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IMPACT OF HIGHLY BIODEGRADABLE SUBSTRATE ON THE BIODEGRADATION OF RECALCITRANT COMPOUNDS

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ABSTRACT

The effects of the presence of an easily biodegradable compound on the biodegradation of a recalcitrant chemical were studied with the preloaded GAC columns. The addition of an easily biodegradable compound increased the total biomass in the GAC columns. Microorganisms might simultantously utilize both the easily biodegradable and recalcitrant compound. However, the presence of an easily biodegradable compound retarded the biodegradation of the recalcitrant compound in the liquid phase and decreased the bioregeneration rate and efficiency of the sorbed recalcitrant compound.

Introduction

Biodegradation of recalcitrant compounds generally occurs at a slow rate. An increase in the total biomass may improve the biodegradation rate of recalcitrant compounds. An increase in the total biomass can be obtained by adding an easily biodegradable compound into the bioreactor, especially when the concentration of the recalcitrant compound is low. The presence of the second carbon and energy source can improve the removal of a biodegradable compound present at very low concentration through the secondary substrate utilization[14]. Secondary substrate utilization has been used to increase the removal of trace contaminants, especially when the concentration of a trace SOC (Synthetic Organic Chemical) is less than its $S_{m\,in}$, below which the concentration is too low to solely support microbial growth. In the presence of a primary substrate, which had a concentration greater than its $S_{m\,in}$, trace organics could be easily removed even if their concentrations were below their minimum concentrations[11, 12].

Klecka and Maier[5] used a biodegradable substrate analog to improve the biodegradation of a recalcitrant compound, PCP. The overall degradation rate was accelerated due to an increase in cell concentration as a result of simultaneous growth on both substrates. Namkung and Rittmann[11] showed that the presence of acetate in a biofilm reactor increased overall phenol removal in comparison with that in the column reactor fed only with phenol. The basic concept was that a cell's growth and maintenance could be supported by more than one compound present in the reactor. Thus, an individual organic compound was utilized not only by the biomass grown from its utilization, but also by the biomass grown from the utilization of a second substrate. Lapat-Polasko et al[7] showed that the rate of dichloromethane utilization at trace concentrations was greater in the presence of acetate than without it. Schmidt et al[15] showed that the addition of glucose enhanced the degradation of PNP, when PNP was present at high concentrations. The enhanced degradation resulted from the simultaneous use of glucose and PNP and the increase rate of microbial growth on glucose. Kim and Maier[6] showed that the degradation rate of 2,4-dichlorophenoxyacetate was enhanced when nutrient broth (beef

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extract and peptones) was added. The enhanced degradation was due to concurrent substrate utilization.

Contradictory results have been observed on the effects to the presence of a second compound on the biodegradation of a recalcitrant compound. Lu and Speitel[8] showed that the presence of the second compound may or may not enhance the biodegradation of a recalcitrant compound. The presence of a second carbon source does not necessarily enhance the biodegradation of a recalcitrant compound. The presence of a preferred substrate repressed the synthesis of inducible enzymes capable of catabolizing the recalcitrant compound, or less preferable compound[2] Papanastasiou and Maier[13] studied the biodegradation of 2,4-dichlorophenxylacet-ate in the presence of glucose and showed that when both substrates were present, concurrent growth took place and the utilization of the one substrate was inhibited by the other. Haller[3] showed that the presence and absence of an easily biodegradable compound did not affect the lag period for biodegradation of chlorinated aromatic substrates.

In this study the effects of the presence of an easily biodegradation compound on the biodegradation of a sorbed recalcitrant compound were studied in GAC columns. PCP (pentachlorophenol) was used as the target compound to examine the effects of the presence of an easily biodegradable, but nonadsorable compound on the biodegradation of a recalcitrant compound. The easily biodegradable, but nonadsorbable compound used in this study was acetate. Acetate has been widely used as the model primary substrate to enhance microbial growth in aerobic and anaerobic reactors [1, 10, 14, 18].

Acetate can also serve as primary substrate to enhance the removal of other biodegradable chemicals through secondary substrate utilization[1, 10, 11, 14]. PNP was selected to study the effect of acetate on the biodegradation of low concentrations of a biodegradable, adsorbable chemical. The presence of acetate is expected to increase the total biomass in the GAC columns and perhaps improve the biodegradation rate of PNP.

Experimental Procedures

1. Granular Activated Carbon(GAC)

Calgon Filtrasorb 400 (F-400)was used in this research. F-400 was ground in an analytical mill (Tekmar Co., Cincinnati, OH) and sieved to recover the 30x40 mesh fraction, which provided an average particle diameter of 0.5 mm.

