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Replacement of sugars to hydrogen production by *Rhodobacter capsulatus* using dark fermentation effluent as substrate

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HIGHLIGHTS

- Hydrogen production was conducted by photo-fermentation using DFE as substrate.
- The provision of lactose and glucose has improved H_2 productivity.
- Hydrogen evolution was negligible in the presence of only organic acids.
- The maximum volumetric hydrogen productivity was 208.40 mmol H_2/L d.

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ABSTRACT

Hydrogen is a promising alternative for the increased global energy demand since it has high energy density and is a clean fuel. The aim of this work was to evaluate the photo-fermentation by *Rhodobacter capsulatus*, using the dark fermentation effluent as substrate. Different systems were tested by changing the type of sugar in the dark fermentation, investigating the influence of supplementing DFE with sugar and adding alternate and periodically lactose and glucose throughout the process. The supplementation of the DFE with sugar resulted in higher H_2 productivity and the replacement of the sugars repeatedly during the photo-fermentation process was important to maintain the cell culture active. By controlling the residual amount of sugar, bacteria inhibition was avoided; lactic acid, that was toxic to the biomass, was consumed and the metabolic route of butyric acid production was predominant. Under optimum conditions, the H_2 productivity reached 208.40 mmol H_2/L d in 52 h.

1. Introduction

In recent years, biofuel production, such as biogas, bio-diesel, bio-oil and bio-hydrogen, received considerable attention by many researchers in the world. In this perspective, hydrogen stands out as a highly energetic and clean fuel. The sustainable production of H_2 may be achieved by biological processes including: biophotolysis, photo-fermentation, dark fermentation and hybrid systems (Das et al., 2008). Considering this aspect, the production of hydrogen by biological pathway proves to be a promising alternative in the area, due to its high purity, potential for recycling and non-polluting nature (Amrouche et al., 2011; Costa et al., 2015).

Due to the benefits that biological routes provide for sustainable development of H_2 synthesis, it is extremely important to study these processes in order to make feasible its production (Cardoso et al., 2014). However, the isolated use of these routes has the limitation of low conversions, and, therefore, it is not yet competitive technology for thermochemical routes. Thus, a proposal to overcome these obstacles is the possibility of using a biological system that combines the use of different microorganisms in order to increase the H_2 production, this process being known as hybrid system. One type of hybrid system, which has been widely studied, is represented by the combination of fermentative and photosynthetic bacteria, in a sequential operation dark fermentation and photo-fermentation, wherein the fermentative bacteria, in the absence of light, utilizes a variety of carbohydrates as substrate to generate H_2 and organic acids, and the resultant organic acids are, in turn, the source for H_2 production by photosynthetic bacteria (Das and Veziroğlu, 2001).

In the metabolic process for the production of hydrogen by purple non-sulfur photosynthetic (PNS) bacteria, nitrogenase is the main enzyme associated, which catalyzes reactions in the medium with limiting nitrogen (N_2) is verified. This kind of enzyme use as

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substrates, organic acids or simple sugars and requires illumination and maintenance of anaerobic inert atmosphere. This is due to the irreversible inactivation that oxygen (O_2) causes in the nitrogenase; however, it is interesting to note that the PNS bacteria do not produce O_2 in their reactions. Besides, in general, studies demonstrated that, when in an environment where there is a limitation of N_2, nitrogenase activity is induced, resulting in the production of H_2 (Oh et al., 2004). In this context, it is important that the fermentation medium contains no nitrogen components in high concentrations able to inhibit the H_2 synthesis catalyzed by nitrogenase (Lázaro et al., 2012; Oliveira et al., 2014).

In order to enhance H_2 production, culture conditions such as the C/N ratios, carbon sources, pH, temperature, operation modes, co-culture systems, ion concentrations and light intensity have been widely studied (Eroglu et al., 1999; Oliveira et al., 2014; Abo-Hashesh et al., 2011; Abo-Hashesh et al., 2013; Özgür et al., 2010b; Zhang et al., 2010; Lázaro et al., 2012).

Hence this study investigated the production of hydrogen in batch and fed-batch processes, using 1.5 L reactor, by purple non-sulfur bacteria (*Rhodobacter capsulatus)* applying dark fermentation effluent as substrate. Thus, it was evaluated the effect of sugar supplementation. The sugars, lactose and glucose, were tested and the effect of alternating the addition of these sugars was also investigated. The response was higher hydrogen productivity based on the analysis of the influence of the concentration and type of carbon source.

2. Methods

2.1. Microorganism and culture condition

The strain of photosynthetic bacterium *Rhodobacter capsulatus DSM 1710* acquired by the DSMZ German Collection of Microorganisms and Cell Culture, was used in the assays of photo-fermentation. The activation and the cultivation of photosynthetic bacteria were performed in basal medium RCV (*R. capsulatus* V minimal medium) as Weaver et al. (1975), in distilled water with an initial pH of 6.8, and kept in germination chamber at 30 °C under illumination of 30 μmol photons m^{-2} s^{-1} by using fluorescent lamps (20 W).

