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Biodegradation of anionic surfactants by *Alcaligenes faecalis*, *Enterobacter cloacae* and *Serratia marcescens* strains isolated from industrial wastewater

Mourad Fedeila[a,*], Zohra Hachaïchi-Sadouk[a], Luis Fernando Bautista[b], Raquel Simarro[c], Farida Nateche[d]

[a] Laboratory of Applied Organic Chemistry, Faculty of Chemistry, University of Sciences and Technology of Houari Boumediene (USTHB), PO Box 32, El Alia, Bab Ezzouar 16111, Algiers, Algeria
[b] Department of Chemical and Environmental Technology, ESCET, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain
[c] Department of Biology and Geology, Physics and Inorganic Chemistry, ESCET, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain
[d] Laboratory of Cellular and Molecular Biology, Microbiology Team, Faculty of Biological Sciences, University of Sciences and Technology of Houari Boumediene (USTHB), PO Box 32, El Alia, Bab Ezzouar 16111, Algiers, Algeria

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ABSTRACT

Pseudo-persistent organic pollutants, such as anionic surfactants (AS), are nowadays among the more complex problems that threaten the aquatic environments and other environmental compartments. The present work describes the identification and efficiency of a consortium, isolated from Algerian industrial wastewater, to remove three anionic surfactants (i.e., sodium dodecylbenzenesulfonate (SDBS), sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES)). The genetic analysis of 16S rRNA indicated that these strains are *Alcaligenes faecalis*, *Enterobacter cloacae* and *Serratia marcescens*. Under aerobic conditions, pH 7.0 and optimum temperature of 30 °C, the mixed consortium allowed to degrade 85.1% of initial SDBS amount after 144 h of incubation with half-life of 20.8 h. While *E. cloacae* and *S. marcescens* pure strains eliminated 46% and 41% less SDBS respectively. Evenly, SDS was degraded at only 23.71% by *A. faecalis* strain. However, the degradation capacity of SDS by the consortium was very high (94.2%) with a half-life of 9.8 h. The SLES anionic surfactant showed a lower biodegradation by the consortium (47.53%) due to the presence of ether oxide units in the chemical structure of SLES which induced toxicity to the medium. The investigation of the biodegradation of this type of organic pollutants by microorganisms has recently become a key issue for the environmental protection area.

1. Introduction

Scientific and industrial research progress in different areas such as detergents, pharmaceuticals, cosmetics and several other fields, have always been the subject of the birth of many synthetic pathways of anionic surfactants (AS) whose physicochemical properties are differently important (Hosseini et al., 2007; Sibila et al., 2008; Caracciolo et al., 2017). Sodium dodecylbenzenesulfonate (SDBS), because of its significant dispersing and emulsifying properties, make up the major part of the synthetic anionic surfactants used in the world for household and cleaning products (Perales et al., 1999; Ying, 2006). Moreover, sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES) have also recently experienced a remarkable presence in cosmetics formulations, essentially, as active component of shampoos and foaming agents for toothpastes and other healthcare products (Hosseini et al., 2007; Sibila et al., 2008; Caracciolo et al., 2017). Likewise, SLES is a primordial component in most commercial products used for soil conditioning in the excavation industry, especially as lubricants for mechanized tunnelling (Baderna et al., 2015; Caracciolo et al., 2017).

Urban and industrial wastewaters are the first destination for synthetic anionic surfactants after their human and industrial applications. Oued El-Harrach is one of the longest industrial effluents with the biggest environmental impact in Algeria due to pollution. This river stretches along 67 km and crosses a number of active industrial zones of the Algerian capital (Algiers). As measured as part of our research, a mixture of anionic surfactants at concentrations above 13.1 mg/L have been detected, thus presenting a problem of pseudo-persistence in wastewater. The consequences are even more serious when these pseudo-persistent organic pollutants, characterized by their short half-life (Bu et al., 2016), are not removed nor degraded by wastewater

* Corresponding author.
E-mail address: mouradfedeila@hotmail.fr (M. Fedeila).

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treatment plants reaching the aquatic environment and causing heaps of foam. Also, these compounds have been reported as contaminants for waterborne organisms in the marine environment and other aquatic environments (Jimenez et al., 1991; Margesin and Schinner, 1998; Eichhorn et al., 2002; Hosseini et al., 2007). More toxic effects of anionic surfactants have been encountered in agricultural soils from recycled wastewater sludge and/or affected irrigation waters (Kuhnt, 1993; Wilke, 1997; Abboud et al., 2007).

The study of the biodegradation of anionic surfactants by microorganisms is of remarkable importance to minimize their environmental impact (Cain, 1994; van Ginkel, 1996; Caracciolo et al., 2017). Few laboratories have studied the biodegradation of SDBS and SLES. However, many researchers have focused on the degradation of SDS by bacterial strains isolated from wastewater treatment plants. A mixed bacterial consortium of two isolates *Pseudomonas beteli* and *Acinetobacter johnsoni* was able to degrade 97.6% of initial SDS quantity after 240 h of growth (Hosseini et al., 2007). Furthermore, a high amount of SDS (4000 ppm) has been completely degraded by a consortium of mixed facultative anaerobes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* within 120 h (Abboud et al., 2007).

In the present work, the biodegradation of SBDS, SDS and SLES anionic surfactants has been studied. Three bacterial strains having the ability to degrade these compounds in pure and mixed cultures under aerobic conditions were isolated from industrial wastewater in Algeria (Oued El-Harrach, Algiers). The bacterial isolates forming this consortium were identified by 16S rRNA microbial sequencing and the degree of degradation of anionic surfactants by the pure bacterial strains and the mixed consortium was evaluated.

