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Phenol Degrading and Chromium (VI) Reducing Biofilm System: Effect of Shock-loading

Mpumelelo T. Matsena, Tony E. Igboamalu, Evans M.N. Chirwa*

Water Utilisation Division, Department of Chemical Engineering, University of Pretoria, Pretoria 0002, South Africa
evans.chirwa@up.ac.za

Hexavalent chromium [Cr(VI)] which are discharged together with organic pollutants in effluent streams from industry pose a health hazard to humans. The present study observed the reduction of Cr(VI) in presence of phenol using microbial culture consortium originally obtained from Brits Waste Water Treatment Works (North West Province, South Africa) immobilised on ceramic beads in a packed bed reactor. The reactor was stabilised under a phenol feed of 1,500 mg/L and 30 mg/L of Cr(VI). After introducing a shock-loading of 40 mg/L of Cr(VI) and 1,500 mg/L of phenol, Cr(VI) concentration approached influent values in less than 3 HRTs (48-51 h). It took about 144 h for the system to recover after the feed conditions were re-established to 10 mg/L Cr(VI) and 400 mg/L of phenol, and the reactor achieved complete removal efficiency at operating condition of 10 mg/L Cr(VI) influent concentration. Results from this study also suggest that intermediates from the degradation of phenol served as electron donors in the reduction of Cr(VI), whilst the primary compound, which is phenol, remained relatively the same, thus, its degradation was inhibited by intermediates. The microbial culture consortium from Brits have the potential for efficient and simultaneous removal of organic pollutants and heavy metals in complex wastewaters.

1. Introduction

Chromium is found in many oxidation states, and has applications which range from use in alloy plating industry, and tanning of leather (Chirwa and Igboamalu, 2016). Its hexavalent form [Cr(VI)] is known to be acutely toxic at high concentrations, carcinogenic and mutagenic at low concentrations to living organisms (Federal Register, 2004). Phenol is found in the environment in lignin, and its uses include manufacturing of resins and plastics for circuit board, and production of fuel and lubricant additives. Phenol is classified as a hazardous substance, and has to be removed from sources of water before the water can be used for human consumption (Lin and Hsien, 2009).

Due to the regulations set on chromium and phenol that one can safely discharge to the environment, more money is being invested in finding ways to efficiently remove phenol and chromium from industrial wastewater, and current methods to remove them include: chromium removal by chemical precipitation, adsorption, and microbial reduction; phenol removal by activated sludge process, and biological techniques (Zhou, 2014). These methods have advantages and disadvantages, but the simultaneous microbial reduction of Cr(VI) and phenol degradation under aerobic conditions has proven to be an eco-friendly alternative to conventional processes (Al-khalid and El-Naas, 2012). However, more studies on the process still need to be done before considering large scale commercial applications (Gupta and Balomajumder, 2016).

Simultaneous reduction of Cr(VI) and degradation of phenol under aerobic conditions involves a process where phenol is converted to carbon dioxide and intermediates, and phenol as the primary substrate is made available for the biomass to grow and provide electrons for Cr(VI) reduction (Chandana Lakshmi et al., 2012). An example of how this process works can be explained by the mass balance and stoichiometric relationship derived by Chirwa and Wang (2001) for simultaneous phenol degradation in Pseudomonas putida DMP-1, and Cr(VI) reduction in Escherichia coli ATCC 33456, where the intermediate 2-HSMA ($C_6H_6O_5$) is used as an electron donor for Cr(VI) reduction as shown in Equation (1) and (2) (Igboamalu and Chirwa, 2014).

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Pseudomonas putida DMP-1:

$$C_6H_6O + 4.5\ O_2 \rightarrow 0.5\ C_6H_6O_5 + 1.5\ H_2O + 3\ CO_2 \quad (1)$$

Escherichia coli ATCC 33456:

$$2\ CrO_4^{2-} + C_6H_6O_5 + 8\ H^+ \rightarrow 2\ Cr^{3+} + 2\ HCO_3^- + 3\ H_2O + HOOC{\cdot}(CH_2)_2{\cdot}COOH \quad (2)$$

Biofilm systems are believed to offer an environment with a variable survivability range due to the creation of a dynamic concentration gradient across the biofilm thickness. Thus, under high loading conditions of Cr(VI), mass transport resistance may create zones inside the biofilm where the concentration levels are low enough for the survival of detoxifying microbial agents (Chirwa and Wang, 2001). Since Cr(VI) and phenol coexist in many industrial wastewaters (Chirwa and Wang, 2001), methods which treat these toxic substances simultaneously would be of high value since they pose severe threats to the environment. A biofilm system was tested in this study for the simultaneous reduction of Cr(VI) and degradation of phenol by conducting batch studies, followed by a packed bed reactor shock-loaded with different Cr(VI) concentrations to evaluate the response.

