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à Pesquisa do Estado da Bahia

SENAI CIMATEC

University Center

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The SENAI CIMATEC (Integrated Manufacturing and Technology Center) is University Center maintained by of The National industries Confederation (Brazil) and industrie Federation of Bahia State..

Preliminary evaluation use of lees generated in the refining vegetable oil blend as a substrate for the production of biosurfactant.

Bioprocess Group:

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Vegetable oil Brazil Cenary:

- Brazil stands out in the scenario of vegetable oils.

- It is responsible for 32% of world soybean production,

With the 2016/17 crop estimated at around 113.8 million tons.

- Brazil produces 8 MM ton soy oil for year and 1.6 MM ton cotton oil.

- In the refinery this oils produces 720 M ton /year lees'soy and cotton.

(BRAZILIAN ASSOCIATION OF VEGETABLE OILS INDUSTRIES, 2017)

Soy and Cotton oil

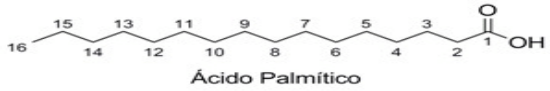
Long carbon chains provide a good culture substrate for microorganisms.

Soybean oil, the most abundant in Brazil, contains about 61% polyunsaturated fatty acids with 18 carbon long chains (linoleic and linolenic).
Cotton oil contains about 60% unsaturated fatty acid and 25% fatty acid saturated with carbon chain ranging from 16 to 18 carbons (ricinoleic and linolenic).

(F O N S E C A, H. 1994).

Fatty Acids:

Fatty acids are organic compounds that have a carboxyl group at one of their ends, with open and long chains, with 4 to 22 carbon atoms, which can be saturated and unsaturated
(JUN, A. et al. , 2016).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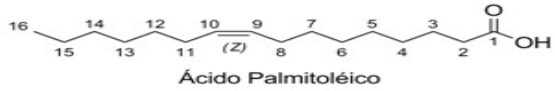
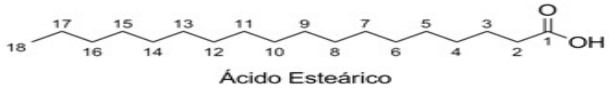
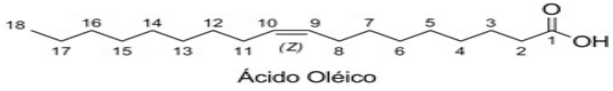

Ácido Palmitoléico


Ácido Esteárico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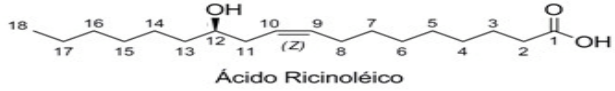
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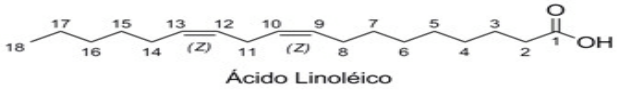
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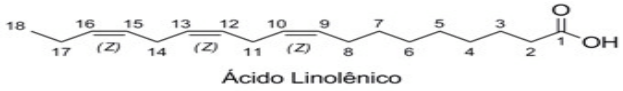
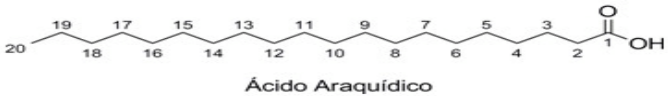

Ácido Linolênico


Ácido Araquídico

Biosurfactant:

- Biosurfactants have the environmental advantage that they can be synthesized from renewable substrates such as oil residues and fully biodegradable (BEZERRA, M. S, 2012).

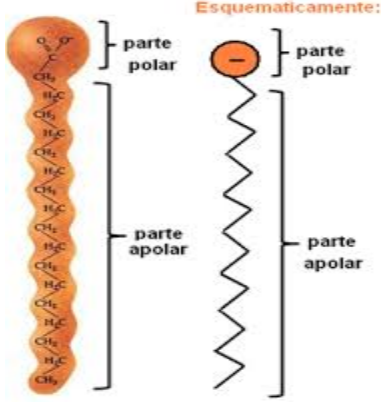
- Uses: detergency, emulsification, lubrication, foaming capacity, wettability, solubilization and dispersion of phases.

