Original Research Article

Biosurfactant Production by *Cunninghamella phaeosphora* UCP 1303 Using Controlled Temperature Through of Arduino

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A B S T R A C T

The objective of this study was to investigate the ability of *Cunninghamella phaeosphora* UCP 1303 to produce biosurfactant converting soybean oil and corn liqueur, using temperature monitoring through the Arduino and DS18B20 waterproof temperature sensor. Initially, a 10^7 cells/mL suspension was inoculated and incubated under 150 rpm shaking at 25, 28 and 35°C during 96 h. Surface and interfacial tensions were determined by the Du Nouyring method and also the emulsification index and the dispersion test in water. The results demonstrated unprecedented use of Arduino in fermentation temperature control, with temperatures of 25°C and 35°C showing a surface tension reduction of 70 mN/m to 36.1mN/m and 70 mN/m for 37.1 mN/m respectively. Fermentation temperature at 28°C presented the best results for reducing surface tension (70 mN/m to 28.1 mN/m) and interfacial tension (2.2 mN/m) with high potential of motor oil emulsion Burned (E_{24} = 100%) and excellent dispersant properties (28.26 cm^2) of motor oil burnt in water. The results showed that C. phaeospora produced biosurfactant in low cost medium using agroindustrial residues; in addition, the excellent properties of the biosurfactant suggest its potential of application in the bioremediation of sites polluted by hydrophobic compounds.

Introduction

Biosurfactants, also known as microbial surfactants, are amphipathic molecules having a hydrophobic (non-polar) and other polar hydrophilic region. Used mainly in the cleaning products industries, such as soaps and detergents, petroleum, cosmetics and hygiene products (Nitschke and Pastore, 2002). Due to its structure, surfactants have several properties, such as reduction of surface and interfacial tension, high emulsifying capacity, dispersant among others. However, because they originate from

petroleum, they have high toxicity. In this context, the biosurfactants have the same properties, are non-toxic and are produced by microorganisms (bacteria, yeasts and filamentous fungi, these being less exploited), from renewable substrates (Barros *et al.,* 2008; Colla *et al.,* 2012).

Cunninghamela is filamentous fungus that is being used for the production of biosurfactants because it has the ability to metabolize xenobiotic compounds; in response to these, the hydrolases enzymes are excreted. This capability reflects its potential in processes of biodegradation, bioremediation and biotransformation (Eustáquio *et al.,* 2003)

The biosurfactants can be used in the bioremediation, being used in the degradation of hydrocarbons in water through its capacity of emulsion and dispersion in biodegradation of pesticides, to emulsify and increase the solubility of hydrophobic contaminants in soil and water (Nitscheke and Pastore, 2002), in the removal of heavy metals such as uranium, cadmium and lead (Mulligan *et al.,* 2001).

The objective of this study was to produce biosurfactant through *Cunninghamella phaeosphora* UCP 1303 converting agroindustrial wastes (soybean oil and corn liqueur) digitally monitoring the temperature by the Arduino.

Materials and Methods

Microorganism and Maintenance media

Filamentous fungi was used *Cunninghamella phaeospora* (UCP1303), isolated from the Caatinga of the State of Pernambuco and deposited in the Collection of Cultures UCP (Catholic University of Pernambuco).The strain was maintained in Potato Dextrose Agar medium at 5°C.

Biosurfactant production medium

The production medium used consisted of a base of salts (NH_4NO_3 - 0,1g; KH_2PO_4 - 0,02g; $MgSO_4.7H_2O$ - 0,02g and glutamic acid – 1g) supplemented with 6% (v/v) of cornflower (CSL) and 2,5% (v/v) of post-fry soybean oil (SHOW), optimized through factorial planning DCCR 2^2 by Lins *et al.,* (2016).

Substrates for the means of production

The post-fry soybean oil (SWO) yielded by the informal trade and maize (CSL) residue obtained from corn processing yielded by Corn Products Brasil, both locate in the state of Pernambuco, were used as substrates in the medium for the production of biosurfactant.