2. Material and methods

2.1 Bacterial culture and Growth media

The microbial culture consortium was collected from Brits Waste Water Treatment Works (North West Province, South Africa). The samples were collected in sterile containers and stored at 4 °C. Basal mineral media was prepared by dissolving: 10 mM NH_4Cl, 30 mM Na_2HPO_4, 20 mM KH_2PO_4, 0.8 mM Na_2SO_4, 0.2 mM $MgSO_4$, 50 µM $CaCl_2$, 25 µM $FeSO_4$, 0.1 µM $ZnCl_2$, 0.2 µM $CuCl_2$, 0.1 µM NaBr, 0.05 µM Na_2MoO_2, 0.1 µM $MnCl_2$, 0.1 µM KI, 0.2 µM H_3BO_3, 0.1 µM $CoCl_2$, and 0.1 µM $NiCl_2$. The media was sterilised before use by autoclaving at 121 °C at 115 kg/cm^2 for 15 minutes. Luria-Bettani (LB) broth, and LB agar were prepared by respectively dissolving 25 g:1 L, and 45 g:1 L of distilled water, and sterilised before use by autoclaving at 121 °C at 115 kg/cm^2 for 15 minutes. The LB broth was stored at 4 °C, and the LB agar was cooled at room temperature and dispensed in petri dishes to form agar plates.

2.2 Aerobic batch reactor studies

All batch studies were done under these conditions: The microbial culture consortium collected from Brits was grown for a desired period of incubation time at a temperature of 30 ± 1 °C at 150 rpm aerobically in Erlenmeyer flasks containing the desired volume of LB broth. Cells were harvested by centrifuging at 6,000 rpm at 4 °C for 10 minutes, the supernatant was discarded, and the pellet was washed three times in a sterile solution of 0.85 % of NaCl whilst centrifuging. Experiments were conducted in 250 mL Erlenmeyer flasks by adding both Cr(VI) and phenol in basal mineral media to give a total volume of 100 mL. Before the experiments, initial measurements were taken, and the harvested cells were then added in the Erlenmeyer flasks. The flasks were then plugged with cotton wool and were incubated at a temperature of 30 ± 1 °C at 150 rpm.

2.3 Packed bed reactor set-up and start-up

The reactor was 70 cm long by 10 cm internal diameter, and it was packed with spherical ceramic beads of a diameter of 10 mm. The schematic diagram of the column is shown in Figure 1. The reactor was operated at an up-flow mode under micro-aerobic conditions, and the hydraulic retention time was 18 h. The feed was sterilised to prevent contamination, and the reactor was stored in a temperature-controlled room of 32 ± 1 °C. Microbial culture consortium collected from Brits Waste Water Treatment Works was grown in a 1 L volumetric flask containing 1 L of LB broth for over 48 h at 34 °C. The reactor was then operated at 18 h of hydraulic retention time (HRT) under micro-aerobic conditions without Cr(VI) feed; only 1,500 mg/L of phenol, and microbial culture was fed from the recycle tank to the reactor until biofilm was observed on the ceramic beads 21 days into operation. In the feed tank, feed concentrations of 30 mg/L Cr(VI) and 1,500 mg/L of phenol in a basal mineral media were then introduced at 18 h in the system indicating the start of the experiment.

2.4 Analysis

Cr(VI) was measured using a UV/Vis spectrophotometer (WPA, Light Wave II, Labotech, South Africa) at a wave length of 540 nm (10 mm path) after acidification of 0.2 mL samples with 1N H_2SO_4 and a reaction with 1,5-diphenylcarbazide (DPC) to produce a purple colour (APHA, 2005), Phenol concentration on the other hand was measured using the Waters Alliance 2695 High Performance Liquid Chromatogram (HPLC) (Meadows Instrumentation Inc, Illinois, USA) equipped with a 717 Plus waters PAH C18 Symmetry Column (4.6 mm x 250 mm, 5 µm stationary phase) and 996 Photodiode Array Detector. For sample injection, 5 ml syringes attached with 0.45 µm pore size filters were used to transfer 10 µm of sample into the column. The mobile phase comprised of a 60 % acetonitrile: 40 % aqua pure water.