Currently, the cost of producing a biosurfactant is much greater than the synthetic surfactant. Mainly due to the cost of the substrate.

Price of biosurfactant has fallen over the years:

1980 $ 10. / 0.01 Kg
2000 $ 10. / 0.02 Kg
2018 $ 10. / 0.05 Kg

But.....$ 10. / 1.0 Kg



Economic Viability:

The economic viability of this project, on an industrial scale, is in the use of an inexpensive substrate or of no economic value, for example refining residue (lees) of vegetable oils, (Figure 1).

Adaptation of microorganisms (Bacillus) to the substrate proposed for the production of biosurfactant.

Figure1

Figure 2

http://www.ibb.unesp.br/Home/Departamentos/Microbiologiaelmunologia/aula_bacillus.pdf
(acess: dezembro/18)

Vegetable oil Industrialization:

In the industrialization of vegetable oils generates three sub-products: gums, lees, originating from the neutralization step and the condensate.

Sodium soaps are formed in the step of neutralizing the chemical refining of crude oil through the reaction with sodium hydroxide to remove the free fatty acids. (alcaline neutralization)

When the oil is heated with an alkali solution, glycerol and a mixture of alkali salts of fatty acids (soaps) are formed.

Free fatty acids also react with alkali resulting in soaps in the neutralization reaction

(FRÉ, N. C., 2009).

Vegetable oil Industrialization:

These soaps produced are insoluble in the oil and concentrate in the aqueous phase which separates from the neutral oil and constitutes the refining slurry (lees), which will be removed from the neutral oil by physical process.

The soaps and most of the non-oily material (lees), are separated by continuous centrifugation. (Figure 3)

Figure 3

The lees:

The sludge (lees) contains: fatty acids, sodium salts, water, triglycerides, saponifiable material and oil degradation products. The lees contains between 35% and 50% of total fatty acids (FRÉ, N. C., 2009).

This lees no have comercial value or very small value (U$ 2,5/ton), and are enviromental problem!

The Brazil produces 720 M ton /year lees'soy and cotton oil

(BRAZILIAN ASSOCIATION OF VEGETABLE OILS INDUSTRIES, 2017).

Methodology:

- The methodology consists of: collection of information from a vegetable oil production industry located in Bahia, covering the technical visit and data collection on lees availability and sample collection for qualitative and quantitative analytical evaluation.

- The visit to the industry occurred on June 26, 2018. During this visit the productive process of refining the blend' soy and cotton oil, in ratio of 1:1, by mass. This process consists in heating the crude oil from temperature (27°C) to 50 °C in a 1-pass hull tube heat exchanger.

- After heating the oil blend reacts with the diluted caustic soda, in pre-prepared concentration, which varies from 10 to 20%, depending on the acidity of the oil.

Oil Refinery Process:

- The heated crude oil and the caustic soda are mixed in a line mixer and proceed to the contact tower (Figure 4), where the neutralizing reaction of the free fatty acids occurs.
- After this contact tower, the neutralized oil and the residues are heated to 75°C and then to the first centrifuge. In this centrifuge the sludge (lees) exits through the upper part (Figure 5).

Figure 4

Figure 5

Collection point of the sludge (lees)' soy and cotton blend sample:

Physically, the sludge leaves the centrifuge at a temperature of 70^{0}C, ranging from a light brown to dark brown color, depending on the initial acidity of the crude oil, ranging from 1.0% to 3.0%. This efluente (lees) contains: triglycerides, saponifiable material, salts of fatty acids, non-hydratable phosphatides, water and sodium salts, (LOTFABAD, TB, 2016).

At the end of the production visit, four samples were collected at the top of the centrifuge as shown in Figure 6, and sent for analysis on CIMATEC's Integrated Laboratory of Applied Chemistry Research (LIPAQ).

Figure 6

Determination of neutral oil:

The oil slurry was homogenized with 60 mL of 50% ethanol solution and 25 ml of petroleum ether in magnetic stirrer shaker. Thereafter, 60 ml of distilled water and then 50 ml of ethyl ether were added. After phase separation, the upper phase (petroleum ether and oil) was maintained and the lower (aqueous) phase was transferred to another separatory funnel. One more extraction was made.

