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Use of *Pseudomonas fluorescens* in the production of single-cell oil and estimate the oil content of fatty acids

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Abstract--The study aimed at producing single-cell oil using *Pseudomonas fluorescens* bacteria and conducting solubility and solubility testing to confirm oil production and estimate the content of oil produced from fatty acids, corn coals were used as a carbon source and applied optimal conditions for the production obtained from the previous study, as used amid liquid corn coals in the production experiment and oil was extracted with the Soxhlet device (Soxhlet)) We obtained the highest oil productivity by 55.47%, the oil produced was fully soluble in chloroform and dissolved to a lesser degree in ethanol and the inability of the oil produced to melt in water, and the oil produced contained the highest percentage of linoleic acid Unsaturated at 69.214%, while stearic acid was 23.62%, palmitic acid 2.92%, propionic acid 1.6% and butyric acid 0.146%.

Keywords--oil, acids fatty, oil production *pseudomonas fluorescens*, single cell oil.

Introduction

Monocellular oils (SCO) are the oils obtained from microorganisms (Yazdanian that the increase in the use of oils for industrial and food purposes led to the search for new microbial sources for it (2011 (El-Naggar *et al.*,... Microorganisms have the ability to produce oils that have advanced advantages compared to soybean and sunflower oils, because it contains fatty acids for any plant or animal such as Gamma-linolenic acid (GLA), Arachidonic acid (ARA) and docosahexaenoic acid (DHA) *et al.* , 2010) Gayathri). Ara and DHA acids in oils produced from

microbiology are very important for human brain development, yazdani *et al.*, 2010 said. In addition, it can be added to some types of infant formula because it increases the growth rate. Chatzifragkou stated that polyunsaturated fatty acids found in oil produced from microbiology are of great commercial importance in preventing atherosclerosis and heart disease and creating conditions that are not suitable for the growth of cancer cells. The state of plant production, especially when using cheap planting circles available in the local environment, in addition, the production of microbial oil is less affected by climatic and environmental conditions, so its production is not determined by a particular agricultural place or separation, in addition there is safety in the absence of contamination of heavy elements and toxic pollutants that can be found in oil extracted from plants due to their use in agricultural processes when developing (Tamilalagan) *et al.*, 2019).

Materials and working methods

Oil Production

The bacterial isolation of *Pseudomonas fluorescens* producing single-cell oil died under optimal production conditions obtained from a previous study, used the liquid medium containing the residues of corn coals and also elected as the best environmental carbon source which consists of 1 g bpton, 20 g corn coals, 2.4 g KH_2PO_4 and 2.4 g K_2HPO_4 in 1000 ml of tweezers. A 250-milliliter conical rotor was used, each with 100ml of liquid medium, pH adjusted at 10 and sterilized in the bumper \times .

Oil Extraction

Use soxhlet according to A.O.A.C., 1990) to extract oil from biomass and the center of production, if you put 20 milliliters of sample in Thimble inside the saxon system and then add 500 milliliters of solvent (oil ether) in the device's course, and adjust the temperature of the device at (60) m $^\circ$. The oil extraction process lasted approximately 4 hours until the yellow oil was not colored at the top of the device and collected in the dourq, after which the oil extracted with the solvent used was withdrawn and transferred to separation funnel and separated the oil in BakerBeaker known weight and then placed in the electric oven for an hour and at a temperature of 60 m $^\circ$. To ensure evaporation of the residue of the solvent and the disappearance of its smell, then go out and leave until cooled and then weigh using the same balance that weighed it before putting oil in it, and extracted the fat ratio according to the following equation:

$$\%100 \times \frac{\text{weight of blanked beaker} - \text{Weight beaker with oil}}{\text{weight of the sample}} = (\%)Oil$$

Confirmed tests of oil produced

Solubility test

The oil produced was distributed at 1 milliliter per glass test tube and three pipes containing water, ethanol, chlorform and the pipes were moved, after

which the nature of the melting of the oil produced in each Murugan *et al* pipeline was observed. , 2012).

Saponification test

This test was conducted by adding 2 ml of NaOH sodium hydroxide solution at a concentration of (2%) to 3 ml of oil produced from bacteria in a test tube to observe the formation of a soap solution (Murugan *et al.* , 2012) .

