# Chapter #

# Possibilities and Challenges for Biosurfactants Uses in Petroleum Industry

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## Abstract

Biosurfactants are a group of microbial molecules identified by their unique capabilities to interact with hydrocarbons. Emulsification and de-emulsification, dispersion, foaming, wetting and coating are some of the numerous surface activities that biosurfactants can achieve when applied within systems such as immiscible liquid/liquid (e.g., oil/water), solid/liquid (e.g., rock/oil and rock/water) and gas/liquid. Therefore, the possibilities of exploiting these bioproducts in oil-related sciences are vast and made petroleum industry their largest possible market at present. The role of biosurfactants in enhancing oil recovery from reservoirs is certainly the best known; however they can be effectively applied in many other fields from transportation of crude oil in pipeline to the clean-up of oil storage tanks and even manufacturing of fine petrochemicals. When properly used, biosurfactants are comparable to traditional chemical analogues in terms of performances and offer advantages with regard to environment protection/conservation.

This chapter aims at providing an up-to-date overview of biosurfactant roles, applications and possible future uses related to petroleum industry.

## Introduction

Petroleum has been driving the modern world for the past 100 years, however the high-quality and easily extractable light crude oils are limited. The ultimate recoverable resources are estimated at between 2-4 trillion barrels,[1] which poses two major issues. Firstly, the high priority need for maximizing the efficiency over all the stages of processing in the current petroleum industry. For example, less than half of the crude oil content of any reservoir can be actually extracted by the current techniques and improvements are sought after. Secondly, the challenge of utilizing heavy crude oils, bitumen and tar sand that are abundant in many parts of the world and which may represent the hydrocarbon-based energy of the future. Such poor-quality cruds being extremely viscous with densities higher than water, some solid at ambient temperature and additionally rich in sulphur and metals, are in need of novel technologies for upgrading. Traditional methods for production, transportation and refining are not suitable for such heavy oils and need to be improved.

In the above reasons, biotechnology may find a special niche within the related research areas as important links between microbiological and biotechnological research and petroleum industry have been built up in the recent years with regard to several areas of interest such as biocorrosion and biofouling, degradation of hydrocarbons within oil reservoirs, enzymes and biocatalysts for petroleum upgrading. Biosurfactants and bioemulsifiers are a novel group of molecules and among the most powerful and versatile bioproducts that the modern microbial biotechnology can offer.

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In this chapter we discuss some roles and applications of these microbial compounds in oil-related sciences, presenting the processes that exploit commercially available biosurfactant technologies and highlighting those in which they may be potentially applied and have a greater impact on in the near future. Recent laboratory-scale researches along with field trials and patents will be described. Where possible information about technical aspects of the marketed systems will be included.

## Surfactants and Biosurfactants in Petroleum Industry

Surfactants are molecules with two functional groups, namely a hydrophilic or polar end and a hydrophobic or nonpolar chain. Due to the affinity towards both polar and nonpolar phases, surfactants present in a mixed system (e.g., oil/water) move from the bulk phase to preferably adsorb at the surface or interface where they cause remarkable changes in surface and interfacial tensions, viscosity, wettability, charge and elasticity.[2]

Most surfactants currently in use are of petrochemical origin and therefore face the increasing environmental awareness and tightening of regulations in this regard. Microorganisms have long been known to be able to produce a variety of surface active compounds that display properties and activities comparable to those of synthetic surfactants. Numerous research describing biosurfactants produced by bacteria, yeasts and fungi have been carried out over the past years and many reviews covering various aspects of the topic are available in literature (see refs. 3-6).

Biosurfactants can potentially replace chemical analogue compounds, even offering additional advantages in all the aspects of petroleum processing including: 1- Extraction, 2- Transportation, 3- Upgrading and refining and 4- Petrochemical manufacturing.

## Microbial Enhanced Oil Recovery

Classical oil production technologies involving 'primary' and 'secondary' can only partially recover the oil present in the field, with an efficiency estimated at 30-40% of the overall amount of oil available. Such efficiencies are expected to decrease during the gradual depletion of light crude reservoirs leaving the viscous crude oils. This requires the development of the 'tertiary' processes which aim at enhancing oil recovery (EOR).[7] Among these, microbially enhanced oil recovery (MEOR) exploiting microbial activities and metabolites, is at present gaining increased attention due to some advantages such as:

- Natural products are generally harmless and less detrimental to the environment;
- Microbial processes do not require large thermal consumption of energy;
- Costs of microbial products are not affected by crude oil price and can be produced using inexpensive raw-substrates or even waste materials;
- Microbial products/activities can be stimulated in situ within the reservoir, potentially allowing both tailor-made and cost-effective treatments.

