International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
Open Access

Original Research Article

Degradation Of Chlorophenolic Compounds By A Moderately Halotolerant Bacterial Consortium Isolated From Saline Environment

Krishnaswamy Veenagayathri[1*] and Namasivayam Vasudevan[2]
[1]Department of Biotechnology, Stella Maris College, Chennai-87, Tamilnadu, India
[2]Microbiology Laboratory, Centre for Environmental Studies, Anna University, Chennai-25, Tamilnadu, India

Received for publication: May 10, 2015; **Revised**: May 20, 2015; **Accepted**: June 05, 2015

Abstract: Chlorophenols are carcinogenic and toxic environmental pollutants which are massively discharged into the environment from uncontrolled industrial activities. This present study focuses on the degradation of different concentrations of substituted chlorophenols by a moderately halotolerant bacterial consortium. The isolated bacterial consortium was able to grow from primary chlorophenol to pentachlorophenol. The moderately halotolerant bacterial consortium was able to utilize up to 100 mg/L of 4-CP to 2,4,6-TCP. Increase in the concentrations of chlorophenol decreased the degradation of chlorophenol due to the recalcitrant nature of the higher substituted chlorophenols. The intermediates produced during the degradation of 4-CP, 2,4- DCP and 2,4, 6 TCP were identified by Gas chromatography Mass- Spectroscopy, which proved the degradation of the chlorophenolic compounds. Such isolated moderately halotolerant bacterial consortium could be employed in the treatment of chlorophenols containing saline wastewater.

Key words: Chlorophenols; Halotolerant Bacterial consortium; Saline Environment; Wastewater

Introduction

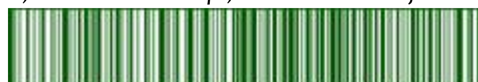
Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds (Annadurai *et al.,* 2000). It is an organic, aromatic compound that occurs naturally in the environment (Prpich and Daugulis, 2005), but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing and the production of paints and varnish (Mahadevaswamy *et al.,* 1997; Banyopadhyay *et al.,* 1998). This aromatic compound is water-soluble and highly mobile (Collins and Daugulis, 1997) and as such wastewaters generated from these industrial activities contain high concentrations of phenolic compounds (Chang *et al.,* 1998) which eventually may reach down to streams, rivers, lakes, and soil, representing a serious ecological problem due to their widespread use and occurrence throughout the environment (Fava *et al.,* 1995). The adverse effects of phenol on health are well documented (Calabrese and Kenyon, 1991) and death among adults has been reported with ingestion of phenol ranging from 1 to 32 g (Prpich and Daugulis, 2005). The low volatility of phenol and its affinity for water make oral consumption of contaminated water the greatest risk to humans (Prpich and Daugulis, 2005).

The degradation of phenolic compounds by both pure and mixed cultures under non-saline conditions, however, very few reports have been documented, especially by moderately halophilic bacteria on biodegradation of phenol (Alva and Peyton (2003), Garcia *et al.,* 2005, Hinteregger and Streichsbier 1997). The degradation potential of a halophilic bacterium *Halomonas organivorans* was studied to catabolise different concentrations of low molecular weight aromatic compounds at 10% NaCl concentration (Garcia *et al.,* 2005). Wang *et al.,* (2009) indicated that there was almost no effect on the growth of *Arthrobacter* sp. and the degradation of mixtures of phenol (200 mg/L) and *p*-cresol (100 mg/L) with less than 5% NaCl in 88 h. However the use of bacterial consortium in degradation phenolic compounds in saline industrial effluents has been ignored. The present study focuses on a moderately halophilic bacterial consortium grown on different phenolic compounds at different concentrations. The ability of the consortium to treat Phenol-contaminated saline wastewater was also studied.

Materials and Methods

Bacterial consortium

Soil samples were collected from different ecosystems in Chennai such as salt pan, Puliket marine back water lake; Sea harbor (Chennai), tannery affected soils and soil from sea food industries. The bacterial consortium was isolated by enrichment culture technique, where the soil sample (300g wet weight) was mixed in sterile distilled water (1:1 w/v) for 1 h at room temperature. During the initial adaptation stage the consortium was enriched with phenol 50 mg/L (Concentration of Phenol) and they were biochemically characterized, having six strains, of which four strains were gram positive and two strains gram negative. Further analyses by cloning and 16S rRNA gene sequence analysis, identified the isolates as *Bacillus cereus, Arthrobacter sp., Bacillus licheniformis,*



