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**Directing mixed cultures for optimized hydrogen dark fermentation**

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Hydrogen is recognized as an ideal future energy carrier for the replacement of fossil fuels. Among hydrogen production processes, mixed culture dark fermentation has been viewed as the most promising and environmental friendly. If waste/wastewaters can be used as raw materials for the production of hydrogen in such process, its overall sustainability can be even augmented. For this purpose, it is of utmost importance to select, e.g. by means of environmental pressure, the seed sludge used to start-up. The aim is to obtain a reliable method for the development of microbial anaerobic mixed communities specialized in hydrogen production, in which methanogenic activity has to be prevented.

In this work, different strategies (heat, BES+Chloroform and BES) were used for promoting the selection of hydrogen-producing microorganisms in anaerobic granular sludge (AGS). Three EGSB reactors ( $R_{Heat}$ ,  $R_{BES/Chlo}$  and  $R_{BES}$ ) were inoculated with the AGS resulting from the 3 different treatments and fed with synthetic sugar-based wastewater. Morphological properties of the sludges during reactors' operation were studied using image analysis techniques. Microbial community dynamics was followed by using 16S rRNA gene based techniques (DGGE, cloning and sequencing).

Hydrogen production in  $R_{Heat}$  was unstable and rather low. No methane was produced and acetate concentrations were high (up to  $1878\text{mg l}^{-1}$ ), suggesting hydrogen consumption by homoacetogenic bacteria.

Homoacetogenic activity in  $R_{BES/Chlo}$  was lower, but an increase on propionate formation decreased the overall hydrogen yield. This AGS showed higher free filaments length per VSS, lower granules density and an evident granule fragmentation. This was coincident with a decrease on microbial diversity.

In  $R_{BES}$  there was an initial period in which homoacetogenic activity could be detected. However, an additional pulse of BES triggered hydrogen production rate to an average value of  $700\text{mlH}_2\text{ l}^{-1}\text{d}^{-1}$ . In terms of morphological integrity of AGS this strategy revealed higher potential. Bacterial communities present during  $R_{BES}$  operation were predominantly composed of bacteria branching the *Clostridiaceae* family.

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