Bioconversion of agro-industrial by-products into microbial lipids and bioemulsifier by *Absidia cylindrospora* var. *cylindrospora* UCP 1301

Bioconversão de subprodutos agroindustriais em lipídios microbianos e bioemulsificante por *Absidia cylindrospora* var. *cylindrospora* UCP 1301

Bioconversión de subproductos agroindustriales en lípidos microbianos y bioemulsionante por *Absidia cylindrospora* var. *cylindrospora* UCP 1301

Received: 05/24/2022 | Reviewed: 06/09/2022 | Accept: 06/10/2022 | Published: 06/19/2022

Rafael de Souza Mendonça
ORCID: https://orcid.org/0000-0001-9226-1627
Catholic University of Pernambuco, Brazil
E-mail: rafa.13souza@hotmail.com
Dayana Montero Rodríguez
ORCID: https://orcid.org/0000-0001-8954-7309
Catholic University of Pernambuco, Brazil
E-mail: dayanamontero87@gmail.com
Adriana Ferreira de Souza
ORCID: https://orcid.org/0000-0002-9527-2206
Catholic University of Pernambuco, Brazil
E-mail: adrife.souza@gmail.com
Rosileide Fontenele da Silva Andrade
ORCID: https://orcid.org/0000-0001-8526-554X
Catholic University of Pernambuco, Brazil
E-mail: rosileide.andrade@unicap.br
Galba Maria de Campos-Takaki
ORCID: https://orcid.org/0000-0002-0519-0849
Catholic University of Pernambuco, Brazil
E-mail: galba.takaki@unicap.br
Sérgio Mendonça de Almeida
ORCID: https://orcid.org/0000-0002-1336-6525
Catholic University of Pernambuco, Brazil
E-mail: sergio.almeida@unicap.br

Abstract

Large amounts of agro-industrial by-products and wastes are generated in Brazil annually. Often, they are underutilized and improperly disposed, causing environmental pollution. In this context, reuse them as raw material to obtain high-added value products, becomes a promising strategy. Hence, this study aimed to investigate the biotechnological potential of the Mucoralean fungus *Absidia cylindrospora* UCP 1301 for production of oleaginous biomass and bioemulsifier (BE), using agro-industrial by-products as low-cost substrates. Firstly, the morphological identification of the strain was performed for confirmation. Then, fermentations were carried out, according to a 2^3 full-factorial design (FFD) in order to evaluate the influence of substrates concentrations on production of biomass, total lipids and BE. The fungus was identified as *Absidia cylindrospora* var. *cylindrospora* and the results showed it as an oleaginous microorganism, since it accumulated lipids above 20 % of dry weight. The highest biomass production (11.38 g/L) was found in condition 3 of the FFD, while the highest lipid yield (32.22%) was reached in assay 6. Moreover, this strain was confirmed as a promising BE-producing microorganism, because of it achieved EI_{24} values above 50% in all conditions of the FFD. The highest EI_{24} with motor oil (93.3%) was obtained in condition 7 and this emulsion remained stable after 150 days of incubation. Statistical analysis showed that the three agro-industrial by-products had a significant influence on the production of biomass, lipids and BE, confirming the suitability of unconventional substrates for obtaining both microbial oil and emulsifier, which makes this bioprocess attractive for several industries.
Keywords: Oleaginous fungus; Emulsifier; Renewable substrates.

Resumo

Grandes quantidades de subprodutos e resíduos agroindustriais são gerados anualmente no Brasil. Frequentemente, eles são subutilizados e descartados de forma inadequada, causando poluição ambiental. Nesse contexto, reutilizá-los como matéria-prima para a obtenção de produtos de alto valor agregado, torna-se uma estratégia promissora. Assim,

este estudo teve como objetivo investigar o potencial biotecnológico do fungo Mucorales *Absidia cylindrospora* UCP 1301 para a produção de biomassa oleaginosa e bioemulsificante (BE), utilizando subprodutos agroindustriais como substratos de baixo custo. Primeiramente, foi realizada a identificação morfológica da cepa para confirmação. Em seguida, as fermentações foram realizadas, de acordo com um planejamento fatorial completo de 2³ para avaliar a influência das concentrações dos substratos na produção de biomassa, lipídios totais e BE. O fungo foi identificado como *Absidia cylindrospora* var. *cylindrospora* e os resultados mostraram-no como um microrganismo oleaginoso, pois acumulou lipídios acima de 20% do peso seco. A maior produção de biomassa (11,38 g/L) foi obtida na condição 3 do planejamento fatorial, enquanto o maior rendimento de lipídios (32,22%) foi alcançado no ensaio 6. Além disso, esta cepa foi confirmada como um micro-organismo promissor produtor de BE, pois alcançou valores de IE_{24} acima de 50% em todas as condições do planejamento. O maior IE_{24} com óleo de motor (93,3%) foi obtido na condição 7 e esta emulsão permaneceu estável após 150 dias de incubação. A análise estatística mostrou que os três subprodutos agroindustriais tiveram influência significativa na produção de biomassa, lipídeos e BE, confirmando a adequação de substratos não convencionais para obtenção tanto de óleo microbiano quanto de emulsificante, o que torna este bioprocesso atrativo para diversas indústrias.
Palavras-chave: Fungo oleaginoso; Emulsificante; Substratos renováveis.

Resumen
En Brasil se generan anualmente grandes cantidades de subproductos y residuos agroindustriales. A menudo, estos son subutilizados y desechados inadecuadamente, provocando contaminación ambiental. En este contexto, reutilizarlos como materia prima para obtener productos de alto valor agregado se convierte en una estrategia prometedora. Así, este estudio tuvo como objetivo investigar el potencial biotecnológico del hongo Mucorales *Absidia cylindrospora* UCP 1301 para la producción de biomasa oleaginosa y bioemulsionante (BE), utilizando subproductos agroindustriales como sustratos de bajo costo. En primer lugar, se realizó la identificación morfológica de la cepa para su confirmación. Luego, se realizaron fermentaciones, según un diseño factorial completo de 2³ para evaluar la influencia de las concentraciones de sustrato en la producción de biomasa, lípidos totales y BE. El hongo fue identificado como *Absidia cylindrospora* var. *cylindrospora* y los resultados la mostraron como un microorganismo oleaginoso, ya que acumuló lípidos por encima del 20% del peso seco. La mayor producción de biomasa (11,38 g/L) se encontró en la condición 3 del diseño factorial, mientras que el mayor rendimiento de lípidos (32,22 %) se alcanzó en el ensayo 6. Además, esta cepa se confirmó como un prometedor microorganismo productor de BE, debido a que alcanzó valores de IE_{24} superiores al 50 % en todas las condiciones de diseño factorial. El mayor IE_{24} con aceite de motor (93,3%) se obtuvo en la condición 7 y esta emulsión se mantuvo estable después de 150 días de incubación. El análisis estadístico mostró que los tres subproductos agroindustriales tuvieron una influencia significativa en la producción de biomasa, lípidos y BE, confirmando la idoneidad de sustratos no convencionales para la obtención tanto de aceite microbiano como de emulsificante, lo que hace que este bioproceso sea atractivo para varias industrias.
Palabras clave: Hongo oleaginoso; Emulsionante; Sustratos renovables.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) estimates that thousands of tons of agro-industrial by-products are generated in Brazil every year. However, most of them do not receive proper treatment, being disposed of incorrectly that causes environmental problems and harm to human and animal health (Ricardino, et al., 2020; Guedes, et al., 2021). An ecological alternative to solve this problem is the use of these underutilized compounds as low-cost substrates to obtain high-added value products, such as microbial lipids and emulsifiers (Lopes, et al., 2020; Santiago, et al., 2021).

