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Concomitant biohydrogen and poly-β-hydroxybutyrate production from dark fermentation effluents by adapted *Rhodobacter sphaeroides* and mixed photofermentative cultures

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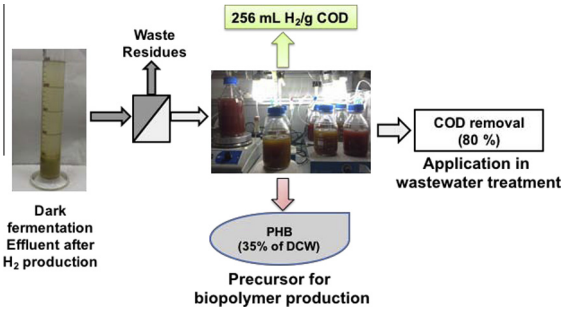
HIGHLIGHTS

- Dark fermentation effluent was valorized via photofermentative H_2 and PHB production.
- *R. sphaeroides* and mixed PNSB cultures were applied.
- H_2 (256 mL H_2/g COD) and PHB (32.5% DCW) production was achieved from *R. sphaeroides*.
- More than 70% COD removal from DFE was achieved by mixed PNSB cultures.

GRAPHICAL ABSTRACT



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ABSTRACT

This work aimed at investigating concomitant production of biohydrogen and poly-β-hydroxybutyrate (PHB) by photofermentation (PF) using dark fermentation effluents (DFE). An adapted culture of *Rhodobacter sphaeroides* AV1b (pH 6.5, 24 ± 2 °C) achieved H_2 and PHB yields of 256 (±2) N mL H_2/g Chemical Oxygen Demand (COD) and 273.8 mg PHB/g COD (32.5 ± 3% of the dry cells weight (DCW)), respectively. When a diluted (1:2) DFE medium was applied to the adapted pure and mixed photofermentative culture, the respective H_2 yields were 164.0 (±12) and 71.3 (±6) N mL H_2/g COD and the PHB yields were 212.1 (±105.2) and 50.7 (±2.7) mg PHB/g COD added, corresponding to 24 (±0.7) and 6.3 (±0) % DCW, respectively. The concomitant H_2 and PHB production from the PF process gave a good DFE post treatment achieving up to 80% COD removal from the initial DFE.

1. Introduction

Biological hydrogen (bio-H_2) processes have gained much interest as they could lead to low cost and renewable hydrogen production technologies which are environmentally benign (Das and Veziroglu, 2008). Biological hydrogen production processes can be categorized into light dependent processes such as biophotolysis and light independent processes such as dark fermentation (DF)

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and bioelectrochemical systems or microbial electrolysis cells (Ghimire et al., 2015a). In light dependent processes, water is broken down into H_2 and O_2 gas by algae and cyanobacteria. Alternatively, cyanobacteria or cyanophytes can also synthesize H_2 from water and inorganic carbon. Moreover, photofermentation (PF) is carried out by photosynthetic bacteria, where photodecomposition of organic compounds into H_2 occurs (Das and Veziroglu, 2008; Hallenbeck and Ghosh, 2009).

DF systems are a promising biological route for H_2 production due to its mild operational requirements (ambient temperature and pressure), higher conversion rates to H_2 and wide range of complex low cost waste biomass that can be used as feedstock (Ghimire et al., 2015a; Guo et al., 2010). However, dark fermentative conversion of complex organic biomass to H_2 produces by-products, mainly volatile fatty acids (VFAs), lactic acids and alcohols as soluble metabolites and un-hydrolyzed solid residues, leaving incomplete conversion of the organic biomass (Xia et al., 2013). Dark fermentative biohydrogen production is strongly correlated with the initial soluble carbohydrate fraction present in the substrates (Alibardi and Cossu, 2016; Guo et al., 2013; Monlau et al., 2012). Nonetheless, the soluble metabolites (organic acids and alcohols) present in DF residues can be further converted to biohydrogen through PF (Chookaew et al., 2015; Ghimire et al., 2015b; Rai et al., 2014).

Under anaerobic conditions, purple non-sulfur bacteria (PNSB) carry out an anaerobic photosynthesis using light as the energy source synthesizing bio-H_2. In PNSB, this takes place with reduced carbon sources such as organic acids by the nitrogenase enzyme in the presence of light (Barbosa et al., 2001). Photofermentative bio-H_2 production systems are attractive owing to their higher substrate to H_2 conversion potential compared to dark fermentative systems (Han et al., 2012).

Moreover, a theoretical H_2 potential of 12 mol of H_2 per mole of hexose could be realized by integrating a PF process with DF systems (Han et al., 2012). Thus, the integration of DF–PF can provide a practical solution to H_2 production along with the enhanced conversion of organic biomass. The integrated DF–PF process has been demonstrated by several studies (Rai et al., 2014; Tawfik et al., 2014; Yang et al., 2015). DF has the unique capability to utilize a wide range of complex waste biomass that can ensure the future supply of feedstock, and combining the two processes (DF + PF) can provide the further conversion of organic substrate in addition to enhanced H_2 yields.

