

## Oil Bioremoval Prototype Controlled by *Arduino* Utilizing the Biosurfactant Produced by *Candida Guilliermondii*

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In 2010, the most catastrophic oil disaster occurred in the Gulf of Mexico: the Deepwater Horizon Accident, spreading at least 780 million liters of petroleum. As an alternative to treat the reported spill, the scientists utilized over than 7 million liters of chemical surfactants without control, however the toxicity had raised 56 times. In this sense, the combination of a machine controlled by a *Arduino* board to remove the oil with a biotechnology of tensoative compounds performed by the biosurfactants was made to ensure the bioremediation. This machine was denominated Oil Bioremoval Prototype (OBP) and was composed by two modulus: one with mechanical agitation made by impellers to mix oil, water, and biosurfactant and other with dissolved air utilized to flotote the mixture. Besides, the biosurfactant was produced by the yeast *Candida guilliermondii* in a medium composed by 4.0 % corn steep, 2.5 % molasses, and 2.5 % residual soybean oil in a bioreactor 50.0 l, during 132 h, under a temperature of 28 °C, an agitation of 250 rpm, and an aeration of 16.5 l/min. Through a Central Composite Rotatable Design (CCRD), three independent variables were applied in the OBP: the concentration of the biosurfactant, which was modified from 1.6 to 18.4 %, the aeration frequency, which was changed from 33.2 to 66.8 l/h, and the aeration time which was analysed from 0.32 to 3.68 min, measuring the turbidity difference as the response surface. Furthermore, the cost to produce the OBP was evaluated in euros. Wherefore, the turbidity differences showed that low fractions of the biosurfactant were required to become a mixture with a less turbid of 300.0 NTU. On the other hand, the aeration time and air frequency ranges were performed with maxima values. Wherefore, it is important to join the biotechnology with robotics to comprehend the involved factors and to remove the oil.

### 1. Introduction

In 2010, the scientists took 87 d to close entirely the Macondo 252 well inside the Gulf of Mexico Sea. After 16 months, 780 million liters of petroleum that were spilled, reaching 790 km from platform. Thus, this dispersion of hydrocarbons and oils is caused: by the wavelengths, wherein a 1.8 m wave becomes the oil remover vessel work difficult, by the speeds and directions from the winds and the marine currents, and by the elevated temperatures, which can raise the evaporation rate of the oil low-fractions and the solubility of oil high-fractions into the water, interfering in the oil removal. Besides these factors, the remediation time is essential, because, if the oil was not removed from the sea at least 24 h, the natural conditions would not help the remediation (Tansel, 2014).

After some days without an effective solution, the scientists sprayed a chemical surfactant cloud onto the oil slick. Initially, the oil disappeared, although the water analysis showed that the toxicity grown up 52 times (Mascarelli, 2011), and the oil slick came back to the surface. An alternative to substitute the high-toxic chemical surfactants is the combination of substrates from industrial residues and potential microorganisms to produce the biosurfactants: the oil bioremediators (Brasileiro et al., 2015).

Furthermore, the microbial surfactants should be applied correctly, excluding the surfactant pulverization. Thus, a tertiary water treatment can be used as a Dissolved Air Flotator (DAF), changing the coagulants as aluminum sulfate to the biosurfactants (Karhu et al., 2014).

In this sense, the work has the objectives of producing the biosurfactant in a 50 l batch bioreactor and of selecting the best conditions of Biosurfactant Concentration (%), Air Frequency (l/h) and Aeration Time (min) through the Oil Bioremoval Prototype. This machine, controlled by the low-cost *Arduino Uno* board, can simulate the steps of a real Dissolved Air Flotator, reducing the time of experiments and choosing the best conditions to recover the oil in an oil spill.

## 2. Materials And Methods

### 2.1 Microorganism and media

There was utilised the yeast *Candida guilliermondii* (UCP0992) for the biosurfactant production, which was obtained from the culture collection of the *Universidade Católica de Pernambuco* (Brazil).

For the Maintenance Medium, there were prepared the Yeast Mold Agar (YMA) slants (w/v) with the composition of yeast extract (0.3 %), malt extract (0.3 %), tryptone (0.5 %), D-glucose (1.0 %), and agar (5.0 %). In the absence of agar, the medium was denominated Yeast Medium Broth (YMB) with the aim to grow up the yeast. Transfers were made to other fresh slants each month to maintain the microorganism alive (Sarubbo et al., 2015).

In order to compose the Production Medium, the waste materials were: molasses (2.5 %), corn steep liquor (4.0 %), and residual soybean oil (2.5 %), in pH 5.5.

