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Application of Biosurfactants as Sustainable Environmental Remediation

C.N. Mulligan[1]

[1] *Professor and Director, Concordia Institute of Water, Energy and Sustainable Systems, Concordia University, Montreal, Canada, email: catherine.mulligan@concordia.ca*

ABSTRACT

Sustainable management options for contaminated soils are required and will be evaluated. In situ remediation could be beneficial over ex situ technologies due to a reduction in costs and lack of solid disposal requirements. Selection of the most appropriate remediation technology must coincide with the environmental characteristics of the site and the ongoing sediment fate and transport processes. To be sustainable, the risk to human health and the environment at the site must be reduced, and not be transferred to another site. Cost-effectiveness and sustainable solutions are significant factors in determining the treatment. Both in situ and ex situ treatment approaches are available but decisions must be made based on the information available. The application of biosurfactants has been evaluated as alternatives to chemical reagents due to their surface active and emulsifying properties, low toxicity, biodegradability, unlimited applicability and relative low production cost for sustainable remediation. Studies showed that for effective application of biosurfactants, they should be selected based on pollutant characteristics and properties, treatment capacity, costs, regulatory requirements, and time constraints. Moreover, understanding of the mechanisms of interaction between biosurfactants and heavy metal and hydrocarbon contaminants or the contaminated environment can assist in selection of the appropriate biosurfactants for sustainable remediation. This paper will include research on various environmental applications of biosurfactants and future directions.

Keywords: contaminated soils, biosurfactants, sustainability, heavy metals, organic contaminants, remediation

1 INTRODUCTION

Contaminants are emitted into the soil environment from various sources including spills, leaks, accidents, improper storage or transport or improper management of wastes and landfills (Yong et al., 2014). Contaminants can be of inorganic or organic forms and some examples include petroleum hydrocarbons, metals, chlorinated hydrocarbons, pesticides, among others.

To restore soils and sediments, a variety of remediation techniques can be considered. The options are based on biological, chemical and/or physical methods (Yong et al., 2014). The selected management methods must not only be effective but must also be sustainable (Mulligan, 2019). Some of the criteria for achieving sustainable remediation should include effective use of resources and costs. Wastes and emissions generated must be minimized. In addition, non-toxic materials should be used as much as possible.

Two options are available for contaminated soil, in situ or ex situ treatment. Disposal after excavation (i.e., dig and dump) of the soil in a secure landfill or disposal facility is not sustainable. Treatment of the contaminated soil and subsequent reuse of the treated soil can be an expensive procedure, especially at large sites.

To determine the most appropriate sustainable technology, the procedure in Figure 1 is suggested. In general, available technologies are classified as physical, chemical and biological. In situ methods reduce transportation requirements compared to ex situ ones where the soil is excavated and treated. In situ processes include (a) bioremediation, (b) air or steam stripping or thermal treatment for extracting volatile compounds or flushing soluble contaminants (c) chemical oxidation treatments for oxidation and (d) stabilization/ solidification with additives such as lime or cement for heavy metals or organics. Phytoremediation involves the selection of the most suitable types of plants according to the pollutant. Ex situ techniques include excavation of the soil followed by biological treatment processes, solidification/stabilization, vitrification, incineration, physical separation, and/or washing. Other technologies related to nanotechnologies are also being developed (Babaee et al., 2018).

Some guidelines for site remediation exist to reduce environmental impacts (ASTM, 2013; ASTM, 2016). An ASTM guide (ASTM E2876-13) includes environmental, social and economic aspects. Reduction in the amounts of materials used, wastes generated and water impact are key elements of best management practices (BMPs). BMPs should be adopted to reduce the environmental footprint of the remediation. Suggestions for a greener cleanup BMPs are included in the guideline. For example, for pump and treatment, biobased products can be used such as biological surfactants. Mulligan (2014) has shown that biodegradable, non-toxic products called biosurfactants (e.g., rhamnolipids and sophorolipids) can be produced from waste materials and can be employed for soil flushing or washing for metal and organic contaminants or for enhanced biodegradation of organic pollutants. Figure 1 shows the process of sustainable remediation.