Several metabolites are of interest for applications in MEOR including gas (e.g., carbon dioxide, methane and hydrogen), acids (e.g., acetate and butyrate), solvents (e.g., acetone, n-butanol and ethanol), biomass for selective plugging and biosurfactants/biopolymers.[8] Biosurfactants in particular have several benefits enhancing oil displacement and movement through oil-bearing rocks by means of three main mechanisms: (i) reduction of interfacial tension between oil-rocks and oil-brine; (ii) modification of the wettability of porous media; (iii) emulsification of crude oil. In addition, biosurfactant production contributes to the metabolism of viscous oils by microorganisms that release lighter hydrocarbon fractions thus making the oil even more fluid. The strategies investigated so far for MEOR involving biosurfactants include:

- Injection of ex situ produced biosurfactants into the reservoirs;
- Injection of laboratory-selected biosurfactant-producing microorganisms into the reservoirs;
- Stimulation of indigenous microbial population to produce biosurfactants in situ through supplying suitable nutrients.

## Injection of ex Situ Produced Biosurfactants into Oil Reservoirs

Biosurfactants can be produced in industrial-scale through fermentation technologies. However, the cost for the final product is still high for applications in this specific area. Several reasons are implicated and include costs for activity and maintenance of bioreactor apparatus, product extraction and purification, production of biosurfactants at generally low yields (1-10 g/l) by natural bacteria, reduced fermentation efficiency due to foaming and other metabolic-associated problems. Thus, while this option is not yet economically sustainable, experimental evidences supported the efficacy of the flooding technique in which biosurfactants replaced or assisted conventional chemical surfactants.

Lichenysin is one of the most powerful biosurfactants ever characterized. It is synthesized by *Bacillus licheniformis* JF-2 (ATCC 39307), isolated from well injection water[9] and recently reclassified as *B. mojavensis*.[10] Lichenysin, even at low concentrations (10-60 mg/l), is able to reduce interfacial tension to ultra low values (less than $10^{-2}$ mN/m) required to release the trapped oil. In addition, it is not affected by temperature (≤140˚C), pH (from 6 to 10), salinity (up to 10% w/v NaCl) and calcium concentrations (≤340 mg/l $CaCl_2$).[11] It has been tested in core flooding experiments in a partially purified form and showed that, when included into the formulation of a flooding solution containing 2, 3-butanediol and 1g/l of partially hydrolyzed polyacrilamide (PHPA), residual oil was recovered from sandstone cores at up to 40%, compared to 10% recovered by the fluid containing chemical surfactants only.[12]

Similar results of improved flooding performance were obtained with rhamnolipid biosurfactants. In particular, it was observed that in the presence of rhamnolipids the adsorption of the surfactant alkylbenzene sulfonate (ORS) to sandstone was reduced by 25-30% and consequently its loss decreased. Thus, the oil recovered increased 7% when biosurfactants were added to the flooding solution. It was suggested that rhamnolipids acted as sacrificial agents by adsorbing preferably to oil sands thus both altering the wettability of porous media and making the chemical surfactant more available for displacement activity.[13]

Even more effective than low-molecular weight biosurfactants are the higher mass bioemulsifiers and biopolymers. For example, emulsan by *Acinetobacter venetianus* RAG-1 (ATCC 31012) used at a concentration of 0.1 mg/ml removed 89% of crude oil pre-adsorbed to limestone samples and up to 98% when used at 0.5 mg/ml.[14]

## Injection of Laboratory-Selected Biosurfactant-Producing Microorganisms into Oil Reservoirs

Most studies focuses on the possibility of introducing biosurfactant-producing bacteria along with nutrients into the oil wells to allow their growth and activity. However to be suitable for this MEOR strategy, bacteria are required to thrive and be metabolically active at the extreme conditions typical of petroleum reservoirs.[15] Although extremophilic microorganisms have been isolated from different environments, native strains from oil reservoirs would be optimal candidates. The use of exogenous strains is disadvantageous due to competition with indigenous bacteria.

Most of biosurfactant-producing bacteria so far described and tested for in situ MEOR applications belong to *Bacillus* genus that commonly includes thermo- and halotolerant, facultative anaerobic strains. Among them, *B. mojavensis* JF-2 has been extensively investigated. This strain can grow while producing lichenysin under both aerobic and anaerobic conditions and at relatively high temperature (40˚C),[16] which makes it a good candidate for in situ activity. Various processes exploiting JF-2 strain for oil recovery applications have been proposed including injection into oil-bearing formations alone[17] or as part of a microbial consortium.[18] An increase of 14% in oil production was observed after flooding with *B. mojavensis* JF-2 and the presence of living cells in the production fluids were detected 6 weeks after injection.[19,20]

Most other biosurfactant-producing microorganisms are not suitable for MEOR applications due to reservoir conditions. However, some thermotolerant *Pseudomonas aeruginosa* strains have been isolated from injection waters and found effective in displacing trapped oil both in laboratory tests and within low-temperature reservoirs.[21,22] Rhamnolipid biosurfactants produced by this species are very active compounds, with a critical micelle concentration (CMC) of 70 mg/liter, stable at high temperatures up to 90˚C, best performing at lower pH and only slightly affected by salinity and calcium ions. The use of *P. aeruginosa* for in situ MEOR techniques is however limited for several reasons: (i) it is classified as risk-group 2 organism with restriction and regulation on its handling and dispersion into the environment; (ii) rhamnolipid synthesis is controlled by a complicated quorum-sensing system related to environmental stimuli; (iii) it is typically an aerobic mesophile that could not be actively growing under reservoir conditions. The possibility to overcome such limitations by engineering microorganisms in order to produce rhamnolipids in situ has been suggested and cloning biosynthetic genes into host organisms was attempted with limited success.[23-25]