2. Substrates

Pentachlorophenol(PCP) and 4-nitrophenol (PNP) were chosen as the model substrates, because both compounds were recalcitrant and both are on the EPA priority pollutant list. Radiolabelled PCP and PNP were used to monitor the bioregeneration rate of GAC and to confirm the mineralization of the synthetic organic chemical as indicated by the production of radiolabelled CO_2. The radiolabelled chemical was obtained from Pathfinder Laboratories Inc. (St. Louis, MO) in an analytical-reagent grade with uniformally ring-labelled carbon-14. The radiolabelled chemical was prepared in an aqueous form at pH 2 to avoid biodegradation during storage. All chemicals, radiolabelled and unlabelled, were stored in the refrigerator at 4℃.

3. Nutrients

In addition to carbon and energy sources for maintenance and synthesis, microorganisms need nutrients in the synthesis of a variety of macromolecules. Nitrate was used as nitrogen source, Instead of ammonia, to avoid nitrifier growth in the reactor. The calcium concentration was purposely maintained at a relatively high concentration, because Turakhia[20] showed that calcium increased the rate and extent of biofilm mass accumulation on attachment surfaces. The compositions of the feed solutions for PCP column study are as following: KH_2PO_4, 600 μ g/L; K_2HPO_4, 545 μ g/L; $CaCl_2$, 23.2 μ g/L; $MgSO_4$, 8.6 μ g/L; KNO_3 13.3 μ g/L; For PNP column study the compositions of the feed solutions are as following: KH_2PO_4, 900 μ g/L; K_2HPO_4, 29.5 μ g/L; $CaCl_2$, 23.2 μ g/L; $MgSO_4$, 8.6 μ g/L; KNO_3, 13.3 μ g/L.

4. Bioregeneration of GAC Columns

GAC was preequilibrated with PCP following the procedures described in elsewhere [9, 16, 17]. In the first study, four columns were run in parallel. The four columns were grouped into two sets. Each set contained one 20-cm and one 0.5 -cm GAC column. Each column contained 0.5g

of radiolabelled GAC which was placed at the effluent end of the column. The 0.5-cm columns contained only the radiolabelled GAC. One set of two columns was fed only PCP at 25 μg/L, while the other set of two columns was fed both PCP at the same concentration and acetate at 1 mg/L. Acetate was injected into the feed line with a 20 -mL syringe by a syringe pump which was operated at 20 mL/day. The experimental design is shown in Figure 1. Acetate was prepared in a stock solution at pH 2 to avoid microbial contamination. The total influent flow rate, including the acetate solution, was 2.5 mL/min during the first three weeks and 1.25 mL/min thereafter. The pH of the influent solution was not affected by the acidified acetate solution, because the acetate solution was fed at a very low flow rate and the buffer strength in the main feed solution was appropriately controlled.

After loading with preequilibrated GAC and PCP-degrading microorganisms, the column reactors were allowed to stand over eight hours to permit microorganisms to attach onto the GAC surface. Feed solution containing PCP at the equilibrium concentration was introduced and fed continuously. Acetate was also injected into the column in one of the two-column sets. Samples were collected to analyze the PCP effluent concentration, and radiolabelled materials were also monitored. The effects of the presence of acetate on the biodegradation of recalcitrant compounds in the liquid and sorbed phases could be observed by comparing the two sets of columns.

In the second PCP/acetate study, the column set-up and operating conditions were the same as those in the phenol-exhausted GAC columns [9, 16, 17], except that acetate was added at 100 μg/L. Three PCP-exhausted GAC columns were run in parallel. The total mass of GAC in each column was 1.8, 1.0, and 0.5g. Each column contained a radiolabelled GAC element with 40 μCi of radiolabelled PCP. The radiolabelled GAC element was placed at the effluent end of the column. The shortest column contained only the radiolabelled GAC. After the incubation period, PCP was continuously fed at the equilibrium concentration, 5 μg/L. Acetate was also simultaneously fed into the columns with a syringe pump at 20 mL/day. Samples were collected for PCP and radioactivity analysis.

The column conditions for the PNP/acetate study were similar to those in the phenol-exhausted GAC columns except for the addition of acetate[9, 16, 17]. The GAC columns were simultaneously fed with PNP at its equilibrium concentration(10 μg/L) and acetate at 2 mg/L.

The procedures of sample collection, sample analysis, data analysis are the same as the previous works[9, 16, 17].

