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Simultaneous metabolism of benzoate and photobiological hydrogen production by *Lyngbya* sp.

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ABSTRACT

Cyanobacteria are an inexpensive and sustainable source of photobiological H_2 production due to their simple nutritional conditions. Combination of toxic contaminant degradation with H_2 production is a promising approach in wastewater treatment. This study demonstrated that *Lyngbya* sp., a filamentous cyanobacterium, can generate H_2 in the presence of benzoate which is a central intermediate during the anaerobic degradation of many aromatic compounds. The highest H_2 production rate of 17.05 μmol H_2/g Chla/h was obtained in the second cycle and 600 mg/L benzoate was depleted within 64 h in H_2 production test. The strain has shown a higher H_2-producing capacity, which was comparable to those of certain noted strains such as N_2-fixing filamentous *Anabaena variabilis* PK84 and non-N_2-fixing filamentous *Microcystis PCC* 7806. The co-metabolism of benzoate for H_2 production by *Lyngbya* sp. makes it an interesting model strain for clean energy production and hazardous pollutant removal.

1. Introduction

Hydrogen is regarded as a clean, renewable and alternative energy to fossil fuels [1]. Microalgae can evolve H_2 from water using sunlight as an energy source under anaerobic conditions [2]. Cyanobacteria are ideal microbes for photobiological H_2 production because they require the simplest nutritional conditions [3]. Some filamentous cyanobacteria and a unicellular diazotrophic were found to produce high amounts of H_2 [4,5]. Photobiological H_2 production of a cyanobacterium is significantly influenced by many environmental factors including oxic/anoxic, light/dark period, light intensity, temperature, pH and salinity [3,6].

Aromatic compounds are widely used as industrial chemicals and consequently have a high potential to become environmental pollutants [7]. Microalgae, the primary producers in many aquatic ecosystems, are capable of removing these hazardous pollutants and can grow photosynthetically without the addition of any organic carbon sources [8,9]. Phenol degradation by green algae, i.e., *Chlorella* spp., *Scenedesmus obliquus* and *Spirulina maxima*, has been well documented by many studies [10]. *Ochromonas danica*, a golden-brown alga, has the metabolic capacities to degrade phenol and phenolics [11]. However, at present, the biodegradative capabilities of aromatic compounds by prokaryotic cyanobacteria remain relatively unknown.

The present work aimed to explore the potential for *Lyngbya* sp., a filamentous cyanobacterium, to degrade aromatic compounds. Benzoate was selected as the modeled hazardous pollutant because it is the simplest aromatic acid and also the product of oxidative catabolism of many aromatic hydrocarbons. Furthermore, the feasibility of harvesting H_2 from wastewater containing benzoate by *Lyngbya* sp. was also investigated.

2. Materials and methods

2.1. Strain and growth conditions

Lyngbya sp. isolated from an upflow anaerobic sludge blanket reactor treating phenol-containing wastewater was used. It was pre-cultured in a basal medium containing (mg/L): benzoate 500; $NaNO_3$ 800; KH_2PO_4 250; $MgCl_2$ 250; $CaCl_2$ 100; $NaHCO_3$ 200; $(NH_4)_6Mo_7O_{24}$ 0.5; $CoCl_2 \cdot 6H_2O$ 0.01; $ZnCl_2$ 0.1; $CuCl_2$ 0.01; H_3BO_3 2; EDTA-2Na 2. The 150 mL culture was grown in 300-mL glass reactors with rubber-stoppers at a temperature of 30 ± 1 °C, pH of 7.4 and light intensity of 1000 lux.

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2.2. Hydrogen production experiment

The strain at the late exponential phase was harvested by centrifugation (4000× *g* for 5 min), washed three times with deionized water and resuspended in fresh H_2 production medium consisting of (mg/L): benzoate 600; KH_2PO_4 50; K_2HPO_4 50; $MgCl_2$ 50; $CaCl_2$ 50; $NaHCO_3$ 50; NH_4Cl 50; $(NH_4)_6Mo_7O_{24}$ 0.5; $CoCl_2 \cdot 6H_2O$ 0.01; $ZnCl_2$ 0.1; $CuCl_2$ 0.01; H_3BO_3 2; EDTA-2Na 2. Approximately 100 mL of the cyanobacterium suspensions was placed in 150-mL glass reactors, flushed with argon for 10 min and sealed with rubber-stoppers. The pH, temperature and light intensity were adjusted to 7.4, 32 ± 1 °C and 4000 lux, respectively.

After the H_2 evolution ceased, the cultures collected from each reactor were used to perform the H_2 production test again under the same conditions described above. The process was repeated three times to evaluate the stability of H_2 production by *Lyngbya* sp.

