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Phenol biodegradation in an aerobic fixed-film process using conductive bioelectrodes: Biokinetic and kinetic studies

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ABSTRACT

The main goal of this article is to present biokinetic of phenol biodegradation in a bioelectrochemical process using steel wool and carbon cloth electrodes. Phenol biodegradation experiments were performed in a batch set up using a plexiglass reactor. Kinetic data were evaluated at pH 7, optimum applied current of 2 mA and laboratory temperature (24 ± 1°C). Optimum phenol concentration of 250 mg L^{-1} was considered for determination of biokinetic parameters in Haldane model. The main kinetic parameters such as K, R^2, μ_{max}, K_s, K_i, Y, b and biofilm density were determined in the bioelectrochemical process. The obtained results showed that the degradation of phenol was following the first order kinetic. Biodegradation and mineralization of phenol were 100% and 95% at 8 h reaction time, respectively. The μ_{max}, K_S and K_i of Haldane model were 0.16 h^{-1}, 0.26 mg L^{-1} and 300 mg L^{-1}, respectively. The yield factor and decay coefficient were achieved 0.324 mg VSS/mg phenol and 0.117 d^{-1}, respectively. The obtained experimental data indicate that the Halden model is applicable for the biokinetic behaviour description of phenol degradation in bioelectrochemical system.

Keywords: Biodegradation; Haldane model; Biokinetics; Kinetic; Bioelectroactive; Phenol

1. Introduction

Phenol is an industrial chemical compound which creates various health and environmental problems. It is highly toxic to human, animals and plants [1]. It can be toxic to humans through skin absorption, vapor inhalation, or gastrointestinal. The acute toxicity can lead to muscle weakness, convulsions, and death [2]. Many industries such as coal-producing industries, refineries, pesticides, plastics, explosives, and herbicides widely used phenolic compounds [3]. Phenolic compounds have been presented on the list of priority pollutants of American Environmental Protection Agency (EPA) [4]. Because of the high toxicity of phenol, EPA has been proposed the strict standards for phenol concentration in drinking and mineral waters, sewer system, and discharge to surface water [3]. Various processes such as ion exchange, solvent extraction, advanced oxidation, adsorption, and burning have been proposed for removal of phenolic compounds from aquatic solutions. Disadvantages of these techniques are high cost, low efficiency, and production of toxic substances. Phenol biodegradation has been considered due to the environmental friendly process, accessibility to the sustainable final products, and its cost-effectiveness [5]. Generally, in the phenol biodegradation process, bacteria utilize phenol as a carbon and energy sources under aerobic and anaerobic conditions [6]. The process has some benefits including more stable biodegradable performing, less sludge production, and higher tolerance against phenol [7]. Recently, bio-electrochemical process is regarded as a novel technology for simultaneous production of electricity and wastewater treatment [8]. In bioelectroactive systems, microorganisms attach to surface of electrodes to carry out the oxidation or reduction [9]. Electric current is used directly in bioelectrochemical reactors, to increase the efficiency of biodegradation by microorganisms [10]. In recent years, the tendency to use bioelectroactive processes is increasing for the organic pollutant treatment

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using a low voltage electric current [11]. The electrodes material, structure and arrangement have an important role in the performance of the process [12]. Carbon cloth utilized as an electrode with high effective surface, which can improve bioelectrochemical system efficiency. It is considered as electrode due to its availability, relative low cost, and easy processing, stability in various pH values and high-temperature range [13]. Also, steel wool could be utilized as an effective electrode because of its high conductivity, low cost, high surface area for biofilm formation, reducing the operating current density, low power consumption, and the ability of high rates oxidation achievement in bioanode [14]. Biokinetic studies will assist the better process control and pollutant biodegradation efficiency in the bioelectrochemical process. One of the most widely proposed models for the biodegradation of pollutants is the Haldane model. This model is used to describe the specific growth rate dependence on substrate concentration. The Haldane model considers the inhibitory effect of toxic pollutants such as phenol. To date, some studies on evaluation of Haldane-model for phenol biodegradation have been reported [15]. However, no study has been reported to evaluate the biodegradation of phenol using Halden model in a bioelectrochemical system. Therefore, the aim of this study was to estimate the main parameters (Ks, Ki, Y, μ, b) of phenol biodegradation based on the Haldane model. It is expected that experimental results could be used as a reference for the biokinetic studies of toxic organic pollutants using bioelectrochemical systems.

