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### ► To cite this version:

Antonella Marone, Olga Ayala, Alessandro Carmona Martinez, Roman Moscoviz, Eric Latrille, et al.. Bio-electrolytic conversion of acidogenic effluents from wastewater fermentation to biohydrogen: an integration strategy for higher substrate conversion into hydrogen. 21. World Hydrogen Energy Conference 2016 - WHEC 2016, Asociación Española del Hidrogeno (AEH2). Madrid, ESP., Jun 2016, Zaragoza, Spain. hal-02738602

**HAL Id: hal-02738602**

**<https://hal.inrae.fr/hal-02738602>**

Submitted on 2 Jun 2020

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# Bio-electrolytic conversion of acidogenic effluents from wastewater fermentation to biohydrogen: an integration strategy for higher substrate conversion into hydrogen

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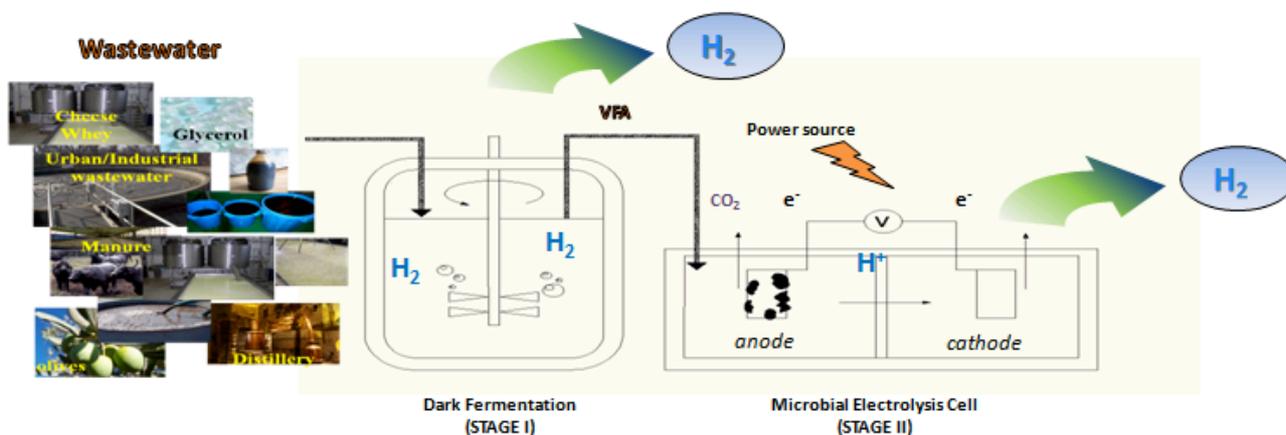
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## Introduction and aim of the work

Wastewater represents an abundant, cheap and available source of biodegradable substrates not yet exploited enough to produce bio-hydrogen by dark fermentation (DF) [1]. Using mixed consortia as biocatalyst for H<sub>2</sub> production, DF can be a practical and promising option for scaling up of technology especially when wastewater is used as substrate. However, fermentative H<sub>2</sub> production provides only a partial oxidation of the organic substrate. It is likely to be industrially viable only if fermentative bioprocesses are integrated within a stream that can utilize fermentation by-products.

Bio-electrochemical systems have been proposed as methods to couple with fermentative hydrogen production [2]. Among these, microbial electrolysis cells (MEC) are based on microbially-mediated oxidation of organic substrates in an anodic compartment, with the aid of an external circuit where a small external power supply is provided for electrochemically-driven hydrogen production [3].

The aim of this research is to individuate the most suitable wastewater for the development of a two-step biohydrogen production process, combining DF and MEC in a biorefinery framework (**Figure 1**).



**Figure 1.** Biorefinery scheme for hydrogen production from wastewater treatment by coupling Dark Fermentation and Microbial Electrolysis Cells technologies.

