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Continuous biohydrogen production from starch wastewater via sequential dark-photo fermentation with emphasize on maghemite nanoparticles

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ABSTRACT

Hydrogen production from starch wastewater via sequential dark-photo fermentation process was investigated. Two anaerobic baffled reactors (ABRs) were operated in parallel at an OLR of 8.11 ± 0.97 g-COD/L/d, and a HRT of 15 h. ABR-1 and ABR-2 was inoculated with pre-treated sludge and sludge immobilized on maghemite nanoparticles, respectively. Better hydrogen yield of 104.75 ± 12.39 mL-H_2/g-COD-removed was achieved in ABR-2 as compared to 66.22 ± 4.88 mL-H_2/g-COD-removed in ABR-1. The effluent of ABR-2 was used for further hydrogen production by photo fermentation in ABR-3. An overall hydrogen yield of 166.83 ± 27.79 mL-H_2/g-COD-removed was achieved at a total HRT of 30 h. 16S rRNA phylogeny showed that *Clostridium* and *Rhodopseudomonas palustris* species were dominant in ABR-1, ABR-2 and ABR-3, respectively.

1. Introduction

In Egypt, dependence on fossil fuels as a primary energy source certainly caused serious problems such as climate change, environmental degradation and human health. Moreover, the rise of oil and natural gas prices may drive the Egyptian economy toward alternative energy sources. Hydrogen energy has been recognized to be environmentally safe and alternative to fossil fuels, since it has triple the energy yield of conventional hydrocarbon fuels [1]. Hydrogen combustion produces only water without carbon monoxide, carbon dioxide, hydrocarbons, or fine particles [2]. Fortunately, starch wastewater has great potential to be used as a sole substrate for hydrogen production via dark fermentation process [3]. According to the reaction stoichiometry, a maximum of 553 mL hydrogen gas is produced from one gram of starch with acetate as by-product [4]. However, the hydrogen fermentative production is less for wastewater rich starch i.e. the maximum specific hydrogen production rate is 237 mL-H_2/g-VSS/d, from corn starch wastes [5]. Zhang et al. [4] found that mesophillic (37 °C) conditions provided a higher specific hydrogen yield of 480 mL-H_2/g-VSS/d, with 4.6 g/L starch as compared to thermophilic one (55 °C) (365 mL-H_2/g-VSS/d) using a mixed culture bacteria.

Although, hydrogen production from starch wastewater is a promising approach, the H_2 yield is still relatively low when only mixed culture bacteria is employed [6]. Several researchers [6,7] investigated the role of nanoparticles for improvement of the hydrogen production i.e. nanoparticles could remarkably enhance the bioactivity of hydrogen producing microbes and modify the bacteria growth as well as their metabolites distribution. The authors claimed that nanoparticles would play as "electron sinks" due to their affinity for electrons, which allows to further reduce protons to hydrogen. Others suggest existence of interactions between iron oxide nanoparticles and hydrogen producing bacteria resulting a faster hydrogen production rate [8]. The effect of hematite nanoparticles concentration and initial pH value on anaerobic mixed culture bacteria for hydrogen production from

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sucrose was investigated by Han et al. [7]. The optimum hematite nanoparticles concentration at an initial pH of 8.48 was 200 mg/L, with the maximum hydrogen yield of 3.21 mol-H_2/mol-sucrose (32.64% higher than the blank test). Zhang and Shen [9] investigated the enhancement effect of nanometer-sized gold particles on fermentative hydrogen production, and found that decreasing the size of gold nanoparticles can enhance the hydrogen production. However, the cost of gold nanoparticles is too much for practical application.

Photo-fermentation process could utilize soluble metabolites products and convert them into H_2 [10]. This process is performed by photosynthetic bacteria e.g. purple non-sulfur bacteria (*Rhodobacter and Rhodopseudomonas*) in the presence of light by using volatile fatty acids (VFAs) such as acetate (HAc) and butyrate (HBu) as substrate [11]. Sequential dark-photo fermentation could enhance the theoretical hydrogen yield from 4 to 12 mol-H_2/mol-glucose, and experimental hydrogen yield from 1.72 to 5.48 mol-H_2/mol-glucose [12].

In this study, a two-step process of sequential dark-photo fermentation was investigated for hydrogen production from starch wastewater. The effect of thermally pre-treated sludge immobilized on maghemite nanoparticles, with a ratio of 25 mg-(γ-Fe_2O_3)/g-VSS, on hydrogen production was investigated. Moreover, phylogenetic microbial analysis for dark and photosynthetic bacteria species was identified and detected in the reactors.

