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Short communication

Scale-down studies of membrane bioreactor degrading anionic surfactants wastewater: Isolation of new anionic-surfactant degrading bacteria

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A B S T R A C T

Sodium lauryl ether sulfate (SLES) is an anionic surfactant used in the formulation of several detergent products. The high concentration of organic materials and SLES present in the industrial wastewater poses a real problem. In the previous study, Dhouib et al. (Dhouib et al., 2003) showed that *Citrobacter braakii* has a large capacity to degrade anionic surfactants in the wastewater treatment of a cosmetics industry. The scale-down has made possible to isolate, from the aerobic membrane bioreactor (MBR) inoculated with *Citrobacter braakii* since after one year, four strains A4, A10, A13 and A14 which were identified as *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Bacillus safensis* and *Staphylococcus arlettae*, respectively. These isolates are able to degrade 100% of SLES at a concentration of 1000 mg l^{-1} in the BSM medium. Physical-chemical parameters have been followed during 104 days in the influent, bioreactor and effluent. Biomass concentration increased slightly in the reactor and reached 13 g VSS l^{-1}. The COD and surfactants average removals were about 92% and 99.6%, respectively.

1. Introduction

Surfactants are amphipathic molecules, which reduce the interfacial tensions conferring excellent detergency, emulsifying and foaming. The surfactants represent a widespread group of organic pollutants that are extensively used in several domains such as textiles, fibers, food, paints, polymers, cosmetics, pharmaceuticals, microelectronic, mining, oil recovery, pulp-paper industries. In 2014, the world market for surfactants reached a volume of more than 33 billion US-dollars. Surfactants are classified according to their charge as anionic, non-ionic, cationic and amphoteric. Non-ionic, anionic and cationic surfactants are the most commonly used classes (Ying, 2006). The class of anionic surfactants is very important, it accounts for 60% of the world production.

Because of extensive application, in both industrial and domestic purposes, large quantities of surfactants and their derivatives are discharged to wastewater treatment plants (WWTP) or directly into the aquatic and/or terrestrial environment (González et al., 2007; Ying, 2006). These compounds are usually toxic to the environment and biologically not degradable. The increasing releases of surfactants by industries can be environmentally hazardous and alarming consequences on various living organisms (Chaturvedi and Kumar, 2010). They can directly affect the biological activity of microorganisms by binding to enzymes, proteins, phospholipids or by changing the hydrophobicity of the bacterial cell (Cserháti et al., 2002). The toxic effect of surfactants on bacteria depends on their chemical structure and their concentration. According to literature data, anionic surfactants give toxic effects to various aquatic organisms at concentrations as low as 0.0025 mg l^{-1} (Bizukojc et al., 2005; Petterson, 2000).

These compounds can act on biological wastewater treatment process and cause problems in sewage aeration and treatment facilities due to their high foaming, lower oxygenation potentials and making the death of waterborne organisms (Eichhorn, 2001). Surfactants removal operations wastewater effluents involve processes such as oxidation, chemical and electrochemical oxidation, chemical precipitation, photocatalytic degradation and adsorption were developed (Aboulhassan et al., 2006; Adak et al., 2005; Kowalska, 2012; Zhang, 2014) Biodegradation is the most frequent technology for the removal of surfactants and other pollutants from

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wastewater (Scott and Jones, 2000). Biological wastewater treatment processes present several advantages when compared to physicochemical processes mainly because of easy application and of relatively low costs (Cserháti et al., 2002). During the biodegradation, microorganisms can either utilize surfactants as substrates for energy and nutrients or co-metabolize the surfactants by microbial metabolic reactions. Divers bacterial surfactant-degraders were isolated from wastewater contaminated with surfactant (Chaturvedi and Kumar 2011a,b; Ojo and Oso, 2008; Shukor et al., 2009). The rate of surfactant biodegradation is dependent on chemical and environmental factors such as temperature, pH, aeration, concentration and the presence of other sources (Sales et al., 1999).

Conventional wastewater treatment processes under optimized conditions are able to eliminate more than 90% of the surfactants, although this efficiency may vary according to the operating characteristics of the WWTP (González et al., 2007; Matthijs et al., 1999). The biological treatment process of wastewater is generally unable to completely eliminate the surfactants when they are present in large quantities in sewage effluent. To improve the degradation of these products, biotechnological approaches have to be developed and bioaugmentation technique, the process of adding selected strain and/or mixed cultures to the reactor to improve the metabolism of specific compounds, may be used as efficient solutions in biological cleanup of industrial wastewater (Dhouib et al., 2005). In order to increase bacterial concentrations in the bulk solution and to enhance biodegradation rates, membrane bioreactors have been used successfully (Dhouib et al., 2003; Friha et al., 2014; González et al., 2008). Membrane bioreactor (MBR) technology which combines biological activated sludge process and membrane filtration have become more popular, abundant and accepted in recent years for the treatment of many types of wastewaters. MBRs offer several advantages in comparison to conventional activated sludge process. Among the advantages of this type of treatment is to have a solid-free effluent and airborne germs. MBR also could produce good quality effluent in terms of biological oxygen demand (BOD) and chemical oxygen demand (COD) due to the retention by membranes. It is well known that in MBRs can grow adapted microorganisms that can improve the elimination of these persistent pollutants present in the raw wastewater (González et al., 2008).

