Microbial process development for fermentation based biosurfactant production

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Abstract

The development of fermentation processes of microbial bio products has come a long way with scientists modifying existing technologies and inventing new and more efficient methods. Surfactants especially those produced by biological systems are highly useful and unique molecules that have a variety of applications in various industries and aspects of human life. Biosurfactants are produced mainly by microorganisms of which the *Pseudomonas sp* was one of the first discovered to produce such molecules as a secondary metabolite. Biosurfactants are generally classified based on the charge they carry on their surfaces as anionic,

cationic, amphoteric or non-ionic surfactants. They can also be classified based on their chemical structures as glycolipids, lipopeptides, oils or polymers. Production of biosurfactants can be achieved by batch, fed-batch or continuous fermentation processes but are however quite expensive. An optimization of the production parameters like the nutrient content can lead to a more efficient production and lesser cost. Varying the carbon to nitrogen content and altering some parameters like the need for oxygen or the pH can greatly affect the production of biosurfactants and lead to a better yield.

1. Introduction

Surfactants are surface active molecules which have the ability to lower the surface tension of liquids due to the molecules' unique structure which contains both hydrophilic and hydrophobic regions (Gutnick and Bach, 2011). This structure enables surfactants to display a variety of surface activities that allow the solubilization of hydrophobic compounds (Satpute *et al.,* 2010) due to the reduction in surface and interfacial tension. Due to this unique property, surfactants have been widely used as detergents, emulsifiers, de-emulsifiers, dispersants,

wetting agents, foam retardants, etc., but these chemically produced surfactants are derived from waste products of the petro-chemical industry and are toxic and not easily degraded in the environment.

2. Biosurfactants

Biosurfactants are receiving increasing interest and attention in the last decade in an attempt to compete with chemically derived surfactants (Satpute *et al.,* 2010; Khopade *et al.,* 2012a; Khopade *et al*., 2012b; Luna *et al.,* 2013). These are a structurally diverse group of surfactants synthesized by microorganisms such as bacteria and yeast, and they are surface active biomolecules known as amphiphilic compounds. They are great detergents with foaming and emulsification properties. Their properties in general are reduction of surface tension between different phases, their ability to reduce critical micelle concentration(CMC) and their ability to reduce interfacial tension between aqueous and hydrocarbon mixtures. Biosurfactants can be classified based on the charge they carry as either negatively charged molecules known as anionic biosurfactants or positively charged molecules known as amphoteric biosurfactants. In between these two groups are the non-

ionic and cationic biosurfactants that are either a polymerization product or possess a positively charged quaternary ammonium group respectively.
Biosurfactants can also be classified based on their molecular weight (Rahman and Gakpe, 2008). These biologically produced surfactants have the same properties as chemical surfactants but they have shown several advantages over the latter such as lower toxicity and biodegradability. In addition to these desirable properties they have also shown high activity and stability at extreme temperatures, pH and salinity and have shown better environmental acceptability (Luna *et al.,* 2013; Jain *et al.,* 2013), these traits have all contributed to the increased interest in biosurfactant production as many industries are looking for greener alternatives to many of their products and processes.

Classification based on their chemical structure is another method that is finding ground. Table 1 below shows this classification mode. Biosurfactants gained their popularity and importance in the area of oil recovery and environmental bioremediation. They have also been used extensively in the food processing industry and as pharmaceuticals. They are produced by bacteria, yeasts

and fungi by fermentation using renewable carbon sources. The increase in interest in the application of biosurfactants is due mainly to their highly desirable characteristics which include higher biodegradability compared to chemical surfactants. This can be said to be one of the singular most important reason why the microbial surfactants have gained their popularity. Another property that is unique to biosurfactants compared to their chemical counterparts is that they have lower toxicity when used especially in the environment. Microbial surfactants are also a better choice as they are effective at extremes of pH, temperature and salinity (Mukherjee et al., 2009).

