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Analysis of Individual and Bacterial Consortium of Broth Culture of *Pseudomonas Aeruginosa*, *Serratia Marscecens* and *Bacillus Subtilis* to Emulsify Oils and Hydrocarbons

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Abstract

Three bacteria namely, *Pseudomonas aeruginosa*, *Serratia marscecens* and *Bacillus subtilis* were screened for biosurfactant activity and consortium was formed to examine the emulsification of oils and hydrocarbons. The bacteria and consortium showed positive blood hemolytic and lipase activity. The bacteria and consortium was able to emulsify vegetable oil effectively as compared to hydrocarbons viz. petrol, Diesel, kerosene and mobil oil.

Keywords:

emulsification, *Pseudomonas aeruginosa*, *Serratia marscecens* and *Bacillus subtilis,* consortium

Introduction

Microorganisms exhibit emulsifying production by producing biosurfactants and utilize hydrocarbons as substrate often mineralizing them or four different oils as substrate and the biosurfactant converting them into harmless products (Priya and Usharani, 2009). Biosurfactants are metabolites, generally secondary, that constitute a group of diverse compounds synthesized by a wide variety of microorganisms (bacteria, filamentous fungi and yeasts) (Rodríguez *et al.,* 2010; Cameotra and Makkar, 2004; Nitschke *et al.,* 2005; Banat, 2010).

Biosurfactants are microbially produced surface-active agents and occur in nature as chemical entities such as glycolipids, phospholipids and lipopeptides. These molecules have attracted considerable scientific attention due to lower toxicity, higher biodegradability, activity at extremes of temperature, pH and salinity and possibility of their production through fermentation using cheap agro-based substrates (Desai and Banat, 1997; Sen *et al.,* 2009). They have the unique property of lowering the interfacial tension between two liquids. Biosurfactants act on the interface and are amphipathic molecules with both hydrophilic and hydrophobic moieties present within the same molecule (Sekhon *et al.,* 2011). In addition biosurfactants have a huge repertoire that enables them to degrade a wide range of organic pollutants [Magdalena *et al.,* 2011). The prospects of biosurfactants have a great potential because of their applications in the petroleum industry [Mulligan, 2005; Banat, 1995; Amedea *et al.,* 2010] and microbial enhanced oil recovery (Okpokwasili and Ibiene 2006; Youssef *et al.,* 2007; Salehizadeh and Mohammadizad, 2009; Amani *et al.,* 2010;

Shavandi *et al.,* 2011; Darvish *et al.,* 2011). The *Rhodococcus ruber* biosurfactants are found to be 1.4 to 2.3 times more efficient then the synthetic surfactants (Tween 20, Tween 60) in enhanced crude oil desorption and mobilization from soil core, with 65-82% crude oil recovery (Philip, 2005). Moreover, esterases and lipases show activity on a great variety of substrates, with no requirement for added cofactors (Schmidt-Dannert, 1999). Thus, they are very interesting biocatalysts for industrial purposes such as detergency, flavor production, paper recycling, chemical synthesis and resolution of racemic mixtures (Jaeger *et al.,* 1999).

The present work is an initial attempt to systematically screen for biosurfactant-producing microorganisms and to evaluate their emulsification activity.

Materials and Methods

Screening of isolates for biosurfactant activity

Cells in the flasks were harvested by centrifugation at 6000rpm for 15 minutes and the supernatant was used as the biosurfactant solution. The test for determines the potency of the biosurfactant was based on the following (Umeji *et al.,* 2010).

Haemolysis on Blood Agar

Blood hemolysis was screened by plating cells on Blood Agar plates containing 5% (v/v) sheep blood/human blood and incubated at room temperature for 24 hours. Haemolytic activity was detected by occurrence of a defined clear zone around a colony which was an indicative of biosurfactants activity.

Drop Collapse assay

Petrol/Diesel oil (2µl) was added to petriplate lid. The lid was pre-equilibrated for 1 hour at room temperature, and then 5µl of the culture supernatant was added to the surface of oil. The shape of the drop on the oil surface was inspected after 1 minute. Biosurfactant containing cultures will give flat drops, thus indicating a positive result.

Lipase assay

Tributyrin agar plates were prepared using Nutrient agar and Tributyrin (1%). The pH of the medium was adjusted to 7.3 - 7.4 using 0.1 N NaOH. The culture was streaked on the Tributyrin agar plates and incubated at 28°C for 7 days. The plates were then examined for zone of clearance around the colonies.

Emulsification index

Sterile biosurfactant solution (2ml) was added into each test-tube (in a set of three) containing the substrate (Petrol/Diesel) 2ml. The content of the tubes was vigorously shaken for uniformity for 2 minutes and left undisturbed for 24 hours. The volume of oil that separated after 24h, 48h and 72h of standing was measured that showed the ability of a molecule to form a stable emulsion. The emulsification activity was defined as the height of the emulsion layer divided by the total height and expressed in percentage.

$$E = \frac{\text{Height of the emulsion layer}}{\text{Total height}} \times 100$$

Where,

- E_0=emulsification index at 0h.
- E_{24}= emulsification index after 24h.
- E_{48}=emulsification index after 48h.

- E_{72}= emulsification index after 72h.

RESULTS

Screening of isolates for biosurfactant activity

Blood hemolysis assay by isolates

Pseudomonas aeruginosa showed α hemolytic pattern on blood agar medium while *Serratia marscecens* and *Bacillus subtilis showed* β hemolysis. Blood hemolysis assay indicates a role of hemolysis caused by biosurfactant producing micro organisms that has been reported in several earlier literatures.

Table: 1 Blood hemolysis assay by isolates

Biosurfactants source	Blood hemolytic pattern
Pseudomonas aeruginosa	**α**
Serratia marscecens	**β**
Bacillus subtilis	**β**
Consortia	**β**

Lipase activity of consortia and individual bacteria

All three bacterium showed a positive lipase activity on Tributyrin agar plates as well as the consortium was able to hydrolyze lipid.

Table: 2 Lipase activities of consortia and individual bacteria

Biosurfactants source	Petrol	Diesel	Mobil oil	Kerosene	Mustard	Soybean	Jasmine	Almond
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+
Serratia marscecens	+	+	+	+	+	+	+	+
Bacillus subtilis	+	+	+	+	+	+	+	+
Consortia	+	+	+	+	+	+	+	+

Emulsification index of *Serratia marscecens* culture in BH broth

Broth culture of *Serratia marscecens* showed highest emulsification in mobil oil (50%) that was stable till 96h of incubation (29.16%). It also emulsified diesel with stability till 96h as29.16%, 22.22, 20.83, 17.39 and 13.63. Kerosene was emulsified till 24h of incubation while there was no emulsification of petrol. Among vegetable oils coconut oil was highly emulsified with stability till 96h. Mustard oil, jasmine and soy bean oil were also emulsified having stability till 96h of incubation. Comparatively the broth culture was found to effectively emulsify vegetable oil as to that of hydrocarbons.

