

Characterization and Application of Sodium Surfactin in Mobilization of Toluene and Perchloroethylene by Batch Configuration Test

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The present work focuses on the remediation of contaminated aquifers by hydrophobic organic contaminants, such as hydrocarbons or chlorinated solvents, following the approach of Surfactant Enhanced Aquifer Remediation (SEAR) technology, based on the use of surfactants for the solubilization and mobilization of pollutants.

In this context, surfactin, a lipopeptide biosurfactant produced by *Bacillus Subtilis*, was selected as reference material for the NAPLs mobilization study. After a preliminary characterization of surfactin for the experimental evaluation of its physicochemical parameters (critical micelle concentration and surface behavior), the study involved in a batch configuration test in which toluene and perchloroethylene adsorption on a porous material was investigated in the presence and in the absence of surfactin as an indirect way to evaluate mobilization ability of surfactin through the reduction of NAPLs adsorption.

Experimental measurements highlighted a relatively low CMC value ($2.45 \times 10^{-2} \text{ g L}^{-1}$) and a great tendency of surfactin to adsorb at the surfaces or interfaces. The batch test showed that the surfactin solution had no effects on the adsorption of toluene and PCE. On the other hand, surfactin led to an important decrease in the affinity between dissolved pollutants and solid surface. In general, these results suggest the possibility of using surfactin in the enhanced surfactant remediation technology.

1. Introduction

Non-aqueous phase liquids (NAPLs) are among the most widespread soil and groundwater contaminants. They are hydrophobic organic pollutants, poorly soluble and immiscible in water with a high tendency to accumulate in the soil organic fraction, classified into dense (DNAPLs) and light (LNAPLs) based on their density. Among the DNAPLs, chlorinated solvents (ethanes, ethenes, and chlorinated methanes) are certainly the most widely identified compounds, while LNAPLs are complex petroleum hydrocarbon mixtures. The physicochemical characteristics of NAPLs, their distribution into the sub-soil, their molecular diffusion and the adsorption in the low permeability layers, and the subsequent back-diffusion and desorption phenomena (particularly relevant in historical contamination cases) make the efficiency of conventional remediation technologies very low. For example, pump-and-treat (P&T) may show strong limitations for NAPLs removal and achievement of low regulatory limits.

In this regard, it is possible to use surfactants as an enhancer for NAPLs recovery in less time and at a lower cost by washing or flushing processes (Ciampi et al., 2021). Surfactants are amphiphilic organic molecules with a hydrophobic tail group and a hydrophilic head group (García-Cervilla et al., 2020). Thanks to their chemical structure, surfactants can adsorb at the interface between immiscible phases (i.e., liquid NAPLs and water) reducing the interfacial tension. Surfactants also increase the apparent solubility of insoluble compounds due to micelles formation when surfactant concentration is equal to or greater than their critical micelle concentration (CMC), that is an important parameter of surfactant because allows to define the minimum amount of surfactant that can guarantee the formation of micelles and the increase of solubilization of hydrophobic compounds (Dussaussoy et al., 2021; Hinton & Alvarez, 2021).

Under the same conditions (e.g., temperature, pressure, pH, ionic strength), micellization strongly depends on the structural molecular properties. It is demonstrated that CMC decreases with the increase in alkyl chain length and increases the increasing size of the hydrophilic group, which is mainly due to steric hindrance (Gaudin et al., 2019; Hinton & Alvarez, 2021).

Surfactants can be classified into synthetic and natural surfactants according to their production method: chemical synthesis or microbial fermentation, respectively (Jahan et al., 2020; Jesus et al., 2021). However, the use of synthetic surfactants as extractive agents in soils can be a problem because, due to their fossil derivation and, on the other hand, they tend to remain partially in the soil leading to a secondary contamination for their toxicity and low biodegradability (Huo et al., 2020; Madrid et al., 2019). Recently, most interest has been focused on surfactants that are 100% bio-based (Pironti et al., 2020), such as sugar-based nonionic surfactants or lipopeptide surfactants synthetically produced from renewable sources (e.g., starch and vegetable oils) or from biological fermentation of an organic feedstock (Gaudin et al., 2019).