The majority of the past studies carried out on combined DF–PF processes for H_2 production have used synthetic pure substrates containing major VFAs and pure microbial cultures (Chen et al., 2010; Tao et al., 2007). However, low cost complex waste biomass such as agricultural residues, organic fraction of municipal solid waste (OFMSW) and industrial wastes are attractive substrates for economically sustainable scaled up applications of dark fermentative bio-H_2 production. A number of studies have recently shown the possibility of combined DF–PF processes using waste biomass as the substrate (Chookaew et al., 2015; Rai et al., 2014; Zong et al., 2009). In this scenario, the use of dark fermentation effluents (DFE) generated from DF of complex organic waste and the application of PNSB for its capability to produce H_2 from DFE is attractive.

In addition to H_2 production, PNSB can synthesize poly-β-hydroxybutyrate (PHB) under certain conditions of physiological stress, such as high Carbon/Nitrogen (C/N) ratio or sulfur deprivation (Eroglu and Melis, 2011; Waligórska et al., 2009). Similar to H_2 production, PNSB synthesize PHB as a way to dissipate the excess reducing power (Waligórska et al., 2009). PHB is a polyhydroxyalkanoate, an interesting biodegradable polymer having applications in bioplastics production and medicine (Kemavongse et al., 2008). The amount of PHB accumulation depends on the PNSB strains and the process operational conditions (De Philippis et al., 1992; Montiel-Corona et al., 2015). In *R. sphaeroides*, Waligórska et al. (2009) found that accumulation of PHB increased 30-fold when the C/N ratio rose from 6 to 120. Although PHB biosynthesis is a H_2 competing pathway, its concomitant production with hydrogen raises future interests, as PHB possesses economic value as a precursor for biodegradable polymers (Koku et al., 2002).

Use of a mixed culture of PNSB is important for practical applications, as it reduces the asepsis costs involved when waste residues from DF systems are utilized. PF by pure cultures using spent DF residues generated from complex waste biomass has been reported in a few studies, i.e. sugarcane bagasse (Rai et al., 2014), glycerol (Chookaew et al., 2015) and cassava (Zong et al., 2009). However, there are limited studies that have been conducted using mixed PNS cultures for DFE conversion to H_2 (Montiel-Corona et al., 2015; Tawfik et al., 2014). In a recent study, Ghimire et al. (2015b) reported the 1.75-fold increase in H_2 yield from the integration of DF and PF processes using adapted *R. sphaeroides* cultures as inoculum. However, a long lag phase for H_2 production was observed, which was attributed to the initial PHB accumulation (Ghimire et al., 2015b).

The aim of this study was to investigate the concomitant production of H_2 and PHB from DFE (with and without dilution) obtained from the thermophilic DF of food waste, using adapted pure and mixed PNSB cultures under sterile and non-sterile conditions, respectively. H_2 production, PHB quantification and COD removal efficiency were the major parameters taken into consideration during this study of DFE valorization. Other hydrogen production performance parameters such as lag phase and time required to achieve 95% of the maximum H_2 production were considered for the evaluation of the photofermentative H_2 production performance.

2. Methods

2.1. Dark fermentative H_2 production

A thermophilic DF process, described elsewhere by Ghimire et al. (2015b), was set-up for continuous hydrogen production from food waste. A semi-continuous stirred 2 L serum bottle with a 1.5 L working volume and 500 mL headspace was used as DF reactor. The culture pH was 4.5 (±0.2). The H_2 yields and production rates were 104 (±17) N mL H_2/g VS and 208 (±35) N mL H_2/L/d at organic loading rates (OLRs) of 2.0 g VS/L/d and hydraulic retention time (HRT) of 4 days.

2.2. Photo fermentative H_2 production

2.2.1. PF inoculum

R. sphaeroides AV1b (kindly provided by professor Roberto De Philippis, University of Florence, Italy), isolated from the Averno Lake (Naples, Italy) by Bianchi et al. (2010), was used as inoculum for PF tests RS-I and RS-D. *R. sphaeroides* AV1b was first grown in RPN medium as described by Bianchi et al. (2010) containing (g/L): DL-malic acid, 2; sodium glutamate, 1.7; K_2HPO_4, 0.5; KH_2PO_4, 0.3; $MgSO_4{\cdot}7H_2O$, 0.4; NaCl, 0.4; $CaCl_2{\cdot}2H_2O$, 0.075; ferric citrate, 0.005; yeast extract, 0.4 and 10 mL of trace metals solution containing (mg/L): $ZnSO_4{\cdot}7H_2O$, 10; $MnCl_2{\cdot}4H_2O$, 3; H_3BO_3, 30; $CoCl_2{\cdot}6H_2O$, 20; $CuCl_2{\cdot}2H_2O$, 1; $NiCl_2{\cdot}6H_2O$, 2 and $Na_2MoO_4{\cdot}2H_2O$, 30 (Bianchi et al., 2010). Similarly, a reddish brown hydrogen producing mixed PNSB culture was obtained from anaerobic digestate, after 7–10 days incubation in the RPN medium.

R. sphaeroides AV1b was adapted in autoclaved (121 °C for 20 min) DFE, centrifuged and supplemented with buffer and other