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Photofermentative production of hydrogen and poly-β-hydroxybutyrate from dark fermentation products

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HIGHLIGHTS

- Photofermentative treatment of a dark fermentation effluent is investigated.
- Performances of a *R. sphaeroides* and a mixed PNSB culture are compared.
- The mixed culture results more efficient in H_2 production (233.8 N mL H_2 g COD^{-1}).
- The pure culture shows a higher PHB productivity (155 mg PHB g COD^{-1}).
- High soluble COD removal from DFE is achieved (>80%).

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ABSTRACT

The aim of this work is to investigate the hydrogen and poly-β-hydroxybutyrate (PHB) production during the photofermentative treatment of the effluent from a dark fermentation reactor fed with the organic fraction of municipal solid waste. Two different inocula, an adapted culture of *Rhodobacter sphaeroides* AV1b and a mixed consortium of purple non sulphur bacteria have been investigated under the same operational conditions. Different hydrogen productivities of 364 and 559 N mL H_2 L^{-1} were observed for the *Rhodobacter sphaeroides* and the mixed culture consortium tests, respectively: the consortium of PNSB resulted 1.5-fold more productive than the pure culture. On the other hand, *Rhodobacter sphaeroides* culture showed a higher PHB productivity (155 mg PHB g COD^{-1}) than the mixed culture (55 mg PHB g COD^{-1}). In all the tests, the concomitant H_2 and PHB production was associated to a dissolved COD removal higher than 80%.

1. Introduction

In energy and environmental field, hydrogen (H_2) has gained considerable interests due to its higher specific energy content (122 MJ kg^{-1}), clean combustion (Balat and Kırtay, 2010) and environmental friendliness in production and use (Lin et al., 2014; Andreottola et al., 2012; Ghimire et al., 2015).

At present, the production of H_2 for industrial applications comes mainly from thermo-catalytic and gasification processes, which in turn are fossil fuels dependent. In comparison to these energy intensive physico-chemical routes for H_2 production, biological processes represent a valid alternative as they can utilize renewable biomasses (Ghimire et al., 2015). However, one of the main challenges arising from the use of low value organic biomass for hydrogen production lies in the maximization of hydrogen yields. The dark fermentation (DF) of waste biomass represents the most explored biological route for the biohydrogen production. However, dark fermentative degradation of carbohydrate rich organic biomass normally leads to incomplete substrate conversion and low H_2 yields due to thermodynamic constrains and accumulation of organic acids and alcohols as by-products (De Gioannis et al., 2013; Urbaniec and Bakker, 2015). These different types of carbon can be used as a reducing energy source by other microbial species to perform diverse biochemical reactions (Mattei et al., 2015a). Therefore, combining the DF with other processes such as photo fermentation (PF) or bioelectrochemical systems could

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lead to higher H_2 yields and enhance the waste biomass valorization (Bastidas-Oyanedel et al., 2015).

Under anaerobic conditions, Purple Non-Sulphur Bacteria (PNSB) carry out an anaerobic photosynthesis using light and reduced carbon sources, such as organic acids and alcohols, to produce H_2. This ability could be exploited for treating dark fermentation effluents (DFE) (Cheng et al., 2015; Rai et al., 2014). Indeed, the combined DF–PF process not only results in a higher hydrogen production (e. g. 4 extra H_2 moles for each mole of acetic acid), but also in the possibility of synthetizing poly-β-hydroxybutyrate, which is a biopolymer precursor of economic interest (Montiel-Corona et al., 2015).

In photofermentative bacteria, PHB is often produced under nutrient starvation and accumulated in the cytoplasm as intracellular carbon and energy storage compounds. Several studies have been conducted on PHB or, generally, on poly-hydroxyalkanoates (PHA) bio-accumulation (Kumar et al., 2016; Korkakaki et al., 2016), as the optimization of the biological production of plastic material may be seen as the way to overpass the environmental and recycling issues deriving from the wide utilization of petrochemical-derived plastic materials. However, their extraction and production procedures do not allow the commercial application due to the high costs required.

H_2 and PHB production strongly depend on the Volatile Fatty Acids (VFAs) present in the DFE used as feedstock for the PF. Based on the type and concentration of VFAs in the culture media, PNSB can differently convert organic sources in biological H_2 by several pathways (Ghimire et al., 2016; Kemavongse et al., 2007). Moreover, the structures of these copolymers directly affect their mechanical properties and thus their feasible applications (Reddy et al., 2003).

