Biosurfactants for sustainable soil management

By

TH. NENGPARMOI

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KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA – 680656

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DECLARATION

I, Th. Nengparmoi (2018-11-137), hereby declare that the seminar report entitled "Biosurfactants for sustainable soil management" has been completed by me independently after going through the references cited here and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara	Th. Nengparmoi
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CERTIFICATE

This is to certify that the seminar report entitled "Biosurfactants for sustainable soil management" has been solely prepared by Th. Nengparmoi (2018-11-137), under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

Vellanikkara
24-01-2020

Dr. P. Prameela
Professor (Agronomy)

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Certified that the seminar report entitled “Biosurfactants for sustainable soil management” is a record of seminar presented by Th. Nengparmoi (2018-11-137) on 16th January, 2020 and is submitted for the partial requirement of the course Agron. 591.

Dr. Anil Kuruvila
Professor
Department of Agricultural Economics
College of Horticulture, Vellanikkara

Dr. Reshmy Vijayaraghavan	Dr. SangeethaKutty M.
Assistant Professor	Assistant Professor
Department of Plant Pathology	Department of Vegetable Science
College of Horticulture, Vellanikkara	College of Horticulture, Vellanikkara

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1. Introduction

Degradation of soil resources is a results of various natural and anthropogenic activities including the loss of organic matter, decline in soil fertility, erosion, and the effects of toxic chemicals is a serious global environmental problem and it may be made worse by climate change and also it can be as a consequence of poor management of our natural resources (soils, water, vegetation, etc.). (DeFries *et al.*, 2012; Lal, 1997). The need to prevent further land degradation and to restore degraded lands is especially important now because the agricultural productivity to meet growing demand is increase with increase in human population. This should involve the adoption of sustainable land use practices as well as the restoration and protection of any degraded or marginal soils to ensuring food security for an increasing population demand. Use of green techniques or compound to achieve sustainable agricultural is the present necessity. Thus, the use of biosurfactants and also biosurfactants producing microorganisms to improve soil health and to bioremediate polluted soils is an emerging approach.

2. Biosurfactants

Biosurfactants are surface-active molecules produced by microorganisms, such as bacteria, yeast and fungi and they have amphiphilic (hydrophilic and hydrophobic) components. Biosurfactants are divided into two groups viz., low molecular mass and high molecular mass. Major classes of surfactants with a low molecular mass include glycolipids, lipopeptides and phospholipids, all of which reduce surface tension and interfacial tension (IFT). In contrast, surfactants with a high molecular mass (i.e. polymer surfactants and particulate surfactants) are effective emulsion stabilizers. Biosurfactants are widely used in food production, cosmetics, pharmaceuticals, detergents, textiles and petroleum. They also have environmental applications due to their properties, such as biodegradability, low toxicity, biocompactibility and digestibility, emulsification, tolerance to high pH, temperature and ionic strength. Furthermore, biosurfactants play an important role in bioremediation, such as treatments of contaminated soils, oil spills at sea and crude oil removal.

3. History

Babylonians 2,800 years ago biosurfactants was used in the production of soap. In 1960s the first biosurfactants was produced by microbes through hydrocarbon fermentation in the form of extracellular compounds. The first biosurfactant name “Surfactin” was produced by Bacillussubtilis(Arima*et al*., 1968). Based on their chemical composition and the microbes source of origin,biosurfactants is classified into five categorisedviz., glycolipids (sophorolipids, rhamnolipids), lipopeptides and lipoproteins (surfactin, lichenysin), phospholipids and fatty acids (corynomycolic acid)polymeric biosurfactants (emulsion, liposan) and particulate biosurfactants (vesicle, whole microbial cell) (Banat, 1995).

4. Sources of biosurfactants

Biosurfactants are mainly produced by microorganisms and plants. However, microbially produced biosurfactants are receiving more attention due to ease in culturing, lower production cost and greater functional properties.

4.1. Plant-based biosurfactants

The most common plant-based biosurfactants are saponins, lecithin, soy protein, and cyclodextrins. Plant-based biosurfactants have excellent emulsification properties, although they are expensive to produce on an industrial scale. Moreover, plant-based surfactants have other issues such as solubility and hydrophobicity (Xu *et al.*, 2011).

Class	Source	References
Saponin	Tea seed	Wang *et al*. (2016), Tang *et al*. (2014), Cao *et al*.(2013), and Liu *et al*. (2011)
	Caryophyllaceae	Cao *et al*. (2013)
	Soybeans, broad beans, and peanuts, kidney beans, and lenticels	Xu *et al*. (2011) Oakenfull (1981)
	Quillaja	Tippel*et al*. (2016: Kilic*et al*. (2011), Chen *et al*. (2008): Hong *et al*. (2002)
Phospholipid	Maize roots and lupin	Read *et al*. (2003); Read and Gregory (1997)
Glycolipids	Bracken fern fronds	Jarvis and Duncan (1974)
Mono- and digalactosyldiacylglycerolipids	Lamk	Wegner and Hamburger (2002)
Humic acid-like substance	Soapnut plant	Song *et al*. (2008)
Cyclodextrins	Maize	Adani *et al*. (2010)
Polymeric	Chicory	Stevens *et al*. (2001)

Table 1: Plant-based biosurfactants and their sources

4.2. Microbial-based biosurfactants

Microorganisms such as yeasts, bacteria, and some filamentous fungi are capable of producing biosurfactantswith different molecular structures and surface activities.

