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Photosynthetic hydrogen production by alginate immobilized bacterial consortium

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HIGHLIGHTS

- Photosynthetic H_2 production from immobilized cells was studied.
- Immobilized cells produced more H_2 than free cells.
- Optimal granule size, cell loadings, and cell ages for granules were reported.
- Minimum substrate concentration and maximum illumination intensity were reported.
- Alginate matrix can provide shield to embedded cells from external challenges.

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ABSTRACT

Photosynthetic hydrogen production from organic wastewaters using immobilized mixed culture with photosynthetic bacteria (PSB) was studied. A PSB consortium was immobilized by alginate matrix to form granules. The so-yielded granules exhibited minimal diffusional resistances to substrates and to illumination penetration but still produced more hydrogen from synthetic wastewater than the free cells at identical experimental conditions. Optimal granule size, cell loadings, and cell ages for granules and the minimum substrate concentration and maximum illumination intensity requited to maximize hydrogen production were studied. The applied alginate matrix can provide shield to embedded cells from external challenges, likely the produced proton gradients from the surroundings.

1. Introduction

Hydrogen (H_2) is an energy carrier with high energy density (122 kJ/g), about 3.8 times to gasoline (Kumar et al., 2016). Biological hydrogen production is one of potentially feasible ways for supplying green hydrogen to sustainable society (Khan et al., 2016; Boboescu et al., 2016). Biological H_2 production can be categorized into dark and photo-fermentative pathways (Kumar and Chowdhary, 2016), in which photo-H_2 production by photosynthetic bacteria (PSB) is considered a promising technology which is driven by light energy (Guo et al., 2015; Lin et al., 2016) and can couple with wastewater treatment processes at ambient temperature and pressure (Hosseini et al., 2015).

Cell immobilization is widely used for enhanced fermentative hydrogen production (Kumar et al., 2016). Compared with free PSB cells, immobilized cells can effectively prolong H_2 production time and improve H_2 production rate. Guevara-Lopez and Buitron (2015) evaluated the different support materials for immobilizing *Rhodopseudomonas palustris* consortium for their capability to photofermentatively produce H_2 from volatile fatty acids as substrates. Another effective immobilization technology is to immobilize functional substances in polymeric matrix (Lai et al., 2016).

Zhu et al. (1999) studied hydrogen production from tofu wastewater by *Rhodobacter sphaeroides* immobilized in agar gels and found that the H_2 production lasted up to 50 h and the yield of hydrogen was 1.9 mL/mL. Ishikawa et al. (2008) used agar gel immobilized *Escherichia coli* to yield 6.7 mL/(L·h) H_2 from glucose

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wastewater. Seon et al. (1993) optimized H_2 production from glucose by immobilized *Rhodospirillum rubum* KS-301, maximizing H_2 production rate at 91 mL/h from glucose wastewaters. Zhang et al. (2016) immobilized *Rhodopsudomonas palustris* as biofilm on optical fiber for producing H_2 at 0.85 mmol/g-h with uniform lighting. Zagrodnik et al. (2015) immobilized Rhodobacter sphaeroides O. U.001 on porous glass plates or glass beads for photo-H_2 production from malic acid. The average H_2 production rate at 12.7 mL/L·h was achieved. All the above-mentioned studies utilized pure culture cells for immobilized photo-fermentative tests. However, to produce H_2 from waste materials cannot be realized in a sterilized environment; the pure culture would be continuously challenged by external strains. The mixed culture consortium always has a better adaption to environmental changes than pure culture systems (Lee et al., 2011).

Sodium alginate is a carbohydrate with chemical formula $(C_6H_7NaO_6)_x$, which is commonly used as immobilized substrate for bacterial cells or other organic/inorganic compounds (Yang et al., 2014). This study used alginate to immobilized cultivated PSB consortium with mixed bacterial species for producing hydrogen from synthetic medium via photofermentative pathway. Effects of substrate concentration, biomass loading, granule size, cell age and illumination intensity on photo-H_2 productivity were studied.

2. Materials and methods

2.1. Microorganisms and medium

The PSB HAU-M1 is a consortium composing of *Rhodospirillum rubrum, Rhodobacter capsulatus* and *Rhodopseudomonas palustris* (Lu et al., 2016). The cultivation medium had the following compositions (g/L): NH_4Cl, 1; $NaHCO_3$, 2; K_2HPO_4, 0.2; CH_3COONa, 3; $MgSO_4{\cdot}7H_2O$, 0.2; NaCl, 2; yeast extract, 1; and micronutrient solution (1 mL/L) with $FeCl_3{\cdot}6H_2O$ (5 mg/L), $ZnSO_4{\cdot}7H_2O$ (1 mg/L), $CuSO_4{\cdot}5H_2O$ (0.05 mg/L), H_3BO_4 (1 mg/L), $MnCl_2{\cdot}4H_2O$ (0.05 mg/L), and $Co(NO_3)_2{\cdot}6H_2O$ (0.5 mg/L). Argon gas purged the solutions to expel oxygen to form anaerobic conditions. The consortium was cultivated anaerobically at 30 °C at illumination of incandescent lamp (3000 lx). The solution pH was adjusted to 7.0 by adding 50% (w/w) KOH solution. The C, N, P and micro-nutrient of the fermentation medium were supplied at (g/L): NH_4Cl (0.4), $MgCl_2$ (0.2), yeast extract (0.1), K_2HPO_4 (0.5), NaC1 (2), and sodium glutamate (3.5).