In the last decades, research on the production of microbial lipids as alternative oil sources has been growing (Uthandi, et al., 2021; Vilas Bôas & Mendes, 2022). Recent studies indicated that the production of microbial oils by bacteria, yeasts and fungi becomes, in fact, an ecological and economically viable alternative, which can replace biodiesel, since these microorganisms can reach high levels of lipids, regardless of the climate, seasonality and high labor costs (Montero-Rodríguez, et al., 2016; Dvoretsky, et al., 2018; Carmona-Cabello, et al., 2021). Several microorganisms have the ability to accumulate more than 20% of lipids in their dry weight, and this value can reach up to 70% during the period of metabolic stress (Bao, et al., 2021; Fazili, et al., 2022). They are commonly called oleaginous microorganisms or *single cell oils* (SCOs), and have an important role in replacing vegetable biodiesel (Berikten & Hoşgün, 2022).

On the other hand, bioemulsifiers (BEs) are amphipathic compounds, high molecular weight, which have the properties of forming stable emulsions between two immiscible liquids with different degrees of polarity (Mendonça, et al., 2019; Morales-Guzmán, et al., 2021; Tavares, et al., 2021). Compared to synthetics ones, they have advantages for being biodegradable and less toxic, being biocompatible and stable to extreme values of pH, salinity and temperature (Tao, et al., 2019; Datta, et al., 2022). In addition, they can be applied in the petrochemical, agricultural and food industries, as dispersants, stabilizers, foams, among others (Adetunji & Olaniran, 2021; Markande, et al., 2021). BEs are produced mainly by bacteria and yeasts; however, studies with the production of BEs by filamentous fungi are still scarce (Rulli, et al., 2018; Marques, et al., 2020). This microbial group have high biotechnological potential for BE production, due to its ability to adapt to different environmental conditions, due to variations in their physiological, biochemical and genetic activities (Silva, et al., 2018).

Although microbial lipids and BEs are promising alternatives for several industrial applications, large-scale production of them is still limited, due to the high production costs and the expensive conventional growth media. In this sense, agro-industrial by-products and waste become an alternative to minimize production costs, making the process less costly and ensuring more profitable production (Prabha, et al., 2019; Saranraij, et al., 2022). Hence, this study aimed to investigate the potential of the Mucoralean fungus *Absidia* sp. UCP 1301 for lipids and BE production, using agro-industrial by-products as unconventional substrates, as a promising alternative to improve large-scale bioprocess.

2. Methodology

2.1 Microorganism

The microorganism used in this study was the Mucoralean fungus *Absidia cylindrospora* UCP 1301, previously isolated from the soil of the Caatinga, Serra Talhada - PE, Brazil. The strain was kindly provided by the Cultures Collection UCP (Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco), registered in the World Federation for Culture Collections (WFCC) under the number 927. The filamentous fungus was maintained at 5°C on Sabouraud Dextrose Agar (SDA; 40 g dextrose, 10 g peptone, 15 g agar in 1000 ml of distilled water).

2.2 Confirmation of morphological identification of *Absidia cylindrospora* UCP 1301

A. cylindrospora UCP 1301 was grown in triplicate on Malt Extract Agar (MEA: malt extract 20 g, peptone 5 g and agar 20 g, in 1000 ml of distilled water) and Potato Dextrose Agar (PDA: peeled potato 200 g, dextrose 20 g and agar 20 g, in 1000 ml of distilled water) and incubated at 15, 20, 25, 28, 30 and 35°C for 5 days (Hoffmann, et al., 2007; Hofmann, 2010). After this time, the growth of the strain at different temperatures was verified, as well as the color and aspect of the colonies. For micromorphological identification, cultures were performed on coverslips for better conservation of reproductive structures. Coverslips were removed from the cultures and a drop of lactophenol blue dye or sterile water was added to a slide and observed under an optical microscope. The morphological identification of the strain was performed according to the description key of the genus *Absidia* described by Hoffman (2010), in order to identify the variety.

2.3 Production of total lipids and BE using agro-industrial by-products

2.3.1 Inoculum preparation

Spores of *A. cylindrospora* UCP 1301, previously grown on SDA for 96 h at 28 °C, were transferred to an Erlenmeyer flask containing sterile distilled water, until obtaining a spore suspension of 10^7 spores/ml, which was used as inoculum in the production media.

2.3.2 Agro-industrial by-products

The agro-industrial by-products used as alternative substrates were crude glycerol (CG), a by-product of the production of biodiesel from cottonseed oil (CETENE-PE, MCT, Brazil); corn steep liquor (CSL), from the corn processing industry Corn Products (Cabo de Santo Agostinho-PE, Brazil) and whey, from a local dairy industry, from the processing of cheese.

2.3.3 Production of total lipids and bioemulsifier

Fermentations were carried out in 250 ml Erlenmeyer flasks containing 100 ml of production medium consisting of saline solution (0.2 g KH_2PO_4, 0.2 g $MgSO_4.7H_2O$, in 1000 ml distilled water), added with 1% trace elements solution ($FeSO_4 \cdot 7H_2O$ 0.63 mg, $MnSO_4$ 0.01 mg, $ZnSO_4$ 0.62 mg, distilled water 100 ml) (Pele, et al., 2019). The agro-industrial by-products (CG, CSL and whey) were added at varying concentrations, according to a full-factorial design (FFD) (topic 2.3.4). The production media in the flasks were adjusted to pH 5.5±0.05 and sterilized by autoclaving for 15 min at 121 °C. Then, they were inoculated at 5% and incubated under orbital rotation (150 rpm) for 96 h at 28 °C. After fermentation, the cultures were subjected to filtration and subsequent centrifugation (10.000 *g* for 15 min) and the biomass and the cell-free metabolic liquids were used for analyses described after.

