

Studies on adsorption, desorption and biodegradation of pentachlorophenol by the anaerobic granular sludge in an upflow anaerobic sludge blanket (UASB) reactor

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Abstract

PCP-degrading anaerobic granular sludge could be formed in an upflow anaerobic sludge blanket (UASB) reactor that was seeded with anaerobic sludge acclimated to chlorophenol. When hydraulic retention time (HRT) was 20–22 h and PCP loading rate was 200–220 mg L⁻¹ d⁻¹, the wastewater containing 170–180 mg L⁻¹ PCP could be treated effectively in UASB reactor, and PCP removal rate reached up to 99.5%. PCP adsorption and desorption by anaerobic granular sludge follow Freundlich isothermal equation and part of adsorption capacity was not reversible. And the isothermal equation could well describe the variation law of PCP adsorption and desorption by anaerobic granular sludge. The results indicated that the principal removal mechanism of PCP was biodegradation by anaerobic granular sludge, but not adsorption or volatilization.

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Keywords: Pentachlorophenol; Bio-degradation; Bio-adsorption; Volatilization; Anaerobic granular sludge

1. Introduction

Pentachlorophenol (PCP) has been used in the past as a pesticide, herbicide, antifungal agent, bactericide and wood preservative [1]. And part of PCP is also produced from the bleaching process with chlorine gas in pulp and paper industry.

PCP is acutely toxic to a variety of microorganisms and mammals, as it is an inhibitor of oxidative phosphorylation [2]. Furthermore, it disrupts the proton gradient across membranes in cells [3,4]. PCP appears to accumulate within the food chains, and is thought to be mutagenic or at least comutagenic [5]. Thus, human exposure to PCP is considered to pose significant health hazards [6].

Due to its environmental significance, it has been the focus of numerous environmental investigations [7–9]. Many

researches have indicated that PCP is very endured to microbial degradation due to its highly chlorinated organic nature [10,11]. However, recent studies show that microbial degradation of PCP is possible, but at a very slow rate [12–14]. In contrast, reductive dechlorination of chlorophenols has been shown to occur under anaerobic conditions [15,16]. The rate of dechlorination under such conditions is actually greater for more heavily chlorinated compounds. Even though PCP has been shown to endure biodegradation, nevertheless several pathways for the microbial degradation of PCP have been found. These pathways are PCP methylation, reductive or oxidative dehalogenation and ring cleavage. And the ridding of PCP with GAC was studied widely [17–19]. GAC is capable of removing many dissolved organic compounds from water and wastewater. Thermal regeneration is the most commonly applied regeneration method, but it is energy intensive, resulting in relatively high costs. There can also be a significant deterioration of the GAC's pore structure, which reduces the specific sur-

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face area available for adsorption [20]. On the other hand, use of an organic solvent extraction regeneration method will lead to the problem of treating the solvent–adsorbate solution. In addition, a loss of 18.5% adsorption capacity in five cycles has been reported for a solvent regeneration technique applied to spend GAC loaded with phenol [21].

For the above reasons and the environmental significance of PCP, the anaerobic degradation of PCP was investigated extensively [22–28]. 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 the glucose utilizing in these systems and the significant inhibition of glucose uptake at the 10 mg L^{-1} PCP and higher was found [2,22]. 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 [23]. Both anaerobic and aerobic biodegradation pathways for PCP and CPs have been reported by investigators over the past decades. Chlorinated phenols are anaerobically biodegraded through the reductive chlorination. Researchers have found that PCP and other chlorinated compounds were degraded by 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 [23]. 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 [24]. 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.

Initially, researchers thought little about the bio-adsorption of target compound in wastewater containing chlorophenols. Most investigations were conducted in the serum bottles with long period, airtight, low biomass and low concentration target compound as sole carbon source. However, bio-adsorption could have an important effect on the fate of these target pollutants because of large biomass in the system of wastewater bio-treatment. In present studies, bio-adsorption has been paid more attention to, but there is no way to differentiate the bio-adsorption and biodegradation. In this paper, with PCP as target compound and basing on the PCP-degrading anaerobic granular sludge, kinetics of adsorption and desorption of PCP by anaerobic granular sludge and treatment effect of wastewater containing PCP in UASB reactor were investigated.