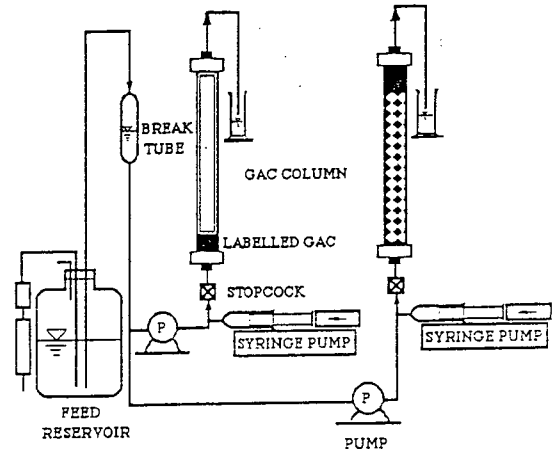

Figure 1. The experimental design of GAC column study.

Table 1. PCP effluent concentrations in the GAC columns fed with both PCP and acetate.

	PCP effluent concentration(μg/L)**			
column	20-cm GAC column		0.5-cm GAC column	
feed condition	PCP only	PCP+acetate*	PCP only	PCP+acetate*
operation time				
15 days	18.3	18.4	18.0	24.8
27 days	16.0	16.8	20.7	20.5

* : acetate concentration=1000 μg/L

**: PCP influent concentration=25 μg/L

Table 2. PCP effluent concentrations in the GAC columns fed with both PCP and acetate.

	PCP effluent concentration(μg/L)*		
column length	20cm	10cm	0.5cm
operation time			
3 days	10.3	12.2	8.5
10 days	11.4	12.5	6.2
13 days	11.9	14.5	15.6
20 days	7.2	7.2	15.4

*:acetate influent concentration=100 μg/L

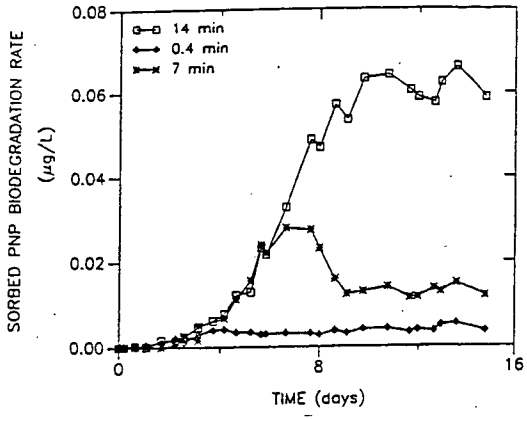

Figure 2. Bioregeneration rate of PNP-exhausted GAC columns in the presence of 2 mg/L acetate.

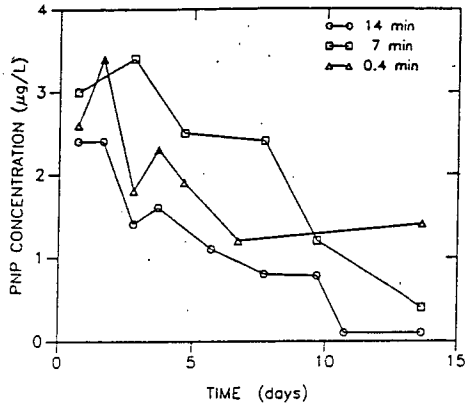

Figure 3. PNP effluent concentrations in the PNP-exhausted GAC columns in the presence of 2 mg/L acetate.

Results and Discussion

1. Biodegradation of PCP in the Presence of Acetate

The results of PCP biodegradation in the presence of acetate are listed in Tables 1 and 2. Table 1 suggests that PCP removal was not affected by the presence of acetate in both 20- and 0.5-cm columns. However, the 20-cm column had a little better PCP removal after 27 days of operation. PCP removal in the 20-cm columns was about 36 percent, while it was 20 percent in the 0.5-cm columns.

Table 2 shows the results of the second study on PCP biodegradation in the presence of acetate with columns of different length. Table 2 suggests that the 20-cm column had the best PCP removal among the three columns. However, the effluent concentrations in all three columns exceeded the influent concentration (5 μg/L) throughout the entire experiment period. This might suggest that the equilibrium concentration was underestimated.

The results of biodegradation of sorbed PCP in the presence of acetate are listed in the Table 3, along with results from experiments in the absence of acetate[16, 17]. The presence of acetate did not increase the biodegradation of soubed PCP as indicated by the $^{14}CO_2$ production.

