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Biosorption and biodegradation of pentachlorophenol (PCP) in an upflow anaerobic sludge blanket (UASB) reactor

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Abstract In order to understand the fate of PCP in upflow anaerobic sludge blanket reactor (UASB) more completely, the sorption and biodegradation of pentachlorophenol (PCP) by anaerobic sludge granules were investigated. The anaerobic granular sludge degrading PCP was formed in UASB reactor, which was seeded with anaerobic sludge acclimated by chlorophenols. At the hydraulic retention time (HRT) of 20–22 h, and PCP loading rate of 200–220 mg l^{-1} d^{-1}, UASB reactor exhibited good performance in treating wastewater which containing 170–180 mg l^{-1} PCP and the PCP removal rate of 99.5% was achieved. Sequential appearance of tetra-, tri-, di-, and mono-chlorophenol was observed in the reactor effluent after 20 mg l^{-1} PCP introduction. Sorption and desorption of PCP on the anaerobic sludge granules were all fitted to the Freundlich isotherm equation. Sorption of PCP was partly irreversible. The Freundlich equation could describe the behavior of PCP amount sorbed by granular sludge in anaerobic reactor reasonably well. The results demonstrated that the main mechanism leading to removal of PCP on anaerobic granular sludge was biodegradation, not sorption or volatization.

Keywords Ananerobic granular sludge · Biosorption · Desorption · Pentachlorophenol (PCP) · Upflow anaerobic sludge blanket reactor (UASB)

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Introduction

Pentachlorophenol (PCP) has been applied widely in the wood preserving industry and agriculture as a fungicide, pesticide and herbicide. Because of improper treatment of PCP, soil and groundwater has been widely contaminated and their toxicity seriously affected living organisms. The discharge of PCP is of great concern because of its toxicity and persistence. In cells, PCP disrupts the proton gradient across membranes (Escher et al. 1996). Because of its environmental significance, biotreatment is of great interested. Both anaerobic and aerobic biodegradation pathways of PCP and other chlorophenols have been widely reported (Armenante et al. 1999; Buitron and Gonzales 1996; Flora et al. 1994; Khodadoust et al. 1997; Nicholson et al. 1992; Suidan et al. 1996). Recent literature has suggested that anaerobic microorganism may be suited to reductively dehalogenate highly chlorinated phenolic compounds while aerobic biological systems are

suited for degrading the less-halogenated phenolic compounds (Buitron and Gonzales 1996; Kafkewitz et al. 1992; Togna et al. 1995). The rate of dechlorination under such conditions is actually greater for more heavily chlorinated compounds. Additional research has indicated that biodegradation of highly chlorinated compounds, such as PCP, is hindered by aerobic bacteria. In batch studies, PCP and other chlorinated compounds have been degraded by both acclimated and unacclimated cultures obtained from sediments and sewage. The studies also show that biodegradation pathways and kinetics are influenced by the source of the culture and the acclimation process.

One essential step in developing a comprehensive soil treatment for soils contaminated with PCP, or developing an effective anaerobic active sludge technique to treat PCP containing wastewater, is to understand the physical and chemical interactions of the pollutants with soil or granular sludge. Several researchers have identified adsorption parameters for PCP in different soils through batch studies, but the reports on adsorption of PCP on anaerobic granular sludge was not much (Gregory et al. 1998). As for biodegradation of PCP, a number of researchers have studied anaerobic PCP degradation at low concentration as a sole carbon source and with low biomass content during the past years. There is little information about the effect of bio-sorption on reducing target pollutant in aqueous environment (Kennedy et al. 1992; Woods et al. 1989). However, in the wastewater treatment system containing these pollutants, bio-sorption may play an important role in ultimate fate of these substances. Several investigations had paid attention to the bio-sorption, but they could not differentiate between bio-sorption and bio-degradation (Wu et al. 1993).

