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Potential use of the liquor from sisal pulp hydrolysis as substrate for surfactin production

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ABSTRACT

Surface-active compounds of microbial origin are more environmentally-friendly, biocompatible and biodegradable when compared with synthetic surfactants therefore, the market interest in such molecules is increasing; however, their production costs are not competitive to chemical surfactants. The use of low-cost substrates such cellulosic-based material is a strategy to reduce economics of biosurfactant (BS) production and also contribute to reducing greenhouse gas emissions. The aim of this work was to evaluate the potential application of the liquor obtained from sisal (*Agave sisalana*) pulp hydrolysis as substrate for biosurfactant production. The liquor deriving from acid and enzymatic hydrolysis of sisal cellulose was utilized as carbon source to provide growth and surfactin production by a *Bacillus subtilis* strain. The surfactin obtained in acid hydrolysate showed a surface tension (ST) of 29.8 mN m^{-1}, interfacial tension (IT) of 5.7 mN m^{-1} and a critical micelle concentration (CMC) of 1394.0 mg L^{-1}, whereas when enzymatic hydrolysate was utilized the product showed a ST of 28.7 mN m^{-1}, IT of 3.8 mN m^{-1} and a CMC of 64.0 mg L^{-1}. The washing of contaminated sand with surfactin obtained in acid and enzymatic hydrolysate removed 80% and 70% of diesel, respectively, suggesting good potential for bioremediation. Mass spectrometry analysis showed that the surfactin obtained from EH has homologous molecules distribution similar to standard product. The results suggest that the liquor from sisal pulp hydrolysis is a feasible and sustainable substrate for surfactin production.

1. Introduction

Surfactants are chemical compounds derived from petroleum feedstock that have a wide range of industrial applications mainly as raw material in the detergent industry (Yu et al., 2008). Some microorganisms as bacteria, yeast and filamentous fungi can synthesize surface-active (Rahman and Gakpe, 2008) molecules known as biosurfactants (BS). These molecules have hydrophobic and hydrophilic moieties that permit them to accumulate between fluid phases, thus reducing surface (liquid–air) and interfacial (liquid–liquid) tension, enhancing the solubility, mobility and bioavailability of hydrophobic organic compounds. Those properties make them adequate for using in several industries as emulsifiers, wetting, foaming and dispersing agents (De Faria et al., 2011; Gharaei-Fa, 2011). Furthermore, biosurfactants present remarkable advantages as promising alternatives for their chemically counterparts, such as lower toxicity, higher biodegradability and effectiveness at extremes of temperatures, pH and salinity (Desai and Banat, 1997) along with their status of "natural or green" compounds.

Surfactin was firstly described by Arima et al. (1968) that reported the presence of a biological active compound in cultures of *Bacillus subtilis* which exhibited remarkable surfactant activity. The chemical structure of surfactin was further elucidated by Kakinuma et al. (1969) as a heptapeptide with a β-hydroxy fatty acid within a lactone ring structure. Surfactin is considered one of the most powerful biosurfactant as a concentration of 20 μM can lower the surface tension of water to 27 mN m^{-1} (Peypoux et al., 1999).

Despite the advantages presented by the BS, they are not currently commercially available due to the low production yield and high production and recovery costs when compared to the chemical surfactants (Banat et al., 2010). The main strategies that have been utilized to reduce production costs comprise the use of renewable and economical substrates, the development of overproducing strains and more efficient recovery operations (Makkar et al., 2011). Different low-cost and renewable substrates have been proposed

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as alternatives to improve economics of biosurfactant production, including okara (O'Toole, 1999), molasses (Saimmai et al., 2011), milk whey (Cagri-Mehmetoglu et al., 2012), cassava flour wastewater (Costa et al., 2009) and orange fruit peeling (George and Jayachandran, 2009).

As described above, food related raw materials are the most explored substrates for BS production an also for the production of sugar-based chemical surfactants. In the last years, with an increase in environmental conscience, cellulosic material has gained relevance as an alternative carbon source for biotechnological processes, due to its renewable and inexpensive nature (Delgenes et al., 1998; Henkel et al., 2012). Recently, Ludot et al. (2014) proposed the transformation of wood hemicellulose into biodegradable decyl pentosides surfactants as alternative to valorization of this substrate using a chemical synthesis approach.

According to Henkel et al. (2012) the biosurfactants, which are produced independently of feedstock derived from human food, may be referred to as next-generation biosurfactants. However, the actual productive use of lignocellulosic material for biotechnological processes is restricted to small-scale production of cellulose ethanol, and its high potential remains currently almost unexplored.

Agave sisalana also known as sisal (Gutiérrez et al., 2008) is native to Mexico and grows all year in hot climate and arid regions, which are often unsuitable for other crops (Basu et al., 2012). Brazil is the largest world producer and exporter of sisal fibers and its derived products as twines, pads, rugs and carpets; however, the amount of value added to sisal is still limited (FAO, 2014). The sisal fibers possess high cellulose content; is very easily cultivated and has a short growing cycle (Megiatto et al., 2007) which makes them a potential alternative as carbon source for the production of high-value bioproducts.

