

The emulsion stability was determined after 24 hours of rest and the emulsification index was calculated by the formula:

$$I_E = \frac{(H_E)}{H_T} \times 100 \quad (1)$$

Where IE is the emulsification index, HE which represents the height of the emulsion and HT which represents the total height of the emulsion

2.6. Evaluation of the stability of the biosurfactant emulsification (effects of pH, addition of NaCl, time under heating and temperature)

The effects of different temperatures (5°C, 70°C, 100°C and 120°C), of different NaCl concentrations (2.0, 4.0, 6.0, 8.0 and 10.0%) and of different pHs (2.0, 4.0, 6.0, 8.0, 10.0 and 12.0) in the activity of the biosurfactant were evaluated in the cell-free metabolic fluid to determine the emulsification activity (Cooper and Goldenberg, 1987) using post-use motor oil as oily substrate. All analyzes were performed in triplicate.

2.7. Biosurfactant isolation and phytotoxicity assessment

The isolation of the *Candida guilliermondii* UCP 0992 biosurfactant was performed according to the methodology described by Daverey and Pakshirajan (2010). The biosurfactant produced was first extracted with ethyl acetate and isopropanol (8:2, v/v) and then the solvent layer containing the biosurfactant was evaporated in a rotary evaporator (Fisatom 804, Brazil) to obtain a quantitative biosurfactant partially purified. The phytotoxicity of the biosurfactant was evaluated at concentrations of ½ CMC, 1x CMC, 2x CMC through seed germination and root growth of cabbage (*Brassica oleracea*) after five days of incubation in the dark, according to Tiquia et al. (1996), being calculated according to the formulas: Relative seed germination (%) = (number of germinated seeds in the extract / number of germinated seeds in the control) x 100, Relative root length (%) = (mean length root length in extract / mean root length in control) x 100, GI= [(% seed germination) x (% root growth)] / 100 %.

2.8. Preparation of commercial formula of natural detergent and application

The formulation procedure was performed as follows: the biosurfactant was added to 10 ml of distilled water for dissolution. Then, 5mL of sodium hydroxide solution were added while stirring. The system remained at rest for 6 hours for the reaction to complete. Afterwards, it was added to the fatty acid diethanolamide in 10 ml of water, always stirring. Then, sodium chloride and potassium sorbate were added and the volume was completed to 50 mL. The sample was left to rest for 24 hours and then stored in previously sterilized bottles.

For application, a sample of clean white cotton fabric (5 cm²) was stained with 1 mL of different post-use consumer products (engine oil, soy oil and automotive grease) on the cloth fabric, which was subjected to drying for 12 hours. The stained fabric samples were subjected to comparative analysis by washing with a commercial detergent solution (OMO) with the formulated natural detergent. The stained cloths were placed in separate bottles, one bottle with tap water and commercial detergent (final concentration of 10 mg/mL) and one bottle with tap water and the formulated detergent (final concentration of 10 mg/mL). In all flasks, the final volume was 100 mL. Then, stained tissue samples were maintained under agitation at 200 rpm at room temperature. After the washing time, the tissue samples were removed, washed with water and dried, and all experiments were performed in triplicates. The percentage of stain removal was calculated according to the formulas:

$$R_M = \frac{(P_{TM})}{P_{TL}} \times 100 \quad (2)$$

Where R_M is Stain Removal, P_{TM} represents the post wash and dry stained fabric weight and P_{TL} represents the clean stain free and dry weight of the fabric.

3. Results and discussion

Knowing that the effectiveness of surfactants is determined by the ability to reduce surface tension which is the measure of free energy of the surface per unit area, necessary to bring a molecule from the interior of the liquid to the surface. The reduction of surface and interfacial tensions is considered the main parameter for detecting a surface-active compound in a given medium (Sarubbo et al., 2022).

According to the studies carried out, the media used as a carbon source provided a reduction in surface tension. The surfactants produced in these media were able to reduce surface tension up to 32.18 mN.m⁻¹, meaning a 55% reduction in relation to the tension of distilled water (72 mN.m⁻¹). Therefore, the reduction in surface tension indicates that *Candida guilliermondii* UCP 0992 managed to degrade the substrates present in the media and

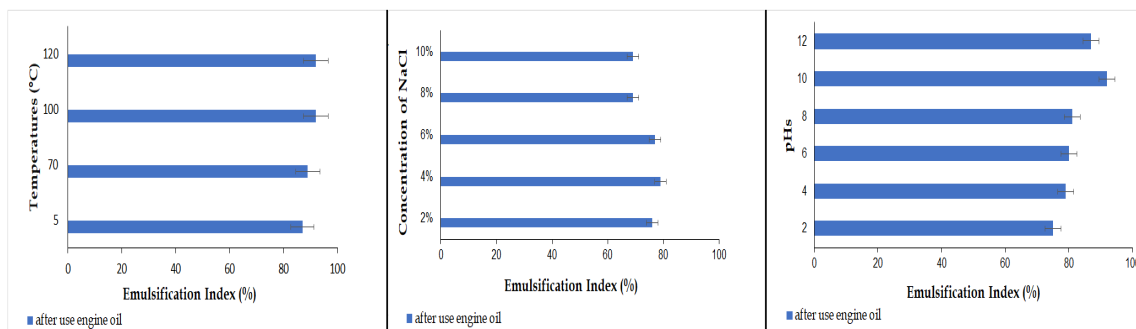


Figure 2: Stability of the *Candida guilliermondii* UCP 0992 biosurfactant, evaluated under different pH, temperature and NaCl addition conditions through the emulsification index.