2. Materials and methods

2.1. Chemicals

All materials used in this study were of analytical reagent grade and were used without further purification. Distilled water was used for all dilutions. Phenol, ammonium hydroxide, amino anti-pyrene, potassium ferricyanide, microbial nutrients were obtained from Merck (Germany).

2.2. Bioelectrochemical reactor set up

The bioelectrochemical reactor consists of a plexiglass cylindrical vessel having a diameter of 8 cm and a height of 30 cm with an effective volume of 1 liter. The reactor was equipped with steel wool as anode electrode. Carbon cloth (2 × 21 cm) was used as a cathode in the center of the reactor. The contents of the reactor were mixed by a magnetic stirrer (300 rpm). Air bubbles were supplied for aeration through a dispenser at the reactor bottom. A power supply (Atten APS3005-3D, China) was used to supply direct current in the electrodes. The setup of bioelectrochemical reactor is illustrated in Fig. 1.

2.3. Experiments

The experiments were carried out in laboratory scale. Prior to the operating experiments, the active bacteria were inoculated to the reactor from activated sludge obtained

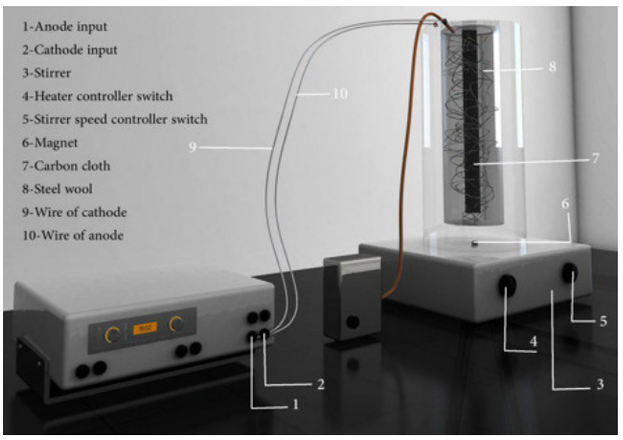

Fig. 1. Schematic of the electrochemical bioreactor.

from a municipal wastewater treatment plant (Tehran, Iran). 200 mL of return sludge and 800 mL of culture medium were introduced into the bioelectrochemical reactor. The culture medium contained the following (per liter of distilled water): KH_2PO_4 10 g, K_2HPO_4 3.0 g, NH_4Cl 1.0 g, $NaHCO_3$ 6.0 g, $MgSO_4{\cdot}7H_2O$ 0.3 g, NaCl 1.0 g, $CaCl_2$ 0.3 g, and $FeCl_2$ 0.2 g. The final MLSS concentration in the bioreactor was 2600 mg/L. At the end of a single cycle, samples were collected and analyzed. Then 90% of the medium was replaced with fresh medium and the power supply was switched to process next cycle. The operating parameters include; initial concentration of phenol (50–300 mg L^{-1}), applied current (2–8 mA) and reaction time (3–8 h) were studied during the experiments. All experiments were performed at least three times at laboratory temperature (24 ± 1°C) and pH 7.

2.4. Analytical methods

Electrical conductivity, pH and redox potential (ORP) were studied during the experiments. The samples were centrifuged (Hettich Universal 320R, Germany) at 5000 rpm for 10 min. The phenol concentrations, VSS, MLSS and COD were measured in accordance with Standard Methods [16]. COD was determined using a UV-Vis spectrophotometer (9200UV/VIS). Microbial growth was determined by measuring optical density (OD) at 600 nm. The threshold of accuracy of this method is 10^7 CFU/ml and equal to 20 mg/L.

2.5. Kinetic and biokinetic studies

2.5.1. Kinetic parameters

The kinetic of phenol degradation was evaluated by the kinetic model as follows:

The phenol degradation was calculated using Eq. (1):

$$\text{Phenol degradation}(\%) = \frac{C_0 - C_t}{C_t} \times 100 \quad (1)$$

where C_0 is the initial phenol concentration in the solution (mg L^{-1}) and C_t is the final phenol concentration in the solution (mg L^{-1}).

