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Biodegradation of Polychlorinated Biphenyls Using Acclimatized Biofilm in a Three-Phase Fluidized Bed Aerobic Reactor

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This paper investigates the ability of microorganisms in a biofilm to degrade polychlorinated biphenyls (PCBs) using a three-phase fluidized bed aerobic biofilm reactor. Water was spiked with PCBs that contain mainly Aroclor 1260 to simulate PCB-contaminated water. The "contaminated" water was batch fed into the reactor to acclimatize the microorganisms in the biofilm. The degradation of PCBs was monitored through the decrease in concentration of Aroclor 1260. Samples were analyzed using gas chromatography equipped with an electron capture detector (ECD). Batch experimental runs with an initial concentration of around 70 ppm showed PCB degradation of up to 93% after 8 h. After the runs that used "contaminated" water, the batch runs using feed that contain easily degradable organic chemicals were performed to determine the effect of contact with PCB on the biofilm. Their latter runs showed that the COD degradation rate had no significant difference with the COD runs before the PCB batch runs. These results showed that biofilm formed in a three-phase fluidized bed Aerobic is capable of degrading PCB in water and that the microorganisms are not significantly affected by exposure to PCB.

Keywords: Acclimatized biofilm, biodegration, (bio)chemical oxygen demand (BOD/COD) reduction, polychlorinated biphenyls (PCBs), *and* three-phase fluidized aerobic bed bioreactor.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are aromatic synthetic chemicals produced in the United States in 1929 by Monsanto Corporation to answer the need for a stable, nonflammable transformer dielectric cooling oil. PCBs have since been marketed worldwide under the trade names Aroclor (US), Fenchlor (Italy), Kaneclor (Japan), Pyralene (France), and Clophen (West Germany) (Erickson 1997). The use of PCBs extend to hydraulic fluids, plasticizers, and solvent extenders, among others. When accidentally released into the environment, however, PCBs can cause a lot of environmental damage due to their chemical stability, lipophilic nature, and resistance to microbial degradation. PCBs bioaccumulate in the fatty tissues of animals and are probable carcinogens to humans. Although the manufacture and use of PCBs have been banned since the 1970s in the United States the Toxic Substances Control Act (ATSDR 1997), a number of PCB-contaminated sites still exist in various parts of the world. Today, under the Chemical Treaty signed at the Stockholm Convention, PCBs have been listed as one of the 12 persistent organic pollutants (POPs) banned worldwide.

Figure 1. Photo of the Reactor System

Several studies worldwide have been focused on the elimination of PCBs. Both chemical and biological methods were explored and demonstrated. The biological method, particularly the biofilm systems, offers a challenge on the degradation of PCBs. Biofilm systems have been used extensively to degrade various types of toxic wastes and the results were successful (Tartakovsky et al. 2001. Villaverde et al. 2000, Gupta and Gupta 1999, Zhang et al. 1998, Hirata et al. 1997, Sanderson and Stewart 1997, Okabe S. et al. 1996, Edgehill 1996, Fathepure and Vogel 1991). Biofilm systems are more resilient to the toxic effects of hazardous wastes than free-floating microorganisms because the microbial colonies inside the biofilm structure are sheltered by extracellular polymeric substances (EPS), which limit the contact between microbes and toxic substances. Moreover, since biofilms contain multispecies colonies of microorganisms, the combined effects of the consortium of microorganisms causes the degradation of very biologically resistive chemicals.

This study aims to investigate the ability of a completely mixed three-phase fluidized bed aerobic biofilm reactor to degrade PCBs. The mixed culture of microbes in the biofilm was acclimatized by exposing the biofilm to PCBs in batch experimental runs.