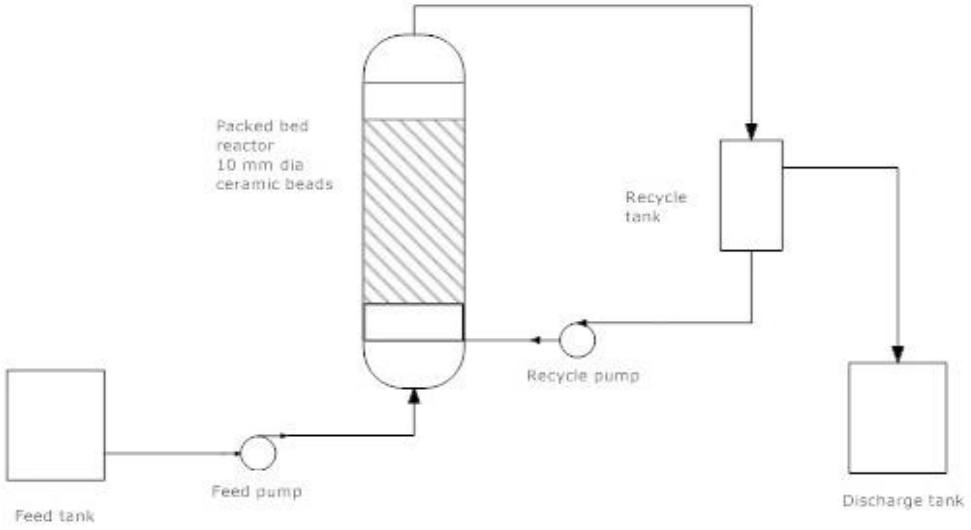

Figure 1: Diagram of a packed bed reactor system.

3. Results and discussion

3.1 Microbial analysis

Before running the experiments, microbial performance and resistivity of the dried sludge collected from Brits Waste Water Treatment Works (North West Province, South Africa) was investigated for Cr(VI) reduction in the presence of phenol under aerobic conditions. The results in Table 1 and Table 2 show that 60.69 % of Cr(VI) was reduced and 23.03 % of phenol was degraded. This signified the capacity of the microorganisms from the dried sludge to reduce Cr(VI) and degrade phenol.

Table 1: Cr(VI) reduction in a dried sludge sample in the presence of 1,500 mg/L phenol

Cr(VI) concentration prepared at t=0 h (mg/L)	Cr(VI) concentration Measured at t=0 h (mg/L)	Cr(VI) concentration Measured at t=144 h (mg/L)	% of Cr(VI) concentration reduced
30	29.97	11.78	60.69

Table 2: Phenol degradation in a dried sludge sample in the presence of 30 mg/L of Cr(VI)

Phenol concentration prepared at t=0 h (mg/L)	Phenol concentration measured at t=0 h (mg/L)	Phenol concentration measured at t=144 h (mg/L)	% of Phenol concentration degraded
1500	1498.98	1153.80	23.03

3.2 The effect of carbon source on the reduction of Cr(VI)

Phenol as a carbon source was required for the reduction of Cr(VI) as shown in Figure 2a and Figure 2b. This means that the biological reduction of Cr(VI) occurred due to the presence of phenol which acted as an electron donor.