Production of biosurfactant

Inoculum

The spores of *C. phaeospora* were collected and counted in a Neubauer chamber for a suspension of 10^7. Subsequently, 2 ml of the spore suspension was transferred to the surface of the Sabouraud medium in the Petri dishes, kept at 28°C for 24h, and then 10 discs were cut and transferred to Erlenmayer flasks containing the production medium, incubated at 150 rpm, temperatures of 25°C, 28°C and 35°C for 96h.

Temperature and pH determination

The temperature was determined by the DS18B20 sensor with the aid of Arduino Uno every 10 min. In the fermentation with temperature at 25°C the power supply was a 5 V charger and a microSD card was used to store the data and in the fermentation with temperature at 35°C was used a computer with power supply and storage. Two libraries were added to the Arduino software, One

Wire and Dallas Temperature. These two libraries worked together to extract the data from the DS18B20 waterproof temperature sensor. The DS18B20 sensor can perform temperature readings in a range from -50ºC to 125ºC with an accuracy up to ± 0.5ºC and send the information to the microcontroller using only one wire. Each sensor has a physical address (identified by the OneWire library), so it was possible to place several sensors on a single bus, using a single microcontroller port (Thomsen, 2015). The pH adjustment to 5.5 was determined by potentiometry.

Separation of metabolic fluid

After the incubation time, the vials were withdrawn from orbital shaking. The metabolic liquid was separated from the biomass through 120 mesh nylon filtration. Samples were centrifuged at 4000 rpm at 10°C for 15 min and the cell free supernatant was stored for future analyzes.

Biosurfactant potential evaluation tests

Determination of surface and interfacial tension

The monitoring of the biosurfactant production in the metabolic liquid was performed by measuring the surface and interfacial tension in the Du Nuoy ring method using the automated tensiometer, according to the methodology of Kuykina *et al.,* (2001). The interfacial tension was evaluated after the addition of n-hexadecane in the supernatant according to Darvishi *et al.,* (2011).

Emulsification Index (IE)

In the test tubes, 1mL of the cell free metabolic liquid and 1mL of the different oils were added: vegetal (Oil of Mamona and Maize Oil) and two oils derived from petroleum (Diesel Oil and Burnt Engine Oil). The tubes were vortexed for 2 min at full speed and allowed to stand for 24 hours according to Cooper and Goldenberg (1987). The percentage emulsification was determined from Equation 1 below.

$$E = \frac{\text{height of the emulsified layer (cm)}}{\text{total height of the liquid (cm)}} \times 100$$

Equation 1

Absorption capacity of biosurfactant

The dispersion capacity of motor burned oil in water by the biosurfactant was investigated. To this end, 20mL of distilled water was contaminated with 0.5mL of burned engine oil and 1mL of cell-free metabolic fluid was added. The diameter of the free zone formed after the addition of the biosurfactant-containing metabolic liquid to indicate the ability to disperse hydrophobic substrates was calculated as an oil displacement area (ODA) according to Morikawa *et al.,* (1993) using equation 2 according to Manocha *et al.,* (1980).

$$ODA = \frac{22 \, x (radius)^2}{7} \, cm^2$$

Equation 2

Results and Discussion

Influence of temperature on the production of biosurfactant using Arduino

The schedules used by the software with the hardware of the Arduino were efficient, being possible to verify more precisely the temperatures of the shaker in the production of the biosurfactant. No significant temperature variations were observed in the biosurfactant production with a temperature of 25°C, varying the average hourly temperatures between 25.46°C and 25.75°C, as shown in Figure 1. However, in fermentation with a temperature of 35°C, occurred significant and distant variations of

the determined temperature, the average temperatures varied between 35.98 and 39.71°C (Figure 2), reaching peaks of 40°C.

Production of biosurfactant by *Cunninghamella phaeosphora* in medium containing corncina and soybean oil after frying

According to the results obtained for the production of biosurfactant, in the medium constituted by 2.5% of oil and 6% of corncine, in the condition of fermentation at 28°C there was the maximum reduction of the surface tension of 70 to 28.1 mN and interfacial from 40 to 2.2 mN / m (Table 1). The biosurfactant produced by *C. phaeosphora* by fermentation at 28°C showed excellent results compared to the surface tension obtained by the biosurfactant synthesized by *Cunninghamella elegans* that was 28.2 mN / m (SOUZA, 2015).

