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Biodegradation of high concentration phenol containing heavy metal ions by functional biofilm in bioelectro-reactor

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Abstract: Functional microorganisms to high concentration phenol containing Cr^{6+} and Pb^{2+} were cultured and biofilm was formed on polypropylene packings in bioelectro-reactor. It was found that the biodegradation capability of such biofilm to phenol changed with the applied voltage. Under the optimal electric field conditions (voltage of 3.0 V, electric field of strength 17.7 V/m and current density of 1.98 A/m^2), biodegradation efficiency of phenol aof concentration of 1200 mg/L increased 33% compared to the instance without applying electric field. However, voltage had inverse effect on biodegradation, as microorganisms were killed under strong electric field. Voltage had little effect on heavy ions elimination. Higher absorption rate of Cr^{6+} and Pb^{2+} was observed when changing pH from acidic to neutral. The experiment results indicated that, after treatment, 10 L phenol of 2400 mg/L was biodegraded completely within 55 h and concentrations of Cr^{6+} and Pb^{2+} dropped to less than 1 mg/L within 12 h and 6 h, from initial values of 50 mg/L and 30 mg/L, respectively.

Keywords: biofilm; biodegradation; phenol; heavy ions; electric field; reactor

Introduction

Phenol is widely used in the preparation of antiseptics, dyes, antirust products, synthetic resin, photographic, chemicals inks, etc. Its derivatives are present in wastewater of many industries such as oil refineries, chemical plants and coke ovens (Sitting, 1997; Nemerow, 1978; Patterson, 1985). Because they are toxic to many biochemical functions (Sufit, 1978) and pose risk to human beings, phenol degradation by microorganism has been studied extensively.

In recent years, industrial utilization of heavy metals such as metal plating, tanning, and preparation of catalysts generates large quantity of aqueous effluent containing high levels of heavy metal (Leung *et al.*, 2000; Chua *et al.*, 1999; Williams *et al.*, 1998), and these heavy metals will get into water system eventually. If not being treated in time, it could bring about serious pollution to human beings. So it is necessary to develop a novel process for efficiently treating this kind of wastewater (Mykola *et al.*, 1999; Yu *et al.*, 1999; Matheickal and Yu, 1999).

Biofilm reactors have been widely used for phenol or heavy metal ions removal from industrial wastewater, but immobilized cell reactor offer several advantages over suspended cell reactors (Livingston and Chase, 1990). These include increasing biomass concentrations, allowing higher loading rates, and resistance to shock loading of inhibitory compounds. Moreover, much attention has been paid to investigate the effect of electric current on the metabolism of bacteria (Jackman *et al.*, 1999; Li *et al.*, 2001) over the past decade. The combination of electrokinetics and biodegradation in environmental protection (Sakakibara and Kuroda, 1993; Kuroda *et al.*, 1997; Islam and Suidan, 1998) has received much attention due to its high efficiency and low cost.

Li *et al.*(2003) has reported purification of wastewater containing glucose and metal ions such as Cr^{3+} and Cu^{2+} whose solubility product constant are smaller than that of water, by a combined process of micro electrolysis and biofilm. However, to the best of our knowledge, it has not been reported in open literature that high concentration, difficultly biodegraded organic matter, such as phenol (concentration over 1000 mg/L) containing heavy metal ions of very different solubility product constant such as Pb^{2+} and Cr^{6+} (5.18×10^{7} mg/L and 3.48×10^{-2} mg/L at pH=6), was biodegraded by immobilized microoganisms under electric field.

In this work, the self-cultured functional microorganisms which could endure high concentration of phenol were used to form biofilm in a bioelectro reactor. The objectives of this work are to study the effect of direct current electric field on biodegradation capability of such biofilm to phenol containing heavy metal ions and to find the optimal electrical field conditions for biodegradation efficiency.

1 Materials and method

1.1 Materials

The activated sludge was obtained from the Wastewater Treatment Plant of Jizhuangzi, Tianjin, China in October 2004. The polypropylene packing was provided by the Tianjin University Packing Co., Tianjin, China (TUPAC2#, specific surface area 250 m^2/m^3) and used for the preparation of biofilm. All of the chemicals were of analytical grade.

