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Effects of Electron Acceptor and Electron Donor on Biodegradation of CCl_4 by Biofilms

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Electron Donor 및 Electron Acceptor의 농도가 생물활성대형성 및 유해폐기물 처리에 미치는 영향

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ABSTRACT

Biodegradation of carbon tetrachloride (CTC) in denitrifying and aerobic columns was investigated under various conditions of electron-acceptor and electron-donor availability. CTC removal increased when the electron-acceptor (nitrate) injection was stopped in the denitrifying column; however, CTC removal decreased when electron donor (acetate) was deleted in the denitrifying and the aerobic column. Small fractions of the CTC removed appeared as chloroform, indicating that reductive dechlorination of CTC was occurring. The results from the denitrifying column support the hypothesis that CTC behaves as an electron acceptor that competes for the pool of available electrons inside the bacterial cells.

INTRODUCTION

The biodegradation of carbon tetrachloride (CTC) was observed in denitrifying environments[1,2]. A probable mechanism for CTC biodegradation is reductive dechlorination, because formation of chloroform (CF) was observed[2]. Because a reduction reaction requires electrons, reductive dechlorination of CTC requires an electron donor. Reductive dechlorination might compete for electrons with other electron-consuming processes, particularly electron consumption by the primary electron acceptor. Therefore, in reductive dechlorination of CTC, the concentration of the primary substrates (electron acceptor and electron donor) could directly affect CTC-removal kinetics. This work summarizes experiments that demonstrate strong effects of electron acceptor and electron donor substances on CTC biodegradation rates in denitrifying and aerobic biofilm reactors.

EXPERIMENTAL METHODS

Bench-scale, porous-medium reactors were constructed as shown in Fig. 1. A special electron-acceptor injection assembly was placed 7.5 cm downstream from the column inlet. Sampling ports were placed along the length of the column. The details of reactor set-up can be found elsewhere[3]. The flow rate was 0.2 mL/min (interstitial flow velocity 0.1 cm/min) of a mineral medium containing 7.5 mg-C/L of acetate, but virtually no O_2 or NO_3^-. A biologically active zone (a region of active biofilms) was developed by injecting NO_3^- or H_2O_2 through the injection assembly at a stoichiometrically sufficient level. Sodium molybdate was added to the mineral medium to suppress the growth of sulfate reducers.

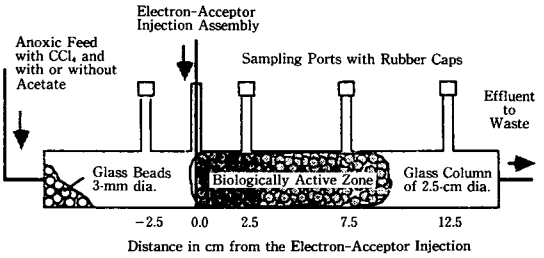

Fig. 1. Schematic of the Biofilm Reactor Used to Create a Biologically Active Zone.

CTC was dissolved into the mineral at two different ranges of influent concentration: namely, low CTC ($18.6\pm8.7\,\mu g/L$) and high CTC ($737\pm128\,\mu g/L$) concentrations. The removal of CTC was measured under normal operating conditions (i.e., electron acceptor and electron donor present as usual), without the electron acceptor, or without the electron donor. All experiments, except under normal operating conditions, were performed on a short-term basis (i.e., for 1~8 days).

Samples were taken from the sampling ports in the order 12.5 cm, 7.5 cm, 2.5 cm, and −2.5 cm. Since samples could not be taken at the electron-acceptor injection port (0 cm location), concentrations at the injection port were assumed to be the same as those at the upstream location (−2.5 cm). CTC and CF were measured by a gas chromatograph equipped with an electron-capture detector (Hewlett-Packard, Model 5710A) after extraction with dodecane[1].

RESULTS

Fig. 2 shows the relative CTC concentrations when low input concentrations ($18.6\pm8.7\,\mu g/L$) were fed. Two trends are clearly observed in this

figure. First, the CTC removal was greatly improved when nitrate (the primary electron-acceptor) injection was stopped. The improvement of removal without nitrate injection suggests that the CTC behaved as an electron acceptor that competed with the primary electron acceptor for electrons. A small amount of CF was always produced, and the amount at the 2.5 cm-location was 3~10% by molar concentration of the CTC removed. CF formation is evidence that reductive dechlorination was taking place in the experiment. The second trend is that the CTC removal declined when acetate (the external electron-donor) was removed from the medium. It again suggests that the removal of CTC requires electrons. The effect of electron-donor starvation became clearer when starvation persisted, because day-36 data, which were taken after 8 days of starvation, gave lower removal than did day-29 data, which were taken after 1 day of starvation. This decrease in CTC removal during electron-donor starvation probably occurred because cellular storage materials, utilized as an alternative electron donor, were depleted during electron-donor starvation.

