BIOMASS CONVERSION TECHNOLOGY

PRINCIPLES and PRACTICE

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PREFACE

Biomass in the form of forestry, agricultural and agro-industrial materials, represents the largest renewable resource in the world. Unlike petroleum, biomass is well-distributed globally. However, this biomass resource is presently under-utilized and, in fact, is often regarded as surplus or waste material.

The basic chemistry of these diverse biomass materials is similar: polymers of five- and six-carbon sugar polymers (hemicellulose and cellulose) and aromatic ring polymers (lignin). An array of industrially important products can be produced from these polymers by various conversion technologies. New and improved techniques, many capitalizing on integrated physical, chemical and biological process strategies, are being developed to produce a wide range of useful products. Efforts are also being directed at improved methods of cultivating biomass materials on the one hand, and of removing them as potential environmental pollutants on the other.

In this monograph, all these aspects of biomass production and utilization are considered, including fundamental principles as well as practical applications. Scientists, engineers and others who are interested in learning or reviewing some of the basics and current developments in biomass conversion technologies are addressed. The manuscripts are organized under the following five sections:

1. Biomass Pretreatment
2. Production of Fuels and Solvents
3. Production of SCP
4. Production and Action of Cellulases
5. Other Biomass Conversion Technologies

In the preparation of this monograph, invaluable assistance in the reviewing of manuscripts was obtained from my associate editors: Jonathan Lamptey, Bernie Glick and Henry Bungay. Proof-reading and production co-ordination was provided by Arlene Lamptey. Finally, I wish to acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and UNESCO in this publication.

Waterloo, Ontario, Canada — Murray Moo-Young

June 1986

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Section 1

Biomass Pretreatment

OLIGOSACCHARIDES PRODUCED BY TREATING WOOD CHIPS WITH GASEOUS HYDROFLUORIC ACID

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ABSTRACT

HPLC analysis of aqueous extracts of poplar wood chips, pretreated with gaseous hydrofluoric acid, shows the presence of oligosaccharides of low molecular weight. The oligosaccharides, which appear to have less than 10 units per chain, are readily extractable from the HF-treated chips and account for more than 85% of the theoretically available sugars. The monosaccharides are formed by acid hydrolysis under mild conditions in almost quantitative yields.

The results suggest that fermentable sugars from wood chips, pretreated with HF(g), may be obtained by subjecting the chips to a relatively mild acid hydrolysis. Alternatively, equally satisfactory results are produced after exhaustive extraction, followed by hydrolysis of the resulting homogeneous solution.

INTRODUCTION

At the end of 1981, the Ethanol-from-Cellulose program was implemented and assigned to Canertech Inc. with headquarters in Winnipeg. The main purpose of the program is to establish an optimum procedure to produce ethanol from lignocellulosics. Thus far, it has used poplar wood chips as the feedstock to produce fermentable sugars by the action of anhydrous hydrofluoric acid (HF) on the lignocellulosic material, followed by a secondary treatment. Although the action of HF on cellulose has been known for decades and early applications of it occurred in Germany in 1938, a complete process was never implemented to the stage of a pilot plant (1).

Presently, the use of HF in treating lignocellulosics has been adopted by several groups, including the Ethanol-from-Cellulose program (2-6). So far, the results obtained by this program are encouraging and strongly suggest that anhydrous HF may be used as the primary reagent to obtain fermentable sugars from wood chips at pilot plant scale.

EXPERIMENTAL

Poplar wood chips were treated with gaseous hydrofluoric acid as described previously (6).

The hydrolysis of HF-treated wood chips and aqueous extract solutions were carried for times that varied between 30 to 60 minutes at temperatures between 110°C and 140°C, and with sulfuric acid concentrations between 0.5 and 1.0%.

The extraction of oligosaccharides was performed by treating HF-treated wood chips with hot water. It was found that a temperature of about 80°C was sufficient to effect the extractions.

Sugars were analysed by HPLC techniques (8), using a Beckman instrument model 334 equipped with a Waters differential refractometer detector model R401, a Biorad HPX-87P cation exchange column (kept at 85°C) and Aminex guard columns. The solvent used was water at a flow rate of 0.6 mL/min.