MATERIALS AND METHODS

The reactor system

The reactor was fabricated from acrylic resin boards. It has an effective volume of 3L. The particulate media used were cement balls (CB) made from coal ash where the biofilm was formed. The CB particles have an average diameter of 235 μm and a density of 1.92 g/cm^3. The reaction temperature was maintained at ambient temperature (25–30 °C). Air was supplied at a rate suitable to fully disperse the bioparticles and to provide excess dissolved oxygen. The substrate used was a complex medium of two types: *simulated wastewater* and *simulated PCB-contaminated water.* The schematic diagram of the reactor is shown in Figure 1.

Simulated wastewater composition

The medium composition was adapted from Auresenia (2002). The medium was composed of the following: 0.402 g/L yeast extract, 0.187 g/L dextrin, 0.402 g/L bacto-peptone, 0.066 g/L NaCl, 0.084 g/L $MgSO_4 \cdot 7H_2O$, 0.186 g/L KH_2PO_4, 0.134 g/L KCl, 0.817 g/L NH_4Cl, and 1.914 g/L $NaHCO_3$.

This *standard feed composition* contained dissolved biochemical oxygen demand (BOD_5) equal to approximately 400 mg/L; chemical oxygen demand (COD) of around 1,000 mg/L; total nitrogen (T-N) of 450 mg/L; and ammonium nitrogen NH_4-N of 250 mg/L, which simulate domestic wastewater.

Simulated PCB-contaminated water preparation

The PCBs were taken from an old transformer in Clark Special Economic Zone (CSEZ), formerly known as the Clark Airbase, in Clarkfield, Pampanga. The oil was analyzed to contain predominantly Aroclor 1260 with a concentration of 720,000 ppm. of this oil, 0.1 g was dissolved in 10-ml methanol. A millimiter of this solution was added to each liter of distilled water, and then left in the fume hood overnight to allow methanol to evaporate. The simulated wastewater additives (without the organics dextrin, bacto-peptone, and

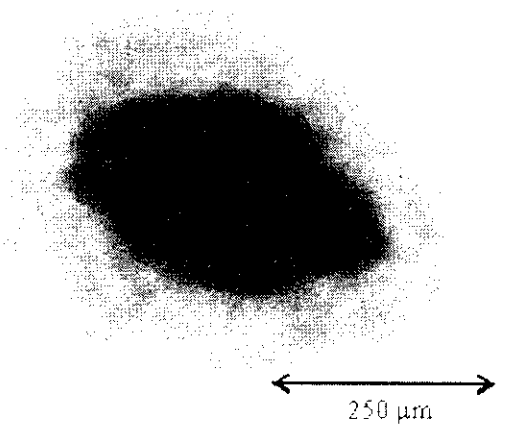

Figure 2. The Bioparticle Used (CB Center and Biofilm Outer Rim)

yeast extract) were added and stirred using a magnetic stirrer to allow complete mixing of the solution.

Biofilm used

The biofilm used in this study was obtained by feeding the completely mixed three-phase fluidized bed reactor, which contain CBs made from coal ash with modified kitchen waste as the substrate, continuously for 6 months (Auresenia et al. 2002). After which, it was switched to the simulated wastewater continuously for another year. The inoculum used was obtained from *leather tanning wastes*, which contain microorganisms of the wild type that are resilient both to toxic chemicals and heavy metals characteristic of tannery waste products. The biofilm has an average thickness of 100 mm (see Figure 2).

Experimental plan

The biofilm was acclimatized with PCBs using batch tests only to avoid PCB-contaminated water buildup. Batch experimental runs on both simulated PCB-contaminated water and simulated wastewater were conducted alternately twice a week. Figure 3 illustrates the experimental plan for these tests. PCB test is done on day 1 from 8:00 AM to 4:00 PM. Samples were taken at 2-h intervals. The samples were either immediately analyzed or stored in a refrigerator until further analysis. After the PCB test, the reactor was then switched to continuous feeding. The COD test was performed the following day. Likewise, sampling was done every two hours but the samples were immediately analyzed. The COD tests were performed after the PCB batch runs to check if the biofilm was affected or not. After each of the batch runs, the reactor was switched to CSTR in order for the microorganisms to recover. The bioparticles were carefully washed each time before the start of the experiment to remove the sludge.

