Fermentation of Lignocellulosic Biomass

FEEDSTOCK CLASSIFICATION

The structural materials that plants produce to form the cell walls, leaves, stems, stalks, and woody portions of biomass are composed mainly of three biobased chemicals called cellulose, hemicellulose, and lignin. Together, they are called lignocellulose, a composite material of rigid cellulose fibers embedded in a cross-linked matrix of lignin and hemicellulose that bind the fibers. Lignocellulose plant structures also contain a variety of plant-specific chemicals in the matrix, called extractives (resins, phenolics, and other chemicals), and minerals (calcium, magnesium, potassium, and others) that will leave ash when biomass is burned.

Lignocellulose material is by necessity resistant to physical, chemical, and biological attack, but it is of interest to biorefining because the cellulose and hemicellulose can be broken down through a process called hydrolysis to produce fermentable, simple sugars. Lignocellulosic biomass is often a waste material of the food processing and forest products industries that may be locally, readily available at low cost.

Cellulose is a very large polymer molecule composed of many hundreds or thousands of glucose molecules (polysaccharide). The molecular linkages in cellulose form linear chains that are rigid, highly stable, and resistant to chemical attack. Hemicellulose consists of short, highly branched, chains of sugars. It contains five-carbon sugars (usually D-xylose and L-arabinose) and six-carbon sugars (D-galactose, D-glucose and D-mannose) and uronic acid. Hemicellulose is amorphous and relatively easy to hydrolyze to its constituent sugars. When hydrolyzed, the hemicellulose from hardwoods releases products high in xylose (a five-carbon sugar). The hemicellulose contained in softwoods, by contrast, yields more six-carbon sugars.

There is more experience fermenting six-carbon sugars than the five-carbon sugars, but both are valuable fermentation feedstocks, especially with recent advances in fermenting five-carbon sugars. Lignin is a polymer constructed of non-carbohydrate, alcohol units that are not fermented, but must be separated from the cellulose and hemicellulose by chemical and other means.

Lignocellulose materials vary in their proportions of cellulose, hemicellulose, and lignin. Typical biomass contains 40% to 60% cellulose, 20% to 40% hemicellulose, and 10% to 25% lignin.[1] Extractives and minerals generally account for less than 10% of the dry biomass weight. The sugar and ash composition of various biomass feedstocks (weight percent) is as follows:[1]

Material	**Six-Carbon Sugars**	**Five-Carbon Sugars**	**Lignin**	**Ash**
Hardwoods	39-50%	18-28%	15-28%	0.3-1.0%
Softwoods	41-57%	8-12%	24-27%	0.1-0.4%
Ag Residues	30-42%	12-39%	11-29%	2-18%

The composition of specific lignocellulose feedstocks is available in several databases.[1,2,3]

FEEDSTOCK EXAMPLES

- alfalfa
- corn stover
- crop residues
- debarking waste
- forage grasses
- forest residues
- municipal solid waste
- paper mill residue
- pomace, scraps & spoilage (fruit & vegetable processing)
- sawdust
- spent grains
- spent hops
- switchgrass
- waste wood chips
- wood chips

FEEDSTOCK RESTRICTIONS

For best economic performance, biomass feedstocks must be available at a consistent price, quality, and quantity, and must be harvested, stored, and transported cost-effectively year-round.

PROCESS DESCRIPTION

Fermentation is a biological process in which enzymes produced by microorganisms catalyze chemical reactions that break simple sugars or amino acids into lower molecular weight materials such as organic acids and neutral solvents such as ethanol. Although organisms exist to break down virtually any organic material, five- and six- carbon sugars are widely available in the plant and animal world. An enormous variety of bacteria, yeasts, and fungi exist to ferment these sugars. These microorganisms digest simple one and two molecule sugars to produce the energy and chemicals they need to live and reproduce, and give off byproducts such as carbon dioxide, organic acids, hydrogen, ethanol, and other products.

Lignocellulose may also be converted to hydrogen and carbon monoxide “syngas” through biomass gasification or black liquor gasification. The syngas can be fermented or converted directly to ethanol or other chemicals. Refer to the descriptions of **biomass gasification** and **black liquor gasification** for further details on those approaches.

Producing commercial products through fermentation of lignocellulose is a multi-step process: pre-treatment and hydrolysis of the lignocellulose to release fermentable simple sugars, fermentation of simple sugars by living microorganisms to produce hydrocarbons such as organic acids or alcohols, recovery from the fermentation broth of the desired fermentation products, and utilization of the byproducts. Although the process steps are described separately, the steps may be integrated to optimize production performance.

