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INDUCTION OF HYDROGEN PRODUCTION AFFECTS MICRO AND MACRO STRUCTURE OF GRANULAR SLUDGE

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KEYWORDS

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ABSTRACT

In this work we study the potential for directing microbial anaerobic mixed communities towards improved hydrogen production. Strategies applied for promoting the selection of hydrogen-producing bacteria in anaerobic granular sludge consisted of heat treatment and chemical treatment with 2-bromo-ethane sulfonate (BES) and with BES+Chloroform. Three expanded granular sludge bed reactors, R_{Heat}, R_{BES} and $R_{BES+Chlo}$, were individually inoculated with the treated sludges. Hydrogen production was monitored and the morphological integrity of the granules and bacterial community composition of granular sludge were assessed. This work demonstrates that different methods applied for directing granular sludge for hydrogen production can cause changes in the macro- and microstructure of granular sludge, which can be incompatible with the long-term operation of high-rate reactors.

INTRODUCTION

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. For this purpose, it is of utmost importance to obtain a reliable method for the development of microbial anaerobic mixed communities specialized in hydrogen production, in which hydrogen consuming microorganisms activity has to be prevented. Sludge treatment processes used for reduction of hydrogen-consuming microorganisms include heating, aeration, addition of chemicals (e.g. bases, acids or specific inhibitors), and application of electric current. Heat treatment has been commonly used for inactivating methanogenic archaea in anaerobic sludges; at the same time, this method allows screening for hydrogen-producing bacteria, as many of these mesophilic bacteria are spore-formers (Lay et al., 1999). Chemicals such BES, an analogue of coenzyme M in methanogens is a specific inhibitor for methane-producing archaea (Dimarco et al., 1990). Chloroform is another chemical, that not only inhibits methanogenesis from both H_2/CO_2 and acetate, but also acetate consumption by sulphate-reducers (Chidthaisong and Conrad, 2000). Chidthaisong and Conrad (2000) suggested that chloroform might inhibit hydrogen-dependent homoacetogenesis as well. Granular sludge allows higher biomass concentration in the system and these systems can be operated at high dilution rates without biomass washout. A major drawback in granular sludge processes is the long start-up period, which generally requires several months for the development of hydrogen-producing granules. Therefore, directing microbial mixed communities present in matured/developed anaerobic granules towards improved hydrogen production, by means of environmental pressure, is an important alternative.

The aim of the present study was to evaluate the effect of different methods generally used for the development of anaerobic microbial communities specialized in hydrogen production, on the macro and microstructure of granular sludge. The compatibility of these effects with long-term operation of high rate reactors was evaluated.

METHODS

Treatment of anaerobic granular sludge with heat (boiled at 100ºC for 15 min (Lay et al., 1999)) and two different chemical treatments, i.e. contacting the sludge with 2-bromo-ethane sulfonate (BES) (15 Mm, 72 h at 37ºC) and with BES+Chloroform (15 mM BES and 30 µM chloroform, 72h at 37ºC), were applied to select hydrogen-producing microorganisms. Three mesophilic EGSB reactors - R_{Heat}, R_{BES} and $R_{BES+Chlo}$ – were inoculated with the respective treated sludges and fed with a synthetic sugar-based wastewater (5 gCOD L^{-1}, HRT 20-12h). Hydrogen production was monitored using gas chromatography. Morphological integrity of the granules and bacterial community composition of granular sludge were assessed by quantitative image analysis and 16S rRNA gene based techniques (DGGE, cloning and sequencing), respectively.

RESULTS

Hydrogen production in R_{Heat} was below 300 $mLH_2L^{-1}d^{-1}$ with the single exception of a transient production of 1000 $mLH_2L^{-1}d^{-1}$, after the decrease in HRT. In $R_{BES+Chlo}$ hydrogen production rate did not exceed 300 $mLH_2L^{-1}d^{-1}$. In this sludge it was possible to identify granule fragmentation, release of free filaments from aggregate structures, and decrease of granule density. This coincided with a decrease in microbial diversity. In R_{BES}, after an initial period with unstable H_2 production, an additional pulse of BES triggered the hydrogen production rate to an average value of 700 ± 200 $mLH_2L^{-1}d^{-1}$, which was stable for 30 days. This strategy did not affect significantly granular sludge morphology and structure. The dominant bacterial ribotypes displayed in the DGGE profiles along the R_{BES} operation were found to be closely related to Clostridium species, e.g. Clostridium ljungdahlii (99% identity), Clostridium drakei (94% identity), and to uncultured microorganisms belonging to Clostridiaceae and Ruminococcaceae.

CONCLUSIONS

The strategies used for selecting hydrogen producing microorganisms affected both, micro and macro structure of granular sludge. Image analysis allowed the identification and quantification of changes on the morphological properties of granular sludge that could be related to bacterial community dynamics and diversity. Comparing with the other strategies studied, pre-treatment and subsequent pulses with BES revealed to be the strategy with higher potential for high-rate reactors start-up and further stable continuous operation, at mesophilic conditions. This strategy not only directed the inoculum for hydrogen production but also allowed the maintenance of the morphological integrity of the granular sludge necessary for long term operation.

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