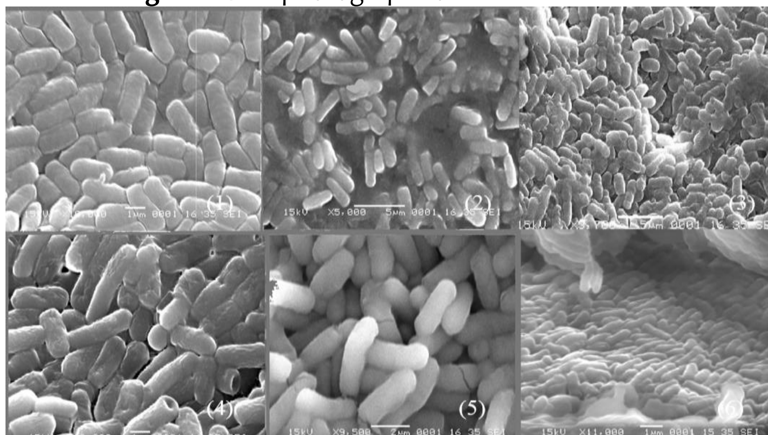
***Corresponding Author:**
Dr. Krishnaswamy Veenagayathri,
Department of Biotechnology,
Stella Maris College, Chennai -87,
Tamilnadu, India.

Halomonas salina, Bacillus pumilus and *Pseudomonas aeruginosa* (VeenaGayathri and Vasudevan 2010).

Scanning electron microscopy

The sample preparation for SEM was carried out according to the method of Prior and Perkins (1974). The isolates were grown on mineral salts medium (MSM) for 24h, centrifuged and pellets were immediately resuspended in 2% glutaraldehyde with 0.05M phosphate buffer and 4% sucrose (pH 7.3). Pellets were placed on aluminium foil disks, air dried, platinum coated and examined under SEM (JEOL JSM-6360)

Figure 1: SEM photographs of the six bacterial strains



(1) KVGNV1 (2) KVGNV2 (3) KVGNV3 (4) KVGNV4 (5) KVGNV5 (6) KVGNV6

Chemicals

The Phenolic compounds were purchased from Sigma Aldrich and all other chemicals (analar grade) were purchased from Merck, India.

Enrichment of a Chlorophenol-degrading bacterial consortium

The bacterial consortium were enriched in mineral salts medium of NaCl 10.0-100.0, KH_2PO_4 0.25, NH_4Cl 1.0, Na_2BO_7 2.0, $FeCl_3$ 0.0125, $CaCl_2$ 0.06 and $MgCl_2$ 0.05 all the salts as gram in one liter. The medium was supplemented with a specified amount of added NaCl, and 10 mg of yeast extract adjusted to pH - 7. Alva *et al.,* (2003). The medium was autoclaved, cooled to room temperature and was amended with phenol (50 mg/L) through a sterile filter (0.45µm) in 250 ml Erlenmeyer flasks. The bacterial consortium was enriched on chlorophenol (50 mg/L) in mineral salts medium with 50 g/L NaCl concentration. The culture was then transferred, four times after every four days. Increase in cell count of the bacterial consortium from 10^5 to 10^6 cfu/mL with phenol was taken as a confirmation for utilization of phenol. Once the consortium was fully acclimatized to phenol, its ability was analyzed to grow on substituted chlorophenols (2-Chlorophenol (2-CP), 4-Chlorophenol (4-CP), 2,4-Dichlorophenol (2,4DCP), 2,4,6-Trichlorophenol (2,4,6-TCP) and Pentachlorophenol (PCP). As the optimum concentration for degradation of chlorophenols was 5% in the range from 3- 10% NaCl, all the experiments were performed at 5% NaCl.

Preliminary studies on chlorophenol degradation by the bacterial consortium

Chlorophenolic compounds were added to the medium at different concentrations. The bacterial consortium isolated from the marine environment was grown and the plate count (cfu/mL) was checked daily. Cell morphology and the motility of cells in exponentially-growing liquid cultures were examined on freshly-prepared wet mounts by light microscopy. Plate counting (cfu/mL) was done on nutrient agar medium. The bacterial consortium was studied for its growth on the phenolic compounds with phenol as the sole carbon source.

For the degradation study, mineral medium containing phenolic compounds was inoculated with the bacterial consortium. Different conditions used for the degradation of phenolic compounds were (i) medium + chlorophenol + bacterial consortium; (ii) medium + chlorophenol and (iii) medium + bacterial consortium, with (ii) and (iii) serving as controls. The bacterial consortium was added to the medium at concentrations of 10^4– 10^5 cfu/mL. The culture, in duplicate, was incubated at 37 °C with shaking at 150 rpm and extracted every 24 h for 5 days. Each culture was extracted twice with dichloromethane (v/v) after acidification to pH 2.5 with 1N HCl. The extracts were filtered through anhydrous sodium sulphate and condensed to 1 mL with a rotavapour (Buchi, Germany) for further GC analysis.

