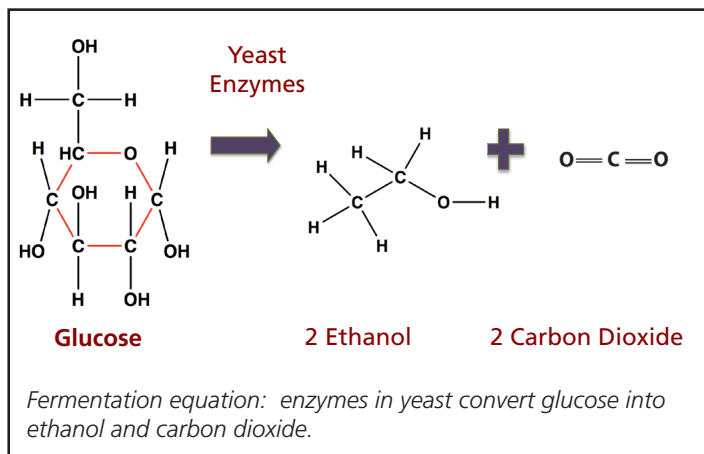


## Why is it so difficult to make cellulosic ethanol?

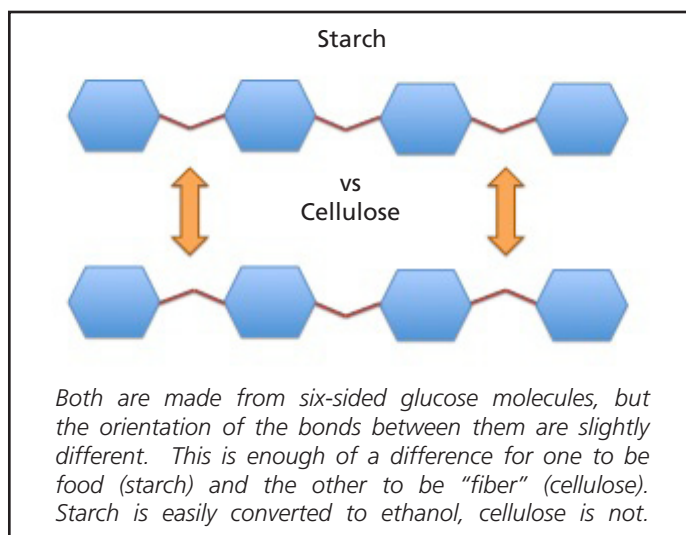
Ethanol can be created from a variety of source materials and through a number of methods. Beer and wine fermentation uses a well-understood biological process in which yeast are fed simple sugars from barley malt or grapes. Yeast digest these sugars to grow and reproduce, and brewers and vintners then harvest the ethanol the microbes create as a waste product. Yeast has special enzymes, or protein catalysts, capable of converting a simple sugar, called glucose, into ethanol as they extract energy from the molecule. Creating ethanol from sugar cane, as they do in Brazil, is fairly straightforward because cane juice contains these simple sugars that yeast can digest. The production of ethanol becomes more difficult when starting with more complex carbohydrates from corn grain or other plant materials.

Starch conversion is also relatively simple. Corn grain and potatoes, for example, are heavy in starches, which are composed of long chains of glucose molecules. Enzymes that chop the long chains of starch into smaller glucose units are readily available. Our digestive systems do this whenever we eat starchy foods with an enzyme called amylase, found in saliva. Once the links between glucose molecules are broken, the yeast can create ethanol. Corn grain ethanol and vodka are two examples of products we make from fermenting this type of carbohydrate.

Because cellulose makes up nearly half of all plant biomass, cellulosic ethanol is considered the largest potential source of biofuel in the near future.

