ISSN : 2350-0743

www.ijramr.com

International Journal of Recent Advances in Multidisciplinary Research
Vol. 05, Issue 12, pp.4275-4291, December, 2018

REVIEW ARTICLE

BIO-SURFACTANTS: A PACKAGE OF ENVIRONMENTAL AND INDUSTRIAL BENEFITS

[1,*]Hassan A.H. Ibrahim, [1]Saher Wefky, [1]Gehan M. Abo Elela, [1]Nermeen A. El Sersy, [2]Dalia El Badan and [2]Aml Hassan

[1]Marine Microbiology Department, National Institute of Oceanography and Fisheries, Alexandria Branch, Alexandria, Egypt
[2]Microbiology Department, Faculty of Science, Alexandria University, Egypt

ARTICLE INFO

Article History:
Received 09th September, 2018
Received in revised form
26th October, 2018
Accepted 14th November, 2018
Published online 26th December, 2018

Keywords:
Bio-surfactants, Environmental Benefits, Industrial Benefits.

ABSTRACT

Bio-surfactants are surface-active compounds are chemically synthesized and commonly used in almost every sector of recent industry that produced by several microorganisms such as; bacteria, fungi, and yeast varying in their chemical properties and molecular sizes. Bio-surfactants are classified into six main types based on their different properties and producer. There are five factors affect practically on the bio-surfactant production, such as; producer organism, carbon and (substrate) nitrogen sources, trace elements, temperature, and aeration. Due to their enormous diversity, they are considered interesting group of materials for many applications in several fields such as; agriculture, public health, food, health care, waste utilization, and in environmental pollution control such as; hydrocarbons degradation present in soil. Herein, these applications are covered and reviewed. Bio-surfactants are the most promising existing alternative products for further prospects in the future depends upon their availability, low-cost production, and stability in various industrial preparations. They present green solutions in food and therapeutics.

INTRODUCTION

Surface-active compounds are chemically synthesized and commonly used in almost every sector of recent industry (Samadi *et al*., 2007). Environmental carefulness has expanded and led to alternative biological surfactants as the most promising existing product (Henkel *et al*., 2012). Bio-surfactants are produced by biological processes, being excreted extracellularly by microorganisms such as bacteria, fungi, and yeast, which vary in their chemical properties and molecular size (Janek *et al*., 2013). Bio-surfactants have attracted attention as alternative surfactants due to their high biodegradability low toxicity. The enormous diversity of bio-surfactants makes them an interesting group of materials for application in many areas such as agriculture, public health, food, health care waste utilization, and environmental pollution control such as hydrocarbons degradation present in soil (Ramana and Karanth, 1989). Further prospects of bio-surfactants in future depend upon their availability, low-cost production, and stability in various industrial preparations. For the demand of green solutions in food and therapeutics, further research should be focused on low-cost production, toxicity assessment, and encouragement to utilize these green surfactants in future developments (Zhang and Wang, 2016). Therefore, the current review will focus on the productivity and several benefits of bio-surfactants to be applied as promising alternatives in vital and valuable products necessary for human.

***Corresponding author:* Hassan A.H. Ibrahim,**
[1]Marine Microbiology Department, National Institute of Oceanography and Fisheries, Alexandria Branch, Alexandria, Egypt.

Definition of bio-surfactants

Bio-surfactants are produced by microorganisms from various taxonomic groups and diverse habitats and their role usually being linked to lipid solubilization (Mao *et al.,* 2015). Bio-surfactants are amphiphilic compounds produced on living surface, mostly microbial cell surfaces, or excreted extracellularly and contain phydrophobic and hydrophilic moieties that reduce surface tension and interfacial tensions between individual molecules at the surface and interface, respectively (Figure 1A and B). Since bio-surfactants and bio-emulsifiers both exhibit emulsification properties. Bio-emulsifiers are often categorized with bio-surfactants, although emulsifiers may not lower surface tension. A bio-surfactant may have one of the following structures: mycolic acid, glycolipids, polysaccharide-lipid complex, lipoprotein or lipopeptide, phospholipid, or the microbial cell surface itself. Bio-surfactants are able to retain their properties even under extreme conditions of pH, temperature, and salinity (Nitschke and Pastore, 2006; Pornsunthorntawee *et al.,* 2008). Bio-surfactants are capable of increasing the bioavailability of poorly soluble polycyclic aromatic hydrocarbons such as phenanthrene (Olivera *et al.,* 2003).Therefore, the use of bio-surfactants should be a promising means of emulsify the polluted oils prior to biodegradation. The robustness of bio-surfactants leads to several potential uses in spanning environmental, food, biomedical, and other industrial applications (Banat *et al*., 2010). The important prerequisities for the competitive production of bio-surfactants include high bio-surfactant yields, alternative low cost substrates, and cost-

effective bioprocesses. Hopefully future's extremophilic microbial surfactants appear to depend specifically on the use of plentiful and cheap substrates for optimization of the operational cultivation conditions, since they have particular adaptations to maintained stability in oppose environments (Makkar *et al*., 2011). The major classes of bio-surfactants that have been studied using alternative low-cost substrates, like vegetable oils and agro-industrial wastes, are rhamnolipids produced by *Pseudomonas aeruginosa* and surfactin produced by *Bacillus subtilis* (Nitschke *et al.,* 2005).

Classification of bio-surfactants: Most of bio-surfactants are either anionic or neutral (Figure 2). The hydrophobic moiety is based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic portion can be a carbohydrate, amino acid, phosphate or cyclic peptide (Okoliegbe and Agarry, 2012). The microbial surfactants are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. Microorganisms also produce surfactants that are in some cases combination of many chemical types referred to as the polymertic microbial surfactants. Many microbial surfactants have been purified and their structures elucidated. While the high molecular weight microbial surfactants are generally polyanionic heteropolysaccharides containing polysaccharides and proteins, the low molecular weight microbial surfactants are often glycolipids. However, a broad classification of bio-surfactants is in the present flowing main groups (Table 1):

Glycolipids: Glycolipids are the most common bio-surfactants with low molecular weights that have been isolated and studied so far. These bio-surfactants contain carbohydrates in combination with long-chain aliphatic acids or hydroxy aliphatic acids. The rhamnolipids secreted by *Pseudomonas aeruginosa* are considered the most actively studied. Several recent works have shown the effects of these compounds on phospholipids membranes of various compositions (Ortiz *et al.,* 2006; Aranda *et al.,* 2007). Another important group of glycolipid bio-surfactants is formed by trehalose-containing glycolipids (Banat *et al.,* 2010). The constituent mono-, di, tri- and tetra saccharides include glucose, mannose, galactose, glucuronic acid, rthamnose, and galactose sulphate. The fatty acid component usually has a composition similar to that of the phospholipids of the same microorganism. The glycolipids can be categorized as follows:

.

The trehalos lipids are mainly produced by different yeast strains (55Gamero-Sandemetrio *et al.*, 2018) with interesting physicochemical and biological properties. Trehalose lipids can reduce the surface tension of aqueous solutions and the interfacial tension between aqueous and oil phases to levels observed with synthetic surfactants, and have low critical micelle concentrations (Lang and Philp, 1999). Thus, a number of industrial applications of these compounds have been proposed, including environmental applications such as microbial enhanced oil recovery, biodegradation of polycyclic aromatic hydrocarbons or oil-spill treatment (Chang *et al.,* 2004; Kuyukina *et al.,* 2005). Because of the structural diversity of trehalose lipids, it is important to elucidate the individual contribution of the major components of the crude extract in order to understand their mechanism of action and to obtain a trehalose lipid with desirable properties for specific uses. There is an increasing interest in the use of bio-surfactants as therapeutic agents (Rodrigues *et al.,* 2006).

Trehalose lipids: Trehalose lipids have been reported to have potential application in the bioremediation technologies (Gandolfi *et al.,* 2010), antidepressants drug aggregation (Banjare *et al*., 2018), combatting mycobacterial infection (O'Neill *et al.*, 2018), antiviral properties (Yuan *et al*., 2017) and immunomodulating activity (Kuyukina *et al.,* 2007). Although the amphiphilic nature of trehalose lipids points to the membrane as their hypothetical site of action, very little is known about the interaction between these bio-surfactants and biological membranes. Recently, it has shown that trehalose lipid increases the fluidity of phosphatidylcholine membranes and forms domains in the fluid state (Aranda *et al.,* 2007). Sophorolipids are produced by several strains of the yeast (Konishi *et al*., 2016)**.** The sophorolipids have different applications as degradation of diesel (2Chandran and Das, 2012) and antifungal agents (Sen *et al.,* 2017)**.**

Rhamnoplipids: Rhamalrpids produced by different *Pseudomonas* sp., in large quantities of as a glycolipid, are consisted of two molecules of rhamnose and two molecules of b-hydroxydecanoic acid (Soberón-Chávez *et al.,* 2005), which show physico-chemical properties that differ from those of single congeners, with the most abundant structure in the mixture having the largest impact on the overall characteristics of the total mixture (Rikalovic *et al*., 2015).

Fatty acids: The fatty acids can be produced by bacteria and Fungi during growth on n alkanes and have importance in medical applications. Gautam and Tyagi, (2006) also suggested that the isolation and cloning of genes involved in the production of surfactants can be used in fermentative processes.

Phospholipids: *Acinetobacter* spp. produce phosphatidyl ethanolamine-rich vesicles form optically clear microemulsions of alkanes in water. The produced bio-surfactants are important to medical applications. It was reported that phospholipid protein complex deficiency is the major cause of respiratory failure in the children born prematurely (Gautam and Tyagi, 2006).

Surface active antibiotics: Contact-active surfaces exhibit antimicrobial activity without releasing biocidal substances. Several mechanisms are believed to take place in contact-active surfaces (Krumm and Tiller, 2014). These are: (i) a so-called spacer effect, where the biocidal group is attached to the surface through a polymer chain, allowing the biocide to reach the cytoplasmic membrane of the bacteria and to perforate them; (ii) alternatively, positively charged QACs, e.g. 3-aminopropyl trimethoxysilane grafted to cellulose nanofibres, can detach phospholipids from the cell membrane and thereby kill the bacteria (Krumm and Tiller, 2014; Saini *et al*., 2016). This approach is also referred to as biomimetic with respect to the activity of chitosan – a polysaccharide derived from exoskeleton of crustaceans or cell walls of fungi. Hydrophobic parts of a surface can act similarly to QACs by deforming the membrane through adhesion (Asri *et al*., 2014). Gramicidin S and polymyxins are small cationic cyclic peptides and act as potent antibiotics against Gram-negative and Gram-positive bacteria by perturbing integrity of the bacterial membranes (Mogi and Kita, 2009). Polymyxin B and colistin (polymyxin E) are bactericidal pentacationic lipopeptides that act specifically on Gram-negative bacteria, first by disrupting their outermost permeability barrier, the outer membrane (OM), and then damaging the cytoplasmic membrane (Vaara, 2018).

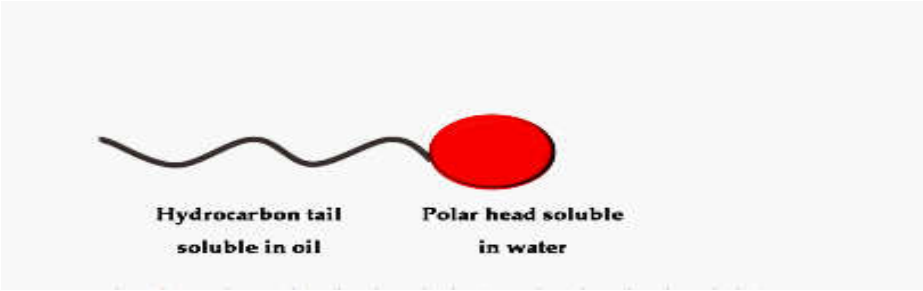

Figure 1A. Hydrophobic and hydrophilic domains of a bio-surfactant molecule (Santos *et al*., 2016)

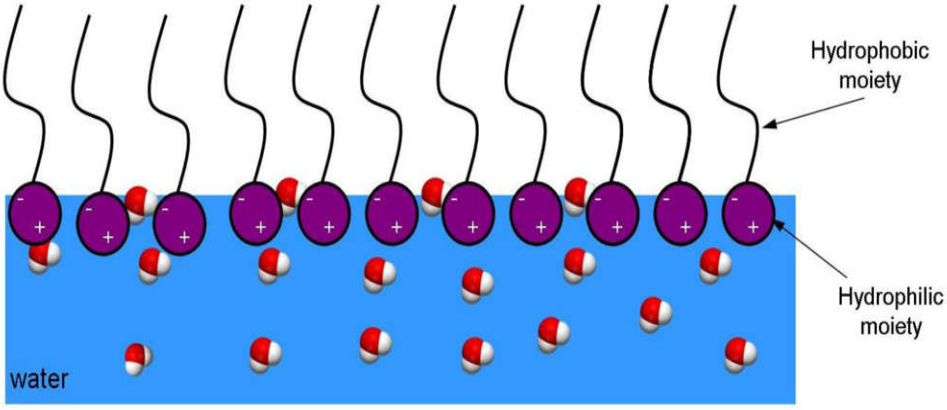

Figure 1B: Accumulation of bio-surfactants at the interface between liquid and air (Pacwa-Płociniczak *et al*., 2011)

