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Enhanced photo-H_2 production by *Rhodopseudomonas faecalis* RLD-53 immobilization on activated carbon fibers

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ABSTRACT

Activated carbon fibers (ACFs) were firstly applied as fluidized solid carrier to immobilize photo-fermentative bacteria (PFB) for H_2 production in batch culture. The observations by scanning electronic microscopy (SEM) demonstrated the close interaction between ACFs and PFB. The amount of immobilized bacteria and the performance of H_2 production were strongly affected by specific surface area, length and amount of ACFs, respectively. Large specific surface area provided more surface attachment sites and more PFB were immobilized. ACFs with proper length avoided intertwining with each other and better fluidized during reactor operation. Excessive amount of ACFs not only limited the light conversion efficiency, but also increased biofilm detachment, resulting in low H_2 yield. The maximum yield (3.08 mol H_2 mol^{-1} acetate) and rate (32.85 ml l^{-1} h^{-1}) of H_2 production were obtained, using specific surface area (1500 m^2 g^{-1}), length (1 mm) and amount (0.8 g l^{-1}) of ACFs. Compared with the conventional solid carriers, ACFs were effective solid carriers to immobilize PFB for improving H_2 production, due to bacteria immobilized on the external surface of fluidized ACFs and formed a layer of dense biofilm.

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1. Introduction

H_2 is considered an attractive energy carrier for the future, due to its potentially higher efficiency of conversion to usable power, low generation of pollutants and high energy density [1]. Photo-fermentation H_2 production as one of the most promising processes for producing a renewable energy source has been studied widely [2,3]. However, one of the main obstacles for the commercialization of the process is low yields and rates of H_2 production [4]. In order to make photo-fermentation process close to practical production, several modes of photo-H_2 production have been employed to improve H_2 production performance, such as combination of the dark and photo-fermentation [5,6], repeated fed-batch mode operation [7] and separation of CO_2 from reaction system [8], etc. In addition, bacterial immobilization technology has been suggested as an effective method to improve photo-H_2 production [9,10]. Immobilized PFB have been applied into photo-H_2 production in batch [11], semi-continuous [12], and continuous [9,10] cultures and H_2 production was significantly improved.

The immobilization of PFB caused higher cell densities with consequent increases in reaction rates and productivity. Most commonly used techniques for bacterial immobilization involve cell entrapment [11] or attachment [9,10]. Entrapment of microbial cell in polymeric matrices allows better biomass retention under low hydraulic retention time and creates a local anaerobic environment for H_2 fermentation [13].

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However, cell entrapment was not feasible in continuous operation, due to mass transfer limitations arising from cell entrapment [14], structural damage caused by biogas production [15] and leakage of cells through instability of polymeric matrices [16]. In addition, the dense packing of immobilized PFB granules limits light penetration, which has an adverse effect on light conversion efficiency [17]. The natural attachment methods can keep the maximum cell viability and biochemical activity after adhesion [18], owing to the natural formation of biofilm. Therefore, for the photo-fermentative H_2 production system, surface attachment seems to be a more reasonable approach than cell entrapment.

Different solid carriers have been used for the immobilization of PFB, such as porous glass [17], optical fiber [19] and granule activated carbon [9]. However, most of these conventional solid carriers, such as porous glass [17], expanded clay and granule activated carbon [9] just deposited on the reactor bottom. And light energy decreased quasi-exponentially upon light path through the fermentation medium and 90% of the incident light energy was absorbed in the light path of 1.0 cm [20]. In that event, most of immobilized bacteria cannot receive light energy and just consume the substrate without H_2 production, even with some transparent solid matrices. This greatly limited the application of immobilization technology for photo-H_2 production. Therefore, the key to further develop immobilization for photo-H_2 production is using the fluidized solid matrices as carriers. In that case, light energy could reach every place of solid carrier in the fluidization, which could greatly reduce the shading effect of solid carrier.

As a novel porous carbon material, the outstanding advantages of activated carbon fibers (ACFs) are the smaller fiber diameter, more concentrated pore size distribution and excellent adsorption capacity [21]. These minimize diffusion limitations and allow rapid adsorption, compared with conventional solid carriers. In addition, carbon based materials are commonly used as biocarriers because of their excellent biocompatibility. So far, the information about ACFs as carrier immobilizing bacteria for enhancing bio-H_2 production has not reported. Thus, we first applied porous ACFs as fluidized solid carrier to improve photo-fermentation H_2 production.