2. Materials and methods

2.1. Chemicals and instruments and equipments

Pentachlorophenol (PCP) of 99.5% purity was supplied from QingPu New Products Institute. 4-CP of 96.0% purity was provided from the third Shanghai chemical agent factory. 3-CP of 96.0% purity was obtained from Fluka AG. Chem. Fabrik CH-9470 Buchs. 2-CP with the purity of 95–98% was produced in Shanghai Laizhe Fine Chemical Institute.

Waters high performance liquid chromatography instruments, rotary rocking bed, chemical oxygen demand (COD) measuring equipments.

2.2. Source of anaerobic activated sludge

Anaerobic activated sludge was obtained from the wastewater treatment station of Hangzhou citric acid plant in Zhejiang Province. The seeded activated sludge for the reactor was acclimated for half a year to PCP, 4-CP, 3-CP or 2-CP, respectively [29]. The control UASB reactor was seeded with the equal amount of anaerobic sludge without acclimation.

2.3. UASB reactor and seeding

Sketch of UASB reactor and process in the experiment was illustrated in Fig. 1. Experiments were performed in the temperature controlled room at $28 \pm 1^\circ \text{C}$. Volume of reactor, components and amount of seeding sludge were illustrated in Table 1.

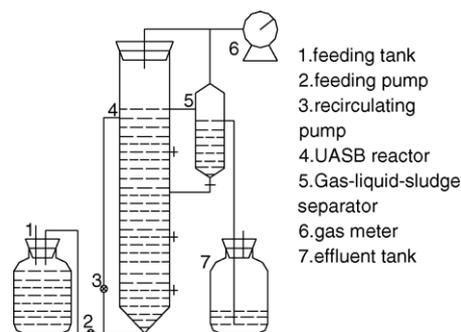


Fig. 1. Schematic diagram of the experimental reactor: (1) feeding tank; (2) feeding pump; (3) recirculating pump; (4) UASB reactor; (5) gas–liquid–sludge separator; (6) gas meter; (7) effluent tank.

Table 1
Volume of UASB reactor and compositions^a of seeding sludge

Reactor	Total volume (mL)	Efficient volume (mL)	Amount of seeding sludge (g-TVS)
#1	1140	866	20.1
#2	1120	865	20.1

The sludge for Reactor #2 was the mixture of anaerobic sludge from citric acid plant separately acclimated with PCP, 4-CP, 3-CP, 2-CP for half a year by 1:1:1:1.

^a The sludge for Reactor #1 was the anaerobic activated sludge from citric acid plant acclimated with PCP for half a year.

Table 2
Compositions of the synthetic wastewater

Constituent	g L ⁻¹
Yeast cream	0.3
Ammonium chloride (NH ₄ Cl)	7.3
Potassium dihydrogen <i>ortho</i> -phosphate (KH ₂ PO ₄)	2.5
Sodium bicarbonate (NaHCO ₃)	33.0
Dipotassium hydrogen <i>ortho</i> -phosphate (K ₂ HPO ₄ ·3H ₂ O)	1.3
Sucrose	27.5

2.4. Components of wastewater

Synthetic wastewater was used in this experiment in order to keep the influent stable. Table 2 shows the detailed components of the synthetic wastewater. 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 (COD = 30,000 mg L⁻¹) and PCP solution were maintained at 4 °C in a refrigerated container before they were fed. The chemical oxygen demand (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 rate was increased stepwise by increasing its concentration in the synthetic wastewater. If toxicity was exhibited by a decrease in COD removal rate less than 80%, feeding of PCP was suspended until the reactor recovered.

2.5. Start-up and operation of UASB reactor

UASB reactor was equipped with a phase separator beside it. Biogas production was monitored with a wet gas flow 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 reactors and formation of PCP-degrading anaerobic granular sludge.