Total protein analysis

For analysis of total cell protein, samples were centrifuged at 12,000 rpm for 10 minutes and washed with fresh (substrate-free) mineral medium, then centrifuged and washed few times to remove the substrate. The pellet from each sample was then disrupted by sonication at 30% amplitude for a total of 3 minutes (1.5min x 2) in an ice-water bath. To 0.5 mL of sample was added to 0.5 mL Coomassie Blue protein dye and the absorbance at 595nm were measured. Total protein concentration was determined by calibration with bovine serum albumin standards according to Bradford (1976).

Gas chromatographic analysis of phenolic compound degradation

The ability of the consortium to utilize phenol as sole carbon source was determined by growing it in the mineral medium containing different concentrations of phenol from 50 mg/L to 250mg/L.

The cell suspensions were clarified by centrifugation at (10,000rpm for 15 min, 6°C). The culture supernatant was extracted with dichloromethane and filtered through a 0.2µm Gelman filter acro disc, prior to analysis in gas chromatograph (Chemito GC Model No 1000) equipped with FID detector and capillary column(Varian Chromopak capillary column CP SIL 8CB, 30m X 0.32mm with detection limit of 10 ppt of the compound. Nitrogen was used as a carrier gas, injector temperature was 220°C, detector temperature was 250°C and the oven temperature of the column was maintained at 150°C. Standard solutions of different phenolic compounds were used for reference. The samples were injected one by one and the utilization rates of phenolic compounds were calculated based on the peak area percent and retention time.

Gas Chromatography-Mass Spectral (GC-MS) Analysis

A GC-MS analysis was performed with GC-MS-QP2010 [SHIMADZU] with an inert mass selective detector and a computer workstation was used for the Phenolic compounds analysis. The samples were silylated before analysis. The GC–MS was equipped with: an Agilent DB-5 capillary column (30m x 0.25mm id x 0.25 µm); with an injection volume of 1 µL, split ratio of 20 injection at 280°C and an ion source temperature at 200°C. Oven operating temperature was 80°C with the holding time of 1 min, 300 °C for 2 mins with the total time of 41.67 mts. The masses of primary and secondary phenolic compound ions were determined by using the scan mode with impact ionization (70 eV, 200°C) for pure phenolic compounds standards (Merck). Qualitative analysis of Phenols was performed by using the selected ion monitoring (SIM) mode. Fragmented products were identified using computer station library search. Retention time of the fragmented products are further compared and confirmed by analyzing authentic standards. Helium was used as the carrier gas.

RESULTS AND DISCUSSION

This present study was conducted to understand the rate of degradation of different chlorophenolic compounds at various concentrations and to identify the metabolites produced by a moderately halotolerant bacterial consortium. Figure 1 shows the morphological identification of the six bacterial strains present in the bacterial consortium observed under scanning electron microscope. The 16S rRNA gene sequence analysis, identified the isolates of the consortium as *Bacillus cereus, Arthrobacter sp., Bacillus licheniformis, Halomonas salina, Bacillus pumilus* and *Pseudomonas aeruginosa* (VeenaGayathri and Vasudevan 2010).

Growth of the halotolerant bacterial consortium on chlorophenols

The isolated bacterial consortium was grown on different substituted chlorophenols (2-CP, 4-CP, 2,4-DCP, 2,4,6-TCP and PCP). The growth of the bacterial consortium was very less on PCP. The growth of bacterial consortium on PCP was very slow, probably because of its recalcitrant structure with five chlorine atoms substitutes attached to the aromatic ring structure.

The isolated bacterial consortium utilized the chlorophenols, where during the utilization of 2-CP and 4-CP the viable cell counts increased to 7×10^6 and 4×10^6 cfu/mL respectively. The maximum cell count of the bacterial consortium with 2,4-DCP increased to 6×10^6 cfu/mL, the viable cell count for 2,4,6-TCP was comparatively less, where the cell count was 5×10^5 cfu/mL on 3rd day. The cell count was very less when the bacterial consortium was grown on PCP ($4{\times}10^5$cfu/mL) Figure 2.

