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Production of biosurfactant by *Cunninghamella elegans* UCP 0542 using food industry waste in 3 L flasks and evaluation of orbital agitation effect

Produção de biossurfactante por *Cunninghamella elegans* UCP 0542 utilizando resíduos da indústria alimentícia em frascos de 3 L e avaliação do efeito da agitação orbital

Producción de biosurfactante por *Cunninghamella elegans* UCP 0542 a partir de residuos de la industria alimentaria en frascos de 3 L y evaluación del efecto de la agitación orbital

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Abstract
In current study, the production of biosurfactant by *Cunninghamella elegans* UCP 0542 in food industry waste-based medium was investigated as a biotechnological strategy to reduce the bioprocess costs. Cultivation of the Mucoralean fungus was performed in 3 L Fernbach flasks containing 1.5 L of effective volume of medium composed by 2% instant noodle waste, 2% corn steep liquor and 0.5% post-frying soybean oil, with carbon/nitrogen ratio of 30:1. Fermentations were carried out during 96 h and 28ºC, at 150, 180 and 200 rpm, in order to evaluate the influence of orbital agitation in biosurfactant production. The properties of surface tension reduction, emulsification and dispersion were investigated. According to the obtained results, *C. elegans* produced biosurfactant in sustainable medium, reducing the surface tension from 71 to 27.5 mN/m after cultivation at 200 rpm. Biotensoactive produced in this condition formed stable emulsions with engine oil and burnt engine oil (emulsification index of 100%) and showed 48.39 cm² of oil dispersion area (ODA) with burnt engine oil. The biomolecule was isolated by different methodologies, reaching a maximum yield of 2.1 g/L with ethanol. The biosurfactant produced by *C. elegans* exhibited excellent potential for application in bioremediation processes considering its promising properties as a surface tension reducing agent, emulsifying and dispersing action of petroderivatives.
Keywords: Biotensoactive; Mucoralean fungus; Renewable substrates; Surface tension reduction; Emulsifying properties; Dispersing action.

Resumo

No presente estudo, a produção de biossurfactante por *Cunninghamella elegans* UCP 0542 em meio à base de resíduos da indústria de alimentos foi investigada como uma estratégia biotecnológica para reduzir os custos do bioprocesso. O cultivo do fungo Mucorales foi realizado em frascos Fernbach de 3 L contendo 1,5 L de volume efetivo do meio composto por 2% de resíduo de macarrão instantâneo, 2% de milhocina e 0,5% de óleo de soja pós-fritura, com relação carbono/nitrogênio de 30:1. As fermentações foram realizadas durante 96 h e 28ºC, a 150, 180 e 200 rpm, a fim de avaliar a influência da agitação orbital na produção do biossurfactante. As propriedades de redução da tensão superficial, emulsificação e dispersão foram investigadas. De acordo com os resultados obtidos, *C. elegans* produziu biossurfactante em meio sustentável, reduzindo a tensão superficial de 71 para 27,5 mN/m após cultivo a 200 rpm. O biotensoativo produzido nesta condição formou emulsões estáveis com óleo de motor e óleo de motor queimado (índice de emulsificação de 100%) e apresentou 48,39 cm² de área de dispersão de óleo (ADO) com óleo de motor queimado. A biomolécula foi isolada por diferentes metodologias, atingindo um rendimento máximo de 2,1 g/L com etanol. O biossurfactante produzido por *C. elegans* apresentou excelente potencial para aplicação em processos de biorremediação considerando suas propriedades promissoras como agente redutor de tensão superficial, ação emulsificante e dispersante de petroderivados.

Palavras-chave: Biotensoativo; Fungo Mucorales; Substratos renováveis; Redução da tensão superficial; Propriedades emulsificantes; Ação de dispersante.

