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Biodegradation of 2,4-dichlorophenol in an air-lift honeycomb-like ceramic reactor

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Abstract

A novel air-lift bioreactor, with a honeycomb-like ceramic column packed in the inner draft tube as the carrier for immobilization of microbial cells, was developed in this laboratory. A microorganism, identified as *Achromobacter* sp. and capable of degrading 2,4-dichlorophenol (2,4-DCP), was immobilized in the ceramic carrier and used for biodegradation of 2,4-DCP. Semi-continuous biodegradation of 2,4-DCP as a single substrate and in the presence of phenol as co-substrate was investigated. The results showed that when 2,4-DCP occurs alone, its biodegradation rate increased gradually from Run 1 to Run 6 and the degradation process could be described with zero-order kinetics model. When phenol was used as co-substrate, the existence of phenol could inhibit the biodegradation of 2,4-DCP and the biodegradation rate of 2,4-DCP decreased gradually. However, the biodegradation of phenol increased with the increase of run number of the batch experiments. In addition, continuous degradation of 2,4-DCP was also investigated. The results indicated that 2,4-DCP at the concentration ranged from 6.86 to 102.38 mg l^{-1} could be degraded at a dilution rate of 0.16 h^{-1} and the removal percentage ranged between 84 and 100%. The effect of interruption of 2,4-DCP supply to the bioreactor on the degradation ability of microbial cells was investigated by replacing 2,4-DCP with sodium acetate as the sole carbon source for 12 days. Intermission of 2,4-DCP supply did not cause the loss of chlorophenol-degrading ability.

Keywords: Biodegradation; Immobilization; Honeycomb-like ceramic carrier; 2,4-Dichlorophenol; Phenol; Co-metabolism; Bioreactor; Wastewater treatment

1. Introduction

Chlorinated phenols 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, recalcitrance, bioaccumulation, strong odour emission, persistence in the environment and suspected carcinogenity and mutagenity, chlorophenols pose serious ecological problem as environmental pollutants [1]. Their fate in the environment is of great importance.

Some physical and chemical methods have been used for the removal of phenols and their derivatives from wastewater including adsorption over activated carbon, air stripping, chemical oxidation, solvent extraction, ultraviolet light, ozone etc. However the high cost and low efficiency of these processes limit their applicability.

Biological treatment of chlorophenols attracts more attention than physical and chemical methods for many different types of microorganism are known to utilize chlorophenols as their sole carbon source or energy source, such as *Pseudomonas pickettii*, *Alcalilgenes eutrophus*, *Desulfomonile tiedjei*, *Phanerochaete chrysosporium* etc. [2–5]. However, conventional activated sludge systems often fail to achieve high efficiency in treating chlorophenols containing wastewater for their toxicity or inhibition to microorganisms. On the other hand, low bacteria growth yield with chlorinated

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phenols result in slow biomass accumulation in treatment system [6].

Recently, the treatment of wastewater containing chlorophenols has focused on employing and exploring new type of bioreactors with high performance, such as fluidized bed bireactor, fixed-bed biofilm reactor, up-flow anaerobic sludge blankets, combined anaerobic-aerobic bioreactor, in which microbial cells were attached or immobilized on the carriers [7–10]. Aerobic methods are more efficient in degrading low chlorinated phenols such as mono-chlorophenols (MCP) and di-chlorophenols (DCP) than anaerobic methods [11,12]. Aerobic biofilm processes such as a fluidized bed reactor demonstrates great potential in degrading chlorophenols because they can maintain and accumulate large amounts of biomass through inoculating and attaching high-performance degrading culture on to the bio-carriers. A variety of biomass carrier such as sand, volcanite, diatomaceous earth, granular activated carbon, tyre chips etc were used to provide surface for microbial attachment [7,10,13,14].

In this laboratory, a new-type of carrier, honeycomb-like ceramic carrier, specially developed for immobilization of microbial cells, was used in an air-lift reactor for attachment of biomass. Previous investigation showed that this kind of porous ceramic carrier had good adsorptive ability and could promote the attachment of microbial cells. Rapid formation of the bio-film on the carrier was observed. The honeycomb-like ceramic carrier was packed in the inner tube of the air-lift reactor, the reactor operated like a fix-bed, but the wastewater up-flowed by air circulation in the inner-loop, which makes it work like a fluidized bed reactor.