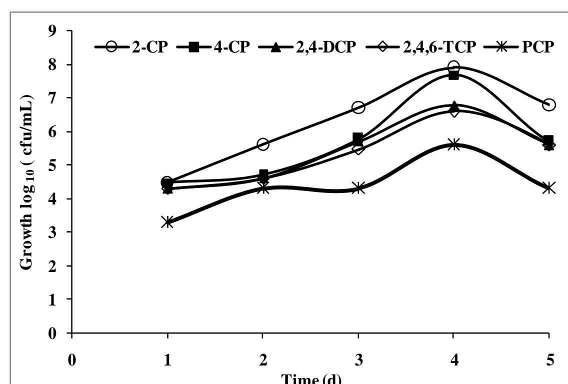


Figure 2: Degradation of different Chlorophenols at 5% NaCl by the bacterial consortium

Garcia *et al.*, (2005) studied the degradation of low- molecular- weight aromatic compounds (benzoic acid, *p*-hydroxy benzoic acid, cinnamic acid, phenylacetic acid, *p*-coumaric acid, ferulic acid, salicylic acid) by a group of halophilic bacteria. When the isolates were enriched on phenol, they were able to utilize a greater number of aromatic compounds than the rest of the isolates enriched on other aromatic compounds other than phenol, showing their wider substrate specificity. But the mixed isolates were unable to utilize *p*-Cresol. In the present work the isolated bacterial consortium was able to grow with most of the substituted chlorophenols under saline conditions.

Degradation of different chlorophenols at various concentrations by the bacterial consortium

To study the degradation of chlorophenols each representive of ring structured compounds one ring- 4-CP (Chlorophenol), two ring-2,4- DCP

(Dichlorophenol), Three ring- 2,4,6- TCP (Trichlorophenol) was studied for degradation at different concentrations.

Batch study performed at 25 mg/L of 4-CP, showed a maximum degradation of 93 %, with a protein yield of 37.5 mg/L end of two days. When the concentration of 4-CP was increased to 50 and 75 mg/L the degradation reached 90% and 88% respectively with a decrease in the protein yield on the 2nd day 35.4 mg/L and 32.1 mg/L respectively. At 100 mg/L, the degradation of 4-CP reduced to 84% with a respective decrease in the protein yield on the second day to 27.5 mg/L. This indicated that the growth of the bacterial consortium reduced with the increase in substrate concentration and there was no further degradation. An increase in the 4-CP concentration to 100 mg/L decreased the degradation, which might be due to the toxic effects of the compound at its higher concentration Figure 3.

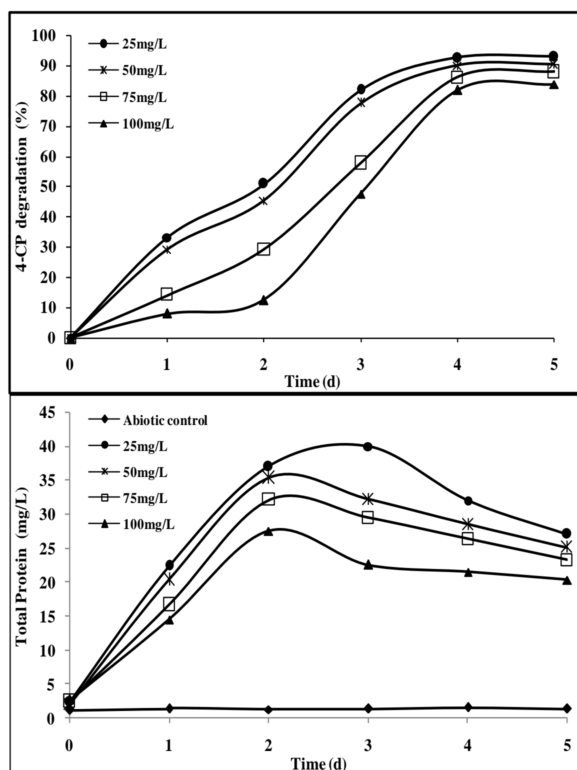


Figure 3: Degradation of different concentrations of 4-CP at 5% NaCl by the bacterial

2,4–dichlorophenol (2,4-DCP) is a key intermediate in the synthesis of chloride-based herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4-dichlorophenoxy propionic acid (2,4 –DP). To our knowledge there are no reports on the degradation of 2,4- DCP by the bacterial consortium under saline conditions. The bacterial consortium in the present study utilised 2,4- DCP, as sole source of carbon and energy source at 25 mg/L to 100 mg/L concentrations at 50 g/L NaCl (Figure 4). Initially at 25 mg/L the degradation of 2,4-DCP was 92% with a maximum protein yield of 32.5 mg/L on the 2nd day. At substrate concentrations of 50 mg/L and 75 mg/L, the degradation decreased to 88% and 86% respectively with the a maximum protein yield on the 3rd day of 30 and 26.5 mg/L respectively. The degradation of 2,4-DCP reduced relatively to 82% with a maximum protein yield of 25.6 mg/L at 100 mg/L of 2,4- DCP. To our knowledge there are no reports on the degradation of 2,4-DCP under saline conditions, However, biodegradation of chlorophenols by a variety of microorganisms have been studied by many authors (Kim *et al.,* 2002, Kargi and Eker 2005) under non-saline conditions. According to these studies, the biodegradability of chlorophenols depends on the number and position of halogens in the aromatic ring. Furthermore, high chlorophenol concentrations are known to be inhibitory to microbial growth.