Pre-treatment and Hydrolysis

After mechanical separation and milling, lignocellulosic biomass must be hydrolyzed to break the cellulose and hemicellulose down into simple sugars. Although hydrolysis technologies such as concentrated acid and dilute acid have long industrial histories, recent government and industrial focus has been on adding enzymatic hydrolysis to the process as the most promising method for reducing costs while improving yields. Research continues on methods for improving dilute-acid and other hydrolysis techniques.[4]

The paper industry has developed numerous, effective thermochemical approaches for separating lignin, hemicellulose, and cellulose. For the biorefinery, one may start with dilute-acid (using <1% sulfuric acid solution) pretreatment of the biomass to hydrolyze the hemicellulose, breaking it down into its component sugars (xylose and others). Because cellulose is naturally wrapped in a sheath of hemicellulose and lignin, pre-treatment makes the cellulose more accessible to further action. The second stage is optimized to hydrolyze the more resistant cellulose fraction, either through additional dilute-acid processing, or the use of biobased enzymes. Biobased enzymes are proteins that help break cellulose into glucose.

Liquid hydrolyzates (the sugars) are recovered from each stage and neutralized for fermentation. Residual cellulose and lignin left over in the solids from the hydrolysis reactors serve as boiler fuel for electricity or steam production. Black liquor gasification of lignin is a recent commercial option.

Fermentation Process

Large, temperature-controlled tanks are used for batch commercial fermentation. The sugars are mixed with water to form a fermentation broth. Sugar concentration in the fermentation broth water is adjusted to meet the needs of the microorganism, and nutrients such as a nitrogen source are added as needed to facilitate the reactions. The broth is brought to the optimum temperature for fermentation, which may be different than the temperatures used in pre-treatment steps.

Fermentation begins as the growing population of microorganisms produces enzymes to break two-molecule sugars into single molecule sugars (if needed or capable), and then convert the single molecule sugars into the commercial chemicals and byproducts. Yields of chemicals approach a limit as the microorganisms either consume all the fermentable sugars or the products and byproducts of fermentation inhibit (or kill off) the organism.

There are inherent challenges to working with living microorganisms in commercial fermentation. Microorganisms ferment a watery broth containing sugars and other nutrients, which in most cases must be aseptic and held to tight temperature and pH conditions. Production rates are low by chemical refinery standards, with upper limits on the amount of dissolved materials in the broth at the start of fermentation and the final concentration of fermentation products. Living organisms are variably sensitive to impurities that inhibit their action, including their own byproducts. The advantage of fermentation processes is that the reactions are highly specific and can be directed toward the production of valuable chemicals.

In many fermentations, the product acts as an inhibitor to the production reactions. Removing the product during fermentation increases the yield by allowing more to be formed from a given amount of biomass, in addition, product removal increases the production rate by reducing the accumulation of an inhibitory product. Using continuous extraction, a side-stream can be pumped out of the unit and the extracted broth returned to it.

Product Recovery Processes

Typically, 50–70% of the total production cost in classical fermentation processes is due to downstream processing.[5] Recovery costs can range as low as 10% to 50% of total production cost for bulk chemicals such lactic acid, or 90% for high purity pharmaceutical products.[5] The high costs are due primarily to the low final concentration of product in the water broth, the complex mixture of cellular materials and chemicals in the final broth, and the purity required of the final product. As fermentation moves into lower-value higher-volume chemicals, it becomes necessary to maximize efficiency and minimize costs and waste byproducts to compete effectively against traditional options. Some recommend that achieving these goals means approaching the design of fermentation and downstream separations as a single, integrated process.[5]

Distillation is an energy-intensive separation process used to recover water-soluble products from fermentation. Distillation separates two liquids by taking advantage of their difference in boiling point temperatures. A distillation "still" consists of a vessel to heat the combined liquids to boiling, a condenser to cool the vapors back into liquid, and receivers to collect the concentrated liquids. Distillation follows one of two main methods. In the first method, the heated mixture may consist of two liquids with significantly different boiling points. The vapor that is given off will be a majority of one or the other liquid, which after condensation and collection effects the separation.

A second method, fractional distillation, is more effective at separating liquids with similar boiling points. This method relies upon a gradient of temperatures existing in the condenser stage of the equipment. Often in this technique, a vertical condenser, or column, is used. By extracting products that are liquid at different heights up the column, it is possible to extract liquids that have different boiling (and condensing) temperatures. Under ideal conditions, distillation can be used to effect a near-complete separation of one type of liquid from another. The fractions can be purified further by a second distillation.

Distillation is not effective for recovering many fermentation products. Even where distillation may work, lower cost, lower energy separation techniques are being developed and used. In the ethanol industry, distillation accounts for about 40% of the total energy needed for corn-to-ethanol conversion.[6] Other recovery methods include precipitation and other chemical based techniques, and various types of membrane separation.

