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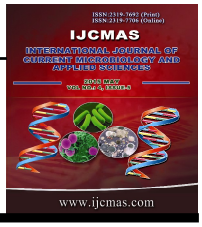

Original Research Article

Degradation of dual phenolics by a moderately halophilic bacterial consortium and its degradation products

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Keywords

Biodegradation, Phenolic compounds, Dual substrates, Halophilic bacterial consortium, Salinity

A B S T R A C T

The biodegradability of phenolic substrates such as phenol and cresols by a moderately halophilic bacterial consortium isolated from saline environment was investigated. The phenolic compounds degrading moderately halophilic bacterial consortium was isolated from a mixture of soil samples collected from saline environments in Chennai, India. The isolated consortium could degrade phenol at different salt concentrations at optimum salinity of 5 % NaCl. The isolated bacterial consortium had the capability to degrade phenolic substrates as carbon source as individual substrates and as well as mixtures under saline conditions. The bacterial consortium degraded phenol from 100 to 300 mg/L, at 300 mg/L the degradation efficiency reduced to 55 %, increase in the concentration of the substrate decreased the degradation under saline conditions. The bacterial consortium was able to degrade the substituted cresols (*o*-,*m*-,*p* cresols) up to 100 mg/L, where *o*-cresol showed a maximum degradation of 93 %. The bacterial consortium degraded 95 % of phenol and 91% of *o*-cresol as dual substrates. Metabolites formed during the degradation of dual substrates showed that the consortium followed ortho cleavage pathway during the degradation.

Introduction

Phenols and its derivatives are the most frequently found pollutants in rivers, industrial effluents and landfill run-off waters. Phenols are commonly employed in many industries, such as coke, refineries, and manufacturers of resin, pharmaceuticals, pesticides, dyes, plastics, explosives and herbicides, as well as occurring in their wastewaters (Lop and Tar 2000; Lows et al. 1994). The main concern with phenolic compounds is the toxicity they pose to public health, aquatic life and humans through bioaccumulation (Fagbote and Olaufekum 2010; Lee and Byeon, 2010).

Saline and hyper-saline environments are frequently contaminated with organic compounds as a result of industrial activities (Margesin and Schinner 2001; Oren et al. 1992). Contamination of these habitats constitutes a serious environmental problem mainly due to the high toxicity exhibited by

the organic compounds. Worldwide, hypersaline effluents are likely to represent up to 5 % of the total global wastewater production. Several industrial processes, such as pesticide, chemical and pharmaceutical as well as gas and oil extraction, generate thousands of millions of litres of saline to highly saline wastewaters (Lefebvre and Moletta 2006). Major organic contaminants of the saline environments are the xenobiotic compounds that include mainly polyaromatic hydrocarbons and phenolic compounds.

Saline effluents are usually treated through physico-chemical means, as the conventional biological treatment is known to be strongly inhibited by salt (mainly NaCl). However, physico-chemical techniques are energy-consuming and their start-up and running costs are high. Some times the level of salt in the effluent is close to 120 g/L (mainly NaCl and KCl), makes it impossible for the operation of a conventional wastewater treatment system (Ludzack and Noran 1965; Zhuang et al. 2010). The conventional micro-organisms are sensitive to abrupt ionic changes and, in addition, they do not tolerate salt concentrations higher than 30 g/L. Lastly, bacterial acclimatization to salt is quickly lost if salinity suddenly drops.

Halophiles are microorganisms, which are included in extremophiles that not only thrive in but also requires saline conditions for their growth. The ability of halophiles/ halotolerants to oxidize pollutants in the presence of NaCl is useful for the biological treatment of saline ecosystems contaminated with organic pollutants (Margesin and Schinner 2001; Mohanan et al. 2007). Halophilic bacteria have adapted to the osmotic stress of high salinity environments by actively accumulating K^+, glycerol, betaine and other compatible solutes within the cell, thus allowing the halophile to balance intra and extra cellular ionic strength to maintain cytoplasmic hydration. (Woolard and Irvine 1995). Hence, halo-adaptation method employed by halophilic and halotolerant bacteria may aid them to more efficiently degrade a wide range of substrates because their intracellular enzymes are conventional, or the same as non-halophiles (Oren et al. 1992). Phenol degradation by pure and mixed cultures has been reported under non-saline conditions. However very few report is available on the degradation of phenol by pure cultures under saline conditions (Hinteregger and Streichsbier 1997; Bastos et al. 2000; Garcia et al. 2001; Munoz et al. 2001; Alva and Peyton 2003; Garcia et al. 2005; Wang et al. 2009).

Previous studies on the degradation of phenol under saline conditions were mainly focused on the biodegradation using single target compound. As phenolic compounds are present as mixtures in pharmaceutical wastewater, the present study of biodegradation of mixed pollutants is very important in treating such saline wastewaters. Hence, it is desirable to use microorganisms that are able to degrade mixed phenolic compounds under saline conditions. To our knowledge, we found that there have been scarce reports on the degradation of mixed phenolic compounds under saline conditions.