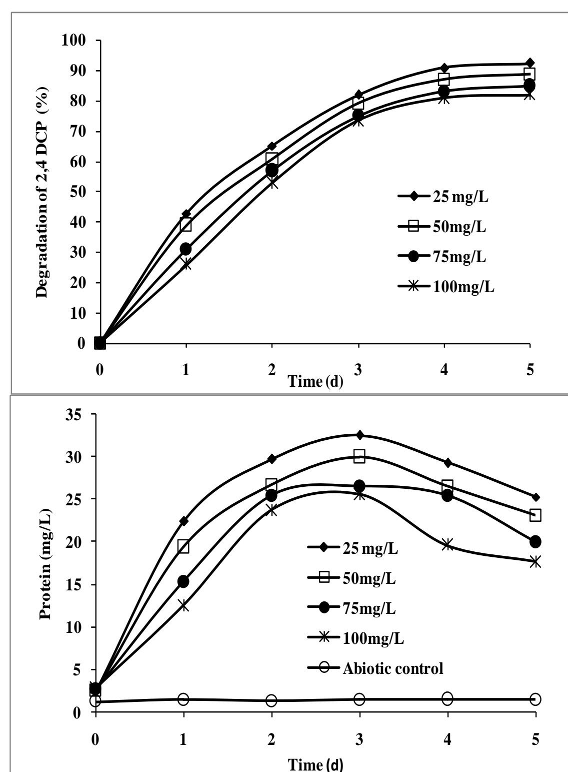


Figure 4: Degradation of different concentrations of 2.4-DCP at 5% NaCl by the bacterial consortium

From the six isomers of trichlorophenols, 2,4,6-TCP has been reported as highly toxic and frequently discharged into the aquatic environment. Hence batch studies were conducted with different concentrations of 2,4,6-TCP by the bacterial consortium at optimum salinity of 5% NaCl. The protein yield of the consortium and degradation was examined, with different concentrations of 2,4,6-TCP ranging from 25 to 100 mg/L. The protein yield of the bacterial consortium on 2,4,6- TCP was comparatively less than

other phenolic compounds used in the study. This might be due to the recalcitrance of the compound. Initially at 25 mg/L of 2,4,6-TCP the degradation was 89% with a maximum protein yield to 30.3 mg/L in 2 days. When the concentrations of 2,4,6-TCP was increased to 50 mg/L and 75 mg/L the degradation reduced to 84% and 80% with the corresponding decrease in the protein yield to 25.4 and 28.4 respectively. The degradation of 4-CP, dropped significantly with 100 mg/L of the substrate to 74% with protein yield increasing on the 2nd day to 21.5 mg/L. Figure 5 illustrates the degradation of 2,4,6-TCP and protein yield by the bacterial consortium at 50 g/L NaCl. The results showed that the increase in the concentration of the substrate reduced the growth of the consortium which was intern represented by the protein yield. When the concentration of the substrate increased to 100 mg/L the degradation reduced to 74 %, this might be due to toxicity of the substrate or unavailability of 2,4,6 -TCP to the consortium or accumulation of toxic metabolites or depletion of nutrients such as nitrogen and phosphate or all these factors together would have inhibited the growth of the bacterial consortium. Another possible reason would be other than the substrate no other carbon source like glucose was supplied in the medium.