In this work, the effects of specific surface area, length and amount of ACFs on bacterial immobilization and photo-H_2 production were studied. Comparison of ACFs with conventional solid carriers in H_2 production was also investigated.

2. Material and methods

2.1. Bacterium and medium

The photo-H_2 producer used in this study was *Rhodopseudomonas faecalis* RLD-53 [22]. Acetate was used as the sole carbon source in the medium for H_2 production. The culture medium of strain RLD-53 consisted of (in g l^{-1}) acetate, 4.1; KH_2PO_4, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 4H_2O$, 0.2; $CaCl_2$, 0.08; NaCl, 0.1; glutamate, 1.69; EDTA-Na, 0.1; $FeSO_4 \cdot 7H_2O$, 0.012; L-cysteine·HCl·H_2O, 0.5; trace element solution, 1 ml; vitamin solution, 1 ml. The trace element solution contained $FeCl_2.4H_2O$ 1.8 g, $CoCl_2.6H_2O$ 0.01 g, $CuCl_2.2H_2O$ 0.01 g, $MnCl_2.4H_2O$ 0.7 g, $ZnCl_2$0.1 g, $H_3BO_3$0.5 g, $NaSeO_3.5H_2O$ 0.01 g, ddH_2O 1.0 l. The vitamin solution contained Biotin 0.1 g, Niacin 0.35 g, Thiamine dichloride 0.3 g, p-Aminobenzo acid 0.2 g, Ca-panthothenate 0.1 g, Vitamin B_{12} 0.05 g, Pyridoxolium hydrochloride 0.1 g, ddH_2O 1.0 l. The initial pH of the medium was adjusted to 7.0 by 0.1 N NaOH or HCl.

2.2. Preparation of ACFs

ACFs were obtained from Sinocarb Carbon Fibers CO., LTD (Anshan, China) with three different specific surface areas, 1000, 1200 and 1500 m^2 g^{-1}. Prior to use, ACFs were washed several times with distilled water and then dried at 105 °C until the weight was invariant.

2.3. Photo-bioreactor operation and data analyses

The H_2 production experiments were carried out in triplicate sealed photo-bioreactor and filled with argon to maintain anaerobic conditions. The photo-bioreactor was 100 ml glass-made vessel with working volume of 80 ml. The photo-bioreactor was illuminated with external light sources (60 W incandescent lamps). The average light intensity on the outside surface of the reactor was maintained at 150 W m^{-2}. The photo-bioreactor was stirred at 120 rpm at constant temperature of 35 °C. *R. faecalis* RLD-53 in the mid-exponential growth phase was inoculated into reactors.

H_2 production performance by immobilized PFB was evaluated by modified Gompertz equation, including H_2 production potential, H_2 production rate and lag times. After cumulative H_2 production curves were obtained over the course of an entire batch experiment, the modified Gompertz equation as shown in Eq. (1) was applied to determine the H_2 production potential, H_2 production rate and lag phase [23,24].

$$H = H_{max} exp\left\{ -\exp\left[\frac{R_{max} e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where t is culture time (h); H is cumulative H_2 production (ml l^{-1} medium); H_{max} is maximum cumulative H_2 production (ml l^{-1} medium); $e = 2.71828$; R_{max} is maximum H_2 production rate (ml l^{-1} h^{-1}); and λ is the lag phase time (h).

The light conversion efficiency was calculated based on Eq. (2) [25].

$$\eta = \frac{33.61 \times \rho_{H_2} \times V_{H_2}}{I \times A \times t} \times 100 \quad (2)$$

where ρ_{H_2} is the density of H_2 (g l^{-1}); V_{H_2} is the volume of H_2 produced (l); I is the light intensity (W m^{-2}); A is the irradiation area (m^2); and t is the duration of H_2 production (h).

Substrate conversion efficiency of acetate was determined as the ratio of moles H_2 produced experimentally to moles H_2 that could be theoretically produced if all the acetate is consumed through the stoichiometric Eq. (3).

$$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (3)$$

2.4. Analytical method

Light intensity was measured at the surface of photo-bioreactor with solar power meter TENMARS TM-207

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