### 2.2 Fermentation

Initially, the inoculum of *C. guilliermondii* was transferred from YMA slants to YMB sterilized Erlenmeyer flasks, being maintained in Shaker under 200 rpm and 28 °C, and during 24 h. After a day, a volume with concentration of  $10^8$  cells/ml was inoculated in a sterilized 50 l batch bioreactor, containing the Production Medium, until a concentration of  $10^4$  cells/ml with 4 % of inoculum. Under 250 rpm, an aeration frequency of 16.7 l/min and 28 °C, and during 132 h, the biosurfactant was produced, centrifuged and filtrated.

### 2.3 Application of the biosurfactant into the Oil Removal Prototype controlled by an *Arduino Uno* board

In order to simulate the Dissolved Air Flotator action of oil removal, there was made a prototype that combines an *Arduino Uno* board and two mainly module of an original DAF: agitation and flotation. The first modulus (figure 1) had 5 impellers to maintain a frequency of 150 rpm and to mix the biosurfactant with the oily water, while the second modulus (figure 2) had a compressor and 5 pumice stones to dole out the air frequency.

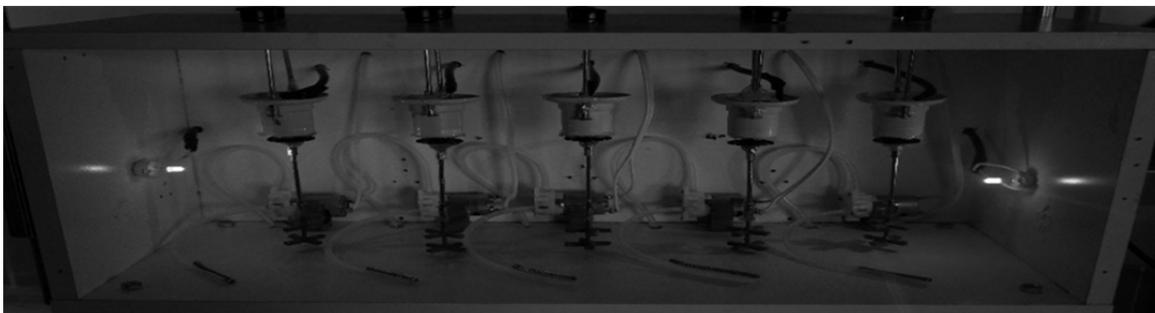


Figure 1: The first modulus of the agitation

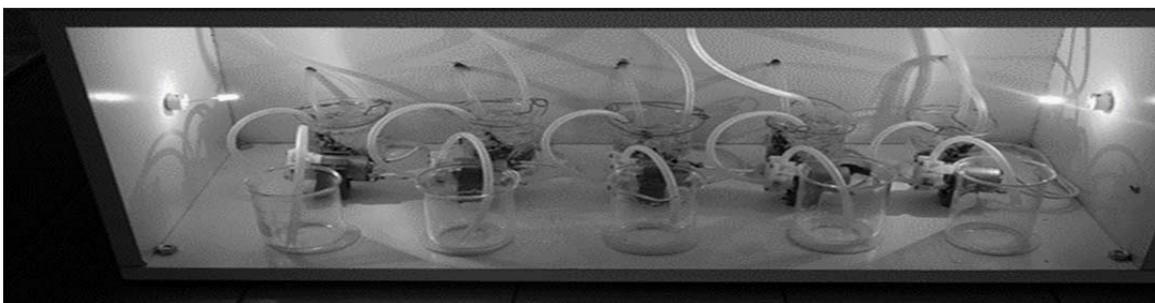


Figure 2: The second modulus of the air frequency

### 2.3 Central Composite Rotatable Design of Oil Removal Prototype conditions

Three factors were essential in the application of the biosurfactant: the air frequency (air flow), the aeration time (time of flotation), and the biosurfactant concentration. Thus, it was necessary to combine ranges concomitantly in order to verify synergisms and antagonisms of the oil removal. Therefore, a Central Composite Rotatable Design (CCRD) was applied in the process with the variables (table 1) and all the experiments (table 2). The response surface of this experimental design was the Difference Between the Turbidities (DBT): the initial and the final (Rodrigues and lemma, 2009).