In the H_2 production assays, the substrate used was the effluent from the dark fermentation, the composition of which was described (Romão et al., 2014) by applying an inoculum from an up-flow anaerobic sludge blanket reactor (UASB), gently donated by a dairy located in Uberlandia, MG (Brazil). For clarification, the substrate of the dark fermentation was a synthetic medium composed of (g/L): 3 KH_2PO_4, 7 K_2HPO_4, 1 $MgSO_4$, 3 yeast extract, 0.5 meat extract, 1 $(NH_4)_2SO_4$, 20 lactose, 0.6 $FeSO_4$, 1.5 $(NH_4)_2SO_4$ and 1 $MgSO_4$. Lactose was from the milk whey permeate, acquired from the *Sooro Concentrado Indústria de Produtos Lácteos Ltda* company (Brazil). The effluent resultant from the dark fermentation, rich mainly in organic acids, was centrifuged and supplemented with the components of the RCV medium. It should be pointed out that ammonium sulfate and malic acid, commonly found in the RCV medium, were not added. Ammonium sulfate could result in the inhibition of nitrogenase due to high ammonia content in the medium and malic acid was not added since the aim of the work was to evaluated the influence of using dark fermentation effluent (DFE) without supplementing it with a high cost raw material. The final pH of the dark fermentation is about 5.0, so it was adjusted to pH 6.8. This medium was heat-treated (121 °C, 20 min) in an autoclave to promote the inactivation of microbial consortium cells and precipitation of any colloidal materials, which could interfere with the penetration of light (Özgür et al., 2010a). After cooling, this treaty medium was inoculated with *R. capsulatus*.

2.2. Conditions for assays of hydrogen production

The experiments were performed in a bioreactor of 1.5 L with working volume of 700 mL, 65.3 mL of inoculum *R. capsulatus* and 634.7 mL of the medium of dark fermentation. The temperature of photo-fermentation was 35 ± 3 °C and was kept under a light intensity of 70 photons μmol m^{-2} s^{-1}, by using fluorescent lamps (25 W), and agitation of 130 rpm.

After inoculation with the *R. capsulatus*, argon gas was bubbled into the system for 15 min to ensure anaerobic conditions. The volume of produced biogas was measured from the use of a volumetric flow meter MilliGas-Counter of Ritter Type MGC-1.

For monitoring cell growth and production of metabolites, samples were withdrawn periodically during the photo-fermentation assay. Cell concentration of each sample was measured by absorbance and then the sample was centrifuged for 15 min at 7730*g*. The supernatant was collected for further analyses of metabolites and sugars by liquid chromatography. The cells, after the centrifugation, were discarded or, if necessary, used to analyze concentration of volatile solids (SV) by the gravimetric method. The biogas samples were stored in Gasometric ampoules (Construmaq LTDA, Brazil) for analysis of biogas composition.

Assays were conducted in the bioreactor in order to verify the behavior of consumption of organic acids and sugars during the photo-fermentative process. The different conditions for H_2 synthesis by photo-fermentation are shown in Table 1. The responses were analyzed in terms of volumetric productivity of hydrogen (H_2 mmol/L d) and specific productivity of hydrogen (mmol H_2/g_{vs} d), cell concentration (g_{vs}/L), accumulated hydrogen (mol), biogas volume (L) and concentrations of metabolites (organic acids and ethanol) and non-converted sugar (g/L).

2.3. Analytical methods

The biogas composition was determined by gas chromatography using the chromatograph Shimadzu model GC 17A equipped with a thermal conductivity detector (TCD) and a capillary column Carboxen 1010 (length 30 m, internal diameter 0.53 mm). The operating temperatures of the injection port, the oven, and the detector were 230, 35 and 230 °C, respectively. Argon was used as carrier gas.

The cell density (g_{vs}/L) was determined by spectrophotometry and the optical density at 660 nm converted to g_{vs}/L by predetermined correlation equation between OD_{660} and grams of volatile solids per liter (Clesceri et al., 1998).

Concentration of lactose, glucose and organic acids were determined using High Performance Liquid Chromatography (HPLC).

Table 1
Photo-fermentative conditions evaluated in the production of H_2.

Photo-fermentation	Characteristic
Condition 1	Only acids coming from DFE (substrate: lactose)
Condition 2	Synthetic medium (RCV) with lactose (15 g/L)
Condition 3	DFE (substrate: lactose) with addition of glucose (10 g/L) in the beginning and after 25 and 60 h
Condition 4	DFE (substrate: glucose) with addition of glucose (10 g/L) in the beginning and after 28 and 70 h
Condition 5	DFE (substrate: lactose) with addition of lactose in the beginning (10 g/L) during photo-fermentation (5 g/L – t = 31 h)
Condition 6	DFE (substrate: lactose) initially supplemented with lactose (10 g/L) and addition of glucose during photo-fermentation (6 g/L – t = 19 h)
Condition 7	DFE (substrate: lactose) initially supplemented with lactose (10 g/L) and alternate addition between glucose and lactose, both maintained at around 7 g/L