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Original Research Article

Isolation of CSMBs for the Biodegradation of Recalcitrant Pollutants

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Keywords

Heterocyclic aromatic compounds. Recalcitrant pollutant. Micro-aerophilic**.** Phenol. Ortho cleavage. Leather industry.

A B S T R A C T

Biodegradation of heterocyclic aromatic constituents is highly challenging due to their recalcitrance. Six bacterial strains that utilize phenol as sole carbon were isolated by selective enrichment at microaerophilic condition from pilot scale Upflow Anerobic Sludge Blanket. Molecular characterization based on 16S rDNA gene sequencing identified CSMB 1 to 6 as *Alcaligenes sp. MH146, Enterobacter cloacae strain SJ 6, Serratia sp. HA1, Alcaligenes faecalis subsp. faecalis strain AE1.16, Bacillus sp. KMSII-3, Klebsiella pneumoniae strain SDM45* respectively and these strains were deposited in Microbial Type Culture Collection, IMTECH. CSMB1 to CSMB6 tolerate the initial phenol concentration of 1500 mg/l with 50 to 60% degradation within 24h. Salinity studies observed them to be moderately halophilic. Presence of 50 mg/l concentration of heavy metals used in leather industry, when tested showed no inhibition on growth by CSMB isolates. In the evaluation of antibiotic activity at microaerobic condition, resistance of CSMBs is more pronounced. Ortho cleavage ring fission was observed by all CSMB strains. The unique feature of the isolates was their capacity to degrade a range of heterocyclic compounds that are used in leather industry to transform the putrescible hide/skin into valuable leather, by utilising them as sole carbon and energy, even without any growth factors including vitamins, amino acids and peptides. Degradation of these compounds was confirmed by the utilization of secondary metabolites of the corresponding compounds. This is the first time utilization of recalcitrant pollutants present in leather industrial wastewater as a growth substrate by a pure bacterial culture at microaerophilic condition is reported.

Introduction

The leather industry plays a significant role in today's global economy, by transforming animal hides/skins into valuable leather goods by subjecting them to chemical and mechanical sequential processes (Ozgunay 2007). Effluent generated from leather

processing contains a high level of organic, inorganic, aromatic chemicals (Song et al. 2003; Manikant Tripathi 2011) and heavy metals (Colak, 2005).

Phenol and its derivatives with different functional groups attached to the benzenoid ring structures such as, hydroxyl, phenyl, methyl, sulphonic or amide are the basic chemicals used in leather processing. Hence, the leather industry poses serious environmental impact with pollution load resulting in high oxygen demand (Marrot, 2006; Chandra et al. 2011) with other heterocyclic aromatic chemicals. About 30–40 m^3 of wastewater is generated per ton of raw material (hide/skin) during leather processing. The wastewater characteristic varies in accordance with variations in raw material, process, chemicals and water consumption. Biodegradation of toxic organic and aromatic constituents is challenging due to their recalcitrance (Shah and Thakur 2002; Cokgor et al. 2008) and hence pose difficulties in meeting the mandatory discharge limits set by the pollution control boards.

The recalcitrant fraction of Chemical Oxygen Demand (COD) in wastewater contributes 10 to 20 % and it remains in conventional wastewater treatment plants as residual COD. At present, regulatory authorities have enforced zero liquid discharge (ZLD). The major limitation of the aerobic process is its high operational costs due to aeration (Daumer et al. 2007; Guo et al. 2010). For cost reduction in aerobic treatment, several researches tried different Dissolved Oxygen (DO) levels with reduced oxygen supply of 0.3 to 0.9 mg /l of DO, in treating domestic wastewater (Wang et al. 2007; Ma et al. 2009; Zheng et al. 2011, 2012). At this DO concentration, there is a reduction in air supply of about 60 to 80 %, resulting in lower operational cost when compared with conventional aerobic treatment where 2 to 2.5 mg/l DO is required. One more disadvantage in aerobic treatment is the bulking of sludge due to proliferation by filamentous bacterial species if DO levels fall below 1 mg/l (Xie et al. 2007; Nielsen et al. 2009). The microorganisms present in microaerophilic conditions provide versatile metabolic activity and involves numerous physiological processes (Zheng and Cui, 2011). The availability of O_2 decides a primary environmental signal for switching between growth modes, allowing the organism to utilize C and N sources by different metabolic strategies (Fuchs G, 2011). Microaerobic condition where dissolved oxygen is very low, finds increasing application in the environmental biotechnology, especially in the biodegradation of recalcitrant chemicals.

Therefore, the focus of the present study is to acclimatise the anaerobic sludge to microaerophilic conditions, with phenol as sole carbon, and to isolate and identify potential bacterial strains to degrade heterocyclic aromatic compounds that are present as recalcitrant pollutants in leather industrial wastewater. Evaluation studies were also done in detail on the isolated strains to find out their efficiency to be employed in the treatment of wastewater.

