Production of biosurfactant from *Candida cruzi* isolated from produce water oil fields in Basrah for microbial enhance oil recovery

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**Abstract**

The produced water of Nahran Omar oil fields used for isolated the biosurfactant produce *Candida cruzi* which enhanced by using carbon and nitrogen sources, the biosurfactant production was followed by measuring the oil spreading, emulsification activity, oil collapse and cleaning oil contaminated vessels. The crud biosurfactant was able to spreading the oil (24) mm with (8%) emulsification activity and oil collapse of (++) the result of cleaning oil contaminated vessels revealed the recovered of oil from the wells of vessels. Under optimization of carbon and nitrogen sources the results revealed that the largest oil displacement was (9) mm with dextrin the maximum emulsification power was (92.8% and 92.5%) for lactose and fructose respectively, these results demonstrated the high potential of emulsifiers produced by *C. cruzi* that can play an important role in application in enhance oil recovery.

**Introduction**

Microorganisms are use a wide range of organic compounds as a source of carbon and energy for their growth produce a variety of substances are known as biosurfactants. They used these substances when the carbon source is in an insoluble form like a hydrocarbon to make possible their diffusion into the cell [1]. Many microorganisms such as bacteria, yeasts and filamentous fungi are produce the biosurfactants [2, 3]. The most commonly isolated biosurfactants are glycolipids which classified as rhamnolipid produce by the genus of *pseudomonas* [4] surfactin and iturin released by *Bacillus subtilis* strains [5].

Relatively fewer fungi are known to produce biosurfactants. *Candida bombicola* [6] and *Candida lipolytica* [7] are among the most commonly studied yeast for the production of sophorolipids [8] also *Candida albicans* [9], *Trichosporon asahii* [10] (and *Aspergillus ustus* [11].

Because of the biosurfactants gained several advantages such as low toxicity, better environmental acceptability, high biodegradability and better functionality under extreme conditions [12, 13, 14] So
they considered as one of the best alternative of chemically synthesized surfactant in different application [15, 16].

Currently the biosurfactants are widely used in petroleum industry, they used in oil spills control, bioremediation of soil and water, removal of oil residue from storage tanks, microbial enhanced oil recovery [17, 18]. as well as facilitate transportation of heavy crude oil by pipeline [19].

The present research aimed to study the potential of *Candida cruzi* isolated from produced water of Nahran Omar oil fields in Iraq for biosurfactant production and optimization of cultural conditions to get a high yield of biosurfactant as initiative steps in order to applicants in oil industry.

**Material and methods**

**Sample collection**

Samples of produce water was obtained from the separator tanks of Nahran Omar oil fields, which located in the north of Basrah, oil reservoirs are located at a depth of ~(2000 – 3000) m below ground and the in situ temperature ~(50-60) °C the samples collected in sterile glass bottles and transport immediately to the laboratory for analysis.

**Isolation and identification of Candida**

The yeast was isolated by spreading of 0.5 ml of produced water on sterile CSB medium plate [20], incubated at 30°C for 7 days. The obtained culture were purified by streaking on Potato Dextrose Agar (PDA). For identification of Candida species using Hirome Candida Differential Agar (Hi Media Laboratories Pvt. Ltd., India according to manufacturer instructions.

**Culture condition and biosurfactant production**

The isolate was activated by aseptically transferred one agar plugs (1 cm²) of the 24 hr. pure cultures to nutrient broth at 30°C and 120 rpm in a shaker incubator for 5-7 days before using it as inoculums for biosurfactant production in mineral salts medium MSM.

The production medium MSM contained (g L⁻¹): Sucrose 40; Yeast extract 18.8; Sodium acetate 0.5; Sodium benzoate 0.1; MgSO₄·7H₂O 0.5; (NH₄)₂SO₄ 3; KH₂PO₄ 2; NaCl 0.9; the medium was supplemented with 1% and 2% of vegetable (Sunflower oil) and crude oil individually used as an additional carbon source. The production medium was inoculated with 9% v/v of activated Candida. The cultivation was performed in 100 ml Erlenmeyer flask at 30 °C and 120 rpm in a shaker incubator.
The cell free supernatant was collected after centrifugation at 6000 rpm for 30 min. in 4°C, and was analyzed for biosurfactant production [21].