2.3.4 Full-factorial design (FFD)

A 2^3 FFD was carried out in order to analyze the effects and interactions between the agro-industrial by-products (CG, CSL and whey) in biomass, lipids and BE production (Table 1), using yield of biomass and total lipids, and emulsification index (EI_{24}) as response variables. A set of 8 assays with 3 replicates in the central point were carried out. Statistical analysis of the data obtained in the experiments was performed using the STATISTICA software package version 12.0 (StatSoft Inc., Tulsa, OK, USA), and the significance of the results was tested at $p \leq 0.05$.

Table 1 - Levels and variables in 2^3 full-factorial design for the production of lipids and bioemulsifier by *Absidia cylindrospora* UCP 1301.

Variables	Levels		
	-1	**0**	**+1**
Crude glycerol (%, *v/v*)	2	2.5	3
Corn steep liquor (%, *v/v*)	3	4	5
Whey (%, *v/v*)	0	2	4

Source: Authors.

2.3.5 Biomass yield

The biomass obtained after filtration and centrifugation was washed three times with distilled water and subjected to lyophilization (Advantage Plus EL-85 lyophilizer, SP Scientific, USA). After that, it was kept in a desiccator until constant weight, determined by gravimetry, and the biomass yield was expressed in g/L.

2.3.6. Total lipids extraction and lipids yield

The lipids content of biomass was determinate by successive extractions steps with methanol:chloroform at different proportions (1:2, 1:1, 2:1; *v/v*) (Manocha, et al., 1980). The extracts were stirring for 5 min and the biomass was submitted to a new extraction. Yield of total lipids present in the biomass was quantified by gravimetric method and the results was expressed as percentage of the biomass weight using equation 1.

Total lipids (%) = (lipids/dry biomass) x 100 (Equation 1)

2.3.7 Determination of emulsification index (EI_{24})

The EI_{24} was determined according to the methodology described by Cooper & Goldenberg (1987), using the cell-free metabolic liquids from the assays of the FFD and motor oil as hydrophobic compound. The condition with best result was used to determine the EI_{24} with castor oil and burned motor oil. The EI_{24} was determined by the equation 2:

Emulsification index (EI_{24}) = (Height of emulsion layer/Total height of mixture) x 100 (Equation 2)

3. Results and Discussion

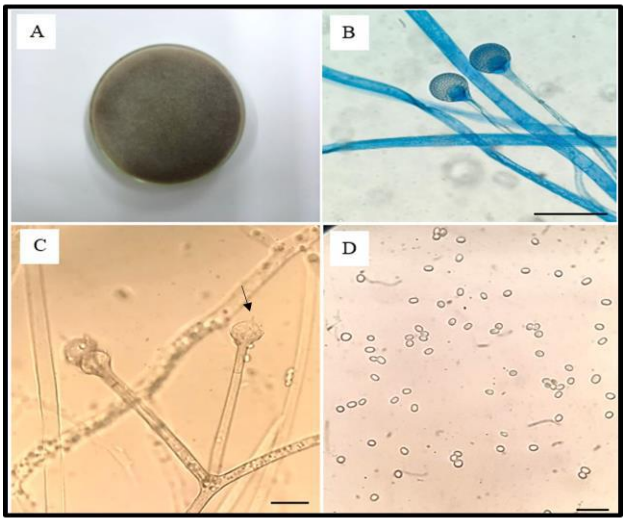
3.1 Morphological identification of *Absidia* sp. UCP 1301

Etymology: *cylindrospora*. Reference to sporangiospores with a slightly wide cylindrical shape (Hoffmann, 2010).

Diagnosis: it differs from *Absidia pseudocylindrospora* for not exceeding 4 whorls and presenting a rounded and sometimes bulbous projection that reaches 4.5 µm (Hoffmann, 2010).

Description: colony initially white, turning gray with age on PDA medium. Slow growth at 15, 20 and 25 °C. Rapid growth colonizing the entire Petri dish (9 cm in diameter) in five days at 28 °C (Figure 1A). Brownish-gray, apophyseal, and piriform sporangia, up to 35 µm in diameter, multispored and smooth-walled (Figure 1B). Sporangiophores slightly brownish, branched, single or with up to 4 whorls (Figure 1C). A septum was observed near the apophysis (Figure 1C). Hemispherical columella; projection in the rounded and sometimes bulbous columella (Figure 1C). Collar usually evident. Cylindrical and smooth sporangiospores slightly wide reaching up to 5.5 x 3.0 µm (Figure 1D). Chlamydospores not evident.

Figure 1 – *Absidia cylindrospora* var. *cylindrospora* UCP 1301. A. Colony surface in PDA at 28°C. B. Sporangiophore with fertile sporangia. C. Branched sporangiophore with piriform columella and projections (arrow)). D. Sporangiospores. Scale bars: **B**:5 0 µm; **C**: 30 µm; **D**: 10 µm.



Source: Authors.

The species *Absidia cylindrospora* has already been recorded in semi-arid soils (De Souza, et al., 2013; Santiago, et al., 2013). The genus *Absidia* is a mesophilic group, presenting an optimal growth range of 25-34 °C, not exceeding 37 ˚C, differentiating itself from the genus *Lichtheimia*, a thermotolerant group (Hoffmann, et al., 2007; Hofmann, 2010). Morphologically, the shape of the sporangiospores is one of the characteristics that differentiate the species of the genus

Absidia, groups that form globose, conical and cylindrical spores. *A. cylindrospora*, *A. anomala*, *A. pseudocylindrospora*, *A. repens*, *A. psychrophilia* and *A. spinosa* develop cylindrical sporangiospores. However, spore sizes vary and are not useful for differentiating between species (Hoffmann, et al., 2007; Hofmann, 2010). Sporangiophore length, shape of sporangia, presence of septation in rhizoids and number of veticilies are morphological characters that differentiate *Absidia* species with cylindrical and elliptical spores. Projections are essential in differentiating from another genus. However, with the discovery of new species of *Absidia* without projections, a character that must be revised (Zhao, et al., 2022).