2.6. Cultivation of anaerobic granular sludge without degradation ability of PCP

The seeded anaerobic activated sludge was obtained from the wastewater treatment station of Hangzhou citric acid plant in Zhejiang Province. The influent was constituted of life sewage and synthetic wastewater without PCP, and the control reactor was started up in the same way as the experimental reactor. The anaerobic granular sludge with no degradation

ability of PCP was incubated in a controlled reactor as control.

2.7. Experiment of PCP adsorption and desorption by anaerobic granular sludge

2.7.1. Batch tests

In the first group pressure tubes, 0.5 g wet granular sludge, centrifuged by 3000 rpm for 30 min, and 50.0 mL synthetic wastewater (COD = 600 mg L⁻¹) without sucrose and PCP were fed into each tube. Then, these tubes were capped with isobutylene rubber plugs after filling nitrogen into each tube. Adding corresponding PCP preparation dilution into them with the injector made PCP concentration 5, 10, 20, 40, 80 mg L⁻¹, respectively (according to the monitoring value by HPLC); the second and third group pressure tubes (without anaerobic sludge feeding) were as controls. 0.1 mL 1% Na₂S dilution used as reducing media was injected into the first and second group tubes, but not injected into the third group. After blending uniformly, 1.0 mL sample, taken out from each tube in the second and third group immediately, was used to analyze initial PCP concentration by HPLC. Then, all three groups of pressure tubes were shaken and incubated under 28 ± 1 °C (150 rpm). After 14 h, 1.0 mL sample, taken out from each tube, was used to analyze PCP by HPLC, respectively. The purpose of sample analysis from the second and third group was for checking variation of PCP concentration during incubation. The sludge and liquid from the first group of pressure tubes were separated by centrifuge. After taking out 1.0 mL liquid sample, supernatant liquor was abandoned and the residual was washed in triple quickly in a little amount of distilling water. Then, residual liquor was sucked out in suction paper and 2.0 mL synthetic wastewater without sucrose and PCP was injected again, and shaken and incubated for 6 h at 28 ± 1 °C to desorb the adsorbed PCP. After incubation, 1.0 mL supernatant liquor from centrifuge separation was offered to analyze PCP by HPLC. Each test was performed in triple.

2.7.2. Measurement of PCP adsorption capacity of anaerobic granular sludge

Different from measurement of PCP in wastewater, the anaerobic granular sludge had to be firstly separated from mixture in order to measure PCP adsorption capacity of anaerobic granular sludge. So MLSS from super-layer, mid-layer and base-layer of UASB reactor respectively was centrifuged by 3000 rpm for 30 min and supernatant was abandoned. The residual was washed in triple in distilling water carefully and the distilling water after centrifuge was abandoned. For the purpose of measurement of PCP adsorbed by the granular sludge, 2.0 g wet sludge was taken out and 2.0 mL NaOH solution with concentration of 1 mol L⁻¹ and 0.5 mL acetonitrile were added to the above wet sludge. Mixture was shaken for 10 min, and then centrifuged. The supernatant was filtrated with Millipore filter and offered to analyze PCP by HPLC. At the same time,

mixture liquid suspended solid (MLSS) concentration was measured.

2.8. Analysis of COD, TS, TVS, pH and components of gas

Analysis of COD, TS, TVS, pH and components of gas was referred in reference [30].

2.9. Determination of PCP

2.9.1. Analysis of PCP in effluent of UASB reactor

A 2.0 mL sample was fed into centrifuge tube and 0.5 mL acetonitrile was added to the same tube, then blended uniformly. Mixture was centrifuged by 3000 rpm for 30 min and the supernatant was filtrated with Millipore filter and offered to analyze PCP by HPLC. Analysis and measurement were performed under the condition that mobile phase was 2% HAC (acetic acid)/CH₃OH (methanol) (15/85), flow rate was 1.0 mL min⁻¹, the wave length was 300 nm and retention time was 3.2 min.

2.9.2. PCP analysis of water phase in the adsorption and desorption experiments

All of test sample in each tube was poured into the corresponding centrifuge tube, respectively. Mixture was centrifuged by 3000 rpm for 30 min and mixture of 2.0 mL supernatant and 0.5 mL acetonitrile was filtrated with Millipore filter and offered to analyze PCP by HPLC. The conditions for analysis and measurement were the same as Section 2.9.1.

