BIODEGRADATION OF 4-CHLOROBIPHENYL BY *PSEUDOMONAS SYNXANTHA*

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ABSTRACT

The stabilization and disposal of polychlorinated biphenyls (PCBs) from soil environment and wetland areas is of great concern for health and safety. Wetland remediation with microorganisms is an approach for treating PCBs. A bacterial strain was isolated from hydrocarbon contaminated soil of Ropar, Punjab, able to degrade PCBs under aerobic conditions. The percentage of degradation with 100 mM/ml of 4-chlorobiphenyl was up to 90%. Degradation was monitored by mass spectrometry, high performance liquid chromatography and spectrophotometrically, showing that 4-chlorobiphenyl was degraded almost completely. The bacterial strain was identified as *Pseudomonas synxantha* by 16sRNA sequencing method. This is the first report of 4-chlorobiphenyl degradation by *Pseudomonas synxantha*.

RÉSUMÉ: La biodégradation du 4-chlorodiphényle par *Pseudomonas synxantha***.**

La stabilisation et l'élimination des diphényles polychlorés (PCB) sont une des préoccupations majeures concernant la protection des sols et des zones humides pour des raisons de sureté et de santé. La restauration des zones humides à l'aide des microorganismes est une approche récente dans le traitement de la pollution aux PCB. À Ropar, Punjab, dans des sols contaminés aux hydrocarbures, une souche bactérienne capable de dégrader le PCB dans des conditions aérobies a été identifiée. Le pourcentage de dégradation du 4-chlorodiphényle pour une concentration de 100 mM/ml a atteint 90%. La dégradation a été étudiée par spectroscopie de masse, par chromatographie liquide de haute performance et par spectrophotométrie. Les résultats de ces techniques ont montré une dégradation quasi complète du 4-chlore diphényle. Par le séquençage de l'ARN 16S, la souche bactérienne a été identifiée comme étant *Pseudomonas synxantha*. Il s'agit de la première mention de la dégradation du 4-chlorodiphényle par *Pseudomonas synxantha*.

REZUMAT: Biodegradarea 4-clorobifenilului de către *Pseudomonas synxantha*.

Stabilizarea și eliminarea bifenililor policloruraţi (PCB) este una din preocupările majore cu privire la protecția mediului pedologic și a zonelor umede din motive de siguranță și sănătate. Reabilitarea zonelor umede cu ajutorul microorganismelor este o abordare recentă în tratarea poluării cu PCB. O tulpină bacteriană capabilă să degradeze PCB în condiții aerobe a fost izolată din solul contaminat cu hidrocarburi din Ropar, Punjab. Procentul de degradare al 4-clorobifenilului la 100 mM/ml a fost de până la 90%. Degradarea a fost monitorizată prin spectroscopie de masă, cromatografie lichidă de înaltă performanță și spectrofotometrie și s-a demonstrat că 4-clorobifenilul a fost degradat aproape complet. Prin metoda secvențierii 16sARN tulpina bacteriană a fost identificată ca fiind *Pseudomonas synxantha*. Acesta este prima mențiune a degradării 4-clorobifenilului de către *Pseudomonas synxantha*.

