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Biomass and pigments production in photosynthetic bacteria wastewater treatment: Effects of photoperiod

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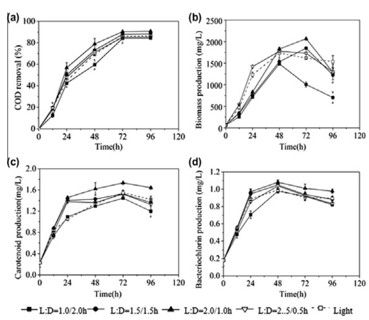
HIGHLIGHTS

- Different photoperiods were used for the cultivation of PSB.
- PSB showed the highest biomass production and COD removal with light/dark of 2 h/1 h.
- PSB showed higher biomass production and biomass yield with higher light frequency.
- Pigments contents were much higher with low light frequency of 2–4 times/d.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed at enhancing the bacterial biomass and pigments production in together with pollution removal in photosynthetic bacteria (PSB) wastewater treatment via using different photoperiods. Different light/dark cycles and light/dark cycle frequencies were examined. Results showed that PSB had the highest biomass production, COD removal and biomass yield, and light energy efficiency with light/dark cycle of 2 h/1 h. The corresponding biomass, COD removal and biomass yield reached 2068 mg/L, 90.3%, and 0.38 mg-biomass/mg-COD-removal, respectively. PSB showed higher biomass production and biomass yield with higher light/dark cycle frequency. Mechanism analysis showed within a light/dark cycle from 1 h/2 h to 2 h/1 h, the carotenoid and bacteriochlorophyll production increased with an increase in light/dark cycle. Moreover, the pigment contents were much higher with lower frequency of 2–4 times/d.

1. Introduction

Photosynthetic bacteria (PSB) widely distribute in soil, water and wastewater (Okubo et al., 2006). Since 1960s, PSB have been used to treat diary wastewater, soybean wastewater, food wastewater, fermented starch wastewater and domestic wastewater (Prachanurak et al., 2014; Chitapornpan et al., 2013; Ela et al., 2010; Hülsen et al., 2014). PSB wastewater treatment has recently achieved increasing attentions. In comparison to traditional methods, PSB wastewater treatment has the advantage of accumulating valuable biomass in together with pollutant removal. PSB biomass is rich in single cell protein, carotenoid, bacteriochlorophyll, biopolymers, antimicrobial agents and pantothenic acid (Kuo et al., 2012). Among those valuable products, the pigments of carotenoid and bacteriochlorophyll, are especially attractive. They have been applied as food coloring agents, precursors of vitamin A in food and animal feed, β-carotene and astaxanthin in industry, and additives to porphyrin (Aksu and Eren, 2005; Tamiaki et al.,

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2014). Non-hazardous wastewater provides nutrients for PSB, and PSB can be used for single cell protein production or pigments extraction.

Previous studies have shown that PSB biomass production could be greatly influenced by the light-oxygen condition, pH, trace-level elements and C/N ratios (Wu et al., 2012; Zhou et al., 2014a). PSB contain rich pigments such as carotenoid and bacteriochlorophyll. Correspondingly, the carotenoid and bacteriochlorophyll production might also be influenced by changing operative conditions. These two pigments are the main light-harvesters in PSB (Chen et al., 2006) and play an important role in capturing and transferring light energy into chemical energy for PSB growth. Hence, light conditions may have great effects on the PSB biomass, carotenoid, and bacteriochlorophyll production. For photosynthetic microorganism culturing, critical components of light conditions include the light source, light intensity and photoperiod. In previous studies, the effects of light source and light intensity on PSB growth were examined and the corresponding parameters were optimized (Zhou et al., 2014b, 2015).

On the other hand, studies about the effects of photoperiod on PSB biomass or pigments production were very limited. Microalgae is an equally important photosynthetic microorganism that utilizes pigments for photosynthesis, and the related studies on microalgae can be used as references. Studies showed that different light/dark cycles and different light/dark cycle frequencies impacted the growth of microalgae (Lee et al., 2015; Wahidin et al., 2013). The biomass of microalgae under intermittent illumination was higher than under continuous illumination (Janssen et al., 2000; Liao et al., 2014; Xue et al., 2011). Similarly, there might be a good chance that the intermittent illumination can enhance the growth of PSB. The selection of correct photoperiod may significantly enhance the PSB growth and pigment production, thus save the cost of light energy.