Materials and Methods

Chemicals

Phenol, Nonyl phenol, 2-(thiocyanomethylthio)-benzothiazole, wattle extract, Phenolic formaldehyde, Melamine resin, Sulfo chlorinated paraffins, Remazol Red RG, Black DB, Yellow 194, Brown 3GV, Remazol blue, Black BG, Blue RGB, Brown DB, Acid green, Reactive orange, Black RB were kindly provided by a tannery

in Chennai and were used without further purification. All medium components were procured from E.Merck Mumbai (India). Phenol, Catechol, Resorcinol, Sodium benzoate, L-Glutamic acid, 2 -hydroxy benzoic acid, 3 -hydroxy benzoic acid, 4 - hydroxy benzoic acid, Metanilic acid and sulfanilic acid, Benzothiazole, 3 Hydroxy benzothiazole, 2-Methyl benzothiazole, 2-Mercaptobenzothiazole 2-Methyl thio benzothiazole, 2-amino benzothiazole, Benzothiazole sulfonate, Naphthalene, and Benzene were procured from Sigma-Aldrich, India. All the solutions were prepared in Milli-Q water.

Microorganisms

Anaerobic sludge collected from a pilot scale Upflow Anaerobic Sludge Blanket reactor (UASB) present in the Environmental Technology division, CLRI, Chennai India, served as the initial inoculum. This anaerobic reactor was fed with raw wastewater generated from leather industry. The Mineral Salt Medium (MSM) with phenol (Atlas 1946) was used as enrichment medium for isolation of microorganisms in microaerophilic condition. For degradation studies of heterocyclic aromatic chemicals, the MSM was modified by substituting nitrate and sulphate of ammonium salts with corresponding chloride salts in order to utilise the aromatic chemicals as sole carbon and energy source. The isolated bacterial strains were maintained on Luria agar and stored at 4°C until further use.

Isolation

About six phenol degrading bacterial strains were isolated at microaerophilic condition in a laboratory scale bioreactor (Hygene Fermentor, Lark), with automatic control biosensors employing the enrichment culture technique as described earlier (Umamaheswari and Rama 2014). Acclimatisation of anaerobic biomass to microaerophilic condition was conducted by controlled oxygen concentration of 0.9 ± 0.2. It was operated with pH constant at 7.0 and temperature at 30°C so as to maintain the conditions similar to conventional leather industrial treatment plants in tropical countries, in the presence of 1g/l phenol as the sole carbon source.

Identification

Thirty six phenol degrading bacteria were isolated from the pilot scale UASB anaerobic biomass after acclimatising them to microaerophilic condition in online controlled Hygene Fermentor, with an initial concentration of 1g/l phenol as sole carbon within 24 h. The screened phenol degrading microaerophilic bacterial strains were examined on heterocyclic aromatic compounds specifically on the recalcitrant pollutants generated from leather industrial wastewater, as sole carbon and energy source. MSM containing the chemicals (Table 1) used in leather processes of 25 mg/l each of Biocide, (TCMTB); Surfactant, (Luwet- 40); Vegetable tannin, (Wattle powder); Phenolic Syntan, (Phenol formaldehyde); Synthetic tannin, Naphthalene sulfonic acid, Melamine resin; Acrylic polymer, (Relugan); Vat dye (Direct black 38), Synthetic Fatliquor - (FB-II) and Solvent (Benzene) were added independently. It was inoculated with 10 % of isolated bacterial cultures and incubated in a screw capped flask until dense growth was obtained. About six bacterial strains utilized all the ten recalcitrant chemicals tested (Fig.1). They were designated as CSMB 1, CSMB 2, CSMB 3, CSMB 4, CSMB 5 and CSMB 6 (Fig.2). Selected isolates were identified through biochemical analysis (Cappuccino and Sherman, 1996).

Molecular identification by 16S rDNA sequencing was done by Xcelris Labs Ltd; Ahmedabad (India). The identified bacterial strains were purified by repeated streaking and were stored at -40°C in 50 mM KH_2PO_4/K_2HPO_4 buffer containing 20% (v/v) glycerol.

Phenol Degradation

At the optimised condition of pH 7.0, temperature 30 ºC and dissolved oxygen of 1.0 mg/l, the phenol degradation potential of all the six bacterial strains, CSMB 1 to CSMB 6 were evaluated. The effect of initial concentration of 1000 to 2000 mg/l of phenol was evaluated in MSM. An initial cell density of 0.034 was inoculated individually into the culture medium in 50 ml screw capped Erlenmeyer flasks and incubated for 24 h. Growth was measured by turbidity at 600 nm and after removing the cells by centrifugation at 8,000 rpm for 10 min, the supernatant was immediately measured for the concentration of phenol by 4-aminoantipyrine method at 510 nm (APHA 2005) using a UV-Vis spectrophotometer (Shimadzu UV2450), at different time intervals until complete degradation.

Effect of Salinity

The composite wastewater discharged in common effluent treatment plants (CETPs) is a mixture of different processes from different tanneries. The salinity of composite wastewater varies between 1 to 1.5%. Salinity hinders the activity of microorganism therefore, substrate degradation is inhibited. So effect of each CSMB strain on salinity was evaluated in 50 ml screw capped Erlenmeyer flasks by inoculating individually into the culture medium containing 0.5% to 1.5% sodium chloride. After 24 h of incubation, growth of CSMBs was measured at 600 nm.

