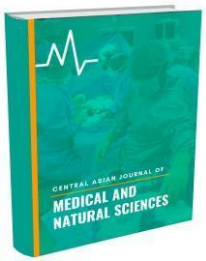

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Article

Comparative Study of Biosurfactant Production by Saccharomyces Cerevisiae Using Various Carbon Sources

Zeena A. Ibrahim[1*], Amal A. Hussein[2], Saad H. Khudhair[3]

1. Collage of Dentistry, Al Mustansiriyah University, Baghdad, Iraq.
2. Applied Science Department , University of Technology, Baghdad, Iraq.
3. Al Karkh University of Science, Baghdad, Iraq.

*Correspondence: Zeeneta36@uomustansiriyah.edu.iq

Abstract: Saccharomyces cerevisiae, a widely used and safe yeast in biotechnology, was evaluated for its ability to produce an environmentally friendly biosurfactant using various carbon sources. This study aimed to compare biosurfactant production from glucose, crude oil, olive oil, and cooking oil, addressing the gap in understanding the impact of insoluble carbon sources. Yeast was activated in YEPG broth and cultured in modified YEP medium with different carbon sources. The biosurfactant activity was assessed through surface tension reduction (ST) and oil spreading method (OSM). Results indicated that glucose did not significantly enhance biosurfactant production (60mN/m ST, 3mm OSM), while crude oil (50mN/m ST, 45mm OSM), olive oil (53mN/m ST, 40mm OSM), and cooking oil (54mN/m ST, 35mm OSM) significantly improved activity. The findings demonstrate that insoluble carbon sources are crucial for effective biosurfactant production by S. cerevisiae, with crude oil yielding the most active biosurfactant. This research highlights the potential of using oils to enhance biosurfactant production, providing insights for industrial applications.

Keywords: Biosurfactant, Carbon sources, Oils, Crude oil, Olive oil.

1. Introduction

Insoluble oils spills are considered one of the major aquatic environment pollutants. Petroleum oils, vegetable oils, and animal fats share common physical properties and produce similar environmental effects, these wastes and spills polymerize and solidify which leads to oxygen reduction, suffocation and toxicity to aquatic animals and organisms [1]. The heavy use and improper disposal of these oily wastes especially the frying oils may lead to drainage systems clogging and significant increase in rats and vermin proliferation rates that are feeding on these solidified oils [2]. The demands for proper and environmental friendly disposal methods of these wastes were solved by biodegradation with microorganisms that are able to produce biosurfactants [3]. The biosurfactants have both hydrophilic and hydrophobic moieties which give its amphipathic structure, these molecules are able to play a key role in emulsification foam formation, detergency, and oil dispersion activities, which are desirable traits in different industries [4]. Biosurfactants have the capacity to reduce surface and interfacial tensions of solutions [5]. Also they increase the bioavailability of substrate to the microorganisms. In addition to environmental applications [6], Biosurfactants showed a great result in many industries such as medical (antibiotics and pharmaceutical products) [7], food products

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(additives and salad dressing) [8], oil recovery [9], agriculture (pesticides) [10] and cosmetics (makeup removals and skin moisturizers) [11,6].

Biosurfactants in the latest century was considered green surfactants i.e environment friendly products due to their great properties such as surface activity, stability and activity under pH and thermal changes, antiadhesive, biodegradability, low toxicity, emulsifying agent, antimicrobial activity, and antioxidant ability [12].

The aim of this study, is to produce extracellular biosurfactant by using crude oil, olive oil and cooking oil as the carbon source for Saccharomyces cerevisiae which.

2. Materials and Methods

Yeast yield

The yeast Saccharomyces cerevisiae was obtained from a previous [13]. The yeast was activated by using100ml of YEPG broth medium (20gL-1 glucose, 20gL-1 peptone, 10 gL-1 yeast extract, pH=5). Culture flasks were incubated at 28^0C for 3 days. A drop of the growth medium examined under microscope to detect any bacterial contamination.

Extracellular biosurfactant production

A modified YEP medium (20gL-1 peptone, 10 gL-1 yeast extract, pH=5) was used for biosurfactant evaluation and carbon sources were added separately as shown in table 1, the medium with glucose as carbon source was used as control. Culture flasks were incubated at 28^0C for 1 week in shaker incubator.

Then the cultures were centrifuged at4000rpm for 15min. Crude extracellular biosurfactant was drawn up by micropipette carefully for further testes.

Table 1. carbon sources used in the research

No.	Carbon source
1	Glucose (2%)
2	Crude oil (2%)
3	Olive oil (2%)
4	Cooking oil (2%)
5	Control (without yeast inoculation)

Screening for biosurfactant activity

Oil spreading method

Two layers of immiscible liquids by pouring 25ml of distil water in a petri-dish and10µl of crude oil on the water surface then 10 µl of biosurfactant added to the center of the crude oil layer. The clear zone was measured in millimeters [14].