3.2 Growth and lipids production by *Absidia cylindrospora* var. *cylindrospora* UCP 1301 using agro-industrial by-products

Efficient growth and accumulation of lipids by an oleaginous microorganism depends, among other factors, on the substrates used as sources of carbon and nitrogen in the culture medium (Lu, et al., 2022; Martinez-Silveira, et al., 2022). In this context, three agro-industrial by-products (CG, CSL and whey) were used in this study as low-cost substrates for the production of biomass and lipids by *A. cylindrospora* var. *cylindrospora* UCP 1301. According to the results showed in Table 2, biomass production was evidenced in all conditions of FFD, standing out the yield obtained in condition 3 (11.38 g/L) in medium containing 2% CG and 5% CSL. This result was higher than those obtained previously for other Mucorales fungi (Table 3) and confirms the suitability of CG and CSL as excellent sources of carbon and nitrogen for the growth of microorganisms (Pele, et al., 2019; Mendonça, et al., 2021).

Table 2 – Results of the 2^3 full-factorial design applied to the production of lipids and bioemulsifier by *Absidia cylindrospora* var. *cylindrospora* UCP 1301, using agro-industrial by-products.

Conditions	CG (%)	CSL (%)	Whey (%)	Biomass (g/L)	Total lipids (%)	Emulsification index (%)
1	2	3	0	4.25	18.35	82.0
2	3	3	0	4.88	23.15	77.4
3	2	5	0	11.38	25.18	83.4
4	3	5	0	6.10	30.78	92.9
5	2	3	4	5.81	20.58	78.3
6	3	3	4	7.59	32.22	89.5
7	2	5	4	5.79	22.92	93.3
8	3	5	4	5.72	19.55	91.6
9	2.5	4	2	5.32	24.80	78.1
10	2.5	4	2	5.14	22.95	78.3
11	2.5	4	2	5.95	23.90	78.3

Source: Authors.

Regarding the production of lipids, the accumulation above 20% was found in most of the FFD assays, indicating that *A. cylindrospora* var. *cylindrospora* UCP 1301 is an oleaginous microorganism. Greater production (32.22%) was achieved in condition 6, in medium composed by 3% CG, 3% CSL and 4% whey. The accumulation of lipids by Mucoralean fungi in agro-based media have been related by several researchers (Table 3), ratifying the high biotechnological potential of these microorganisms.

Table 3 - Comparison of production of biomass and total lipids by *Absidia cylindrospora* var. *cylindrospora* UCP 1301 with the results obtained by other Mucorales fungi in agro-industrial substrates-based media.

Microorganism	Agro-industrial substrates	Biomass (g/L)	Total lipids (%)	References
Cunninghamella echinulata ATHUM 4411	Waste glycerol	3.9	25.6	Moustogianni, et al. (2015)
Mortierella isabelina ATHUM 2935		2.9	11.5	
M. ramanniana MUCL 9235		4.9	32.9	
Mucor sp. LGAM 365		1.7	15.6	
C. echinulata UCP/WFCC 1299	Sugarcane molasses and CSL	10.1	47.86	Lima, et al. (2017)
M. circinelloides URM 4182	Sugarcane molasses	11.2	29.0	Bento, et al. (2020)
C. phaeospora UCP 1303	Cashew apple juice and cheese whey	10.15	-	Berger, et al. (2020)
C. elegans UCP 1306		13.06	-	
Lichtheimia hyalospora UCP 1266	CSL and cassava wastewater	11.87	- -	Souza, et al. (2020)
A. cylindrospora var. *cylindrospora* UCP 1301	CG, CSL and whey	11.38	32.22	Present study

Source: Authors.

3.3 Production of bioemulsifier by *Absidia cylindrospora* var. *cylindrospora* UCP 1301 using agro-industrial by-products

The determination of the EI_{24} has been used as a parameter for the identification of high molecular weight surfactants that have ability to stabilize emulsions between liquids with different degrees of polarity (Souza, et al., 2016; Rahman, et al., 2019). This property is commonly evaluated by its ability to maintain at least 50% of the original emulsion volume after 24 hours after its formation (Pele, et al., 2018; Nogueira, et al., 2020). In this context, *A. cylindrospora* var. *cylindrospora* UCP 1301 showed high potential to produce BE using the agro-industrial by-products in all conditions of the FFD; however, the highest value of EI_{24} (93.3%) was achieved in condition 7 (Table 2). This result was similar to those previously reported by other Mucoralean fungi (Table 4); however, to our knowledge, this is the first study reporting the BE production by an *A. cylindrospora* strain.

Table 4 - Comparison of emulsification index obtained by *A. cylindrospora* var. *cylindrospora* UCP 1301 in this study with the results of other Mucoralean fungi cultivated in agro-industrial substrates.

Microorganism	Agro-industrial substrates	EI_{24} (%)	References
Cunninghamella echinulata UCP 1299	Instant noodle waste, CSL and postfrying oil	81.4	Andrade, et al. (2018)
Rhizopus microsporus var. *chinensis* UCP 1296	Post-frying oil	91.7	Pele, et al. (2018)
R. microsporus var. *microsporus* UCP 1304		94.8	
R. arrhizus UCP 1607		82.6	
Absidia sp. UCP 1144	CG, CSL and whey	79.17	Mendonça, et al. (2019)
Mucor hiemalis UCP 0039	Post-frying soybean oil	96.0	Ferreira, et al. (2020)
M. circinelloides UCP 0001	CSL and soybean oil	100	Marques, et al. (2020)
M. circinelloides UCP 0005	Jatobá (*Hymenaea stilbocarpa*) husks and CSL	94	Santiago, et al. (2021)
C. elegans UCP 0542	Instant noodle waste, CSL and soybean post-frying oil	100	Medeiros, et al. (2022)
A. cylindrospora var. *cylindrospora* UCP 1301	CG, CSL and whey	93.3	Present study

Source: Authors.

In addition, emulsion formed from condition 7 of the FFD was monitored up to 150 days at room temperature, showing high stability (90.1%) (Figure 2). The ability to form stable emulsions is one of the most important characteristics to be considered in a biological surface-active compound, and suggest its application in formulation of detergents, cosmetics, and food products (Rulli, et al., 2019). Moreover, BE produced in this condition showed good emulsifying properties against castor oil and burned motor oil, with EI_{24} of 57.3% and 53.8%, respectively. These results show that *A. cylindrospora* var. *cylindrospora* UCP 1301 produced a BE capable of emulsifying several hydrophobic compounds and keeping these emulsions stable above 50%.

