Rhamnolipid biosurfactants—past, present, and future scenario of global market

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RHAMNOLIPIDS—BRIEF OUTLINE

Biosurfactants, widely known as surfactants of biological origin, have carved a niche for themselves in the market due to their unique environmentally-friendly properties. They have come a long way since first biosurfactant “surfactin” was purified and characterized by Arima et al. (1968). Biosurfactants have been researched thoroughly and satisfactorily since then by many research groups across the world yet there are aspects that elude our understanding. There are five major categories of biosurfactants viz. glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric biosurfactants and particulate biosurfactants that have found applications in agricultural, pharmaceuticals, food, cosmetics, and detergent industries. Data reveals there are more than 250 patents obtained on these wonder biodegradable molecules so far (Shete et al., 2006; Rahman and Gakpe, 2008). It has also been observed that microbial biosurfactants are advantageous over plant-based surfactants due to the scale-up capacity, rapid production, and multifunctional properties. Several plant-based biosurfactants for example saponins, lecithins, and soy proteins have excellent emulsification properties but are expensive to produce at industrial scale and have other debatable issues such as solubility and hydrophobicity (Xu et al., 2011).

Among the various categories of biosurfactants the glycolipid biosurfactants “rhamnolipids” stand apart. Rhamnolipid, primarily a crystalline acid, is composed of β-hydroxy fatty acid connected by the carboxyl end to a rhamnose sugar molecule. Rhamnolipids are predominantly produced by Pseudomonas aeruginosa and classified as: mono and di-rhamnolipids. Other Pseudomonas species that have been reported to produce rhamnolipids are P. chlororaphis, P. plantarii, P. putida, and P. fluorescens. Some bacteria are known to produce only mono-rhamnolipids while some produce both. The ratio of mono and di-rhamnolipid can also be controlled in the production method. There are enzymes available that can convert mono-rhamnolipids into di-rhamnolipids. In 1984, the first patent for the production of rhamnolipids was filed by Kaeppeli and Guerra-Santos (US 4628030) and obtained in 1986 for their work on Pseudomonas aeruginosa DSM 2659 (Kaeppeli and Guerra-Santos, 1986). Subsequently, Wagner et al. filed a patent (US 4814272) in 1985 for the biotechnical production of rhamnolipids from Pseudomonas sp. DSM 2874 and obtained the same in 1989 (Wagner et al., 1989). In the past close to three decades, there has been a great body of research work carried on rhamnolipids revealing many of their astonishing applications and making them reach the pinnacle of popularity among all the categories of biosurfactants in the global market. The reason behind the current global interest in rhamnolipid production owes to their broad range of applications in various industries along with many spectacular “eco-friendly” properties.

The current critique articulates to present opinion on rhamnolipid research and is an attempt to retrospect what brings rhamnolipids in the forefront. This article is a bird’s eye view on a timeline of rhamnolipids story so far and also a critical analysis on why despite so many patents and research work rhamnolipids still do not rule the global biosurfactant market.

INIMITABLE APPLICATIONS OF RHAMNOLIPIDS

Over the years rhamnolipids are becoming broadly pertinent in various industries and are posing a serious threat to the synthetic surfactants. Before venturing into the current production economics of rhamnolipids it is imperative to evaluate the major applications of rhamnolipids that make them noticeable among other biosurfactants. A list of five major applications of rhamnolipids that cater to the wide range of industrial demands includes:

1) Bioremediation and enhanced oil recovery (EOR): Rhamnolipids show excellent emulsification properties, efficiently remove crude oil from contaminated soil and facilitate bioremediation of oil spills (Rahman et al., 2003; Costa et al., 2010).