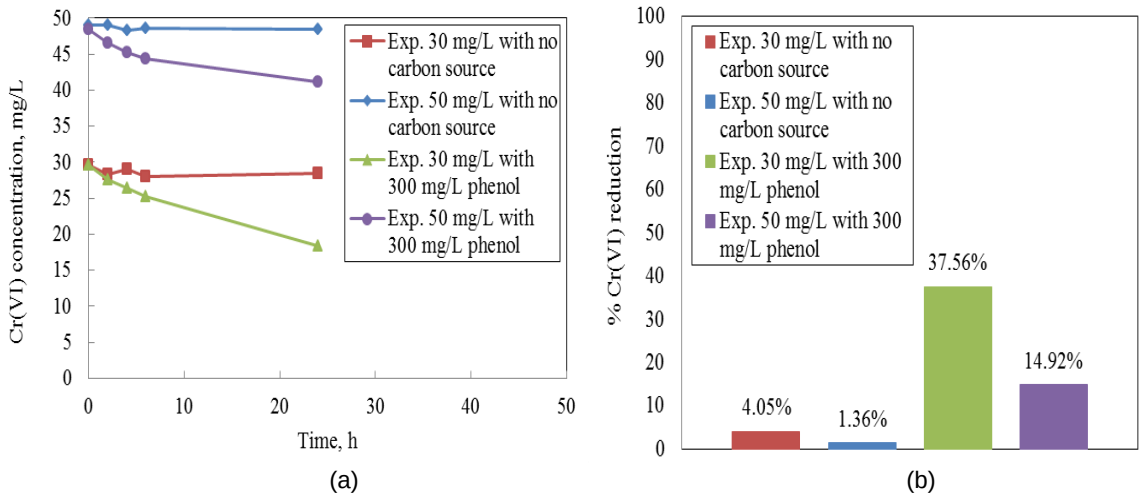

Figure 2: (a) Cr(VI) reduction with and without carbon source at 300 mg/L of phenol. (b) Percentage of Cr(VI) reduction with and without carbon source at 300 mg/L of phenol.

3.3 The effect of Cr(VI) and phenol concentration

Cr(VI) reduction was checked at initial concentration of 30 – 50 mg/L with phenol as sole carbon source. A near complete Cr(VI) reduction was seen when the solution was amended with 30 mg/L and 900 mg/L of Cr(VI) and phenol Figure 3a, with Cr(VI) removal efficiency of 87.53 % Figure 3b. 60.78 % removal efficiency was observed when phenol concentration was increase up to1,500 mg/L Figure 3b. In addition, 52.4% Cr(VI) removal efficiency was observed when the solution was increase to Cr(VI) and phenol concentration of 50 mg/L and 900 mg/L. The drop-in Cr(VI) reducing efficiency suggests an inhibition effect on the microbial activities at higher Cr(VI) and phenol concentration. A near complete phenol removal was seen at 30 mg/L and 900 mg/L Cr(VI) and phenol concentration Figure 4a, with 100% phenol removal efficiency after 150 days of incubation. Like Cr(VI) removal Figure 3a, lower phenol removal efficiency was observed at 30 mg/L and 1500 mg/L Cr(VI) and phenol concentrations Figure 4b. When Cr(VI) and phenol concentration was also increased to 50 mg/L and 1500 mg/L phenol degradation decrease with phenol removal efficiency of 26 % Figure 4b. There is a significant drop in phenol removal efficiency and this means that phenol degradation was also inhibited at higher concentration which is a result of inhibition on microbial growth and metabolism.

