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Integral production and concentration of surfactin from *Bacillus* sp. ITP-001 by semi-batch foam fractionation

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ABSTRACT

Foam fractionation is an adsorptive separation method that occurs on the liquid-foam interface, when rising bubbles removes active-surface compounds distributed throughout the continuous liquid phase. The formed foam is allowed to build up above the liquid, where, a drainage process results in further enrichment of the surface-active species. In this work, foam fractionation was used the concentration of surfactin from a fermentation broth. The biosurfactant was produced in a culture of *Bacillus* sp. ITP-001 and the bioreactor was integrated to a fractionation column aiming at the saving of steps in the purification of the product. The production of foam was controlled by fixed aeration and agitation rates. An experimental planning design method of type 2^2 was used in the experiments set up. The maximum value of surfactin concentration was acquired in the operation with minimum values of aeration and agitation rates. The average surfactin enrichment in these operation conditions reached the value of 28 times and the mass recovery was 94%. The integration of the bioreactor and the separation device was demonstrated as a simple and practical method for surfactin concentration using *Bacillus* sp. ITP-001, a production method. Assays of antimicrobial activity indicated that the produced surfactin is a powerful antimicrobial agent against several pathogenic microorganisms (*Escherichia coli*, *Candida albicans*, *Fusarium* sp.).

1. Introduction

Biosurfactants are surface-active compounds produced in fermentation by microorganisms such as bacteria (*Bacillus subtilis* [1], *Lactobacillus* [2], molds (*Candida* species [5]) and yeasts (*Saccharomyces cerevisiae* [3]). Just like the chemical surfactants, biosurfactants have the ability to lower surface and interfacial tension of liquids, and to form micelles and microemulsions between two different phases [4]. Their main beneficial characteristics include low toxicity, high biodegradability and environmental compatibility [5,6]. These characteristics make them powerful agents for subsurface pollution remediation and enhancement of the availability of hydrophobic compounds (e.g., oil, polyaromatic hydrocarbons, and pesticides) [7]. Nowadays, they are widely used in industries such as cosmetics, specialty chemicals, food, pharmaceutics, agriculture, cleansers, enhanced oil recovery and bioremediation of contaminated oil [8].

Biosurfactants are categorized by their chemical composition and microbial origin. They include glycolipids, lipopeptides, polysaccharide-protein complexes, protein-like substances, lipopolysaccharides, phospholipids, fatty acids and neutral lipids [9]. The most commonly isolated biosurfactants are glycolipids and lipopeptides. They include rhamnolipids released by *Pseudomonas aeruginosa* [10], sophorolipids from *Candida* species [11], as well as surfactin and iturin produced by *B. subtilis* strains [12]. Surfactin is a biosurfactant usually produced by various strains of genus *Bacillus* [13]. Its chemical structure consists of a ring of amino acids and C13–C15 side chain, as shown in Fig. 1. It belongs to the lipopeptide family which are powerful biosurfactants with antimicrobial, antiviral and anti-inflammatory properties that make them a good drug for solving some health issues [14,15]. Surfactin preparations have extreme surface-activity power in terms of minimum surface tension (typically 30 mN m^{-1}), and are effective at low concentrations (with a typical minimum critical micelle concentration – CMC–of 0.1 g L^{-1}) [16,17].

Despite the advantages previously mentioned, the downstream processes (which involve recovery, concentration and purification) of biosurfactants, including the surfactin, from the fermentation broths reveal to be challenging as it is highly diluted [18]. Most of

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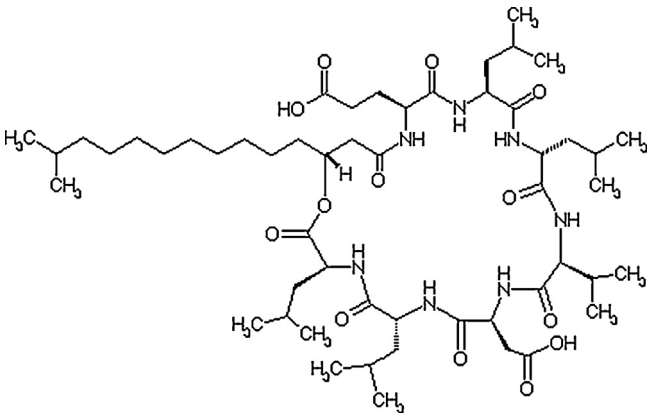

Fig. 1. Structural of the surfactin molecule.