In the present study phenol degrading bacterial consortium was enriched under saline conditions and was used to degrade the phenolic compounds individually and as dual substrates. The intermediates formed during the degradation of the phenolic compounds showed that they followed ortho-cleavage for their degradation.

Materials and Methods

Medium

The bacterial consortium was grown in mineral salts medium of (g/L) NaCl 50.0, 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 with 10 mg/L of yeast extract, adjusted to pH -7 and distilled water – 1L (Alva and Peyton 2003). The medium was autoclaved, cooled to room temperature and was amended with respective phenolic compound through a sterile filter (0.45 µm) in 250 ml Erlenmeyer flasks. The chemicals and reagents (Analar grade) used in the studies were purchased from Merck, India.

Bacterial consortium

The bacterial strains used in the present study were *Bacillus cereus, Arthrobacter sp.,Bacillus licheniformis, Halomonas salina, Bacillus pumilus* and *Pseudomonas aeruginosa* (Veena gayathri and Vasudevan 2010).

Studies on the degradation on phenolic compounds

The bacterial consortium was isolated from soil samples collected from different habitats of Chennai, such as phenol contaminated sites and from places having proximity to saline environment. The bacterial consortium was enriched in the mineral salts medium. The medium was autoclaved, cooled to room temperature and was amended with phenol (50 mg/L) through a sterile filter (0.45 µm) in 250 ml Erlenmeyer flasks. After 1 month of cultivation, the consortium was spread on agar plates containing the same medium and phenol. During all these experiments, after observing the turbidity at an interval of 24 h, 5ml aliquots were aseptically inoculated into 100 ml of liquid medium supplemented with increasing phenol concentrations. Increase in protein yield was taken as a confirmation of the ability of the consortium for utilization of phenol. Cell morphology and the motility of cells were examined by light microscopy. Alternatively, the cells were also plated on nutrient agar and viable cells (cfu/mL) were counted. The consortium was studied for its growth and degradation of phenol, cresols and mixture of the substrates as the sole carbon source.

For the degradation study, mineral salts medium containing phenolic compounds were inoculated with the bacterial consortium. Different conditions used for the degradation of phenol were (i) medium + Phenolic compound + bacterial consortium; (ii) medium + Phenolic compound 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 interval for 5 days. Each culture was acidified to pH 2.5 with 1N HCl and extracted twice with dichloromethane (v/v). The extracts were filtered through anhydrous sodium sulphate and condensed to 1 mL in a rotavapour (Buchi, Germany) for further gas chromatographic analysis.

Protein analysis

For analysis of total cell protein, samples were centrifuged at 12,000 rpm for 10 mins 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 (1.5 min x 2) on an ice-water bath. Sample (0.5 ml) was added to 0.5 mL Coomassie Blue protein dye and

the absorbance was measured at 595 nm. The total protein concentration was determined by calibration with bovine serum albumin standard according to (Bradford 1976).

Phenolic compounds

Initially the bacterial consortium was acclimatized with 50 mg/L of phenol, then their concentrations were increased up to 300 mg/L. Substituted phenols like *o*-cresol, *m*-cresol, *p*-cresol were used at concentrations of 50 mg/L, 75 mg/L and 100 mg/L. To study the degradation of dual substrates, optimum concentration of phenol (100 mg/L) with *o*-cresol (50 mg/L) were used in the study.

Gas Chromatographic analysis

The ability of the consortium to utilize the phenolic compounds as sole carbon source was determined by growing it in the mineral salts medium containing Phenol (100, 150, 200, 250, 300 mg/L), *o*-, *m*- and *p*-Cresol (50, 75 and 100 mg/L). The cell suspensions were clarified by centrifugation at 10,000 rpm for 15 min, at 6°C. The culture supernatant was extracted with dichloromethane, condensed and filtered through a 0.2mm Gelman filter acro disc, before analysis in gas chromatograph (Chemito GC Model No 1000) equipped with FID detector and capillary column (Varian Chromopak capillary column CP SIL 8 CB, 30 m X 0.32 mm . Nitrogen was used as a carrier gas, with injector temperature 220°C, detector temperature 250°C and the oven temperature of the column at 150°C. Standard solutions of different phenolics were as standards. The samples were injected and the rate of utilization of phenolic compound was calculated based on the peak area percent and retention time.

Analysis of metabolites by GC-MS

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 280ºC and an ion source temperature at 200ºC. Oven operating temperature was 80ºC with the holding time of 1 min, 300 ºC for 2 mins with the total time of 41.67 mins. The masses of primary and secondary phenolic compound ions were determined by using the scan mode with impact ionization (70 eV, 200ºC) for pure phenolic compound 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. Standards from Sigma Aldrich were used for the phenolic compounds and their metabolites. A GC–MS library search was used to confirm the metabolites without standards.

