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Surface tension technique as a strategy to evaluate the adsorption of biosurfactants used in soil remediation

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ABSTRACT

This study investigated the adsorption of two biosurfactants, non-ionic saponin and anionic Reco-10 (a mixture of rhamnolipids). The experiments were performed with three different soils (sandy clay loam, clay loam, clay) and at two soil/biosurfactant ratios, m/V=1/10 and 1/40. Using a tensiometer, surface tension in aqueous biosurfactant solutions and their supernatants was measured and the critical micelle concentration (CMC) was determined. The amount of adsorbed biosurfactants was calculated based on the CMC values. Adsorption of both biosurfactants depended on soil type and m/V ratio. The highest saponin and Reco-10 adsorption was in the soil with the greatest content of clay and organic matter, the highest cation exchange capacity and a m/V of 1/40. Thus, clay soils may need a higher concentration of biosurfactants than sandy or loamy soils for effective pollutant removal.

INTRODUCTION

Surfactants are amphiphilic compounds (containing hydrophobic and hydrophilic portions) that reduce the free energy of a system by replacing bulk molecules of higher energy at an interface (Mulligan 2005). Due to their ability to lower surface/interfacial tension, and to increase solubility, detergency power, wetting ability and foaming capacity, surfactants have a wide range of applications in many fields, such as the petroleum or pharmaceutical industries. In addition, surfactants monomers aggregate in micelles at a specific concentration, which not only reduces surface and interfacial tension, but also facilitates the desorption of pollutants and increases their bioavailability in soils or sediments. These properties mean that surfactants can be used in many surfactant-enhanced remediation systems like soil washing (Mao et al. 2015; Mulligan 2009), electrokinetic processes (Saichek and Reddy 2005), phytoremediation (Liu et al. 2013) and bioremediation (Pacwa-Plociniczak et al. 2011).

Up to now, different ionic (anionic, cationic) and non-ionic surfactants have been tested for soil remediation. Anionic synthetic surfactants that have been tested include sodium dodecyl sulphate (SDS), bis(2-ethylhexyl) sulfosuccinate sodium (AOT) and linear sodium alkene sulfonates (Spolapon AOS). As a cationic surfactant, cetyltrialkyl ammonium bromide (CTAB) has been used. In contrast to ionic surfactants, nonionic surfactants have lower toxicity and greater capacity to solubilize contaminants, so they are more commonly used in remediation projects than ionic (Zheng et al. 2012). Although ionic surfactants are highly efficient at removing various pollutants

such as PCBs, petroleum, NAPLs and BTEX, their toxicity can limit their usefulness (Mao et al. 2015).

Currently, biosurfactants appear more attractive than synthetic surfactants for surfactant-based soil remediation. Biosurfactants are natural surface active agents produced by bacteria, fungi and yeast, or extracted from plants (Paria 2008). They have a larger molecular structure and more functional groups than synthetic surfactants, which enables them to remove both hydrophobic organics and heavy metals. The biosurfactants commonly used in soil remediation are anionic rhamnolipids secreted by *Pseudomonas aeruginosa* (Juwarkar et al. 2007; Muligan 2009) and non-ionic saponin of plant origin (Hong et al. 2002).

Biosurfactants differ in their properties and can behave in soil in different ways. Although biosurfactants have a low environmental impact, and can be left in soil after treatment (Wouter et al. 2004), their adsorption can lower the efficiency of surfactant-based soil remediation. The degree of their adsorption depends primarily on soil properties, i.e. its organic carbon content and cation exchange capacity, and on the chemical nature of the surfactant. Anionic surfactants are generally adsorbed less than nonionic surfactants and much less than cationic surfactants (Lee et al. 2004). As a result of surfactants being adsorbed to soil, the hydrophobicity of the soil can be increased, and previously removed pollutants, especially organic ones, can be re-adsorbed on the soil surface (Paria 2008). In many remediation projects, the biosurfactant concentration is chosen based on the critical micelle concentration (CMC) (Zhang et al. 2011). If the degree of adsorption is great, surfactant concentrations could drop below the CMC and pollutants will not be solubilized (Chu 2003). Therefore, selection of the proper biosurfactant concentration for soil remediation should be preceded by determination of the CMC in the soil-surfactant solution system.