Biosurfactants	**Biosurfactant-Producing Microorganisms**	**References**
Rhamnolipids	*Pseudomonas aeruginosa* NCIM 5514	Varjani and Upasani (2016)
Trehalose lipids	*Mycobacterium* spp., *Rhodococcus* spp.	Franzetti*et al*. (2010)
Sophorolipids	*Candida bombicola* ATCC 22214	Kang *et al.* (2010)
	Starmerellabombicola	Kurtzman *et al.* (2010)
Lipopeptides	*Pseudomonas aeruginosa* CB1, *B. subtilis* CN2	Bezza and Chirwa (2016)
Emulsan	*Acinetobactercalcoaceticus* BD413	Kaplan and Rosenberg (1982)
	Acinetobactercalcoaceticus RAG-1	Goldman *et al*. (1982)
Alasan	*Bacillus mojavensis*	Ben Ayed*et al*. (2014)
Liposan	*Candida lipolytica*	Cirigliano and Carman (1984)
Surfactin	*Bacillus subtilis* CMB32	Kim *et al.* (2010)

Table 2: Microbially produced biosurfactants

5. Properties of biosurfactants

5.1. Surface and Interfacial Activity

Efficiency and effectiveness are essential characteristics of a good surfactant. Efficiency is measured by the CMC, whereas effectiveness is related to surface and interfacial tensions. The CMC of biosurfactants ranges from 1 to 2000 mg/L, whereas interfacial (oil/water) and surface tensionsare respectively approximately 1 and 30 mN/m. Good surfactants are able to reduce water surface tension from 72 to 35 mN/m and the interfacial tension of n-hexadecane from 40 to 1 mN/m.

5.2. Tolerance to Temperature, pH and Ionic Strength

Many biosurfactants can be used at high temperatures and pH values ranging from 2 to 12. Biosurfactants also tolerate a salt concentration up to 10%, whereas 2% NaCl is enough to inactivate synthetic surfactants.

5.3. Biodegradability

Biosurfactants are easily degraded by microorganisms in water and soil, making these compounds adequate for bioremediation and waste treatment.

5.3. Low Toxicity

Low degree of toxicity allows the use of biosurfactants in foods, cosmetics and pharmaceuticals. Low toxicity is also of fundamental importance to environmental applications.

5.5. Biocompatibility and Digestibility

These properties allow the use of biomolecules in different industries, especially the food, pharmaceutical and cosmetic industries.

5.6. Emulsion Forming/Breaking

Biosurfactants can be either emulsifiers or de-emulsifiers. An emulsion is a heterogeneous system consisting of an immiscible liquid dispersed in another liquid in the form of droplets, the diameter of which generally exceeds 0.1 mm. There are two basic types of emulsion: oil-in-water (o/w) and water-in-oil (w/o). Emulsions have minimal stability, but the addition of biosurfactants can lead to an emulsion that remains stable for months or even years. Liposan, which is a water-soluble emulsifier synthesised by *Candida Lipolytica*, has been used with edible oils to form stable emulsions. Liposan is commonly used in the cosmetic and food industries for producing stable oil/water emulsions.

5.7. Specificity

Biosurfactants are complex molecules with specific functional groups and therefore often have specific action. This is of particular interest in the detoxification of different pollutants and the de-emulsification of industrial emulsions as well as specific food, pharmaceutical and cosmetic applications.

6. Mechanisms of biosurfactants

1. Mechanism of hydrocarbon removal
2. Biosurfactant-assisted phytoremediation
3. Mechanism of heavy metal removal by biosurfactant

6.1. Mechanism of hydrocarbon removal by biosurfactants

Biosurfactants have been employed to reduce the interfacial tension between oil/water and oil/rock, which leads to a reduction in the capillary forces that impede oil from moving through rock pores.Biosurfactants also form an emulsion at the oil-water interface, which stabilises the desorbed oil in water and allows oil removal along with the injection water.

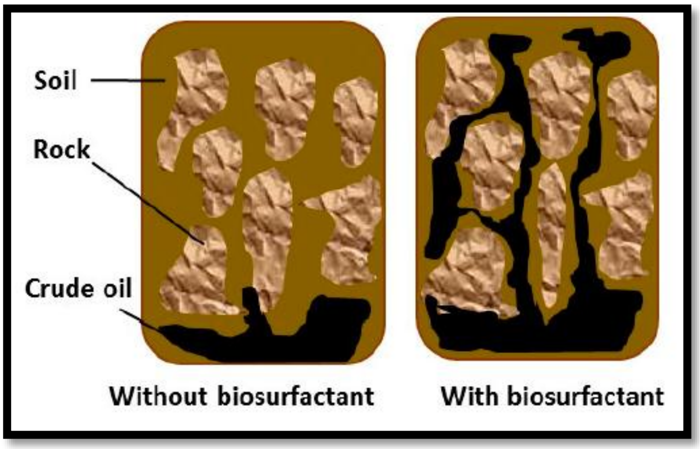

Figure 1: Biosurfactants for bioremediation of hydrocarbon contaminated soil

6.2. Biosurfactants-assisted phytoremediation

It increase in the bioavailability of the hydrophobic substrate to microorganisms, with a consequent reduction in surface tension of the medium around the bacterium as well as a reduction in interfacial tension between the cell wall and hydrocarbon molecules.