1.2 Reactor set-up

The schematic diagram of the experimental apparatus is shown in Fig.1. In order to study biodegradation capability of the electro-biofilm

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process in fixed quantify, circular loop was used to deal with wastewater, this can be conveniently turned into continuous operation mode in actual application. The bioelectro-reactor consisted of a rectangular organic glass vessel with dimensions of 21cm × 30 cm × 20 cm. The gas distributor consisted of four tubes (full of pinholes) at the bottom of the reactor,

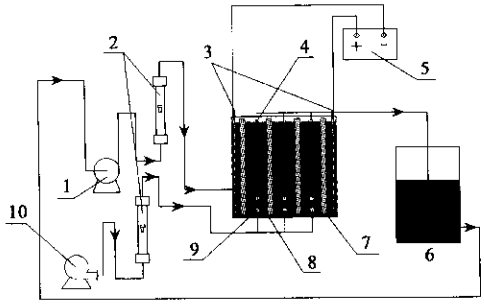

Fig.1 Schematic diagram of the experimental apparatus
1. peristaltic pump; 2. rotameter; 3. cathode and anode; 4. water inlet; 5. regulated power supply; 6. water container; 7. polypropylene packings; 8. reactor; 9. air distributor; 10. air compressor

dissolved oxygen was supplied to the biofilm adhering to polypropylene packings. The size of the packing is 15 cm × 10 cm. Two 20 cm × 15 cm stainless steel electrodes were placed in the vessel, 17 cm apart. Type WYJ-0 direct current power was supplied by Quanli Electrical Appliance Co., Shanghai, China.

1.3 Experimental

The activated sludge had been enriched in a 10-L container, using glucose as carbon source at beginning. Phenol was substituted for glucose gradually until it became the sole carbon source, the concentration of phenol increased continuously. At the end of the 12th weeks, the microorganisms in activated sludge could endure phenol of 2100 mg/L. The concentrations of Cr^{6+} and Pb^{2+} were also increased during the course. The compositions of culturing or acclimating materials at the beginning and end of the course are shown in Table 1.

After cultured and acclimated, the functional bacteria were enriched and inoculated on poly-

Table 1 Composition of culturing or acclimating materials at beginning and after 12 weeks

	$C_6H_{12}O_6$, mg/L	$(NH_4)_2SO_4$, mg/L	$FeSO_4 \cdot 7H_2O$, mg/L	$MgSO_4 \cdot 7H_2O$, mg/L	KH_2PO_4, mg/L	Cr^{6+}, mg/L	Pb^{2+}, mg/L	Phenol, mg/L
Beginning	800	500	25	10	30	0	0	
After 12 weeks		500	25	10	30	50	30	2100

propylene packings to form biofilm in the bioelectro-reactor as shown in Fig. 1. The formation of the biofilm would take for two weeks. During this period, heavy metal ions and mineral medium were supplied every 24 h so as to keep their initial concentrations as listed in Table 1 and phenol concentration was also adjusted to 1200 mg/L every 24 h. The solution was circulated between reactor and container. Water flux was kept at 0.2 L/h during the first 5 d, then increased from 0.2 to 0.5 L/h regularly in the next 9 d. Air flux was kept at 200 L/h during the whole 14 d. On the day 14, the biofilm on one of the ten polypropylene packings at settled station in the reactor was taken out for measuring its dry weight. The others were kept at their primary stations for electro-biodegradation experiment. Electric field strength and current density were changed by varying the applied voltages, and the optimal electric field condition could be found at initial concentration of 1200 mg/L. The biodegradation capability of the biofilm to phenol was measured at different phenol concentrations ranging from 1600 to 2800 mg/L under the obtained optimal electric field condition. At the beginning of each experiment, 10 L water container was filled with solution containing mineral medium in fixed quantity and phenol at concentration that experiment needed.