In conventional biological treatment systems where contact time is insufficient for biodegradation to occur, bio-sorption plays an important role in removing toxic compounds from the aqueous phase and accumulating hazardous pollutants in the microbial sludge. Final disposal of the sludge may cause new environmental risk because the toxic pollutants may be reversibly adhered to the microbial biomass. The upflow anaerobic sludge blanket (UASB) reactor is an advanced anaerobic treatment which is characterized by an anaerobic granular sludge with a notably high metabolic activity and good biosolids settling ability. These characteristics allow the reactor to maintain a high biosolids content while operating at relatively low hydraulic retention time (HRT) (Kennedy et al. 1992; Droste et al. 1998; Shen et al. 2005). There have been numerous investigations regarding the inhibition of anaerobic degradation due to elevated chlorophenol concentrations, but little is known on the bio-sorption of halogenated aromatics to anaerobic granular sludge. Additionally, although a few studies compared the bio-sorption and de-sorption of hazardous organic pollutants on to microbial biomass, they have limited to specific microbial species and aerobic sludge (Tsezos and Bell 1989). The aim of this study was to investigate the sorption and de-sorption of PCP on the anaerobic granular sludge, and to evaluate the performance of UASB reactor in treating PCP containing wastewater after developing successfully the anaerobic granular sludge degrading PCP.

Materials and methods

Chemicals

All chlorophenolic compounds [PCP (98.5%), 2-CP (96.0%), 4-CP (96.0%)] used in this study were purchased from ShangHai Reagent factory, ShangHai, China, except 3-CP, which was obtained from Fluka AG. Chem. Fabrik CH-9470 Bucks. The CPs was used without further purification. Stock solution (10,000 mg l^{-1}) of PCP was prepared in 0.05 mol l^{-1}NaOH because PCP is much easier to be dissolved in alkaline solution. pH adjustment was not done before adding to the anaerobic sludge in bioreactors because the volume of PCP additions was not much and pH of the feed did not changed greatly.

Source of anaerobic sludge

The initial anaerobic enrichment culture used was the anaerobic digested sludge of Hangzhou citric acid factory in Hangzhou. The sludge contained

total solid (TS) and volatile solid (VS) contents of 9.6 and 7.6%, respectively. The specific methane production rate was 1.12 g CH_4-COD (gVSS d)$^{-1}$. The sludge were acclimated with PCP, 4-CP, 3-CP, and 2-CP, respectively, in the laboratory for 6 months, and then the acclimated sludge with PCP was used as inoculum of the reactor R1. The acclimated sludge with 4-CP, 3-CP, and 2-CP were mixed under 1:1:1 ratio and used as inoculum of the reactor R2 (Table 1). During the acclimation, the synthetic wastewater with carbon and electron donor was feed. The procedure and results of acclimation were shown in another paper (Ye and Shen 2004). The unacclimated sludge was inoculated directly into the control reactor.

Experimental set-up

Start-up and operation of UASB reactors

Experiments were performed in a temperature-controlled room at 28 ± 1°C with two same glass UASB reactors (Fig. 1). The capacity of the reactor is 1.1 l. UASB reactors were equipped with a phase separator at the top. After passing the gas through a concentrated sodium hydroxide solution to remove carbon dioxide, methane production was monitored with a wet gas meter. Synthetic wastewater containing PCP was pumped into the bottom of the reactors with a peristaltic pump. Another peristaltic pump was used to recycle the effluent. The sludge of upper-layer, middle-layer and bottom-layer were taken from three sludge sampling ports which located evenly along the side of each reactor. Effluent was collected in a food grade glass bucket.

Concentrated synthetic wastewater and PCP solutions were maintained at 4°C in a refrigerated container before they were fed. The chemical oxygen demand (COD) of the wastewater was about 30,000 mg l^{-1}, but it was diluted to a desirable concentration (3,000–6,200 mg l^{-1}) before use. Table 2 shows the detailed composition of the synthetic wastewater. The organic COD in wastewater was provided by peptone, sucrose and meat extract and other nutrients and trace elements including nitrogen, phosphorus, sulfur, calcium, iron and magnesium were also added. The concentration of sodium bicarbonate was high enough to maintain the buffering capacity of synthetic wastewater.

After being acclimated for 6 months, the sludge all became suspended and the settling ability was very poor, so the reactors were operated in closed recycle when the initial influent was fed into the reactors until the COD removal rate was more than 80% and the settling ability of the sludge was improved. Initially, the upflow rate was very low (5 ml h^{-1}). PCP, COD and hydraulic loadings were progressively increased and HRT was decreased depending on effluent COD and PCP concentrations as well as biogas production.

Incubation of anaerobic granular sludge without degrading PCP capacity

The control reactor was seeded with anaerobic sludge from Hangzhou citric acid plant and fed with the same synthetic wastewater, but without PCP. The control reactor was started up by the similar style to that of the test reactors to culture anaerobic granular sludge without capacity of degrading PCP.