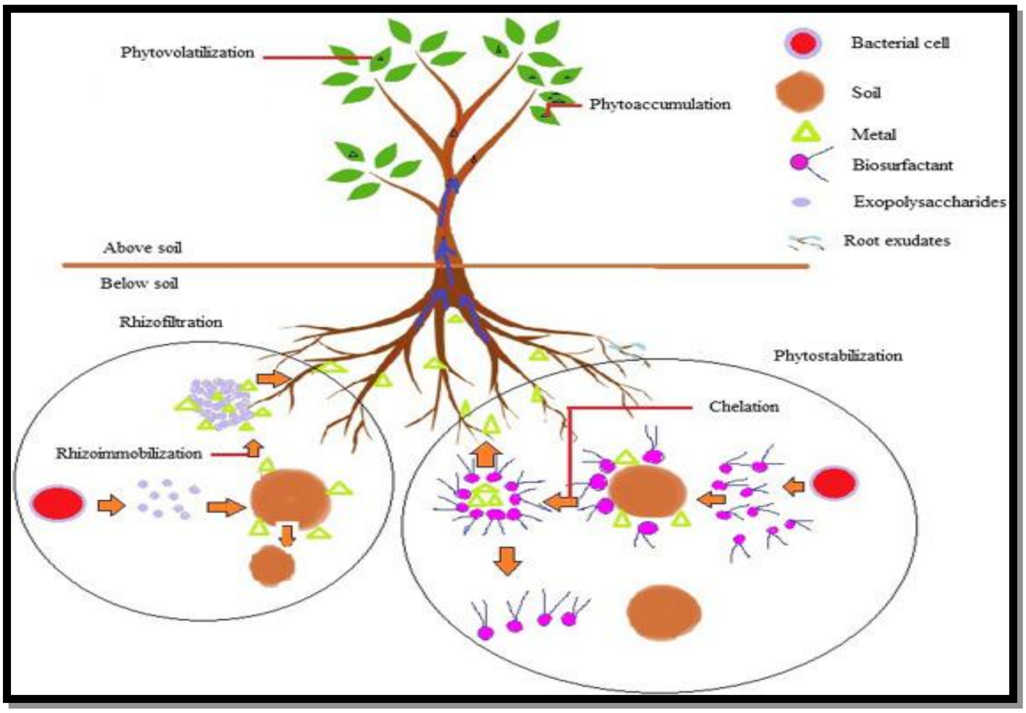

Figure 2: Biosurfactants-assisted phytoremediation of problem soil

6.3. Mechanism of heavy metal removal by biosurfactants

The removal of metals by ionic biosurfactants is thought to occur in the following order:

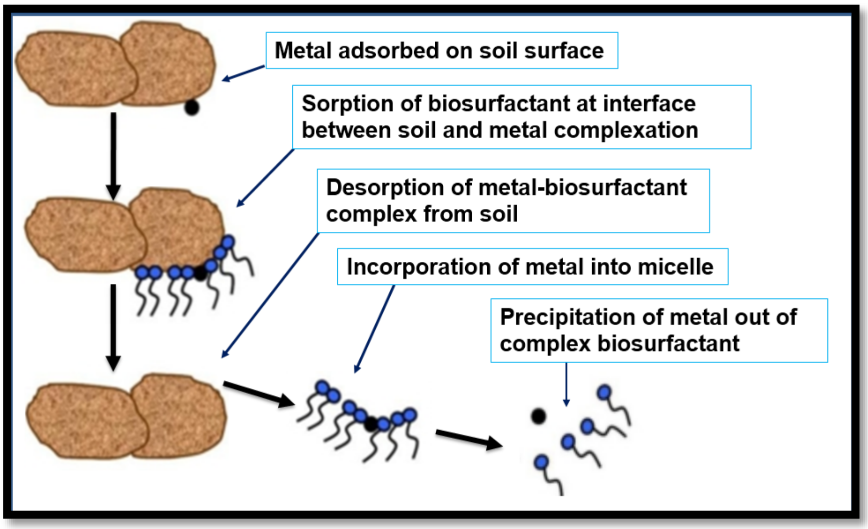

Figure 3: Biosurfactants for bioremediation of heavy metal contaminated soil

7. Effect of biosurfactants on soil properties

7.1. Physical properties

- Reduced the equilibrium water content of the soil and the leaching loss of sandy soil
- Modify contact angle and increase wettability of soil

Lin *et al*. (2017). Studied on chemical agents were utilized to evaluate the influence of chemical leaching on permeability. They observed that chemical solutions of CaCl2, FeCl3 and rhamnolipid reduced soil permeability due to the dissolution of carbonates and oxides and the dispersion of soil colloids.

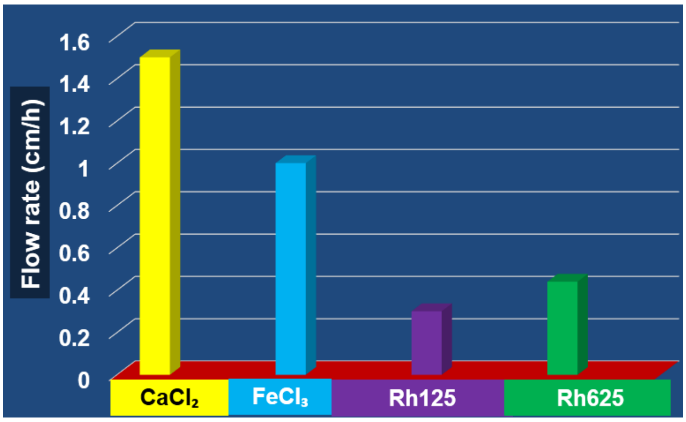

Figure 4: Effect of biosurfactant on leaching loss in sandy loam soil

Hallmann and Medrzycka, (2015) measured the effectiveness of rhamnolipid concentration on contact angle on soil surfaces. It was found that at a very low concentration of rhamnolipid (up to about 0.003 gl^{-1}) the contact angle remained constant and equal to the value for pure water. With further increase in the surfactant concentration, the contact angle decreased. Contact angle values were about 40 degree at 0.005 gl^{-1}rhamnolipid and complete wetting (contact angle: 0 degree) was achieved at 0.5 gl^{-1}

