms and conditions of this program.

There is a need to ensure that the requirements for a Final Report are well understood by the Industry Association. This need should be outlined in the letter to the association advising it that their AIMS plan has been accepted by the Steering Committee. The specific requirements for the Final Report of a specific project should be set out in the letter sent by the project officer to when the project funding has been approved.

The letter to the Association will also set out the administrative regulations related to the government's financial contribute (eg. that the government will reimburse certain transportation and other costs). See the specimen to an industry association Appendix 4).

Project officers should indicate that the government's financial contribution under EEF is contingent on all participants fulfilling their obligation to contribute to the Final Report.

EXPENSES COVERED BY EEF FUNDS

EEF monies may be used, subject to the limitations of the relevant Treasury Board directives, to cover:

- (1) travel, accommodation and living expenses for individuals who fall into the following categories:
 - 1) Employees of Agriculture Canada and other Federal or Provincial government departments;
 - 2) industry association representatives who are directly participating as contributing members to the project;
 - 3) contracted experts; and,
 - 4) invitees from foreign countries.
- (2) support costs related to missions such as meeting room rentals, costs of interpreter and translation services, printing, photocopying and other office services, vehicle rentals and related expenses;
- (3) development production and distribution costs for promotional material such as brochures, posters and videos;
- (4) contract costs for professional services to complete reports or studies.





IRAP

The Industrial Research Assistance Program

The National Research Council's, Industrial Research Assistance Program (IRAP) is designed to help small and medium firms (fewer than 500 employees) to be more technologically competitive in domestic and international markets. The program involves a network of over 250 Industrial Technology Advisors (ITAs) across Canada. ITAs provide technical advice and help firms access technical expertise and other sources of federal and provincial assistance. The program also provides financial contributions to R&D projects of individual firms.

Small and medium-size firms are one of the main driving forces of the Canadian economy. Success in these firms is achieved not only by desire, hard work and business skills, but also by effectively adapting technologies to the firms' needs.

Through IRAP, the National Research Council aims to help Canadian firms put appropriate technical expertise to work. Technology can stimulate productivity, profitability and international competitiveness. IRAP assists manufacturing, resource, construction and service industries. Vital linkages between companies and sources of appropriate technology and expertise are provided through the IRAP Technology Network.

NRC - IRAP