Effect of heavy metals

Chromium is the major heavy metal used in chrome tanning of leather processes. Zirconium (Zr) aluminium (Al) are used in tanning and retanning as a substitute for chromium salts, and certain metal salts are used in dyeing processes (Basaran et al. 2006). Effect of heavy metal resistance with initial concentration of 25 mg/l of metal salts such as chromium, aluminium, zirconium, zinc, barium and Magnesium were tested. The growth of each strain was determined individually on all CSMB strains.

Sensitivity to Antibiotics

At present due to the widespread usage of antibiotics, microbial species have developed numerous mechanisms that render them resistant to them. According to Russell, (1996), for an antibiotic to be effective against bacteria, the existence of a susceptible antibiotic target must be present in the cell, quantity of the antibiotics to the target should be sufficient, and the antibiotic should be active. Susceptibility for all CSMBs to different antibiotics namely erythromycin, neomycin, penicillin, ampicillin, polymixin-B, cephaloridine, tetracycline, Ciprofloxacin, Co-trimazole, Gentamycin, Kanamycin and Streptomycin was determined by disc diffusion method (Bauer 1996).

The antibiotic impregnated discs (Oxoid) were placed on freshly prepared lawn of bacterial isolate on Mueller Hinton agar plates, and incubated at 30 ± 1°C for 24 h. The bacterial isolate was classified as resistant or susceptible by examining the zone of inhibition on the lawn of bacterial culture, according to the criteria recommended by the national committee for clinical Laboratory Standards, 2001 (Barry, 1981).

Metabolic versatility

The experiments on metabolic versatility of six CSMB strains as consortium were carried out with MSM supplemented with different synthetic chemicals used in leather processing as sole carbon and energy at a final concentration of 25 mg/l (Table 4). Recalcitrant chemicals used in leather processing, specifically pesticide, acid and basic dyes, synthetic chemicals used in finishing units and its probable secondary metabolites, secondary amines, were also evaluated to confirm the degradation of recalcitrant chemicals. The six CSMB strains were mixed in equal proportion with an initial cell density of 0.034 and inoculated as consortium into the above chemicals. The culture medium was incubated at 30 °C on an orbital shaker in 50 ml screw capped Erlenmeyer flasks at 50 rpm for a period of 1 to 2 days until dense growth was observed.

The negative and abiotic controls were conducted during every set of experiments. The reported values are the average of three replicate measurements.

Cleavage pathways

To detect meta or ortho ring fission pathways, production of the yellow product α-hydroxy muconic semialdehyde (α HMS) or β-ketoadipate from catechol was analysed. (Ambujom, S. 2001). A 10 ml suspension of 24 h culture of each of CSMB1 to CSMB6 strains was concentrated to 2 ml by centrifuging 10,000 g for 15 minutes at 4 °C. From the concentrate, 0.5 ml was re-suspended in 2 ml of 0.2 M Tris buffer (pH 8.0). To solublize the cell membrane, 0.5 ml of toluene was added, and then the sample was shaken with 0.2 ml of 1.0 M catechol solution. Appearance of yellow colour within a few minutes indicated meta cleavage activity. To test for ortho cleavage, 1 g of $(NH4)_2SO_4$ was added to 2.5 ml of cell suspension and incubated for 1 h at 30 °C. The sample pH was adjusted to 10 with 0.5 ml ammonia (5 N) and a drop of 1% sodium nitroprusside was added to the mixture. Appearance of a deep purple colour indicates ortho cleavage activity.

Results and Discussion

Isolation and Identification

About six bacterial strains that can degrade all the ten chemicals tested (Table 1) (Fig.1) were selected for identification. They were labelled as CSMBs. Biochemical characterisation showed that all the six bacterial strains are closer to *Alcaligenes, Bacillus* and *Enterobacteriaceae* families. The CSMBs are flocculent in nature with high settleability, resulting in a compact sludge. They were adopted in such a way that either they were capsulated or sporulated or highly motile to face the stressed conditions. The morphological and biochemical identities of the bacterial isolates are given in Table 2. The CSMBs were easily cultivable in Luria broth reaching exponential phase (OD 1.267 at 600 nm) within 6 h of incubation. Molecular characterization based on 16S rDNA gene sequencing showed that CSMB 1, CSMB 2, CSMB 3, CSMB 4, CSMB 5 and CSMB 6 were identified as *Alcaligenes sp. MH146, Enterobacter cloacae strain SJ 6, Serratia sp. HA1, Alcaligenes faecalis subsp. faecalis strain AE1.16, Bacillus sp. KMSII-3, Klebsiella pneumoniae strain SDM45* with accession number FJ626617.1, EU779827.1, HM136579.1, HM136579.1, GQ284565.1 and GQ468395.1 respectively (Table 3). These CSMBs were deposited in Microbial Type Culture Collection, Institute of Microbial Technology; Sector 39-A,

Chandigarh-160 036 (India) for the invention entitled, "A microaerophilic bacterial consortium and use thereof for the simultaneous biodegradation of mixture of recalcitrants present in water" Indian Patent application No.3437, DEL2012 (Umamaheswari et al., 2012).