Biosurfactant	Conc.(g/l)	Contact angle
Rhamnolipid	0.003	90
	0.005	40
	0.5	0

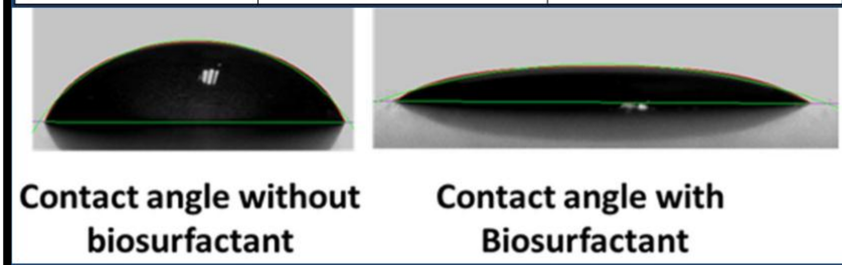

Figure 5: Effect of biosurfactant on contact angle of water

7.2. Effect on soil biological properties

- Ability to stimulate growth of microorganisms in soil
- Increase decomposition of organic matter in soil and microbial activities
- Sufficient levels of biosurfactants in the rhizosphere, plants could be able to extract water and nutrients from micropores

Ali and Sandouka (2012) extracted a biosurfactant from sunflower seed meal and studied its effect on the soil microbial community. Biosurfactant was applied to the rhizosphere and non-rhizosphere soil which had been artificially polluted with crude petroleum. In the absence of a biosurfactant, the total heterotrophic bacteria (THB) in the rhizosphere and non-rhizosphere soil were 15.0 ±1.40 X 107 (cfu) and 5.43 ± 0.20 X 107 per g dried soil, respectively. However, upon application of biosurfactant, THB were 43.2 ± 1.4 X 107 (cfu) and 155.7 ± 18 X 107 per g dried soil,respectively.They observed that THB population was more in a treatment containing biosurfactants, due to reduction in surface tension of soil solution that leads to releasing more water n nutrients to microbes.

Treatments	Microbial population after 90 days	
	CFUx10[7] /g dried soil	
	Biosurfactant	No biosurfactant
Polluted non-rhizosphere soil	43.2±1.4	5.43±0.20
Polluted rhizosphere soil	155.7±18.0	15.0±1.40

Table 3: Effect of biosurfactants on soil microorganisms

Jin *et al*. (2006) investigated the effect of biosurfactants (rhamnolipid) on microbial community. They found larger populations of bacteria, actinomycetes and fungi in the soil with biosurfactant treatment than in the control soil during thewhole composting process.

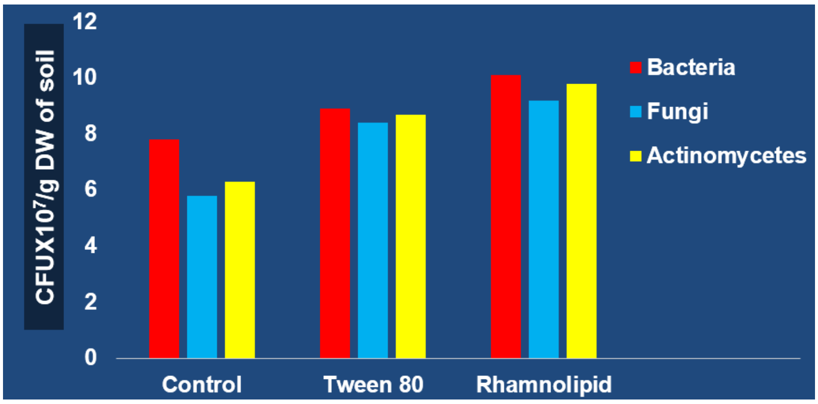

Figure 6: Effect of biosurfactants on microbial community

8. Role of biosurfactants in soil fertility management

- Reduce amount of phosphate adsorption on sandy loam soil
- Enhance phosphorus desorption from soil

Read *et al*. (2003) conducted a study to assess the effect of a plant produced biosurfactant (lecithin) on adsorbed P in two soils with different textures, i.e., Bullionfield soil (sandy loam, slightly acidic) and sonning soil (sandy loam, neutral to slightly acidic). They found that lecithin decreased the amount of phosphate adsorption on soil and enhanced phosphate concentration in solution by 10% by blocking the phosphate-adsorption site.

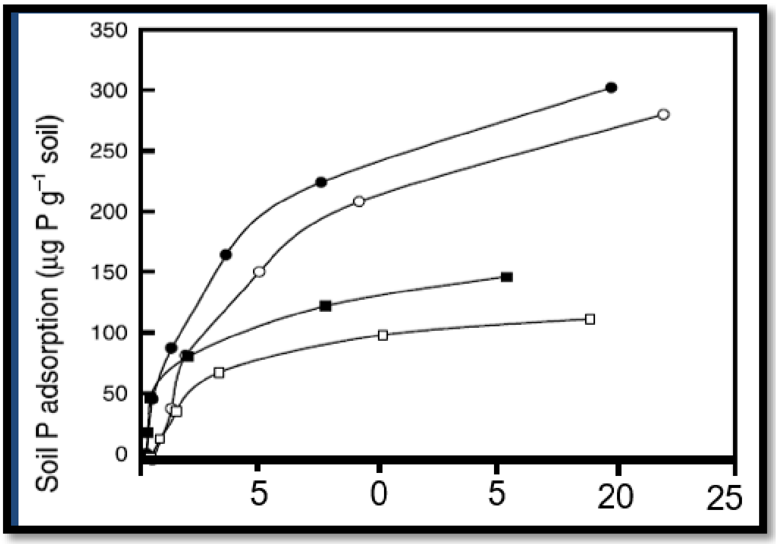

Figure 7: Effect of biosurfactant on phosphate adsorption of soil

Marschner (1995) study the Effect of biosurfactants on phosphate desorption from soil matrix.

