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Bioaugmentation of halophilic consortia for the degradation of petroleum hydrocarbons and petroleum wastewater treatment

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Abstract

The present study, details about a halophilic bacterial consortium enriched from water and sediment samples from Red sea, Jeddah, Saudi Arabia analyzed for PAHs degradation potential under saline condition (40 g/L NaCl concentration). The bacterial consortium was able to growth in halophilic mineral salt medium with PAHs (Polycyclic Aromatic Hydrocarbons) as sole carbon source. Different types of selected LMW (Low Molecular Weight) PAHs such as phenathrene (PHN) and fluorine (FLU) at different concentrations (25, 50, 100, 200, 500 ppm) and pyrene (PY) from HMW(High Molecular Weight) PAH at 50 and 100 ppm concentrations was used in the study under saline condition. The results recorded 90% degradation of phenanthrene and fluoreneupto 500 ppm. HMW pyrene revealed 79% and 69% degradation at 50 ppm and 100 ppm concentration under saline condition by the halophilic consortium. Co-metabolic study performed by adding FLU with PY enhanced the biodegradation process and reduced the time required for biodegradation. Addition of FLU (100 ppm) along with the PY (50 ppm) recorded 85% of PY degradation in 8 days and 98% degradation in 12 days. Addition of yeast extract during PY degradation recorded complete degradation of PY (50 ppm) in 12 days. Lab Scale reactor study with CSTR (continuous stirred tank reactor) used to investigate the PAHs degradation and petroleum refinery wastewater treatment efficiency of in thehalophilic bacterial consortium. The results recorded 95% COD removal in 32 days with complete degradation of LMW PAHs in 12 days under saline condition. HMW PAHs recorded 91% degradation in 28 days by the bacterial consortium. Bacterial strains in the consortium were identified by using molecular techniques such as DNA isolation and next generation sequencing. Phylogenetic analysis revealed *Ochrobactrum* sp dominated the PAHs degrading halophilic consortium. Next generation sequencing results of both PAHs degradation and reactor study contained the dominance of *Ochrobactrum* sp. *Marinobacter* sp occupied the second position in the consortium, followed by *Pseudomonas* sp and *Stenotrophomonas maltophilia* in third and fourth position. Thus the potential halophilic bacterial consortium can be recommended to be employed in the treatment of saline petroleum refinery wastewater.

Keywords: Halophiles, biodegradation, petroleum hydrocarbons, wastewater treatment

1. Introduction

Petroleum hydrocarbons are ubiquitous environmental pollutants present in the environment with high toxicity. Petroleum hydrocarbons present in the crude oil enters the environment through different routes. Around 50% of crude oil was transported by the sea. Saudi Arabia produces 10 million barrels per day and support 22% of world crude oil requirement. Crude oil spillage is the major problem faced by the coastal ecosystem. Due to high spillage of the crude oil, the hydrocarbons with more benzene persist for longer period in the environment and cause toxic effects to marine and terrestrial ecosystem. Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic in nature which cause cytotoxic, mutagenic and carcinogenic (Shetaia et al. 2016). USEPA (United states Environmental Protection Agency) identified 16 PAH compounds as highly toxic released from industrial sectors. PAHs are divided into two major based on the number of benzene rings and molecular weight of the compound as low and high molecular weight PAHs (Kanaly and Harayama 2000). The low molecular weight (LMW) PAHs consist of compounds with less than three benzene ring (naphthalene, anthracene, phenanthrene and fluorene) and high molecular weight (HMW) PAHs with more than four benzene ring compounds (pyrene, chrysene, benzo(a)pyrene, benzo(e)pyrene and benzo(k)fluoranthen). PAHs exposed to human leads affect different parts of the body (lung, stomach, skin, breast, kidney and bladder) cause cancer (Kim et al. 2013).

The crude oil enters by various anthropogenic activities such as oil exploration or drilling, refining, storage and transportation process (Gong et al. 2014). Based on the number of fused benzene rings in the PAHs records the potential toxicity level, ranged from two benzne rings to six benzene ring compounds (Abdel-Shafy and Mansour 2016). Various treatment methods was employed in the treatment of PAHs such as physical (radio frequency heating, incineration), chemical (chemical precipitation, photolysis, electrolysis and adsorption) and biological (biodegradation, bioaugmentation, phytoremediation, rhizofilteration) for PAHs contaminated water, soil and sediments (Lamichhane et al. 2017). Even though there are different types of treatment methods such as photocatalysis (Liu et al. 2016), ionizing radiation (Almeida et al. 2006) and natural process of solublization and evaporation (Fingas 2013), bioremediation act as the promising treatment technology for the removal of hydrocarbons. Still the process is limited with environmental factors such as salinity, temperature and availability of nutrients (Atagana et al. 2003, Lefebvre and Moletta 2006, Fang et al. 2016).

Among the treatment methods bioremediation posses the attraction with special features of mineralizing the toxic PAHs to harmless form of CO_2 and water (Pugazhendi et al. 2017). The positive aspects of the bioremediation process are cost effective and sustainable ecofriendly cleaning technology. Naturally occurring microorganisms such as bacteria, fungi and algae was employed in the biodegradation of PAHs at different conditions (Vila et al. 2015). Extensive novel researches were performed using different microbial strains for treating petroleum contaminated sites due to the ecofriendly and economic value of the technology. Bioremediation was performed more than three decade under normal condition, under extreme condition limited studies were performed. Petroleum refining industries produce wastewater generally referred as produced water which consists of high range of salinity (Diaz et al. 2002). Major limiting factors of PAHs biodegradation under saline conditions are salinity, temperature and nutrients. Salinity highly influence the biodegradation process by damaging the cell membranes, enzyme production and desiccating osmotic force leads to lysis of bacterial cell (Kargi and Dincer 2000). Unavailability of nutrients such as phosphate and nitrogen leads to the death (decline phase) of bacterial cell. Thus the treatment of PAHs was ineffective with conventional biodegradation process under saline condition without nutrients (Atagana et al. 2003).

Many petroleum refinery company branches present all around the kingdom face the problem of PAHs removal. Due to high saline condition and temperature the treatment of petroleum refinery wastewater was a major challenge for the effluent treatment operators (Lefebvre and Moletta 2006). Halophiles capable of degrading PAHs draw immense attention of the scientific community for biotechnological applications in industrial wastewater treatment, due to their high level of tolerance towards different saline concentration (Dassarma and Dassarma 2015). Halophiles handle the challenge to treat the petroleum wastewater with different pollutants from oil and gas extraction process (Dillon 2003, Arulazhagan and Vasudevan 2009). Recent studies using single or group of bacterial strains for PAHs degradation explore the importance of halophiles in the field of petroleum hydrocarbon biodegradation (Mnif et al.