Sorption and de-sorption test of PCP on anaerobic granular sludge

(1) *Batch test method*: Sorption experiments were conducted in the serum bottles by mixing the

Table 1 Characteristics of the seed sludge in each reactor

Reactors	Total volume (ml)	Working volume (ml)	Source of sludge	Acclimated substance	Content of inoculum (g VSS)
R1	1,240	946	Anaerobic sludge from citric acid plant	PCP	20.9
R2	1,220	935	Anaerobic sludge from citric acid plant	PCP, 4-CP, 3-CP, 2-CP	20.9

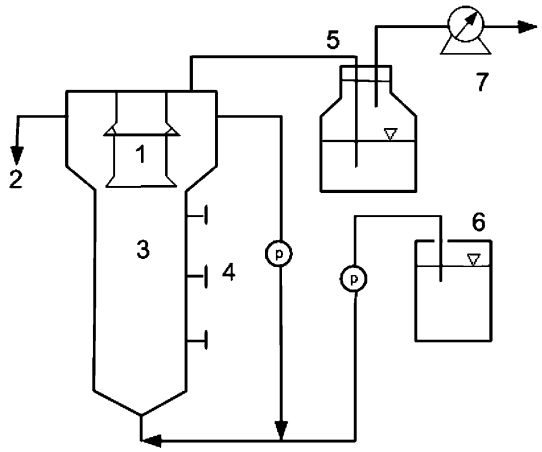

Fig. 1 Schematic diagram of UASB system. 1, Gas–liquid–sludge separator; 2, effluent; 3, sludge bed; 4, sampling ports; 5, CO_2 uptake tank; 6, feeding tank; 7, wet gas meter; P, pump

anaerobic sludge with different PCP concentrations. In the first group of serum bottles, 0.5 g wet granular sludge was centrifuged 30 min under 3,000 rpm, and 10 ml wastewater without sucrose were added into each tube (Sucrose could serve as easily degraded carbon source). Every tube was filled with nitrogen and sealed with a butyl rubber stopper. Afterwards, PCP solution was added into each tube to obtain 5, 10, 20, 40 and 80 mg l^{-1} PCP, by using 1 ml sterile plastic syringes fitted with 24×0.5 mm hypodermic needles, respectively. The second group of serum bottles containing the same amount of biomass as the first group of serum bottles, but no PCP, was used as background samples. The third group of serum bottles containing media and PCP but no anaerobic sludge was served as the controls. In the first and second groups of tubes, 0.1 ml 0.1% Na_2S solution was added as reducing reagent, but Na_2S solution was not added into the third group of tubes. Being mixed completely, 1.0 ml sampler was taken out immediately from each tube of the second and third groups for analyzing its original PCP concentration. Then all serum bottles of three groups were incubated with shaking (150 rpm) at 28 ± 1°C for 14 h. Then 1.0 ml sampler was removed from all serum bottles in three groups for analysis. The samplers from the second and third groups of tubes were used to analyze variation of PCP concentration during the incubating period. 1.0 ml supernatant was drawn from the first group of tubes after being centrifuged, and the sludge in the tubes was immediately cleaned with distilled water for three times, and the remaining liquid was absorbed up. 2.0 ml synthetic wastewater without sucrose and PCP was added into these tubes again and incubated with shaking (150 rpm) at 28 ± 1°C for 6 h to de-sorb PCP which had been adsorbed in the sludge. After incubating, the tubes were centrifuged and 1.0 ml supernatant was drawn out for analyzing its PCP concentration. All tests were conducted in triplicate.

(2) Adsorption measurement of PCP on the anaerobic granular sludge: The sludge from upper, middle, and lower part of reactors were withdrawn and centrifuged under 3,000 rpm for 30 min. The supernatant was discharged and the sludge was cleaned with distilled water for three times. After being centrifuged again and discharged the supernatant, 2.0 g wet sludge was taken out, 2.0 ml NaOH (1 mol l^{-1}) solution and 0.5 ml acetonitrile were added. The mixture was oscillated for 10 min and centrifuged. The supernatant was filtered through 0.45 μm filter to be analyzed for PCP.