Considering the use of surfactants in the context of environmental remediation, this current work investigated the ability of sodium surfactin produced by the secondary metabolism of *Bacillus subtilis*, to dissolve and mobilize Non-Aqueous Phase Liquids (NAPLs), i.e., toluene and perchloroethylene (PCE) selected for the experimentation as reference pollutants. An important contribution of our work is the use of bio-based surfactants derived from renewable resources or organic substrate fermentation, which are non-toxic and 100% biodegradable, a relevant aspect of the field of sustainable remediation approaches.

The study concerned a preliminary physicochemical characterization of surfactin to evaluate the CMC (critical micelle concentration) and surface activity parameters. Then, selecting toluene (LNAPL) and perchloroethylene (DNAPL) as reference pollutants, an indirect mobilization study was carried out through a batch thermodynamic study (adsorption isotherms) to evaluate the effect of surfactin, employed with a concentration equal to 5 times the CMC (5x CMC) for NAPLs adsorption process on a reference sorbent material.

2. Material and Methods

2.1 Materials

2.1.1 Sodium Surfactin

The surfactin sodium salt was supplied by the company Chimec s.p.a. Surfactin belongs to the group of cyclic lipopeptide biosurfactants produced by *Bacillus Subtilis*. The hydrophilic fraction contains an oligopeptide ring with seven amino acids. The hydrophobic part is composed of a C11-C13 fatty acid (Hoffmann et al., 2021). The surfactin structure is shown in Figure 1.

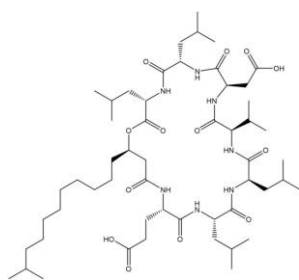


Figure 1. Chemical structure of surfactin

2.1.2 Pine-Wood Biochar

Biochar from pine wood wastes (PWB) was used as sorbent reference material for batch tests. PWB was obtained from the gasification of wood at approximately 850°C in V 3.90 Burkhardt and ECO 180 HG wood gas generator (Plößberg bei Tirschenreuth, Germany). This microporous material with a total pore volume of 0.383 cm³ g⁻¹, has a high specific surface area (343 ± 2 m² g⁻¹) and a high carbon content (95.84 wt%) (Rossi et al., 2021).

2.2 Critical Micelle Concentration (CMC) determination

This study began with the experimental determination of surfactin's critical micelle concentration (CMC). A series of surface tension measurements of the surfactants were performed, according to the ring detachment method, by a MWG LAUDA tensiometer, using the Du-Nouy ring (platinum-iridium alloy ring with a diameter of 0.95 cm) (Lira et al., 2021). During the experiment, a surfactin aqueous solution was prepared using high-

purity milli-Q water with a surfactant concentration equal to 1 g L⁻¹. Each sample was prepared by diluting the previous one with a concentration step of 0.2 g L⁻¹.

Surface tension data were plotted against the surfactant concentration and against the logarithm of the surfactant concentration (two plots were made) and CMC value was calculated from breakpoints of the two plots.

2.3 Batch configuration experiment

A thermodynamic study was performed to indirectly assess the mobilization capacity of surfactin by evaluating the reduction of adsorption of contaminants by PWB in the presence of surfactants. Two organic compounds were selected as target contaminants, toluene and tetrachloroethylene (PCE), which belong to the LNAPLs and DNAPLs classes, respectively. A series of isothermal batch experiments with PWB/contaminant/surfactant were conducted at room temperature (23 ± 2 °C) and pressure in 20 mL batch reactors (VWR International glass vials, Milan, Italy), using different amounts of sorbent material (10, 20, 50, 80, 100 and 200 mg), and 50 mg L⁻¹ of contaminant. For the tests, a surfactant concentration five times greater than CMC (5x CMC) was adopted to balance the economic aspects and the generation of surfactant micelles. In parallel, a reference tests were performed without surfactant solutions. Each test was performed in duplicate to evaluate reproducibility. The initial concentration of the contaminant was analyzed at time zero (C₀) and after 24 hours (C_e), which is the time required to reach the thermodynamic equilibrium condition (Rossi et al., 2021).