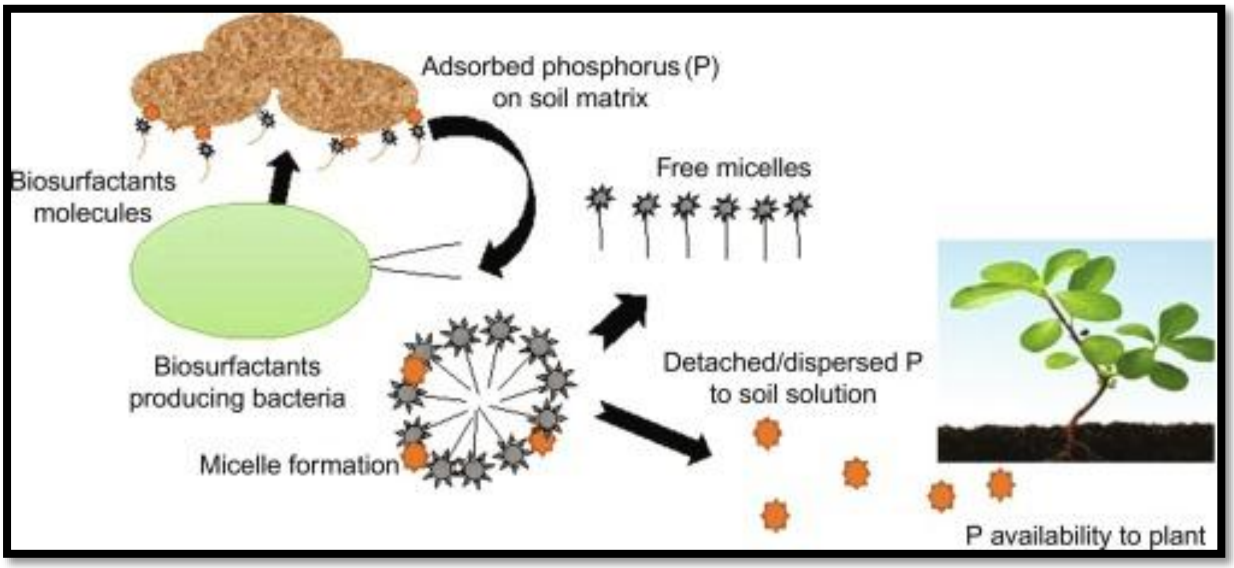

Figure 8: Effect of biosurfactant on phosphate desorption from soil

9. Biosurfactants in management of contaminated soils

1. Bioremediation agents for

i) Pesticide-contaminated soils

ii) Hydrocarbon-contaminated soils

iii) Heavy metal-contaminated soils

vi) Salt affected soil

2. Biosurfactant-assisted phytoremediation of soils

9.1.1. Bioremediation agents for pesticide contaminated soils

In recent years, biosurfactants have been applied in soils as emulsifying agents for treating pesticide-contaminated soils. Biosurfactants increase the solubility and degradation of pesticides in soil by improving their desorption from soil colloids.

Singh *et al*. (2016) observed that solubility of chlorpyrifos in the aqueous phase increased from 25% (control) to more than 87% in the medium supplemented with biosurfactant produced by Pseudomonas sp. (ChlD).

Chemical	Treatments	Bacterial population
Chlorpyrifos (50mg/kg of soil)	Bacterial consortium (Control)	$1x10^{7}$ CFU /g soil
	Bacterial consortium + Rhamnolipid @20(mg/l)	

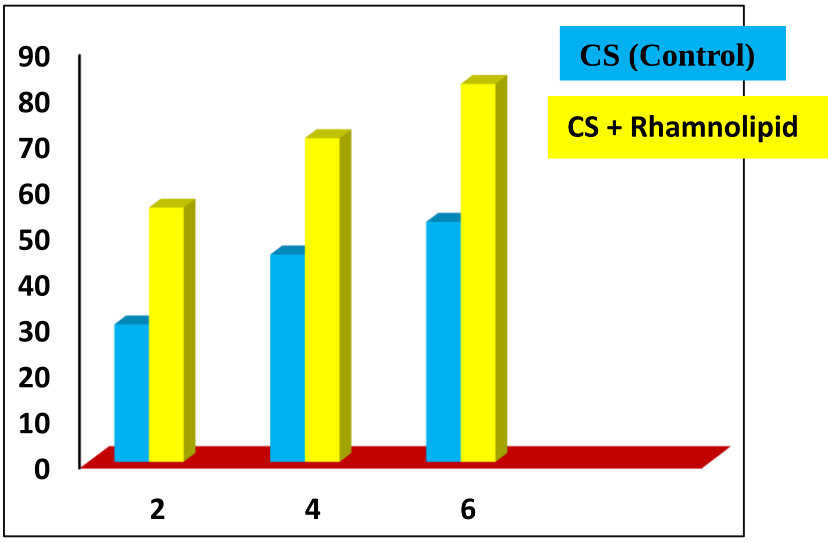

Figure 9: Effect of biosurfactant on chlorpyrifos contaminated soil

Some others example of biosurfactant-assisted bioremediation of soils contaminated with pesticides.

Types of pesticides	Carbenazim	Endosulfan	Trifluralin
Medium	Liquid	Soil	Soil-slurry
Pesticide conc.(ppm)	200	320	200 - 400
Biosurfactants	*Rhodococcus sp*. D-1 + rhamnolipid	*Pseudomonas aeruginosa* + rhamnolipid	*Streptomyces* PS1
Treatment time (days)	2	7	22
Biodegradation (%)	97.3	90	30
References	Bai *et al*.(2017)	Mani *et al.* (2011)	Sandoval *et al*.(2001)

Table 4: Biosurfactant-assisted bioremediation of soils contaminated with pesticides.

