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Mechanism of 2, 4, 6-Trichlorophenol Degradation in Microbial Fuel Cells System with Microbe Isolated from Submarine Sediment

YI Aifei[1,2], *FENG Yali*[1,*], *DU Zhuwei*[2], *LI Haoran*[2,*]

[1] School of Civil and Environment Engineering, University of Science and Technology Beijing 100083;
[2] State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, P.R.CHINA.
[*]E-mail: ylfeng126@126.com; hrli@home.ipe.ac.cn

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This study explores the use of MFCs to anodic dechlorination of aromatic chlorides such as 2,4,6-trichlorophenol (2,4,6-TCP). Mixed microbe, isolated from submarine sediment, was used to detect its dechlorination performance in microbial fuel cells device. Moreover, It was confirmed that exoelectrogen microbe accelerated the 2,4,6-TCP degrading in MFCs. Dechlorination efficiency of 2,4,6-TCP in the anode of MFCs could reach 86.51%, accompanying with power generation. Besides, the pathway of 2,4,6-TCP degradation in MFCs was proved. Dichlorophenol, a major intermediate product for TCP dechlorination, was degraded into CO_2.

Keywords: 2, 4, 6-trichlorophenol degradation, dechlorination, UV–visible spectra, microbial fuel cells

1. INTRODUCTION

2, 4, 6-Trichlorophenol (2,4,6-TCP), used widely for a pesticide or a wood preservative, is a persistent organic pollution widely existed in water, soil, sometimes in the air [1-4]. For its three chloride groups linked to the phenol ring, TCP accumulating to certain levels is harm to wildlife and human health. In terms of its high toxicity and persistency characteristic, 2,4,6-TCP is listed as a precedence-controlled pollutant by the US EPA [5, 6]. The C-Cl bond and the position of chlorine atoms relative to the hydroxyl group are responsible for their toxicity, carcinogenic properties, structural stabilization and persistence in the environment, making the removal of 2,4,6-TCP from the environment very crucial.

For the treatment of chlorinated phenols, several methods of physical, chemical, and biological such as activated carbon adsorption, incineration, and biological degradation [7, 8]. Generally, biological treatment is superior to physicochemical methods with high treatment costs and possibilities of secondary pollution. Moreover, it can benefit the environment. However, microorganisms may easily be inhibited by toxic chlorophenols when conventional biological treatments are used [7, 9]. Being energy saving and clean production, 2,4,6-TCP removal through biological degradation is considered as a significant and sustainable pathway for the future [10-12]. Biodegradability of chlorophenol depends on the number and position of chlorine groups on the aromatic ring. So our research focused on exploring an exoelectrogen culture from submarine hydrothermal sediment to fit the toxic chlorophenol environment and to degrade it.

Microbial fuel cells (MFCs) [14, 15] considered as a novel system for energy generation, are attracting wide attention as a new type of energy recovery technology and waste water treatment. Air cathode MFCs, not needing proton exchange membrane and aeration system, were taken as the degradation reactor to study ocean bacteria's performance on dechlorination.

2. MATERIALS AND METHODS

2.1 MFCs configuration

Air-cathode MFCs with a 400 mL plate anode and air cathode is utilized in the experiments described in this study. Cathode and anode electrodes having 95.0 cm^2 (3.14×5.5cm×5.5cm) and 43.0 cm^2 (3.14×3.7cm×3.7cm) surface area respectively, were made of 1.5-mm and 5-mm thick graphite felt (Sanye Carbon Co., Ltd). Electrochemical treatment for the graphite felt was carried out with a constant current density of 30 $mA*cm^{-2}$ for 12 h (based on the projected area of the anode). Subsequently, treated felts were washed by distilled water, and then were dried at 100^o C for 4 h in vacuum drier [16]. Titanium wire was used for the connection of the external circuit with a resistance of 510 ohm to the electrodes. The schematic diagram of MFCs device equipment was shown in Fig.1.

