Biodegradation of 2,4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *Achromobacter* sp

Xiangchun Quan a,b,*, Hanchang Shi a, Yongming Zhang c, Jianlong Wang a, Yi Qian a

*State Key Joint Laboratory of Environment Simulation and Pollution Control, Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, China*

b State Key Joint Laboratory of Environment Simulation and Pollution Control, Department of Environmental Science and Engineering, Beijing Normal University, Beijing, 100875, China

c Nanchang Institute of Aeronautic Technology, Nanchang 330000, China

**Abstract**

An airlift inner-loop bioreactor packed with honeycomb-like ceramic as the carrier was developed and its capacity to immobilize microorganism was studied through adding bacteria, *Achromobacter* sp., capable of degrading 2,4-dichlorophenol (2,4-DCP), directly to the reactor under continuous operation. Effects of phenol in the feed with 2,4-DCP on 2,4-DCP removal were investigated under fed-batch and continuous operations. The results showed that the pure strain could be easily immobilized on the carrier and proliferated using 2,4-DCP as the sole carbon source. In the process of fed-batch operation, removal rate of 2,4-DCP decreased with the increase in run number, while that of phenol was just to the contrary. In the continuous operation, 2,4-DCP loading rate was kept at 29.72–32.23 mg/(l day), but phenol loading rate was increased stepwise from 325.56 to 602.79 mg/(l day). The results showed that with the increase of phenol loading rates, the removal efficiency of 2,4-DCP declined from 100 to 87.9%, while that of phenol remained at about 99.6%. Presence of phenol in feed inhibited the biodegradation of 2,4-DCP and caused the major carbon source shift from 2,4-DCP to phenol.

© 2003 Elsevier B.V. All rights reserved.

**Keywords:** Biodegradation; Phenol; 2,4-Dichlorophenol; Airlift inner-loop bioreactor; Immobilized bacteria

1. Introduction

Chlorophenols constitute an important class of pollutants because of their wide use in the production of wood preservers, pesticides and biocides. Due to their high toxicity, strong odor emission, persistence in envi- ronment and suspected carcinogen and mutagen to the living, chlorophenols as environmental pollutants pose a serious ecological problem [1]. Biological treatment of chlorophenols attracts more attention than physical and chemical methods, because a variety of microorganisms are known to utilize chlorophenols as the sole carbon or energy source, such as *Pseudomonas picketti*, *Alcaligenes eutrophus*, *Desulfomonile tiedjei*, *Phanerochaete chrysosporium*, etc. [2–5]. However, conventional activated sludge systems often fail to achieve high efficiency in
removing chlorophenols from wastewater due to the toxicity or inhibition of chlorophenols to microorganisms. In addition, chlorophenol-degrading populations in bio-treatment plants are likely small for chlorophenols, are poor-growth substrates and often account for a small fraction of the total organics in influent [6].

Recently, the treatment of wastewater containing chlorophenols focuses on employing and exploring new type of bioreactors with high performance, such as fluidized bed bioreactor, fixed-bed biofilm reactor, up-flow anaerobic sludge blanket, combined anaerobic–aerobic bioreactor, in which microbial cells were attached or immobilized on the carriers [7–10].

A variety of biomass carriers such as sand, volcanite, diatomaceous earth, granular activated carbon, tire chips, etc. were used to provide the surface for microbial attachment [7,10–12]. Our previous investigation showed that honeycomb ceramic had good adsorptive ability and could promote the attachment of microbial cells [13,14].

Many strains have been reported to degrade 2,4-DCP effectively, when 2,4-DCP exists alone. However, under practical condition, 2,4-DCP generally exists with other organic compounds, such as phenol [15]. Phenol is a biodegradable pollutant and generally can be biodegraded by microorganisms more easily than 2,4-DCP. The performance of a bioreactor in degradation of 2,4-DCP in feed with phenol has rarely been studied.

The objective of the present study is to investigate the performance of a novel bioreactor, an airlift inner-loop bioreactor, with honeycomb ceramic as carrier to degrade 2,4-dichlorophenol (2,4-DCP) alone or mixed with phenol under fed-batch and continuous operation.