Synthesis of biosurfactants under anaerobic conditions is of particular interest for application of MEOR processes, though most biosurfactant-producing microorganisms are strictly aerobic or facultative anaerobes. Few strictly anaerobic bacteria have been so far characterised as biosurfactant-producers. *Anaerophaga thermohalophila* strain Fru22$^T$ (DSM 12881$^T$) for example, is a strictly anaerobic bacterium able to grow at elevated temperature (50˚C) and high salinity (7.5% w/v NaCl) while producing a surface active compound preliminary characterized as a low-molecular weight lipopeptide (<12 kDa) which may include sugar moieties. Although no further attempt of investigating oil displacing activity has been reported, on the basis of its unique physiological properties strain Fru22$^T$ appears to be a good candidate for in situ MEOR.[26]

Mixed microbial consortia can be particularly effective for in situ treatments as they offer a broader range of activities and products in comparison with single species. A recently patent "MMMAP" (Multi-strain Mixed Microbial Application) consisting of thermophilic, barophilic, acidophilic and anaerobic strains belonging to *Thermoanaerobacterium* sp., *Thermotoga* sp. and *Thermococcus* sp. isolated from oil well water is claimed to be active in producing biosurfactants, fatty acids, alchools, methane and carbon dioxide at in situ temperature up to 90˚C. Its injection into wells supplemented with specific nutrients resulted in 3-fold increased oil recovery.[27]

## Stimulation of Indigenous Biosurfactant-Producing Microorganisms Within Oil Reservoirs

The third strategy of MEOR is based on the concept that oil reservoirs are inhabited by indigenous microbial communities able to grow or survive under extreme conditions. Knowledge of such microbial ecosystems is still limited due to obvious difficulties in collecting representative samples as well as carrying out in situ analyses. Therefore whether indigenous microorganisms are native or contaminants exogenously introduced through water flooding, drilling or other oil well operations is still to be confirmed as well as their metabolism and activities established.[15]

Technologies involving injection of nutrient solutions (e.g., carbon substrates and minerals) into the oil well to stimulate the resident microbial communities have long been known and are available on a commercial basis. Benefits such as enhanced oil recovery, reduced oil viscosity and prolonged well lifetime are generally claimed, though a scientific monitoring of in situ activities is difficult and untreated controls are impossible to include. For example, in recent field trials, Youssef et al[28] provided direct proof that the presence of biosurfactant-producing bacteria in a nutrient-stimulated oil well was likely due to exogenous contamination and therefore could not be maintained over the duration of the treatment. As a result, in the wells treated with only nutrients no significant surface activities were detected.

## MEOR Field Trials

The real potential of biosurfactants in MEOR applications can however be fully assessed only in field-scale. Several yet sporadic trials have been carried out during the past years and tentatively reviewed.[29-31] The real impact of biosurfactant-based MEOR techniques however has never been estimated because of lack of both quantitative information regarding microbial processes in situ and consistency in data collection and processing. Only recently a small field-scale MEOR experiment provided for the first time data of in situ metabolism and activities. Molecular techniques combined with traditional methods showed that *Bacillus* strains injected into oil wells maintained activity, consuming the glucose and nutrients supplied and releasing $CO_2$ and fermentation products including a lipopeptide biosurfactant leading to an increased production estimated as one barrel of oil/day over 7 weeks after the treatment.[28]

## Crude Oil Transportation in Pipeline

Crude oil often needs to be transported over long distances from the extraction fields to the refineries. One of the major factors affecting pipelining is oil viscosity that slows the flow. Heavy oils in particular are characterised by viscosities ranging from 1000 cP to more than 100,000 cP at 25°C and cannot be transported through conventional pipelining systems that optimally requires viscosities of <200 cP. Heating or diluting with solvents were the traditional methods applied to reduce oil viscosity. However, a promising technology consisting of producing a stable oil-in-water emulsion that facilitates oil motility has been recently developed and introduced new routes to the application of the bioemulsifier-type of biosurfactants which have been found particularly suitable for this application. They are high-molecular weight surfactants characterised by different properties compared to glycolipids and lipopeptides. They are not effective in reducing interfacial tensions, but have excellent capability to stabilize oil-in-water emulsions. Due to the high number of reactive groups in the molecule, bioemulsifiers bind tightly to oil droplets and form an effective barrier that prevents drop coalescence. Among the bioemulsifiers, emulsan (Fig. 1) and its analogs synthesised by *A. venetianus* RAG-1, are certainly the most powerful, yet others such as alasan and biodipersan produced by different *Acinetobacter* strains have been extensively studied.[32]

