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Effect of easily degradable substrate on anaerobic degradation of pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor

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Abstract

The effect of microbial easily degradable substrate (MEDS) on the anaerobic degradation of pentachlorophenol (PCP) in two upflow anaerobic sludge blanket (UASB) reactors was investigated. The results indicated that glucose-utilizing activity decreased with the increase of PCP concentration in the mixed culture, and MEDS promoted PCP-dechlorination and degrading activities. The concentration of MEDS increased from 917 to 4580 mg L^{-1} with the increase of PCP concentration from 100 to 181 mg L^{-1}in influent, which was necessary for maintenance of steady operation of the experimental reactors, the removal rate of PCP and COD ranged up to 99.5 and 90.0% and the concentration of PCP in the effluent was less than 0.5 mg L^{-1}. The concentration of PCP in effluent was linearly or logarithmically related to sucrose concentration in the influent while PCP was less than the maximum permissible PCP concentration. The activity of anaerobic sludge in the reactor decreased as the concentration of PCP increased, but it could be recovered step by step as time progressed. In the lowest layer of the reactor, the activity of sludge was the highest. So it is feasible to accelerate the degradation of the organic toxic compounds like PCP, by adding suitable quantities of microbial easily degradable substrate to the system.

Keywords: Microbial easily degradable substrate; Pentachlorophenol (PCP); Anaerobic degradation

1. Introduction

Pentachlorophenol (PCP) has been widely used as the wood preservative, antifungal agent, pesticide and herbicide in the related sections. Large quantities of PCP are also produced from the bleaching process with chlorine gas in pulp and paper industry. Because of its toxic properties, PCP is an environmentally significant chemical that acts on a variety of organisms as a potent inhibitor of oxidative phosphorylation. In cells, it disrupts the proton gradient across membranes [1]. Now it has been designated as a priority pollutant and is a probable human carcinogen [2].

Because of its environmental significance, it has been the target of a number of investigations focused on its possible biotreatment [3–15]. From these investigations, mono- and, to a less extent, dichlorophenols can be metabolized by aerobic microorganisms, but aerobic attack becomes less effective with more highly chlorinated compounds. Additional research has indicated that biodegradation of highly chlorinated compounds, such as PCP, by aerobic bacteria is hindered. In contrast, reductive dechlorination of chlorophenols has been shown to occur under anaerobic conditions [5,10,11]. The rate of dechlorination under such conditions is actually greater for more heavily chlorinated compounds. Even though PCP has been shown to resist biodegradation, several pathways for the microbial degradation of PCP have been identified. These pathways are PCP methylation, reductive or oxidative dehalogenation and ring cleavage.

For the above reasons and the environmental significance of PCP, the anaerobic degradation of PCP was investigated extensively [16–22]. Some investigations studied on the toxicity and the fate of PCP in the anaerobic acidogenic systems. In these investigations, the toxicity of PCP was shown by

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the glucose utilizing in these systems and the significant inhibition of glucose uptake at the 10 mg L^{-1} PCP and higher was found. Biodegradation of PCP may be expected since an accumulation of excess reducing equivalents in the form of hydrogen is characteristic of acidogenic systems and this should stimulate reductive dechlorination. Otherwise, anaerobic/aerobic biodegradation of PCP has also been shown to be an effective treatment process when used as part of an integrated system [17]. Both anaerobic and aerobic biodegradation pathways for PCP and CPs have been reported by investigators over the past few decades. Chlorinated phenols are anaerobically biodegraded through the reductive chlorination. Researchers have degraded PCP and other chlorinated compounds with both acclimated and unacclimated cultures obtained from the sediments and sewage in batch tests. Therefore, they have also shown biodegradation pathways are influenced by the culture's source and acclimation process [17]. Fixed film bioreactors are able to remove up to 90% of PCP from the mineral solution in the presence of glucose and 60% without glucose addition [18]. Possibility of anaerobic biodegradation of PCP was demonstrated by the observation that an accumulation of less chlorinated phenols occurred with PCP disappearance in the anaerobic sewage sludge. Reductive dechlorination, or direct removal of chlorinate atoms from the ring of aromatic compounds at a first step is a significant process, because dechlorinated products are usually less toxic and more readily degraded either anaerobically or aerobically.

A thorough investigation on the metabolism and variation of anaerobic microbes has shown that their potentials have not been shown under aerobic conditions. The detoxification and degradation of the poisonous and noxious organic pollution were found, such as the reductive dechlorination of polychlorinated alkane and aromatic hydrocarbons [11–13]. The anaerobic microbes can degrade the most halogenated organic compound by the mean of co-metabolism (as the second substrate), and reduction or oxidation co-metabolism is the first step of biodegradation. Therefore, attention to utilizing anaerobic microbial co-metabolism to deal with the aerobic difficultly degradable organic compound was paid [12]. This paper reported that sucrose, used as the microbial easily degradable substrate, was found to affect anaerobic biodegradation of PCP and that the relationship between sucrose and PCP was drawn to find the key factor influencing the anaerobic biodegradation of the toxic organic pollution.

