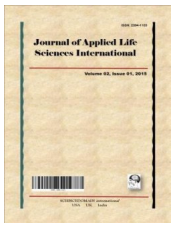

Journal of Applied Life Sciences International
7(2): 1-19, 2016; Article no.JALSI.27308
ISSN: 2394-1103

SCIENCEDOMAIN *international*
www.sciencedomain.org

Biosurfactants: Current Perspectives in Environmental Remediation

Kuldeep Nigam[1], Sanjay Gupta[1] and Nidhi Gupta[1*]

[1]Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/27308
Editor(s):
(1) Martin Koller, University of Graz, Research Management and Service, c/o Institute of Chemistry, Austria.
Reviewers:
(1) Ojeda Morales Marcia Eugenia, Autonomous Juarez University of Tabasco, Mexico.
(2) Saima Fazal, Huazhong University of Science and Technology, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/15772

Review Article

Received 27th May 2016
Accepted 2nd August 2016
Published 13th August 2016

ABSTRACT

Biosurfactants are diverse group of surface active compounds synthesized by microorganisms. These molecules have gained environmental significance as they are known to increase the degradation of pollutants by increasing their bioavailability with high specificity and low toxicity. They are preferred over their chemical counterparts because they are biodegradable and non-hazardous. Because of these advantages, biosurfactants have also gained importance in other industries like oil, agriculture, paint and pharmaceutics. Despite so much of importance and demand, large scale production of these compounds is not achieved as yet. This review provides the comprehensive overview of the properties, classification and factors limiting the commercialization of biosurfactant.

Keywords: Biosurfactant; bioremediation; classification; commercialization; pollutant.

1. INTRODUCTION

Industrialization, population growth and globalization are accompanied with the production of large scale pollutants leading to irreparable damage to earth. The pollutants produced are either organic or inorganic in nature. Various physical and chemical processes

**Corresponding author: E-mail: nidhi.gupta@jiit.ac.in, nidhi112000@yahoo.com;*

are used for their remediation but they don't prove as promising technologies as they involve various additional risks incorporating them. Since, the growth of pollution is at alarming rate there are need of bio-based eco-friendly treatments or commonly known as "Bioremediation" which are environment friendly, cost-effective and provide more promising future. Bioremediation techniques involve lowering the pollutants level either by involving enzymatic alteration to comparative nontoxic compounds or by intracellular accumulation. Varieties of microbes and their products are reported for degradation and detoxification of such pollutants. With the help of upcoming technologies they are widely used onsite or offsite, in forms of pure microbial strains or in microbial consortia.

Disturbing ecosystems by various series of courses such as releasing large amount of pollutants in the atmosphere, acid rains, oil spills etc., proves as a man-made cause of global warming. Remediation of contaminated sites is attained generally by conventional physicochemical methods that helps in removing pollutants but during this process basically transfer the contaminant from one to other form in the environment. During the process they produce toxic by-products which in turn simply add on the pollutant levels as they can't be completely cleared off the ecosystem. Here biologically derived alternatives prove as excellent substitutes among which are "Biosurfactant" or "biologically derived surfactants".

Surface active molecules (bio + surfactants) are amphiphilic molecules i.e. they have both hydrophilic and hydrophobic moieties. The hydrophilic region forms the "head" of the molecule classifying it to cationic, anionic, zwitter-ionic and non-ionic forms while the hydrophobic moiety of surfactant forms a "tail" consisting of long hydrocarbon chain. This diverse structure offers variety of properties such as lowering of surface or interfacial tension between various liquids, increasing contact area of insoluble compounds (e.g. Hydrocarbons) improving their bioavailability & mobility thus is helping in biodegradation of such compounds. Also, they have ability to form micro-emulsions & micelles making them industrially useful. Biosurfactants thus plays a crucial role in bioremediation over their synthetic counterparts in environment friendly way with relative low toxicity, simple chemical structure, high biodegradability and applications in larger domains by being highly selective & specific and working under variable range of pH, salinity and temperature. Biosurfactants are generally classified as low molecular weight molecules & high molecular weight molecules. The major classes of low molecular weight biosurfactants include glycolipids, phospholipids and lipopeptides which help in lowering surface and interfacial tensions while higher molecular weight biosurfactants include particulate and polymeric surfactants which are effective in providing stability by forming emulsions. Currently, majorly biosurfactants are used in petroleum industry for clearing oil spills, cleaning of storage tanks, microbial enhanced oil recovery etc.

Since the chemically synthesized surfactants have a major drawback of toxicity, effectiveness and environment compatibility, the exploration of surfactants from natural world is currently being explored. Biosurfactants are diverse group of surface active molecules which are produced by microorganisms. Their ability of reducing surface tension with high specificity, low toxicity, biodegradability and environment friendly nature, lead to a keen interest on these microbial products as alternatives to chemical surfactants. Biosurfactants are even beginning to acquire a status as potential performance for their multiple roles in degradation [1], enhanced oil recovery [2], bioremediation [3].

Many microorganisms such as bacteria and fungi are reported to produce biosurfactants especially *Acinetobacter* spp., *Bacillus* spp., *Candida* spp. and *Pseudomonas* spp. [4]. Their vast structural diversity, specificity towards broad substrate attracts them for variety of industrial applications [4,5,6]. In past two decades there are numerous reports supporting biosurfactant mediated bioremediation. Microorganisms have the genes for the degradation of these compounds but the major bottleneck in the degradation is their poor bioavailability. The chief interest is attracted because of their property of increasing bioavailability due to enhanced solubilization of toxic pollutants such as Poly aromatic hydrocarbons (PAHs). The main proposed mechanism of action of these biosurfactants facilitating biodegradation includes processes above and below critical micelle concentration (CMC). Process above the critical micelle concentration involves solubilization where the pollutants are separated from the contaminated soil and is attached to hydrophobic part of surfactant micelles. At concentration below the critical micelle concentration or CMC, individual

units of surfactant molecules increases the contact angle between the pollutant and soil particles thus, promoting the separation of pollutant from soil particles and finally displaces it from soil. The modern approach lies using the combination of surfactants where different types of surfactants are intermixed to attain wide-ranging properties which can't be attained by using single biosurfactant alone. Reports show the using of cationic and anionic biosurfactants not only facilitate solubilization of the pollutant but also increases the efficacy of microorganism by increase of bioavailability of pollutant [7,8].

1.1 Merits of Biosurfactants

Biosurfactants are attracting so much attention due to its following properties and at the same time has several limitations that restricts its use at a commercial scale

- Biodegradability: Biosurfactants are easily degraded by various organisms and hence don't possess any risk of pollution in future
- Low toxicity: Biosurfactants being biologically derived are comparatively very less toxic than their chemical counterparts
- Biocompatibility: Biosurfactants are specific in action and they can be used simultaneously with other chemical compounds without hindering their process
- Availability of raw material: Biosurfactants can be generally produced using cheap raw materials
- Acceptable production economics: Biosurfactants when needed in bulk production, can be produced by cheap industrial wastes and by-products
- Use in environmental remediation: Biosurfactants have been reported for numerous processes in environmental remediation such as oil spill degradation, detoxification of certain pollutants and their further biodegradation
- Specificity: Biosurfactants are molecules with specific highly functional groups which are generally highly specific in action. This property makes them highly useful in pollution control, emulsification and de-emulsification of industrial compounds, etc.

1.2 Demerits of Biosurfactants

- Large scale productions of biosurfactants are currently very expensive. This problem can be overcome by co-production of biosurfactants using industrial by-products simultaneously [4,5].
- Purification of biosurfactants is a major hurdle currently. Biosurfactants are required in pure form in pharmaceutical, cosmetic and food industries. For purification there is requirement of additional downstream processes which simply adds up the manufacturing costs [9].
- Production yields are comparatively low. There is a current requirement for overproducing strains to increase the productivity [10].
- Increased biosurfactant productions lead to foam formation and hence diluted media is to be applied [9].

2. CLASSIFICATION OF BIO-SURFACTANTS

Biosurfactants are classified chiefly based on their chemical composition. They are amphiphilic in nature including a hydrophobic moiety consisting of saturated or unsaturated fatty acids and hydrophilic moiety which can range from mono-, di- or polysaccharides or cationic/anionic peptides [11]. Based on these moieties biosurfactants are broadly classified as (i) glycolipids, (ii) lipopeptides (e.g. Surfactin, Liposan), (iii) Fatty acids & phospholipids (e.g. corynomycolicacid), (iv) polymeric surfactants (e.g. Emulsan, Liposan) and (v) particulate biosurfactants (vesicles, whole cells). They are also categorized as higher molecular weight surfactants (HMW) constituted by polysaccharides, lipoproteins & lipopolysaccharides and Lower Molecular Weight Surfactant (LMW) constituted by various classes of glycolipids. HMW biosurfactants help in stabilizing oil-in-water emulsions whereas LMW surfactants help in efficiently lowering surface and interfacial tensions [12]

2.1 Glycolipids

Glycolipids (e.g. rhamnolipids, sophorolipids, trehalose lipids, Mannosylerythritol and Cellobiose Lipids) have a carbohydrate moiety with a long chain of aliphatic acids or its derivatives. They are of different subtypes: Rhamnolipids, Trehalolipids, Sophorolipids, Mannosylerythritol and Cellobiose Lipids based on their chemical composition [4].