Biosurfactants in plant diseases management

Plant disease	**Crops**	**Pathogen**	**Biosurfactant**	**References**
Damping-off	Cucumber	*Pythium aphanidermatum*	Surfactin A	Peng *et al. (*2017)
Early blight	Tomato	*Alternaria solani*	Rhamnolipid	Lahkar*et al*. (2015)
Late blight	Potato	*Phytophthora infestans*	Ten	Bengtsson*et al.* (2015)
Root rot	Wheat	*Rhizoctonia solani*	Cycliclipopeptide	Yang *et al*.(2014)
Mold	Potato	*Aspergillus parasiticum*	NI	Mule and Bhathena(2012)

Table 5: Control of plant diseases through Biosurfactant

Peng *et al*. (2017) studied the Inhibition of damping-off in cucumber by biosurfactants. They found that disease incidence percentage was lowest in a treatment containing biosufactants, due to suppressing the release and lysis of Pythium zoospores and reduced damping-off incidence and Pythium sporangium is unable to release zoospores after being treated with suspension culture of *Bacillus mycoides*.

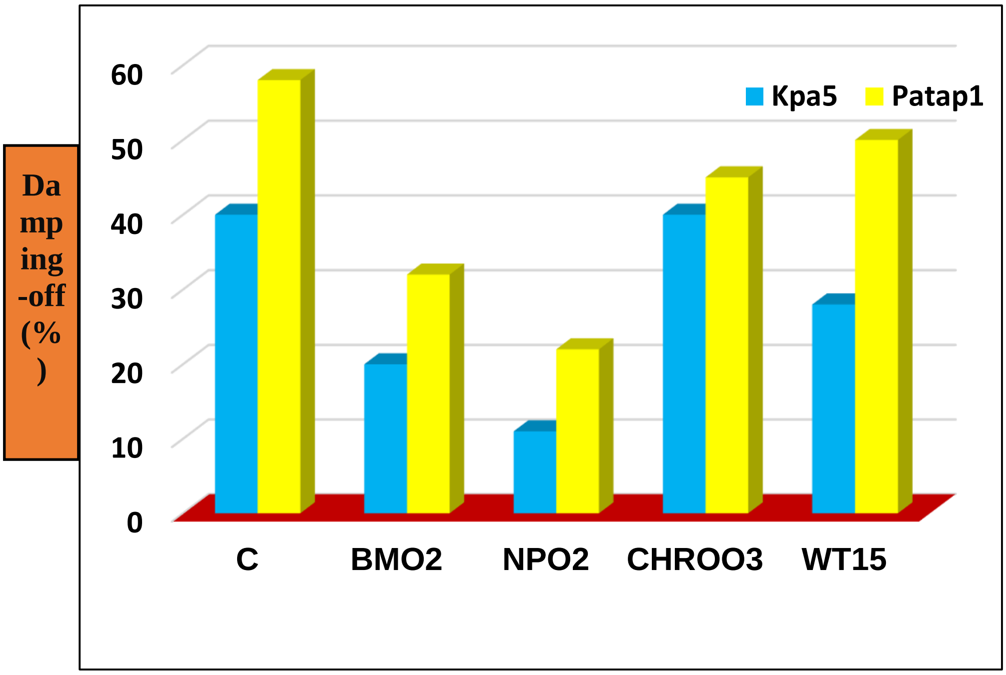

Figure 10: Inhibition of damping-off in cucumber by biosurfactants

Lahkar*et al.* (2015) studied the inhibition of *Alternaria solani* in tomato by biosurfactant. This is the first report showing complete inhibition of early blight disease of tomato caused by *Alternaria solani* using a rhamnolipidbiosurfactant in field condition. They observed that with increased in concentration of biosurfactants the diseased was completed inhibited, by destructing the growing hyphae during the initial stage of spore germination thereby preventing appearance or progress of disease symptoms.

