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Sustainable Biotransformation of Barley and Milk Whey for Biosufactant Production by *Penicillium sclerotiorum* UCP1361

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Surfactants are amphipathic molecules of petrochemical origin used in various industrial segments, mainly in the petroleum industry. However, they are toxic and produced from non-renewable sources. Biosurfactants are natural, biodegradable and less toxic molecules synthesized by bacteria, yeast, and filamentous fungi, however, their cost of production is still high. In the present study, agro-industrial by-products were used as renewable sources to evaluate their influence on the production of biosurfactant by *Penicillium sclerotiorum* UCP1361. A central composite rotated design (CCRD) 2^2 was performed to optimize the concentrations of milk whey and barley on the synthesis of biosurfactant. The filamentous fungus *P. sclerotiorum* was inoculated in the assays according to the experimental design and incubated in orbital agitation at 150 rpm, pH 5.0 for 96 h at 28 °C. Then, the cell-free metabolic liquid was obtained and the surface tension was measured. The emulsification index was also analyzed with different hydrophobic substrates. The dispersing capacity of the biosurfactant and its viscosity were tested on burnt engine oil. The extraction of the biomolecule was performed using chloroform and methanol as solvent system. The results showed that the biosurfactant reduced the surface tension from 72 to 27 mN/m and the emulsification index was 68% with burnt engine oil. The dispersing capacity detected was 33.15 cm^2 and further reduced the oil viscosity from 279.6 to 48.5 cP. This study indicated that the biosurfactant produced by *P. sclerotiorum* may be promising as a bioremediation agent in oil spilled sites.

1. Introduction

Biosurfactants are metabolic byproducts of microbial origin defined as amphipathic molecules with hydrophobic and hydrophilic parts which have properties of reducing surface and interfacial tension, solubilization, dispersion, emulsion stabilization and foaming (Fenibo et al., 2019). These microbiological surfactants have stood out against chemical surfactants due to their broad industrial applicability combined with the fact that they are more beneficial (Sari et al., 2019). They have diversified structures, low toxicity, greater biodegradability, effective in environmental conditions with different pH, temperature and salinity, as well as the use of renewable sources for their production, such as industrial waste and industrial by-products (Pinto et al., 2018; Ribeiro et al., 2019).
However, considering the high cost in the biosurfactant production process, industrial waste bioconversion can be used as an economically attractive alternative for cost reduction in the production of this biomolecule (Nurfarahin et al., 2018). Whey stands out for being the main byproduct of the dairy industry and contains nutrients such as lactose, soluble proteins, lipids and minerals. (Das et al., 2016). Barley agro-industrial by-products from beer can be used as an alternative carbon source for the cultivation of microorganisms in biotechnological process (McCarthy et al., 2013).

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The objective of this work was to develop a strategy in the formulation of an economical culture medium for maximizing the production of a biosurfactant by *Penicillium sclerotiorum* UCP1361 using low cost renewable sources.

2. Material and Methods

2.1 Microorganism

Penicillium sclerotiorum UCP1361 isolated from soils of the semi-arid in the state of Pernambuco, Northeastern Brazil, is deposited at the Cultures Bank of the Center for Research in Environmental Sciences and Biotechnology (NPCIAMB) of the Catholic University of Pernambuco. The fungus was kept in Sabouraud (agar, dextrose and peptone) pH 5.5 medium.

2.2 Biosurfactant production

For the production of the biosurfactant, thirty 6 mm diameter discs containing young spores of *P. sclerotiorum* were transferred to each test containing 100 ml of production medium in different concentrations of whey and barley in 12 tests according to the factorial design. The submerged culture was incubated for 96 h at 28 ° C, pH 5.0 at 150 rpm orbital shaking.

2.3 Experimental planning

A Central Composite Rotational Design (CCRD) 2² with 4 axial points and 4 central points (Table 1) was applied to analyze the influence of nutritional parameters (whey and barley) on biosurfactant production. Experimental data were performed using Statistica® software version 9 (Statsoft Inc, USA) and adjusted for a polynomial model.

Table 1: Variables and levels of Central Composite Rotational Design (CCRD) for biosurfactant production by P. sclerotiorum.