2.1.1 Rhamnolipids

Among these the Rhamnolipids are the best studied and industrially important class of biosurfactants mostly obtained from *Pseudomonas* sp [13,14,15,16]. Rhamnolipid are of two types R-1 and R-2 with R-1 containing two rhamnose units linked to two â-hydroxydecanoic acid units by a glycosidic linkage. R-2 is similar in structure as R-1 except that it contains only one rhamnose unit. Furthermore, two more types of Rhamnolipids are reported which contain one â-hydroxydecanoyl moiety with one or two rhamnose units, but may be considered as degradation derivatives of types R-1 & R-2 [17]. Rhamnolipids show antiviral and antifungal properties along with bactericidal properties towards gram positive bacteria. Since they have effective detergent properties, they are being explored rapidly to be produced commercially as soil remediation agents. Thermophillic non-pathogenic bacteria belonging to genera *Thermus* and *Meiothermus* have wide range of Rhamnolipid molecular arrangements where mono- & di- Rhamnolipid homologues one or two fatty acids. *Myxococcus sp*. is also reported to produce two unusual Rhamnolipids, Myxotyrosides A and B where rhamnose unit is linked to tyrosine and C16 fatty acid.

2.1.2 Trehalolipids

Trehalolipid or trehalose lipids are type of glycolipid biosurfactants which contain trehalose sugar as hydrophilic moiety where two units of glucose are linked by glycosidic linkage. They are lower molecular weight biosurfactants efficient in lowering surface and interfacial tensions. These are reported mainly from gram positive *Actinomyceties* bacteria including genera of *Arthrobacter, Brevibacteria, Corynebacterium, Gordonia, Micrococcus, Mycobacterium, Nocardia, Rhodococcus* etc. [18]. Of these trehalose lipids reported, one of the best known is 'cord factor' found in cell wall of *M. tuberculosis* comprising of trehalosedimycolates. The complexity and variability arise due to different chain length, cyclopropane rings and various oxygenated functional groups leads to roughly 500 distinct molecular species making this genus highly pathogenic. *Rhodococcus erythropolis* produce different trehalolipids, trehalose-6-monocorynomycolates and trehalose-6,6'-dicorynomycolates. The only trehalolipid reported till date from animal source is 'maradolipids' which is present in the larva of *Caenorhabditis elegans* where the trehalose is esterified to two C15 –C19 fatty acids.

2.1.3 Sophorolipids

This class includes extracellular glycolipids reported to be produced mainly by fungal species including *Candida apicola, Candida bombicola, Candida batistae, Rhodotorula bogoriensis and Wickerhaminella domercqiae* [18]. Their general structure includes a sophorose sugar dimer linked to hydroxyl group of a C18 saturated or monoenoic fatty acid. The terminal fatty acid carboxyl group can in lactonic form (where 6 & 6' hydroxyl groups are acetylated) or in hydrolyzed anionic form [19]. Sophorolipid biosurfactants are reported to reduce the surface and interfacial tensions but have poor emulsification activity. Although the clear biological role of sophorolipid is not known in yeast species but it is believed that they serve as extracellular carbon storage along with providing defense against competing microorganisms.

2.1.4 Mannosylerythritol lipids and cellobiose lipids

Mannosylerythritol lipids are chemically composed either of 4-O-β-D-mannopyranosylerythritol or 1-O-β-D-mannopyranosylerythritol as hydrophilic head

Table 1. Classification of biosurfactants

Biosurfactant class	Microbial source
Glycolipids	
Rhamnolipids	*Pseudomonas* sp., *P. aeruginosa,*
Trehalolipids	*R. erythropolis, Mycobacterium* sp.
Sophrolipids	*T. bombicola, T. apicola, T. apicola*
Cellobiolipids	*U. zeae, U. maydis*
Lipopeptides and lipoproteins	*B. licheniformis, B. subtilis, S. marcescens, Azospirillum, Azotobacter*
Fatty acids and neutral lipids phospholipids	*P. spiculisporum*
Polymeric biosurfactants	*S. cerevisiae, Acinetobacter* sp.
Particulate biosurfactants	*Acinetobacter* sp., *Pseudomonas aeruginosa, Pseudomonas marginalis*

group linked to fatty acids and are found in various fungal species. *Pseudozyma antarctica* is reported to produce higher yields of extracellular mannosylerythritol lipids. However, these lipids were first reported to be found in *Ustilago maydis* and hence named 'ustilipids'. Here the hydroxyl group of both mannose residue are esterified, 2-hydroxyl group with a c2-c8 fatty acid and 3-hydroxyl group with a c12 – c20 fatty acid. Interestingly, *Ustilago maydis* also has different glycolipid called cellobiose lipid or ustilagic acid in which cellobiose disaccharide is linked to hydroxyl group of long chain fatty acid di- or trihydroxyhexadecanoic acid with a glycosidic linkage. *Pseudozyma flocculosa* which is reported fungal biocontrol agent, is also reported to have flocculosin (2-(2',4'-diacetoxy-5'-carboxypentanoyl) octadecylcellobioside) which is actually responsible for its antifungal activities.

2.2 Lipopeptides

Lipopeptides are generally isolated from *Bacillus* and *Pseudomonas* strains and are cyclic in structure. Their main constituent is hydrophilic peptides which are usually 7 and 10 amino acid long linked to hydrophobic fatty acid structures. Among them Surfactin is most studied heptapeptide lipopeptides attached to 3-hydroxy fatty acid within a lactone ring [20]. In both air-water interface or in aqueous solution surfactin folds into a beta-sheet structure hence resembling a horse saddle [21]. Also, due to presence of two negative charges on aspartate and glutamate residues it has unique ability to bind with variety of metal ions such as magnesium, cobalt, manganese etc. [22]. Surfactin is reported as a powerful biosurfactant reducing surface tension to 27 mN/m even in low concentration (0.005%). The surface active properties and solubility of this biosurfactant is totally dependent on the orientation of its residues. Till now, major application of surfactin is seen in the field of biochemical research such as blood coagulation inhibitor, preventing protein denaturation etc. [23]. In a recent study, Ojeda et al. [24] reported production of lipopeptide biosurfactant from *A. brasilense* and *A. lipoferum*.

2.3 Phospholipids and Fatty Acids

Various bacterial and fungal species when growing on n-alkanes are reported to produce large quantities of fatty acid and phospholipid biosurfactants. *Acinetobacter sp.* HON when growing on hexadecane has reported to produce phosphatidylethanolamine rich vesicles [25]. Also, phosphatidylethanolamine is produced by *Rhodococcus erythropolis* which lowers the interfacial tension between water and hexadecane when grown on n-alkanes [26]. Certain *Pseudomonas* strains produce viscosin which is a peptidolipid proficient of lowering the surface tension [27].

Fatty acids that are produced as a result of microbial oxidation from alkane generally have surface active ability [28]. Microbes produce straight chain and complex fatty acids containing alkyl and hydroxyl branches. One such example is Corynomucolic acid which is having surfactant activity [26]. Most active saturated fatty acids for lowering interfacial and surface tensions are in range of C12-C14 [12]. Basically, the hydrophillic-lipophillic balance (HLB) of fatty acids is related to hydrocarbon chain length.

2.4 Polymeric Surfactants

Polymeric surfactants which are best studied include emulsan, liposan and other protein-polysaccharides complexes. Emulsan which is generally reported to be produced from *Acinetobacter calcoaceticus* [29] consist of trisaccharide backbone of D-galactominouronic acid, D-galactosamine and deoxyaminohexose, to which C10-C22 chain length fatty acids are linked via amide and ester bonds. It has been proved as a very effective emulsifying agent even at low concentrations (0.001% to 0.01%) and powerful emulsion stabilizer [30]. Liposan on the other hand is an extracellular emulsifier which water soluble and reported to be synthesized by *Candida lipolytica*. It majorly contains carbohydrate (~83%) and protein (~17%) [31].

2.5 Particulate Biosurfactants

Gram negative bacterial cell wall contains proteins, phospholipid, lipopolysaccharide (LPS) and a growing cell secretes these complexes in the form of vesicles. These spherical extracellular membrane vesicles form micro-emulsion manifesting the emulsifying activity of broad range of aliphatic and aromatic hydrocarbon, thus, easing the microbial uptake. Vesicles of *Acinetobacter sp*. strain H01-N is composed of LPS, proteins and phospholipids [32]. Similarly, surface active particulate polymeric biosurfactant PM-factor by *Pseudomonas marginalis* PD 14-B is reported for affecting PAH degradation by bacterial cells and prevent flocculation [33].

3. ROLE OF BIOSURFACTANTS

Hydrophobic pollutants, due to their insoluble nature are difficult to be degraded compounds and biosurfactant facilitate their degradation. Mode of action of biosurfactant vary depending on both the bacterial species producing the biosurfactant and the property of the biosurfactant but broadly biosurfactant facilitate the access of hydrocarbon either by solubilizing the substrate and thus increasing the substrate bioavailability for microorganisms or by increasing the cell hydrophobicity and thus allowing bacterial cells to associate more easily with these hydrophobic substrates [34]. Bioavailability is increased either by emulsification or micellar solubilization. During emulsification, interfacial tension decreases resulting in the formation of small droplets of the NAPL particles and the presence of these particles in the aqueous layer where as solubilization results in the self-aggregation of the biosurfactant and partitioning of the pollutant in its hydrophobic core.