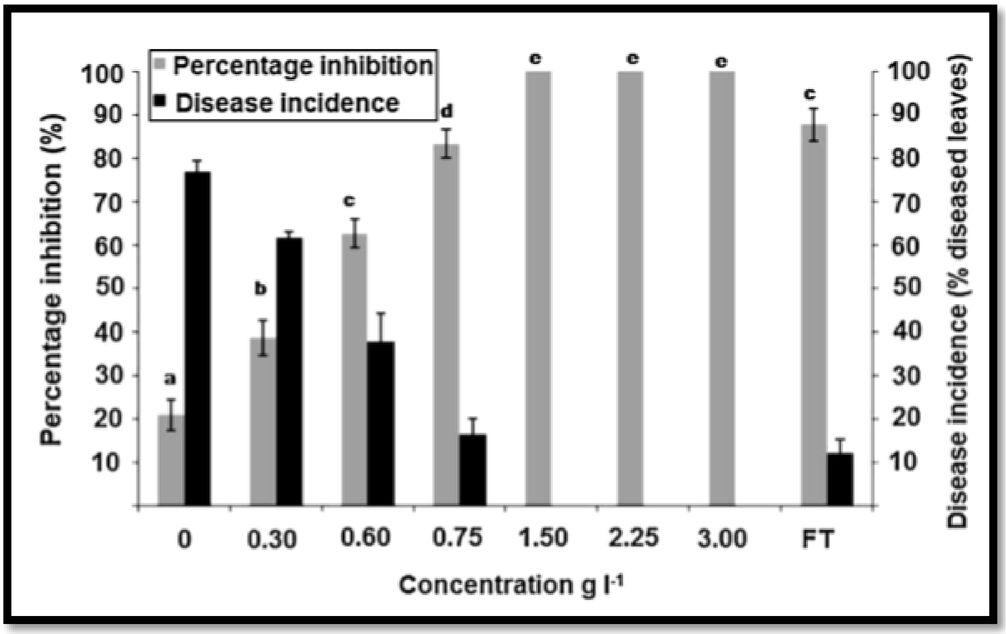

Figure 11: Inhibition of *Alternaria solani* in tomato by biosurfactant

9.1.2. Biosurfactants for bioremediation of hydrocarbon-contaminated soil

Biosurfactant application to soil increases solubility and bioavailability of hydrocarbons as they increase their desorption from soil colloids. Hydrocarbons in soil solution are either removed through soil washing or can be degraded by soil microorganisms. As hydrocarbons are mostly insoluble in water, bacterial cultures capable of producing biosurfactants are helpful in solubilizing and emulsifying hydrocarbons, leading to desorption and thereby enhancing biodegradation and removal rate (Bezza and Nkhalambayausi-Chirwa, 2015; Kuyukina*et al.*, 2005; Liu *et al.*, 2017; Rogers *et al.*, 1993).

Kuyukina*et al*. (2004) study the effect of biosurfactants on crude oil desorption and mobilization in a soil system, This result suggests that oil mobilized by biosurfactants could be easily biodegraded by soil bacteria *Rhodococcus* biosurfactants can be used for in situ remediation of oil-contaminated soils.

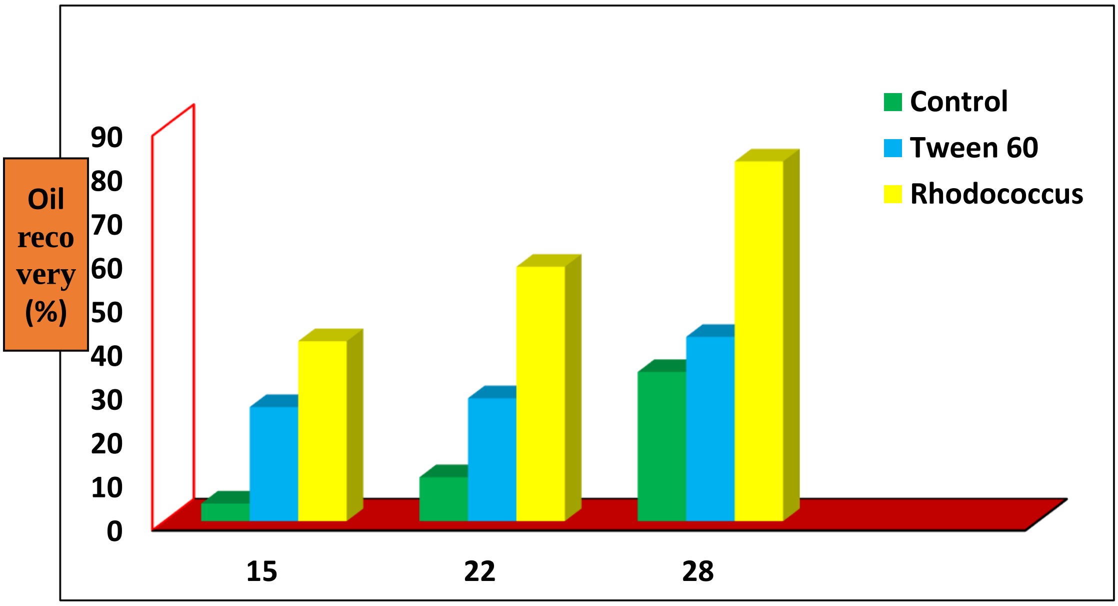

Figure 12: Effect of biosurfactants on crude oil desorption and mobilization in soil system

Wang *et al*. (2017) Study the effect of biosurfactant on polycyclic aromatic hydrocarbon contaminated soil. They observed that the percentage of biodegradation was more in a treatment containing biosurfactants compared to control.

Type of hydrocarbon	Treatments	Biodegradation (%)
Hydrocarbon (0.690 ppm)	**Control**	**6.3**
	Anthrobacterglobiformis	**35.6**
	***Anthrobacterglobiformis* + Rhamnolipid @ 5mg/l**	**64.3**

Table 6: Effect of biosurfactant on polycyclic aromatic hydrocarbon contaminated soil

9.1.3.Biosurfactants for bioremediation of heavy metals contaminated soils

Biosurfactants enhances solution of heavy metal concentration through desorption from soil colloid.

Heavy metals	Medium	Conc. (ppm)	Biosurfactants	Removal Efficiency (%)	References
Zn	Soil	57000	Rhamnolipid	68	Diaz *et al.* (2015)
As	Soil	560	*Burkholderia* sp. Z-90-glycolipid	31.6	Yang *et al.* (2016)
Pb	Soil	100	Rhamnolipid	76	Kim and Vipulanandan(2008)
Cu	Sand	26.28	Rhamnolipid	45	Haryanto and Chang (2015)
Cd and Ni	Soil	1706-2010	Rhamnolipid	68.1-73	Wang andMulligan (2004)

Table 7: Biosurfactants-assisted bioremediation of soils contaminated with heavy metals

9.1.4. Bioremediation agents for salt affected soils

Halotolerent bacteria Halovivax (A21) and Haloarcula (D21) produced biosurfactants at very high salt concentration. Biosurfactants produced by microorganisms have been found stable over a wide range of salinity levels

da Silva et al. (2015). Study the effect of biosurfactants produced by Bacillus spp. at wide range of salinity levels. They observed that biosurfacant were found to be active in solutions containing up to 175g L -1NaCl.

