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MICROBIAL DEGRADATION OF PHENOL BY ACTIVATED SLUDGE IN A BATCH REACTOR

Biodegradation of phenol in a batch reactor was investigated using activated sludge. The sludge was able to degrade phenol of initial concentrations up to 1.500 mg/dm^3. The optimum temperature and pH for the reaction were determined in extensive tests. The optimum pH was around 6, whereas the temperature showed no significant impact on the biodegradation rates over the investigated conditions. This activated sludge degraded phenol at the maximum rate of 0.048 g phenol/(g VSS·h) at pH 6 and 30 °C, whereas inhibitory effects existed at concentrations higher than 100 mg/dm^3. The Haldane kinetic model was used to elucidate the kinetics of phenol degradation in an activated sludge. The kinetic parameters were estimated to be q_{max} = 0.4695 g phenol/(g VSS·h), K_I = 28.4860 mg/dm^3, and K_S = 603.9869 mg/dm^3, with the correlation coefficient (R^2) of 0.9599. The high q_{max} value for phenol biodegradation shows that the activated sludge exhibited high resistance to phenol.

1. INTRODUCTION

Phenols and phenolic compounds originated from oil refineries, pulp and paper manufacturing plants, resins and coke manufacturing, steel and pharmaceutical industries are toxic to human beings, fish and to several biochemical functions [1–5]. Increasing presence of phenols represents a significant environmental toxicity hazard. Wastewaters including phenols and phenolic compounds must be treated in order not to be a threat with human health and not to lead to serious ecological risks [6–8]. Increasingly stringent restrictions have been imposed on the concentrations of these compounds in wastewaters for safe discharge. Thus, the approach for the removal of phenols from industrial wastewater has generated significant interest [9–14].

Several methods with different removal performance and cost levels are available for the treatment of phenolic wastewaters. Either conventional physicochemical [9, 10] or biological [15–19] techniques may be used. However, these treatments are very complex and expensive. This situation is triggering the development of new

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treatment technologies for phenolic wastewater. Biological treatment has been shown to be economical, practical and the most promising and versatile approach as it leads to complete mineralization of phenol with low possibility of the production of byproducts [15, 16, 19]. In this viewpoint, the application of biodegradation process to metabolize the phenolic wastewater appears to be an alternative to conventional treatment processes.

However, the toxicity of phenolic compounds may inhibit or even reduce microorganisms in municipal biological wastewater treatment plant [20]. The presence of phenols strongly reduces biological biodegradation of other components which makes the process of degradation of phenols so difficult. It is the reason why activated sludge reactors have been widely used for phenol removal from industrial wastewater. Batch reactor processes employing either suspended or immobilized cultures are in use for the degradation of toxic compounds. The activated sludge process is generally a preferred biological process for the treatment of industrial wastewaters including phenols [15, 16, 19, 21–24].

Presently, great restrictions to application of biological processes are related to the acclimation of the biomass to phenol biodegradation and variability of wastewater composition. A simple and effective method to obtain a specified biomass from activated sludge for phenols treatment is highly desired, which provides selection and multiplication of specialized microorganisms. Furthermore, phenol degradation seems to be influenced by some environmental factors such as temperature and pH. This issue should also be explored for its full application, as well as the substrate inhibition effect and the related biodegradation kinetics. Thus, to acclimatize bacteria to phenol under experimental conditions easier to implement and to investigate the possibility of phenol biodegradation at high initial concentrations and to study the phenol biodegradation kinetics motivates this work.

Therefore, the aim of this work was to evaluate the microbial degradation of phenol by activated sludge in a batch reactor. The kinetics of phenol removal in a batch reactor was investigated in order to develop activated sludge process that could effectively treat phenols. The microbial process reported here could potentially be applied to remove phenols and phenolic compounds in industrial effluents.