Biosurfactant's effectiveness depends upon three major features: lowering of surface tension which is determined by CMCs, stabilizing emulsions and studying hydrophilic lipophilic balance (HLB). Activity of a biosurfactant is greatly influenced by the concentration of surface-active compounds, up until the critical micelle concentration (CMC) is attained. Critical micelle concentration (CMC) could be defined as the concentration of these surface active compounds exceeding which micelles form and all extra surfactant molecules added to the system go to micelles. CMC determines the efficiency of a particular surfactant. The biosurfactant which have low CMC means that they are required in fewer amounts for lowering the surface tension and hence are more efficient.

Another feature is hydrophilic lipophilic balance (HLB) which determines whether biosurfactant is water in oil or oil in water based emulsion. Micelle formation plays an important role in microemulsion formations which are liquid and stable mixtures of oil and water separated by aggregates or layer of biosurfactants. These microemulsions are characterized as "direct microemulsions" where oil is dispersed in water while on other hand in "reversed microemulsions" water is dispersed in oil [4]. Biosurfactants of higher molecular weights which are generally termed as "bio-emulsifiers" show remarkable ability to stabilize the emulsions of hydrocarbons and water which results in increase in contact area for bacterial degradation. On the other hand, biosurfactants with lower molecular weight functions differently. The hydrophobic hydrocarbon pollutant partitions with biosurfactant micelle above the critical micelle concentration (CMC), thus increasing the bioavailability of pollutant for microbe to act upon it. Low molecular weight surfactants like glycolipid are more effective in lowering the interfacial tension where as high molecular weight surfactants are effective stabilizer of oil in water emulsion. It is important to note that biosurfactants while being used in environmental remediation can not only interact with abiotic pollutant particles but can also interact with microorganism itself. Though the entire function of biosurfactants in bacteria is not fully understood but its involvement in various physic-chemical processes postulates another mechanism of hydrocarbon intake which includes breaking down of insoluble substrates majorly hydrocarbons along with changing cell surface hydrophobicity by exposing the different parts of cell bound biosurfactants [35,36,37]. Thus, the effect of biosurfactant on the degradation rate is the result of the unique interactions between microbial cells, surfactant molecules and hydrophobic substrates. On one hand where the pollutant contaminant is degraded with mechanism of emulsification and micelle formation, certain contaminants are attached to bacterial cell surface ultimately leading to change in cell membrane composition and its hydrophobicity. Zhao et al. [38] reported the effect of rhamnolipid on *Pseudomonas aeruginosa* ATCC9027 and *Bacillus subtilis* BUM. It was observed it reduced the CSH of *Bacillus* BUM but significantly increased the CSH and enhanced the biodegradation for the *Pseudomonas* strain. Sotirova et al. [39] studied the effect of rhamnolipid produced by *Pseudomonas* sp PS – 17 on cell surface structure and reported that the biosurfactant below CMC affected the OMP composition of the *Pseudomonas* cell and above CMC resulted in the reduction of the total LPS component of 22% resulting in the increase in the hydrophobicity of the cell to 31% adherence. It must be also noted that this mechanism can on one hand increase the biodegradation of certain poorly soluble pollutants while on other hand hinder the degradation process in general.

Although the chemical surfactants and biosurfactants are both known to increase the rate of degradation but their mode of action can

be different. Mohanty et al. [40] studied the effect of biosurfactant JBR-515 and a chemical surfactant Triton X-100 on the degradation of NAPLs through *B. multivorans* and demonstrated that biosurfactant enhanced the rate of degradation by increasing the bioavailability due to micellar solubilization and not emulsification whereas Triton X-100 improved the degradation rate by emulsifying and supported direct interfacial uptake due to changes in the cell hydrophobicity. Microorganisms belonging to the group of *Pseudomonas* or *Torulopsis* sp. produce biosurfactant like Rhamnolipid and sophorolipid respectively and result in emulsification where as *Rhododcoccus sp., Mycobacterium sp*., *Arthrobacter sp*. synthesize cell bound lipopolysaccharide and trehalose [41].

Biosurfactants are generally known to enhance degradation of contaminants but at the same time there are reports showing no effect or even negative impact on the removal of contaminants. The inability of the biosurfactant to increase the degradation rate has been attributed to surfactant toxicity, substrate toxicity due to increase in the availability of the substrate, unavailability of the substrate due to its entrapment into micelle, utilization of biosurfactant as a growth substrate and interference with membrane uptake process by formation of barrier between the microbe and organic molecules. The inhibition is similar to that of chemical surfactants as seen in Witconol SN70 which is an alcoholic ethoxylate surfactant, reducing the degradation rate of phenanthrene and hexadecane [42]. Similar inhibitory activity was seen by other synthetic surfactants such as Sodium dodecyl sulfonate, tetra decyl trimethyl ammonium bromide, Citrikleen and Tween 20 at concentrations more than or equal to CMCs [7].

4. GENETIC REGULATION OF BIOSURFACTANTS

4.1 Genetic Regulation of Rhamnolipid Biosurfactants

Rhamnolipids are most studied glycolipid biosurfactants composing one or two L-rhamnose with mono or dimer of β-hydroxy fatty acid. Rhamnolipid biosynthesis and gene regulation can be understood in three simple steps: Biosynthesis of (dTDP)-L-rhamnose via *rmlBDAC* operon, rhamnolipid biosynthesis via *rhlAB* operon and role of quorum sensing and other factors in gene regulation of *rhlAB* operon.

4.1.1 Biosynthesis of (dTDP)-L-rhamnose

Rhamnose is a hexose sugar is widely found in bacteria but is absent in humans. Synthesis of deoxy-thymidine di-phospho-L-rhamnose involves four sequential steps involving the gene products of *rmlBDAC* operon. The first enzyme involved is RmlA EC2.7.7.24 (glucose-1-phosphate thymidylyltransferase) which is responsible for transfer of TMP (thymidyl-monophosphate) to glucose-1-phosphate generating dTDP-glucose. dTDP-glucose is oxidized C4 hydroxyl group of D-glucose immediately followed by dehydration by second enzyme RmlB EC 4.2.1.46 (dTDP-D-glucose 4,6-dehydratase) leading to formation of dTDP-4-keto-6-deoxy-D-glucose [43]. Next step involves double epimerization of dTDP-4-keto-6-deoxy-D-glucose at C3 and C5 positions by RmlC EC 5.1.3.13 (dTDP-4-keto-6-deoxy-D-glucose 3,5 epimerase) forming dTDP-4-keto-6-deoxy-L-mannose which is finally reduced at C4 keto group by RmlD EC 1.1.1.133 dTDP-4-keto-6-deoxy-L-mannose reductase) forming dTDP- L-rhamnose [44].

4.1.2 Rhamnolipid biosynthesis

dTDP-L-rhamnose synthesized via *rmlBDAC* pathway can allosterically inhibit enzyme RmlA and its own synthesis [45] and additionally it is also channeled to other extracellular structures consisting L-rhamnose. RhlA enzyme is involved in coupling of rhamnose and fatty acid moiety which is generated from FAS-II cycle to form free 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA). RhlC and RhlB rhanmosyltransferases further catalyze the transfer of dTDP-L-rhamnose to previously generated monorhamnolipid to yield dirhamnolipid or to other HAA respectively [46]. Recent studies shows involvement of RhlA in fatty acid synthesis cycle by directly competing with FabA and FabI for β-hydroxydecanoyl-ACP intermediate leading to diversion of FAS cycle providing substrate for RhlAB enzyme for rhamnolipid molecule synthesis [47,48].

4.1.3 Role of quorum sensing systems and other factors

Quorum sensing is observed in bacteria as a method of communication and coordination by secretion and detection of certain signal molecules (autoinducers) within a population. The major components of quorum sensing system involve a signal receptor or regulatory protein, QS signal synthase and signal molecule [49]. One such QS system reported in

P. aeruginosa is *rhl* system including *rhl*R and *rhl*I which are responsible for regulation of *rhl*AB gene expression. RhlI proteins act as autoinducers (N-acylhomoserine lactones, BHL, C4-HSL or PAI-2) and also influence RhlR regulator protein. Other quorum sensing system is coded by *las*R and *las*I influencing rhamnolipid synthesis. The *las* system, both upregulates and downregulates *rhl* system which is in turn responsible for rhamnolipid biosynthesis by producing N-oxododecanoyl homoserine lactone (OdDHL 3OC12-HSL or PAI-1) [50,51]. Also, *rhl*AB transcription also involves σ 54 factor or RpoN where *rhl* QS system is activated by RpoN in high cell densities [51].