RESULTS AND DISCUSSION

It has been demonstrated that gaseous hydrofluoric acid reacts with cellulose (I) to produce a glucopyranosyl fluoride derivative (III) via an oxonium ion intermediate (II). Removal of HF induces polymerization to yield oligosaccharides (IV) with molecular weights that depend on the experimental conditions employed (7). This process is illustrated in Figure 1, where it is shown that oligosaccharides are also produced from glucose (V), demonstrating the reversible nature of the reaction. The same general reaction mechanism applies also to other monosaccharides, including pentoses, which form oligomeric compounds when treated with HF(g).

Thus, when lignocellulosics such as wood chips are treated with HF(g), oligosaccharides are produced. However, the complexity of the material treated makes possible several undesired side reactions and the optimal reaction conditions changes accordingly. In addition, both cellulose (mainly hexosans) and hemicellulose (mainly pentosans) serve as substrates that yield oligosaccharides of varied structures.

Analysis of water extracts of poplar wood chips, pretreated with gaseous hydrofluoric acid as previously described (6) showed that the size of the oligosaccharides formed is quite small. The extracts were obtained by filtration, after treating the HF-treated wood chips with hot water (ca 80°C). These solutions were analysed by HPLC, using an HPX-87P Biorad column and as described in the Experimental. The chromatograms show a sharp single peak at a position that would correspond to a chain length of about nine thousand monosaccharide units, as seen in the upper HPLC chromatogram of Figure 2. These and other results, such as solubility characteristics, strongly suggest that the oligosaccharides produced contain less than ten sugar units. However, it is not possible to rule out the presence of oligosaccharides of higher molecular weight, which may not be adequately separated by the HPLC column used in the present work.

The structural characteristics of these oligosaccharides are complicated by two additional factors. The first one refers to their mixed monosaccharide nature, since there would be both hexoses (mainly glucose) and pentoses (mainly xylose) in their chains. The second factor refers to the mixed nature of linkages between monosaccharide units in each chain: it is expected that the beta-1-4 bond type, originally present in cellulose, as well as the readily hydrolysable alpha-1-6 bond would coexist in these oligomers.

Exhaustive extractions of HF-treated wood chips demonstrate that oligosaccharides are readily extractable. Calculations show that more than 85% of the theoretically available saccharides from the wood chips becomes water soluble as short-chain oligosaccharides. Moreover, these oligomers are readily hydrolysed, under mild acidic conditions, to yield monosaccharides in almost quantitative yields. This is illustrated in Figure 2, where the HPLC chromatogram at the bottom belongs to the extract solutions after undergoing acid hydrolysis.

CONCLUSIONS

The results obtained to date indicate that fermentable sugars from poplar wood chips, pretreated with gaseous hydrofluoric acid, may be obtained by different means. Thus, high yields of monosaccharides may be obtained by subjecting the chips to a relatively mild acid hydrolysis. Alternatively, equally satisfactory results are produced after exhaustive aqueous extraction, followed by hydrolysis of the resulting homogeneous clear solution.

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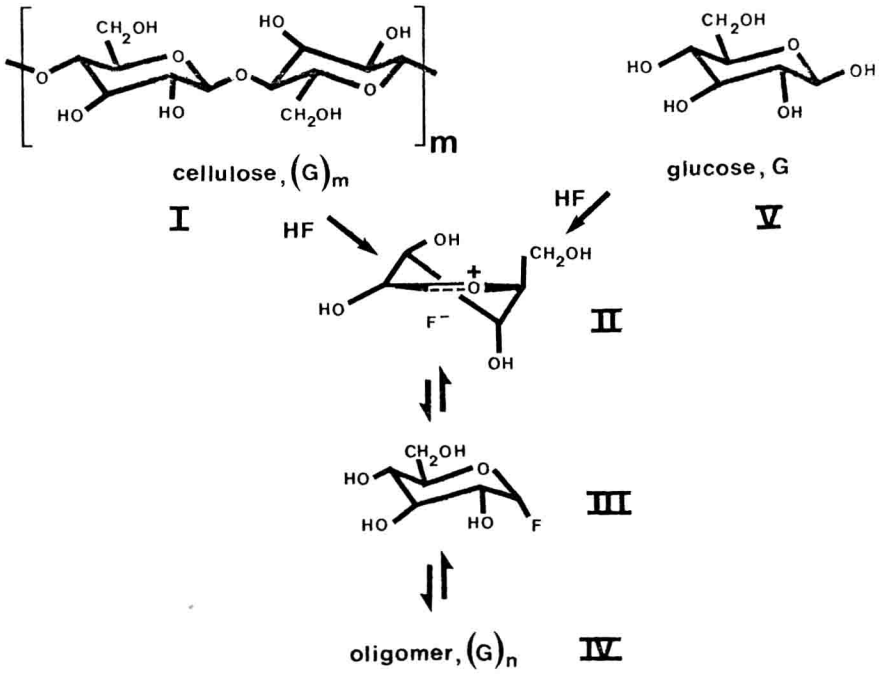

Figure 1: Reaction of HF with cellulose (I) or glucose (V) to yield oligosaccharides of low molecular weight (IV) via intermediates II and III.

