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Enhancement of carotenoid and bacteriochlorophyll by high salinity stress in photosynthetic bacteria

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ABSTRACT

The variation of carotenoid and bacteriochlorophyll in photosynthetic bacteria (PSB) by high salinity stress (20–100 g L^{-1}) was investigated. Results showed that PSB could grow in high salinity wastewater and the productions of two pigments were affected by the salinity significantly. Suitable salinity stress could enhance their productions while excessive salinity would impede the productions. When the NaCl concentration was 50 g L^{-1}, the PSB were stimulated continuously during the whole culture period, and the productions of carotenoid and bacteriochlorophyll were 1.17 and 1.45-fold of the control group. The variation of dehydrogenase activity indicated that high salinity changed the metabolic activity greatly, and the highest level of dehydrogenase activity appeared at 50 g L^{-1} NaCl condition. Mechanisms analysis showed that the variations of pigments and dehydrogenase were related to self-protection. At the same time, pollutants in high salinity wastewater were removed by PSB. The COD and NH_3-N removals under 20–100 g L^{-1} NaCl concentration were around 33.5–66.1%. The biomass yields were over 0.2 mg-biomass (mg-COD-reduction)$^{-1}$ under all situations and salinity benefitted the biomass yield.

1. Introduction

Photosynthetic bacteria (PSB) are a group of phototrophic anoxygenic prokaryotic organisms distributing widely in nature. They contain redundant valuable substances such as pigments, 5-aminolevulinic acid and vitamin B_{12} (Kuo et al., 2012; Sasaki et al., 2005). Among these compounds, carotenoid and bacteriochlorophyll are of especial attraction because these two pigments are valuable for various industries. They can be applied to food, pharmaceutical and cosmetic products. For example, carotenoid has been used as food coloring agents and cosmetic additives; and bacteriochlorophyll is a promising substance to be applied to photodynamic therapy (Paliwal et al., 2015; Aksu and Eren, 2005; Rudolf and Grammel, 2012). These natural bio-chemicals are in increasing industrial demands nowadays. To meet this demand, enhancement of carotenoid and bacteriochlorophyll in PSB is a key point in researches. In previous studies, optimizing the medium and regulating operative parameter (light conditions) were used to improve the productions and contents of the two pigments, and these methods achieved effective results (Chen et al., 2006; Zhou et al., 2014; Saejung and Apaiwong, 2015; Wu et al., 2015).

According to literature, some physiological and biochemical mechanisms related to microbial growth could be influenced by high salinity, because many organisms would alter their metabolism to adapt to the extreme environment (Mohan and Devi, 2014). Studies have shown that high salinity could induce pigments production in microalgae (Paliwal et al., 2015; Gómez et al., 2003). PSB are phototrophic microorganisms, and there is similarity between PSB and microalgae. Therefore, it is possible that carotenoid and bacteriochlorophyll productions in PSB can also be enhanced under high salinity stress. Providing saline condition will be more convenient and economical than other optimization, which is worth investigation.

Culturing PSB to obtain the pigments often used culture medium (Chen et al., 2006; Saejung and Apaiwong, 2015), which increased the cost. Therefore, in order to cut down the cost of complex culture medium, wastewater might be an appropriate alternative. Large amount of high salinity wastewater is produced from food-processing, leather, petroleum and many other industries, which contains plenty of salts (mainly NaCl) and organic

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matters (Lefebvre and Moletta, 2006). According to researches and analysis above, abundant organics and hypersaline environment in food processing wastewater are appropriate conditions for PSB to produce more carotenoid and bacteriochlorophyll. What's more, the wastewater can be purified to avoid serious environmental pollution (Li et al., 2011). So, the aim of this study was to demonstrate the feasibility of enhancement of carotenoid and bacteriochlorophyll in PSB by salinity stress. The effect of NaCl concentration on the carotenoid and bacteriochlorophyll productions in PSB was determined. PSB's growth and pollutants removal were inspected. Moreover, the mechanisms of carotenoid and bacteriochlorophyll productions under the high salinity with different NaCl concentration were investigated, too.

2. Materials and methods

2.1. Materials

In previous study, a PSB strain (*Rhodopseudomonas*) was isolated from a local fish farm and named Z16. The strain Z16 could tolerate high salinity and was used in this study. It was cultured in a thermostatic shaker (90 rpm, 28 ± 2 °C) with the improved RCVBN medium (Lu et al., 2011). After 48 h growth in pure culture, Z16 reached the exponential growth stage and was inoculated to high salinity wastewater.

