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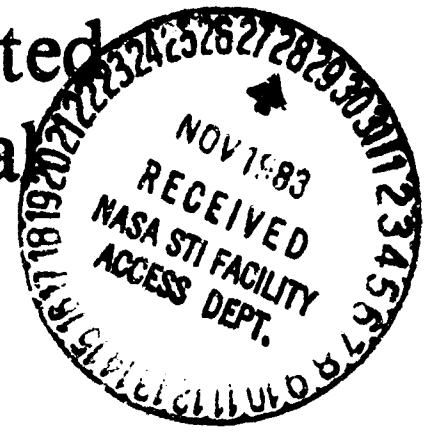

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ABSTRACT

A laboratory experiment was conducted to determine if polychlorinated biphenyls (PCB's) found in an industrial sewer sludge can be effectively degraded by mutant bacteria. The aerated sludge was inoculated daily with mutant bacteria in order to augment the existing bacteria with bacteria that were considered to be capable of degrading PCB's. The pH, nitrogen, and phosphorus levels were monitored daily to maintain an optimum growing medium for the bacteria. A gas chromatographic method was used to determine the PCB concentrations of the sludge initially and also throughout the experiment. Results and discussion of the bacterial treatment of polychlorinated biphenyls are presented in this paper.

SUMMARY

A 90-day laboratory sized experiment was conducted to determine the viability of using mutant bacteria to decontaminate an industrial type sludge that was contaminated with polychlorinated biphenyls (PCB's). The contaminated sludge samples were obtained from an industrial waste sewer lagoon system which is an integral part of a water pollution control facility. Four representative samples of sludge were selected for treatment with mutant bacteria. The samples originally contained the PCB Aroclor 1260 in concentrations ranging from 135 to 232 parts per million (ppm). In addition to the four samples, a fifth sludge containing only three ppm of Aroclor 1260 was spiked with nearly 1000 ppm of Aroclor 1260. This spiked sample was used to determine whether high concentrations of PCB's in sludge would be affectd by the bacteria.

The five samples were inoculated daily with mutant Pseudomonas aeruginosa bacteria that had a potential for degrading PCB's. The nitrogen, phosphorus, and pH levels were monitored daily to maintain a growing medium for the bacteria.

At the completion of the 90-day augmentation period, the concentrations of the PCB's in the sludge samples were found to be higher than the original concentrations. The cause of the apparent increase in concentrations has not been fully understood. One possibility is the biodegradation of the sludge in which the sludge is metabolized and broken down into lighter molecules. The lighter sludge increases the concentration of PCB on a weight-to-weight basis.

The apparent lower PCB concentration found around 20 days after inoculation suggests that the PCB was absorbed by the bacteria, thereby causing the apparent decrease in the PCB concentration of the sludge. After 20 days, the bacteria began to die, perhaps caused by the build up of toxic metabolic wastes, and subsequently released PCB back into the sludge without having metabolized the PCB molecules. Thus, the experiment can be summarized as that the PCB in the sludge was absorbed by the mutant Pseudomonas aeruginosa bacteria for 20 days. After 20 days, the bacteria began to release the absorbed PCB's back into the sludge which by now has been degraded into lighter molecules. Thus, the PCB concentration appeared to decrease at around 20 days, then increase after 20 days. Under the constraints of this experiment, the PCB was absorbed by the bacteria, but was not degraded.

Section 1

INTRODUCTION

Polychlorinated biphenyls (PCB's) are chlorinated aromatic organic compounds that are commonly used as dielectric fluids in capacitors and transformers. Their physical and chemical properties are such that they are chemically and thermally stable, fire resistant, essentially non-conductive, and have a low solubility in water.

Because of their chemical and physical stability, they are virtually indestructible when spilled into the environment, whether accidently or discarded for disposal purposes. High temperature combustion processes and special chemical treatment methods are evolving which are used to destroy the PCB's or to degrade the components into non-toxic materials. The U.S. Environmental Protection Agency has established a level of 50 parts-per-million above which materials must be disposed of in a Federally approved landfill or incinerator. Other treatment and cleanup methods are under investigation, and one of the most appealing concepts is biological treatment in which bacteria is used to degrade PCB's in situ.