2) Pharmaceuticals and therapeutics: Rhamnolipids show low toxicity, surface active properties and antimicrobial activities against several microbes ( Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes) thereby showing promising applications in pharmaceuticals.
| S. No. | Company                          | Location(s) | Product(s)                                                                 | Focus on                                                                                     |
|-------|---------------------------------|-------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| 1     | TeeGene Biotech                 | UK          | Rhamnolipids and Lipopeptides                                              | Pharmaceuticals, cosmetics, antimicrobials and anti-cancer ingredients                      |
| 2     | AGAE Technologies LLC           | USA         | Rhamnolipids (R95, an HPLC/MS grade rhamnolipid)                           | Pharmaceutical, cosmeceutical, cosmetics, personal care, bioremediation (in situ & ex situ), Enhanced oil recovery (EOR) |
| 3     | Jeneil Biosurfactant Co. LLC    | USA         | Rhamnolipids (ZONIX, a bio-fungicide and RECO, a rhamnolipid used in cleaning and recovering oil from storage tanks) | Cleaning products, EOR                                                                     |
| 4     | Paradigm Biomedical Inc.        | USA         | Rhamnolipids                                                              | Pharmaceutical applications                                                               |
| 5     | Rhamnolipid Companies, Inc.     | USA         | Rhamnolipids                                                              | Agriculture, cosmetics, EOR, bioremediation, food products, pharmaceutical                |
| 6     | Fraunhofer IGB                  | Germany     | Glycolipids, Cellulose lipids, MELs                                      | Cleansing products, shower gels, shampoos, washing-up liquids, pharmaceutical (bioactive properties) |
| 7     | Cognis Care Chemicals           | China, Germany, USA | Alkyl polyglucoside APG®, Plantacare 1200 GLY (green surfactant for use in oral-dental formulations), Rheocare TTA (for cleansing formulations) | Used in formulations for household cleaners, bath/shower gels, dish washing, laundry detergents and in agrochemicals |
| 8     | Saraya Co. Ltd.                 | Japan       | Sophorolipids (Sophoron, a low-foam dishwasher detergent)                 | Cleaning products, hygiene products                                                        |
| 9     | Ecover Belgium                  | Belgium     | Sophorolipids                                                             | Cleaning products, cosmetics, bioremediation, pest control, pharmaceuticals               |
| 10    | Groupe Soliance                 | France      | Sophorolipids                                                             | Cosmetics                                                                                  |
| 11    | MG Intobio Co. Ltd.             | South Korea | Sophorolipids (Sopholine—functional soap with Sophorolipids secreted by yeasts) | Beauty and personal care, bath supplies e.g., soaps with new functions                     |
| 12    | Syntexyme LLC                   | USA         | Sophorolipids                                                             | Cleaning products, cosmetics, food products, fungicides, crude oil emulsification          |
| 13    | Allied Carbon Solutions (ACS) Ltd | Japan     | Sophorolipids (ACS-Sophor—first bio-based surfactant from Indian mahua oil) | Agricultural products, ecological research                                                  |
| 14    | Henkel                          | Germany     | Sophorolipids, Rhamnolipids, Mammoslyerthritol lipids                    | Glass cleaning products, laundry, beauty products                                         |
| 15    | Lion Corporation                | Japan       | Methyl ester sulfonates (MES)                                             | Detergents formulations, cleaning products                                                 |
| 16    | Lipo Chemicals                  | USA         | Lipomulse Luxe (high-temperature resistance emulsifier)                   | Skin care, sun-lots hair care formulations, thickening polymers, rheological modifiers, natural gums |
| 17    | Kaneka Co.                      | Japan       | Sophorose lipids                                                          | Cosmetics and toiletry products                                                           |
and therapeutics (Magalhaes and Nitschke, 2013).

(3) **Cosmetics:** Rhamnolipid as an active ingredient is found to be effective for several skin treatments i.e., wound healing with reduced fibrosis, cure of burn shock, treatment of wrinkles hence are in demand in the health and beauty industry (Piljac and Piljac, 2007).

(4) **Detergents and cleaners:** Rhamnolipids are natural emulsifiers and surface active agents leading to their wide spread usage in detergent compositions, laundry products, shampoos and soaps (Parry et al., 2013).

(5) **Agriculture:** Rhamnolipids are already used for soil remediation for improving soil quality and are now further getting explored for plant pathogen elimination, for aiding the absorption of fertilizers and nutrients through roots and as biopesticides (Sachdev and Cameotra, 2013).

