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# Study of the Sorption of Biosurfactants from *l. Pentosus* on Sediments

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***Abstract*****—**Losses of surfactant due to sorption need to be considered when selecting surfactant doses for soil bioremediation. The degree of surfactant sorption onto soil depends primarily on the organic carbon fraction of soil and the chemical nature of the surfactant. The use of biosurfactants in the control of the bioavailability of toxicants in soils is an attractive option because of their biodegradability. In this work biosurfactants were produced from a cheap raw material, trimming vine shoots, employing *Lactobacillus pentosus*. When biosurfactants from *L. pentosus* was added to sediments the surface tensión of the water containing the sediments rapidly increase, the same behaviour was observed with the chemical surfactant Tween 20; whereas sodyum dodecyl sulphate (SDS) kept the surface tension of the water around 36 mN/m. It means, that the behaviour of biosurfactants from *L. pentosus* is more similar to non-ionic surfactatns than to anionic surfactants.

***Keywords*****—**Biosurfactants, *L. pentous*, sediments, surface tension

## I. Introduction

REMEDIATION of soil contaminated with petroleum and its derived products is a major environmental concern due to their toxic, mutagenic and carcinogenic properties [1]. These products are introduced to the environment due to various anthropogenic activities, such as accidental spills from transportation processes, leaking underground storage tanks, and poor waste disposal practices. These compounds are commonly found in soil groundwater aquifers in industrialized areas.The presence of surfactants or biosurfactants can lead to an increase in the concentration of hydrophobic compounds in the water phase. This is achieved through the formation of hydrocarbon/water emulsions and solubilisation, where, above the Critical Micelle Concentration (CMC), biosurfactant molecules aggregate to form micelles. CMC is that concentration of surfactant favouring micelle formation. Micelles arise when the lipophilic part of the surfactant molecule that is unable to form hydrogen bonding in an aqueous phase causes an increase in the free energy of the system. One way to alleviate this free energy increase is for the hydrocarbon tail to be isolated from water by adsorption onto surfaces, absorption into an organic matrix or the formation of micelles vesicles where the hydrocarbon moiety of the surfactant become situated towards the centre with the hydrophilic part in contact with water.The adsorption of surfactants on rock/soil/sediment solid matrix may result in the loss and reduction of their concentration, which may render them less efficient or ineffective in practical treatment. Adsorption of surfactants from aqueous solutions in porous media is very important in enhanced oil recovery of oil reservoirs because surfactant loss due to adsorption on the reservoir rocks impairs the effectiveness of the chemical solution injected to reduce the oil–water interfacial tension and renders the process economically unfeasible [2]On the other hand, biosurfactants are biological compounds that exhibit high surface-active properties. Microorganisms, plants and animals, including humans, produce them. Biosurfactants are significantly less toxic than synthetic petroleum-based surfactants [3]. In this way biosurfactants can be obtained by fermenting hemicellulosic sugars from trimming vine shoots by *L. pentosus* [4, 5, 6, 7]. For instance, Bustos et al. [6] carried out continuous production of lactic acid and biosurfactants from hemicellulosic sugars of trimming vine shoots. Moreover, Portilla et al. [8] found that there is an optimum carbon source ratio (glucose/xylose) to produce biosurfactants from *L. pentosus*. They found that trimming vine shoots, after hydrolysis with sulphuric acid, produce hemicellulosic sugars with xylose/glucose ratio accurately to obtain biosurfactants using *L. pentosus.* Trimmings of vine shoots from agriculture industry, related with wine elaboration, usually are field burnt; releasing cancerous compounds like polycyclic aromatic hydrocarbon as well as greenhouse gases. Therefore, the utilization of trimming vine shoots as carbon source to produce biosurfactants can decrease the environmental impact produced by this kind of residue, when it is field burnt.In this work biosurfactants obtained after fermentation of trimming vine shoots with *L. pentosus* were added to soil sediments, and the increase of surface tension in the water extract of sediments was evaluated in order to know if the biosurfactant produced by *L. pentosus* is adsorbed onto the sediments or remains in the aqueous phase. Moreover additional assays were also carried out using chemical surfactants (Tween 20, and sodium dodecyl sulphate) in order to compare the behaviour of biosurfactants from *L. pentosus* with the chemical surfactants.