2. MATERIALS AND METHODS

Experimental system. The experimental system was a fully mixed bench scale reactor with a constant temperature water circulator and monitoring device for temperature, dissolved oxygen (DO) and pH. The working volume of the reactor was 2 dm^3. The reactor had a water jacket for controlling the temperature which was kept constant at each test. The air velocity of 0.3 $m^3 \cdot h^{-1}$ was applied to the reactor. The DO concentration of the mixed liquor was controlled using the aeration ON/OFF controller. Air

was introduced by an air pump from the reactor bottom. The dissolved oxygen (DO) level was kept over 4 mg/dm^3 in the aeration phase. The reactor was operated under batch operation and the stirring speed was adjusted to 100 rpm. The reactor was operated sequentially within a 6 h cycle, including 5 min of influent filling, 325 min of aeration, 20 min of settling, and 10 min of effluent discharging. Effluent was drawn from the middle port of the reactor column (1 dm^3), and the resulting hydraulic retention time (HRT) was 12 h. Samples were taken from the reactor at predetermined time intervals using a peristaltic pump. The desired SRT was set by controlling the amount of sludge wasted from the reactor in each cycle. About 1.5 dm^3 of seeding sludge were inoculated into the SBR, resulting in a mixed liquor suspended solid (MLSS) concentration of about 4.5 g/dm^3 in the reactor, and correspondingly the loading rate was about 0.1 kg phenol/(m^3·d).

Source of sludge and wastewater. The phenol removing sludge used in the experiments was developed from an activated sludge collected from a wastewater treatment plant in China. The seeding sludge had a sludge age of 10 d, a mixed liquor suspended solids (MLSS) concentration of 6.0 g/dm^3 and the sludge volume index (SVI) of 70 cm^3/g. The sludge was acclimatized for 40 days in aerobic and phenol containing wastewater before being used in experiments. The acclimatized culture was brown in colour and had fluffy, irregular and loose structure floc morphology, and filamentous bacteria could be well observed in the flocs. The synthetic contaminated wastewater was prepared from distilled water using phenol as the contaminant. The synthetic wastewater used in the batch growth studies comprised (dm^{-3}): 227 mg $(NH_4)_2SO_4$, 100 mg $MgSO_4{\cdot}7H_2O$, 0.5 mg $FeCl_3$, 100 mg $MnSO_4{\cdot}H_2O$, 7.5 mg $CaCl_2$, 250 mg KH_2PO_4, and 500 mg K_2HPO_4.

Phenols biodegradation tests. The presence of phenols in wastewater varied widely from over ten to thousands in concentrations (mg/dm^3). In addition, the toxicity of phenols that inhibit microorganisms might increase upon increasing concentration of phenols [1]. Thus, in this work, the concentrations of phenol for biodegradation tests were selected in a wide range based on reported literature values. The tests were started by inoculating synthetic wastewater containing 50–1500 mg/dm^3 of phenol and the acclimatized sludge to the reactor at 30 °C. Thus, the initial phenol /biomass (F/M) ratios were 0.007–0.2 mg phenol /mg VSS (VSS is volatile suspended solid). The pH, biomass amount, and phenol concentrations of samples in reactors were measured at predetermined time intervals. The optimum temperature and pH for the biodegradation were determined in extensive tests. The pH values adjusted by phosphate buffer were in the range 5.0–8.0. The phosphate buffers were prepared by mixing various volume fractions of 0.375 g/dm^3 of K_2HPO_4 and 0.42 g/dm^3 of KH_2PO_4 solutions in deionized water (Millipore, Milli-Q). Control experiments, in a sludge-free medium which only included phenol and other constituents, were also done in order to evaluate the possi-

ble degree of phenol removal with volatilization and it was found that the phenol concentration almost remained unchanged. All the experiments were run in triplicate and the averaged results were reported here.

Analytical methods. Phenol concentration in suspension was measured using 4-aminoantipyrine colorimetric approach on supernatant drawn from samples centrifuged at 8000 rpm for 10 min. pH was determined by using a pH meter. Measurements of VSS were conducted in accordance with the standard methods [25]. Determination of biodegradation rates and parameters of the Haldane equation were done on a personal computer by using a spreadsheet program (Microsoft Excel 2003).