Phenol degradation

The potential of bacterial strains CSMB 1 to CSMB 6 was evaluated at 24h for phenol degradation in MSM, with different initial concentrations of phenol at optimum conditions (Fig 3). Phenol degradation was evaluated at microaerophilic condition by growing the six bacterial strains independently in 1000, 1250 1500 and 2000 mg/l of phenol as sole carbon. 98 to 99 % degradation of phenol was observed by CSMB 1 to CSMB 6 for the initial concentration of 1000 mg/l. Similar results were described by Margesin et al. (2005); Clintia E.Paisio (2012). At concentration of 1250 mg/l phenol, CSMB 1 to CSMB 6 degraded 81%, 73%, 75%, 72%, 80% and 74% respectively within 24 h. CSMBs degraded between 50 to 60 % within 24h when the concentration of phenol was 1500 mg/l. However, inhibition began from the concentration of 1750 mg/l where CSMB 1 to CSMB 6 degraded 44%, 37%, 34%, 30%, 41% and 30% of phenol respectively.

Effect on salinity

The inhibitory effect of salinity from 0.1 to 1.5% was evaluated by the growth of the six bacterial strain CSMBs (Fig 4). By the results obtained, they were observed to be moderately halophilic. Each one of the isolates were able to tolerate well up to 0.8 % NaCl with the absorbance value of 0.5 at 600 nm. 0.5% of NaCl was observed to be optimum with maximum growth at 0.1 % NaCl. However, the phenol degradation was inhibited at 1.5 %.

Effect on heavy metals

The growth potential of CSMB strains with heavy metals that are predominantly present in leather industrial wastewater was evaluated as presented in Fig.5. No significant inhibition on growth was observed on all tested metals at 25 mg/l concentration. Maximum growth was observed in the presence of Mg. Next in order were chromium, barium and zinc. Alumium scored better growth when compared with zirconium. Microorganisms that are effective in sequestering heavy metals, (Shuttleworth, 1993) are useful to remove metals from polluted wastewater. The observed results proved that the bacterial strains CSMBs may be exploited for industrial wastewater treatment.

Sensitivity to Antibiotics

CSMB bacterial strains were tested against disks of commonly used antimicrobials to evaluate their sensitivity at microaerophilic conditions (data not shown). Zone diameter against antimicrobial disks was significantly narrower for erythromycin, neomycin, penicillin, ampicillin and tetracycline (about 1 to 2 mm each) and for chloramphenicol with 3 mm.

However, zone was comparatively larger around Streptomycin with 7.5 mm, Kanamycin with 7.5 mm and Gentamycin 8 mm. The zone diameter against polymixin-B, cephaloridine, Ciprofloxacin, Co-trimazole was between 6 to 7 mm. But with vancomycin it was observed to be extreme resistant with nil zone of inhibition. According to Mayer (2007), if the zone diameter is □ 15, it is interpreted as (R) resistant. Similar to our results, Bhoj Raj Singh (2012) also reported that microaerobic bacteria showed more resistant when compare to aerobic bacteria.

Cleavage pathways

Production of the yellow product from catechol was tested for detecting meta or ortho ring fission pathways. Since there was no appearance of yellow colour within a few minutes it indicated absence of meta cleavage activity in the bacterial strains. A deep purple colour appeared with all CSMB cultures with a drop of 1% sodium nitroprusside, confirming ortho cleavage ring fission. (Fig.6)

Metabolic Versatility

Effect on growth of CSMBs as consortium to a variety of structurally different heterocyclic aromatic compounds, 32 in number (Table 4), used in leather processes was studied. For confirmation of degradation of the tested chemicals, the probable secondary metabolites were tested utilising them as sole carbon and energy. It was confirmed that CSMBs grew very well with a wide spectrum of recalcitrant chemicals with an initial concentration of 25 mg/l, present in leather industrial wastewater. Growth was observed within 24 h in the range of 0.8 to 1.4 as absorbance (OD 600 nm). It may due to the ortho cleavage pathway of CSMBs which releases more energy than meta pathway.

To conclude, recalcitrant chemicals are a common contaminant of industrial wastewaters at present. The biological treatment of these waste streams is strongly inhibited by high salt and heavy metal concentrations. Very few studies have been cited in relation to biodegradation of recalcitrant pollutants. It is more energy-efficient than conventional aerobic systems, requiring less energy for aeration and producing minimum sludge. This process can be utilised for the removal of recalcitrant aromatic substances under oxygen-limited conditions, which is an important step in wastewater treatment. It is an economically viable process and can be installed easily in an existing treatment plants. The isolated CSMB strains are moderately halophilic, highly motile, either capsulated or sporulated to withstand the stress conditions. The unique feature of the isolates was their capacity to degrade a range of heterocyclic compounds that are used in the leather industry to transform the putrescible hide/skin into valuable leather, by utilising them as sole carbon and energy, even without any growth factors including vitamins, amino acids and peptides. Degradation of these compounds was confirmed by the utilization of secondary metabolites of the corresponding compounds.. Resistance to heavy metals is an added feature for CSMBs. In the evaluation of antibiotic activity, resistance of CSMBs is more pronounced at microaerobic condition. Ortho cleavage ring fission was observed by all CSMB strains resulting in more energy. This is the first time utilization of recalcitrant pollutants present in leather industrial wastewater as a growth substrate by a pure bacterial culture at microaerophilic condition is reported.