2.2. Immobilization of bacterial cells

After PSB HAU-M1 consortium was cultivated in batch reactor to its exponential growth phase with cell concentration of 1.25 ± 0.02 g/L, the cells were washed with sterilized deionized water and were rapidly mixed with sodium alginate solution (at final sodium alginate concentration of 3%) at 30 °C. A peristaltic pump extruded the mixed solution as drops into 5% $CaCl_2$ solution for forming 6.5 ± 0.1 mm PSB granules. The formed granules were washed thoroughly with physiological brine and were ready for use. The entire immobilization protocol was under sterile condition.

2.3. Bio-H_2 production tests

Fresh swine manure was collected from farms in the eastern suburbs of Zhengzhou City, China. The physical and chemical characteristics of swine manure were analyzed following Standard Methods for chemical oxygen demand (COD), nitrogen, and total phosphorus (APHA, 2012), water 79% (w/w), COD 179 mg/g, nitrogen 8.4 mg/g, phosphorus 2.9 mg/g, pH 7–8. Prior to tests, the fresh pig manure was exposed to air in 120 rpm oscillator at 25 °C for four days. And then the suspension was screened with 40-mesh sieve and the filtrate was sterilized at 121 °C for 30 min. 1 g/L glucose was added to enhance growth of H_2-producing bacteria.

250-mL conical flasks sealed with rubber stoppers were applied in batch tests for photo-fermentation of the pre-prepared swine wastewater. The flasks were placed in thermostat at 30 °C with two incandescent lamps being placed at opposite sides of each flask to assure uniform incident light distributions.

The PSB granules formed in Section 2.2 were cultured with cultivation medium for 4 h before being fed into the photobioreactor. The control tests were conducted with the same ingredients (40 mL sodium alginate solution +60 mg cell (dry basis) +40 mL cell-free fermentation medium) at pH 7.0. Effects of cell age (cultivation time before immobilization) (36–108 h), biomass quantity in sodium alginate matrix (1–3 mg cells/mL), substrate concentration (1200–8000 mg COD/L), granule diameter (0.5–2.5 mm), and illumination intensity (2000–10000 lx) on H_2 production were studied (Table 1).

All runs were done in triplicate to assure the data reproducibility. Using glucose as model compounds, the COD decrease equivalent is assumed as follows: 8 g COD reduced = 1 g H_2 produced.

2.4. Analytical methods

The compositions of generated gas from the flasks were measured every 12 h using a gas chromatography (6820GC-14B, Agilent, USA). Nitrogen at a flow rate of 45 mL/min was the carrier gas; the temperatures of the injector, detector and column were 100, 80 and 150 °C, respectively. The solution pH value was measured by a pH meter (PHS-3C, Shanghai, China). The optical density of cell biomass was determined at 660 nm using a spectrophotometer (HP8453 Ultraviolet Spectrophotometer, Agilent, USA) (Pattanamanee, 2012).

3. Results and discussion

3.1. Immobilized cells on photo-H_2 production

In this section the photo-H_2 production using immobilized granules at 30 °C, 5500 mg COD/L, pH 7, 2.0 mm granules with 72 h cell age and the control test with free suspended cells (with identical quantity of alginate and other ingredients) is compared (Table 2). As this table shows, the immobilized cells produced 1.37–1.43 times H_2 at identical conditions with free cells. For instance, at 120 h, the granules produced 152.1 mL H_2 (=reduction of 1120 mg COD/L) while free cells yielded 977 mL H_2 (=reduction of 977 mg COD/L).

3.2. Single-factor photo-hydrogen tests

3.2.1. Effects of immobilized biomass on H_2 production

The hydrogen production rate at 30 °C, pH 7, 8000 lx, 2 mm granule size, 72 h cell age, 5500 mg COD/L is increased with immobilized biomass quantity, but not in proportionality (Fig. 1a). For instance, at 144 h, the H_2 production quantity was 142 mL for 1 mg/mL granules and was 186 mL for 2 mg/mL. At 2.5 mg/mL, the H_2 production peaked to 193 mL at 144 h; but at 3 mg/mL, the H_2 production reduced to 164 mL. Restated, there is an optimal biomass loading in immobilized matrix, 2.5 mg/mL in the present case.

During the tests, the solution COD was declined with time, while the pH was reduced from 7 to 5.5 in the first 20-h testing. The solution pH was declined owing to the production of volatile fatty acids in substrate hydrolysis. The COD decreasing rate was