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Repeated batch cycles as an alternative for hydrogen production by co-culture photofermentation

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ABSTRACT

The use of hydrogen as an energy source represents an alternative to reduce the environmental impact since water is the only major by-product in its combustion. It can be used as an on-board fuel for motive power through internal combustion engine or fuel cell that converts chemical energy (an electrochemical device) and can be applied in several transportation devices. The hybrid system for hydrogen production by combining the dark fermentation followed by photofermentation has great potential. In the current work, the production of hydrogen was investigated in repeated-batch processes using dark fermentation effluent as substrate to pure culture (*Rhodopseudomonas palustris*) and co-culture purple non-sulfur bacteria (*Rhodopseudomonas palustris* and *Rhodobacter capsulatus*). The influence of glucose and milk whey permeate (MWP) on the hydrogen production was studied. The results showed that the system employing the co-culture and glucose kept the culture activity for a long time (679 h) and the amount of accumulated hydrogen was 0.98 ± 0.02 mol and maximum productivity reached 287.39 ± 5.75 mmol of H_2/L·day. The findings from alternating feeding between glucose and milk whey permeate, showed higher results for the amount of accumulated hydrogen (1.41 ± 0.01 mol). Besides, maximum productivity was of 266.60 ± 5.33 mmol of H_2/L·day.

1. Introduction

Environmental problems such as the aggravation of global warming caused by the burning of fossil fuels motivated several researchers to search for inexhaustible and sustainable energy sources [1]. In addition, these green sources might have their production processes with satisfactory cost and benefit. In this context, hydrogen is shown as a very promising alternative, being called by many authors as the fuel of the future, since it is an ideal energy carrier with a high-energy density of 1600 Wh/kg of tank system weight [2,3].

Besides that, its high energy density (141.86 kJ/g) makes it attractive compared to hydrocarbon-based fuels. In addition, its combustion releases only water as a by-product ($H_2 + 1/2\ O_2 \rightarrow H_2O$). Finally, hydrogen is also able to generate electricity through fuel cells [4].

Winter [5] discussed the transition of energy source based on the decarbonization and hydrogenation trends, that is, the shift from oil and gas to operationally non-carbon renewables and hydrogen. Trend analysis shows that the continuous shift from solid via liquid to gaseous energy carriers over the last century and a half and a prospective into the 21st century. The relative percentage of the share market of solids (wood, hay and coal) constantly decreased; there will a maximum production for liquids and after it will decline, and gases are on a permanent rise from methane-containing natural gas to hydrogen.

Currently, natural gas reform, hydrocarbon oxidation, coal gasification and water electrolysis are the main methods used to produce hydrogen [6]. However, the mentioned methods require high temperatures and pressures for operation, which demands a high amount of energy, besides these processes are of high cost [7]. In contrast, the synthesis of hydrogen *via* biological processes, which corresponds to only 1% of the total hydrogen production, is a promising alternative because it is performed at room temperature and ambient pressure, representing a reduction in energy consumption and, consequently, favorable energy balances [8]. Microorganisms like algae, cyanobacteria (photobiolysis of water), photosynthetic bacteria (photodecomposition of organic compounds) can produce hydrogen by using renewable sources such as solar energy, biomass or water and fermentative anaerobic bacteria (dark fermentation) degrade organic compounds into hydrogen without light [9].

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Among the biological systems for hydrogen production, dark fermentation has been highlighted due to its fast hydrogen conversion rates and the possibility of using different renewable sources as substrate [10–13]. However, this technology has some limitations, such as the large number of by-products (mainly organic acids) generated and, therefore, low hydrogen yields [14,15].

An alternative to minimize the high organic load remaining in the medium of the dark fermentation (in the form of organic acids) and increase the final hydrogen yield is to use a hybrid system, and the most common is the dark fermentation process followed by the photofermentation process [16,17]. In the photofermentation, from organic volatile fatty acids (VFAs), the non-sulfur purple (PNS) bacteria are capable of produce hydrogen in an anoxic environment and in the presence of light [18–20]. In particular, these non-sulfur purple bacteria are able to produce hydrogen efficiently, presenting high theoretical yields of substrate conversion [19].

In addition, the PNS bacteria are also capable of degrading sugars such as glucose, lactose, sucrose and soy molasses to produce hydrogen [20–22]. It is important to note nitrogenase is the enzyme responsible for catalyzing the hydrogen formation reaction by PNS bacteria and its performance is improved in the absence of oxygen and nitrogen [20].

The comparison of the three systems (dark fermentation, photofermentation and hybrid) developed by Manish and Banerjee [23], considering sugarcane as substrate, showed that photofermentation and two-stage process required 65% less sugarcane than in dark fermentation process since the hydrogen yields for those processes were higher. Consequently, with the decrease of sugarcane, the electricity expenses during milling stage were also reduced. Moreover, the hybrid system presented the highest reduction in greenhouse gas emission, energy efficiency and net energy ratio (ratio of hydrogen output to the non-renewable energy input). It is evident that this analysis depends on the substrate that is used. Nevertheless, this study proved the importance of improving photofermentation process in order to incorporate it to dark fermentation resulting in an effective and competitive process to overcome conventional methods.