Organic acid fermentation products may be recovered as salts of calcium and other metals. Recovery involves concentration of the salt, and conversion back into the acid. Other techniques use caustic chemicals to precipitate fermentation products. Recovery through these methods results in large volumes of waste salt materials such as gypsum.

Membrane separation technology applies to the use of a membrane, an engineered barrier with special properties that restricts the transport of various chemicals in a selective manner. Transport through the barrier by selective chemicals may be driven by convection, diffusion, or electric charge (electrodialysis); or by pressure, temperature, or concentration differences. Membranes can provide substantial energy savings over distillation, and offer flexibility and modularity in design.

A relatively new technology called "pervaporation" may provide considerable energy savings over traditional distillation technologies. Pervaporation is a membrane-based process used to separate and concentrate volatile compounds from a liquid mixture by selective permeation through a non-porous membrane into a vacuum permeate stream. The remaining liquid thus becomes depleted in the compound that permeates the membrane.[6]

PRIMARY BIOBASED PRODUCTS

An enormous variety of bacteria, yeasts, and fungi are of interest for biorefining through fermentation because they are able to produce dozens of chemicals with significant market potential.[7] In addition to **ethanol**, products that are already being produced through commercial fermentation include therapeutic and research enzymes, bulk enzymes, antibiotics, lysine, monosodium glutamate, gluconic acid, **lactic acid**, **acetic acid**, citric acid, malic acid, and whole-cell yeast biomass.

Of special interest in biorefining are platform intermediate chemicals from fermentation that can be converted into numerous consumer and industrial products, including **succinic acid**, **butanol**, **itaconic acid**, **1,3 propanediol**, **polyhydroxyalkanoates**, and **3-hydroxypropionic acid**.

PROCESS BYPRODUCTS

Residual cellulose and lignin left over in the solids from the hydrolysis reactors serve as boiler fuel for electricity or steam production. **Black liquor gasification** of lignin is a recent commercial option.

The fermentation process releases gases such as carbon dioxide that may be captured for sale, for example, to the beverage industry. Fermentation may also produce volatile organic compounds that may need remediation prior to release.

Distillation and membrane separation processes result in large volumes of wastewater that contain a high biological oxygen demand that requires treatment. Some recovery methods generate large volumes of solid materials, such as calcium sulfate (gypsum), that must be recycled if possible, sold as a soil amendment, or disposed of properly.

MAJOR EQUIPMENT

As described above, the major components of the process may be carried out in separate process steps. Major equipment includes the pre-treatment process that may include cleaning and milling operations; the hydrolysis process that may involve a dilute-acid reactor to process the hemicellulose, and a separate reactor for enzymatic hydrolysis of the cellulose; the fermentation process that may have separate vessels for five-carbon and six-carbon sugar fermentation; and product recovery equipment.

An important process modification made for the enzymatic hydrolysis of biomass was the introduction of simultaneous saccharification and fermentation (SSF), which has recently been improved to include the cofermentation of multiple sugar substrates.[8] In the SSF process, cellulose hydrolysis and fermenting microbes are combined. As sugars are produced, the fermentative organisms convert them to ethanol.

ENERGY REQUIRED

Energy used in ethanol production is representative of commercial fermentation. One study examined the question in detail, from growing corn on farms and lignocellulosic “crops” to production of ethanol and byproducts, with results that follow.[9] Assuming a lignocellulosic feedstock of short rotation woody crops and an average efficiency ethanol plant, the total energy used in growing the woody crop and processing it into a gallon of ethanol and other products is 76,093 Btus. Ethanol contains 84,100 Btus per gallon and the energy value for combustion for the other co-products is 115,400 Btus. Thus, the total energy output is 199,500 Btus and the net energy gain is 123,407 Btus, for an energy output to input ratio of 2.62 to 1.

CAPITAL AND OPERATING COST

The Iogen demonstration facility cost CN $45 million to process 40 tons per day of feedstock, to produce 3 to 4 million liters of fuel per year. [10]

COMMERCIALIZATION STATUS

Large scale process technology capable of fermenting ethanol from lignocellulose has been around for decades, but it has not proven commercially attractive other than during times of shortage, such as wartime.[8] Intense research and development work is ongoing to improve the process, especially on two critical needs: 1) the need to develop cost-efficient processes for hydrolyzing fermentable sugars from cellulose, and, 2) the need for robust microorganisms to ferment non-glucose sugars, especially five-carbon sugars from hemicellulose.

Biotechnology advances in lignocellulose hydrolysis and fermentation have opened new commercialization opportunities.[8,11,12,13,14] For example, researchers have been locating novel microorganisms in the wild, using genetic engineering to express desirable traits, and patenting robust new organisms that carry out hydrolysis and fermentation.