2014, Ghosal et al. 2016, Wang et al. 2017). Limited number of research studies was focused on the degradation and treatment of petroleum hydrocarbons in refinery wastewater under saline condition. The present study is focused to degrade low and high molecular weight PAHs under saline condition. The study also aimed to detail the importance of co-metabolism and utilization of additional substrate during PAHs degradation by halophiles under saline condition. The potential of the consortium to degrade the PAHs and treatment of wastewater was studied in continuous stirred tank reactor (CSTR) with optimized operational parameters under saline condition.

2. Materials and Methods

Chemicals

Low molecular weight (LMW) namely phenanthrene (PHN), fluroene (FLU) and high molecular weight (HMW) pyrene (PY)used in the study with high purity (98-99%) was purchased from Sigma Aldrich Chem. Co., USA. All other chemicals used in the mineral salt medium preparation were of analytical grade and purchased from Himedia, India. Reagents and Enzymes used for molecular microbial studies were purchased from Qiagen (Germany) and Invitrogen (USA).

Sample collection

The samples were collected from different place in Red sea, Jeddah coast, Saudi Arabia. The samples were containing sediment and water. The sample was transported to sterile glass bottles in the lab to be used for further analysis.

Halophilic Mineral Salt Medium (HMSM)

HMSM used in the present study consist of the following: Ammonium chloride (2.5 g/L), Disodium hydrogen phosphate (4.76), Potassium dihydrogen phosphate (5.46 g/L) and magnesium sulphate (0.2g/L) and sodium chloride (40 g/L). The pH of the medium was adjusted to 7.4 before sterilization. The medium was sterilized in autoclave at 121 °C for 15 min.

Experimental design for biodegradation of petroleum hydrocarbons

The experimental design consist of two control flasks namely abiotic (HMSM+PAH), biotic (HMSM+BC) and a test flask (HMSM+PAH+BC). All the experiments performed in duplicates. The samples present in control and test flasks were extracted with ethyl acetate (v/v) twice using a glass separating funnel. The organic phase (Ethyl acetate + PAH) was subjected to filtration using whatmann filter paper holding anhydrous sodium sulphate to remove the water content in the sample. After filtration, 1 mL of the filtrate was procured and filtered again with syringe filter into a glass vial for HPLC (High Performance Liquid Chromatography) and GCMS (Gas Chromatograph Mass Spectrometry) analysis.

HPLC and GCMS

HPLC (Agilent, USA) was used to analyze the residual hydrocarbons present in the experimental flasks. Acetonitrile at the flow rate of 1mL/min was used as mobile phase. C_{18}general purpose column was used as stationary phase with UV detector (254 nm) was used to detect the residual hydrocarbons in the flasks. GCMS was used to identify the metabolites formed during PAH biodegradation by the halophilic consortium. GCMS with fuse-silica capillary column (30m x 0.25mm I.D x 0.25µm) and performed analysis as reported by Pugazhendi et al. (2017).

Co-Metabolism and role of additional substrate study

PHN (100 ppm) used as co-substrate along with high molecular weight pyrene (50 ppm) to accelerate the biodegradation process. Yeast extract (100 ppm) was also used along with PY (50 ppm) to analyze the role of additional substrate in PAHs degradation.

Contact Angle and Zeta potential Measurement

Contact angle was measured to indicate the hydrophobicity of the halophilic bacterial consortium. Zeta potential magnitude indicates the degree of electrostatic repulsion between adjacent and similarly charge particles in a colloidal dispersion. Hydrophobic stability of the bacterial cell to attach with PAHs was confirmed using the zeta potential measurement (Harms et al. 2010). Samples for contact angle (CA, θ) and zeta potential analysis was prepared as detailed by Furuno et al. (2012).

Lab scale Reactor Study

Evaluation of the treatment potential of the bacterial consortium was executed in lab scale CSTR (continuous stirred tank reactor). The reactor designed with 10 L of total volume and 7 L as working volume capacity. Petroleum contaminated refinery wastewater was obtained from petrorabigh, Jeddah, Saudi Arabia (Fig. 1). The reactor contained 6.5L of wastewater and 0.5L of the consortium operated under saline condition (4%). OLR (organic loading rate) of the reactor under continuous mode was optimized based on the COD reduction. Different OLRs such as 0.154 kg/m3.d, 0.11 kg/m3.d, 0.085 kg/m3.d, 0.077 kg/m3.d with respective HRT (Hydraulic Retention Time) of 10, 14, 18 and 20 days. The optimized condition for the reactor was 18 days HRT with 0.085 kg/m3.d OLR. Increase or decrease in the OLR greatly influenced the COD removal and PAHs degradation in the reactor. HPLC analysis confirmed the presence of different both low and high molecular weight PAHs such as PHN (144.5 ± 0.3 ppm), FLU (182±1.1 ppm), Naphthalene (248.6±1.5 ppm), Anthracene (37.1±2.4) and PY (803.5±1.6). Chemical parameters for wastewater analysis such as COD (chemical oxygen demand), MLSS (mixed liquor suspended solids), MLVSS (mixed liquor volatile suspended solids), total nitrogen and total phosphate were analyzed based on the methods detailed in standard methods (APHA 2005).

