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Evaluation of aeration pretreatment to prepare an inoculum for the two-stage hydrogen and methane production process

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HIGHLIGHTS

- Aerobic stress was assessed for selecting a H_2-producing inoculum.
- The biochemical hydrogen potential was tested on waste of the food industry.
- The produced inoculum was effective in H_2 and CH_4 production in a two-stage process.
- A high energy recovery as H_2 and CH_4 from waste was achieved.

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ABSTRACT

This study evaluates the effect of aeration pretreatment to prepare an inoculum for H_2 and CH_4 production in a two-stage process. Moreover, the biochemical hydrogen potential and biochemical methane potential of waste from the food industry in a two-stage process was assessed. The results confirmed the possibility of using an aerobic stress for selecting a hydrogen-producing inoculum. The inoculum was fairly stable since no hydrogenotrophic–methanogenic activity was observed in 25 days. The yields measured using glucose as substrate were of approximately 160 and 280 $N mL_{H_2} g_{COD}^{-1}$ of glucose for hydrogen and methane, respectively, which are in agreement with other studies using heat-shock for the pretreatment of the inoculum. When waste of the food industry (wheat milling) was used as substrate, a lower H_2 yield was achieved by the aerobically-pretreated inoculum if compared to heat-shock; however, when combined with methane production in a two-stage process, much higher CH_4 yield was achieved.

1. Introduction

Over the last decade, considerable efforts have been spent to develop environmentally sustainable technologies for hydrogen production; in fact, whilst hydrogen is a clean energy carrier, nowadays it is primarily produced using non-renewable fossil fuels (Das and Veziroglu, 2001).

Biological hydrogen production can be performed through photosynthetic or dark-fermentative processes (Das and Veziroglu, 2001). Several biotechnological processes which apply different operating conditions (pure and mixed substrates, pure and mixed cultures, batch and continuous reactors, etc.) have recently been proposed for biological hydrogen production (Das and Veziroglu, 2001). The use of mixed cultures seems to be more promising as compared with pure cultures because they can be easily adapted to different substrates belonging to renewable resources or organic wastes. Moreover, well-developed and largely applied technological systems for the anaerobic treatment of wastewaters and organic solid wastes could be easily adapted for hydrogen production (Li and Fang, 2007).

Although numerous bacteria are capable of producing hydrogen, during anaerobic processes hydrogen production is usually not detected due to its rapid utilisation by other micro-organisms (Das and Veziroglu, 2001). In performing biohydrogen production tests, thus, the inoculum is usually thermally (mainly) or chemically pretreated in order to remove hydrogen-consumers whereas hydrogen-producing bacteria are capable of surviving due to their ability to produce spores (Li and Fang, 2007). However, these

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pretreatments are very expensive hindering the application of the process. Furthermore, the regrowth of hydrogenotrophic methanogens has sometimes been observed (Doungmanee et al., 2007; Spagni et al., 2010), precluding or limiting the application of continuous biohydrogen-producing systems. Spagni et al. (2010) demonstrated the regrowth of methanogens can be hindered by controlling the organic loading rate to the reactor. On the contrary, Doungmanee et al. (2007) proposed to control the growth of methanogens by repeated exposure of the sludge to high temperature (90 °C for 20 min).

Aeration has also been evaluated as pretreatment for harvesting hydrogen producing biomass (Chang et al., 2011; Ren et al., 2008; Wang and Wan, 2008; Zhu and Beland, 2006). Although aeration is also an energy-demanding process it could easily be implemented in many plants since blowers are already available in many sites (e.g., in wastewater treatment plants). Moreover, the use of aeration for controlling the process if the shift of H_2-producing to hydrogenotrophic–methanogenic conditions is observed seems to be easier than the use of thermal pretreatment. In fact, aeration requires blowers and diffusers that could be easily moved or are even already available on-site, whilst thermal treatment also requires equipment that must be resistant to heat-shock.

Since dark-fermentative processes produce volatile fatty acids (VFAs) together with hydrogen, recently a two-step process has been proposed where the produced VFAs are used as substrate for biological methane production (Antonopoulou et al., 2008a, 2008b; Chu et al., 2012; Cooney et al., 2007; Hawkes et al., 2008; Jung et al., 2013; Lu et al., 2009; Venkata Mohan et al., 2008; Xie et al., 2008a, 2008b; Zhu et al., 2008). Some studies have also demonstrated that the two-stage process for H_2 and CH_4 production can increase the methane yield in the second stage likely due to the improvement of hydrolysis in the first stage (Liu et al., 2006; Pakarinen et al., 2011).