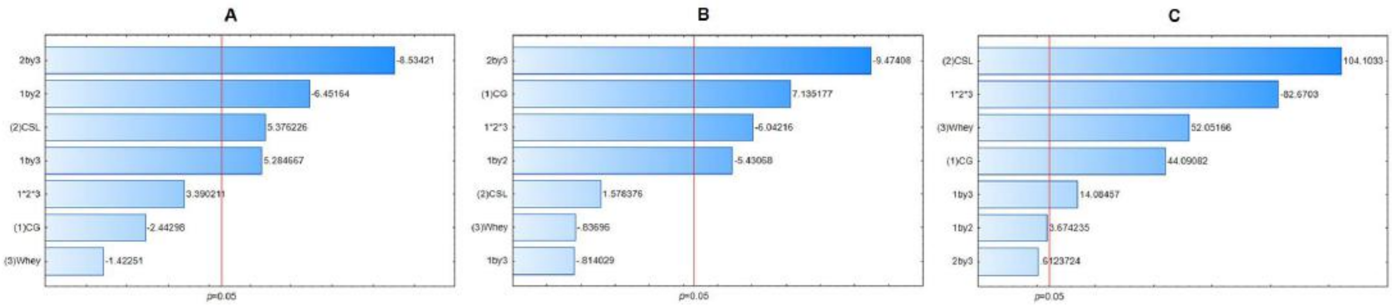
Figure 2 – Production of bioemulsifier by *A. cylindrospora* var. *cylindrospora* UCP 1301 using agro-industrial by-products. Emulsion obtained from condition 7 of FFD after 24 h (A) and 150 days (B).

Source: Authors.

3.4 Influence of agro-industrial by-products on growth and lipids and bioemulsifier production.

The reuse of agro-industrial waste and by-products as raw material to obtain value-added products aims to ensure the principles of the circular economy required for the sustainability of any bioprocess (Kapoor, et al., 2020). In this context, this study focused into the valorization of three agro-industrial by-products as alternative sources for production of oleaginous biomass and BE by *A. cylindrospora* var. *cylindrospora* UCP 1301. The influence of CG, CSL and whey was investigated by application of 2^3 FFD and statistical analysis, and the Pareto charts obtained are represented in Figure 3.

Figure 3 - Pareto chart of the standardized effects of crude glycerol (1), corn steep liquor (2) and whey (3), and their interactions on production of biomass (A), lipids (B) and bioemulsifier (C) by *Absidia cylindrospora* var. *cylindrospora* UCP 1301. The point at which the effect estimates were statistically significant (p = 0.05) is indicated by dashed lines.



Source: Authors.

According to Pareto chart in Figure 3A, CSL and the interaction of CG and whey demonstrated positive effects on biomass production, indicating them as promising sources of carbon and nitrogen that favored the growth of this strain. However, only CG showed positive influence on lipids production (Figure 3B), *i.e.*, an increase in the concentration of CG in the production medium induced a greater accumulation of lipids in the fungal biomass. Previously, several researches reported CG as suitable substrate for production of microbial lipids by Mucoralean fungi (Moustogianni, et al., 2015; Papanikolau, et al., 2016; Siramon, et al., 2016; Mendonça, et al., 2021).

Regarding to BE production, the statistical analysis showed that the three by-products and the interaction between CG and whey had positive effects on the EI_{24}, from a statistical point of view (Figure 3C). This means that an increase in the concentrations of these alternative substrates led to higher EI_{24}, suggesting the production of BE in the culture medium.

Therefore, the results obtained here verified the suitability of CG, CSL and whey as unconventional substrates for the production of biomass and accumulation of lipids, as well as in the obtention of emulsifying compound. The valorization of agro-industrial by-products as renewable feedstocks becomes a promising strategy, since the feasibility of the large-scale production of these microbial metabolites depends on greater economic development.

4. Conclusion

The present study showed the biotechnological potential of *A. cylindrospora* var. *cylindrospora* UCP 1301 in the production of lipids and bioemulsifier, with high industrial and environmental applicability. In addition, the use of agro-industrial by-products as low-cost substrates was verified as a sustainable alternative for the cultivation and growth of the species, which may be economically viable for biotechnological processes.

Acknowledgments

The authors are grateful for the financial support of FACEPE (APQ.0291-2.12/15), CAPES and CNPq (Process nº 314422/2018-8).

References

Adetunji, A. I., & Olaniran, A. O. (2021). Production and potential biotechnological applications of microbial surfactants: An overview. Saudi Journal of *Biological Sciences*. https://doi.org/10.1016/j.sjbs.2020.10.058

Alphy, M. P., Anjali, K. B., Vivek, N., Thirumalesh, B. V., Sindhu, R., Pugazhendi, A., Pandey, & Binod, P. (2021). Sweet sorghum juice as an alternative carbon source and adaptive evolution of *Lactobacillus brevis* NIE9.3.3 in sweet sorghum juice and biodiesel derived crude glycerol to improve 1, 3 propanediol production. *Journal of Environmental Chemical Engineering*. https://doi.org/10.1016/j.jece.2021.106086

Andrade, R. F. S., Silva, T. A. L.; Ribeaux, D. R., Rodriguez, D. M., Souza, A. F., Lima, M. A. B., Lima, R. A., Silva, C. A. A., & Campos-Takaki, G. M. (2018). Promising biosurfactant produced by *Cunninghamella echinulata* UCP 1299 Using renewable resources and Its application in cotton fabric cleaning process. *Advances in Materials Science and Engineering*. https://doi.org/10.1155/2018/1624573

Bao, W., Li, Z., Wang, X., Gao, R., Zhou, X., Cheng, S., Men, Y., & Zheng, L. (2021). Approaches to improve the lipid synthesis of oleaginous yeast *Yarrowia lipolytica*: A review. *Renewable and Sustainable Energy Reviews*. https://doi.org/10.1016/j.rser.2021.111386

Bento, H. B. S., Carvalho, A. K. F., Reis, C. E. R., & Castro, H. F. (2020). Single cell oil production and modification for fuel and food applications: Assessing the potential of sugarcane molasses as culture medium for filamentous fungus. *Industrial Crops and Products*. https://doi.org/10.1016/j.indcrop.2020.112141

Berikten, D., & Hoşgün, E. Z. (2022). Ksiloz ve gliserol ortamlarinda çeşitli mayalarin lipit üretim kapasitelerinin belirlenmesi. *Journal of the Institute of Science and Technology*. https://doi.org/10.21597/jist.1034410

Cândido, T. R. S., Mendonça, R. S., Lins, U. M. B. L., Souza, A. F., Rodriguez, D. M., Campos-Takaki, G. M., & Andrade, R. F. S. (2022). Production of biosurfactants by Mucoralean fungi isolated from Caatinga bioma soil using industrial waste as renewable substrates. *Research, Society and Development*. https://doi.org/10.33448/rsd-v11i2.25332