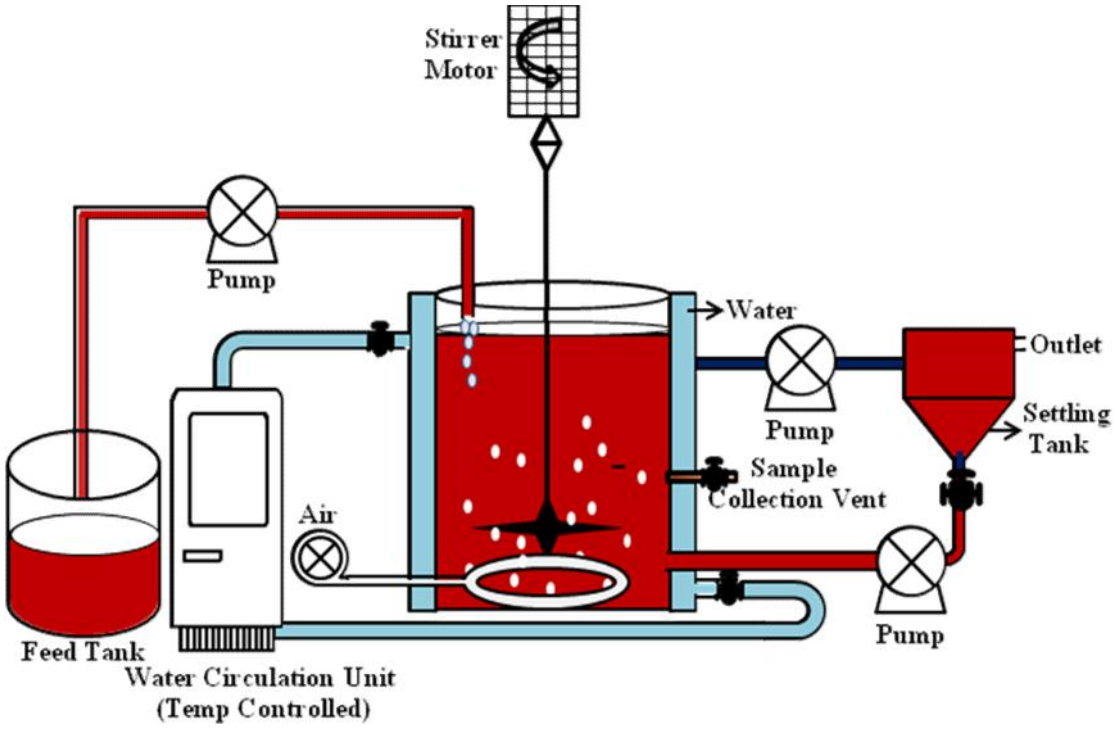

Fig. 1 CSTR for the treatment of petroleum wastewater under saline condition

Phylogenetic analysis

DNA isolated during different degradation experiments and reactor study using Qiagen DNA isolation kit. The extracted DNA was amplified in PCR machine (Applied Biosystems) with 27F and 1492R primers. PCR supermix (Invitrogen, USA) was used for DNA amplification as per the protocol reported by Arulazhagan and Vasudevan (2009). The potential halophilic bacterial strains present in the consortium was identified using high through put sequencing techniques. Primer set (515-532U and 909-928U) targeting at V4-V5 region of 16S rRNA was used in the high through put sequencing. The high through put sequencing reactions were performed as detailed by Pugazhendi et al. (2017).BLASTN search confirm the genus of the bacterial strain.

3. Results

Preliminary screening study on PAHs degradation

Initial study of PAHs degradation study conducted with PHN in HMSM agar medium showed PAH clearing zone under UV light. Zone of clearance by the consortium confirmed the utilization of PHN under saline condition (40 g/L NaCl concentration).

Biodegradation of PAHs

PHN and FLU was used as the model compound from low molecular weight PAHs. PHN was used to study the degradation potential of the bacterial consortium. The study started with 25 ppm concentration of PHN under saline condition (40 g/L NaCl concentration). The results showed 85% degradation of PHN in 4 days and complete degradation in 6 days (Fig.2).

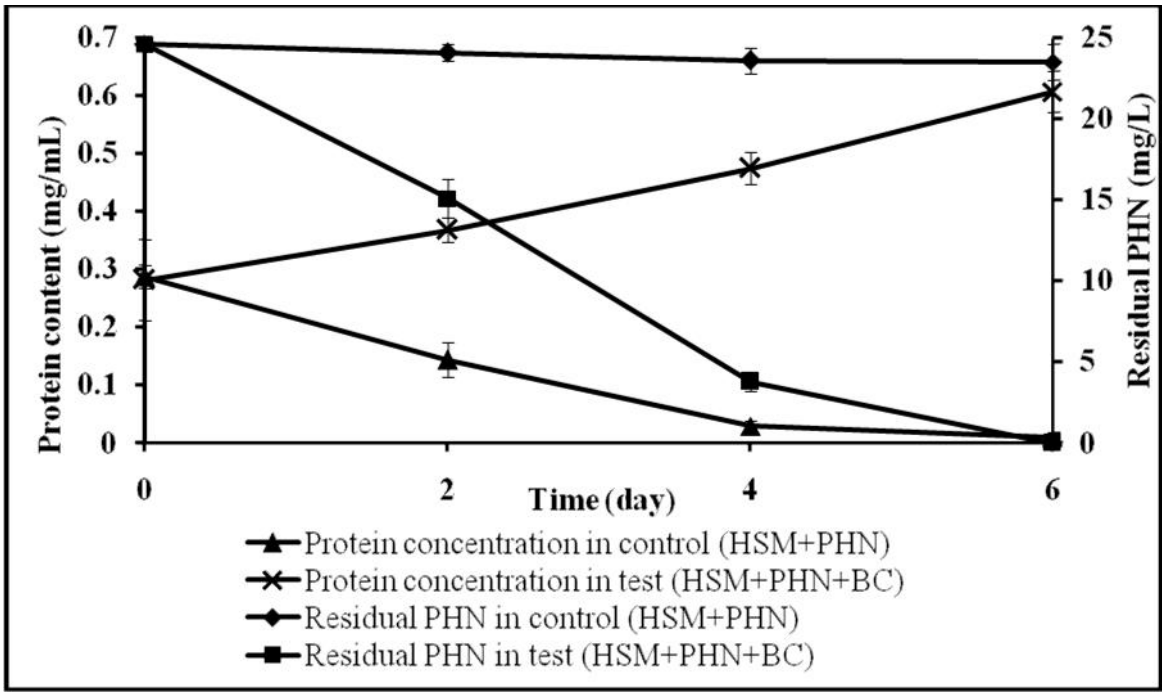

Fig.2 Degradation of PHN (25 ppm) by the halophilic bacterial consortium

GCMS analysis in LMW and HMW PAHs (PHN and FLU) degradation

PHN and FLU represented common metabolites such as benzoic acid, 1-Hydroxy-2-naphthalene carboxylic acid, oxalic acid and pentanoic acid (Table1). Pyrene was used to represent the HMW PAHs under saline condition to analyze the degradation efficiency of the halophilic consortium. 3-Benzenedicarboxylic acid, oxalic acid, phenanthrene-4-carboxylic acid, naphthalene carboxylic acid and benzoic acid were the metabolites highly matching with the metabolic pathway proposed for pyrene degradation under saline condition to an end-product of CO_2 and water (Table 2). Thus the consortium potently degraded both LMW and HMW to non-toxic form which reveals the importance of employing the halophilic consortium in PAHs degradation and petroleum wastewater under saline condition.

Table.1 GCMS analysis of metabolites formed during LMW PAHs degradation by halophilic bacterial consortium