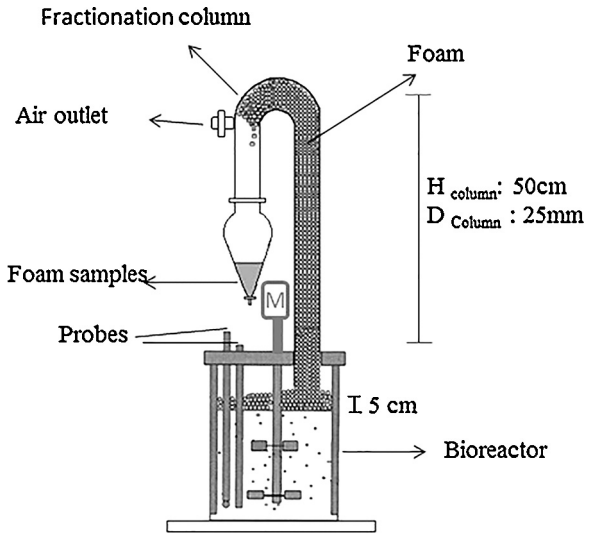

Fig. 2. Experimental module used for the integrated production and concentration of culture *Bacillus* sp. ITP-001.

the biosurfactants can be easily recovered from the culture medium by using a combination of traditional techniques such as precipitation, crystallization, centrifugation and solvent extraction [19,20]. However, there are drawbacks in using these conventional methods, due to the fact that in addition to being time consuming, the organic solvents commonly used (such as acetone, methanol, and chloroform) are expensive, toxic, as well as harmful to health and the environment [19]. The searches of the economic and efficient method of recovery begin to be an urgent task, because the application of any other inefficient methods would only significantly increase the cost of production. A modern approach for concentration of surfactin is the foam fractionation integrated with the production scheme. It is an adsorptive bubble separation technique for recovery and concentration of surface-active compounds like proteins, amino acids and surfactants and works based on the principle that the soluble surface-active compounds can be adsorbed by a layer of gas–liquid interface [21]. This phenomenon allows the foaming through the reduction of surface tension that consequently, rises in the column and is concentrated in the foam phase at the expense of drainage and coalescence processes [22,23]. Since, the microbial fermentation produces foam, especially the aerobic fermentations, which in turn constitutes an obstacle for the production of surface-active compounds, this separation technique is a better choice. It also avoids the use of chemical antifoams that are costly, lower than the oxygen transfer rate and have adverse effects on cell physiology.

Nevertheless, the information concerning the integrated production/concentration of foam for isolation of surfactin from the fermentation broths is very scant even if the use of the foam fractionation column [24,6], linked to production stage, could allow the efficient recovery of biosurfactants [24]. This work aims to fill this gap by linking the foam fractionation column to the bioreactor to simultaneously produce and concentrate the surfactin from culture of *Bacillus* ITP-001. So the foam produced in the fermentation is allowed to flow out of the bioreactor toward to the column top in a space, where, liquid drainage occurs forming a dry foam. This foam breaks in the descendent part of the apparatus and is collected in a separate vessel for analysis purposes. The performance of foam fractionation during batch culture is expressed in terms of enrichment and recovery and has also been investigated.

2. Materials and methods

2.1. Microorganisms and cultivation conditions

Bacillus sp. ITP-001 used in the present work, was isolated from petroleum-contaminated soil by the Institute of Research and Technology (Aracaju-Sergipe, Brazil) and was identified as *Bacillus* sp. ITP-001 by ADNVISION (Belgian). It was maintained in nutrient agar slants and stored at 4 °C. The culture media for the bioreactor consisted of (%, w/v): starch (2.0), peptone (0.13), yeast extract (0.6), $MgSO_4{\cdot}7H_2O$ (0.05), $NaNO_3$ (0.3), KH_2PO_4 (0.1). The pH initial value was always adjusted to 7.0 with pHmeter Digimed®/DM-20, using HCl 0.1 M and NaOH 0.1 M. and then sterilized in the autoclave at 121 °C for 20 min. The bioreactor was pre-inoculated with 10% (v/v) of an inoculum (0.3 g L^{-1}) of 48 h old. All fermentations were carried out to 120 h, at 37 °C in the absence of chemical antifoam.

2.2. Integrated production/concentration systems

The surfactin concentration was obtained by collecting the foam produced during fermentation. In order to remove the foam, a foam fractionating column was integrated on to one of the outputs of the bioreactor and then connected to the foam collection container (Fig. 2). The experiments were carried out in a bioreactor (Tecnal/Tec Bio V) of 4.5 L (total capacity) containing 3.0 L culture media working volume. The foam fractionation column had a height of 50 cm and an internal diameter of 25 mm, and its lower end penetrated the headspace of the fermenter such that its base was 5 cm above the level of the culture liquid. On the top of the column there was a pronounced curvature region of 180° which allowed the collection of the air entrained foam. Foam cells rupture spontaneously (due to gravity effect only) and liquid or top product is directed to the bottom of the container and then collected as a sample for analysis.

2.3. Analysis

2.3.1. Biomass, protein, glucose, and starch

Initially, an aliquot of 8 mL was removed from the fermented broth and centrifuged at 2500 rpm for 15 min. The biomass was dry and the cell-free broth was used to determine the concentrations of glucose, starch and protein.

The biomass was measured, followed by drying in the oven (105 °C) until a constant weight. Biomass was expressed as mg of cell dry weight per milliliter.

Glucose concentration was determined using the glucose-oxidase method (Glicose Liquiform Assay Kit Labtest, Lagoa Santa-Minas Gerais, Brazil). The kit, utilizing a spectrophotometric method in absorbance at 510 nm, was used according to the manufacturer's instructions. Protein concentration were performed according to the Bradford method [25], and the concentration of

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