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Coupled anaerobic/aerobic biodegradation of 2,4,6 trichlorophenol

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Abstract: Degradation of 2, 4, 6-trichlorophenol(TCP) with co-immobilizing anaerobic granular sludge and isolated aerobic bacterial species was studied in coupled anaerobic/aerobic integrated reactors. The synergism of aerobes and anaerobes within co-immobilized granule might facilitate degrading the TCP and exchange of anaerobic metabolites 4-CP, which promoted system organic removal efficiency and recovered from organic shock-loads more quickly. The biomass specific activities experiment further confirmed that strict anaerobes be not affected over the course of this experiment by the presence of an oxic environment. aerobic activity predominated in the outer co-immobilized granule layers, while the interior was characterized by anaerobic activity. The co-immobilized granule could thus enable both aerobic and anaerobic microbes function in the same reactor and thereby integrate the oxidative and reductive catabolism.

Keywords: co-immobilizing granule; 2, 4, 6-trichlorophenol(TCP); reductive dechlorination; mineralization

Introduction

Highly chlorinated aromatic pollutions are difficult to be decomposed biologically, and are mutagenic as well as carcinogenic in nature, sequence anaerobic-aerobic process had been proved to be a successful degradation process to deal with these substances(Evans, 1996). Although the reductive dehalogenation resulted in incomplete biodegradation(Natarajan, 1998), less chlorinated or dechlorinated compounds were more amenable to aerobic degradation. The rate of anaerobic degradation of these compounds by methanogenic population decreased with the chloride removal from the molecule while the degradation rate under aerobic conditions by methanotrophs increased with the decreasing extent of chlorination. Thus coexistence of aerobic and anaerobic zones in a co-immobilized granule might be of paticular interest when highly substituted compounds had to be fully biodegradation.

Shen(Shen, 1996) had confirmed that high tolerance of methanogens in the granular biofilm to the extensive presence of oxygen in the reactor. Tartakovsky(Tartakovsky, 1998) co-immobilized the aerobic methanotroph methylosinus sporium with anaerobic methanogens and applied for the dechlorination of tetrachloroethylene(PCE), the mineralization efficiency of the commensal system was 88% as opposed to 38% in the control system, which was not inoculated with the methanotrophs.

Chlorophenol is a typical constituent of petrochemical, oil refinery, plastic, pulp and wood preservative(Liu, 1991). In this experiment, we studied the coupling anaerobic and aerobic microbial populations to degrade chlorophenol with 2, 4, 6-trichlorophenol(2, 4, 6-TCP) as a model substrate, the objective of our research was to determine if the coupled reactor had more advantages over the UASB reactor in the complete mineralization and degradation of chlorinated aromatic substance.

1 Materials and methods

1.1 Microorganism and immobilization procedure

Four strains aerobes that could degrade CP of pseudomonas, enterobacter cloacae, corynebacterium xerosis, klebsiella pneumoniae were isolated from an existing upflow aerobic fluidized reactor in our laboratory(Chen, 2000), anaerobic granules sludge were obtained from a municipal UASB reactor. Co-immobilization of anaerobic sludge with the 4 strain aerobic microorganism was achieved using 8%

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polyvinyl alcohol(PVA) and 2% sodium alginate. First, 10 ml of mixed 4 strain aerobic microorganism were collected with centrifuge operated at 6000 r/min, which were mixed thoroughly with 150 ml of an 8% PVA and 2% sodium alginate. Next, 150 ml of anaerobic granule sludge were added to this solution and stirred with a magnetic mixer for 10 min, then pumped pressurized air, the air stream caused the mixture to break into droplets. The droplets were added to 4% solution of $CaCl_2$ for five hours. The resulting beads of 3 mm diameter were transferred into the refrigerator, stayed there overnight at −15℃, then thawed at room temperature, afterwards, they were washed with sterilized water.

1.2 Reactor setup and operation

The coupled anaerobic/aerobic bioreactors(Fig.1a) consisted of 890 ml upflow anaerobic sludge bed (UASB) type reactors connected to 420 ml oxygen contactor(Fig.1), through which the UASB effluent was recirculated in a down flow mode, for the purpose of oxygen transfer to the entire system. In the control UASB reactor(Fig.1b), the bulk liquid was recycled from the top back to the base of the reactor by a bump. The reactors were operated with a hydraulic retention time(HRT) of 1d, a liquid upflow velocity of 5—6 m/h of the sludge bed. In the aerated column reactors the aeration rate was 5.5—6.5 $L/(V_R \cdot h)$, where V_R is the reactor volume.

