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Physiological and Biochemical Responses of *Synechocystis* sp.PCC 6803 to Stress of Phenol

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Abstract. In this study, the physiological and biochemical responses of the freshwater microalgae *Synechocystis* sp. PCC 6803 to phenol were evaluated using 96h growth tests in a batch-culture system. The results showed that low concentration of phenol (<100 mg•L⁻¹) nearly had no effects on the physiological and biochemical characteristics during the cell cultivation. However, the cell growth, Chlorophyll a (Chl-a) and soluble carbohydrate increases in the culture of *Synechocystis* sp. PCC 6803 were significantly inhibited by higher concentration of phenol (>200 mg•L⁻¹). These results help the further study of the effects of phenolic compounds on microalgae growth and environment.

1. Introduction

CO₂ emission from industrial productions leads to serious greenhouse effects, which is related to global climate change. Some microorganisms including microalgae could capture CO₂ through photosynthesis and the harvested microalgal biomass is available for the next conversion to chemicals or biofuels[1]. Cyanobacteria is a prokaryote, and its cell wall has the characteristics of the gram-negative bacteria, and it's a simple, unanchored nucleus[2]. It can not only perform photosynthesis, but also have nitrogen fixation and hydrogen metabolism. The cyanobacterium *Synechocystis* sp. PCC 6803 is widely used as a model organism for the study of photosynthetic processes.

Polycarbonate resins, paints, paper, explosives, inks, perfumes, textiles and antibacterial agents contain organic pollutants such as phenol[3]. Phenolic compounds are known to be toxic and carcinogenic by ingestion, contact or inhalation, and are highly stable hazardous pollutants. In recent years, because of the increasing industrial and agricultural wastewater, contamination of aquatic environments by phenol has become a focus of researchers and environmentalists. Phenol is a typical pollutant in wastewater and its toxicity to microalgae is very complex. Phenol is also an alternative substrate for microalgae. In recent study, it was shown that the low phenol concentration stimulated cell growth and lipid accumulation. However, high concentration phenol was harmful to
photosynthesis of microalgae\textsuperscript{4}. In addition, Reactive Oxygen Species (ROS) could be induced by phenol\textsuperscript{5}. It could increase the level of peroxide in the cell. In severe cases, it can lead death of cells.

In order to examine the effect of phenol on the microalage, the unicellular microalgae \textit{Synechocystis} sp. PCC 6803, ubiquitous in freshwater, was used in this study to explore the physiological and biochemical responses of cyanobacteria to stress of phenol, including the evaluation of the toxic effects of phenol on the growth characteristics, the increases of Chlorophyll-a (Chl-a) and soluble carbohydrate in the cultivation of \textit{Synechocystis} sp. PCC6803.

2. Materials and Methods

2.1. Chemicals and solutions

Phenol was purchased from Solarbio Science & Technology Co., Ltd.(Beijing, China). BG-11 formula is as follows (g \cdot L\textsuperscript{-1}): NaNO\textsubscript{3} 1.5, K\textsubscript2HPO\textsubscript{4}\cdot3H\textsubscript{2}O 0.04, MgSO\textsubscript{4}\cdot7H\textsubscript{2}O 0.075, CaCl\textsubscript{2}\cdot2H\textsubscript{2}O 0.036, citric acid 0.006, Ferric ammonium citrate 0.006, EDTA (diantrium-salt) 0.001, Na\textsubscript2CO\textsubscript{3} 0.02, 1mL A5 trace element stock solution and ddH\textsubscript{2}O 919 mL. Reagent purchased from Sinopharm Chemical Reagent Co.,Ltd (ShangHai, China). Test solutions were prepared by adding a certain amount of phenol in BG-11 medium, and the phenol concentrations used were 0, 50, 100, 200, 300, 400,500 and 600 mg \cdot L\textsuperscript{-1} respectively for the cytotoxicity tests.

2.2. Microorganism

\textit{Synechocystis} sp. PCC 6803 was provided by Dr. Chen Gao of Shandong Academy of Agricultural Sciences. The stock culture and inoculum were grown in BG-11 medium. The inoculum was precultured aseptically in 250 mL Erlenmeyer flasks with 150 mL of BG-11 medium. The flasks were placed in a 30°C illuminated incubator for 4 days under 12h light/12h dark photoperiod and a light density of 50 uE/m \cdot s\textsuperscript{-1}.

2.3. Microalgal growth analysis

Daily microalgal cell density was determined turbidometrically at 730 nm using a spectrophotometer (UVmini-1240, Shimadzu Corporation, Kyoto, Japan).

