Fermentative hydrogen production enhancement by microbial community selection and enrichment through biostimulation

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Introduction

Hydrogen is the most attractive alternative fuel due to its high energy content and clean combustion properties. Over the last few years, among the different hydrogen production processes, the scientific community has principally focused on biological ones. In particular, the dark fermentation process represents a key technology for hydrogen production due to its environmental friendly characteristics. However, in order to achieve proper conditions for the process scale-up from lab to pilot/full scale application, it is necessary to reduce operating costs and enhance hydrogen yield (Ghimire *et al*., 2015). Currently, the main challenge for researchers is to obtain high hydrogen yield by the conversion of waste cheap materials, using mixed cultures that do not require sterile conditions (Pérez *et al*., 2015). However, since the hydrogen is produced during the acidogenesis and acetogenesis steps of the anaerobic fermentation, the inhibition of hydrogen consuming microorganisms fromthe mixed culture should be required in order to properly enhance the hydrogen production. For this aim, different inocula pre-treatments have been investigated (such as thermal, acid or alkaline treatments) (Garcìa and Cammarota, 2019). Nonetheless, despite their potential effectiveness., these procedures can entail significant energy or chemicals consumption. According to this, a more economical and environmentally feasible approach for microbial selection could represent a promising alternative to the previously reported pre-treatments. Then, the present work focused on the potential hydrogen production yield enhancement from dark fermentation tests with mixed microbial culture by selecting hydrogen producing species through a biostimulation technique. In general, the biostimulation of microbial communities is performed through different nutrient typologies addition. In this case, the biostimulation technique was carried out by sequential re-inoculation of a porous support (mimicking a feasible ecological niche for the microbial community growth) in further dark-fermentation batch reactors. This approach was aimed at providing a novel and more economical/environmental friendly biostimulation methodology for proper hydrogen production yield enhancement from dark fermentation process.

Materials and Methods

The dark fermentation batch tests were carried out by using as substrate the cheese whey obtained from the production of mozzarella cheese. Digestate from an anaerobic digestion real scale plant, treating the organic fraction of urban waste, was involved as microbial inoculum. The experiments were conducted in triplicate, using 500 mL glass airtight closed reactors in mesophilic conditions (36±1 °C) with a working volume of 400 mL. Each reactor top was equipped with two ports for gas and liquid sampling operations. The produced gas was analysed to measure its composition. Liquid samples were collected for organic acid concentration measurement and microbiological analysis. The porous support, was included into a polyethylene net and fixed to the reactor top with an inert nylon thread. Each test was characterized by a start-up reactor and 7 sequential re-inoculums. After 24 h (i.e. the selected re-inoculation time), the porous support was moved to a new reactor representing the following re-inoculum step. In the start-up reactors, the working volume was prepared with the addition of not pre-treated inoculum, substrate, and 0.1 M phosphate buffer solution. The start-up tests were conducted assigning a food to microorganisms ratio (F/M) of 1 gCOD/gCOD. For the following re-inoculum tests, instead, the inoculum addition was provided by the microbial community from the porous support. The quantitative composition of reactors is reported in Table 1.

Table 1. Quantitative composition of reactors.

Test	Digestate (mL)	Cheese whey (mL)	Buffer solution (mL)
Start-up	67	83	250
Re-inoculum	-	83	305

Results and Discussion

In general, dark fermentation tests are carried out by digestate addition as inoculum. Since it contains both acidogenic and methanogenic species, a high F/M ratio and an inoculum pre-treatment for methanogens inhibition should be required (Luongo *et al*., 2019). In this study, in fact, during the start-up dark fermentation test, both hydrogen and methane were produced suggesting the presence of a mixed consortium (Figure 1).

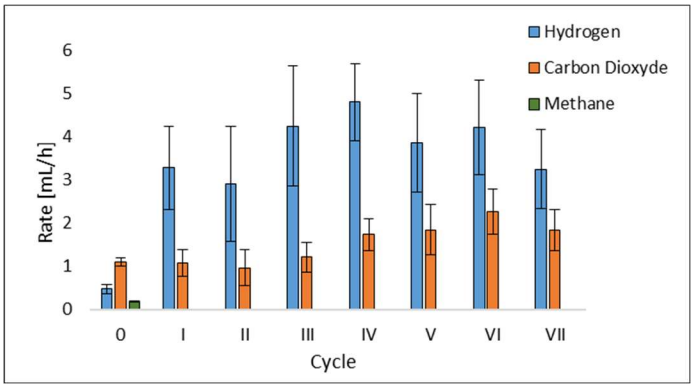

Figure 1. hydrogen, carbon dioxide and methane production during the different dark fermentation cycles.

Despite the high F/M ratio, methanogenic species were not inhibited, confirming the need of digestate pre-treatment step or microbial selection technique in order to improve the hydrogen production yield. However, after the start-up test, it was possible to observe a null methane production rate and a significant increase of the hydrogen production. This potentially confirmed that the involved biostimulation operating conditions (i.e. 24 h fermentation time and sequential re-inoculums) strongly disadvantaged the methanogens community growth while consistent selection of hydrogen producing species was favoured. From the obtained results, it was possible to observe that the hydrogen production rate in the re-inoculum tests was ranging between 2.9 and 4.8 mLH_2/h. These values were similar compared to other studies on cheese whey fermentative conversion to biohydrogen, conducted using similar operating conditions but with the involvement of physical-chemical pre-treatments (De Gioannis *et al.*, 2014).

Conclusions

The results achieved in the present work represent an interesting base for future studies in this field. In order to assess the effectiveness of the investigated biostimulation technique, ongoing studies are focusing on the microbial characterization of the collected samples. This further data can provide more detailed information about the adopted microbial selection methodology. Moreover, further experimental activities are also focusing on the application of the same approach to the light fermentation process to potentially assess the feasible applicability of the suggested biostimulation approach to different treatments aimed at the hydrogen production from organic wastes.

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