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Aerobic Degradation of Aroclor 1242 by *Pseudomonas mendocina strain* CL-10.4.

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Abstract
Polychlorinated biphenyl (PCBs), a family of 209 aromatic compounds (congeners) containing 1 to 10 chlorines, are toxic, recalcitrant and ubiquitously distributed contaminants. Aroclors are trade name given to commercial mixtures of PCB's. In the current study, *Pseudomonas mendocina strain CL-10.4.* Was a reported potential PCB degrader was analysed for Aroclor degradation? It was enriched using Mineral Salt Medium supplemented with inducer benzoate. For degradation studies, above isolate was inoculated in medium with biphenyl and Aroclor 1242 to check its degrading/ catabolizing ability which was confirmed by GC-MS analysis. The results obtained from GC-MS analysis proved that the above isolate was capable of completely metabolizing Aroclors into simple degradation products like acetic acid and galactose. The highlight of this study remains complete aerobic degradation of Aroclors which usually occurs under anaerobiosis.

Keywords: pcb's, aroclors, biodegradation, aerobic.

1. Introduction
Polychlorinated biphenyl (PCBs), a family of 209 aromatic compounds (congeners) containing 1 to 10 chlorines, are toxic, recalcitrant and ubiquitously distributed contaminants. Commercial preparations of Aroclor are specified with a four-digit code. The first two numbers in the code refer to the parent structure (12 indicating biphenyl) and the second two digits refers to the weight percentage of chlorine. For example, Aroclor 1242, 1248, 1254 and 1260 refer to PCB mixtures with an average weight percentage of chlorine of 42%, 48%, 54% and 60%, respectively [1]. PCBs are invaluable in the industries because they have properties like high boiling points, a high degree of chemical stability, low flammability and low electrical conductivity. These properties make them highly recalcitrant to biodegradation and they persist in the environment for a long time [2]. They are hydrophobic chemicals and their strong adsorption to organic matter leads to the accumulation in soil and aquatic sediments causing health hazards to human and aquatic life [3, 4]. Prevention of PCBs into the environment and removal of PCB contamination that already exists is necessary to reduce exposure to wildlife. Incineration, thermal desorption, chemical dehalogenation, solvent extraction, soil washing and solidification are some of the established remedial technologies for their removal from the environment [5]. Other potential technologies for PCB remediation are chemical oxidation, vitrification and phytoremediation [5, 6]. Most of these technologies are not cost effective hence the industries are tempted to discard the waste residues without treatment. Therefore, bioremediation using microorganisms can be used as a biofriendly method for the removal of PCB from the environment. The main objective of this study was to study microbial aerobic degradation of Aroclor 1242.

2. Materials & Methods
2.1. Materials: Arocolor 1242 (Sigma Aldrich Ltd.), Biphenyl, Nutrient Agar, Luria agar, Cetrimide agar. All the other material used was AR grade.

2.2. Methods

2.2.1. Enrichment of Aroclor 1242 degrading bacteria: *Pseudomonas mendocina strain CL-10.4* Potential PCB-118 degrader isolated earlier was analysed for its Aroclor 1242 degrading activity. The bacterial isolate was grown on Nutrient Agar, Luria agar and Cetrimide agar plate for enrichment and purity tests.

2.2.2. Study of Aroclor 1242 degrading ability of the isolate: Mineral salt (MS) media, containing yeast extract (50 mg/L), 1.0 g/L KNO3, 0.02 g/L $FeCl_3$, 0.2 g/L $MgSO_4$, 0.1 g/L NaCl, 0.1 g/L $CaCl_2$ and 1.0 g/L K_2HPO_4 was supplemented with benzoate (2.5 mM) as sole carbon source for enrichment of the cells. An uninocluated control was set up to confirm the purity of the MS medium. The isolates were cultivated in 150ml Erlenmeyer flasks and incubated aerobically at 25˚C on an orbital shaker (100 rpm). After 48 hrs of incubation, the broths were centrifuged for 20 min at 3000 rpm. The pelleted cells were washed twice in MS medium lacking benzoate, and resuspended in MS medium without benzoate at an optical density (OD) of 0.5–0.6 at 600 nm. 1 ml (approximately 10^5–10^6 cells/ml) of this washed-cell suspension was used to inoculate 10 ml of assay medium. The assay was carried out in 3 sets containing 3 different sole sources of carbon:

Set 1: The assay medium was MSM supplemented with 1mg/10ml of biphenyl dissolved in 1.0 ml acetone.