Early studies in the use of bacteria for the degradation of specific chemicals reported in 1970, have shown that biphenyl can be degraded by gram-negative bacteria isolated from soil (Ref. 1). In that study, biphenyl was converted to phenylpyruvate in a salt medium. Other studies of biphenyl degradation involved bacteria such as Pseudomonas putida, Beijerinckia species, and a strain of Mucor. For the metabolism of pure chlorinated biphenyl (not PCB), Rhizopus japonicus was used to convert 4-chlorobiphenyl to 4-chloro-4-hydroxybiphenyl. Two species of Achromobacter, isolated from sewage effluent, degraded biphenyl to benzoic acid, and 4-chlorobiphenyl to 4-chlorobenzoic acid. However, no natural bacteria was found to successfully degrade polychlorinated biphenyls, which are mixtures of many chlorinated biphenyls.

If bacteria that can degrade PCB's are found, they most likely will be developed through genetic engineering. T use of such mutant bacteria would be particularly effective in the treatment of soils, sludges, and waste water for the decontamination of PCB's in those environments. Contaminated waste water lagoons would not

require the subsequent draining and physical removal of PCB contaminated soil and sludge. The lagoon could be conveniently treated by adding a mutant bacteria in situ and effect a clean up process at a relatively low cost and with little effort.

To determine the feasibility and effectiveness of bacterial augmentation, a laboratory experiment was conducted using PCB contaminated sludge samples from an industrial sewer lagoon. By using the actual sludge samples from the lagoon, this experiment was to provide insight as to whether the lagoon can be decontaminated by mutant bacteria. This paper describes the experiment and discusses the results of using mutant Psuedomonas aeruginosa bacteria with PCB contaminated sludge samples.

Section 2

EXPERIMENT

The industrial sewer lagoon used for this study is part of a six-lagoon water pollution control facility which receives decante waters generated from waste tanks that process rinse waters and liquid wastes generated by metal plating and tube cleaning facilities. This lagoon serves as an equalization pond holding about 3.5 million gallons of waste water. An earlier investigation indicated widespread, low-level PCB contamination in the lagoon. Few sites were found to be contaminated with PCB's in excess of 50 parts-per-million (ppm) of Aroclor 1260. The EPA regulated concentration is 50 ppm.

In order to fully determine the magnitude and extent of PCB contamination in the lagoon, an extensive sampling program was conducted. Sampling equipment was loaded in a boat and towed to the selected sampling points. Core samples of sludge and the sediment were collected. Average depth of the sediment was about six inches with the base material of clay. These samples were analyzed for PCB concentrations which ranged up to the highest concentration of 467 ppm. A definite water flow pattern was observed in which the inlet of the lagoon contained the highest concentrations and toward the discharge outlet the concentrations gradually declined.

After contamination levels were determined, many alternatives and possibilities were considered for decontamination of the sewer lagoon. The alternatives ranged from closing the lagoon; permanently fixing the sediment into concrete-like material; biological and physiochemical methods; to physically dredging the sediment for disposal, treatment, or incineration. Many other possibilities were also considered, but one of the most attractive alternatives was the in-place degradation of PCB by microorganisms. If this microbiological method proved valid, draining of the lagoon water and physically removing the sludge would not be required. Using the sludge samples that had been analyzed for PCB, a small laboratory experiment was carried out to determine whether biological degradation of PCB's would be feasible for the industrial waste lagoon. Glass dessicators with approximately 2-liter capacities were used as experimental tanks. Five such sludge tanks were charged with the first tank containing about 450 grams of sludge with

205 ppm concentration of PCB. Water was added to bring the aqueous level up to one liter. The second tank contained 50 grams of sludge with 222 ppm PCB diluted to one liter. The third tank was two liter tank containing 100 grams of sludge in which 980 ppm of Aroclor 1260 was added to the sludge originally containing 3 ppm. This tank was started to determine whether high concentration of PCB in the sludge would be affected by the bacteria, and to roughly determine the degradation rate to see how long the treatment would be needed to bring the concentration below the 50 ppm level. The fourth tank contained 50 grams of sludge with 135 ppm, and the fifth tank had 50 grams of sludge with 232 ppm diluted to one liter.