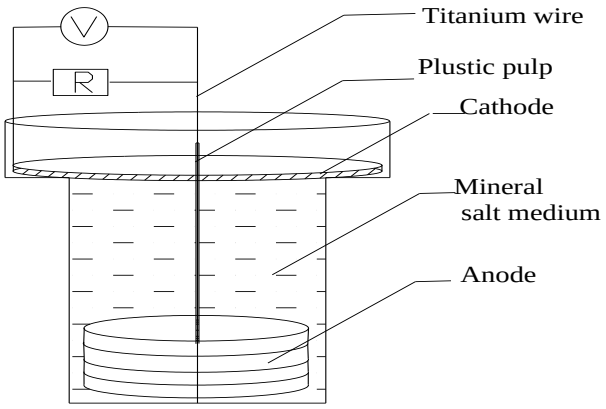

Figure 1. Schematic diagram of air-cathode MFCs configuration

2.2 Inoculation and operation

MFCs were inoculated with a mixed bacterial culture from submarine hydrothermal sediment sample (China Ocean Biologic Sample Repository). The salt medium fed in the anode of MFCs, contained $Na_2HPO_4.12H_2O$ (4.09 gL^{-1}) and $NaH_2PO_4.2H_2O$ (2.93 gL^{-1}), NH_4Cl (0.31 gL^{-1}), KCl (0.13 gL^{-1}), metal salt (12.5 mLL-1), vitamin (5 mLL^{-1}) solutions [17] and acetate 20 $mmolL^{-1}$ with a certain concentration of 2,4,6-TCP as substrates. At a fixed external resistance of 510 ohm, with different TCP concentration (50 mgL^{-1}, 100 mgL^{-1}, 150 mgL^{-1}) in anolyte, at each TCP concentration MFCs reactors run 3 cycles each 10-days around to make anode electrode form a stable biofilm. When the voltage dropped below 40 mV, feed solutions were replaced to initiate a new cycle. All tests were operated at room temperature. Each experiment was done in duplicate. Under the optimal conditions of biodegradation, the concentration of 2,4,6-TCP was monitored every 24 h.

2.3 Analysis and calculation

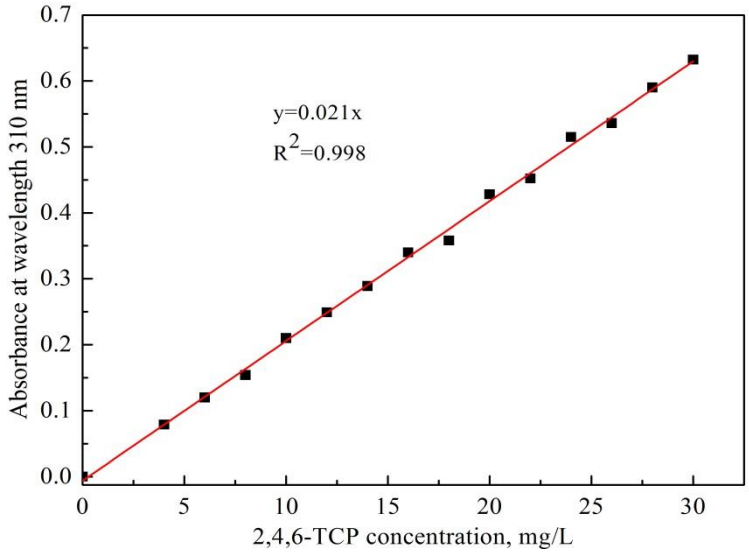

Figure 2. The standard curve of 2,4,6-TCP concentration correlated with absorbance at 310nm, salt medium as reference solution.

COD was measured according to potassium dichromate method [18]. Cell voltages were measured using a data acquisition system (AD8201H, Ribohua Co., Ltd.). The 2,4,6-TCP concentration was measured through absorbance value at wavelength of 310 nm (fig.7) by ultraviolet spectrophotometer (UV-1750, Shimadzu Co., Ltd). The standard curve of 2,4,6-TCP was drawn in Fig.2. 2,4,6-TCP concentrations were calculated by formula: $C = (A-A_0) / B$, where A is the absorbance value of the sample, A_0 is the absorbance value of blank control group and B is a constant value of 0.021 (Fig.2). 2,4,6-TCP degradation efficiencies were calculated by the following formula: efficiency (%) = $[(C_1-C_2) / C_1]\times100$, where C_1 and C_2 are the initial and residual concentration of 2,4,6-TCP (mg/L), respectively; COD removal efficiency were calculated by formula: efficiency (%) = $[(C_3-C_4) / C_3]\times100$, where C_3 and C_4 are the initial and residual concentration of COD (mgL^{-1}).