Iogen operates a demonstration facility of newer technology in Ontario Canada where ethanol fuel is made from agricultural residues. The CN $45-million plant is the final step before the construction of full-scale, CN $250+ million commercial plants. The demonstration plant is designed to prove the feasibility of Iogen's EcoEthanol™ process by validating equipment performance and identifying and overcoming production problems prior to the construction of larger plants. Iogen's EcoEthanol process uses an enzyme hydrolysis to convert the biomass into sugars. These sugars are fermented and distilled into ethanol fuel using conventional ethanol distillation technology. The plant can handle all functions involved in the production of cellulose ethanol, including receipt and pretreatment of up to 40 tons per day of feedstock, conversion of cellulose fiber into glucose, fermentation, and distillation. It employs approximately 20 people to produce 3 to 4 million liters of ethanol per year. The plant uses wheat, oat, and barley straw as raw materials.[10]

BC International has patented new organisms that have the ability to ferment five-carbon sugars to ethanol as well as offering the opportunity to hydrolyze the cellulose with enzymes. Commercialization is ongoing with a large scale plant under construction.[12]

COMMERCIAL SUPPLIERS

Noteworthy commercialization efforts to produce ethanol from biomass are being carried out by Iogen[11] and BC International.[12] Numerous companies, including Genencor[15] and Novozymes,[16] are involved in commercial development of enzymes, as summarized by Kerr.[13]

REFERENCES

[1] U.S. Department of Energy. Understanding Biomass as a Source of Sugars and Energy. Biomass Program. http://www.eere.energy.gov/biomass/understanding_biomass.html#biomass (May 28, 2004).

[2] Scurlock, Jonathan, Bioenergy Feedstock Characteristics, Oak Ridge National Laboratory, Bioenergy Feedstock Development Programs, P.O. Box 2008, Oak Ridge, TN 37831-6407. **http://bioenergy.ornl.gov/papers/misc/biochar_factsheet.html** (May 28, 2004).

[3] PHYLLIS the composition of biomass and waste. ECN-Biomass. Energy Research Centre of the Netherlands (ECN). **http://www.ecn.nl/phyllis/** (May 28, 2004).

[4] Towler, Gavin, Oroskar, Anil, and Smith, Suzanne, July 2003. Development of a Sustainable Liquid Fuels Infrastructure Based on Biomass, Prepared for presentation at AIChE Annual Meeting, November 18, 2003, Session 143. UOP Innovation GroupDes Plaines, IL.

[5] Van Hoek, Pim and Aristos Aristidou, Cargill Dow LLC; J. J. Hahn and Alex Patist, Cargill, Inc., Process Solutions, Technology Development Center. January 2003. Fermentation Goes Large Scale **http://bio.aiche.org/pdfs/Articles/FERMEN~1.PDF** (April 27, 2004).

[6] Vane, Leland. November 10, 2003. Pervaporation–Membrane Process for Bioethanol Recovery, Solvent Dehydration, and Contaminant Removal. Environmental Protection Agency, Washington DC.

[7] Energetics Incorporated. 2003. Industrial Bioproducts: Today and Tomorrow. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Office of the Biomass Program, Washington, D.C.

[8] U.S. Department of Energy. Sugar Platform. Biomass Program. **http://www.eere.energy.gov/biomass/sugar_platform.html** (May 28, 2004).

[9] Lorenz, David and David Morris. August 1995. How Much Energy Does It Take to Make a Gallon of Ethanol? Institute for Local Self-Reliance, National Office, Washington, DC.

[10] Iogen Corporation, Marketing and Communications, 8 Colonnade Rd., Ottawa, Ontario, Canada K2E 7M6, tel: (613) 733-9830, **http://www.iogen.ca/**

[11] Gantz, Rachel May 5, 2003. Genencor, Iogen Make Headway on Cellulose to Ethanol Technology. Chemical Week Associates. Vol. 15 No. 18 New Coverage available from Iogen at **http://www.iogen.ca/HTML/5000.html** (May 28, 2004)

[12] BC International Corporation, 980 Washington Street, Dedham, Massachusetts 02026 , P: 781.461.5700 **http://www.bcintlcorp.com/index.htm** (May 28, 2004).

[13] Kerr, Ellyn. March 15, 2004. Broadened Applicability of Use for Industrial Enzymes. Genetic Engineering News, Volume 24, Number 6. Reprint available at **http://www.genencor.com/pdf/GEN_031504.pdf**

[14] LaPlaza, Jose, and Jeffries, Thomas, May 12, 2004, Engineering Yeasts for Better Ethanol Production, Institute for Microbial and Biochemical Technology, USDA Forest Products Laboratory, Madison, WI.

[15] Genencor International. **http://www.genencor.com/** (April 27, 2004).

[16] Novozymes North America Inc., 77 Perry Chapel Church Road, Franklinton, N.C. 27525, Phone: +1 919 494 3000, **http://www.novozymes.com/** (April 27, 2004)