Carmona-Cabello, M., García I. L., Papadaki, A., Tsouko, E., Koutinas, A., & Dorado, M. P. (2021). Biodiesel production using microbial lipids derived from food waste discarded by catering services. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2020.124597

Carvalho, A. K., Bento, H. B. S., Rivaldi, J. D., & de Castro, H. F. (2018). Direct transesterification of *Mucor circinelloides* biomass for biodiesel production: Effect of carbon sources on the accumulation of fungal lipids and biofuel properties. *Fuel*. https://doi.org/10.1016/j.fuel.2018.07.029

Chotard, M., Mounier, J., Meye, R. Padel, C., Claude, B., Nehmé, R., da Silva, D., Floch, S. L., & Lucchesi, M.-E. (2022). Biosurfactant-Producing Mucor Strains: Selection, Screening, and Chemical Characterization. *Applied Microbiology*. https://doi.org/10.3390/applmicrobiol2010018

Cooper, D. G., & Goldenberg, B. G. (1987). Surface-Active Agents from Two *Bacilllus* Species. *Applied And Environmental Microbiology*. https://doi.org/10.1128/aem.53.2.224-229.1987

Cordeiro, T. R. L., Nguyen, T. T. T., Lima, D. X. da Silva, S. B. G., Lima, C. F., Leitão, J. D. A., Gurgel, L. M. S., Lee, H. B., & Santiago, A. L. C. M. A. (2020). Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest. *Acta Botanica Brasilica*. https://doi.org/10.1590/0102-33062020abb0040

Datta, P., Pannu, S., Tiwari, P., & Pandey, L. (2022). Core Flooding Studies Using Microbial Systems. *Microbial Enhanced Oil Recovery*. https://doi.org/10.1007/978-981-16-5465-7_10 de Souza, C. A. F., Costa, C. M. C., Maia, L. C., & Santiago, A. L. C. M. A. (2013). Mucorales (Mucoromycotina). Parque Estadual Mata da Pimenteira: Riqueza Natural e Conservação da Caatinga, 51-63.

Dvoretsky, D. Dvoretsky, S., Temnov, M. Markin, I., Bushkovskaya, A., Golubyatnikov, O., & Ustinskaya, Y. (2018). Technology of using municipal wastewater for obtaining *Chlorella vulgarisviomass* with high lipid content for biofuel production. *Chemical Engineering Transactions.* https://doi.org/10.3303/CET1864082

Fazili, A. B. A., Shah, A. M., Zan, X. Naz, T., Nosheen, S., Nazir, Y., Ullah, S., Zhang, H., & Song, Y. (2022). *Mucor circinelloides*: a model organism for oleaginous fungi and its potential applications in bioactive lipid production. *Microbial Cell Factories*. https://doi.org/10.1186/s12934-022-01758-9

Ferreira, I. N. S., Rodríguez, D. M., Campos-Takaki, G. M. & Andrade, R. F. S. (2020). Biosurfactant and bioemulsifier as promising molecules produced by *Mucor hiemalis* isolated from Caatinga soil. *Eletronic Journal of Biotechnology*. https://doi.org/10.1016/j.ejbt.2020.06.006

Guedes, E. H. S., Santos, A. L., Ibiapina, A., Aguiar, A. O., Soares, C. M. S., Vellano, P. O., Santos, L. S. S., & Junior, A. F. C. (2021). Resíduos agroindustriais como substrato para a produção de lipases microbiana: uma revisão. *Research, Society and Development*. http://dx.doi.org/10.33448/rsd-v10i2.12537

Hashem, A. H., Abu-Elreesh, G., El-Sheikh, H., & Suleiman, W. B. (2022). Isolation, identification, and statistical optimization of a psychrotolerant *Mucor racemosus* for sustainable lipid production. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-022-02390-8

Hoffmann, K. (2010). Identification of the genus *Absidia* (Mucorales, Zygomycetes): a comprehensive taxonomic revision. In: Molecular identification of fungi. [s.l.] *Springer*. https://doi.org/10.1007/978-3-642-05042-8_19

Hoffmann, K., Discher, S., & Voigt, K. (2007). Revision of the genus *Absidia* (Mucorales, Zygomycetes) based on physiological, phylogenetic, and morphological characters; thermotolerant *Absidia* spp. form a coherent group, Mycocladiaceae fam. nov. *Mycological research*, 111(10), 1169-1183.

Kapoor, R., Ghosh, P., Kumar, M., Sengupta, S., Gupta, A., Kumar, S. S., & Pant, D. (2020). Valorization of agricultural waste for biogas based circular economy in India: a research outlook. *Bioresource Technology*, *304*, 123036.

Kong, T. K., Zhao, H. ; Liu, X. L., & Ren, L. Y. (2021). Taxonomy and phylogeny of the *Absidia* (Cunninghamellaceae, Mucorales) introducing nine new species and two new combinations from China. Research Square. https://doi.org/10.21203/rs.3.rs-820672/v1

Lima, M. C., Silva, T. C. D. M., Souza, A. F., Luna, M. A. C., Andrade, R. F. S., Silva, C. A. A., & Okada, K. (2017). Produção simultânea de biomassa e lipídeos utilizando meios contendo resíduos agroindustriais por *Mucor subtilíssimus* UCP/WFCC 1262, *Cunninghamella echinulata* UCP/WFCC 1299 e *Rhizopus microsporus* UCP/WFCC 1304 isolados do solo da Caatinga de Pernambuco, Engevista, v. 19(5), pp. 1417-1430.

Linas, A. B., Bione, A. P., Fonseca, T. C. S., Silva, T. C., Silva, P. H., Morante, K. V., Andrade, R. F. S., & Campos-Takaki, G. M. (2017). Biosurfactant production by *Cunninghamella phaeosphora* UCP 1303 using controlled temperature through of Arduino. International Journal of Current Microbiology and Applied Sciences, 6(12), pp. 2708-2715. https://doi.org/10.20546/ijcmas.2017.612.314

Lu, H., Yadav, V., Zhong, M., Bilal, M., Taherzadeh, M., & Iqbal, H. M. N. (2022). Bioengineered microbial platforms for biomass-derived biofuel production - A review. Chemosphere, 288(Pt 2). https://doi.org/10.1016/j.chemosphere.2021.132528

Manocha, M. S. (1980). Lipid composition of *Paracoccidioides brasiliensis*: comparison between the yeast and mycelial forms. *International Society for Human and Animal Mycology*. https://doi.org/10.1080/00362178085380481

Markande, A. R., Patel, D., & Varjani, S. (2021). A review on biosurfactants: properties, applications and current developments. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2021.124963