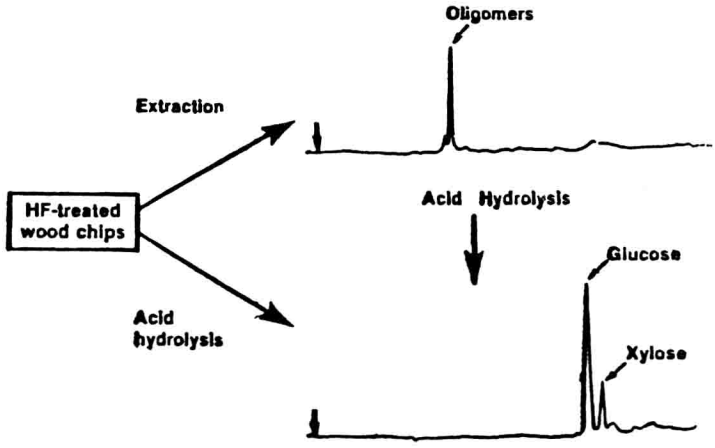

Figure 2: HPLC chromatograms of aqueous extract of HF-treated wood chips (top) and hydrolysate of HF-treated chips (bottom). The same chromatogram is also obtained after the aqueous extract is subjected to mild hydrolysis.

BIOTECHNOLOGY IN CROP PRODUCTION

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This manuscript presents an overview of Agriculture Canada's biotechnology activities relating to crop improvement. While virtually all our efforts are directed towards cultivar development of traditional food and fibre crops, namely cereals, legumes and forages, the principles and approaches being used are equally applicable to energy crops like Jerusalem artichokes, Kochia and fodder beets, all of which are potential sources of biomass for fuel production. The strategy of any crop development program is to generate useful genetic variability, screen for the optimum combination of desirable traits, and then fix or stabilize this combination through several generations of inbreeding. While biotechnology is not expected to replace existing technology, it will certainly complement it. It will do this by making available new ways of generating genetic variation, by facilitating gene transfer and by providing new and more efficient systems for selection, thereby reducing substantially the time required to produce new varieties.

Applications of biotechnology in crop improvement involve the techniques of in vitro organ, tissue and cell culture, protoplast fusion and regeneration into hybrid plants as well as recombinant DNA methodology (Table 1). An integral part of each group of techniques listed is the ability to regenerate plants from cultured cells or tissues. It is important to note that many species, including most of the cereals and grain legumes, are generally recalcitrant to standard tissue culture protocols and thus are not at the present time amenable to cellular and molecular manipulations.

Some of the direct uses of in vitro culture includes asexual propagation of horticultural crops, elimination of viral diseases via meristem culture, preservation of valuable germ plasm generated asexually, generation of somaclonal variants and in vitro selection for economically important traits. Several of these techniques are already under commercial exploitation.

The production of haploid plants through another culture provides an efficient method for obtaining homozygous lines. This technique involves the culturing of the male reproductive structures found in flowers. Under appropriate conditions, developing pollen grains within the anthers can be induced to undergo plant regeneration. Plants obtained in this manner have been termed "haploids" because they possess only a single, rather than the normal double copy of the chromasomes, or units of DNA which form the genetic blueprint of the plant. Haploids can be utilized in crop improvement in a number of ways. These include the rapid breeding of homozygous diploid crops, the rapid development of pure lines for hybrid seed production and the section of novel genetic traits or mutations in cell culture. Desirable mutants would include those that are resistant to disease or tolerant to low temperature, drought, saline conditions, pollutants and herbicides.