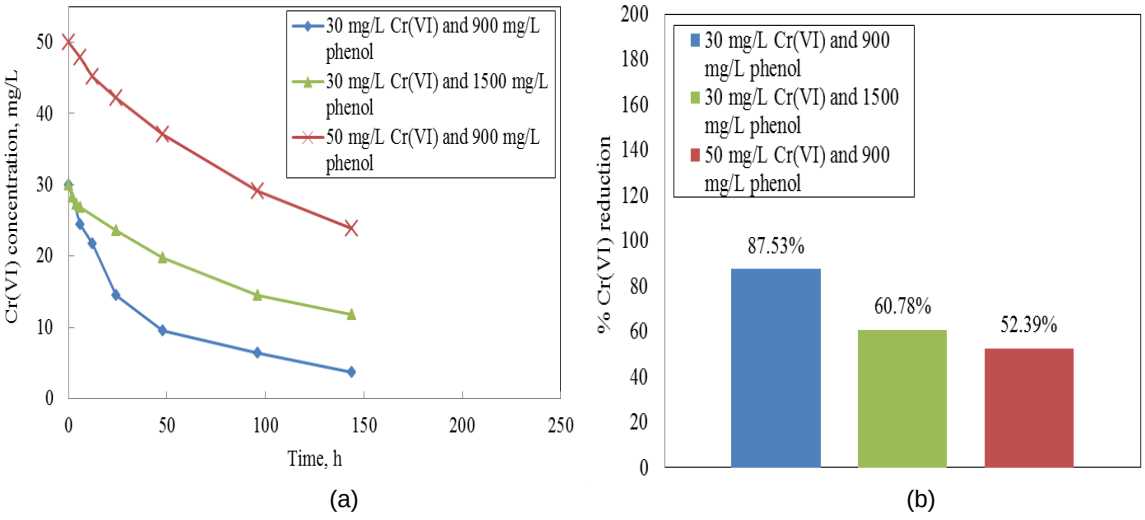

Figure 3: (a) Cr(VI) reduction at different Cr(VI) and phenol concentrations. (b) Percentage of Cr(VI) reduction at different Cr(VI) and phenol concentrations

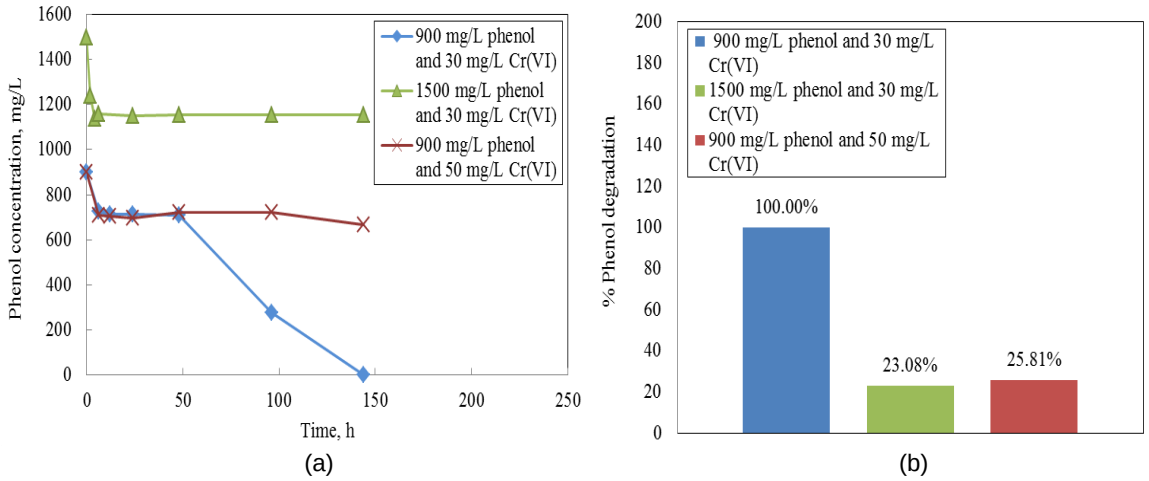

Figure 4: (a) Phenol degradation at different Cr(VI) and phenol concentrations. (b) Percentage of phenol degradation at different Cr(VI) and phenol concentrations.

3.4 The effect of metabolites

It should be noted that the profile of phenol degradation in Figure 4a which is relatively constant after the initial degradation might be due to the formation of metabolites which inhibit the degradation of phenol but act as electron donors to the reduction of Cr(VI). This is because even though the concentration of phenol was relatively constant, Cr(VI) reduction was still taking place. The degradation rate of phenol between 50-150 h for phenol concentration of 900 mg/L and 30 mg/L of Cr(VI) in Figure 4a is significant but Cr(VI) response in Figure 3a for the same experiment doesn't exhibit significant reduction. This is attributed to the fact that the microbial culture consortium also oxidized phenol and used molecular oxygen rather than Cr(VI) as an electron acceptor to degrade phenol (Shen and Wang, 1995). Therefore between 50-150 h, the degradation rate of phenol is largely increased due to minimal inhibitory effects on phenol degradation by phenol and Cr(VI) concentrations, and with oxygen and Cr(VI) both acting as electron acceptors.