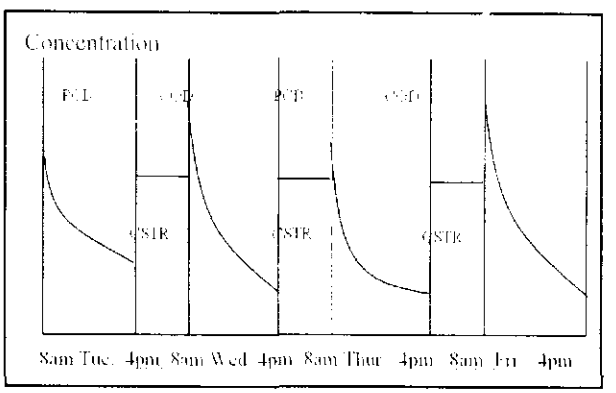

Figure 3. The Experimental Plan

Analytical Methods

For water quality, COD was measured according to APHA standards (1992). The average biofilm thickness was measured by the

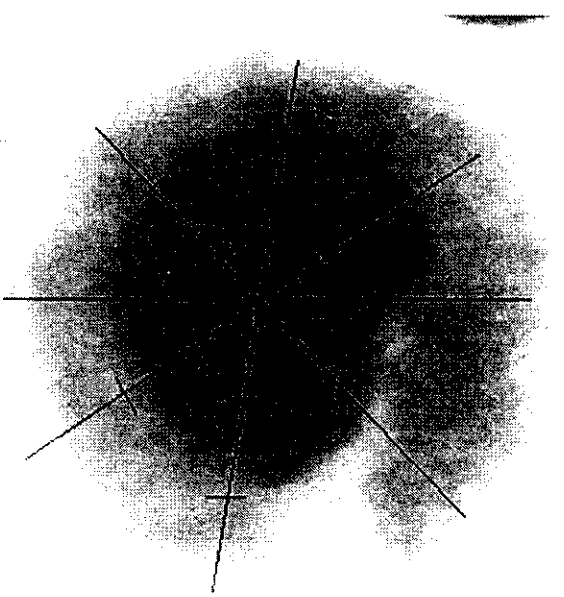

Figure 4. Electron Micrograph of Biofilm Diameter Computation

images taken from an Olympus BX51 microscope. Line were drawn across the center of the bioparticle. The average length of the darker portion of the image represents the particle diameter, while the average length from one periphery to the other represents the bioparticle diameter. Since the average particle diameter was known to be 235 μ from the sieve analysis, the average diameter of particle with an attached biofilm can be computed by ratio and proportion. The difference of the diameters shows the average biofilm thickness (see Figure 4). The biofilm was also examined using a scanning electron microscope (SEM). The bioparticles were dehydrated by aqueous acetone solution and then sputter-coated with gold using a JEOL 1200 Fine Coater and viewed in a JEOL 5310 SEM.

PCB was analyzed using the solid-phase extraction (SPE) method. A 10mL sample was taken from the reactor and treated with methanol. The sample was allowed to pass through the C-18 cartridge. The cartridge was then dried for 20 min. under 15-mm Hg vacuum. PCB was then eluted using 1 ml of 80:20 mixture of hexane and ethyl acetate. A 100 ml of the extracts were taken and diluted to 1 ml using hexane. The extracts were then analyzed in a Shimadzu GC–14B equipped with an electron capture detector (ECD). The extracts were injected into a SPB™-5 fused silica capillary column (30 m x 0.32 mm ID x 0.25 μm film thickness). The column oven temperature programming was 180°C then increased to 260°C at 4°C/min, then 10°C/min to 280°C, and maintained at 280°C for 12 min giving a total running time of 34 min. The injector temperature was 300°C, the detector temperature was 300°C, and the carrier gas was ultrahigh purity nitrogen. The chromatogram of Aroclor 1260 was used as the basis for the computation (Estrellan 2003).