### BIOSURFACTANT PRODUCING COMPANIES—WITH FOCUS ON RHAMNOLIPIDS

Rhamnolipids are highly applicable in various activities with some researchers advancing the technology from laboratory to higher scale. However, there still are very limited companies in the field which are producing biosurfactants at a marketable scale. We tried to compile a list of biosurfactant producing companies around the globe (Table 1). The compilation evidently defines which biosurfactants are mostly researched and produced at higher scale.

### AVAILABILITY OF FEEDSTOCK AND ITS IMPACT ON BIOSURFACTANTS

Biodiesel is produced by the trans-esterification of vegetable oils and fats with methanol in the presence of a catalyst. Glycerol is received as a by-product from this reaction. The production of 1 ton biodiesel generates about 100 kg of glycerol. Hence, the European biodiesel industry might release about 600 Kiloton glycerol per year with an increasing tendency in Europe and worldwide. Oversupply of glycerol, essentially due to increasing biodiesel production, leads to decreasing prices and weak markets.

The price of pure glycerol varied from $0.50 to $1.50/lb and crude glycerol from $0.04/kg to $0.33/kg over the past few years. The price of glycerol in the market will continue to drop in such an over saturated market. Currently, the main supply of glycerol coming into the market is from the rapidly growing biodiesel industry. Estimated production of glycerol would reach 5.8 billion pounds in 2020. This is due to demand of biodiesel that is projected at 8 billion gallons in 2020 (Ayoub and Abdullah, 2012). Hence new outlets for glycerol are urgently needed, particularly in the case of crude glycerol released by the biodiesel processes. As glycerol is a nontoxic, edible, biodegradable compound, it will provide important environmental benefits to the new platform products.

In case of biosurfactant production, dramatically rising in biodegradable, nontoxic and eco-friendly alternative for chemical surfactants and the re-discovered opportunity of biosurfactants that gave rise to invention and investment ahead of the typical rigors of techno-economic modeling for the use of glycerol as a feed stock, leading typically to unmet expectations. Bacteria produce biosurfactants if grown on carbon sources such as glucose, glycerol, and various vegetable oils. Our research on biosurfactant production by bacteria indicates that glycerol can be used efficiently for biosurfactant production (Rahman et al., 2002).

The considerable interest in biosurfactants in the recent years is also due to their low toxicity, biodegradable nature and specificity, which would be very suitable to meet the European Surfactant Directive. Regulation EC No.: 648/2004 requires clear and precise description of the biodegradability of the surfactant and test methods to give assurance of its aerobic biodegradability. This regulation establishes rules designed to achieve the free movement of detergents and surfactants for detergents in the internal market while, at the same time, ensuring a high degree of protection of the environment and human health.

Surfactants constitute an important class of industrial chemicals and are widely used in almost every sector of modern industry. Most of the commercially available surfactants are chemical surfactants mainly, petroleum-derived. However, rapid advances in biotechnology and increased environmental awareness among consumers combined with expected new environmental legislation has provided further impetus for serious consideration of biological surfactants as possible alternatives to existing products.

### BIOSURFACTANT’S ECONOMIC FEASIBILITY—WHAT IT TAKES TO BECOME A MARKET LEADER?

As described in the previous section, there is enormous awareness among the consumers these days with regard to sustainability and global warming. The demand for bio-based technologies is ever increasing and “green solutions” are sought for every process. Rhamnolipids have promising properties and fulfill the eco-friendly criteria, one of the main

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### Table 2 | Cost of biosurfactant per liter of solution (diluted and the CMC based cost calculation carried out by Connolly et al., 2010).

| Biosurfactant | Origin | Supplier | ST mN/m | CMC (%) | Cost (£/L) |
|---------------|--------|----------|---------|---------|------------|
| BioFuture     | Bacterial rhamnolipid | BioFuture Ltd. Dublin | 28      | 0.08    | 0.02       |
| Citrasolv     | Orange peel | Cleveland Biotech Ltd., Teesside | 30      | 0.9     | 0.01       |
| EC601         | Bacterial rhamnolipid | Ecochem Ltd., Canada | 29      | 0.2     | 0.23       |
| EC1800        | Bacterial consortium | Ecochem Ltd., Canada | 28      | 0.04    | 0.01       |
| Petrosolv     | Bacterial unknown | Enzyme Technologies Inc., USA | 34      | 0.2     | 0.01       |
| Saponin       | Plant bark | Sigma UK | 45      | 0.1     | 0.50       |

