**Waste2bioHy (MC-FP7-PEOPLE-2010-IEF-326974 – Final Report)**

“Sustainable hydrogen production from waste via two-stage bioconversion process: an eco-biotechnological approach”

Among renewable H2-producing biotechnologies, dark fermentation (DF) of Organic Waste Streams (OWS) has received significant attention in recent years, since it combines sustainable waste management with pollution control and concomitant generation of a valuable clean energy product. Fermentative H2 production provides only partial oxidation of the organic substrate, and therefore, H2-producing bioprocesses are likely to be industrially viable only if they are integrated within a process that can utilize the end-products. Microbial Electrolysis Cell (MEC) is an emerging technology that could utilize the metabolic byproducts generated by DF to produce more H2. Combining DF with MEC in a cascade twosteps process can result in a complete exploitation of OWS, with maximization at the same time of the energy recovery and effluent depollution efficiency.

The aim of the Waste2bioHy project is to investigate fundamentals of microbial communities involved in fermentative/electrogenic bioprocesses dedicated to the conversion of organic matter to energy.

The project relies on a highly interdisciplinary approach, connecting microbial ecology principles to mixed cultures biotechnology, as well as functional characterization. The specific techniques used, such as enrichment culture, metabolic characterization, culture-free molecular methods, phylogenetic characterization, functional characterization were combined to enable and to improve each other to target maximum overall system performance in biological H2-production system. After having well characterized the ecosystems and the type of OWs suitable for H2 production, the possibility of controlling accurately mixed culture fermentation bioprocesses in dark fermentation (DF) as well as microbial electrochemical cells (MEC) was investigated. Besides the acquisition of fundamental knowledge on microbial metabolic interactions occurring in mixed cultures, the technical aim of the project was the development of a sustainable, cascade two-step process for BioH2 production from organic waste streams (OWS), by combining dark fermentation (DF) and microbial electrolysis cell (MEC). Such coupling of DF and MEC constitutes a technological brick within the larger concept of “environmental biorefinery”.

The specific objectives of the project were:

1. Select a proper OWS for coupling DF and MEC.
2. Select and test easily available and high yielding inocula issued from natural ecosystems in DF and MEC processes,.
3. Manipulate biologically the microbial fermentative communities in order to set up a stable and efficient DF process, in terms of substrate conversion and H2 production performances, from the selected OWS in scalable bioreactor (Stage I).
4. Manipulate biologically the microbial exoelectrogenic community in order to set up a stable and efficient process, in terms of conversion into H2 and depollution of organic compounds from Stage I (DF) effluent in a MEC (Stage II).
5. Coupling Stage I and Stage II in a lab scale system

More than 20 OWSs were successfully collected from a wide range of agro-industrial sectors (food, manufacturing, biofuel, wastewater treatment, among others). They were evaluated in terms of H2 potential in DF, and were fully characterized before and after dark fermentation. These OWSs were also evaluated for coupling DF and MEC processes to maximize the hydrogen recovery. The data obtained from DF tests were processed in a statistical multivariate analysis of factors. The results were used to develop a predictive model based on the compositional features of OWSs. This model allows to predict and to describe the hydrogen production potential from wastewater fermentation. Thus, it provides a powerful tool to evaluate rapidly an effluent for H2 production which help making DF process to be truly industrially viable.

In terms of microbial communities, several natural inocula were evaluated and some were selected and characterized both for their high efficiency in both DF and MEC processes. Advanced multivariate analysis of the metagenomics data of the initial and final microbial community together with the data about fermentative metabolic behaviour considering both H2, metabolites and kinetic of process, showed correlations existing between the initial origin of inocula, the final structure of the fermentative communities and their metabolic behaviour. These new findings represent an important brick of knowledge for determining which microbial criteria/structure may predict with accuracy expected metabolic pathways.

In addition, innovative methods were performed to direct the microbial selection toward specific enrichment of functionally active microorganisms for both DF and MEC processes. These procedures are effective in selecting rapidly and specifically microbial communities highly efficient for H2 production in batch and in continuous mode, and in preventing the growth of hydrogen-consuming microorganisms. In particular, an active control of the redox potential was implemented to select specifically useful microorganisms that has never been tested before. A special focus was made on halophilic communities since many OW streams present high quantity of salts, which makes them difficult to be treated by conventional biological routes. Highly successful and innovative enrichment procedures were developed for both halophilic DF and MEC, allowed to make biological H2 production from OWSs feasible in halophilic conditions. As an illustration, wastewater generated from table olive processing (TOPW) was used as model substrate in MEC process. As accomplishment of this experimentation, the feasibility to apply bioelectrochemical technology to treat TOPW and simultaneously recover energy in a sustainable way was successfully demonstrated for the first time. An innovative and effective method for treating this wastewater and simultaneously recover energy using MEC systems was developed. A successful enrichment of specific anodic electroactive biofilm enhancing energy (methane) production and substrate conversion in bioelectrochemical treatment of TOPW was achieved. Since no biological processes are currently available to treat TOPW (the most current treatment of TOPW is its direct discharge into evaporation ponds), the treatment of this effluent in Bioelectrochemical Systems is of high industrial interest. The exploitation of this method is of high relevance for EU, being European countries, especially in Mediterranean regions, the principal table olives world producers.

Finally, the most promising OWSs DF effluents were selected on the basis of fermentative conversion efficiency (i.e., both H2 and metabolic by-product accumulation) to further recover H2 in MEC cells. Results of the coupling are very innovative since several DF effluents, coming from the fermentation of real OWSs, were tested and compared in MEC reactors for the first time. By considering both processes concomitantly (and not separately), it was shown that the conversion of OWSs into bioenergy can be maximized. These findings suggest the existence of complex interaction mechanisms existing between fermentative and electroactive bacteria present in the microbial communities of anodic biofilm, which cooperated together to the degradation of organic matter. As overall achievement of the project, an increase up to three fold of the total H2 recovery was obtained from real OWS by considering both processes, in comparison to a DF step alone.

In conclusion, the coupling of DF and MEC in a cascade two-step process for BioH2 production for organic waste streams (OWS) treatment, constitutes a feasible and highly promising route in the environmental biorefinery framework. The project has generated new insights as well as practical methods for the control of the metabolism of microbial communities to further optimize both DF and MEC processes on real organic waste, which could then be extended to other mixed culture biotechnologies in the near future. Waste2bioHy project developed and consolidated specific competences that can turn these challenges into an opportunity for Europe to become a leading player in the field of biotechnologies and distributed generation. The achievement obtained by Waste2bioHy project increase the EU scientific and technical excellence of the anaerobic mixed culture.