3.5 Shock-loading response of Cr(VI) in a packed bed reactor system

In respect to the batch experiment, it was seen that the at 30 mg/L and 1500 mg/L Cr(VI) and phenol concentrations inhibits the microbial activities resulting to lower removal efficiencies. Because of the resilience of the continuous flow system, it is expected that higher Cr(VI) and phenol removal efficiency to be achieve. The response of a packed bed reactor system was investigated by initially using a feed of 1,500 mg/L of phenol, 30 mg/L of Cr(VI), and Basal mineral media. Phenol was the sole carbon source present in the media. The hydraulic retention time of the reactor was 18 h, and the reactor was operated in a temperature-controlled room of 32 ± 1 °C under micro-aerobic conditions. During phase I (day one to four) of operation with the recycle pump switched off to stop the recycle of microorganisms back into the reactor system, the reactor initially showed a Cr(VI) removal efficiency of 33.6 % as shown in Figure 5. However, when the reactor was operated with the recycle pump switched on during phase II (day four to ten), the reactor system showed an increase in Cr(VI) removal efficiency to 50 %. The increase in the removal efficiency is attributed to the fact that since the recycle pump was in operation, some cells lost in the system were recycled back to the reactor causing the Cr(VI) reduction efficiency to improve.

During phase III (day ten to fourteen), the feed concentration of Cr(VI) was increased to 40 mg/L and the response is shown on Figure 5. The increase in feed concentration of Cr(VI) led to a 40 % decrease in the removal efficiency and Cr(VI) concentration approached influent values in less than 3 HRTs (48-51 h). However, during phase IV (day fourteen to twenty), complete Cr(VI) removal was achieved in 144 h when the feed conditions were re-established to a phenol concentration of 400 mg/L and 10 mg/L of Cr(VI) as shown in Figure 5. By reducing the feed concentration of Cr(VI) from 40 mg/L to 10 mg/L, the system was able to recover. With system overload conditions characterised by high phenol and Cr(VI) concentrations, this demonstrated the resilience of the system since recovery was achieved after the system was off-loaded from high Cr(VI) and phenol feed concentrations.

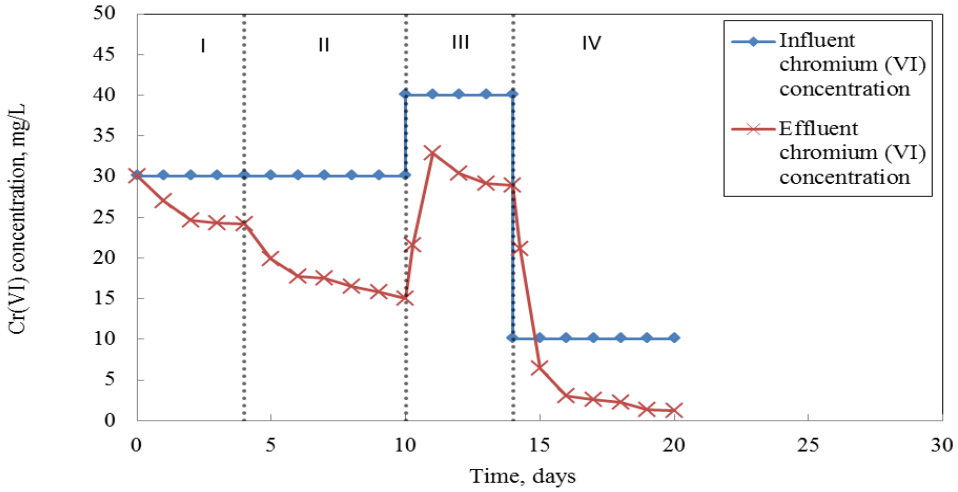

Figure 5: Shock loading response of Cr(VI) reduction in the packed bed reactor system

4. Conclusions

The microbial culture consortium obtained from Brits Waste Water Treatment Works (North West Province, South Africa) removed Cr(VI) and phenol by utilising phenol as its electron donor. High concentrations of phenol and Cr(VI) both inhibited the reduction of Cr(VI) and the degradation of phenol. The profile of phenol degradation in Figure 4a remained relatively constant after the initial degradation, whilst the reduction of Cr(VI) was still taking place. This suggests that the formation of metabolites inhibit the degradation of phenol but act as electron donors to the reduction of Cr(VI). Complete recovery of the system and removal of Cr(VI) was achieved in a reactor packed with ceramic beads at 400 mg/L of phenol and 10 mg/L of Cr(VI) under the operating conditions of hydraulic retention time of 18 h, and a temperature of 32 ± 1 °C. The recycle of cells back in the system led to an improved Cr(VI) reduction efficiency. However, future work is required to establish the profile of phenol concentration in the continuous flow process at this concentration.

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