International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:9, No:6, 2015

# Bioremediation of Sewage Sludge Contaminated with Fluorene Using a Lipopeptide Biosurfactant

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***Abstract*****—**The disposal and the treatment of sewage sludge is an expensive and environmentally complex problem. In this work, a lipopeptide biosurfactant extracted from corn steep liquor was used as ecofriendly and cost-competitive alternative for the mobilization and bioremediation of fluorene in sewage sludge. Results have demonstrated that this biosurfactant has the capability to mobilize fluorene to the aqueous phase, reducing the amount of fluorene in the sewage sludge from 484.4 mg/Kg up to 413.7 mg/Kg and 196.0 mg/Kg after 1 and 27 days respectively. Furthermore, once the fluorene was extracted the lipopeptide biosurfactant contained in the aqueous phase allowed the biodegradation, up to 40.5% of the initial concentration of this polycyclic aromatic hydrocarbon.

***Keywords*****—**Fluorene, lipopeptide biosurfactant, mobilization, sewage sludge.

## I. Introduction

POLYCYCLIC aromatic hydrocarbons (PAHs) are persistent organic compounds as a result of the incomplete combustion of organic matter and are distributed in the environmental (water, soil, sediments and air) mainly by anthropogenic activities. PAHs have received much attention over the years because they have well-known carcinogenic and mutagenic properties [1], [2]. Fluorene is 3-ring PAHs of the 16 PAHs priority pollutants listed by US Environmental Protection Agency (EPA) [3], which is present in sewage sludge.

Sewage sludge is a waste that results from the wastewater treatment. Recycling and use of wastes are the preferred options for sustainable development rather than incineration or landfilling [4]. The utilization of sewage sludge as fertilizer or soil amendments is the best alternative for sludge disposal, which contains nutrients and organic matter; however due to the physico-chemical processes involved in the wastewater treatment, the sewage sludge tends to concentrate heavy metals and poorly biodegradable trace organic compounds as well as potentially pathogenic organisms [5]. Thus, a 3rd draft of a European Union (EU) directive has regulated the total concentration of 11 PAHs in sewage sludge for agriculture use has to be lower than 6 mg $kg^{-1}$ dry mass [6]. An ecofriendly alternative for the treatment of sewage sludge is the mobilization and biodegradation of PAHs in presence of biosurfactants [7].

Biosurfactants are surfactants produced mainly by microorganisms that stimulate the cracking of hydrocarbons molecules by micelle formation, increasing their mobility, bioavailability and exposure to bacteria, thus favoring hydrocarbon biodegradation [8]–[12]. However, the major obstacle to the large scale industrial application of biosurfactants is their higher production cost and lower production rates in comparison with commercially available synthetic surfactants, thus some authors have focus their research on reducing the biosurfactant cost using low-cost feedstock or agricultural byproducts as carbon sources [13]. In this study, corn steep liquor (CSL) is proposed as a source of low-cost biosurfactants. CSL is an aqueous industrial stream from corn wet-milling industry, whose properties like the sugar content, the acidity of the stream and the presence of lactic bacteria, promote the spontaneous production of biosurfactants [14].

The aim of this work was the utilization of a cost-competitive biosurfactant obtained from corn steep liquor for the simultaneous mobilization and biodegradation of fluorene in sewage sludge samples.

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## II. Materials and Methods

### A. Polycyclic Aromatic Hydrocarbons (PAHs)

Fluorene (Lot A0313026, 98%) was purchased by Acros Organics (New Jersey, USA).

### B. Sewage Sludge

Sewage sludge sample, coming from secondary treatment, was provided by a municipal treatment plant in Vigo (Spain). Prior to its utilization it was dried a room temperature in order to reduce the water content up to 11%, and stabilize the sewage.

### C. Contamination of Sewage Sludge

For the bioremediation experiments sewage sludge was contaminated with 484.4 mg/Kg of fluorene. Thus, 250 g of sewage were contaminated with 100 mL of a solution containing 1.21 mg of fluorene/mL of acetone and stirred vigorously for 30 min to promote the homogeneous distribution of fluorene. Before starting the extraction of with the biosurfactant, the sewage sludge samples were left to rest for 48 h at 28ºC to eliminate acetone in a shaker at 150 rpm.

### D. Corn Steep Liquor (CSL)

Corn steep liquor (Lot #MKBN0183V, liquid brown, 50% solid content and pH 4.4) was supplied by Sigma-Aldrich (St. Louis, MO, USA).