Table 2 Composition of the synthetic wastewater of 30,000 mg COD l^{-1}

Constituents	mg l^{-1}
Peptone	8,000
Sucrose	2,700
Meat extract	3,000
Sodium bicarbonate, $NaHCO_3$	33,000
Calcium chloride, $CaCl_2{\cdot}2H_2O$	380
Magnesium sulfate, $MgSO_4{\cdot}7H_2O$	420
Ammonium Chlorid, NH_4Cl	7,300
Ferrous sulfate, $FeSO_4{\cdot}7H_2O$	320
Potassium dihydrogen ortho-phosphate, KH_2PO_4	2,500

Analytical methods

Wastewater was taken from the reactors for the purpose of monitoring the variation of residual PCP concentrations, COD and pH values. COD, pH, TS and total volatile solid (TVS) were measured according to standard method (APHA 1992).

For the determination of PCP and CPs, 2.0 ml wastewater sample from reactor, were centrifuged for 30 min at 3,000 rpm. 2.0 ml supernatant was saved and added with 0.5 ml acetonitrile to extract PCP, and passed through 0.45 μm filter. Extracts were analyzed via high performance liquid chromatography (HPLC) (Waters 510). The HPLC was equipped with a C-18 reverse-column and a variable wavelength UV absorbance detector operated at 300 nm. The mobile phase used 2% mixture of acetic acid and methanol (15:85). The retention time was 3.2 min (Ye and Shen 2004).

Hydrogen and methane was quantified with a gas chromatograph (Shimadzu 14B, Japan) equipped with a packed column and flame ionization detector. Column, injector and detector temperature were maintained at 55, 90 and 90°C, respectively. Nitrogen was employed as carrier at a flow rate of 20 ml min^{-1}.

Results and discussion

PCP-degrading activity of anaerobic granular sludge

At the end of about 120 d operation after acclimation, it was indicated from Table 3 that the anaerobic granular sludge in the reactors inoculated with the seed sludge acclimated to PCP for half a year could degrade PCP faster, and it could be designated the PCP-degrading anaerobic granular sludge. Otherwise, the anaerobic granular sludge in the control reactor inoculated with the unacclimated sludge exhibited hardly any PCP-degrading activity, and so it could be designated the non-degrading PCP anaerobic granular sludge. In the same reactor, the sludge at bottom of the reactor exhibited PCP-degradation activity best, and the upper-layer was lowest in the activity. Based on the results and reference information, it is very difficulty to obtain a precise sorption or de-sorption isotherm of PCP with PCP-degrading anaerobic sludge. Therefore the non-degrading PCP anaerobic sludge must be selected to investigate its sorption and de-sorption characteristics of PCP and to evaluate the possibility of PCP concentration decreased in the liquid phase due to the physical and chemical effect of anaerobic sludge.

Table 3 PCP-degrading activity of anaerobic granular sludge in UASB reactors (mg PCP g^{-1} TVS d^{-1})

Reactors	Upper-layer	Mid-layer	Bottom-layer
R1	2.32	4.22	10.05
R2	1.78	3.31	9.88
Control	Not detected	Not detected	0.06

Table 4 shows that two UASB reactors can treat steadily wastewater containing 170–182 mg l^{-1} PCP. PCP loading was 200–220 mg $(l\ d)^{-1}$ accordingly. The performance of the reactor R1 inoculated with sludge acclimated by PCP was a little better than the reactor R2 which was inoculated with mixed sludge acclimated by 4-CP, 3-CP and 2-CP.

To evaluate the metabolism of PCP, 20 mg l^{-1} PCP was introduced into reactor R1 (Fig. 2). PCP was observed in the effluent at the 4th day after the introduction of PCP into the influent. 2,3,5,6-TeCP was detected at 10th day and maintained low level. At 23rd day a sharp peak of 2,3,5-TCP was observed after introduction of PCP in the influent, but this intermediate was relatively short lived in the reactor R1 as well. Coinciding with the decrease of 2,3,5-TCP, a higher peak of 3,5-DCP was observed at 34th day and a highest peak of 3-CP was observed at 60th days after PCP introduction. The results were consistent with the work using continuous cultures acclimated to PCP for 6 months (Nicholson et al. 1992), but contrasted with some other observations that monochlorophenols are relatively recalcitrant to reductive dechlorination (Bryant et al. 1991; Duff et al. 1995). In our continuous reactor system, we observed a series of daughter products from reductive dechlorination of PCP, which indicated that our acclimated anaerobic consortium carried out para-dechlorination to produce 2,3,5,6-TeCP followed by ortho-dechlorination, producing 2,3,5-TCP, a daughter product. This dechlorination pathway accorded with previous reports (Christiansen et al. 1995; Hendriksen et al. 1992). Different organisms are responsible for the different dechlorination pathway and the rate of dechlorination of PCP. In our investigation, the