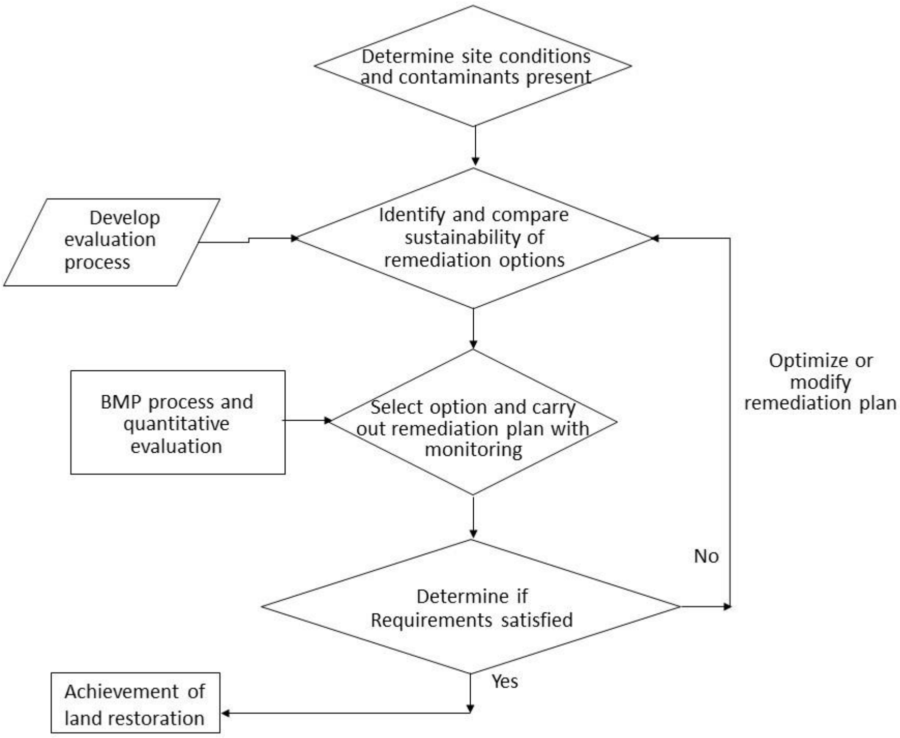

Figure 1. *Steps in a sustainable remediation process (adapted from Mulligan 2019)*

EPA (2009) has summarized how to incorporate sustainability objectives into the project. The core elements are:

- Protection of land and ecosystems
- Minimization of water use and the impact on water resources
- Reducing energy use and maximizing renewable energy
- Minimization of air pollutants and GHG emissions
- Reduction, reuse and recycling of wastes and materials.

Various types of footprint analysis or lifecycle assessment (LCA) can be performed to determine more sustainable options for remediation. The references of ISO 14044 (2006) or EPA documents (USEPA Life Cycle Assessment, Principles and Practice, EPA/600/R-06/060 (May 2006) or USEPA Methodology for Understanding and Reducing a Projects Environmental Footprint, EPA 540-R-12-002 (Feb. 2012b) can be used for these procedures.

Biosurfactants have shown potential for environmental applications for organic and/or inorganic contaminants. Therefore, the objectives of this paper are to demonstrate the use of biosurfactants for sustainable environmental remediation technologies and waste materials for their production and to identify future research directions.

2 BIOSURFACTANTS

2.1 Characteristics of biosurfactants

Remediation of contaminated soil and water with biosurfactants is promising due to their high biodegradability, metal affinity and effectiveness for promoting biodegradation, and low toxicity and critical micelle concentration (CMC). Various mechanisms of ion exchange, solubilization, mobilization and complexation enhance metal removal. Biosurfactants are produced by bacteria or yeast. The most common ones are anionic or neutral that are rhamnolipids, sophorolipids or lipopeptides (Biermann et al., 1987). CMCs typically vary between 1 and 200 mg/L and from 500 to 1500 daltons in molecular mass (Lang and Wagner, 1987). Production is from soluble carbohydrates, or hydrophobic, insoluble substrates such as oils.