Results and Discussion

Degradation of Phenol by the bacterial consortium at different salt concentrations

The influence of salt on the degradation of phenol by the bacterial consortium was studied at different salt concentrations from ((1-15 %) i.e 10, 30, 50, 70, 100 and 150 g/L). At 3 % NaCl, the degradation of phenol was 95 % with a maximum protein concentration of 37.6 mg/L and the

degradation reached maximum of 99 % at 5 % NaCl in 4 days (Fig 1). The total protein concentration reached a maximum of 43.2 mg/L on the 2nd day. When the NaCl concentration was increased to 7 %, 10 % and 15 %, the degradation decreased to 93 %, 89 % and 14 % with a decrease in protein concentration to 15, 7.6 and 5.2 mg/L respectively. The reduction in the degradation of phenol above 7 % NaCl may be due to higher salt concentrations, which affected the metabolic rate of bacterial cells; this was also supported by few studies (Ventosa et al. 1998; Diaz et al. 2000; Diaz et al. 2002). Similar observations were also made by few researchers on polyaromatic hydrocarbons (Rambeloarisoa et al. 1984; Mille et al. 1991; Oren et al. 1992; Bertrand et al. 1993).

The utilization of phenol by the bacterial consortium was as low as 10 % at 1% NaCl, this was also indicated by the very less protein concentration of 7.4 mg/L which emphasizes that the bacterial consortium needed salinity for utilizing phenol as the substrate; this proved that the bacterial consortium is moderately halophilic in nature. The optimum growth of the bacterial consortium in utilizing phenol was at 5 % NaCl. Previous literature on the degradation of phenol under saline conditions by individual strains showed that optimum degradation was achieved at 5 % NaCl (Woolard and Irvine 1995; Hinteregger and Streichsbier 1997).

Degradation of different concentrations of phenol

The bacteria consortium was studied for the degradation of different concentrations of phenol from 100 mg/L to 300 mg/L at the optimum salinity of 50 g/L NaCl (Fig 2). Phenol at 100 mg/L, 150 mg/L and 200 mg/L concentrations was almost completely degraded (> 93 %) by the bacterial consortium in 4 days. At optimum salinity of 50 g/L, the bacterial consortium utilized 100 mg/L with increase in protein concentration of 45.2 mg/L in 2 days. The degradation reached to a maximum of 99 % in 4 days. At 150 mg/L of phenol, the degradation was 96 % with maximum protein yield of 37.6 mg/L. When the concentration of phenol was increased to 200 mg/L, the degradation reduced to 93 % with corresponding decrease in the protein yield of about 34.5 mg/L. Further, phenol degradation reduced to 90 % and 55 % at 250 mg/L and 300 mg/L of phenol and with a maximum protein yield of 29.5 and 24.3 mg/L respectively. Optimum degradation of phenol was achieved at 100 mg/L concentration. Similar results were reported Hinteregger and Streichsbier (1997), where *Halomonas* sp. could degraded phenol (100 mg/L) as sole source of carbon and energy at 5 % NaCl concentration. Woolard and Irvine (1995) showed that halophilic mixed cultures degraded only up to 100 mg/L of phenol at 140 g/L NaCl with 99 % degradation in 150 h. The bacterial consortium used in the present study, could effectively utilize up to 300 mg/L of phenol with an optimum degradation at 100 mg/L, in 4 days. Thus when the concentration of phenol was increased to 300 mg/L, it affected the growth of the bacterial consortium and the degradation of phenol. This behaviour is characteristic of toxic substrate metabolism as suggested by (Hill and Robinson 1975), who reported that, as concentration of toxic substance increases, the more detrimental it becomes to the organism to degrade the substrate.

Peyton et al. (2002) reported biodegradation of phenol by halophilic mixed cultures, in which one of the cultures utilized up to 300 mg/L phenol at 100 g/L NaCl in 70 h. Hinteregger and Streichsbier (1997) showed

that an unidentified *Halomonas* sp. degraded phenol only up to100 mg/L concentration at 140 g/L of NaCl in 13h. Alva and Peyton (2003) studied the biodegradation of phenol (130 mg/L) by *Halomonas campisalis*, which was able to completely remove phenol in 100 h. While in the present study the bacterial consortium was able to grow up to 300 mg/L of phenol within 4 days.

Degradation of different concentrations cresols

Degradation of *o*-cresol at different concentrations with growth in terms of protein yield is depicted in the Fig 3. *o*-cresol at 50 mg/L, 75 mg/L and 100 mg/L concentrations were almost completely (93 %) degraded by the bacterial consortium in 4 days. At 50 mg/L and 75 mg/L of o-cresol, the bacterial growth in terms of protein yield was 42.3 mg/L and 35.6 mg/L respectively in 2 days. At the end of 4 days, the degradation of 50 mg/L and 75 mg/L *o*-cresol was 99 % and 95 %. When the concentration of *o*-cresol was increased to 100 mg/L, the degradation reduced to 93 % in 4 days and with a decrease in the protein yield to 32 mg/L in the log phase (2 days).