Table.1 Structure of Heterocyclic aromatic Chemicals used in leather processing

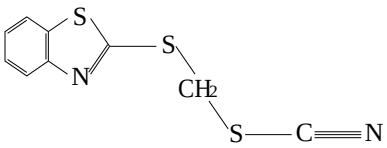
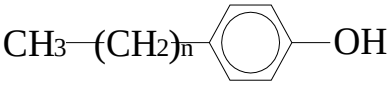
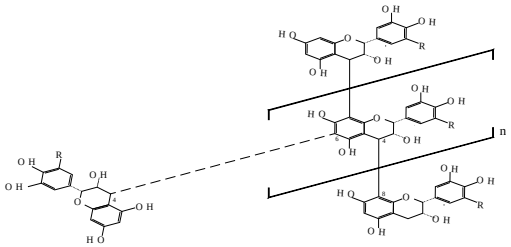
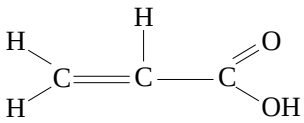
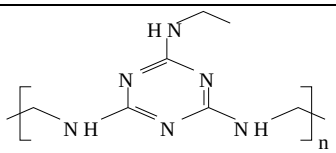
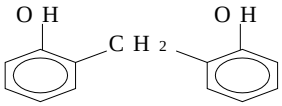
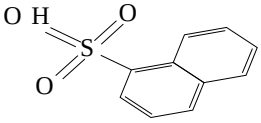
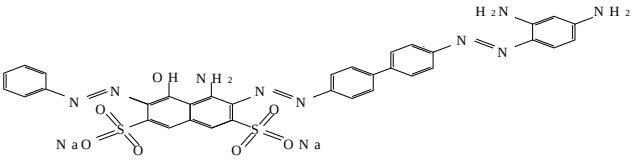
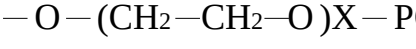
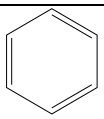
Name of the process - Application - Name of the aromatic chemical	Structure
Beam house – Preservative - TCMTB	
Beam house - Wetting Agent - Luwet 40	
EI.Tanning - Vegetable tanning-Wattle	
Retanning-Acrylic polymer– Relugan RE	
Retanning - Resin- Melamine formaldehyde	
Retanning - Syntan - Phenol formaldehyde	
Retanning – synthetic tanning - Naphthalene sulphonic acid	
Dyeing & fatliquoring - Vat dye - Direct Black38	
Dyeing and fatliquoring - Synthetic fat Liquor- FB II	
Finishing unit- spraying - Benzene	

Table.2 Morphological and Biochemical characteristics of *CSMB1 to CSMB 6*

Test	CSMB 1	CSMB 2	CSMB 3	CSMB 4	.CSMB 5	CSMB 6
Colony morpholog	Irregular	Circular	circular	Irregular	Circular	Mucoid
Gram reaction	G-	G-	G-	G-	G+	G-
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	-
Endospore s	+	+	-	+	+	+
Habitat	Facultative aerobe	Facultative aerobe	Facultat ive	Facultativ e anaerobe	Facultati ve	Facultativ e aerobe
Acid from						
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Arabinose	+	+	+	+	+	-
Lactose	+	+	-	-	+	+
Mannitol	+	+	+	+	+	-
Sorbitol	+	+	+	-	-	+
Rhamnose	+	+	+	-	-	-
Adonitol	+	-	-	-	-	-
Hydrolysis of						
Casein	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+
Starch	+	+	+	+	+	+
Citrate utilisation	+	+	+	+	+	+

Voges Proskauer	-	+	+	-	+	+
Indole formation	-	+	-	+	-	-
Methyl Red	+	-	-	+	-	-
Nitrate to Nitrite	+	+	+	-	+	+
Phenyl Alanine	-	+	-	-	-	-
Ornithine decorboxyla	+	+	+	+	+	-
Lysine Utilization	+	+	+	+	+	+
Urease Detection	+	-	-	-	+	+
Oxidase reaction	+	+	+	+	-	-
Catalase reaction	-	-	+	-	+	+
H_2S Production	-	-	-	-	-	+
Name of the isolate	*Alcaligenes sp*. MH146	*Enterobacter cloacae*	Serratia sp.	*Alcaligenes faecalis*	*Bacillus* sp.	*Klebsiella pneumoni*

Table.3 Molecular identification of Isolates deposited in MTCC for filing the patent