2.4 Analytical Methods

Toluene and PCE were determined by a Dani master gas-chromatograph (GC) equipped with a Flame Ionization Detector (FID), a TRB624 capillary column (30 m × 0.53 mm ID × 3 μm), and an HSS Dani 86.50 headspace auto-sampler (Dani Instrument, Contone, Switzerland). The injection temperature was set at 180 °C, and the FID temperature at 300 °C.

2.5 Calculation

To evaluate the surface properties of surfactin, efficiency, and effectiveness parameters were evaluated both for the adsorption to the liquid-air surface and for the reduction of surface tension. The Gibbs adsorption isotherm equation (Eq. 1) was used to calculate the maximum (saturated) surface excess concentration.

$$\Gamma_{max} = -\frac{1}{2.303nRT} \left(\frac{\partial \gamma}{\partial \log C} \right) \quad (1)$$

Where Γ_{max} is the maximum excess surface concentration (mol cm⁻²), n is the number of the solute species which for nonionic surfactant is equal to 1, R is the gas constant value (8.314 J mol⁻¹ K⁻¹), T is the ambient temperature (K), and $(\partial \gamma / \partial \log C)$ is the slope of the linear fit of the data below the CMC. The surface tension reduction efficiency at the CMC, the surface pressure parameter (π_{CMC}) was calculated according to Eq. 2

$$\pi_{CMC} = \gamma_0 - \gamma_{CMC} \quad (2)$$

Where γ_0 and γ_{CMC} are the surface tension of water ($\gamma_0 = 72.8$ mN m⁻¹) and surfactant solution at CMC, respectively.

In the context of the thermodynamic batch experiment, equilibrium concentration data, both in the liquid (C_e) and solid (q_e) phases were first calculated. C_e was directly measured by gas-chromatographic analysis, and q_e was calculated from C_e using the following formula (Eq. 3):

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (3)$$

Where C₀ is the initial concentration of pollutant in the solution (50 mg L⁻¹), V is the total volume of the liquid phase (20mL) and m is the mass (in grams) of PWB in each reactor. Langmuir isotherm model (Eq. 4) was used for equilibrium data fitting purposes.

$$q_e = q_{max} \frac{K_L C_e}{1 + K_L C_e} \quad (4)$$

Where q_{max} (mg g⁻¹) is the maximum quantity of adsorbed species, K_L is the Langmuir thermodynamic constant (L mg⁻¹). Parameters optimizing were obtained by Sigma Plot 12 to calculate both q_{max} and K_L.

3. Results and Discussions

3.1 Critical Micelle Concentration (CMC) determination

The plots of the surface tension vs concentration and log of concentration are reported in Figs. 2a and 2b, respectively show the typical pattern with two slopes, whose intersection provides the CMC value. This value is reported in Table 1, along with the values of surface tension at CMC (γ_{CMC}), maximum surface excess concentration (Γ_{max}), and surfactant effectiveness in surface tension reduction (π_{CMC}).

From surface tension measurements, a CMC = $2.45 \times 10^{-2} \text{ g L}^{-1}$ and a corresponding surface tension $\gamma_{CMC} = 30.63 \text{ mN m}^{-1}$ were calculated. The maximum excess surface concentration (Γ_{max}), which measures the effectiveness of surfactant adsorption at the interfaces or surfaces, was equal to $3.12 \times 10^{10} \text{ mol cm}^{-2}$. All experimental values are within the range reported in the scientific literature for lipopeptide surfactants (CMC = $2 \times 10^{-2} - 5 \times 10^{-2} \text{ g L}^{-1}$; $\gamma_{CMC} = 27 - 32 \text{ mN m}^{-1}$; $\Gamma_{max} = 2.7 \times 10^{10} - 6 \times 10^{10} \text{ mol cm}^{-2}$) (Hoffmann et al., 2021; Jahan et al., 2020).