- The upper phases were placed in the same funnel and washed with 50 mL of distilled water until the pH was neutral, (Figure 7).

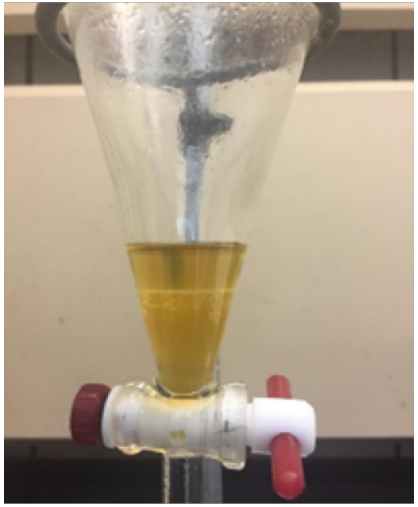


Figure 7.

- Filtration was then performed with filter paper and anhydrous sodium sulfate. The filtrate was collected in a pre-weighed bottom flask. The flask was brought to the rotary evaporator at 25°C until all the solvent evaporated. One milliliter of acetone was added to the oil in the flask and the drying was done at room temperature. Then the balloon containing only oil was weighed, (Figure 8).

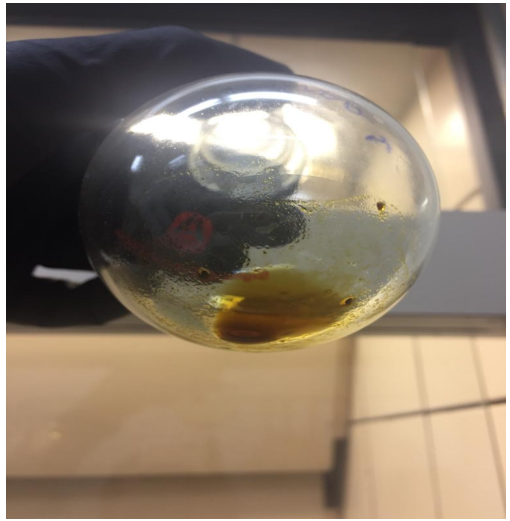


Figure 8

Determination of the residue content of soap

- Analysis of the soap residue content was determined in which 0.4 g of slurry was homogenized with 60 ml of distilled water and 1 ml of bromotinol blue. The sample was titrated with 0.1 N HCl, previously standardized, until turning from blue to yellow.
- The calculation was performed according to Equation:

$$\text{Soap (ppm)} = (V * N * 304 * 1000)/m.$$

- Where: V = titrated acid volume (mL), N = normality of 0.1 N HCl, 304 = molecular weight of oleic acid (282) plus 22, because the soap content refers to other fatty acids besides acid oleic in = sample mass (g)

Results:

Neutral oil **(7,1%)**

Soap residue **(32 ppm)**

pH **(9,3)**

Total Fatty Acids **(36,2%)***

Moisture **(33,4%)**

Conclusion:

This lees contains fatty acids, sodium salts, water, triglycerides, saponifiable material and oil degradation products.

Reference literature informs biosurfactant production, through Pseudomonas, using this similar substrate.

The lees originated soya and cotton oil blend is viable substrate to produce biosurfactant.

Next Steps:

Chromatographic Analysis of Fatty Acids by Gas Chromatography with Mass Spectrum Detector

Define the type of Bacillus to use: B. subtilis or B. licheniformis

Adapt the microorganism (Bacillus) to the substrate

Producing the biosurfactant: (Surfactin / Lipid Peptide)

Thank You for your attention!

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Cromatógrafo - CG com detector espectro de massa.

GCMS – QP2010SE GC-2010 Plus

Preparo das Amostras [12]

A determinação dos ácidos graxos pode ser feita através de cromatografia gasosa. Para tanto deve-se fazer uma reação de transesterificação. onde é realizada a extração dos ésteres dos ácidos graxos. Pois os AG tem ponto de ebulição alto e os ésteres destes AG, apresentam PE menor, compatível para GC