RESULTS AND DISCUSSION

The biofilm was developed by Auresenia et al. (2002) using modified kitchen waste for 6 months using an inoculum from tannery waste. After which, the biofilm was then switched to the simulated wastewater medium. Early attempts on the acclimatization of this biofilm were conducted for 6 months; however, the results were never taken into account because the GC at that time gave unstable results. A paper presented by Taleon et al. in the ARRPET National Workshop 2003 showed a 67% degradation in a 6-h run.

During the fourth months of PCB exposure, a few bioparticles were taken from the reactor and examined under the SEM (see Figure 5). The Examination revealed the following observations: (a) Various types of microorganisms adhered to the cement ball; (b) excessive amounts of extracellular polymer totally surrounded rod-shaped as well as spherical bacteria; and (c) filamentous organisms were present in the biofilm, in which formed corallike branches that protruded from an uneven surface that is marked by ventlike structures.

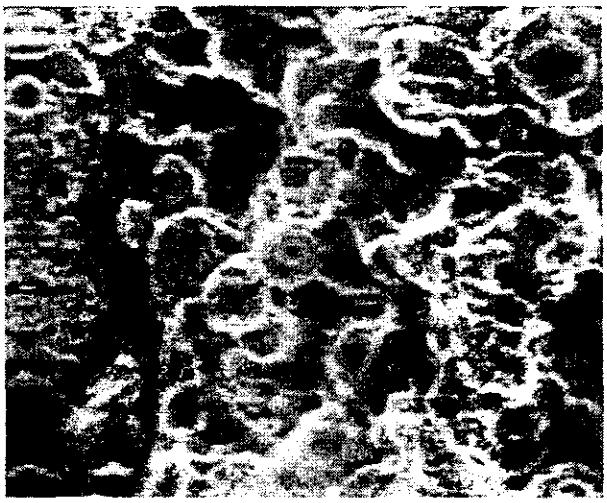

Figure 5. Electron Micrograph of Biofilm During the Fourth Month of the PCB Exposure

The biofilm reactor was acclimatized with PCBs in batch runs only for 8 h. Samples were taken at 2-h intervals. The initial concentrations of PCBs in terms of Aroclor 1260 (in mg/L) fed in the reactor were as follows: Run 1, 71.66; run 2, 70.17; run 3, 77.15; run 4, 72.15; run 5, 71.97; and run 6, 76.61 (see Figure 6a). All of the runs were conducted at room temperature and at the same fluidization condition. The biofilm measured at these runs averaged to 100 μm.

A series of batch experimental runs were performed to determine the effect of exposing the biofilm to PCB-contaminated water. Run 1 was performed 6 months after batch feeding the reactor with simulated PCB-contaminated water. Run 1 achieved an 84.7% degradation of PCBs