2.4. Chlorophyll a (Chl a) determination

Triplicate 5 ml of well-blended cultures were centrifuged at 4500 rpm for 10 min to discard the supernatants. And the pellets were homogenized with 80% (V/V) acetone for Chl a extraction. The mixtures were vigorously shaken using a vibrator, and then placed in a refrigerator in the dark at 4 °C for 24 h. Subsequently, the extracted samples were centrifuged at 12,000 r/min for 10 min to remove the pellets. Supernatants were transferred into 1\times1 cm glass cuvettes, and measured for Chl a at 663 nm and 645 nm using a spectrophotometer. All absorbance values were corrected using the 80% acetone as control. The concentration of Chl a was calculated by the following equation\textsuperscript{6}(Eq. 1):

\[
\text{Chl a(mg/L)} = 12.71A_{663} - 2.59A_{645}
\]

2.5. Soluble carbohydrate determination

Triplicate 5 ml of well-blended cultures were centrifuged at 4500 r/min for 10 min to discard the supernatants, and the pellets were homogenized with 1mL PBS. Finally, the mixtures were treated by ultrasonic wave. When there are no intact cells in algal fluid under the observation by microscopy, the soluble carbohydrate was extracted under 90°C water bath for 3 hours, then the samples were centrifuged at 12,000 r/min for 10 min, the supernatants were used for the determination of soluble carbohydrate. Soluble carbohydrate determination was carried out using phenol-sulfuric acid colorimetric method.
3. Results

3.1. Microalgal growth characteristics
Phenol is a harmful compound for the microalgal growth. Its effects on cyanobacterium cells and growth characteristics of Synechocystis sp. PCC 6803 were analyzed in this study. As shown in Figure 1, when the concentration of phenol>200 mg•L\(^{-1}\), the growth of cyanobacteria was significantly inhibited, and when the phenol concentration is less than 100 mg•L\(^{-1}\) (P>0.05), phenol has no apparent effect on algae growth. In addition, in the process of cultivation, high phenol concentrations make cells to secrete large amounts of viscous material, which may be due to cell leakage resulting from destruction of cell membrane structure.

![Figure 1. Growth profiles of Synechocystis sp. PCC 6803 under different phenol concentrations (mg/L) during 120 h exposure. The points represent the means of three replicates (n = 3); error bars represent standard deviations.](image1)

3.2. Chlorophyll a content
Figure 2. shows the changes of Chl a content in Synechocystis sp. PCC 6803 with phenol contents. When the concentration of phenol>200 mg•L\(^{-1}\), the synthesis of Chl a in the cytoplasm was obviously inhibited. When the phenol concentration is less than 100 mg•L\(^{-1}\), the phenol had no significant effect on Chl a content (P>0.05). Phenol had great inhibitory effect on the Chl a content in Synechocystis sp. PCC 6803 when its concentration came to 400 mg•L\(^{-1}\). The Chl a nearly stopped increase while the phenol concentration was higher than 400 mg•L\(^{-1}\), which indicated the cell almost died because of the toxicity of phenol.

![Figure 2. Effects of phenol on chlorophyll a content of Synechocystis sp. PCC 6803 during 96 h of exposure. The points represent the means of three replicates (n=3); error bars represent standard deviations.](image2)
3.3. Soluble carbohydrate content

As an important source of energy and carbon, carbohydrate has played a very important role in the process of cellular metabolism [7]. Figure 3. shows the changes of soluble carbohydrate content in the culture medium of Synechocystis sp. PCC 6803 with phenol treatments. When the concentration of phenol was greater than 200 mg•L⁻¹, the growth of cyanobacteria was inhibited, but there was no significant change in the carbohydrate content. When the concentration of phenol was over 400 mg•L⁻¹, the synthesis of the carbohydrate in the cell was significantly inhibited.

![Fig.3. Effects of phenol on soluble carbohydrate content of Synechocystis sp. PCC 6803 after 96 h of exposure. Points represent means of three replicates (n=3); error bars represent standard deviations](image)

4. Discussion

Phenol is a toxic, carcinogenic and mutagenic organic pollutant, which is typically present in industrial effluents from refineries, coking operations, coal processing plants and petrochemical industries. Based on literature, the low concentration phenol stimulated cell growth and lipid accumulation for Dunaliella salina [8], while high concentration phenol was harmful to photosynthesis of Chlamydomonas reinhardtii. It was reported that the resulting or evolved strain Chlorella sp. L5 was able to degrade 500–700 mg•L⁻¹ phenol [9]. In the present study, we also found a similar phenol stress on unicellular microalgae, Synechocystis sp. PCC 6803. When the phenol concentration is less than 100 mg•L⁻¹, the phenol had no significant effect on it. However, high concentration of phenol (>200 mg•L⁻¹) had serious inhibition effect on the growth characteristics and physiological properties of Synechocystis sp. PCC 6803. The cells were poisoned and decolorized and the growth was completely inhibited when the phenol concentration was higher than 400 mg•L⁻¹, and the toxicity increases with the extension of time, because organic pollutants first destroy the cell membrane and increase the permeability of the cell membrane. The toxin can enter the cell more smoothly and react with some life necessary substances [10]. When the phenol concentration was increased to a certain point, the concentration of chlorophyll a is close to zero, which may because high concentrations of phenol in the cell destroyed the structure of the chlorophyll a and stopped the synthesis of chlorophyll a; similarly, the high phenol concentration also inhibits the synthesis of soluble carbohydrate in the algae cell.

5. Conclusion

The stress of phenol on freshwater microalgae Synechocystis sp. PCC 6803 was evaluated in this study. The results showed that low concentration of phenol (<100 mg•L⁻¹) nearly had no effect on the physiological and biochemical characteristics during the cell cultivation. However, the cell growth, Chl a and soluble carbohydrate increases in the culture of Synechocystis sp. PCC 6803 were significantly inhibited by higher concentration of phenol (>200 mg•L⁻¹). This helps the further study of the effects of phenolic compounds on microalgae growth and environment.
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