< Insert Table 1 >

In addition to the environmental benefits of biosurfactants, they also have other benefits over chemical surfactants such as their stability in extreme conditions which would make them suitable replacements for conventional chemical surfactants. Microorganisms produce biosurfactants in order to assist in solubilization of hydrophobic compounds in the environment to facilitate their use as substrates by the microorganism. A few examples of biosurfactants and their structures can

be seen in Figure 1, this figure includes, mono-Rhamnolipid, Di-Rhamnolipid, Lipopeptide and Sophorolipid. In order for biosurfactants to become a commercially viable product, they need to compete with currently utilised chemical surfactants in terms of cost, function and production capacity (Rocha e Silva *et al.,* 2014). Therefore, to successfully meet these demands and successfully compete with chemical surfactants, microorganisms with suitable metabolic pathways that are easily culturable and are capable of producing a high yield of effective biosurfactant need to be identified through bioprospecting. Bioprospecting for a suitable microorganism to meet the current industrial demand may be a lengthy and costly process but a lot of research is currently being conducted into this regard (Najafi *et al.,* 2010; Satpute *et al.,* 2010; Khopade *et al.,* 2012a; Khopade *et al.,* 2012b).

(Insert figure 1)

The marine environment presents an enormous diverse environment and it is estimated that less than 0.1% of the marine microbial world has currently been explored (Satpute *et al.,* 2010). This gives enormous potential for unique and important microorganisms which may contain

unique metabolic pathways to be discovered, which could not only influence the production of biosurfactants but could potentially have a huge impact on all areas of bioprocessing. Due to the complexity of the marine environment and the extreme conditions that some marine microorganisms exist under, not all microbes currently collected are culturable in a lab environment for analysis and have only been identified using molecular methods. Due to the necessity during oil recovery and remediation of oil spills of a bacteria and its biosurfactant to be halotolerant, it is mandatory to screen and develop potential biosurfactant producers from marine environments (Khopade *et al.,* 2012b). Different screening methods are currently utilised in order to identify biosurfactant producing microorganisms but as each biosurfactant has different functional and chemical properties it is difficult to obtain biosurfactant producing microorganisms using only one screening method (Satpute *et al.,* 2010). Several marine microbial identification strategies and their effectiveness as marine biosurfactant producers have been reviewed. Khopade *et al.,* (2012a), isolated marine *Streptomyces* species B3 and characterized its biosurfactant after optimization of the culture medium and fermentation process. Khopade *et al.,* (2012b), isolated marine *Nocardiopsis* species B4

and characterized its biosurfactant after optimization of the culture medium and fermentation process. Identification of the strains studied in Khopade *et al.,* (2012a) and Khopade *et al.,* (2012b), was done using 16S rDNA technology, Scanning Electron Microscopy, biochemical and cultural characterisation.

3. Characteristics of Biosurfactants

Microbial surfactants act by reducing interfacial and surface tensions in much the same way as chemical surfactants. The ability to reduce critical micelle concentration (CMC) is another characteristic of biosurfactants as stated previously. These molecules are usually produced by microorganisms where they either remain adherent to the cell surface of microbial cells or are secreted into the culture broth. Microbial surfactants are diverse and can occur as a variety of chemical structures like glycolipids or lipopeptides; the two most common ones. They can also occur as fatty acids, phospholipids or particulate structures (Müller *et al.*, 2012).

The advantages of using microbial biosurfactants far outweighs using chemical surfactants as the latter is

produced from petroleum feedstock which has effects detrimental to the environment and moreover, the source is deemed expendable. On the other hand, microbial surfactants are from sustainable sources and the technology to produce them in large quantity is readily available.

An important advantage of using renewable microbial agents that utilize low cost feedstock is another upper hand microbial surfactants have over chemical surfactants.

4. Fermentation Requirements

There are three different types of fermenter operation processes which are frequently used for culturing bacteria. These are batch, fed-batch and continuous fermentation processes. Batch fermentation is the process of culturing with all of the required nutrients provided at the start of the fermentation process and the process is run until all of the nutrients are exhausted and the broth is then harvested, all of the ingredients required for fermentation are added to the fermenter before inoculation with the seed culture. Batch fermentation has the advantage of being simple and having low risk of

external contamination as no further additions are required except for pH stabilisers. The process is best for fermentations of cultures with high yield and for substances that can tolerate high initial nutrient conditions. Fed-batch fermentation is similar to batch fermentation but only starts with some of the required nutrients at the inoculation stage in order to prevent inhibition of product production at high concentrations of substrate, further nutrients are added as the fermentation progresses in order to maintain substrate concentration for the production of the desired product. The advantages of fed batch fermentation include reduction of substrate and product inhibition and can decrease overall fermentation time, this then allows higher concentration of product without being inhibited by high levels of nutrients in the broth (Anderson, 2009; Chang *et al.,* 2012). Fed-batch fermentation however, carries the risk of potential contamination due to the addition of nutrients through a steriliser, and the increased costs for specialized sterilization equipment. Batch and fed batch fermentations can be repeated using the same fermenter system after harvesting the culture by leaving a small amount of the previous batch in the fermenter as inoculum, this adds the risk of contamination, and degradation of the culture limits the number of repeat