Name of the isolates	Closest sequence to the Isolate	Gen Bank Accession Number	MTCC No.	Date of Deposit
CSMB 1	*Alcaligenes sp. MH146*	FJ626617.1	5608	21.03.2011
CSMB 2	*Enterobacter cloacae strain SJ 6*	EU779827.1	5600	29.12.2010
CSMB 3	*Serratia sp. HA1*	HM136579.1	5602	29.12.2010
CSMB 4	*Alcaligenes faecalis subsp. faecalis strain AE1.16*	GQ284565.1	5601	29.12.2010
CSMB 5	*Bacillus sp. KMSII-3*	GQ468395.1	5611	19.04.2011
CSMB 6	*Klebsiella pneumoniae strain SDM45*	GQ417303.1	5609	21.03.2011

Table.4 Utilization of heterocyclic aromatic compounds and its secondary metabolites as sole carbon source by CSMB strains as consortium

(a) Pesticide and its Secondary metabolites	**Presence of growth**
2-thiocyanomethylthiobenzothiazole (TCMTB)	+++
Benzothiazole	+++
3 Hydroxy Benzothiazole	+++
2 Methyl Benzothiazole	+++
2 MercaptoBenzothiazole	+++
2 Methyl thioBenzothiazole	+++
2 amino Benzothiazole	+++
Benzothiazole sulfonate	+++
(b) Leather dyes and its Secondary metabolites	**Presence of growth**
Remazol Red RG	+++
Black DB	+++
Yellow 194	++
Brown 3GV	++
Remazol blue	+++
Black BG	++
Blue RGB	+++
Brown DB	++
Acid green	++
Reactive orange	+++
Black RB	+++
Metanilic acid	+++
Sulfanilic acid	+++
(c) Synthetic Chemicals used in leather processes	**Presence of growth**
Phenol	+++
Nonyl phenol	+++
Benzene	++
Naphthalene	++
Catechol	+++
Resorcinol	+++
Sodium benzoate	+++
L-Glutamic acid	+++
2 -hydroxy benzoic acid	+++
3-hydroxy benzoic acid	+++
4- hydroxy benzoic acid	++