3. RESULTS AND DISCUSSION

3.1. Effect of 2,4,6-TCP initial concentration on 2,4,6-TCP degradation and voltage output

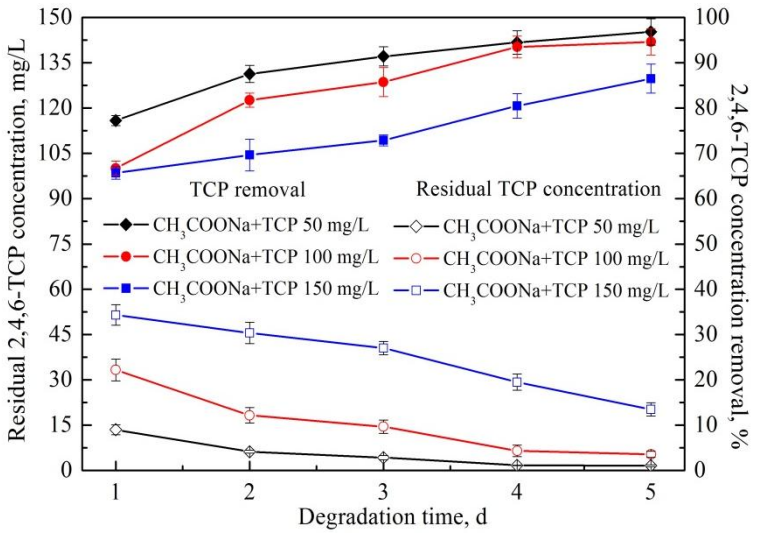

Figure 3. The effect of 2,4,6-TCP initial concentration on 2,4,6-TCP degradation efficiency (pH=7.0 at room temperature, concentration of sodium acetate 20 mmolL^{-1}).

Experiments were carried out to confirm different initial 2,4,6-TCP concentration affected on the bacteria degrading characteristics. Residual 2,4,6-TCP concentration and its degradation efficiency were shown in Fig.3. As its initial concentration increases, residual 2,4,6-TCP concentration increases and 2,4,6-TCP degradation efficiency decreases. This implies that there is negative correlation between residual 2,4,6-TCP concentration and initial 2,4,6-TCP concentration, which is in line with the regularities of the existing research [19]. At low initial 2,4,6-TCP concentration, the 2,4,6-TCP degradation efficiency can be almost 97.45%. The higher initial 2,4,6-TCP concentration, the lower degradation efficiency it is.

3.2 Effect of COD removal in MFCs

Under the condition of different initial 2,4,6-TCP concentration in MFCs, changes of the residual chemical oxygen demand (COD) concentration, were observed as shown in Fig.4. At initial stage, high concentration of the 2,4,6-TCP makes COD removal decrease. However, 2 days later, no obvious change happens in both COD removal and residual COD concentration. So it stands to reason that toxicity of high 2,4,6-TCP concentration in the anolyte of MFCs has great negative effect on COD removal of initial stage. COD concentration decreases with the increasing time shows that organics are degraded into CO_2.

