Cadmium transfer and distribution in a multi-species biofilm and the impact on naphthalene removal

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Abstract Biofilm technology is being introduced into bioremediation of contaminated soils and underground water, in the form of biofilm barriers. However, the low availability of polycyclic aromatic hydrocarbons (PAHs) and toxicity of the frequently accompanying heavy metals may limit its application. The objective of this study was to investigate the mass transfer and distribution of cadmium (Cd) into the biofilm matrix and the impact caused by it. The influences of pH value, presence of an alternative substrate and increased dissolved oxygen were examined. As the pH value increased, cadmium sorption in the biofilm increased and naphthalene removal decreased. Ten mg/L of cadmium was enough to show a significant impact on biofilm when the pH was above 7.5. The cadmium minimum inhibition capacity was determined to be 5μgCd/mgVS. Acetate, added as an alternative substrate, competed with naphthalene and did not demonstrate the ability to reduce cadmium toxicity. Hydrogen peroxide, added to supplement the dissolved oxygen, accelerated the cadmium uptake/efflux cycle, making the biofilm more vulnerable to cadmium attack. Cadmium was shown to transfer faster than naphthalene into biofilm, and the removal of naphthalene in the presence of cadmium was retarded and reduced.

Keywords Biodegradation; biofilm; cadmium; mass transfer; metal distribution; naphthalene

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of several hundred individual compounds containing at least two condensed rings (Wilcke, 2000). PAHs are produced through incomplete combustion or volcanic eruptions. PAHs are receiving increased attention and are of concern because of their toxicity to human health and to natural ecology. Although they are not readily biodegradable and are persistent under natural conditions, biological processes have been applied to remediate PAH contaminated soils and ground water.

Subsurface biofilm barriers (biowalls) are constructed by injection or establishment of selected microorganisms suitable for biodegradation of concerned contaminants into the subsurface, and development of a well functioning biofilm matrix for contaminant entrapment and degradation. This technology may be potentially useful at locations with restricted access to the subsurface, since excavation is not necessary and there is no obvious depth limitation (Cunningham *et al.*, 2003).

Heavy metal wastes are often produced simultaneously with PAHs in mining, ore refinement, coal combustion, and so on. They are also present with PAHs at many hazardous waste sites due to co-disposal. Heavy metals will not only influence human health, but may also inhibit microbial activity. Heavy metal cations, e.g. Hg^{2+} and Ag^{+}, form strong toxic complexes with cellular components, which make them too dangerous for any physiological function (Nies, 1999). However, the biofilm matrix provides diversity of sorption sites for heavy metal ions, which could reduce the chance for them to enter the microbial cells (Flemming, 1995). Although some researchers have shown higher affinity of bacterial surfaces for metal binding compared to extracellular polymeric substances (EPS) (Wuertz *et al.*, 2001; Spath *et al.*, 1998), others have demonstrated that

EPS may also play a crucial role in biosorption of heavy metals (Fukushi *et al.*, 1996; Liu *et al.*, 2001).

In order to facilitate the application of biofilm processes in bioremediation of contaminated soils and ground water, further research is needed to illuminate the mechanisms of heavy metal transfer and distribution in the biofilm matrix. The objective of this study was to investigate the interaction between cadmium and biofilms when PAHs are the only carbon source, and the impact of transfer and distribution of cadmium on biofilm systems.

Materials and methods

Biofilm was developed using a seed of activated sludge from an aeration tank at a municipal wastewater treatment plant in order to produce a multi-species biofilm system. A multi-species biofilm represents the natural ecological environment more than a single species one and provides diversity of EPS, which may be critical to metal transport and distribution. In this study, biofilm was developed on glass slide surfaces for further examination.

In order to support biofilm growth, naphthalene, which has the highest solubility of the common PAHs, was selected as the carbon source. Cadmium, which is not only of concern due to its danger to human health, but also because it is known to be toxic to some PAH degraders (Malakul *et al.*, 1998), was selected as the heavy metal. Acetate, used as an alternative substrate, was introduced not only to increase the growth of bacterial cells, but also to change the structure of the biofilm system and consequently change the interaction between heavy metals and biofilms. Hydrogen peroxide was supplied to provide molecular oxygen when necessary. Since pH is critical to cation sorption, the experiments were conducted at several pH values.

Each glass slide with biofilm attached was put into a glass container which contained a mixed solution of naphthalene (15–20 mg/L), cadmium (10 mg/L), mineral nutrients and pH buffer (Tris (hydroxymethyl) aminomethane). The glass containers were sealed without any head space. The pH value was adjusted to a selected level, and hydrogen peroxide was supplied to provide dissolved oxygen when necessary. Aqueous and biofilm samples were taken and measured after 24 hours, which was believed enough for cadmium transport into the biofilms to equilibrate. Gas chromatography and atomic absorbance spectroscopy were used to measure naphthalene and cadmium, respectively. Biofilm blanks were killed with sodium azide overnight.