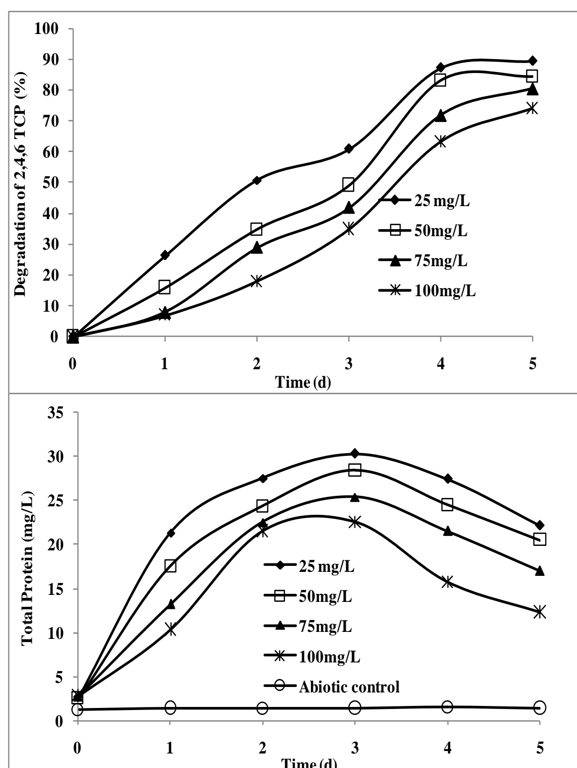


Figure 5: Degradation of different concentrations of 2,4,6-TCP at 5% NaCl by the bacterial consortium

Herrera *et al.*, (2008) reported the degradation 2,4-DCP by a *Bacillus* consortium (CPHY1).Where GC-MS of the culture supernatant showed only 4-CP was produced as the intermediate, which indicated that 2,4-DCP was incompletely degraded. In the present study the detection of the 3,5-dichlorocatechol in the supernatant showed that the substrate 2,4-DCP was degraded to form its intermediate metabolite. Dichlorocatechol is the central metabolite in the aerobic degradation of a wide range of chlorinated aromatic compounds (Farell and Quilty 1999). Degradation of 2,4-DCP by pure cultures and mixed cultures was reported by many other researchers with dichlorocatechol as the intermediate (Quan *et al.*, 2004, Matafonova *et al.*, 2006, Sahinkaya *et al.*, 2007).

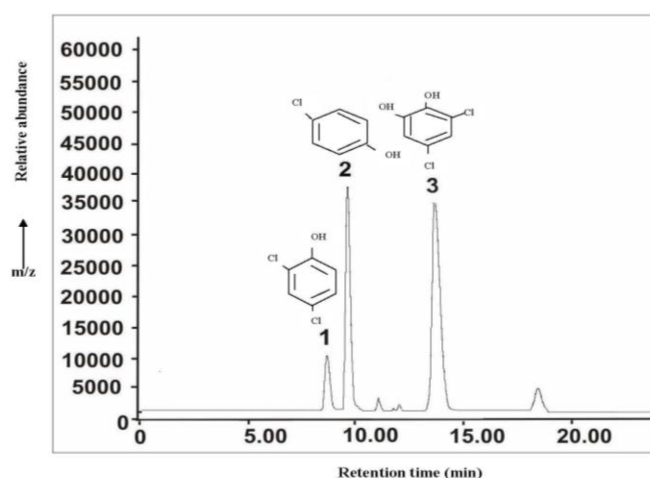


Figure 6: GC-MS Spectrum of the metabolites formed during degradation of 2,4-DCP

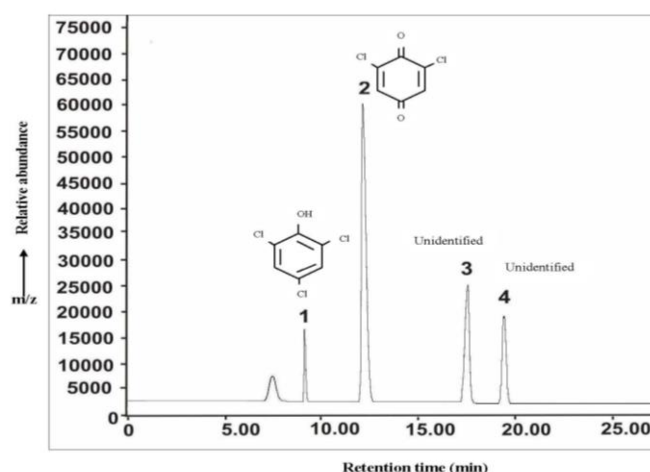


Figure 7: GC-MS Spectrum of the metabolites formed during degradation of 2,4,6-TCP

Kharoune *et al.*, (2002) reported the degradation of 2,4,6-TCP (200 mg/L) under non-saline conditions by a microbial consortium containing *Sphingomonas paucimobilis*, *Burkholderia cepacia*, *Chryseomonas luteola* and *Vibrio metschnikovii*, where only of about 51 mg of 2,4,6-TCP g/L cell protein/h was degraded in 160 h.