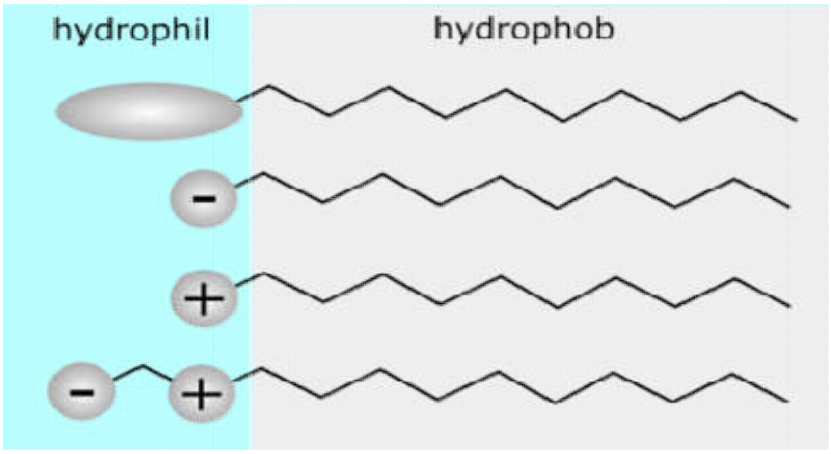

Figure 2. Surfactant classification according to the composition of their head: nonionic, anionic, cationic, and amphoteric

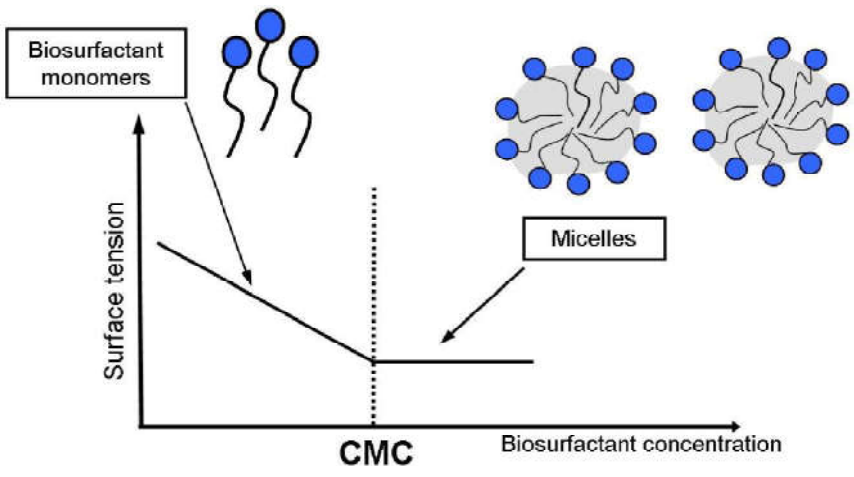

Figure 3: The relationship between bio-surfactant concentration, surface tension and formation of micelles ((Pacwa-Płociniczak *et al*., 2011)

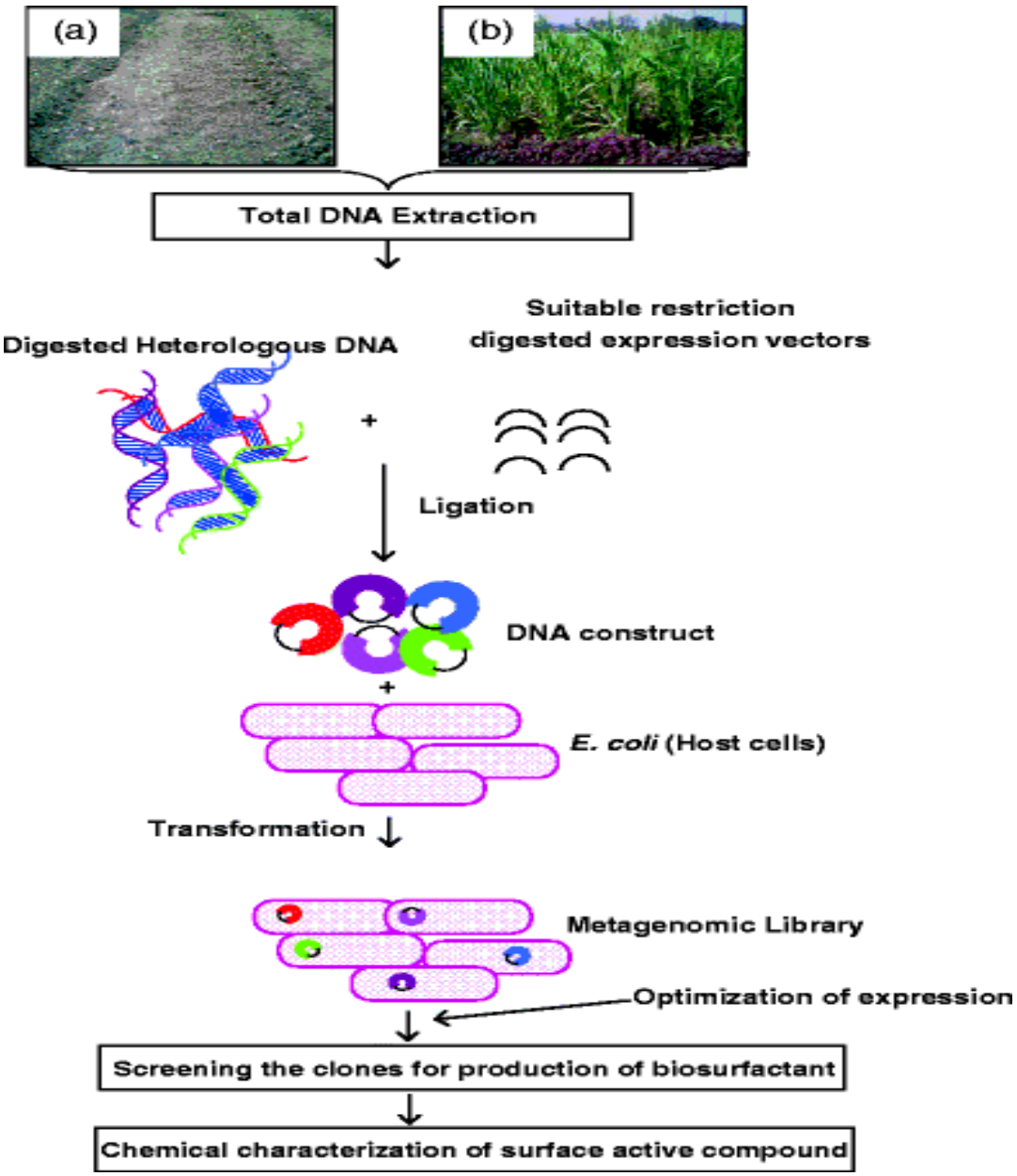

Figure 4: Schematic representation of construction and screening of metagenomic libraries from contaminated agriculture soil (a) and rhizosphere (b) for novel bio-surfactant from uncultured bacteria (55Sachdev and Cameotra, 2013

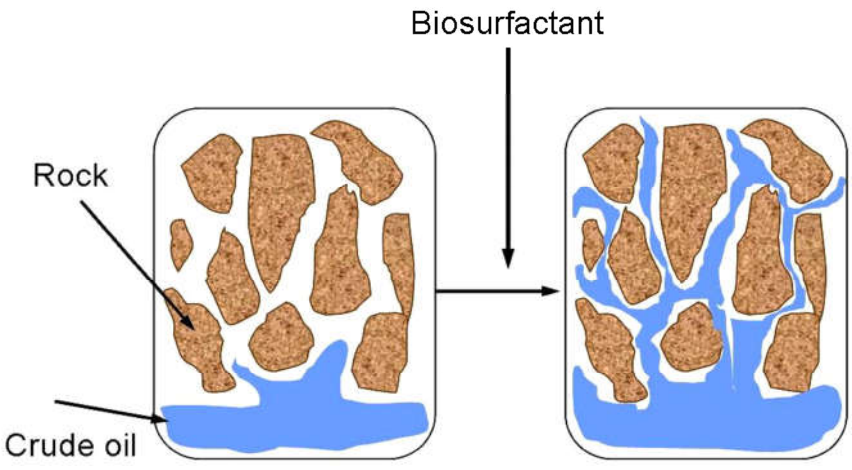

Figure 5.: Applications of MEOR (Pacwa-Plociniczak *et al*., 2011)

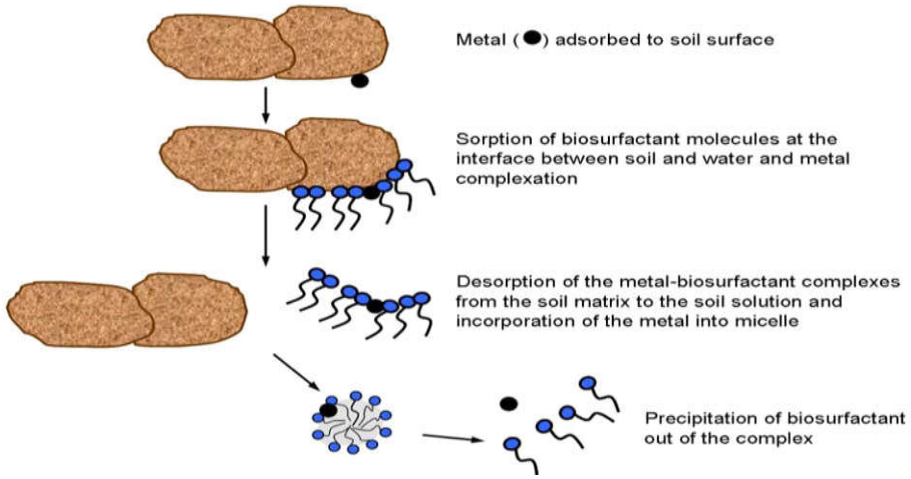

Figure 6. Mechanism of bio-surfactant activity in metal-contaminated soil (Mulligan, 2005)

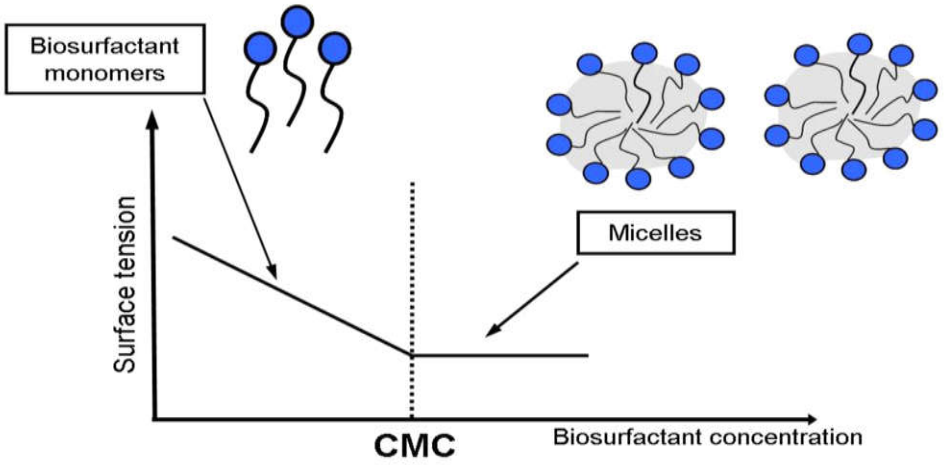

Figure 7. The relationship between bio-surfactant concentration, surface tension and formation of micelles (Whang *et al*., 2008)

Table 1. Major bio-surfactants classes and microorganisms involved (Desai and Banat, 1997).