Degradation of *m*-cresol at concentrations of 50 mg/L, 75 mg/L and 100 mg/L with the protein yield is shown in the Fig 4. The degradation of *m*-cresol was 94 % at 50 mg/L of *m*-cresol with a maximum protein yield of 37.6 mg/L. When the *m*-cresol concentration was increased to 75 mg/L there was a drop in the degradation (92 %) with a corresponding protein yield of 30 mg/L. At highest concentration of *m*-cresol (100 mg/L) the bacterial consortium gave a lowest degradation of 82 % with a maximum protein yield of 26.3 mg/L. Such decrease in the degradation might be due to the position of functional group of *m*-cresol. Fig 5 shows the degradation of *p*-cresol at different concentrations of 50 mg/L, 75 mg/L and 100 mg/L. When compared to *o*- and *m*-cresol, the degradation of *p*-cresol was 93 % at 50 mg/L with a maximum protein yield of 33.2 mg/L. While the degradation decreased to 90 % and 79 % at 75 mg/L and 100 mg/L with the maximum protein yield of 33.2 mg/L and 25.6 mg/L respectively. A decrease in the degradation of *m*-cresol and *p*-cresol compared with o-cresol could be due to the methyl group, which reduces the solubility of the substrate. Among the cresols, *p*-cresol proved to be more recalcitcrant than the other Cresols.

The bacterial consortium in the present study was able to degrade > 92 % of o-cresol under saline conditions which was achieved after 4 days. This is higher than the degradation (87 %) by the biofilm of *Arthrobacter viscosus* (Quintelas et al. 2006) and also with the fungal population (84 % degradation) previously reported by (Atagana 2004) under non-saline conditions.

Garcia et al. (2005) studied the catabolic versatility of many low molecular weight aromatic compounds including phenols and *p*-cresols. Most of the isolates belonging to the genus *Halomonas* were able to grow on most of the aromatic compounds except for *p*-cresol. The isolates enriched with phenol were able to utilize a greater number of aromatic compounds than the rest of the isolates enriched with other aromatics. In present study, the bacterial consortium enriched with phenol had the ability to grow on various cresol substituents with more than 90 % degradation. There are several reports on the degradation of phenol under saline conditions (Hinteregger and Striechbier 1997; Alva and Peyton 2003), but there are no reports on the degradation of cresols by individual strains or by a bacterial consortium under saline conditions.

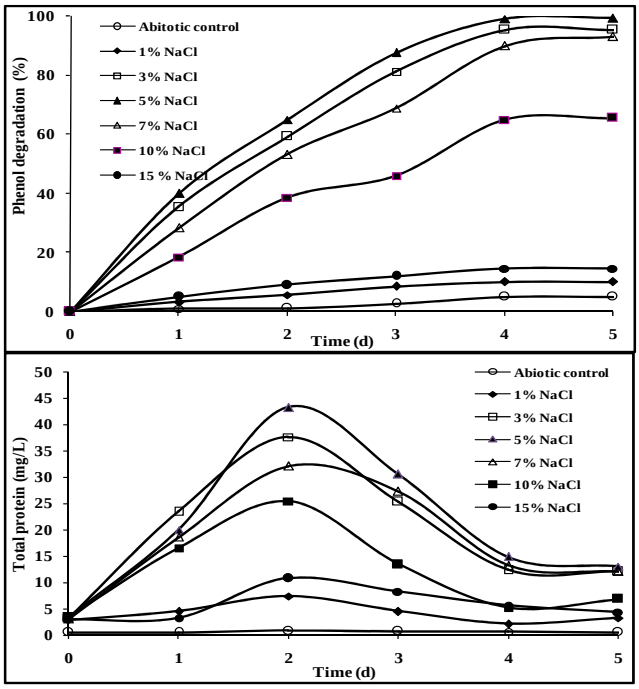

Fig. 1 Degradation of phenol and protein yield by the consortium at different NaCl concentrations