Table 1: Oil Removal Prototype Variables

| Level | Biosurfactant Concentration (%) | Aeration Time (min) | Air Frequency (l/h) |
|-------|---------------------------------|---------------------|---------------------|
| -1.68 | 1.6                             | 0.32                | 33.2                |
| -1.00 | 5.0                             | 1.00                | 40.0                |
| 0.00  | 10.0                            | 2.00                | 50.0                |
| 1.00  | 15.0                            | 3.00                | 60.0                |
| 1.68  | 18.4                            | 3.68                | 66.8                |

Table 2: Oil Removal Prototype Experiments

| Experiment | Biosurfactant Concentration (%) | Aeration Time (min) | Air Frequency (l/h) |
|------------|---------------------------------|---------------------|---------------------|
| 1          | 5.0                             | 1.00                | 40.0                |
| 2          | 5.0                             | 1.00                | 60.0                |
| 3          | 5.0                             | 3.00                | 40.0                |
| 4          | 5.0                             | 3.00                | 60.0                |
| 5          | 15.0                            | 1.00                | 40.0                |
| 6          | 15.0                            | 1.00                | 60.0                |
| 7          | 15.0                            | 3.00                | 40.0                |
| 8          | 15.0                            | 3.00                | 60.0                |
| 9          | 1.6                             | 2.00                | 50.0                |
| 10         | 18.4                            | 2.00                | 50.0                |
| 11         | 10.0                            | 0.32                | 50.0                |
| 12         | 10.0                            | 3.68                | 50.0                |
| 13         | 10.0                            | 2.00                | 33.2                |
| 14         | 10.0                            | 2.00                | 66.8                |
| 15         | 10.0                            | 2.00                | 50.0                |
| 16         | 10.0                            | 2.00                | 50.0                |
| 17         | 10.0                            | 2.00                | 50.0                |
| 18         | 10.0                            | 2.00                | 50.0                |

### 2.4 Economic evaluation

The cost of the Oil Bioremoval Prototype construction was calculated in base of the Recife city prices.

### 3. Results and Discussion

#### 3.1 Utilization of a Central Composite Rotatable Design to find the highest Difference Between the Turbidities

The CCRD showed the equation 1 that correlated the response surface Difference Between the Turbidities (DBT, NTU (Nephelometric Turbidity Units)) with the Air Frequency ( $a$ , l/h), the Biosurfactant Concentration ( $b$ , %) and the Aeration Time ( $t_0$ , min).

$$DET = 1211.9 + 9.2 \cdot b - 0.4 \cdot b^2 - 235.1 \cdot a + 24.2 \cdot a^2 - 39.1 \cdot t_0 + 0.3 \cdot t_0^2 - 4.4 \cdot b \cdot a - 0.2 \cdot b \cdot t_0 + 3.4 \cdot a \cdot t_0 \quad (1)$$

Each independent variable was selected in the middle point of the respective range to perform the response surface graphs. Thus, the figure 3 correlated the Difference Between the Turbidities, the Aeration Time and the Biosurfactant Concentration, demonstrating that the highest Aeration Time and the lowest Biosurfactant Concentration indicate the maximum DBT of 300.0 NTU.

The figure 4 combined the maximum Air Frequency and the minimum Biosurfactant Concentration to obtain 200.0 NTU. As well as the figure 3, the figure 4 showed that lower concentrations of the biosurfactant are required to reduce the turbidity of the water, however the presence of microbial surfactant is necessary, because the reduction without the bioremediator demonstrated a DBT of 115.5 NTU.

Finally, a medium Biosurfactant Concentration with the variations of the Aeration Time and the Air Frequency (figure 5) reflected higher indexes in both elevated levels.

