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# Biodegradation and COD removal of *p*-Cresol in a denitrification baffled reactor: Performance evaluation and microbial community

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ABSTRACT

A denitrification baffled reactor (DnBR) was developed to treat *p*-Cresol-laden wastewater. The effects of different operational parameters including *p*-Cresol initial concentration, hydraulic retention time (HRT), COD/$NO_3^-$, salinity and nitrate-free influent were studied on biodegradation and COD removal of *p*-Cresol. The results show that complete degradation of 1000 mg/L *p*-Cresol could be reached at HRT of 24 h. Also, in this condition, corresponding COD and nitrate concentration (2500 mg/L for both) were decreased to about 83 mg/L and 4.5 mg N/L, respectively. There was not a significant failure in the performance of the bioreactor at HRT value of 4 h and nitrate removal rate of 1.65 kg N/$m^3$ d was reached. The highest dehydrogenase activity of the DnBR was 17.65 μgTF/gVS d for organic loading rate (OLR) of 7.5 kgCOD/$m^3$ d. DnBR could tolerate salinity concentration of 10 g NaCl/L, however increasing of salinity from 10 to 20 g/L caused moderate failure in the reactor performance. The optimum COD/$NO_3^-$ was 0.9. Microbial community was also investigated and PCR results revealed that *Bacillus megaterium*, *Bacillus aryabhattai*, and *Bacillus cereus* were the predominant bacteria in degradation and mineralization of *p*-Cresol. Accordingly, DnBR could be considered as a promising technique for biodegradation and COD removal of *p*-Cresol in wastewater.

## 1. Introduction

p-Cresol is one of the toxic phenolic compounds which is known as a priority pollutant and classified as a pollutant of Group C (possible human carcinogens) [1]. It is found in different effluents such as landfill leachate, wastewater from oil refineries, coal conversion and coal coking processes, textile industry, pulp and paper treatment facilities, polymeric resins, and pharmaceutical plants. *p*-Cresol can cause irritation and burning skin and eyes and adversely affect the respiratory tract, mood and mental health, and it can damage liver and kidneys [2–4]. In addition, *p*-Cresol is persistent against chemical and biological degradation in the environment. Removal of phenolic compounds including *p*-Cresol by physicochemical processes such as solvent extraction, adsorption, chemical oxidation, incineration, etc. suffer from serious disadvantages such as the high operation cost and the formation of hazardous by-products. Biodegradation is the most appropriate method of choice for the complete destruction of organic pollutants at lower costs [2–5] and thus might be an attractive method for the removal of *p*-Cresol from polluted water to reach the effluent requirements with lower cost.

The anaerobic baffled reactor (ABR) can be considered as a series of up-flow anaerobic sludge blanket (UASB) reactors separated by standing and hanging baffles. It consists of a series of vertical baffles that force the wastewater to flow under and over them as it passes through the length of the reactor [6–8]. The ABR has many potential advantages i.e. it does not require the sludge granulation for effective performance (although granulation can occur over time), the reactor is very stable against organic and hydraulic shock loads due to its compartmentalized structure, lack of concern about biomass with weak settling properties, low capital and operating costs and mechanical simplicity [9,10]. Many researchers investigated the denitrification process in different baffled reactors; however, none of them studied single denitrification in the presence of toxic contaminants. Liu et al. [11] proposed a baffled bioreactor for advanced nitrogen removal (separated nitrification and denitrification) for small flow wastewater. They [11] used the non-toxic organic content of wastewater as the carbon source for denitrification. Hu et al. [12] used a hybrid aerating membrane-anaerobic baffled reactor for the simultaneous nitrogen and organic carbon removal from synthetic wastewater. Glucose, a readily biodegradable organic compound, was used as carbon source for denitrification. Barber et al. [5] studied nitrogen removal in a modified anaerobic baffled reactor. They [5] used an eight-compartment ABR

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that two last compartments were aerated for conducting nitrification and the nitrate-containing effluent was returned back to the ABR inlet. Sucrose and protein were used as the denitrification carbon source. Nonetheless, investigating the single denitrification in the presence of a toxic compound as sole carbon and energy source can be a substantial step in extending the ABR application [13].

In an ABR, the upflow velocity of wastewater (hydraulic retention time, HRT) and consequently reactor's hydrodynamic are effective parameters in mixing and contact between biomass and substrate, which affect the mass transfer rate and thus reactor performance [6,7]. The high hydraulic loading rate expands better the biomass accumulated in upward compartments resulted in affecting the treatment efficiency. Therefore, evaluating of the reactor performance under different hydraulic loading rates is necessary. The COD/$NO_3^-$ ratio is one of the other most critical operational parameters affecting the rate of denitrification; regulating the reaction at optimum value allow to attain sufficient COD removal without remaining nitrate in the effluent. The ratio of COD/$NO_3^-$ may vary depending on the type of the organic compound used as the carbon source [14]. Wastewater salinity is another parameter detrimentally affect the denitrification process by imposing salt stress on the denitrifiers resulting in inhibiting the involved enzymes, losing cellular activities and eventually causing cell plasmolysis [15,16].

Accordingly, the aim of this study was to investigate the effect of main operational variables of initial *p*-Cresol concentration, HRT, COD/$NO_3^-$ ratio and salinity on the biodegradation and chemical oxygen demand (COD) removal of *p*-Cresol as a model of phenolic compound in a denitrification baffled reactor (DnBR). In addition, the occurrence of the denitrification process in the DnBR was proved with a series of experiments without nitrate addition as the final electron acceptor. Finally, microbial community involved in biodegradation of *p*-Cresol in DnBR was identified.