Cadmium was extracted from the biofilms in two ways. The biofilm was washed off the glass surface with DI water and centrifuged at 11,000 g for 30 minutes. The cadmium then present in the supernatant was defined as loosely attached cadmium. The settled biofilm was extracted with concentrated nitric acid, and the cadmium extracted was defined as tightly attached cadmium.

Results and discussions

Impact of pH values

Since hydrogen ions are much smaller than cadmium ions, they are important competitors of cadmium ions for sorption in the biofilm matrix. In order to reflect the common pH values in natural environments, pH was set at 6.5, 7.0, 7.5 and 8.0 and buffered with tris buffer.

No significant difference was shown for naphthalene removal by the biofilms at different pH values when no cadmium was added. However, when the bulk cadmium concentration was 10 mg/L, naphthalene removal and cadmium sorption were significantly different at the selected pH levels (Figures 1 and 2). As pH increased, cadmium sorption

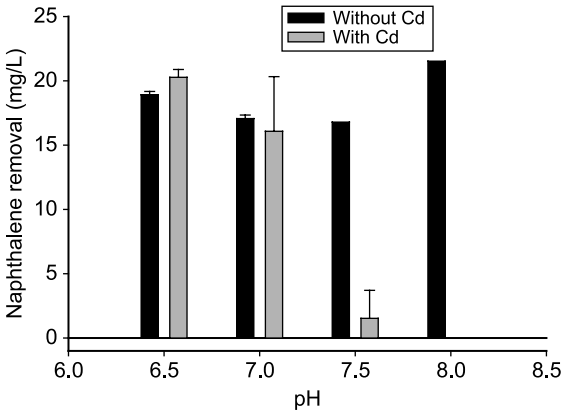

Figure 1 Naphthalene removal with or without cadmium at different pH values

increased. This may be a result of less competitive sorption of hydrogen ions at higher pH values or of changes in speciation of the cadmium. Consequently, both naphthalene removal and the amount of biofilm mass (data not shown) were reduced due to the increased cadmium concentration in the biomass and the resulting increased cadmium toxicity. At pH 6.5 and 7.0, most of the naphthalene (initially around 15–20 mg/L) in the bulk liquid was removed, while at pH 7.5 and 8.0 almost no removal occurred at all. Mergeay *et al.* (1985) showed that at pH 7.0, the cadmium minimum inhibitory concentration (MIC) for *Escherichia coli* was about 50 mg/L, while in this study, a 10 mg/L bulk concentration was enough to stop biofilm from functioning. This may be because the Cd concentration in the biofilm in contact with the microorganisms was much higher at pH 7.5 than at pH 7.0 due to less adsorption competition between Cd ions and hydrogen ions.

The toxicity of cadmium to the biofilms can be demonstrated quantitatively by incorporating the biofilm function with the cadmium sorption. As the cadmium sorption increased, the biofilm behavior toward naphthalene removal can be divided into three phases (Figure 3). When the cadmium sorption was less than 5 μgCd/mg VS, no negative impact was found. When the cadmium sorption was more than 10 μgCd/mg VS, there was no further reduction in naphthalene removal as the cadmium sorption increased. When the cadmium sorption was between 5 and 10 μgCd/mg VS, the naphthalene removal decreased proportionally as the cadmium sorption increased. Cadmium sorption

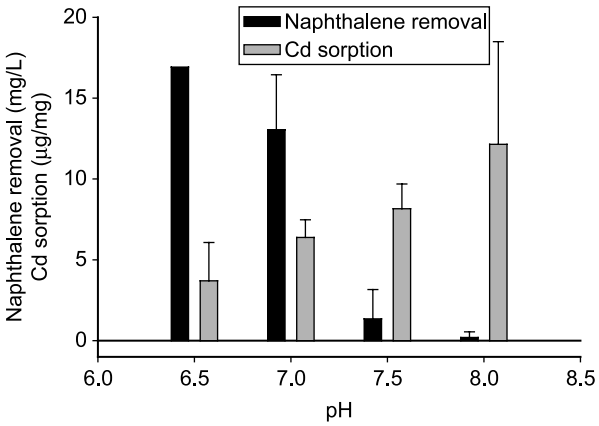

Figure 2 Naphthalene removal and cadmium sorption at different pH values

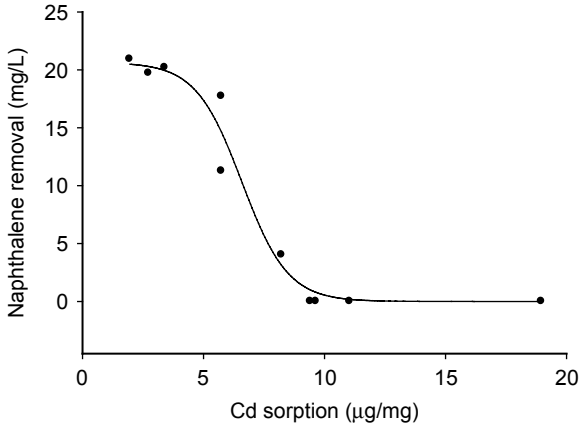

Figure 3 Naphthalene removal and cadmium sorption relationship

of 5 μgCd/mg VS was defined as the minimum inhibition capacity, and the range 5–10 μgCd/mg VS was defined as the cadmium effective inhibition range.