INTRODUCTION

The stabilization and disposal of polychlorinated biphenyls (PCBs) from soil and wetland areas is very important for health and safety. Wetland improvement with microorganisms is a way to deal with PCBs. Polychlorinated Biphenyls (PCBs) are often encountered as contaminants of soil environments and wetlands areas, usually originating from electrical transformer leaks or improper disposal of wastes containing PCBs. PCBs have been used as dielectric fluids in capacitors, flame retardants, transformers, ink solvents and plasticizers. PCBs have been sold under trade names such as Clophen (Bayer, Germany), Aroclor (Monsanto, USA, Canada and UK), Phenoclor (Prodelec, France and Spain), Kanechlor (Kanegafuchi, Japan) and Sovol and Sovtol (Orgsteklo, Orgsintez, former Soviet Union). More than 1.7 million tons of PCBs were produced globally, and a substantial amount of these compounds have been discharged into the environment. PCB exposure can cause liver damage, respiratory disorders, thyroid gland disorders, muscle and joint pain, headaches, loss of appetite, nausea, vomiting and abdominal pain, chloracne (a severe, persistent acne-like rash), reproductive problems including increased spontaneous abortion rates, still births, underweight births and decreased post-natal survival, and cancer as it is a Class 2 Carcinogen (probable human carcinogen). These complications are due to traded mixtures which typically consist of 40-70 congeners. These congeners have been reported to cause serious effects on endocrine, immune, nervous and reproductive systems and cancer. The National Occupational Health and Safety Commission (NOHSC) has determined a short term exposure limit (STEL) for PCBs containing 42 % chloride up to 2 mg/m^3 and PCB's containing 54 % chloride up to 1 mg/m^3. In recent years, PCBs have been detected in aquatic systems in Central and Southern Chile.

Global reduction and withdrawal of persistent organic pollutants (POPs) discharge into the environment has been build up by the Stockholm Convention in 2001. In this context, bioremediation is an attractive technology to decontaminate affected sites. Microorganisms play a major role in the removal of these organic pollutants from the environment. The biodegradation of 4-chlorobiphenyl and its complex products were studied with *Achromobacter* sp. strain and a *Bacillus brevis* strain. *Pseudomonas* sp. strain DJ-12 was used due to genetic organization of genes responsible for the crucial steps of the catabolic degradation of 4-chlorobiphenyl. Bacterial strains can oxidize mono- and dichlorinated biphenyls to the corresponding chlorobenzoic acid and several other minor chlorinated metabolites. This study describes bacterial degradation of PCBs and analyzes the strategies to optimize bioremediation of these organic pollutants.

Bio-stimulation of the native micro-flora and bio-augmentation with selected microorganisms has been applied for the removal of PCBs from contaminated environments. PCB bioremediation, specifically in soil or sediments, is limited by a number of factors including PCB availability, incomplete catabolic breakdown, low expression of catabolic genes, and toxicity of PCBs and their metabolic intermediates).

MATERIAL AND METHODS

4-Chlorobiphenyl of analytical grade was procured from Sigma-Aldrich and used for the present study. All other chemicals used were of analytical grade. Millipore water was used for preparation of various solutions. For the isolation of potential bacterial strains able to degrade PCBs, soil was taken from the hydrocarbon contaminated area of Ropar, Punjab, India.

Instruments

The spectra were taken with UV–Vis spectrophotometer (Shimadzu 1650). HPLC analyses were performed on Shimadzu CTO- 10ASVP instrument equipped with a UV-Vis detector and C-18 column (symmetry, 4.6×150 mm). The mobile phase was acetonitrile: water (78:22 v/v). The injection volume was 20 µl, with a flow rate of one ml/min and wavelength of 254 nm.

Biodegradation Studies

Media and culture conditions

Minimal media with the following constituency were used for growth of bacterial cultures with an incubation temperature of 30°C. Minimal media used in the study contained 1.6 g disodium hydrogen phosphate, 0.2 g potassium dihydrogen orthophosphate, 1 g ammonium sulphate, 0.2 g magnesium sulphate, 0.01 g ferrous sulfate, 0.02 g calcium chloride and 0.1 g sodium chloride in 1 L of the solution.

Isolation and characterization of bacteria

Serial dilution and plating techniques were utilized for the isolation of bacteria from hydrocarbon contaminated soils. Four different isolates obtained were screened for their ability to grow on PCB. Minimal media with 100mM 4-Chlorobiphenyl as the sole source of carbon and energy were used for primary screening with growth being monitored spectrophotometrically by taking OD at 600nm at 24 h intervals. From these four strains, only the potential strain designated as M1(S) was chosen for further studies on the basis of its growth at high 4-chlorobiphenyl concentrations.