Table 4 Operation performance of UASB reactors

Reactors	Operation time (d)	Influent CODcr (mg l^{-1})	COD removal rate (%)	Influent PCP (mg l^{-1})	Effluent PCP (mg l^{-1})	PCP removal rate (%)	HRT (d)
R1	10–30	3,000–3,100	73.6–95.8	2–4	0–0.212	89.4–100	5.8–1.2
	35–80	4,500–6,200	97.0–97.8	7.60–65.94	0–0.258	99.6–100	1.1–0.9
	103–118	5,580	97.4–97.6	183.0	0	100	0.9
R2	10–30	3,000–3,100	77.7–96.1	2–4	0–0.200	90–100	4.6–1.1
	35–80	4,500–6,200	96.2–97.7	7.55–66.31	0–0.189	97.6–100	1.0–0.87
	91–100	5,750	90.3–89.9	135–172.1	0	100	0.90–0.89

rate of dechlorination of PCP was rather high, which may be due to the long acclimation and operation period of reactor R1.

Sorption of anaerobic sludge to PCP

Because PCP is a weak organic acid, pH, organic solvent and ion intensity influenced its distribution between solid and liquid phases (Kennedy et al. 1992). In order to make the conditions comparable with the conditions in the USAB reactor, the test employed the same synthetic wastewater as the media of sorption and de-sorption. In each test, equilibrium conditions were determined after 14 h of contact time because other researchers have reported that bio-sorption is a rapid process which will take less than 1 day to reach equilibrium (Tsezos and Bell 1989), and that observed uptake by anaerobic biomass at 24 h of contact time is very similar to the 2 h contact time (Kennedy et al. 1992). Figure 3 shows that bio-sorption and de-sorption of PCP were fitted to Freundlich isothermal equation very well, with correlation coefficients more than 0.9. Sorption isotherm is $X/M = 0.567C_e^{1.113}$, $r = 0.9619$. De-sorption isotherm is $X/M = 0.282C_e^{0.965}$, $r = 0.996$ (where X/M = equilibrium concentration of adsorbate on anaerobic granular sludge; C_e = equilibrium concentration of adsorbate in solution). From the above equations, Freundlich constant of sorption isotherm is $K = 0.567$ mg (g TVS)$^{-1}$, $1/n = 1.113$; Freundlich constant of de-sorption isotherm is $K = 0.282$ mg (g TVS)$^{-1}$, $1/n = 0.965$. The results illuminated that sorption capacity of PCP on anaerobic sludge is limited. The difference

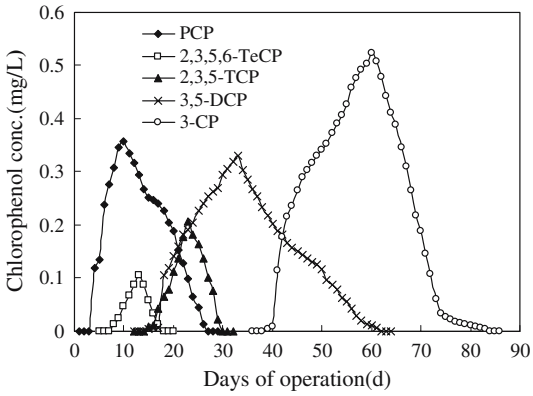

Fig. 2 Sequential dechlorination of PCP after 20 mg/l PCP introduction in R1 UASB reactor

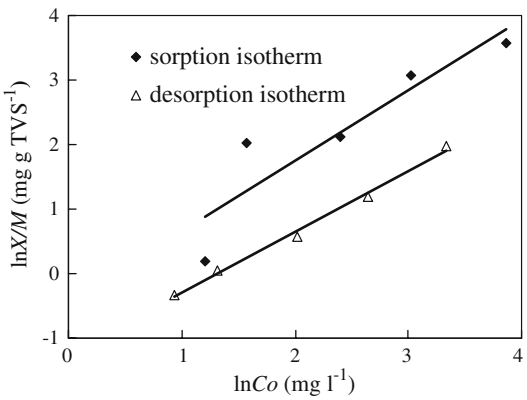

Fig. 3 The sorption and desorption isotherm of PCP on anaerobic granular sludge

between adsorption and de-sorption capacity shows that sorption of PCP on anaerobic sludge is irreversible partly because it is impossible for unacclimated anaerobic sludge to degrade PCP completely in such short time (14 h) (other chlorophenolic compounds were not observed by HPLC).