4.2 Genetic Regulation of Surfactin Biosurfactants

Surfactin is a lipopeptide biosurfactant and its biosynthesis is controlled by non-ribosomal peptide synthetase complex called as surfactin synthetase. Surfactin synthetase consists of three protein subunits known as SrfA, SrfB or ComA and SrfC. SrfA operon consist of four ORFs- SrfAA, SrfAB, SrfAC and SrfAD in which SrfAA, SrfAB and SrfAC is required for synthesis of surfactin. For activation of surfactin synthetase, phosphopantetheinyl transferase is required which is encoded by *sfp* gene. Another uncharacterized gene also plays an important role producing acyl transferase for transferring hydroxyl fatty acid group to SrfAA [52]. ComX which is a signal peptide pheromone is activated by gene product of *com*Q, controls the signal transduction mechanism via quorum sensing and srfA expression interacting with ComP and ComA. ComA which is a response regulator gets activated upon phosphorylation at histidine residue by ComP or Histidine Protein kinase and results in stimulation of *srf* operon. Other activators of *srf* transcription machinery include extracellular pheromone CSF, ComR and SinR influencing *srf* expression [53,54,55,56].

4.3 Genetic Regulation of Mannosylerythritol Biosurfactants

Mannosylerythritol lipids or MELs are generally produced extracellularly by variety of fungi such as *Ustilago maydis, Pseudozyma* sp. etc. MELs are classified on the basis of acetyl groups present on mannosyl moiety at R4 and R6 positions – MEL-A (fully acetylated) , MEL-B, MEL-C and MEL-D (completely deacetylated) [57]. Synthesis of MELs is majorly studied in *Ustilago maydis* which comprises gene cluster of five genes- *emt1, mat1, mac1, mac2* and *mmf.* Gene product of *emt1* is glucosyltransferase which carries mannosylation of meso-erythritol at C4 position. Specific acylation is carried by acyltransferases transcribed by *mac1* and *mac2* at R2 and R3 positions respectively. Thereafter, acetyltransferase encoded by *mat1* transfers acetyl group at R4 and R6 positions of mannose. Secretions of these MELs are dependent on fifth gene *mmf1* encoding family of MEL transporters. Both dual acylation and *mmf1* MEL protein transporters are required for extracellular secretions of MELs [58].

5. COMMERCIALIZATION OF BIO-SURFACTANT

Surfactants are in great demands due to their high global applications and utilization. In a recent survey by Van Bogaert et al. [59], the global production of surfactants crossed 10 million tons per year. Despite such a huge demand for surfactants and biosurfactants being more environmental friendly, industrial production of biosurfactants has not yet started. The costs of biosurfactant production range around 3-5 $/lb compared to the costs of synthetic surfactants which is around 1$/lb [5]. Surfactin, a biosurfactant manufactured by Sigma (98% pure) costs around $150 for 10 mg vial while the estimate from Ron and Rosenberg's work shows, Emulsan containing broth produced by RAG-1 costs around $50/ kg hence denoting about increased costs due to extraction, purification and other downstream processes. Uptill now only two companies viz Jeneil Biotech Inc. and Rhamnolipid companies Inc. have been able to market the production of biosurfactant profitably.

For carrying out large scale production of any metabolite main parameters that decide the economy of the process includes the selection of raw material, carrying out the production, and extraction and purification process in an economical way. Keeping these points in mind, lot of research is going on for economical and sustainable production of biosurfactant. Three basic strategies adopted include using:

i) Alternative cheap raw material
ii) Better bioprocess techniques for production, extraction and purification
iii) Recombinant strains for overproduction [6,60,61]

Raw material contributes up to 75% of the total selling cost of the byproduct [62]. Thus the first step to decrease the cost of biosurfactant is to use waste material for its production. Variety of

agro-industrial, distillery wastes, starchy substances and oil wastes are reported as cheap substrates for biosurfactant production [61]. First attempt to use waste material (olive oil mill effluent) for production was demonstrated by Mercade et al. [63] using *Pseudomonas aeruginosa* 47T2 and was the first one to propose the utilization of industrial effluent as substrate for biosurfactant production albeit with decreased enzymatic activities like surface tension, emulsification and interfacial tension. The properties of the biosurfactant are dependent on composition of raw material and more specifically on the ratio of carbon and nitrogen. They further observed that biosurfactant produced by the same microorganism using olive oil mill effluents from different industries had different enzymatic properties. Since then several groups have proposed the use of alternative substrates for biosurfactant production. Benincasa et al. [64] reported the rhamnolipid production (concentration range of about 15.9 g/l) from *Pseudomonas aeruginosa* LBI. Same strain was also used to produce Rhamnolipid biosurfactant from soybean, corn, babassu, palm and corn oil refinery wastes as substrates where the production yield of 75% was achieved [65]. *Pseudomonas aeruginosa* A41 is reported to produce biosurfactant with different yields in different type of carbon sources as seen in olive oil (6.58 g/l), coconut oil (2.93 g/l) and palm oil (2.91 g/l) [66]. Dairy industries producing various marketable products generate large amounts of waste by-products. Most common among them is whey which is rich in lactose sugar providing perfect carbon source for microbes. Whey is a nitrogen source and not carbon source [67] cultivated *Candida* sp. on oil refinery wastes (which oil refinery) such as soap stock or fatty acids to produce biosurfactant. Soybean associated wastes such as soy molasses were used to produce sophorolipid biosurfactants using *Candida bombicola* [68]. Production of various types of biosurfactants such as sophorolipids, Rhamnolipids and mannosylerythritol lipids are reported to be produced from sunflower, soybean, corn, rapseed and babassu oils [69,70,71,72,73]. Reports of Daverey et al. [62] shows sophorolipid (maximum yield 34 g/l) production in mixed medium of deproteinized whey, glucose, yeast extract and oleic acid using *Candida bombicola*. In some developing countries such as India, major by-product obtained from sugarcane and beet root industries is molasses. Molasses are not only rich in sugar contents (sucrose 48-56%) but also have vitamins, proteins and several other compounds proving a cheap substrate for biosurfactant production. Desai and Patel [91] reported production of Rhamnolipid biosurfactants using molasses and corn steep liquor as carbon and nitrogen sources by *Pseudomonas aeruginosa* GS3. Ghurye et al. [74] in 1994 first used stirred batch bioreactors for producing biosurfactants using molasses as substrate. In order to maximize biosurfactant production using cheap substrates, a mixture of two or more types of substrates can be used. One such effort includes works of Rodrigues et al. [75] where they used whey and molasses as substrates producing 1.2-1.5 times increase in biosurfactant production and 75% cost reduction using *Lactococcus lactis* 53 and *Streptococcus thermophiles*. Lignocellulose which contains cellulose, hemicellulose and lignin can also be used as substrate for biosurfactant production using species of *Lactobacillus* [76,77,78]. Starch which is a major agriculture residue is produced in abundance and large solid residues are dumped in waste waters. Reports show various biological starch rich wastes are suitable for biosurfactant production. Bala and Fox [79] showed production of biosurfactant using potato associated residues with high surface activity [79]. Wang et al. (2008) used solid state fermentation for production of biosurfactant from sweet potato and soybean residues using *Bacillus subtilis* B6-1.

While the major focus earlier relied upon production of biosurfactants on various substrates, the recent approach emphases on coproduction of biosurfactants along with other metabolites. One such remarkable approach was shown by Ramnani et al. [80]. They coproduced commercially important enzyme proteases and biosurfactants using cornstarch and soy as carbon and nitrogen sources. Although, the main idea behind coproduction of both the enzymes was to replace the commercial surfactant used in textile industry. However, more than 2 fold increase in both enzyme and biosurfactant production was observed, suggesting cost effective production of biosurfactant. In a different approach PHA (polyhydroxyalkanotes) were produced either simultaneously or by using wastes after biosurfactant production. Füchtenbusch et al. [81] used the remaining oil after PHA production for producing rhamnolipid biosurfactants. Marsudi et al. [82] showed by using palm oil PHA and rhamnolipids can be simultaneously produced using *Pseudomonas aeruginosa* IFO3924 where fatty acids and

glycerol where used as carbon sources for PHA and rhamnolipid productions respectively.

The second reason due to which biosurfactants are not been able to compete with synthetic surfactants is the cost associated with production, recovery and purification. Biosurfactants are secondary metabolites and hence their production varies greatly on process parameters including media constituents such as nitrogen and/or carbon sources; elements such as iron, manganese; growth factors and environmental factors such as temperature, dissolved oxygen, pH, agitation rate etc. Also, it has been seen the ratios of various components such as C:N, C:Fe, C:P etc. also affect biosurfactant production by microorganisms. The conventional strategy for process optimization involved varying single variable at a time and obtaining the corresponding results and henceforth evaluating the results. Since this method is time consuming, tedious, exhausting and sometimes become impractical carrying out vast number of experiments. Thus, an alternative approach is developed using statistical methods based upon Response surface methodology (RSM) is being used which uses regression and factorial designs for evaluating multifactor experiments. To achieve optimized process controls various statistical techniques such as Fractional Factorial Designs (FFD), Central Composite Designs (CCD), Monte Carlo Optimization, Plackett–Burman statistical experimental designs etc. are used for designing experiments, developing models and evaluating effects of various factors at the same time [83]. Sen et al. [84] studied the effect of four different variables viz. pH, temp, agitation and aeration on surfactin production using surface response methodology. The optimum conditions for maximum surfactin conc. were reported as pH 6.7, temp 37.4 C, agitation 140 rpm and aeration 0.75 vvm. SRM was also used for optimizing culture conditions for the maximum production of rhamnolipid from *Pseudomonas*. But without efficient down streaming processes and purification techniques commercialization of biosurfactants can't be achieved. Also, downstream processing covers the major cost in production of biosurfactants in large scale thus becoming the major bottleneck in commercialization of biosurfactants. Conventional strategies include ammonium sulfate precipitation, solvent extraction, acid precipitation, centrifugation etc. The application of biosurfactants is highly dependent upon its purity with efficient recovery. Crude biosurfactants can be applied for environmental remediation while pharmaceutical or cosmetic industries require highly pure biosurfactants. Foam produced during biosurfactant production can directly be applied on contaminated soils. Mulligan & Wang [85] showed effective usage of rhamnolipid foam, enhancing remediation of heavy metals in contaminated soils. With time certain advancements in technologies lead to development of certain techniques such as ultrafiltration, ion exchange chromatography, ultra-filtration, adsorption- desorption on polystyrene resins or activated carbon etc. Using such methods resulted in more efficient downstream processes with better purity in the products which ultimately increases the costs. To reduce toxicity and wider environmental applications organic solvents such as acetone, chloroform are effectively replaced by using methyl tertiary-butyl ether (MTBE) in recovery of biosurfactants. Currently, there is a great demand in having more proficient recovery techniques with involvement of multi-step recovery processes to get varied degree of purities [86,87].