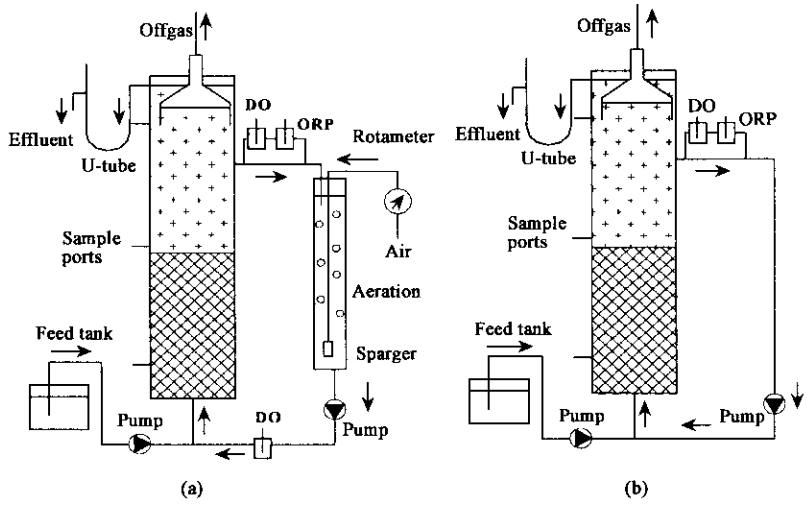

Fig.1 Set up of anaerobic/aerobic coupled (a) and UASB(b) reactor

The composition of the feed(in mg/L): K_2HPO_4, 40; KH_2PO_4, 30; NH_4HCO_3, 340; $(NH_4)_2SO_4$, 30; yeast extract, 8500; $NaHCO_3$, 2720; $KHCO_3$, 3470; $FeSO_4 \cdot 7H_2O$, 1400; H_3BO_3, 0.25; $ZnSO_4 \cdot 7H_2O$, 52; $MnSO_4 \cdot H_2O$, 1; $CuCl_2 \cdot 2H_2O$, 0.08; $CoSO_4 \cdot 7H_2O$, 0.3; $Ni_2SO_4 \cdot 6H_2O$, 0.2; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.1; $AlK(SO_4)_2 \cdot 12H_2O$, 0.07; $MgSO_4 \cdot 7H_2O$, 20.

The coupled anaerobic/aerobic bioreactor was inoculated with 300 ml co-immobilized granule, the control UASB bioreactor was inoculated with the same amount of anaerobic granules sludge described above. Both reactors had similar operational strategies.

There were two stages in each reactor run. An enrichment and activation of the microbial population in aerobic 4-CP degraders was first achieved. This involved a first stage in which both reactors were fed with a solution of 4-CP. For the next stage of operation, the reactors were fed with a solution of 2, 4, 6-TCP.

1.3 Analytical methods

The oxidation reduction potential(ORP) was monitored with pHS-3C/ORP meter, dissolved oxygen were determined from HACH sension 6, portable dissolved oxygen meter.

Chlorophenol were analyzed using a gas chromatograph extracted with CH_2Cl_2 and hexane. The extracts were analyzed using a gas chromatograph coupled to an electron capture detector(Autosystem XL, Perkin-Elmer). The extracts were injected into a DB-5 capillary column(15 m × 0.32 mm i.d.)(J & W

Scientific, Follsom. CA). The temperature program was started at 80℃ for 1 min, then ramped 10℃/min to 250℃. The injector temperature was 28℃, the detector temperature was 350℃ and the carrier gas was nitrogen. Injections of pure chlorophenol were used to identify degradation intermediates.

The volume of cumulative offgas was measured by means of displacement of acidified water, gas composition was analyzed by means of a gas chromatograph, equipped with thermal conductive detectors (TCDS).

Substrate anaerobic activity tests were carried out based on the method of Guiot (Guiot, 1995), through measuring the depletion rate of a nonlimiting concentration of a singled defined substrate as the sole carbon source under strict anaerobic conditions. The substrate utilization value per biomass concentration was used as an indicator of the relative biomass content of the targeted trophic groups

The oxygen utilization activity of the microbes was assessed through oxygen depletion rate in a BOD measuring vessel with a portable dissolved oxygen meter.

2 Results and discussion

2.1 Start up of the reactor

Two months of continuous operation were required in order to reach a pseudo-steady state. During the start up of two reactor, the reactors were fed with yeast extract and mineral nutrients described above, organic loading rate increased gradually from 1 kgCOD/(m^3·d) to 3.7 kgCOD/(m^3·d), 4-CP and 2, 4, 6-TCP were initially introduced to the two reactors at 10 mg/L after 35 d of operation. Organic loading rate increased only when daily COD removal over 3 d was more than 80% at a certain organic loading.