9.2. Biosurfactant-assisted phytoremediation of soils

- It provide benefits of phytoremediation and microbial remediation of contaminated soils
- Enhance bioavailability and apparent solubility of metals, hydrocarbons and pesticides

It facilitate their accelerated absorption by plants

Wang *et al*. (2017a) observed that biosurfactant-producing *Pseudomonas* sp. SB helped to phytoremediate soil contaminated with DDT by tall fescue plants. DDT residues in soil planted with tall fescue plants were 576 μgkg^{-1} which decreased to 498μgkg^{-1} in the presence of a biosurfactant-producing bacterium

Treatments	**DDT (μg/kg)**		**Treatment time(days)**	**Removal efficiency (%)**
	before	**after**		
Control	**1417**	**847**	**150**	**40.3**
Tall Fescue		**576**		**59.4**
Tall Fescue +*Pseudomonas*		**498**		**65.6**
Rye grass+*Pseudomonas*		**483**		**65.9**

Table 8: Biosurfactant-assisted phytoremediation of DDT-contaminated soil

10. Advantages

- Ecologically safe
- Biodegradable
- Wastes can be used as raw material
- Increase wettability of soil
- Increasing solubility of water-immiscible substances
- Able to work in extreme condition
- Wide applications

11. Manufacturers of biosurfactants

WORLD		**INDIAN**
Companies	Countries	Evonik India Pvt. Ltd.
BASF	Germany	
Evonik	Germany	Mitsubishi India, Vetline (Unit of Simfa Labs Pvt. Ltd.)
Ecover	Belgium	
Evonik	China	Altinbio scientific Pvt. Ltd.
BioFuture Ltd.	Ireland	

12. Conclusion

Biosurfactants have much more potential to be used in agriculture and thus can play a major role in the sustainable food production. Much of the research carried out on biosurfactants in the last decade has sought to increase their cost competitiveness over synthetic compounds. Availability of commercial products at lower cost can help in their wide application for a greener future.

13. Dicussion

1.What is the range molecular mass of biosurfactant ?

The molecular mass of biosurfactants generally ranges from 500 to 1500 Da.

2. Why microbial population increase, when biosurafctant applied in polluted soil ?

Biosurfactants reduce tension of soil solution, that leads to releasing more water and nutrients to microbes. In addition to the surface active property of the biosurfactant, bacteria present is soil may be able to utilize the biosurfactant and to protect themselves from the toxic polutants present in soil.

3. Is there any negative effect in using biosurfactants in soil ?

No, there is no negative effect in soil.

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KERALA AGRICUTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agronomy

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Name	**: Th.Nengparmoi**	**Venue**	**: Seminar Hall**
Admission No	**: 2018-11-137**	**Date**	**:16-01-2020**
Major Advisor	**: Dr.P. Prameela**	**Time**	**: 10.45 am**

Biosurfactants for sustainable soil management

Abstract

Degradation of soil resources is a serious global environmental issue and it may be made worse by climate change. It is important that soils should be handled with a careful consideration for sustainable agricultural production using environmental friendly techniques. The use of biosurfactants to improve soil health and bioremediate polluted soils is an emerging area in agriculture.

Biosurfactants are amphiphilic surface-active molecules which are mainly derived from microorganismsandplants. They are of great benefit because of their unique properties and environment friendly nature. A biosurfactant named "surfactin"produced by *Bacillus subtilis*, was first purified and characterized by Arima et al. (1968). Based on their chemical composition and microbial source of origin, biosurfactants are classified into five categories *viz.,* glycolipids, lipopeptides and lipoproteins, hospholipids and fatty acids, polymeric and particulate biosurfactants (Banat, 1995).

The amphiphilic properties of biosurfactants enable them to solubilize hydrophobic substances by the formation of micelles and emulsions. Thesecompounds increase the bioavailability of organic pollutants for microbial degaradation, while some biosurfactants can be used to extract heavy metals from soil. The sources of plant based biosurfactants include crops like soybean, maize, castor etc. Many microbes like *Pseudomonas* spp, *Candida* spp, *Rhodococcus* spp, *Flavobacterium* spp, *Bacillus* spp. have been identified as sources of biosurfactants. The biosufactants produced by them include rhamnolipids, sophorolipids, surfactins, fatty acids and an array of other biochemical compounds.

Biosurfactants have varied applications like removal of hydrocarbons, heavy metals, pesticide residues etc., from soil, increasing bioavailability of nutrients, enhancing phytoremediation potential of plants and controlling plant diseases. Biosurfactants modify physical and biological properties of soil and there are reported claims of their effect on soil fertility improvement. Read *et al*. (2003) reported that biosurfactants reduce amount of phosphate adsorption on soil by blocking phosphate adsorption sites. They reduce infiltration and hydraulic conductivity of sandy loam soil by reducing the soil permeability due to the dissolution of carbonates and oxides and also increase the wettability of soil by decreasing the contact angle of water. Biosurfactants have the ability to stimulate the growth of microorganisms in the rhizosphere and also increase the decomposition of organic matter in soil (Ali and Sadouka, 2012). The action of bioagents in plant protection is mainly due to their production of

biosurfactants. For example, *Pseudomonas aeruginosa* controls the early blight of tomato by producing rhamnolipid (Lahkar *et al*., 2015). The principle behind the use of *Bacillus subtilis* in diesel oil remediation is production of surfactin (Manif *et al*., 2017).

Biosurfactants have much more potential to be used in agriculture and thus can play a major role in sustainable food production. Much of the research carried out on biosurfactants in the last decade has sought to increase their cost competitiveness over synthetic compounds. Availability of commercial products at lower cost can help in their wide application for a greener future.

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