Emulsan was applied in a field trial for pipeline transportation of a Boscan heavy crude oil of viscosity of about 200,000 cP. The bioemulsifier was used at a surfactant-oil ratio of 1:500 and produced a 70% w/w oil-in-water stable emulsion named hydrocabosol with viscosity reduced to 70 cP which was pumped through 380 miles over 64 hours. It was estimated that under optimal conditions the emulsion could have been transported for 26,000 miles.[33] Once transported to the refinery, hydrocarbosols can be either de-emulsified and utilized directly without de-watering or treated with specific enzymes called emulsanes to depolymerise the bioemulsifier thus breaking the emulsion before use.[34] To our knowledge there are no commercial applications of bioemulsifiers yet. Low-molecular weight biosurfactants can also be effective emulsifying agents. Rhamnolipids produced by *P. aeruginosa* strain USB-CS1 for example were able to emulsify a viscous crude oil to give an emulsion with viscosity reduced to less than 500 cP and stable for 14 days.[35]

In the case of waxy crude oils, their transportation is generally affected by the problem of paraffin precipitation that can cause numerous negative consequences from reduction and eventually block of the internal diameter of pipes to changes in the oil composition. Traditional techniques for treating wax included thermal, mechanical and chemical methods but all they failed to be fully successful as energy consuming, detrimental to the pipes and highly toxic respectively. Thus, over the past decade microbial treatments became an increasing valuable alternative.[36] Many bacteria are known to be able to grow on paraffinic hydrocarbons while producing biosurfactants that act as dispersing and solubilizing agents and make the paraffinic fractions more available for the up-take by cells. In this way not only wax deposits can be dissolved and prevented but also heavy crude oil fractions can be degraded by bacteria to lighter fractions.

Bacteria capable of degrading *n*-paraffins belong predominantly to *Pseudomonas* and *Bacillus* species and a mixed consortium was found particularly effective in the treatment of two paraffinic oils by Lazar et al.[37] Laboratory pilot tests were carried out by using a flow equipment containing ten liters of paraffinic oil to simulate a pipeline system. Bacterial consortium supplemented with brine and essential microelements (nitrogen and phosphorous) was circulated along with the oil for 5 days alternating flowing and stationary periods. Microbial activity was monitored and biosurfactant production was detected all through the experiment. As a result, the authors reported a decrease of total paraffin content up to 10% and consequently of the freezing points up to 7-9°C. The viscosities also resulted much lowered especially at low temperatures.

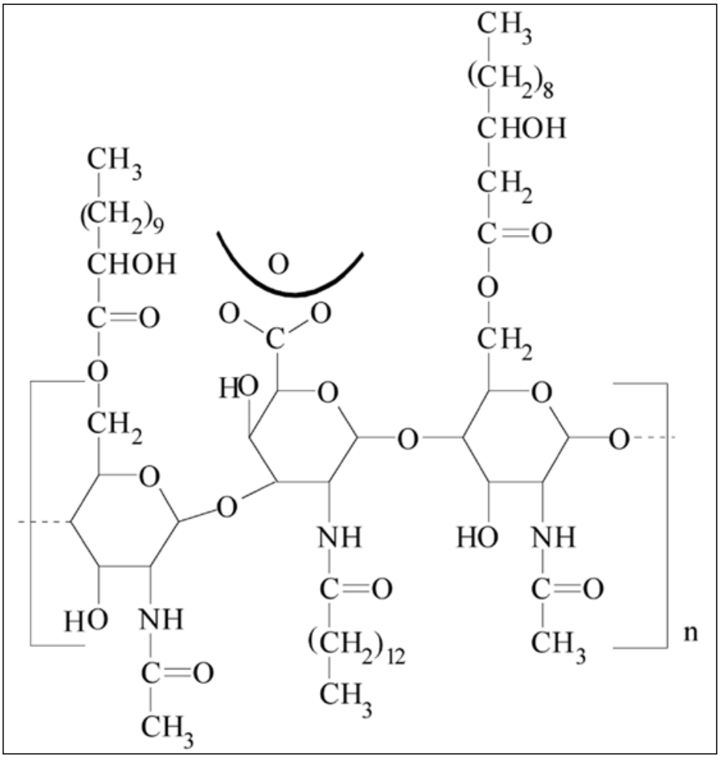

Figure 1. Structure of emulsan bioemulsifier produced by *A. venetianus* RAG-1. It is composed of a backbone of a repeating trisaccharide motif bound to fatty acid chains. Redrawn from ref. 3.

Biological solutions to paraffin control problem find nowadays concrete application. Several commercial bioproducts have been formulated over the past few years and are currently available in the market. Micro-Bac International for example (Round Rock, TX) is manufacturer of a wide product line containing a proprietary combination of natural microorganisms able to control paraffins of chain length ranging from $C_{16}$ up to $C_{60}$ through the production of biosurfactants and other metabolites.