Table: 3 Emulsification index of *Serratia marscecens* culture in BH broth

Emulsification index of *Serratia marscecens* culture in BH broth

	Substrate	E0	E24	E48	E72	E96
Hydrocarbons	**Kerosene**	11.53	9.094	4	0	0
	Petrol	0	0	0	0	0
	Diesel	29.16	22.22	20.83	17.39	13.63
	Mobil oil	50	40.9	40	36	29.16
Vegetable oils	**Mustard oil**	40	30.76	34.37	32.25	30
	Coconut oil	60	54.16	50	47.82	45.45
	Soy bean oil	45.83	43.75	35.71	28.57	23.07
	Jasmine oil	53.84	52	46	44	41.66

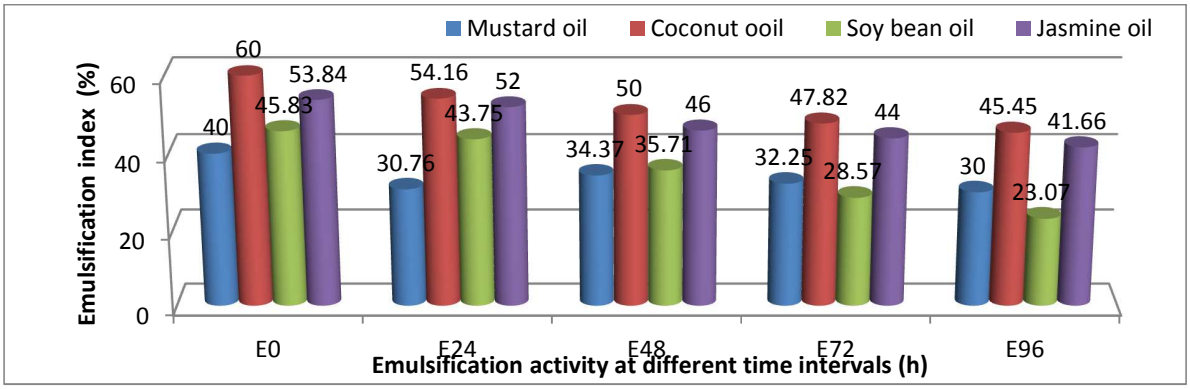


Fig:1 Emulsification index of *Serratia marscecens* culture in BH broth

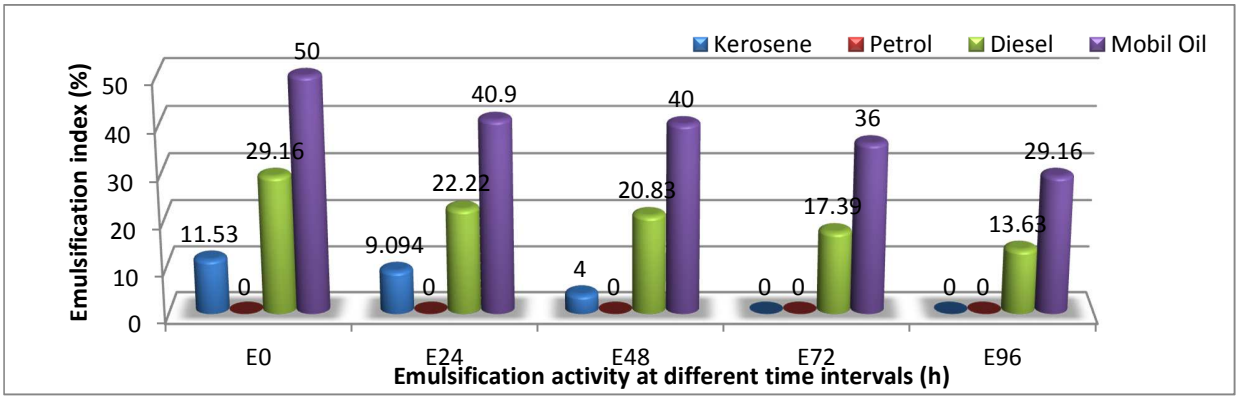


Fig:2 Emulsification index of *Serratia marscecens* culture in BH broth

Emulsification index of *Bacillus subtilis* in BH broth

Broth culture of *Bacillus subtilis* showed highest emulsification in mobil oil (76.92%) that was stable till 96h of incubation (33.33%). It also emulsified diesel with stability till 96h as 53.84%, 52%, 46.15%, 45.83% and 42.85%. Kerosene was emulsified as 28%, 25%, 20%, 12.5% and 9.69% while for petrol emulsification was as followed 45.45%, 42.1%, 40%, 38.88% and 35.29%. Among vegetable oils jasmine was highly emulsified (76.92%, 75%, 68%, 58.33% and 54.16%) with stability till 96h. Mustard oil, jasmine and soy bean oil were also emulsified having stability till 96h of incubation. Comparatively the broth culture was found to effectively emulsify vegetable oil as to that of hydrocarbons.

Table: 4 Emulsification index of *Bacillus subtilis* in BH broth

	Emulsification index of *Bacillus subtilis* in BH broth					
	Substrate	**E_0**	**E_{24}**	**E_{48}**	**E_{72}**	**E_{96}**
Hydrocarbons	**Kerosene**	28	25	20	12.5	9.69
	Petrol	45.45	42.1	40	38.88	35.29
	Diesel	53.84	52	46.15	45.83	42.85
	Mobil oil	76.92	62	48	37.5	33.33
Vegetable oils	**Mustard oil**	75.86	68	68.96	67.85	66.66
	Coconut oil	42.85	41.66	37.03	33.33	30.76
	Soy bean oil	58.62	57.14	53.84	52	50
	Jasmine oil	76.92	75	68	58.33	54.16

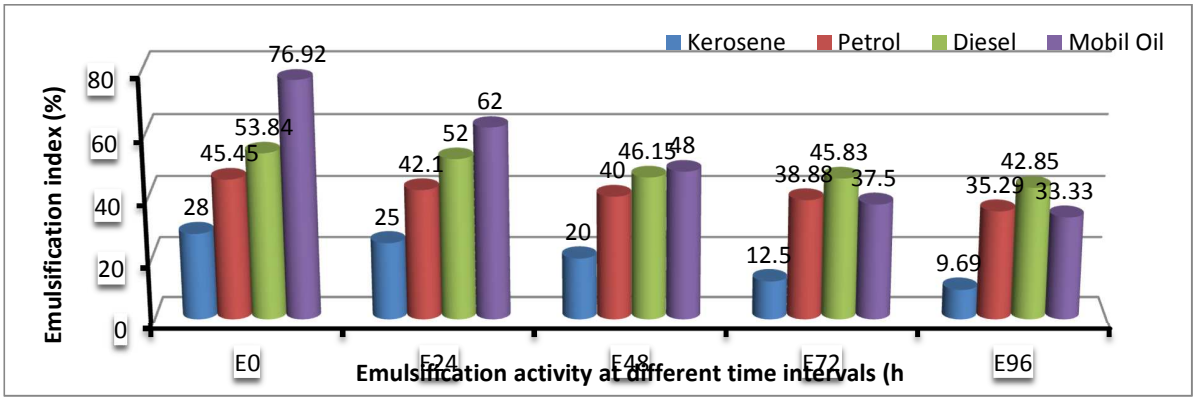


Fig:3 Emulsification index of *Bacillus subtilis* in BH broth

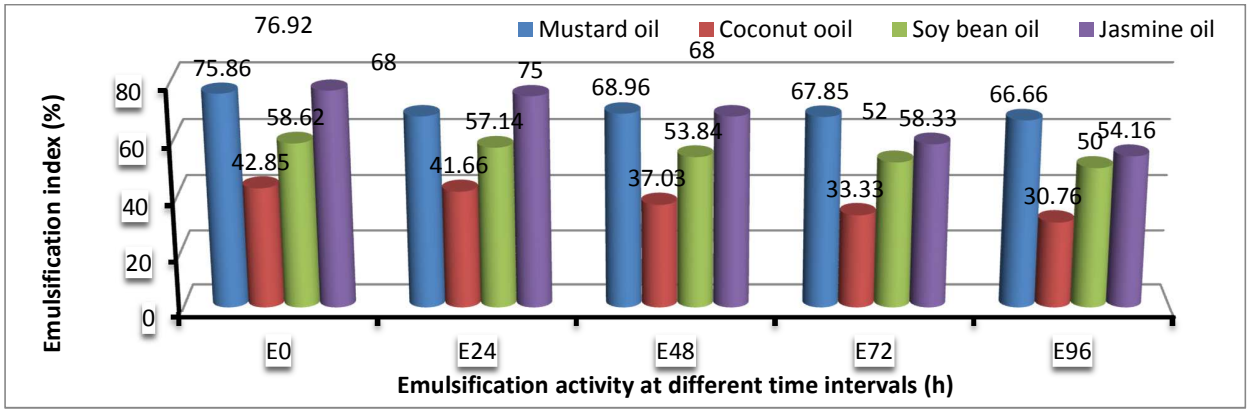


Fig:4 Emulsification index of *Bacillus subtilis* in BH broth

Emulsification index of *Pseudomonas aeruginosa* in BH broth

Broth culture of *Pseudomonas aeruginosa* showed highest emulsification in mobil oil (50%) that was stable till 96h of incubation (29.16%). It also emulsified diesel with stability till 96h as 88%, 64.28%, 50%, 47.82% and 45.45%. Petrol was emulsified till 24h of incubation while there was no emulsification of Kerosene and diesel oil. Among vegetable oils mustard oil, was highly emulsified

with stability till 96h. Coconut oil, jasmine and soy bean oil were also emulsified having stability till 96h of incubation. Comparatively the broth culture was found to effectively emulsify vegetable oil as to that of hydrocarbons.