2.2 Rhamnolipids

Most remediation studies have been performed with rhamnolipids. Positively charged metals can be removed by rhamnolipids added to soil and sediment as reviewed by Mulligan (2014b). Juwarkar et al. (2007) showed the rhamnolipid decreased toxicity and enhanced microbial activity of *Azotobacter* and *Rhizobium*, thus indicating improved soil quality. Toxicity reduction of the rhamnolipid treated soil was shown by the increase in biomass levels and survival of two species of worms (*Eisenia fetida* and *Lumbricus terrestris*) (Slizovsky et al., 2011).

It has also been demonstrated that anions of chromium and arsenic can also be removed by rhamnolipids. Hexavalent chromium extraction and reduction to Cr(III) was achieved in kaolinite, soil and water (Massara et al., 2007; Ara and Mulligan, 2015). Mining residues were also studied for removal of the As(V) form, at high pH by rhamnolipids (Wang and Mulligan, 2009b, Arab and Mulligan 2020). Cu, Zn, and Pb removal is also positively correlated with that of arsenic.

Surfactant can also be added as a foam (surfactant solution with injected air) which is more suitable of low permeability soil. It was found that a 0.5% rhamnolipid foam solution was beneficial for cadmium and nickel removal from a contaminated sandy soil (Mulligan and Wang, 2004) and for fresh water sediment treatment co-contaminated with polycyclic aromatic hydrocarbons (PAHs), Pb, Zn, and Ni (Alavi and Mulligan, 2011).

For batch experiments with rhamnolipids, the oil remediation efficiency was up to 84%, based on the experimental conditions. Optimum conditions to achieve the highest oil remediation performance included a rhamnolipid biosurfactant: nanoparticle ratio of 10:1 (wt%: wt%), pH 7, room temperature, and shaking speed of 60 rpm for 60 min. The remediation rate was improved by higher temperature and lower ionic strength. In the presence and absence of nanoparticles, rhamnolipid biosurfactant demonstrated a higher remediation efficiency than sophorolipid biosurfactant and ultraplex surfactant (Vu and Mulligan, 2022a). Other experiments with rhamnolipid and nanoparticles enabled maximum oil removal by the biosurfactant foam/nanoparticle, biosurfactant/nanoparticle, and biosurfactant-amended soil was about 3 times, 2.5 times, and 2 times more than the control, respectively (Vu and Mulligan, 2022b).

In another application, rhamnolipid with isolated microbial cultures from weathered oil could enhance the flocculation of the oil sands tailings compared to the control by 2.70 times (Mulligan and Roshtkhari, 2016). The mechanism involved an increase in hydrophobicity of the tailings particles, followed by adsorption of the biosurfactants and other organic compounds that enabled the formation of a bridge between particles prior to sedimentation. The sedimentation of the tailings would enable reduction in the volume of the ponds. The surface and interfacial tension reduction and encapsulation in micelles of the oil was the main dispersion mechanism. Another study evaluated the biodegradability of these petroleum products by indigenous oil degrading bacteria in the presence of biosurfactants (Saborimanesh and Mulligan, 2015). 16S rRNA pyrosequencing indicated that *Firmicute* was the dominant phylum.

2.3 Sophorolipids

The yeast *Starmella bombicola* (formerly known as *Candida* or *Torulopsis bombicola*) produces sophorolipids (Cooper and Paddock, 1984). The high yields make it a potentially the most cost-efficient biosurfactant. A crude sophorolipid enhanced metal removal from soils and sediments (Mulligan et al., 1999b, 2001). Washing mining tailings by the sophorolipids (Arab and Mulligan, 2020) removed increasing amounts of arsenic, copper, and iron, as the temperature increased from 15 to 23°C. Sophorolipid dispersion of biodiesel, diesel, and light crude-oil was studied by Saborimanesh and Mulligan (2018). *Actinobacteria* dominated in the diesel and *Proteobacteria* and *Actinobacteria in* the light crude oil. Addition of the sophorolipid with these cultures enabled dispersion and biodegradation of the hydrocarbons.