1.4 Analytical methods

After cultured and acclimated, the SEM pictures of functional biofilm samples were taken at Centre Laboratory of Nankai University by scanning electron microscope (SEM) (Japanese Electronic Company, Japan). Before analysis, the samples were centrifuged at 4500 r/min for 10 min and the supernatant fraction was filtrated with G3 glass acid-resistant funnel. The concentration of phenol undegraded in the samples was determined by a modified colorimetric technique using 4-aminoantipyrine as a reagent at 510 nm by UV spectrophotometer (UV-9100, Beijing Ruili Analysis Apparatus Company, China), based on a standard method for phenol. Biofilm was harvested from polypropylene packing by washing and vacuum filtration. After washing twice with the same volume of deionized water to remove salts, biofilm was dried to constant weight at 105℃. The concentration of Cr^{6+} and Pb^{2+} were measured by the polarized zeeman atomic adsorption spectrophotometer (Hitachi 180-80, Japan).

2 Results and discussion

2.1 SEM pictures of functional biofilm samples

The SEM pictures of acclimated biofilm are shown in Fig.2.

In these two pictures, a kind of bacterium intimate intergrowth with filamentous fungus. A mass of this kind of bacteria adhered to the "frame" of filamentous fungus. With increasing of phenol concentration, pH value decreased and biological oxygen demand increased in the wastewater, so the filamentous fungus grow in a large amount. It is the

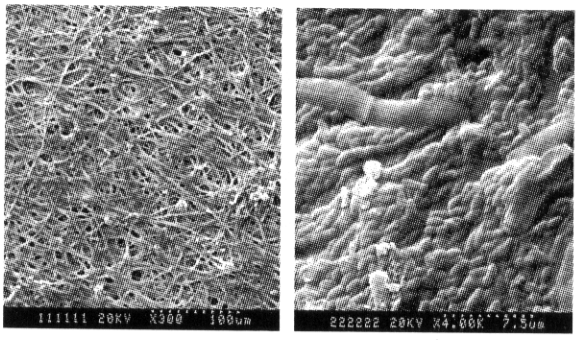

Fig.2 SEM morphology of acclimated biofilm
a. ×300; b. ×4000

support and immobilized function of the fungus "frame" that made phenol biodegraded by these two kind of microorganisms become more availably. Beyond doubt, the filamentous fungus and another kind of bacterium, that had been acclimated to 2100 mg/L phenol solution were the functional strains.

2.2 Influence of voltage on phenol biodegradation process

With Cr^{6+} and Pb^{2+} present (50 and 30 mg/L, respectively), the effect of voltage on phenol degradation efficiency is shown in Fig.3. As shown in Fig.3a, voltage has a significant effect on the time needed to degrade phenol. Without applying voltage, it took abour 28 h to fully degrade phenol from 100 mg/L to about 0 mg/L. At 3 V, 10 L phenol of 1200 mg/L was entirely degraded by biofilm within 21 h, so the biodegradation efficiency to phenol was increased 33% compared to 0 V. However, further increase of voltage inversely affect the phenol degradation. At 10 V, the phenol concentration only decreased a little over 32 h. This is due to large numbers of the microorganisms were killed at such high voltage. Evidence from experiment is that, in a few hours, biofilm sheded from packings in pieces and plenty of dead microbes floated over the water. The influence of voltage can be ignored at voltage below 1 V, as observed from Fig.3a and 3b.

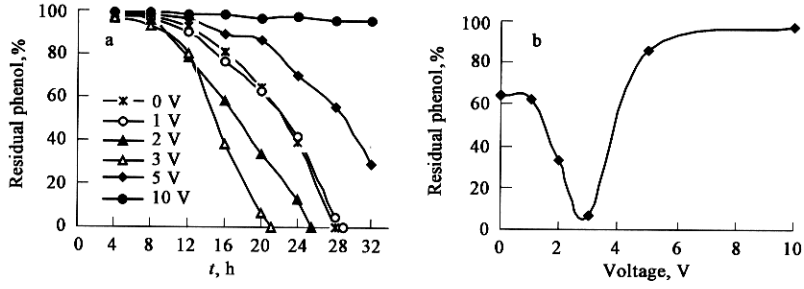

Fig.3 Phenol containing heavy metal ions degraded by functional biofilm under voltage from 0 to 10 V (a) and voltage from 0 to 10 V at 20 h treatment (b)