Table 1 Biotechnology Objectives (Goals) in Relation to Crop Improvement

Objective	Technique(s)
Plant propagation	- meristem culture
Pathogen elimination/detection	- meristem culture
	- cell fusion (monoclonals)
Germplasm preservation	- protoplast, cell, meristem culture
Homozygous lines (haploids)	- anther, pollen, ovule, embryo culture
Genetic variants (mutants)	- protoplast, cell culture
	- mutagenesis
Interspecific hybrids	- embryo culture
	- cell fusion
Genetic transformation	- cell fusion
	- microinjection
	- rDNA methodology

Production of interspecific and intergeneric sexual hybrids in cereals is now possible through embryo culture techniques. At the Central Experimental Farm in Ottawa we have a well-established program in this area. It has led to the production and cytological characterization of a range of hybrids, including wheat x barley, barley x rye, and wheat or barley x several wild species. At our Ste. Foy Research Station, wheat x Agropyron hybrids have been obtained via embryo culture, an important step in our long term objective of transferring specific genes for viral resistance into cereals.

Protoplast fusion is another technique which can be employed in plant improvement. By fusing cells from different species it is possible to produce hybrid cells that can in turn be used to regenerate hybrid plants. Thus, the barriers which normally prevent cross fertilization between remotely related plant species can be bypassed and novel hybrids can be developed. We have generated a large number of tobacco interspecific hybrids which are being used at our tobacco breeding station in Delhi, Ontario. One particular hybrid cell fusion has resulted in a variant that is unusually high in nicotine, low in tar, and exhibits complete immunity to blue mold disease. This hybrid which is fertile has been backcrossed to common tobacco for several generations and a new variety is expected to be released with the next year.

Recently, we obtained seed from a somatic cell fusion of eggplant (Solanum melongena) which is a distant relative of S. sisymbriofolium. The latter carries resistance to root-knot nematode and Verticillium wilt. Derived cell lines are currently being screened for disease resistance in collaboration with colleagues at the U.S.D.A. A number of new somatic hybridization projects have been initiated in Agriculture Canada laboratories. One example is the cell fusion experiments between alfalfa and bird's food trefoil with the aim of transferring bloat resistance genes into alfalfa.

Plant genetic engineering, or recombinant DNA technology is the newest and most exciting area of plant biotechnology. Back in the late 1970's, it was shown that the T-region of the Ti plasmid from the soil bacterium Agrobacterium tumefaciens is inserted into the genome of infected plants. The inserted piece of DNA encodes for both opines production and tumor induction. This bacteria has been shown to naturally infect a broad spectrum of dicotyledonous plants. Transformed cells produce opines, undergo proliferation and possess the property of being autotrophic for growth hormones. In other words, they will continue to divide in vitro without an exogenous source of growth regulators. Recently, Monsanto scientists have used the Ti plasmid as a vector to transfer bacterial antibiotic resistance genes into plant cells of petunia. Mature plants have been regenerated and shown to be stably transformed. Genes for herbicide resistance and the small protein subunit of ribulase bisphosphate carboxylase have also been tranferred to plant cells using the Ti vector.

Investigators at Agriculture Canada in Ottawa have recently initiated a research project involving the genetic transformation of Brassica spp. by the Ti plasmid and are presently characterizing regenerants derived from transformed (crown gall) tissue. Another project established by the Ottawa group and involving collaboration with a group from Carleton University involves the development of microinjection techniques for introducing foreign DNA directly into plant cell nuclei. Within the past few months, this technique has been used to introduce Ti plasmid into alfalfa protoplasts. Evidence for transformation of microinjected cells (based on the detection of opine synthesis), has recently been obtained in a number of protoplast-derived colonies.

Gene transfer programs involving recombinant DNA techniques require major, long term commitments involving teams of skilled personnel. A major problem in Canada is the lack of scientists skilled in plant molecular genetics. Another general constraint in this field relates to our limited knowledge of gene expression and regulation in higher plants. At present, investigators have access to only a very limited number of plant genes for potential use in genetic engineering. Furthermore the technology, as it presently exists, has been established to transfer single genes. Unfortunately, many agronomic traits (i.e., yield, tolerance to environmental stress, etc.) are polygenically inherited. However, over the long term, plant breeding will certainly benefit immensely from the development and application of molecular techniques. Even now, molecular approaches to genome characterization, independent of genetic engineering, have been found to be powerful tools.

In summary, biotechnology could have a major impact on agriculture and food production, particularly in plant and animal strain improvement. To succeed the technology will have to be judiciously integrated into conventional technologies and approaches. We have already witnessed some impressive achievements with this new technology in terms of crop improvement, which today is deriving economic benefits for Canada.