The table also gives the origin of biosurfactant along with surface tension (ST) and critical micelle concentration (CMC) values.
Table 3 | A timeline and the major patents and grants obtained on rhamnolipids so far.

| S. No. | Patent or Application No. | Filed      | Issued     | Title                                                                 | Inventors                           |
|--------|---------------------------|------------|------------|----------------------------------------------------------------------|-------------------------------------|
| 1      | 4628030                   | Aug 1984   | Dec 1986   | Process for the production of rhamnolipids                           | Kaeppeli and Guerra-Santos          |
| 2      | 4814272                   | Feb 1985   | March 1989 | Process for the biotechnical production of rhamnolipids including rhamnolipids with only one. Beta-hydroxydecanoic acid residue in the molecule | Wagner et al.                       |
| 3      | 4933281                   | March 1987 | June 1990  | Method for producing rhamnose                                       | Daniels et al.                      |
| 4      | 4902512                   | Jan 1988   | Feb 1990   | Rhamnolipid liposomes                                                | Ishigami et al.                     |
| 5      | 5417879                   | Sep 1993   | May 1996   | Synergistic dual-surfactant detergent composition containing sophoroselipid | Hall et al.                         |
| 6      | 5455232                   | April 1994 | Oct 1995   | Pharmaceutical preparation based on rhamnolipid                      | Piljac and Piljac                   |
| 7      | 5550227                   | May 1994   | Aug 1996   | Method for the preparation of rhamnose monohydrate from rhamnolipids  | Mixich et al.                       |
| 8      | 5466675                   | July 1994  | Nov 1995   | Immunological activity of rhamnolipids                               | Piljac and Piljac                   |
| 9      | 5520839                   | Jan 1995   | May 1996   | Laundry detergent composition containing synergistic combination of sophorose lipid and non-ionic surfactant | Hall et al.                         |
| 10     | 5501966                   | Jan 1995   | March 1996 | *Pseudomonas aeruginosa* and its use in a process for the biotechnological preparation of L-rhamnose | Giani et al.                       |
| 11     | 5658793                   | June 1995  | Aug 1997   | *Pseudomonas aeruginosa* and its use in a process for the biotechnological preparation of L-rhamnose | Giani et al.                       |
| 12     | 5514661                   | Aug 1995   | May 1996   | Immunological activity of rhamnolipids                               | Piljac and Piljac                   |
| 13     | 5767090                   | Jan 1996   | June 1998  | Microbiologically produced rhamnolipids (biosurfactants) for the control of plant pathogenic zoospore fungi | Stanghellini et al.                |
| 14     | 7129218                   | Aug 2000   | Oct 2006   | Use of rhamnolipids in wound healing, treatment and prevention of gum disease and periodontal regeneration | Stipcevic et al.                   |
| 15     | 7262171                   | Aug 2000   | Aug 2007   | Use of rhamnolipids in wound healing, treating burn shock, atherosclerosis, organ transplants, depression, schizophrenia and cosmetics | Piljac and Piljac                   |
| 16     | 20040224905               | May 2002   | Nov 2004   | Use of rhamnolipids in wound healing, treatment and prevention of gum disease and periodontal regeneration | Stipcevic et al.                   |
| 17     | 20060233935               | Nov 2003   | Oct 2006   | Rhamnolipids in bakery products                                      | Hæsendorf and Vanzeveren           |
| 18     | 7202063                   | Aug 2005   | April 2007 | Processes for the production of rhamnolipids                        | Gunther et al.                      |
| 19     | 20070191292               | Feb 2006   | Aug 2007   | Antimycotic rhamnolipid compositions and related methods of use       | Gandhi et al.                      |
| 20     | 20070155678               | Feb 2007   | July 2007  | Use of rhamnolipids in wound healing, treating burn shock, atherosclerosis, organ transplants, depression, schizophrenia and cosmetics | Piljac and Piljac                   |
| 21     | 20070207930               | Feb 2007   | Sep 2007   | Rhamnolipid compositions and related methods of use                   | Gandhi et al.                      |
| 22     | 7968499                   | Feb 2007   | June 2011  | Rhamnolipid compositions and related methods of use                   | Gandhi and Skebba                  |
| 23     | 20080213194               | July 2007  | Sep 2008   | Rhamnolipid-based formulations                                       | Keith DeSanto                       |