In this study, 2,4-DCP was chosen as the target pollutant, because it has been detected dominantly in chlorophenol-containing industrial effluents. It can also be formed as a breakdown product of some herbicides such as 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. The objective of this study was to investigate the biodegradation of 2,4-DCP in this kind of novel bioreactor, that is, an air-lift inner-loop bioreactor using honeycomb-like ceramic as immobilizing carrier. The biodegradation kinetics of 2,4-DCP in the presence or absence of phenol as co-substrate was also studied.

2. Materials and methods

2.1. Microorganism

The pure culture used in this study was isolated from activated sludge acclimated up to 50 mg l^{-1} of 2,4-DCP for 6 months. This strain was capable of utilizing 2,4-DCP as sole carbon source. It was identified as *Achromobacter* sp. according to the report of Biolog Microstation system.

2.2. Synthetic wastewater

Tap water spiked with 2,4-DCP was used to simulate contaminated wastewater. 2,4-DCP alone or together with phenol was used as carbon source. The composition of the synthetic wastewater is shown in Table 1.

2.3. Reactor

An air-lift inner-loop reactor packed with the carrier of honeycomb-like ceramic was used throughout the experiment. The volume of the reactor was 15 l, a removable draft-tube (10 × 35 cm) was located concentrically inside the reactor (18 × 59.4 cm). A honeycomb-like ceramic column was packed in the inner draft tube. Air was introduced through the diffuser placed at the bottom of the inside tube at the flow rate of 8.33 l min^{-1}.The configuration of the bioreactor is shown in Fig. 1A. The profile of the ceramic honeycomb support is shown in Fig. 1B. The honeycomb-like ceramic carrier was a column, 9 cm in diameter and 35 cm in height. It was similar to a hollow-fiber, but the hole sizes were much large. It was much up to 0.5 × 0.5 cm square hole on its section, and there are a lot of micro-pores inside the ceramics, which provide a large surface area for attachment of microbial cells. The hygroscopic rate of ceramics was 62.0%.

Synthetic wastewater was introduced into the reactor from an influent port and lifted by the flow of air. In the reactor the pollutant contacted the biomass attached on to the wall of the ceramic carrier and was biodegraded.

2.4. Immobilization of biomass

The pure culture was grown in broth culture and induced by mineral salt medium containing 10 mg l^{-1} of 2,4-DCP for 24 h before immobilization. Then it was inoculated into the reactor. To promote the sufficient immobilization of biomass onto the carrier, the reactor was operated in batch mode for 3 days.

Table 1
Composition of synthetic wastewater

Component	Concentration (g l^{-1})
$(NH_4)_2SO_4$	0.1
KH_2PO_4	0.5
Na_2HPO_4	0.5
$MgSO_4 \cdot 7H_2O$	0.5
Yeast extract	0.02
2,4-DCP	6–102 mg l^{-1}
Phenol	80–400 mg l^{-1}

pH was adjusted to 7.2–7.8.

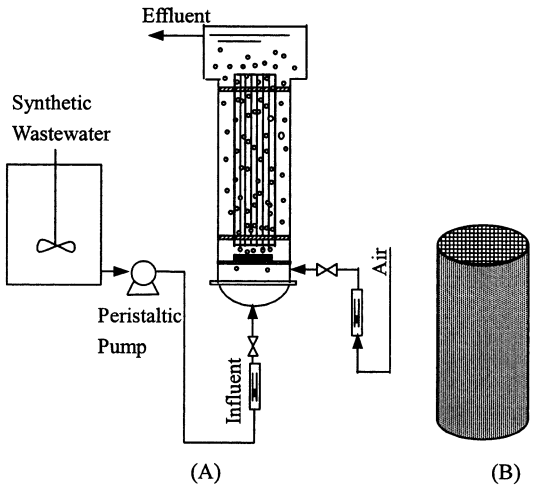

Fig. 1. Schematic diagram of the novel bioreactor (A) and the profile of the honeycomb-like ceramic carrier (B).