Emulsifying property

Some biosurfactants have the ability to form and stabilize emulsions. In addition, biosurfactants with emulsifying properties are those capable of forming emulsions with percentages above 50% (Willumsen and Karlson, 1996). In this context, the biosurfactant produced by *C. phaeosphora* in the fermentation condition at 28°C was able to form a stable emulsion, with IE_{24} of 93.62% using as the hydrophobic substrate the motor burned oil (Figure 3).

The biosurfactants obtained in the fermentations at 25°C and 35°C presented satisfactory indices, 51.9157% and 54.708%, 53.448%, respectively.

Dispersibility of engine oil burned by the biosurfactant produced by *Cunninghamella phaeosphora*

The biosurfactant of *C. phaeosphora* produced in the fermentation condition at 28°C dispersed 28.26 cm^2 of the burned motor oil (Figure 4), presenting a similar result to Silva *et al.,* (2014) which reached a maximum dispersion index of 37.36 cm^2, considering that *Cunninghamella echinulata* was used in the production of the biosurfactant and both occupied the entire region of the plaque.

Fig.1 Variation of the means of the fermentation temperatures of the biosurfactant production process at 25°C
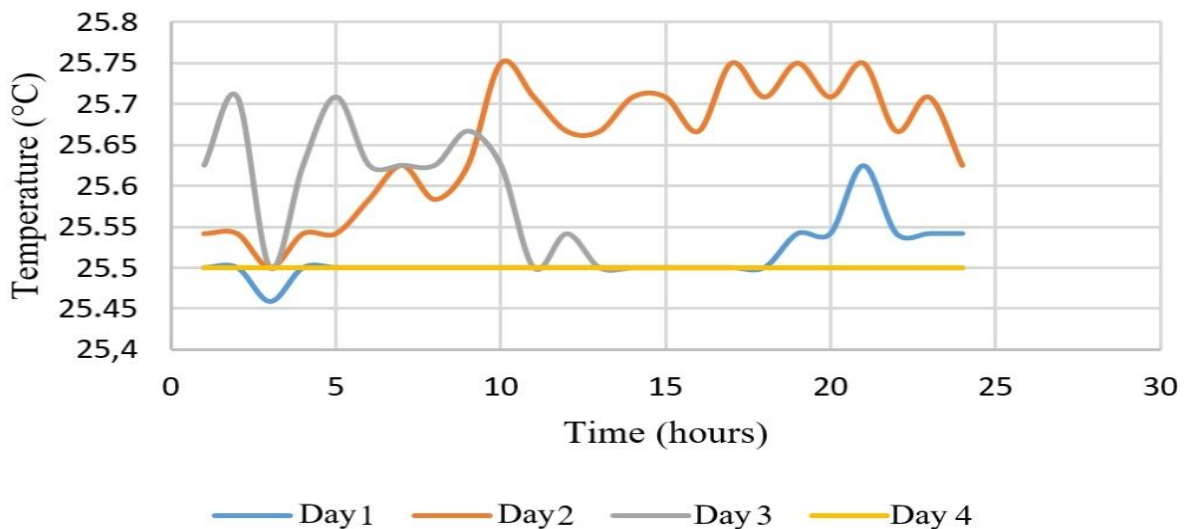
Fig.2 Variation of the means of fermentation temperatures of the
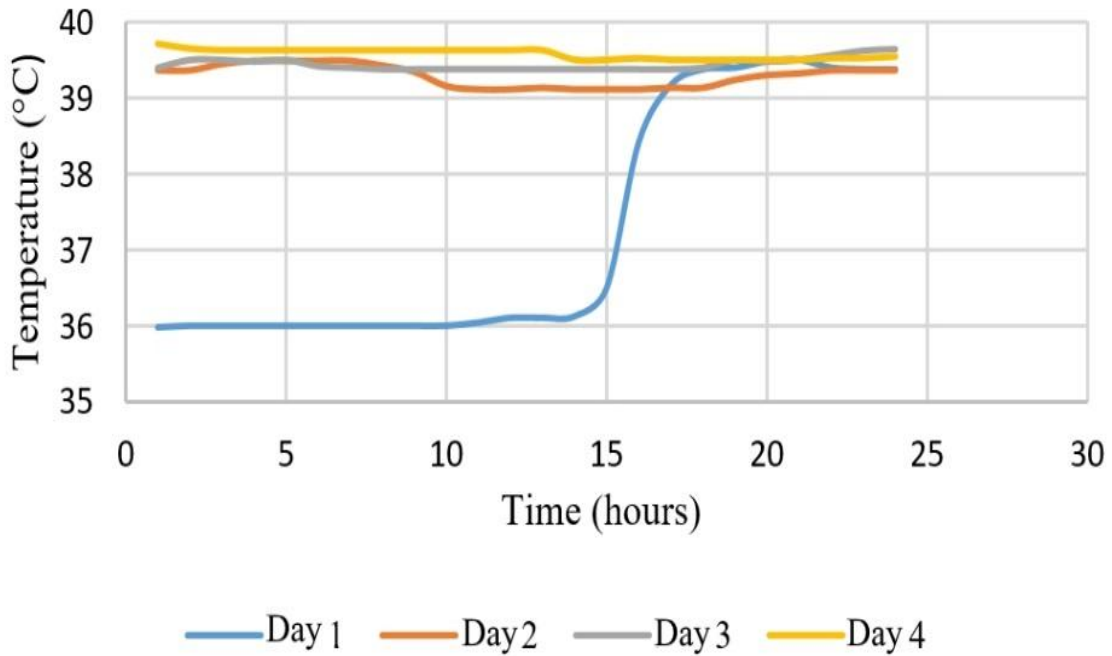biosurfactant production process at 35°C