Resumen

En el estudio actual, se investigó la producción de biosurfactante por *Cunninghamella elegans* UCP 0542 en un medio basado en residuos de la industria alimentaria como una estrategia biotecnológica para reducir los costos del bioproceso. El cultivo del hongo Mucorales se realizó en matraces Fernbach de 3 L que contenían 1,5 L de volumen efectivo de medio compuesto por 2% de residuo de fideos instantáneos, 2% de licor de maceración de maíz y 0,5% de aceite de soya post fritura, con una relación carbono/nitrógeno de 30:1. Las fermentaciones se realizaron durante 96 h y 28ºC, a 150, 180 y 200 rpm, con el fin de evaluar la influencia de la agitación orbital en la producción de biosurfactante. Se investigaron las propiedades de reducción de la tensión superficial, emulsificación y dispersión. De acuerdo con los resultados obtenidos, *C. elegans* produjo biosurfactante en medio sustentable, reduciendo la tensión superficial de 71 a 27.5 mN/m después del cultivo a 200 rpm. El biotensoactivo producido en esta condición formó emulsiones estables con aceite de motor y aceite de motor quemado (índice de emulsificación del 100%) y mostró 48,39 cm² de área de dispersión de aceite (ADO) con aceite de motor quemado. La biomolécula fue aislada por diferentes metodologías, alcanzando un rendimiento máximo de 2.1 g/L con etanol. El biosurfactante producido por *C. elegans* exhibió un excelente potencial para su aplicación en procesos de biorremediación considerando sus prometedoras propiedades como agente reductor de la tensión superficial, acción emulsionante y dispersante de los petroderivados.

Palabras clave: Biotensoativo; Hongo Mucorales; Sustratos renovables; Reducción de la tensión superficial; Propiedades emulsionantes; Acción dispersante.

1. Introduction

Currently, with the advent of industrial sustainability, technologies are becoming increasingly essential for the industries around the world (Andrade, et al., 2018; Silva, et al., 2020). In this sense, the interest in compounds produced by sustainable technology and the new environmental legislation has encouraged the search for biosurfactants instead of chemical surfactants (Hisham, et al., 2019; Cândido, et al., 2022).

Biosurfactants are natural and biodegradable amphiphilic compounds produced by microorganisms. On the other hand, chemical surfactants are synthesized from petroleum derivatives, and represent an important source of pollution, causing adverse biological effects to aquatic organisms (Rubio, et al., 2017; Andrade et al., 2018; Sá, et al., 2019; Durval et al.,2021; Silva, et al., 2022). The use of biosurfactants has received great interest due to the characteristics that make them advantageous in relation to their chemical counterparts, such as low toxicity, biodegradability and the possibility of production from renewable and low-cost materials. These factors make them desirable to replace the widely used chemical surfactants (Andrade, et al., 2018; Bezerra & Sarubbo, 2021; Silva, et al., 2022).

The efficiency of a bioprocess is the basis for any biotechnological industry, including that focused on the production of biosurfactants. In this case, the search for increased productivity demands the addition of components to the culture medium induces maximum or optimal productivity (Makkar, et al., 2011). The formulation of production media based on agro-

industrial wastes for the biotechnological production of metabolites, has high value-added (Fonseca, et al., 2018; Das, Kumar, 2019; Marcelino, et al., 2020; Melanouri et al., 2022). Several studies have described techniques for reusing these residues in industrial production processes, because in addition to reducing environmental damage, they represent an economic alternative for companies, due to the large amount of macro and micronutrients present in their composition (Sant'Anna, et al., 2012; Ashour, et al., 2014; Sun, et al., 2018). In this sense, the present work contributes to the study of biotechnological strategies for the production of biosurfactant in large scale using alternative and sustainable technology through the use of food industry waste, generating a high value-added bioproduct for industries and enabling its industrial production.

2. Methodology

Microorganism and mycelial growth

The Mucoralean fungus *Cunninghamella elegans* UCP 0542 used in this study was isolated from Caatinga soil in Pernambuco, Brazil, and was obtained from the Culture Collection UCP, Catholic University of Pernambuco registered in the World Federation for Culture Collections (WFCC). Mycelial growth of *C. elegans* was performed in Petri dish containing Sabouraud agar medium during 72 h and 28ºC.

Preparation of inoculum

Spores of *C. elegans* were transferred to sterile distilled water until reaching a concentration of 10^7 spores/mL. Then, this spore suspension was used as inoculum for biosurfactant production.

Components of the production medium

The medium consisted of instant noodle waste (INW), kindly provided from instant noodle industry (Cabo de Santo Agostinho, PE, Brazil), corn steep liquor (CSL) obtained from corn products and post-frying soybean oil (PFSO) from informal food commerce. Chemical composition of the substrates was provided by Andrade et al. (2018).

Production of biosurfactant in 3 L Fernbach flasks

The production was performed in 3 L Fernbach flasks containing 1.5 L of effective volume of medium composed by 2% INW, 2% corn steep liquor CSL and 0.5% PFSO, with carbon/nitrogen ratio of 30:1 (Andrade et al., 2018). The medium was adjusted to pH 5.5 and autoclaved at 121°C for 15 min and then, it was inoculated with 3% of spore suspension of *C. elegans*. Fermentations were carried out at 28ºC and 150 rpm, during 96 h and after this period, the medium was filtered to separate the biomass from the metabolic liquid.