Note: ++ OD $\geq$ 0.8; +++ $\leq$ 1.2

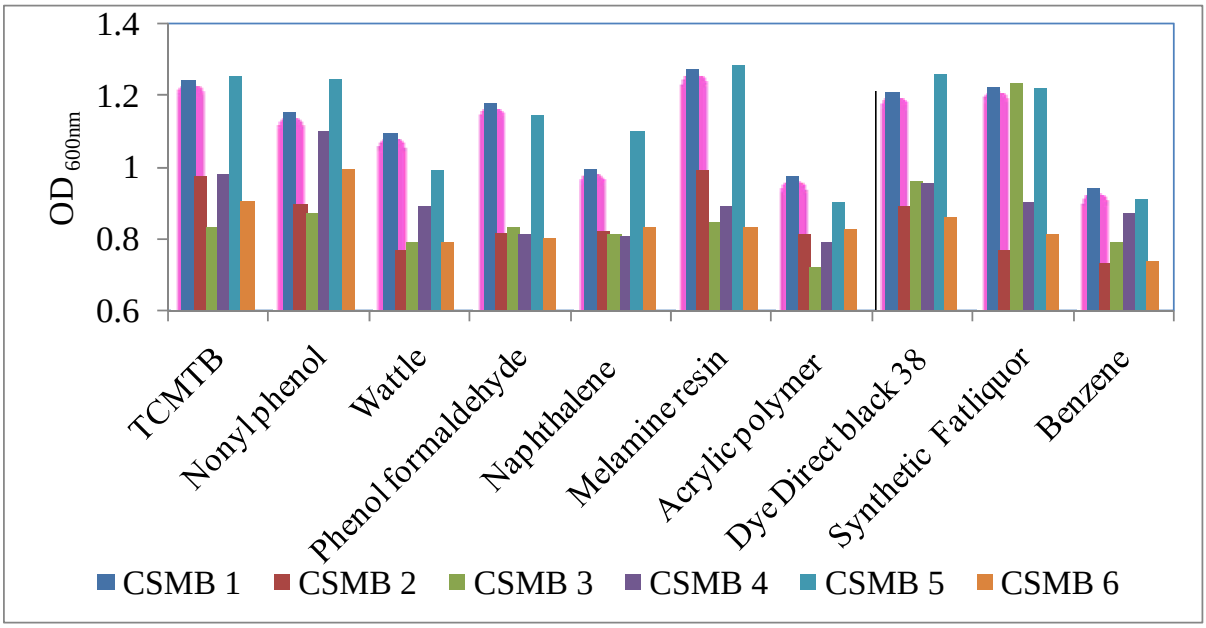


Fig.1 Growth of CSMB strains on heterocyclic aromatic compounds

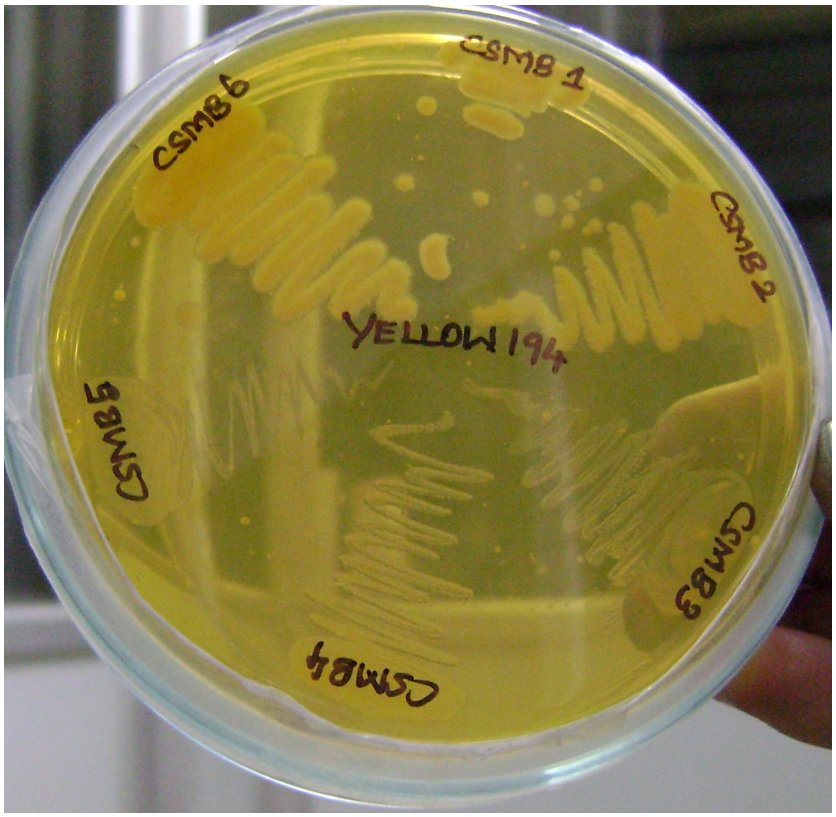


Fig.2 Pure colony of CSMBs in mineral agar

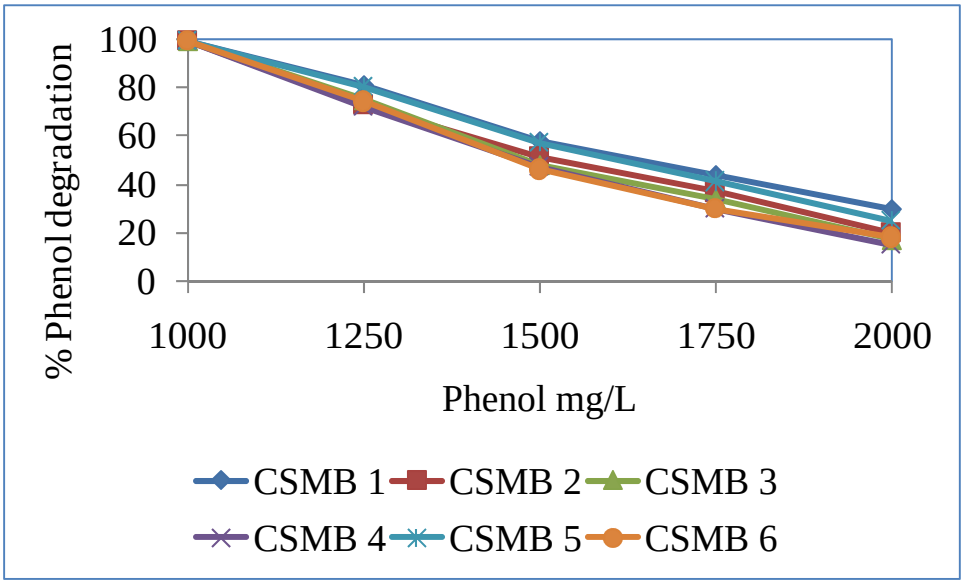


Fig.3 Effect of initial phenol concentration

