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Biohydrogen production from algal biomass (*Anabaena* sp. PCC 7120) cultivated in airlift photobioreactor

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ABSTRACT

The present study deals with the optimization of pretreatment conditions followed by thermophilic dark fermentative hydrogen production using *Anabaena* PCC 7120 as substrate by mixed microflora. Different airlift photobioreactors with ratio of area of downcomer and riser (A_d/A_r) in range of 0.4–3.2 were considered. Maximum biomass concentration of 1.63 g L^{-1} in 9 d under light intensity of 120 μE m^{-2} s^{-1} was observed at A_d/A_r of 1.6. The mixing time of the reactors was inversely proportional to A_d/A_r. Maximal H_2 production was found to be 1600 mL L^{-1} upon pretreatment with amylase followed by thermophilic fermentation for 24 h compared to other methods like sonication (200 mL L^{-1}), autoclave (600 mL L^{-1}) and HCl treatment (1230 mL L^{-1}). The decrease of pH from 6.5 to 5.0 during fermentation was due to the accumulation of volatile fatty acids. Amylase pretreatment gave higher reducible sugar content of 7.6 g L^{-1} as compare to other pretreatments. Thermophilic fermentation of pretreated *Anabaena* biomass by mixed bacterial culture was found suitable for H_2 production.

1. Introduction

The rapid growth of the world population and speedy industrialization have led to sharp increase in global energy consumption using conventional fossil fuel [1]. However, the use of fossil fuels is associated with environmental pollution mainly due to the greenhouse effect [2]. Thus researchers around world now increasing their efforts for developing renewable energy sources, which are not only economic but also environmentally friendly [3–5]. Biomass is one of the most promising renewable resources used to generate different types of biofuels, such as biodiesel, bioethanol, biogas and biohydrogen [6–8]. Hydrogen is widely recognized as clean and potential candidate as it has highest energy density among any known fuels (143 GJ $tonne^{-1}$) and is the only common fuel that does not produce CO_2 as a by-product when used in fuel cells for electricity generation. Biological hydrogen production from biomass is considered one of the most promising alternatives for sustainable green energy production [9]. With the development and commercialization of fuel cells, hydrogen production from biomass is being considered as an alternative energy source for decentralized power generation.

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Currently, the sucrose and starch crops (e.g., sugarcane and corn, first generation) as well as lignocellulosic materials (e.g., rice straw and switch grass, second generation) are being used as biomass feedstocks for biofuel. However, using agricultural crops or agricultural waste as feedstock for bioenergy production has problems mainly due to the limited arable lands and water supply [10]. Furthermore, the high cost involved in hydrolysis of lignocellulosic materials is one of weakness of the process due to high lignin content in biomass, making the saccharification process very difficult [11,12]. Microalgae and cyanobacteria have recently been considered as a third generation feedstock for biofuel production [13,14]. However, since some microalgae/cyanobacteria species have high carbohydrate content, in terms of starch/glycogen and cellulose, they are also excellent substrates for biohydrogen production [15]. The cyanobacterial biomass-based carbohydrates are mainly in the form of glycogen, cellulose and exopolysacharide (with the absence of lignin), are thus much easier to convert to monosaccharides when compared with lignocellulosic materials [16]. Cyanobacteria like *Anabaena* sp., *Synechocystis* PCC6803, *Synechococus*, *Spirulina* sp. have been shown to accumulate a large amount of carbohydrates (20–30% of the dry weight) [17,18]. *Anabaena* sp. PCC 7120 is nitrogen fixing cyanobacteria which requires minimal nutrient requirements and fix nitrogen from atmospheric nitrogen. The carbohydrates in *Anabaena* sp. PCC 7120 mostly come from glycogen stored in cytoplasm and several polysaccharides associated with cell envelop, which are not readily fermentable for hydrogen production by microorganisms [19]. Therefore, prior to fermentation, the polysaccharides of microalgae should be hydrolysed to fermentable sugars. Several methods for algal cell pretreatment methods have been used with individual advantages and disadvantages by earlier studies chemical (acid and alkaline) or enzymatic hydrolysis is common methods used for this purpose [20,15]. However, the most efficient method for cyanobacteria has not yet been settled.