Bio-surfactant class	Microorganism
•Glycolipids	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalolipids	*Rhodococcus erythropolis*
	Arthrobacter sp.
Sophorolipids	*Candida bombicola, Candida apicola*
Mannosylerythritol lipids	*Candida antartica*
•Lipopeptides	
Surfactin/iturin/fengycin	*Bacillus subtilis*
Viscosin	*Pseudomonas fluorescens*
Lichenysin	*Bacillus licheniformis*
Serrawettin	*Serratia marcescens*
•Phospholipids	*Acinetobacter sp.*
	Corynebacterium lepus
•Surface-active antibiotics	
Gramicidin	*Brevibacterium brevis*
Polymixin	*Bacillus polymyxa*
Antibiotic TA	*Myxococcus xanthus*
•Fatty acids/neutral lipids	
Corynomicolic acids	*Corynebacterium insidibasseosum*
•Polymeric surfactants	
Emulsan	*Acinetobacter calcoaceticus*
Alasan	*Acinetobacter radioresistens*
Liposan	*Candida lipolytica*
Lipomanan	*Candida tropicalis*
•Particulate biosurfactants	
Vesicles	*A. calcoaceticus*
Whole microbial cells	*Cyanobacteria*

Table 2. Application of bio-surfactant (Kapadia and Yagnik, 2013)

Role of bio-surfactant	Application	Industry
Improving oil drainage into bore-well, stimulating release of oil entrapped within the capillaries, wetting of solid surfaces, reduction of oil viscosity and oil pour point, lowering of interfacial tension, and dissolution of oil.	Enhanced oil recovery	Petroleum
De-emulsification of oil emulsions, oil solubilization, viscosity reduction, wetting agent.	De-emulsification	
Emulsification of hydrocarbons, lowering of interfacial tension, metal sequestration.	Bioremediation	Environmental
Emulsificationthroughadherenceto hydrocarbons, dispersion, foaming agent, detergent, and soil flushing.	Soil remediation and flushing	
Emulsifier, solubilizer, demulsifier, suspension, wetting, foaming, defoaming, thickener, and lubricating agent.	Emulsification and de-emulsification	Food
Interactionwithlipids,proteinsand carbohydrates, protecting agent.	Functional ingredient	
Physiological behavior such as cell mobility, cell communication, nutrient accession, cell–cell competition, plant and animal pathogenesis.	Microbiological	Biological
Antibacterial, antifungal, antiviral agents, adhesive agents, immune modulator molecules, vaccines, gene therapy.	Pharmaceuticals and therapeutics	
Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, induced systemic resistance and hypovirulence.	Biocontrol	Agricultural
Biocatalysisinaqueoustwo-phase systemsand microemulsions,bio-transformations,recoveryof intracellular products, enhanced production of extracellular enzymes and fermentation products.	Down-stream processing	Bioprocessing
Emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agents, mediators of enzyme action.	Health and beauty products	Cosmetic

Polymeric microbial surfactants: Polymeric microbial surfactants are high molecular weight surfactants. The most important surfactants are also an, lipomanan, Emulsan, lipomanan, liposan Emulsan is an emulsifier for hydrocarbons in water at concentrations as low as 0.001% to 0.01%. Liposan is an extracellular water soluble emulsifier synthesized by *C. lipolytica* and is made up of 83% carbohydrates and 17% different applications of liposan in cosmetic industries and food were reported (Chakrabarti, 2012).

Surfactants of *Acinetobacter* spp: *Acinetobacter* spp. are known as bio-surfactant producer. Effects of the bio-surfactants produced by *A. calcoaceticus* on the solubility and biodegradation of PAHs were investigated in batch systems. Bio-surfactants produced by *A. calcoaceticus* BU03 at 25 times their CMC significantly increased the apparent aqueous solubility of phenanthrene (PHE), pyrene (PYR) and benzo(a)pyrene (B[a]P) to 54.3, 6.33 and 2.08mg L^{-1}, respectively. In aqueous system, the bio-surfactants at concentrations of 0.5 CMC and 1 CMC slightly enhanced the biodegradation of PHE (Zhao and Wong, 2009).

Sophorolipids

Sophorolipids are mainly produced by *Candida* spp. (Cortés-Sánchez *et al.,* 2013). These glycolipids have a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid through glycosidic bonds and are preferable in many applications (Gautam and Tyagi, 2006).

Surfactants of yeast: Different species of yeast produce low molecular weight bio-surfactants, with demulsifying properties (Joshi-Navare *et al*., 2013). Bio-surfactants crude extracts, produced by yeasts (*Candida guilliermondii*, *Candida lipolytica* and *Candida sphaerica*) grown in industrial residues, were tested for demulsifcation capacity in their crude and pure forms. About 35-40% of the seawater emulsified with motor oil was recovered (Silva *et al*., 2017).

Surfactants of *Pseudomonas* sp: Coutinho *et al.* (2013) found that the cells and metabolites produced by *P. aeruginosa* have demulsifying characteristics for W/O and O/W emulsions, with a demulsifcation rate.

The surface tension of the bio-surfactants produced by *P. cepacia* and *P. aeruginosa* presented 25 and 26 mN/m (Silva *et al.,* 2017).

Surfactants of *Cyanobacterium phormidium:* The change in cell surface hydrophobicity of *Cyanobacterium phormidium* was correlated with the production of an emulsifying agent; emulcyan (Fattom and Shilo, 1984). The partially purified emulcyan has a MW greater-than 10,000 Da and contains carbohydrate, protein and fatty acid esters. Addition of emuicyan to adherent hydrophobic cells resulted in their becoming hydrophilic and detach from hexadecane droplets or phenyl sepharose beads.

Particulate: Particulate bio-surfactants partition extracellular membrane vesicles to form a microemulsion that exerts an influence on alkane uptake in microbial cells. The *Acinetobacter* spp. have vesicles with a diameter of 20 to 50 nm and a buoyant density of 1.158 cubic gcm composed of proteins, phospholipids and lipo-polysaccharides (Chakrabarti *et al*., 2012; Vijayakumar *et al*., 2015). They are produced by most hydrocarbon-degrading microorganisms, many non-hydrocarbon degraders; some species of *Cyanobacteria* (Fattom and Shilo, 1984).

Production of bio-surfactants: Various fermentation strategies are adopted for the production of bio-surfactant. In addition genetic engineering and immobilized cultivation are followed to enhance the surfactin production. Cheap raw materials used for the bio-surfactant production are oil waste, soap stock and other waste from food industries and vegetable oil refineries. Amongst the entire carbon sources, vegetable based oil is found to have higher bio-surfactant yield ((Vasantharaj *et al*., 2016). Shake flask, batch, fed-batch, continuous and integrated microbial/enzymatic process may be used for bio-surfactant production. In batch cultivation, growth limiting substrates such as plant oil or glucose are used for bio-surfactant production. However in glycerol or plant oil serves as a growth limiting substrate for fed batch cultivation. In continuous cultivation mode glucose and hydrocarbon are used as substrates. Yeh *et al.* (2006) developed a novel bioreactor to avoid the foam spillage during the production of bio-

surfactant. The application of bubbleless bioreactor using a hollow fiber membrane as an air-liquid contactor was reported to produce surfactin and fengycin by *Bacillus subtilis* (Coutte *et al*., 2010). Most bio-surfactant-producing microorganisms are cultivated under aerobic conditions, so aeration is required in the bioreactor systems. Due to the high foam production of some bio-surfactants, a significant amount of microbes can be carried with the foam coming out of the bioreactor, leading to a decrease in the bioreactor's performance. In some cases, mechanical foam breakers and chemical antifoam agents are used, but the addition of chemical antifoam agents can impact downstream processing (Frederico *et al*., 2010). Therefore, foaming can result in high expenditure for foam controlling techniques and even in decreased productivity.

Although sequencing batch reactors (SBRs), which are filling-and draw systems, is widely used for the treatment of both domestic and industrial wastewaters, the SBR technology can be employed to produce bio-surfactants from appropriate substrates by using selected bio-surfactant producing microorganisms. Cassidy *et al.* (2000) compared the performance of an SBR with a continuous tank reactor (CSTR), and found that the SBR showed better reactor performance and gave greater bio-surfactant production. Interestingly, much more foam was found to be generated in the SBR than in the CSTR, and foaming could be easily controlled by using the proper feed strategy. **It** has also been reported that the SBR encouraged microbial growth, resulting in the enhancement of the bio-surfactant production (Cassidy and Hudak, 2001). Therefore, the SBR technology is of great interest for adaptation for bio-surfactant production from wastewaters on a large productivity sc ale.
Microbes produce bio-surfactants as a mixture of various isoforms. These isoforms vary in the carbohydrate and peptide part or in the chain length or branching of the lipid part of the molecule (Mukherjee and Das, 2005; Costa *et al.,* 2006; Perfumo *et al.,* 2006).

It was reported earlier that specific bio-surfactant isoforms confer some kind of competitive advantage to the producer strains in their parent habitats such as utilization of hydrophobic substrates and/or antibiotic action against competing microorganisms (Mukherjee and Das, 2005). Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (C_xH_y), microorganisms facilitate their diffusion into the cell by producing the bio-surfactants. Some bacteria and yeasts excrete ionic surfactants which emulsify the CxHy substrate in the growth medium. Some examples of this group of bio-surfactants are rhamnolipids which are produced by different *Pseudomonas* sp. (Mazaheri Assadi *et al*., 2004; Mazaheri Assadi and Tabatabaee, 2008). Microorganisms can carry out bio-surfactant production when grown either on insoluble substrates (such as hydrocarbons, oils and waxes) or on soluble ones (carbohydrates). Hence, the isolation of microbial strains capable of bio-surfactant production using soluble substrates is of interest. The screening of bio-surfactant-producing microorganisms is generally carried out by monitoring parameters that estimate surface activity, such as surface tension, interfacial tension and the ability to emulsify oils or hydrocarbons. Moreover, the applied screening medium and conditions will influence whether or not surfactants are produced (Bodour *et al.,* 2003). Although this process guarantees the detection of producing strains, it is also very arduous, time-consuming and expensive. To overcome these shortcomings, different selection criteria have been assayed.

A sensitive rapid method, a drop collapsing test, was advised for screening bacterial colonies that produce surfactants. Drops of cell suspensions of surfactant-producing colonies collapsed an oil coated surface (Jain *et al.,* 1991). Bento *et al.* (2005) used the reduction of surface tension and the emulsify capacity to screen bio-surfactant-producing microorganisms.
Bio-surfactant activities depend on the concentration of the surface-active compounds until the critical micelle concentration (CMC) is obtained. CMC is the concentration of the surfactants where the reduction in surface tension reached a level at which supramolecular micelles or vesicles start to be formed and no further reduction in surface tension occurred. At concentrations above the CMC, bio-surfactant molecules associate to form micelles, bilayers and vesicles (Figure 3). Micelle formation enables bio-surfactants to reduce the surface and interfacial tension and increase the solubility and bioavailability of hydrophobic organic compounds. The CMC is commonly used to measure the efficiency of surfactant. Efficient bio-surfactants have a low CMC, which means that less bio-surfactant is required to decrease the surface tension. Micelle formation has a significant role in microemulsion formation (Pacwa-Płociniczak *et al*., 2011). Microemulsions are clear and stable liquid mixtures of water and oil domains separated by monolayer or aggregates of bio-surfactants. Microemulsions are formed when one liquid phase is dispersed as droplets in another liquid phase, for example oil dispersed in water (direct microemulsion) or water dispersed in oil (reversed microemulsion) (Desai and Banat, 1997).

Molecular methods for profiling of bio-surfactant producing communit: The conventional methods used for screening microbes for bio-surfactant production are well complied (Satpute *et al*., 2010b; Walter *et al*., 2010). Ecological niches contaminated with hydrocarbon are the most recommended sites for the isolation of bio-surfactant producing microbes. Techniques for purification of bio-surfactant includes thin layer chromatography, high pressure liquid chromatography and phase separation technology (Baker and Chen, 2010) followed is the characterization of the biomolecule by infrared, gas chromatography mass spectrometry, nuclear magnetic resonance and fast atom bombardment mass spectrometry (Satpute *et al*., 2010). High throughput methods are also developed by automation and miniaturization for screening of bio-surfactant producers (Walter *et al*., 2010). Recently, MALDI-TOF mass spectrometry is reported for detection and separation of bio-surfactants (Kurtzman *et al*., 2010). Along with the traditional methods, molecular techniques are being implemented to detect presence of bio-surfactant producing bacteria. Techniques such as PCR, cloning, sequencing, homology analysis, and transposon mutagenesis appear in the literature. PCR based techniques targeting genes involved either in synthesis of bio-surfactant (for e.g., srfA3, sfp, coma, licA3, rhlA, rhlB, rhlC, swrW) or regulation of bio-surfactant production (for ,e.g., rhlR, rhlI, dnaK) have been employed (Simpson *et al*. ---2011; Neilson *et al*., .---2010; Hommais *et al*., .---2008), mainly for *Bacillus* spp., *Pseudomonas* spp., and *Serratia* spp. Bioinformatics approach such as “mine” the genome of are used in few studies which has led to identification of a non-ribosomal peptide biosynthetic gene cluster that codes for proteins involved in the production of structurally related linear

lipopeptides (Berti *et al.*, 2007). The molecular approach is concentrated for very few bacterial strains and there is need to explore novel bio-surfactant from uncultured microbes by using advanced methodologies like functional metagenomics. This approach will also append tremendous knowledge of genes pool related to bio-surfactant production; still undiscovered. The data generated from such high throughput studies will accelerate application of bio-surfactant in agriculture as well as other fields. The following are the steps that can be employed for molecular characterization of bio-surfactant production in bacteria from selected habitat especially hydrocarbon/crude oil/ heavy metal-contaminated agricultural soil:

- Total DNA directly can be extracted from soil samples and subsequently analyzed either by characterizing particular sequences targeted and amplified by PCR.
- PCR products can be analyzed by cloning or genetic fingerprint. Genetic fingerprint consists in a rapid and simple electrophoretic analysis of the PCR products enabling the analysis of the genetic structure of the community.
- Characterization of cloned sequences enables assessment of the genetic diversity of a community and can reveal the phylogenetic affiliation of the community members.
- Similarly, the sequencing of bands of fingerprint profiles can lead to identification of particular populations and/or type of bio-surfactant dominant in the selected niche.
- Functional community can be analyzed by FISH, SIP, DNA microarray technology and can also help to assess the genetic structure of bio-surfactant producing communities.

Apart from the above methodologies, real time PCR can also be employed to understand the bio-surfactant producing population present in a particular niche in comparison with the total bacterial community profile (55Sachdev and Cameotra, 2013).