At the end of this stage, the pH, redox potential(ORP) and the CH_4 : CO_2 ratio in the two reactors, and the dissolved oxygen concentration(DO) just before entry to the base of the reactor(DO_{in}) and outlet of the reactor(DO_{out}) were measured, the results are shown in Table 1.

Results in Table 1 indicated that the pH of the coupled reactor was higher than that for the UASB reactors due to the gas stripping of the CO_2 during the aeration process. The DO concentration in coupled reactor declined from inlet of 3.6 ± 1.0 mg/L to outlet of 0 mg/L, the oxygen consumption suggested significant activity of aerobic and facultative organisms at the surface of co-immobilized sludge granule and in the bulk liquid. The low ORP and DO concentration in the outlet of the coupled reactor also indicated that the coupled reactor was oxygen limited. The CH_4 : CO_2 ratio was 1.2—1.7 and 0.3—1.1 for the UASB and coupled reactor respectively, which showed that oxygen addition could decrease methane production.

Table 1 Comparison of coupled versus UASB reactor(mean *SD*)

	UASB	Coupled
pH	7.1 ± 0.1	7.3 ± 0.1
DO_{in}, mg/L	- -	3.6 ± 1.0
DO_{out}, mg/L	0	0
ORP_{out}, mV	- 133 ± 24	- 110 ± 12
CH_4 : CO_2	1.2—1.7	0.3—1.1

2.2 4-CP degradation in the reactor

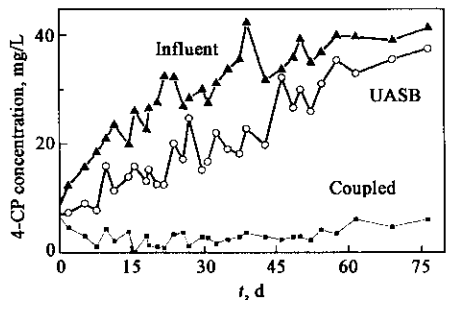

Fig. 2 4-CP degradation in coupled and UASB reactor

In this stage(1—80 d) the reactors were fed with a solution of 4-CP and nutrients and microelements described above. 4-CP was gradually increased to 42 mg/L, this stage was aimed at confirming the viability of aerobe CP microbial cells entrapped outside the co-immobilized bead. As shown in Fig.2, the degradation of 4-CP in a coupled reactor system was more complete than in an anaerobic system alone. When 4-CP concentration increased to 40 mg/L, the effluent 4-CP concentration in UASB increased to 30—37 mg/L, while in the coupled reactor 4-CP was degraded to only 3—8 mg/L. This result confirmed that

less chlorinated compounds be more amenable to aerobic degradation, and the aerobes of outer layers can function under limited oxygen.

2.3 2, 4, 6-TCP degradation in the reactor

For the third part of the experiment(81—163d), the reactors were fed with a solution of 2, 4, 6-TCP and nutrients and microelements described above. 2, 4, 6-TCP concentration gradually increased to 42 mg/L, the result is shown in Fig. 3.

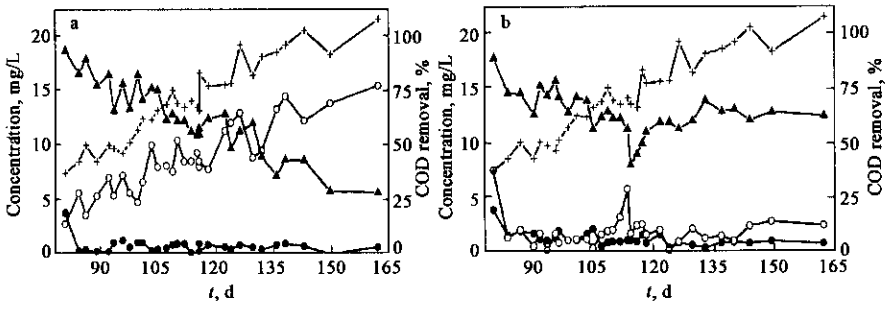

Fig.3 Concentrations of influent TCP and metabolites in coupled(a) and UASB(b) reactors
COD removal rate(▲), influent 2, 4, 6-TCP concentration(+), and effluent 2, 4-DCP concentration (●), 4-CP concentration(○)

In both reactors, the degradation of 2, 4, 6-TCP, 2, 4-DCP were observed with an effluent concentration of 2, 4, 6-TCP and 2, 4-DCP below 1 mg/L(effluent 2, 4, 6-TCP concentration were not shown) which indicated the complete degradation of TCP and DCP in both reactors. Detection of 4-CP in effluent was found in both reactor, at the end of this stage, the effluent concentration of 4-CP and COD removal rate in coupled reactor were 2.2 mg/L and 64% compared to 15.7 mg/L and 27% in UASB reactor, it revealed that both aerobic and anaerobic microorganisms were functioned in the coupled reactors.