Table: 5 Emulsification index of *Pseudomonas aeruginosa* in BH broth

	Emulsification index of *Pseudomonas aeruginosa* in BH broth					
	Substrate	**E_0**	**E_{24}**	**E_{48}**	**E_{72}**	**E_{96}**
Hydrocarbons	**Kerosene**	0	0	0	0	0
	Petrol	9.52	5.55	0	0	0
	Diesel	0	0	0	0	0
	Mobil oil	88	64.28	50	47.82	45.45
Vegetable oils	**Mustard oil**	79.31	60.71	57.14	55.55	53.84
	Coconut oil	48	47.82	47.82	45.45	42.85
	Soy bean oil	48	47	41.66	40.9	40
	Jasmine oil	54.54	52.38	45.45	45	38.88

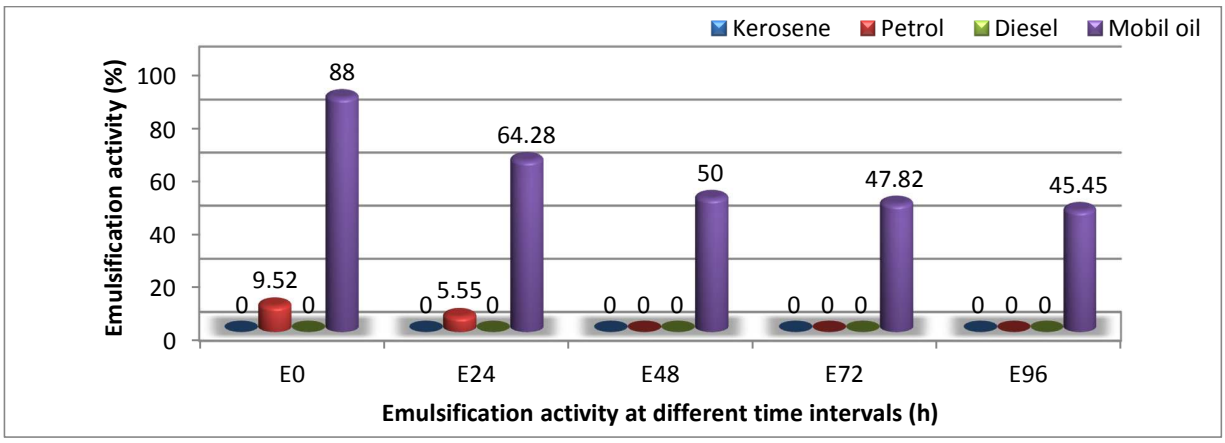


Fig:5 Emulsification index of *Pseudomonas aeruginosa* in BH broth

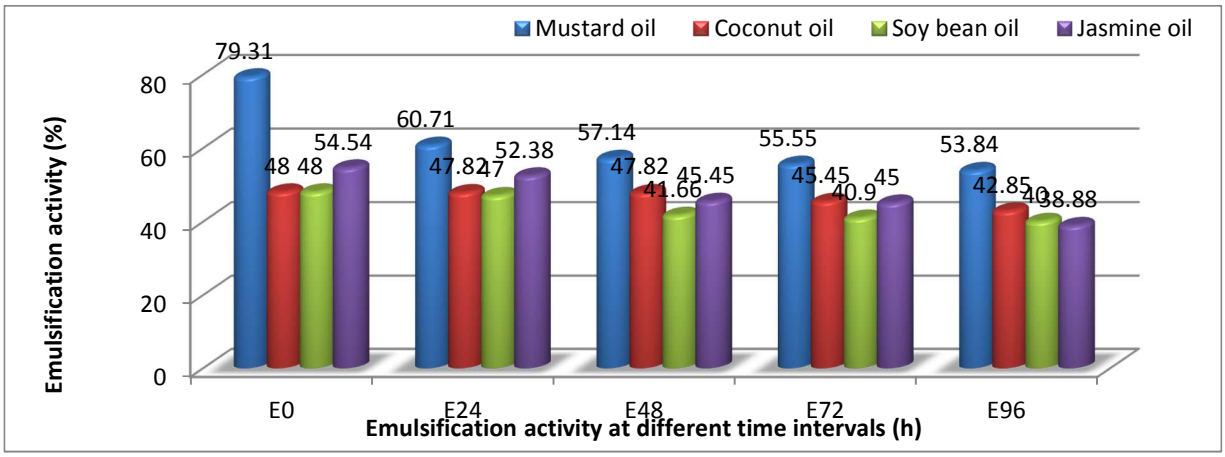


Fig:6 Emulsification index of *Pseudomonas aeruginosa* in BH broth

Emulsification index of consortium in BH broth

Broth culture of consortium showed highest emulsification in mobil oil (88%) followed by petrol that was stable till 96h of incubation (78.26%). Bacterial consortium was not able to emulsify Diesel and kerosene. Among oils jasmine was highly emulsified folloed by coconut mustard and soy bean oil. Comparatively the broth culture was found to effectively emulsify vegetable oil as to that of hydrocarbons.

Table: 6 Emulsification index of consortium in BH broth

	Emulsification index of consortium in BH broth					
	Substrate	**E_0**	**E_{24}**	**E_{48}**	**E_{72}**	**E_{96}**
Hydrocarbons	Kerosene	0	0	0	0	0
	Petrol	40.9	34.78	30.43	22.22	13.33
	Diesel	0	0	0	0	0
	Mobil oil	88	81.81	80	79.16	78.26
Vegetable oils	Mustard oil	35.48	33.33	30	20.68	17.85
	Coconut oil	42.3	37.03	32	29.16	25
	Soy bean oil	16	13.04	8	4.16	0
	Jasmine oil	48	47.82	40	34.78	33.33