The amount of phenol mineralization was calculated using Eq. (2):

$$\text{Phenol mineralization}(\%) = \frac{\text{COD}_0 - \text{COD}_t}{\text{COD}_t} \times 100 \quad (2)$$

where COD_0 is the COD concentration in the solution before mineralization (mg L^{-1}) and COD_t is the COD concentration in the solution after mineralization (mg L^{-1}).

The rate constant (k) was obtained by fitting the data with the first-order kinetic model. The model fitted to the experimental data was assessed based on the amount of R^2 value. It was found that the degradation of phenol, chlorophenol, nitrophenol by the bioelectrochemical system follows the first order kinetic [17,18]:

$$Ln\left(\frac{C_t}{C_0}\right) = -K_1 t \quad (3)$$

where t is the reaction time and K_1 is the reaction rate constant first-order reaction rate constant.

The second order reaction was calculated as follows:

$$\frac{1}{C_t} - \frac{1}{C_0} = K_2 t \quad (4)$$

where C_0 is the initial phenol concentration in the solution (mg L^{-1}), C_t is the final phenol concentration in the solution (mg L^{-1}) and K_2 is the reaction rate constant of second-order reaction

2.6. Biokinetics parameters

The Haldane's inhibitory growth kinetics equation was used for biokinetic studies as follows [19]:

$$\mu = \frac{\mu_{\max} S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \quad (5)$$

where K_i is the inhibition constant, K_s is the half saturation constant, μ_{max} is maximum of growth rate, and S is the substrate.

The yield coefficient (Y) for phenol-utilizing bacteria can be determined by the following equation [20]:

$$Y = -\frac{\Delta X}{\Delta S} \quad (6)$$

where ΔX is the change in biomass concentration, ΔS is the change in substrate concentration.

The decay coefficient (b) was calculated from the following equation [20]:

$$b = -\frac{\ln\ln\left(\frac{x2}{x1}\right)}{t2 - t1} \quad (7)$$

where t_1 and t_2 are the initial and final time, X_1 and X_2 are the initial and final biomass concentration.

3. Results and discussion

It was reported that degradation of phenol is performed in several steps [17]. The phenol is oxidized by oxidizing agents such as hydroxyl radical. Without the strong oxidizing agent of free radicals, the by-products would accumulate in the system. The hydroxyl radical generation would provide a sufficient non-selective oxidizing capacity that could attack the aromatic rings more effective [21]. Finally, the phenol rings were converted into sustainable products and inorganic such as CO_2 and H_2O, after formation of enough hydroxyl radicals on the anode. In order to study the phenol degradation using the bioelectrochemical system, COD analysis was considered during 3–8 h. The percentages of phenol degradation and mineralization were calculated using Eqs. (1) and (2). The obtained results showed that the highest phenol mineralization was achieved in 250 mg L^{-1} and 2 mA applied current during 8 h (Fig. 2). The highest sCOD removal (98.5%) was obtained with steel wool as anode and carbon cloth as cathode using 6 mA applied current at 24 h. The high COD removal efficiency could be related to production of hydroxyl radicals (·OH), which it could remove phenol from aqueous solution [1,22]. An integrated system using a pair of stainless iron meshes-graphite plate electrodes has been reported for removal of phenol due to phenol hydroxylase activity and microorganism synergistic effect [23]. In a similar study, the high performance of an electrode of metallic base with a carbon coating electrode was reported because of the high conductivity and suitability for microorganisms [24]. Also, phenol removal (250 mg L^{-1}) was reported at 50°C, electrical voltage of 5 V, and pH 2 using Ti/PbO_2 electrode. The COD reduction of Ti/PbO_2 and Ti electrodes were 78 and 22%, respectively. The COD removal rate increased at pH 2, because of conversion of CO_2 is increased substantially [25].