Screening unculturable microbes for bio-surfactant production: A metagenomic approach: Metagenomics is the culture-independent genomic analysis of microbial communities. The term was derived from the statistical concept of meta-analysis (the process of statistically combining separate analysis) and genomics (the comprehensive analysis of an organism's genetic material) (Schloss and Handelsman, 2003). Thus, this technique is a powerful tool for exploring novel compounds from uncultured bacteria associated with natural ecosystems. No PCR is involved in the metagenomics and hence PCR biases can be ruled out. Other advantage is that whole soil DNA is cloned and sequenced, thus metagenomics offers the opportunity to capture operons or genes encoding pathways that may direct the synthesis of complex molecules such as bio-surfactants. It is observed that the genes that encode for proteins/enzymes involved in the pathway of bio-surfactant synthesis are usually clustered in a region of chromosome. The gene related to biosynthesis of bacterial surfactants lie on gene cluster of approximately 3,000-7,000 bp. Hence it is possible to imply the metagenomic approach to obtain novel bio-surfactant from uncultured bacteria associated with contaminated agricultural soil and rhizosphere. Most of the reports on commercially significant bio-surfactant are from pathogenic bacterial strains and thus metagenomic approach is must for production of bio-surfactants and in supplement there is greater possibility to search for novel bio-surfactant by this technique.

However, Figure. 4 summaries the steps for functional metagenomic to mine the novel bio-surfactants from uncultured bacteria. The location for extraction of total metagenomic DNA should be selected which may include hydrocarbon, pesticides or heavy metal-contaminated agricultural soil and/or rhizosphere. The total DNA extracted should be fragmented by restriction enzymes and inserted in a suitable expression vector. The DNA construct should be transformed in host like *E. coli* and all the metagenomic clones should be screened for production of bio-surfactant by known conventional and/or high through put techniques. The novel surface active compound should be further chemically characterized. The sequence of the clone positive for bio-surfactant production should be sequenced and analyzed. The functional metagenomic approach seems a promising technique for mining novel green surfactants which can replace the harsh chemical surfactants widely employed in agriculture as well as other sectors (55Sachdev and 55Cameotra, 2013).

Factors affecting bio-surfactant production: Although the type and amount of the microbial surfactants produced depend primarily on the producer organism, factors like carbon and nitrogen, trace elements, temperature, and aeration also affect their production by the organism. The yield of microbial surfactants varies with the nutritional environment of the growing microorganism. Intact microbial cells that have high cell surface hydrophobicity are themselves surfactants. In some cases, surfactants themselves play a natural role in growth of microbial cells on water-insoluble substrates. Exocellular surfactants are involved in cell adhesion, emulsification, dispersion, flocculation, cell aggregation, and desorption phenomena.

- Producer organism.
- Carbon and (substrate) nitrogen sources.
- Trace elements.
- Temperature.
- Aeration.

Because bio-surfactants are amphiphilic compounds, they contain a hydrophobic and hydrophilic moiety. The polar moiety can be a carbohydrate, an amino acid, a phosphate group, or some other compound. The non-polar moiety is mostly a long- carbon-chain fatty acid. Although the various bio-surfactants possess different structures, there are some general phenomena concerning their biosynthesis. For example, bio-surfactant production can be induced by hydrocarbons or other water-insoluble substrates (Silva *et al*., 2014, Mao *et al*., 2015). Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a-hydrocarbon, microorganisms-facilitate their diffusion into the cell by producing a variety of substances, the bio-surfactants. The activity of microbial types naturally present can be enhanced by bioremediation technique; which include increased aeration of the polluted area and, nutrient additions (Chakraborty *et al.*, 2014; Marchant *et al.*, 2014). Another striking phenomenon is the catabolic repression of bio-surfactants synthesis' by glucose and other primary

metabolites. For example, in the case of *Arthrobacter paraffineus,* no surface-active agent could be isolated from the medium when glucose was used as the carbon source instead of hexadecane.

Similarly, a protein-like activator for n-alkane oxidation was formed by *P. aeruginosa* S7B I from hydrocarbon, but not from glucose, glycerol, or palmitic acid. *Torulopsis petrophilum* did not produce any glycolipids when grown on a single-phase medium that contained water-soluble carbon source when glycerol was used as substrate. Rhamnolipid production by *P. aeruginosa* was sharply reduced by adding glucose, acetate, succinate or citrate to the medium. The type, quality and quantity of bio-surfactant produced are influenced by the concentration of N, P, Mg, Fe, and Mn ions in the medium and the culture conditions, such as pH, temperature, agitation and dilution rate in continuous culture (Al-Araji *et al*., 2007). Olive oil mill effluent, a major pollutant of the agricultural industry in Mediterranean countries, has been used as raw material for rhamnolipid bio-surfactant production by *Pseudomonas* sp. JAMM. Many microorganisms are known to synthesize different types of bio-surfactants when grown on several carbon sources. However, there have been examples of the use of a water-soluble substrate for bio-surfactant production by microorganisms (Al-Araji *et al.,* 2007). Bio-surfactant production from *Pseudomonas* strains MEOR171 and MEOR172 are not affected by temperature, pH, and Ca, Mg concentration in the ranges found in many oil reservoirs. Their production, on the other hand, in many cases improves with increased salinity. Thus, they are the bio-surfactants of choice for the Venezuelan oil industry and in the cosmetics, food, and pharmaceutical markets. The nitrogen source can be an important key to the regulation of bio-surfactant synthesis. *Arthrobacter paraffineus* ATCC 19558 preferred ammonium to nitrate as inorganic nitrogen source for bio-surfactant production. Urea also results in increased bio-surfactant production. In some cases addition of multivalent, cations to the culture medium can have a positive effect on bio-surfactant production by penicillin influenced the formation of interfacially active compounds. The regulation of bio-surfactant production by these compounds is either through their effect on solubilization of non-polar hydrocarbon substrates or by increased production of water-soluble (polar) substrates (Al-Araji *et al*., 2007). In some cases, bio-surfactant synthesis is regulated by pH and temperature. For example in rhamnolipid production by *Pseudomonas* sp. in cellobioselipid formation by *Ustilago maydis* (Frautz, 1986), pH played an important role, and in case of *Arthrobacter paraffineus* ATCC 19558, *Rhodococcus crythropolis* temperature was important. In all these cases however the yield of BS production was temperature dependent (Al-Araji *et al*., 2007). Barakat *et al.* (2017) studied the effect of different factors on the production of bio-surfactant by marine *Bacillus amyloliquefaciens* SH20 and *B. thuringiensis* SH24 and concluded that, a typical time course profile of maximum bio-surfactants production by both strains was performed at pH 11, 30^{o}C and 15% (w/v) salinity. Also, Elazzazya *et al*. (2015) observed that the maximum bio-surfactant production by *V. salarius* (KSA-T) strain was shown when pH increases to 9.

Valuable applications of bio-surfactants: The identification and characterization of bio-surfactant produced by various microorganisms have been extensively investigated. Because properties of bio-surfactants, they have been used in several applications. The famous and potential applications are as in following topics (Table2):

- In microbial enhanced oil recovery (MEOR)
- In hydrocarbon degradation
- In hydrocarbon degradation in aquatic environment
- In bio-remediation of heavy metal
- Pesticide-specific bio-surfactants
- As antimicrobial agents
- In food additives
- As antioxidant agents
- In phytoremediation of heavy metals

In microbial enhanced oil recovery (MEOR): In microbial enhanced oil recovery (MEOR), bio-surfactants are used in microbially-enhanced oil recovery, in the cleaning of contaminated vessels and to facilitate transportation of heavy crude oil in pipelines (Ghurye *et al.,* 1994*).* Microorganisms in reservoir are stimulated to produce polymers and surfactants which aid MEOR by lowering interfacial tension at the oil-rock interface as shown in Figure 5 to produce bio-surfactants *in situ,* microorganisms in the reservoir are usually provided with low-cost substrates, such as molasses and inorganic nutrients, to promote growth and surfactant production. To be useful for MEOR *in situ,* bacteria must be able to grow under extreme conditions encountered in oil reservoirs such as high temperature, pressure, salinity, and low oxygen level. The effectiveness of MEOR has been reported in field studies carried out in US, Czechoslovakia, Romania, USSR, Hungary, Poland, and The Netherlands.

In hydrocarbon degradation: Pollution of the sea by crude oil, mostly caused by stranding of tankers, is one of the urgent and serious environmental issues over the world. Ship operations also produce wastes that are collected in the lowest part of the hull, called the bilge area. This oil-containing bilge waste must be managed properly to avoid environmental pollution (Olivera *et al.,* 2003). The ordinary self-cleaning of the sea involving; evaporation, photochemical oxidation or sedimentation of certain oil components, as well as biodegradation by marine microorganisms is overburdened due to the additional hydrocarbons, especially large oil spills (Harayama *et al.,*1999). Biodegradation of a given hydrocarbon depends on its dispersion state. The biodegradation is generally maximized when the water-insoluble substrate is solubilized or emulsified (Mattei *et al.,* 1986).

Synthetic detergents used to clean up these spillages have often led to more destruction of the environment. From an environmental viewpoint, it is important that all substances released into the environment should be degradable. Their potential for causing environmental damage should be assessed and the possibility of future harm due to build-up in the environment should be taken into consideration. On the other hand, several bacteria and yeasts excrete ionic surfactants which emulsify the hydrocarbon substrate in the growth medium (Deleu *et al.,* 1999). These molecules reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures making them potential agents for bioremediation (Banat *et al.,* 2000). Bio-surfactants affect the biodegradation processes of hydrocarbons because of their efficacy as dispersion and remediation agents and their environment-friendly qualities such as low toxicity and high biodegradability (Mulligan, 2005).

Both mono and mixed-cultures can be used for bioremediation. However, higher rates of hydrocarbons degradation are often achieved with a bacterial enrichment consortium isolated from the environment that needs bio-restoration (Juhasz *et al.,* 2000). Bacterial consortia display a wide array of metabolic mechanisms in the breakdown of diesel oil components, including production of surface-active agents and emulsifiers (Willumsen and Karlson, 1997). A wide variety of metabolic and physiological factors are required for the degradation of different compounds in diesel oil (Frielo *et al.,* 2001). All of such properties are not found in one organism. Monocultures can be adversely affected by negative interaction. So, the best approach would be the use of a consortium of microorganisms. By selecting a consortium from a contaminated environment, the negative interactions could be minimal. Moreover, the microorganisms will readily adjust to the contaminated environment. Really, the best effect on lowering the surface tension was observed when bacterial isolates were mixed. Practically, bio-surfactant activity can be measured by changes in surface and interfacial tensions and emulsification/emulsion stabilization. Microbial candidates for bio-surfactant production are expected to decrease surface tension to about 35 mNm^{-1} (Desai and Banat, 1997). Liu *et al.* (2016) reported that the bio-surfactant produced by strain Y-1 had the capability to decrease the surface tension of water from 74.66 to 27.26 mNm^{-1}, with the critical micelle concentration (CMC) of 40 mgl^{-1}. The bio-surfactant performed not only excellent stabilities against pH, temperature and salinity, but also great emulsifying activities to different kinds of oil, especially the crude oil.

Among the major types of bio-surfactants produced by microorganisms, surfactin is one of the most known products with commercial application. Only *B. subtilis* and *B. pumilus* have been reported as surfactin producers (Banat *et al.,* 2000). *Bacillus pumilus* produces a lipopeptide of the surfactin family, named pumilacidin that decreased the surface tension to 49 mNm^{-1} and increased emulsification to 59% (Peypoux *et al.,* 1999). Bioremediation is considered the best approach for restoring diesel oil contaminated soils in which, the technology is cost-effective and environmentally desirable. The success of bioremediation is dependent upon the microbial ability to degrade these complex mixtures and their rate limiting kinetics (Margesin and Shirnner, 2001). Amongst hydrocarbon pollutants, diesel oil (a complex mixture of alkanes and aromatic compounds) is frequently reported soil contaminant leaking from storage tanks and piplines or released in accidental spills (Gallego *et al.,* 2001). The mechanism behind surfactant-enhanced removal of oil from soil has been proposed to occur in two steps mobilization, solubilization (Mulligan *et al.,* 2001). In contrast to chemical dispersants, which caused ecological damage after application for abatement of spilled oil in marine ecosystems, bio-surfactants from soil or freshwater microorganisms are less toxic and partially biodegradable (Poremba *et al.,* 1991). Biodegradation is dependent on the presence of soil microorganisms, hydrocarbon-degrading hydrocarbon composition, oxygen availability, water, temperature, pH, and inorganic nutrients. The physical state of hydrocarbon can also affect the biodegradation. Addition of synthetic surfactants or bio-surfactants resulted in increased mobility and solubility of hydrocarbon, which is essential for effective microbial degradation (Morgan, and Watkinson, 1989).