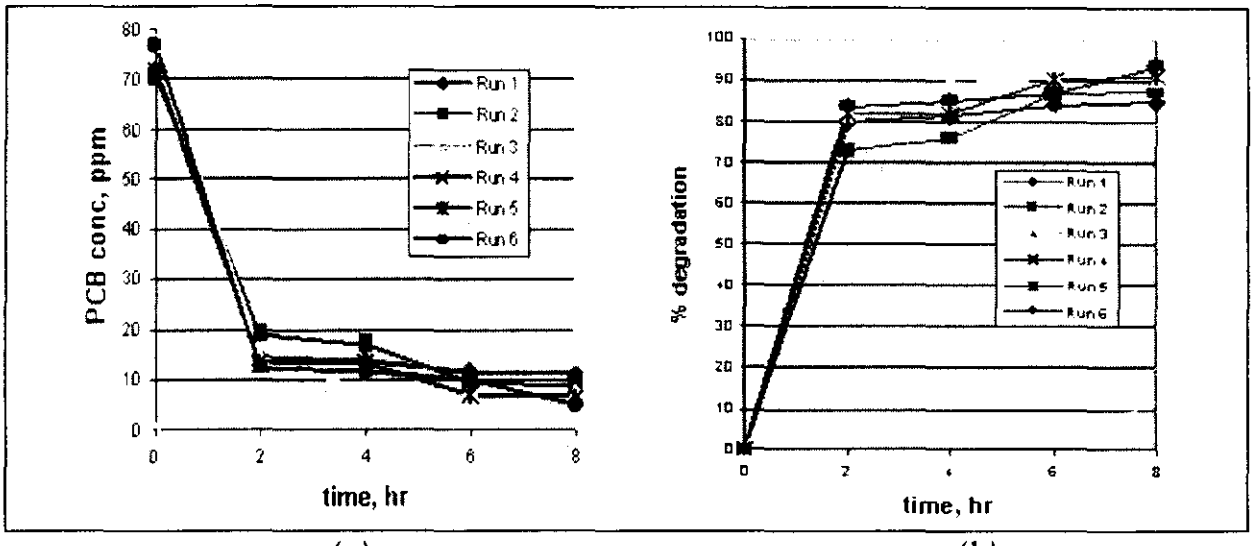

Figure 6. (a) Concentration Profile of Aroclor 1260 During Batch Runs and (b) In Terms of Percent Degradation

in terms of Aroclor 1260. Run 2, which was conducted a week after the first run, showed an increase in degradation by 2.8%. Run 3 was performed 5 days after. At this time point, sloughing of the biofilm was observed. Nevertheless, the system was able to degrade PCBs by 90.6%. Run 4 which was conducted 2 days after the third run; registered only a slight increase in degradation at 90.7%. This result, however, was expected because the condition of the biofilm at this point was the same as Run 3. In Run 5, 91% degradation resulted from the batch test conducted. This was done 2 weeks after the fourth run was to patch the sloughed biofilm. No PCB tests were done during this period. Only a slight increase was observed from Run 4. Consequently, the biofilm was exposed to PCB in batch runs and after a week, Run 6 was performed. The reactor at this point was able to degrade simulated PCB-contaminated water by up to 93.4%. Degradation of PCBs proceeded in a manner similarly from runs 1 to 6 as shown in figure 6. Slight decrease of PCB concentration was observed in all the runs after the 2nd hour of PCB tests. From the results obtained, the biofilm was acclimatized to degrade PCBs.

COD tests were conducted after each PCB-batch run to determine if the PCB tests affected the activity of the microorganisms. Initial concentration for all runs was 830 mg/L (see Figure 7). Before Run 1 was conducted on COD

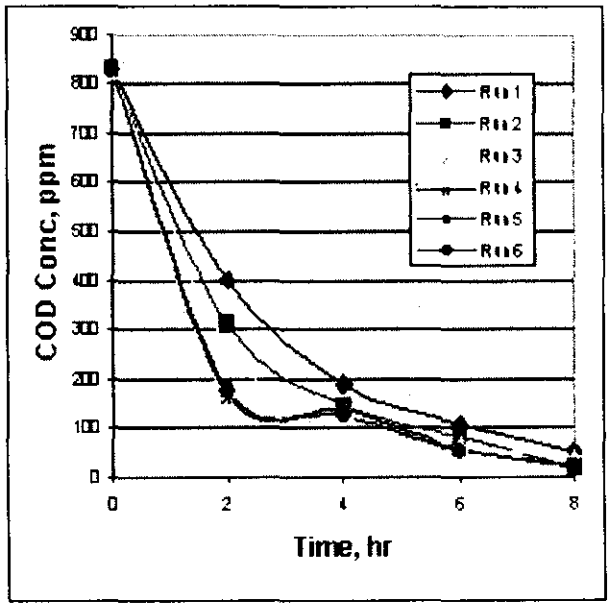

Figure 7. COD Concentration After Each Batch Run

test, the reactor was able to achieve a 98% COD reduction. After Run 1 in the PCB test, COD reduction was only 94%. This result was expected because the concentration of PCB fed in the reactor was higher compared to the concentration of PCB in the previous experiments done. After Run 2, the microorganisms recuperated and were able to achieve a 97.8% COD reduction. The feeding of simulated PCB-contaminated water was still conducted in the batch test although the results were not reported. This process