Fig:7 Emulsification index of consortium in BH broth

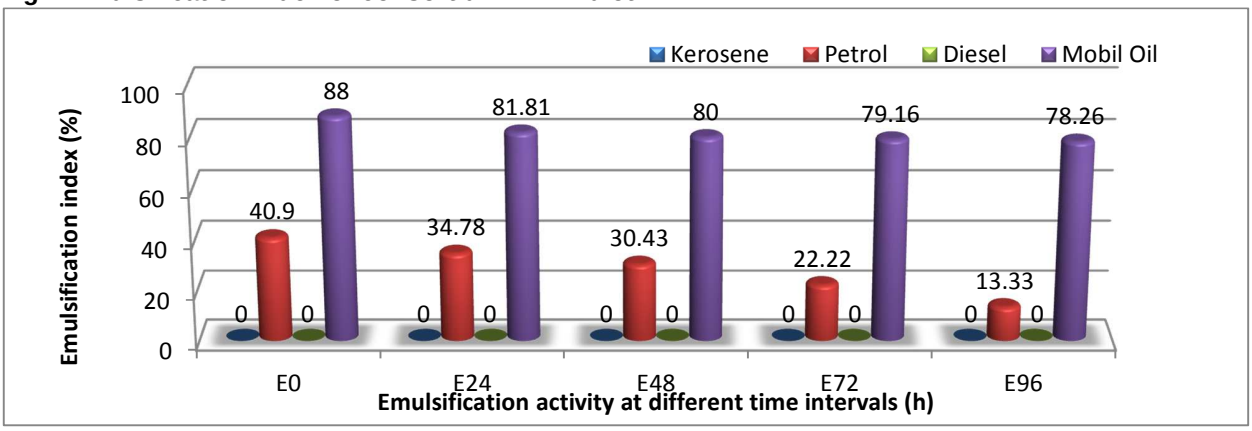
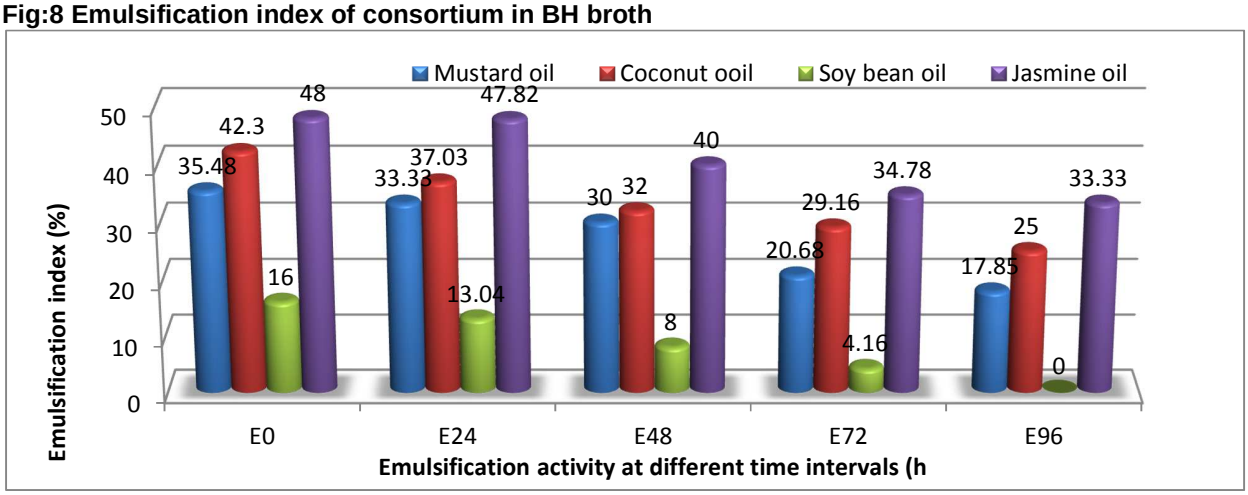