Standardised methods for the determination of the biochemical methane potential (BMP) of organic compounds have been applied for a long time (Angelidaki et al., 2009) and similar batch tests have also been proposed for evaluating the biochemical hydrogen potential (BHP) of several substrates (e.g., Liu et al., 2013). Moreover, a batch test has recently been suggested for the determination of the hydrogen and methane potential for the two-stage fermentative process (Giordano et al., 2011).

To the best knowledge of the authors, no studies have been performed to assess aerobic pretreatment of inoculum for H_2 and CH_4 production in the two-stage process, and, in addition, to specifically evaluate the "stability" of the hydrogen-producing biomass (i.e., if hydrogen-consuming bacteria regrowth is observed). Therefore, the aims of the present study are to: (i) evaluate the use of the aerobic stress to develop a hydrogen-producing inoculum; (ii) evaluate the "stability" of the hydrogen-producing inoculum (i.e., evaluate if hydrogenotrophic methanogens regrowth is observed); and (iii) assess the BHP and BMP of organic wastes as a simulated two-stage process (i.e., biohydrogen production followed by biomethane production on the produced VFAs) by using the prepared inoculum.

2. Methods

2.1. Substrates

Glucose (Carlo Erba reagents, Italy) was used as substrate for both BHP and BMP tests. Moreover, two wastes from a typical Italian food industry (i.e., flour mill processing) were used as feedstock: (i) wheat bran of *Triticum durum* for the production of the durum wheat of the typical Italian pasta (produced in a flour mill in the province of Pavia, Northern Italy); (ii) wheat bran of *Triticum aestivum* for the production of the common wheat bread (produced in a flour mill in the province of Alessandria, Northern Italy). Before use, the wastes were stored at −20 °C.

Total solids (TS) and volatile solids (VS) of the wheat bran (both common and durum) were 88% and 84%, respectively.

2.2. Inoculum and aerobic pretreatment

Granular mesophilic sludge originating from a full-scale upflow anaerobic sludge blanket (UASB) treating waste of the food industry (potato processing) was used as inoculum for the estimation of the biochemical hydrogen and methane potential (Giordano et al., 2011). The sludge had a TS and VS content of 124 ± 20 and 83 ± 8 g L^{-1}, respectively, whilst the pH was 7.3 ± 0.5.

Aerobic pretreatment was carried out maintaining approximately 2 L of the granular sludge continuously aerated in a Plexiglas vessel using an aquarium blower and a porous stone. Aeration was maintained at approximately 100 L_{air} L_{sludge}^{-1} h^{-1}. After 2, 3, 4, 5, 6, 8, 10, 12 and 14 days of aeration, the granular sludge was used for the determination of the BHP.

The same granular sludge without pretreatment was used for the determination of the BMP.

2.3. Hydrogen production using aerobic pretreated sludge

The experiments were conducted in batch mode, in 1 L Pyrex-glass bottles maintained at constant temperature (35 ± 1 °C) in a thermostatic cabinet.

For every duration (2, 3, 4, 5, 6, 8, 10, 12 and 14 days) of the aerobic pretreament, two bottles were prepared with 120 g (drip-wet weight) of inoculum and 6.9 g of glucose. pH was corrected to 7.2 ± 0.1 by using sodium carbonate monohydrate (approximately 0.25–0.6 g). Tap water was added to reach the final volume of 600 mL, resulting to a glucose concentration of 11.5 g L^{-1} (12.305 g of COD (chemical oxygen demand) L^{-1}; 64 mmol L^{-1}).

The bottles were airtight sealed with screw caps ("Omnifit C series") which contained three valves for sampling. Anaerobic conditions were achieved by flushing the bottles with N_2 for approximately 5–10 min before the beginning of the experiments.

Two "blank" bottles were prepared as described above but without the addition of glucose. All results have been expressed as net values (subtracting the blank).

More details of the procedures, including the addition of macro- and microelements, are reported elsewhere (Giordano et al., 2011).

2.4. Stability of the aerobic pretreated inoculum for biological hydrogen production

For this experiment, three bottles (prepared as described in Section 2.3) were filled with 120 g (drip-wet weight) of aerobically (for 4 days) pretreated sludge, 6.9 g of glucose and tap water to a final volume of 600 mL. When glucose was depleted, also highlighted by the end of the biological hydrogen production, another 6.9 g of glucose was added twice consecutively (without any additional sludge).

The tests were performed at 35 ± 1 °C.

Two blank bottles were also arranged as described in Section 2.3.

2.5. Biochemical hydrogen potential (BHP) and subsequent biochemical methane potential (BMP)

The experiments were conducted according to the method for the estimation of BHP and BMP described in Giordano et al. (2011).

Three 1-L bottles were filled with 120 g of granular sludge aerobically pretreated (4 days of aeration), the substrates and tap