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## II. Materials And Methods

### A.Hydrolysis of trimming vines shoots

Ground samples of trimming vine shoots were hydrolysed under selected conditions (3 % $H_2SO_4$, 15 min, 130 ºC, liquid/solid ratio 8:1 g/g) and neutralized with $CaCO_3$ to a final pH of 6.5. The $CaSO_4$ precipitated, was separated from the supernatant by filtration.

*B.Microorganism*

*L. pentosus* CECT-4023 T (ATCC-8041) was obtained from the Spanish Collection of Type Cultures (Valencia, Spain). The strain was grown on plates using the complete media MRS agar. Inocula were prepared by solubilisation of cells from plates with 5 mL of sterilized hydrolysate.

*C.Fermentation of hemicellulosic sugars from trimming vines shoots by L. pentosus*

The clarified hydrolysates were supplemented with nutrients (10 g/L of yeast extract and 10 g/L of corn steep liquid), sterilized and used directly as fermentation media. The chemostat fermentation was carried out in a 2 L Applikon fermentor at 200 rpm with 1.6 working volume at 31 ºC and pH controlled to 5.85 during 48 hours. Once the fermentation has finished, *L. pentosus* biomass was separated from the fermentation medium by centrifugation to biosurfactant extraction.

*D.Extraction of biosurfactants*

Cells were recovered by centrifugation, washed twice in demineralized water, and resuspended in 50 mL of phosphate-buffer saline (PBS: 10mM $KH_2PO_4$/$K_2HPO_4$ and 150 mM NaCL with pH adjusted to 7.0). The bacteria were left a room temperature up to 2 hours with gentle stirring for biosurfactant release. Biosurfactants were obtained in the PBS and bacteria were removed by centrifugation. The remaining supernatant liquid was tested for surface activity.

*E.Surface activity determination*

The surface activity of biosurfactants produced by the bacterial strains was determined by measuring the surface tension of the samples with the ring method. The surface tension of PBS extract containing the biosurfactants from *L. pentosus* was measured using a KRUSS K6 Tensiometer equipped with a 1.9 cm Du Noüy platinum ring. To increase the accuracy an average of triplicates was used for this study.

*F.CMC of bisourfactant from L. pentosus*

The biosurfactant concentration was determined using a calibration curve mg/L of biosurfactant=(surface Tension (mN/m)-72.6)/(-8.64). The calibration curve was calculated for a commercial biosurfactant produced by *Bacilli* (surfactin) using different concentrations of biosurfactant solutions, below the critical micelle concentration with known surface tension. In this biosurfactant concentration range the decrease of surface tension is linear and it is possible to establish a relationship between the biosurfactant concentration and the surface tension [9].

*G.Chemical characterization of sediments*

The organic matter content (%) in sediments was determined by oxidation with a mixture of $K_2Cr_2O_7$ and $H_2SO_4$ and titration with Mohr Salt, following the method proposed by Sauerlandt and modified by Guitián and Carballas (1976). Grain size distribution was measured by the pipette method according to Guitián and Carballas [10]. N was determined by wet digestion with $H_2SO_4$, by using the Kjeldhal method as described in Guitián and Carballas [10]. Total phosphorus in sediments was determined by means of an acid digestion (HF, $H_2SO_4$, HCl, 10:1:10) followed by colorimetric determination with molibdenum blue, as described by Murphy and Riley [11].