**Optimization of biosurfactant production**

Two factors were chosen aiming to obtain higher productivity of biosurfactant carbon and nitrogen source which were employed at a concentration of 20 g/l in MSM medium with 2% oil vegetable. The carbon source used were Glucose, Lactose, Fructose and Dextrin, for evaluation of the most appropriate nitrogen source for the production of biosurfactant Aspargin, L-Arginine, peptone and sodium nitrate were used, the media were autoclaved, inoculated with activated Candida broth at 30 °C and 120 rpm in a shaker incubator for 7 days. The cell free supernatant was collected after centrifugation at 6000 rpm for 30 min. at 4°C, and were analyzed for biosurfactant production.

1- **Oil displacement test**

The oil displacement test was carried out by slowly dropping of 100µl of petroleum crude oil into the surface of 50ml of distilled water in a Petri dish (9cm in diameter). 10 µl of cell free metabolic liquid (crude biosurfactant) was then added to the surface of oil layer [22]. bio7 clear zone displacing the oil was measured in mm. distilled water was used as control.

2- **Emulsification index E24**

In this test 2ml of crude biosurfactant was added to 2ml of vegetable oil in a test tube and by vortexing at high speed for 2min. After 24 h the Emulsification index (E24) was calculated by dividing the measure height of the emulsion layer on the total height of solution by 100 to expressing in percentage [23].

3- **Drop collapsing test**

This test is carried out by added two microliters of mineral oil into the Petri dish and equilibrate for one hr. at room temperature. five microliters of the culture supernatant was added to the surface of oil. The shape of drop on the oil surface was noted after one min. the culture supernatant that collapse the oil drop was indicated as positive (+) to (+++) corresponding partial to completed spreading on the oil surface while the culture supernatant which field to collapse the oil drop was indicated as negative (−). Distilled water was used as negative control. The procedure is discussed with some modification [24, 25].
4- Microcosm test for cleaning of oil contaminated vessels

The test was conducted by using two beakers (100)ml to behave as oil tanks the beakers were filled with crude oil and left stagnant after 24h they emptied, wall and bottoms were contaminated with residual oil. Some drops of culture supernatant softly introduced to one of the beakers the same drops of water was used as control. After 15 min the result were observed and reported as (+) to (+++) [26].

Result and discussion

Isolation and identification of Candida

Although bacteria have been extensively studied for biosurfactant production, yeasts are also potential biosurfactant-producing microorganisms. Because of their unique structures, biosurfactants may have a greater range of properties that can be exploited commercially [27]. In the present study, Candida cruzi isolated from produced water of oil field was used for production of biosurfactant, Yeast can be preferred to bacteria as a source for biosurfactants because of its producing biosurfactant in higher concentration than bacteria and they do not present risk of inducing toxicity or pathogenic reaction [28]. The isolated colonies of Candida was appear after 7-10 days of incubation at 30°C on CSB medium, colonies was white round as shown in figure (1) these colonies identified as Candida cruzi after growing on Hicrome Candida Differential Agar where the colonies appear in purple color figure (2).

![Fig. (1) Candida on CSB medium](image1)

![Fig. (2) Candida cruzi on Hicrom Candida Differential Agar](image2)
1-Oil displacement test

The yield of the crude biosurfactant produced by *Candida cruzi* isolate grown on sucrose as main carbon source and yeast extract as nitrogen source with 1% and 2% vegetable and crude oil was able to displaced the crude oil, the result of oil spreading showed that the largest zone 24 mm was observed with 2% vegetable oil as displayed in table (1) and figure (3). These result was compatible with previous studies of Sarubo [29] and Konishi [30] who used olive oil and canola oil for production of biosurfactant from *Candida batistae* CBS550 and *C. lipolytica* UCP0988 respectively. The used of vegetable oil as carbon source to produced biosurfactant seem to be an interesting alternative and low cost by the yeast [31, 32, 33, 34] found that incorporated in to aqueous solution the different stabilization properties of biosurfactant producing was when using the vegetable and mineral oils and the better stabilization properties when vegetable oil was used.