Miura and Iiyama (2002) isolated a new bio-surfactant-producing microorganism, strain 1E-I058 of *Gordonia* sp., from soil and identified it as a potential bio-surfactant producer with a strong emulsifying action on heavy oil. When the strain was cultivated using hydrocarbons as the carbon source, an extracellular bio-surfactant complex was produced. Lindley and Heydeman (1986) grew the fungus *Cladosporium resiuae,* on alkane mixtures, produced extracellular fatty acids and phospholipids, mainly dodecanoic acid and phosphatidylcholine. Supplement of the growth medium with phosphatidylcholine enhanced the alkane degradation rate by 30%. Foght *et al.* (1989) reported that the emulsifier, Emulsan, stimulated aromatic mineralization by pure bacterial cultures, but inhibited the degradation process when mixed cultures were used. Addition of bio-surfactants, such as some sophorolipids, increased both the extent of degradation and final biomass yield Oberbremer and Muller-Harting (1990). Berg *et al.* (1990), using the surfactant from *Pseudomonas aeruginosa* UG2, reported an increase in the solubility of hexachlorobiphenyl added to soil slurries, which resulted in a 31% recovery of the compound in the aqueous phase. This was about 3-times higher than that solubilized by the chemical surfactant sodium ligninsulfonate (9.3%). When the *P. aeruginosa* bio-emulsifier and sodium ligninsulphonate were used together, additive effect on solubilization (41.5%) was observed (Banerjee *et al.,* 1983). Thus, this emulsifier can be used to enhance bacterial degradation of organochlorine compounds.

In hydrocarbon degradation in aquatic environment: Microorganisms that are able to degrade hydrocarbons have been isolated from aquatic environment. The microorganisms which exhibit emulsifying activity as well as the soil microorganisms which produced bio-surfactants may be useful in aquatic environment. Providenti *et al.* (1995) studied the effects of *P. aeruginosa* UG2 bio-surfactants on phenanthrene mineralization in soil slurries and detected an increase in phenanthrene mineralization combined with reduced lag period prior to the onset of mineralization Applying surfactants as immobilizing agents might be one way to enhance the solubility of PAHs. Surfactants help degradation by solubilization or emulsification, to release hydrocarbons sorbed to soil organic matter and increase the aqueous concentrations of hydrophobic compounds, resulting in higher mass transfer rates (Banat *et al*., 2000).

In bio-remediation of heavy metal: The usefulness of bio-surfactants for bioremediation of heavy metal contaminated soil is mainly based on their ability to form complexes with metals. The anionic bio-surfactants create complexes with metals in a nonionic form by ionic bonds. These bonds are stronger than the metal's bonds with the soil and metal-bio-surfactant complexes are desorbed from the soil matrix to the soil solution due to the lowering of the interfacial tension. The cationic bio-surfactants can replace the same charged metal ions by competition for some but not all negatively charged surfaces (ion exchange) (Figure 6). Metal ions can be removed from soil surfaces also by the bio-surfactant micelles. The polar head groups of micelles can bind metals which mobilize the metals in water (Aşçı *et al*., 2008) as shown in Figure 7.

Pesticide-specific bio-surfactants: Because of biodegradation property of bio-surfactants; they are ideally suited for environmental applications, especially for removal of the

pesticides, an step in bioremediation. Use of bio-surfactants for degradation of pesticides in soil and water environment has gained importance. However, there were some of the earlier works in India have initiated studies on bio-surfactants. They were; Banarjee *et al.* (1983) on 2, 4, 5-tricholoacetic acid, Patel and Gopinath on Fenthion (1986), and Anu Appaiah and Karanth (1991) on alpha HCH. Moreover, reports on production and exploitation of microbial bio-surfactants for the removal of pesticides from the environment have been done by Ramesha *et al.* (1995) and Veenanadig and Karanth (1997). These bio-surfactants can replace the harsh surfactant presently used in pesticide industries as these natural surfactants are found to be utilized as carbon source by soil inhabiting microbes (Takenaka *et al.,* 2007; Lima *et al.*, 2011).

The example we focus on, here, is Lindane (HCH) and fenthion using bio-surfactants. Lindane or Hexa-chlorocyclohexane (HCH) is the higher ranking pesticide used in many countries. There are eight known isomers of HCH. The poor solubility is one of the limiting factors in the microbial degradation of alpha-HCH. Presence of six-chlorines in the molecule is another factor that renders HCH lipophilic and persistent in the biosphere. Even though there are several reports available on biodegradation of specific isomers of HCH in animals, plants, soil and microbial systems. Furthermore, the exact mechanism of translocation of HCH to the site of destruction and degradation of alpha-HCH in bacteria is not well understood. One of the strains efficient in alpha-HCH degradation was characterized as *Pseudomonas Ptm*$^{+}$ strain. This isolate has produced extracellular bio-surfactant in a mineral medium containing HCH. While these bio-surfactants emulsified the solid organochlorine HCH to a higher extent, it emulsified other organochlorines such as DDT and cyclodienes to a lesser extent (Anu Appaiah, 1992), implying thereby, the specificity of the bio-surfactant in dispersing HCH. It was also demonstrated that the peak in production of the emulsifier appeared before the onset of HCH degradation by the *Pseudomonas* growing in liquid culture. The production of bio-surfactants for Fenthion, a liquid OP insecticide, has also received attention. *Bacillus subtilis* excreted the bio-surfactants both in liquid as well as in solid state fermentation system (Veenanadig, 1995; Veenanadig and Karanth, 1996). The microbial surfactant produced by these two organisms also shows properties of a good cleansing agent for dislodging the pesticides from used containers, mixing tanks, cargo docks, etc.
Attempts have also been made to standardize parameters for bio-surfactants production both in liquid and solid state fermentations.

As antimicrobial agents: There is a high demand for new antimicrobial agents, because of the increased resistance shown by pathogenic microorganisms against the existing antimicrobial drugs. Das *et al*. (2008) produced bio-surfactant from marine *Bacullus* had a potent antimicrobial activity against Gram-positive and Gram-negative pathogenic and semi-pathogenic microbial strains including MDR strains. Only one of the HPLC fractions of the crude bio-surfactants was responsible for its antimicrobial action. The antimicrobial lipopeptide bio-surfactant fraction was also found to be non-haemolytic in nature. Bio-surfactants have also found applications in therapeutic, health, and biomedical fields. Their antiviral, antifungal, makes them suitable compounds for use as therapeutic agents, and owing to their biological origin, they are largely considered safer than synthetic pharmaceuticals (Rodrigues *et al*., 2006). Bio-surfactants general abilities to disrupt membranes resulting in increased membrane permeability, metabolite leakage and cell lysis, and hence, antimicrobial activity are of relevance in these applications. Moreover, due to their ability to partition at the interfaces properties such as adhesion of cells microorganisms on surfaces are also affected. Numerous literature describing such biomedical applications of bio-surfactants have been published (Rodrigues *et al*., 2006; Seydlova *et al*., 2008; Banat *et al*., 2010; Rodrigues Rodrigues *et al*., 2010; Fracchia *et al*., 2012).

Several bio-surfactants display antiadhesive and antimicrobial activities (Remichkova *et al*., 2008; Sotirova *et al*., 2008). Zeraik and Nitschke (2010) recently reported antiadhesive activity against attachments of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Micrococcus luteus* on polystyrene surfaces using rhamnolipids and surfactin. Lunasan produced by the yeast *Candida sphaerica* UCP0995 also completely inhibited the adhesion of several *Streptococcus*, *Staphylococcus, Pseudomonas,* and *Candida* strains on plastic tissue culture plates (Luna *et al*., 2001). The same research group also described antiadhesive and antimicrobial activities of Rufifisan; a bio-surfactant produced by the yeast *Candida lipolytica* (Gudina *et al*., 2010). Similar observations were reported for bio-surfactant produced by the strain *Lactobacillus*, which isolated from a Portuguese dairy plant (Gudina *et al*., 2010) and from fresh cabbages (Fracchia *et al.,* 2010) and other sources (Brzozowski *et al*., 2011). Such antiadhesive activity of bio-surfactants against bacteria indicates their potential application either as coating agents for food related utensils and surfaces or to decrease antifouling rate or occurrence.

In food additives: Food additives are ingredient with no nutritional value added to food to modify physical, chemical, biological, or sensory characteristics during the manufacturing, processing, preparation, treatment, packaging, storage, transport or handing. A fundamental principles of additive use is safety therefore before approval for use, an additive must comply with adequate toxicological evaluation, taking into account any accumulative, synergic, and protective effects stemming from its use. Additives confer many properties such as thickening, gelling, stabilization, and emulsifying. Monostearate and carboxymethyl cellulose for example are synthetic emulsifiers that are widely used in the food industry, these additives have been subject to restrictions particularly by consumers' demands for less use of "artificial" or chemically synthesized additives in favor of more natural ingredients. Emulsifiers are essential ingredients in many foods particularly those containing oils and fats. They are surface active agents, which facilitate the formation of an emulsion due to their capacity to reduce interfacial tension between two immiscible phases and subsequently stabilize the emulsion formed (Kralova and Sjöblom, 2009) and improving texture and shelf life. For example, during foam fractionation gas bubbles are introduced into liquid containing surface active substances that lead to foam formation when surface active molecules attach to the gas-liquid interface of the introduced bubbles becoming stabilized (Burghoff, 2012). Discovering new microbial surfactants has been a highly sought after accomplishment in many industries to secure new ingredient additives with thickening and stabilizing abilities similar to xanthan gum or a new gelling emulsifier like emulsan. This combined with the

desire to reduce dependency on plant emulsifiers. Availing of other favorable properties, including antioxidants, anti-adhesives, antimicrobial, and biofilm disruption capacity has resulted in an increased interest in finding alternative natural sources for bio-surfactant amphiphilic molecules suitable for used in new and advanced formulations in food and other industries. Emulsification plays a role in consistency and texture as well as phase dispersion and the solubilization of aromas in most food industry products (Radhakrishnan *et al*., 2011). The function of an emulsifier is to stabilize the emulsion by controlling the clustering of globules and stabilizing aerated systems (Patino *et al*., 2008; Campos *et al.*, 2013).

Moreover, Campos *et al.* (2013) reviewed and discussed the potential future applications of bio-surfactants as food additives acting as thickening, emulsifying, dispersing or stabilizing agents in addition to the use of sustainable economic processes utilizing agro-industrial wastes as alternative substrates for their production. By definition, an emulsion is a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplets. The stability of such systems can be enhanced by surfactants, which reduce the interfacial tension, thereby diminishing the surface energy between the two phases and preventing the coalescence of particles through the formation of steric and electrostatic barriers (Muthusamy *et al*., 2008). The emulsification index (E24) is a fast and qualitative method to determine the emulsifying properties of a surfactant (Desai and Banat, 1997). Emulsifying and dispersing agents used in food products do not necessarily need the ability to reduce the surface tension of water or of hydrocarbons. Lipson has been shown not to reduce the surface tension of water and yet has been used successfully to emulsify commercial edible oils (Shepherd *et al*., 1995).

As antioxidant agents: Bio-surfactants show some potential as antioxidant agents; Mannosylerythritol lipids (MELs) are versatile bio-surfactants known for their versatile interfacial and biochemical properties using free-radical and superoxide anion-scavenging assay. Takahashi *et al*. (2012) reported antioxidant activity *in vitro*. They concluded that MEL-C has highest antioxidant and protective effects in cells and suggest potential use as anti-aging skin care ingredients. Similar observations were reported for a bio-surfactant obtained from *B. subtilis* RW-I showing antioxidant capacity to scavenge free radicals and suggesting potentials use as alternative natural antioxidants (Yalcin and Cavusoglu, 2010). Some time ago a polysaccharide emulsifier from *Klebsiella* was also shown to have potent inhibition of the autooxidation of soybean oil (Kawaguchi *et al*., 1996). The emulsifier suppressed soybean oil peroxidation by encapsulation, thereby isolating the oil from the surrounding medium. This polysaccharide was under development in France, as a source of rhamnose for the manufacture of furaneol, a flavor precursor.

In phytoremediation of heavy metals: Efficiency of phytoremediation of heavy metal contaminated soils can be increased by inoculation of plants by bio-surfactant-producing and heavy metal-resistant bacteria. Bio-surfactant-producing *Bacillus* sp. J119 strain was investigated for its capability to promote the plant growth and cadmium uptake of rape, maize, sudangrass and tomato in soil contaminated with different levels of Cd (Sheng *et al*., 2008). The study demonstrated that the tested strain could colonize the rhizosphere of all studied plants but its application enhanced biomass and Cd uptake only in plant tissue of tomato. This means that root colonization activity of the introduced strain is plant type influenced. However, further analyses of interactions between the plants and bio-surfactant-producing bacterial strain J119 may provide a new microbe assisted-phytoremediation strategy for metal-polluted soils. Further work on the applications of bio-surfactants and bio-surfactants-producing bacteria in phytoremediation, especially in sites co-contaminated with organic and metal pollutants is required.

In other applications: By virtue of properties of biodegradability, substrate specificity, chemical and functional diversity, and rapid/controlled inactivation, bio-surfactants are gaining importance in various industries like agriculture, food, textiles, petrochemicals, etc. (Prince, 1993).