2.5. Reactor operation

After biomass immobilization, the reactor was operated in semi-continuous mode to study the biodegradation kinetics of 2,4-DCP alone or co-existing with phenol. After each run of the experiments, all the wastewater in the reactor was discharged and the reactor was washed twice with tap water to remove the suspended biomass. 2,4-DCP in the influent ranged between 15 and 50 mg l^{-1}, and the phenol concentration in the mixed substrate ranged between 80 and 400 mg l^{-1}.

2.6. Analytical methods

Chlorophenol and phenol was analyzed by high performance liquid chromatography (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) at a flow rate of 1 ml min^{-1}.

Microorganisms immobilized on the ceramic were observed by Scanning Electron Microscopy. Specimen preparation was as follows. Firstly, it was fixed with 2.5% glutaraldehyde fixing solution and 1% osmic acid solution, and then washed with phosphate Millonig buffer for three times. Secondly, the specimen was exposed to sequential ethanol dehydration from 30 to 100% in 20% increment with 20 min exposure at each concentration, and then it was replaced by acetate isoamylester. After dehydration, it was dried at CO_2 critical point. The specimen was sputtered with gold by ion-coater for 2 min at an applied current of 50 mA (Eiko, IB-3 Ion-coater) and then examined under a Scanning Electron Microscope (HITACHI S-570).

3. Results and discussion

3.1. Abiotic loss of 2,4-DCP

In order to estimate the removal of 2,4-DCP in the reactor by abiotic processes, such as air stripping and physical adsorption to the ceramic carrier, the following experiment was performed before bacterial immobilization. The reactor was filled with water. Air was introduced through a spherical fine bubble diffuser placed at the bottom of the ceramic carrier. The flow rate of air was 8.33 l min^{-1}. The abiotic loss of 2,4-DCP from wastewater containing various initial concentrations of 2,4-DCP in the reactor was investigated. Samples were taken after 36 h to analyze the concentration of 2,4-DCP. The removal percentage of 2,4-DCP by stripping and adsorption to the ceramic was calculated through the following equation:

$$r\% = \frac{c_i - c_e}{c_i} \times 100\% \quad (1)$$

Where r% is the removal percentage of 2,4-DCP by stripping and adsorption; c_i is the initial 2,4-DCP concentration, c_e is the 2,4-DCP concentration after 36 h operation.

When the initial concentrations of 2,4-DCP varied between 10 and 60 mg l^{-1}, the abiotic loss was within 5%, which indicated that abiotic loss of 2,4-DCP accounted for a small part of removal efficiency compared with removal by biodegradation Fig. 2.

3.2. Biodegradation of 2,4-DCP

The start-up of the bioreactor was operated in batch mode. In order to promote the sufficient immobilization of biomass onto the carrier, the reactor was operated in batch mode for 3 days. The honeycomb-like ceramic carrier possesses lots of micro-pores and cavities, which

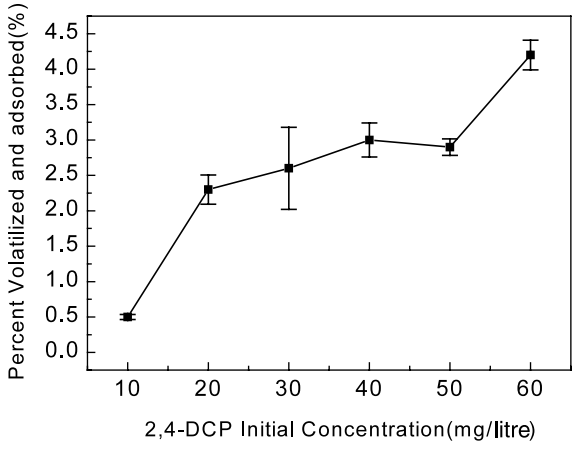

Fig. 2. Abiotic loss of 2,4-DCP at various initial concentrations in the reactor.

