International Biodeterioration & Biodegradation 127 (2018) 10–16

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biosurfactant production from industrial wastes with potential remove of insoluble paint

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ARTICLE INFO

Keywords:
Biosurfactant application
Emulsifying activity
Ionic character
Surface tension

ABSTRACT

Biosurfactants are amphipathic compounds formed by a hydrophilic and hydrophobic component, this characteristic confers to these compounds the possibility of several applications. The aim of this study was to produce biosurfactants from different industrials wastes using *Corynebacterium aquaticum* and *Corynebacterium* spp. CCT 1968 and study the application in paint removal. The production of biosurfactants was evaluated through surface tension, emulsifying activity and character ionic. The analyses were carried out at 0, 24, 48 h. The biosurfactants that presented lower surface tension and higher emulsifying activity were applied in insoluble paint. The microorganism *Corynebacterium aquaticum* showed efficient biosurfactant production when using fish and bagasse residues as carbon source. The surface tension obtained for these treatments was 27.8 and 33.9 mN/m and emulsifying capacity was 87.6 and 61.6%, respectively. The *Corynebacterium* spp. CCT 1968 produced biosurfactants only in the medium containing fish waste (28.5 mN/m). The biosurfactants produced by both microorganisms showed anionic character. The applied biosurfactants showed potential use in solubilization and paint removal. Therefore, the residues of fish and sugarcane bagasse showed efficient as carbon sources to obtain biosurfactants. In addition, the preliminary paint removal application presented great results that can be explored in the future.

1. Introduction

Biosurfactants are surface-active compounds synthesized by microorganisms, which have the capacity to reduce surface and interfacial tensions of solutions (Franzetti et al., 2011). They are an alternative for the replacement of chemical surfactants produced from petroleum (Nitschke et al., 2004; Luna et al., 2012; Ismail et al., 2013). These compounds are produced by a wide variety of bacteria and fungi. Thus, several types of structures are formed as phospholipids, glycolipids, lipopeptides, polymeric surfactants and others. Depending on the structure of biosurfactant the interaction with pollutants could be different (Sriram et al., 2011).

Biosurfactants have several advantages over the surfactants chemically produced, such as lower toxicity, higher biodegradability, improved environmental compatibility, higher selectivity and specific activity in conditions of adverse temperatures, pH, salinity, and finally, ability to be synthesized from renewable raw materials (Luna et al., 2012; Nalini and Parthasarathi, 2013; Souza et al., 2014b).

A major problem in biosurfactant production is the costs involved in the process. The carbon source is responsible by considerate part of biosurfactant production costs (Li et al., 2016). However, this may be significantly reduced by using alternative sources of nutrients, which are easily available and inexpensive. The use of industrial wastes as an energy source for biosurfactants production is an attractive alternative to decrease the production costs, making the process viable (Al-Bahry et al., 2013). Agro-industrial wastes with high content of carbohydrates, lipids and proteins are attractive to be used as substrate, they usually have the necessary nutrients for it (Nitschke and Pastore, 2002). Wastes such as glycerol (Souza et al., 2014a), petroleum sludge (Pirôllo, 2006; Cameotra and Singh, 2008; Ibrahim et al., 2013), sugarcane bagasse (Rakeshkumar et al., 2013) and fish waste (Aguiar et al., 2014) can be used such carbon source in fermentative process. In general, these raw materials can be used as distinct energy sources for different microorganisms with the capacity to produce biosurfactants. Several microorganisms are capable to biosurfactants production, among genus bacteria such as *Bacillus, Corynebacterium, Pseudomonas, Rhodococcus* (Souza et al., 2014b). Bacteria such as *Corynebacterium* spp. (Pinto et al., 2009; Deon et al., 2012; Decesaro et al., 2013). and *Corynebacterium aquaticum* (Pinto et al., 2009; Aguiar et al., 2014) are reported in the literature as producers of biosurfactants with low surface

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https://doi.org/10.1016/j.ibiod.2017.11.005
Received 3 October 2016; Received in revised form 10 October 2017; Accepted 4 November 2017

tension and with emulsifying capacity.

Petroleum-based compounds are highly pollutant when released to the environment and are considered largely responsible for the main causes of global pollution. A large number of these compounds are toxic and carcinogenic and may cause harm to human and animal health (Nalini and Parthasarathi, 2013). Among these compounds the solvents used for paint removal generally adhered to the surfaces which showed environment adverse impacts. For reduction of theses impacts is extremely important to search techniques with less aggressive toward pollutants (Young et al., 2015). According to Mulligan (2005) the biosurfactants are compounds that have several advantages compared with conventional surfactants which may be used in bioremediation processes (water and soil), enhanced oil recovery, solubilization of insoluble material, sequestering metals and removal and solubilization of paints.

Thus, the aim of this study was produce biosurfactants by *Corynebacterium aquaticum* and *Corynebacterium* spp. CCT 1968 using different carbon sources (fish waste, sugarcane bagasse, petroleum sludge and glycerol) and promotes biosurfactants application in paints removal from metallic surface.