Metabolite	Retention time (min)	M/z of fragment ions (% relative abundance)
		Phenanthrene
9-Phenanthrol	2.63	69.5(20), 81.3(35), 82.5(55), 135.7(92), 177(53), 186.3(28), 194 (35)
Octanoic acid	2.81	30(100), 42(16.8), 68(20.4), 98(17.2), 124(22.0).
1-Hydroxy-2-Naphthalenecarboxylic acid	3.33	127(60), 128(15), 155(55), 168(100)
1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	3.6	69.3 (15), 81.5(20), 128(15), 155(55), 168(100)
Pentanoic acid, 2-hydroxy-, ethyl ester	4.45	55(60), 73(100),104(13)
4-phenanthrol	4.8	69.2 (15), 81.2(26), 82.5(64), 83(98), 97.3(56), 139.8(75) 163(15), 165.5(22), 166.2(45), 167.2(78), 194.3(12).
Oxalic acid, hexylneopentyl ester	5.33	41(26.8), 57(54.4), 71(88.8), 43(100)
Benzoic acid, 4-ethoxy-, ethyl ester	6.78	121(100), 138(34), 149(58),166(15),194.3(30)
2-Propenoic acid, pentadecyl ester	9.86	55(61.6), 57(63.6), 70(69.6), 43(82.4), 71(97.2) 85(100).
Terephthalic acid, di(2-methoxyethyl) ester	12.35	58(83.2), 149(34.4), 207(100)
		Fluorene
Octanoic acid	2.78	30(100), 41(24.0), 55(31.6), 56(29.6)
Naphthalene	4.100	102.1(11.8), 127.15(14.49), 128.15(100), 129.15(11.58), 207.05(20.62)
Pentanoic acid	4.45	55(60), 73(100),104(13)
9-Fluorenone	7.63	43(59.2), (55.2) (66.3) 71(100.0)
Furanone, dihydro-3-hydroxy-4,4-dimethyl	8.55	41(40.0), 29(40.4), 43(59.2), 71(100.0)
Furanone	8.55	43(59.2), 71(100.0)
Trihydroxybiphenyl	9.23	39(15), 95(100), 105(22.3), 202(38.7)
Dihydronaphthalene	10.11	39(10), 51(22.3), 64(20.2), 77(15.2), 89(21.3), 102(18), 115(65.3), 130.2(100)
o-Phthalate	10.21	149(34.4), 58(83.2), 114(100)
Hydroxylaminobenzene	10.6	39(25.3), 51(21.2), 65(80.3), 92(100), 109(78.3)
1-Hydroxy-2-Naphthalenecarboxylic acid	10.98	127(60), 128(15), 155(55), 168(100)
Methyl Salicylate	11.3	39(20.1), 65(24.6), 92(78), 120(100), 152.2(41.3)
4-Hydroxy-9-fluorenone	12.3	41(40.0), 43(59.2), 71(100.0)

Table 2 GCMS analysis of metabolites formed during HMW PAH degradation by halophilic bacterial consortium

Metabolite	Retention time (min)	M/z of fragment ions (% relative abundance)
	Pyrene	
Pyrene-1,2-oxide	12.35	58(83.2), 65.3(22), 89(25), 149(34.4), 222(100)
3-Benzenedicarboxylic acid	3.01	200.75(98.85), 207(100)
1-Hydroxypyrene	4.36	47(10), 63(12), 74(5), 81(19), 94(22), 95(55), 109(20), 186(15), 187(16), 188(21), 189(75), 220(100)
Oxalic acid	5.33	41(26.8), 57(54.4), 71(88.8), 43(100)
Phenanthrene-4-carboxylic acid [33,34]	12.35	200.75, (98.8), 207(100)
1-Hydroxy-2-Naphthalenecarboxylic acid	3.98	127(60), 128(15), 155(55), 168(100)
Benzoic acid	6.77	121(100), 138(34), 149(58),166(15),194.3(30)
3,4-Dihydroxybenzoate	12.90	109(25.6), 168(40.0), 137(100).

Degradation of LMW PAHs

Phenathrene

Study at 50 ppm and 100 ppm concentration of PHN revealed 100% degradation in 8 days and 95% degradation in 8 days under saline condition (Fig. 3). Negligible amount of abiotic loss of PAHs was recorded in the control flasks. PHN degradation and the corresponding increase in protein concentration clearly depict the mineralization of PHN under saline condition by the halophilic consortium. The results were evident to prove the PHN degradation potential by the consortium. Further increase of PHN concentration to 200 ppm and 500 ppm recorded 95% and 93% degradation in 10 and 12 days respectively. Degradation of PHN at 200 ppm and 500 ppm showed 92% and 80% degradation in 8 days. Increase in PHN concentration slightly influenced the degradation potential of the consortium, thus the slow degradation of PHN under saline condition was recorded (Table 3).

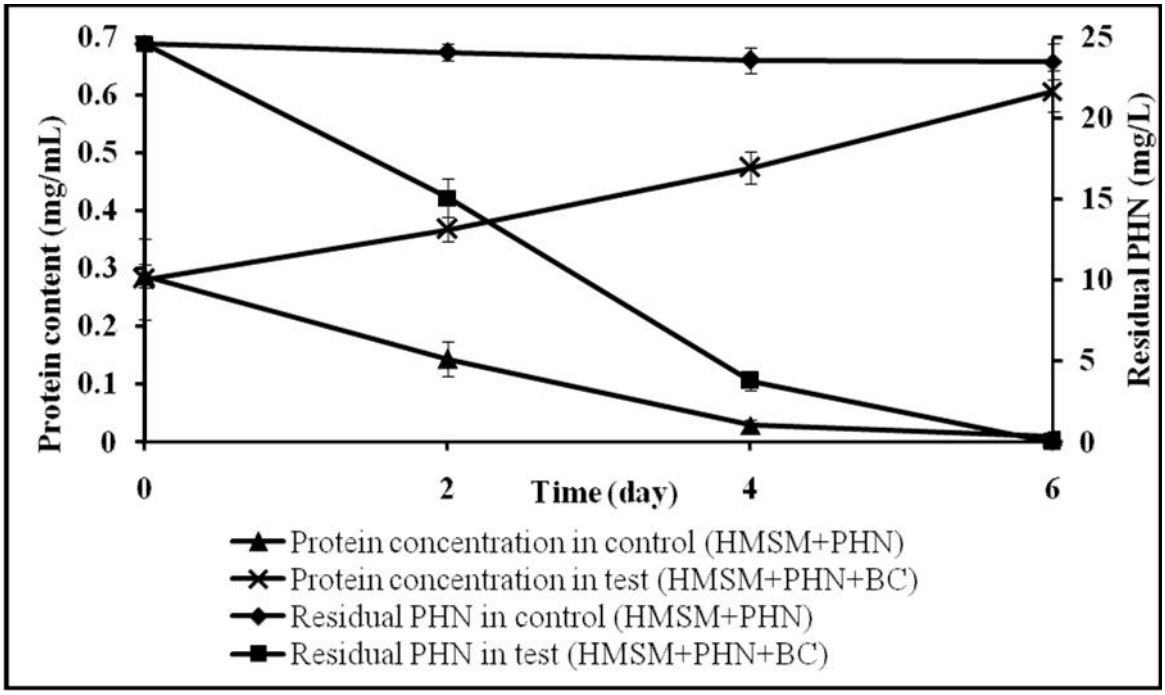

Fig. 3 Degradation of PHN (50 ppm) by the halophilic bacterial consortium

Fluorene

Biodegradation of FLU was studied at different concentration ranging from 50 ppm to 500 ppm. The results confirmed potential degradation efficacy of the halophilic consortium. Initial analysis on FLU degradation was performed at 50 ppm concentration (Fig. 4). The consortium degraded 96% of FLU in 6 days and complete degradation in 8 days under saline condition (40 g/L NaCl concentration).