Fig.3 Determination of the emulsification index (IE_{24}) in the metabolic liquid
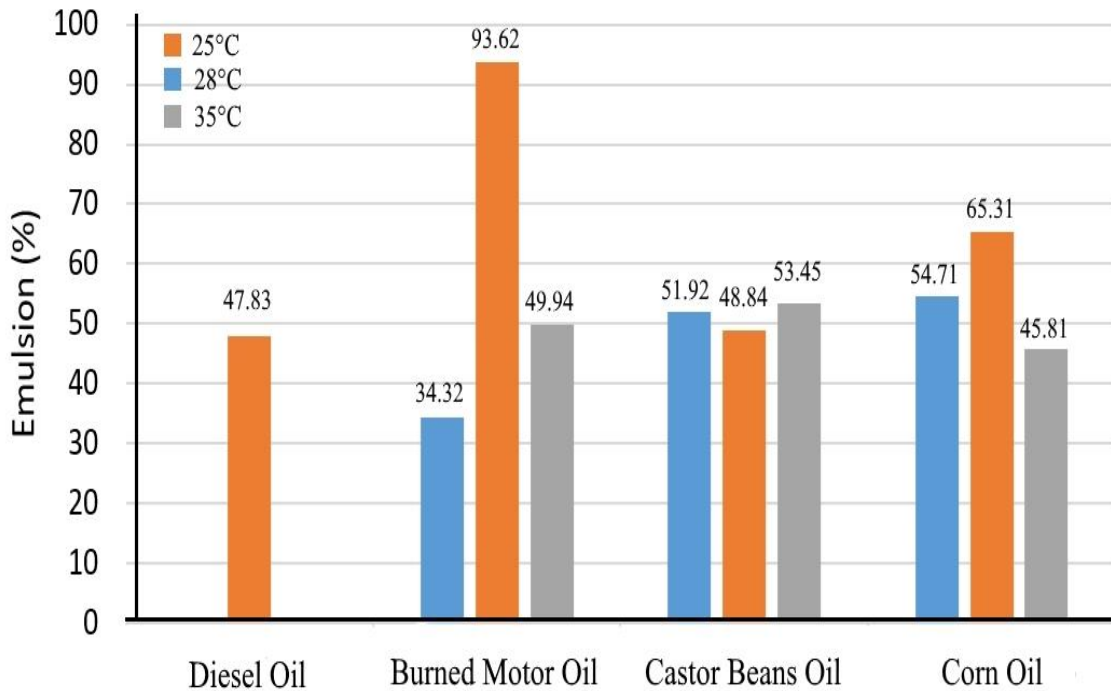of *C. phaeosphora* using different hydrophobic substrates