batches to about 2 or 3 before the fermenter must be cleaned and sterilized. Continuous fermentation processes start with the medium and inoculum in the fermenter, after the culture has grown, the broth is withdrawn at the same rate as the fermenter is fed nutrients in order to maintain a constant volume of broth in the fermenter. Under ideal conditions the dilution rate will be the same as the culture growth rate, when this balance is maintained for long enough, there are no changes in the conditions within the reactor; this is called steady state operation (Brethauer and Wyman, 2010). Compared to batch fermentation processes, continuous fermentation reduces down time for cleaning and sterilization between batches, although continuous fermentation cannot be run indefinitely, fermentations of several hundred hours can be completed under aseptic conditions. Continuous fermentation has better control at steady state operation which in turn reduces costs (Brethauer and Wyman, 2010), but contamination from adapted cultures is difficult to avoid as they can grow back through the continuous harvest line (Anderson, 2009). Khopade *et al.*, (2012b) completed their investigations using shake flasks in batch fermentation.

When designing and optimising a fermentation process, the optimum growth conditions of the isolated microorganism need to be identified, this is most effectively achieved at small scale using shake flasks by measuring optical density of the culture medium throughout the culture time to produce a growth curve for each of the variables such as temperature, salinity and medium composition. Parameters such as pH, O_2 content and O_2 uptake and other environmental factors cannot be as easily monitored and controlled at small scale (Smith, 2009). Following this, optimum conditions can be established for culture of the microorganism. It should be noted that optimum conditions for growth of the microorganism may not be the optimum conditions for the production of the desired product. Following the optimization in shake flasks, the process can be scaled up to larger volumes for further optimization and development for potential use at an industrial scale.

The development of a suitable growth medium depends on the nutritional requirements of the microorganism to be cultured. In order to ensure that the production of biosurfactants is economical, low cost substrates with sufficient nutritional value need to be used as this can account for 10-30% of the overall costs (Silva *et al.,*

2010). Khopade *et al.,* (2012b) chose to optimize the carbon and nitrogen source available for utilization in order to obtain higher productivity of the biosurfactant. This was done using several carbon sources whilst keeping the nitrogen source constant, then using the optimum carbon source, varying nitrogen sources were compared and the optimums were chosen. The optimal growth conditions required for high cell density is not the same as the optimum conditions for biosurfactant production as previously noted, and in the case of *Pseudomonas aeruginosa*, when producing Rhamnolipid, fed batch fermentation with the carbon source in the feed produces a very low dry cell weight concentration (g/l) whereas the Rhamnolipid concentration is at its highest producing over 3.5g/L (Ghomi Avili *et al.,* 2012).

5. Production of Biosurfactants

Biosurfactants, in general, are diverse in nature with about 60 different congeners and homologues and are produced at different concentrations by various species of bacteria, yeast and fungi especially those with filaments. The major group of microorganisms that produce biosurfactants are the *Pseudomonas* species

that were the first to be discovered to produce such secondary metabolites. Other microorganisms that produce biosurfactants are the *Bacillus* sp, *Candida* sp. *Acinetobacter* sp. and *Arthrobacter* sp.

Pseudomonas species are well known as potential marine and terrestrial bacteria that produce a variety of bioactive metabolites. A report by Bhatnagar and Kim (2012) showed that these novel bacteria produce about 800 bioactive molecules ranging mainly from antibiotic agents to others with diverse properties. The main biosurfactant produced as exoproducts by *Pseudomonas* sp. are the glycolipid-type surfactants known as rhamnolipids (Bhatnagar and Kim (2012)

Biosurfactants can be produced in large scale using bioreactors and cheap substrates as sources of nutrient. A lot of studies have shown that biosurfactants can be produced by growing bacteria like *Pseudomonas* known for their production of rhamnolipids as shown by rhamnolipids experts like Rahman and Gakpe (2008).