Alternative substrate

Because of the low solubility of naphthalene, acetate was added in some experiments as an alternative substrate in order to modify biofilm structures, to improve the performance of biofilm for naphthalene removal and to reduce the cadmium toxicity. The biomass produced by acetate-utilizing species appeared to have a high affinity for PAH (similar to biomass produced by PAH-utilizing species) and would improve the capture of PAHs in the subsurface environment. However, acetate neither improved the naphthalene removal nor reduced cadmium toxicity. In Figure 4, it is shown that compared to about 4 mg/L/mg naphthalene removal when no acetate was added, no naphthalene removal was found when 4 mg/L acetate was added. Total oxygen consumption also decreased, which means that the phenomenon of no naphthalene removal was not a result of insufficient oxygen. It may take some time before the microbes switch from acetate degrading to naphthalene degrading, and the experimental period was not long enough to recognize it. Ebihara (1999) and Ebihara and Bishop (2002) also showed that the addition of acetate as an alternative substrate in batch experiments did not increase the PAH degradation rate,

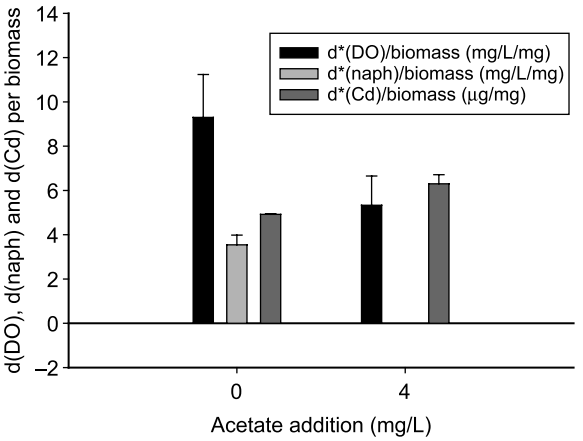

Figure 4 Impact of acetate addition on cadmium sorption and naphthalene removal (d* signifies differences between initial and final concentrations)

although it did decrease the time needed to reach equilibrium. Substrate competition may be an explanation for the unexpected results.

Hydrogen peroxide supplement

Oxygen is critical to the growth and function of PAH degraders. However, the dissolved oxygen concentration in solution without any additional sources may not be enough to maintain aerobic conditions. Hydrogen peroxide was added in some experiments to supply needed dissolved oxygen. The H_2O_2 concentration in bulk solution was about 0.0043%, which will not cause toxicity to microorganisms. However, in Figure 5, it is shown that with the addition of hydrogen peroxide, the cadmium toxicity increased. Nies (1999) showed that the cadmium uptake and efflux cycle is energy dependent. Addition of hydrogen peroxide increased the dissolved oxygen concentration and increased the growth rate of cells. More energy was thus produced and this accelerated the uptake of cadmium by the cells, enhanced the cadmium transport rate in the biofilms, and as a result, increased the cadmium toxicity to the bacterial cells in the biofilm. Hydrogen peroxide and Cd concentrations in the biofilms will be detected with microelectrodes in future work.

Mass transfer and transport

The bulk profiles of naphthalene, cadmium and dissolved oxygen (no H_2O_2 added) were investigated with respect to time. In Figures 6 and 7, naphthalene removal or dissolved oxygen consumption profiles matched very well, and naphthalene removal stopped when no dissolved oxygen remained. Naphthalene was probably not completely mineralized, because less oxygen was consumed than the theoretical requirement. No significant naphthalene removal and dissolved oxygen consumption were found when the biofilms were inhibited by sodium azide, which indicated that naphthalene removal is a degradation dependent process and sorption is not important. It is also shown in Figures 6 and 7 that naphthalene removal was retarded for about 12 hours by the presence of cadmium and the removal rate was reduced from 0.75 to 0.43 mg/L/h. Figure 8 demonstrates that the sorption of cadmium into the biofilm was much faster than naphthalene removal. Sorption of cadmium happened very quickly in the first hour, at a rate of about 7 μg/mg/h, and most cadmium was sorbed in the first two hours. The profiles for loosely and tightly attached cadmium were similar.