Pamukoglu and Kargi (2008) reported the degradation of 2,4,6-TCP under non –saline conditions by *Rhodococcus rhodochrous* (DSM 43241) where 150 mg/L of 2,4,6-TCP inhibited the growth and degradation, in the presence of glucose as the additional carbon source. The bacterial consortium effectively degraded mono- and di-chlorophenols when these were used as the only sources of carbon and energy. The biodegradability of the compounds decreased in the order: 4-CP >2,4-DCP >2,4,6-TCP. In studies of chlorophenol biodegradation, it can be noted 4-CP, 2,4-DCP were degraded readily than 2,4,6-TCP.

Identification of metabolites formed during biodegradation of Chlorophenols

The intermediates formed during the degradation of 2,4- DCP was analyzed by GC-MS and is presented in the Figure 6. The mass spectrum from the 72 h culture filtrate were compared with the standards (4-CP and 2,4-DCP). The chromatogram showed 3 peaks, first peak at a retention time of 9.314 represented the parent compound 2,4-DCP with masses m/z (40,49,63,73,97,98,125,126,145,162), peak 2 at retention time 9.717 represented 4-CP with masses m/z (14,18,39,46,65,73,93,100,128) followed by peak 3 representing 3,5-dichlorocatechol at retention time of 14.131 with masses m/z (40,51,63,83,87,98,115,126,144,170,185,200,259,274). A comparison of the mass spectra from intermediates of sample-extracted compounds with the standards (2,4,6- TCP) showed that the peaks in Figure 7 are 2,4,6-TCP (peak 1) at retention time of 9.825 the corresponding mass yielded m/z (36,48,62,73,97,99,125,132,160,169,196), the peak 2 represented the intermediate 2,6-dichlorohydroquinone with m/z (53,60,88,120,141,158,176) at retention time of 12.025. The peak 3 and peak 4 were unidentified products. Peak 2 showing 2,6-dichlorohydroquinone indicated that 2,4,6 TCP was dechlorinated to form its intermediate. Figure 7 represents the GC-MS analysis results of 2,4,6-TCP, where the structure of the compounds are represented at the top of the peak. From the results it was obvious that 2,6 –dichlorohydroquinone was the intermediate formed from 2,4,6-TCP degradation which may further undergo oxidation to the corresponding quinone compound and is only detectable when it is present in appreciable amounts. Sanchez and Gonzalez (2007) reported that the 2,6–dichloroquinone was produced during the degradation of 2,4,6-TCP.

Xun and Webster (2003), studied the degradation of 2,4,6-TCP by *Ralstonia eutropha* JMP134 and described that 2,4,6-TCP monooxygenase catalyzes sequential dechlorination of 2,4,6-TCP to 6-chlorohydroxyquinol. The monooxygenase converts 2,4,6-TCP to 6-chlorohydroxyquinone by means of two different reactions first it oxidizes 2,4,6-TCP to 2,6-dichloroquinone, and then it hydrolyzes 2,6-dichloroquinone to 6-chlorohydroxyquinone.

Conclusion

The bacterial consortium enriched from the saline environment was capable of degrading substituted chlorophenols at 50 g/L of NaCl concentration. The intermediates produced during the degradation proved the utilization of chlorophenols by the halotolreant bacterial consortium under saline conditions. Thus, the present study gives promising idea on the promising degradation of chlorophenol contaminated environment by a moderately halotolerant bacterial consortium under saline condition.

References

1. Alva VA, Peyton BM Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: infl uence of pH and salinity. Environ Sci Technol 2003, 37: 4397-4402.
2. Annadurai G, Balan SM, Murugesan T. Design of experiments in the biodegradation of phenol using immobilized *Pseudomonas pictorium* (NICM- 2077) on activated carbon. Bioproc. Eng. 2000, 22: 101-107.
3. Bandyopadhyay K, Das D, Maiti BR. Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. Bioproc. Eng. 1998,18: 373-377.
4. Bradford M.M., A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem, 1976, 72, 248-254.
5. Chang YH, Li CT, Chang MC, Shieh WK. Batch phenol degradation by *Candida tropicalis* and its fusant. Biotechnol. Bioeng. 1998, 60: 391-395.
6. Collins LD, Daugulis AJ. Biodegradation of phenol at high initial concentration in two-phase partitioning batch and fed-batch bioreactors. Biotechnol. Bioeng. 1997, 55: 155-162.
7. Farrell A. and Quilty B., Degradation of mono-chlorophenols by a mixed microbial community via a meta-cleavage pathway, Biodegradation, 1999, 10, 353-362.
8. Fava F, Armenante PM, Kafkewitz D, Marchetti L. Influence of organic and inorganic growth supplements on the aerobic biodegradation of chlorobenzoic acid. Appl. Microbiol. Biotechnol. 1995, 43: 171-177.
9. Felsenstein J Confi dence limits on phylogenies: An approach using the bootstrap. Evolution 1985,39: 783-791.