<Insert table 2>

6. Monitoring of Biosurfactant production:

Optimization of the production of biosurfactant can be achieved by testing the biosurfactant production throughout the fermentation process whilst changing the variables accordingly. Using a tensiometer in order to monitor any changes in surface tension is a good indicator of biosurfactant production, foaming in shake flasks during culturing is also a good indicator of the presence of biosurfactants in the media and this can be analysed further by testing the emulsification index of the biosurfactant produced as described in Shavandi *et* al., (2011). It can be noted that fed batch fermentation is more effective than batch fermentation processes in order to produce higher concentrations of Rhamnolipid by *P. aeruginosa*, when the carbon source is limited by the feed process (Ghomi Avili *et al.,* 2012; Shavandi *et al.,* 2011). This higher concentration does not mean that the biosurfactant produced cannot be isolated and studied at lower concentrations when using the batch fermentation process however, which may be more cost effective during initial screening for suitable biosurfactants.

7. Downstream Processing of biosurfactants

In order to successfully characterize the biosurfactant produced by the fermentation process, it must be separated from the cells and the broth and then purified. Separation from the cells can be achieved by centrifugation at 5000-10,000g for 10-20 minutes (Nayak et al., 2009; Shavandi *et al.,* 2011; Luna *et al.,* 2013; Roche e Silva *et al.,* 2014), the cell pellet can then be removed and dried in order to measure the dry cell weight for the culture and the supernatant can be further purified in order to characterize the biosurfactant more accurately. Purification of the biosurfactant from the supernatant can be achieved by acidifying the supernatant with hydrochloric acid to pH 2.0 and then precipitating the biosurfactant with methanol. The precipitate can then be centrifuged before being separated. The precipitate is then washed with methanol and dried at 37^{o}C, this method is described in Luna *et al*., (2013) and yields pure biosurfactant for further study. Another separation method described recently by Ismail *et al*., (2013) used solid phase extraction (SPE) by centrifuging the broth followed by filtration of the supernatant to remove excess biomass, then the leftover solution was loaded onto SPE cartridges and the crude biosurfactant was eluted from the cartridge using methanol. Both of these extraction methods are able to

produce crude biosurfactant for further analysis and have both proven to be effective in estimating yield by weighing the residual biosurfactant (Ismail *et al.,* 2013; Luna *et al.,* 2013). Characterizing the purified biosurfactants can be done in a number of ways in order to identify structure, functional groups and properties. When using a tensiometer with a Du-Nouy ring, the critical micelle concentration (CMC) can be calculated, as seen in Khopade *et al.,* (2012b), the surface tension and interfacial tension (mN/m) are determined by the maximum force exerted by the solution and at the CMC a sudden change in surface tension can be observed (Shavandi *et al*., 2011). This value can be determined by plotting a graph showing the surface tension observed for different concentrations of biosurfactant (log of mg/l or g/l). Fourier transform infrared spectroscopy (FTIR) analysis can be completed to characterize structure of the biosurfactant produced, the sample is freeze dried and then analysed using an infrared spectrophotometer, the resulting spectrum can then be analysed. The bands and peaks on this spectrum can be used to indicate the functional groups and chemical bonds in the molecular structure of the biosurfactant (Aparna *et al.,* 2012; Khopade *et al.,* 2012b; Jain *et al.,* 2013).

Biosurfactants such as those shown in **Table 3.0** have the potential to be utilized in a number of different processes including land and water remediation, oil extraction, medical processes and a number of other industrial applications. In order for them to be successfully integrated into current industrial processes they must be produced at a reasonable price and have a yield high, enough to compete with currently utilized chemical surfactants. Even though biosurfactants are greener alternative which is an attractive attribute, they need to be more cost effective in order to secure their place in the industrial marketplace. Some scientists are now trying to overcome the cost and capital issues by not only focusing on biosurfactants being more attractive as a greener alternative, but by showing their higher productivity and ability to outperform current chemical surfactants even in extreme environments such as high salinity, variable pH and extremes in temperature (Aparna *et al.,* 2012; Khopade *et al.,* 2012a; Khopade *et al.,* 2012b; Marti *et al*., 2014).

<Insert table 3>

8. General Applications of biosurfactants

Biosurfactants as mentioned earlier are produced in different quantities by a variety of microorganisms. These molecules are produced by microorganisms to fulfil a number of functions like self-defence or the ability to feed. The same principles are used in developing them for various applications. As stated above, the diversity in their chemical structures gives rise to a variety of functions which include the reduction of surface tension and thereby the reduction of interfacial tension. They also increase surface areas which have been proven useful in water insoluble-hydrophobic compounds. Their ability to disperse or dissolve hydrophobic compounds has made them useful in a variety of industries like pharmaceutical, food and energy.