The absence of toxicity is of fundamental importance for the application of an ambient product. Eco-toxicity bioassays are analytical methods that allow characterizing the toxicity of chemical substances to be used in domestic and industrial products (detergents) (Almeida et al., 2019). Therefore, the evaluation of the biosurfactant toxicity test was carried out using seeds of the Cabbage-Oxheart (*Brassica oleracea*) and cherry tomato (*Solanum lycopersicum*) vegetables, in order to guarantee that the biosurfactant will not be toxic to the environment. The biosurfactant produced by the yeast, at the concentrations tested ($\frac{1}{2}$ CMC, 1x CMC and 2x CMC), showed satisfactory results, since there was a germination index between 86 and 98% in the vegetables used for the tests (Table 3).

Table 3: Phytotoxicity test at different concentrations of biosurfactants produced by *Candida guilliermondii* UCP 0992.

Germination Index (%)				
Vegetable Seeds	Biosurfactant Concentration			
	$\frac{1}{2}$ CMC	CMC	2X CMC	Água
Cherry Tomato (<i>Solanum lycopersicum</i> var. <i>cerasiforme</i>)	98,66 ± 0,20	94,64 ± 0,30	84,41 ± 0,10	100 ± 0,20
Oxheart Cabbage (<i>Brassica oleracea</i>)	92,64 ± 0,10	86,66 ± 0,30	94,46 ± 0,30	100 ± 0,20

Biosurfactants are used in various industrial processes due to their different structures and properties (Rocha e Silva et al., 2020). Preservative compounds are chemical substances whose function is to preserve the initial conditions of the product so that there are no significant changes in its properties over time. The formulation of the natural detergent using the biosurfactants produced by the isolates of *Candida guilliermondii* UCP 0992 showed a very stable behavior with the organoleptic characteristics, pearly white color, pleasant odor, fluid and homogeneous consistency, as well as, it did not cause changes in its surface tension, since they were recorded as 32 mN/m⁻¹. The formulated natural detergent was evaluated in dirt removal tests (soybean oil, post-use engine oil and automotive grease) on cotton fabric, to study the influence of the product (the formulated one) on cleaning, in relation to the removal efficiency, of fabric stains. The results obtained were promising, since the formulated natural detergent showed removal of dirt in 95% for motor oil, 93% for soybean oil and 75% for automotive grease, similar to commercial detergent (OMO), demonstrating that the product remained viable in immediate removal of most contaminants. Thus, the results point to a potential application of the biosurfactant from the strain *Candida guilliermondii* UCP 0992 in the formulation of commercial detergents (Figure 3).

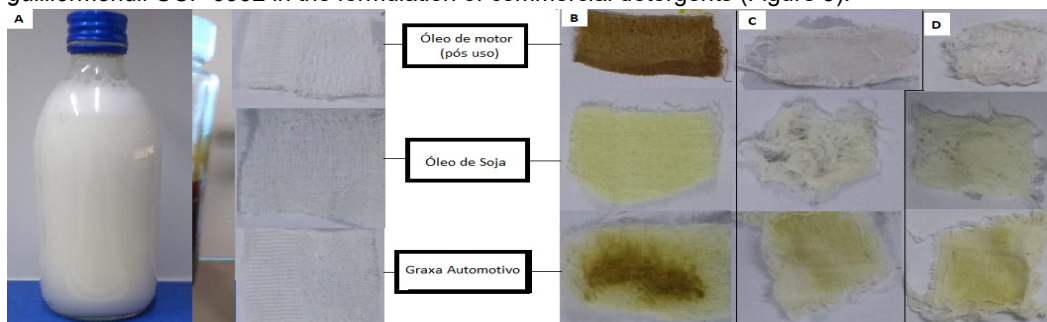


Figure 3: Ilustração da fórmula comercial do detergente natural e o tecido de algodão utilizado (A), bem como, as sujidades no tecido (B), e sua remoção pelo formulado com o biossurfactante de *Candida guilliermondii* UCP 0992 (C), comparando com o detergente comercial – OMO, pós-lavagem (D).

4. Conclusion

Candida guilliermondii UCP 0992, demonstrated a great biotechnological potential in the production of biosurfactant. In addition, the results of the emulsification, stability, toxicity and formulation experiments clearly demonstrate the viability of applying the biosurfactant produced by yeast as a biotechnological additive for remeasurement processes that consider the preservation and reduction of environmental impacts as essential aspects for maintaining the quality of life and social well-being.

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