Fig:8 Emulsification index of consortium in BH broth



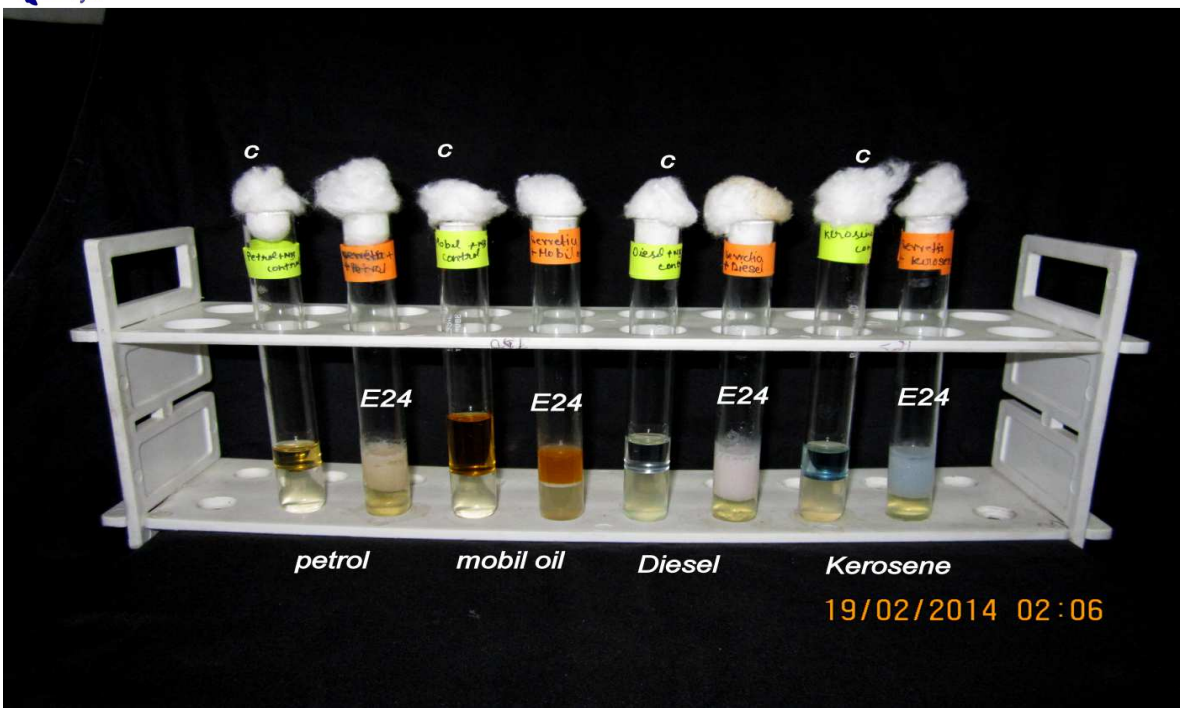


Plate: 1 Emulsification activity of cells of *Serratia marscecens* in BH broth

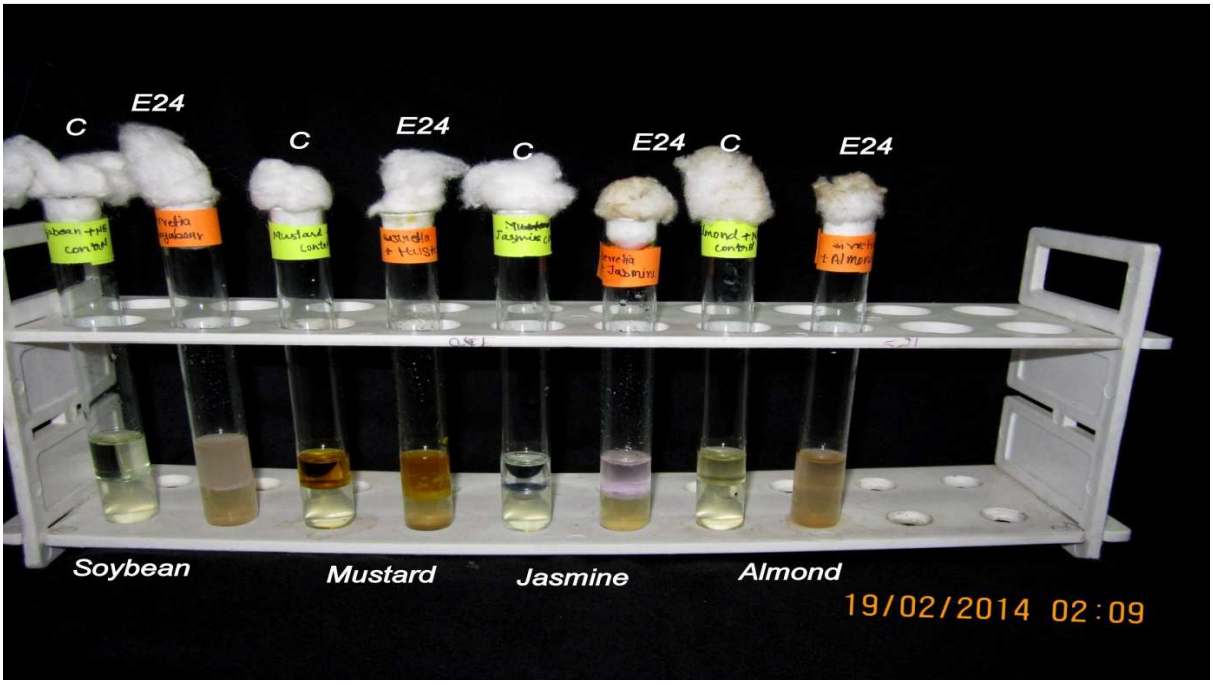


Plate: 2 Emulsification activity of cells of *Serratia marscecens* in BH broth

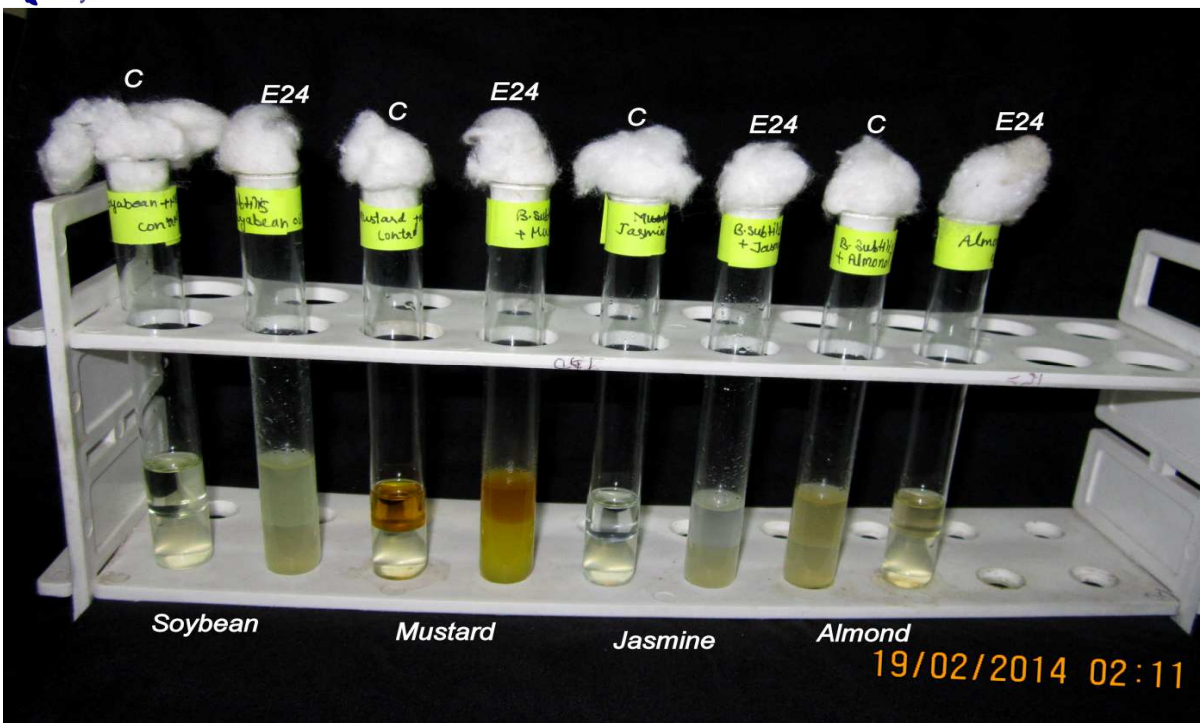


Plate: 3 Emulsification activity of cells of *Bacillus subtilis* in BH broth

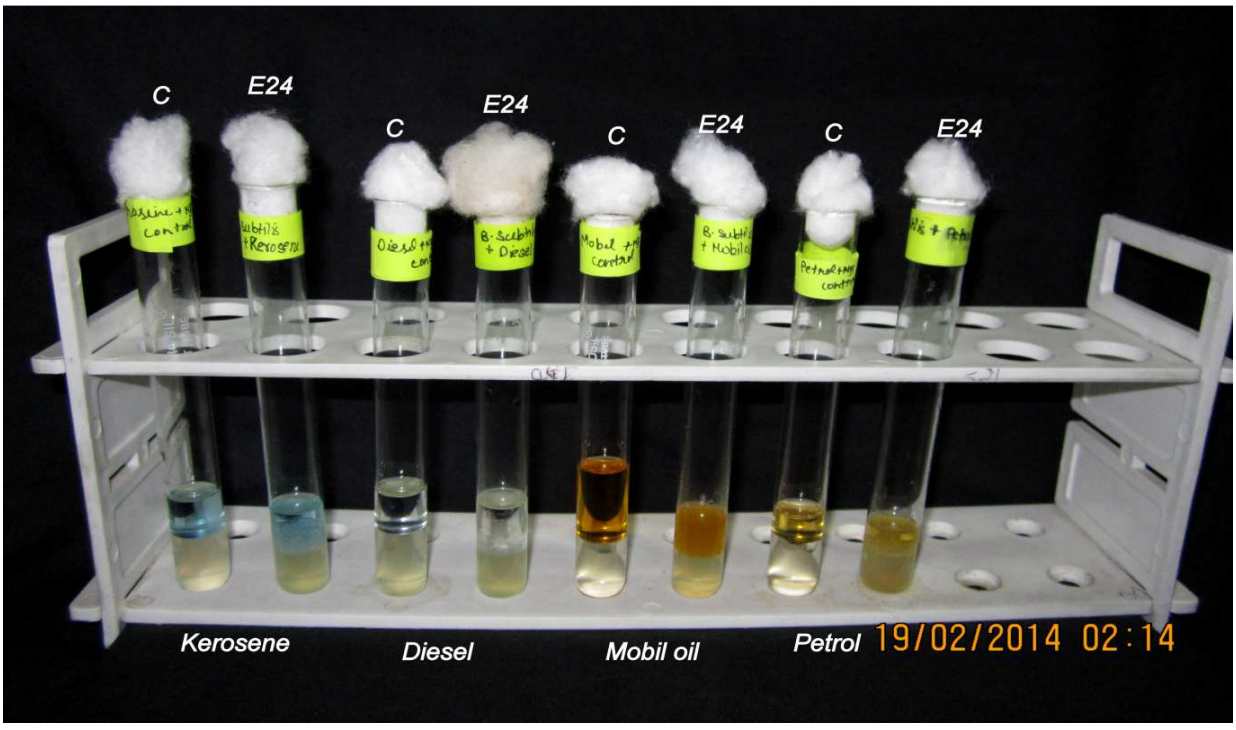