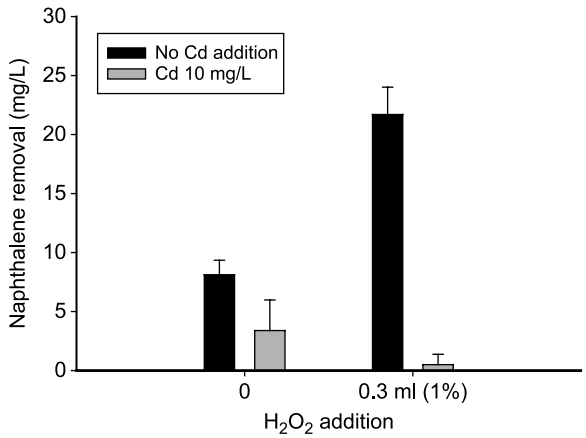

Figure 5 Impact of H_2O_2 and cadmium addition on naphthalene removal

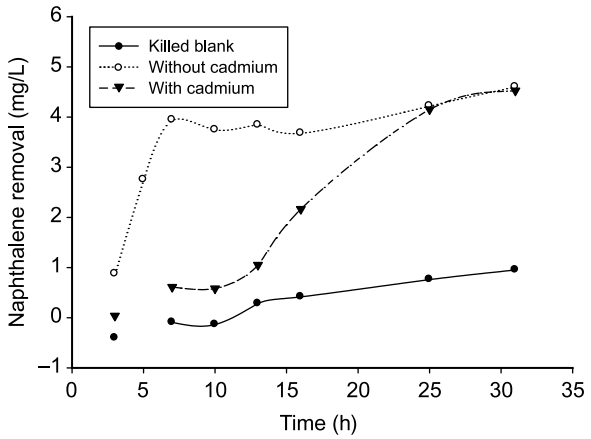

Figure 6 Naphthalene removal with time

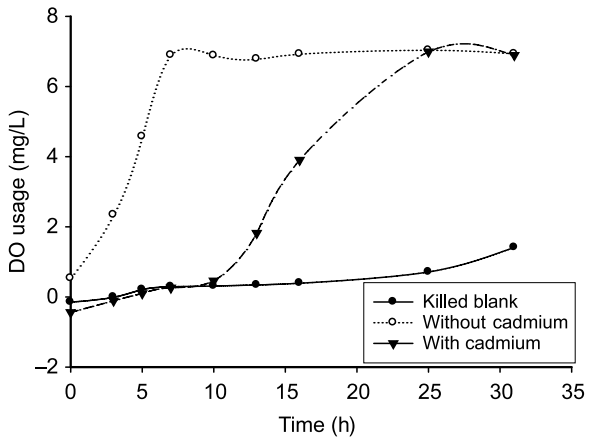

Figure 7 Dissolved oxygen consumption with time

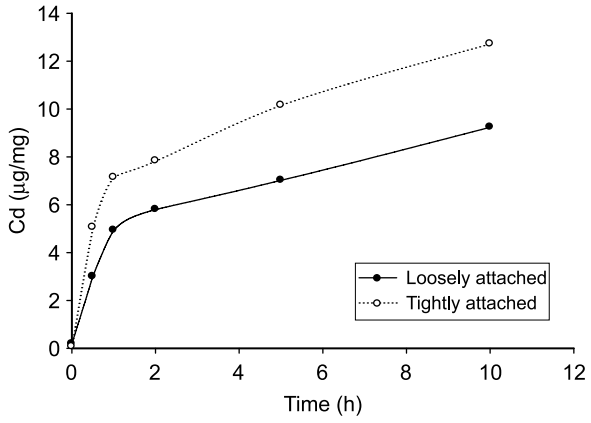

Figure 8 Cadmium sorption with time

Conclusions

The presence of heavy metals may be a crucial factor influencing the application of biofilm processes for bioremediation of contaminated soils and ground water. Environmental conditions will also have a significant impact on bioremediation. As the pH value increased, cadmium sorption in the biofilm increased and naphthalene removal decreased. Addition of 10 mg/L of cadmium produced a significant impact on biofilm when the pH was above 7.5. The minimum inhibition capacity for Cd was about 5 μgCd/mg VS, and the range 5–10 μgCd/mgVS was defined as the cadmium effective inhibition range. Acetate, added as an alternative substrate, competed with naphthalene and did not reduce the cadmium toxicity. Hydrogen peroxide, added as supplement for dissolved oxygen, improved not only the cell growth, but also the cadmium uptake/efflux cycle, which made the biofilm more vulnerable to cadmium attack. Cadmium was shown to transfer faster than naphthalene into biofilms, retard naphthalene removal and reduce the removal rate. In the future, studies on the micro-environmental profiles in the biofilm matrix need to be conducted to help explain the mechanisms of cadmium mass transfer and distribution in biofilm processes.

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