The advancement of field of biotechnology totally relies on altering the genomic machinery of an organism. By using recombinant technology or producing a mutant variety of a strain one can exploit the commercial advantage of particular gene of industrial application. Thus using efficient bioprocess techniques, cheap substrate and raw materials and a hyperproducing strain biosurfactant production can be achieved in a profitable approach. Apart from natural biosurfactant producers various mutant and recombinant strains are developed using radiations, chemical mutagens such as N-methyl-N'nitro-N-nitrosoguanidine, transposons and selective screening on ionic detergents. Development of mutant *Bacillus subtilis* SD901 using N-methy-N'nitro-N-nitrosoguanidine is reported to produce highest amount of Surfactin ranging from 8-50g/L (Yoenda et al. US patent no. 7,011,969). In other studies, different recombinant of *Pseudomonas aeruginosa* was developed by incorporating *E. coli lacZY* genes which naturally cannot utilize lactose as substrate [88]. Similarly, various recombinant strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were developed which produces *Pseudomonas aeruginosa* rhamnolipid without any toxicity and have wider biotechnological applications without any risks and hazards what was the strategy that was used [89]. Also, with such a rapid pace in

development and advancement of various biotechnological methods we can expect even better hyperproducer strains for biosurfactant production.

Table 2. Substrates used by microorganisms producing biosurfactants

Microorganisms	Substrate	Biosurfactant class	Scientist group	Year	Reference
P. aeruginosa 47T2	Olive oil mill effluent	Rhamnolipid	Mercade et al.	1993	[90]
P. aeruginosa GS3	Molasses and corn steep	Rhamnolipid	Patel et al.	1997	[91]
P. aeruginosa EBN-8	Molasses	Rhamnolipid	Raza et al.	1997	[92]
P. aeruginosa UW-1	Mixture of vegetable oil	Rhamnolipid	Sim et al.	1997	[93]
Tsukamurella sp. DSM44370	Sunflower oil	Glycolipid	Vollbrecht et al.	1999	[94]
P. aeruginosa UG2	Corn oil	Rhamnolipid	Mata-Sandoval et al.	1999	[95]
P. aeruginosa 47T2	Sunflower and olive oil	Rhamnolipid	Haba et al.	2000	[96]
P. aeruginosa BS2	Distillery and whey wastes		Dubey et al.	2001	[97]
P. aeruginosa DS10-129	Soybean oil, safflower oil and glycerol	Rhamnolipid	Rahman et al.	2002	[98]
P. aeruginosa LB1	Soap stock	Rhamnolipid	Benincasa et al.	2002	[64]
Pseudomonas sp. DSM 2874	Rapseed oil	Rhamnolipid	Trummler et al.	2003	[69]
Penicillium citrinum	Olive oil	Glycolipid	Camargo de Morais et al.	2003	[99]
Candida apicola/ *Candida antartica*	Soap stock	Glycolipid	Bednarski et al.	2004	[67]
Candida bombicola	Soy molasses	Sophorolipid	Solaiman et al.	2004	[68]
B. subtilis ATCC 6633	Commercial sugar, sugarcane juice and cane molasses, glycerol, mannitol and soybean oil		Reis et al.	2004	[100]
Pseudoxanthomonas kaohsiumgenis sp. nov strain J36	Olive oil		Chang et al.	2005	[101]
P. aeruginosa A41	Vegetable oil (oilve oil, palm oil, cocnut oil)		Thaniyavarn et al.	2006	[66]
Candida lipolytica	Groundnut oil	Lipopeptide	Ruffino et al.	2007	[102]
Pichia anomal P41	Soyabean oil	Sophorolipid	Thaniyavarn et al.	2008	[103]
P. aeruginosa LRM 10	Soybean oil, soap stock, fish oil and glycerol	Rhamnolipid	Prieto et al.	2008	[104]
Candida lipolytica	Soyabean oil		Ruffino et al.	2008	[105]
Candida sphaerica	Groundnut oil	Glycolipid	Sobrinho et al.	2008	[106]
B. subtilis BS5	Molasses	Surfactin	Abdel-Marvgoud et al.	2008	[107]
P. fluorescens Migula 1895 DSM2	Olive oil	Rhamnolipid	Abouseoud et al.	2008	[108]
P. alealigenes	Palm oil		Oliveira et al.	2009	[109]
Candida bombicola	Sugarcane molasses and soybean/sunflower/olive oil	Sophorolipid	Daverey et al.	2009	[62]
P. aeruginosa Bs20	Soyabean oil	Rhamnolipid	Abdel-Marvgoud et al.	2009	[110]
Trichosporon montevideense CLOA 72	Sunflower oil	Glycolipid	Monteiro et al.	2009	[111]

Microorganisms	Substrate	Biosurfactant class	Scientist group	Year	Reference
P. fluorescens	Vegetable oil	Glycolipid	Stoimenoral et al.	2009	[112]
Candida bombicola	Deproteinized whey and glucose	Sophorolipid	Daverey et al.	2010	[113]
Lactobacillus delbrueckii	Peanut oil cake	Glycolipid	Thavasi et al.	2011	[114]

Certain parameters should be taken in account for biosurfactant production. These include Carbon source, Nitrogen source and other environmental factors. For growth of a microorganism, suitable carbon source is one of the most important culture condition to be taken in account. Various carbon sources such as glucose, glycerol, mannitol etc. are supplemented to culture mediums which are used as water soluble substrates while various hydrocarbons, vegetable oils etc. are also used for biosurfactant production. The type of carbon source in turn affects the composition of biosurfactant [115,116,117]. Also, various reports showed enhanced biosurfactant production once water soluble carbon source is consumed [118, 119]. In reports of Daverey et al. [62,113] sophorolipid production was obtained from *Candida bombicola* using various substrates such as sugarcane molasses, soybean and various vegetable oils, deproteinized whey along with glucose. In another study by Kosaric et al. [119], *Arthrobacter paraffineus* ATCC 19558 along with D-glucose with supplementation of hexadecane showed increased biosurfactant yield [121].

Apart from carbon source, nitrogen source is also very important for biosurfactant production. Various sources such as urea, ammonium salts and nitrate are used depending upon the microorganism used. Kosaric et al. [119] reported enhanced biosurfactant production on addition of various essential amino acids in the medium which also effects structure of biosurfactant produced. Reports show increased biosurfactant production and change in biosurfactant structural composition as soon as culture media becomes nitrogen limiting [120,121,122,123]. Various reports show increased biosurfactant production once the microorganism is subjected to nitrogen limiting conditions. Reports show enhanced biosurfactant production in *Pseudomonas aeruginosa, Candida tropicalis* etc [120,121,122].

Biosurfactant production is seen to depend of variety of other factors in culture medium. Temperature, dissolved oxygen content, pH and rate of agitation greatly effect growth of microorganism and thus in turn have a significant role in production of various metabolites such as biosurfactants. In a recent study by Ojeda et al. [124], maximum biosurfactant production was seen at 25°C by *A. lipoferum* and viscosity slightly more than water which decreased with increase in temperature. Certain studies show change in biosurfactant composition with effect of temperature [117,125]. Also, in some biosurfactants properties such as emulsification activity and lowering surface tension remain unchanged even at high temperatures and autoclaving [126]. As stated, biosurfactant production depends upon growth of microorganism. When operating in culture conditions high rate of agitation decreases growth of microorganism due to high shear stress and thus reduces the production yield of biosurfactant [127,128]. Other factors, such as oxygen content and salt concentration also play important role in biosurfactant production depending on cellular activity [129]. Generally, no considerable effect with slight decrease in CMC values is seen on biosurfactant production upto 10% salt concentration [126]. Ojeda et al. [124] showed decrease in emulsification activity with increase in NaCl concentration.