3.1. Kinetic analysis of phenol degradation

Kinetic analysis was applied to investigate the efficiency of applied currents for the considered phenol concentration degradation using 2–8 mA applied current to the electrodes. Kinetic data for phenol degradation was analyzed during 3, 6, 8 h retention time. Fig. 3 represent the phenol degradation at different electrical currents during the reaction time. As shown in Fig. 3, the degradation of phenol increased from 65% to 96.26% by increasing the applied current from 0.26 mA/m^2 to 0.79 mA/m^2 during 3 h. Higher applied current could make the better electrical stimulation and increasing the oxidation of phe-

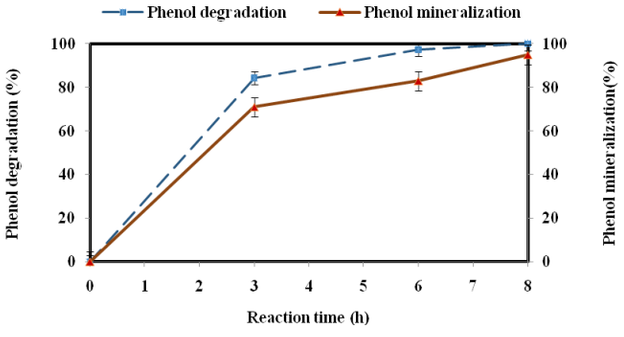

Fig. 2. Phenol degradation and mineralization (pH = 7, initial concentration of phenol = 250 mg L^{-1}, applied current = 2mA).

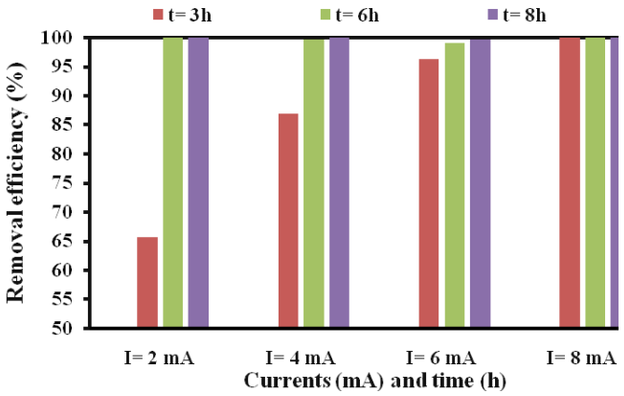

Fig. 3. Effect of applied electrical current on phenol degradation efficiency during the experiments (pH = 7).

Table 1
First order and second order kinetics of phenol degradation at various applied currents; initial phenol concentration = 250 mg L^{-1}

Electrical current (mA)	First order kinetic		Second order kinetic	
	K_1 (h^{-1})	R^2	K_2 (h^{-1})	R^2
2	1.240	0.923	10.36	0.687
4	1.218	0.958	12.05	0.668
6	0.937	0.877	7.009	0.339
8	0.960	0.616	9.488	0.616

nol in the steel wool electrode. Mashkour and Rahimnejad reported that the carbon cloth is an appropriate electrode according to the generated power density, and the high surface area [13]. The obtained results showed that high applied current leads to decrease in phenol concentration and increase in the reaction rate constants of degradation. Table 1 lists the respective values of first order and second order kinetics of phenol degradation at applied electrical currents. The R^2 values given in the first order kinetics indicate that the kinetic model have fitted in applied current of 2, 4 mA. The second order kinetic model represents the lower linear regression coefficient in all applied current ranges. According to the obtained results, the phenol degradation data were well fitted by the first order model. The phenol degradation rate was higher at lower electrical current (2 and 4 mA). Biofilm sloughing was observed in the high-applied currents. Ailijiang et al. reported that the phenol biodegradation was stimulated by applying 2 mA current in the biofilm attached to the electrodes [26]. The peak value of *K* obtained in this study was similar to the Ailijiang report. The phenol degradation rate constant of electrobioreactor with Ti/PbO_2 as anode and stainless steel as cathode was higher than present bioelectrochemical reactor [27]. The BDD electrode has lower rate constant compare to this study [17]. The results show that biological and electrochemical processes have a synergistic effect. Hence, the bioelectrochemical process could increase the production of the active substances [27].