3. RESULTS AND DISCUSSION

3.1. PERFORMANCE OF BIODEGRADATION OF PHENOLS

The activated sludge was grown in the presence of phenol as the sole carbon source and adapted to increasing concentrations of phenol over a period of 40 days; the reactor was continuously operated during this period. The sludge is supposed to be acclimated to the system when phenol is completely degraded in repeated uses in fixed time intervals. The performance of the activated sludge to degrade phenol was evaluated by monitoring phenol disappearance at its various concentrations in the batch reactor. Figure 1 shows results of batch tests for phenol degradation by activated sludge at 30 °C. At initial phenol concentrations of 50–1500 mg/dm^3, phenol concentration quickly decreased for all tests when phenol concentration was sufficient. For example, at an initial phenol concentration of 200 mg/dm^3, the degradation reached 75% in 3 h. However, phenol concentrations in the batch reactor decreased with time rather steeply at low initial phenol contents as compared to those containing high levels of phenol. Since the phenol contents of the control reactors did not change significantly (<3%) during the course of experiments, the extent of non-biological degradation of phenol was negligible. These observations are in accordance with previous results [12, 26–28].

3.2. EFFECT OF INITIAL CONCENTRATION OF PHENOLS

Batch tests for phenol biodegradation using the activated sludge were conducted in the phenol containing synthetic wastewater with phenol concentrations of 50–1500 mg/dm^3 at pH 6 and 30 °C. Based on Fig. 1, the average phenol biodegradation rates with various initial phenol concentrations (were determined to be 60, 60, 41, 40, 36, and 35 mg/(dm^3·h), respectively. The sludge began degrading phenol without a time lag at an initial phenol concentration of 50 and 100 mg/dm^3, and completely degraded phenols

in 0.8 and 1.8 h, respectively. At 200, 400, and 800 mg/dm^3, the time lag of 2, 4, and 8 h was observed, after which phenol was completely degraded. At the initial concentration of 1500 mg/dm^3, the lag time was 15 h and the time needed for complete phenol biodegradation was prolonged to 40 h. Furthermore, the average phenol biodegradation rates decreased upon increasing initial phenol concentrations from 50 mg/dm^3 to 1500 mg/dm^3, suggesting the inhibition to bacteria by high initial concentrations of phenols.