6. CONCLUSIONS

Biosurfactants are biologically derived surface active compounds which have huge potential and wide applicability in environmental remediation. Biosurfactants provide suitable alternative over their chemical counterparts for their use in oil spill remediation, cleaning of oil transportation pipelines and storage tanks, microbial enhanced oil recovery (MEOR) etc. Their advantages include biodegradability, biocompatibility, lower toxicity and high selectivity. Their usage though currently limited can undoubtedly benefit more after more advanced research on their molecular interactions and mechanisms of actions on pollutants and producer cells. Comprehensive studies are required for analyzing biosurfactants production and their natural roles for wider applications. Currently, two important problems are still associated with biosurfactants. Firstly, genetic regulations of various types of

biosurfactants are still not fully understood. Secondly, commercialization of biosurfactants and their bulk production is highly limited because of lack of efficient bioprocess techniques for their cheap market availability. This problem can be overcome by using cheap and renewable waste substrates for production of biosurfactants involving multi-step downstream processing methods which are more cost-effective and economically feasible. Also, development of various mutant and recombinant microbial strains which are adaptive and able to produce biosurfactants in varied conditions are also required.

ACKNOWLEDGEMENT

This work, in part was supported by generous financial grant received from Department of Science and Technology, Government of India (Project No. SB/YS/LS-223/2013).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhang F, She Y. Quantitative and qualitative analysis of biosurfactant by oil spreading method. Chem Eng. 2005; 1(38):14-15
2. Wang Q, Fang X, Bai B, Liang X, Schuler PJ, Goddard WA, et al. Engineering bacteria for production of rhamnolipid as an agent for enhanced oil recovery. Biotechnology and Bioengineering. 2007; 98(4):842-853
3. Nguyen TT, Youssef NH, McInerney MJ, Sabatini DA. Rhamnolipid biosurfactant mixtures for environmental remediation. Water Res. 2008;42:1735-1743.
4. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. R. 1997; 61:47-64.
5. Makkar RS, Cameotra SS, Banat IM. Advances in utilization of renewable substrates for biosurfactant production. AMB Express. 2011;1:5.
(Accessed 16 April 2016)
Available:http://www.amb-express.com/content/1/1/5
6. Kosaric N. Biosurfactants in industry. Pure Appl Chem. 1992;64:1731-1737.
7. Billingsley KA, Backus SM, Ward OP. Effect of surfactant solubilization on biodegradation of polychlorinated biphenyl congeners by Pseudomonas LB400. Appl Microbiol Biotechnol. 1999;52:255-260.
8. Mulligan CN. Recent advances in environmental applications of bio-surfactants. Current Opinions Colloid Interface Sciences. 2009;14:372-378.
9. Kosaric N. Biosurfactants in industry. Pure and Applied Chemistry. 1992;64(11):1731-1737.
10. Ron, Eliora Z, Rosenberg E. Biosurfactants and oil bioremediation. Current Opinion in Biotechnology. 2002;13(3):249-252.
11. Pacwa M, Plaza GA, Seget P, Cameotra SS. Environmental applications of biosurfactants: Recent advances. Int. J. Mol. Sci. 2011;12(1):633-654.
12. Rosenberg E, Ron EZ. High- and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology. 1999;52(2):154-162.
13. Burger MM, Glaser L, Burton RM. The enzymatic synthesis of a rhamnose-containing glycolipid by extracts of *Pseudomonas aeruginosa*. J. Biol. Chem. 1963;238:2595-2602.
14. Shreve GS, Inguva S, Gunnam S. Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by *Pseudomonas aeruginosa*. Molecular Marine Biology and Biotechnology. 1995; 4(4):331-337.
15. Arino S, Marchal R, Vandecasteele JP. Identification and production of a rhamnolipidic biosurfactant by a Pseudomonas species. Appl. Microbiol. Biotechnol. 1996;45:162-168.
16. Zhang Y, Miller RM. Effect of rhamnolipid (biosurfactant) structure on solubilization and biodegradation of n-alkanes. Applied and Environmental Microbiology. 1995;61: 2247-2251.
17. Slydatk C, Lang S, Matulovic U, Wagner F. Production of four interfacial active rhamnolipids from n-alkanes or glycerol by resting cells of Pseudomonas species DSM 2874. Zeitschrift für Naturforschung C. 1985;40(1-2):61-67.
18. Franzetti A, Tamburini E, Banat IM. Application of biological surface active compounds in remediation technologies. In: Sen R, editors. Biosurfactants, Berlin: Springer. 2010;672.
19. Van Bogaert INA, Baccile N, Nassif N, Malfatti L, Soetart W, Babonnaeu F, et al. Sophorolipids: A yeast-derived glycolipid as greener structure directing agents for

self-assembled nanomaterials. Green Chem. 2010;12:1564-1567.

20. Rosenberg E, Ron EZ. High- and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology. 1999;52(2):154-162.
21. Ishigami Y, Osman M, Nakahara H, Sano Y, Ishiguro R, Matsumoto M. Significance of β-sheet formation for micellization and surface adsorption of surfactin. Colloids and Surfaces B: Biointerfaces. 1995; 4(6):341-348.
22. Thimon L, Peypoux F, Michel G. Interactions of surfactin, a biosurfactant from *Bacillus subtilis*, with inorganic cations. Biotechnology Letters. 1992;14(8): 713-718.
23. Vollenbrioch D, Ozel M, Vater J, Kamp RM, Pauli G. Mechanism of inactivation of enveloped viruses by the biosurfactant surfactin from *Bacillus subtilis*. Biologicals. 1997;25:289-297.
24. Ojeda-Morales ME, Dominguez MD, Rivera MA, Cruz JZ. Biosurfactant production by strains of *Azospirillum* isolated from petroleum-contaminated sites. Water Air Soil Pollution. 2015; 226:400-415.
DOI: 10.1007/s11270-015-2659-0
25. Kaeppeli O, Finnerty WR. Characteristics of hexadecane partition by the growth medium of *Acinetobacter* sp. Biotechnol. Bioeng. 1980;22:495-501.
26. Kretschmer A, Bock H, Wagner F. Chemical and physical characterization of interfacial-active lipids from *Rhodococcus erythropolis* grown on n-alkanes. Appl. Environ. Microbiol. 1982;44:864-870.
27. Neu TR, Poralla K. Emulsifying agent from bacteria isolated during screening for cells with hydrophobic surfaces. Appl. Microbiol. Biotechnol. 1990;32:521-525.
28. Rehn HJ, Reiff I. Mechanisms and occurrence of microbial oxidation of long chain alkanes. Adv. Biochem. Eng. 1981;19:175-216.
29. Rosenberg E, Zuckerberg A, Rubinovitz C, Gutnick DL. Emulsifier Arthrobacter RAG-1: Isolation and emulsifying properties. Appl. Environ. Microbiol. 1979;37:402-408.
30. Belsky I, Gutnick L, Rosenberg E. Emulsifier of Arthrobacter RAG-1: Determination of emulsifier-bound fatty acids. FEBS Lett. 1979;101:175-178.
31. Cirigliano MC, Carman GM. Purification and characterization of liposan, a bioemulsifier from Candida lipolytica. Appl. Environ. Microbiol. 1984;50:846-850.
32. Kaeppeli O, Finnerty WR. Partition of alkane by an extracellular vesicle derived from hexadecane – grown Acinetobacter. J Bacteriology. 1979;140(2):707-712.
33. Burd G, Ward OP. Bacterial degradation of polycyclic aromatic hydrocarbons on agar plates: The role of biosurfactants. Biotechnology Techniques. 1996;10(5): 371-374.
34. Mulligan CN, Gibbs BF. Types, production and applications of biosurfactants. In Proc. Indian Nat. Sci. Acad. 2004;1:31-35.
35. Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM. Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: Effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol. 2000; 66:3262-3268.
36. Rosenberg E, Rubinovitz C, Legmann R. Ron EZ. Purification and chemical properties of *Acinetobacter calcoaceticus* A2 biodispersan. Appl Environ Microbiol. 1987;54:323-326.
37. Franzetti A, Bestetti G, Caredda P, Colla PL, Tamburini E. Surface-active compounds and their role in the access to hydrocarbons in Gordonia strains. FEMS Microbiol. Ecol. 2008;63:238-248.
38. Zhao Z, Selvam A, Wong JWC. Synergistic effect of thermophilic temperature and biosurfactant produced by *Acinetobacter calcoaceticus* BU03 on the biodegradation of phenanthrene in bioslurry system. Journal of Hazardous Materials. 2011; 190(1-3):345-350.
39. Sotirova A, Spasova E, Tonkova V, Galabova D. Effects of rhamnolipid-biosurfactant on cell surface of *Pseudomonas aeruginosa*. Microbiol. Res. 2009;164:297-303.
40. Mohanty M, Potential applications of biosurfactant from marine bacteria in bioremediation. MSC. Thesis, National Institute of Technology, Rourkela, India; 2013.
41. Ortiz A, Teruel JA, Espuny MJ, Marqués A, Manresa A, Aranda FJ. Interactions of a *Rhodococcus* sp. biosurfactant trehalose lipid with phosphatidylethanolamine membranes. Biochimica et al. Biophysica Acta (BBA) – Biomembranes. 2008; 1778(12):2806-2813
42. Colores GM, Macur RE, Ward DM, Inskeep WP. Molecular analysis of

surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl Environ Microbiol. 2000;66:2959-2964.