*H.Surfactants*

The surfactants employed in this work consisted on Tween 20; sodium dodecyl sulphate (SDS) and biosurfactants from *L. pentosus*. Tween 20 is a polysorbate surfactant whose stability and relative non-toxicity allows it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. It is a polyoxyethylene derivative of sorbitan monolaurate, and is distinguished from the other members in the polysorbate range by the length of the polyoxyethylene chain and the fatty acid ester moiety. Tween 20 is a non-ionic surfactant and its CMC is 8.04 x $10^{-5}$ M at 21 ºC.Sodium dodecyl sulfate (SDS or NaDS), sodium laurilsulfate or sodium lauryl sulfate (SLS) ($C_{12}H_{25}SO_4Na$) is an anionic surfactant used in many cleaning and hygiene products. SDS is a highly effective surfactant and is used in any task requiring the removal of oily stains and residues. It is not carcinogenic when either applied directly to skin or consumed [12]. It has however been shown to irritate the skin of the face with prolonged and constant exposure (more than an hour) in young adults [13]. The CMC of SDS is about 0.0082 M in pure water at 25 ºC. Biosurfactants from *L. pentosus* were produced by fermentation of hemicellulosic sugars from trimming vine shoots with *L.pentosus*. This biosurfactants are cell bound to the plasmatic membrane of *L. pentosus* cells and they were extracted with PBS. Biosurfactants from *L. pentosus* are no toxic for animal sor plants because Lactic acid bacteria are considered GRAS microorganisms.

*J.Surfactant sorption onto sediments*

10 g of sediments were added to different solutions of surfactans (Tween 20, SDS, biosurfactant from *L. pentosus*) at different liquid /solid ratio (1:5; 1:10; 1:20) and the surface tension of the water was measured employing a KRUSS K6 Tensiometer equipped with a 1.9 cm Du Noüy platinum ring. Surface Tension was measured up to 200 min. To increase the accuracy an average of triplicates was used for this study. The concentration of surfactants employed in the sorption experiments was two times over their CMC.

## III.RESULTS AND DISCUSSION

Sediments employed in this work has pH=6; 3 % of organic matter and a C/N ratio of 12; whereas the P content is about 702 mg /Kg. Table I shows the general characterization of the sediments used for the surfactant sorption experiments. On the other hand, Figure 1 shows the minima surface tension of water in presence of biosurfactans from *L. pentosus* (55 mN/m) and the dilution rate that it is nedded to reach its CMC. Biosurfactants from *L. pentosus* can be diluted about 6 times without decreasing the surface tension of water. The total concentration of biosurfactants produced by *L. pentosus* was about 11 mg/L and its CMC was around 2 mg/L; measured as equivalents of surfactin.

TABLE I
PROPERTIES OF SEDIMENTS EMPLOYED IN THIS WORK

| **Properties of sediments** | |
|---|---|
| Texture (%<63 μm) | < 1 |
| pH | 6.14 |
| Organic matter (%) | 3.03 |
| C/N | 12 |
| Total P mg/Kg | 9.8 |

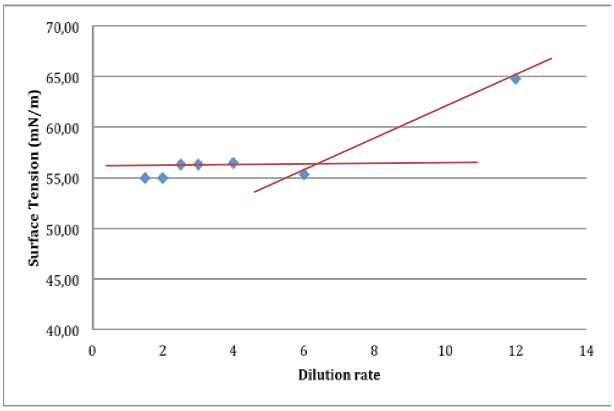


Fig. 1 Calculation of the dilution ratio for achieving the CMC concentration by the bisourfactant produced by *L. pentosus*