To determine biosurfactant adsorption, there are some methods based on the measurement of selected surfactant properties, i.e. surface tension, absorbance or chemical oxygen demand (COD) (Liu et al. 1992). However, methods based on measurement of absorbance or COD can be problematic, because compounds released from the soil can affect the extract color and concentration of organics. As a result, surfactant adsorption may be overestimated. Zhou et al. (2013) confirmed that, after soil sorption experiments, it is difficult to accurately quantify by UV spectrometry the total concentration of Sapindus saponin in aqueous solution. Thus, methods using measurement of surface tension seem to be more adequate.

Although the adsorption of various synthetic surfactants has been determined, little is known about biosurfactant adsorption on soil, especially plant-biosurfactants. Therefore, the aim of the present study was to determine the adsorption of two commercially available biosurfactants (saponin and rhamnolipids) at their CMC, using a surface tension technique. The experiments were performed with three soils with different properties and at two ratios of soil to biosurfactant solution.

MATERIALS AND METHODS

Biosurfactants

Two different biosurfactants were used. Chemically-pure saponin (Product No. 16109), a non-ionic plant-derived biosurfactant, was purchased as a powder from Riedel-deHaën, Switzerland. Saponin is an acidic biosurfactant (pH 4.5–5.5) with a density of 1.015-1.020g·mL^{-1} at 20°C (5% in H_2O). It is a mixture of triterpene-glycosides extracted from the bark of the tree *Quillaja saponaria*, and its hydrophilic part is composed of sugar chains with functional groups. Purum saponin contains 42.3% carbon (C), 6.2% hydrogen (H), 0.2% nitrogen (N), and 51.3% oxygen (O).

Reco-10, a 10% mixture of two major rhamnolipids, RLL (R1, $C_{26}H_{48}O_9$) and RRLL (R2, $C_{32}H_{58}O_{13}$) was purchased from the Jeneil Biosurfactant Co LLC, USA. Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and p-hydroxydecanoic acid. The rhamnolipids were produced from sterilized and centrifuged fermentation broth. The commercially available product is in the form of a dark brown solution. In contrast to that of saponin, the pH of rhamnolipids ranges from 6 to 7.

The chemical structure of both biosurfactants is given in Figure 1.

Soils

Three soils were collected from different sites in Warmia and Mazury province, Poland: sandy clay loam, SCL-B (Baranowo), clay loam, CL-W (Wanguty) and clay, C-W (Wiktorowo). The soils were air-dried and ground to pass through a 1-mm sieve. The physico-chemical properties of the soils are given in Table 1.

Determination of biosurfactant adsorption on soils

To determine biosurfactant adsorption on soils, the surface tension of fresh biosurfactant solutions at concentrations from 1 to 10 000mg·L^{-1} was measured with a Krüss K100 tensiometer employing the Wilhelmy plate method. Then, each biosurfactant solution at a given concentration was shaken with soil (SCL-B, CL-W, C-W) at soil/biosurfactant ratios of 1/10 and 1/40 (m/V) on a rotary shaker at 150 rpm for 24h. The supernatants were centrifuged at 8000 rpm for 1h, filtered, and then the surface tension was measured again. The surface tension values were plotted vs. the logarithm of the surfactant concentration. The point of intersection of the two regression lines made on the basis of the experimental data indicates the critical micelle concentration (CMC). The CMC is the lowest aqueous concentration of surfactant at which the surface tension of the solution shows the smallest tensional force (Urum and Pekdemir 2004). The amount of biosurfactant adsorbed on soil at the critical micelle concentration was calculated using the following formula (Zheng and Obbard 2002):