Identification of strains

The organism designated as M1(S) was identified as *Pseudomonas synxantha* by 16sRNA sequencing method. Primers used for identification of microbes in this method are 27F (5’ AGAGTTTGATCCTGGCTCAG 3’) and 1492R (5’ CAGCATTGTTCCATYGGCAT 3’).

RESULTS AND DISCUSSION

Isolation and screening of micro-organism

Four pure bacterial cultures were isolated from hydrocarbon contaminated soil sample area of Ropar, Punjab, India. Potential biodegradation studies showed that only one bacterial isolate, M1(S) was able to metabolize 4-chlorobiphenyl at 100 mM/ml concentration in minimal media. This bacterial strain was identified as *Pseudomonas synxantha* and deposited in NCBI Genebank with the accession number JQ406550.

Biodegradation experiment

The biodegradation studies were carried out with potential isolate *Pseudomonas synxantha* and the persistence of 4-chlorobiphenyl was monitored by UV-Visible Spectrophotometer, HPLC and MALDI analysis. Degradation of 4-Chlorobiphenyl was observed during different time intervals. It took about 96 h for almost 87% of mineralization (Tab. 1; Fig. 1).

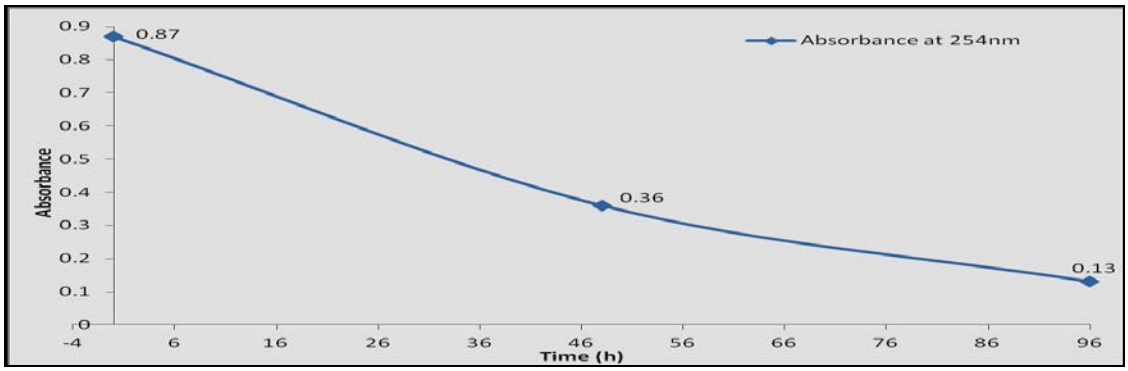

Figure 1: Percentage degradation of 4-Chlorobiphenyl with respect to time interval.

Table 1: Percent degradation of 4-CBP (100 mM/ml) by *Pseudomonas synxantha*.