acclimatized the biofilm to higher concentrations of PCB in water enabling the biofilm to perform normally instead. Sloughing was observed in Run 3, resulting in a decrease in performance of the reactor to reduce COD. Reduction was at 95.7% in Run 3. Run 4 gained a COD reduction from Run 3 by 1%. In runs 5 and 6, the reactor operated again with 98% COD reduction. From the COD data obtained, it was evident that the microorganisms present in the system were not affected by the PCB tests conducted.

Additional runs were conducted to verify the results, but this time the initial concentrations (in mg/L) were as follows: Run 7, 53.8; Run 8, 53.32; and Run 9, 52.35. Run 7 was conducted 3 weeks after Run 6. The same pattern noted for runs 1 to 6 was observed in experimental runs 7 to 9. The results obtained were consistent in reducing Aroclor 1260 by 4.9 mg/L in Run 7; 4.18 mg/L in Run 8; and 4.06 mg/L in Run 9; giving a percentage degradation of 90.09%, 92.16%, and 92.24% respectively in 8h (Fig. 8). These results confirmed that the reactor could achieve 92 to 93% of aqueous PCB solution degraded in 8-hour batch experimental runs.

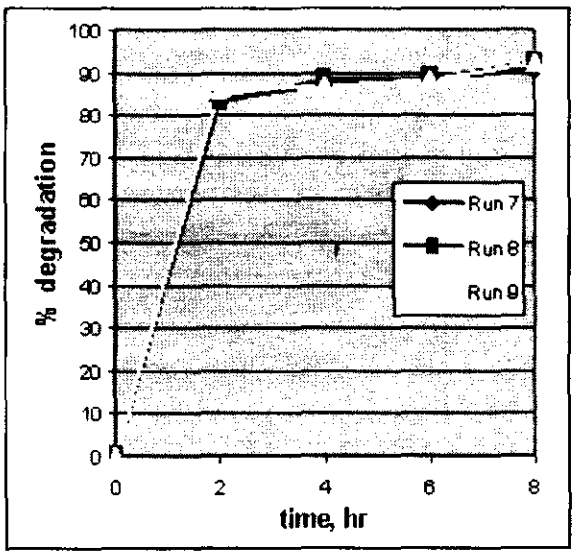

Figure 8. Additional Runs of PCB Tests Show the Same Pattern as that in Previous Experiments