Bio-surfactants from some other bacterial taxa may be of public health concern. Lipopolyglycans from mycoplasmas show endotoxic properties, potentially inducing procoagulant activity in human leukocytes (Miragliotta *et al.,* 1987). The toxicity and antigenic properties of mycobacterial glycolipids produced by pathogenic mycobacteria such as; *M. aviumintracellure, M. scrofulaceum* and *M. fortulitum,* which habitat of water polluted with industrial and domestic residues, are well known (Jardine *et al.,* 1989). The varied uses of bio-surfactants also imply scope for bio-surfactants, and the need to strengthen the research in this emerging area. An important group of bio-surfactants is mycolic acids which are very long-chain fatty acids contributing to some characteristic properties of a cell such as acid fastness, hydrophobicity, adherability, and pathogenicity. Enriching waters- and soils with long- and short-chain mycolic acids may be potentially hazardous. Daffe *et al.* (1988) reported trehalose polyphthienoylates as a specific glycolipid in virulent strains of *Mycobacterium tuberculosis.* Kaneda *et al.* (1986) reported that granuloma formation and hemopoiesis could be induced by C_{36}-C_{48} mycolic acid-containing glycolipids from *Nocardia rubra.*

Conclusion

Upon the knowledge presented in the current review and because the bio-surfactants have several benefits such as; their availability, low-cost production, and stability in various industrial preparations, they are recently considered the most promising existing alternative products for further prospects in the future depends upon. Nowadays, bio-surfactants give green solutions in many fields specifically in food, therapeutics and environmental sustainability. Therefore, more efforts for their developing and productivity should be carried out.

REFERENCES

Al-Araji L., Raja Noor Zaliha Raja Abd RN., Rahman, Mahiran Basri M., Salleh A. 2007. Microbial Surfactant. *AsPac J. Mol. Biol. Biotechnol*., 15 (3): 99-105.

Anisworth S.J. 1990. Move to Natural Cosmetics Challenges Ingredient Suppliers. Chem. Eng News, 68(48): 25-48.

Anu Appaiah, K.A., Karanth, N.G.K. 1991. Insecticide specific emulsifier production by hexachlorocyclohexane utilizing *Pseudomonas tralucida* Ptm^+ strain. *Biotechnol. Lett*., 13: 371-374.

Aranda F.J., Teruel J.A., Espuny M.J., Marques A., Manresa A., Palacios Lidon E., Ortiz A. 2007. Domain formation by a *Rhodococcus* sp. biosurfactant trehalose lipid incorporated into phosphatidylcholine membranes. *Biochim Biophys Acta* 1768:2596- 2604.

Aşçı Y., Nurbaş M., Açıkel Y.S. 2008. A comparative study for the sorption of Cd(II) by soils with different clay contents and mineralogy and the recovery of Cd(II) using rhamnolipid biosurfactant. *J. Hazd. Mater.* 154: 663–673.

Asri L.A.T.W., Crismaru M., Roest S., Chen Y., Ivashenko O., Rudolf, P. et al. 2014. A shape-adaptive, antibacterial-coating of immobilized quaternary-ammonium compounds tethered on hyperbranched polyurea and its mechanism of action. *Adv Funct Mater*, 24: 346-355.

Baker S.C., Chen C.Y. 2010. Enrichment and purification of lipopeptide biosurfactants. *Adv Exp Med Biol* 672: 281–288.

Banat I.M., Franzetti A., Gandolfi I., Bestetti G., Martinotti M.G., Fracchia L., Smyth T.J., Marchant R. 2010. Microbial biosurfactants production, applications and future potential. *Appl Microbiol Biotechnol*. 87: 427–444.

Banerjee S., Duttagupta S., Chakrabarty A.M. 1983. Production of emulsifying agent during growth of *Pseudomonas cepacia* with 2,4,5-trichlorophenoxyacetic acid. *Arch. Microbiol*. 135(2): 110-114.

Banjare M., 3Behera K., Kurrey R., 3Banjare R., Manmohan L., Pandey S., Ghosh K. 2017. Self-aggregation of bio-surfactants within ionic liquid 1-ethyl-3-methylimidazolium bromide: A comparative study and potential application in antidepressants drug aggregation. Pure Appl Chem. 89(9): 1223–1249.

Barakat K.M., Hassan S.W.M, Darwesh O.M. 2017. Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, *Egypt. Egypt. J. Aquat. Res*. 43 (3): 205-211.

Basta N. 1983. New biopolymer vies for many surfactant uses. *Chem. Eng*. 90, 20-22.

Beeba J.L., Umbrell W.W. 1971. Extracellular lipid of Thiobacillus thiooxidans. J. Bacteriol. 108: 612-614.

Bento F.M., de Oliveira Camargo F.A., Okeke B.C., Frankenberger Jr W.T. 2005. Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. *Microbiol. Res*. 160 : 249-255.-23

Berg G., Seech A.G., Lee H., Trevors J.T. 1990. Identification and characterization of a soil bacterium with extracellular emulsifying activity. *J Environ. Sci. Health* 25: 753-764.

Berti A.D., Greve N.J., Christensen Q.H., Thomas M.G. 2007. Identification of a biosynthetic gene cluster and the six associated lipopeptides involved in swarming motility of Pseudomonas syringae pv. tomato DC3000. *J. Bacteriol*. 189: 6312–6323.

Biosurfactants and their use in upgrading petroleum vacuum distillation residue: A Review Mazaheri Assadi, M ., and Tabatabaee, M. S.

Bodour A., Drees K., Maier R. 2003. Distribution of biosurfactant-producing bacteria in undisturbed and contaminated arid southwestern soils. *Appl Environ Microbiol*. 69 6. :3280–3287

Brady R.O., Trams E.G. 1964. The Chemistry of Lipids. Annu. Rev. Biochem. 33: 75-78.

Brzozowski B., Bednarski W., Golek P. 2011. The adhesive capability of two Lactobacillus strains and physicochemical properties of their synthesized biosurfactants. *Food Technol. Biotechnol*. 49: 177–186.

Burghoff B. 2012. Foam fractionation applications. J. Biotechnol. 161: 126–137.

Cameotra S.S., and Makkar R.S. 2004. Recent applications of biosurfactant as biological and immunological molecules. *Curr. Opin.Microbiol*. 7: 262-266.

Campos JM., Stamford TLM., Sarubbo LA., de Luna JM., Rufino RD., Banat IM. 2013. Microbial Biosurfactants as Additives for Food Industries. *Biotechnol. Prog*., 29 5. : 1097-1108.

Cassidy D.P., Efendiev S., White D.M. 2000. A comparison of CSTR and SBR bioslurry reactor performance. *Water Research* 34: 4333-4342.

Cassidy D.P., Hudak A.J. 2001. Microorganism selection and biosurfactant production in a continuously and periodically operated bioslurry reactor. *J. Haz. Mat.* 84: 253-264.

Chakrabarti S. 2012. Bacterial biosurfactant: Characterization, antimicrobial and metal remediation properties. Ph.D. *Thesis, National Institute of Technology, Surat, India*.

Chakraborty J., Das S. 2014. Biosurfactant-based bioremeditaion of toxic metals. In: Das S., editor. Microbial Biodegradation and Bioremediation. 1st ed. Elsevier; Rourkela Odisha, India: pp. 167–201.

Chandran P., 2C+Nilanjana"Das N. 2016. Role of Sophorolipid Biosurfactant in Degradation of Diesel Oil by Candida tropicalis. Biorem. J. 16 1. :

Chang J.S., Radosevich M., Jin Y., Cha D.K. 2004. Enhancement of phenanthrene solubilization and biodegradation by trehalose lipid biosurfactants. *Env. Toxicol. Chem*. 23: 2816-2822.

Cirigliano M.C., Carman G.M. 1984. Isolation of a bioemulsifier from Candida lipolytica. Appl. Env. Microbiol. 48: 747-750.

Cortés-Sánchez A.J. Sánchez HH., Jaramillo-Flores ME. 2013. Biological activity of glycolipids produced by microorganisms: New trends and possible therapeutic alternatives. *Microbiol. Res*. 168: 22–32.

Costa S.G.V.A.O., Nitschke M., Haddad R., Eberlin M.N., Contiero I. 2006. Production of Pseudomonas aeruginosa LBI rhamnolipids following growth on Brazilian native oils. *Proc. Biochem*. 41: 483-488.

Coutinho JOPA., Silva MPS., Moraes PM., Monteiro AS., Barcelos JCC., Siqueira EP., Santos LL. 2013. Demulsifying properties of extracellular products and cells of Pseudomonas aeruginosa MSJ isolated from petroleum-contaminated soil. *Bioresour. Technol*. 128:646–654.

Coutte F., Lecouturier D., Yahia S A., Leclere V., Bechet P. et al. 2010. Production of surfactin and fengycin by Bacillus subtilis in a bubbleless membrane bioreactor. *Appl Microbiol Biotechnol*. 87: 499-507.

Daffe M., Lacave C., Laneelle M., Gillois M., Laneele G. 1988. Polyphthienoyl trehalose, glycolipids specific for virulent strains of the tubercle bacillus. *Eur. J. Biochem*. 172: 579-584.

Das P., Mukherjee S., Sen R. 2008. Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. *Chemosphere* 72 9. : 1229-1234.

Deleu M., Paquot M., Jacques P., Thonart P., Adriaensen Y., Dufrene Y.F. 1999. Nanometer scale organization of mixed surfactin/phosphatidylcholine monolayers. *Biophys. J.* 77: 2304-2310.

Desai J.D., Banat I.M. 1997. Microbial production of surfactants and their commercial potential. *Microbiol.Mole. Biol. Rev.* 61: 47-64.

Deshpande S., Shiau B.J., Wade D., Sabatini D.A., Harwell J.H. 1999. Surfactants Selection for *Enhancing Ex-Situ Soil Washing, vol. 33, Pergamon* pp.551-560.

Elazzazya A.M., Abdelmoneima T.S., Almaghrabi O.A. 2015. Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi J. Biolo. Sc*. 22 4. : 466–475.

Ellman G.L. 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys*. 82: 72-77.

Fattom A., Shilo M. 1984. Hydrophobicity as an Adhesion Mechanism of Benthic Cyanobacteria . *Appl. Env. Micobiol*. 47: 135-148.

Fernanda C.P., Silva R., Bruno A.C., Roque, Nathalia M .P., Silva R., Raquel D., Rufno, Juliana M. Luna, Valdemir A. Santos, Ibrahim M. B., Leonie A., Sarubbo. 2017. Yeasts and bacterial biosurfactants as demulsifers for petroleum derivative in seawater emulsions. *AMB Exp*r. 7:202.

Foght J.M., Gutnick D.L., Westlake D.W.S. 1989. Effect of emulsan on biodegradationof crude oil by pure and mixed bacterial cultures. *Appl. Env. Microbiol*. 55: 36–42.

Fracchia L., Cavallo M., Allegrone G., Martinotti M.G. 2010. A Lactobacillus-derived biosurfactant inhibits biofilm formation of human pathogenic Candida albicans biofilm producers. *Appl. Microbiol. Biotechnol*. 2:827–837.

Fracchia L., Cavallo M., Giovanna Martinotti M., Banat I.M. 2012. Biosurfactants and bioemulsifiers biomedical and related applications. In: Ghista DN, editor, Present Status and Future Potentials, Biomedical Science, Engineering and Technology. *Shanghai: InTech*. 325–70.

Franzetti A., Gandolfi I., Smyth T.J., Banat I. 2010. Production and application of trehalose lipid biosurfactants. *Eur.J. Lipid.Sci.Technol*. 112 6. :617 - 627

Frautz B., Lang S., Wagner F. 1986. Formation of cellobios lipids by growing and resting cells of Ustilago maydis. *Biotechnol. Lett*. 8: 757-762.

Frederico A., Kronemberger , Cristiano P., Borges, Denise MG., Freire. 2010. Fed-batch Bbiosurfactant production in a bioreactor .*Int.Review.Chem. Engin*. 2 4. 74-80.

Frielo D.A., Mylroie J.R., Chakrabarty A.M. 2001. Use of genetically engineered multi-plasmid microorganisms for rapid degradation of fuel hydrocarbons. *Inter. Biodeter. Biodegrad*. 48: 233-242.

Gallego L.R., Loredo J., Lamas IF., Vasquez F., Sanchez J. 2001. Bioremediation of diesel-contaminated soils: evaluation of potential in situ techniques by study of bacterial degradation. *Biodegradation* 12: 325-335.

Gamero-Sandemetrio E., %23x000e1%3B-Tormo%20L%5 BAuthor%5D&cauthor=true&cauthor_uid=29610760"Payá-Tormo L., %23x000f3%3Bmez-Pastor%20R% 5BAuthor%5 D&cauthor=true&cauthor_uid=29610760" Gómez-Pastor R., 5BAuthor%5 D&cauthor= true&cauthor_uid=29610760" Aranda A.,5 BAuthor%5D&cauthor=true&cauthor_ uid=29610760" Matallana E. 2018. Non-canonical regulation of glutathione and trehalose biosynthesis characterizes non-Saccharomyces wine yeasts with poor performance in active dry yeast production. Microb Cell. 5 4. : 184–197.

Gautam KK., Tyagi VK. 2006. Microbial Surfactants: A review. J. Oleo Sci. 55: 155–166.

Georgiou, Lin S.C., Sharma, M.M. 1992. Surface-active compounds from microorganisms. Biotechnology 10: 60-65.

Ghurye G.L., Vipulanandan C., Willson, R.C. 1994. A practical approach to biosurfactant production using non aseptic fermentation of mixed cultures. *Biotechnol. Bioengin.* 44: 661- 666.

