Isolation and diagnosis of SCO-producing bacteria and improved optimal production conditions

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Abstract:

This study was carried out for the purpose of isolating and diagnosing oil-producing bacteria from different soil samples in Anbar province and determining the optimal conditions for production. Used for isolation amid oil dens, if 10 bacterial isolations were obtained, four of which were characterized by their ability to collect oil at a high rate and used the black sudan dye B to choose the most efficient isolation of them, namely isolation U isolated from soil planted with bamia plant, then diagnosed isolation through morphological and chemical tests and showed that it belongs to the bacteria *Pseudomonas fluorescens* . The results showed that corn coals were the best carbon source in oil production and that 0.1% of the nitrogen source was the best in oil production at 40.3%, The best hydrogen number at 10 was an oil production rate of 47.5%, the best lap temperature at 30 m if the oil ratio was 50.7%, and the best concentration of carbon source 2% with an oil production rate of 50.7%, The pepton was the best nitrogen source used in oil production with a 48-hour lap with a production rate of 50.7 percent.

Keywords: *Pseudomonas fluorescens* , Oil, Corn cobs, Oil Production , Single Cell Oil
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Introduction:

SCO single-cell oils are oils produced by microbiologists that are stored inside microbial cells or secreted in the middle of production andconsist of tag trichloride **(**Kapahi *et al.* , 2021**).** Oily microorganisms have been known to collect a minimum of 20% of their biomass as those of Patel *et al.* , 2020) **) .** The property of producing or assembling oil is relatively rare, as less than 100 types of yeasts, rots, bacteria and algae are oil-producing Ma *et al.* , 2018) **).** These organisms differ in their ability to collect oil within them as some microorganisms are able to collect 25% of their dry weight, while others are able to produce 50% of the oil and assemble it and very few are able to assemble approximately 80% oil (Behera *et al).* , 2019**).** Oil-producing microorganisms begin to collect oils within their cells under special conditions, including a necessary ingredient, usually nvad, the source of nitrogen and sometimes phosphorus. (Ukegbu *et al.* , 2021) The aggregation of oils in oil-producing microbes occurs at the growth stage and when the necessary nutrients are available and when nitrogen becomes limited and the source of carbon is abundant, microbes go through an unbalanced growth phase known as Idiophase production phase and during this phase the reproduction stops due to the lack of nitrogen and the lack of protein construction and therefore cells tend to continue to use the available carbon and assemble it oil by possessing an enzyme Adenosine triphosphate citrate lyase stimulating the production of Acetyl Co enzyme, which is essential in the formation of fatty acids and thus the formation of oils that use it as an energy storage material to take advantage of them in the post-growth stages Liu *et al.* , 2021)). Some studies have also indicated that the presence of phosphorus, magnesium, zinc or iron elements can lead to increased oil accumulation in oil-producing microbiology cells Dzurendova *et al.* , 2020b) **).** The oil production process

by oil microbes is due to the genetic and genetic traits they possess or obtain through gene transfer and cell fusion ko *et al.* , 2020) **).**

Materials and methods:

Isolation of Oil Production Bacteria:

Twenty-four soil samples were collected from different areas in Anbar province , including soil contaminated with oil and soil grown with various field crops in order to isolate oil-producing bacteria from the soil. 10 Each was placed in sterile petri dishes and then added amid the sterile oil dens, which consists of 40 g pepperon, 5 g sucrose, 15 g acar and 5 ml sunlight oil in 1000 ml of distilled water and moved the dishes towards and reverse the clock hand for the purpose of ensuring the distribution of the sample harmoniously and broodingat 30 m temperature for 72 hours was re-purified those isolations . Sub-culturing) with several moves on the center of the oil dens to get pure single colonies.