Removals of CTC for the high-concentration feed (737±128 μg/L) are shown in Fig. 3. The percentage removals at the downstream sampling ports (i.e., at 2.5 cm, 7.5 cm, and 12.5 cm) were averaged for each experimental condition and are given in the figure for comparison to Fig. 2. Fig. 3 confirms the two trends that were observed in Fig. 2: more removal without nitrate injection and less removal without acetate feeding, compared to the removal under normal operating conditions. However, the removal under acetate-starved conditions decreased from the beginning. Chloroform production with CTC removal was clearly observed in this experiment. The amount at the 2.5 cm-location ranged from 0.7 μg/L (on day 121) to 11.6 μg/L (on day 76), which corresponded to 4.7~12.8% (molar) of CTC removed.

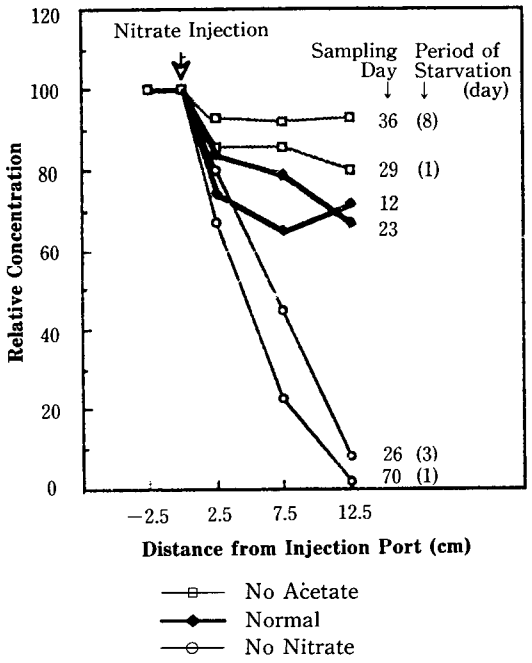

Fig. 2. CTC Removals with Low Input Concentrations in the Denitrification Column at Different Operating Conditions.

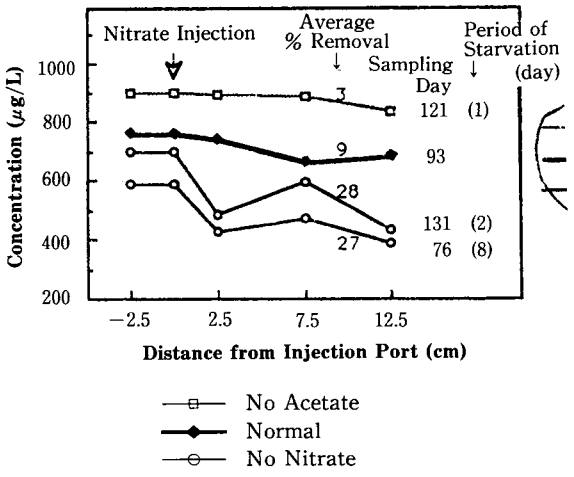

Fig. 3. CTC Removals with High Input Concentrations in the Denitrification Column at Different Operating Conditions.

Substantial removal of CTC was observed in the H_2O_2-injection column when low input concentrations were fed (Fig. 4). The effects of electron donor availability could be seen as in the denitrifying column: when acetate starvation persisted, removal of CTC decreased substantially. Unlike the denitrification column, however, the deletion of H_2O_2 did not increase CTC removal. Similar trends were observed in the high-CTC experiment (data not shown). These results indicate that the mechanisms involved in CTC removal in the aerobic column were probably different from those in the denitrifying column. Although the effect of the external electron-acceptor concentration was not apparent, reductive dechlorination appeared to take place with the CTC removal, because CF was formed. The production of CF at the 2.5 cm -sampling port ranged from 2.6% to 12.4% (molar) of CTC removed. Thus, the reductive dechlorination of CTC was not eliminated by H_2O_2 or the O_2 produced by decomposition of H_2O_2.

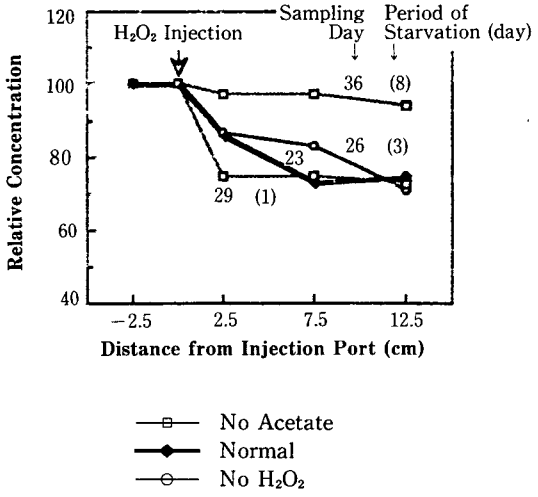

Fig. 4. CTC Removals with Low Input Concentrations in the Aerobic Column at Different Operating Conditions.