3.2. Biokinetic studies

A range of 50–300 mg L^{-1} was used as initial phenol concentration. The number of bacteria was measured by optical density at 600 nm. The growth rate of bacteria as a function of time is represented in Fig. 4. The suspended biomass increased from 50 to 250 mg L^{-1} of phenol concentrations, but it decreased in 300 mg L^{-1} of phenol concentration. Therefore, the inhibition effect of phenol was revealed at 300 mg L^{-1}. This decreasing of biomass growth confirms that phenol is an inhibitory compound over the 300 mg L^{-1} concentrations [28]. Fig. 5 shows the relation between the phenol concentration and the growth curve of the mixed microorganisms over time at the applied current of 2 mA. Because of the inhibitory of phenol at high concentrations, the microorganisms need to acclimate to the phenol concentrations. In this study, we have acclimated the applied microorganisms via co-metabolism process during one-month. In the first step, glucose was used as the carbon source, then phenol was added as carbon source. After acclimation period, the suspended biomass reached to the stationary phase after 6 h. Also, the complete removal of phenol occurred during 6 h. In a similar study, the stationary phase and optical density of suspended growth were reported 5 h and 578 nm, respectively [19]. Haldane model is a modified version of monode model which represents inhibitory effects of toxic materials [29]. It was determined by Eq. (5). The adapted mixed microbial to the inhibitory phenol at a specified inhibitory concentration μ tended to decrease due to the toxic effect of phenol. In order to estimate of specific growth rate, initial phenol concentrations were utilized from 50 to 300 mg/L. The specific growth rate for studied phenol concentrations was obtained from the slope of linear logarithmic plots of optical density against time [19]. Fig. 6 shows the specific growth rate (μ) in various phenol concentrations (S). The μ values were obtained 0.079, 0.092, 0.104, 0.111 and 0.094 h^{-1} within 50–300 mg L^{-1} at 2 mA during 8 h. The maximum value of μ was achieved at substrate concentration of 250 mg L^{-1}. By increasing phenol concentration more than 250 mg L^{-1}, the cell growth rate was decreased. High concentrations of phenol lead to toxicity effects for microorganisms [31]. Zeyoudi et al. has reported lower of μ in applied current of 5 A m^{-2} compare to results of this study [22]. The lower amount of applied current and lower biostimulation could be reason of different growth rate. The model parameters including μ_{max}, K_s and K_i were obtained using the non-linear regression and fitting the experimental data with specific growth rate as a phenol concentration function [30]. The μ_{max}, K_s and K_i, were determined to be 0.16 h^{-1}, 0.26 and 300 mg L^{-1}, respectively. No previous studies were found which use the Haldane model for bioelectrochemical process, but some biological studies were reported regarding this model. The value of maximum specific growth rate (μ_{max}) has determined in the range of 0.051–0.656 h^{-1} for phenol degradation in bioelectrochemical system. Pishgar et al. reported the same values for aerobic condition [31]. The obtained value of K_s, 0.26 mg L^{-1}, shows the microorganisms were able to grow at low phenol concentration. While K_s value in the previous studies was more than the present study [32]. The high tendency to toxic substrate could be achieved by acclimatization during electrical stimulation in a bioelectrochemical process. The obtained K_i value shows that the mixed cultures

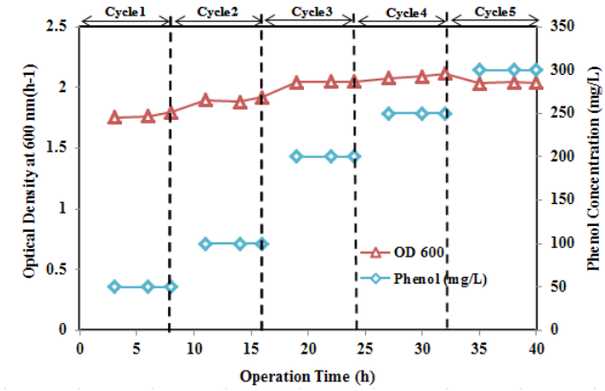

Fig. 4. Optical density of the suspended biomass as a function of time.

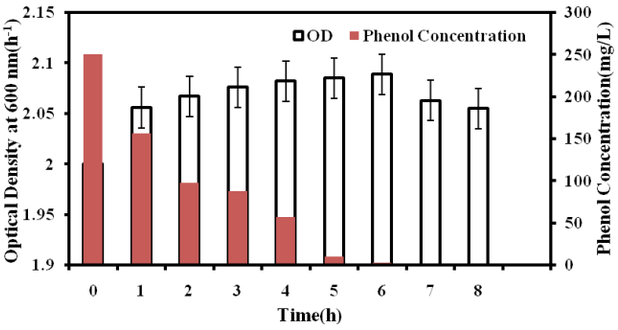

Fig. 5. Relation between phenol concentration and the cell growth.