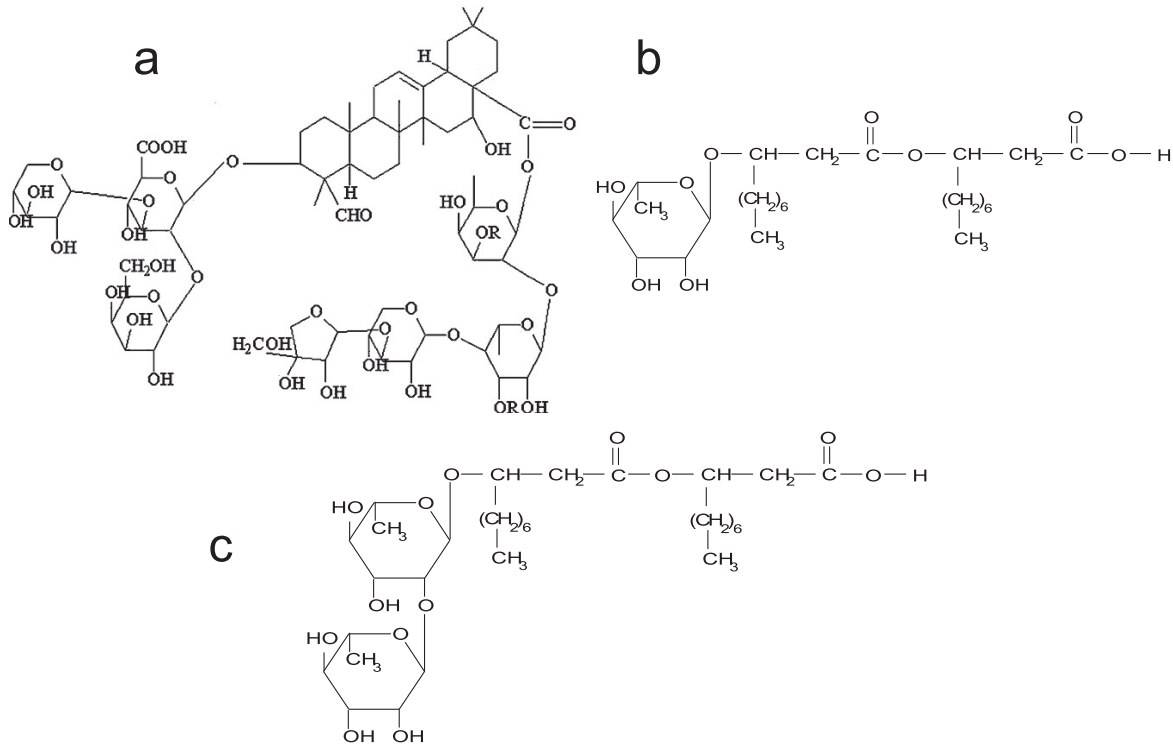

Figure 1. Chemical structure of biosurfactants: a) saponin (Higuchi 1987), b) rhamnolipid R1, c) rhamnolipid R2 (JENEIL 2006).

Table 1. Physico-chemical characteristics of the soils.

Characteristic	Unit	Soil		
		SCL-B	CL-W	C-W
Sand	%	64	44	6
Silt	%	16	18	33
Clay	%	20	38	61
Organic matter	%	1.6	2.1	10.3
CEC	$cmol \cdot kg^{-1}$	12.3	20.3	53.3
pH (in water)	-	7.1	7.2	6.1
Bulk density	$kg \cdot L^{-1}$	1.69	1.58	1.07
Porosity	%	34.7	42.3	53.7
Iron	%	1.08	2.03	2.39

SCL-B sandy clay loam, CL-W clay loam, C-W clay
CEC cation exchange capacity
- not applicable

$$Q_{surf}=\left(\frac{CMC_{SS}-CMC_{FS}}{m}\right)\cdot V \qquad (1)$$

where Q_{surf} is the amount of surfactant adsorbed on soil ($mg \cdot g^{-1}$); CMC_{SS}, the critical micelle concentration of the soil/surfactant supernatant ($mg \cdot L^{-1}$); CMC_{FS}, the critical micelle concentration of fresh surfactant solution ($mg \cdot L^{-1}$); V, the volume of the surfactant solution (L); m, the weight of the soil (g).

