EFFECT OF ANAEROBIC SLUDGE SOURCE AND CONDITION ON BIOSORPTION OF PCP

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Abstract—Biosorption isotherms for pentachlorophenol (PCP), on granular and dispersed anaerobic sludge from five different industrial sources fit well to the Freundlich equation. No significant difference was observed in biosorption capacity between dispersed and granular anaerobic sludge. Some variation in biosorption capacity was observed among the different industrial sources of sludge. Comparison with results reported by other investigators suggested that biosorption by anaerobic biomass is lower than that by aerobic microorganisms.

Key words—biosorption, pentachlorophenol, anaerobic, granular, Freundlich

INTRODUCTION
Pulp bleaching and wood preservation processes produce a variety of chlorinated organic aromatics that are known to resist biodegradation in conventional biological treatment systems. Studies have shown that these chlorinated aromatics are recalcitrant (Leuenberger et al., 1985) and tend to bioaccumulate in the aquatic environment (Craig et al., 1990). These environmental problems are of increasing public concern. To address these concerns, government regulations are lowering the maximum allowable levels of adsorbable organic halogen (AOX) in pulp mill effluents. Process modifications to reduce AOX formation are available; however, total elimination of chlorine is often more expensive than waste water treatment options (Randle et al., 1991).

Anaerobic processes are reported to be better suited for the dechlorination of polychlorinated aromatic compounds than aerobic processes. Concomitantly, Upflow Anaerobic Sludge Bed (UASB) reactors, which have gained acceptance for treatment of various industrial waste waters, are now being applied to the treatment of chlorinated aromatics. Anaerobic sludge in the form of granules are employed in these reactors. The granules contain a consortia of anaerobic microorganisms that are organized to give micropores and microchannels, as seen under the electron microscope (MacLeod et al., 1990; Wiegant and De Man, 1986).

While the actual mechanism of dechlorination is unknown, biosorption of AOX onto anaerobic biomass is an essential step in the dechlorination process. Biosorption of organic compounds have been correlated to the lipid contents of biosorbents used (Grimes and Morrison, 1975; Canton et al., 1977). It was demonstrated that high biosorption capacities correspond to biosorbents having high lipid contents. The type of membrane lipids present has also been reported to be an important factor (Kennedy et al., 1992).

Many investigators have successfully used the Freundlich equation to construct biosorption isotherms for various hazardous organic pollutants, including PCP. Bell and Tsezos (1987) determined the Freundlich biosorption isotherms of several organic pollutants by inactive (dead) aerobic activated sludge and Rhizopus arrhizus, at 5° and 20°C. These authors also compared biosorption by live and dead biomass (Tsezos and Bell, 1989). They reported that PCP uptake by dead R. arrhizus was higher than that by live R. arrhizus, while the opposite was observed for activated sludge. Higher sorption by dead biomass was suggested to be influence by such factors as the absence of metabolic protection against cellular transport of pollutants, increased permeability of dead cell membrane, and the change in the surface adsorptive properties of the cells following death. Biodegradation of the PCP was believed to be responsible for the higher biosorption level exerted by live activated sludge. The authors explained that R. arrhizus, being a pure and unacclimated culture, could not metabolize PCP readily; whereas, activated sludge had been reported to be capable of degrading PCP.

Biosorption of PCP, along with other chlorophenols, by granular anaerobic sludge from a laboratory UASB reactor was studied in detail by Kennedy et al. (1992). The granular sludge was taken from the UASB reactor which was used to treat sucrose and acetic acid based waste water at 35°C, and was not acclimated to chlorophenols. Biosorption (at 35°C) of
PCP was reported to be lower than those reported by Tsezos and Bell (1989). The lower level of PCP uptake by anaerobic biomass was believed to be influenced by the higher test temperature and the difference in the type of membrane lipids present in aerobic and anaerobic biomass. Increased temperatures have often been demonstrated to reduce sorption capacities of biomass. According to Balch et al. (1979) aerobic sludge, mainly eubacteria, possess membrane lipids consisting of fatty acids that are esterified to glycerol. The membrane of anaerobic sludge, mainly archaeobacteria (methanogens), consists of squalene and ether linked polar lipids.