They are also capable of quorum sensing with the ability to initiate cell-cell signalling. These novel substances have the ability to bind-heavy metals. They can be pathogenic to bacteria and also have the ability to form bio-films. The sectors where they are used are presented below with examples of their applications.

8.1. Environmental applications

Since their discovery and isolation in 1965 to date, biosurfactants have been employed in a variety of applications chief amongst which is environmental clean-up of oil contaminated areas as bioremediation agents. This technique is known as microbial enhanced oil recovery (MEOR) as they are surface active agents with low toxicity and excellent emulsifying capabilities which are stable even at extreme conditions as shown by research. A study by Xia *et al.* (2011) showed that biosurfactants from three bacteria were compared for their bioremediation activity and they all showed good promise in oil recovery even when used at extreme levels of pH, temperature, metal ions and salinity. The three bacteria used were *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Rhodococcus erythropolis* where *P. aeruginosa* showed the highest emulsification index of 80%. These highly efficient bioremediation agents are being used to clean up land contaminated by hydrocarbon and heavy metals as it has been found that only about 30% of oil contaminants can be removed by conventional primary and secondary techniques.

8.2. Agricultural applications

Biosurfactants have been used in agriculture as a measure against pests. This use has been made possible due to their antimicrobial effects especially against plant pathogens. An added advantage of using biosurfactants in agriculture is their safety margin compared to synthetic surfactants that have residual effects left on agricultural produce. This use has been demonstrated by the application of rhamnolipids extracted from *Pseudomonas* sp. EP-3 as a pesticide against the green peach aphid (Kim *et al.*, 2011). Another study on the use of biosurfactants in agriculture showed that rhamnolipids are recognised as impacting immunity to plants by triggering signalling pathways known as microbe-associated molecular patterns (MAMPs). This activation then confers immunity to the plants in a way similar to how the pathway works in mammals (Vatsa *et al.*, 2010).

8.3. Food industry applications

Another area where biosurfactants are being used is the food industry where they are used as antimicrobial

agents to prevent food-borne pathogenic infections. A recent study published in 2013 showed that rhamnolipids in particular are active against a wide range of gram-negative and gram-positive bacteria which include *Salmonella typhimurium*, *E.coli*, *Enterobacter aerogenes, Staphylococcu aureus*, *Clostridium perfringens* and *Bacillus suptilis*. Rhamnolipids also showed fungicidal activity against *Phytophthora infestans*, *P. capsici*, *mucor spp, Botrytis cinerea* and *Fusarium graminearum*. The research showed the remarkable effect of rhamnolipids against *Liesterai monocytogenes,* a gram positive bacterium that is one of the most virulent food-borne pathogens responsible for an unprecedented number of deaths related to food contaminated by this organism. The study, however, showed that although the rhamnolipids effect was bacteriostatic, the addition of Nisin remarkably increased the effectiveness of the biosurfactant (Magalhães and Nitschke, 2013).

8.4. Cosmetic Industry applications

Biosurfactants have been used in the cosmetic industry for quite some time and they have been found to be safer alternatives compared to some chemical surfactants.

These novel molecules are used in the cosmetics industry due to their excellent wetting, emulsifying, dispersing, solubilizing, foaming and most especially detergent effects. A number of cosmetic products containing rhamnolipids have been patented for use as anti- ageing products, anti-acne products. Their use has been successful as they are compatible with the skin and cause very low or no irritation when used as personal care products (Lourith and Kanlayavattanakul, 2009).

8.5. Application as antimicrobials

Biosurfactants have been found to be excellent algaecides as shown by a research on their algicidal activity. The harmful algal bloom (HAB) species of algae, *H. akashiwo*, *P. dentatum* and *Gymnodinium* sp. are known to be harmful to human health and research using rhamnolipids from *P. aeruginosa* showed that it was able to inhibit their growth at lower doses (0.4–3.0 mg/L) causing cell lysis. The study showed that rhamnolipids were able to inhibit these harmful organisms by breaking down their plasma membranes thereby irreversibly damaging their inner structures leading to loss of function (Wang *et al.*, 2005)

