Effects of Dibutyl Phthalate on Physiological and Biochemical Behaviors of Electroactive Biofilm

Yue Dong, Peifang Wang, Yixuan Zhang

Key Laboratory of Integrated Regulation and Resource Development on Shallow Lake of Ministry of Education, College of Environment, Hohai University, Nanjing 210098, China
Email: dongyuehohai@hhu.edu.cn

Abstract. Microbial electrochemical systems (MESs) are a promising clean energy source to directly convert waste chemicals in domestic wastewater to available electric power with synchronous pollutant removal. The physiological and biochemical behaviors of electroactive microorganisms (EAMs) is the key factor of the MESs. Dibutyl phthalate (DBP), as the emerging pollutant, widely exists in municipal wastewater which has strong ecological toxicity. So, utilization of MESs in the engineering practice of wastewater treatment must consider the impact of DBP on the physiological and biochemical behaviors of electroactive biofilm. The output voltage and power density of the MES decreased significantly with the increase of DBP in the synthetic wastewater. In addition, the EAMs resisted the stimulation of DBP by secreting more extracellular polymer substances (EPS) by microscopic observation. The composition and structure of the EPS also changed, mainly reflected in the increase of β-polysaccharide content and the expansion of its coverage area.

1. Introduction
Dibutyl phthalate (DBP) is a kind of Phthalate Esters (PAEs), which is commonly used as plasticizer in plastic products. It continuously releases to the environment through the decomposition of plastic products, which is ubiquitous in water, air and food [1-3]. Investigation and research on the content of DBP in surface water and municipal wastewater in some areas of China revealed that most surface water bodies were polluted by DBP. The average daily influent DBP concentration in municipal wastewater is higher in developed areas, as shown in Figure 1 [4-5]. It can be judged that factors including emission sources, seasons, population densities will cause large differences in DBP content in the surface water and municipal wastewater.

Most PAEs exhibit triple effect, namely, mutagenicity, carcinogenicity, and teratogenicity. Among them, DBP shows stronger toxicity to the human body [6]. It is generally believed that DBP belongs to environmental endocrine disruptors (EDs), which can affect the endocrine process of an organism by inhibiting the synthesis of steroid hormones. The reproductive and developmental toxicity of DBP is another risk of DBP. A series of experiments have shown that DBP can cause reduced testicular marker enzyme activity and weight loss [7-8].

Microbial electrochemical systems (MESs) are a type of device that utilizes electroactive microorganisms to recover biomass energy. In MES, a layer of electrochemically active biofilm (EAB) is grown on the anode, which is mainly composed of microorganisms and extracellular polymer substances (EPS) secreted by the microorganisms. The electroactive microorganism can directly transfer electrons to the anode and form current in the circuit. EAB has unique research value in wastewater treatment due to its function of electron transfer on solid electrodes. It can be used as an effective
biocatalyst for electricity generation of MES by degrading organic pollutants through metabolism [9-10].

Figure 1. The average DBP concentrations in surface water and municipal wastewater in some parts of China (μg/L).

The physiological and biochemical behaviors of EAB is the key factor affecting the operation performance of MESs. The municipal wastewater is the important reservoir of DBP which inevitably has impact on the physiological and biochemical behaviors of EAB. The use of MES in the engineering practice of municipal wastewater treatment must take the existence of DBP into consideration. In this study, the effects of DBP on EAB was investigated by analyzing the electricity generation performance of MES and the morphological structure of EAB under different DBP concentrations.

2. Material and methods

2.1. Wastewater samples
The wastewaters were collected from Jiangxinzhou municipal wastewater treatment plant in Nanjing. Wastewater samples from each processing section was collected, and then filtered through a 0.45 μm microporous membrane and stored at 4 ℃ for use. Solid-phase extraction-high performance liquid chromatography was used to detect the DBP concentration in the wastewater samples.

2.2. Reactor design and operation
The double-chamber MES reactor was shown in Figure 2. The reactor was made of plexiglass and was divided into an anode chamber and a cathode chamber. The cathode and anode chambers were separated by an ion exchange membrane. The membrane was fixed by nylon screws and sealed by a silicone rubber pad. The dimensions of two chambers were 12 × 4 × 4 cm. The anode electrode was carbon fiber brush with a length of 10 cm and a diameter of 4 cm. A piece of graphite sheet (Length: 4 × Height: 9 × Thickness: 0.1 cm) was used as cathode. Two holes with a diameter of 1 cm were opened in the upper part of the anode and cathode chambers for medium refreshment.

The anode was inoculated and fed with the effluent from an existing MES operated at ambient temperature. The MES was placed in a 30 ℃ incubator and operated with an external resistance of 200 Ω. The composition of anolyte was: CH₃COONa: 1 g/L, NH₄Cl: 0.31 g/L, KCl: 0.13 g/L, Na₂HPO₄·12H₂O: 10.32 g/L, Na₂HPO₄·2H₂O: 3.32 g/L, trace elements: 1 mL/L, trace minerals and vitamins. The DBP concentrations was set as 0 mg/L, 1 mg/L, 10 mg/L in the anolyte. The catholyte was K₃[Fe(CN)₆]: 16.462 g/L, KCl: 3.725 g/L. The anolyte and catholyte were replaced when the output voltage was lower than 60 mV.
Figure 2. Reactor design: (A) reactor configuration; (B) anode electrode; (C) cathode electrode.