Influence of orbital agitation in biosurfactant production

The influence of orbital agitation in biosurfactant production was investigated by incubating the production medium at 150, 180 and 200 rpm, under the conditions described above. After 96 h, the media were filtered in order to separate the biomass from the metabolic liquids and they were subjected to surface tension measurement.

Measurement of surface tension (ST)

Surface tension (ST) was measured on the cell-free metabolic liquid using a digital tensiometer equipped with Du Noüy ring, following the methodology of Kuykina et al. (2001).

Determination of emulsification index (EI_{24})

The emulsifying potential of the biosurfactant was investigated by determination of emulsification index (EI_{24}) using the cell-free metabolic liquid and the hydrophobic substrates (burnt engine oil, sunflower oil, corn oil, canola oil, engine oil, soybean oil, PFSO and n-hexadecane). The EI_{24} was calculated after 24 h, according to the methodology described by Cooper and Goldenberg (1984), as the height of the emulsion layer divided by the total height of the liquid column and expressed as percentage. The measurements were taken in triplicate.

Dispersing capacity

The ability of the biosurfactant as dispersing agent was investigated by oil spreading test, using the cell-free metabolic liquid and burnt engine oil as the hydrophobic compound. The diameter of the clear zone on the oil surface was measured and expressed as oil displacement area (ODA), following the method established by Morikawa et al. (1993). Distilled water was used as negative control and the chemical surfactant sodium dodecyl sulfate (SDS) was used as positive control.

Extraction of the biosurfactant

The biosurfactant was isolated from cell-free liquid using different methods, as described in Table 1. All samples were centrifuged at rotation of 3600 *g* at 5ºC. The yield of crude biosurfactant was determined and the results were expressed in g/L.

Table 1- Methods of extraction of the biosurfactant produced by *C. elegans* UCP 0542 using food industry waste.

Method	Ratio	Reference
Ethanol P.A.	2:1	Bueno, et al. (2010)
Ethanol 70%	2:1	Bueno, et al. (2010)
Acid precipitation + ethanol P.A.	2:1	Present study
Acid precipitation + ethanol 70%	2:1	Present study
Acid precipitation + acetone	1:1	Present study

Source: Authors.

3. Results and Discussion

3.1 Production of biosurfactant in 3 L flasks and evaluation of orbital agitation effect

The eco-friendly strategy for biosurfactant production in large scale by *C. elegans* performed in this study was the use of food waste as substrates in 3 L flasks of capacity at different rotation speeds. According to the results summarized in Table 2, the Mucoralean fungus was able to reduce the ST in volume of 1.5 L of sustainable medium (2% INW, 2% CSL and 0.5% PFSO). However, the higher reduction was verified in culture incubated at orbital agitation of 200 rpm, from 71 to 27.5 mN/m. Previously, Andrade et al., (2018) and Mnif and Ghribi (2015) indicated that microorganisms considered excellent biosurfactant producers are those who can reduce ST of the production medium to values below 30 mN/m. Recently, Pele et al. (2019) observed that the biosurfactant produced by *Rhizopus arrhizus* could reduce the ST to 28.8 mN/m.

Table 2- Production of biosurfactant by *C. elegans* in medium containing food waste.

Volume (L)	Orbital agitation (rpm)	Surface tension (mN/m)
1.5	150	34.8
1.5	180	34.2
1.5	200	27.5

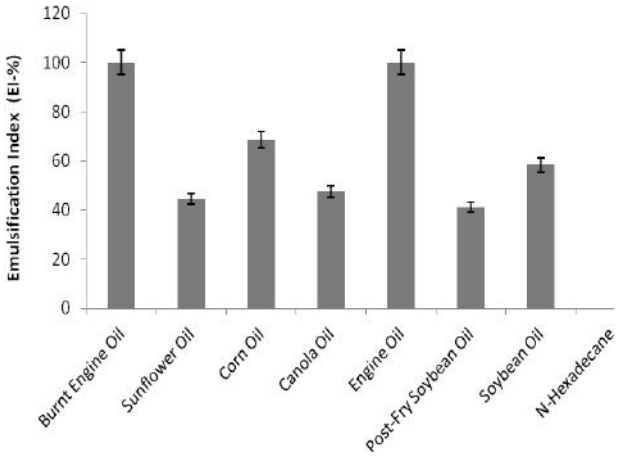
Source: Authors.