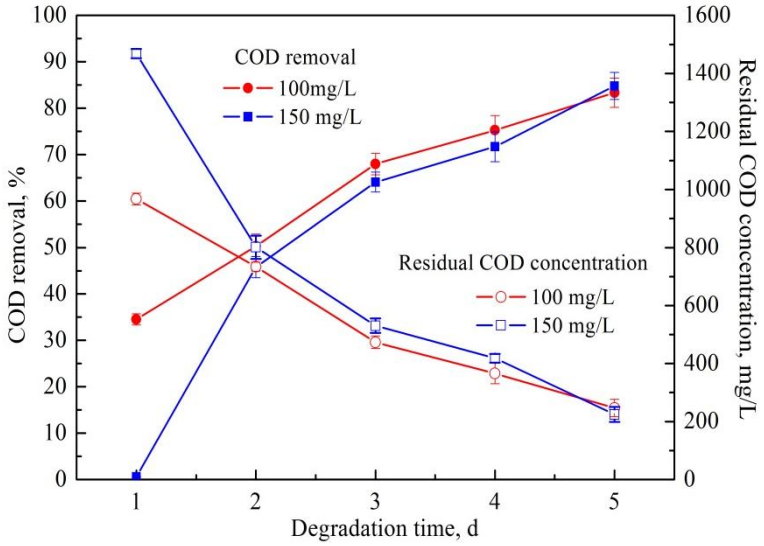

Figure 4. The effect of 2,4,6-TCP initial concentration on COD residual concentration and COD removal (pH=7.0 at room temperature, concentration of sodium acetate 20 mmolL^{-1}).

3.3 Open circuit controls efficiency compared to that of close circuit

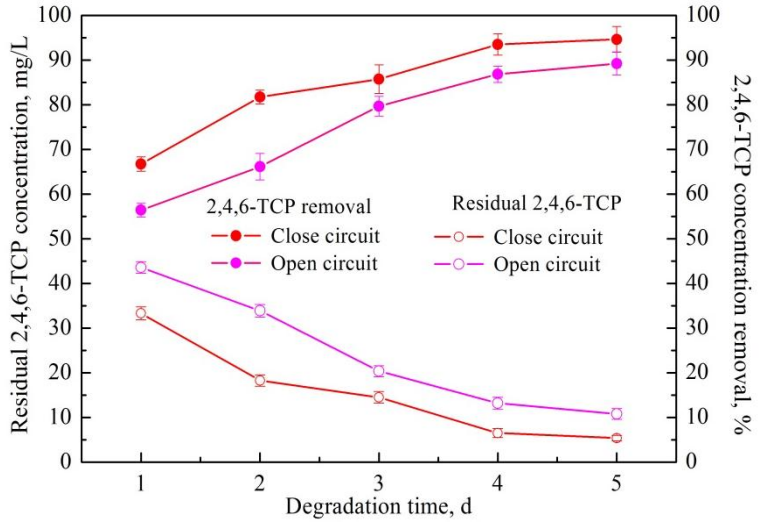

Figure 5. The efficiency of TCP removal in close circuit and in open circuit, where CH_3COONa concentration is 20 mmolL^{-1}

To investigate microbe's effect on TCP removal efficiency in MFC or in anaerobic biological treatment, under the same condition, microbe's performance on TCP removal ratio in open circuit and in close circuit were investigated. As results show in Fig.5, when initial TCP concentration is 100mgL^{-1}, TCP removal in close circuit reaches 66.72% and TCP removal in open circuit reaches 57.43%. The former TCP removal ratio is nearly 9% higher than the latter. At any other time duration, TCP removal ratio in close circuit is always higher than the one in open circuit. It indicates that TCP removal in MFC is better than the tradition way of anaerobic biological treatment. It is due to current generated in MFC stimulating the microbe utilizing TCP.

3.4. Electrochemical performance

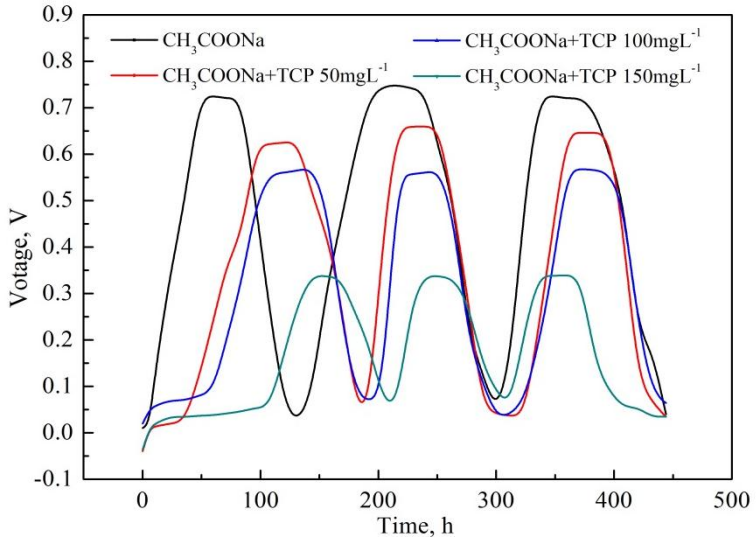

Figure 6. The effect of 2,4,6-TCP initial concentration on the voltage output of MFCs