Set 2: The assay medium was MSM supplemented with 1mg/10ml of Aroclor1242 (Sigma Aldrich Ltd.) dissolved in 0.1 ml acetone.

Set 3: The assay medium was MSM supplemented with 1mg/10ml of Aroclor1242 and 1mg/10ml of biphenyl dissolved in 0.1 ml acetone.

Aroclors and biphenyl are relatively insoluble in water, the compounds were added to the MS medium in acetone. In order to evaluate any possible loss of the xenobiotics during the incubation, these analyses were also performed on replicate uninoculated controls, which were prepared and incubated under the same conditions of the samples. All the cultivations were performed in 150ml Erlenmeyer flasks and incubated aerobically at 25 ˚C on an orbital shaker (100 rpm) and the flasks were inoculated for 12 days. Reactions were stopped after 12 days by adding 5 ml of hexane, thereafter, mixed continuously on a tube rotator for 12 hrs. Hexane extraction lyses cells and identical congener recoveries are obtained using uninoculated or killed-cell controls. Since no solid phase was present other than biomass, our extraction method allowed almost complete PCB recovery. The organic phase was collected and taken for GC-MS analysis.

2.2.3. GC-MS Analysis: Samples were analyzed on a Hewlett -Packard column Model: GCD - HP1800A Mass range: 10 - 425 amu integrated gas chromatograph - electron ionization detector operated through a data system. HP 1 column has 30m long with internal diameter 0.25mm. The temperature program was set at an initial temperature of 150^0 C (2 min). This was increased by 6^0 C/min to 260^0 C (2 min) and then by 2^0C/min to 290^0C.

3. Result and Discussion

3.1. PCB Degrader isolation: The PCB degrading culture isolated and identified previously as *Pseudomonas mendocina strain CL-10.4.* previously [5] was rechecked for purity using media like Nutrient Agar, Luria agar plate and Cetrimide agar plate. *Pseudomonas* genera is a metabolically versatile microorganism that can catabolize aromatic pollutants. The ability to utilize a wide variety of xenobiotic and aromatic compounds has made *Pseudomonas* genera an attractive research candidate in the field of biodegradation. [7]

3.2. Enrichment of Aroclor degrader:

The isolate was first enriched with benzoate as a sole source of carbon and incubated for 48 hours on shaker.

Fig 1: Growth of the isolate in the enrichment media with benzoate.

For the purpose of successful microbial biodegradation of PCBs to occur; PCB-degrading pathways in aerobic bacteria need to be induced [8] Benzoate is the simplest aromatic salt and is an intermediate of the biodegradation of many aromatic compounds, like PCBs. Thus in this study benzoate was used as an inducer for the Aroclor 1242 degradation pathway. Also knowing that there exists a similarity among PCB degrading micro-organisms who are biphenyl utilising bacteria, which metabolize PCBs with the same suite of enzymes employed in biphenyl catabolism [9, 10], pre-growing the organisms on benzoate rather than biphenyl, suggested constitutive expression of the enzymes of the upper biphenyl degradative pathway [11]. After the enrichment *Pseudomonas mendocina strain CL-10.4* it was evaluated for degradation of Aroclor 1242 using washed, benzoate-grown cells. No carbon sources other than Aroclor 1242 was provided.

3.3. GC – MS Analysis of Control and Isolated Sample: To understand the PCB degradation capabilities of the organisms, activities in the presence of biphenyl as primary growth substrate were analysed. The above isolate was inoculated in three different sets. Set 1: Biphenyl as the sole carbon source; Set 2: Aroclor 1242 as the sole carbon source; 3: Mixture of biphenyl and Aroclor 1242. the biodegradation analysis of Aroclor 1242, metabolites formed were monitored by GC-MS analysis. Degradation of each peak was calculated

by comparison of the area under individual peaks in inoculated reactors with areas of corresponding peaks in uninoculated controls [12],disappearance of the standard Aroclor peaks. The lack of significant disappearance of Aroclor 1242 in replicate, uninoculated control tubes demonstrated that the removal was not due to physical–chemical losses. Since our prolonged hexane extraction lyses cells and also extracts congeners sorbed to cells, disappearance of Aroclor 1242 was not a result of cell sorption. The controls of each set were established to compare the degradation of Aroclor 1242.The GC-MS chromatograms show that the peaks obtained during the analysis. The mass spectrums show the components of the sample eluted out during different retention times and an area report gives us an estimate of the total quantity of that particular separated component. The results obtained by GC-MS analysis were compared with the control of the respective set. The obtained result showed degradation abilities of the organism.