Estimate of fatty acids in oil produced **Estimate of long-chain fatty acids**

Long-chain fatty acids were estimated according to the standard method (Raslan, 2010), if the size of 200 microliters of bacterial oil was taken and added 2 ml of potassium hydroxide (KOH) with concentration M) 4) and put the mixture in a rocking water bath at a temperature of 50 m° for 30 minutes and left to cool at laboratory temperature, after which he was added 1 ml of distilled water and placed on the foam for one minute, Then add 1 ml of hexan n-Hexan solvent and place the centrifuge on 10,000 rpm for 10 minutes, The upper layer (organic) was then withdrawn and filtered with Millipore Filter membrane filters measuring μ 0.45 and injected with the gas chromatography device (GC in which the Column column type (DN 10) was used, and the analysis conditions were Flow rate: 1 ml/min and temperature 105 M° for one minute and then fixed at 220 m° and pressure 4 psi). The area occupied by each long-chain fatty acid in the sample was obtained through the device and compared with the area and standard pre-prepared concentration of each fatty acid to obtain the concentration of fatty acids in the sample.

Estimate of short-chain fatty acids

Short-chain fatty acids have been estimated according to the Torii *et al method*. Standard 2010, If he took the size of 1 g of bacterial oil and added 3 ml of hexan solvent n-Hexan and put on the concussion for 3 minutes, then put in the centrifuge for 10 minutes on a rotation score of 10,000 cycles / minutes and pulled the chip layer and dried with nitrogen gas, then added 1 ml of methanol and injected with a device (HPLC) with which he used mobil phase detector: Water (0.1% H₃PO₄) and column column used in the device were type (4.6mm* 250 mm *5um) C18, analysis conditions flow rate:0.8 ml/min, temperature 30 m° and Detector: UV-210 and Injection volume: 20 μ ll . Through this device, the area occupied by each short-chain fatty acid was obtained in the sample and compared with the area and standard pre-prepared concentration of each fatty acid to obtain the concentration of fatty acids in the sample.

Results and Discussion

Single-cell oil produced

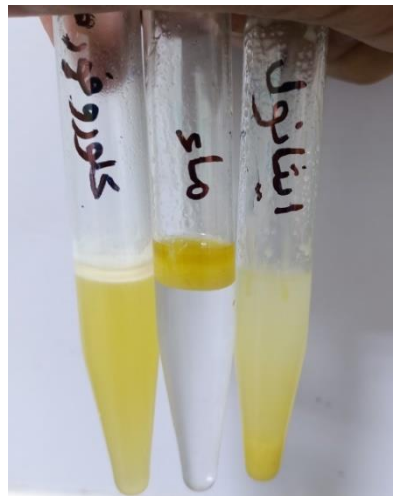
The productivity of oil after 48 hours, using the middle of corn and *bacteria Pseudomonas fluorescens*, was 55.47%, which is good compared to similar studies. This finding agreed with gaykawad *et al.* (2021) findings which produced

single-celled oil from the nematodes *Mucor circinelloides* and *Mortierella alpina* and the oil ratio for the product was 54.1% and 55.8%, respectively.

Confirmed tests of oil produced

Solubility test

After the process of extracting single-cell oil, the oil solubility test produced from *Pseudomonas fluorescens* bacteria showed high meltability in chloroform solution and less ethanol lysis, while no water solubility shown in form (4-3). These results are due to the insolubleness of oil in polar solvents such as water and its meltability in non-polar organic solvents such as chloroform and ethanol (Zhao *et al.* , 2022) .



Form 1. test the melting of single-cell oil in water, ethanol and chloroform

Saponification test

The result of the soap test for the oil produced showed that it is a soapy solution as shown in the figure (4-4). This result is generally due to the susceptibility of oils to the composition of soapy solution when interacting with maotsela *et al.* , 2019) .



Form 2. one-cell oil greenhouse test

Fatty acids in oil produced:

The oil produced from *Pseudomonas fluorescens* bacteria was liquid at room temperature, yellow (shape 3) and unsaturated type. The results of the analysis of long-chain fatty acids using GC and short fatty acids using HPLC (Table 1) showed that the oil produced contained the highest percentage of unsaturated linoleic acid and a rate of 69.21 4% while stearik acid was 23.62%, palmetic acid 2.92%, propionic acid 1.6% and butarik acid 0.146%.

Table 1
Oil content produced from fatty acids andtheir proportions

to	Fatty acid	Percentage (%)
1	Linoleic acid	69.214
2	Stearic acid stearic acid	23.62
3	Palmitic acid palmitic acid	2.92
4	Propionic acid propionic acid	1.6
5	Butaric acid butaric acid	0.146



Form 3. oil produced from *bacteria Pseudomonas fluorescens*

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