2. Materials and methods

2.1. Material

The raw materials used as a carbon source for cultivation were sugarcane bagasse (SCB), fish waste (FW), crude glycerol (GL) and petroleum sludge from storage tanks (PS). The bagasse was obtained from farmers of Santo Antônio da Patrulha/RS, Brazil. The fish waste was provided by Pescal Company in the city of Rio Grande/RS, Brazil, the residue obtained was composed by heads, bones, skin, scales, muscles and viscera. Crude glycerol was provided by BS BIOS company, located in the city of Passo Fundo/RS, Brazil. The petroleum sludge remaining of storage tanks was donated by Oil Refinery Riograndense, located in Rio Grande/RS, Brazil.

2.2. Raw material preparation

The fish waste was reduced size using a mechanical removing device (HIGH TECH, HT/2500, Brazil). The fibers of bagasse were dried in an oven (Model Q-314 D 242 - Quimis) at 40 °C for 24 h and grounded (Mill Type Willye TE-650 - Tecnal) to reduce the particle size (100 mesh). The glycerol and sludge were homogenized manually.

2.3. Microorganisms

The microorganism *Corynebacterium aquaticum* was donated by Biochemical Engineering Laboratory (LEB) located at Federal University of Rio Grande (FURG) Brazil. This microorganism was isolated from washer trucks that carried hydrocarbons products. *Corynebacterium* spp. CCT 1968 was obtained by Tropical Culture Collection (CCT) donated by Foundation André Tosello, located in São Paulo/RS, Brazil.

The microorganisms were stored at 4 °C and propagated in Erlenmeyer flasks containing Agar Brain Heart Infusion (BHI) for cultivating of *Corynebacterium* spp. CCT 1968 and Agar Plate Count (PCA) for *Corynebacterium aquaticum*. Both were incubated at 37 °C for 48 h.

2.4. Cultivation medium

The aerobic cultivation was performed in erlenmeyer flasks (500 mL) at 30 °C and orbital shaking (Incubator Model TE-420 -Tecnal) 200 rpm for 48 h. The mineral medium was prepared as described by Yeh et al. (2005), consisting of NH_4NO_3 (50 mM), Na_2HPO_4 (3 mM), KH_2PO_4 (3 mM), $CaCl_2$ (7 μM), $MgSO_4.7H_2O$ (0.8 mM), EDTA sodium (4 μM), and $FeSO_4.7H_2O$ (2 mM). Carbon sources were used in concentrations of 3, 5 and 7%. After preparation of the culture medium, it was sterilized (AV Vertical Autoclave Model 30 - Phoenix) to eliminate some pre-existing contamination. The mineral medium was checked of surface tension by tensiometer (Kruss Processor Tensiometer K-9, Germany).

The addition of microorganisms was performed as described by Makkar and Cameotra (1998). The microorganisms presented in the surface of the agar in the erlenmeyer was removed and diluted in mineral medium until optical density between 0.8 and 0.9 (600 nm). The suspension was added in proportion of 2% (v/v). The microorganisms were added separately.

2.5. Evaluation of biosurfactants production

The biosurfactants production was evaluated at 0, 24 and 48 h of cultivation. The samples were taken from the reactor and centrifuged (Centrifuge Model MDW - 350 - Biosysten) for 30 min at 8667 g to remove cells and solid residues. Then, analysis were performed as described below.

2.5.1. Determination of surface tension

The surface tension (ST) of extract containing the biosurfactants was evaluated using a tensiometer (Kruss Processor Tensiometer K-9, Germany). This equipment uses the method of Du Nouy ring. This ring comes into contact with the liquid forming a film liquid/surface that is elongate to the breaking, measuring the maximum force used.

2.5.2. Emulsifying activity

The determination of oil emulsifying activity ($EA_{o/w}$) was analyzed according to the method described by Broderick and Cooney (1982). The extract containing the biosurfactants was homogenized with soybean oil using vortex and then, the samples rested for 24 h. The emulsifying activity was calculated by dividing the height of the emulsion layer by the total height of the mixture and multiplying by 100.

2.5.3. Character ionic of biosurfactant extract

The ionic character was determined as described by Meylheuc et al. (2001), using the technique of double diffusion in agar. This technique consists in the evaluation of two wells of the same distance created in low hardness agar (1% agar), one of them was added an ionic charge solution known and in the other, the biosurfactant. The anionic compound used was sodium dodecyl sulfate 20 mM and cationic solution used was barium chloride 50 mM. The appearance of the lines between the wells containing the biosurfactants and the ionic compound is able to suggest the ionic character of biosurfactants. The reading was made after 24 h of rest.

2.6. Biosurfactant application

The biosurfactants produced by *Corynebacterium aquaticum* using 3% of fish waste and 3% sugarcane bagasse were used to study the paint removal from metallic surface.

Synthetic enamel paint was used in the application process. The paint was composed of alkyd resin, organic and inorganic pigments, additives and solvent. The paint was insoluble in water and suitable for coating metal parts. The object used to support the paint was a metal plate (iron) 3 × 3 cm and 3 mm thickness. The metal plates were immersed in a container with paint, after it was removed from the container and suspended for remove the excess of paint. Thus, the plate containing the still wet layer of paint was placed into petri dish and covered with the respective solution for each treatment. The solutions used to cover the plates were previously selected. Four treatments were used, distilled water, solvent (turpentine solvent consisting of mixture of hydrocarbons), biosurfactant produced with fish waste and biosrufactant produced with sugarcane bagasse. The metal plates remained