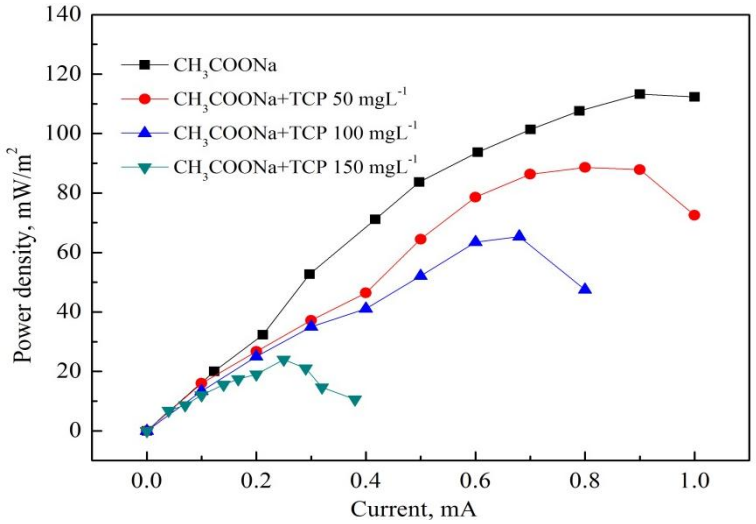

Figure 7. Effect of TCP on MFCs performance (concentration of sodium acetate 20 mmolL^{-1})

As can be seen in Fig.6, it shows electrochemical performance of different TCP concentration in MFCs. When TCP concentration is 50 mgL^{-1}, voltage output was lower than that of the one added sodium acetate only. After two cycles more domestication, bacterium on the anode of MFCs adapt to the toxic environment and voltage output is stable. The maximum voltage output (0.72V) of MFCs containing sodium acetate and TCP is about 80 mV lower than that (0.64 V) at condition of containing sodium acetate only. The maximum voltage output decreases with the increasing TCP concentration. It can be inferred that TCP has negative effect on voltage output.

As it shown in Fig.7, TCP has a great effect on MFCs performance. When sodium acetate is the only substrate, its power density can steadily reach 116 mW/m^2. As concentration of TCP in MFCs increases, power density value decreases. At TCP concentration of 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-}

[1], power density reach 88 mW/m^2, 65 mW/m^2 and 24 mW/m^2, respectively. It clearly shows that TCP restrains MFCs performance.