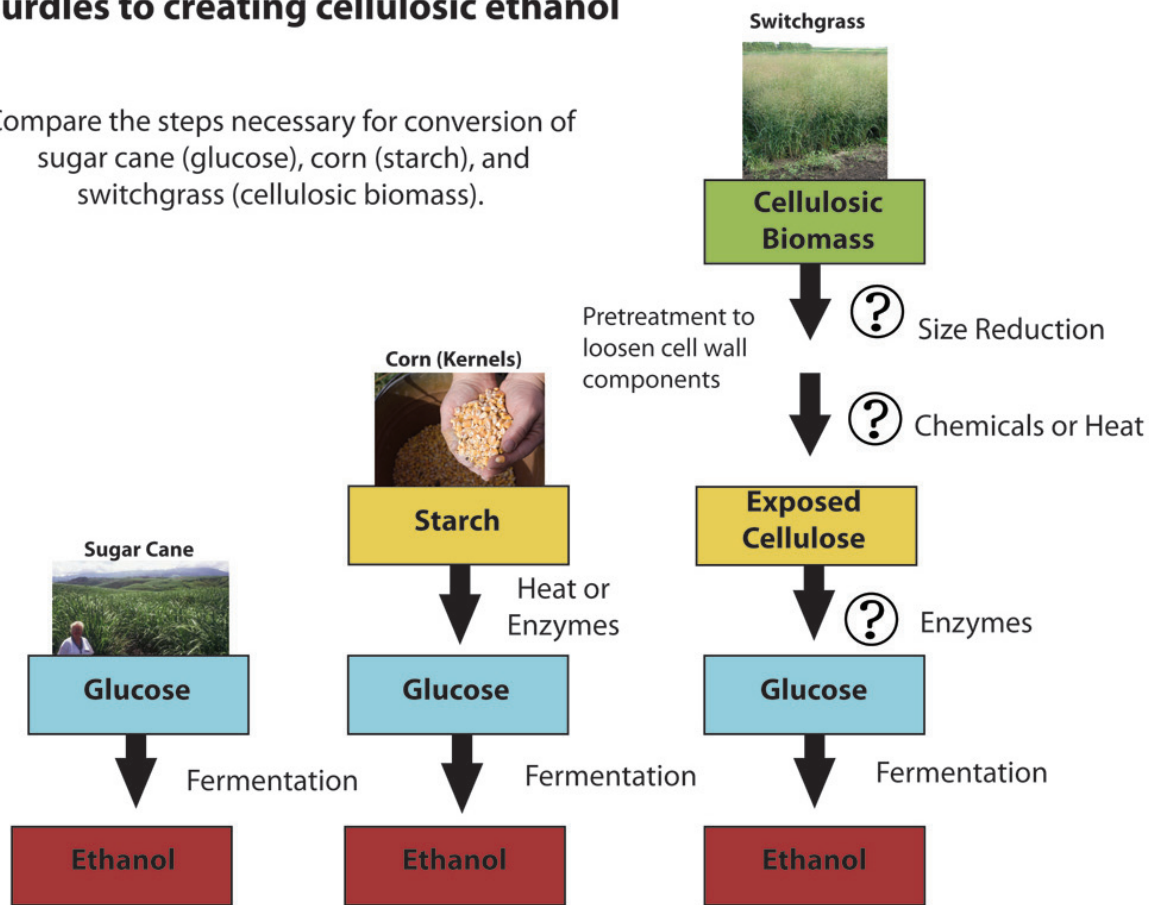


Cellulose, like starch, is a complex carbohydrate made up of chains of glucose. However, the nature of the links holding the glucose together is different in cellulose, and there are fewer identified organisms with enzymes that are capable of breaking down cellulose. Enzymes work in a lock and key system; each enzyme matches a particular molecule—without the right enzyme you cannot build or degrade a molecule biologically.



## Hurdles to creating cellulosic ethanol

Compare the steps necessary for conversion of sugar cane (glucose), corn (starch), and switchgrass (cellulosic biomass).

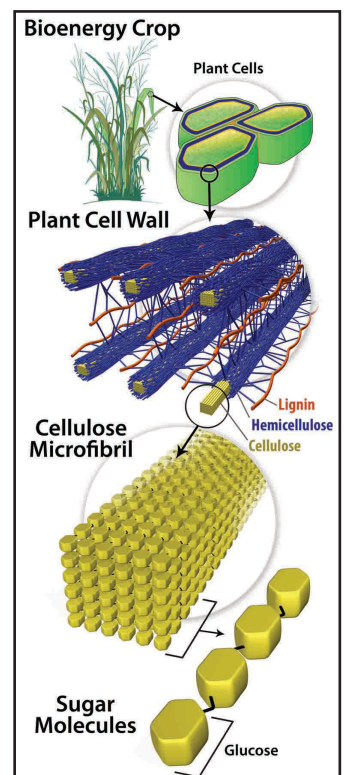


Steps with a question mark are still being researched and we do not know what the final process will be.

To complicate matters even further, the cellulose molecules are packed into a tight crystalline form and then wrapped up in lignin and hemicellulose, two groups of molecules that work with cellulose to create sturdy plant cell walls and protect plants from disease. In order to access the cellulose we first have to disentangle it from the other molecules and unpack the crystals. This process is called “pretreatment” and is currently the most expensive part of the conversion process.