2.3. Analytical methods

The light intensity, chlorophyll *a* amount, cell dry weight and H_2 production were measured according to the methods described in previous studies [12]. The morphology of *Lyngbya* sp. was observed using a light microscope (Olympus CX41, Japan). The evolved gas collected from the headspace of the reactors using a gas-tight lockable syringe was injected into a gas chromatograph (GC, SP-6800A, China) to monitor H_2 and O_2 contents. The operational parameters for GC could be found in our previous work [13]. The concentration of benzoate was determined by high-performance liquid chromatography (Agilent 1100, USA) using a C-18 column, with a UV detector at 225 nm. The mobile phase used was 0.3% H_3PO_4 in 60% methanol at a flow rate of 1.2 mL/min.

3. Results and discussion

3.1. Cyanobacterium growth

The morphology of *Lyngbya* sp. is shown in Fig. 1, which is classified as a non-N_2-fixing filamentous cyanobacterium due to the lack of heterocysts. The growth of *Lyngbya* sp. was examined in liquid medium in the presence of 500 mg/L benzoate.

As shown in Fig. 2, the cyanobacterium concentration increased with the incubation time. To describe the growth of *Lyngbya* sp. in detail, the following modified logistic model was used [14]:

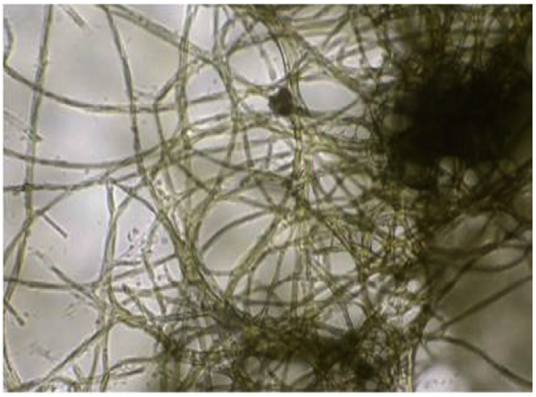

Fig. 1. Morphology of *Lyngbya* sp. under a light microscope.

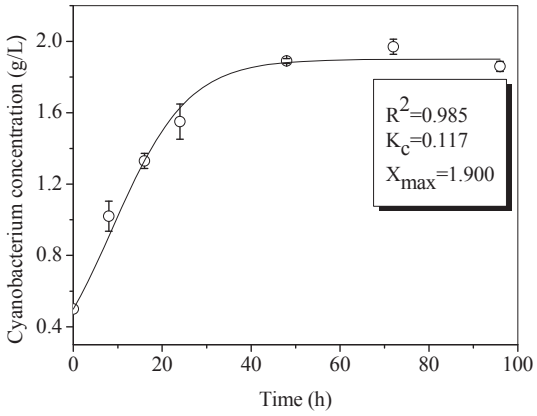

Fig. 2. Growth of *Lyngbya* sp. in liquid medium in the presence of 500 mg/L benzoate.

$$X = \frac{X_0 \times \exp(k_c \times t)}{1 - (X_0/X_{max})(1 - \exp(k_c \times t))} \quad (1)$$

where X is the cyanobacterium dry weight (g/L), X_o is the initial cyanobacterium dry weight (g/L), K_c is the apparent specific growth (h^{-1}) and X_{max} is the maximum cyanobacterium dry weight (g/L). A high correlation coefficient (R^2) of 0.985 showed that the experimental data fitted the modified logistic model well (Fig. 2). The maximum apparent specific growth (K_c) reached 0.0395 h^{-1}, suggests that the cyanobacterium number of *Lyngbya* sp. could be doubled after incubation for 8.54 h. The maximum cyanobacterium dry weight of 1.90 g/L was achieved in a 48 h incubation period and 500 mg/L benzoate was consumed completely. The results also indicate that *Lyngbya* sp. is capable of metabolizing both an inorganic carbon source and an organic carbon source to support its growth. However, no H_2 generation was observed in growth phase of *Lyngbya* sp. with 800 mg/L $NaNO_3$ as the nitrogen source.

3.2. Photobiological H_2 production

Fig. 3 shows the H_2 production profiles in two reactors under nitrate-deprivation conditions, the control reactor without the addition of benzoate and the other reactor with 600 mg/L benzoate as the carbon source. No H_2 generation was observed from the control reactor, whereas H_2 evolution was detected from the other

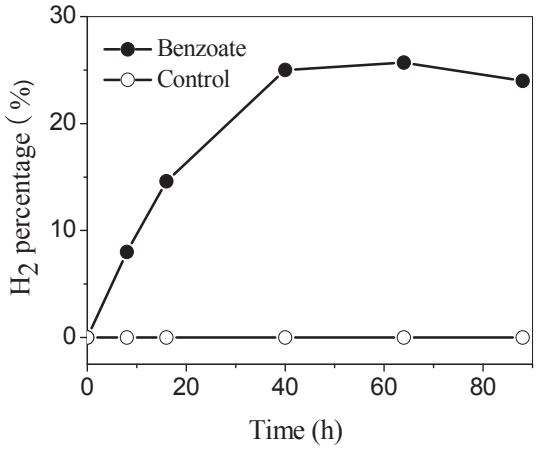

Fig. 3. H_2 percentage profiles in two reactors: (●) with benzoate; and (○) without benzoate.