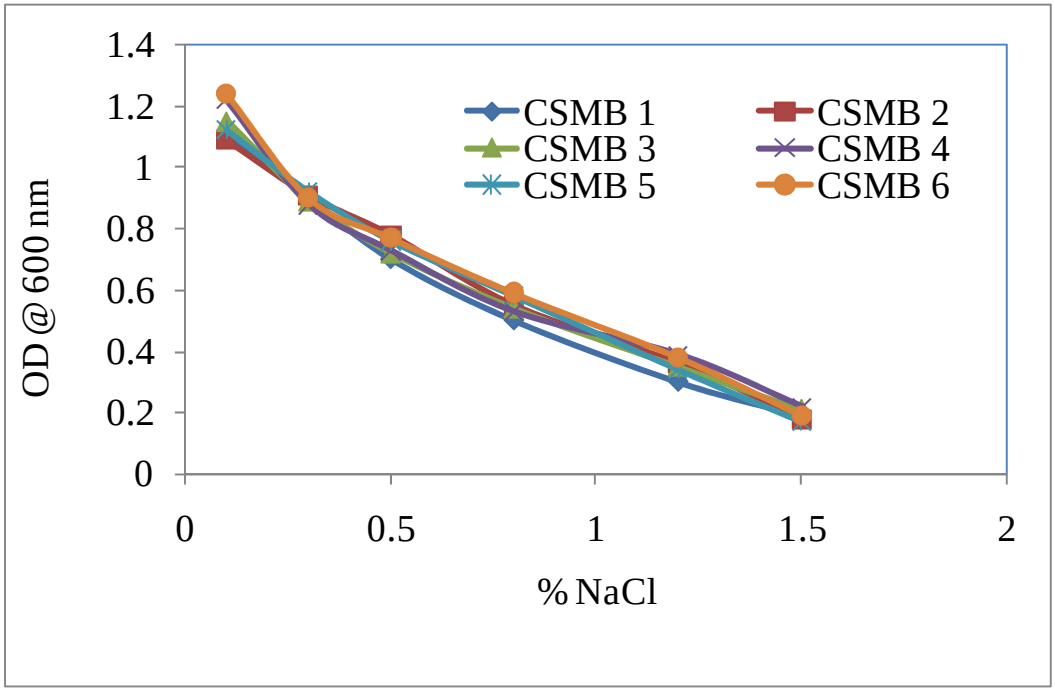


Fig.4 Effect of Salinity tolerance

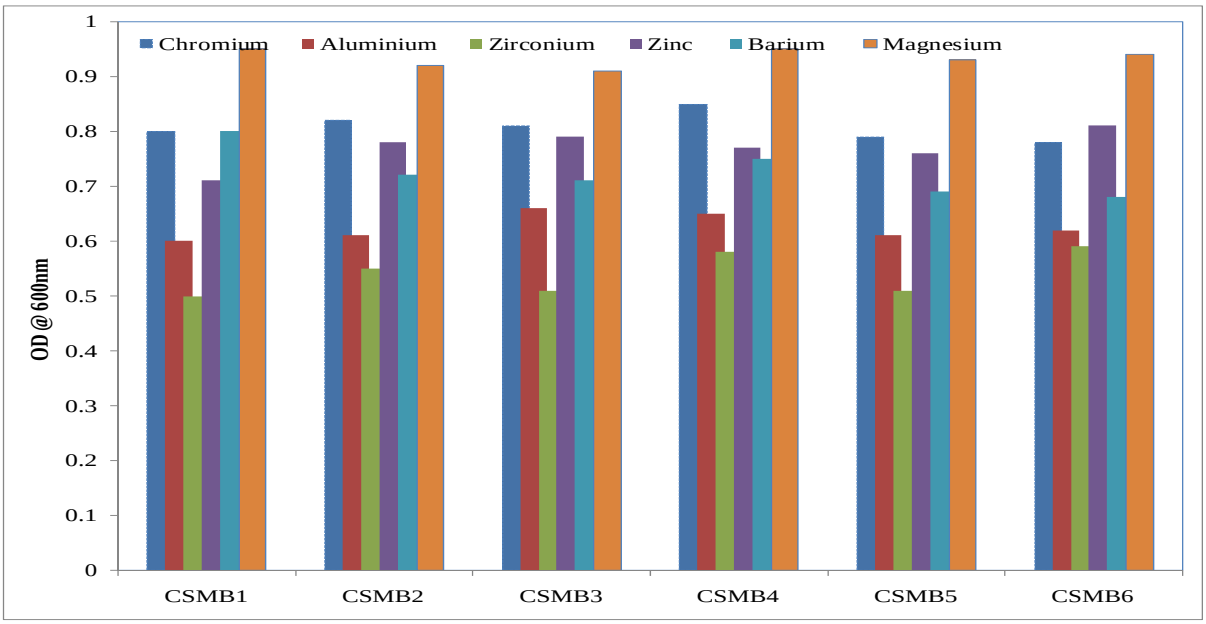


Fig.5 Effect of Heavy metal resistance

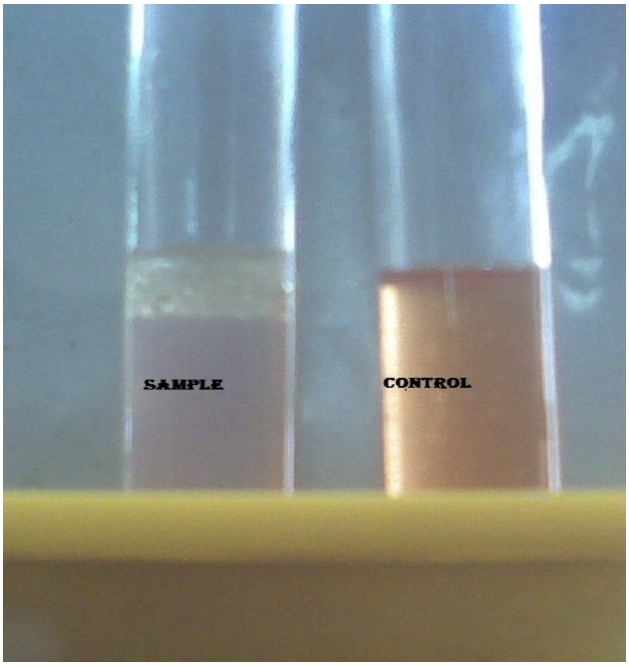


Fig.6 Ortho cleavage ring fission by CSMBs

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