10. García MT, Mellado E, Ostos JC, Ventosa A *Halomonas organivorans* sp. nov., a moderate halophile able to degrade aromatic compounds. Int J Syst Evol Microbiol 2004, 54: 1723–1728.

11. Garcia MT, Ventosa A, Mellado E Catabolic versatility of aromatic compound- degrading halophiic bacteria. FEMS Microbiol Ecol 2005, 54: 97-109.

12. Herrera Y., Okoh A.I., Alvarez L., Robledo N., Trejo-Hernandez T.H., Biodegradation of 2,4-dichlorophenol by a *Bacillus* consortium World, J. Microbiol. Biotechnol., 2008, 24, 55-60.

13. Hinteregger C, Streichsbier F *Halomonas* sp., a moderately halophilic strain, for biotreatment of saline phenolic waste-water. Biotechnol Lett 1997, 19: 1099–1102.

14. Kargi F. and Eker S., Removal of 2,4 -Dichlorophenol and toxicity from synthetic wastewater in a rotating perforated tube film reactor, Proc. Biochem., 2005, 40, 205-211.

15. Kharoune L., Kharoune M. and Lebeault J.M., Aerobic degradation of 2,4,6-trichlorophenol by a microbial consortium – selection and characterization of microbial consortium, Appl. Microbiol. Biotechnol., 2002, 59, 112-117.

16. Kim J.H., Oh K.K., Lee S.T., Kim S.W. and Hong S.I., Biodegradation of phenol and chlorophenol with defined mixed culture in shake-flasks and a packed bed reactor. Proc. Biochem., 2002, 37, 1367-1373.

17. Mahadevaswamy M, Mall ID, Prasad B, Mishra IM. Removal of phenol by adsorption on coal fly ash and activated carbon. Pollut. Res. 1997, 16 (3): 170-175.

18. Matafonova G., Shirapova G., Zimmer C., Giffhorn F., Batoev V. and Kohring G.W., Degradation of 2,4-dichlorophenol by *Bacillus* sp. isolated from an aeration pond of Baikalsk pulp and paper mill (Russia), Int. Biodeterio. Biodeg., 2006, 58, 209-212.

19. Pamukoglu M.Y. and Kargi F., Biodegradation kinetics of 2,4,6-trichlorophenol by *Rhodococcus rhodochrous* in batch culture. Enz.Microb.Technol., 2008, 43, No. 1,43-47.

20. Prior and Perkins, Artifacts induced by preparation for SEM in *Proteus mirabilis* exposed to carbenicillin, Can. J. Microbiol., 1974, 20, 794-795.

21. Prpich GP, Daugulis AJ. Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two-phase partitioning bioreactor. Biodegradation 2005,16: 329-339.

22. Quan X., Shi H., Hong L., Wang J. and Qian Y. Removal of 2,4-dichlorophenol in a conventional activated sludge system through Bioaugmentation. Process Biochem., 2004, 39, 1701-1707.

23. Sahinkaya E. and Dilek F.B., Biodegradation kinetics of 2,4-dichlorophenol by acclimated Mixed cultures. J. Biotech. 2007, 127, 4, 716-726.

24. Sanchez M.A. and Gonzalez B., Genetic Characterization of 2,4,6-Trichlorophenol degradation in *Cupriavidus necator* JMP134. Appl. Environ. Microbiol., 2007, 73, 9, 2769-2776.

25. Veena Gayathri K and Vasudevan N. Enrichment and Isolation of Phenol degrading Moderately Halophilic Bacterial Consortium from Saline Environment. J Biorem Biodeg; (2010, 1(1) 1-6.

26. Wang P, Qu Y, Zhou J Biodegradation of Mixed Phenolic Compounds Under High Salt Conditions and Salinity Fluctuations by *Arthrobacter* sp. W1. Appl Biochem Biotechnol 2009, 159: 623-633.

27. Xun L., and Webster C.M. A Monooxygenase Catalyzes Sequential Dechlorinations of 2,4,6-Trichlorophenol by Oxidative and Hydrolytic Reactions. J.Biol.Chem. 2003, 279, 8, 6696–6700.

CITE THIS ARTICLE AS:

Krishnaswamy Veenagayathri1 and Namasivayam Vasudevan, Degradation Of Chlorophenolic Compounds By A Moderately Halotolerant Bacterial Consortium Isolated From Saline Environment, International Journal of Bioassays, 2015, 4 (07), 4102-4108.

Source of support: Nil
Conflict of interest: None Declared