Gilewicz M., Ni'matuzahroh, Nadalig T., Budzinski H., Doumencq P., Michotey V., Bertrand J.C. 1997. Isolation and characterization of a marine bacterium capable of utilizing 2-methylphenanthrene. *Appl. Microbiol. Biotechnol*. 48:528–533.

Grula E.A., Russell H.H., Bryant D., Kenaga M., Hart M. 1982. Isolation and screening of Clostridium for possible use in MEOR. In: E.C. Donaldson and J.B. Clark Editors. , Proceedings of International Conference on Microbial Enhancement of Oil Recovery, Afton, OK, May 16–21. *Springfield*, VA: NTIS, pp. 43-47.

Gudiña E.J., Teixeira J.A., Rodrigues L.R. 2010. Isolation and functional characterization of a biosurfactant produced by Lactobacillus paracasei. *Colloids Surf. B. Biointer*. 76: 298-304.

Guerra-Santos. L, H., Kappeli, O. and Fiechter, A., 1986. Dependence of Pseudomonas aeruginosa continous culture biosurfactant production on nutritional and environmental factors. *Appl. Env. Microbiol.* 24: 443-448.

Gutnick D.L., Bayer E.A., Rubinowitz C., Pines O., Shabtai Y., Goldmann S, ,Rosenberg E. 1981. Emulsan production in Acinebacter RAG-1. *Adv. Biotechnol*. 3: 455-459.

Haferburg D., Hommel R., Claus R., Kleber H.P. 1986. Extracellular microbial lipids as biosurfactants. Adv. Biochem. *Eng. Biotechnol*. 33: 53-93.

Harayama S., Kishira H., Kasai Y., Shutsudo K. 1999. Petroleum biodegradation in marine environments. *J. Mol. Microbiol. Biotechnol*. 1: 63-70.

Henkel M., Müller M.M., Kügler J.H., Lovaglio R.B., Contiero J., Syldatk C. 2012. Rhamnolipids as biosurfactants from renewable resources: concepts for next-generation rhamnolipid production Process. Biochem. 47 8. : 1207–1226.

Hommais F., Oger-Desfeux C., Van Gijsegem F., Castang S., Ligori S., Expert D., Reverchon S., Nasser W. 2008. PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium Erwinia chrysanthemi 3937. *J. Bacteriol*. 190: 7508–7522

Ivanov V.T. 1977. Peptides: In Proceeding of the 5th American Peptides Symposium M. Goodman and J. Meinhofer, eds.. , John Wiley, New York, p. 307.

Iwabuchi N., Sunairi M., Urai M., Itoh C., Anzai H., Nakajima M., Harayama S. 2002. Extracellular polysaccharides of Rhodococcus rhodochrous S-2 stimulate the degradation of aromatic components in crude oil by indigenous marine bacteria. Appl. Env. Microbiol. 68: 2337-2343.

Jain D.K., Collins-Thomson D.L., Lee H., Trevors J.T. 1991. A drop-collapsing test for screening. surfactant-producing microorganisms. J. Microbiol. Methods 13: 271-279.

Janek T., Łukaszewicza M., Krasowska A. 2013. Identification and characterization of biosurfactants produced by the Arctic bacterium Pseudomonas putida BD2. Colloids Surf. B 110: 379–386.

Jardine I., Scanlan G., McNeil M., Brennan P.J. 1989. Plasma desorption mass spectrometric analysis of mycobacterial glycolipids. Anal. Chem. 61 5. : 416-422.

Jarvis F.G., and Johnson M. 1949. A Glyco-lipid produced by Pseudomonas aeruginosa., J. Am. Chem. Soc. 71: 4124-4126.

Joshi-Navare K., Khanvilkar P., Prabhune A. 2013. Jatropha oil derived sophorolipids: production and characterization as laundry detergent additive. Biochem. Res. Int. 3:133–143.

Juhasz A., Stanley G.A., Britz M.L. 2000. Microbial degradation and detoxification of high molecular weight polycyclic aromatic hydrocarbons by Stenotrophomonas maltophilia strain VUN 10,003. Lett. Appl. Microbiol. 30 5. : 396-401.

Kachholz T., Schlingmann M. 1987. Possible food and agricultural application of microbial surfactants. In "Biosurfactants and Biotechnology" N. Kosaric, W. L. Cairns, and N.C.C. Grey, eds . , pp. 183-210.

Kaneda K., Sumi Y., Kurano F., Kato Y., Yano I. 1986. Granuloma formation and hemopoiesis induced by C36-48-mycolic acid-containing glycolipids from HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/3781628"Nocardia rubraHYPERLINK "https://www.ncbi.nlm.nih.gov /pubmed/3781628". Infect. Immun. 54 3. : 869-875.

Kapadia S.G., Yagnik B.N. 2013. Current Trend and Potential for Microbial Biosurfactants. Asian Journal of Experimental Biological Sciences 4 1. :1-8

Kappeli O., Finnerty W. R. 1979. Partition of alkane by an extracellular vesicle derived from hexadecane-grown Acinetobacter. J. Bacteriol. 140: 707-712.

Kawaguchi K., Satomi K., Yokoyama M., Ishida Y. 1996. Antioxidative properties of an extracellular polysaccharide produced by a bacterium Klebsiella sp. isolated from river water. Nippon Suisan Gakkaishi. 42: 243–251.

Kjelleberg S., Stenström, T.A. 1980. Lipid surface films: Interaction of bacteria with free fatty acids and phospholipids at the air-water interface. *J. Gen. Microbiol*. 116:417–423.

Konishi M.,2C+Manako" Fujita M.,2C+Yu" Ishibane Y.,2C+Yuki"Shimizu Y.,2C+Yusuke" Tsukiyama Y., 2C+Masashi" Ishida M. 2016. Isolation of yeast candidates for efficient sophorolipids production: their production potentials associate to their lineage. Microbiol. Ferment. Technol. 80 10. 2058–2064.

Kralova I., Sjöblom J. 2009. Surfactants used in food industry: a review. *J. Dispers. Sci. Technol*. 30: 1363–1383.

Kretschmer A., Block H., Wagner F. 1982. Chemical and Physical Characterization of Interfacial-Active Lipids from Rhodococcus erythropolis Grown on n-Alkanes. *Appl Env. Microbiol.* 44 4. : 864-870.

Krumm, C. Tiller J.C., Kontaktaktiv oder durch Biozide. 2014. *Nachrichten aus der Chemie*. 62:984e7.

Kurtzman C.P., Price N.P., Ray K.J., Kuo T.M. 2010. Production of sophorolip biosurfactants by multiple species of the Starmerella Candida. bombicola yeast clade. *FEMS Microbiol. Lett*. 311: 140–146.

Kuyukina M.S., Ivshina I.B., Gein S.V., Baeva T.A., Chereshnev V.A. 2007. In vitro immunomodulating activity of biosurfactant glycolipid complex from Rhodococcus ruber. Bullet. *Exper. Bio. Med*. 144 3. : 326–330.

Kuyukina M.S., Ivshina L.B., Makarov S.O., Litvinenko L.V., Cunningham C.J., Philp J.C. 2005. Effect of biosurfactants on crude oil desorption and mobilization in a soil system. *Environ. Int.* 31 2. : 155–161

Lang S., Philp J. 1999. Surface active lipids in Rhodococci. Antonie van Leeuwenhoek 74: 59–70.

Lee B.S., Kim E.K. 2004. Lipopeptide production from Bacillus sp. GB16 using a novel oxygenation method. *Enz. Microb. Technol*. 35: 639–647.

Lima T.M., Procópio L.C., Brandão F.D., Leão B.A., Tótola M.R., Borges A.C. 2011. Evaluation of bacterial surfactant toxicity towards petroleum degrading microorganisms. Bioresour. Technol. 102: 2957–2964.

Lindley N.D., Heydemann M.T. 1986. The uptake of n-alkanes from alkane mixtures during growth of the hydrocarbon-utilizing fungus Cladosporium resinae. *Appl. Microbial. Biotechnol*. 23 5. : 384-388.

Liu B., X16302211?via%3Dihub"Liu J., X16302211?via%3Dihub"Ju M., X16302211?via%3Dihub" Li X. 2016. Purification and characterization of biosurfactant produced by Bacillus licheniformis Y-1 and its application in remediation of petroleum contaminated soil. *Mar Pollut Bull*. 107 1. : 46-5.1

Luna J.M., Rufino R.D., Sarubbo L.A., Rodrigues L.R., Teixeira J.A., Campos-Takaki G.M. 2011. Evaluation antimicrobial and antiadhesive properties of the biosurfactant lunasan produced by Candida sphaerica UCP 0995. *Curr. Microbiol*. 62: 1527– 1534.

M. Banat, R. S., Makkar S. S., Cameotra. 2000. Potential commercial applications of microbial surfactants. *Appl Microbiol Biotechnol*. 53: 495-508.

Macdonald C.R., Cooper D.G., Zajic J.E. 1981. Surface-Active Lipids from Nocardia erythropolis Grown on Hydrocarbons. *Appl. Env. Microbiol*. 41 1. : 117–123.

Madigan M.T. 2005. Anoxygenic phototrophic bacteria from extreme environments. In: Discoveries in Photosynthesis. Govyindjee J.T., Beatty H., Gest H., Allen J.F. Eds.. , pp: 969-983.

Makkar S.R., Cameotra S.S.C., Banat I.M. 2011. Advances in utilization of renewable substrates for biosurfactant production. AMB Express. 1 5. : 1-19.

Mao X., Jiang R., Xiao W., Yu J. 2015. Use of surfactants for the remediation of contaminated soils: A review. J. Hazard. Mater.;285:419–435. doi: 10.1016/j.jhazmat.2014.12.009.

Mao X., Jiang R., Xiao W., Yu J. 2015. Use of surfactants for the remediation of contaminated soils: A review. J. Hazard. Mater. 285:419–435.

Marchant R., Funston S., Uzoigwe C., Rahman P.K.S.M., Banat I.M. Biosurfactants. Taylor & Francis; New York, USA: 2014. Production of biosurfactants from nonpathogenic bacteria; pp. 73–81.

Margesin R., Schinner F. 2001. Biodegradation and bioremediation of hydrocarbons in extreme environments. Appl. Microbiol. Biotechnol. 56: 650-663.

Mattei G., Rambeloarisoa E., Giusti G., Rontani J.F., Betrand J.C. 1986. Fermentation procedure of a crude oil in continuous culture on seawater. *Appl. Microbiol. Biotechol*. 23: 302-304.

Milena G.,Rikalovic., Mairoslav M., Ivanka M., Karadzic. 2015. Rhamnolipid biosurfactant from Pseudomonas aeruginosa from discovery to application in contemporary technology. Review. J. Serb. Chem. Soc. 80 3. 279–304.

Miragliotta G., Barone G., Monno R.A., Fumarola, D., Smith, P.F. 1987. Induction of procoagulant activity in human leukocytes with lipoglycans from Mycoplasmas, *J. Clin. Microbiol*. 25: 1295-1297.

Miura A., Iiyama, T. 2002. New biosurfactants and methods for their production. JP Patent Office Publication No. JP2002–239368.

Mogi T., Kita K. 2009. Gramicidin S and polymyxins: the revival of cationic cyclic peptide antibiotics . *Cell. Mol. Life Sci*. 66: 3821–3826.

Morgan P., Watkinson R.I. 1989. Hydrocarbon degradation in soils and methods for soil biotreatment. *CRC Crit Rev. Biotechnol*, 4: 305-333.

Mukherjee A.K., Das K. 2005. Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by Bacillus subtilis strains in a ticular habitat. FEMS. Microbiol. Eparcol. 54: 479-489.

Mulligan C.N. 2005. Environmental applications for biosurfactants. *Environ. Pollut*. 133: 183-198.

Mulligan C.N., Yong R.N., Gibbs B.F. 2001. Remediation Technologies for Metal-Contaminated Soils and Groundwater An Evaluation. Engineering Geology 60: 193-207

Muthusamy K., Gopalakrishnan S., Ravi T.K., Sivachidambaram P. 2008. Biosurfactants: properties, commercial production and application. *Curr. Sci*. 94: 736–747.

National Research Council. 2005. Oil Spill Dispersants: Efficacy and Effects. Washington, DC: The National Academies Press. https://doi.org/10.17226/11283.

Neilson J.W., Zhang L., Veres-Schalnat T.A., Chandler K.B., Neilson C.H., Crispin J.D., Pemberton J.E., Maier R.M. 2010. Cadmium effects on transcriptional expression of rhlB/rhlC genes and congener distribution of monorhamnolipid and dirhamnolipid in Pseudomonas aeruginosa IGB83. *Appl. Microbiol. Biotechnol*. 88: 953–963.

Neufeld R.J., Zajic J.E., Gerson D.F. 1980. Cell surface measurements in hydrocarbon and carbohydrate fermentations. *Appl. Environ. Microbiol.* 39: 511-17.

Nitschke M., Costa S.G.V.A.O., Contiero J. 2005. Rhamnolipid surfactants: An update on the general aspects of these remarkable biomolecules. Biotechnol. Prog. 21: 1593–1600.