*E. Extraction of Lipopepetide Biosurfactant from CSL*

The lipopeptide biosurfactant was extracted with chloroform from corn steep liquor using the methodology proposed by [14]. After extraction, the biosurfactant was separated from the chloroform by rotary evaporation (Buchi R-210, Switzerland) and finally, the biosurfactant was dissolved in water by vortex and sonication process.

*F. Mobilization Process*

Sewage sludge (10 g) contaminated with 484.4 mg/Kg of fluorene were placed in 250 mL erlenmeyer flasks, and 100 mL of an aqueous solution containing the lipopeptide biosurfactant, at 2.5 times its critical micelle concentration (CMC), were added. The experiments were conducted in a shaker at 150 rpm, for 27 days at 30ºC. Moreover, acetonitrile (AcN) was used as solvent control extractant under the same conditions.

*G.Analysis of Fluorene in the Liquid Phase*

In order to analyze the fluorene mobilized to the liquid phase, sewage sludge samples were removed from the solution (AcN or biosurfactant) by filtration (PTFE syringe filter), and the fluorene mobilized to the liquid phase was identified and quantified by high performance liquid chromatography (HPLC) with diode array detection (DAD) and fluorescence detection (FLD) (Agilent Technologies 1200 Series, Germany) using Envirosep PP column (125 × 4.60 mm) (Phenomenex, USA) maintained at a constant temperature of 35°C. The fluorene was analysed using an isocratic elution, with Milli-Q water:acetonitrile (1:1) at a flow rate of 2 mL/min.

*H.Analysis of Fluorene that Remain in the Sewage Sludge*

Sewage sludge samples resulting from the bioremediation process were collected in a 10 mL glass centrifuge tubes and washed twice in water. Then, samples were submitted to solid-liquid extraction using 5 mL of AcN and shaken horizontally (200 rpm), during 3 days at 30ºC. Following sewage sludge was separated from the AcN by filtration through a 0.45 µm filter, and the fluorene contained in the liquid phase was detected by HPLC-DAD-FLD following the protocol described above.

A flowchart of experimental process carried out in this work is shown in Fig. 1.

## III. Results and Discussion

During the treatment of wastewater in municipal treatment plants, PAHs are often strongly adsorbed onto the sludge solids during sedimentation treatment because of their low water solubilities and medium-high octanol–water distribution coefficients [15]. Hence, the addition of biosurfactants in the sewage sludge treatment can increase their mobilization from sludge to the aqueous phase and thus improve the availability of PAHs for its microbial biodegradation [16].

In this work sewage sludge contaminated with 484.4 mg/Kg of fluorene was submitted to a bioremediation process using a natural biosurfactant. Fig. 2 shows the concentration of fluorene mobilized by the AcN and the biosurfactant to the liquid phase. After 24h of treatment the biosurfactant from CSL was able to mobilized fluorene that achieved a concentration of 7.1 mg/L in the aqueous biosurfactant solution, although the maximum solubility of fluorene rendered a concentration of 28.8 mg/L after 27 days of treatment. Moreover, it was observed that AcN was able to solubilize all the fluorene from sewage sludge achieving around 48.0 mg/L in the liquid phase after 9 days of treatment. On the other hand, Fig. 3 shows the percentage of fluorene extracted from sewage sludge using AcN or biosurfactant aqueous solution.

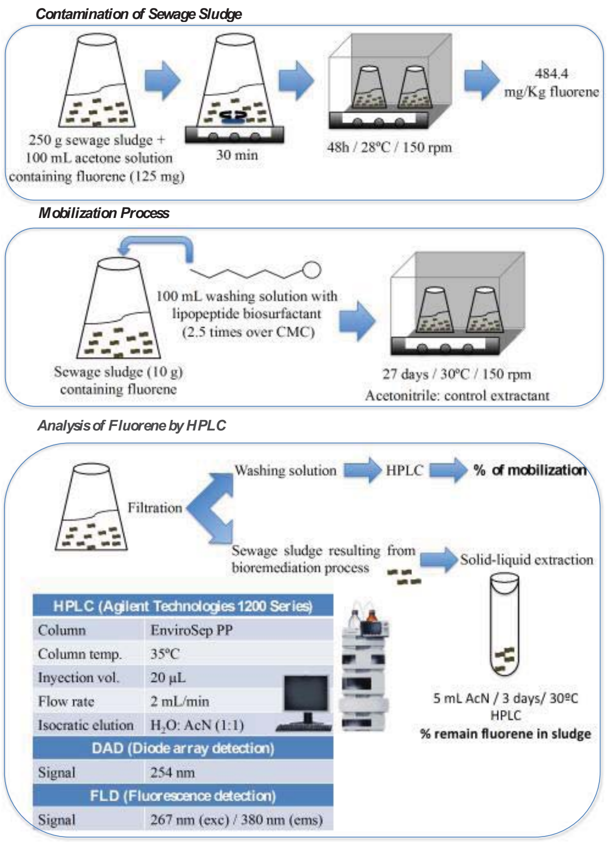


Fig. 1 Flowchart of bioremediation process of sewage sludge contaminated with fluorene using a lipopeptide biosurfactant