Nanoparticles such as zero valent iron (nZVI) have been used in combination with biosurfactants such as sophorolipids to remediate contaminated soil. The large specific surface area of nanoparticles allows excellent interaction with contaminants such as oil to improve the solubility and enhance removal rates (Vu and Mulligan, 2022a). The oil pollutants adsorb on the nanoparticle surfaces. The biosurfactant reduces aggregation of the nanoparticles which can reduce their effectiveness by decreasing the surface area and active sites of nanoparticles. The biosurfactants also enhances the solubilization of the oil. The combination of nZVI and sophorolipid was able to remove 83% of the oil in an hour. In the form of a foam the same combination reduces the oil concentration on the soil by 67% in 30 min (Vu and Mulligan, 2022a). A summary of selected biosurfactant studies is shown in Table 1.

Table 1. *Soil washing/flushing studies with biosurfactant addition*

Biosurfactant	Medium	Contaminant	References
Rhamnolipid, MEL, saponin	Soil, sediment	Zn, Cu, Pb, Oil	Mulligan et al. (2007)
Rhamnolipid	Soil, water	Cr	Ara and Mulligan (2015)
Rhamnolipid	Mining residues	As	Wang and Mulligan (2009a,b)
Rhamnolipid	Sediments	PAH, Pb, Zn, Ni	Alavi and Mulligan (2011)
Rhamnolipids + NPs*	Soil	Oil	Vu and Mulligan (2022b)
Sophorolipids +NPs	Soil	Oil	Vu and Mulligan (2022a)
Sophorolipids	Mining residues	As	Arab and Mulligan (2020)

*NPs denotes nanoparticles

3 DISCUSSION

The concept of industrial ecology is shown in Figure 2 and is based on protection of the environment and resource conservation (Mulligan, 2019). The overall process of production from wastes can be seen in Figure 3. Sustainable production of biosurfactants and their application for remediation should be considered this way. The approach of LCA as previously mentioned can be utilized to quantify emissions and wastes throughout the production process as seen in Figure 4 and to determine where the impacts can be minimized. As indicated by Marchant (2019), a full life cycle analysis (LCA) is necessary to identify where costs and impacts in the remediation process can be reduced. Recovery of the biosurfactants for reuse can also enhance process sustainability.

Despite the advantages of biosurfactants over synthetic surfactants for remediation, the high cost of the biosurfactants has limited full scale application. Crude forms and production from inexpensive or waste substrates can be employed (Mulligan and Gibbs, 1993). Waste materials will also improve the sustainability of the biosurfactants through cost reduction and waste reduction.

For rhamnolipid production, various soluble sugars, hydrocarbons and vegetable oils (Liu et al. 2018). A disadvantage of using waste substrates, however, is their inconsistent quality. Molasses, whey milk or distillery waste peels, various fruits and vegetables, wastes from coffee and tea, and cooking oils are examples of wastes for biosurfactant production (Mulligan, 2014). Olasanmi and Thring (2018) reviewed the role of biosurfactants towards environmental sustainability. Potential avenues of using renewable by-products or waste materials have been identified to reduce costs and waste that would otherwise need further management. Banat et al. (2014) and Makkar and Cameotra (2002) have indicated that agroindustrial by-products, agricultural residues and food by-products and wastes can be used as substrates for biosurfactant production. Another advantage of using wastes is that they do not competed with food (Henkel et al. 201 Low-cost substrates can reduce the costs by up to 30%. Saisa-Ard et al. (2013) indicated that the raw materials account for 30 to 50% of the final product cost.

Jimoh and Lim (2019) indicated that corn, for example, is much less expensive where it is produced. They summarized various renewable and industrial wastes that could be used as substrates for biosurfactant. The oil industry represented the largest proportion (35%), followed by agro-industrial wastes (20%), dairy products (18%), the food industry (15%) and then industrial wastes (12%).