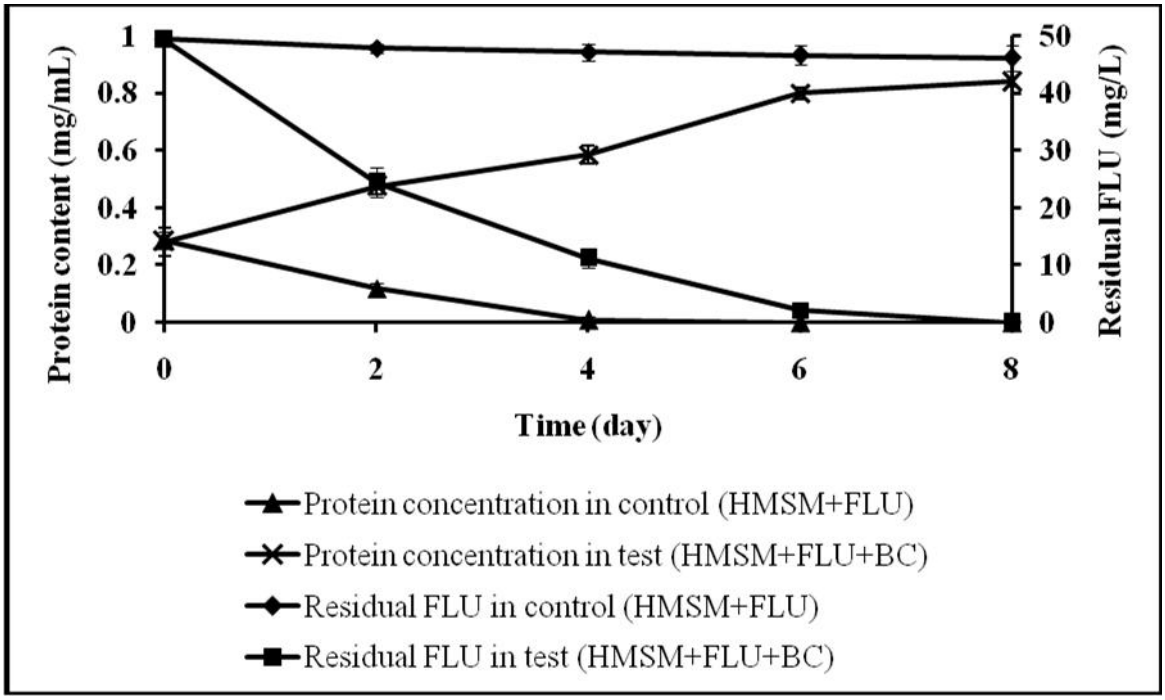

Fig. 4 Degradation of FLU (50 ppm) by the halophilic bacterial consortium

FLU concentration was increased in the HMSM to analyze the degradation potential of the bacterial consortium. At 100 ppm concentration, complete degradation was achieved in 8 days. Further increase in FLU concentration to 200 and 500 ppm showed excellent degradation pattern of 99% and 96% degradation in 10 and 12 days respectively (Table 3). Further increase in concentration showed decrease in degradration under saline condition. Thus the results from low molecular weight PAHs were promising for the degradation potential of the halophilic bacterial consortium.

Table 3 Degradation of LMW PAHs at different concentrations by the consortium

LMW PAHs concentration	Phenanthrene degradation (%)	Day	Fluorene degradation (%)	Day
50	100	8	100	8
100	95	8	100	8
200	95	10	99	10
500	93	12	96	12

Degradation of HMW PAH

Pyrene

Pyrene with four benzene rings selected to represent the HMW PAHs for biodegradation by the halophilic consortium under saline condition (40 g/L of NaCl concentration). PY degradation was started with 50 ppm concentration as sole carbon source for the bacterial consortium. The consortium was able to degrade 79% of PY in 12 days due to increased number of benzene ring and stressed salinity (Fig.5a).

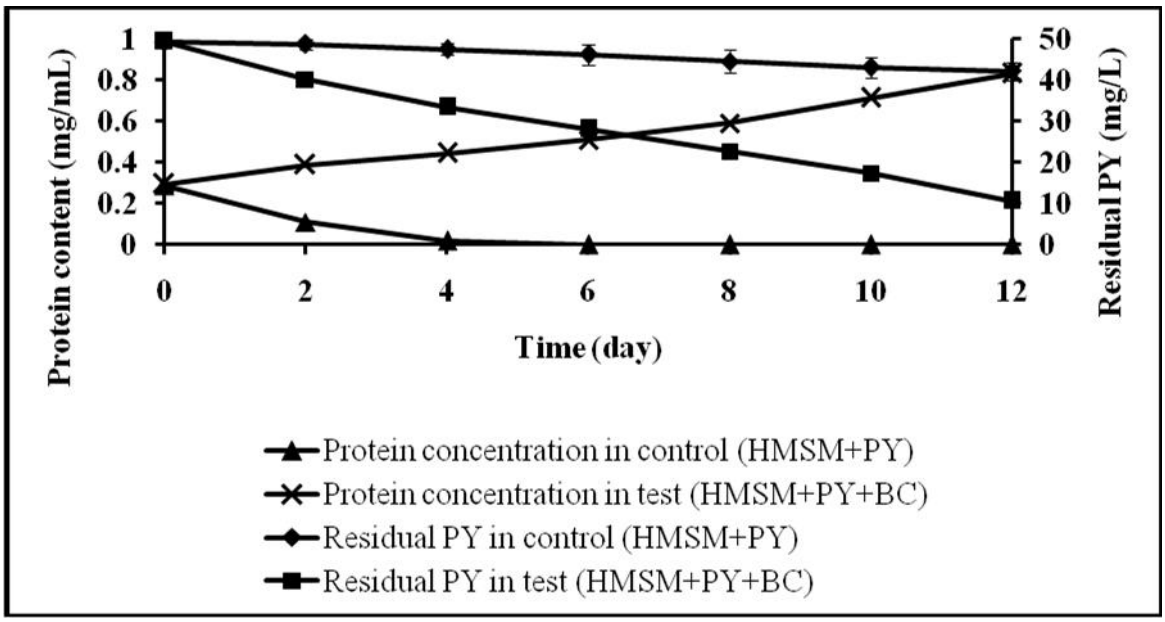

Fig. 4a Degradation of PY (50 ppm) by the halophilic bacterial consortium

Increase in PY concentration to 100 ppm recorded decline phase in the degradation process. Degradation of PY at 100 ppm was 69% in 12 days at 40 g/L of NaCl concentration (Fig. 5b).