9. Conclusion:

Biosurfactants are unique biomolecules with a variety of functions that are fast becoming a more efficient and greener alternative to their predecessor chemical surfactants. These molecules occur in various forms and they can be characterized based on either the charge they carry on their surfaces, chemical structures or even their molecular weight. They occur as rhamnolipids, glycolipids, sophorolipids, Surfactin and viscosin. They can also occur as lipopolysaccharides and fatty acids etc. They are surface active biomolecules that act by reducing surface tension between different layers of liquid surfaces. They are great detergents and have excellent foaming properties, as such they are used in a number of industries that include the food, agricultural and pharmaceutical industries. The environment and the oil industry are the two areas where the impact of biosurfactants have had the most effect as they have been used and studied extensively in bioremediation and environment clean-up of oil spillages. They are also used in the cosmetic industry due to their history of safety and low toxicity. Biosurfactant production can be a costly process which can be made less so by varying

production modalities and parameters. Production can be either by batch, fed-batch or continues fermentation methods when down streaming and growth controlling factors especially the nutrient can be altered to optimize production. Biosurfactants can therefore be produced in adequate quantities using bioreactors and cheap feedstock as nutrient sources. Knowledge of biosurfactants, their characteristics and uses is expanding and more invaluable research is being conducted into optimizing productivity and reducing costs. The benefits of biologically produced surfactants cannot be denied and they surpass conventional chemical surfactants in many ways but there are major limitations still facing their industrial application. Their low yield and high cost when compared to chemical surfactants has started to receive more biotechnological research in order to successfully overcome these limitations. If momentum is maintained, we will start to see commercially available biosurfactant products being utilized by industries such as oil recovery, fuel extraction and medicine within the next decade.

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Table 1: Examples of biosurfactants based on their chemical structure classification (Müller *et al.*, 2012)

Structural classification	**Examples**
Glycolipids	Rhamnolipids(RL), Sophorolipids(SL), Mannosylerythritol lipids (MELs), Trehalose lipids (TL)
Lipopeptides/lipoamino acids	Surfactin, Ornithine lipids, Lysin lipids
Polymers	Polysaccharides, Lipopolysaccharides , Proteins, lipoproteins
Oil/membranes	Fatty acids, glycerolipids and phospholipids

Table 2 Different biosurfactants and their microbial sources (adapted from (Rahman and Gakpe, 2008)

Type of biosurfactant	**Source microorganism (s)**
Alasan	*Acinetobacter radioresistens*
Arthrofactin	*Arthrobacter sp*
Biosur PM	*Pseudomonas maltophila*
Cellobose lipids	*Ustilago maydis*
Diglycosyl diglycerides	*Lactobacillus fermentii*
Fatty acids (corynomycolic acid, spiculisporic acid)	*Penicillium spiculisporum* *Corynebacterium lepus* *Arthrobacter paraffineus* *Talaramyces trachyspermus* *Norcadia erythropolis*
Glycolipids	*Alcanivorax borkumensis* *Arthrobacter sp.,* *Serratia marcescens* *Tsukamurella sp.*
Lichenysin A lychenysin B	*Bacillus licheniformis*
Lipopolysaccharides	*Acinetocbacter calcoaceticus(RAG1)* *Pseudomonas sp., Candida*

	lipolytica
Ornithine, lysine peptids	*Thiobacillus thiooxidans* *Streptomyces sioyaensis*
Particulate surfactant(PM)	*Pseudomonas marginalis*
Phospholipids	*Acinetocbacter sp.*
Polyol lipids	*Rhodotorula glutinous* *Rhodotorula graminus*
Rhamnolipids	*Pseudomona aeruginosa* *Pseudomonas sp., Serratia rubidea*
Sophorolipids	*Candida apicola, Candida bombicola, Candida lipolytica* *Candida bogoriensis*
Streptofactin	*Streptomyces tendae*
Sulphoryl lipids	*T. thiooxidans* *Corynebacterium alkanolyticum* *Capnocytophaga sp.*
Surfactin	*Bacillus subtilis, Bacillus pumilus*
Trehalose lipids	*Arthrobacter paraffineus* *Corynebacterium sp.* *Mycobaceterium sp* *Rhodococcus erythropolis,* *Norcardia sp.*
Viscosin	*Pseudomonas flourescens*

Table 3. Examples of biosurfactant producing microorganisms, culture process in batch condition and biosurfactant properties