DISCUSSION

Although the production of CF and the effects of the electron donor and acceptor provide strong evidence of reductive dechlorination of CTC, a quantitative analysis of the data indicates that there may be another pathway for CTC biodegradation. For instance, if all CTC removed between the −2.5 cm and 2.5 cm ports was transformed to CF and the CF was further reduced to dichloromethane, the removal of produced CF would have been more than 90% in most cases. This removal ratio is far higher than the removal ratios for CTC in the same region (1 ~33%). Since reduction of chlorinated compounds is generally expected to be more difficult as the level of chlorination becomes lower[4], faster reduction of CF than of CTC is unlikely. Therefore, there seems to be another degradation pathway that might be equally or more important in terms of the CTC degradation rate. One possibility is that a biologically mediated hydrolysis of CTC occurred, producing CO_2 or hydroxylated intermediates, such as chlorinated methanol[2], which could not be detected in GC analysis for CTC and CF. Hydrolysis of CTC is a favorable reaction energetically[2].

Electron budgets in the denitrification and aerobic columns are presented in Table 1. The electrons available directly from acetate were calculated from the measured soluble organic carbon concentrations at the −2.5 cm port and the flow rate, assuming from the theoretical basis of stoichiometry that 43% (for denitrification reaction) and 41% (for aerobic reaction) of acetate-C were oxidized to CO_2-C, while the remaining fractions were incorporated into cell mass for new synthesis. The electrons available

from cell oxidation were estimated from CO_2 productions measured under acetate starvation and from the cell-decay coefficients (0.1/day for the denitrification column, measured; 0.2/day for the aerobic column, assumed). The electron-consumption potentials by NO_3^- and H_2O_2 were taken as the maximum values from full reduction of all input primary electron acceptors. The electron-consumption potentials by CTC were computed for reduction of CTC to dichloromethane, since complete reduction to methane is unlikely. Two different concentrations of CTC, e.g., 5 μg/L and 100 μg/L which were the approximate maximum amounts of CTC removed under normal operating conditions at low and high input concentrations respectively, were chosen for computation.

Table 1 illustrates two important points. First, the electrons available from cell oxidation are substantial in quantity compared to acetate, and second, the electron consumption by CTC reduction is a small fraction compared to electron availability. These two points might explain the insignificant initial effect of acetate starvation in the low-CTC experiment, while a significant effect occurred for the high-CTC experiment. When the CTC concentration was low, cell oxidation provided ample electrons to reduce CTC at the beginning of acetate starvation. With time, the electron storage decreased, and CTC reduction was affected accordingly, because competition for electrons with the primary electron acceptors became greater. For high CTC, on the other hand, the cellular electron storage was not sufficient to provide enough electrons to maintain CTC reduction at a rate about 20 times faster than for the low CTC experiment. Thus, CTC reduction without acetate decreased from the beginning. In summary, it appears that when the electron availability was low, or when the electron requirement for CTC reduction was high, CTC was in greater competition for electrons with the primary electron acceptors, and CTC reduction decreased.

Table 1. Electron Budgets in the Column Reactors (unit 10^{-6} e^- equivalent/day)

Sources and Sinks	NO_3^- column	H_2O_2 column
Electron sources		
Acetate fed	268	256
Cell oxidation at		
t=1 day*	79	187
t=8 day*	39	46
Electron sinks (potential)		
NO_3^- or H_2O_2	783	1376
CTC reduction (to CH_2Cl_2)		
5 μg/L	0.04	0.04
100 μg/L	0.8	0.8

*t indicates the period of acetate starvation.

CONCLUSIONS

In a denitrification column, CTC removal increased when the electron-acceptor (nitrate) injection was stopped, but it decreased when electron donor (acetate) was deleted from the feed. A small fraction of the CTC removed appeared as CF. These results support the hypothesis that CTC behaves as an electron acceptor that competes for the pool of available electrons inside the bacterial cells. Quantitative analysis of CTC removed and CF formed suggested that another mechanism, perhaps microbially catalyzed hydrolysis, also might be acting.

25~30% of the input CTC was biodegraded in the biologically active zone in the H_2O_2-injection column when the electron donor was present. When the electron donor was deleted, CTC removal decreased. Formation of CF indicated that reductive dechlorination of CTC was pos-

sible in an aerobic environment.

ACKNOWLEDGEMENT

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