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Investigation of hydrogenase molecular marker to optimize hydrogen production from organic wastes and effluents of agro-food industries

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In recent years policy makers have started looking for alternatives to fossil fuels, not only to counter the threat of global warming, but also to reduce the risk of overdependence on imported oil and gas supplies. By contrast with hydrocarbon fuels, hydrogen (H_2), whether burned directly or used in fuel cells, is intrinsically a clean energy vector with near zero emissions. However the main current method of producing hydrogen, steam reforming of methane, involves the release of large quantities of greenhouse gases. So although hydrogen already accounts for around 2 % of world consumption of energy, its more widespread adoption is limited by several challenges. Therefore new processes are investigated, especially those using renewable raw material, e.g. woods and organic wastes, and/or involving microorganisms. Indeed, for some algae and bacteria, the generation of 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* genera. 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 …). 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 $mol_{H2}/mol_{glucose}$, 699 ml H_2/l.h). Molecular metabolic markers were designed to study the metabolic role of [Fe] hydrogenases and to optimise culture conditions by testing their expression via the mRNA directly extracted from pure culture bioreactor samples.

Ke**ywords: biohydrogen, dark fermentation, *Clostridium butyricum*, [Fe] hydrogenases.**