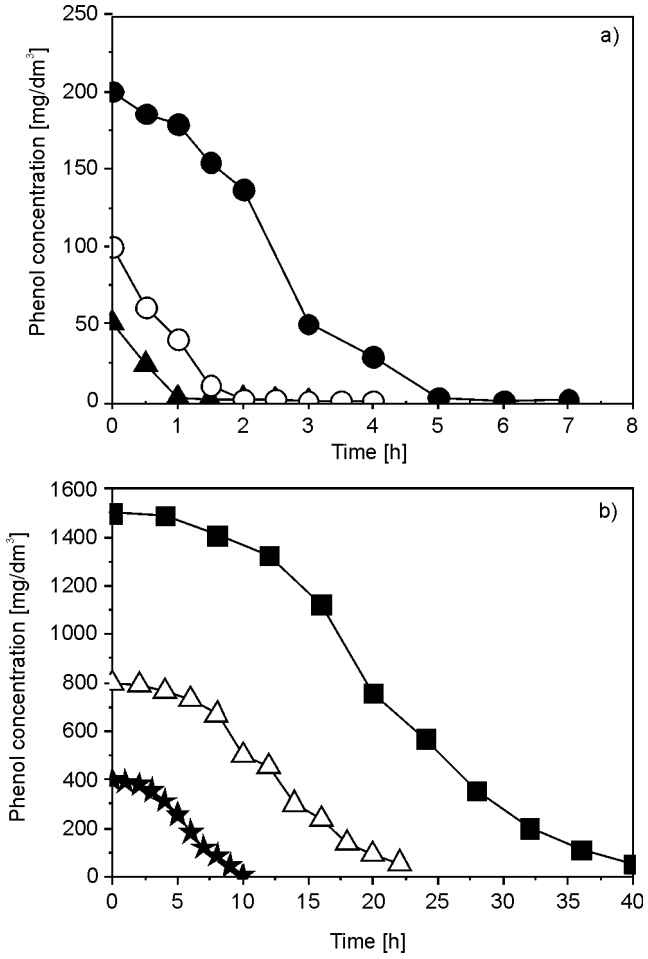

Fig. 1. Batch tests results of phenol biodegradation by activated sludge with various initial phenol concentrations at 30 °C and pH of 6: a) 50, 100, 200 mg/dm^3, b) 400, 800, 1500 mg/dm^3

Figure 1 also shows that for the same initial biomass concentration the higher the concentration of phenol is the more time it takes to be consumed. The phenol concen-

tration in the culture medium decreases clearly due to utilization by microorganisms as they grow. The growth rate should be higher when phenol concentration decreased because of a substrate inhibition phenomenon. The extent of phenol degradation and the time required depends on the initial phenol concentration in the medium.

3.3. EFFECT OF TEMPERATURE AND PH TO BIODEGRADATION OF PHENOLS

Degradation of phenol seems to be dependent on some environmental factors such as temperature and pH [2, 29, 30]. Effect of temperature and pH was performed to explore their optimum values for biodegradation of phenols by the activated sludge in the batch reactor.

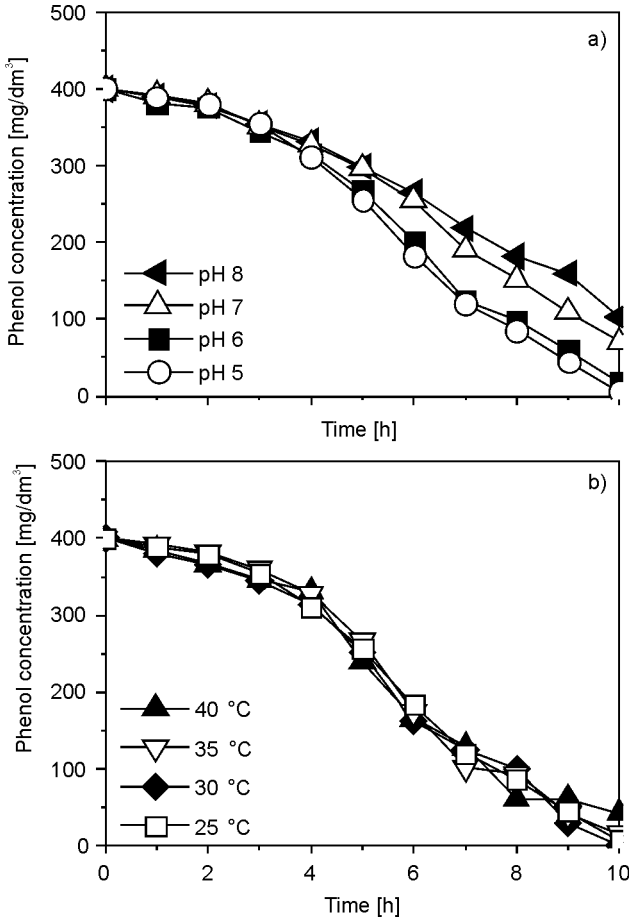

Fig. 2. Batch tests for phenol biodegradation by the activated sludge at the initial phenol concentration of 400 mg/dm^3: a) effects of pH (5–8), b) effects of temperature (25–40 °C)

Figure 2 shows the results of batch tests at various pH and temperatures at the initial phenol concentration of 400 mg/dm^3. Phenol was completely biodegraded at pH 5–6 and at 30 °C while not depleted at pH 7–8. In comparison, the biodegradation of phenols performance did not change much at 25–40 °C. The optimum pH for phenol degradation is around 6, whereas the temperature has shown no significant impact on the reaction rates over the investigated conditions.