RESULTS AND DISCUSSION

The changes in surface tension depending on saponin and Reco-10 concentration are presented in Figures 2 and 3, respectively. For both biosurfactants, the surface tension curves were composed of two characteristic regions. In the first region, the surface tension decreased sharply as surfactant concentration increased to the critical micelle concentration (CMC). In this phase, both biosurfactants occurred as monomers.

In the second region, above the CMC, surface tension remained constant. This means that the biosurfactant monomers aggregated in micelles. At m/V = 1/40, changes in surface tension of the supernatants showed a similar trend, but the point of intersection of the two regression lines was at different concentrations (data not shown). Anionic Reco-10 decreased surface tension more than nonionic saponin. The minimum value of surface tension for Reco-10 averaged 26.7mN·m^{-1} (Figure 3), whereas for saponin it was 37.6mN·m^{-1}

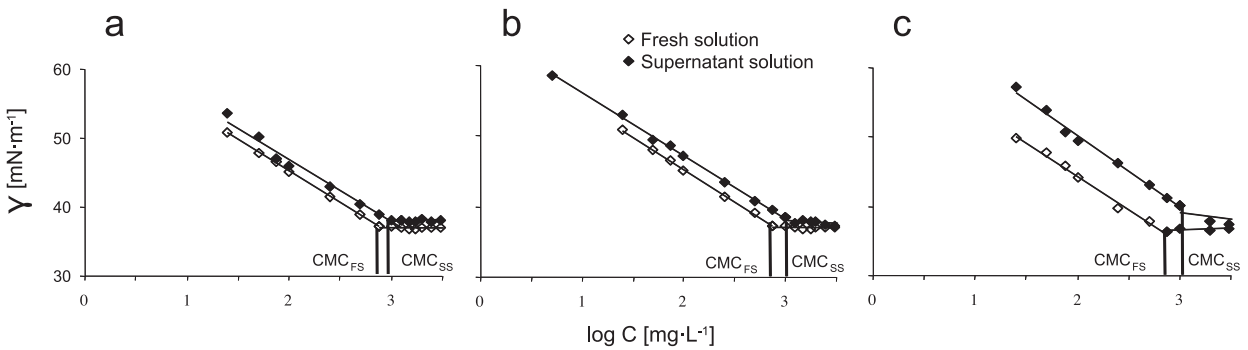

Figure 2. Changes in surface tension depending on saponin concentration C (biosurfactant concentration in mg·L^{-1}) (expressed as log C) in fresh and supernatant biosurfactant solutions at soil (m, grams) to biosurfactant (V, litres) ratio m/V=1/10: a) SCL-B sandy clay loam, b) CL-W clay loam, c) C-W clay; CMC_{FS} stands for CMC in fresh biosurfactant solution, CMC_{SS} stands for CMC in supernatant biosurfactant solution.

(Figure 2). Similar values of surface tension for both biosurfactants were indicated by Urum and Pekdemir (2004). For each biosurfactant, linear regions of the surface tension curve below and above the CMC were described using the equations for the regression lines. For example, for the fresh saponin solution these equations were γ_1=-9.148x+63.567 and γ_2=-0.0295x+37.17, respectively.

By finding the intersection of both lines, the CMC in fresh solutions and in supernatant solutions with different soils, at various m/V ratios was determined. The calculated values are given in Table 2.

The critical micelle concentration (CMC) of a surfactant is an important physical characteristic. When surfactants are present at a concentration above the CMC they can act

Table 2. The Critical Micelle Concentration (CMC) values for the biosurfactants.