<table>
<thead>
<tr>
<th>dispersant of oil / mm</th>
<th>Vegetable oil</th>
<th>Crude oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% 2% 1% 2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 24 10 11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (1) Dispersant of the crude oil by biosurfactant extracted from *candida*.

Fig. (3) Dispersion of crude oil by biosurfactant.
2-Emulsification activity E24

The results of this test appear *Candida cruzi* ability to produce emulsifiers, the better emulsification activity E24 was about 25% with 2% crude oil table (2) and figure (4). This compatible with previous Studies by Cirigliano and Carman [35] who showed the produce of emulsifiers liposan by *Candida lipolytica* cultivated in hexadecane. also De Luna et al [36] showed the ability of *Candida glabrata* for production emulsifiers. The emulsifying property determines the strength of biosurfactant in retaining the emulsion of hydrocarbons or oil in water [23].

<table>
<thead>
<tr>
<th>Emulsification activity %</th>
<th>Vegetable oil</th>
<th>Crude oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>2%</td>
<td>18</td>
<td>25</td>
</tr>
</tbody>
</table>

3-Oil drop collapse

A rapid drop collapsing test was also employed to screen the *Candida cruzi* isolate for biosurfactant production, a drop of culture supernatant was flattened on oil surface leading to positive collapse reaction as (++) figure (5). This was agreed with previous studies [38,39].
4-Microcosm test for cleaning of oil contaminated vessels

For cleaning of oil contaminated vessels the result showed that when the supernatant of the *Candida* was applied to the surface wall of beaker a few second after the application of biosurfactant small clear zone free from oil were developed in the wall (++), when water was used, negligible result was observed. Heavy oil fractions and the waste that buildup on the walls and at the bottom of storage tanks are highly viscous and become solid deposits, solvents and manual cleaning are used to removal of this material which is expensive procedure, time consuming [40, 41]. Microbial biosurfactants form oil- water emulsion by decrease the viscosity of sludge and oil deposits which facilitates the pumping of waste [14]. The supernatant can be used for cleaning oil contaminated vessels and tanks, compared to chemical methods such clean process represent economically less hazardous this result agreed with Diab and Gamal Eldin [26] who evaluated the effect of *P. aeruginosa* SH 29 supernatant in cleaning of oil contaminated vessels and Chamanrokh et al. [42] who found that washing the oil contaminated vessels with 10 to 20 mg/l of biosurfactant solution readily form an oil- water emulsion.

5-Optimization of biosurfactant production in different carbon sources

The production of surfactant by the yeast in combination of a carbohydrate plus vegetal oil as a very interesting alternative has been demonstrated studied in previous works [32, 43, 44]. The medium optimization was perform by growing the selected *Candida* under study in different carbon and nitrogen source. The influence of the carbon source in biosurfactant production has been extensively studied in some microorganisms. Depending on the yeast strain different type of carbon source have been used [27].

The result of optimization of biosurfactant production in different carbon source were displayed in table (3), the high oil displacement was 9 mm with dextrin while the oil displacement was 73%. The
lactose and fructose was the best carbon source for surfactant producing which gave the effectiveness of emulsification activity E2492% with low displacement oil 2-3 mm. Yarrowia lipolytica have high emulsification activity in the presence of glucose as a carbon source [45]. this was compatible with Sarubbo et al. [32]. Who observed the maximum biosurfactant production by Candida galbrata by using carbon source, [46]. shown that the sophorolipid yield from C. bombicola ATCC22214 increase with use carbon source also this was agreed with Khopade et al. [47]. who showed that carbon sources such as trehalose,hexadecane and olive oil supported the growth of Nocardiopsis.