It is interesting to note a relatively low CMC and a relatively high maximum surface excess concentration (Γ_{max}) if we consider the big size of the polar head group of surfactin (CMC and Γ_{max} of surfactin are comparable with those of other biosurfactants which have a smaller polar group, i.e. sophorolipids, and rhamnolipids (Jiménez-Peñalver et al., 2020)). This observation indicates that surfactin molecules are in a very packed form during surface adsorption or micellization processes, with the polypeptide ring oriented in the same direction of the hydrophobic chain (perpendicular to the liquid air surface or the interface between two phases) which does not inhibit the aggregation of surfactin molecules (Maget-Dana & Ptak, 1992).

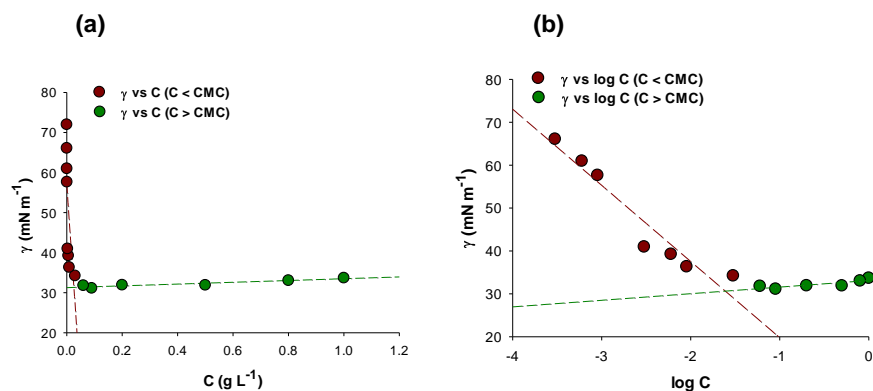


Figure 2. Variation of surface tension with surfactant concentration (a) or log of surfactant concentration (b)

Table 1. Micellar and surface properties of surfactin at 298.15 K and atmospheric pressure

Surface Parameters	CMC (g L ⁻¹)	γ_{CMC} (mN m ⁻¹)	π_{CMC} (mN m ⁻¹)	$\Gamma_{max} \times 10^{10}$ (mol cm ⁻²)
	2.45×10^{-2}	30.63	42.17	3.12

3.2 Batch Configuration Experiment

The adsorption isotherm tests were further conducted to evaluate the mobilization capacity of surfactin in the presence of different organic pollutants, i.e., toluene from the LNAPLs group and PCE representing the chlorinated solvent family (DNAPLs). Organic compounds mobilization was studied indirectly by evaluating the reduction of their adsorption in the presence of an aqueous solution of surfactin, with respect to the reference test conducted with only distilled water.

Fig. 3a and Fig. 3b show the evolution of the adsorption isotherm of toluene and PCE on PWB, respectively. Each figure shows the adsorption isotherms obtained in the absence (Reference) and in the presence of surfactin solution with a surfactant concentration 5 times greater than CMC.

As can be seen in Fig. 3 and Table 2, the adsorption capacity of toluene and PCE on PWB was not affected by the presence of surfactin. It is possible to note that q_{max} parameter, which quantifies the maximum adsorption capacity, is almost the same between the reference test and that one in the presence of surfactin. In fact, in the presence of surfactin q_{max} changed from 79.71 mg g^{-1} to 87.97 mg g^{-1} for toluene, and from 119.12 mg g^{-1} to 124.37 mg g^{-1} for PCE. This observation can be explained by the great and irreversible adsorption of surfactin on PWB surface (Penfold et al., 2012). Consequently, the effective surfactant in the

solution resulted lesser than the expected amount, leading to an incapacity to successfully solubilize NAPLs and to reduce their adsorption on PWB.

At the same time, the surface properties of PWB changed reducing the affinity between dissolved NAPLs and PWB. Indeed, Table 2 shows that in the presence of surfactin solution, the Langmuir constant (K_L), that is the indicator parameters of the affinity between NAPLs and PWB, was decreased from $15.4 \times 10^{-2} \text{ L mg}^{-1}$ and $29 \times 10^{-2} \text{ L mg}^{-1}$ to $86.1 \times 10^{-3} \text{ L mg}^{-1}$ and $67.1 \times 10^{-3} \text{ L mg}^{-1}$ for toluene and PCE, respectively.