Another approach is to biostimulate the microorganisms to produce the biosurfactants *in situ*. This reduces soil transportation costs and reduces risk of contaminant exposure and degrades the organic contaminants. *In situ* biosurfactant production could be sustainable and cost effective due to the lower labor, material, energy, and transport requirements and thus stimulating *in situ* production could be beneficial. This has shown by the growth of anaerobic indigenous bacteria in oil sands tailings (Rezaeitamijani and Mulligan, 2021). In this study, 13 indigenous bacteria were isolated and identified. Eight of the isolated bacteria were facultative or anaerobe tolerant. Their biosurfactant production and their effect of surface tension was monitored in both aerobic and anaerobic conditions. Then the potential isolates were added to the stirred tank reactor, STR (with agitation) and anaerobic bottle (no agitation) to simulate the MFT condition. The bioremediation of residual hydrocarbons was examined for 8 weeks after bacterial addition. Both STR and anaerobic bottle showed hydrocarbon degradation 58.7% and 55.1% respectively which shows agitation won't be necessary in the degradation. The role of *in situ* biosurfactant production could also enhance natural attenuation processes in the soil and groundwater (Yong and Mulligan, 2019).

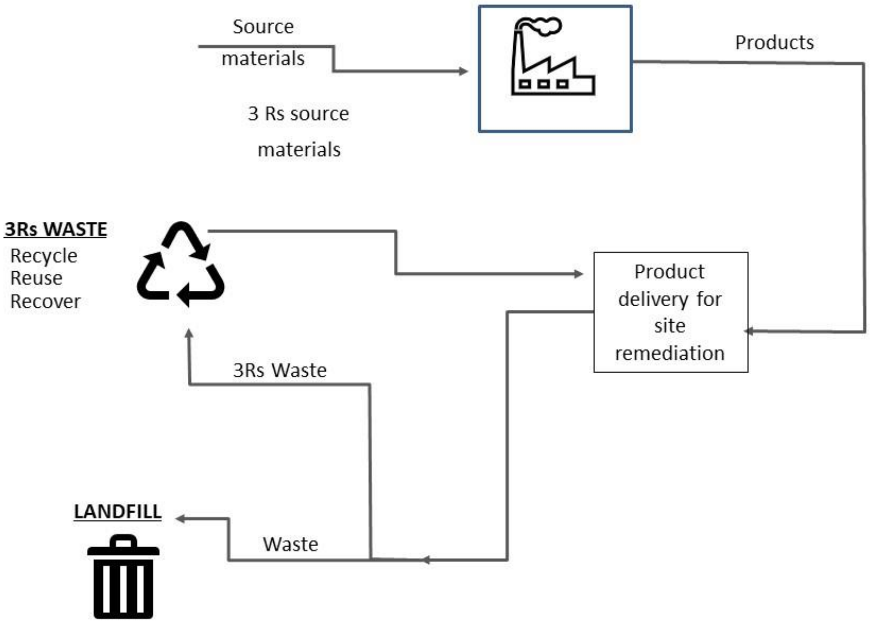

Figure 2. *Concept of industrial ecology (adapted from Mulligan 2019)*

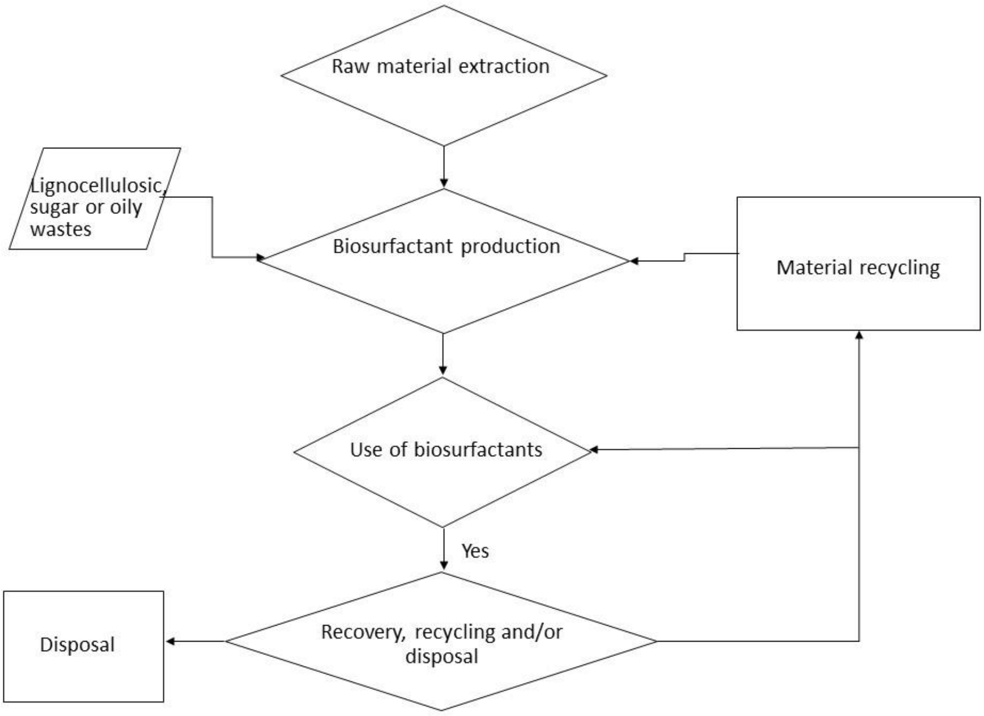

Figure 3. *Biosurfactant production using waste materials*

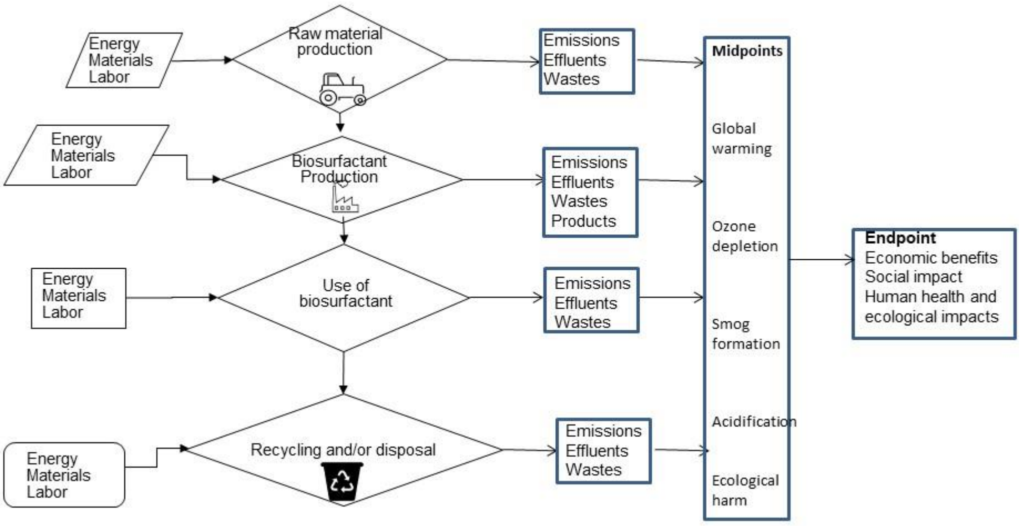

Figure 4. *Life cycle assessment of biosurfactant production process (modified from Mulligan, 2019)*

In summary, biosurfactants have the potential for sustainable remediation of contaminated soils, sediments, and wastes (e.g., tannery and mining) due to their low toxicity, biodegradability, and effectiveness. However, the entire life cycle of the biosurfactant needs to be optimized for material, energy, and cost requirements. *In situ* stimulation for production of the biosurfactants is potentially a sustainable approach.

Scale-up studies are needed for future full-scale production. In addition, further studies on in situ biosurfactant production are needed to facilitate contaminant degradation and avoid the need for surfactant addition.

The costs of biosurfactants need to be reduced to compete with synthetic surfactants. As substrates for biosurfactant production contribute substantially to the production cost, waste materials could be used due to their low costs. In addition, the sustainability of the process is improved as this will provide waste management with a circular economy concept. For determining the most suitable waste, availability, adequate nutrient and mineral contents, transportation costs and pre-treatment requirements must be taken into consideration. Waste pre-treatment (e.g., particle size reduction, ozonation, acid or enzyme hydrolysis) should be avoided if possible as this step can add extra costs for materials and processing. The selected wastes must contain nutrients and minerals to avoid the need for supplementation.

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