Amaral et al. [48] reported that the use of glucose as a carbon source was important for the production of Yansan by Yarrowia. Also Joice and Parthasarathi [49]. reported that isolate of Pseudomonas aeruginosa PBSCI was produce higher amount of biosurfactant in presence of glucose and glycerol.

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>Emulsification %</th>
<th>Displacement mm</th>
<th>Oil recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>71.4%</td>
<td>2mm</td>
<td>++</td>
</tr>
<tr>
<td>Lactose</td>
<td>92.8%</td>
<td>3mm</td>
<td>+++</td>
</tr>
<tr>
<td>Fructose</td>
<td>92.8%</td>
<td>3mm</td>
<td>+</td>
</tr>
<tr>
<td>Dextrin</td>
<td>73.3%</td>
<td>9mm</td>
<td>+++</td>
</tr>
</tbody>
</table>

Proteins are essential for the growth of microbes, the fermentation process and production of enzymes so the nitrogen is important in biosurfactant production medium [50]. The effect of nitrogen sources affected on production of biosurfactant showed that L-Arginine was the best source of nitrogen for biosurfactant synthesis its gave the large zone of oil displacement 10 mm and the maximum emulsification activity was 73.3% table (4) figure (6) similar results were found with study of Santos et al. [51]. who showed that rhamnolipids are mainly produced by Pseudomonas species using various carbon sources such as vegetable oils and nitrogen source to achieve improve productivity.

The important factors in production of biosurfactant are carbon and nitrogen sources which have great influence their production cost [52]. The supernatant of Candida cruzi product was emulsifiers than the surfactant because emulsification power was better than oil displacement this was compatible with Alburquerque et al. [53] who showed that most of the biosurfactant produced by yeasts are better emulsifiers than biosurfactants, mainly because of the chemical structure Fontes et al. [54] reported that
nitrogen source were important for cell growth and biosurfactant synthesis and the glucose and glycerol were the most efficient carbon source to produce biosurfactant by *Yarrowia lipolytica*.

**Table (4) Effect of nitrogen sources affected on production of biosurfactant**

<table>
<thead>
<tr>
<th>Nitrogen sources</th>
<th>Emulsification %</th>
<th>Displacement mm</th>
<th>Oil recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>73.3%</td>
<td>10mm</td>
<td>-ve</td>
</tr>
<tr>
<td>Asparagine</td>
<td>66.6%</td>
<td>6mm</td>
<td>+++</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>66.6%</td>
<td>5mm</td>
<td>++</td>
</tr>
<tr>
<td>Peptone</td>
<td>71.4%</td>
<td>6mm</td>
<td>+</td>
</tr>
</tbody>
</table>

**Fig. (6) Emulsification activity of optimization of biosurfactant with carbon and nitrogen source.** (1) Glucose, (2) Lactose, (3) Fructose, (4) L-Arginine, (5) Asparagine, (6) Sodium nitrate, (7) Peptone and (8) Dextrine.

**Conclusion**

The biosurfactant produced by *Candida cruzi* showed biosurfactant production and emulsification activity. We have shown that this Candida can be induced to produce high emulsification activity when it's grown with lactose and fructose. It's reduced the cost of production of biosurfactant because the cell free broth containing surfactant can be used directly without purification test. So it's could be applied in enhanced oil recovery operations specially this Candida isolated from produced water of oil fields.
References


35. Juliana Moura de Luna1, Leonie Sarubbo2,3 and Galba Maria de Campos Takaki 2,3 A New Biosurfactant Produced by *Candida glabrata* UCP1002: Characteristics of Stability and Application in Oil Recovery Vol.52, n.4: pp.785-793, July-August 2009. BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY.