Factors	Levels				
	-1.41	-1	0	+1	+1.41
Milk Whey (%)	3.18	4	6	8	8.82
Barley (%)	1.18	2	4	6	6.82

2.4 Determination of surfactant activity

To detect the presence of biosurfactant in the medium, its surface tension was measured. Cell-free metabolic fluid was used for this measurement after a 96 h incubation period. A Sigma 70 tensiometer was used (KSV Instruments Ltd., Helsinki, Finland), according to the Du Nouy ring methodology. (Kuyukina et al., 2001).

2.5 Determination of Emulsifying Property

The hydrophobic substrates tested for induction of emulsion formation were: canola oil, corn oil, post-frying soybean oil, sunflower oil and burnt motor oil. Samples were centrifuged at 10,000 rpm for 15 min. 1 mL of hydrophobic substrate and 1 mL of cell free metabolic fluid were added to the test tubes. Subsequently, the tubes were vortexed for 2 min and allowed to stand for 24 h to measure the emulsion index. The percentage of emulsification index (E_{24}) is given as the percentage of emulsion layer height divided by the total height of the liquid column (Cooper and Goldenberg, 1987).

2.6 Viscosity effect on engine oil burnt

To assess the effect on reducing hydrocarbon viscosity, 6 mL of flared engine oil was transferred to test tubes containing the biosurfactant present in the metabolic liquid (2 mL). Water was used as negative control in reducing viscosity and chemical surfactant SDS (sodium dodecyl sulfate) was used as positive control. The tubes were vortexed and the emulsions formed were read on a Brookfield viscometer (Middleboro, MA, USA, TC 500). Results were expressed as centipoise (cP).

2.7 Application of biosurfactant in the dispersion of hydrophobic

The biosurfactant ability to disperse burnt engine oil was performed by adding 20 mL of distilled water at a petri dish, 1 mL of engine burnt oil, and 1 mL of metabolite liquid containing biosurfactant. The positive dispersion control used was commercial detergent and the negative was water. The average diameter of the light zones in the center of each plate was measured and calculated as the petri dish diameter rate (Morikawa et al., 2000).

3. Results and discussion

3.1 Biotransformation of barley and whey for biosurfactant production by *P. sclerotirum* UCP1361

Environmental legislation constantly requires industries to carry out a waste treatment or reuse plan. In this context, this work appears as an alternative for the reuse of barley and whey, both residues of industrial origin, as renewable sources for the production of microbiological surfactants.
In addition, problems in the production of biosurfactants arise mainly from the use of expensive substrates, as they represent about 30-40% of the total industrial scale production cost, and the purification of the bioproduct, resulting in an expensive product (Syldatk and Hausmann, 2010; Nurfarahin et al., 2018). To overcome these problems, the use of cheaper alternative substrates and the optimization of production conditions are reported in the literature (Amodu et al., 2014).
The data obtained in this study demonstrate that *P. sclerotiorum* produced a biosurfactant with excellent surface activity capable of reducing the surface tension from 72 mN/m to 27 mN/m in a culture medium composed of 4% whey and 2% barley, corresponding to condition 1 factorial design (table 2). These results confirm the ability of *P. sclerotiorum* to synthesize a more efficient biosurfactant when compared to other fungi grown in agro-industrial residues, such as *Fusarium* sp. (32 mN / m) (Qazi et al., 2013), *Cunninghamella echinulata* (36 mN / m) (Andrade Silva et al., 2014) and *Penicillium* sp. (42 and 62 mN/m) (Mendez-Castillo et al., 2017). Furthermore, there are no reports in the literature reporting the production of biosurfactant using barley as a microbial nutritional source.

Table 2: Surface tension (ST) as a parameter to evaluate biosurfactant production by P. sclerotiorum in medium containing whey and barley.