Regarding the temperature effect, there are opposite experimental results in previous work [2, 19, 26, 31]. It has been reported that temperature could play an important role in the degradation of phenol [31]. Many authors find higher efficiency of phenol removal near 30 °C [2, 26, 28]. Chung et al. [28] found an optimum temperature of 30 °C for the two processes for immobilized cells and free cells. It is probably due to the higher production of metabolites at this temperature [29]. However, the rate and the extent of degradation is relatively sensitive to deviations outside the optimum range [26]. Adav et al. [19] found that temperature of 25–40 °C had no noticeable effect on the growth rate when using a strain with maximum ability to degrade phenol and a high tolerance to phenol toxicity isolated from an aerobic granule identified as *Candida tropicalis*. In short, it appears that biodegradation of phenol could occur at room temperature. Different sludge system might be a reason for different experimental results. Additionally, different wastewater characteristics, reactor operating conditions and microbial communities also are responsible for the differences.

The follow-up of the medium pH can be an indicator of the phenol degradation and one of the factors significant in the success of the biological treatment. A slight reduction is observed as biomass grows and pH variation increases when the initial phenol concentration increases [12, 32]. The decrease in pH suggests that biological degradation of phenol occurs and with a stable pH phenol is successfully degraded as shown in this work. pH significantly affects biochemical reactions required for phenol degradation. For instance, pure *P. putida* could not efficiently resist pH change [2]. Consequently, phenol degradation may be deteriorated as medium pH deviates. Aksu and Gonen [33] found that pH affects the surface charge of cells of the activated sludge biomass. Thus, the electrostatic attraction between phenol and activated sludge biomass would be impacted [33]. In conclusion, it seems that the best pH range for the phenol degradation does exist. It is possible that enzymes for phenol degradation have their optimum enzymatic activities at an optimum pH. However, the optimum pH for differs from one bacterium to another. For example, pH ranges between 8 and 11 were found for the bacterium *Halomonas campisalis* and for the biodegradation of phenol by *Klebsiella oxytoca*, pH was 6.8 [34].

3.4. KINETICS OF BIODEGRADATION OF PHENOLS

Kinetic analysis of the biodegradation data was performed based on the Haldane equation for describing biodegradation of an inhibitory substrate [5]

$$q = \frac{q_{\max} S}{K_S + S + \dfrac{S^2}{K_I}}, \tag{1}$$

where q and $q_{\max}$ are the specific and the maximum specific substrate degradation rates (g phenol/(g VSS·h) in this work), respectively, and S, K_S and K_I are the substrate concentration, half-saturation constant, and inhibition constant (mg/dm^3), respectively.

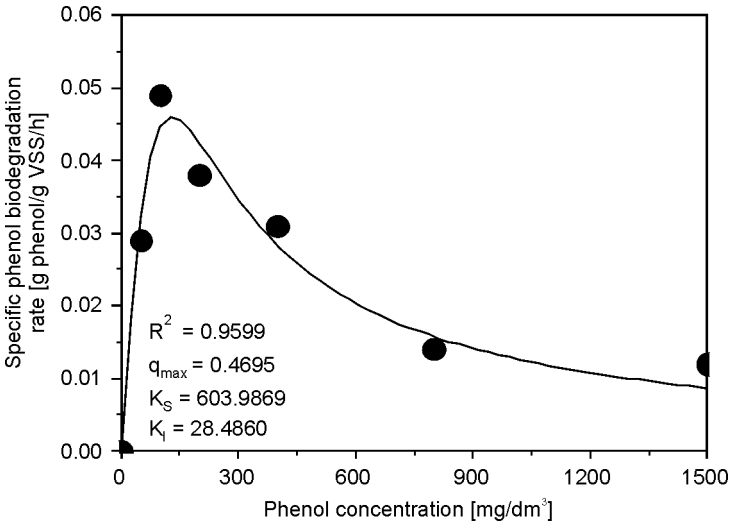

Fig. 3. Specific phenol biodegradation rates of the activated sludge at various phenol concentrations