2. Materials and methods

2.1. Culture and medium

The microorganism used in this study was a pure strain capable of degrading 2,4-DCP. It was isolated from the activated sludge of wastewater treatment plant by enrichment shaking culture at 30 °C. The strain was purified by successive streak transfers on agar-plate medium and maintained as slant cultures on peptone-glucose extract agar. It was identified as Achromobacter sp. according to the report of Biolog Microstation System. The cultures were cultivated in 1000 ml flask containing 250 ml growth medium in an orbital shaker at 250 rpm and 30 °C. The growth medium was composed of 983 mg/l K2HPO4; 840 mg/l KH2PO4; 840 mg/l MgSO4·7H2O; 13.2 mg/l FeSO4·7H2O; 60 mg/l CaCl2; 488 mg/l (NH4)2SO4; 60 mg/l NaCl and 1 ml trace element solution. Trace element solution contained: 366 mg/l CoCl2·6H2O, 19 mg/l CuSO4·5H2O, 34 mg/l MnSO4·H2O, 35 mg/l Na2MoO4·2H2O, 100 mg/l ZnSO4·7H2O, 300 mg/l H3BO3, and 29 mg/l NiSO4·6H2O.

2.2. Synthetic wastewater

The composition of the synthetic wastewater was shown in Table 1. The concentration of 2,4-DCP and phenol varied according to experimental plan.

2.3. Reactor

An airlift inner-loop reactor with the carrier of honeycomb-like ceramic was used throughout the experiment. The effective volume of the reactor is 15 l, and a removable draft-tube (10 × 35 cm) was located concentrically inside the reactor (18 × 59.4 cm). Honeycomb-like ceramic column was packed in inner draft tube. Air was introduced through a diffuser placed at the bottom of the inside tube at the flow rate of 8.33 l/min. The outside diameter increased from 18 to 24 cm at the top end of the outside tube. The configuration of the bioreactor was shown in Fig. 1(A). The profile of ceramic honeycomb support was shown in Fig. 1(B). The honeycomb-like ceramic carrier was a column, with 9 cm in diameter and 35 cm in height. It is like hollow-fiber, but the hole sizes

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH4)2SO4</td>
<td>0.1</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.5</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>0.5</td>
</tr>
<tr>
<td>MgSO4·7H2O</td>
<td>0.5</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>0.02</td>
</tr>
<tr>
<td>2,4-DCP/phenol</td>
<td>Varied</td>
</tr>
</tbody>
</table>

Note: pH was adjusted to 7.2–7.4.
are much larger, about 0.5 × 0.5 cm square hole on its section, and there are a lot of micro-pores inside the ceramics, which provide large amount of surfaces for attachment of microbial cells.

2.4. Experimental procedures

First of all, a single inoculation experiment was conducted to investigate the property of the ceramic carrier to immobilize bacteria. The bioreactor was once inoculated with the culture of *Achromobacter* sp., and was filled with 2,4-DCP containing synthetic wastewater in full quickly. Then it was changed to continuous operation through pumping synthetic wastewater to the reactor continuously. The flow rate was 1.875 l/h, and the HRT was 8 h. The initial total biomass inoculated was 1.134 g. 2,4-DCP concentrations in influent ranged 12.8–31.5 mg/l, and the reactor operated at about 25°C. Our previous study had demonstrated that the removal percentage of 2,4-DCP by air stripping and adsorption to the ceramic at the above operation conditions can be neglected [13], hence it can be deduced that the removal of 2,4-DCP from wastewater was mainly by biodegradation of the inoculated bacteria. In addition, a control experiment was done at the same conditions as above except that the ceramic carrier was taken out from the bioreactor. After the above single inoculation experiment, synthetic wastewater containing both 2,4-DCP and phenol was fed to the reactor in fed-batch mode. Generally the 2,4-DCP concentration in the contaminated water is relatively low at about 5–20 mg/l, and phenol concentration is relatively high at about 100–400 mg/l. Therefore, in this study, the influent concentration of 2,4-DCP ranged 15.6–22.5 mg/l, and phenol ranged 80.4–400.1 mg/l.

Finally, the reactor was switched to continuous flow conditions with HRT being 8 h. Influent 2,4-DCP concentration was kept at 15.6–22.5 mg/l corresponding to the loading rate about 29.72–32.23 mg/l day), while phenol concentration was increased stepwise from 239.5 to 400.1 mg/l, corresponding to the loading rate about 325.56–602.79 mg/l day).

Samples of the mixed liquor were taken at a certain interval throughout all the experiments. The concentration of phenol, 2,4-DCP and TOC were measured.

2.5. Analytical methods

Chlorophenol and phenol concentrations were analyzed by a high performance liquid chromatograph (Hewlett Packard 1050) equipped with a RP C-18 column (4.6 × 250 mm) and a diode array detector set at 280 nm. The mobile phase was a mixture of methanol/2% acetic acid water solution (77:23, v/v) and the flow rate was 1 ml/min. Total Organic Carbon (TOC) was measured by TOC automatic detector SHIMADZU TOC-5000. Bacteria immobilized on the ceramic were observed by Scanning Electron Microscope (HITACHI S-570).