2.3. Measurements and analysis
The voltages of MDECC were recorded every 30 minute by a data acquisition system (PISO-813, ICP DAS Co., Ltd.). The polarization and power curves were obtained by changing the external resistances from open circuit to 10Ω. The current and power densities were calculated by normalizing the current and power by sectional area of 48 cm².

The micromorphology of the EAB was observed and measured using Hitachi SU8220 scanning electron microscope (SEM). Multiple fluorescent staining and confocal laser scanning microscopy (CLSM) were used to visualize the spatial distribution of the main components of protein (PRO) and polysaccharide (PS) in EPS of EABs.

3. Results and discussion

3.1. DBP concentration in wastewaters
The DBP concentrations and removals after each processing section were shown in Table 1 and Figure 3, respectively. It can be found that the DBP concentration decreased along the processing section. The DBP removal of the secondary sedimentation tank was the highest, which was determined by the degradation of microorganisms and the adsorption of excess sludge. After passing through the aeration tank, the DBP concentration slightly increased. It was considered that the DBP was a fat-soluble substance that was easy to enrich and adhere to the inorganic particles. When passing through the aeration tank, hydraulic shear resulted in the release of DBP. Notably, the concentration of DBP in wastewater at the aerobic phase increased instead of the anaerobic phase. The possible reason was that a dynamic dissolution-adsorption balance existed between wastewater and sludge. There was no obvious change of DBP concentration in the advanced treatment process due to the significant removal of DBP after above processing sections.

| Processing Sections     | Concentration (μg/L) | Removal |
|-------------------------|----------------------|---------|
| Municipal wastewater    | 13.29                |         |
| Aeration tank           | 13.87                | -4.4%   |
| Anaerobic tank          | 5.41                 | 63.7%   |
| Aerobic tank            | 5.86                 | -3.4%   |
| Secondary sedimentation | 1.93                 | 29.6%   |
| tank                    | Advanced treatment   | 2.01    | -0.6%   |
Figure 3. The removals of DBP after each processing section in Jiangxinzhou municipal wastewater treatment plant.

3.2. Electricity generation performance
The voltage outputs of MES reactors with various addition of DBP were shown in Figure 4. It could be seen that the MES reactor without DBP had the best electricity generation performance with maximum voltage of ~0.59 V at the cycle time up to about 210 h. The electricity generation performance of the MESs reactor with 1 mg/L and 10 mg/L DBP significantly decreased. The maximum voltage output was about ~0.36 V with DBP concentration of 1 mg/L, and cycle of MES operation was shortened to about 140 h. The reactor with 10 mg/L DBP had the worst electricity generation performance, and the voltage output was only ~0.24 V. The cycle of MES operation was further shortened to about about 128 h.
Figure 4. Voltage output of the MES reactors at different DBP concentrations: (A) DBP, 0 mg/L; (B) DBP, 1 mg/L; (C) DBP, 10 mg/L.

The power outputs of MES reactors with various addition of DBP were shown in Figure 5. The internal resistance of the MES also gradually increased from 200 Ω to about 400 Ω with the increase of DBP concentration, which limited the power output of the MES reactor. The above results showed that DBP would affect the electricity generation performance of MES. With the increase of DBP concentration, its toxicity would inhibit the activity of EAB, bring worse electricity generation performance and shorten the electricity generation cycle. Thus, it could be concluded that the addition of DBP had a adverse impact on the power generation capacity of microorganisms, with limited the power output performance.
3.3. The micromorphology of the EAB

The SEM was used to observe the micromorphology of the EAB. In Figure 6, it can be clearly seen that the biofilm on the anode carbon brush without DBP has less EPS, while the biofilm with DBP of 1 mg/L and 10 mg/L was thick with more coverage of EPS. Celmer et al. [11] found more EPS yields could resist the transport of toxic substances and reduce reactions between toxic substances and biofilms [12-13]. Therefore, it is speculated that the stimulation of the toxic substance DBP caused the electroactive microorganisms to secrete more EPS to counteract its ecotoxicity.

In addition, the spatial composition and structure of the biofilm obtained by CLSM showed that the content of protein and α-polysaccharide in the EAB is large and scattered throughout the field of view. The content of β-polysaccharide is the lowest and scattered in a few points. The proportions of the protein and α-polysaccharides were relatively stable, while the amount of β-polysaccharides increased significantly with the increase of DBP concentration. This result demonstrated that the β-polysaccharides played important role in resistance of DBP ecotoxicity.

**Figure 5.** Power output and electrode potentials of the MES reactors at different DBP concentrations: (A) DBP, 0 mg/L; (B) DBP, 1 mg/L; (C) DBP, 10 mg/L.

**Figure 6.** SEM and CLAM of the EAB at different DBP concentrations: (A and D) DBP, 0 mg/L; (B and E) DBP, 1 mg/L; (C and F) DBP, 10 mg/L.
4. Conclusions
In summary, the effects of DBP on EAB was investigated. The conclusion are: (1) the DBP concentration decreased along the processing section in municipal wastewater treatment plant; (2) the addition of DBP had an adverse impact on the power generation capacity of microorganisms, with limited the power output performance; (3) the β-polysaccharides in EPS played important role in resistance of DBP ecotoxicity.

5. References

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