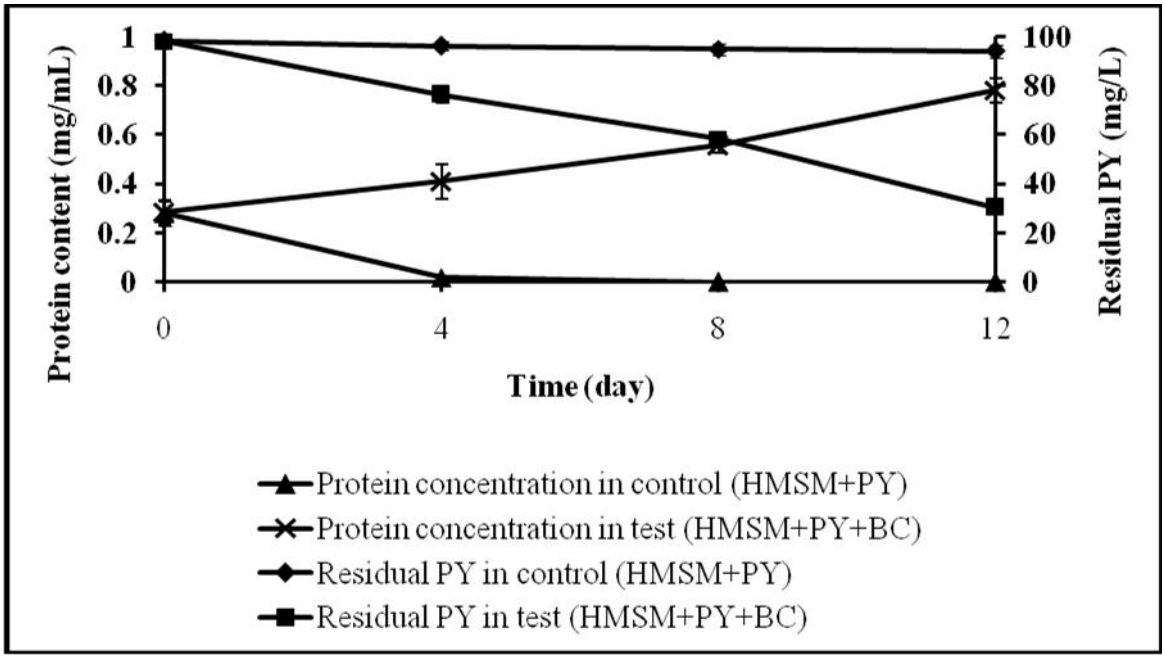

Fig. 5b Degradation of PY (100 ppm) by the halophilic bacterial consortium

Bacterial cell interaction with PAHs

Halophilic bacterial consortium cell hydrophobicity was analyzed in goniometer or contact angle measurement analyzer and zeta potential value. The contact angle (θ) for the consortium was 87º and zeta potential value was -28 ± 1.1 mv. The results confirm the bacterial cell interaction with hydrophobic PAHs by forming biofilm.

Co-metabolism in PAHs degradation

During PY degradation the consortium was unsuccessful under saline condition due to dual stress with high benzene ring compound and salinity. To overcome this problem addition of LMW PAHs along with HMW PAHs was trialed. In the present study FLU (100 ppm) was used along with PY (50 ppm) under saline condition. Co-metabolism process potentially enhanced the biodegradation process. The results of co-metabolism process depicted 85% of PY removal in 8 days. FLU was degraded completely in 8 days and 98% of PY was degraded in 12 days during co-metabolic process (Fig.6).

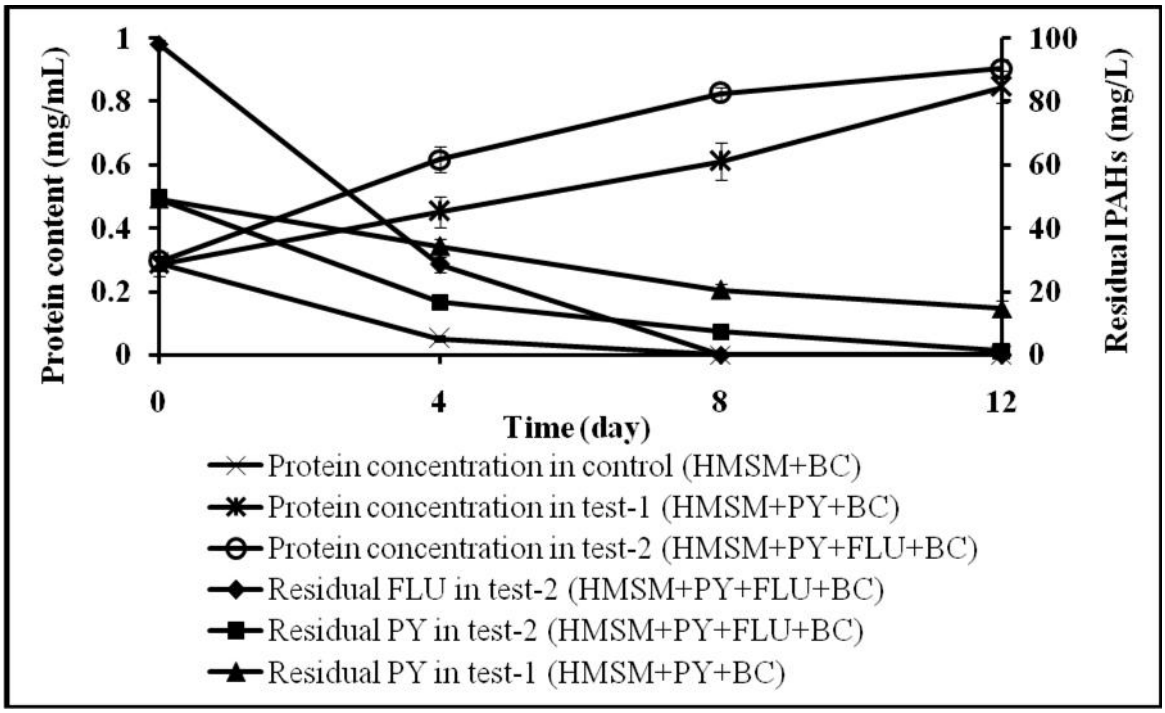

Fig.6 Co-metabolic degradation of PY with and without FLU under saline condition.

Role of additional substrate in PAHs degradation

Similar to co-metabolism to accelerate the biodegradation process yeast extract was added as additional substrate in the study along with PY. Addition of yeast extract enhanced the biodegradation process, recorded 83% of PY degradation in 10 days and completes degradation in 12 days respectively (Fig. 7).