These experimental sludge tanks were inocculated with the mutant Pseudomonas aeruginosa bacteria obtained through a biochemical firm that has considerable experience and expertise in genetic engineering. The bacteria culture which was contained in a bran base was prepared for augmentation by soaking the culture in water for six to nine hours with a pinch of sodium bicarbonate. The initial dosage rate was 0.1 grams of the culture per one liter, then the dosage rate was gradually decreased over 20 days to a maintenance level of 20 milligrams per liter. Along with daily supplementation of bacteria, a nutritional balance for biological activity was maintained each day. The dissolved oxygen was kept at about 7 ppm level by aeration of the tank and agitation of the slurry. The pH level was maintained at 7.5, and nitrogen as ammonia determined by the Nesslerization method was over 5 ppm level. The phosphorus as ortho-phosphate was determined by the molybdate method and maintained at 1 ppm. The temperature of the tanks was kept at about 75° F.

Section 3

RESULTS AND DISCUSSION

In monitoring the sludge tanks daily, the general trend has been the drop in the pH and nitrogen, while phosphorus concentration remained above 1 ppm level. No particular pattern was observed other than a need for almost daily addition of sodium bicarbonate to bring the pH up and an ammonium compound to supplement the nitrogen level. To compensate for nitrogen loss, ammonium hydroxide was used if the pH dropped to about 6.8, and ammonium nitrate was used if the pH was near 7.5 level.

During the augmentation period, biological activity in the tanks was monitored biweekly. An ordinary microscope at 100 magnification and a phase contrast microscope at 400 magnification were used to insure the presence of active organisms in the tanks. As activated sludge is formed and ages, there is a successive predominance of protozoans and rotifers which correlates with the bacterial population (2). In general, as the bacteria population increases, flagellates become predominant. When the bacteria population is at the maximum, free swimming ciliates are the predominant organisms. As the bacteria population declines, rotifers become predominant. In the sludge tanks, the protozoans and rotifers were observed with the flagellates and free swimming ciliates as the predominant organisms. At about the middle of the augmentation period, the number of the higher organisms appeared to have sharply declined. Toward the end of the augmentation period, the higher organisms were almost absent.

In the last 12 days of augmentation, a large amount of bacteria was added to each tank along with supplemental food to provide an additional carbon source. The population of microorganisms correlates with the sludge conditions, in that the sludge starts to age and breaks down when the population of microorganisms is at the maximum. The sludge becomes digested and the food source for the bacteria becomes diminished. With further digestion of sludge, the organisms eventually utilize the internal material through endogeneous process, resulting in the diminished population. Near 12 days toward the end of the augmentation period, the sludge appeared emulsion-like and the higher organisms were almost absent.

This indicated that the microorganism population had severely declined due to lack of an external food source. Although a large amount of bacteria was added along wit i supplemental food, no improvement was observed.

The results of bacterial augmentation over a 90-day period are presented in the Table. These results were obtained by analyzing the sludge samples by a gas chromatographic method. A sample of sludge was weighed, then PCB was extracted with 1:1 acetone and hexane mixture using an ultrasonic bath. Extracted PCB was analyzed by a gas chromatograph equipped with an electron capture detector. This analytical instrument was fitted with a two-meter glass column packed with 3% OV-1 on 80-100 mesh Chromosorb W-HP and maintained at 200° C. The carrier gas was ultrapure nitrogen at a flow rate of 25 milliliters per minute. The injection port was kept at 250° C. and the detector was maintained at 300° C. The output of the gas chromatograph was recorded on a 1 mv strip chart recorder. The PCB chromatogram of the sample was quantitated from the standard chromatograms.