Table 3. Summary of biodegradation of PCP and PNP in the presence of acetate.

substrate	initial GAC loading (μg-sub/mg-GAC)	feed conc. (μg/L)	total GAC mass (g)	total biodegr. sorbed mass (μg)	preloaded substrate (μg)	% biodegr sorbed sub (%)
	32.0	15	19.4	2710	16000	16.90
PNP	32.0	15	10.8	3020	16000	18.90
	31.0	15	0.5	1260	16000	7.90
PNP	23.3	10(2)*	19.0	756	11600	6.50
+	23.3	10(2)*	10.0	265	11600	2.30
Acetate	23.3	10(2)*	0.5	67	11600	0.60
PCP	141	5(0.1)*	18.0	447	70600	0.63
+	141	5(0.1)*	10.0	323	70600	0.46
Acetate	141	5(0.1)*	0.5	207	70600	0.29
PCP	181	25(1)*	21.0	387	90700	0.43
+Acetate	181	25(1)*	0.5	897	90700	0.99
	181	25	20.1	359	90700	0.40
PCP	181	25	0.5	502	90700	0.55
PCP	157	15	24.6	481	78400	0.61

*:acetate influent concentration(mg/L)

Although the presence of acetate increased the total biomass in the columns, the biodegradation of sorbed PCP did not increase because acetate did not improve PCP removal in the liquid phase.

The $^{14}CO_2$ production was negligible in both PCP/acetate studies.

2. Biodegradation of PNP in the Presence of Acetate

Figure 2 shows the bioregeneration rate of PNP-exhausted GAC columns in the presence of 2 mg/L acetate. In comparison with the columns fed with PNP alone [16, 17] biodegradation of sorbed PNP was not enhanced in the presence of acetate. Actually, the opposite result was observed. Biodegradation of sorbed PNP was hindered by the presence of acetate. The presence of acetate reduced the biodegradation of PNP. For the 20-cm columns, 17 percent of the sorbed PNP was biodegraded in the column fed with PNP, while only 6.5 percent was biodegraded in the column fed with both PNP and acetate (Table 3).

The effluent PNP concentrations are shown in Figure 3. In comparison to columns fed with PNP alone[16, 17], the presence of acetate did not reduce the time required for the columns to reach steady state (about 0.2 μ g/L). For the 20-cm columns, 11 days were required to reach steady state in both cases. The results suggested that the presence of acetate did not enhance the biodegradaton of PNP. In addition, the lag period in the columns fed with both PNP and acetate was about 20 hours longer than that in the columns fed only with PNP[16, 17] The microbial population in the columns fed with both PNP and acetate should be much larger than that in the columns fed only with PNP. However, the larger microbial population did not reduce the lag period. The smaller bioregeneration rate, longer lag period, and smaller percentage of bioregeneration in the columns fed with both PNP and acetate suggest that the presence of an easily biodegradable compound does not neces-sarily enhance the removal of a recalcitrant compound in the liquid and sorbed phases. Acetate increased the number of microorganisms but apparently did not enhance the growth of PNP degraders or caused them to biodegrade acetate, but not PNP.

The concept of secondary substrate utilization has been used to explain the biodegradation of a trace SOC when a primary substrate is available. Acetate has been used as the primary substrate, which has concentration higher than the S_{min}, to support the microbial activity to degrade the secondary substrate, which has concentration less than its Smin. When the concentration of the secondary substrate is low, the secondary substrate itself cannot support the microbial activity. The basic assumption of secondary substrate utilization is concurrent utilization. The microorganisms grown on the primary substrate also can utilize the secondary substrate. However, if the primary and secondary substrate are biodegraded by different microorganisms, concurrent utilization (or secondary substrate utilization) does not exist. This is especially true when the primary and secondary substrates are structurally unrelated, or their biodegradabilities are extremely different. In other words, the microorganisms grown on an easily biodegradable compound do not necessarily carry the degradative enzymes for the biodegradation of a recalcitrant compound. In this study, the results apparently showed that concurrent utilization did not exist. The microbial population grown on acetate did not utilize PNP.

The decreased biodegradation of PNP in columns fed with both PNP and acetate might be also due to the lower influent PNP concentrations in comparison with the previous works[16, 17] Influent PNP concentrations in columns fed only with PNP and with both PNP and acetate were 15 μ g/L and 10 μ g/L, respectively. The initial GAC loading in these two experiments also was different, 32 μ g-PNP/mg-GAC for columns fed only with PNP and 23 μ g-PNP/mg-GAC for columns fed with both PNP and acetate. The lower bioregeneration rate in the columns fed with both PNP and acetate might be due to the lower initial GAC loading . As noted previously, for the 14-minute EBCT GAC columns, 6.5 percent of sorbed PNP was biodegraded for the column fed with both PNP and acetate and 17 percent for the column fed only with PNP. In the shortest column (0.4-, cm EBCT), only 0.6 percent of sorbed PNP was biodegraded for the column fed with both PNP and acetate and 8 percent for the column fed only with PNP. The differences beween the two experiments are quite large and it seems unlikely that differences of this magnitude would result solely from the relatively small change in PNP concentration. The results

indicated that the presence of acetate decreased the biodegradation of PNP in the liquid phase and decreased the bioregeneration rate of sorbed PNP.