In addition, using carbohydrate-rich cyanobacterial biomass for biohydrogen production is advantageous, since it grows faster and fixes CO_2 at a higher rate than terrestrial plants in pneumatic photobioreactors. Airlift photobioreactors are a special type of pneumatic photobioreactors which currently are receiving much attention for potential application to various cyanobacterial cultivation systems [21]. Their self-generated liquid circulation has been shown to give them added advantages, of improved heat transfer and mixing, compared to the bubble columns due to the presence of draft tube. The liquid circulation is the result of the difference in hydrostatic pressure between the riser and the downcomer section [22]. The hydrostatic pressure is function of liquid velocities which is mainly dependent on the ratio of the cross-sectional area of the two reactor zones in raiser and downcomer [23,24].

Accordingly, the present study aimed to investigate the influence design configuration of airlift system, i.e. downcomer-to-riser cross-sectional areas ratio (A_d/A_r) on production of *Anabaena* sp. PCC 7120 biomass and subsequent use of the same as substrate to produce H_2 in dark fermentation using mixed consortia of thermophilic bacteria.

2. Materials and methods

2.1. Microbial culture development

Anabaena sp PCC 7120 was inoculated into 250 mL Erlenmeyer flasks that contained 100 mL of the $BG11_0$ medium and incubated in a shaking incubator at 30 °C and 150 rpm. The $BG11_0$ medium contains: 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 6.0 mg citric acid, 6.0 mg ferric ammonium citrate,1.0 mg Na_2EDTA, 0.02 g Na_2CO_3, and 1.0 mL trace metal solution A5 per 1 L. The trace metal solution A5 of contained 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.39 g $Na_2MoO_4 \cdot 2H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, and 49.4 mg $Co(NO_3)_2 \cdot 6H_2O$ per 1 L. The culture was illuminated from the top of the shaking incubator by fluorescent lamps, and the light intensity was adjusted to various levels by changing the number of fluorescent bulbs used.

An enriched thermophilic mixed culture (collected from the anaerobic digester of M/s. IFB AGRO Industries Ltd., Noorpur, India) capable of producing hydrogen was used in the present study. Media used for this study consist of Na_2HPO_4 (4.2 g L^{-1}), KH_2PO_4 (1.5 g L^{-1}), NH_4Cl (1.95 g L^{-1}), $MgCl_2$ (0.18 g L^{-1}), yeast extract (2.0 g L^{-1}), glucose (10 g L^{-1}), cysteine HCl (1 g L^{-1}), vitamins solution (DSMZ medium No. 141, German Collection of Microorganisms and Cell Cultures). An inoculum size of 10% v/v and inoculum age of 6 h was used for all the experiments. Nucleotide sequences reported in this microbial consortium have been assigned following GeneBank accession numbers JQ997158–JQ997161. This microbial consortium has the ability of utilizing different complex sugars for production of hydrogen [25].

2.2. Construction and operation of photobioreactors

Airlift photobioreactors were made of clear acrylic plastic of 5 mm thick, an internal diameter of 7.2 cm, height of 45 cm and nominal working volume of 1.4 L and equipped with internal draft tubes as depicted in Fig. 1 [19]. The ratio between the downcomer and riser cross-sectional areas (A_d/A_r) was varied from 0.7 to 3 by changing the diameter of draft tube (Table 1). The dispersion system for the reactors consisted of a 1.0 cm diameter spherical air sparger located at the centre of the column. The dispersion system was made up of a perforated tube of internal diameter 4 mm. The reactors were continuously illuminated with 10 × 40 W fluorescent lamps. Various intensity of light was achieved either by changing the distances between the photobioreactor and the light source or by number of fluorescent lamps used at a time. Rotameters were used to monitor the flow rate of filtered air and pure CO_2. The microalgae were cultivated in the three parallel photobioreactors in batch mode for experimental study of single parameter. The designed experimental set ups were run for 9 days. All parameters were maintained constant unless mentioned otherwise.

2.3. Algal biomass pretreatment

Prior to pretreatment the elemental composition of *Anabaena* sp. PCC 7120 was determined using CHN analyzer