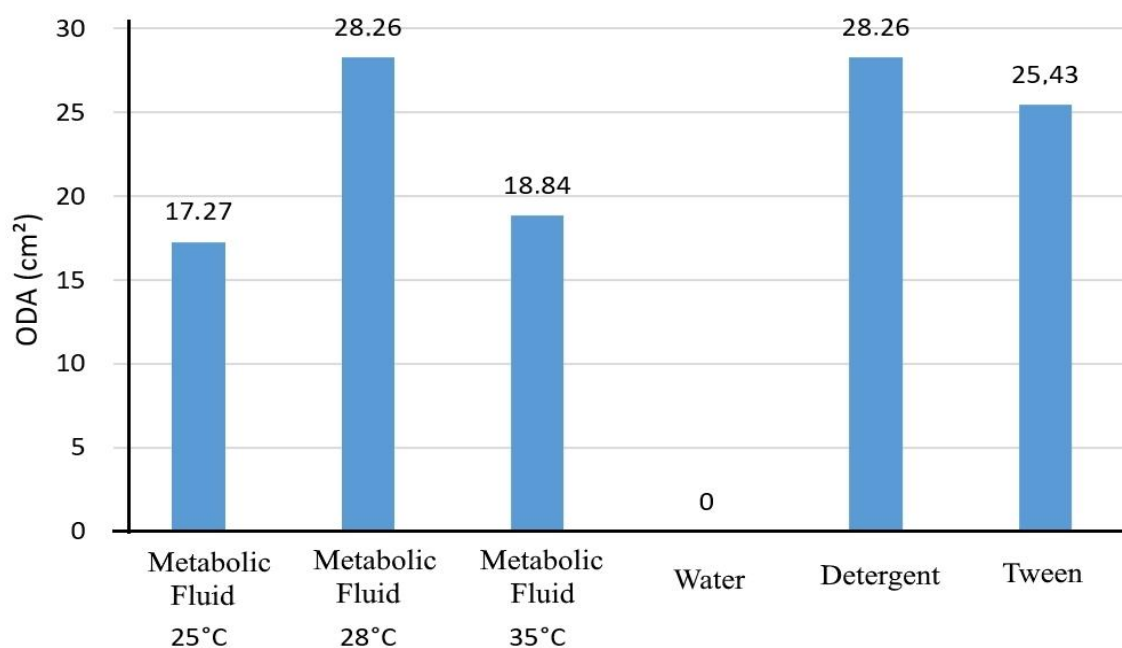**Fig.4** Determination of ODA in the metabolic liquid of *C. phaeosphora* using different hydrophobic substrates

Table.1 Produção de biossurfactante por *Cunninghamella phaeospora* UCP 1303 determinada através das médias das tensões superficiais no líquido metabólico, após as 96 horas de cultivo

Conditions	Average surface tension (mN/m)	Mean of interfacial tension (mN/m)
25 °C	36.1	21.4
28 °C	28.1	2.2
35 °C	37.1	14.9

At the end, the ability of the microorganism *Cunninghamella phaeosphora* to metabolize the SWO and CSL substrates of the low cost production medium with the production of biosurfactant (extracellular compound) was verified. It was also possible to evaluate that 28 ° C was the best fermentation temperature for the production of biosurfactant. This biosurfactant that was produced by *C. phaeospora* demonstrated potential of use in industrial applications and bioremediation of petroleum products.

The unprecedented use of programming and arduino in temperature monitoring has been shown to be effective throughout the biosurfactant production process. The DS18B20 waterproof temperature sensor did not interfere with the process and did not contaminate the microbial cultures.

Finally, the results obtained in this study are promising, their applicability is diverse, either in the use of a system of accurate temperature monitoring, cheap and that does not interfere in the system, or in environmental treatments of ecosystems contaminated with oils, or in industrial processes with the production and commercialization of a natural biosurfactant.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Acknowledgments

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