Synthetic high salinity wastewater was used in this study. It contained sucrose (5 g L^{-1}), DL-malic acid (3 g L^{-1}), NH_4Cl (1.15 g L^{-1}), KH_2PO_4 (0.4 g L^{-1}), $NaHCO_3$ (1 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.2 g L^{-1}) and NaCl. The chemical oxygen demand (COD), NH_3-N, and total phosphorus (TP) of the wastewater was about 6000, 400 and 90 mg L^{-1}. Three levels of high salinity (NaCl concentration of 20, 50 and 100 g L^{-1}) along with a control condition without extra NaCl were set. The pH of wastewater was adjusted to 7.0–7.2 with HCl and NaOH solution.

2.2. Methods

Z16 was cultivated in high salinity wastewater with inoculum dose of 400 mg L^{-1} in a 500 mL Erlenmeyer flask as the bioreactor, and the final volume was 400 mL. The bioreactors were put in a thermostatic shaker (90 rpm, 28 ± 2 °C) and light-anaerobic condition was chosen for the PSB's growth. The illumination was provided by incandescent lamps with light intensity of 3200 lux. The dissolved oxygen (DO) was kept under 0.2 mg L^{-1} by flushing N_2 into the bioreactors and sealing the bioreactors with parafilms.

2.3. Analysis methods

Samples (5 mL every day) were taken from the bioreactors and were centrifuged at 11,000 rpm for 10 min. The supernatant was used to measure the final COD, NH_3-N, and TP according to the national standard methods (HJ/T 399-2007, HJ 535-2009, and GB 11893-89), and the sediments (PSB) were used to test the microbial indexes. The biomass was measured according to Wu et al. (2015). The carotenoid and bacteriochlorophyll were extracted according to Liu et al. (2016) and calculated as in Eqs (1) and (2):

$$\text{Carotenoid production}\left(\text{mg L}^{-1}\right) = (A_{473} \times V_1 \times N \times 10)/(2500 \times V_2) \quad (1)$$

$$\text{Bacteriochlorophyll production}\left(\text{mg L}^{-1}\right) = (A_{771} \times V_3 \times N)/(760 \times V_4) \quad (2)$$

where A_{473} and A_{771} were the absorbances of the extracts at 473 and 771 nm; V_1 and V_3 were the volumes of the extracts; N was the dilution ratio; V_2 and V_4 were the volumes of the initial samples.

The dehydrogenase activity was tested according to Filipič et al. (2012). The pH was measured by a pH tester (PHS-3C, Inesa Instrument Inc., Shanghai, China). The DO was measured by a dissolved oxygen meter (JPB-607A, Inesa Instrument Inc., Shanghai, China).

The biomass yield (mg-biomass (mg-COD-reduction)$^{-1}$) was calculated as in Eq. (3):

$$\text{biomass yield} = \text{biomass increase(mg)}/\text{COD reduction(mg)} \quad (3)$$

2.4. Statistical analysis

Parallel experiments, samples and measurements were carried out. Each reported value was the average one. Statistical comparisons were made with Tukey's test and was considered at $p < 0.05$.

3. Results and discussion

3.1. PSB's growth in high salinity wastewater

PSB's growth in high salinity wastewater is the premise for enhancement of the pigments. Thus, the feasibility of using high salinity wastewater to culture PSB was studied first and the results are shown in Fig. 1. As Fig. 1 shows, the biomass proliferation of PSB occurred in every experimental run. Although PSB's growth was inhibited to different extent under different NaCl concentration, they appeared to possess strong salt tolerance. When the NaCl concentration was 20–50 g L^{-1}, the growth situations were a little behind that of the control group. When the NaCl concentration was up to 100 g L^{-1}, there was a lag phase before 72 h; but after that, PSB acclimatized to the circumstance and accumulated biomass. Other researchers have found that some PSB species could grow at 0–85 g L^{-1} NaCl (Nunkaew et al., 2015; Panwichian et al., 2010). The phenomenon in this study was consistent with the literature.

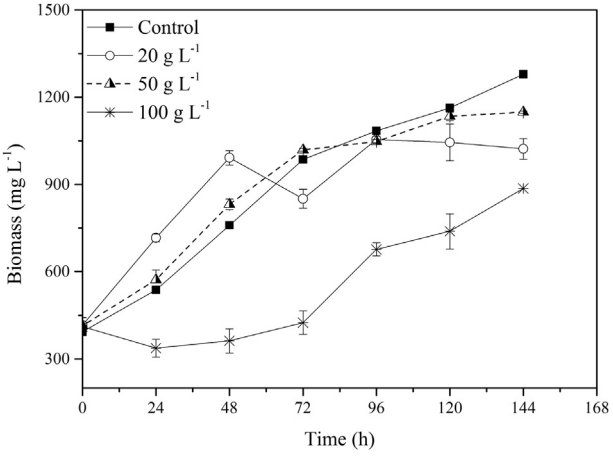

Fig. 1. PSB's growth in high salinity wastewater with different NaCl concentrations.