Sample	m/V	CMC [mg·L^{-1}]	
		Saponin	Reco-10
Control (aqueous solution without soil)	–	785.0	24.5
SCL-B (sandy clay loam)	1/10	851.1	169.8
	1/40	1024.9	138.0
CL-W (clay loam)	1/10	870.9	380.2
	1/40	1122.0	195.0
C-W (clay)	1/10	1096.5	1548.8
	1/40	1698.2	660.7

- not applicable

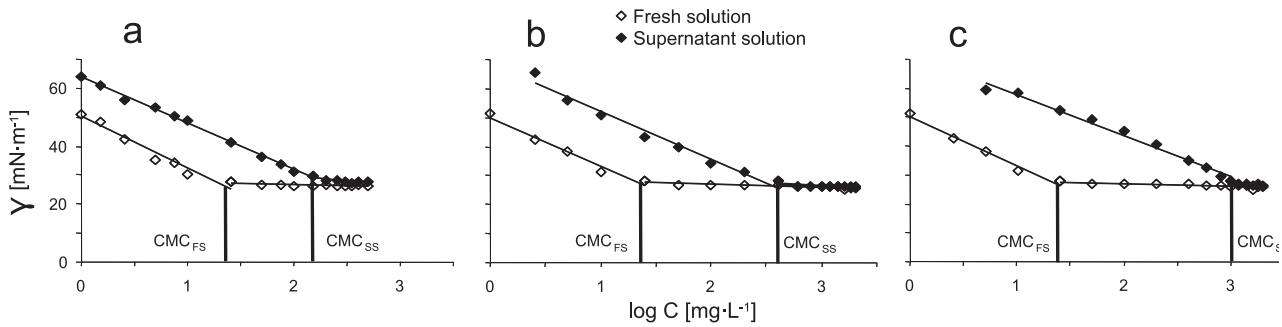

Figure 3. Changes in surface tension depending on Reco-10 concentration C (biosurfactant concentration in mg·L^{-1}) (expressed as log C) in fresh and supernatant biosurfactant solutions at soil (m, grams) to biosurfactant (V, litres) ratio m/V=1/10: a) SCL-B sandy clay loam, b) CL-W clay loam, c) C-W clay; CMC_{FS} stands for CMC in fresh biosurfactant solution, CMC_{SS} stands for CMC in supernatant biosurfactant solution.

as emulsifiers, allowing normally immiscible compounds to dissolve in the solvent (Held 2014).

Saponin had a higher CMC than Reco-10, except with C-W soil at a soil/biosurfactant solution ratio of 1/10 (m/V). The CMC of both biosurfactants was higher with soils than without, which is in accordance with the literature (Chu and Chan 2003). With soil, the surfactant dose required for micelle formation is greater because the surfactant is partitioned onto soil. Therefore, the CMC is higher in soil-water systems than in aqueous solutions. Chu and Chan (2003) reported that the CMC for nonionic Brij 35 increased from $1.6 \cdot 10^{-4}$mol·L^{-1} in aqueous solution to $1.0 \cdot 10^{-3}$mol·L^{-1} in a surfactant/soil system. Sorption of saponin and two types of rhamnolipids (JBR 515 and JBR 425) was also studied by Kuczajowska-Zadrożna et al. (2015). They used the same technique for CMC determination as in the present study, but another type of sorbent, i.e. grains of activated sludge immobilized in a mixture of alginate with polyvinyl alcohol. Despite the difference in types of sorbent, the CMC for saponin increased from 707 to 1206mg·L^{-1}, for JBR 515 from 63 to 279mg·L^{-1}, and for JBR 425 from 107 to 234mg·L^{-1}. As a result, the loss of biosurfactants by partitioning onto the sorbent amounted to 41.3%, 77.4%, and 54.3%, respectively.

The changes in CMC depended on soil type. In the present study, more of both biosurfactants were adsorbed on soils with higher CEC, and higher content of clay and organic matter (Figure 4). Ochoa-Loza et al. (2007) stated that sorption of rhamnolipids can be predicted based on the mineral and chemical composition of the soil. The authors tested sorption of rhamnolipid R1 by six different soils. The lowest sorption of R1 was in soil with the lowest clay (33g·kg^{-1}), iron (2.9g·kg^{-1}), and total organic content (3.2g·kg^{-1}). In contrast, the greatest R1 sorption was in soil with a high content of iron (230g·kg^{-1}) and clay (569g·kg^{-1}). In the present study, sorption of both biosurfactants was the highest in clay soil (C-W), and the lowest in sandy clay loam (SCL-B). This confirms the findings of Ochoa-Loza et al. (2007) that there is a relationship between soil composition and biosurfactants sorption, and that the higher the organic and clay content in soil, the greater the surfactant dose required for soil remediation.