On other hand, stirring speed between 350 and 550 rpm has been commonly used for biosurfactant production by different fungi after scale-up (Souza et al., 2018). In this work, a lower agitation speed (200 rpm) favored the maximum production of biosurfactant by *C. elegans*, which contributes to the reduction of production process costs and the viability of commercialization of biosurfactants. Therefore, the results obtained in this study indicate that *C. elegans* is a promising microorganism for biosurfactant production.

3.2 Emulsifying properties of biosurfactant produced by *C. elegans*

The metabolic liquid from culture medium with orbital agitation of 200 rpm was selected for further studies due to the reach of the minimum surface tension (27.5 mN/m). According to Figure 1, it is possible to state that the biosurfactant produced in the selected condition showed emulsifying properties because it was capable of forming homogeneous and stable emulsions with most of the hydrophobic compounds used. The EI_{24} varied depending on the oil used, highlighting the EI_{24} of 100% with engine oil and burnt engine oil.

Figure 1 - Emulsifying capacity of the biosurfactant produced by *C. elegans* in 1.5 L of sustainable medium at orbital agitation of 200 rpm

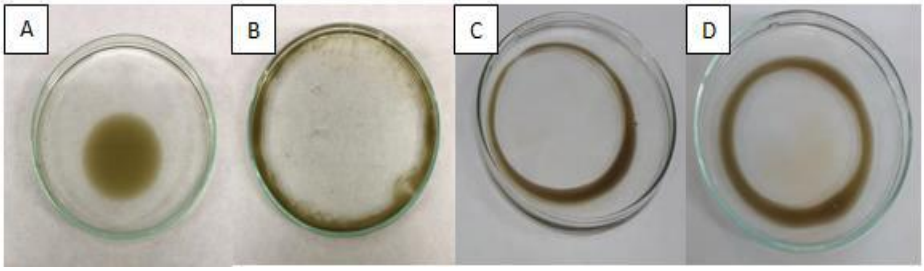


Source: Authors.

3.3 Dispersing properties of biosurfactant produced by *C. elegans*

The area of displacement formed by presence of biosurfactant in solution is directly proportional to the potential of activity this biomolecule (Morikawa, et al., 1993). In this context, Figure 2 illustrates the potential of the biosurfactant produced by *C. elegans* in disperse burnt engine oil. According to Figure 2A, the distilled water did not present dispersion activity (negative control), while Figure 2B showed the excellent dispersant property of the commercial detergent (positive control), with a clear zone covering the entire Petri dish (ODA= 72.35 cm^2). SDS and biosurfactant produced by *C. elegans* showed similar dispersing activity with burnt engine oil, resulting in ODA of 47.93 cm^2 and 48.39 cm^2, respectively.

Figure 2 –Dispersion potential of the biosurfactant produced by *C. elegans* in 1.5 L of sustainable medium at orbital agitation of 200 rpm. Dispersion test using: distilled water (A), commercial detergent (B), SDS (C) and biosurfactant produced by *C. elegans* (D).



Source: Authors.

3.4 Yield of the biosurfactant produced by *C. elegans*

One of the biggest disadvantages of producing a microbial surfactant is the recovery cost, which often makes unfeasible its large-scale production and application (Andrade et al., 2018). In order to determine the most effective method for the isolation of the biosurfactant produced by *C. elegans*, five methodologies were tested in this work (Table 1). The results showed in Table 3 demonstrated that the extraction method with ethanol PA resulted in maximum biosurfactant yield (2.1 g/L).

Table 3- Selection of the extraction method for biosurfactant produced by *C. elegans*.

Method	Biosurfactant yield (g/L)
Ethanol P.A.	2.1
Ethanol 70%	1.7
Acid precipitation + ethanol P.A.	1.8
Acid precipitation + ethanol 70%	1.2
Acid precipitation + acetone	1.3

Source: Authors.

4. Conclusion

The production of the biosurfactant in large scale using food industry waste showed to be biotechnological strategy excellent for enhance the competition of biosurfactants in the world market. In addition, *Cunninghamella elegans* UCP 0542 proved to be a promising microorganism of industrial and commercial interest by excellent potential in produce biosurfactant with surface-active, emulsifying and dispersing properties.

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