Sudan Black B stain efficiency test:

The elected and oil-producing insulation was planted on the center of the nourishing dens in the form of a circle of diameter (1 cm) in the center of the dish and a brood for 48 hours at a temperature of 30 m, after which the surface of the dish was flooded with the solution of sudan black dye B and prepared with melting (1 0.3 g dye powder in 100 ml of ethanol alcohol at a concentration (70%) and left the dishes for (30 minutes) after which the dish was gently washed by ethanol concentration alcohol (96%) Murugan *et al.* , 2012)) .

Identification of Bacterial Isolates:

She was diagnosed with the highest productivity of the elected bacterial cells based on their appearance qualities (colony shape, edges, height, color and strength) and then studied the characteristics of microscopic bacterial cells after being diagnosed with Gram's stain, according to Holt *et al*., (1994), and conducted chemical tests of these isolations for diagnosis (MacFaddin, 2000). The diagnosis was then confirmed bythe use of the Vitek 2 compact system equipped by the French company BioMerieux.

Elect the best local carbon source for oil production:

To determine the best local carbon source for oil production, three local carbon sources have been used: crack, date pepper and corn. If prepared in the middle of the crack and the middle of the dens of the dates and the middle of the corn-coated dens by adding 2 g of corn and pepper dates and 2 ml of shrank to 4 g pepper, 0.5 g sucrose, 1.5 g dens in 100 ml of distilled water and then sterilized the circles with the bumper except the crack if sterilized with a jacket The circles were poured after cooling in petri dishes and left to harden, then planted the elected bacterial isolation by making a circle of 1 cm in diameter in the center of the dish at three duplicates per source and incubated at a temperature of **30** m^{o} and for 48 hours, then elected the best successor as a carbon source for oil production according to the measurement of the countries of the bacterial colonies.

Improving optimal environmental conditions for the growth of elected bacterial isolation and producing single-cell oil:

The liquid medium containing corn-coated residues was used as the best environmental carbon source of **20 g bp, 20 g corn coal, 2.4 g** KH_2PO_4 **and 2.4 g** K_2HPO_4 **in 1000 ml of tweezed water** in determining the factors affecting the production of single-cell oil, using a 250 ml conical rotor placed in each 1000 ml. A liter of liquid medium and sterilized the circles in the bumper. Soxhlet **by** A.O.A.C., 1990.

Nitrogen source concentration:

Use pepton as a nitrogen source to produce oil if added to the liquid medium at differentratios (1.5,1.0.5,0.25.0.1 %) to determine the optimal concentration of oil production.

PH:

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Adjust the pH of the medium of growth on each of the following hydrogen figures , 10,9,8,7,6,5(11) to determine the optimal pH for oil production in the liquid medium.

The size of themeeting h:

Various vaccine volumes were used for the purposes of inoculation of the liquid medium of the elected pect isolation to test the effect of the size of the vaccine added in the production of single-cell oil and the volume of the vaccine used was4,3,2,1,0.5 m/l**/**100 ml medium**.**

Temperature:

I tested different temperatures of 45,40,35,30,25,20)) to determine the optimum temperature for oil production in the middle of liquid production.

Carbon source concentration:

Corn conch was chosen as a local carbon source for the production of single-cell oil because it gave the best production of single-cell oil andhigher than the rest of the residues (crack and date pepper) when sifting between wastes using solid circles and itis available as agricultural residues in abundance and obtained it is not economically expensive as it was added to the middle byratios Different 5,4,3,2,1,0.5%) to determine the optimal concentration of oil production.

Nitrogen source type**:**

For the purpose of determining the best nitrogen source for oil production, various sources of nitrogen were used, including organic aldr sucking, namely pepton,urea, and anorticdraconian nitrate and ammonium sulphate.

Incubation duration:

I tested the elected oil-producing plant on the liquid medium containing corn coals as a carbon source, at temperature, pH and concentration identified in previous steps and the type and concentration of nitrogen source and fora different lap length (24,48,72,96 120,) an hour to get the best lap duration for oil production. .