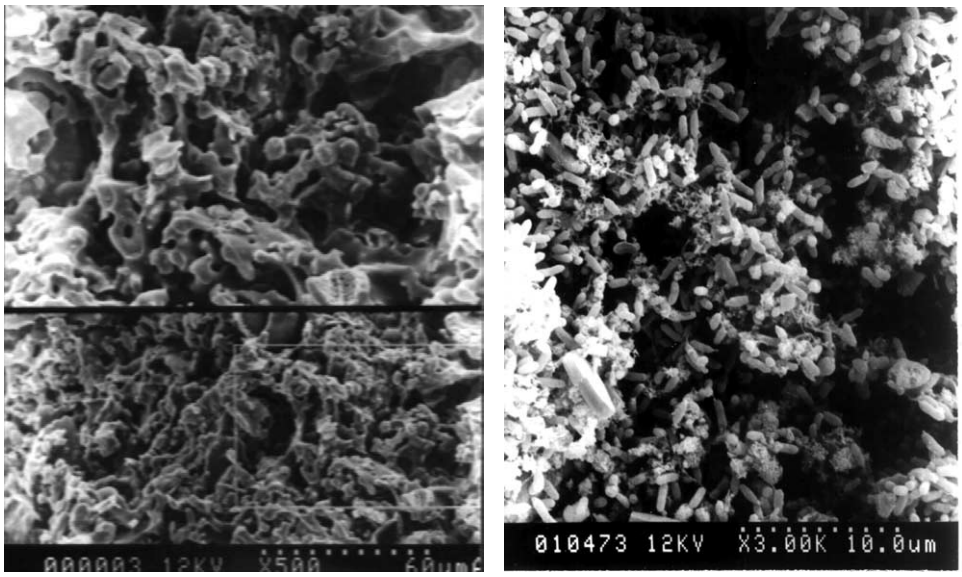

Fig. 3. Scanning electron micrograph of (A) Honeycomb-like ceramic carrier without microbes and (B) Honeycomb-like ceramic carrier immobilized with 2,4-DCP-degrading microbes.

are beneficial for microbial immobilization. The scanning electron micrographs of the ceramic carrier before and after attaching the biomass were shown in Fig. 3.

The biodegradation of 2,4-DCP was carried out in semi-continuous mode after the completion of the reactor start-up. The reactor operated for seven runs with 2,4-DCP alone, which lasted 110 h. The experimental results are depicted in Fig. 4.

Fig. 4 showed that biodegradation rate of 2,4-DCP increased with feeding cycle. In run 1, 33 h was needed for complete degradation of 17.5 mg l^{-1} 2,4-DCP, while it only took 14 h for complete removal of 22 mg l^{-1} 2,4-DCP in run 2. In run 7, although 2,4-DCP concentration was increased to as high as 50 mg l^{-1}, no lag phase was observed and the removal rate was greatly improved. This suggested that microbes had adapted to relatively high concentration of 2,4-DCP.

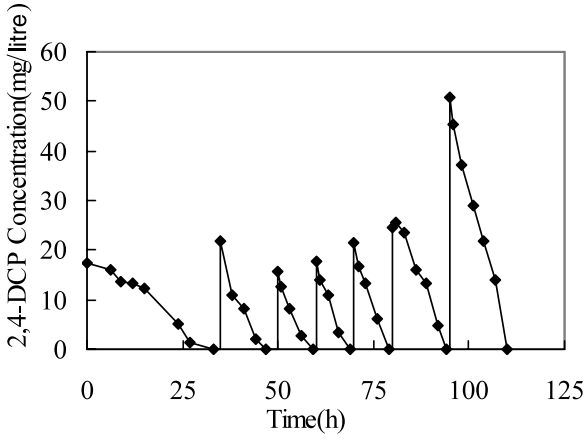

Fig. 4. 2,4-DCP degradation as a single substrate in the reactor operated in fed-batch mode.

The biodegradation rate of organic by microorganisms is often described by the equation:

$$\gamma = \frac{\gamma_m \cdot c}{k + c} \tag{2}$$

Where γ is biodegradation rate, γ_m is maximum specific biodegradation rate, c is the substrate concentration and k is half-saturation constant.If $c \ll k$, Eq. (2) can be reduced to

$$\gamma = \frac{\gamma_m \cdot c}{k} \tag{3}$$

Eq. (3) describes a typical first-order model. Assuming $k_1 = \frac{\gamma_m}{k}$and integrating Eq. (3) the following relation of substrate concentration to time can be obtained:

$$\ln c = a + k_1 t \tag{4}$$

If $c \gg k$, another simplified equation can be got from Eq. (2):

$$\gamma = \gamma_m \tag{5}$$

This biodegradation is zero-order and the biodegradation constant $k_0 = \gamma_m$.Thus the relation of substrate concentration to time is

$$c = b + k_0 t \tag{6}$$

Through the analysis of the data shown in Fig. 4, the biodegradation of 2,4-DCP was shown to follow a zero-order kinetics model. Table 2 summarizes the kinetic