(Continued)
drivers, but are still struggling to become market leaders. The economics of production is a major bottleneck in the outburst of commercialization of rhamnolipids and other biosurfactants (Table 2). There is still no downstream technology economical and convincing enough to recover and purify rhamnolipids at industrial scale. In case of biosurfactant production the downstream processing accounts for 70–80% of the entire production costs.

It is a no-brainer that in order to gain higher profit at commercial scale it requires access to very cheap feedstock. There are some other key parameters that need thorough consideration in order to make any product economically feasible. Technological fit and process optimization are among the main drivers. Fermentation time is another key to success. Fermentation performance and scale impact process economy as it is directly related to the yield, titer, and productivity. High cost of production especially because of the expensive substrates and downstream processes makes it difficult
to bring down the price of these environment-friendly biomolecules. In order to compete with the synthetic detergents or surfactants the cost of production must be brought down to £1.70 per liter which is in itself a challenging task. As there are many barriers in the commercialization of biosurfactants, there seems no dearth of opportunities in this field. Cost comparison of various technologies viz. enzymatic, continuous, shake flask, batch, and fed-batch used for biosurfactant production pinpoint the requirement of innovative methods wherein rhamnolipids can be produced in static conditions to reduce the fermentation cost. The operating costs can be brought down by robust wild-type strains or recombinant mutant strains. Testing the possibility of co-products and/or enzymes is another attractive solution to surge the net profit—for example: esterases released during the production of lipopeptides by Bacillus strain and its recombinants (Sekhon et al., 2011, 2012). Co-products and by-products are value drivers and increase the economic viability of any business. The search of cheap and easily accessible raw material or substrate for biosurfactants production has been going on for years. The utilization of by-products, even if from a different process could be another smart solution—for example: glycerol, which is a by-product of biodiesel production, is available in surplus amount in the global market (Albarelli et al., 2011) which might be a cheap alternative for biosurfactant production.

Rhamnolipids are well-characterized and scientifically proven biosurfactants which are slowly and steadily becoming highly sought after biomolecules. Among other biosurfactants rhamnolipids have the highest number of patents (Table 3) and research publications. However, cost-competitiveness is one of the major factors that is holding rhamnolipids back from becoming the champions of their field. Research needs to be focused on suitable vigorous production strains, cheap substrates and nominal bioreactor technology. The current market price of rhamnolipid (R-95, 95%) is $227/10 mg (Sigma-aldrich) and $200/10 mg (AGAE technologies, USA) calling for strenuous research. Rhamnolipids have favorable applications in various sectors and if made economically sustainable nothing can stop these biomolecules to rule the surface-active compounds market.

CONCLUDING REMARKS
As the Health and Safety in the bioprocessing become paramount for large scale production there are significant interests in the search for novel non-pathogenic rhamnolipid producers. The numbers of cultured organisms from the environmental samples are only a tiny fraction (0.001%) of the actual microbial diversity. There are significant number of microbial isolates that needs to be explored and exploited for rhamnolipid and other bioprocess manufacturing. Biosurfactant producing probiotic organisms will play a key role in the future of biosurfactant market. Edible emulsifiers from these processes would be applicable to many applications including food, cosmetic, environmental clean-up, biomedical and natural therapy. Rhamnolipid could be a potential alternative for the synthetic surfactant molecules and an important platform chemical cluster with the market value of $2.8 billion in 2023 (Grand View Research Inc., 2014). There is a significant need for the discovery of novel non-pathogenic rhamnolipid producers with enhanced production capacity and efforts to scale up through bioprocess engineering are important to meet the future predictions of biosurfactants market.

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