Results and Discussion: Results and Discussion

Isolation of oil-producing bacteria:

The results of the initial insulation shown in table (1) showed the acquisition of 10 bacterial isolations capable of growing highly efficiently on the center of oil out of 35 bacterial isolations isolated from 24 soil samples brought to the laboratory, and these isolations varied in their ability to collect oil based on the density of their growth on the center of the insulation after the transplant and incubation of those bacterial isolations on the middle of the oil densities used as a source of carbon and energy the colonies appeared in clear growth diameters on this medium Bacteria showed a high susceptibility to analyzing oil in the middle and exploiting it as a single source of carbon and a variation in the diameter of the colonies appeared on the said medium. The different growth of these insulation may be due to their varying susceptibility to oil consumption as a single source of carbon and energy, as well as to the nature of the source from which these bacteria have been isolated.

Four isolations were elected that were the most efficient in oil analysis and assembly, which bore local symbols U-X3 N2-D2 and had a growth rate on the center of the oil dens (8-8-8.4-8.5 cm) respectively.

Table (1) Rate of insulation growth diameters on the center of insulation (cm)

1	D2	8.5
2	H1	4.8
3	L	5.5
4	N2	8.4
5	P2	6.5

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6	T3	7.5
7	U	8.1
8	X1	7.2
9	X3	8
10	Z2	7

Testing the efficiency of elected isolations using Sudan's black B dye **:**

The most efficient isolation in the collection of oil was elected using the black sudan b dye, if the four elected isolations were replanted on the center of the nourishing dens by a circle of 1 cm in the center of the dish and incubated the dishes for 48 hours and at the temperature of 30 m after which the surface of the dish was flooded with black Sudan B dye for 30 minutes and then washed dishes with ethanol (96%) the isolations appeared in a dark blue color slanted to black color and the more intense the color indicated the efficiency of isolation in the oil, The isolation with the local symbol U was elected out of the four elected isolations (Figure 1) depending on the severity of its pigmentation and its appearance in dark blue, which is black . , 2014; Kulvinder and Bishnoi, 2016).

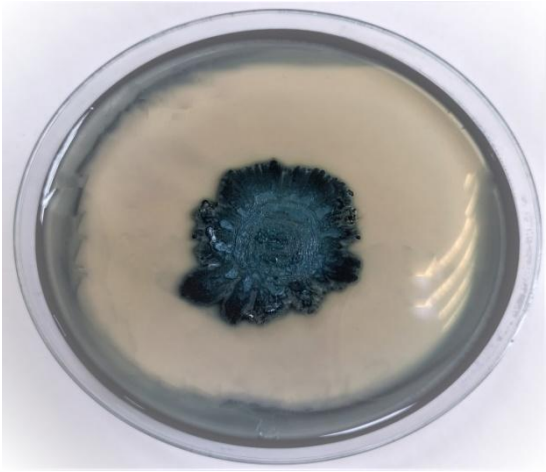

Figure 1 Dyes The Elected U Isolation with Black Sudan B

Diagnosis:

The results of transplantation, microscopic and chemical tests of elected bacterial isolation based on Holt *et al*., (1994) showed that the bacterial isolation that gave the local symbol U has the characteristics of *pseudomonas fluorescens* table (2) and (3), this was confirmed by the diagnosis of isolation using the Vitek VITEK2 Compact.

Table (2) Implant and microscopic specifications for elected bacterial isolation.

Shape	**Color**	**Textures**	**Edge**	**Transparen**	**Height**	**Bacillus**	**Single or double**	**Negative**
Pie	**White**	**Snotty**	**Pretty**	**Dark**	**Convex**			

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Table (3) Results of chemical tests for elected bacterial isolation.