3. Results and discussions

3.1. The PCP-degrading activity of anaerobic granular sludge

Results were presented in Table 3. The anaerobic granular sludge from UASB reactor seeded with the anaerobic sludge acclimated to chlorophenol for half a year had activity of PCP dechlorination and quick degradation, so called PCP-degrading anaerobic granular sludge; the anaerobic granular sludge from UASB reactor seeded with the anaerobic sludge without acclimated to chlorophenol had little activity of PCP dechlorination, so called PCP non-degradable

Table 3
Activity of PCP degrading anaerobic granular sludge (mg-PCP g⁻¹-TVS d⁻¹)

Reactor	Layers of anaerobic granular sludge		
	Super-layer	Mid-layer	Base-layer
#1	2.20	4.10	9.95
#2	1.66	3.21	9.78
Control	Not detected	Not detected	0.07

Note: The data were even values of triple repetitions.

anaerobic granular sludge. Whereas, it was difficult to use PCP-degrading anaerobic granular sludge to acquire exact adsorption and desorption isothermal curves. It is essential to substitute PCP non-degrading anaerobic granular sludge for it and investigate the characteristics of adsorption and desorption of PCP in order to ascertain the impossibility of decrease of PCP concentration caused by physical–chemical process of anaerobic sludge from UASB reactor.

3.2. PCP adsorption and desorption by anaerobic granular sludge

Distribution of PCP as a kind of weak organic acid in the solid and liquid phase was influenced by pH, organic solvent and ion strength. In order to satisfy the comparison of experimental results and complexions within UASB reactor, the dilution similar as synthetic wastewater was used media of adsorption and desorption in experiment. Results are shown in Fig. 2 and they indicate that bio-adsorption and desorption of PCP followed Freundlich isothermal equation completely. The adsorption isothermal curve was:

$$\frac{X}{M} = 0.567C_e^{1.113}, \quad r = 0.9619^{**};$$

the desorption isothermal curve was:

$$\frac{X}{M} = 0.282C_e^{0.955}, \quad r = 0.9996^{**}.$$

From the above equations, the Freundlich constants of the adsorption isothermal curve were 0.567 mg g⁻¹-TVS for K and 1.113 for $1/n$, respectively.

The Freundlich constants of the desorption isothermal curve were 0.282 mg g⁻¹-TVS for K and 0.965 for $1/n$, respectively.

It showed that PCP adsorption capacity of anaerobic granular sludge was little because anaerobic granular sludge without acclimation could not degrade PCP thoroughly in so short time (no other chlorophenols were noted by HPLC detection). The difference of capacity of PCP adsorption and desorption

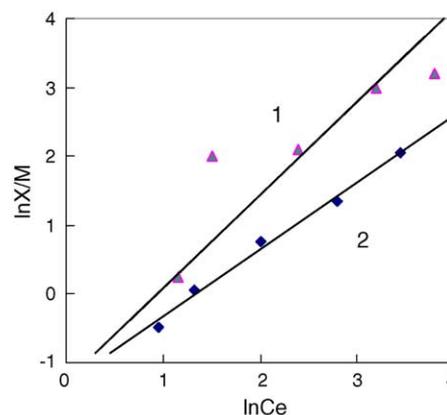


Fig. 2. Anaerobic granular sludge adsorption and desorption of PCP isothermal curves: (1) adsorption isothermal curves; (2) desorption isothermal curves.

Table 4
Results of UASB reactor operation

Reactor	Operation periods (d)	COD _{Cr} in influent (mg L ⁻¹)	COD _{Cr} removal rate (%)	PCP in influent (mg L ⁻¹)	PCP in effluent (mg L ⁻¹)	PCP removal rate (%)	HRT (d)	Remark
#1	10–30	3050–3080	86.72	2–4	0–0.216	89.2–100	5.8–1.2	Recovery of activity
	35–75	4600–6250	97.56	7.50–66.04	0–0.264	99.6–100	1.1–0.9	Formation of granular sludge
	103–118	5570	97.45	181.0	Undetected	100	0.9	Stable operation
#2	10–30	3000–3080	85.75	2–4	0–0.200	90–100	4.5–1.1	Recovery of activity
	35–75	4600–6250	96.35	7.50–66.01	0–0.195	97.4–100	1.0–0.85	Formation of granular sludge
	91–98	5740	90.15	137–170.1	Undetected	100	0.90–0.88	Stable operation