Surface tension measurement

Tenisometer was used to measure the surface tension of solution at room temperature by using platinum NOUY ring socked in the surface of 20ml of biosurfactant in a clean dry glass vessel, the platinum ring was gradually pulled up and the surface

tension was measured in mN/m. Distill water was used as a control and to test the accuracy of the device.

3. Results and Discussion

Pure Saccharomyces cerevisiae was used to produce biosurfactant. Biosurfactant production was enhanced by using olive oil, cooking oil and crude oil as carbon source in modified YPD broth medium. The screening of biosurfactant production of the cell free medium by using glucose as carbon source showed a reduction of the surface tension to (60 mN/m) while showed a minimum surface oil spread about (3mm) as compared to the biosurfactant produced by using crude oil as carbon source the surface tension reduced to (50 mN/m) while showed a great result in spreading the surface oil about (45 mm). The surface tension and oil spreading method for control medium (non-inoculated) was (68 mN/m and 0 mm respectively) as shown in figure 1 and 4.

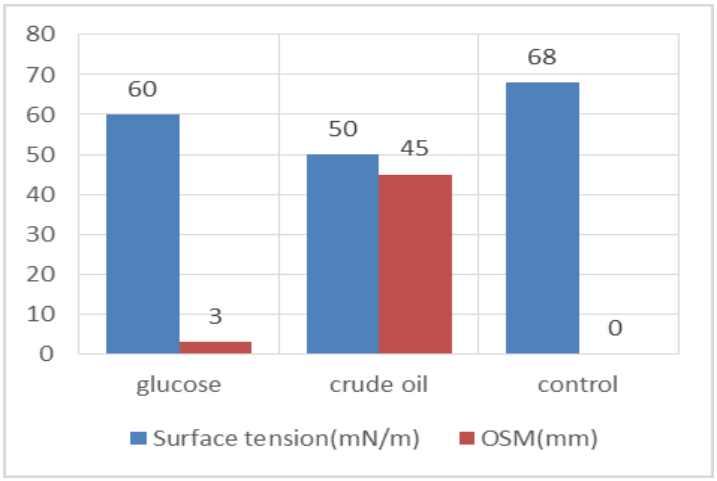

Figure 1. Biosurfactant screening by Surface tension and crude oil spreading method for the medium that contains glucose and crude oil as carbon source.

Olive oil also used as carbon source in the medium which enhanced the production of biosurfactant by Saccharomyces cerevisiae, the estimated result of surface tension for the cell free medium was reduced to (53mN/m). While, the estimated result of oil spreading method for the cell free medium was about (40mm) the result was great as compared to the biosurfactant produce form glucose as carbon source which didn't show a significant result as shown in figure 2 and 4.

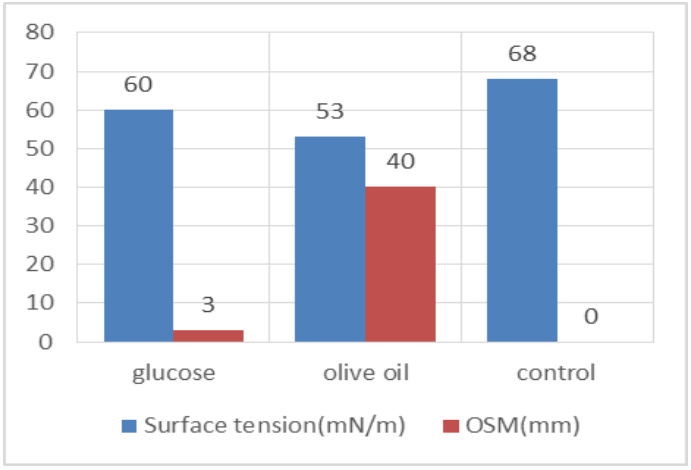

Figure 2. Biosurfactant screening by Surface tension and oil spreading method for the medium that contains glucose and olive oil as carbon source

The estimated result of biosurfactant screening by surface tension reduction and oil spreading method for the cell free medium that contains cooking oil as carbon source also proved the production of biosurfactant the reduction of surface tension was about (54mN/m) and surface oil was spread about (35mm). which also showed good results when compared to the biosurfactant that was produced by using glucose as carbon source as shown in figure 3 and 4.

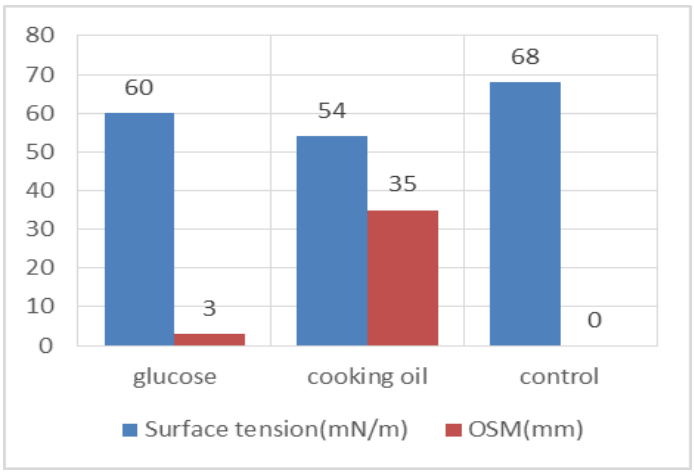

Figure 3. Biosurfactant screening by Surface tension and oil spreading method for the medium that contains glucose and cooking oil as carbon source