When voltage is fixed, the electric field could be treated as steady one because parameters such as electrode area, solution conductance, lead resistance and space between electrodes are all fixed value, and so the relationship between voltage (U), electric field strength (E) and current density(j) exists. That means that E and j are directly proportional to U. We considered the experimental result as the conjunct action of electric field strength and current density together. Mass transfer of substrate fluxes was intensified through directional movement of culture medium ions with the increasing of electric field strength. Cao *et al.* (2003) had demonstrated that the thickness of biofilm and flux of NH_4^+ increases are proportional with increasing electric field strength when current density is constant, and the increasing of current density decreases the biofilm thickness and substrate fluxes.

On the other hand, because there was weak current exists in all of the biology, the stimulation action to cells growth was strengthened with increasing electric field strength. The cells facing anode-end grew faster than the cells facing the cathodes ends under direct current electric field (Rajnicek *et al.*, 1994). Moderate current density can adjust metabolizability of microorganisms. It leads to increasing of cell exponential, shortening periods of karyokinesis and increasing mitosis rate so that the biology grow faster under the electric field (Jia *et al.*, 1988). The imbalance of microorganism metabolism would be induced by immoderate current density, and cells were even killed directly when voltage was too high. However, the mechanism of how current density act on the biology is still unknown. The phenomena in our experiment could be explained as directional growth of cells in response to electric field. In this study, the optimal electric field conditions include: voltage of 3 V; electric field strength at value of 17.7 V/m and current density of 1.98 A/m^2.

The average dry weight of biofilm on the polypropylene packing was 633 mg. The maximal average error was less than 1.5% in thrice experiments.

2.3 Biodegradation capability of functional biofilm under the optimal electric field conditions

Under the optimal electric field conditions,

experimental results of biodegradation capability to phenol at different initial phenol concentrations are shown in Fig.4. Phenol solutions with different initial concentrations from 1600 to 2400 mg/L were treated by biofilm. The biodegradation process needs longer time for solution with higher initial phenol concentration. At initial phenol concentration of 2400 mg/L, 10 L phenol solution was degraded completely in 55 h only. We can conclude that high concentration phenol could be degraded by electro-bilfilm technique effectively.

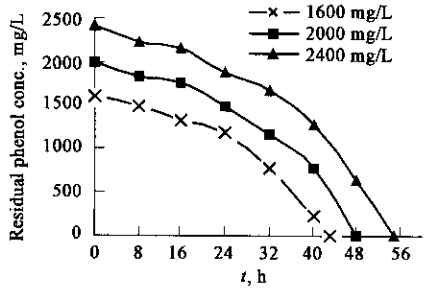

Fig.4 Biodegradation capability to phenol at different initial concentrations

2.4 Effect of voltage on heavy metal ions adsorption process

Fig.5 presents the results of treating organic wastewater containing Cr^{6+} and Pb^{2+} by biofilm at different applied voltages varying from 0 to 10 V.

It can be seen that the influence of voltage to Cr^{6+} and Pb^{2+} adsorption process is negligible. Only very small difference among experiment results for voltages at 0 V (without electric field), 3 V (the biofilm has the strongest activities) and 10 V (plenty of microorganism dead, biofilm sheded from packings in pieces and floated on the wastewater). As we thought, the main mechanism to heavy metal ions biodegradation is adsorption. The adsorbing capability of the biofilm to specific kind of metal ions is dependent on its structure and is not related to microorganism biological activities. This may explain why the applied voltage has no effect on the heavy metal ions adsorption process.