Sodium lauryl ether sulfate (SLES) is a mixture of linear primary alcohol ether sulfate (AES) commonly used in the formulation of several detergent products. Sodium lauryl ether sulfate consists of straight carbon chains, usually 12 to 14, and, due to its more simple structure, it can be more easily biodegradable than other surfactants such as linear alkyl sulfate (LAS). Many studies have been performed on the degradation of anionic surfactants in municipal wastewater treatment plants. However, few reports on the biodegradation of SLES compounds at high concentrations, such as those present in wastewater generated from surfactant manufacturing were studied. In previous studies conducted by Dhouib et al. (Dhouib et al., 2005, 2003), developed a high-performance process for the treatment of anionic surfactants containing wastewater. We described a powerful *Citrobacter brakii* strain able to degrade a wide range of anionic surfactants.

The treatment of cosmetic industry wastewater (JASMINAL-HENKEL company, Sfax, Tunisia) by *Citrobacter braakii* strain was investigated at a pilot scale using continuous stirred tank reactor (CSTR) connected to an external tubular cross-flow ultrafiltration membrane unit (Dhouib et al., 2005). The sodium lauryl ether sulfate (SLES) was the highest concentration of surfactant used in this factory.

SLES is toxic to microorganisms in the bioreactor to some degree. However, the use of bacteria and/or consortia which tolerate or degrade completely a wide range of surfactants at different concentrations for the treatment of wastewater surfactant-rich in the bioreactor was successfully performed. In addition, molecular analysis of many SDS-degrading bacteria revealed the presence of *sdsA* gene coding for alkyl sulfatase enzyme, the primary enzyme responsible for SDS degradation to sulfate and 1-dodecanol (Jovcic et al., 2010). Other studies suggest that transferable plasmids contributed to surfactant degrading properties in bacteria and play an important role in the adaptation of microbial communities to the presence of xenobiotics in their environments (Yeldho et al., 2011). Furthermore, the use of MBR system enhances the extension of the contact time of the microbial system with SLES growing adapted microorganisms and facilitates efficient removal of slowly biodegradable pollutants and thus upgrading the effluent quality.

The objective of this paper is to isolate aerobic bacterial or consortia strains capable of degrading anionic surfactants. The performance of the membrane bioreactor (MBR) treating industrial effluents containing a high amount of anionic surfactants was investigated after one year of the operational period.

2. Materials and methods

2.1. Chemicals and culture media

Sodium lauryl ether sulfate (SLES), methylene blue and other solvents were purchased from Sigma Chemical Company (St. Louis, MO) and Aldrich Chemical Company (Milwaukee, WI). Luria-Bertani (LB) medium contained (g l^{-1}): tryptone 10.0, NaCl 5.0, yeast extract 5.0, pH 7.0.

Basal salt medium (BSM): The basal salt medium contained (per liter): 1.36 g KH_2PO_4, 1.388 g Na_2HPO_4, 0.5 g KNO_3, 0.01 g $MgSO_4$, 0.01 g $CaCl_2$, 7.7 g $(NH_4)_2SO_4$ and 10 ml SL4 a trace elements solution containing (per liter distilled water): 0.5 g EDTA, 0.2 g $FeSO_4$, 100 ml SL6 solution containing (per liter distilled water): 0.1 g $ZnSO_4$ ($7H_2O$), 0.03 g $MnCl_2$ ($4H_2O$), 0.3 g H_3BO_4, 0.2 g $CoCl_2$ ($6H_2O$), 0.01 g $CuCl_2$ ($2H_2O$), 0.03 g Na_2MoO_4 ($2H_2O$). SLES was added separately after autoclaving the medium in the appropriate concentration. Twenty gram per liter agar was added in the solid medium.

2.2. Sampling

The membrane bioreactor (MBR) used in this study was followed during 104 days after an experimental period of one year. Samples were collected every 7 days from the MBR-influent, the bioreactor itself and MBR-effluent. All samples were stored at 4 °C until analysis.

2.3. Analytical techniques

COD, BOD_5, anionic surfactant, pH-value, Electrical conductivity (EC), Volatile suspended solids (VSS), and Total suspended solids (TSS) were measured as previously described (Aloui et al., 2009). Microbial biomass was monitored by measuring the optical density OD in a 1-cm cell at 600 nm on a Shimadzu UV160U spectrophotometer.

2.4. Toxicity test

The toxicity tests were performed using Gram-negative marine bioluminescent bacteria of the species *Vibrio fischeri* LK 480 of the Vibrionaceae family. The toxicity of different samples before and after treatment was tested with the LUMIStox system (Dr. Lange GmbH, Duesseldorf, Germany) following the standard procedure according to ISO 11348-2 (ISO 11348-2, 1998). Percentage inhibition