After the adsorption experiments it was observed that SDS almost did not was not adsorbed onto the sediments whereas tween 20 and bisourfactants from *L. pentosus* were absorbed at few minutes. It can due to de differences between the nature of surfactants and biosurfactants. Surfactant adsorption by soils/sediments depends on the type of surfactant and the soil properties. In this sense, the results of different studies addressing the adsorption of anionic and non-ionic surfactants by soils/sediments with different compositions have revealed the relative importance of soil/sediment organic matter and clay minerals on the adsorption of anionic and non-ionic surfactants [14]. Furthermore, Non-ionic surfactants were found to adsorb extensively to pure clay minerals, however, adsorbed amounts of anionic surfactant where significantly lower [15].Muherei and Junin [14] found that non-ionic surfactants tended to be strongly adsorbed to shale compared to sandstone. The possible sorption mechanism for non-ionic surfactant is the adsorption by hydrogen bonding and it strongly seems to show correlation with clay minerals. Anionic surfactant, SDS, on the other hand showed minor adsorption capacities to both shale and sandstone. It has been shown that the maximum adsorption amounts of SDS on both shale and sandstone were lower compared with that of TX100. This was attributed to repulsion forces between the negatively charged SDS and the negatively charged shale/sandstone. The same behaviour was observed in this work with the SDS, which showed minor adsorption capacities on the sediments tested than tween 20 and biosurfactants obtained from *L. pentosus.* Figure 2, Figure 3 and Figure 4 shows the increase of surface tension of water solution in presence of sediments and Tween 20 at different liquid/solid ratios (1:5; 1:10; 1:20 respectively).

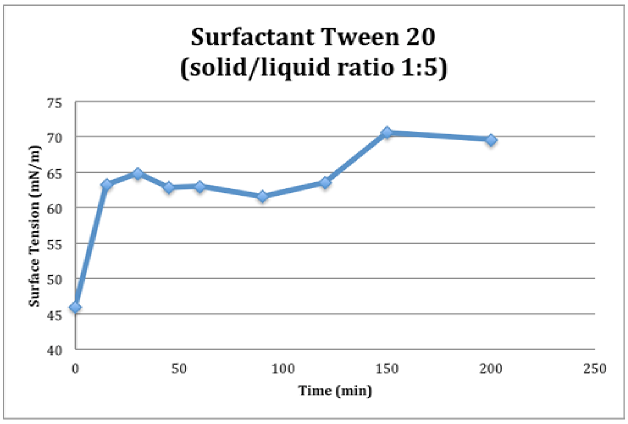


Fig. 2 Evaluation of surface tension of water in presence of Tween 20 and sediments using a solid/liquid ratio of 1:5

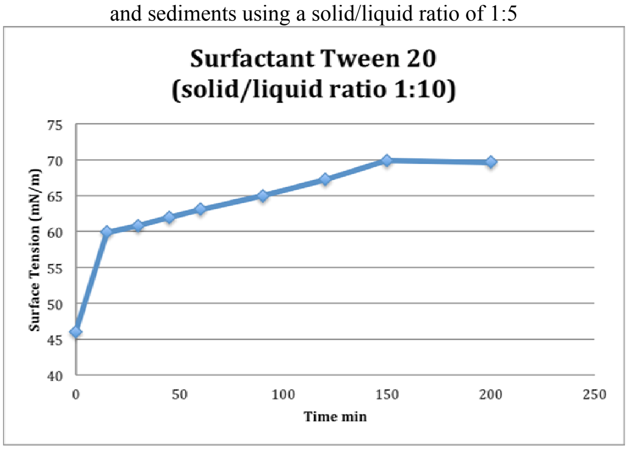


Fig. 3 Evaluation of surface tension of water in presence of Tween 20 and sediments using a solid/liquid ratio of 1:10