2. Materials and methods

2.1. Chemicals, instruments and equipments

Pentachlorophenol (PCP) of 98.5% purity was supplied from QingPu New Production Institute. Anaerobic activated sludge was obtained from the wastewater treatment station of HangZhou citric acid plant in Zhejiang Province. The seeding activated sludge for the reactors was acclimated for half a year to PCP, 4-CP, 3-CP or 2-CP, respectively.

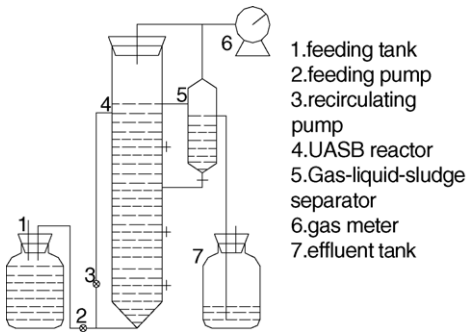

Fig. 1. Schematic diagram of the experimental reactor: (1) feeding tank; (2) feeding pump; (3) recirculating pump; (4) UASB reactor; (5) gas–liquid-sludge separator; (6) gas meter; (7) effluent tank.

Waters high performance liquid chromatography instruments, 721 model spectrophotometer, chemical oxygen demand (COD) measuring equipments.

Reactor and process in this experiment are illustrated in Fig. 1. The experiments were performed in a temperature controlled room at the $28 \pm 1\,^{\circ}C$. The total volume of each reactor was 1.1 L and the efficient volume was 866 mL. Reactor #1 was seeded with the anaerobic activated sludge that was acclimated to PCP for half a year and Reactor #2 was seeded with the equal amount of anaerobic activated sludge that was acclimated to PCP, 4-CP, 3-CP or 2-CP for half a year. The amount of seeding sludge was 20.1 g-VSS.

2.2. Components of wastewater

Synthetic wastewater was used in this experiment in order to make the influent stable. Table 1 shows the detailed components of the synthetic wastewater. Sucrose was added at the specified concentrations (before use). Stock solution for PCP was prepared at concentration of 10 g L^{-1} in methanol, but was diluted to a desired concentration before use. Concentrated synthetic wastewater and PCP solution were maintained at $4\,^{\circ}C$ in a refrigerated container before they were fed. The organic COD in wastewater was provided by peptone, sucrose, meat extract, other nutrients and trace elements including nitrogen, phosphorus, sulfur, calcium, iron and magnesium were also added. The buffering capacity was maintained by the addition of sodium bicarbonate. At each stage, the PCP loading was increased stepwise by increasing its concentration in the synthetic wastewater. If toxic-

Table 1
Compositions of the synthetic wastewater

Constituent	g L^{-1}
Yeast cream	0.05
Ammonium chloride (NH_4Cl)	1.2
Potassium dihydrogen *ortho*-phosphate (KH_2PO_4)	0.42
Sodium bicarbonate ($NaHCO_3$)	5.5
Dipotassium hydrogen *ortho*-phosphate ($K_2HPO_4 \cdot 3H_2O$)	0.22

ity was exhibited by a decrease in COD removal rate less than 80%, feeding of PCP was suspended until the reactor recovered.

2.3. Experimental set-up

UASB reactor was equipped with a phase separator beside it. Biogas production was monitored with a wet gas meter. Synthetic wastewater containing PCP was pumped into the bottom of the reactor with a peristaltic pump. Another peristaltic pump was used to recycle effluent.

Initially, the sequencing batch influent and closed recycling were adopted in this experiment. When the removal rate of substrate was above 80% and sedimentation of sludge was improved partly, the influent flow was slightly increased and increasing hydraulic load and substrate load (organic compound and PCP load) with time. Then shortening hydraulic retention time (HRT) was implemented to accelerate start-up of reactor and formation of PCP-degradation anaerobic granular sludge. After start-up, HRT of the two reactors was 20–22 h and the highest influent PCP concentrations were 181 mg L^{-1} for Reactor #1 and 160 mg L^{-1} for Reactor #2.

2.3.1. Continual dynamic test

After start-up of the experiment reactors, the test was continuously operated and changes of PCP and COD concentration in effluent were measured at the selected time.

