Degradation of Di- Through Hepta-Chlorobiphenyls in Clophen Oil Using Microorganisms Isolated from Long Term PCBs Contaminated Soil

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Abstract Present work describes microbial degradation of selected polychlorinated biphenyls (PCBs) congeners in Clophen oil which is used as transformer oil and contains high concentration of PCBs. Indigenous PCBs degrading bacteria were isolated from Clophen oil contaminated soil using enrichment culture technique. A 15 days study was carried out to assess the biodegradation potential of two bacterial cultures and their consortium for Clophen oil with a final PCBs concentration of 100 mg kg^{-1} . The degradation capability of the individual bacterium and the consortium towards the varying range of PCBs congeners (di-through hepta-chlorobiphenyls) was determined using GCMS. Also, dehydrogenase enzyme was estimated to assess the microbial activity. Maximum degradation was observed in treatment containing consortium that resulted in up to 97 % degradation of PCB-44 which is a tetra chlorinated biphenyl whereas, hexa chlorinated biphenyl congener (PCB-153) was degraded up to 90 % by the consortium. This indicates that the degradation capability of microbial consortium was significantly higher than that of individual cultures. Furthermore, the results suggest that for degradation of lower as well as higher chlorinated PCB congeners; a microbial consortium is required rather than individual cultures.

Keywords Clophen · Polychlorinated biphenyls (PCBs) · Congeners · Dehydrogenase activity · Degradation

Introduction

Increasing industrialization and rapid growth of population has led to introduction of numerous xenobiotic chemicals in the environment. Some of these chemicals show properties like high toxicity, strong bioaccumulation, and low biodegradability. The natural system gets polluted due to long term exposure to these chemicals termed as *persistent organic pollutants* (POPs). On May 23, 2001, Stockholm Convention described POPs as “one of the greatest environmental challenges the world faces today” [1]. Polychlorinated biphenyls (PCBs) are one of the toxic environmental contaminants included in the group of POPs which are found in air, water, soil, sediments and biota as well as in human adipose tissues [2]. PCBs synthesis started in 1881 and was commercialized in 1920s. Reported data on production of PCBs revealed that over 1.5 million tons of PCBs were produced worldwide [3]. The production and usage of PCBs was banned in the United States in 1979 [4]. The global ecosystem becomes pool of most of the persistent and toxic organic compounds and PCBs are one of the strong contributors. PCBs are categorized under industrial POPs by the Stockholm Convention owing to their highly notorious chemical nature. Due to their super hydrophobicity and low chemical reactivity, PCBs tends to be adsorbed on natural organic matter in soil, sediments and sludge [5].

The contamination caused by PCBs in environment is increasing day by day due to inappropriate handling and disposal practices of PCBs and PCBs containing articles and also some unintentional generation occurs because of open burning, vaporization etc. In spite of the regulations on handling, use and disposal of PCBs and PCBs containing articles, they are illicitly dumped due to ignorance and negligence. The release of PCBs into the environment

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has caused public concern for several decades because of its recalcitrance and potential carcinogenicity. The debate over the danger that PCBs pose to human health, wildlife and the environment continues to be a great concern owing to its dioxin like toxicity [6].