Nitschke M., Pastore G. M. 2006. Production and properties of a surfactant obtained from Bacillus subtilis grown on cassava wastewater. *Biores. Technol*. 97 2. : 336-341.

Noah KS., 5BAuthor%5D&cauthor=true& cauthor_ uid=15920256"Bruhn DF., 5BAuthor%5D &cauthor= true&cauthor_uid=15920256"Bala GA. 2005. Surfactin production from potato process effluent by Bacillus subtilis in a chemosta. *Appl Biochem Biotechnol*. 121-124:465-73.

Noordman W.H., Janssen D.B. 2002. Rhamnolipid stimulates uptake of hydrophobic compounds by Pseudomonas aeruginosa. *Appl. Env.. Microbiol.* 68 9. : 4502–4508.

O'Neill M., 5BAuthor%5D &cauthor=true&cauthor_u id=29225379"Piligian B., 5BAuthor%5D&ca uthor=true&ca uthor_uid=29225379"Olson C.5B Author% 5D&c author= true& cauthor_uid=29225379. Woodruff P., 5BAuthor%5D &cauthor=true & c author_uid=29225379"Benjamin M., Swarts. 2017. Tailoring Trehalose for biomedical and biotechnological applications. *Pure. Appl. Chem*. 89 9. : 1223–1249.

Oberbremer A., Muller-Hurtig R., Wagner F. 1990. Effect of the addition of microbial surfactants on hydrocarbon degradation in a soil population in a stirred reactor. *Appl. Microbiol. Biotechnol.* 32 3. : 485-489.

Oberbremer, A., Muller-Hurtigm, R., App!. Microbiol. *Biotechnol.,* 1989,31, 582-586.

Okoliegbe I.N., Agarry O.O. 2012. Application of microbial surfactants a review. Scholarly J. Biotechnol. 1 1. : 15-23.

Olivera N.L., Commendatore M.G., Delgado O., Esteves J.L. 2003. Microbial characterization and hydrocarbon biodegradation potential of natural bilge waste microflora. J. Ind. Microbiol. Biotechnol. 30: 542-548.

Ortiz A., Teruel J.A., Espuny M.J., Marqués A., Manresa A., Aranda F.J. 2006. Effects of dirhamnolipid on the structural properties of phosphatidylcholine membranes. *Int. J. Pharm.* 325 1-2. : 99-107.

Pacwa-Płociniczak M., Płaza G.A., Piotrowska-Seget Z., Cameotra S.S. 2011. Environmental applications of biosurfactants: recent advances. *Int. J. Mol. Sci.* 12: 633-654.

Patel M.N., Gopinathan K.P. 1986. Lysozyme sensitive bioemulsifier for immiscible organophosphorus pesticides. Appl. Environ. Microbiol. 52:1224-1226.

Patino J.M.R., Sanchez C.C., Niño M.M.R. 2008. Implications of interfacial characteristics of food foaming agents in foam formulations. *Adv. Colloid Interface Sci*. 140:95–113.

Perfumo A., Banat I.M., Canganella F., Marchant R. 2006. Rhamnolipid production by a novel thermophilic hydrocarbon-degrading Pseudomonas aeruginosa AP02-1. *Appl. Microbiol. Biotechnol*. 72: 132-138.

Peypoux F., Bonmatin J.M., Wallach J. 1999. Recent trends in the biochemistry of surfactin. *Appl. Microbiol. Biotechnol.* 51 5. :553-63.

Poremba K., Gunkel W., Lang S., Wagner F. 1991. Marine biosurfactants, III. Toxicity testing with marine microorganisms and comparison with synthetic surfactants. Z Naturforsch 46 3-4. :210-216.

Pornsunthorntawee O., Wongpanit P., Chavadej S., Abe M., Rujiravanit R. 2008. Structural and physicochemical characterization of crude biosurfactant produced by Pseudomonas aeruginosa SP4 isolated from petroleum-contaminated soil. *Biores. Technol*. 99: 1589-1595.

Prince R.C. 1993. Petroleum spill bioremediation in marine environments. *Crit. Rev. Microbiol*. 19 4. : 217-242.

Providenti MA., Fleming CA., Lee H., Trevors JT. 1995. Effect of addition of rhamnolipid biosurfactants or rhamnolipid producing Pseudomonas aeruginosa on phenanthrene mineralization in soil slurries. FEMS *Microbiol Ecol*. 17: 15-26.

Radhakrishnan N., Kavitha V., Madhavacharyulu E., Gnanamani A., Mandal A.B. 2011. Isolation, production and characterization of bioemulsifiers of marine bacteria of coastal Tamil Nadu. *Indian J. of Geo-Marine Sciences*. 40: 76–82.

Ramana K.V., Karanth N.G. 1989. Production of biosurfactants by the resting cells of Pseudomonas aeruginosa CFTR-6. Biotechnol. Lett.11: 437–442.

Ramesha N., Athiya Afshana, Parvathi, Anu Appaiah, K.A., Karanth N.G.K. 1995. Proceedings of the Symposium on Relevance of Biotechnology in Industry, 4-5 March, Cochin University of Science and Technology, Cochin, pp. 27-28.

Ratledge C. 1986. Lipids. In: Rehm H.J., Reed G eds. Biotechnology, vol 4. In: Pape H, Rehm HJ eds. Microbial products II. Verlag Chemie, Weinheim, pp. 185–213.

Remichkova M., Galabova D., Roeva I., Karpenko E., Shulga A., Galabov A.S. 2008. Anti-herpesvirus activities of Pseudomonas sp. S- 17 rhamnolipid and its complex with alginate. Z Naturforsch C. 63:75–81.

Rodrigues L., Banat I.M., Teixeira J., Oliveira R. 2006. Biosurfactants: potential applications in medicine. J. Antimicrob. *Chemother*. 57: 609–618.

Rodrigues L.R., and Teixeira J.A. 2010. Biomedical and therapeutic applications of biosurfactants. *Adv. Exp. Med. Biol*. 672: 75–87.

Sachdev D., Cameotra S. 2013. Biosurfactants in agriculture. *Appl. Microbiol. Biotechnol*. 97: 1005–1016.

Saini S., Belgacem MN., Salon CB., Bras J. 2016. Non leaching biomimetic antimicrobial surfaces via surface functionalisation of cellulose nanofibers with aminosilane Cellulose. 23: 795-810.

Samadi N., Fazeli M.R., Abadian N., Akhavan A., Tahzibi A., Jamalifar H. 2007. Biosurfactant production by the strain isolated from contaminated *soil. J. Biol. Sci*. 7: 1266–1269.

Santos D., Rufino R., Luna J.,. Santos V., Sarubbo L. 2016. Biosurfactants: Multifunctional Biomolecules of the 21st Century. *Int. J. Molecular Sci*. 17, 401: 1-31.

Satpute S.K., Bhuyan S.S., Pardesi K.R., Mujumdar S.S., Dhakephalkar P.K., Shete A.M., Chopade B.A. 2010. Molecular genetics of biosurfactant synthesis in microorganisms. Adv. Exp. Med. Biol. 672:14–41.

Schloss P.D., Handelsman J. 2003. Biotechnological prospects from metagenomics. *Curr. Opin. Biotechnol*. 14: 303–310.

Sen S., 5BAuthor%5 D&cauthor= true&cauthor_uid =28558761" Borah S., 5BAuthor%5 D&cauthor= true& cauthor_uid=28558761" Bora A., 5BAuthor%5D& cauthor=true& cauthor_uid =28558761" Deka S. 2017. Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. Microb Cell Fact. 16: 95.

Seydlova G., Svobodova J. 2008. Review of surfactin chemical properties and the potential biomedical applications. *Cent. Eur. J. Med.* 3: 123–133.

Sheng X., He L., Wang Q., Ye H., Jiang C. 2008. Effects of inoculation of biosurfactant-producing Bacillus sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J. Hazard. Mat. 155: 17–22.

Shepherd R., Rockey J., Shutherland I.W., Roller S. 1995. Novel bioemulsifiers from microorganisms for use in *foods. J Biotechnol*. 40: 207–217.

Silva RCFS., Almeida DG., Luna J M., Rufino RD, Santos VA, Sarubbo LA. 2014. *Applications of biosurfactants* in the petroleum industry and the remediation of oil spills. Int. J. Mol. Sci. 15: 12523–12542.

Simpson D.R., Natraj N.R., McInerney M.J., Duncan K.E. 2011. Biosurfactant-producing Bacillus are present in produced brines from Oklahoma oil reservoirs with a wide range of salinities. *Appl. Microbiol. Biotechnol*. 91: 1083–1093.

Soberón-Chávez G., 3%A9pine%20F%5BAuthor%5D&cauthor=true&cauthor_uid=16160828"Lépine F., 3%A9ziel%20E%5BAuthor%5D &cauthor=true&cauthor_uid=16160828" Déziel E. 2005. Production of rhamnolipids by Pseudomonas aeruginosa. Appl Microbiol Biotechnol. 68 6. :718-25.

Sotirova A.V., Spasova D.I., Galabova D.N., Karpenko E., Shulga A. 2008. Rhamnolipid–biosurfactant permeabilizing effects on Gram positive and Gram-negative bacterial strains. Curr. Microbiol. 56: 639–644.

Takahashi M., Morita T., Fukuoka T., Tomohiro I., Kitamoto D. 2012. Glycolipid biosurfactants, mannosylerythritol lipids, show antioxidant and protective effects against H2O2—induced oxidative stress in cultured human skin fibroblasts. J. Oleo. Sci. 61: 457–464.

Takenaka S., Tonoki T., Taira K., Murakami S., Aoki K. 2007. Adaptation of Pseudomonassp. strain 7–6 to quaternary ammonium compounds and their degradation via dual pathways. *Appl. Env. Micro*. 73: 1797–1802.

Vaara M. 2018. New polymyxin derivatives that display improved efficacy in animal infection models as compared to polymyxin B and colistin. *Med Res Rev*. 2018.

Veenanadig N.K., and Karanth N.G.K. 1997. Proceedings of Annual Meetings of Society of Biochemist India. , Andhra University, 22-24 December, Vishakapatnam, p p. 177.

Veenanadig N.K., Anu Appaiah K.A., Karanth N.G.K. 1995. Proceedings of the Symposium on Relevance of Biotechnology in Industry, 4-5 March, *Cochin University of Science and Technology*, Cochin, p p. 28.

Veenanadig N.K., Karanth N.G.K. 1996. Proceedings of Annual Conference of Association of Microbiology, IIT Chennai, 4-6 December, Chennai, pp.107.

Vigneshwaran C., Jerold M., Vasantharaj,K., Sivasubramanian V. 2016. A Review on biosurfactants and its environmental applications. 10 8. : 152-160.

Walter V., Syldatk C., Hausmann R. 2010. Screening concepts for the isolation of biosurfactant producing microorganisms. *Adv. Exp. Med. Biol*. 672:1–13

Vijayakumar S., Saravanan V. 2015. Biosurfactants-types, sources and applications. Res. J. Microbiol. 10: 181–192.

Whang L.M., Liu P.W.G., Ma C.C., Cheng S.S. 2008. Application of biosurfactant, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *J. Hazard. Mater*. 151: 155–163.

Willumsen P.A., Karlson U. 1997. Screening of bacteria, isolated from PAH contaminated soils, for production of biosurfactant and bioemulsifiers. *Biodegradation*. 7: 415-423.

Yalcin E., Cavusoglu K. 2010. Structural analysis and antioxidant activity of a biosurfactant obtained from Bacillus subtilis RW-I. Turkish J. Biochem-Turk Biyokimya Dergisi. 35: 243–247.

Yeh M S., Wei Y H., Chang J S. 2006. Bioreactor design for enhanced carrier-assisted surfactin production with Bacillus subtilis, *Process Biochem*. 41: 1799-1805.

Yeh M.S., Wei Y.H., Chang I.S. 2006. Bioreactor design for enhanced carrier-assisted surfactin production with Bacillus subtilis. *Process Biochemistry* 41 8. : 1799-1805.

Zhang Z., Li Z . 2017. Tailoring Trehalose for biomedical and biotechnological applications. *Front Cell Infect Microbiol.* 7: 402.

Zajic J.E., Supplisson B., Volesky B. 1974. Bacterial degradation and emulsification of No. 6 fuel oil. *Env. Sci. Technol*. 8: 664-668.

Zeraik A.E., and Nitschke M. 2010. Biosurfactants as agents to reduce adhesion of pathogenic bacteria to polystyrene surfaces: effect of temperature and hydrophobicity. *Curr. Microbiol*. 61: 554–559.

Zhang M., and Wang H. 2016. Preparation of immobilized sulfate reducing bacteria SRB. granules for effective bioremediation of acid mine drainage and bacterial community analysis Minerals Engineering. /92/supp/C"92: 63-71.

Zhang Y., Miller R.M. 1992. Enhanced octadecane dispersion and biodegradation by a Pseudomonas rhamnolipid surfactant biosurfactant. *Appl. Env. Microbiol*. 58 10. : 3276-3282.

Zhao Z., 2C+Jonathan+WC"Wong J.W. 2009. Biosurfactants from Acinetobacter calcoaceticus BU03 enhance the solubility and biodegradation of phenanthrene. *Environ. Technol.* 30 3. :

****