## Clean-Up of Oil Containers/Storage Tanks

Large amounts of crude oil are daily moved and distributed to refineries with oil tankers, barges, tank cars and trucks, thus increasing the problem of the clean-up and maintenance of the containers.

A process for cleaning tanks used in oil transportation and storage by means of microbial bioemulsifiers was proposed for the first time in 1981 in a patent by Gutnick and Rosenberg.[38] The process included: (i) a washing phase with an aqueous solution of emulsan derivatives (α- and β-emulsans) produced by *A. venetianus* ATCC 31012 where an oil-in-water emulsion was induced by vigorous agitation into the tank; (ii) removal of such emulsion from the clean tank and (iii) recovering of the hydrocarbon residues by breaking the emulsion by physical or chemical methods. However, this potential application remained limited to this report as we are not aware of further development into a commercially available technology.

In 1991, Banat et al[39] described the application of microbial biosurfactants for the clean-up of oil storage tanks. Sludge and oil deposits normally accumulate at the bottom and on the walls of storage tanks thus requiring periodical cleaning operations. Traditional methods are generally manual, hazardous, time-consuming and expensive. Biosurfactants can effectively drive the cleaning activity as demonstrated in a field trial conducted at the Kuwait Oil Company. Two tonnes of rhamnolipid (Fig. 2) biosurfactant-containing culture broth were produced, sterilised and added to an oil sludge tank along with fresh crude oil and water and circulated continuously for 5 days at ambient temperature of 40-50°C. The oil sludge was effectively lifted and mobilised from thebottom of the tank and solubilised within the emulsion formed. The treatment recovered 91% of hydrocarbons in the sludge. The value of the recovered crude covered the cost of the cleaning operation.

Since then, long and accurate researches and experiments carried out over the years lead to a substantial improvement of such technique and the development of the BioRecoil® process patented in 2004 by Idrabel Italia (Italy) and Jeneil Biosurfactant Company (USA).[40]

The process consists of three main steps:

i. **Feasibility study.** Data collection, tank survey, evaluation of sludge composition and concentration, laboratory tests as well as risk assessment, environmental impact and cost analysis are initially carried out in order to set-up the optimal working conditions and design a tailor-made treatment.

ii. **Oil tank treatment.** A mixture composed of water, biosurfactant and fluidizing agent is circulated onto the tank until obtaining an uniform emulsion (Fig. 3a, b). Rhamnolipid biosurfactants are preferably used to this end as capable of efficiently dispersing heavy hydrocarbon fractions by means of both micro- and macro-emulsions, with consequent reduction of the sludge viscosity. When the circulation is stopped, the emulsion breaks and separates in an upper phase containing hydrocarbons and a lower phase containing water, while inorganic residual matter and sand sink to the bottom (Fig. 3c). The hydrocarbon fraction is recovered, analysed and, according to its specific characteristics, transferred to other storage tanks or alternatively to refining plants to be processed.

iii. **Disposal of wastes and residues.** The treatment ends with the safe disposal of the wastes (Fig. 3d). The water used in the process or extracted from the sludge, is sent to the wastewater facilities of the refinery and analysed for oil content, organic content (e.g., COD) and temperature before being discharged or reused. The inorganic phase that remains at the bottom of the tank and that is mainly composed of sediments, metal residues, sand or gravel is in practice the only material that needs to be disposed.

This process can offer numerous benefits including recovery of oil (generally >90%) and reduction of material to be disposed of (<5%), safer in situ operations, use of natural biosurfactant products hence high environmental compatibility and reduction in the tank downtime and risk of damage.

## Formulation of Petrochemicals

A totally unexplored area for potential applications of biosurfactants is the formulation of petrochemical products. Biotechnological alternatives to the existing bulk petroleum-derived products have generally failed for various reasons and mostly for not satisfying economic criteria.

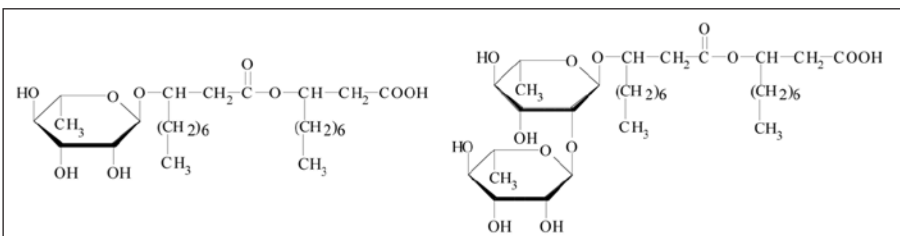

Figure 2. Structure of mono- and di-rhamnolipid produced by *P. aeruginosa* species. The predominant compounds are composed of one or two rhamnose units linked to two units of β-hydroxy-decanoic acid. Some minor congeners are also synthesised as part of a mixture.