Assays	Components Culture medium		Surface tension (mN/m)
	Whey	Barley	
1	+1(8)	+1(6)	27,0
2	+1(8)	-1(2)	30,8
3	-1(4)	+1(6)	35,7
4	-1(4)	-1(2)	33,1
5	0(6)	+1.41(6.82)	32,5
6	+1.41(8.82)	0(4)	33,3
7	0(6)	-1.41(1.18)	37,8
8	-1.41(3.18)	0(4)	32,9
9	0(6)	0(4)	34,1
10	0(6)	0(4)	33,7
11	0(6)	0(4)	37,7
12	0(6)	0(4)	31,5

3.2 Effect of the interaction between barley and whey on biosurfactant production evaluated by Pareto Diagram

The standardized Pareto diagram with a 95% confidence level (Figure 1) shows that barley (linear function) was the most statistically significant variable, followed by whey (quadratic function). However, the association of barley with whey at the concentrations used in this study (linear function) was the variable that had the most significant effect on the reduction of surface tension. Barley, in quadratic function, was the only independent variable that did not influence the reduction of surface tension.
Pareto graph analysis showed that only the quadratic effect of barley concentration was not statistically significant within a 95% confidence interval. The linear effect of barley and the quadratic effect of whey are significant for the increase of surface tension values while the values of linear interaction between factors and the linear effect of whey showed statistical significance for ST reduction. The interaction should be analyzed because it is the most accountable for the decrease of the ST values.

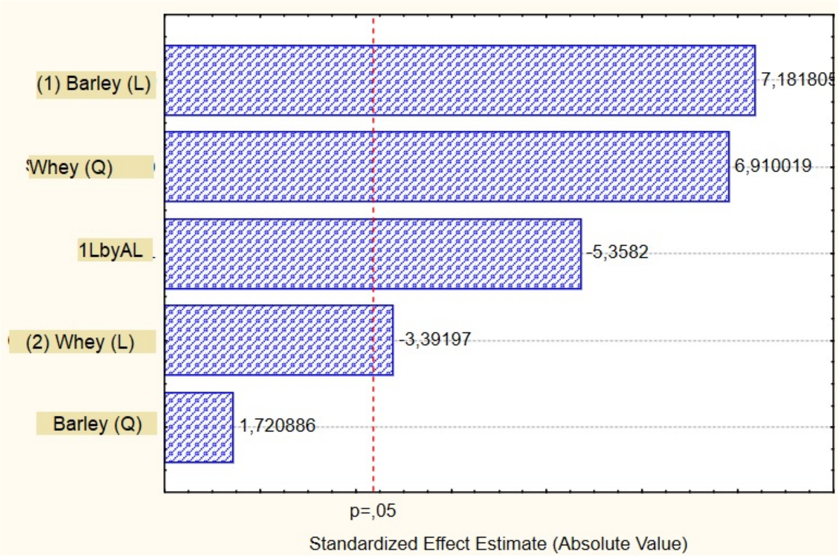

Figure 1: Pareto diagram to investigate the effects of whey and barley concentrations on biosurfactant production by P. sclerotiorum.

3.3 Emulsifying property of *P. sclerotiorum* biosurfactant

The main role of surfactants is to reduce the interfacial tension, which allows the formation of small droplets. Emulsifiers, on the other hand, although they also have an active surface, absorb more slowly on the surface of the drop and provide long-term stability of the emulsion droplets (Salek and Euston, 2019). Emulsifying properties were observed for the *P. sclerotiorum* biosurfactant produced in condition 1 of the factorial design. The formation of stable emulsions was found from the use of burnt motor oil as a hydrophobic substrate, resulting in emulsification of 68%, thus demonstrating its significant emulsifying action, since Willumsen and Karlson (1997) consider significant values above 50% of emulsification. This result was similar to the surfactant synthesized by the bacterium *Streptomyces* sp that emulsified the hydrocarbon by 65% (Colin et al., 2013). In addition, a significant emulsification result was also obtained using corn oil as a hydrophobic substrate (57%) (Figure 2).