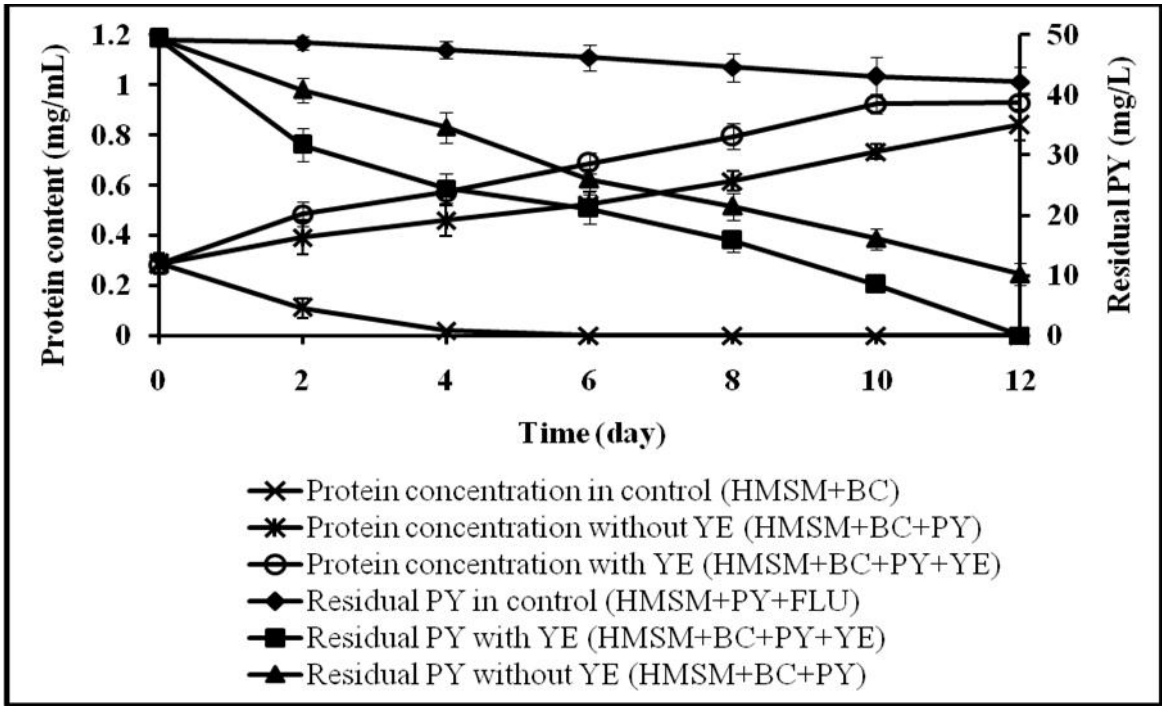

Fig. 7 Biodegradation of PY with and without yeast extract under saline condition.

Lab scale CSTR study for petroleum wastewater treatment

Petroleum wastewater was treated by the halophilic consortium under optimized reactor condition with 18 days HRT and 0.085 kg/m3.d OLR. The results recorded 95% COD removal in 32 days with complete degradation of LMW PAHs in the wastewater under saline condition. MLSS and MLVSS were maintained in the reactor in the range of 3.5 to 4.5 g/L and 3 to 3.3 g/L respectively (Fig. 8). Total nitrogen and total phosphate reduced to negligible concentration (<10 ppm) from 100 ppm, corresponding to the degradation of PAHs and COD removal in the reactor. Complete degradation of LMW PAHs in 12 days and 91% of HMW PAHs in 28 days was recorded.

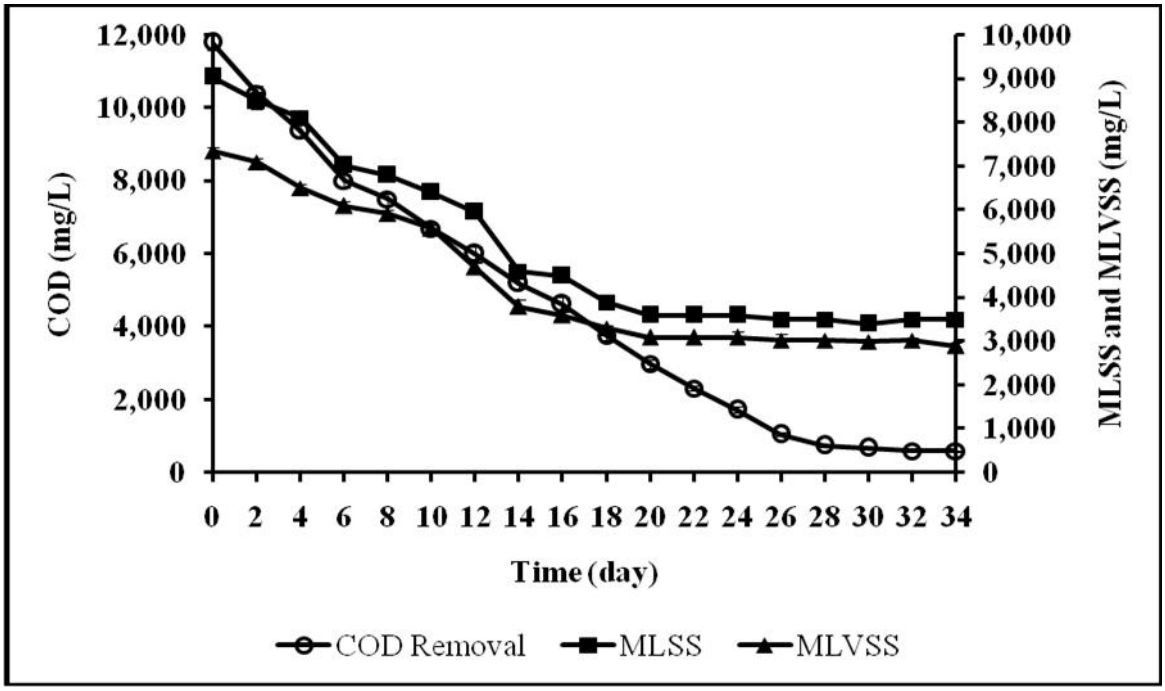

Fig. 8COD removal during the treatment of petroleum wastewater in CSTR under saline condition

Phylogenetic analysis of halophilic bacterial consortium

PAHs degrading bacterial strains in the consortium were identified using molecular techniques such as DNA isolation and next generation sequencing. Phylogenetic analysis performed in different experimental conditions. Bacterial community analysis was performed with samples from PAHs degradation study and CSTR. The results during PAHs degradation and reactor study revealed the dominance of *Ochrobactrum* sp. in the consortium followed by *Marinobacter* sp., *Pseudomonas* sp. and *Stenotrophomonas maltophilia* (Table 4).

Table 4. Bacterial community analysis during PAHs degradation and petroleum wastewater treatment in CSTR

Bacterial Strains in the Consortium	Biodegradation study on both LMW and HMW PAHs (%)	Reactor Study (%)
Ochrobactrum sp.	61	65
Marinobacter sp.	22	26
Pseudomonas sp.	10	5
Stenotrophomonas maltophilia	7	4

Thus the research study details the PAHs degradation and treatment of petroleum wastewater under saline condition by the novel halophilic consortium. Further research may be performed in pilot scale study which will pay way for technology transfer to petroleum wastewater treatment plant.