In this study, more than 20 wastewaters were collected from a wide range of agro-industrial sectors (food, manufacturing, biofuel, wastewater treatment, among others). They were evaluated in terms of H<sub>2</sub> potential in DF and were fully characterized before and after of the fermentation process. These wastewaters were also evaluated for coupling DF and MEC processes to maximize the hydrogen recovery.

## Dark fermentation experiments

DF experiments were carried out in batch reactors (pH 5.5, 37°C) using heat treated anaerobic sludge as inoculum.

Depending on the type of wastewater, hydrogen production was linearly correlated with the initial content of soluble sugars ( $R^2= 0.9$ ) and glycerol ( $R^2= 0.7$ ) present in wastewater. H<sub>2</sub> production obtained from wastewater fermentation ranged from 0 to 296 mLH<sub>2</sub>/L and cheese whey showed the best H<sub>2</sub> production yield of  $120 \pm 31$  mLH<sub>2</sub>/gVS. Acetate, butyrate and ethanol were the main metabolic by-products in all the experiments.

The data obtained from dark fermentation tests were processed in a statistical multivariate analysis of factors using a partial least square regression to investigate the critical factors that can influence the overall process performance. The results were used to develop a predictive model based on the compositional features of wastewater. This model allowed to predict and describe hydrogen production potential from wastewater fermentation. Thus, it provides a powerful tool to evaluate rapidly an effluent for H<sub>2</sub> production which help making dark fermentation process to be truly industrially viable.

### Microbial electrolysis experiments

The most promising dark fermentative effluents, selected on the basis of fermentative conversion efficiency (i.e., both H<sub>2</sub> and metabolic by-product accumulation), derived from the fermentation of six different wastewaters coming from cheese (CW), fruit juice (IWW), paper (PW), sugar (WWS), distillery (VB2) and fruit processing (WC) factories. These effluents were tested to further recover H<sub>2</sub> in MEC cells. Such experiments were conducted in two-chambers (400 mL wV) potentiostatically controlled systems (anode applied potential + 0.2V vs SCE, pH 7, 37°C) using an electroactive biofilm as biocatalyst previously enriched from anaerobic sediments using acetate as carbon source.

MEC results showed satisfactory performances in terms of electrical current production, with a maximum of  $7 \pm 1$  A/m<sup>2</sup> (up to 373 A/m<sup>3</sup><sub>reactor</sub>). The CE (Coulombic Efficiency) reached a maximum value of about 80% and the cathodic hydrogen recovery of about 70%. Interestingly, a complete conversion of metabolic by-products into acetate was first observed before acetate consumption started and current began to increase.

### Coupling dark fermentation and Microbial electrolysis cells

Results of the coupling are very innovative since several dark fermentation effluents, coming from the fermentation of real agro-industrial wastewater, 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 wastewater into bioenergy can be maximized.

Wastewater	YIELDS (mL H <sub>2</sub> /g COD)			OVERALL COD REMOVAL (%)
	Dark fermentation	Microbial electrolysis	Overall	
CW	93	307	400	79
IWW	75	918	993	72
WC	58	332	390	74
PW	19	26	45	67
WWS	19	60	79	66

As overall achievement of the work was obtained an increase up to 13 times of total H<sub>2</sub> recovered from real wastewater considering both processes concomitantly, in comparison to the DF step alone and the total removed COD ranged from 66 to 79% (Table 1).

**Table 1.** H<sub>2</sub> production yields ( mLH<sub>2</sub>/gCOD) of Dark Fermentation step, Microbial Electrolysis step and of the two process and total COD removal (%).

### Conclusions

This work demonstrated that the coupling of dark fermentation and microbial electrolysis cells in a cascade two-step process for BioH<sub>2</sub> production for organic wastewater treatment, constitutes a feasible and highly promising route in the environmental biorefinery framework.

### Acknowledgements

This research was supported by Waste2bioHy project (Marie Curie - IEF – 326974) and BITA Project (Marie Curie IRSES -295170) within the EU FP7.

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