2. Material and methods

2.1. Lab scale anaerobic baffled reactors (ABRs)

Continuous hydrogen production experiments were carried out in anaerobic baffled reactors (ABRs) (Fig. 1) [13,14]. The reactors were designed and manufactured from Perspex material. The ABRs were rectangular shape consisting of five compartments of equal volume, with a total capacity of 30 L. Each reactor was provided by baffles to increase the contact time between mixed culture bacteria and the influent substrate. The bioreactors were continuously fed with starch wastewater collected from starch manufacturing company situated in 10th Ramadan city, Egypt. Anaerobic baffled reactors (ABR-1 and ABR-2) were operated at an organic loading rate (OLR) of 8.11 ± 0.97 g-COD/L/d, temperature of 30 °C, and a HRT of 15 h. The evolved gas was separately collected via porthole at the top of the reactors. The volume of hydrogen gas was daily measured by gas meter (drum type- thermometer-packing fluid). Sufficient mixing was provided to the feeding tank to prevent starch particles from settling. The pH of the influent was controlled and adjusted at values of 6.6–6.8 using sodium bicarbonate. Influent COD, carbohydrate, ammonia (NH_4-N), total Kjeldahl nitrogen (TKj-N) and total phosphorus were 5.07 ± 0.61 g/L, 4.63 ± 0.58 g/L, 33.70 ± 2.11 mg/L, 54.82 ± 6.72 mg/L, and 14.67 ± 2.9 mg/L, respectively.

2.2. Experimental set-up

In this investigation three experiments were conducted, (1) the effect of sludge immobilization on maghemite nanoparticles on fermentative hydrogen production (2) biohydrogen production via sequential dark-photo fermentation process and (3) identification and detection of microbial consortium bacteria in dark and photo fermentation processes.

2.2.1. 1st Experiment

Two anaerobic baffled reactors (ABR-1 and ABR-2) were operated in parallel. The ABR-1 was inoculated with pre-treated sludge at 90 °C for 30 min to inactivate non spore forming methanogens [15,16]. ABR-2 was seeded with pre-treated sludge immobilized on maghemite nanoparticles at a ratio of 25 mg-(γ-Fe_2O_3)/g-VSS.

Magnetite nanoparticles were prepared in two beakers. In beaker-1, 6.2 g $FeCl_3 \cdot 6H_2O$ is added to 4 g-$FeCl_2 \cdot 4H_2O$, and then dissolved in 25 mL deionized water with 1 mL-HCl (12 mol/L). In beaker-2, 250 mL of 1.5 mol/L NaOH solution was heated at a temperature of 80 °C. Afterwards, argon was flowed in beaker-2 for 10 min. with vigorous stirring. Then drops from beaker-1 were added to the former solution drop-wise under vibration for a few minutes providing smaller and homogenized particles. A black precipitate was quickly formed, which was allowed to crystallize completely after 30 min. Hematite nanoparticles were prepared by dissolving 3.2 g-$FeCl_3$ in 500 mL distilled water, and kept in the oven at 100 °C for 72 h. Two solutions were mixed and the color was slowly changed from light yellow to red. Maghemite nanoparticles were washed by distilled water several times, until the pH of the suspension was in a range of 7.0–7.5. The average particle dimension was 55 ± 5 nm. The maghemite nanoparticles were observed by scanning electron microscopy (SEM) (Fig. 2). For the SEM analysis, samples were dried then mounted on the stub by double-sided sticky tape and sputtered with gold. As seen in Fig. 2, maghemite nanoparticles are clearly situated on the surface of mixed culture bacteria.

2.2.2. 2nd Experiment

The effluent of the dark fermentation in ABR-2 was continuously used as a substrate for photo-fermentation process in ABR-3. The source of photosynthetic non-sulfur (PNS) bacteria was the same culture inoculated in ABR-1. The photo-reactor was illuminated by a light intensity of 3500 lx.

2.3. Batch experiments

During continuous experiments, 50 mL-sludge was collected from ABR-1 and ABR-2, and tested for hydrogen production in batch experiments. Four serum bottles with a capacity of 250 mL were used as shown in Table 1. The air was removed from the

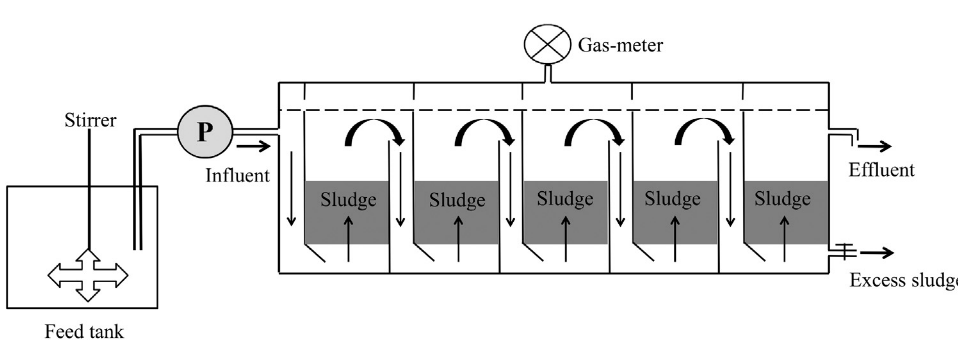

Fig. 1. Schematic diagram of ABR used for H_2 production from starch wastewater.