Marques, N. S. A. A., Silva, I. G. S., Cavalcanti, D. L., Maia, P. C. S. V., Santos, V. P., Andrade, R. S. F., & Campos-Takaki, G. M. (2020). Eco-friendly bioemulsifier production by *Mucor circinelloides* UCP0001 isolated from mangrove sediments using renewable substrates for environmental applications. *Biomolecules*, 10(3), pp. 365. https://doi.org/10.3390/biom10030365

Marques, N. S. A. A., Silva, T. A. L., Andrade, R. F. S., Júnior, F. B., Okada, & K., Campos-Takaki, G. M. (2019). Lipopeptide biosurfactant produced by *Mucor circinelloides* UCP/WFCC 0001 applied in the removal of crude oil and engine oil from soil. *Acta Scientiarum. Technology*, 41(1), pp. e38986. https://doi.org/10.4025/actascitechnol.v41i1.38986

Martinez-Silveira, A., Garmendia, G., Rufo, & Vero, S. (2022). Production of microbial oils by the oleaginous yeast *Rhodotorula graminis* S1/2R in a medium based on agro-industrial by-products. *World Journal of Microbiology and Biotechnology*, 38(46). https://doi.org/10.1007/s11274-022-03236-1

Medeiros, A. D. M., Silva Junior, C. J. G., Souza, A. F., Cavalcanti, D. L., Rodriguez, D. M., Silva, C. A. A., & Andrade, R. F. S. (2022). Production of biosurfactant by *Cunninghamella elegans* UCP 0542 using food industry waste in 3 L flasks and evaluation of orbital agitation effect. *Research, Society and Development*, v. 11(4), pp. e50311427438. https://doi.org/10.33448/rsd-v11i4.27438

Mendonça, R. S., Sá, A. V. P., Rosendo, L. A., Santos, R. A., Marques, N. S. A. A., Souza, A. F., Rodríguez, D. M., & Campos-Takaki, G. M. (2021). Production of biosurfactant and lipids by a novel strain of *Absidia cylindrospora* UCP 1301 isolated from Caatinga soil using low-cost agro-industrial by-products. *Brazilian Journal of Development*, v.7(1). pp. 8300-8313. https://doi.org/10.34117/bjdv7n1-564

Mendonça. R. S., Alves, M. F., Souza, A. F., D. M., & Campos-Takaki, G. M. (2019). Bioemulsifier produced by a promising fungus *Absidia* sp. UCP 1144 isolated from Caatinga soil in the northeast of Brazil. *Brazilian Journal of Development*, v. 5(11), pp. 25402 – 25414. https://doi.org/10.34117/bjdv5n11-204

Montero-Rodríguez, D., Andrade, R. F. S., Lima, R. A., Silva, G. K. B., Ribeaux, D. R., Silva, T. A. L., Araújo, H. W. C., & Campos-Takaki, G. M. (2016). Conversion of agro-industrial wastes by *Serratia marcescens* UCP/WFCC 1549 into lipids suitable for biodiesel production. *Chemical Engineering Transactions*, 49:307-312. https://doi.org/10.3303/CET1649052

Morales-Guzmán, D., Martínez-Morales, F., Bertrand B., Rosas-Gálvan, N. S., Curiel-Maciel, N. F., Teymennet-Ramírez, K. V., Mazón-Román, L. E., Licea-Navarro, A. F., & Trejo-Hernández, M. R. (2021). Microbial prospection of communities that produce biosurfactants from the water column and sediments of the Gulf of Mexico. *Biotechnology and Applied Biochemistry*, v. 68(6), pp. 1202-1215. https://doi.org/10.1002/bab.2042

Moustogianni, A., Bellou, S., Triantaphyllidou, I. E., & Aggelis, G. (2015). Feasibility of raw glycerol conversion into single cell oil by zygomycetes under non-aseptic conditions. *Biotechnology and bioengineering*. https://doi.org/10.1002/bit.25482

Nogueira, I. B., Rodríguez, D. M., da Silva Andradade, R. F., Lins, A. B., Bione, A. P., Silva, I. G. S., Franco, L. O., & Campos-Takaki, G. M. (2020). Bioconversion of agroindustrial waste in the production of bioemulsifier by *Stenotrophomonas maltophilia* UCP 1601 and application in bioremediation process. *International Journal of Chemical Engineering*. https://doi.org/10.1155/2020/9434059

P. Siramon, V. Punsuvon, & A. Riengsilchai. Optimization of lipid production by *Mortierella isabellina* using glycerol, a by-product of biodiesel production as a carbon source. *Journal of Pure and Applied Microbiology*, 10 (2016), pp. 865-871.

Pele, M. A., Rubio-Ribeaux, D., Vieira E. R., Souza, A. F., Luna, M. A. C., Rodríguez, D. M., Andrade, R. F. S., Alviano, D. S., Alviano, C. S., Barreto-Bergter, E., Santiago, A. L. C. M. A., & Campos-Takaki, G. M. (2019). Conversion of renewable substrates for biosurfactant production by *Rhizopus arrhizus* UCP 1607 and enhancing the removal of diesel oil from marine soil. *Electronic Journal of Biotechnology*, v. 38, pp. 40-48. https://doi.org/10.1016/j.ejbt.2018.12.003

Pele, M. A., Montero-Rodríguez, D., Rubio-Ribeaux, D., Souza, A. F., Luna, M. A. C., Santiago, M. F., Andrade, R. S. F., Silva, T. A. L., Santiago, A. L. C. M. A., & Campos-Takaki, G. M. (2018). Development and improved selected markers to biosurfactant and bioemulsifier production by *Rhizopus* strains isolated from Caatinga soil. *African Journal of Biotechnology*., 17(6), pp. 150-157. https://doi.org/10.5897/AJB2017.16230

Prabha, S., Verma, G., Pnadey, S., Singh, B., & Dwivedi, V. (2019). Utilization of Agro-industrial By-products for Production of Lipase Using Mix Culture Batch Process. *Bioscience Biotechnology Research Communications*, 12(3), pp. 748-756. http://dx.doi.org/10.21786/bbrc/12.3/30

Rahman, P. K., Mayat, A., Harvey, J. G. H., Randhawa, K.S., Relph, L.E., Armstrong, M.C. (2019). Biosurfactants and Bioemulsifiers from Marine Algae. *In The Role of Microalgae in Wastewater Treatment*, pp. 169-188. https://doi.org/10.1007/978-981-13-1586-2_13

Ricardino, I. E. F., Souza, M. N. C., & NETO, I. F. S. (2020). vantagens e possibilidades do reaproveitamento de resíduos agroindustriais. *Alimentos: Ciência, Tecnologia e Meio Ambiente*.

