molecular hydrogen is an essential part of their energy metabolism. The approach with the greatest commercial potential is fermentative hydrogen generation (dark fermentation) by bacteria from the *Clostridium* genus. This biological process, as a part of the methane-producing anaerobic digestion process, is very promising since it allows the production of hydrogen from a wide variety of renewable resources such as carbohydrate waste from the agricultural and agro-food industries or processed urban waste and sewage. To date most publications on hydrogen production by *Clostridium* strains have focused on the effects of operating parameters (such as temperature, pH, dilution rate, etc.). We now need to extend this knowledge by identifying and monitoring the various different metabolic agents involved in high H_2 activity. Consequently the aim of this research at the CWBI in the University of Liege is to investigate the role of [Fe] hydrogenases, the key enzymes that remove excess electrons accumulating during fermentation. *C. butyricum* CWBI1009, the strain used for these investigations, is a particularly efficient biohydrogen producer (3.4 $mol_{H2} \cdot mol_{glucose}^{-1}$, 699 ml $H_2 \cdot l^{-1} \cdot h^{-1}$). Molecular metabolic markers were designed to study the metabolic role of [Fe] hydrogenases and to optimize culture conditions by testing their expression via the mRNA directly extracted from pure culture bioreactor samples.
Keywords. Biohydrogen, dark fermentation, *Clostridium butyricum*, [Fe] hydrogenases.

Bioconversion of potatoes residues or surplus potatoes to ethanol under non axenic conditions

Stéphane Lamaudière [(1)], Sylvain Scarceriaux [(1)], Bruno Godin [(2)], Jérôme Delcarte [(2)], Patrick A. Gerin [(1)]

[(1)] Université catholique de Louvain (UCL). Unit of Bioengineering. Place Croix du Sud 2/19. B-1348 Louvain-la-Neuve (Belgium). E-mail: stephane.lamaudiere@uclouvain.be
[(2)] Centre wallon de Recherches agronomiques (CRA-W). Département Valorisation des Productions. Unité Biomasse, Bioproduits et Énergies. Chaussée de Namur, 146. B-5030 Gembloux (Belgium).

Biofuels can offer an alternative to fossil fuels in the context of climate change and fossil reserves depletion. With 3 million tons of potatoes produced in 2007 and a high yield per hectare of 47 tons, Belgium is the 19th largest producer in the world. The residual and surplus potatoes could be used to produce bioethanol by fermentation. We examined the feasibility of a simple ethanol fermentation processunder non axenic conditions. The substrate was pretreated with commercial amylases or by adding as low as 10% FM (Fresh Matter) barley malt. It was then fermented with *Saccharomyces cerevisiae*. Ethanol and volatile fatty acids were analyzed by GC-FID and soluble sugars were analyzed with the Anthrone method. Starch from potatoes was hydrolyzed to soluble sugars. Hydrolysis seems to continue with 10% FM of barley malt after 48 h while the hydrolysis stopped or decelerate with commercial enzymes. With 10% FM of malt, 3 h of hydrolysis and 7 days of fermentation, an ethanol concentration of 42 $g \cdot l^{-1}$ was obtained and the conversion yield was 139 $g_{ethanol} \cdot kg^{-1}$ DM. The fermentation conversion yield of soluble sugars to ethanol was > 82% and the endogenous competition was limited. However, starch hydrolyzing seems to be a limiting step under the conditions tested. Commercial enzymes did not provide better results under the same conditions.
Keywords. Biofuel, bioethanol, starch, barley malt, fermentation, *Saccharomyces cerevisae*.

Optimization of hydrogen production from carbohydrates by *Clostridium butyricum* CWBI1009

Julien Masset, Serge Hiligsmann, Christopher Hamilton, Laurent Beckers, Philippe Thonart

Univ. Liege (ULg). Walloon Center of Industrial Biology (WCIB). Bd du Rectorat, 29/40 – P.70. B-4000 Liege (Belgium). E-mail: j.masset@ulg.ac.be

The dependence on fossil fuels as our primary energy source is a significant cause of global warming, environmental degradation, and health problems. Hydrogen (H_2) is a promising energy vector for the future since CO_2 is not released during its combustion. At present hydrogen is produced by chemical methods, such as steam reforming or partial oxidation of fossil fuels, which involve the release of large quantities of greenhouse gases into the atmosphere. Biological hydrogen production by "dark-fermentation" of organic waste or effluents is a promising mean of producing renewable energy from waste products. The genus *Clostridium* is known to produce hydrogen from carbohydrates using various different metabolic pathways which are promoted or inhibited by the prevailing culture conditions. The metabolic pathway has a maximum yield of four mol of hydrogen per mol of glucose. Each pathway is characterized by the specific metabolite such as acetate, butyrate, ethanol, lactate or formate. The acetate and butyrate pathways are the only ones which involve the release of molecular hydrogen, *i.e.* 4 mol hydrogen per mol glucose with acetate production and 2 mol hydrogen per mol glucose with butyrate production. Although the *Clostridium* genus is promising for fermentative hydrogen production, few investigations have used pure strains to make a detailed study of the optimal conditions for hydrogen production. Many authors have reported that pH, temperature and stirring has a marked effect on hydrogen production from carbohydrate substrates. Since most of these investigations were carried out using mixed cultures of micro-organisms or using pure cultures without pH control, little is known about the precise impact of pH on the hydrogen production rate and yield and the metabolic pathways involved. Investigations were carried out to determine the optimum culture conditions for the production of hydrogen with *Clostridium butyricum* CWBI1009 and to identify some other related limiting factors. Two substrates were used, glucose, the most used substrate in the literature for the characterization of *Clostridium*, and starch an abundant, inexpensive and reliable raw material.
Keywords. Biohydrogen, dark fermentation, *Clostridium* sp., anaerobic fermentation.