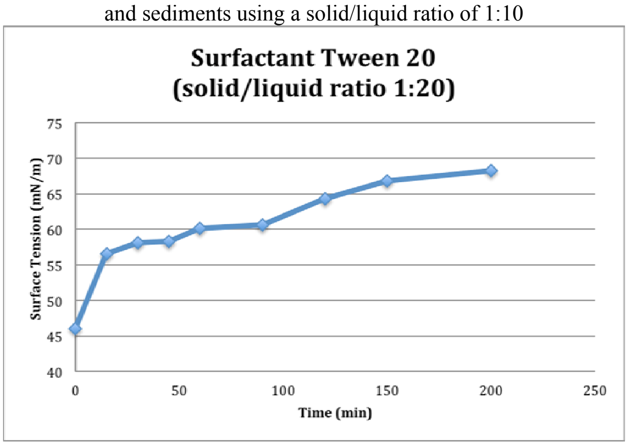


Fig. 4 Evaluation of surface tension of water in presence of Tween 20 and sediments using a solid/liquid ratio of 1:20

When experiments were run using biosurfactants from *L. pentosus*, like in the case of tween 20, the surface tension of water increase few minutes after contacting the bisourfactant with the sediments.Figure 5, Figure 6 and Figure 7, represents the variation of the water surface tension in presence of sediments and biosurfactants from *L. pentosus*, using different liquid/solid ratios (1:5; 1:10; 1:20 respectively).

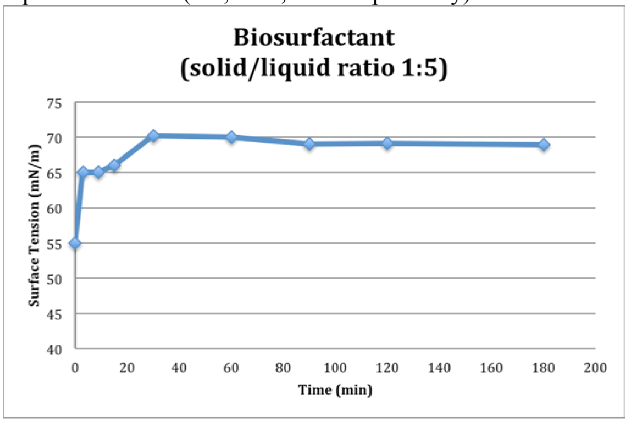


Fig. 5 Evaluation of surface tension of water in presence of Biosurfactant from *L. pentosus* and sediments using a solid/liquid ratio of 1:5

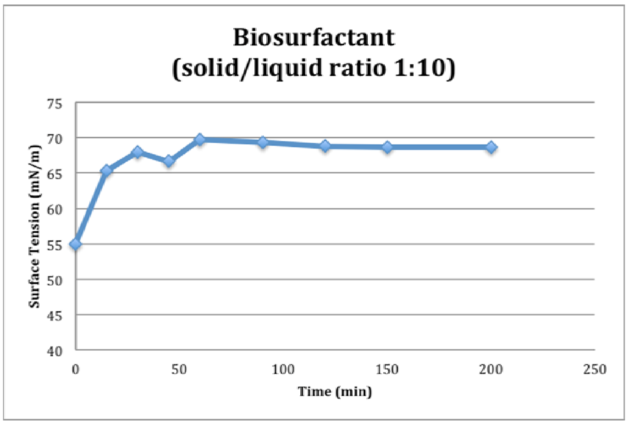


Fig. 6 Evaluation of surface tension of water in presence of Biosurfactant from *L. pentosus* and sediments using a solid/liquid ratio of 1:10

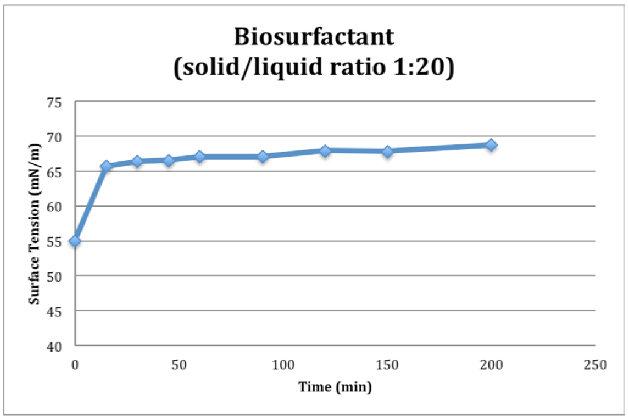


Fig. 7 Evaluation of surface tension of water in presence of Biosurfactant from *L. pentosus* and sediments using a solid/liquid ratio of 1:20

Contrary, when experiments were carried out using SDS the surface tension of the water remained invariable for the entire solid/liquid ratio tested. **Figure 8, Figure 9** and **Figure 10** represent the variation of surface tension of water in presence of sediments and SDS for 1:5; 1:10 and 1:20 solid/liquid ratios respectively.