3. Results and discussion

3.1. Single inoculation experiment

Fig. 2 shows the results of removal of 2,4-DCP by the newly inoculated pure culture under continuous operation. 2,4-DCP was completely removed and the system reached pseudo-steady state after 28.5 h at the initial influent 2,4-DCP concentration of 12.8 mg/l. Increasing influent 2,4-DCP concentration to 28.5 mg/l resulted in a small effluent fluctuation in 2,4-DCP concentration, but the reactor reached steady state again quickly and the removal efficiency of 2,4-DCP ranged 96.5–99%. A similar experiment to that shown in Fig. 2 was carried out in the bioreactor
without the ceramic support as control. It was found that the removal efficiency of 2,4-DCP was nearly negligible after 1 day and the inoculum was washed out almost completely from the reactor after 20 h. The results demonstrated that the honeycomb ceramic carrier played an important role in maintaining the supplemented bacteria in the reactor.

To prove the function of the special bacteria immobilized on the ceramic carrier in degrading 2,4-DCP, the reactor operation was stopped and the wastewater in the reactor was depleted. The inner wall of the reactor was washed with chlorine bleach to reduce and kill the biomass attached on it. Then the honeycomb ceramic support was put back to the reactor again and a batch study of biodegradation of 2,4-DCP was conducted. The results showed that 2,4-DCP at the concentration 25.0 mg/l was completely degraded within 5 h, which further proved the function of the bacteria immobilized on the carrier in the removing 2,4-DCP.

In addition, the surfaces of the ceramic carrier before and after bacteria immobilization were observed by SEM (Scanning Electron Micrograph) (Fig. 3(A) and (B)). Fig. 3(A) shows that the honeycomb-like ceramic carrier possesses lots of micro-pores and cavities, which are beneficial for microbial immobilization. Fig. 3(B) reveals the presence of the bacteria immobilized on the carrier.

3.2. Co-biodegradation of 2,4-DCP and phenol in fed-batch mode

Phenol, known as a common pollutant, exists widely in industrial wastewater and is regarded as a biodegradable organic compound. Compared with chlorophenol, microorganisms can mineralize it more easily. Impacts of phenol presence in the mixed substrates on the biodegradation of 2,4-DCP in the bioreactor were studied first in fed-batch mode, Fig. 4.
It can be seen in the first run, 2,4-DCP at the concentration of 15.5 mg/l was completely degraded within 6 h. As for phenol biodegradation, a 12 h lag phase was observed before phenol biodegradation began, and only 36.2% removal efficiency was obtained for phenol at the initial concentration of 80.0 mg/l within 36 h. It showed that the special bacteria required a period for adaptation to phenol when first meeting with phenol. In the second run, phenol was utilized first but 2,4-DCP had about 3 h lag period, then both were biodegraded gradually. This suggests the successful inducement of enzyme of the special bacteria for phenol biodegradation, and also showed that the presence of phenol had inhibition effect on the enzyme responsible for 2,4-DCP biodegradation. 2,4-DCP and phenol were removed simultaneously in run 3, which suggested that compromise was achieved in the enzyme systems of the special bacteria. It was interesting to observe that after 2,4-DCP was degraded, the biodegradation rate of phenol increased rapidly, which demonstrated that relatively high concentration of 2,4-DCP inhibited the biodegradation of phenol. In the fourth run, when the concentration of phenol was set to 400.1 mg/l and 2,4-DCP was kept at 21.2 mg/l, the biodegradation of phenol lagged about 9 h for inhibition of itself at relatively high concentration, while 2,4-DCP was degraded in the same pattern as the third run.

The removal rate of phenol and 2,4-DCP in the fed-batch operation was determined through calculating the slope rate of the biodegradation curve except lag period. As for the removal rate of 2,4-DCP, it was 2.59 mg/(l h) in the first run, as the reaction went on, it decreased to 1.91, 1.07 and 1.43 mg/(l h), respectively, in the following three batches, respectively. One possible explanation for the variation of the removal rate of 2,4-DCP might be that the special bacteria prefer utilizing phenol to 2,4-DCP, resulting in the main carbon source for the bacteria changed from 2,4-DCP to phenol. It was also observed that the removal rate of 2,4-DCP slightly increased in run 4, which might be due to the fact that there existed about 9 h of lag phase for phenol degradation, which was beneficial to the biodegradation of 2,4-DCP when the phenol concentration was relatively high (400.1 mg/l).

3.3. Co-biodegradation of phenol and 2,4-DCP under continuous operation

It has been found that in fed-batch test the removal rate of 2,4-DCP demonstrated a trend to decrease with the run number. The primary objective of this part was to evaluate the potential capacity of the reactor to degrade phenol and 2,4-DCP under continuous operation. The reactor performance during 30 days continuous operation is presented in Fig. 5.