Table 2
2,4-DCP degradation kinetics as a single substrate in fed-batch mode

Run number	Initial 2,4-DCP concentration (mg l^{-1})	Kinetic equations	Rate constants (mg $l^{-1}h^{-1}$)	Correlation coefficient (r^2)
1	17.5	$C=-0.59t+19.111$	0.59	0.97
2	22.0	$C=-1.76t+19.192$	1.76	0.93
3	15.5	$C=-1.73t+14.456$	1.73	0.97
4	17.6	$C=-1.96t+16.656$	1.96	0.98
5	21.5	$C=-2.29t+20.305$	2.29	0.99
6	24.5	$C=-1.82t+27.031$	1.82	0.97
7	50.8	$C=-3.13t+48.812$	3.13	0.99

equations and rate constants of 2,4-DCP biodegradation.

Table 2 indicates that the kinetic rate constants increased with increase of reaction batches. In the run 1, the rate constant was about 0.59 mg l^{-1} h^{-1}, it increased to 1.76 mg l^{-1} h^{-1} in the second run. A gradual increase trend for the kinetic rate constant was observed in the above runs, which may be due to microbial growth through degrading 2,4-DCP and increased biomass in the reactor. In addition, better adaptation to the compound was another important factor for the increased biodegradation rates. Since it is difficult to measure the microbial quantity immobilized on the carrier, it is hard to evaluate the specific biodegradation rate of 2,4-DCP.

3.3. Biodegradation of 2,4-DCP using phenol as co-substrate

Phenol, known as a common pollutant, exists widely in many industrial wastewaters and is regarded as a biodegradable organic compound. Compared with chlorophenols, microorganisms can more easily mineralized it. The effect of co-existence of 2,4-DCP and phenol on their biodegradation was investigated and the results are shown in Fig. 5.

In the first run, 2, 4-DCP at the concentration of 15.5 mg l^{-1} was completely degraded within 6 h. As for the phenol degradation, about 12 h of lag phase was observed before phenol biodegradation began and only 36% removal percentage was obtained for phenol at an initial concentration of 80 mg l^{-1} within 36 h. In the second run, phenol was utilized first but there was about 3 h of lag period for the biodegradation of 2,4-DCP. After the lag phase, both phenol and 2,4-DCP were degraded, which suggested the successful induction of enzyme for the biodegradation of phenol and the presence of phenol inhibition effect on the enzyme responsible for 2,4-DCP biodegradation. In the third run, 2,4-DCP and phenol was degraded simultaneously. It was interesting to observe that after 2,4-DCP was degraded, the biodegradation rate of phenol increased rapidly, which suggested that compromise was achieved in the enzyme-system and a 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 mg l^{-1} and 2,4-DCP was kept at 21 mg l^{-1}, the biodegradation of phenol lagged about 9 h, while 2,4-DCP was degraded in the same pattern as the previous run. The above phenomena may be explained by that the enzymes responsible for the biodegradation of phenol and 2,4-DCP in *Achromobacter* sp. being different and their activities being affected when 2,4-DCP and phenol co-exist. The effect of co-existence of phenol and chlorophenols on the biodegradation of chlorophenols was also reported by other researchers, for example, Lu and Tsai reported that presence of phenol prolonged the lag phase and retarded biodegradation of 2,4-DCP [15].