Kennedy et al. (1992) used only one type of granular anaerobic sludge which had been exposed to waste water in a laboratory setting while Bell and Tsezos (1987) and Tsezos and Bell (1989) used inactive and active aerobes to study biosorption of a variety of hazardous organic pollutants. Application of aerobic biosorption isotherms to anaerobic systems cannot be done with certainty. Although the former authors studied biosorption by anaerobes, laboratory conditions are highly controlled; thus, the results may not completely reflect conditions in actual anaerobic treatment plants. In this study, PCP biosorption isotherms for anaerobic sludge from actual waste treatment plants were determined. Comparison of the results with those of the mentioned authors will give further insights into the complex biosorption process. The effects of granule dispersion into individual anaerobic microorganisms and concomitant increased microbe/water surface area on microbial PCP accumulation can indicate the relative importance and relevance of the spatial relationships present in anaerobic microbial granules.

EXPERIMENTAL

Materials and equipment
Chemicals used in the experiments were purchased from Aldrich Chemicals and were of +99% purity. Aqueous solutions were made with distilled/deionized water prepared in the laboratory. Organic solvents used were HPLC grade. Hewlett Packard 1090 HPLC equipment with a diode array detector and an HP-300 work station for peak area integration was employed for PCP analysis. Granular anaerobic sludge were obtained from five different industrial sources in the period of January 1992~January 1993:

1. Anaheim Citrus Products (Citrus sludge);
2. Micro-Crystalline Cellulose Production (Cellulose sludge);
3. Clos du Bois Winery (Winery 1 sludge and Winery 2 sludge);
4. Pigment Manufacturing (Pigment 1 sludge and Pigment 2 sludge); and
5. G. Heileman Brewery (Brewery sludge).

Volatile Suspended Solids (VSS) determination
Duplicate samples were done according to Standard Methods (APHA, 1980).

Dispersion of granular sludge
Dispersion was carried out under nitrogen atmosphere. Granular sludge was diluted with three parts (three volumes) of 0.01 N NaOH, pH 7.5 and the pH of the mixture was adjusted to 7.5. The mixture was then placed in a Waring blender and blended at low speed for 2 min. Final biomass concentration was 15-20 g VSS/l.

Biosorption experiment
Biosorption tests were done in duplicates under nitrogen and at room temperature (22 ± 1°C). Granular sludge was diluted with 0.01 N NaOH, pH 7.5, in the same manner as that used for dispersed sludge, to give a biomass concentration of 15–20 g VSS/l. Sample bottles were prepared by placing 15 ml aliquots of the diluted sludge (granular or dispersed) into 60 ml serum bottles. The bottles were sparged with nitrogen and then capped. Sample solutions of PCP, pH 7.5 were injected into sample and control bottles to give final PCP concentrations in the range of 5–120 mg/l. Sample bottles, controls, and blanks were then placed on a shaker set at 150 rpm for 3 h. One milliliter samples from each bottle were centrifuged in a microcentrifuge for 5 min. The supernatant was filtered through a Millipore 0.22 µm Millex-GV<sub>k</sub> filter and stored in glass tubes at 4°C, pending HPLC analysis. HPLC analysis was carried out as described by Kennedy et al. (1992).

Granule size
Approximately 20 ml of anaerobic granules and granules that had been dispersed were placed in a petri dish. Using a dissecting microscope with a scaled eyepiece, the size and number of particles in each sample were determined.

Treatment of data
Since biosorption was done on a heterogeneous surface, the isotherms were determined by the Freundlich equation (Young and Crowell, 1962). Equilibrium concentrations of PCP in the liquid and solid (anaerobic granular biomass) phases were plotted on log-log scales to test their fit to the Freundlich equation. Linear least square analysis was run on the equilibrium data to determine the Freundlich constants. The constants, K and 1/n were determined by using the true dependent variable, \(c_{eq}\), and taking the chlorophenol associated with the biomass as the independent variable.

\[ q = K \times \frac{1}{c_{eq}} \]

where \(q\) = sorbate concentration associated with biomass, \(\mu g/g\) VSS; \(K = \) Freundlich constant, \(1/g\ VSS\); \(c_{eq} = \) equilibrium concentration of sorbate in solution, \(\mu g/l\); \(1/n = \) exponent constant