Incubation period (hours)	Percentage degradation
0 h	0%
48 h	59%
96 h	87%

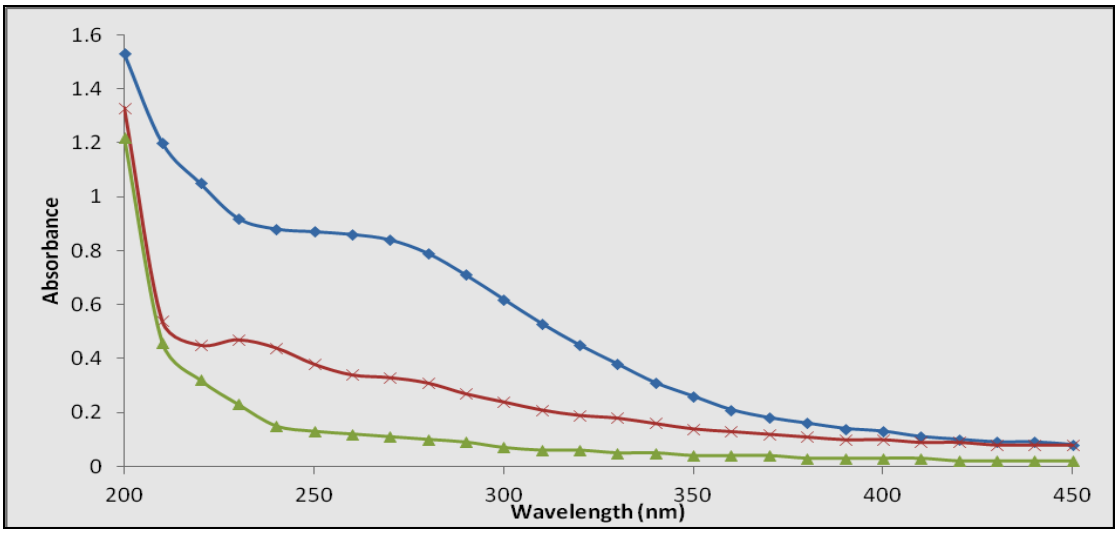

Figure 2: UV-Vis Scanning of 4-Chlorobiphenyl at 0 h, 48 h and 96 h.

UV-Vis scanning - UV-Vis scan (200-450 nm) at different time intervals showed degradation and decrease in concentration. Peak observed at 254 nm (0 h) was decreased without any shift in λ_{max} up to complete degradation of 4-Chlorobiphenyl (96 h), as clearly shown in figure 2.

HPLC analysis - The HPLC analysis of 4-CBP sample collected at the 0 h incubation showed one major peak at 5.467 min. As the biodegradation completed after 96 h, the parent compound was observed with decreased absorbance, thus concentration was observed at 5.483 min (Fig. 3).

MALDI analysis - MALDI analysis was carried out to investigate the metabolites formed during the biodegradation process. Peak at 190.059 m/z was observed in control while in degraded compound the peak at 190.059 was decreased with formation of metabolites at 211.9603 m/z (Figs. 4 and 5).