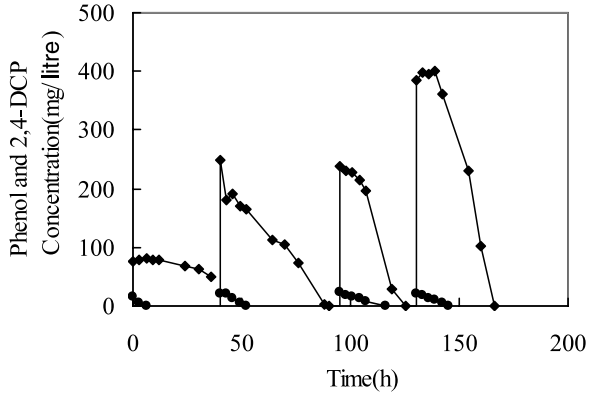

Fig. 5. Biodegradation of 2,4-DCP using phenol as co-substrate (◆) phenol concentration, (●) 2,4-DCP concentration.

The data shown in Fig. 5 also can be fitted to a zero-order kinetic model. The kinetic equations and the rate constants could be obtained using a linear regression method. The variation of rate constants for the biodegradation of phenol and 2,4-DCP at each run is depicted in Fig. 6.

It was apparent that the rate constants of phenol biodegradation were in increasing trend. In run 1, the rate constant was 1.09 mg l^{-1} h^{-1}, as the reaction went on, it gradually increased to 4.30, 11.09 and 14.55 mg l^{-1} h^{-1} in the following three batches, respectively. This may be due to the increased biomass density and the adaptation to toxic inhibition of 2,4-DCP. In contrast,

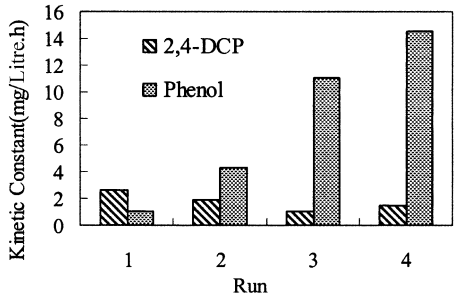

Fig. 6. The variation of rate constants for the biodegradation of phenol and 2,4-DCP.

the rate constants for the biodegradation of 2,4-DCP decreased gradually from 2.59 in the first run to 1.91 and 1.07 mg $l^{-1}h^{-1}$ in the next two runs, but slightly increased in the fourth run. One possible explanation for the variation of rate constants for the degradation of 2,4-DCP might be that phenol was more easily utilized by the isolate than 2,4-DCP, the co-existence of phenol could inhibit the biodegradation of 2,4-DCP. It was also observed that the rate constant of 2,4-DCP degradation slightly increased in run 4, which might be due to the fact of that when the phenol concentration was relatively high (400 mg l^{-1}), there existed about 9 h of lag phase for phenol degradation, this lag phase is beneficial to promote the biodegradation of 2,4-DCP.

3.4. Continuous biodegradation of 2,4-DCP

In the most wastewater treatment plants, treatment is normally operated in a continuous mode, so it is of great significance to investigate the biodegradation of 2,4-DCP under continuous operation.

The continuous biodegradation experiment was performed through switching the semi-continuous operation mode to continuous operation mode, no additional culture was inoculated to the bioreactor again because a thick layer of biofilm had formed in the process of the above-mentioned fed-batch experiments. The operation of the reactor in continuous mode involved in three stages, that is, start-up, feeding sodium acetate as carbon source instead of 2,4-DCP for a certain period, and restart up. The dilution rate was kept at 0.16h^{-1} throughout the experiment. In the start-up process, the initial concentration of 2,4-DCP fed to the bioreactor was relatively low and ranged from 6.86 to 14.92 mg l^{-1} in the first 3 days. The continuous degradation of 2,4-DCP is shown in Fig. 7.

Fig. 7 indicated that the operation of the reactor reached steady state within 54 h in the first stage for start-up. When the 2,4-DCP concentration in the influent were increased to 34.67 mg l^{-1} after 152 h, an obvious decline in the removal percentage was observed, however, it recovered to the previous level soon. The reactor had an increasing capability for biodegradation of 2,4-DCP, which could be verified by the fact that higher 2,4-DCP removal percentage range of 93–99% was reached when the concentration of 2,4-DCP increased to 48.1 mg l^{-1} at the end of the stage 1.

The effect of interruption of 2,4-DCP supply on the performance of the reactor was investigated. After 334 h of continuous operation in the first stage, synthetic wastewater containing sodium acetate as the sole carbon source instead of 2,4-DCP was fed to the reactor continuously for 12 days in the second stage and then replaced with 2,4-DCP containing wastewater in the third stage. Results are also shown in Fig. 7.