Values of \(1/n\) close to 1 indicate a linear sorption isotherm and suggests a constant partitioning sorption mechanism. This type of isotherm can occur when the sorbate readily penetrate into the sorbent (in this case the microbial granule). The availability of sorption sites remains constant at all concentrations up to saturation, typical of the partitioning of a solute between two immiscible solvents. Linear sorption isotherms commonly occur when relatively pure, porous sorbents are used and the sorption is carried out over a relatively small concentration range. In this study the sorbent concentration range was narrow, and anaerobic granules are known to be porous (Wiegant and De Man, 1986; MacLeod et al., 1990) allowing penetration to sorption sites in the inner portion of the granule.

RESULTS AND DISCUSSION

Granule dispersion
Granular sludge was mechanically dispersed in a blender in order to increase cell surface area. Figure 1 shows the typical appearance of granular and dispersed sludge used in this study. Granular sludge contained granules up to 2.5 mm diameter while
dispersed sludge consisted mostly of particles less than 0.2 mm in diameter. Care was taken to ensure that the dispersed sludge used in biosorption tests did not consist of granules larger than 0.25 mm in diameter.

An average size distribution for typical granular and dispersed samples is shown in Table 1. The microbial granules were assumed spherical in order to estimate the surface area of each sample. Using a basis of 100 mm$^3$ total cell volume, it was estimated that there was a 9-fold increase in surface area resulting from cell dispersion (an increase from 678 mm$^2$ to 5820 mm$^2$ per 100 mm$^3$ total cell volume).

**Effects of contact time on biosorption**

The biosorption process has been reported to be very rapid, where equilibrium is reached in less than 24 h (Weber *et al.*, 1987; Bell and Tsezos, 1987; Lu, 1992). Since the types of sludge used in this study were different from those used by the above mentioned investigators, it was necessary to verify that their observation is still valid and applicable. Biosorption capacity of dispersed Cellulose and Citrus sludge at two different sampling times (3 and 24 h) were measured and compared. As indicated by Fig. 2, there is essentially no difference in biosorption capacity at 3 and 24 h for both types of sludge; thus, it can be concluded that equilibrium is reached after 3 h.

From the results presented in Fig. 2, as well as results reported by others (Weber *et al.*, 1987; Bell...
Effect of anaerobic sludge on PCP biosorption

and Tsezos, 1987; Lu, 1992), it was assumed that biosorption by sludge (both granular and dispersed) from the other industrial sources would also reach equilibrium quickly. Therefore, a contact time of 3 h was considered adequate for biosorption to reach equilibrium; subsequent biosorption experiments were performed using a contact time of 3 h. It is interesting to note that most UASB reactors operate at hydraulic retention times (HRT) of 8–36 h (Lu, 1992), indicating that sufficient time is available for biosorption equilibration.

Another important factor that must be considered in biosorption studies is the possibility of biodegradation of the sorbate. While none of the sludge was acclimated to PCP, there still exists this possibility of biodegradation. However, reductively dechlorinated intermediates of PCP (tetra, tri, di and mono chlorophenols; Lu, 1992) were not detected during the course of the sorption tests. Thus, biosorption was considered to be solely responsible for the difference in PCP concentration of the controls and the samples.

Biosorption isotherms

Biosorption isotherms were determined based on the Freundlich equation. The experimental results for both granular and dispersed sludge fit well to this equation. Figure 3, 4 and 5 show granular biosorption data and corresponding Freundlich equation isotherms, while Figs 6 and 7 show those for dispersed sludge.

The grouping of biosorption data as shown in the 5 figures was based on regression results obtained for each type of sludge. Data sets giving relatively similar slopes and intercepts (on a log–log scale) were grouped together to represent a single category. Granular sludge were grouped into three main categories based solely on similarities of biosorption:

1. Pigment sludge consisting of Pigment 1 and Pigment 2 sludge (Fig. 3),
2. Wine sludge consisting of Winery 1 and Winery 2, and Brewery sludge (Fig. 4),
3. Plant sludge consisting of Cellulose and Citrus sludge (Fig. 5).
Dispersed sludge were grouped into two main categories:

1. Wine sludge consisting of Pigment 1 and Pigment 2, Winery 1, Winery 2, and Brewery sludge (Fig. 6).
2. Plant sludge consisting of Cellulose and Citrus sludge (Fig. 7).