As shown in the Table, PCB in the industrial sewer sludge appears not to have been degraded by the bacteria over 90 days period. In these sludge tanks, the final concentrations of PCB at the end of 90 days are higher than the starting concentrations, although in Tank 2 the concentration is decreased slightly. This small decrease in Tank 2 cannot be regarded as an indication of bacterial degradation of PCB, since the fluctuation between the anlysis dates and the precision of sampling and analytical method could cause this small difference.

An interesting observation from the results is the apparent dip in the concentration around 20 days from the beginning of the experiment. After this 20-day period, the concentration rises and remains at an elevated level. Although it is possible that the sampling and the analysis may be in error, this initial dip in the PCB concentration of the sludge might indicate an effective biological activity in the first 20 days, after which the biological activity appears to have ceased. The apparent lower concentration suggests that the PCB was degraded or absorbed by the organisms present in the tanks. Since the PCB concentration increases after 20 days, the absorbed PCB may be subsequently released back into the sludge.

Perhaps, after about 20 days, the sludge tanks become saturated with materials such as the bacterial metabolites that may attack or cause the bacteria to decompose and release the absorbed PCB back into the environment. This is difficult to substantiate because limited data is available. With so many variables that can adversely affect the microorganism population, it is conceivable that the

organisms have decomposed since the microorganism population declined as the augmentation period progressed. Another supportive factor is that the experimental sludge tanks are essentially batch reactors in which no water flows in or out of the tanks on a continuous basis. All added materials and metabolic wastes are accumulated in the tanks. If PCB was metabolized or degraded by the bacteria in order to gain energy and enhance growth, the PCB peaks in the gas chromatogram may show an indication. Possibly, one or more gas chromatographic peaks may be decreasing faster than the other peaks since PCB contains a mixture of chlorinated biphenyls. This hypothesis could not be fully tested because of apparent rise in all PCB peaks after the 20-day period.

The apparent rise in the PCB level cannot be explained at this poirt, but one possible explanation is that the organisms may be preferentially attacking the abundant carbon source of the sludge before attacking the PCB. This may have the effect of increasing the PCB concentration, on a weight-to-weight basis, in the biodegraded sludge. Therefore, when a sample of sludge is weighed out and analyzed, the concentration of PCB would appear to be higher as shown in the results.

Section 4

CONCLUDING REMARKS

In each of the five experimental sludge tanks, the bacterial augmentation over a 90-day period resulted in an apparent increase in the PCB concentration. At the end of 90 days, the final concentrations of PCB in the industrial sewer sludges were higher than the original concentrations. These results may be due to the sludge being degraded prior to PCB and due to the sludge tanks becoming toxic to the bacteria by the bacterial metabolites that cause the bacteria to decompose and release absorbed PCB back into the sludge.

It appears that around 20 days after the augmentation, microorganisms released the absorbed PCB back to the sludge without metabolizing or breaking down the molecules. In this case, if a continuous water system was used to allow a flow of water in and out of the augmentation system, the PCB concentration in the sludge would be decreased by the organisms with absorbed PCB being carried away with the water flow.

At any rate, the mutant bacteria of Pseudomonas aeruginosa used in this experiment did not degrade the PCB's found in the sludge from an industrial sewer lagoon. It is important to test each PCB contaminated material to determine the feasibility and effectiveness of bacterial augmentation prior to an actual field demonstration.

Section 5

REFERENCES

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TABLE: RESULTS OF PCB CONCENTRATIONS
IN BACTERIA AUGMENTED SLUDGE, ppm

No. Days	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
0	205	222	983	135	232
21	94	65	821	---	---
24	---	---	---	110	210
30	145	206	962	---	---
36	---	---	---	131	258
42	195	154	1449	---	---
50	---	---	---	165	275
57	255	162	1160	---	---
75	230	174	1443	---	---
82	253	157	1225	---	---
90	262	181	1476	---	---
91	---	---	---	163	287