It may be seen from Fig. 7 that after an intermission of 2,4-DCP supply to the reactor for 12 days, the performance of the reactor could recover quickly when the 2,4-DCP containing wastewater was fed again. The reactor could reach steady state within 20 h when 2,4-DCP was supplied again at an initial concentration of

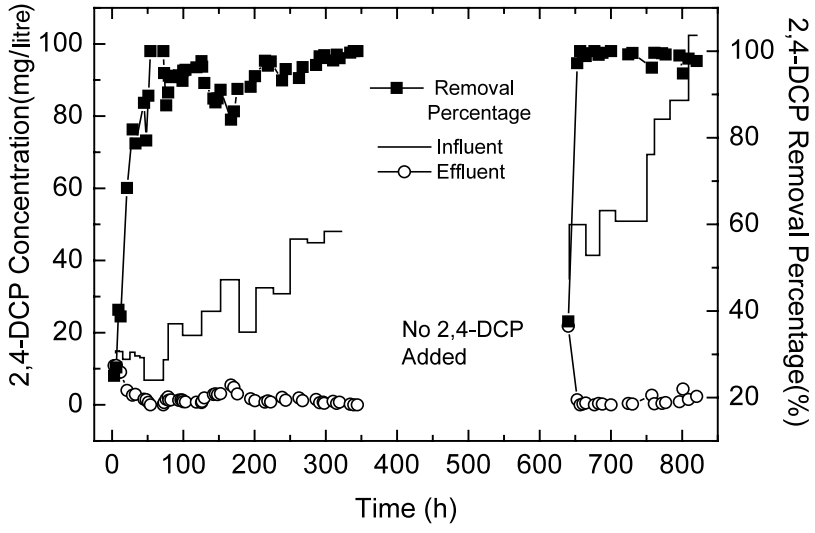

Fig. 7. Continuous biodegradation of 2,4-DCP.

49.96 mg l^{-1}, the restart-up of the reactor was much faster than in stage 1. No obvious loss of capability of degradation of 2,4-DCP was found. Moreover, the reactor system demonstrated a stronger ability for the degradation of 2,4-DCP. The removal percentage for 2,4-DCP was always in the range of 94–99% even when the influent concentration of 2,4-DCP increased from 41.41 to 102.38 mg l^{-1}. This phenomenon could be explained by an increase in immobilized cell density during growth on sodium acetate leading to improved degradative capability.

4. Conclusions

The novel bioreactor, in which a honeycomb-like ceramic was used as carrier for immobilization of microbial cells, was developed and used for the biodegradation of 2,4-DCP. The experimental results indicated that the bioreactor was advantageous for microbial immobilization, microbes could attach onto the surfaces of the carrier effectively and quickly. The biodegradation of 2,4-DCP as a single substrate or in the presence of phenol was performed by the pure culture immobilized on the honeycomb-like ceramic carrier. The semi-continuous degradation of 2,4-DCP followed a zero-order kinetic model when the initial concentration of 2,4-DCP was in the range of 15.5–50 mg l^{-1}, and the degradation rate constant gradually increased from 0.59 (in the first run) to 3.13 mg l^{-1} h^{-1} (in the seventh run). When the synthetic wastewater containing both 2,4-DCP and phenol was fed to the reactor in fed-batch mode, for the biodegradation of phenol, there existed a lag phase in the first run, then the degradation rate increased gradually in the following runs. Whereas the degradation rate of 2,4-DCP decreased, which suggested that the co-existence of phenol could inhibit the degradation of 2,4-DCP. Continuous degradation was operated at a dilution rate of 0.16 h^{-1}. The results showed that the reactor was efficient in degrading 2,4-DCP, when the initial concentration of 2,4-DCP ranged from 6.86 to 102.38 mg l^{-1}, the removal efficiency could reach 88–100%. The experiment replacing 2,4-DCP with sodium acetate as sole carbon source revealed that interruption of 2,4-DCP supply to the reactor did not affect the enzyme activity responsible for the degradation of 2,4-DCP, that is, when 2,4-DCP was supplied again, the immobilized microbes could degrade it quickly.

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