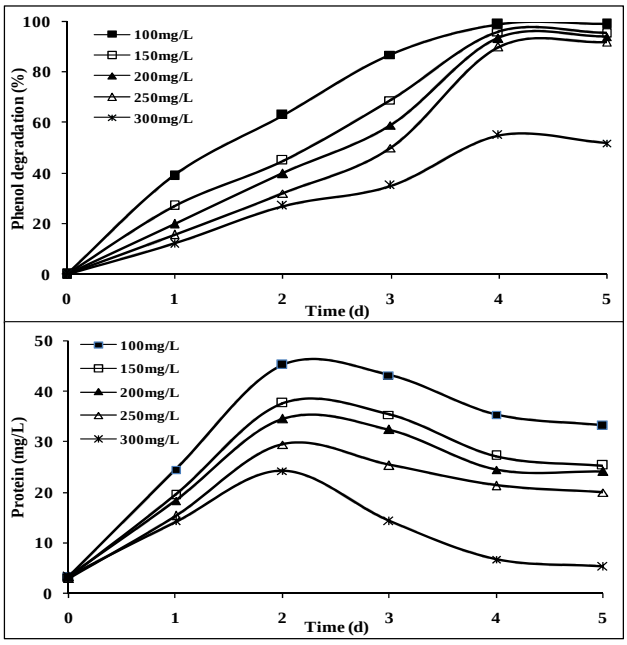

Fig. 2 Degradation of different concentrations of phenol and protein yield by the bacterial consortium at 50 g/ L NaCl

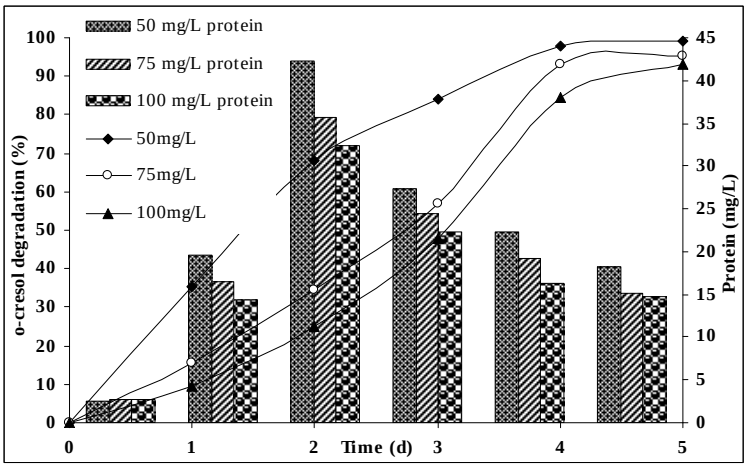

Fig. 3 Degradation and protein yield by the bacterial consortium at different concentrations of *o*-cresol

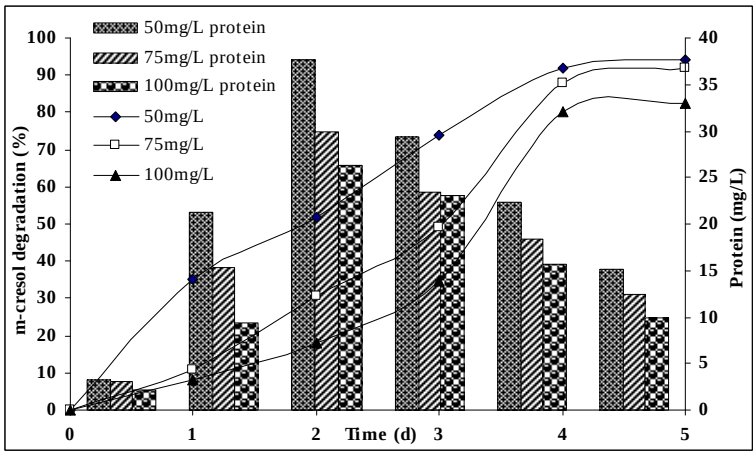

Fig. 4 Degradation and protein yield by the bacterial consortium at different concentrations of *m*-cresol

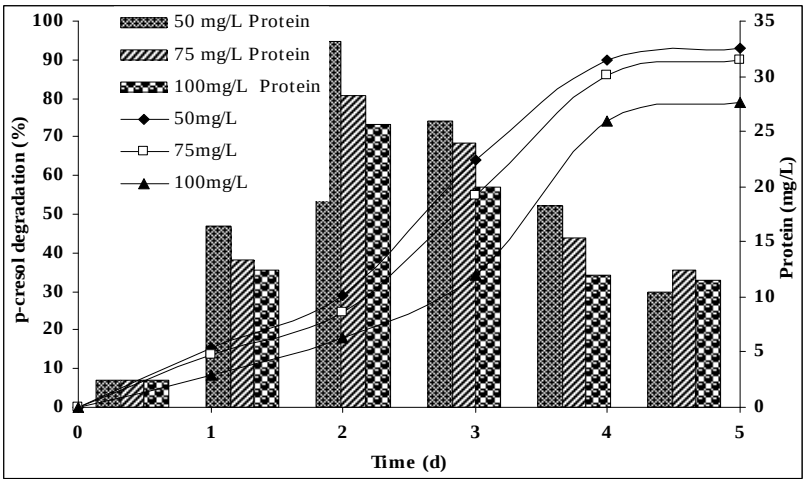

Fig. 5 Degradation and protein yield by the bacterial consortium at different concentrations of *p*-cresol