The microorganisms seeded onto the GAC columns were adapted to the compound of interest, PNP or PCP, and could use the compound as the sole carbon and energy sources. However, the presence of an easily biodegradable compound, such as acetate, interferes with the synthesis of enzymes required for the uptake or metabolism of the other carbon source, especially recalcitrant compounds. When acetate is present, utilization of acetate is energetically more favorable for most microorganisms than utilization of low concentrations of PNP or PCP.

Kim and Maier[6] found that the presence of a second carbon source enhanced the biodegradation of 2,4-dichlorophenoxyacetate and attributed the increased biodegradation to the production of higher concentration of active biomass. However when the concentration of 2,4-dichlorophenoxyacetate was low, they found that the addition of a second substrate inhibeted the degradation of the target compound. Topp et al.[19]showed that the specific activity of PCP-degrading cells was decreased by 40 percent in the presence of supplementary carbons, glutamate and glucose. However, by ascribing to an increase in total PCP-degrading biomass, Topp et al.[19] claimed that the biodegradable compounds in polluted environments may increase the decontamination rate of recalcitrant compounds. In this study, although the total biomass was greater in the bisubstrate (PNP and acetate or PCP and acetate) columns than it in the single substrate columns, the larger number of microorganisms did not increase the removal of the recalcitrant or the moderately biodegradable compound. This suggests that the microorganisms grown from the utilization of acetate cannot simultaneously utilize PCP or PNP. Although the total biomass increased in the presence of acetate, the total PNP- or PCP-degrading microorganisms were not increased, rather decreased. Therefore, the presence of an easily biodegradable compound may not increase the biodegradation of recalcitrant com-pounds in the sorbed and liquid phases.

Generally, metabolism is controlled in a way that microorganisms can grow at a high rate under substrate-sufficient conditions in which the easilly degradable compound is preferred. When the concentration of an easily biodegradable compound is high, inducer exclusion prevents the synthesis of enzymes that are not immediately required. However, under substrate-limited conditions, concurrent utilization occurs. When the substrate is limited, all the available substrates may be simultaneously used for growth as a result of induction or derepression[4]. Concurrent utilization implies that microorganisms can simultaneously utilize more than one substrate, or that the biodegradation pathways are related. In this study, the concentrations of acetate were 100 μg/L and 1 mg/L for two PCP/acetate experiments under the same operating conditions. When the acetate concentration was high (1 mg/L), concurrent utilization did not occur. If the concurrent utilization could have occurrd when acetate concentration was low (100 μg/L), the presence of acetate could have improved PCP removal. However, acetate did not improve PCP removal in either case. The results suggest that acetate-degrading microorganisms did not simultaneously degrade PCP. Therefore, the presence of acetate did not improve PCP biodegradation. From the previous works[16], it was demonstrated that the low bioregeneration rate and efficiency of PCP-exhausted GAC columns were due to the microbial recalcitrance of PCP, rather than its desorption rate. However, an increase of total biomass in the column reactors through the addition of an easily biodegradable compound, acetate, did not enhance the total removal efficiency of PCP and its bioregeneration rate. Therefore, the presence of an easily biodegradable compound does not necessarily increase the biodegradation of a recalcitrant compound, even if the total biomass is increased.

Summary

The presence of an easily biodegradable but structurally unrelated compound does not necessarily enhance the biodegradation of a recalcitrant compound and may result in preferential utilization of the easily biodegradable compound, retarding the biodegradation of a recalcitrant compound. Acetate did not enhance, but rather repress, the biodegradation of PCP, a highly recalcitrant compound. The presence of acetate also did not increase the bioregeneration rate of adsorbed PNP and decreased the

bioregeneration efficiency of PNP-saturated GAC columns.

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易分解化合物對難分解性化合物生物分解的影響

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關鍵詞：生物分解，難分解性化合物，易分解化合物

摘　要

以活性碳生物反應槽中難分解性化合物在易分解化合物存在時之生物分解速率，生物再生率，及去除率以探討易分解化合物對難分解性化合物之影響。易分解化合物之添加增加活性碳生物反應槽中之總微生物量。微生物可同時分解利用易分解及難分解性化合物。然而，微生物量之增加並未增加難分解性化合物之去除率。相反的，易分解化合物之添加延遲難分解性化合物之分解，同時降低生物再生率與再生總量。