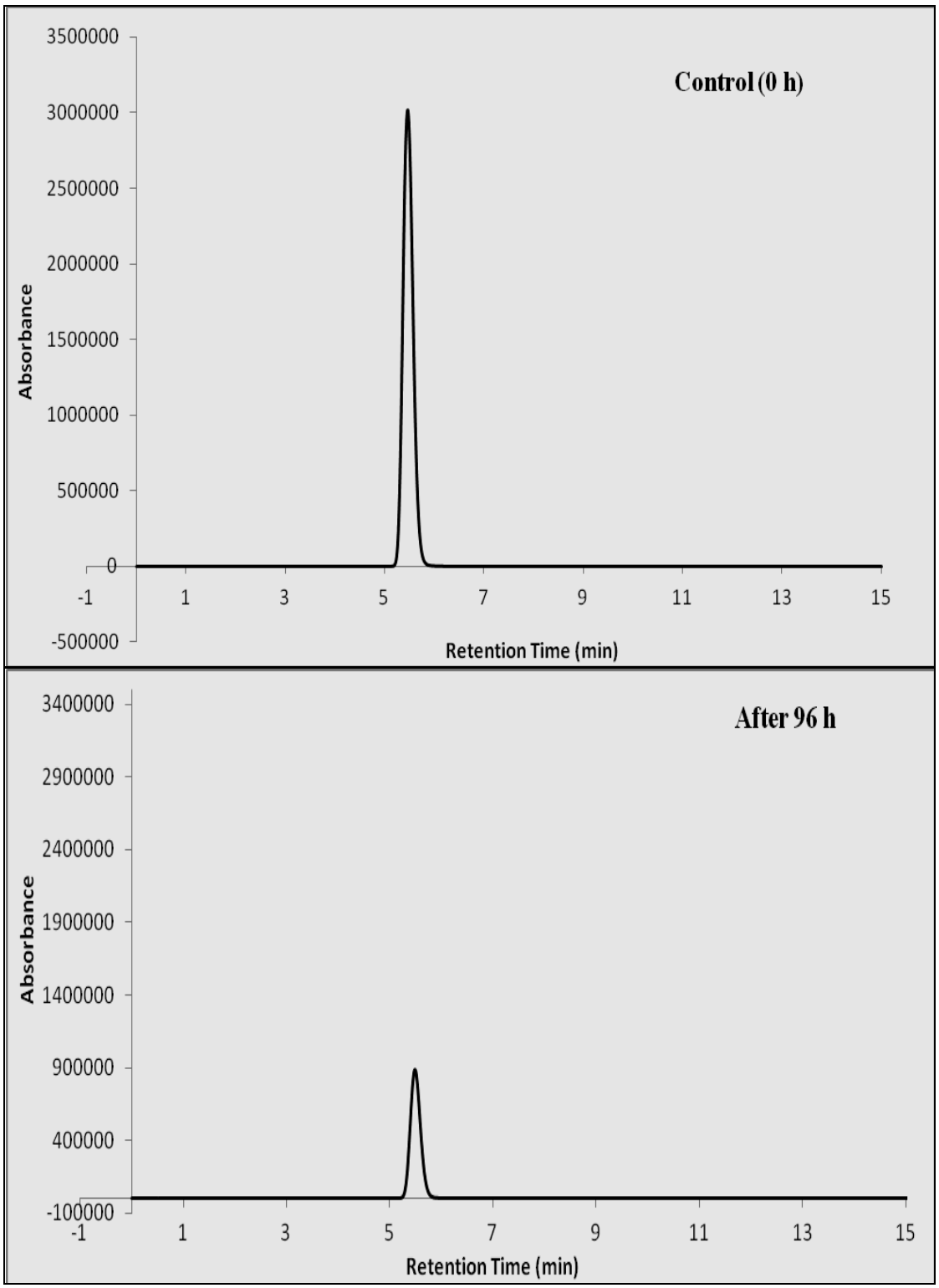

Figure 3: HPLC scan of 4CBP at 0 h (control) and after 96 h (biodegraded).