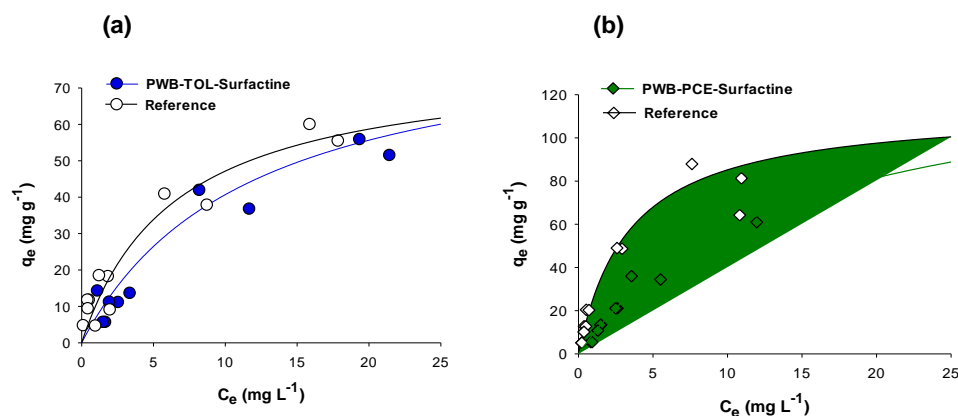


Figure 3. Isotherm curves of toluene (a) and PCE (b) on PWB.

Table 2. Optimized parameters of Langmuir model of toluene and PCE on PWB. All parameters were calculated in this study by using Sigma Plot 2.0 software.

Toluene Isotherms			
	$q_{\max} (\text{mg g}^{-1})$	$K_L (\text{L mg}^{-1})$	R^2
PWB-TOL-Surfactin	87.97 ± 18.4	$86.1 \times 10^{-3} \pm 3.7 \times 10^{-3}$	0.93
Reference TOL	79.71 ± 11.7	$15.4 \times 10^{-2} \pm 5.5 \times 10^{-2}$	0.92
PCE Isotherms			
	$q_{\max} (\text{mg g}^{-1})$	$K_L (\text{L mg}^{-1})$	R^2
PWB-PCE-Surfactin	124.37 ± 22.9	$67.1 \times 10^{-3} \pm 1.84 \times 10^{-3}$	0.97
Reference PCE	119.12 ± 7.8	$29 \times 10^{-2} \pm 4.8 \times 10^{-2}$	0.98

4. Conclusions

This study evaluates the interfacial and surface properties of surfactin, a lipopeptide biosurfactant from *B. Subtilis*. The final purpose of this work is the evaluate the technical applicability of surfactin in the remediation of polluted aquifers. The investigated surfactant, despite its chemical structure with a big hydrophilic moiety, shows a very low CMC ($2.45 \times 10^{-2} \text{ g L}^{-1}$), a great tendency to adsorb at the liquid-air surface ($\Gamma_{\max} = 3.12 \times 10^{10} \text{ molecule cm}^{-2}$) and great efficiency in surface tension reduction ($\gamma_{\text{CMC}} = 30.63 \text{ mN m}^{-1}$ and $\pi_{\text{CMC}} = 42.17 \text{ mN m}^{-1}$). However, the results of the thermodynamic test didn't confirm the evidence of physicochemical characterization. The presence of surfactin solution was not able to reduce efficiently NAPLs adsorption on PWB probably due to an important loss of surfactant that was adsorbed on PWB. But probably also surfactin changed the surface properties of PWB leading to a great reduction of the physicochemical affinity between dissolved NAPLs and solid surfaces.

Further investigations are necessary to better understand the behavior of surfactin toward solid surface of PWB and to evaluate directly NAPLs mobilization by surfactin taking advantage of the great reduction in the affinity between organic pollutants and adsorbent solid found in this study.

Acknowledgments

Chimec SpA is thankfully acknowledged for its collaboration and for supplying the surfactant materials.

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