According to phenol loading rate, the whole process can be divided into four periods. At the beginning of the operation start-up, the loading rate of 2,4-DCP and phenol were 29.72 and 325.56 mg/(l day), respectively. Both phenol and 2,4-DCP were completely removed within 1 day. In the period of day 3–15, when the phenol loading rate increased to 444.72 mg/(l day) and 2,4-DCP loading rate was set to 30.17 mg/(l day), the removal efficiency of phenol and 2,4-DCP both dropped down slightly to 99.7%. When phenol loading rate increased to 542.37 and 602.79 mg/(l day), its removal efficiency remained stable at previous level, while the removal efficiency of 2,4-DCP declined to 93.1 and 88.0%, respectively. For TOC removal, except relatively low removal efficiency (66.1%) in the beginning, high and stable removal efficiency (87.7%) was achieved despite the stepwise increase in TOC loading rate. These phenomena can be explained by that the special bacteria required some time for adaptation to the intermediates produced in the process of phenol and 2,4-DCP biodegradation, so TOC removal efficiency was relatively low in the beginning.

When the bioreactor reached steady state, the removal rate of 2,4-DCP and phenol could be determined by a material balance:

\[ V_r = F(S_i - S_e) \]
Table 2
The removal rate of 2,4-DCP and phenol under continuous operation

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>2,4-DCP</th>
<th>Phenol</th>
<th>TOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loading rate (mg/l day)</td>
<td>Removal rate (mg/h)</td>
<td>Loading rate (mg/l day)</td>
</tr>
<tr>
<td>1–2</td>
<td>29.72</td>
<td>1.24</td>
<td>325.56</td>
</tr>
<tr>
<td>3–15</td>
<td>30.17</td>
<td>1.25</td>
<td>444.72</td>
</tr>
<tr>
<td>16–23</td>
<td>32.23</td>
<td>1.20</td>
<td>542.37</td>
</tr>
<tr>
<td>24–30</td>
<td>31.28</td>
<td>1.15</td>
<td>602.79</td>
</tr>
</tbody>
</table>

where \( V_t \) is the removal rate (mg/h), \( F \) the flow rate (l/h), \( S_i \) the compound concentration (mg/l) in the reactor inlet, and \( S_e \) is the compound concentration in the reactor outlet (mg/l). Removal rates of 2,4-DCP, phenol and TOC were obtained following the above equation and listed in Table 2.

The results in Table 2 showed that phenol removal rates increased from 13.57 to 25.04 mg/h in spite of the increase of its loading rate, whereas, 2,4-DCP removal rate decreased slightly. These results suggested that although the microorganisms immobilized in the airlift bioreactor possessed the capacity to degrade both 2,4-DCP and phenol during 30 days operation, it demonstrated stronger potential to remove phenol than 2,4-DCP. The removal rate of 2,4-DCP was easily affected by the loading rate of phenol. As for removal rate of TOC, it increased with the increase of phenol removal rate, which demonstrated that no large quantities of intermediates accumulated in the system. To achieve high removal efficiency of a more inhibitory compound when a less inhibitory substrate co-exists in feed, it is necessary to control the loading of the less inhibitory substrate.

The results obtained under continuous operation were similar to those under fed-batch mode, i.e. *Achromobacter* sp., the 2,4-DCP degrading pure culture, may shift its main carbon source from 2,4-DCP to phenol when they co-exist in influent. A similar result was also reported by Lu and Chen [16] who studied the effects of phenol on the biodegradation of 2,4-DCP in column biofilm reactors. It was found that 2,4-DCP started to degrade as phenol was almost completely removed although they were fed simultaneously.

4. Conclusions

This study showed that 2,4-DCP degrading pure culture *Achromobacter* sp. could be easily immobilized in the airlift bioreactor packed with honeycomb
ceramic carrier, and maintained itself using chlorophenols as the sole carbon source. When the reactor was fed with the mixed substrates of phenol and 2,4-DCP and operated in fed-batch mode, phenol inhibited the biodegradation of 2,4-DCP resulting in the decrease of 2,4-DCP removal rate. In continuous operation, the bioreactor immobilized with the strain demonstrated stronger potential to degrade phenol than 2,4-DCP, and the phenol loading rate in the system had important influence on 2,4-DCP removal rate. In the mixed substrate system, to achieve high removal efficiency of the more recalcitrant compound, such as 2,4-DCP, it is necessary to control the relatively easy-to-degrade compound, such as phenol at an appropriate level.

Acknowledgements

The authors gratefully acknowledge the financial support from National Natural Science Foundation of China.

References