Plate: 4 Emulsification activity of cells of *Bacillus subtilis* in BH broth

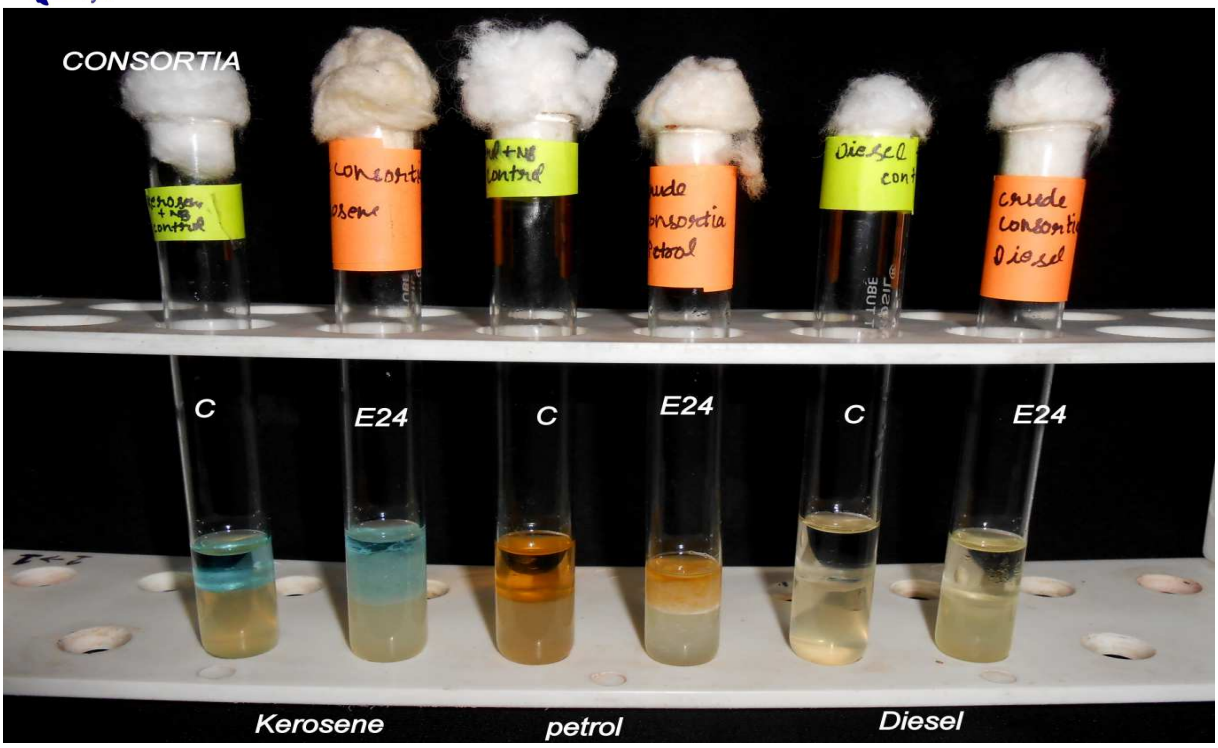


Plate: 5 Emulsification activity of cells of consortia in BH broth

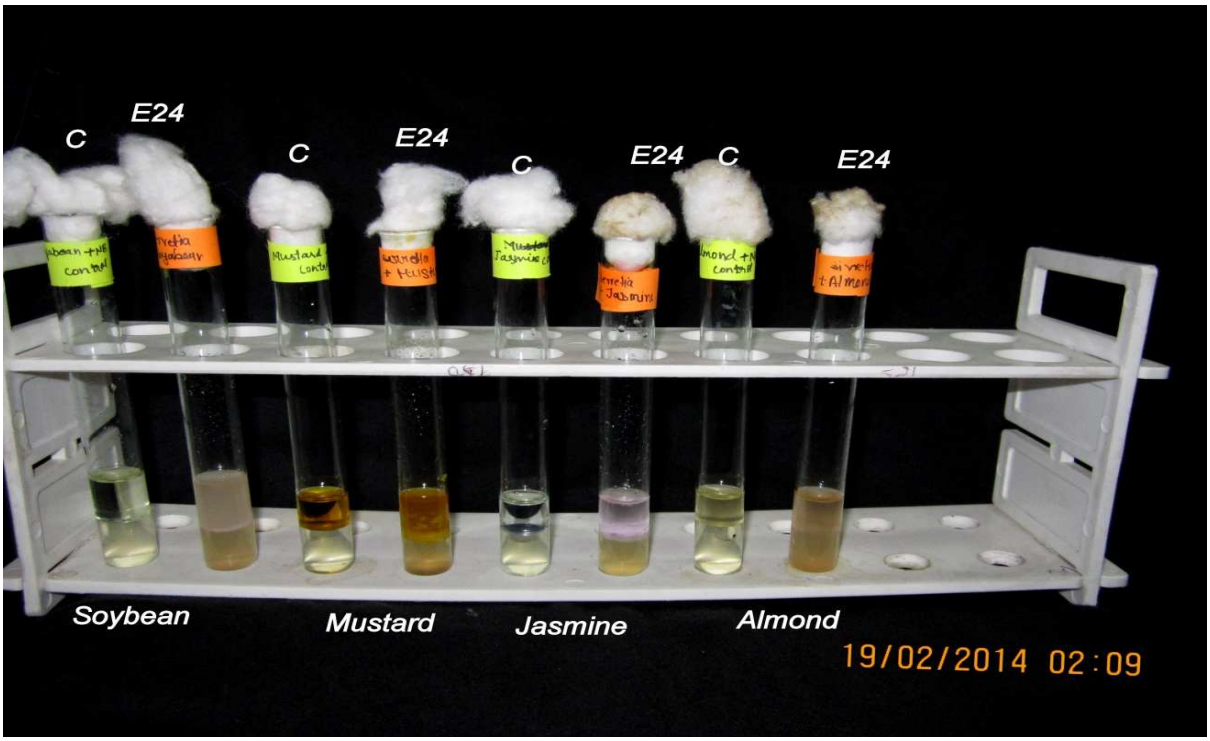


Plate: 6 Emulsification activity of cells of consortia in BH broth

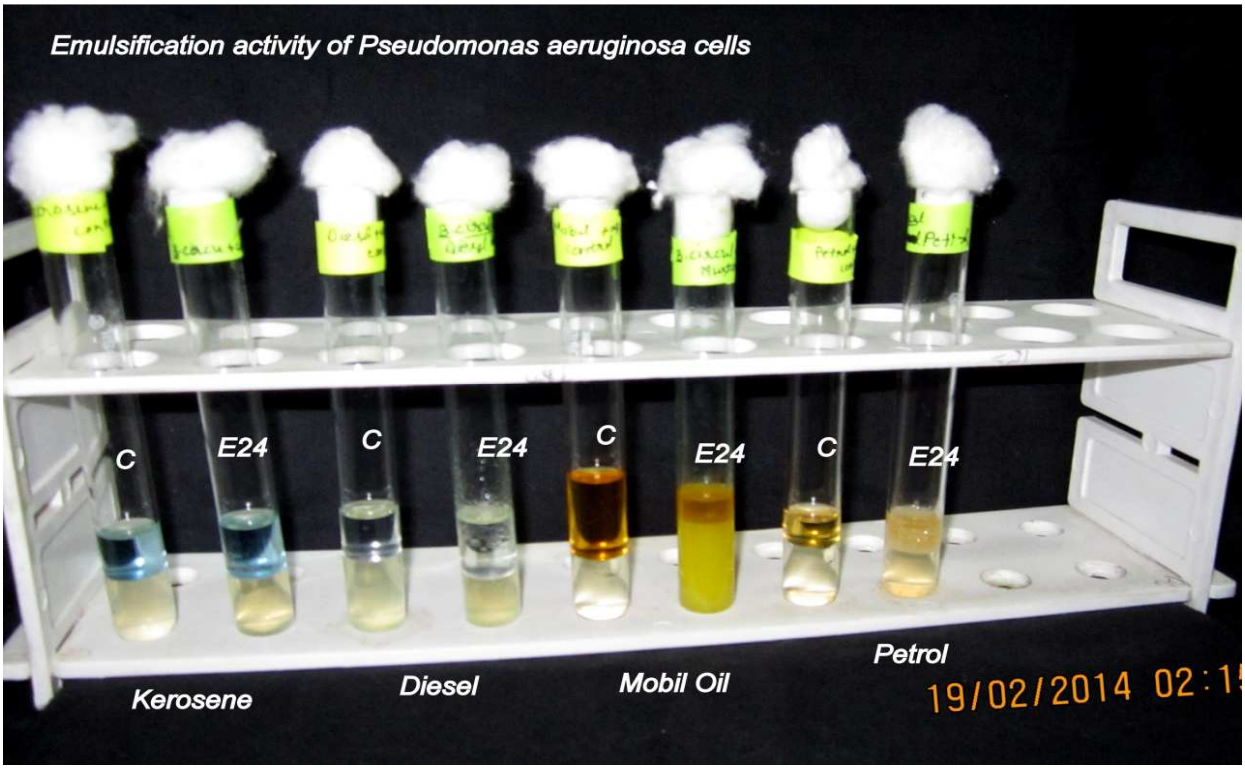


Plate: 7 Emulsification activity of cells of *Pseudomonas aeruginosa* in BH broth