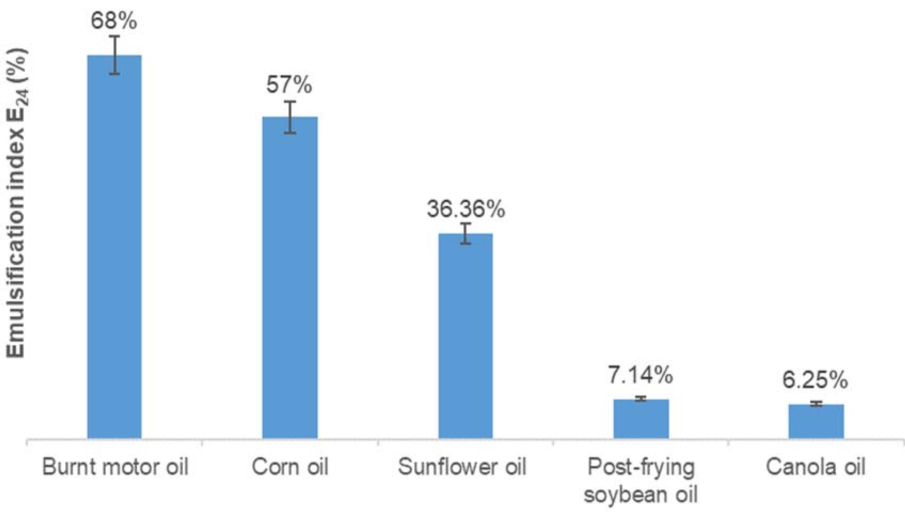

Figure 2: Emulsifying capacity of biosurfactant produced by P. sclerotiorum using whey and barley.

3.4 Biosurfactant as a dispersing agent and hydrocarbon viscosity reducer

Dispersing agents are surfactants that aid in the dissolution of the oil slick into smaller droplets (Pinto et al., 2018). In this context, the biosurfactant produced by *P. sclerotiorum* demonstrated its ability to disperse 33.15 cm2 of the burnt engine oil in water (Figure 3A). Lower dispersion result was obtained with commercial detergent which dispersed 19.52 cm2 (Figure 3B), using water as negative control the dispersion result was zero (Figure 3C). The result obtained in this study was similar to that found by Marques et al. (2019) using biosurfactant produced by *Mucor circinelloides* UCP0001 in medium containing soybean oil and corn steep liquor, in which it dispersed 37.36 cm^2 in the burned motor oil.

P. sclerotiorum crude biosurfactant was able to reduce the burnt engine oil viscosity from 279.6 cP to 48.5 cP, achieving a reduction percentage of 82.3%. On the other hand, in the control using an SDS synthetic surfactant, it reduced the engine oil viscosity to 24.1 cP, exhibiting a reduction percentage of 91.4%. These results indicate that the tensoactive ability of the fungi surfactant was similar to the chemical surfactant.

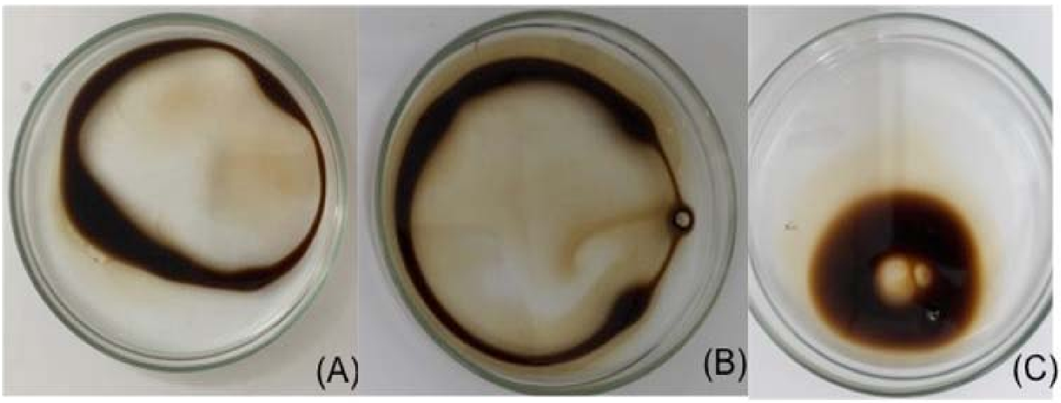

Figure 3: Biosurfactant with dispersant function produced by P. sclerotiorum: (A) Biosurfactant; (B) Positive Control (Commercial Detergent); (C) Water (negative control).

Conclusions

Penicillium sclerotiorum demonstrated the ability to biotransform whey and barley residues into essential sources for effective biosurfactant, reducing surface tension to 27 mN/m, as well as being able to emulsify 68% of burnt engine oil. In addition, it has a dispersing ability and viscosity reducer of hydrocarbons, making it promising for bioremediation processes. In addition, this biomolecule emerges as a sustainable alternative and viable option for industrial scale production due to the low cost in the production process.

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