PCBs contaminate sediments in lakes, rivers and harbors where they bioaccumulate and biomagnify in the food chain [7]. Few studies reported that some mixtures of PCBs have been shown to increase the development of hepatic tumors in rats [8]. Contamination of drinking water, sediment, wastewater, foods, and aquatic organisms by PCBs has also been documented [9]. In natural environment, PCBs degradation is mainly dependent on key factors such as the number and positions of chlorine atoms on the biphenyl ring. Higher chlorinated biphenyls are more resistant to degradation as compared to lower chlorinated biphenyls. Half-lives of PCBs undergoing photolytic degradation vary from 10 days to 1.5 years depending on the degree of chlorination. Bioaccumulation of PCBs depends on partition coefficients of different congeners ($\log K_{OW}$ = octanol water partition coefficient) which ranges from 4.3 to 8.26. Classical remediation techniques such as land filling, incineration, etc. are not only expensive and inefficient, but may also lead to secondary pollution. Several remediation and disposal technologies of PCBs are reported by various researchers which include incineration, landfilling, hydride reduction, hydrodechlorination, dechlorination using metals, photolysis and γ -radiation, disposal through plasma arc, chemical oxidation, electrolysis, mechano-chemical degradation, etc. [10]. Most of these processes adopt harsh reaction conditions such as high temperature, pressure, radiation and strong basic conditions. Many of the remediation methods have not been reported to achieve complete degradation of PCBs. Therefore, bioremediation can prove to be a cost effective and eco-friendly tool for the remediation of PCBs contaminated sites [11, 12]. PCBs are highly stable and hydrophobic compounds and there are some studies which report the degradation of several PCBs congeners using microbes [3]. Different bacterial species show different pattern of degradation with respect to specific PCB congeners [13]. The degradation of different PCB congeners is dependent on the complexity of PCB congener and the type of microorganism in addition to the interaction among the microorganisms in polluted environments [14]. It is also reported that the degradation of various organic pollutants can be enhanced by the use of biosurfactants which emulsify the hydrophobic contaminant and increases its bioavailability to the microorganisms [15]. The present study explicates aerobic degradation of di- through hepta-chlorobiphenyls using indigenous bacterial cultures isolated from PCB contaminated soils and their consortium. The study after further scale-up to a pilot level might play a pivotal role in development of an effective and efficient

bioremediation technology for remediation of PCBs contaminated sites.

Materials and Methods

Minimal Salt Medium

Minimal salt medium (MSM) used for screening and enrichment of bacterial cultures was as reported by Kaczorek et al. [16] with few modifications. 0.1 % Triton X-100 was added to the medium as an emulsifier. The culture enrichment was performed using MSM supplemented with 10 mg kg⁻¹ of Clophen A-40 (Dr. Ehrenstorfer, Germany) as a sole carbon source. The MSM containing flasks were incubated at 37 °C in an orbital shaker at 160 rpm for 72 h. The enriched MSM was used for the isolation of Clophen A-40 acclimatized bacterial cultures. The isolated cultures were then grown on MSM agar plates with Clophen A-40.

Chemicals

The degradation studies were carried out using Clophen oil with high concentration of PCBs (12,456 mg kg⁻¹). Certified reference standards from Dr. Ehrenstorfer, Germany were used for GCMS analysis. The solvents and chemicals were procured from Merck, Germany.

Screening of Bacterial Cultures

Bacterial cultures were isolated by selective enrichment of Clophen oil contaminated soil collected from industrial premises located in central India, which has a history of PCB contamination [17]. The bacterial cultures were grown in MSM using Clophen oil as sole carbon source and the cultures selected were then subjected to formation of consortium. The isolates were characterized and identified on the basis of biochemical tests and 16S rRNA sequencing from SCIGENOME lab, Cochin, India. The sequence were submitted to gene bank using sequin (accession numbers: KF724906 and KF724907). The selected isolates were identified as *Bacillus foraminis* (AAJ5) and (AAJ6). The two cultures were grown till mid log phase separately and then mixed in equal amounts to form a consortium.

Analytical Method

A range of PCB congeners from di- through hepta-chlorobiphenyls (PCB-8, PCB-18, PCB-28, PCB-44, PCB-52, PCB-76, PCB-101, PCB-105, PCB-138, PCB-151, PCB-153 and PCB-180) in Clophen oil were analyzed using GCMS (Varian, 450-GC/240-MS). A DB-5 capillary column (30 m length, 0.32 mm internal diameter with 1.8 μ m

Fig. 1 GC-MS chromatogram for microbial of degradation of di-through hepta-chlorobiphenyls PCB congeners in Clophen oil

Fig. 2 Microbial degradation profile of di-through hepta-chlorobiphenyls PCB congeners in Clophen oil using individual bacterial cultures and consortium

Fig. 3 Microbial dehydrogenase activity for different treatments

film thickness) was used for separation of the PCB congeners. GCMS Programme for analysis was set as: initial temperature, 50 °C with hold time of 1 min, ramping at 10 °C/min up to 150 °C with hold time of 1 min, ramping at 5 °C/min up to 200 °C with hold time of 1 min and final ramping at 25 °C/min up to 280 °C with hold time of 15 min. Total run time was 38.74 min.