Discussion

Microorganisms synthesize an extensive array of biosurfactants, amphipathic molecules that typically concentrate at the interfaces between hydrophobic and hydrophilic phases or surfaces, be they solids, liquids or gasses. As with chemical surfactants, they function to reduce surface or interfacial tensions to form emulsions, and they have the ability to form molecular aggregates including micelles. They may also facilitate cell uptake of extracellular natural organic or indeed inorganic nutrients, or nutrients associated with other living cells through various types of cell-cell interaction including pathogenesis. These interfacial processes may result in formation of microbial cellular aggregates, including microbial biofilms and microbial pellets. These interfacial phenomena may occur naturally and passively in the environment or may be promoted or engineered in bioprocesses, most notably in bioremediation and biological waste treatment processes. Biosurfactants may also impact on the physiology of microbes by exhibiting toxic or inhibitory effects, either directly or indirectly, through pseudosolubilisation of chemicals which may be toxic to specific microbial species. In a different scenario, where microbes have hydrophobic surfaces which enable them to interact directly by surface contact with hydrophobic contaminants addition of biosurfactants or chemical surfactants can counteract this interaction with potential to reduce rates of uptake and

transformation of the contaminants by the microbes. The amphipathic nature of microbial biosurfactants and/or the hydrophobic properties of microbial cell surfaces may be exploited to displace emulsifiers present at the oil-water interface of petroleum emulsions to break the emulsion. The microbial cultures applied to the emulsions utilize hydrocarbon components to support growth and biosurfactant production. Indeed such biodegradations of hydrocarbon components at the water/oil interface may also contribute to the de-emulsification process.

Literature survey illustrates that detailed studies of BS/BE production have been carried out in *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Serratia*, *Candida* spp. BS producing microbes from different resources, *viz.*, fresh water, soil, marine, oil wells and industrial effluents have been studied extensively. Among these natural resources, marine environment is attracting interest from many researchers due to its vastness and novelty with respect to products that can be obtained. However, this survey clearly illustrates that the maximum reports are focused on rhamnolipid and surfactin production from *Pseudomonas* and *Bacillus* spp. respectively. Few researchers have reviewed the enormous data generated on BS/BE production in microorganisms, briefing molecular biological aspects. The mystery why microbes produce BS/BE is still unknown. Justifications include survival on various hydrophobic substrates and desorption from the hydrophobic substrates allowing direct contact with cell, thereby increasing the bioavailability of insoluble substrates. However, few microbes produce BS/BE on water soluble substrates. Different biosynthetic pathways and specific enzymes are involved. Synthesis takes place by de novo pathway and/or assembly from substrates. BS/BE producing microbes may harbour plasmids. However, genes responsible for BS production are located on chromosomal DNA. Interacellular communication and production of enzymes, pigments and BS occurs by QSS which depends on the production of diffusible signal molecules termed autoinducers. The regulatory machinery is different for different BS/BE producers. *Serratia*, a Gram-negative organism is known to produce extracellular surface active and surface translocating agents. *S. marcescens* produces a cyclic lipopeptide BS 'Serrawettin' which contains 3-hydroxy-C10 FA side chain. BS production is correlated with populational surface migration. Techaoei *et al.* (2007) did preliminary screening of biosurfactant producing microorganisms isolated from hot spring and garages in northern Thailand and reported the emulsification at 24, 36 and 48 h of incubation. In this concept, the biosurfactant molecules act as mediators, which increase the mass transfer rate by making hydrophobic pollutants more bioavailable for microorganisms (Inakollu *et al.* 2004; Whang *et al.* 2009). Alternatively, biosurfactants may also induce changes in the properties of cellular membranes, resulting in increased microbial adherence. Hydrocarbons are organic compounds made of carbon atoms bound to each other forming a backbone with hydrogen atoms attached to the remaining sites on carbon. The carbon backbone can be straight or normal, branched, or cyclic (Olah and Molnar, 1995). The specific degradation mechanisms are determined by the compound structure. Linear alkanes degrade through b-oxidation in which the backbone is broken up two carbons at a time and the resulting acetyl-CoA is

mineralized in the TCA cycle. Some cyclic alkanes degrade through cometabolism (Juhasz *et al.* 1996). Aromatic compounds are generally degraded via a dioxygenase enzyme, which converts the compound to a catechol followed by ring fission in the ortho or meta positions (Prince, 1993). Emulsification is a process that forms a liquid, known as an emulsion, containing very smalldroplets of fat or oil suspended in a fluid, usually water. The high molecular weight biosurfactants are efficient emulsifying agents.

Conclusion

In conclusion, the research revealed the emulsifying potential of broth cuture of *Pseudomonas aeruginosa*, *Serratia marscecens* and *Bacillus subtilis* along with the consortium of the three mentioned bacteria potent to show biosurfactant activity and emulsification at oil water interphase. The consortium was even potent of producing stable emulsion in the aforementioned hydrocarbons and vegetable oils. Comparatively vegetable oils were emulsified effectively than hydrocarbons.

Therefore it could be recommended from the study that the crude consortium or individual crode biosurfactants could be used as strong emulsifying agents.

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References

[1] Amani H, Sarrafzadeh MH, Haghighi M, Mehrnia MR: Comparative study of biosurfactant producing bacteria in MEOR applications. *J Pet Sci Eng* 2010, 75:209-214.

[2] Amedea P, Ivo R, Banat IM: Possibilities and challenges for biosurfactants use in petroleum industry. *Adv Exp Med Biol* 2010, 672:135-145.

[3] Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R, Microbial biosurfactants production, applications and future potential. *Applied Microbiology Biotechnology* 2010; **87**(2):427-444.

[4] Banat IM: Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. *Biores Technol* 1995, 51:1-12.

[5] Bodour AA, Maier RM. Biosurfactant: types, screening methods and applications. In: Bitton G, ed. Encyclopedia of Environmental Microbiology. New York: John Wiley & Sons, 2002:750-770.

[6] Bonilla M, Olivaro C, Corona M et al. Production and characterization of a new bioemulsifier from *Pseudomonas putida* ML2. *J Appl Microbiol* 2005; 98:456-463.

[7] Boþgelmez-Tinaz G. Quorum sensing in Gram-negative bacteria. *Turk J Biol* 2003; 7:85-93.

[8] Cameotra SS, Makkar RS. Recent applications of biosurfactants as biological and immunological molecules. *Current Opinion Microbiology* 2004; **7** (3): 1–5.

[9] Darvishi P, Ayatollahi S, Mowla D, Niazi A: Biosurfactant production under extreme environmental conditions by an fficient consortium, ERCPPI-2. *Colloids and Surfaces B: Biointerfaces* 2011.

[10] Das M. Characterization of de-emulsification capabilities of a *Micrococcus* sp. *Bioresource Technol.* 2001; 79:15-22.

[11] Desai AJ, Patel RN. Advances in biosurfactant production: A step forward to commercial applications. *J Sci Ind Res.* 1994; 53:619-629.

[12] Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. *Microbiol Mol Biol Rev* 1997; 61(1):47-64.

[13] Dubey K, Juwarkar A. Determination of genetic basis for biosurfactant production in distillery and curd whey wastes utilizing *Pseudomonas aeruginosa* strain B2. *Indian J Biotechnol* 2004; 3(1):74-81.