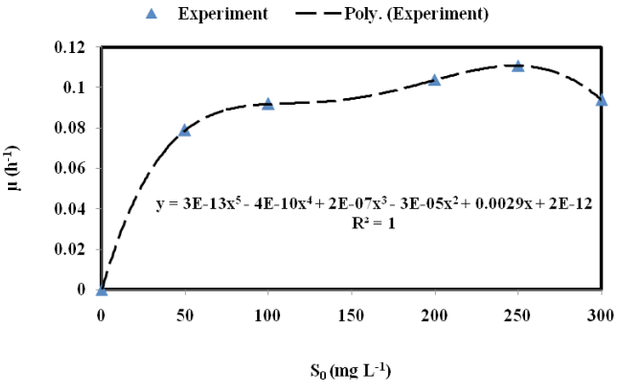

Fig. 6. Relationship between the specific growth rate (μ) and phenol concentration (S).

of microorganisms are less sensitive to substrate inhibition as compared to pure cultures [29]. The yield coefficient was determined in 250 mg L^{-1} initial phenol concentration using Eq. (6). The yield coefficient was determined from slope of linearized plot of the suspended biomass production (X–X_0) versus phenol consumption (S_0–S). The obtained yield coefficient was 0.324 mg VSS/mg phenol (Fig. 7). It was reported that the yield coefficient (Y) is approximately independent of over the range of phenol concentrations for suspended biomass experienced in the growth phase [19]. The theoretical yield coefficient in microbial electrolysis cells was reported to be 0.1–0.3 gVSS $gCOD^{-1}$ at pH 7–9, which is lower than yield coefficient of aerobic microorganisms in activated sludge systems but is similar to yield coefficient of anaerobic microorganisms from anaerobic digester. The yield coefficient of biological phenol treatment from wastewater was reported 0.58 mgVSS/mg phenol [20]. The value of the decay coefficient was obtained 0.117 d^{-1} using Eq. (7) (Fig. 8). This value is approximately equal to biological phenol treatment from wastewater.

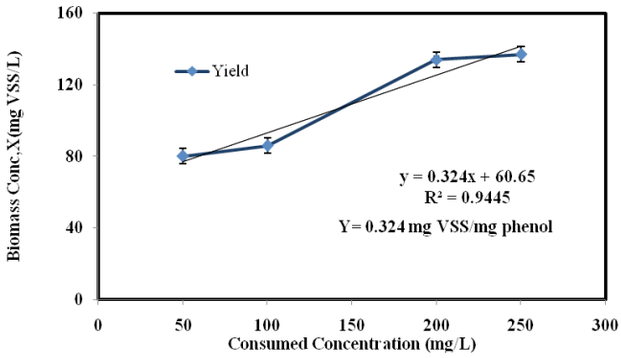

Fig. 7. Yield coefficient of suspended biomass.

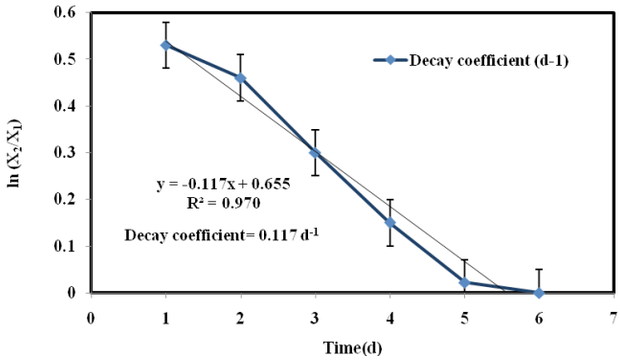

Fig. 8. The true decay coefficient.

4. Conclusions

Based on the results, we reached the following conclusions:

- The Haldane model is an appropriate model for phenol as a toxic pollutant in a bioelectrochemical system.
- Biokinetics parameters such as μ_{max}, K_s and K_i, were determined to be 0.16 h^{-1}, 0.26 mg L^{-1} and 300 mg L^{-1}, respectively.
- The value of K_s 0.26 mg L^{-1}, shows the applied microorganisms were able to grow at low phenol concentration.
- The steel wool as an anode can be provided higher reaction rate, uniform distribution, and stable biofilm at neutral conditions.
- Complete degradation and 95% mineralization of phenol were obtained in the batch bioelectrochemical process during 8 h.

- The small amount of the yield coefficient (0.324 mg VSS/mg phenol) was obtained in the bioelectrochemical system.

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