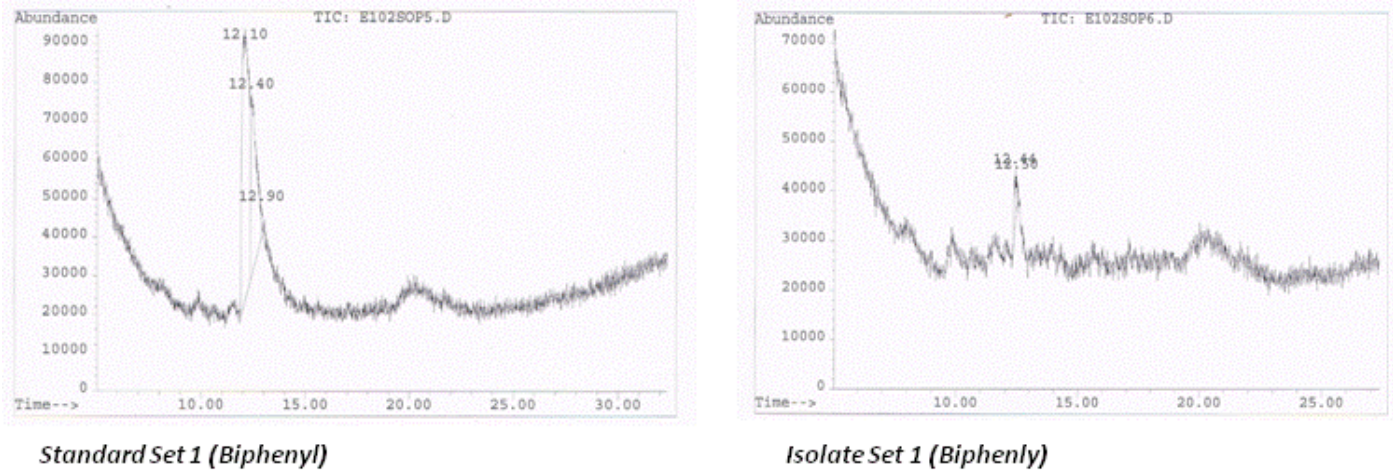

Fig 2: Chromatogram for SET I analysis

In Fig 2.analysis of Set1 shows that the standard peak of biphenyl seen in the standard chromatogram was absent indicating that the organism *Pseudomonas mendocina strain CL-10.4.* is capable of degrading biphenyl. Because there was no standard set I peak of biphenyl seen in the Isolate Set I chromatogram