1	Catalase	+
2	Oxidase	+
3	Indole	-
4	Methyl red	-
5	Voges- Proskauer	-
6	Motility	+
7	Urease	+
8	Simmon Citrate	+
9	Hymolysis	γ
10	Gelatin fill	+
11	Arginine Analysis	+
12	Growth on the center of the Maconkey	+
13	Growth on the center of the Acar straimide	+
14	Production of bioverdin dye	+
15	Biosianin dye production	-
16	Growth at 4 m temperature	+
17	Growth at 42 m temperature	-

(+) positive for testing (-) negative for testing (γ) is not analyzed by blood

Electing the best carbon source in oil production:

The results shown in figure 2 showed that the isolation of *Pseudomonas fluorescens* elected as the most efficient oil production isolation in its ability to exploit the used carbon sources of the spray, corn and dates, was found to be the best carbon source used in oil production, which gave the highest rate of bacterial colony diameter and reached (8.5 cm).

This result is due to the high content of corn coals of fat, which bacteria can analyze and assemble in the cell in the form of fatty acids, in addition to the fact that corn coals are characterized by a high percentage of C/N ratio, which is an important indicator required by the process of producing single-cell oils Prabhu *et al.* , 2019)) . These results agreed with what ma *et al*. (2014) to use corn coals as a carbon source in the production of oil from *cryptococcus curvatus* yeast . (2021) produced for oil by *mortierella isabellina* mushrooms and using corn quails sourced with carbon.

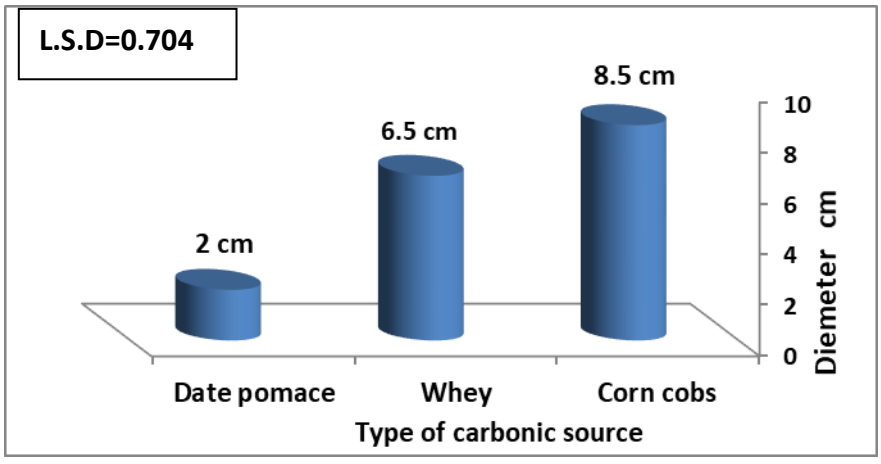

Figure (3) Rate of bacterial colony diameters on different carbon sources (cm)

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Improving optimal environmental conditions for the growth of elected bacterial isolation and producing single-cell oil:

Nitrogen source concentration:

The concentration of the nitrogen source in the middle of production is one of the factors that determines the success of the oil production process depending on the method of obtaining it and its availability, and the results shown in (Figure 4) obtain the highest productivity of single-cell oil at concentration 0.1% and the oil production rate was 40.3% after a lap of 48 hours at pH 7 and temperature of 30 m, while oil productivity decreased at other concentrations. These results are due to the fact that reducing the nitrogen source leads to the induction of oil production by oil-producing bacteria, while reducing the nitrogen source and the abundance of carbon source in the productive environment halt the division of oil-producing bacteria cells and convert carbon into oil stored in their gaps by possessing adenosine triphosphate citrate lyase, a catalyst for the production of Acetyl Co enzyme, which is essential in the ironing of n Fatty acids and thus the formation of oils (Leong *et al.* , 2018).

