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Induction of hydrogen production affects micro and macro structure of granular sludge

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Mixed-culture dark fermentation is an environmentally friendly bio-hydrogen production process. 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 granules consisted of Heat treatment and chemical treatment with 2-bromo-ethane sulfonate (BES) and with BES+Chloroform. Three EGSB reactors, R_{Heat}, R_{BES} and $R_{BES+Chlo}$, where inoculated with each treated granules and fed with synthetic sugar-based wastewater. Hydrogen production was monitored. Morphological integrity and microbial diversity of the granules were studied using image analysis technique and 16S rRNA gene based techniques, respectively. Hydrogen production in R_{Heat} was below 300 $mLH_2L^{-1}d^{-1}$, with the exception of a single transient production of 1000 $mLH_2L^{-1}d^{-1}$, after decrease the HRT. In $R_{BES+Chlo}$ hydrogen production rate never exceeded 300 $mLH_2L^{-1}d^{-1}$. In this sludge, a physical deterioration of the granules was observed along with a decrease of their density and microbial diversity. In R_{BES}, a transient period of unstable H_2 production was observed but an additional pulse of BES triggered hydrogen production rate to an average value of 700 ± 200 $mLH_2L^{-1}d^{-1}$, which was kept for 30 days. This strategy did not affect significantly granules structure. Dominant bacterial ribotypes found in R_{BES} were closely related to *Clostridium* species and to uncultured microorganisms belonging to *Clostridiaceae* and *Ruminococcaceae*. 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.