3.5 The mechanism of 2, 4, 6 -TCP degradation

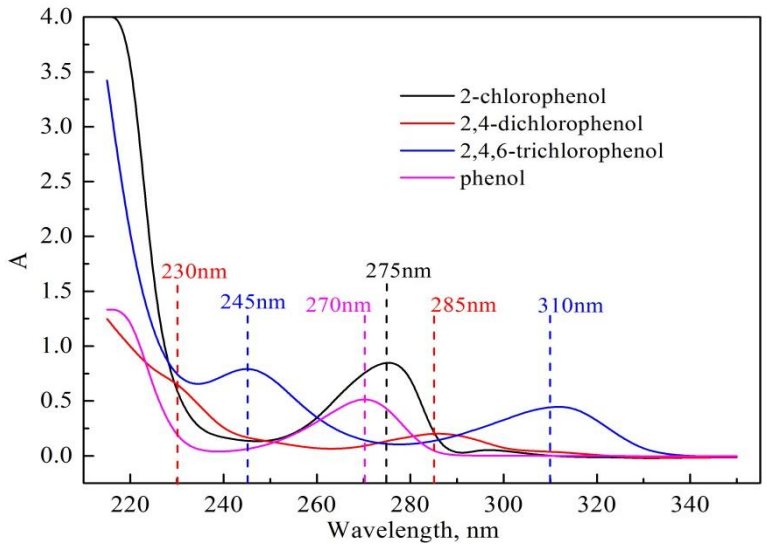

Figure 8. The UV-visible spectra of pure 2,4,6-trichlorophenol, 2,4-dichlorophenol, 2-chloriphenol and phenol (scan rate 0.5 div, an interval 5 nm and scan range 215-350 nm)

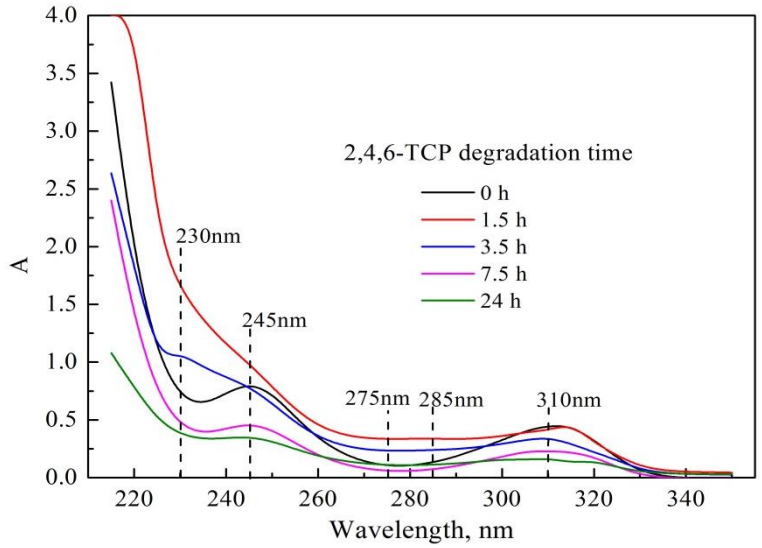

Figure 9. The UV-visible spectra of samples obtained for different degrading time in 2,4,6-TCP degradation process (scan rate 0.5 div, an interval 5 nm and scan range 215-350 nm)

At room temperature, spectral curve of 2,4,6-TCP (99%) and its possible metabolites (dichlorophenol (DCP), 99%; chlorophenol (CP), 99%; phenol, 99%) at wavelength (200–600 nm) are shown in Fig.8. It shows that the peak absorption positions of 2,4,6 -TCP, 2,4-DCP, 2-CP and phenol

were at $\lambda_{(TCP)}$ = 310 nm and 245 nm, $\lambda_{(DCP)}$ = 285 nm and 230 nm, $\lambda_{(CP)}$ = 275 nm, and $\lambda_{(Phenol)}$ = 270 nm respectively. The degradation pathway of 2,4,6-TCP was uncovered relied on the characteristic wavelength. The UV-visible spectra of water samples are shown in Fig.8. No obvious absorbance change during wavelength 350-600nm, so datum of that weren't filled into the Fig.7-Fig.9).

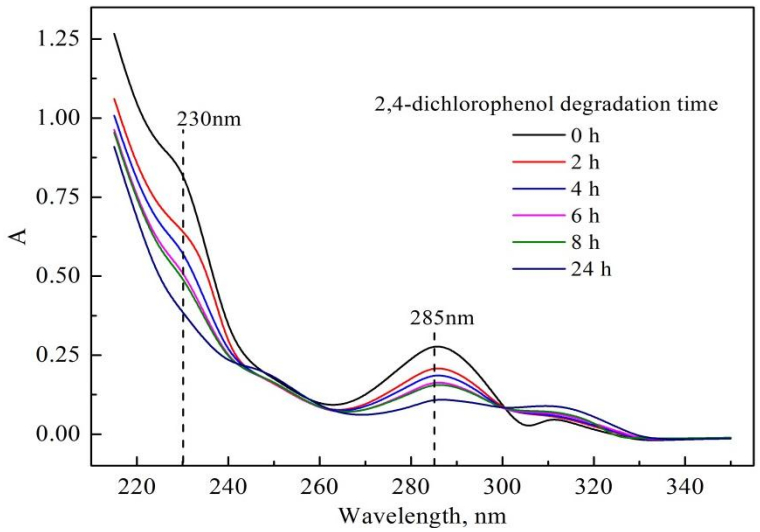

Figure 10. The UV-visible spectra of samples obtained for different degrading time in 2,4-DCP degradation process (scan rate 0.5 div, an interval of 5 nm and scan range 215-350 nm)