Figures 3–7 can be used to estimate biosorption capacities for known equilibrium concentration of aqueous PCP. However, it is often desirable to represent these results by a mathematical relationship. Based on the general distribution of experimental data relative to the Freundlich sorption isotherms (Figs 3–7), it can be concluded that the Freundlich sorption model adequately describes biosorption of PCP by anaerobic sludge. The best fit Freundlich parameters for the different types of anaerobic sludge studied are presented in Table 2.

Inspection of Table 2 indicates that biosorption capacities vary among types (sources) rather than forms (granular vs dispersed) of biomass. To see this behavior more clearly, biosorption capacity at an equilibrium concentration of 1000 μg/l PCP in the aqueous phase was determined, using the Freundlich parameters shown. The results obtained are presented in Table 3. From these results, it is much easier to quantitatively compare the sorption capacity of the various types of sludge.

Considering errors in analytical analysis associated with the biosorption capacities presented in Table 3, it can be concluded that there was essentially no difference in PCP uptake by granular and dispersed sludge, although the surface area of dispersed sludge was estimated to be nine times higher than that of granular sludge (described previously). It is expected that dispersed biomass would accumulate more PCP than granular sludge. This was not observed in this study. This behavior may be due to the underestimation of granular biomass surface area.

Granular anaerobic sludge has been shown to possess a large number of micropores and microchannels (MacLeod et al., 1990; Wiegant and De Man, 1986). As a result, the actual surface area available for biosorption of these microbial granules may be larger than that of spheres having the same diameter (similar to activated carbon). Our results suggest that the actual surface area of granular sludge may be very close to that of dispersed sludge, resulting in very similar sorption capacities.

Another factor which may have an effect on the biosorption capacity is the specific interactions...
Fig. 8. Comparison of biosorption by live anaerobic granular sludge from this study (Pigment, Wine, and Plant; Freundlich parameters from Table 2) to that by live granular anaerobic sludge from laboratory UASB reactor (Kennedy et al., 1992) and by live R. arrhizus and aerobic Activated Sludge (Tsezos and Bell, 1989).

among the various anaerobic microbes within the microbial granules. The specific arrangement of the various species of anaerobes in these granules may offer a mechanism whereby pollutants are cooperatively and actively transported into the cells; thus, PCP uptake can be higher than expected. This, however, does not seem to be the case. From inspection of Table 2, it is noted that the value of $1/n$ is between 0.5 and 0.75 for the two types of sludge studied. The value of $1/n$ has been shown to be indicative of the sorption mechanism (Lu, 1992). Values of $1/n$ close to one is indicative of a constant partitioning sorption mechanism, where sorbate can easily penetrate into the sorbent. Since there was no significant change in the value of $1/n$ for granular and dispersed sludge, it can be concluded that the sorption mechanism for the two sludge classes are similar.

Thus, the spatial configuration of microbial consortia in anaerobic microbial granules did not seem to play an important role in the possible active transport aspect of the PCP sorption process.

The variation in biosorption capacity for sludge from different sources is not based on the sorption mechanism. According to Table 2, the values of $1/n$ were relatively similar. It can be assumed that the mechanism of sorption of one type of granule is the same as that of another granule. However, biosorption has been shown to be influenced by the lipid content of the biosorpent (Grimes and Morrison, 1975; Canton et al., 1977) which may vary for one type of sludge to another, based on waste acclimation. The type of membrane lipids present has also been shown to affect biosorption (Kennedy et al., 1992). The unique lipids associated with strict anaerobic methanogens (Sprout et al., 1983) could affect biosorption. This suggests that while the mechanism of sorption may be the same, the microbial composition of the various types of sludge may be a contributing factor for the observed difference in sorption capacity.

PCP biosorption isotherms determined in this study were compared with those of Kennedy et al. (1992) and Tsezos and Bell (1989) (Figs 8 and 9). Kennedy et al. (1992) reported on PCP sorption by live anaerobic granular sludge from a laboratory UASB reactor (35°C); Tsezos and Bell (1989) reported on PCP sorption by live fungi (R. arrhizus) and aerobic activated sludge (20°C). As presented in Figs 8 and 9, sorption isotherms from this study (room temperature) fall between those for aerobes and UASB laboratory sludge. Similarity between Figs 8 and 9 was expected since there was no significant difference in sorption capacity of granular and dispersed anaerobic sludge studied.