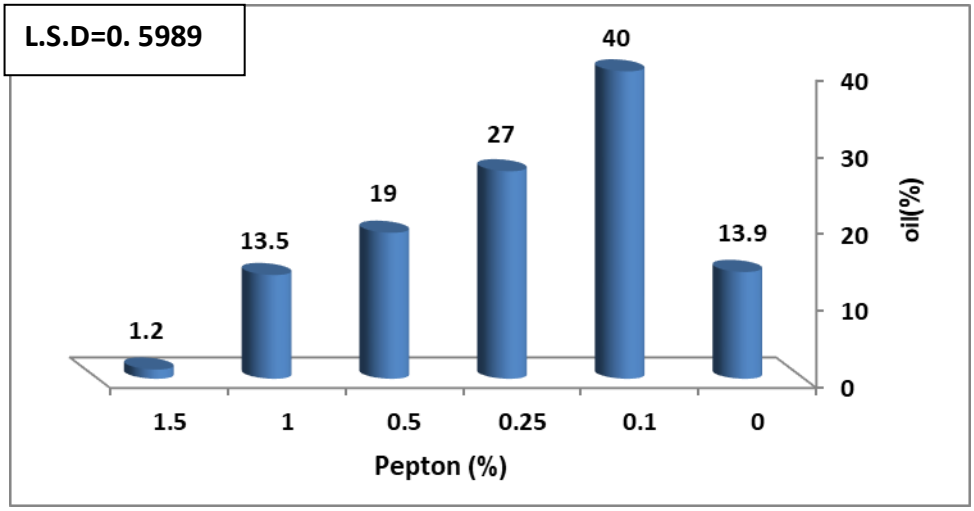

Figure 4 The effect of nitrogen source ratio in the production of single-cell oil

PH:

The results shown in figure 5 showed that the productivity of oil using *Pseudomonas fluorescens* bacteria at pH 10 gave the highest production compared to the rest of the other hydrogen figures, if the proportion of oil produced was 47.5% followed by The hydrogen number 9 was the oil production ratio of 45.5% while there was a decrease in oil productivity when other hydrogen figures. The pH is one of the factors specified in oil production through its effect on the melting of food and its readiness to the microorganism produced, Because this will reflect on the growth of microbiology and lead to its production of oil, as well as the pH of the medium of production affects the enzyme processes and the transfer of nutrients and various substances through the cellular membrane and affects the metabolism of the microorganism produced, the pH must be compatible with the highest level of metabolism of the microorganism produced to obtain the best oil production (Moradi *et al.* , 2020).

This result is almost consistent with jiang and Chen's findings (2000), which was able to produce oil from *crypthecodinium cohnii* microalgae at different hydrogen figures ranging from (4-10).

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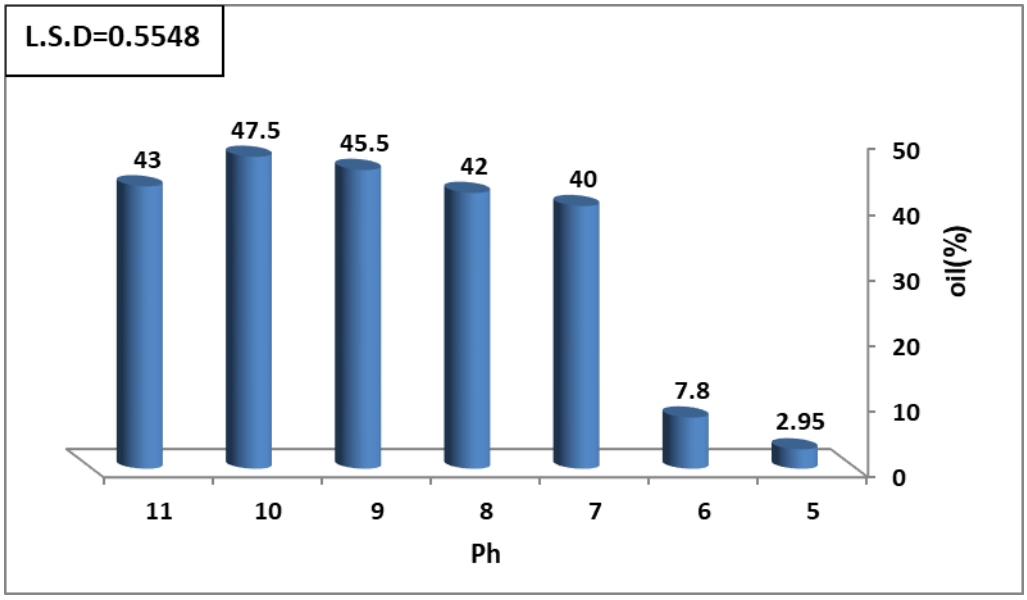