4. Discussion

Degradation of PAHs and treatment of petroleum wastewater under saline condition is the major challenge faced by most of the researchers and operators of petroleum refinery effluent treatment plant. The present study is focused to find a novel solution with a effective treatment technology. Sediment and water samples collected from red sea, Jeddah region enriched in HMSM with PHN as sole carbon source. Initial screening study confirmed the utilization of PHN by the halophilic bacterial consortium with zone of clearance in the PAH coated agar medium. The results of the present study based on Kiyohara protocol (Kiyohara et al.1982) were in agreement with previous studies by Hilyard et al. (2008), Arulazhagan and Vasudevan (2009 and 2011) and Arulazhagan et al. (2014). The degradation potential of the consortium was analyzed with LMW PAHs such as PHN and FLU at different concentrations.Yu et al. (2005) stated a bacterial consortium from marine sediments potentially utilized PHN and FLU completely in 14 days under saline condition. Degradation of PHN and FLU recorded more than 90% upto 500 ppm concentration with variation in time duration under saline condition. Zhao et al. (2009) reported PHN degradation under different saline conditions with yeast extract using a halophilic bacterial consortium. Feng et al. (2012) reported PHN degradation by a halophilic bacterial strain *Martella* sp.AD-3. Studies employed bacterial consortium provided potential degradation of PAHs to non-toxic form compare to research with single strain (Arulazhagan and Vasudevan 2009, Pugazhendi et al. 2017). Several studies performed on PHN degradation under saline condition where percent degradation was limited by concentration of PHN (Cui et al.2014, Erdogmus et al 2013, Arulazhagan et al. 2010). Moghadam et al. (2014) reported 64% and 58% of FLU and PHN degradation in 7 days by the consortium enriched from mangrove sediment supplemented with yeast extract and tween 80. In the present study the consortium pertained complete degradation of FLU and PHN in 8 days without any additional carbon or nitrogen source. Thus the consortium used in the present study potentially degraded LMW PAHs at different concentrations under saline condition (40 g/L of NaCl concentration).

Metabolites formed during PAHs degradation was analyzed in GCMS. Common metabolites such as benzoic acid, 1-Hydroxy-2-naphthalene carboxylic acid, oxalic acid and pentanoic acid were identified during the degradation process. These metabolites were in agreement with the previous reports by Keum et al. (2008), Tsai et al. (2009) and Pugazhendi et al (2017). Oxidization of PAHs by insertion of both oxygen atoms to from cis-dihydrobiols and further metabolized (oxidized) to dihydroxy compounds. This dihydroxy compounds enter TCA cycle by the ortho or meta cleavage pathway (Smith 1990, Roy et al. 2012). Degradation of pyrene (HMW PAHs) by the halophilic consortium revealed the presence of metabolites such as 3-Benzenedicarboxylic acid, oxalic acid, phenanthrene-4-carboxylic acid,

naphthalene carboxylic acid and benzoic acid also enter the TCA cycle. The metabolites identified during PY degradation were similar to the previous study by Liang et al. (2006) and Pugazhendi et al. (2017). The results confirmed the degradation of PY to harmless end product (CO_2 and Water).Thus the mineralization study on LMW and HMW PAHs clearly indicated the absence of toxic intermediates during the process. Bacterial cell interaction with PAHs was confirmed by the hydrophobicity measurement with goniometry and zeta potential analysis. The results indicated 87º contact angle for the bacterial consortium which is rated under moderate hydrophobicity. Zeta potential was -28 mv also reconfirmed the hydrophobicity of the consortium. The hydrophobic stability of the consortium potentially enhanced the bacterial cell interaction with PAH by reducing the distance between them (Harms et al. 2010). Thus the studies confirm the attachment of PAHs to the cell surface of the halophilic consortium for potential degradation.

Degradation of PY (HMW PAH) performed at two concentration 50 and 100 ppm concentration. The results showed 79% and 69% degradation of PY by the consortium under saline condition. To overcome the stress caused by salinity and increase in benzene rings to the consortium co-metabolism was used as a tool. Co-metabolism is the process used in PAHs degradation to enhance to biodegradation and reduce the time required for degradation (Arulazhagan et al. 2014). During co-metabolic process, the consortium initially feed on LMW PAH leads to increase the bacterial growth and followed by mineralization of HMW PAHs. Addition of FLU (100 ppm) along with the PY (50 ppm) was studied which accelerated the biodegradation process and recorded 85% of PY degradation in 8 days and 98% degradation in 12 days. The results were similar to previous studies performed with LMW PAH as co-substrate (Arulazhagan et al. 2014). Role of additional substrate in the degradation of PAHs was analyzed using yeast extract along with PY(HMW PAH). Depletion of nutrients (phosphate and nitrogen) is normal under saline condition which greatly influences the biodegradation of hydrocarbons (Kargi and Dincer 1996). Yeast extract in posses nitrogen, vitamins, carbon and amino acids to support bacterial growth under saline condition. Thus yeast extract was employed as additional substrate which increases the bacterial growth and record corresponding PAH degradation (Nicholson and Fathepure, 2004). In the present study the consortium degraded PY completely in 12 days. Thus addition of yeast extract accelerated the biodegradation process which is similar to previous study by Arulazhagan et al. (2010), Arulazhagan and Vasudevan (2011), Pugazhendi et al (2017).

Petroleum refinery wastewater treatment potential of the halophilic bacterial consortium was evaluated in lab scale CSTR. The study revealed 95% COD removal in 32 days and complete degradation of LMW PAHs and 91% of HMW PAHs under saline condition. Thus the halophilic consortium can be readily employed in the treatment of petroleum wastewater under saline condition. Recently use of extremophiles (Halophiles, thermophiles, acidophiles and alkalophiles) in the treatment of petroleum wastewater attracted the scientific community to provide a novel treatment technology for the petroleum industrial sector. Phylogenetic analysis of the PAHs degrading bacterial consortium revealed potential bacterial strains. *Ochrobactrum* sp dominated in both PAHs degradation study and petroleum wastewater treatment. *Ochrobactrum* was studied in the degradation of organic compounds such as phenols hydrocarbons (Kilic 2009, Chandrasekaran et al. 2017), pesticide (Wang et al. 2013, Talwar et al. 2014) and petroleum (Arulazhagan Vasudevan 2009, Bhattacharya 2015 and Pugazhendi et al 2017). *Marinobacter* sp covered 22 to 26% of the consortium actively participated in the PAHs degradation process. Previous researchers reported the petroleum hydrocarbon degradation potential of Marinobacter (McGowan et al. 2004, Al-mailem et al. 2013 and Yuan et al. 2015). In the present *Pseudomonas* sp. shared 5 to 10% of the consortium also widely known to degrade PAHs under different environmental conditions. Several evident scientific reports were published on PAHs degradation by *Pseudomonas* strains (Mnif et al. 2011, Pasumarthi et al 2013, Patowary et al. 2015 and Pugazhendi et al.2017). *Stenotrophomonas maltophilia* in the PAHs degrading halophilic consortium covered a least portion of 4 to 7%. *S. Maltophilia* was also known as PAH degrading strain evident from the previous reports (Singh et al. 2015, and Pugazhendi et al.2017).Thus all the strains present in the consortium were reported PAHs degrading strains. The present study detailed the PAHs degradation efficiency and petroleum wastewater treatment potential of halophilic bacterial consortium enriched from marine environment. Thus the consortium can be recommended as potential candidate for petroleum wastewater under saline condition in the petrochemical industry.

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