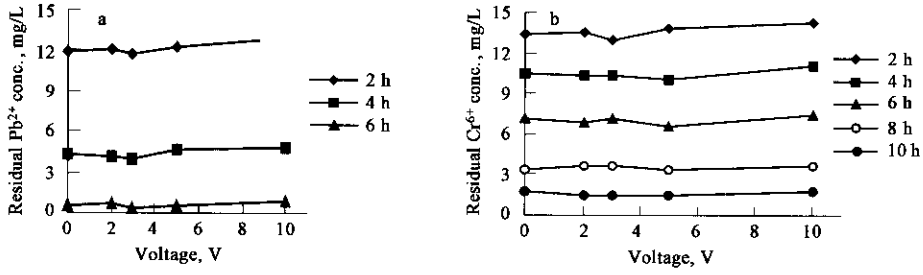

Fig.5 Influence of voltage changing on the adsorption process Pb^{2+} (a) and Cr^{6+} (b)

2.5 Effect of pH value on heavy metal ion adsorption process

Fig.6 illustrates that wastewater pH had an important effect on the removal rate of Cr^{6+} and Pb^{2+}. Much higher absorption rate of heavy metal ions was observed when the pH was increased from acidic wastewater (pH=2.36) to neutral wastewater (pH= 6.50). Usually, microbes will lose activity in the environment with pH below 3.0, but physical adsorption functions caused by netlike structure of biofilm to metal ions do predominant action. At low pH (pH=4.53), heavy metal removal was inhibited, possibly as a result of a positive charge density on metal binding sites due to high concentration of protons in solution. With an increase of pH, the negative charge density on the cell surface increases due to deprotonation of metal binding sites, thus increases biosorption. In addition, network structure of the functional biofilm could also promote adsorption. Under the condition of neutral pH, 50 mg/L Cr^{6+} and 30 mg/L Pb^{2+} were degraded to less than 1 mg/L

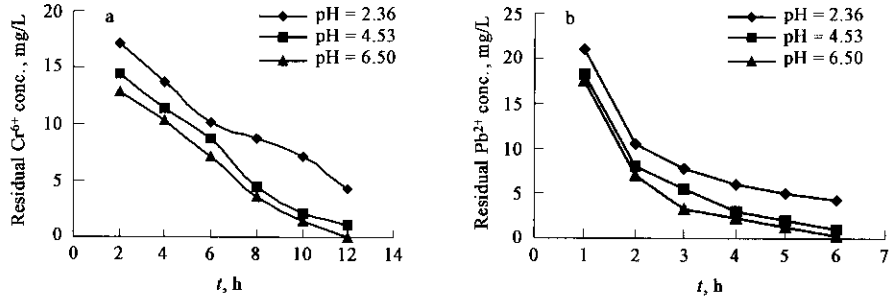

Fig.6 Effect of pH on biosorption to Cr^{6+}(a) and Pb^{2+}(b)

within 12 h and 6 h, respectively.

3 Conclusions

The wastewater containing high concentration phenol and heavy metal ions can be effectively biodegraded by biofilm in bioelectro-reactor. The biodegradation capability to phenol improved obviously under suitable electric field conditions. At 3.0 V, 17.7 V/m and 1.98 A/m^2, biodegradation efficiency of phenol was increased to 33% at concentration of 1200 mg/L compared with that without applying electric field. However, the degradation process was inhibited at a high voltage, and a mass of microorganisms could be killed by a high voltage over 10 V. We consider the phenomena as the conjunct action of electric field strength and current density together. Mass transfer of the substrates fluxes was intensified through directional movement of culture medium ions with the increasing of electric field strength, so the biofilm could grow faster. With the increasement of current density, the activity of microorganisms were strengthened at first, then were inhibited when current density reached to definite range and lose biodegradation capability for imbalance of metabolism at last. Obviously, there is an optimal condition existing between electric field strength and current density. The experiment results indicated that, after treatment, 10 L phenol of 2400 mg/L was biodegraded completely within 55 h under the optimal electric field conditions.

The main mechanism of heavy metal ions elimination was though to be biosorption. There is little effect of electric field on heavy metal ion treatment. But the effect of pH value was significant on heavy metal ion elimination. In pH range of 2.36 to 6.50, removal rates of Cr^{6+} and Pb^{2+} were increased as pH increased. When pH was below 3.0, microbes will lose activity, but physical adsorption functions caused by netlike structure of biofilm to metal ions did predominant action. Under the condition of neutral pH, 50 mg/L Cr^{6+} and 30 mg/L Pb^{2+} were degraded to less than 1 mg/L within 12 h and 6 h respectively.

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