Biosorption capacity of PCP for granular anaerobic sludge developed in a laboratory UASB reactor (Kennedy et al., 1992) was lower than that for industrial granular anaerobic sludge developed in pilot or demonstration units that were used in this study. For an equilibrium liquid phase PCP concentration of 1000μg/l, biosorption onto laboratory USAB biomass (270μg/g VSS) is about two to three fold less than for anaerobic sludge used in this study (405-709μg/g VSS). This difference in biosorption capacity may be related to the difference in temperature that the different biosorption studies were conducted at. Higher temperatures have been shown to decrease sorption capacities of biomass. Also, there may be differences in the microbial composition of the various anaerobic sludge. Pilot and full-scale UASB reactors tend to develop denser granules than those that develop in laboratory units. It is unknown if this is the result of a hydraulic phenomena, the proliferation of different anaerobic consortia in full-scale vs laboratory units or a combination of both.

A preliminary comparison of the results obtained with those reported by Tsezos and Bell (1989) and
Kennedy et al. (1992) suggests that biosorption capacity for aerobes is higher than for anaerobes. Although a comparison of straight results from different laboratories is difficult, the large difference in the sorption capacities should be of importance. Based on an equilibrium aqueous PCP concentration of 1000 µg/l, biosorption onto live granular anaerobic sludge in this study (405–709 µg/g VSS) is about two to four times less than for the fungi R. arrhizus (1536 µg/g VSS) and about 8–13 times less than for aerobic activated sludge (5369 µg/g VSS). Although Grimes and Morrison (1975) and Canton et al. (1977) reported that biosorption of organic compounds are influenced by the lipid content of microbial cells, this factor should not be of major importance here since the total lipid content of aerobes is very similar to that of anaerobes (Sprott et al., 1983). The types of membrane lipids present in anaerobes (squalene and ether linked polar lipids) are quite different from those present in aerobic microbes (fatty acids esterified to glycerol). This may have some effects of the surface adsorptive properties of the anaerobic microbial membrane, leading to the observed results.

CONCLUSIONS

Biosorption data fit well to the Freundlich sorption model. Sorption of PCP by anaerobic biomass was rapid; equilibrium was reached in 3 h (might have been reached at a shorter time but this was not tested). Reductive biodegradation of PCP as monitored by HPLC analysis was not detected during the course of the biosorption tests. There was very little difference between the biosorption capacity of granular and dispersed sludge studied. It was estimated (based on the assumption that granules are spheres) that the method of dispersion used resulted in a nine-fold increase in surface area for the biomass (granules vs free cells). However, anaerobic microbial granules may have higher surface area than estimated due to the presence of micropores and microchannels in the granules. Based on the similarity in the values of 1/n, both forms of sludge (granular and dispersed) employed a similar mechanism for PCP sorption. The exact mechanism or mechanisms remains unknown.

Some variation in PCP biosorption capacity was observed among the different sources of industrial anaerobic granular sludge. Since the 1/n values are similar, the difference in microbial composition of the various sludge may be a contributing factor for this difference in biosorption capacity.

Comparison of the results obtained with those reported by other investigators suggests that PCP sorption by anaerobic sludge is less than that by aerobic biomass. Since total lipid content of anaerobes is similar to that of aerobes, the difference in biosorption capacity may be linked to differences in the types of membrane lipids present. Comparison with biosorption by granular anaerobic sludge done at a higher temperature (Kennedy et al., 1992) indicates that biosorption capacity decreases with increasing temperature.

The Freundlich parameters obtained in this study can be used to determine the PCP sorption capacity for anaerobic granular sludge at various equilibrium concentrations of PCP in the liquid phase. This information can be used in computer programs such as TOXCHEM Version 1.10 (EnvirOmega Ltd, Hamilton, Ontario, Canada) for predicting the fate of PCP in wastewater; they can also be used in optimization calculations.

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REFERENCES


