There is a worldwide shift away from total dependence on fossil fuels - coal and oil - towards renewable energy sources such as bioethanol produced from plant biomass. This has been brought about by the finite reserves of fossil fuels, the escalating price of petroleum products and, more recently, the contribution of fossil fuel usage to global warming.

The interest of Prof James du Preez, chairperson of the Department of Microbial, Biochemical & Food Biotechnology, in bioethanol dates back to the early 1980s, when the oil crisis in the late 1970s sparked research and development aimed at the sustainable production of fuels and chemicals from renewable feedstocks (i.e. plant biomass) from agriculture and forestry. At that time in South Africa the liquid fuel crisis was exacerbated by an oil embargo, and grants became available for research towards the development of alternative fuels. Participating in a national research programme, Prof du Preez focused on the fermentation of the pentose sugar D-xylose a principal sugar of lignocellulosic (plant) biomass that was previously regarded as unfermentable by yeasts. Prof du Preez demonstrated that by using a novel yeast, Candida shehatae, and special fermentation conditions, it was possible to produce ethanol.

Could the humble prickly pear hold the secret to sustainable biofuel feedstock in semi-arid regions?

‘A strong biotechnology industry can contribute significantly to the national economy of any country. In South Africa, a modern fermentation biotechnology industry is in the early stages of development. The UFS is one of very few tertiary institutions to provide a sound training in fermentation biotechnology, filling a need for suitably qualified graduates required by this upcoming industry.’

Prof James du Preez
The conversion of lignocellulosic biomass to bioethanol differs considerably from ethanol production from a food crop, because the starting material is significantly more chemically complex, consisting of cellulose, hemicellulose - quite different from cellulose, despite the name - and lignin as the major components. Cellulose is a linear homopolymer of the hexose sugar (6-carbon) glucose, whereas hemicellulose is a highly branched heteropolymer of various sugars, with the pentose sugar (5-carbon) D-xylose as the most abundant. The crystalline structural conformation of cellulose, as well as its close association with the lignin and hemicellulose, renders cellulose highly recalcitrant to hydrolysis to produce fermentable sugars. The lignin fraction, an aromatic polymer, does not contribute fermentable carbon substrates and is, in fact, a source of inhibitors of microbial fermentation. The relative proportions of the individual sugars depend on the type of plant: the hemicellulose fraction of hardwoods and agricultural waste materials is rich in pentose sugars. In some respects the composition of O. ficus-indica biomass resembles typical hardwood biomass, but with some substantial differences. Due to the unique composition of the O. ficus-indica biomass, pre-treatment and hydrolysis procedures developed for other lignocellulosics to render the constituent sugars need to be adapted.

Now, because of the renewed interest in biofuels, Prof du Preez has again engaged in research on ethanol production from lignocellulosic biomass. The first generation biofuels are based on sugar and starch agricultural crops. Currently, nearly all fuel ethanol is produced by the fermentation of glucose from maize starch in the USA or sucrose from sugar cane in Brazil. However, maize is regarded as the least sustainable biofuel feedstock due to the competition for prime agricultural land and because the competition between usage as a fuel feedstock or for food/feed drives up food prices. The prices of crops that can be used as both food and feedstock for biofuel are now determined by their value as a biofuel feedstock rather than their value as human food or livestock feed. Even the use of sugar cane as a bioethanol feedstock has a detrimental effect due to its production encroaching on agricultural land suitable for other food crops or resulting in the clearing of forests to expand production, which is counterproductive in terms of CO₂ recycling. As a consequence, there is a worldwide research effort to target non-food plant biomass as feedstock for the production of the second generation biofuels.

Whereas the subject of research in the South African national programme of the 1980s was sugar cane bagasse, Prof du Preez is now focusing on using the biomass of the prickly pear cactus, Opuntia ficus-indica. The choice of a lignocellulosic feedstock fell on O. ficus-indica due to the efforts of colleagues Prof HO de Waal (Department of Animal, Wildlife & Grassland Sciences) and Dr Herman Fouche (Department of Soil, Crop and Climate Sciences), who are promoting this plant as a crop that has tremendous potential for cultivation in semi-arid regions unsuitable for the production of conventional food crops. A vast tract of South Africa, specifically the western regions, is predominantly characterised as semi-arid and arid. Thus, the use of O. ficus-indica biomass for bioethanol production will not compete for agricultural land or replace significant natural vegetation, nor will it lead to increased prices of conventional food crops. A further advantage in using O. ficus-indica biomass as feedstock for bioethanol production is that the stillage from the ethanol distillation plant would have a high yeast protein content, rendering the stillage suitable for use as livestock feed. By enriching the protein content of the cactus biomass directly through yeast cultivation, its nutritional quality for use as an animal feed would be greatly improved.

Did you know?
As early as 1925, Henry Ford envisioned a world which would grow its own fuel.
Whereas the large scale ethanolic fermentation of hexoses using baker’s yeast, *Saccharomyces cerevisiae*, is well established, the conversion of the pentoses to ethanol is a challenge. There are no known naturally occurring yeast strains that can ferment all of the pentose sugars from lignocellulosic biomass or that are suitable for commercial large scale ethanol production. In recent years major advances towards this goal has been made through the metabolic engineering of *S. cerevisiae*. For ethanol production from *O. ficus-indica*, however, the focus of the UFS group is not on *S. cerevisiae*, but on another yeast strain isolated recently in the department. This yeast strain has been characterised and shown to have the advantages of being able to grow at a higher temperature than *S. cerevisiae*, grows significantly faster and under aerobic conditions it utilises D-xylose efficiently (whereas *S. cerevisiae* cannot), although it does not ferment this pentose. In collaboration with departmental colleagues Prof Koos Albertyn and Dr Michel Labuschagne, this yeast isolate is being subjected to metabolic engineering to facilitate pentose fermentation. It is anticipated that the engineered strain will eventually be able to compete with the best engineered strains of *S. cerevisiae*, which would widen its industrial application to lignocellulosic biomass feedstocks other than *O. ficus-indica*.

Various aspects of yeast research have been a strength of the department over three decades. Apart from the above project, Prof du Preez’s group is currently also involved in fundamental research on *S. cerevisiae*, namely an investigation of the physiological role of the five different alcohol dehydrogenase iso-enzymes in this yeast. In *S. cerevisiae* the fermentation of glucose is mediated by alcohol dehydrogenase isozymes that catalyse the reduction of acetaldehyde to ethanol, or the oxidation of ethanol to acetaldehyde when the yeast uses ethanol as carbon substrate. By joining forces with Prof Albertyn, thereby combining expertise in yeast physiology and fermentation biotechnology with genetic engineering, new insight regarding the role of these enzymes have been gained by investigating different multiple deletion mutants that were engineered, each with only one intact alcohol dehydrogenase (*Adh*) gene. Using continuous cultures operated in chemostat mode to determine growth kinetic and stoichiometric parameters, it was conclusively demonstrated that *Adh1* alone was sufficient for aerobic growth on ethanol, in contrast to the general belief that *Adh2* was necessary for ethanol metabolism. Furthermore, by engineering a null mutant in which
all five Adh genes have been knocked out and investigating its growth and product formation in aerobic and anoxic cultures, experimental data was collected that suggested the existence of an alternative unknown pathway for the production of ethanol by S. cerevisiae. This research has already resulted in presentations at several national and international congresses and a published review article; several more articles are to follow.

Prof J C du Preez
Microbial, Biochemical & Food Biotechnology
+27 (0)51 401 2396/2679
dpreezjc.sci@ufs.ac.za
www.ufs.ac.za/biotech