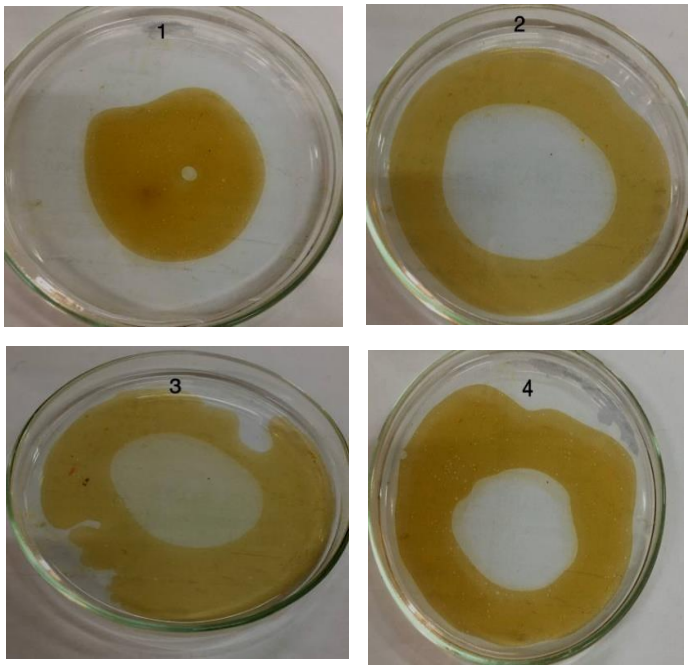

Figure 4. Oil spreading method of the extracellular biosurfactant produced from S. cerevisiae by using different types of carbon sources: 1. Glucose, 2. Crude oil, 3. Olive oil and 4. Cooking oil

The production of extracellular biosurfactant was at best rates by using the insoluble carbon sources (crude oil, olive oil and cooking oil) respectively were better as compared to the medium that contains glucose as carbon source which didn't show any significant results. Oil spreading method was considered the most reliable method for screening biosurfactant production as mentioned by (Hussain and Shawkat et al.,2019,

Dhivya et al., 2014)[15,16]. Hussain Ali, L., and Shawkat Ali, W. A. used different kind of oils which obtained from the local market (olive oil, almond oil, fenugreek oil and castor oil) added to mineral salt medium, maximum activity of biosurfactant was obtained by OSM was (31 mm in diameter) in the presence of olive oil in the production medium [15].

Derguine-Mecheri, L. et al. Was able to enhance the production of extracellular biosurfactant by Rhodotorula sp.YBR by using crude oil as carbon source it was able to reduce the surface tension from72 to 35 mN m−1 and used it later to remove oil from contaminated sand [17]. Olive oil and cooking oil were reliable substrate due to their low cost and good insoluble carbon source to enhance biosurfactant production [18]. While during the process of crude oil biodegradation the enhancement of biosurfactant production occurs [19,20]. In another study glycerol, soybean oil and diesel oil were used to enhance the production of biosurfactant, pH levels and temperatures were optimized to increase the production of extracellular biosurfactant. The results that obtained the maximum emulsifying production were found in the concentration of 5 g L−1 of glycerol, a pH 5.5 and a temperature of 30 °C [21]. Many studies also reported intracellular biosurfactant production by Saccharomyces cerevisiae, such as the study of Raham and Mahmood 2017 [22]. Intracellular biosurfactant was produced by Saccharomyces cerevisiae strains which displace oil about 20 mm by boiling or heat treatment for 30 minutes. While in another study intracellular biosurfactant was produced by autoclave treatment for 20 minutes which displaced oil about 20 mm by Saccharomyces cerevisiae that was isolated from vegetables from Iraqi local markets [13].

4. Conclusion

In conclusion, this study demonstrated that Saccharomyces cerevisiae can produce extracellular biosurfactants with desirable properties when cultivated with various insoluble oil-based carbon sources, highlighting the yeast's adaptability and potential for biotechnological applications. The results revealed that while glucose was ineffective in enhancing biosurfactant production, crude oil significantly outperformed other sources, achieving the highest reduction in surface tension and oil spreading efficiency. These findings suggest that the insolubility of the carbon source plays a crucial role in activating the biosurfactant production pathways in S. cerevisiae. The study underscores the potential for utilizing waste oils in sustainable biosurfactant production processes, contributing to greener industrial practices. Further research should explore the genetic and metabolic mechanisms underlying this enhanced biosurfactant production, as well as the scalability and economic feasibility of using different oil substrates in commercial applications.

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