**Scale up strategy to enhance hydrogen production from wastewater by dark fermentation process**

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**Highlights**

* Continuous H2 production in bioreactors inoculated by synthetic consortium or sludge
* Optimization of HRT in both bioreactors gave the same results
* The recirculation ratio has a huge influence on H2 production and metabolites’ concentration profile

**1. Introduction**

Hydrogen represents a promising clean alternative to fossil fuels, because its combustion produces only energy and water and it is a powerful energy carrier. Among the processes that are commonly used to produce H2, dark fermentation is considered one of the most attractive, because it employs the ability of anaerobic bacteria to produce H2 from renewable feedstock such as wastewater. The technical feasibility of H2 production from wastewater has been largely investigated in upflow anaerobic biofilm reactors (UABR) [1]. Indeed, biofilm provides a good protection to microorganisms against potential changes of operating parameters, thus allowing more flexible operating conditions. However, many studies found low H2 yields (HY) and instable H2 production, probably because of the variability of microbial communities and metabolic pathways. The experimental study described here focusses on a multi-scale experimental approach to improve the scale up the process of H2 production via dark fermentation using wastewater as a substrate. First results from Benomar et al. [2] that have shown stable biofilm formation and improved HY using cocultures of *C. acetobutylicum* and *D. vulgaris* Hildenborough. Based on these results, two continuous UABR were designed, inoculated with the coculture and a natural sludge and operated in different conditions to decipher biological mechanisms of hydrogen production.

**2. Methods**

A 1L-reactor filled with Kaldnes K1 as biofilm carriers was inoculated with a synthetic consortium of *C. acetobutylicum* and *D. vulgaris* Hildenborough ratio 1:1. In parallel another 1L-UASB was inoculated with a pre-heated sludge from a municipal WWTP. These reactors were operated in continuous for several weeks and fed with a solution of glucose (1 g/L) as the main organic carbon source and micronutrients (ratio of C/N/P was 100/21.4/1.9, which is in the range of typical C/N/P molar ratios of municipal wastewater) [3,4]. Biogas production was recorded by a Milligas counter (Ritter, Germany) set at the gas outlet of the reactor. H2 content in biogas was determined using a gas chromatograph Agilent 7820A GC equipped with a 30m column GS-CarbonPLOT (Agilent Technology, USA). Liquid was sampled daily at the outlet of the reactor to follow pH and metabolites concentrations by HPLC system Agilent 1260 Infinity LC (Agilent Technology, USA). After stabilization of bioreactors performances the influence of HRT and inlet/outlet recirculation ratios were studied on hydrogen production rate (HPR) and metabolites’ profiles.

**3. Results and discussion**

First experiments conducted on the consortium showed that the best HRT was 2 hours [3]. These results were confirmed by the experiments conducted with the sludge-inoculated bioreactor (figure 1a).



**Figure 1a.** Influence of HRT on HY and Acetate(HAc)/Butyrate(HBu) ratio [4] –

**Figure 1b**. Influence of recirculation ratio (in blue) on HY

Experiments conducted on the bioreactor inoculated with the synthetic consortium to determine the best liquid recirculation rate gave interesting results plotted in figure 1b. The recirculation ratio between inlet and outlet of the reactor is an important parameter to optimize HPR. This could be explained by its influence on the profiles of pH and metabolites’ (to be shown on the conference).

**4. Conclusions**

The combination of experiments with a synthetic consortium and WWTP sludge gave interesting results leading to an optimization of hydrogen production via dark fermentation in a labscale reactor. This approach combining synthetic and natural consortium studies is promising for an efficient scale-up of the process.

**References**

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