43. Allard AS, Neilson AH. Bioremediation of organic waste sites: a critical review of microbiological aspects. Int. Biodeterior. Biodegrad. 1997;39:253-285.
44. Graninger C, Rahim R, Ochsner UA, Olvera C, Messner P, Lam JS, et al. Cloning and functional characterization of the *Pseudomonas aeruginosa* rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. Molecular Microbiology. 2001;40(3):708-718.
45. Blankenfeldt W, Giraud M, Leonard G, et al. The purification, crystallization and preliminary structural characterization of glucose-1-phosphate thymidylyltransferase (RmlA), the first enzyme of the dTDP- L - rhamnose synthesis pathway from *Pseudomonas aeruginosa*. Acta Crystallogr D Biol Cryst. 2000;56(11):1501-1504.
DOI:10.1107/s0907444900010040
46. Deziel E, lepine F, Milot S, Villemur R. rhl A is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs), the precursors of rhamnolipids. Microbiology. 2008;149(8):2005-2013.
47. Zhu K, Rock CO. RhlA converts b-hydroxyacyl-acyl carrier protein intermediates in fatty acid synthesis to the b-hydroxydecanoyl-b-hydroxydecanoate component of rhamnolipids in *Pseudomonas aeruginosa*. J Bacteriol. 2008;190(9):3147-54.
48. Lopez-Lara IM, Geiger O, Sohlenkamp C. Amino acid-containing membrane lipids in bacteria. Progress in lipid research. 2010;49(1):46-60.
49. Williams P, Camara M, Steve A. N-Acylhomoserine lactones, quorum sensing and biofilm development in Gram negative bacteria. The biofilm mode of life. Mechanisms and Adaptations. 2007;95-122.
50. Pesci EC, Pearson JP, seed PC, Iglewski BH. Regulation of las and rhl quorum sensing in *Pseudomonas aeruginosa*. Journal of bacteriology. 1997;179(10): 3127-3132.
51. Pearson JP, Pesci C, Iglewski BH. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. Journal of Bacteriology. 1997; 179(18):5756-5767.
52. Peypoux F, Bonmatin JM, Wallach J. Recent trends in the biochemistry of Surfactin. Applied Microbiology and Biotechnology. 1999;51(5):553-563.
53. Liu L, Nakano MM, Lee OH, Zuber P. Plasmid-amplified coms enhances genetic competence and suppresses sinr in *Bacillus subtilis*. Journal of Bacteriology. 1996;178(17):5144-5152.
54. Luttinger HJA, Dubnau D. Regulatory inputs for the synthesis of comk, the competence transcription factor of *Bacillus subtilis*. Molecular Microbiology. 1996; 21(4):763-775.
55. Cosby WM, Vollenbroich D, Lee OH, Zuber P. Altered srf expression in *Bacillus subtilis* Resulting from changes in culture pH Is dependent on the spook Oligopeptide Permease and the Comqx system of Extracellular Control. Journal of Bacteriology. 1998;180(6):1438-1445.
56. Das P, Mukherjee S, Sen R. Genetic regulations of the biosynthesis of microbial surfactants: An overview. Biotechnology and Genetic Engineering Reviews. 2008; 25(1):165-186.
57. Kurz M, Eder C, Isert D, Li Z, Paulus EF, Schiell M, et al. Ustilipids, acylated beta-Dmannopyranosyl D-erythritols from Ustilago maydis and Geotrichum candidum. J Antibiot. 2003;59:91-101.
58. Hewald S, Linne U, Scherer M, Marahiel MA, Kamper J, Bolker M. Identification of a gene cluster for biosynthesis of mannosylerythritol lipids in the basidiomycetous fungus Ustilago maydis. Applied and Environmental Microbiology. 2006;72(8):5469-5477.
59. Van Bogaert INA, Saerens K, Muynck CD, Develter D, Soetaert W, Vandamme EJ. Microbial production and application of sophorolipids. App. Microbiology and Biotech. 2007;76(1):23-34.
60. Bognolo F. Biosurfactants as emulsifying agents for hydrocarbons. Colloids and Surfaces A: Physiochemical and Engineering Aspects. 1999;152:41-52.
61. Mukherjee S, Das P, Sen R. Towards commercial production of microbial surfactants. Trends Biotechnol. 2006; 24:509-515.
62. Daverey A, Pakshitajan K. Production, characterization, and properties of

sophorolipids from the yeast Candida bombicola using a low-cost fermentative medium. Appl Biochem Biotechnol. 2009;158:663-674.

63. Mercade ME, Maresa MA, Robert M, Espuny MJ, De Andres C, Guinea J. Olive oil mill effluent (OOME) New substrate for biosurfactant production. Bioresour Technol. 1993;43:1-6.
64. Benincasa M, Contiero J, Manresa MA, Moraes IO. Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source. J Food Engg. 2002;54:pp283-288.
65. Nitschke M, Costa SG, Haddad R, Goncalves LA, Eberlin MN, Contiero J. Oil wastes as unconventional substrates for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* LBI. Biotechnol Prog. 2005;21:1562–1566.
66. Thaniyavarn J, Chongchin A, Wanitsuksombut N, Thaniyavarn S, Pinphanichakarn P, Leepipatpiboon N, Morikawa M, Kanaya S: Biosurfactant production by *Pseudomonas aeruginosa* A41 using palm oil as carbon source. J Gen Appl Microbiol. 2006;52:215–222.
67. Bednarski W, Adamczak M, Tomasik J, Plaszczyk M. Application of oil refinery waste in the biosynthesis of glycolipids by yeast. Bioresour Technol. 2004;95:15-18.
68. Solaiman DKY, Ashby RD, Nuñez A, Foglia TA. Production of sophorolipids by *Candida bombicola* grown on soy molasses as substrate. Biotechnol Lett. 2004;26:1241–1245.
69. Trummler K, Franz E, Syldatk C. An integrated microbial/enzymatic process for production of rhamnolipids and L-(+)-rhamnose from rapeseed oil with *Pseudomonas* sp. DSM 2874. European J of Lipid Science and Technology. 2003; 105(10):563-571.
70. Kim HS, Jeon JW, Kim BH, Ahn CY, Oh HM, Yoon BD. Extracellular production of a glycolipid biosurfactant, mannosylerythritol lipid, by Candida sp. SY16 using fed-batch fermentation. Applied Microbiology and Biotechnology. 2004;70(4):391-396.
71. Nitschke M, Ferraz C, Pastore GM. Selection of microorganisms for biosurfactant production using agroindustrial wastes. Brazilian J of Microbiol. 2004;35:81–85.
72. Vance-Harrop, Mabel H, de Gusmão NB, de Campos-Takaki GM. New bioemulsifiers produced by *Candida lipolytica* using D-glucose and babassu oil as carbon sources. Brazilian Journal of Microbiology. 2003;34(2):120-123.
73. Pekin G, Vardar-Sukan F. Production of sophorolipids from *Candida bombicola* ATCC 22214 using turkish corn oil and honey. Eng Life Sci. 2005;5:357–362.
74. Ghurye GL, Vipulanandan C, Willson RC. A practical approach to biosurfactant production using nonaseptic fermentation of mixed cultures. Biotechnology and Bioengineering. 1994;44(5):661-666.
75. Rodrigues LR, Teixeira JA, Van der Mei HC, Oliveira R. Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. Colloids and Surfaces B: Biointerfaces. 2006;49:79–86.
76. Bustos G, Moldes AB, Cruz JM, Domínguez production of lactic acid from vine-trimming wastes and viticulture lees using a simultaneous saccharification fermentation method. J Sci Food Agricul 2005;85:466-472.
77. Moldes AB, L AJ, C PJ. Strategies to improve the bioconversion of processed wood into lactic acid by simultaneous saccharification and fermentation. J Chem Technol Biotechnol. 2001;76:279–284.
78. Sreenath HK, Moldes AB, Koegel RG, Straub RJ. Lactic acid production from agriculture residues. Biotechnol Lett. 2001;23:179–184.
79. Fox SL, Bala GA Production of surfactant from *Bacillus subtilis* ATCC 21332 using potato substrates. Bioresour Technol. 2000;75:235-240.
80. Ramnani P, Kumar SS, Gupta R. Concomitant production and downstream processing of alkaline protease and biosurfactant from *Bacillus licheniformis* RG1: Bioformulation as detergent additive. Process Biochem. 2005;40:3352–3359.
81. Füchtenbusch B, Wullbrandt D, Steinbüchel A. Production of polyhydroxyalkanoic acids by *Ralstonia eutropha* and *Pseudomonas oleovorans* from an oil remaining from biotechnological rhamnose production. Appl Microbiol Biotechnol. 2000;53:167–172.
82. Marsudi S, Unno H, Hori K. Palm oil utilization for the simultaneous production of polyhydroxyalkanoates and rhamnolipids by *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol. 2008;78:955-961.