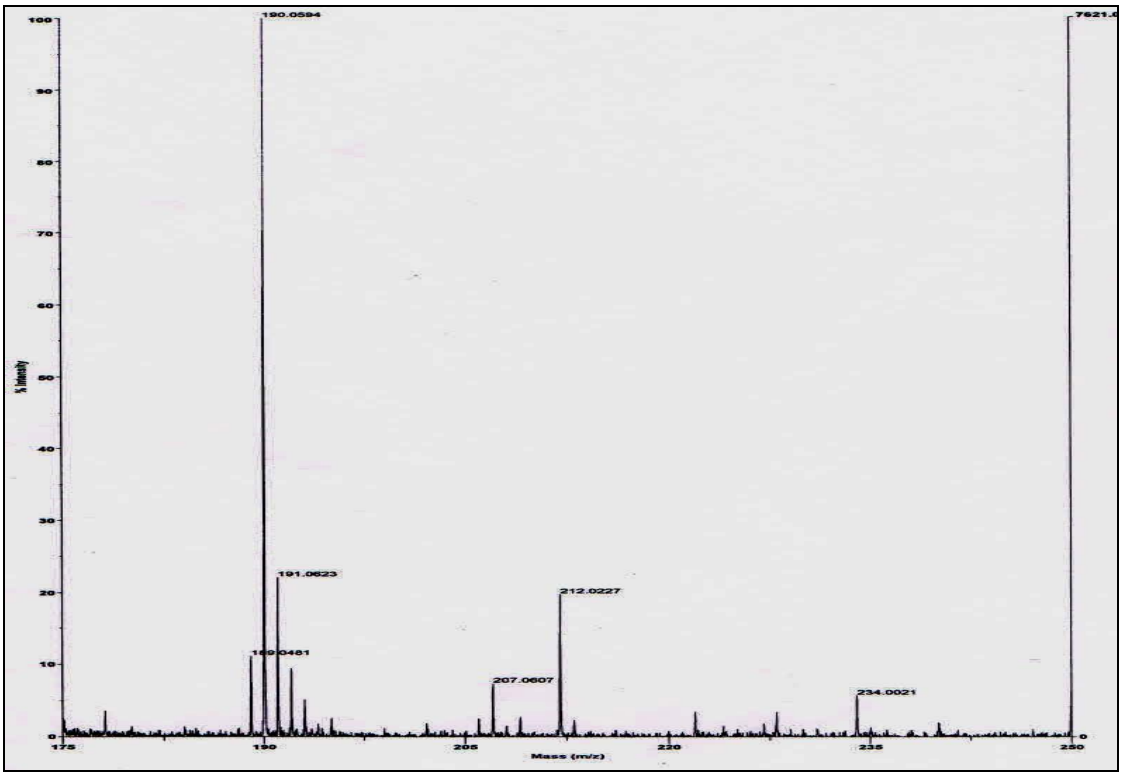

Figure 4: MALDI of 4-Chlorobiphenyl (Control).

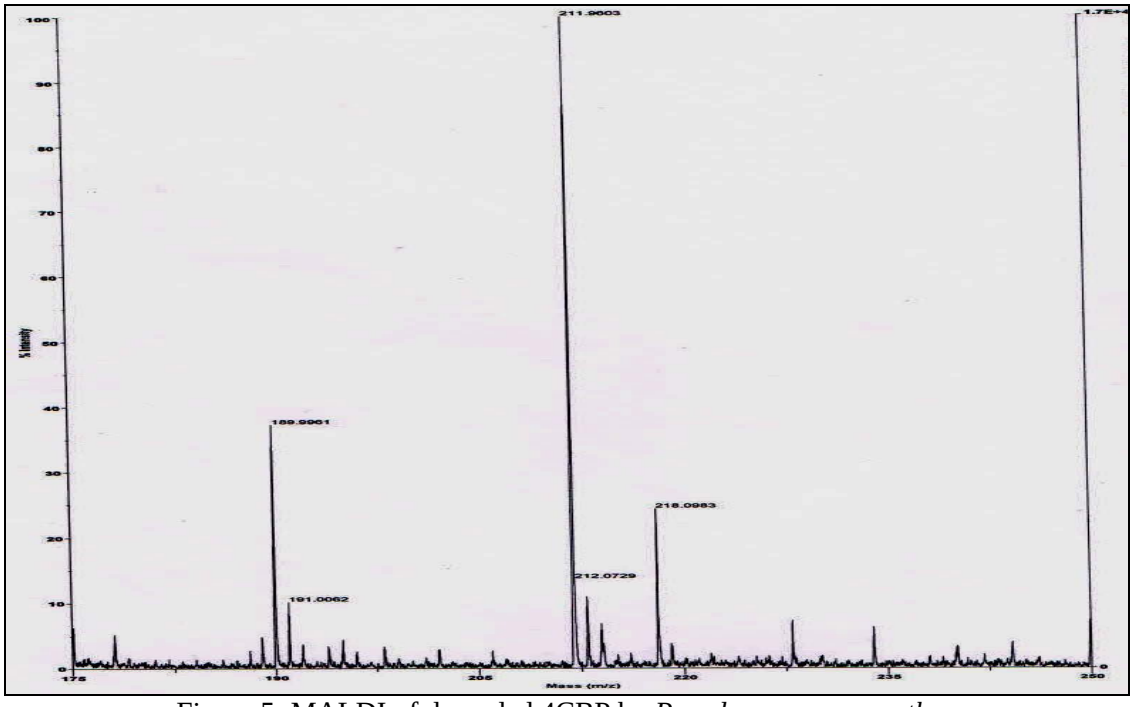

Figure 5: MALDI of degraded 4CBP by *Pseudomonas synxantha*.

CONCLUSIONS

With the help of techniques such as MALDI, HPLC, Uv-Vis spectroscopy it is clear that bacterial strain *Pseudomonas synxantha* isolate was able to degrade PCBs under aerobic conditions.

The use of the bacteria is an important and significant means to degrade contamination caused due to chlorobiphenyls. It can be used to treat this environment pollutant.

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