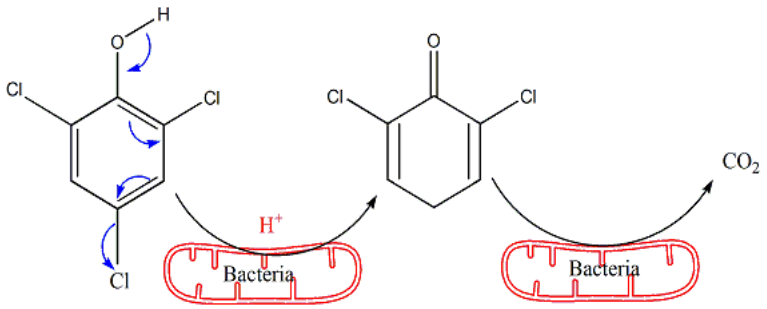

Figure 11. The pathway of 2,4,6-TCP degradation in MFCs

Fig.9 shows that a decrease in absorbance at 310 nm and 245 nm implies the decreasing 2,4,6-TCP concentration. And it is interesting to note that, at wavelength of 230 nm, 2,4-DCP's characteristic peak position, the absorbance value (for sample of degradation time duration 1.5 h) is obviously larger than the one (for sample of degradation time duration 0 h). Moreover, the absorbance values (for samples of degradation time duration over 1.5 h) subsequently decreases as the time increasing. It can be inferred that during degradation process 2,4,6-TCP was converted into 2,4-DCP. But whether CP and phenol are the intermediates of 2,4,6-TCP degradation or not, there is no obvious evidences.

To rule out these, in the same reactor where 2,4-DCP initial concentration was 100 mgL^{-1} without 2,4,6-TCP, experiments were carried out to reveal the degradation pathway of 2,4-DCP. The UV-visible spectroscopies of samples for different degradation time duration are shown in Fig.9. It

shows the absorbance value at 275 nm (2-CP's characteristic peak position in Fig.7) and absorbance value at 270 nm (phenol's characteristic peak position in Fig.7) have no change in MFCs system, which can be inferred that CP and phenol are not intermediates of 2,4,6-TCP degradation. From above discussion, it was certified that under the effect of the microbe, 2,4,6-TCP was converted to intermediate dichloride benzoquinone (easily mutual transformation with DCP) and then was degraded into CO_2 (Fig.10).

4. CONCLUSIONS

Degradation performance of 2,4,6-TCP in MFCs was investigated at initial 2,4,6-TCP concentrations in range of 50 mgL^{-1} - 200 mgL^{-1}. After time duration 120 h, 2,4,6-TCP removal ratio decreased with increasing initial 2,4,6-TCP concentrations, which is due to high 2,4,6-TCP concentration toxic effects on the microorganisms. The time duration of voltage generation at initial TCP concentration of 150mgL^{-1}, is longer than that of the other at initial TCP concentration 50mgL^{-1}. It shows that high initial TCP concentration makes voltage generation time lags. Through the UV-visible spectra of samples at different degradation time duration, it was indicated that 2,4,6-TCP subsequently was converted into an intermediate dichloride benzoquinone and then was degraded into CO_2, which is similar with the existing research [20].

Utilization of marine bio-resource to treat landfill leachate is not only the most significant measure but also the most fundamental initial stage of an effective way to waste water treatment, as the success of the latter is based on the former. The paper has established this not only by defining the baseline study in chlorophenol but also indicating implications of marine bio-resource exploitation.

ACKNOWLEDGEMENTS
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