2. Materials and methods

2.1. Reagents and apparatus

SDBS and SDS (purity ≥ 98.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). SLES (purity of 68.0–72.0%) was procured from the unit for the production of detergents and cleaning products (Groupe Aigle. Algiers, Algeria). Chloroform (purity ≥ 99.8%) and methylene blue (extra pure) were supplied by Riedel-de Haën (Seelze, Germany). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buches, Switzerland). Microbial growth was measured as optical density (OD) at 600 nm on an Optizen 2120UV Plus Split Beam UV/Vis spectrophotometer (Mecasys. Daejeon, South Korea). Anionic surfactant concentrations were quantified using a Jasco V-630 UV/Vis spectrophotometer (Pfungstadt, Germany). Biodegradation tests were carried out using an orbital shaker incubator (Heidolph Unimax 2010. Walpersdorfer. Schwabach, Germany). Bacterial cells were observed in a FEG Nova NanoSEM 230 (Fei Europe. Eindhoven, Netherlands) scanning electron microscope.

2.2. Collection and isolation of a bacterial strains

Water samples (pH 7.1) were collected from industrial wastewater of Oued El-Harrach in Algiers, Algeria. The bacterial strains were isolated by an enrichment technique developed by Sadouk et al. (2009). The samples were placed in a minimal medium (MM) with the following composition (per liter): 3 g K_2HPO_4, 10 g KH_2PO_4, 2 g NH_4NO_3, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.1 g NaCl, 0.01 g $CaCl_2{\cdot}2H_2O$, 0.01 g MoO_3, 0.001 g $MnCl_2$, 0.001 g $CuCl_2$, 0.001 g $FeSO_4$, 0.001 g $ZnSO_4$. The pH of the medium was adjusted to 7.0 before autoclaving by addition of NaOH basic solution (0.2 N). SDBS ($C_{18}H_{29}SO_3Na$, molecular weight 348.48 g/mol) was used as sole carbon source after filtration using microfilters of 0.45 μm. Three bacterial strains were isolated following three enrichments in the MM then purified by successive subcultures and preserved on the nutrient agar solid media (NA) at temperature of 4 °C.

2.3. Bacterial growth conditions

The mixed bacterial consortium was grown in the MM, in separated cultures, containing SDBS, SDS and SLES as sole carbon sources at concentrations of 10 mg/L under aerobic conditions. The growth of the mixed consortium was improved by examining the effect of pH in the medium and incubation temperature. Growth tests were carried out at 30 °C at various pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5). Also, the influence of incubation temperature on the growth of the mixed bacterial culture was studied at pH 7.0 at three different incubation temperatures: 20, 30 and 40 °C. The incubation temperature of 30 °C and pH 7.0 were subsequently applied to study the influence of additional carbon sources on bacterial growth. Series of experiments were conducted at concentrations of 0.2 g/L of glucose, fructose, sucrose and maltose separately as co-substrates in the presence of anionic surfactants. Samples were withdrawn at regular intervals to follow bacterial growth by measuring cell density through the optical density (OD) of the mixed bacterial culture at 600 nm as a function of the incubation time.

2.4. Analytical procedure

Residual surfactant samples were analyzed according to the standard method using methylene blue to determine anionic surfactants in wastewater (ISO, 7875-1, 1996), further improved as described in EN ISO (1173)3: (2004). This method has been also simplified by Jurado et al. (2006) to reduce the quantity of chloroform used to extract the ionic pair methylene blue-anionic surfactant formed and the time and the amount of sample necessary to perform the analysis. The separated organic phase is measured by absorption spectrophotometry at wavelength of 650 nm. The following solutions were prepared:

Buffer solution: 2.5 g of sodium bicarbonate ($NaHCO_3$), and 3 g of anhydrous sodium carbonate (Na_2CO_3) were dissolved in pure water and diluted to 100 mL. The pH was adjusted to 10.2.

Neutral methylene blue solution: 0,03 g of methylene blue was dissolved in pure water and diluted to 100 mL.

Acidic methylene blue solution: 0.03 g of methylene blue was dissolved in pure water and mixed with 0.65 mL of H_2SO_4 (density 1.84 g/mL) and then diluted to 100 mL.

The samples were diluted to 100 mL with pure water, and mixed with 10 mL of buffer solution, 5 mL of neutral methylene blue solution and 10 mL of chloroform in a 250 mL separating funnel. The mixture was shaken for 5 min. After phase separation, the chloroform layer was placed into a second separating funnel, containing 110 mL of pure water and 5 mL of acidic methylene blue solution. The mixture was shaken for 5 min and the chloroform layer was filter and put into a 50 mL graduated flask. After three subsequent extractions, the combined chloroform extracts were filtered and diluted to a final volume of 50 mL with chloroform. The concentration of the anionic surfactant in the chloroform solution was then measured spectrophotometry by absorbance at a wavelength of 650 nm.

2.5. Biodegradation tests and kinetic studies

The biodegradation tests were carried out in 100 mL sterile Erlenmeyer flasks, closed with air-permeable cotton, and containing sterile solution of MM with anionic surfactant concentrations of 10 mg/L as sole carbon sources. The pH was maintained at 7.0 and the solutions were inoculated with the three bacterial strains. The Erlenmeyer flasks were then incubated at 30 °C and a stirring rate of 150 rpm for 144 h under aerobic conditions. Samples were withdrawn regularly and the concentration of anionic surfactants was determined spectrometrically at 650 nm as described above.

2.6. Identification of the strains

Initial identification of *Alcaligenes faecalis*, *Enterobacter cloacae* and