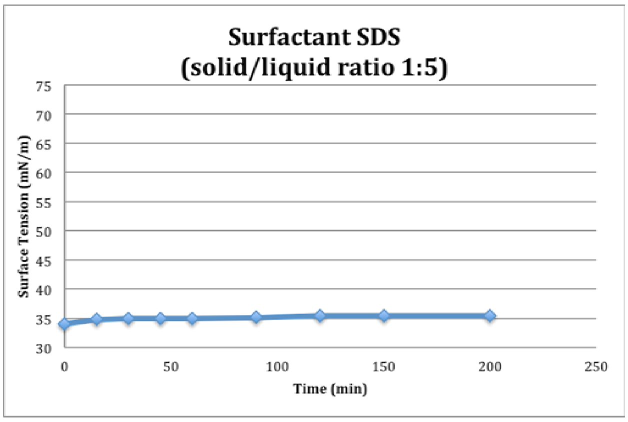


Fig. 8 Evaluation of surface tension of water in presence of SDS and sediments using a solid/liquid ratio of 1:5

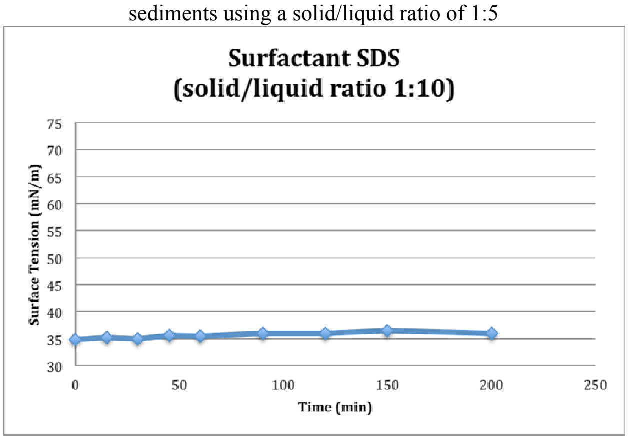


Fig. 9 Evaluation of surface tension of water in presence of SDS and sediments using a solid/liquid ratio of 1:10

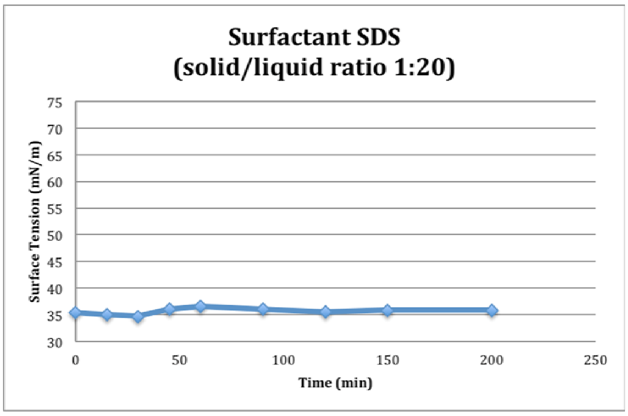


Fig. 10 Evaluation of surface tension of water in presence of SDS and sediments using a solid/liquid ratio of 1:20

## IV. CONCLUSION

Biosurfactants from *L. pentosus* in presence of sediments are adsorbed very fast; with a similar behaviour than that observed for tween 20, a non-ionic surfactant; whereas the sediments almost did not adsorb SDS, probably because SDS is an anionic surfactant. Consequently it can be speculated that biosurfactants from *L. pentosus* has similar properties than non-ionic surfactants like tween 20.

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