Under anaerobic methanogenic conditions, TCP undergoes reductive dechlorination via 2, 4-dichchlorophenol(2, 4-DCP) resulting in the formation of parachlorophenol(4-CP). 4-CP was often the end production of TCP anaerobic degradation. Therefore 4-CP, the metabolites of 2, 4, 6-TCP anaerobic treatment might also be inhibitory to the methanogens themselves, resulting in declining anaerobic treatment efficiencies. The aerobic metabolic pathway for TCP was different from anaerobic, the para-Cl group is replaced by another OH-group forming 2, 6-dichlorohydroquinone (Chaudhry, 1991; Li, 1991; Liu, 1991a; 1991b). Therefore no detection of 2, 6-dichlorohydroquinone and presence of low amounts of 4-CP in the effluent of coupled anaerobic/aerobic bioreactor suggested 2, 4, 6-TCP reductive dechlorination occurred prior to aerobic oxidation. The obvious reduction of accumulation of 4-CP in the coupled reactor compared to the UASB reactor confirmed the coupled reactor's higher capacity of mineralizing the anaerobic intermediates 4-CP that otherwise would accumulate as end products.

From Fig. 3 we could also see that coupled systems had achieved higher COD removal rate than conventional anaerobic process and recovered from organic shock-loads more quickly, thus through proper partitioning between aerobic and anaerobic metabolic activity, the coupled reactor could achieve higher and more complete

Table 2 Comparison of specific anaerobic and aerobic biomass activities of coupled reactor compared to UASB

	Specific substrate activity, g substrate/(gVSS·d)			
	Glucose	Acetate	Propionate	Oxygen
Coupled reactor	2.21	0.54	0.15	0.21
UASB	2.02	0.39	0.23	0.08

degradation of 2, 4, 6-TCP than in an anaerobic system alone. Not only was the sequential biodegradation process accomplished in a single reactor system, but also the synergism of aerobes and anaerobes within granular biofilm might facilitate degrade the TCP and exchange of anaerobic metabolites 4-CP, thus promote system removal efficiency and stability.

2.4 Specific activity of the biomass

By the end of this run, the biomass specific activities of coupled reactors were compared with the control activities for anaerobic UASB reactors (Table 2). Glucose for facultative and obligate anaerobic acidogenic fermenters, propionate for proton-reducing acetogens, acetate for acetotrophic methanogens aerobic specific activities were measured by the oxygen depletion rate per unit mass of biomass under a nonlimiting concentration of glucose (Stephenson, 1999).

The oxygen could be consumed either by the aerobes or the facultative anaerobes in the coupled reactor, adding oxygen to the reactor should decrease the substrate uptake by the acetogens and methanogens, while results in Table 4 indicated that the strict anaerobes were not affected over the course of this experiment by the presence of an oxic environment, since glucose, acetate, propionate anaerobic activities of the coupled reactors biomass were comparable to or higher than the activities of the UASB systems. This confirmed that the aerobes and the facultative anaerobes in the solution or in the peripheral of anaerobes could limit the penetration of O_2 and to shield the strict anaerobes against O_2 in the granule core (Guiot, 1993).

The slightly higher anaerobic glucose activity confirmed that the facultative anaerobes was slightly increased in the coupled reactor, while the aerobic specific activity of co-immobilized granule in the coupled reactors at the end of the experiment was 0.21 $gO_2/(gVSS \cdot d)$ compared to 0.08 $gO_2/(gVSS \cdot d)$ in the UASB reactor, this suggested that aerobes growth occurred in coupled reactor.

3 Conclusions

Degradation of 4-CP and 2, 4, 6-TCP in a coupled system was more complete than in an anaerobic UASB system alone, it revealed that both aerobic and anaerobic microorganisms were functional in the coupled reactors, and the synergism of aerobes and anaerobes within co-immobilized granule might facilitate degrading the TCP and exchange of anaerobic metabolites 4-CP, thus promote system removal efficiency and stability.

Coupled systems achieved higher COD removal than conventional anaerobic processes and recovered from organic shock-loads more quickly. They are potentially more energy-efficient than conventional aerobic systems, requiring less energy for operation.

The biomass specific activities experiment further confirmed that strict anaerobes were not affected over the course of this experiment by the presence of an oxic environment, aerobic activity predominates in the co-immobilized granule outer layers, while the interior was characterized by anaerobic activity. A co-immobilized granule could thus enable both aerobic and anaerobic microbes to function within the same reactor and thereby integrate the oxidative and reductive catabolisms.

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