Microorganism	**Culture time (h)**	**Fermenter type**	**Biosurfactant**	**Surface tension (mN/m)**	**Yield (g/L)**	**Reference**
Pseudomonas cepacia	144	SF	NR	27.5	5.2	Rocha e Silva *et al.,* 2014
Nocardiopsis B4	96	SF	Rhamnolipid	30	NR	Khopade *et al.,* 2012a
Pseudomonas sp. 2B	168	SF	Rhamnolipid	29.7	4.97	Aparna *et al.,*2012
Streptomyces sp. B3	216	SF	Glycolipid	29	NR	Khopade *et al.,* 2012b
Bacillus subtilis	72	SF	Surfactin	27.4	6.2	Marti *et al.,* 2014
B. subtilis	33	BR	Surfactin	27.4	2.5	Marti *et al.,* 2014
Bacillus licheniformis	10-72	BR	NR	28	NR	Joshi *et al.,* 2013
*Pseudoxanthomonas sp.*PNK-04	up to 120	SF	Rhamnolipid	29	2.8	Nayak *et al*., 2009

NR = Not Reported; SF = Shake Flask; BR = Bioreactor

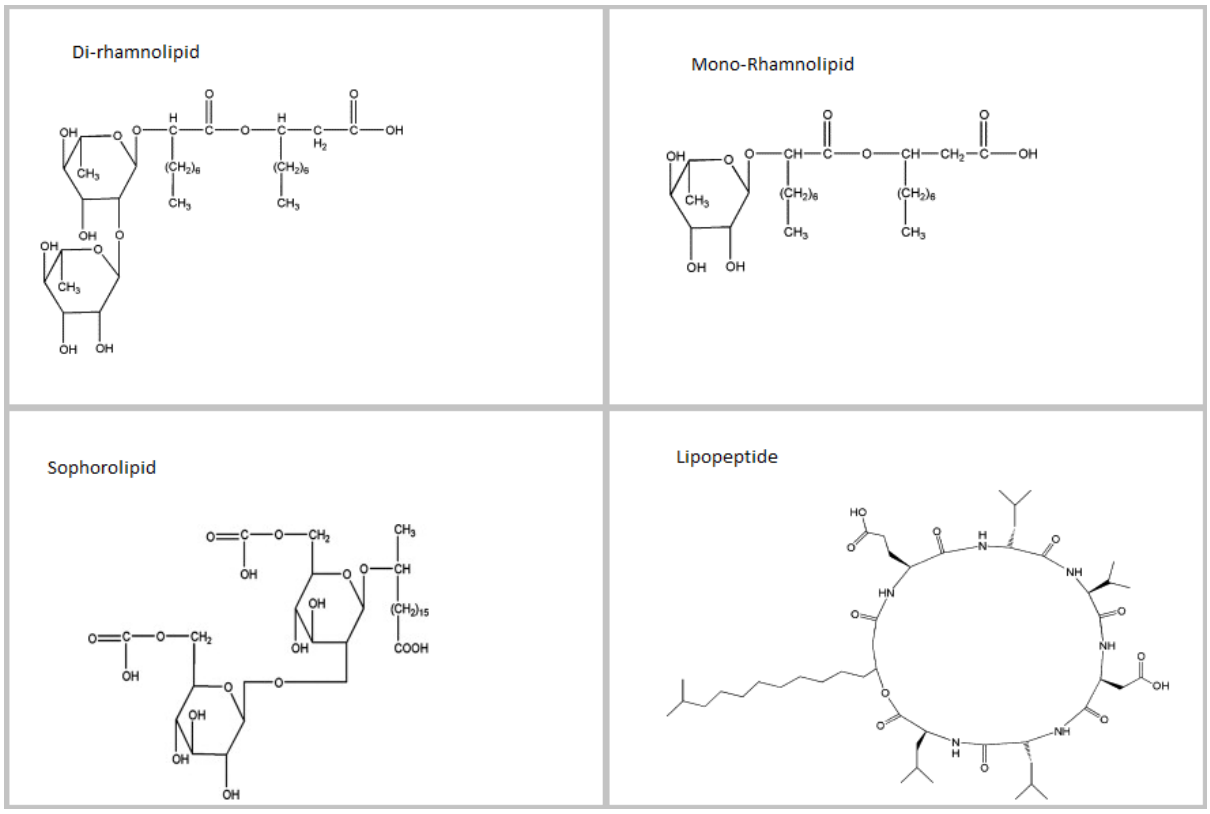

Figure 1 Different biosurfactant molecules (Gutnick and Bach, 2011)