Table 5
Prediction values and actual measurement values of anaerobic granular sludge adsorption of PCP in UASB reactor

Reactor	#1			#2				
PCP concentration in effluent (mg L ⁻¹)	0.04	0.20	0.493	4.0	0.10	0.357	1.474	4.0
Actual measurement total absorption capacity, X _T (mg)	0.618	2.81	7.73	–	1.037	4.51	24.67	80.00
Prediction total absorption capacity (mg)	0.487	2.903	79.0	80.44	1.21	4.95	23.84	72.17
Error (%)	21.2	3.3	2.1	–	16.4	9.7	3.4	9.8

Notes: When PCP concentration in influent was between 170.1 and 180.0 mg L⁻¹, the PCP concentration in effluent from Reactor #2 reached up to 4.0 mg L⁻¹, but the PCP concentration in effluent from Reactor #1 was still less than 0.5 mg L⁻¹, so the actual measuring value of adsorption capacity of the anaerobic granular sludge from Reactor #1 does not exist at 4.0 mg L⁻¹ PCP concentration.

demonstrated that PCP adsorption by the anaerobic granular sludge was not all reversible.

3.3. Experiments on the operational efficiency of UASB reactor

Operational results of experimental UASB reactor treating wastewater containing PCP were illustrated in Table 4. The conclusions can be drawn that wastewater containing 170–181 mg L⁻¹ PCP could be treated stably by two UASB reactors with formation of PCP-degrading anaerobic granular sludge, and corresponding PCP loading rate was 200–220 mg L⁻¹ d⁻¹. Operation behaviors of Reactor #1 seeded with sludge acclimated to PCP was super to that of Reactor #2 seeded with equal amount of mixed sludge acclimated to PCP, 4-CP, 3-CP and 2-CP.

3.4. Application of PCP bio-adsorption kinetics

In order to test the PCP bio-adsorption kinetics model, other four PCP adsorption capacity of anaerobic granular sludge were measured under the different PCP concentration in influent and effluent and then the actual measuring values were compared with the predicted values from the model. The contrast of actual concentration and prediction value was presented in the Table 5.

It indicated that the Freundlich model basing on PCP non-degradable anaerobic granular sludge incubated under similar conditions still could be used to predict the PCP adsorption by anaerobic granular sludge within UASB reactor although it is impossible to attain a true balance of solid and liquid phase in actual UASB reactor due to PCP degradation by anaerobic granular sludge.

From the contrast of Tables 4 and 5, conclusions were drawn that PCP concentration in effluent was very little when UASB reactor was normally operated, and PCP adsorption by the granular sludge was not the principal mechanism of PCP removing in UASB reactor; but if PCP concentration was too high and UASB reactor operation was abnormal, PCP concentration in effluent was increased steeply, and at that time adsorption could play an important role in PCP removing at the initial period of abnormal operation (about 1–2 d).

3.5. Volatilization of PCP in UASB reactor

In the airtight anaerobic reactor, gas phase and liquor phase were mostly balanceable, and especially, PCP concentration decreased with the elevation in UASB reactor, so drive of volatilization could approach zero. In this experiment, the biogas adsorption test was conducted for six days and PCP was not detected in the solution of adsorption (0.06 mol L⁻¹ NaOH). According to the conclusion, the probability that PCP was removed by volatilization was very little in UASB reactor.

4. Conclusions

It is probable to treat the wastewater containing PCP in UASB reactor; the principal mechanism of PCP removing is biodegradation, not adsorption or volatilization.

Adsorption of PCP by anaerobic granular sludge follows the Freundlich isothermal equation and adsorption is not all reversible. The Freundlich isothermal equation can better describe the variation rules of adsorption of PCP by granular sludge in UASB reactor.

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