Application of bio-sorption kinetics of PCP

To review the adsorption kinetics in UASB reactor treating wastewater containing PCP, PCP content sorbed by anaerobic sludge under different effluent PCP concentrations was measured. The measured value and predicted data were illustrated in Table 5. The comparison suggested that the real balance between solid and liquid phases could not achieved due to PCP degradation of anaerobic granular sludge in practical reactor, but the Freundlich model proposed by non-degrading PCP anaerobic sludge which was inoculated in a similar condition can provide a good prediction of PCP sorption of anaerobic sludge in reactor.

Comparing Table 4 with Table 5, it was found that during natural operation state, effluent PCP concentration was very low, and the main mechanism of PCP removal was not PCP sorption of anaerobic sludge. However, when influent PCP concentration was high and reactor operation was not good, effluent PCP concentration increased sharply and PCP sorption may play a significant role in removal of PCP during the earlier days (1–2 day).

Compared with aerobic sludge to adsorb PCP, the adsorption capacity of anaerobic sludge was lower (Table 6). The difference may be due to the test condition and sludge type, but cellular lipid in anaerobic microbes may also attribute to the difference. Generally, the membrane lipid content of aerobic and anaerobic microorganisms was comparable, but two kinds of lipid were different in nature. The aerobes were presumably mostly eubacteria, while the anaerobes were largely archebacteria (methanogens). The former have membrane lipids that contain fatty acids esterified to glycerol, while the latter contain unique neutral squalenes and ether linked polar lipids. Different lipid might have different sorptive capacity (Kennedy et al. 1992).

Volatile effect of PCP in anaerobic reactor

In airtight anaerobic reactor, there is balance between gas and liquid phases. Especially in upstream reactor, PCP concentration decreases gradually with height of reactor, so the forces driving to volatile is about zero. During the biogas sorption test of 6 days, PCP was not detected in the sorbed liquid (0.06 mol l^{-1}), which suggested that it is impossible to remove PCP in anaerobic reactor through volatilization.

Conclusions

(1) It was possible to treat PCP containing wastewater by UASB reactor; the main removal mechanism of PCP in UASB reactor was bio-degradation of sludge, rather than sorption or volatilization. The reactors exhibited stable operation over the range of PCP concentrations tested (170–182 mg l^{-1}). After 40 days of introduction of 20 mg l^{-1} PCP, the reactor produced an effluent containing 3-CP as the only stable daughter product of PCP decomposition.

(2) Sorption by anaerobic sludge granules could be described using Freundlich model. The sorption of PCP from anaerobic sludge was found to be irreversible partly. Compared to reported capacities of aerobic activated

Table 5 Predicted and experimental PCP adsorbed amount by granular sludge in UASB reactors

Reactors	R1				R2			
Equilibrium concentration (Effluent PCP concentration, mg l^{-1})	0.04	0.20	0.493	4.0	0.10	0.367	1.468	4.0
Experimental adsorbed amounts X_T (mg)	0.622	2.77	7.67		1.032	4.49	24.56	
Predicted adsorbed amounts (mg)	0.491	2.89	7.98	79.41	1.23	4.88	23.98	71.97
Error (%)	21.2	3.3	2.1		16.3	9.6	3.3	

Table 6 Conditions of PCP sorption test and Freundlich parameters

Sorbent	Temperature (°C)	Parameters		References
		K	1/*n*	
Rhizopus arrhizus (dead)	20	32.1	0.56	Kennedy et al. (1992)
(active)	20	28.8	0.90	Kennedy et al. (1992)
Aerobic sludge (dead)	20	85.1	0.60	Kennedy et al. (1992)
(active)	20	10.1	0.80	Kennedy et al. (1992)
Anaerobic sludge (dead)	28	1.15	0.47	Wu et al. (1993)
(active)	28	0.567	1.113	This study

sludge and other species, PCP bio-sorption capacity of anaerobic granular sludge was low, which may be due to the differences in lipids characteristics and test conditions. The Freundlich equation could provide a good interpretation of sorption status of PCP by anaerobic granular sludge in UASB reactor.

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