[14] Fiechter A. Biosurfactant moving towards industrial applications. *Trends Biotechnol.* 1992; 10:208-217.

[15] Gautam KK, Tyagi VK. Microbial surfactants: *A review. J Oleo Sci*. 2006; 55(4):155-166.

[16] Gunther NW, Nun˜ez A, Fett W et al. Production of rhamnolipids by *Pseudomonas chlororaphis*, a nonpathogenic bacterium. *Appl Environ Microbiol* 2005; 71(5):2288-2293.

[17] Hommel RK, Ratledge C. Biosynthetic mechanisms of low molecular weight surfactants and their precursor molecules. In: Kosaric N, eds. Biosurfactant: Production, Properties, Applications. New York: Marcel Dekker, Inc., 1993:3-63.

[18] Horng YT, Deng SC, Daykin M et al. The LuxR family protein SpnR functions as a negative regulator of N-acylhomoserine lactone-dependent quorum sensing in *Serratia marcescens*. *Mol Microbiol* 2002; 45:1655-1671.

[19] Inge NA, Bogaert V, Saerens K et al. Microbial production and application of sphorolipids. *Appl Microbiol Biotechnol* 2007; 76:23-34.

[20] Jaeger KE, Dijkstra BW, Reetz MT: Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. *Annual Rev Microbiol* 1999, 53:315-351.

[21] Kitamoto D, Isoda H, Nakahara T. Functions and potential applications of glycolipid biosurfactant—from energy-saving materials to gene delivery carriers. *J Biosci Bioengg* 2002; 94:187-201.

[22] Kokare CR, Kadam SS, Mahadik KR *et al.* Studies on bioemulsifier production from marine Streptomyces sp. S1. *Indian J Biotechnol.* 2007; 6(1):78-84.

[23] Lang S, Wullbrandt D. Rhamnose lipids-biosynthesis, microbial production and application potential. *Appl Microbiol Biotechnol* 1999; 51:22-32.

[24] Magdalena P-P, Grazyna AP, Zofia P-S, Cameotra SS: Environmental applications of biosurfactants: Recent advances. *Int J Mol Sci* 2011, 12:633-654.

[25] Maier RM, Soberon-Chavez G. *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. *Appl Microbiol Biotechnol* 2000; 54:625-633.

[26] Maier RM. Biosurfactant: evolution and diversity in bacteria. *Adv Appl Microbiol* 2003; 52:101-121.

[27] Maneerat S, Takeshi B, Kazuo H et al. A novel crude oil emulsifier excreted in the culture supernatant of a marine bacterium, *Myroides* sp. strain SM1. *Appl Microbiol Biotechno* 2006; 70(2):254-259.

[28] Margesin R, Schinner F. Bioremediation (natural attenuation and biostimulation) of diesel-oil contaminated soil in an alpine glacier skiing area. *Appl Environ Microbiol* 2001; 67:3127-3133.

[29] Mulligan CN: Environmental applications for biosurfactants*. Environ Pollution* 2005, 133:183-198.

[30] Nitschke M, Siddahartha GVAO, Contiero J**.** Rhamnolipid Surfactants: An Update on the General Aspects of these Remarkable Biomolecules. *Biotechnology Progress.* 2005; **21** (6): 1593-1600.

[31] Okpokwasili GC, Ibiene AA: Enhancement of recovery of residual oil using a biosurfactant slug. *African J Biotech 2006, 5:453-456.*

[32] Olivera NL, Commendatore MG, Delgado O et al. Microbial characterization and hydrocarbon biodegradation potential of natural bilge waste microflora. *J Ind Microbiol Biotechnol* 2003; 30:542-548.

[33] Palashpriya Das, Soumen Mukherjee, Ramkrishna Sen. 2009. Substrate dependent

production of extracellular biosurfactant by a marine bacterium. *Bioresource Technology*. 100 (2009) 1015–1019.

[34] Pedroza-Rodríguez, Aura M. Leonardo Sastoque-Cala, Alba M. Cotes-Prado, Refugio Rodríguez Vázquez. 2010. Effect of nutrients and fermentation conditions on the production of biosurfactants using rhizobacteria isolated from fique plants. *Universitas Scientiarum. 15 N° 3: 251-264.*

[35] Peypoux F, Bonmatin JM, Wallach J. Recent trends in the biochemistry of surfactin. *Appl Microbiol Biotechnol* 1999; 51:553-563.

[36] Philp JC: Effect of biosurfactants on crude oil desorption and mobilization in a soil system. *Environ Int.* 2005, 31:155-161.

[37] *Priya, T. and Usharani, G.* 2009. Comparative Study for Biosurfactant Production by Using *Bacillus subtilis* and *Pseudomonas aeruginosa. Botany Research International* 2 (4): 284-287.

[38] Ron E, Rosenberg EZ. Natural role of biosurfactants. *J Environ Microbiol* 2001; 3(4):229-236.

[39] Salehizadeh H, Mohammadizad S: Microbial enhanced oil recovery using biosurfactant produced by *Alcaligenes faecalis*. *Iranian J Biotechnol* 2009, 7:216-223.

[40] Satpute SK, Bhawsar BD, Dhakephalkar PK et al. Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Indian J Marine Sciences.* 2008; 37(3):243-250.

[41] Satpute SK, Dhakephalkar PK, Chopade BA. Biosurfactants and bioemulsifiers in hydrocarbon biodegradation and spilled oil bioremediation. Indo-Italian brain storming workshop on technology transfer for industrial applications of novel methods and materials for environmental problem. 2005; 1-18.

[42] Schmidt-Dannert C: Recombinant microbial lipases for biotechnological applications. *Bioorg Med Chem* 1999, 7:2123-2130.

[43] Sekhon, K K., Khanna S. and Singh, S. Cameotra. 2011. Enhanced biosurfactant production through cloning of three genes and role of esterase in biosurfactant release. *Microbial Cell Factories*. 10:49 http://www.microbialcellfactories.com/content/10/1/49

[44] Shavandi M, Mohebali G, Haddadi A, Shakarami H, Nuhi A: Emulsification potential of a newly isolated biosurfactant producing bacterium, *Rhodococcus* sp. strain TA6. *Colloids and Surfaces B: Biointerfaces* 2011, 82:477-482.

[45] Shete AM. Studies on isolation, biochemical and physiological characteristics, antibiotic and bioemulsifier production and plasmid genetics of marine *Acinetobacter* 2003. A Ph.D. Thesis submitted to the University of Pune, Pune, India.

[46] Soberon-Chavez G, Aguirre-Ramirez M, Ordonez L. Is *Pseudomonas aeruginosa* only "Sensing Quorum"? *Crit* Rev Microbiol. 2005; 31(3):171-182.

[47] Syldatk C, Wagner F. Production of biosurfactants. In: Kosaric N, Cairns WL, Gray, NCC, eds. BioSurfactants and Biotechnology. New York: Marcel Dekker, Inc., 1987:89-120.

[48] Turkovskaya OV, Dmitrieva TV, Muratova AY. A biosurfactant-Producing *Pseudomonas aeruginosa* Strain. *Appl Biochem Microbiol* 2001; 37(1):71-75.

[49] Wei Y, Lai HC, Chen SU et al. Biosurfactant production by *Serratia marcescens* SS-1 and its isogenic strain SMΔR defective in SpnR, a quorum-sensing LuxR family protein. *Biotechnol Lett* 2004; 26:799-802.

[50] Wei YH, Chu IM. $Mn2^{+}$ improves surfactin production by *Bacillus subtilis Biotechnol Lett* 2002; 24:479-482.

[51] Youssef N, Simpson DR, Duncan KE, McInerney MJ, Folsbee M, Fincher T, Knapp RM: *In situ* biosurfactant production by *Bacillus* strains injected into a limestone petroleum reservoir. *Appl Environ Microbiol* 2007, 73:1239-1247.