Several studies report that the synthesis of PHB competes with the H_2 production, as both functions constitute the way to dissipate the excess reducing power (Wu et al., 2012). Nonetheless, a concomitant production of H_2 and PHB is possible, as shown by Montiel-Corona et al. (2015) and Ghimire et al. (2016), but it depends on several operating conditions, such as nutrients availability (C/N, carbon to nitrogen ratio), PNSB strains (mixed and pure culture), pH, light intensity and presence of physico-chemical stress, e.g. major H_2 inhibitor, ammonium in the culture medium, sulphur deprived conditions (Eroglu and Melis, 2011; Adessi and De Philippis, 2014; Chen et al., 2011; Fermoso et al., 2015). Depending on the aim of the process, PF can be directed towards H_2 production, suppressing the PHB synthesis by genetic modifications of the PNSB (Kim et al., 2011) or towards PHB accumulation in photosynthetic bacteria by controlling acetate and nitrogen availability in the growth medium.

The majority of the studies on both photofermentative H_2 production and PHB accumulation involved the use of pure cultures and simple organic substrates. While the use of pure strains usually results metabolically advantageous, one of the main drawbacks in the scale-up of the PF process relies on the presence of inhibitory compounds or competitive species that can affect the purity of the cultures, reducing the efficiency of the system (Ghosh et al., 2016). These problems could be addressed by the use of mixed cultures, as the synergic interactions of the H_2 producing PNSB in the consortium might enhance the efficiency and the effectiveness of PF in terms of H_2 production.

In this work, the ability of PNSB to produce H_2 and PHB from the DFE obtained from the thermophilic DF of the organic fraction of municipal solid wastes (OFMSW) has been investigated. In particular, two different inocula, i.e. *Rhodobacter sphaeroides* AV1b and an enriched mixed culture of PNSB obtained from an anaerobic digestate, were tested under different operating conditions in order to examine the parameters affecting H_2 and PHB productivities. The performances of the different inocula were evaluated in terms of H_2 and PHB production and removal of soluble organic compounds.

2. Materials and methods

2.1. Dark fermentation effluent

The DFE utilized in this study was collected after 110 working days from a thermophilic semi-batch continuous stirred tank reactor with a 0.7 L working volume, a 300 mL headspace and an operating pH of 5.0 (±0.3). The H_2 yields and production rates were 105 (±28) N mL H_2 g VS^{-1} and 205 (±40) N mL H_2 L^{-1} d^{-1} at organic loading rate of 2 g VS L^{-1} d^{-1} and hydraulic retention time of 4 days. The DFE was characterized in terms of total Kjeldhal nitrogen (211 ±4.0 mg L^{-1}), nitrogen ammonium concentration (1.89 ±0.3 mg L^{-1}), COD (4672 ±136 mg L^{-1}) and organic acids concentration (acetic acid 575.90 mg L^{-1}, butyric acid 1117.32 mg L^{-1}, propionic acid 477.90 mg L^{-1} and lactic acid 36.11 mg L^{-1}).

In order to separate the liquid fraction, rich in organic acids, DFE was settled for 30 min, centrifuged at 4500 rpm for 20 min and finally diluted 1:2 with distilled water to obtain a clear medium for PF tests. This enhances the light penetration and reduces the potential hydrolysis of particulate organic materials which might occur otherwise during PF tests.

2.2. Photo fermentation tests

Two different cultures were compared in this study: an adapted culture of *Rhodobacter sphaeroides* AV1b (RS) isolated from the Averno Lake (Naples, Italy) and a mixed consortium (MC) of PNSB enriched in a lab-scale reactor under continuous illumination. In particular, the MC was obtained from the digestate of an anaerobic digestion full-scale plant treating buffalo manure as main substrate for methane production. After the clarification procedure, the digestate was inoculated in synthetic VFAs medium under continuous illumination to stimulate the selection of the PNSB species.

The experiments were carried out in triplicate by using 500 mL reactors with a 400 mL working volume, operated in batch conditions. The reactors were equipped with thin tubing on the top for sampling and gas extraction. The light was continuously provided through fluorescent lamps with constant illumination of 4000 lx, in accordance with other studies investigating the light effects on growth and H_2 production of photofermentative bacteria (Koku et al., 2002; Sevinç et al., 2012; Androga et al., 2014; Akman et al., 2015). The stirring conditions were fixed to 300 rpm through IKA RT 5 stirrer stations (Sevinç et al., 2012; Androga et al., 2014). The experiments were executed at fixed room temperature (25 °C), flushing the headspace of the reactors with argon gas for different times (0, 10 and 20 min). The PF reactors were fed with the real DFE previously defined or with a synthetic culture medium (preliminary tests only) reproducing the same characteristics of the real DFE. The pH of the medium culture for all the PF tests was initially adjusted to 6.0 with 1 M NaOH to prevent any low pH inhibition due to the presence of organic acids as substrates (Chen et al., 2011; Akroum-Amrouche et al., 2011). Total dissolved nitrogen concentration was kept low by removing the particulate organic components from the DFE. In this way, the protein hydrolysis and further release of ammonium, which usually occurs at high pH values, was limited to avoid nitrogen inhibition on PNSB activity (Keskin et al., 2011). Moreover, high C/N ratios have been found to enhance the production of PHB (Koku et al., 2003; Argun et al.,