Ruli, M. M., Alvarez, A., Fuentes, M. S. & Colin, V. L. (2019). Production of a microbial emulsifier with biotechnological potential for environmental applications. *Colloids and Surfaces B: Biointerfaces*. https://doi.org/10.1016/j.colsurfb.2018.11.052

S. Papanikolaou, M. Rontou, A. Belka, M. Athenaki, C. Gardeli, A. Mallouchos, et al. Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. *Engineering in Life Sciences*, 17 (2016), pp. 262-281

Santiago, A. L. C. M. D. A., Santos, P. J. P. D., & Maia, L. C. (2013). Mucorales from the semiarid of Pernambuco, Brazil. *Brazilian Journal of Microbiology*, 44(1), 299-305.

Santiago, M. G., Lins, U. M. B. L., Campos-Takaki, G. M., Filho, L. O. C., & Andrade, R. F. S. (2021) Produção de biossurfactante por Mucor circinelloides UCP 0005 usando novo meio de cultura formulado com cascas de jatobá (*Hymenaea courbaril L*.) e milhocina. *Brazilian Journal of Development*.

Santiago, M. G., Lins, U. M. B. L., Campos-Takaki, G. M. Filho, L. O. C., & Silva, R. F. S. (2021). Biosurfactant production by *Mucor circinelloides* UCP 0005 using new culture medium formulated with jatoba (*hymenaea courbaril*) bark and corn steep liquor. *Brazilian Journal of Development*, v. 7(5), pp. 51292 – 51304. https://doi.org/10.34117/bjdv7n5-497

Saranraij, P., Sivasakthivelan, P., Hamzah, K., & Hasan, M. S. (2022). Microbial Fermentation Technology for Biosurfactants Production. n book: *Microbial Surfactants* pp. 25 -43. https://doi.org/10.1201/9781003247739-2.

Silva, A. A. D., Oliveira, J. M., & Cazetaa, M. L. (2022). Exopolysaccharyde production by *Cryptococcus laurentii* SD7 using molasses and corn steep liquor as substrates. *Acta Scientiarum. Biological Sciences*. https://doi.org/10.4025/actascibiolsci.v44i1.58543

Silva, A. C. S., Santos, P. N., Silva, T. A. L., Aandrade, R. F. S., & Campos-Takaki, G. M. (2018). Biosurfactant production by fungi as a sustainable alternative. *Arquivos Do Instituto Biológico*. https://doi.org/10.1590/1808-1657000502017

Silva, N. R. A. ; Luna, M. A. C. ; Santiago, A. L. C. M. A., Franco, L. O., Silva, G. K. B., Souza, P. M., Okada, K., Albuquerque, C. D. C., Silva, C. A. A., & Campos-Takaki, G. M. (2014). Biosurfactant-and-Bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from Caatinga soil in the northeast of Brazil. *International Journal of Molecular Sciences*, v. 15(9), pp. 15377 –15395. https://doi.org/10.3390/ijms150915377

Son, J., Baritugo, K. A., Lim, S. H., Lim, H. J., Jeong, S., Lee, J. Y., Cheoi, J., Joo, J. C., Na, J. G. & Park, S. J. (2022). Microbial cell factories for the production of three-carbon backbone organic acids from agro-industrial wastes. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2022.126797

Souza, A. F., Rodríguez, D. M., Ribeaux, D. R., Luna, M. A. C., Silva, T. A. L., Andrade, R. F. S., Gusmão, N. B., & Campos-Takaki, G. M. (2016). Waste soybean oil and corn steep liquor as economic substrates for bioemulsifier and biodiesel production by *Candida lipolytica* UCP 0998. *International journal of molecular sciences*, v. 17(10), pp.1608. https://doi.org/10.3390/ijms17101608

Tao, W., Lin, J., Wang, W., Huang, & H., Li, S. (2019). Designer bioemulsifiers based on combinations of different polysaccharides with the novel emulsifying esterase AXE from *Bacillus subtilis* CICC 20034. *Microbial Cell Factories*. https://doi.org/10.1186/s12934-019-1221-y

Tavares, J., Alves, L., Silva, T. P. & Paixão, S. M. (2021). Design and validation of an expeditious analytical method to quantify the emulsifying activity during biosurfactants/bioemulsifiers production. *Colloids and Surfaces B: Biointerfaces*, v. 208. https://doi.org/10.1016/j.colsurfb.2021.112111

Tomaszewska-Hetman, L., Rymowicz, W., & Rywniska, A. (2020). Waste conversion into a sweetener-development of an innovative strategy for erythritol production by *Yarrowia lipolytica*. *Sustainability*. https://doi.org/doi:10.3390/su12177122

Uthandi, S., Kaliyaperumal, A., Srinivasan, N., Thangavelu, K., Muniraj, I. K. M, Gathergood, N., & Gupta, V. K. (2021). Microbial biodiesel production from lignocellulosic biomass: New insights and future challenges. Critical Reviews in Environmental Science and Technology, 1–30. https://doi.org/10.1080/10643389.2021.1877045

Vilas Bôas, R. N., & Mendes, M. (2022). A review of biodiesel production from non-edible raw materials using the transesterification process with a focus on influence of feedstock composition and free fatty acids. *Journal of the Chilean Chemical Society*.

Zadeh, P. H., Moghimi, H., & Hamedi, J. (2018). Biosurfactant production by *Mucor circinelloides*: Environmental applications and surface-active properties. *Engineering in Life Sciences*. https://doi.org/10.1002/elsc.201700149

Zhang, T. Y., Yu Y., Zhu H., Yang S. Z., Yang T. M., Zhang M. Y., & Zhang, Y. X. (2018). *Absidia panacisoli* sp. nov., isolated from rhizosphere of Panax notoginseng. *International journal of systematic and evolutionary microbiology*, v. 68(8), pp. 2468–2472. https://doi.org/10.1099/ijsem.0.002857

Zhao, H., Nie, Y., Zong, T., Dai, Y., & Liu, X. (2022). Three new species of *Absidia* (Mucoromycota) from China based on phylogeny, morphology and physiology. *Diversity*, 14(2), 132.

Zininga, J. T., Puri, A. K., Govender, A., Singh, S., & Permaul, K. (2018). Concomitant production of chitosan and lipids from a newly isolated *Mucor circinelloides* ZSKP for biodiesel production. *Bioresource Technology*, 272, pp. 545 - 551. https://doi.org/10.1016/j.biortech.2018.10.035