Researchers are looking for more efficient, less expensive ways to easily separate the cellulose from the lignin and hemicellulose and also to find enzymes or organisms that efficiently chop the cellulose into smaller pieces of glucose for fermentation. Solutions may come from research into chemically separating these compounds. Other scientists are trying to genetically engineer plants capable of producing different forms of these compounds that may break down more easily. Answers may come from organisms in nature that are already capable of completing some of these activities.

Department of Energy Genome Programs  
<http://genomics.energy.gov>



## “Bioprospecting” for Natural Biomass Degraders

Picture a dead tree that falls in the woods or the compost pile of leaves in your backyard. Does the biomass stay intact forever? No, because fungi and bacteria depend on this biomass for food and are able to break down tough plant cell walls to access the stored energy. For this to be possible, these microbes must produce enzymes capable of breaking down lignin, hemicellulose and cellulose.

“Bioprospecting,” or searching for natural examples of microbial biomass degraders is one way to discover enzymes capable of carrying out the activities we need to improve our biofuel processing dilemmas. The leaf-cutter ant, *Atta columbica*, is an example of an insect with a 50 million year old symbiotic relationship with a fungus, *Leucoagaricus gongylophorus*. *L. gongylophorus* grows exclusively in *Atta* colonies, living on leaf matter cut from the forests’ trees, carried to them by the ants. In return, the fungus exudes a mixture of lipids, proteins and carbohydrates in a bulb-like swelling called gongylidia, formulated just for the ants.



*Leaf-cutter ant with symbiotic fungus.*

Scientists discovered that this particular fungus does not consume the lignin and cellulose from the leaves, so to keep the colony clean, the ants must remove the unused biomass from the fungus garden. The ants deposit these waste products in “dumps”, where another set of bacteria and fungi go to work degrading the left over plant material. GLBRC researchers are working to harvest, cultivate and study these newly discovered microbial strains in hopes of finding new enzymes that may work for ethanol production.

Other opportunities for bioprospecting can be found anywhere plants are being broken down and providing significant amounts of energy to microbes. The guts of termites, bark beetles and wood wasps, cow rumen and even herbivore droppings provide rich fodder for bioprospectors.



*GLBRC scientists bioprospecting for microbes at Yellowstone National Park.*

# For more information on...

## Biofuels Production

Video: Converting Biomass to Liquid Fuels. (2008) NREL (National Renewable Energy Laboratory).

[http://www.nrel.gov/learning/re\\_biofuels.html](http://www.nrel.gov/learning/re_biofuels.html)

A 5 minute video that gives a nice overview of what is cellulosic ethanol, the production process, and the areas of scientific research needed to create the fuel. GLBRC is working in these same areas.

Biofuels for Transportation. (2007) US Department of Energy Office of Science.

<http://genomicsgtl.energy.gov/biofuels/transportation.shtml>

A FAQ-style page with overview material such as "What is biomass?", "How much ethanol can we get from an acre of Bioenergy crops?", "Can one gallon of ethanol displace one gallon of gasoline?" Links to many other quality resources available from the Department of Energy.

Video: What is cellulose and how it used to make ethanol? (2009) Great Lakes Bioenergy Research Center.

<http://www.greatlakesbioenergy.org/2009/08/19/what-is-cellulose-and-how-is-it-used-to-make-ethanol/>

A 5 minute video that gives a nice overview of what is cellulosic ethanol, the production process, and the areas of scientific research needed to create the fuel. GLBRC is working in these same areas.

## Ant Symbiosis

Visit the PBS evolution website

[http://www.pbs.org/wgbh/evolution/library/01/3/l\\_013\\_01.html](http://www.pbs.org/wgbh/evolution/library/01/3/l_013_01.html)

Ancient Fungal Farmers of the Insect World. (2008) in Microbiology Today. Suen, G. and Currie, C.

[http://www.sgm.ac.uk/pubs/micro\\_today/pdf/110802.pdf](http://www.sgm.ac.uk/pubs/micro_today/pdf/110802.pdf)

Both resources provide background on non-biofuel-related symbiotic relationships between leaf-cutter ants and microbes. The PBS site includes video footage of the ants and interviews with scientists.

## Microbes and biofuels

Genomics of cellulosic biofuels. (2008) Rubin, E. M. Nature 454: 841-845

An overview of the known microbes for possible use in different stages of biofuels production.

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