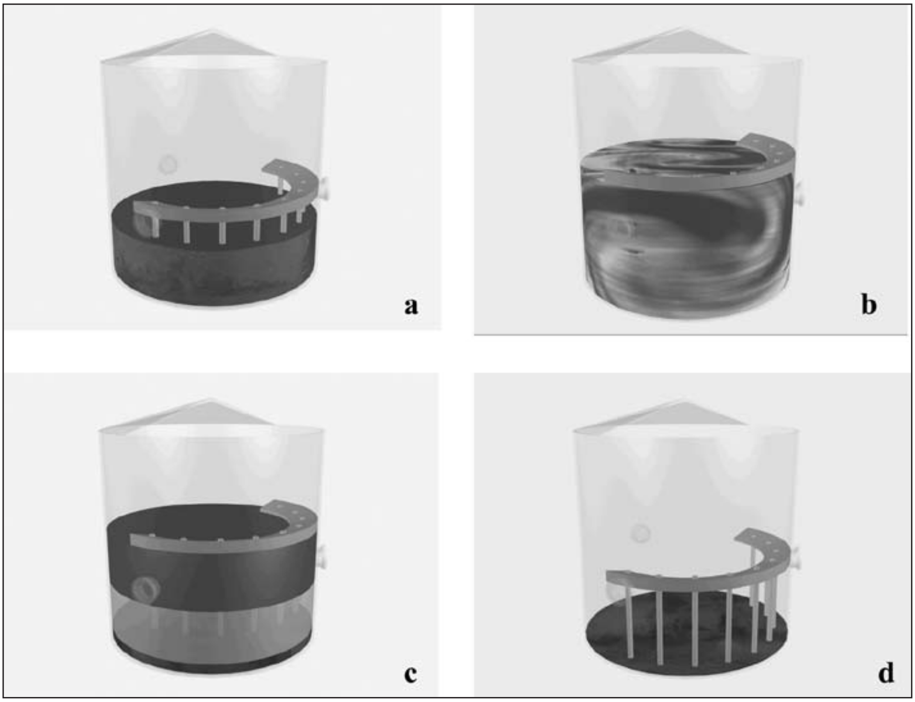


Figure 3. BioRecoil® process for the clean-up of oil storage tanks. Before the treatment, aged oil and residues are deposited at the bottom and on the walls of the tank (a). A rhamnolipid-containing solution is circulated and oil is mobilized and entirely emulsified (b). To end the treatment, emulsion separates in a hydrocarbon-containing upper phase and a lower water phase (c); the former is recovered, while the latter is discharged or reused in the refinery plant. Inorganic materials are safely disposed (d) and a final make-up of the tank can be applied if necessary. Courtesy of Idrabel Italia.

However, those market niches where environmental concern is a major factor might look at biotechnological solutions with increasing interest in the near future. One such area includes the manufacturing of emulsified fuels.

Diesel fuel blended with water has been known since the early 1900's and is currently applied especially in Europe for public transport fleets, marine engines, locomotives but also heat facilities in industrial and institutional complexes. The advantages of diesel emulsions are:

- Improved combustion efficiency due to the microexplotions of water particles;
- Reduction of emission of hazardous pollutants such as nitrogen oxides (≤25%), carbon oxide (≤5%), black smoke (≤80%) and particulate matter (≤60%);
- Reduction of diesel consumption.

An additional aspect is that such fuels are easily applicable without need of engine modification.

Emulsified fuels are technically water-in-diesel emulsions with a typical content of water of 10-20% (v/v). They are prepared using specific surfactant packages along with a variety of additives (e.g., detergents, lubricity enhancers, anti-foaming agents, ignition improvers, anti-rust agents and metal deactivators). Surfactants are expected to stabilize the emulsion and ensure that the finely dispersed water droplets remain in suspension within the diesel fuel (Fig. 4). Non-ionic surfactants such as alcohol ethoxylates, fatty acids ethoxylates and sugar esters of fatty acids are currently the most used.[41,42]

We investigated the possibility to replace traditional chemical compounds with microbial biosurfactants to formulate fuel or diesel emulsions. Preliminary experiments (unpublished data) were carried out in collaboration with Idrabel Italia (Genoa, Italy), in which rhamnolipid mixture produced by *P. aeruginosa* AP02-1 were used in order to prepare a water-in-diesel emulsion consisting of 15% water and 85% diesel (v/v). Five major parameters were evaluated: stability, density (optimally 0.76 to 0.79 g/cm$^3$ at 15°C), viscosity (optimally 2 to 4.5 mm$^2$/s at 40°C), water and sulphur content (optimally less than 2 mg/kg). Among them, emulsion stability was the most relevant factor as phase separation should not occur over 4 months. Stability depends on many factors both physicochemical (e.g., temperature, energy supply, order of mixing the components) and distinctively related to the surfactant properties. Bio-surfactant potential candidate was required to satisfy the following basic criteria:

- The molecule should contain only carbon, hydrogen and oxygen and be free of sulphur and nitrogen atoms. The absence of aromatic rings is further requisite;
- The hydrophilic-lipophilic balance (HLB) should be in the range of 3-6;
- It should be used in a very pure form. This may limit the potential use of biosurfactants from microorganisms due to the difficulties of achieving high-grade purification;
- It should have a very low critical micelle concentration (CMC);
- It should burn readily without release of soot.