Hydrolysis of lignocellulosic fibers to convert cellulose to glucose can be performed via enzymatic or acid treatment and the obtained sugars utilized as substrate for the production of ethanol or other bioproducts whereas, the remaining non-hydrolyzed celluloses fibers can be employed as biomaterials (Lacerda et al., 2013). The application of such combined biorefinery process can increase profits because the raw materials for both micro/nanofibers and fermentation would be generated simultaneously.

In this work we evaluate the use of the liquor obtained from enzymatic (EH) and acid hydrolysis (AH) of sisal pulp as substrates to produce surfactin by a *B. subtilis* strain. The physical–chemical properties of the synthesized BS and their potential for bioremediation were also determined.

2. Materials and methods

2.1. Microorganism

The surfactin producer strain *B. subtilis* ATCC 21332 (Cooper et al., 1981) was preserved at −20 °C in glycerol 20% and kept on nutrient agar (Himedia) slants at 4 °C.

2.2. Culture media

The production medium used was based on synthetic medium described by Sheppard and Cooper (1991), contained approximately ($g\,L^{-1}$): carbon source (EH or AH) 10, NH_4NO_3 1.0, KH_2PO_4 1.02, Na_2HPO_4 1.42, $FeSO_4{\cdot}7H_2O$ 3.75×10^{-3}, $MgSO_4{\cdot}7H_2O$ 4.93×10^{-2}, $MnSO_4{\cdot}7H_2O$ 5.00×10^{-4}, $CaCl_2{\cdot}2H_2O$ 2.50×10^{-4}. Reducing sugar concentration for the enzymatic and acid hydrolysate were $10.65 \pm 1.06\,g\,L^{-1}$ and $9.96 \pm 1.34\,g\,L^{-1}$, respectively. These substrates were sterilized separately. Final pH was adjusted to 6.8–6.9.

2.2.1. Hydrolysis of sisal cellulose

Delignified sisal pulp was provided by Lwarcel (Lençóis Paulistas, São Paulo, Brazil).

The sisal pulp was previously submitted to mercerization with a solution 20% of NaOH at 50 °C for 3 h. This treatment proved be important for increasing the efficiency of the sisal pulp hydrolysis reaction (De Paula et al., 2012). After mercerization, α-cellulose content of pulp increased from 85% to 97.4%; the hemicellulose content was decreased from 15% to 2.6%; the crystallinity index decreased from 74% to 68%; molar mass average from 119,357 g mol^{-1} to 94,618 g mol^{-1} and the ash content of pulp decreased from 1.3% to 0.53%. Lignin was not detected.

For acid hydrolysis 90 g of pulp was reacted with 1800 mL of H_2SO_4 4.6 mol L^{-1} at 100 °C during 6 h (Lacerda et al., 2013). For the enzymatic hydrolysis, 1 g of cellulose was added to 50 mL of citrate buffer (0.01 mol L^{-1}) and sterilized at 121 °C for 20 min. The solution was stirred in rotary shaker at 50 °C for 2 h; the enzymatic complex Accellerase®1500 (Genencor) was added at a ratio of 0.5 mL g^{-1} of cellulose and the reaction maintained during 6 h. The acid hydrolysate was neutralized with KOH 17 M and filtered. Enzymatic hydrolysate was also filtered and pH was adjusted to 7.0. Both substrates were sterilized in autoclave at 121 °C for 20 min and aseptically added to the media.

2.3. Inoculum and culture conditions

The microorganism was streaked on nutrient agar and incubated for 24 h at 30 °C. The pre-inoculum was done by transferring a loop of cells to 20 mL tube containing 8 mL of nutrient broth that was incubated in a rotary shaker (Thermo Scientific) at 30 °C, 120 rpm for 12 h. The OD (610 nm) of the bacterial suspension was adjusted to 0.5 using a spectrophotometer (Thermo Scientific). Subsequently, 1 mL of the standardized solution was added to 150 mL erlenmeyer flask containing 20 mL of nutrient broth and incubated in a rotary shaker at 30 °C, 120 rpm for 9 h. Further, 1 mL of the inoculum was transferred to 250 mL erlenmeyer flask with 20 mL of production medium that was incubated at 30 °C, 120 rpm during 72 h. Samples were withdrawn at time-defined intervals for analytical measurements.

2.4. Analytical measurements

2.4.1. Viable cell numbers

Samples of production medium were serially diluted and viable counts performed by drop method (Miles and Misra, 1938).

2.4.2. Surface activity

Culture samples were centrifuged at $8.000 \times g$ for 20 min for cell removal and the supernatant was used to evaluate surface activity. Surface activity measurements were performed on Sigma 700/701 tensiometer (Attension) using Du Nouy ring method. Critical micelle dilution (CMD^{-1} and CMD^{-2}) was measured using 10 and 100-fold-diluted supernatant, respectively. The critical micelle concentration was calculated using Attension Sigma Software. Interfacial tension was measured against hexadecane.

2.4.3. Reducing sugar

Total reducing sugars were determined by DNS method (Miller, 1959).

2.4.4. High-performance liquid chromatography (HPLC)

Analysis of the sugar and related decomposition products was done by high-performance liquid chromatography (HPLC) using a Shimadzu instrument. Glucose, xylose and arabinose were analyzed using a refractive index detector (RID-6A), AminexHPX 87H column (300×7.8 mm BIO-RAD), 0.005 mol L^{-1} H_2SO_4 as