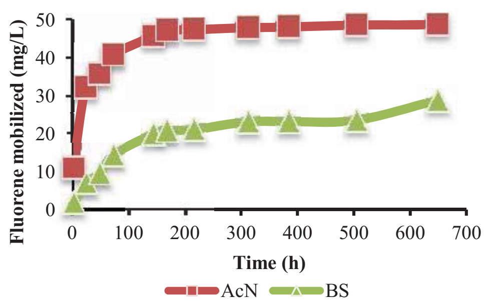


Fig. 2 Concentration of fluorene achieved in the liquid phase consisting of AcN or biosurfactant (BS) aqueous solution during the mobilization of fluorene from sewage sludge

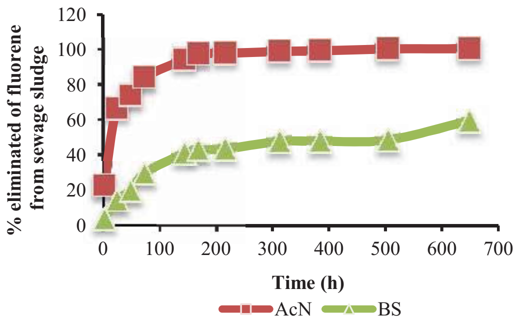


Fig. 3 Percentage of fluorene eliminated from sewage sludge

Biosurfactant used in this work was over its critical micelle concentration (CMC), because concentrations above the CMC will enhance the solubilization of the fluorene promoting the biodegradation of hydrophobic contaminants [12], [17]. Thus, Fig. 4 shows the kinetic biodegradation of fluorene, the percentage of biodegradation was calculated taking into account the percentage of fluorene mobilized from the sewage sludge minus the percentage of fluorene that remained in the biosurfactant solution; observing that 40.5% of the fluorene from the sewage sludge was biodegraded. This biodegradation only occurred in the experiments carried out with the biosurfactant, whereas no biodegradation was observed in presence of AcN, probably due to the toxic effect of this organic solvent on the microbial biomass. Other researchers [18] have observed that for instance the biodegradation of 50 mg/L of fluorene by *Pseudomona* sp. JM2 depends on the pH. Thus, these authors found that 94% of fluorene was biodegradated by *Pseudomona* between pH 6 and 8, whereas at pH=9.3, 40% of fluorene was biodegraded and a pH=4.5 the fluorene content was reduced up to 60%. In this work the biodegradation of fluorene in the biosurfactant extract was carried out at pH=7.3 that it is in the range of the optimum pH. Other authors [19] observed that the addition of Tween 60 and Triton X100 in aqueous phases enhanced the fluorene biodegradation rates by *Rhodococcus rhodochrous* VKM B-2469.

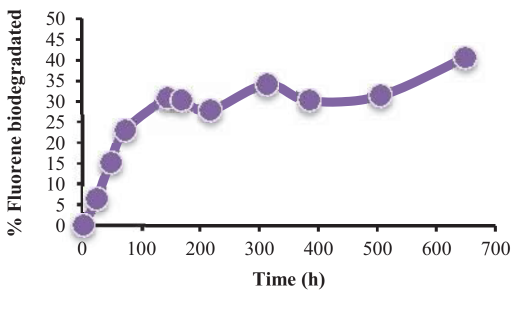


Fig. 4 Variation in the percentage of fluorine bio degradation during the treatment of sewage sludge with the biosurfactant solution

## IV. Conclusion

Biosurfactant extracted from corn steep liquor is an interesting alternative to chemical surfactants or organic solvents for the mobilization of contaminants from sewage sludge. This biosurfactant not only promoted the mobilization of fluorene from the solid matrix but also allowed the biodegradation of this contaminant once it was solubilized in the liquid phase.

## Acknowledgment

The authors thank the Spanish Ministry of Economy and Competitiveness, this work was funded by FEDER funds under the project CTM2012-31873.

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