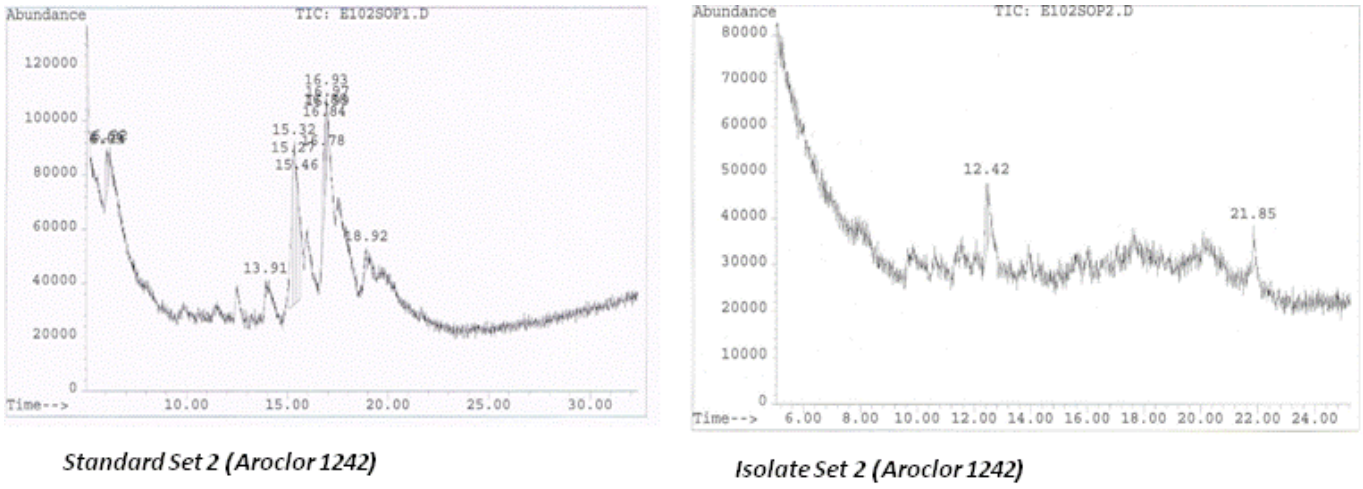

Fig3: Chromatogram for SET II analysis

Fig 3.For Set 2 analysis complete degradation of Aroclor 1242 was observed. Standard peaks for Aroclor congeners were absent in Isolate SET II chromatogram, indicating the elimination of virtually all the detectable peaks and thus concluding that *Pseudomonas mendocina strain CL-10.4.* is capable of complete Aroclor1242 degradation without biphenyl as a primary substrate. The mass spectrum in Isolate Set II obtained did not show presence of any standard congener peaks.

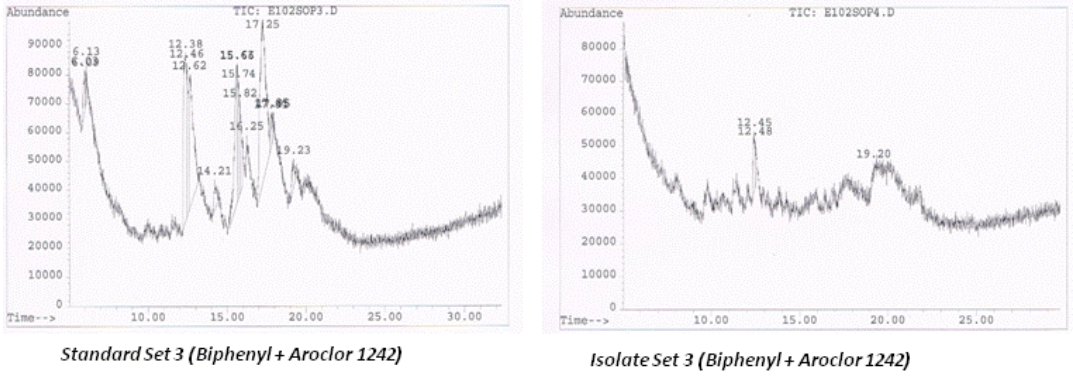

Fig4: Chromatogram for SET III analysis

In Fig.4 for Set 3, complete degradation of Aroclor 1242 was observed. Neither biphenyl nor any of the Aroclor standard peaks were seen in Chromatogram of Isolate Set III indicative of its utilization by the culture. The results obtained were in agreement with the fact that enzymes expressed during growth on biphenyl were involved in PCB catabolism demonstrating better degradation with biphenyl as a primary substrate.

4. Conclusion

Over the past decades, both aerobic and anaerobic microorganisms have been isolated that degrade aromatic compounds. Out of which many isolates such as gram-negative strains of *Pseudomonas, Alcaligenes, Achromobacter, Janibacter*, *Burkholderia*, *Acinetobacter, Comamonas, Sphingomonas,* and *Ralstonia, Enterobacter* and Gram-positive strains, such as *Corynebacterium, Rhodococcus, Bacillus, Paenibacillus,* and also *Arthrobacter* and *Micrococcus* [16,17,18] have shown effective polychlorinated biphenyl degradation. The results obtained are in agreement with the above literature wherein Pseudomonas species is seen effectively degrading the available Aroclor mixtures.The most significant part of *Psudomonas mendocina strain CL-10.4.* as a potential degrader was that during the degradation process in both set 2 and set 3 the organism,formed products like enoic acid, 3,7 benzofurandiol, 2,3-dihydro-2,2-dimethyl and galactose. Enoic acid is the key intermediate that is formed in PCB degradation. After their formation pathways are diverted to catechol/ protocatachuate degradation. Acetic acid is the end product expected after further utilizations of the substrate which was not observed instead galactose was observed which showed even further metabolism of the substrate. The utilisation of benzoate, biphenyls and PCB's indicates that the organism isolated harbours presence of extradiol dioxygenase activity which are related to enzyme families of 2, 3 and 3, 4 di-oxygenases. [19] These enzymes are supposed to be key enzymes in PCB degradation pathway. The potential of such isolates can be applied for PCB and Aroclors degradation in industrial effluents, waste management, water management and thus save the environment from hazards of PCB intake.

5. References

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