2.3.2. Statistic test

The control test was performed in the serum bottle with the volume of 100 mL. Three milliliters of granular sludge mixture from the super-layer, mid-layer and base-layer of the above UASB reactors and 36 mL synthetic wastewater was added to each experimental serum bottle, then was capped with isobutylene rubber material. Each serum was injected into 0.2 mL glucose solution with concentration of 120 g L^{-1}, incubating in a temperature controlled room at the $28 \pm 1\,^{\circ}C$ for a week to ensure anaerobic condition completely. Then these serum bottles were numbered at random to supply the test. PCP was added to each serum bottle with definite quantity according to the experimental aim.

2.4. Analytical methods

To determine the residual PCP concentration, effluent was extracted with acetonitrile, centrifuged for 10 min at 1000 rpm, and filtered through 0.45 um filters. Extracts were analyzed using an Agilent 1100 serial HPLC system. The HPLC system contains a vacuum degasser, quaternary pump, autosampler, column compartment, diode array and multiple wavelength detectors (DAD). The column was Hypersil reversed-phase ODS-C-18 supplied by Agilent, USA. PCP measurement conditions were mobile phase of acetic acid (2%) 10% and methanol 90% at a flow rate of 1.00 mL min^{-1}, signal wavelength at 220 nm, with 20 nm bind width, reference wavelength at 300 nm, with 50 nm bind width. PCP concentration was quantified using an HPLC method. Sucrose analytic and measuring method was referred in Ref. [5], COD was analyzed using the acidity potassium dichromate method.

3. Results and discussion

3.1. The activity of anaerobic granular sludge degrading PCP

After the acclimation, microbes can degrade faster, then the target compound was added again. Results indicated that the anaerobic granular sludge acclimated to PCP for half a year could degrade PCP faster, and it could be called the PCP-degrading anaerobic granular sludge. Otherwise, the anaerobic granular sludge that was not acclimated had a little activity in PCP degradation, and it could be called the PCP difficultly degrading anaerobic granular sludge. In the same reactor, the sludge at the base of reactor was the highest in the activity of PCP-degradation, the mid-layer was second and the super-layer was the lowest in the activity (Table 2).

3.2. Effect of microbial easily degradable substrate on anaerobic degradation of PCP

After start-up of reactors, sucrose concentration (or sucrose load rate) was decided by the concentration of PCP in the influent. When the concentration of PCP was 100 mg L^{-1} (PCP load rate was 120 mg L^{-1} d^{-1}), PCP removal rate of 99.8% was attained when the sucrose concentration was 1146 mg L^{-1}, and residual COD concentration was 121 mg L^{-1} in the effluent. If the concentrations of COD and PCP in effluent were not more than 300 and 0.5 mg L^{-1}, respectively, and at the same time, the concentration of PCP was as above, the sucrose concentration in influent could be controlled between 6880 and 1150 mg L^{-1}. When PCP

Table 2
PCP degradability of anaerobic sludge granules in UASB (mg PCP g^{-1}-VSS d^{-1})

Sludge characteristics	Sludge source	Super-layer	Mid-layer	Base-layer
PCP-degrading sludge	Reactor #1	2.20	4.10	9.95
	Reactor #2	1.66	3.21	9.78
Granular sludge with no PCP-degradation ability		Not detected	Not detected	0.07

Table 3
Effect of microbial easily degradable substrate on PCP anaerobic degradation in UASB reactor (unit: mg L^{-1})

Sucrose conc. in influent	PCP conc. in influent					
	100.4		151.6		180.8	
	Residual COD	Residual PCP	Residual COD	Residual PCP	Residual COD	Residual PCP
9167.0	532.6	0.027	659.4	0.024	–	–
6875.0	379.5	0.052	458.4	0.061	2889	5.15
4583.0	184.2	0.066	291.6	0.196	295.6	ND
2291.7	156.2	0.144	220.8	0.288	671.9	1.60
1145.8	120.7	0.160	176.8	1.730	368.9	14.90
916.7	261.9	0.854	–	–	–	–

Note: data in this table are the mean values for 3–7 days.

concentration was 152 mg L^{-1} in influent, PCP removal rate could reach only 99% with the sucrose concentration of 1150 mg L^{-1} and COD concentration was up to 177 mg L^{-1} in effluent. In the case, if the above effluent demand was satisfied, the highest concentration of sucrose was allowed above 4580 mg L^{-1}, and the lowest concentration should be increased up to 2290 mg L^{-1}. When the concentration of PCP was up to 181 mg L^{-1}, the concentration of sucrose in influent should be maintained about 4580 mg L^{-1} (Table 3).

From Table 3, conclusions could be drawn that maintaining the certain substrate concentration was essential for the optimization of PCP degradation in the wastewater. Too low concentration of primary substrate was not enough to offer the enzyme that was needed for the co-metabolism of dechloration microbes; otherwise, if the concentration of primary substrate was too high, it was assumed that the inhibition of PCP to the anaerobic microbes (methane production bacteria) was large enough to result in the metabolism disorder in the system.