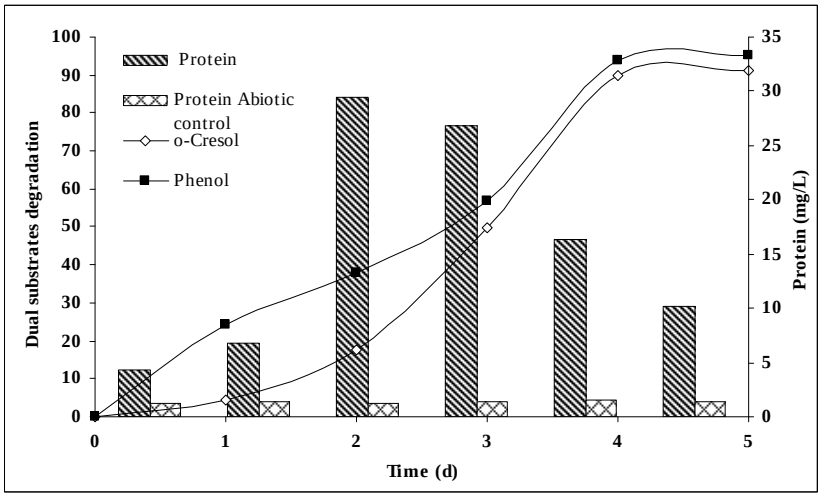

Fig.6 Degradation and protein content by the bacterial consortium on dual substrates

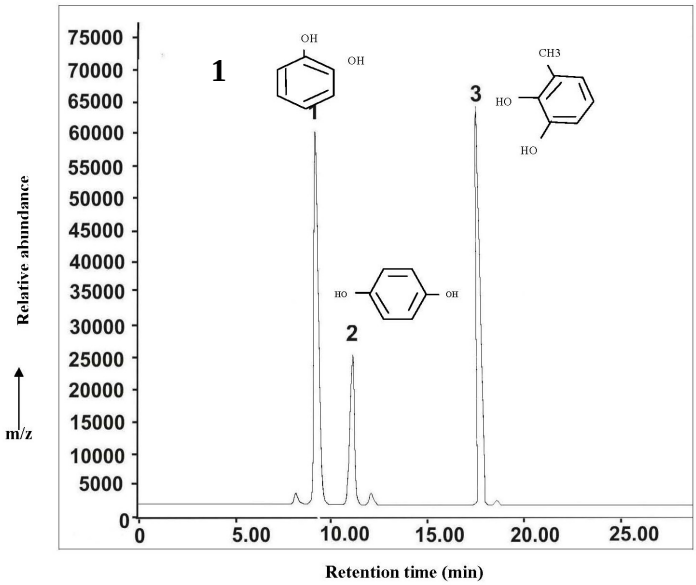

Fig.7 GC-MS Spectrum of the metabolites formed during degradation of dual substrates

In present study, the bacterial consortium enriched with phenol had the ability to grow on all the cresol substrates with more than 90 % degradation efficiency.

Degradation of dual substrates

As industrial wastewater contains complex mixtures of phenolic compounds and salts, it is particularly desirable to use the microorganisms, which are previously acclimatized to the individual substrates, so that they could efficiently degrade phenolic compounds under saline conditions. The bacterial consortium was studied for its ability to degrade optimum concentration of dual substrates (Phenol (100 mg/L) and *o*-Cresol (50 mg/L)) at 50 g/L NaCl. The results from the experiments showed that phenol was degraded 95 % and *o*-Cresol was degraded 91 % with the maximum protein yield of about 29.5 mg/L on 2nd day (Fig 6). The consortium could degrade both the substrates (phenol and *o*-cresol) as a mixture simultaneously. The simultaneous metabolism of phenol and cresol has been

reported in aerobic and anaerobic conditions (Kar et al. (1997); Tawfiki et al. (1999); Fang and Zhou (2000) under non- saline conditions, where the degradation of phenol was inhibited by the presence of alkyphenols (*o*-, *m*- or *p*- cresols).

Paraskevi and Polymenakou (2005) reported that the addition of *o*-cresol strongly inhibited phenol transformation during the co-metabolism of phenol and *o*-cresol. In the present study it was observed from the Fig 6 that irrespective of the concentration levels of the compounds, phenol was degraded higher than that of *o*-cresol. The disparity observed in the growth of the bacterial consortium on phenol and *o*-cresol may be due to the presence of methyl group on the compound, which affects some of their physical properties, such as solubility. This may be due to the fact that phenol, compared to *o*-cresol, is a much simpler carbon source and therefore is easily metabolized by the bacterial consortium. Another reason would be that the bacterial consortium was primarily enriched on phenol as the carbon source. 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; and concentration above 5 % NaCl, the growth was inhibited at late lag phase and complete degradation was achieved in 88 h. Whereas in the present study, it could be seen the degradation of mixture of substrates was more than 90 % within 4 days in the presence of 5 % NaCl without any lag phase.