Batch Culture Experiment and Analysis

A batch experiment was conducted to study the degradation potential of isolated cultures and the consortium. The experiment was conducted in 250 ml Erlenmeyer flasks. Flasks containing individual cultures as well as consortium of the isolated bacterial cultures were inoculated in 100 ml MSM containing PCB oil with a final concentration of 100 mg kg⁻¹ and 1 ml of 0.1 % Triton X-100 as an emulsifier. The flasks were incubated at 37 °C for 15 days with continuous shaking at 160 rpm on an orbital shaker. The study was carried out for a period of 15 days and the samples were collected at a regular interval of 3 days. The samples were extracted using standard liquid–liquid extraction with hexane as extracting solvent. The degradation of PCBs congeners was analyzed using GCMS. Dehydrogenase enzyme was also estimated as an indicator of microbial activity [18].

Results and Discussions

Quantification of PCBs Degradation

Selected PCBs congeners in extracted samples for all the treatments i.e. individual bacterial cultures as well as consortium were analyzed on GCMS and the results are reported in Fig. 1. The GCMS analysis showed that PCB-44 which is a tetrachloro-biphenyl was degraded up to 97 % whereas PCB-153, a hexachloro-biphenyl was degraded up

to 90 % by the consortium. Individual bacterial cultures AAJ5 and AAJ6 on the other hand, were able to degrade PCB congeners, PCB-44 by 67 and 82 % respectively as compared to consortium. A highly chlorinated congener heptachloro-biphenyl (PCB-180) was degraded up to 60, 70 and 75 % by AAJ5, AAJ6 and consortium respectively. Figure 2 represents the degradation profile of selected PCB congeners in different treatments. From the results, it can be inferred that for degradation of PCBs, a selected bacterial consortium is required rather than a single bacterium. Zermeno-Eguia et al. [19] have also reported that the consortium have a better capability to degrade PCBs as compared to individual cultures. It was also reported that the lower chlorinated biphenyls were degraded to a greater extent as compared to the higher chlorinated congeners [6, 20]. It can also be inferred from the results of GCMS analysis that the degree of chlorination is an important factor for degradation of PCB congeners. These results are in conformity with the results obtained by Hatamian-Zarmi et al. [21]. The study also confirmed that the consortium is capable of degrading lower as well as higher chlorinated PCB congeners.

Dehydrogenase Enzyme Activity

The bacterial cultures derive energy for their maintenance by oxidation of the organic compounds initially before they can start multiplying. The multiplication of bacterial cultures starts when the energy of maintenance is excess due to supplementation of higher concentration of organic compounds [22]. In this case, the organic compound supplemented was Clophen oil which acted as sole carbon source for growth and multiplication of bacterial cultures. Also, during the degradation process, dehydrogenase enzyme serves as a catalyst in the oxidation of hydrocarbons by breaking the carbon double bond and transferring the adjacent hydrogen atom to the hydrogen acceptor [23]. Furthermore, the dehydrogenase activity generally corresponds to metabolically active cells present in the system

[24]. Dehydrogenase activity can therefore be used as an indicator for increased microbial population that in turn can indicate increased degradation process. The test was performed at an interval of every 3 days during the course of experiment for all the treatments inoculated with individual cultures as well as consortium and the results are depicted in Fig. 3. Maximum enzymatic activity was observed in the treatment inoculated with consortium as compared to that inoculated with individual bacterial cultures. Figure 3 shows the variation in dehydrogenase activity with time and maximum activity was observed on 12th day of the experiment for all the treatments. The decrease in dehydrogenase activity after 12th day of experiment might be attributed to depletion of substrate required by the microorganisms (i.e. PCBs) in the medium, which is necessary for their growth. Kaimi et al. [25] in their studies on biodegradation of diesel contaminated soils have reported similar results where the dehydrogenase activity decreased after 91 days which was attributed to the decrease in the substrate concentration.

Conclusions

Bioremediation is an attractive and emerging technology which has recently been harnessed for the cleanup of PCBs contaminated sites. Exploration of microbes for in situ bioremediation of PCBs contaminated soils is a cost-effective and eco-friendly option over conventional chemical and mechanical methods. The results suggested better degradation of PCBs by the consortium than the individual cultures and can be used to develop a successful bioremediation technology. However, individual bacterial cultures can be used for conducting studies to decipher various mechanisms involved in the process of PCBs degradation. While the results are encouraging in flask experiment, the degradation potential of consortium will be tested in PCBs contaminated soil also. The authors are optimistic to get encouraging results for degradation of PCBs in soils as well.

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