3.3. The quantity relationship between the initial concentration of primary substrate and PCP degradation in the wastewater

When PCP concentration was certain, PCP degradation would be accelerated with the increase of the initial sucrose concentration within the extent of permission in influent. The relationship can be expressed by the regression equations:

$[S]_{influent} = 1150–917\ mg\ L^{-1}$, $[PCP]_{effluent} = (0.172–1.656) \times 10^{-5}[S]_{influent}$, $r = -0.9629^{**}$ when $[PCP]_{influent} = 100\ mg\ L^{-1}$,

$[S]_{influent} = 1150–9170\ mg\ L^{-1}$, $[PCP]_{effluent} = (0.561–4.73) \times 10^{-4}[S]_{influent}$, $r = -0.971^{**}$ when $[PCP]_{influent} = 152\ mg\ L^{-1}$

In these equations, [PCP] means the pentachlorophenol concentration in influent or effluent and the unit is mg L^{-1}; $[S]_{influent}$ means the concentration of sucrose in the influent and the unit is mg L^{-1}, and ** means marks difference (1% marked level).

From the above regression equations, the concentration of PCP in effluent was marked linearly or semi-logarithmically related to sucrose concentration in influent. Suitable quantity microbial easily degradable substrate can promote the dechlorination and degradable metabolism.

Table 4
Effect of PCP on glucose-utilizing rates in anaerobic sludge granules (relative activity)[a]

	Location of sludge											
	Super-layer				Mid-layer				Base-layer			
	2	4	6	8	2	4	6	8	2	4	6	8
Reactor #1												
1 h	84.2	73.7	62.8	60.0	92.4	78.8	71.7	61.2	100	91.0	83.3	64.7
2 h	91.5	77.7	76.2	73.7	98.5	88.9	74.3	75.9	100	95.4	94.8	78.5
4 h	94.9	77.8	88.1	75.5	100	93.0	87.3	85.7	100	98.3	98.5	87.7
6 h	100	92.1	95.9	91.5	99.4	95.8	92.8	89.3	100	100	100	94.8
Reactor #2												
1 h	70.0	69.5	58.4	49.8	86.5	85.1	62.9	48.0	86.6	89.3	78.6	66.8
2 h	79.3	75.4	62.9	55.4	82.1	76.1	67.3	59.2	91.9	89.8	76.0	68.8
4 h	85.6	80.0	61.8	52.9	80.0	82.0	67.8	70.2	95.8	90.0	79.5	74.0
6 h	98.6	98.6	79.6	79.9	95.4	97.0	90.3	86.6	100	100	96.2	92.4

Notes: when PCP concentration was zero, the rate of glucose utilizing was defined to 100%; the data were even value of three parallels and two repetitions for each initial PCP concentration.

[a] 2, 4, 6 and 8 denote the initial PCP concentrations in mg L^{-1}.

3.4. Effect of PCP on glucose-utilizing rates in anaerobic sludge granules (relative activity)

The activity of anaerobic granular sludge could be partly reflected by the rate of glucose degradation. In the static system, the activity of sludge utilizing glucose decreased with the increase in initial PCP concentration and it could be recovered as time progressed. If initial PCP concentration was lower, the recovery time of activity was shorter in comparison. The influence of PCP on the sludge in Reactor #1 was less than in Reactor #2, the recovering rate of activity in the Reactor #1 was much faster. In the same reactor, the influence of PCP on the sludge utilizing glucose increased with the elevation in the reactor and the recovery time of sludge activity would be correspondingly prolonged (Table 4). It was assumed that it was probably related to the difference in the activity of microbe utilizing glucose, the activity of PCP degradation, and the concentration of sludge. The sludge with high activity had greater density, so it would be predominant in the lower section, and the lighter sludge would be predominant in the higher situation. Otherwise, PCP-degrading microbes were easy to be accumulated in abundance and in this condition, the microbes of PCP degrading had been priority community in the ecology composed of many kinds of microbes. The results from Table 4 also interpret the phenomenon.

4. Conclusions

(1) The granular sludge that was acclimated to chlorophenol for half a year could degrade PCP faster.
(2) The activity of anaerobic sludge utilizing glucose was inhibited by PCP, but the inhibition would disappear as time progressed.
(3) Suitable quantity microbial easily degradable substrate was beneficial to promote the dechlorination and degradation metabolism; the amount of the microbial easily degradable substrate would increase with the concentration of PCP increasing in influent; the concentration of PCP in effluent was marked linearly or semilogarithmically related to sucrose concentration in influent while it was less than the maximum permissible PCP concentration. So it is feasible to accelerate the degradation of the organic toxin like PCP adding suitable quantity of microbial easily degradable substrate to the system.

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