## 2. Materials and methods

### 2.1. Composition of synthetic wastewater

For the preparation of synthetic wastewater, *p*-Cresol, $C_7H_8O$, (Sigma-Aldrich Co.) and $NaNO_3$ (Ghatran Shimi Co., Iran) were dissolved in tap water as the sole carbon and energy source and the electron acceptor (in the case of denitrification conditions), respectively. Required nutrients were supplied by diluting aliquots of the stock nutrient solution to get a C/N/P ratio of 100/5/1 in the wastewater fed to the bioreactor. The composition of stock nutrient solution (in 1 L) was as follow; 120 g $NH_4Cl$, 5 g $K_2HPO_4$, 15 g $KH_2PO_4$, 12 g $(NH_4)_2HPO_4$ and 10 g $NaHCO_3$. All chemicals were purchased from Merck Co. pH of the influent wastewater was regulated at 7.5 ± 0.2 over the course of study.

### 2.2. DnBR setup

Fig. 1 shows schematic of the DnBR used in this study. The DnBR was composed of a Plexiglas rectangular box-shaped reactor with hanging and standing baffles dividing the reactor into six similar compartments (C1-C6). Each compartment was separated into two parts, a down-flow and an up-flow part. The last compartment was used to trap the biomass particles and thus clarification of the effluent. The volume ratio of up-flow to down-flow parts was two in all the compartments. At the bottom of the hanging baffles, there was a 45° guide baffle (2 cm) for effective and adequate mixing and consequently better contact between the substrate and the biomass across the upflow part. The dimensions of DnBR was 42 × 24 × 21 cm (L × W × H) with the effective volume of 13.7 L. Synthetic wastewater was pumped to the bioreactor through a solenoid dosing pump (ETATRON Co.). The V-shaped weir was used as the influent and effluent structure in order to uniform distribution of the flow across the width and thus to prevent the short-circuiting phenomenon.

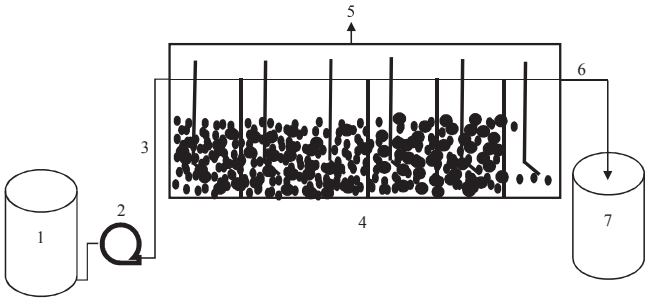


**Fig. 1.** Schematic of the experimental DnBR (1: Feed tank, 2: solenoid dosing pump, 3: influent line, 4: bioreactor, 5: cap, 6: effluent line, 7: effluent tank).

### 2.3. Seed biomass and reactor start-up

The bioreactor was inoculated with activated sludge taken from a laboratory-scale baffled reactor efficiently biodegrading 2-chlorophenol under anoxic condition [13]. The volumetric ratio of wet concentrated sludge to total liquid in the DnBR was 65% at the beginning of the study. Total solids (TSS) and volatile suspended solids (VSS) concentrations of the concentrated sludge in all compartments were between 36 and 56 g/L and 12–21 g/L, respectively, during the whole course of the study. In the given intervals, a part of the wet concentrated sludge was wasted to adjust the sludge volume ratio in the reactor at 65%.

### 2.4. Experimental procedure

The reactor was operated in 6 steps for 424 days as shown in Table 1. The bioreactor was started up with feeding wastewater containing 25 mg/L *p*-Cresol in continuous-flow mode at HRT of 24 h and COD/$NO_3^-$ of 1. The purpose of this step was the acclimation of microorganisms in the seed sludge for biodegradation of *p*-Cresol. Then, the effect of *p*-Cresol concentration, HRT, COD/$NO_3^-$ and salinity were investigated on the performance of the DnBR in biodegradation of *p*-Cresol. Finally, nitrate as the final electron acceptor was removed from influent to prove the biodegradation of *p*-Cresol using denitrifiers. In each step, the biodegradation and COD removal of *p*-Cresol, denitrification rate and dehydrogenase activity (DHA) were determined. The concentration of TSS and VS were measured under the steady-state operation of the bioreactor at each experimental step.

### 2.5. Analysis

The influent and effluent samples were analyzed for the concentrations of *p*-Cresol, COD, nitrate, and nitrite. The concentration of *p*-Cresol was determined using HPLC (Agilent Technologies) coupled with UV detector. The mobile phase was a mixture of methanol and water (60%: 40%) injected at a flow rate of 1 mL/min. Peak wavelength was at 277 nm with the retention time of 5 min. Nitrate (4500-$NO_3^-$ B), nitrite (4500-$NO_2^-$ B) and COD (5220 D) were analyzed according to standard methods for the examination of water and wastewater [17]. For determination of the biomass concentration (TSS and VS) in the reactor, the feeding was stopped, the supernatant (35%) was discharged from each compartment, the content of each compartment was thoroughly mixed with a glass bar and then 10 mL of mixed sludge was taken from each compartment. The concentration of TSS (2540 B) and VS (2540 E) were measured as per described in the standard methods [17]. The pH of influent and effluent streams was determined using Jenway Co. 3505 pH meter.