Therefore, in the current work the hydrogen production by photofermentation was studied using the non-sulfur purple bacteria *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* in systems of pure culture and co-culture. The effluent from the dark fermentation supplemented with different carbon sources (glucose and milk whey permeate (MWP) were used as substrate. Besides, the type of the carbon source feeding strategy was evaluated, being carbon sources added isolatelly, alternately and simultaneously during photofermentation. The aim of investigating the repeated-batch using different cycles of substrate addition was to extend the duration of the hydrogen production processes.

2. Materials and methods

2.1. Microorganism and culture condition

The strains of non-sulfur purple (PNS) bacteria, specifically *Rhodopseudomonas palustris* and *Rhodobacter capsulatus* were used in the assays of photofermentation. The PNS bacteria were acquired by German Collection of Microorganisms and Cell Culture (DSMZ). The cultivation of photosynthetic bacteria were performed in basal medium RCV [24] and kept in a germination chamber at $30 \pm 1\ ^{\circ}C$ under illumination of 30 μmol photons $m^{-2}\ s^{-1}$ by using fluorescent lamps (20 W). Every 10 days, the cells were centrifuged at 8000 rpm for 15 min. The supernatant was discarded and the pellet was suspended in the fresh culture medium.

The fermentable medium used in the photofermentation experiments was the effluent from the dark fermentation by microbial consortium in a UASB reactor, composed by (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 (MWP), acquired from the company of *Sooro Concentrado Indústria de Produtos Lácteos Ltda* (Brazil).

A procedure was followed to make the effluent suitable for the photofermentation experiments. The firstly, the effluent was the centrifuged to remove the biomass (microbial consortium) present in the medium. Subsequently, it was supplemented with the components of the RCV basal medium, except malic acid, ammonium sulfate and thiamine. Ammonium sulfate was not added since ammonia is present in the medium from the dark fermentation. It is important to note the excess of ammonia into the medium would be impracticable as it would increase nitrogen concentration and would lead to inhibition of nitrogenase activity [20]. Thiamine was added after the heat treatment to avoid its degradation. The malic acid was not added since the purpose was to study only the sources of carbon from the organic acids that were present in the effluent and the added sugars.

The pH was adjusted to 6.8 with NaOH (1 M) and the fermentable medium was heat-treated in an autoclave (121 °C, 1 atm and 20 min). After cooling to room temperature, thiamine and the PNS bacteria were added as pure culture or as co-culture systems. The co-culture system used in the experiments was composed by *R. palustris* and *R. capsulatus* grown in RCV medium, in the proportion 1:1 (v/v).

2.2. Photofermentation experiments for hydrogen production

The photofermentation experiments were performed in a 1.5 L bioreactor - Fermenter module TE-2003/1.5-E1. The reaction volume was 700 mL, composed of 65.3 mL of inoculum and 634.7 mL of fermentable medium (from the dark fermentation). The pH of the medium was adjusted and maintained at 6.8 during the process by the addition of NaOH (1 M). The temperature, light intensity and agitation were $32 \pm 1\ ^{\circ}C$, 70 μmol photons $m^{-2}\ s^{-1}$ and 130 rpm, respectively. Argon gas was bubbled in the medium to ensure an anaerobic environment.

The monitoring of the photofermentation was performed by the collection of biogas samples and the fermentable medium. Besides, the cell growth, formed metabolites and the sugar consumption were also measured.

Experiments evaluated the production of hydrogen from the use of pure culture (*R. palustris*) and co-culture (*R. palustris* and *R. capsulatus*) systems, using glucose and lactose from MWP as sources of sugar. All evaluated conditions used the dark fermentation effluent as substrate. Thus, the following conditions were assessed:

- **Pure culture system (*R. palustris*):** assays of hydrogen production were carried out using glucose and lactose from MWP, respectively, with concentration of 10 g/L. The sugar (10 g/L) was replenished whenever its total consumption was detected.
- **Co-culture system (*R. palustris* and *R. capsulatus*):** analyses of hydrogen production were performed using a co-culture system composed of the bacteria *R. palustris* and *R. capsulatus* in a ratio of 1:1 (v/v). In the first mode, the strains were cultured separately in RCV medium and were mixed in the beginning of the photofermentation assay. This mode was studied applying cycles of addition of glucose or MWP. In the second mode, the effect of using the inoculum with the co-cultivated strains, maintained for 7 days prior to the preparation of the experiment, was evaluated as well, thereby creating an acclimated environment, and this acclimated co-culture mode was