We produced several diesel emulsions generally satisfying some of the test factors (density, viscosity and sulphur content); however they lacked in stability and had inadequate consistency. An excess of air content likely due to an inappropriate mixing was the main cause of the destabilization of the phase equilibrium. It is important to note however that rhamnolipid may not have been a suitable choice of biosurfactant in order to achieve a stable emulsion. One of the longer chain heteropolysaccharides and proteins emulsifying-type biosurfactants may have been a better candidate. Although further investigations will be needed, to the best of our knowledge this aspect of biosurfactant applications has not been reported before.

## Conclusions and Future Perspectives

During the past 20 years microbial biosurfactants and bioemulsifiers have been extensively investigated and their potential in most fields of the petroleum industry highlighted by the large number of related patents. Only few however had successful commercialization mainly due to the well known problem of the high production costs. Several other aspects should be taken into consideration to realise their potentials. Though many different types of biosurfactants have been described from a variety of microorganisms, the literature focused predominantly on *Bacillus* sp.,

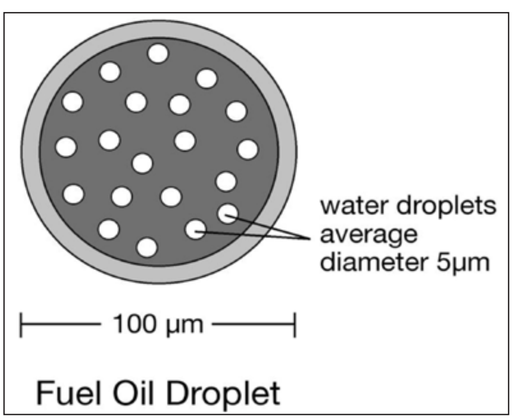


Figure 4. Typical aspect of a drop of emulsified diesel with dispersed microdroplets of water. Surfactants control the water droplets size and prevent their coalescence.

*Pseudomonas* and *Acinetobacter* sp. A number of other promising genera are known and should be closely examined. For example, *Rhodococcus* sp. produces trehalose lipid-type biosurfactants mainly during the growth in presence of hydrocarbons but limited efforts to evaluate their potential utility in petroleum industry have been carried out. More attention should also be directed towards extremophilic and hyper-extremophilic biosurfactant-producing microorganisms to allow use in oil field conditions. Although the biotechnological importance of such microbial groups is well documented with regards to enzymes (extremozymes) in particular, lack of information about production of bioactive compounds remains.

Further progress is expected to be achieved when more advanced methods are developed and applied. Molecular techniques and in particular gene expression monitoring would significantly contribute to the detection and control of activities and processes in situ and in real time. To this end the current knowledge of biosurfactant genes is still insufficient and needs to be explored with the aim of gaining better control of the production technologies and improvement of products yields.

## References

1. Hall C, Tharakan P, Hallock J et al. Hydrocarbons and the evolution of human culture. Nature 2003; 426:318-322.
2. Kanicky JR, Lopez-Montilla JC, Pandey S et al. Surface chemistry in the petroleum industry. In: Holmberg K, ed. Handbook of applied surface and colloid chemistry. John Wiley and Sons Ltd, 2001:251-267.
3. Desai J, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol Mol Biol Rev 1997; 61:47-64.
4. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 2000; 53:495-508.
5. Van Hamme JD, Singh A, Ward OP. Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv 2006; 24:604-620.
6. Singh A, Van Hamme JD, Ward OP. Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnol Adv 2007; 25:99-121.
7. Planckaert M. Oil reservoirs and oil production. In: Ollivier B, Magot M, ed. Petroleum Microbiology. Washington DC: ASM Press, 2005:3-19.
8. Van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 2003; 67:503-549.
9. Jenneman GE, McInerney MJ, Knapp RM et al. A halotolerant, biosurfactant-producing bacillus species potentially useful for enhanced oil recovery. Dev Ind Microbiol 1983; 24:485-492.
10. Folmsbee M, Duncan K, Han SO et al. Re-identification of the halotolerant, biosurfactant-producing bacillus licheniformis strain JF-2 as Bacillus mojavensis JF-2. Syst Appl Microbiol 2006; 29:645-649.
11. McInerney MJ, Javaheri M, Nagle DP Jr. Properties of the biosurfactant produced by bacillus licheniformis strain JF-2. J Ind Microbiol 1990; 5:95-101.
12. McInerney MJ, Maudgalya SK, Knapp R et al. Development of biosurfactant-mediated oil recovery in model porous systems and computer simulations of biosurfactant-mediated oil recovery. Topical report accessed January 2008 from http://www.osti.gov/bridge/servlets/purl/834170-BP4QI4/native/834170.pdf
13. Daoshan L, Shouliang L, Yi L et al. The effect of biosurfactant on the interfacial tension and adsorption loss of surfactant in ASP flooding. Colloids and Surfaces A: Physicochem Eng Aspects 2004; 244:53-60.
14. Gutnick D, Rosenberg E, Belsky I et al. ψ-emulsans. US Patent 4,380,504 1983.
15. Magot M. Indigenous microbial communities in oil fields. In: Ollivier B, Magot M, ed. Petroleum Microbiology. Washington DC: ASM Press, 2005:21-33.
16. Javaheri M, Jenneman GE, McInerney MJ et al. Anaerobic production of a biosurfactant by bacillus licheniformis JF-2. Appl Environ Microbiol 1985; 50:698-700.
17. McInerney MJ, Jenneman GE, Knapp RM et al. Biosurfactant and enhanced oil recovery. US Patent 4,522,261, 1985.
18. Bryant RS. Microbial enhanced oil recovery and compositions therefor. US Patent 4,905,761, 1990.
19. Bryant RS, Burchfield TE, Dennis DM et al. Microbial-enhanced waterflooding: Mink unit project. SPE Reservoir Eng 1990; 5:9-13.
20. Bryant RS, Stepp AK, Bertus KM et al. Microbial-enhanced waterflooding field pilots. Dev Petrol Sci 1993; 39:289-306.
21. Rocha C, San-Blas F, San-Blas G et al. Biosurfactant production by two isolates of pseudomonas aeruginosa. World J Microbiol Biotechnol 1992; 8:125-128.
22. Li Q, Kang C, Wang H et al. Application of microbial enhanced oil recovery technique to daqing oilfield. Biochem Eng J 2002; 11:197-199.