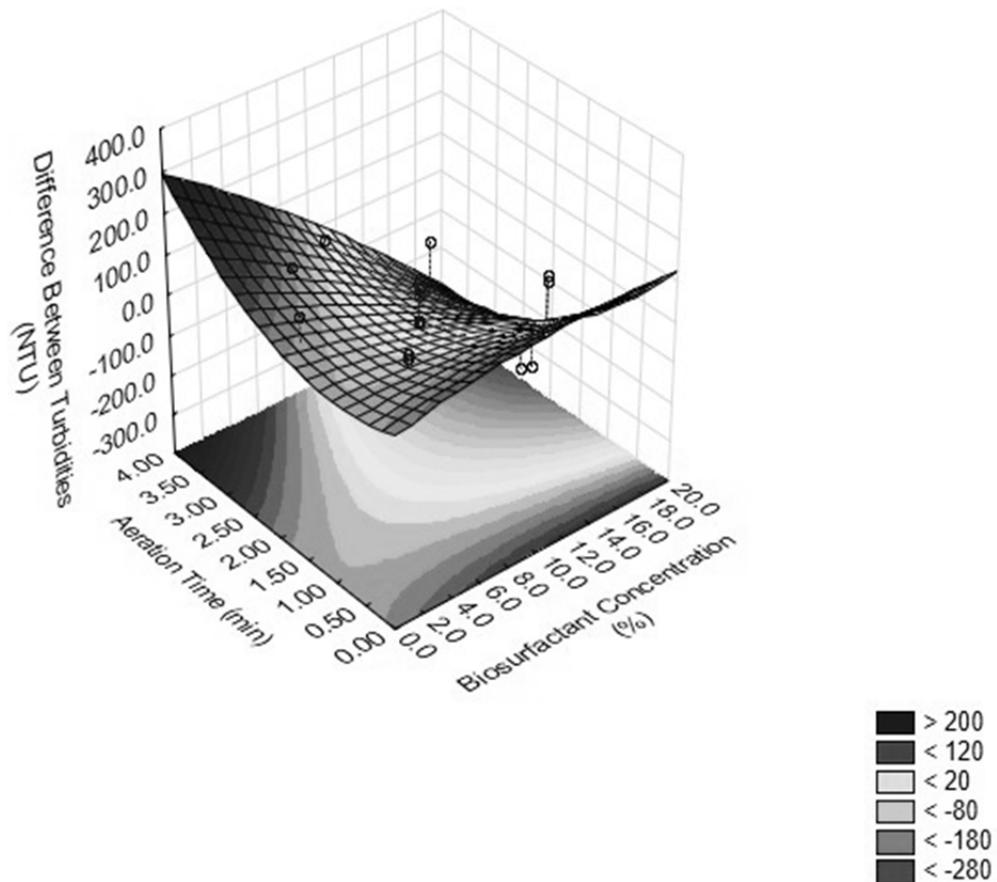


Figure 3: Comparison between the Difference Between the Turbidities, the Aeration Time and the Biosurfactant Concentration

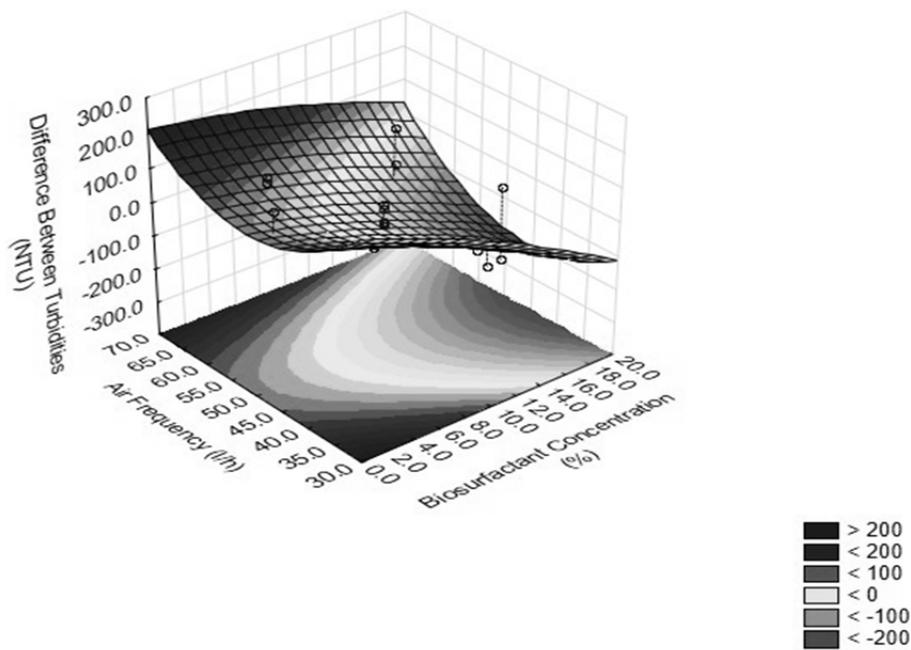


Figure 4: Comparison between the Difference Between the Turbidities, the Air Frequency and the Aeration Time