Figure 9 shows the pictures of the chromatograms taken. The samples matched the

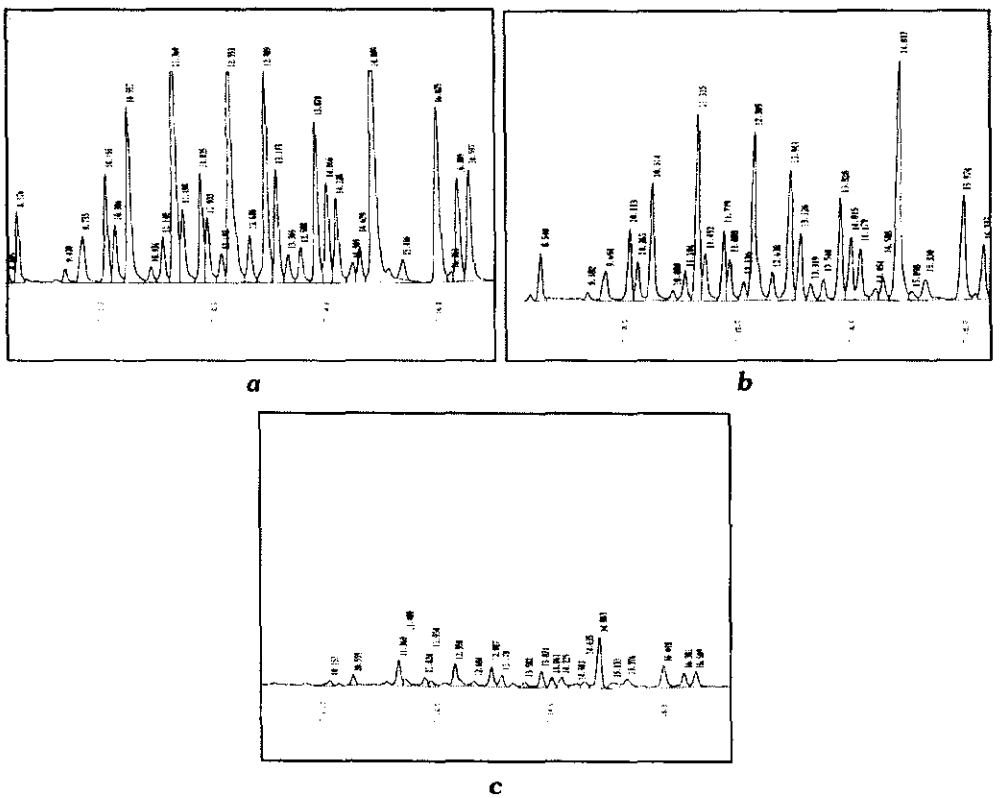

Figure 9. Chromatogram of Aroclor 1260: (a) Standard 1 mg/l Aroclor 1260, (b) Sample taken at initial condition with 10x dilution, and (c) Sample, also at 10x dilution, taken at the final condition showing a decrease in concentration.

peaks of the standard Aroclor 1260. It is evident from the peaks that degradation proceeded in the experiment conducted.

For each run, pH monitoring was conducted. The initial pH of 8.3 registered at 8.1 after 8 h. The presence of sodium bicarbonate in the medium provided the buffering effect in the reactor. A study by Mukerjee-Dhar et al. (1998) showed that the optimum degradation of Aroclor 1242 was at pH 8.4–8.8. At pH 7.5, there was poor degradation observed even for dichlorobiphenyl and trichlorobiphenyls.

The average thickness of the biofilm used was 100 mm. According to Shen and Guiot (1996), the thickness of aerobic biofilm fall within the range of 50–200 μm. The biofilm reactor in this system acts as the two-stage anaerobic–aerobic reactor. The thick layer of the aerobic biofilm acts as a shield to the toxic effect of oxygen for the anaerobes. Because aerobe and anaerobe microorganisms are coupled in one biofilm system, reductive dechlorination can also be achieved since the highly chlorinated biphenyls in Aroclor 1260 undergo reductive dechlorination first followed by aerobic mineralization. In a study conducted by Tartakovsky et al. (2001) using a single-stage coupled anaerobic–aerobic biofilm reactor, Aroclor 1242 had a near complete mineralization with intermediates detected in the system to contain biphenyl, benzoic acid, and mono-hydroxybiphenyls. This study, however, is limited only to lowering the concentration of Aroclor 1260. Thus, the other Aroclor present, specifically Aroclor 1242, was not quantified.

CONCLUSIONS

The present research demonstrated how the biofilm that formed on CB particles using inoculum from tannery wastes could be acclimatized to degrade PCBs in a biofilm reactor system. A maximum of 93% from the initial concentration of Aroclor 1260 in aqueous solution was successfully degraded in an 8h experimental batch run. Consequently, the COD tests conducted confirmed that the biofilm in the reactor was not affected by the PCB tests performed. A 98% COD reduction was still achieved in an 8h batch run after being exposed to wastewater containing PCBs. The results showed that the mixed culture of sheltered microorganisms inside the biofilm is capable of degrading PCBs, that constitute of Aroclor 1260, a highly chlorinated biphenyl. Highly chlorinated biphenyls are known to be toxic to free-floating microorganisms. With the results presented in this paper, it can be concluded that biofilms can resist the toxic effects of persistent organic chemicals due to their sheltered environment.

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