The Kluyver Centre of Genomics for Industrial Fermentation in cooperation with BIRD Engineering and alcohol-producer NEDALCO has developed a yeast variety that is capable of making alcohol out of xylose, an important component of hemi-cellulose, one of the structural components of plants. According to Prof. Jack Pronk from the Kluyver Centre, fermentation of xylose can boost existing bio-ethanol production. Expectations are that the production of ethanol from hemi-cellulose will be possible on an industrial scale within the next five years.

The development of xylose-fermenting yeast can help accomplish the goal of the European Union: the percentage of fuel for transportation derived from biomass, should increase from 2% in 2006 to 5.75% in 2010. Bio-ethanol is already used on a large scale for transportation purposes. In the United States, millions of litres of bio-ethanol made from cornstarch are added to gasoline. In the state of California for instance up to 10 percent is added as a substitute for MTBE (methyl-t-butyl-ether, antiknock). In Brazil, the Proalcool program is picking up speed again as the price of bio-ethanol from sugar now is lower than that of gasoline from crude. This year, sales of cars that run on any mixture of gasoline and up to 85 percent of bio-ethanol have exceeded 300,000.

The bio-ethanol of today is produced by fermenting starch and sugar. These components make up less than one third of the total biomass of plants. In his inaugural lecture in 1922, the famous Dutch microbiologist Kluyver already mentioned that fermenting pentose sugars like xylose would be very welcome as it would help to get rid of corn cobs, while at the same time producing much-wanted alcohol. In Kluyvers' view, the use of cellulose and hemi-cellulose was necessary to meet the increasing demand for motor fuel. As far back as 1918, the British Government had appointed an *Alcohol Motor Fuel Committee* that was charged with making a study into how to expand the alcohol industry, so that alcohol could replace gasoline as the main motor fuel, as the latter would run out in a few decades.

Other types of fuel needed

Although oil and gas have not run out yet, there is certainly a need for other types of fuel, says Hans van Dijken, Director of BIRD Engineering and part-time professor at the Delft University of Technology. The reason is that burning fossil fuels has a severe impact on the world's climate. The Kyoto Protocol and all subsequent agreements force us to develop motor fuels that consume as much carbon dioxide as they produce. Van Dijken: 'If we can use the whole plant, the impact of bio-fuel on the climate will become even less than when we use only part of the plant. On top of that we thus limit the amount of land needed for growing bio-fuel.'

Fermenting a large part of the plant into bio-ethanol has come a lot nearer thanks to the Dutch discovery and subsequent development of the new yeast variety. The story of its discovery actually starts in the early eighties. At that time Van Dijken was a young researcher at the Delft University of Technology. Working on yeasts he found a variety capable of degrading xylose into ethanol. Only it had a very low rate, as it was a two-step process, involving several co-factors.
Although it had its drawbacks, the variety looked promising and was even patented and sold to an industrial company. It was never commercially exploited though, because of the biochemical complications, and in 1986 the research project was terminated. Van Dijken: 'There have been other attempts to ferment xylose into ethanol, both with yeast and with bacteria, but none of them were very successful, due to problems with the metabolism.'

Dutch designer yeast boosts bio-ethanol production

Hans van Dijken
[Director of BIRD Engineering and part-time professor at the Delft University of Technology]

Jack Pronk
[Scientific Director of Kluyver Centre of Genomics for Industrial Fermentation]

Wim de Laat
[Head of Research at Royal NEDALCO]

Indian elephant

Almost fifteen years later, in 2000, Jack Pronk was approached by colleagues from the Radboud University of Nijmegen. From the dung of an Indian elephant they had isolated a mould capable of making ethanol from xylose under anaerobic conditions. They also had sequenced the genes responsible for the enzyme xylose-isomerase that catalyses the reaction. Bacteria had previously been identified with the same capacity, but transferring the genes to yeast had been unsuccessful. As moulds and yeast have more in common than bacteria and yeast, the mould gene held the promise of a successful gene transfer.

In cooperation with BIRD Engineering, the Kluyver Centre succeeded in transferring the gene from the mould to the yeast. The modified yeast showed the capacity to ferment xylose into ethanol, but only just. It had a very low activity, but 'enough to convince me that the invention was worth a patent application', says Wim de Laat, Head of Research at Royal NEDALCO, which is a leading producer of alcohol from molasses and starch. 'The further development of the modified yeast was a high-risk investment but with the possibility of high rewards. That is why we took our chance.'

Technically feasible

BIRD Engineering modified the yeast into a highly productive workhorse. This modified yeast, which is now collectively owned by the Delft University of Technology, BIRD Engineering and NEDALCO, attracts worldwide interest from companies involved in bio-ethanol production. But before the fruits can be reaped, a lot of development work still has to be done. Technically it is all feasible but it has to be developed into an efficient industrial process.

Research continues

At the Kluyver Centre the changes in the genome of the new yeast variety are still being investigated. Pronk explains why. 'Apart from the mould gene we have introduced several changes into the yeast genome, among them the already mentioned over-expression of five or six genes. With genomics you can chart these changes and make an analysis of their effects on the functioning of the genome. For that we have used DNA arrays that show which of the 6000 or so yeast genes are active under different circumstances. We have not found any negative effects of the changes.'

Great potential

The conclusion is that the Dutch designer yeast has a great potential to enhance the efficiency of agriculture and bio-fuel production. Van Dijken: 'If you start from wheat, you can use the flour for bread, while the rest of the plant can be used for the production of bio-fuel and green electricity. It is a major step towards sustainable energy production.'

From elephant to factory

Two lines of development were followed to transform the low-producing modified yeast cell into a highly productive industrial variety. One was an evolutionary approach whereby generations of the yeast were grown on xylose, each time selecting spontaneous mutants that grew best. Within nine months and around 100 generations, a variety evolved with the capacity to produce ethanol under anaerobic conditions. 'Yields were still poor, though', says Pronk. 'That is why we also followed the designer approach whereby the yeast genome was altered.'
Ron Winkler from BIRD Engineering was involved in further modifying the yeast. 'First we changed the promoter gene', he says, 'the gene that activates the whole pathway. Then we changed several other genes so that they over-express themselves. The result is a yeast variety that grows a lot faster, even under anaerobic conditions, and also produces a lot more ethanol from xylose. It still has the capacity to ferment other sugars like glucose and fructose.'

A very important step in industrial production is to break down the structural carbohydrates from plants into sugars. Wim de Laat from NEDALCO has experience with unlocking sugars from starch and sugar beet. 'You start with chopping the plant and grinding it, followed by steam treatment. That is all standard procedure. The next step is different and involves the enzymatic degradation of the structural plant material with enzymes. One part of it, the enzymatic breakdown of cellulose is already being done on an industrial scale, with straw as a raw material. The breakdown of hemi-cellulose will result in even more bio-ethanol from straw or any other raw material.'

NEDALCO is currently preparing a demonstration project in the Netherlands for the production of alcohol from various wheat residues, roadside grass and wood chips. The remaining fraction, lignin, will be used for the production of steam and green electricity. Compared to traditional methods of producing bio-ethanol, the new factory uses mainly green waste as its raw material. As long as transportation distances remain, the energy balance is very much on the positive side. Since xylose-fermentation makes it possible to use a large part of the plant, even future energy plantations could be worthwhile.