83. Abalos A, Maximo F, Manresa MA, Bastida J. Utilization of response surface methodology to optimize the culture media for the production of rhamnolipids by *Pseudomonas aeruginosa* AT10. J of Chemical Technology and Biotechnology. 2002;77(7):777-784.
84. Sen R, Swaminathan T. Application of response surface methodology to evaluate the optimum environmental conditions for the enhanced production of Surfactin. Applied Microbiology and Biotechnology. 1997;47(7):358-363.
85. Mulligan, Catherine N, Wang S. Remediation of a heavy metal-contaminated soil by a rhamnolipid foam. Engineering Geology. 2006;85.1:75-81.
86. Reiling HE, Thanei-Wyss U, Guerra-Santos LH, Hirt R, Käppeli O, Fiechter A. Pilot plant production of rhamnolipid biosurfactant by *Pseudomonas aeruginosa*. Applied and Environmental Microbiology. 1986;51(5):985-989.
87. Kuyukina MS, Ivshina IB, Philp JC, Christofi N, Dunbar SA, Ritchkova, MI. Recovery of *Rhodococcus biosurfactants* using methyl tertiary-butyl ether extraction. Journal of Microbiological Methods. 2001;46(2):149-156.
88. Koch AK, Reiser J, Käppeli O, Fiechter A. Genetic construction of lactose-utilizing strains of *Pseudomonas aeruginosa* and their application in biosurfactant production. Nature Biotechnology. 1988; 6(11):1335-1339.
89. Ochsner UA, Reiser J, Fiechter A, Witholt B. Production of *Pseudomonas aeruginosa* rhamnolipid biosurfactants in heterologous hosts. Applied and Environmental Microbiology. 1995;61(9):3503-3506.
90. Mercade ME, Manresa MA, Robert M, Espuny MJ, de Andres C, Guinea J. Olive oil mill effluent (OOME). New substrate for biosurfactant Production. Bioresour Technol. 1993;43:1–6.
91. Patel Desai R. Surface-active properties of rhamnolipids from *Pseudomonas aeruginosa* GS3. J Basic Microbiol. 1997;37:281–286.
92. Raza ZA, Khan MS, Khalid ZM. Physicochemical and surface-active properties of biosurfactant produced using molasses by a *Pseudomonas aeruginosa* mutant. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2007;42:73–80.
93. Sim L, Ward OP, Li ZY. Production and characterisation of a biosurfactant isolated from *Pseudomonas aeruginosa* UW-1. J Ind Microbiol Biotechnol. 1997;19:232-238.
94. Vollbrecht E, Rau U, Lang S. Microbial conversion of vegetable oils into surface-active di-, tri-, and tetrasaccharide lipids (biosurfactants) by the bacterial strain *Tsukamurella* sp. Lipid – Fett. 1999;101: 389–394.
95. Mata-Sandoval JC, Karns J, Torrents A. High-performance liquid chromatography method for the characterization of rhamnolipid mixtures produced by *Pseudomonas aeruginosa* UG2 on corn oil. J Chromat. 1999;864:211–220.
96. Haba E, Espuny M, Busquets M, Manresa A. Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. J Appl Microbiol. 2000;88:379-387.
97. Dubey K, Juwarkar A. Distillery and curd whey wastes as viable alternative sources for biosurfactant production. World J Microbiol Biotechnol. 2001;17:61-69.
98. Rahman KSM, Rahman TJ, McClean S, Marchant R, Banat IM. Rhamnolipid Biosurfactant Production by Strains of *Pseudomonas aeruginosa* Using Low-Cost Raw Materials. Biotechnology Progress. 2002;18(6):1277-1281.
99. Camargo-de-Morais M, Ramos SAF, Pimentel M, de Morais Jr. M, Lima Filho J. Production of an extracellular polysaccharide with emulsifier properties by *Penicillium citrinum*. World J Microbiol Biotechnol. 2003;19:191-194.
100. Reis FA, Servulo EF, De Franca FP: Lipopeptide Surfactant Production by *Bacillus subtilis* grown on Low-cost raw materials. Appl Biochem Biotechnol. 2004; 115:899–912.
101. Chang JS, Chou CL, Lin GH, Sheu SY, Chen WM. *Pseudoxanthomonas kaohsiungensis* sp. nov. A novel bacterium isolated from oil-polluted site produces extracellular surface activity. Syst Appl Microbiol. 2005;28:137-144.
102. Rufino R, Sarubbo L, Campos-Takaki G. Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. World J Microbiol Biotechnol 2007;23:729–734.
103. Thaniyavarn J, Chianguthai T, Sangvanich P, Roongsawang N, Washio K, Morikawa M, et al. Production of sophorolipid biosurfactant by *Pichia anomala*. Biosci Biotechnol Biochem. 2008;72:2061–2068.

104. Prieto LM, Michelon M, Burkert JFM, Kalil SJ, Burkert CAV. The production of rhamnolipid by a *Pseudomonas aeruginosa* strain isolated from a southern coastal zone in Brazil. J Chemosphere. 2008;71:1781–1785.
105. Rufino RD, Sarubbo LA, Neto BB, Campos-Takaki GM. Experimental design for the production of tensio-active agent by *Candida lipolytica*. J Ind Microbiol Biotechnol 2008;35:907–914
106. Sobrinho HBS, Rufino RD, Luna JM, Salgueiro AA, Campos-Takaki GM, Leite LFC, Sarubbo LA. Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. J Process Biochem. 2008;43:912–917.
107. Abdel-Mawgoud A, Aboulwafa M, Hassouna N. Optimization of surfactin production by *Bacillus subtilis* isolate BS5. Appl Biochem Biotechnol. 2008;150:305-325
108. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination. 2008;223:143-151.
109. Oliveira FJS, Vazquez L, de Campos NP, de França FP. Production of rhamnolipids by a *Pseudomonas alcaligenes* strain. J Process Biochem. 2009;44:383–389.
110. Abdel-Mawgoud A, Aboulwafa M, Hassouna N. Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs 20. Appl Biochem Biotechnol. 2009;57:329-345.
111. Monteiro AS, Coutinho JOPA, Júnior AC, Rosa CA, Siqueira EP, Santos VL. Characterization of new biosurfactant produced by *Trichosporon montevideense* CLOA 72 isolated from dairy industry effluents. J Basic Microbiol. 2009;49:553–563.
112. Stoimenova E, Vasileva-Tonkova E, Sotirova A, Galabova D, Lalchev Z. Evaluation of different carbon sources for growth and biosurfactant production by *Pseudomonas fluorescens* isolated from wastewaters. Z Naturforsch. 2009;64:96–102.
113. Daverey A, Pakshirajan K. Kinetics of growth and enhanced sophorolipids production by *Candida bombicola* using a low-cost fermentative medium. Appl Biochem Biotechnol. 2010;160:2090-2101.
114. Thavasi R. Microbial Biosurfactants: From an Environmental Application Point of View. J Bioremed Biodegrad. 2011;02(05). DOI: 10.4172/2155-6199.1000104e
115. Edmonds P, Cooney JJ. Lipids of *Pseudomonas aeruginosa* cells grown on hydrocarbons and on trypticase soybean broth. J. Bacteriol. 1969;98:16-22.
116. Syldatk C, Lang S, Wagner F. Chemical and physical characterization of four interfacial-active rhamnolipids from Pseudomonas sp. DSM 2874 grown on n-alkanes. Z. Naturforsch. 1985;40C:51-60.
117. Banat IM, Characterization of biosurfactants and their use in pollution removal-state of the art. Acta Biotechnology. 1995;15:251-267.
118. Banat IM, Samarah N, Murad M, Horne R, Benerjee S. Biosurfactant production and use in oil tank clean up. World J. Microbiol. Biotechnol. 1991;7:80-84.
119. Kosaric N, Gray NCC, Cairns WL. Microbial emulsifiers and de-emulsifiers. In Rehm HJ, Reed G. (ed.), Biotechnology, Verlag Chemie, Deerfield Beach, Fla. 1983;3:572-592.
120. Singh M, Saini V, Adhikari DK, Desai JD, Sista VR. Production of bioemulsifier by SCP producing strain *Candida tropicalis* during hydrocarbon fermentation. Biotechnol Lett. 1990;12:743-746.
121. Ramana KV, Karanth NG. Factors affecting biosurfactants production using *Pseudomonas aeruginosa* CFTR-6 under submerged conditions. J. Chem. Technol. Biotechnol. 1989;45:249-257.
122. Suzuki T, Tanaka H, Itoh S. Sucrose lipids of Arthrobacteria, Corynebacteria and Nocardia grown on sucrose. Agric. Biol. Chem. 1974;38:557-563.
123. Therisod M, Klibanov AM. Facile enzymatic preparation of monoacylated sugars in pyridine. J. Am. Chem. Soc. 1986;108:5638-5640.
124. Ojeda-Morales ME, Dominguez MD, Hernandez-Rivera MA, Alvarez-Ramirez JG. Biosurfactant synthesized by *Azospirillum lipoferum* ALM1B2: Characterization and application for environmental protection. Water Air Soil Pollut. 2016;227:181
DOI: 10.1007/s11270-016-2871-6

125. Drouin CM, Cooper DG. Biosurfactant and aqueous two phase fermentation. Biotechnol. Bioengg. 1992;40:86-90.
126. Abu R, Banat IM, Haditirto S, Salem S, Kadri M. Isolation of biosurfactant producing bacteria-product characterization and evaluation. Acta Biotechnol. 1991;11:315-324.
127. Margaritis A, Kennedy K, Zajic JE. Application of an air lift fermenter in the production of biosurfactants. Dev. Ind. Microbiol. 1980;21:285-294.
128. Margaritis A, Kennedy K, Jazic JE, Gerson DF. Production and surface active properties of microbial surfactants. Biotechnol. Bioeng. 1979;21:1151-1162.
129. Sheppard JD, Cooper DG. The effect of biosurfactant on oxygen transfer in a cyclone column reactor. J. Chem. Technol. Biotechnol. 1990;48:325-336.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15772