Figure 3 shows the dependence of initial phenol biodegradation rate on its initial concentration. It increases upon the increasing initial phenol content up to 100 mg/dm^3, reaches maximum at 0.048 g phenol/(g VSS·h) and then decreases for the initial phenol contents between 100 and 1500 mg/dm^3 for the inhibitory effect of phenol at concentrations above 100 mg/dm^3. The Haldane equation was used to model the phenol biodegradation data to evaluate the phenol biodegradation kinetics. The least-square error method was used to estimate the kinetic parameters to be $q_{\max}$ = 0.4695 g phenol/(g VSS·h), K_I = 28.4860 mg/dm^3, and K_S = 603.9869 mg/dm^3, with the correlation coefficient (R^2) of 0.9599.

These values are different from those obtained by Adva et al. [19]. In the study involving strains isolated from aerobic granules for phenol removal by Adva et al. [19], the kinetic parameters of $q_{\max}$, K_I and K_S were found to be 0.385 g phenol/(g VSS·h), 185 mg/dm^3 and 7.1 mg/dm^3, respectively. The maximum specific phenol degradation rate was 0.47 g phenol/(g VSS·h) in this work, higher than that in studies using strains

isolated from aerobic granules [4, 19]. The high q_{max} value for phenol biodegradation shows that activated sludge exhibits high resistant ability to phenol.

3.5. IMPLICATIONS TO INDUSTRIAL TOXICITY REMOVAL

Ultimately this research aims to develop an effective activated sludge process to remove phenols and phenolic compounds in industrial effluents. This work demonstrates that the mixed culture (activated sludge from WWTP) can grow in a simple batch reactor using phenol as the limiting substrate, which is convenient, uncostly and easily operated. The experimental results show that it is possible to treat effluents containing high phenol concentration (up to 1500 mg/dm^3) by activated sludge, indicating that the developed process can potentially be applied to removal of high extent of relate industrial toxicity. Furthermore, it is well known for the importance of the acclimatization for supporting the microorganisms which have the enzymatic material necessary to the degradation of phenol and revealing a new population which is adapted to this toxic agent and is able to consume it like substrate. This acclimatization may relatively easy realize the conditions of temperature and pH which is the most favourable for the development of these microorganisms and the increasing degradable phenol concentration. Thus, the results of this work might significantly benefit the real industry in terms of the extent of toxicity removal and cost of treatment. In addition, the model developed in this study has been applied successfully to phenol biodegradation data obtained from short-term batch experiments. The obtained kinetics for phenol biodegradation can be used for better understanding the activated sludge processes in full scale WWTPs. The high q_{max} value for phenol biodegradation shows that activated sludge can exhibit high resistance to industrial toxicity with proper acclimatization as demonstrated in his work. To this end, a particular emphasis should also be given to develop a practical and easy implement methodology to facilitate its full scale application. The model and the obtained kinetics of phenol biodegradation can deliver a better insight into the system, which can be used to improve design, operation and control of the activated sludge processes for industrial toxicity removal. For example, the approaches and kinetic model presented in this paper could be employed for the design of a batch reactor system for the biodegradation of phenolic wastewater in petrochemical and oil refining industries.

4. CONCLUSIONS

This study demonstrates that activated sludge could be successfully applied in a batch reactor for phenol biodegradation. The sludge was able to degrade initial phenol concentrations up to 1500 mg/dm^3. Results of batch tests show that the optimum pH was around 6, whereas the temperature showed no significant impact on phenol

biodegradation rates over the investigated conditions. Specific phenol biodegradation rates in the sludge followed the Haldane model because of substrate inhibition, and reached maximum at 0.048 g phenol/(g VSS·h) at the phenol concentration of 100 mg/dm^3 at pH 6 and 30 °C. The least square error method was used to estimate the kinetic parameters of the reaction. The high q_{max} value for phenol biodegradation shows that activated sludge exhibited high resistance to phenol. Therefore, this study demonstrated the technical feasibility of utilization of activated sludge for the effective biodegradation of phenols or phenolic compounds.

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