This study aimed to enhance the biomass growth, carotenoid and bacteriochlorophyll production in together with pollution removal in PSB wastewater treatment via the use of different photoperiod. Moreover, this work investigated the mechanism of PSB growth, carotenoid and bacteriochlorophyll production with different light/dark cycles and different light/dark cycle frequencies.

2. Methods

2.1. Materials

Rhodopseudomonas was a strain isolated from a local pond. The Gene Bank accession number for this PSB is CP001151.1. The cells were cultured in a thermostat shaker (120 r/min, 26–30 °C) using improved RCVBN medium with a light intensity of 2000 lux (Lu et al., 2013). After 48 h, the cell concentration in the exponential growth stage reached about 6×10^8 CFU·mL^{-1}. Then cells were used for wastewater treatment.

Since one aim of PSB wastewater treatment is to generate valuable biomass in together of pollutant removal, high-concentration-non-hazardous wastewater is preferred. Sugar wastewater is a typical non-hazardous wastewater with high COD and the amount of sugar wastewater is huge. Synthetic sugar wastewater was used in this study (ingredients see Zhou et al., 2015). The concentration of COD, NH_4^+-N and total phosphate of 6000 mg·L^{-1}, 400 mg·L^{-1}, and 90 mg·L^{-1}, and initial pH of 7.0. The PSB cells were inoculated to the artificial sugar wastewater at a volume ratio of 1:5.

2.2. Methods

Experiments were carried in 1 L glass flasks. Each time, 600 mL wastewater was added to the bioreactor. The reactors with wastewater (in together with PSB) were put into a shaker for wastewater treatment and PSB culturing.

In the light/dark (light hours: dark hours) cycle experiments, the light/dark cycle of 1 h/2 h, 1.5/1.5 h, 2 h/1 h, 2.5 h/0.5 h, and full light exposure were set every three hours. So the changing light/dark cycle frequency was 8 times per day. In the light/dark cycle frequency experiments, the optimal light/dark cycle from above tests was used. PSB were cultured with five different light/dark cycle frequencies: 48, 24, 8, 4 and 2 times per day. Other culturing conditions were as follows: 26–30 °C, with a light intensity of 2000 lux, dissolved oxygen concentration (DO) of less than 0.5 mg/L. Each experiment was repeated three times.

2.3. Analysis methods

COD and biomass production were tested by APHA standard methods (Eaton et al., 2005). The pH, DO and light intensity were tested by pH meter (PHS-3C), DO meter (HACH sensION5) and light meter (TES 1332A).

The carotenoid and bacteriochlorophyll contents were tested using spectrophotometer (Thermoelectron, Rochester, NY) at a wavelength of 473 nm and 771 nm according to Kuo et al. (2012), as shown in Eqs. (1) and (2):

$$\text{Carotenoids content} = A_{473} \times 10000/(250 \times S \times W) \quad (1)$$

$$\text{Bacteriochlorophyll content} = A_{771} \times 10000/(76 \times S \times W) \quad (2)$$

where A_{473} and A_{771} were the absorbances of the extracts at 473 nm and 771 nm; S was the path-length of the cuvette used in the spectrophotometer; and W was the initial amount (g) of samples divided by the final volume (mL) of extracts obtained.

2.4. Statistical analysis

Three repetitions of each experiment were performed. Three parallel measurements were conducted to ensure the accuracy of data. Tukey's test was adopted to analyze the significance of data. The level of significance of different groups exceeded 95% ($P < 0.05$).

3. Results and discussion

3.1. Effects of light/dark cycle

The effects of light/dark cycle on COD removal, PSB biomass production, and pigments production were examined. The results are summarized in Figs. 1 and 2.

3.1.1. Effects of light/dark cycle on COD removal and biomass production in PSB wastewater treatment

As Fig. 1a shows, the COD removal was influenced by the light/dark cycle. The highest and fastest COD removal was achieved with light/dark = 2 h/1 h (Tukey's test $P < 0.05$). Under all conditions, the COD removal increased with time in the first 72 h, and then nearly remained the same from the 72th hour to 120th hour. To shorten the hydraulic retention time, 72 h were chosen, and the corresponding COD removal was 90.3% with light/dark = 2 h/1 h.

At the same time, as Fig. 1b shows, the highest biomass production was achieved with light/dark = 2 h/1 h at the 72th hour, and the corresponding biomass production was 2068 mg·L^{-1}. PSB biomass production increased with the increase of light/dark cycle from 1 h/2 h to 2 h/1 h (Tukey's test $P < 0.05$), which was reasonable since more light provided more energy for PSB growth. With the increase of light/dark cycle from 2 h/1 h to full exposure of light, the biomass production did not increase (Tukey's test