23. Cabrera-Valladares N, Richardson AP, Olvera C et al. Monorhamnolipids and 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs) production using escherichia coli as a heterologous host. Appl Microbiol Biotechnol 2006; 73:187-194.
24. Ochsner UA, Koch AK, Fiechter A et al. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in pseudomonas aeruginosa. J Bacteriol 1994; 176:2044-2054.
25. Wang Q, Fang X, Bai B et al. Engineering bacteria for production of rhamnolipid as an agent for enhanced oil recovery. Biotechnol Bioeng 2007; 98:842-853.
26. Denger K, Warthmann R, Ludwig W et al. Anaerophaga thermohalophila gen nov, sp nov, a moderately thermohalophilic, strictly anaerobic fermentative bacterium. Int J Syst Evol Microbiol 2002; 52:173-178.
27. Lal B, Reddy MRV, Agnihotri A et al. Process for enhanced recovery of crude oil from oil wells using novel microbial consortium. US Patent 20070092930, 2007.
28. Youssef N, Simpson DR, Duncan KE et al. In situ biosurfactant production by bacillus strains injected into a limestone petroleum reservoir. Appl Environ Microbiol 2007; 73:1239-1247.
29. Banat IM. Biosurfactant production and possible uses in microbial enhanced oil recovery and oil pollution remediation—a review. Bioresour Technol 1995; 51:1-12.
30. Bryant R. Potential use of microorganisms in petroleum recovery technology. Proc Okla Acad Sci 1987; 67:97-104.
31. McInerney MJ, Nagle DP, Knapp RM. Microbially enhanced oil recovery: past, present and future. In: Ollivier B, Magot M, ed. Petroleum Microbiology. Washington DC: ASM Press, 2005:215-237.
32. Gutnick DL, Shabtai Y. Exopolysaccharide bioemulsifiers. In: Kosaric N, Cairns WL, Gray NCC, ed. Biosurfactants and biotechnology. New York: Marcel Dekker Inc, 1987: 211-246.
33. Hayes ME, Hrebenar KR, Murphy PL et al. Bioemulsifier-stabilized hydrocarbosols. US Patent 4,943,390, 1990.
34. Hayes ME, Hrebenar KR, Murphy PL et al. Combustion of viscous hydrocarbons. US Patent 4,684,372, 1987.
35. Rocha CA, Gonzalez D, Iturralde ML et al. Production of oily emulsions mediated by microbial tenso-active agent. US Patent 6,060,287, 2000.
36. Etoumi A. Microbial treatment of waxy crude oils for mitigation of wax precipitation. J Pet Sci Eng 2007; 55:111-121.
37. Lazar I, Voicu A, Nicolescu C et al. The use of naturally occurring selectively isolated bacteria for inhibiting paraffin deposition. J Pet Sci Eng 1999; 22:161-169.
38. Gutnick D, Rosenberg E. Cleaning oil-contaminated vessels with $\alpha$-emulsans. US Patent 4,276,094, 1981.
39. Banat IM, Samarath N, Murad M et al. Biosurfactant production and use in oil tank clean-up. World J Microbiol Biotechnol 1991; 7:80-84.
40. Pesce L. A biotechnological method for the regeneration of hydrocarbons from dregs and muds, on the basis of biosurfactants. European Patent EP1427547, 2004.
41. Clark RH, Morley C, Stevenson PA. Diesel fuel compositions. US Patent 7,229,481, 2007.
42. Lif A, Holmberg K. Water-in-diesel emulsions and related systems. Adv Colloid Interface Sci 2006; 123-126:231-239.