Identification of metabolites during the degradation of dual substrates

The analyses of the 72 h culture extracts of the bacterial consortium grown on mixtures of phenol and *o*- cresol, produced intermediate metabolites as shown by GC-MS (Fig 7). The chromatogram showed three peaks, on comparison of the mass spectrum from extracted samples with the standards it proved that the first peak was catechol the intermediate compound of phenol at retention time of 9.88 min; corresponding mass analyses yielded m/z (40,53,63,81,95,110,112), followed by peak 2-Hydroquinone with a retention time of 11.525 min where the m/z (25,27, 39,55, 63,81, 92, 110,112). At the retention time of 18.35 peak 3 was observed which represented the metabolized product of *o*-cresol, 3-methyl catechol with masses (33,42,52,67,85,110,124,134). Ahamad et al. (2001) reported the presence of 3-methylcatechol and 2-ketohex-*cis*-4-enoate during *o*-Cresol degradation. Saravanan et al. (2008) showed hydroquinone, benzoquinone, catechol, oxalic acid, maleic acid, acetic acid, formic acid and tartaric acid were formed as intermediates during the degradation of phenol and *m*-Cresol as mixed substrates under non-saline conditions.

Mesophilic bacteria have been widely reported to catabolize phenol and the isomeric Cresols by converting them before aromatic-ring fission into catechol and methylcatechols, respectively. The present study results indicated that the bacterial consortium was able to convert the dual substrates to their intermediates such as catechol and 3-methyl catechol, in 4 days under saline conditions.

Furthermore, many studies have shown that phenol and p-cresol were metabolized by the *β*-ketoadipate pathway through ortho-fission of catechol (Kolomytseva et al. 2007; Santos et al. 2003). The formation of intermediates like catechol and 3–methylcatechol suggest that the bacterial consortium degraded the dual substrates by *ortho*- cleavage pathway

The results have demonstrated the isolated bacterial consortium was able to degrade phenol at different NaCl concentrations with optimum concentration at 50 g/L NaCl. The consortium was able to degrade phenol at different concentrations from 100 mg/L to 300 mg/L at 5 % NaCl, where optimum degradation of phenol was achieved at 100 mg/L. The bacterial consortium was also able to degrade different cresol substrates at different concentrations from 50 mg/L - 100 mg/L. The optimum degradation was obtained with *o*-Cresol at 50 mg/L concentration. During the degradation of dual substrates the bacterial consortium degraded both the substrates simultaneously.

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References

Ahamad PYA, Kunhi AAM and Divakar S 2001. New metabolic pathway for o-cresol degradation by *Pseudomonas* sp. CP4 as evidenced by 1H NMR spectroscopic studies, 17 (4) : 371-377

Alva VA and Peyton BM 2003. Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: influence of pH and salinity. Environ. Sci. Technol. 37**:** 4397–4402.

Atagana HI 2004. Biodegradation of phenol, *o*-cresol, *m*-cresol and *p*-cresol by indigenous soil fungi in soil contaminated creosote. W. J. Microbiol Biotechnol. 20: 851-858.

Bastos AER., Moon DH and Rossi DHMA, Trevors JT, Tsai SM 2000. Salt-tolerant phenol-degrading microorganisms isolated from Amazonian soil samples. Arch. Microbiol. 174: 346–352.

Bertrand JC, Bianchi M , Mallah MA, Acquaviva M and Mille G 1993. Hydrocarbon biodegradation and hydrocarbonoclastic bacterial communities composition grown in seawater as a function of sodium chloride concentration. J. Exp. Mar. Biol. 168: 125-138.

Bradford MM 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Diaz MP, Boyd KG, Grigon SJW and Burgess JG 2002. Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. Biotechnol. Bioeng. 79: 145-153.

Diaz MP, Grigon SJW, Peppiatt C and Burgess JG 2000. Isolation and characterization of novel hydrocarbon degrading euryhaline consortia from crude oil and mangrove sediments. Mar. Biotechnol. 2: 522-532.

Fagbote EO and Olanipekun EO, 2010. Levels of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in sediment of bitumen deposit impacted area. Int. J. Environ Sci. Tech. 7 (3): 561-570.

Fang H and Zhou G, 2000. Degradation of phenol and p-Cresol in reactors. Wat. Sci. Technol. 42 : 237-244.

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

Garcia MT, Ventosa A and Mellado E 2005. Catabolic versatility of aromatic compound- degrading halophiic

bacteria. FEMS Microbiol. Eco. 54 (11): 97-109.

Hill GA and Robinson CW, 1975. Substrate inhibition kinetics: Phenol degradation by *Pseudomonas* putida. Biotech. Bioeng. 17 (11): 1599-1615.