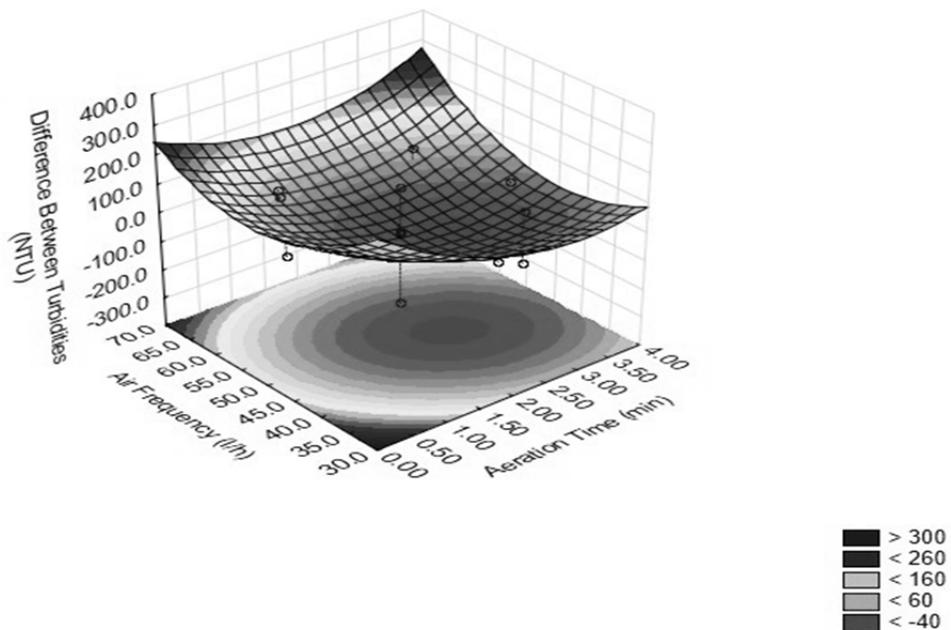


Figure 5: Comparison between the Difference Between the Turbidities, the Air Frequency and the Biosurfactant Concentration

The negative turbidities are caused possibly by the excessive biosurfactant concentration which can emulsify the mixture between water, oil and biosurfactant.

The Pareto's Diagram in the figure 6 ratifies the description of the figures 3, 4 and 5 and characterizes the system as significantly acceptable.

Therefore, the Oil Bioremoval Prototype showed effective results of the bioremediation and a lower cost of manufacture (€ 345,64). The costs were divided by the Agitation and Flotation Module and the Electric Circuit as seen in the figure 7.

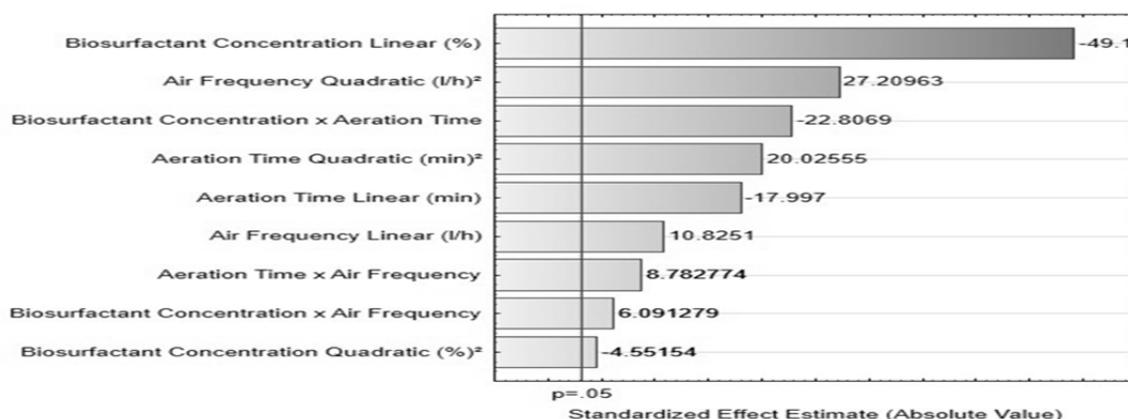


Figure 6: Pareto's Diagram

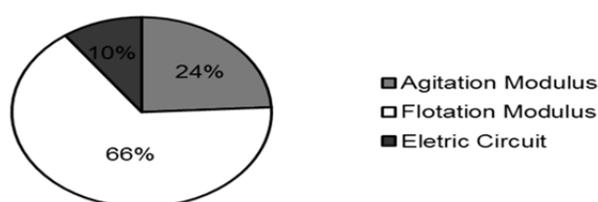


Figure 7: Economic Distribution

#### 4. Conclusion

The Oil Bioremoval Prototype indicated that the best conditions are the lowest Biosurfactant Concentration (1.6 %), the highest Aeration Time (3.68 min) and the middle Air Frequency (50 l/h) with a cost of € 345,64, and these values should be studied with other CCRD to validate the system.

New studies of biosurfactant with the Dissolved Air Flotator should be applied to reach the results of 100 % of oil recover in order to have a defined method to clean the environment.

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