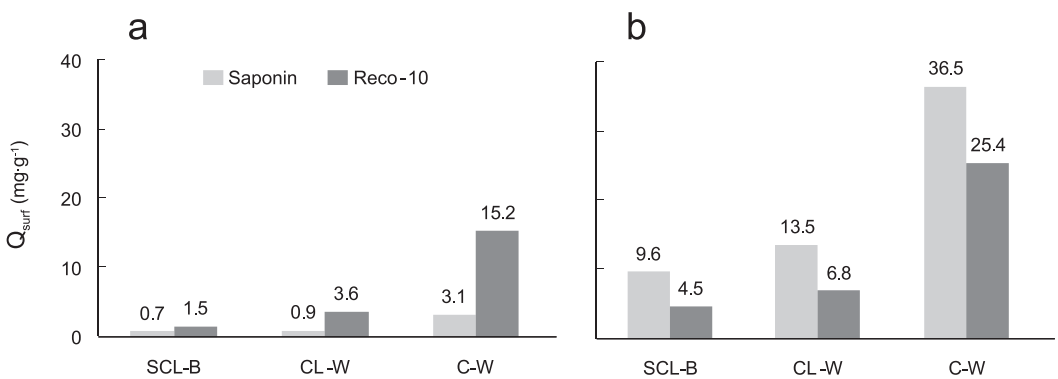

Figure 4. The amount of biosurfactants adsorbed in soils (Q_{surf}) at different soil (m, grams) to biosurfactant solution (V, litres) m/V ratios: a) m/V=1/10, b) m/V=1/40. SCL-B sandy clay loam, CL-W clay loam, C-W clay.

Biosurfactant sorption also depended on m/V ratio. At the lower m/V ratio (1/40), more saponin than Reco-10 was adsorbed in soils. Similarly, Liu et al. (1992) found that as the m/V ratio decreased, higher amounts of surfactant had to be added to decrease the surface tension by a given amount. In the present study, saponin showed higher sorption at lower m/V ratio, in contrast to Reco-10. Because, at both ratios, the mass of soil was constant and the volume of biosurfactant solution was different, at the lower m/V ratio a greater electrostatic attraction between saponin and soil could have occurred due to the higher amount of H^+ ions in the reaction vessel. Saponin is more acidic than Reco-10, which may be why its sorption to soil was greater, especially at m/V=1/40. The adsorption of saponin is known to increase under acidic conditions (Hong et al. 2002).

Generally, adsorption of nonionic surfactants is higher than that of anionic surfactants. Hydrogen bonding (known as hydrophobic bonding) is responsible for adsorption of nonionic surfactants (Muherei and Junin 2009). These surfactants were found to adsorb extensively not only to organic matter in soil, but also to clay minerals. In contrast, adsorption of anionic surfactants to clay minerals was significantly lower (Rodriguez-Cruz et al. 2005, 2006). Rhamnolipid sorption involves cation bridging between anionic polar groups and sorbed cations on soil components. Hydrophobic interactions between nonpolar tails and hydrophobic components in the soil (e.g. organic matter) are also important for rhamnolipid sorption (Ochoa-Loza et al. 2007; Torrens et al. 1998). The observation that, at the lower m/V ratio in the present study, less of the rhamnolopid was absorbed to the soil than saponin can be attributed to the fact that soil in aqueous solution often obtains a negative charge, which repels the anionic rhamnolipid (Muherei and Junin 2009).

SUMMARY AND CONCLUSIONS

A surface tension technique enabled determination of the adsorption of biosurfactants in soil at their critical micelle concentration. Adsorption of saponin and Reco-10 was higher in soils with more clay and organic matter. On each soil, less saponin was adsorbed than Reco-10 at the higher soil/biosurfactant solution ratio (1/10, m/V), whereas more saponin was adsorbed than Reco-10 at the lower ratio (1/40, m/V). Taking into account the sorption of saponin and Reco-10, their application in soil remediation would be more efficient for sandy clay loam than for clay.

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