Hinteregger C and Streichsbier F 1997. *Halomonas* sp., a moderately halophilic strain, for biotreatment of saline phenolic waste-water Biotechnol. Lett. 19 (11): 1099–1102.

Kar S, Swaminathan T and Baradarajan A 1996. Biodegradation of phenol and cresol isomer mixtures by *Arthrobacter.* W.J. Microbiol. Biotechnol. 13: 659-663.

Kolomytseva MP, Baskunov BP and Golovleva LA 2007. Intradiol pathway of *para*-cresol conversion by *Rhodococcus opacus* 1CP Biotechnol J. 2 (7): 886–893.

Lee KH and Byeon SH, 2010. The biological monitoring of urinary hydroxypyrene by PAH exposure among smokers. Int. J Environ. Res. 4 (3): 439-442.

Lefebvre O., Vasudevan N., Torrijos M., Thanasekaran K. and Moletta R. 2006 Microbial diversity in hypersaline wastewater: the example of tanneries Extremophiles 10: 505-513.

Lop KC and Tar CP 2000. Effect of additional carbon sources on biodegradation of phenol. *Bull.* Environ. Contam. Toxicol. 64: 756-763.

Lows FJ, Fulbright DW, Taylor SC and de Bruijn FJ 1994. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repet-protein-due-binding. Anal. Biochem. 72: 248-254.

Ludzack FJ and Noran PK 1965. Tolerance of high salinities by conventional wastewater treatment process. J.Wat.Poll.Con. Fed. 37, (10): 1404-1416.

Margesin R and Schinner F, 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles 5 (2): 73-83.

Mille G, Almallah M, Bianchi M, Wambeke FV and Bertrand JC 1991. Effect of salinity on petroleum biodegradation. Fresenius J. Anal. Chem. 339: 788-791.

Mohanan S., Maruthamuthu S., Muthukumar N., Rajasekar A. and Palaniswamy N. 2007. Biodegradation of Palmarosa oil (Green oil) by *Serratia marcescens*. Int. J. Environ. Sci.Tech., 4 (2), 277-281.

Munoz JA, Perez-Esteban B, Esteban M, de la Escalera S and Gomez MA, Martinez-Toledo MV and Gonzalez-Lopez J (2001) Growth of moderately halophilic bacteria isolated from sea water using phenol as the sole carbon source. Folia Microbiol. 46: 297–302.

Oren A, Gurevich P, Azachi M and Henis Y (1992) Microbial degradation of pollutants at high salt concentrations, Biodegradation 3: 387–398.

Paraskevi N, Polymenakou C and Euripides GS, 2005. Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil Pseudomonad. Biodegradation 16: 403–413.

Peyton BM, Wilson T and Yonge DR 2002. Kinetics of phenol biodegradation in high salt solutions. Wat. Res. 36: 4811-4820.

Quintelas C, Sousa E, Silva F, Neto S and Tavares T 2006. Competitive biosorption of ortho-cresol, phenol, chlorophenol and chromium (VI) from aqueous solution by a bacterial biofilm supported on granular activated carbon. Process Biochem. 41: 2087-2091.

Rambeloarisoa E, Rontani JF, Giusti G, Duvnjak Z and Bertrand JC (1984) Degradation of crude oil by a mixed population of bacteria isolated from sea-surface foams. Mar. Biol. 83: 69-81.

Santos VL, Heilbuth NM, Braga DT and Linardi VR 2003. Phenol degradation by a *Graphium* sp. FIB4 isolated from industrial effluents. J. Basic Microbiol. 43 (3): 238–248.

Saravanan P, Pakshirajan K and Saha P 2008. Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor. Biores. Technol. 99: 205–209.

Tawfiki K, Le´pine F, Bisaillon J and Beaudet R 1999. Simultaneous removal of phenol, *o*- and *p*-cresol by mixed anaerobic consortia. Can J. Microbiol. 45: 318–325.

Veena Gayathri K and Vasudevan N. 2010. Enrichment and Isolation of Phenol degrading Moderately Halophilic Bacterial Consortium from Saline Environment. J. Biorem. Biodeg. 1(1) 513-524.

Wang P, Yuanyuan Q and Zhou J 2009. Biodegradation of Mixed Phenolic Compounds Under High Salt Conditions and Salinity Fluctuations by *Arthrobacter* sp. W1. Appl. Biochem. Biotechnol. 159 (3) : 623-33.

Woolard CR and lrvine R, 1995. Treatment of hypersaline wastewater in the sequencing batch reactor. Wat. Res. 29 (4): 1159-68.

Zhuang X., Han Z., Bai Z., Zhuang G. and Shim H. 2010. Progress in decontamination by halophilic microorganisms in saline wastewater and soil. Environ. Poll 158 (5): 1119-1126.