Figure (5) PH effect in the production of single-cell oil

Vaccine size:

The results shown in figure 6 show that the highest oil production of *P.fluorescens* bacteria when adding the size of the vaccine is 3 ml/100 ml medium density $9\times^{7\text{-}10}$ colonies/ml and the oil production rate was 50.7% after 48 hours and then the production rate decreased when the size of the vaccine increased or decreased further, The lowest oil production when using the vaccine volume was 0.5 ml/100 ml medium and the oil ratio was 18%. Zainuddin *et al.* (2022) The best volume of a vaccine for the production of oil from *Yarrowia lipolytica* yeast was when using the size of a vaccine of 10 ml/100 ml medium. The low oil production at the few volumes of the vaccine is due to the fact that the low concentration of the vaccine is not enough to give a vital mass of bacterial isolation and therefore negatively affects oil production, but the decrease in oil productivity with the increased volume of the vaccine used may be due to the state of intense competition for nutrient exploitation in the middle (Cantu-Jungles and Hamaker, 2020)). Moreover, the volume used in the vaccine should be proportional to the amount of the medium to the size prepared for fermentation, which will reduce the phase of printing and start production (El-Mansi *et al.* , 2018).

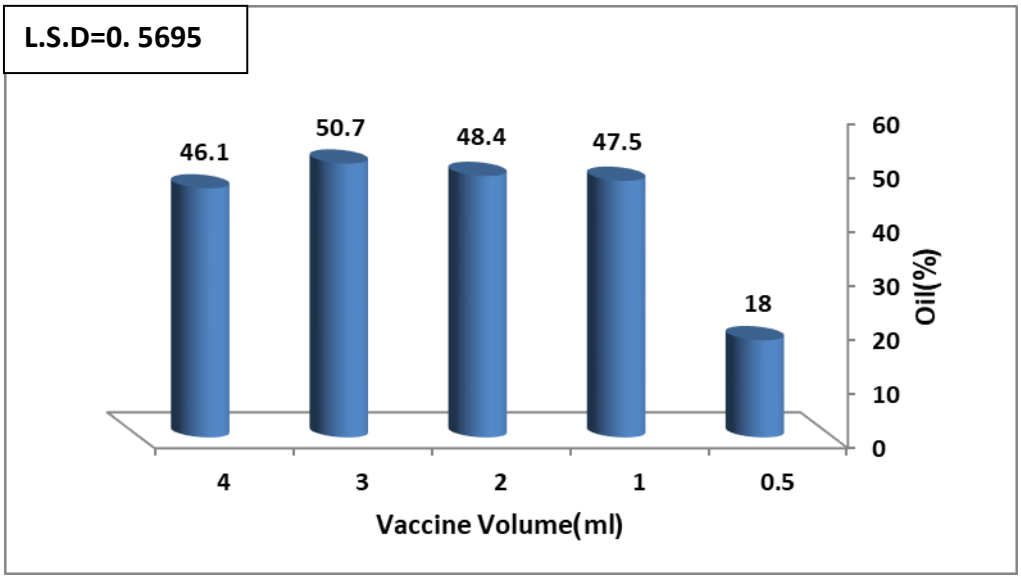

Figure 6 Effect of vaccine size in single-cell oil production

Temperature:

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The results shown in Figure 7 showed that the highest production of single-cell oil from *P.fluorescens* bacteria was at 30m, if the proportion of oil produced was 50.7% of the average production. Increased productivity of single-cell oil due to lower temperatures if increased oil production is associated with an adaptive response to the cellular membrane when temperatures drop to a certain extent that is compatible with the nature of the source from which the bacteria were isolated, which increases the production of unsaturated fatty acids to maintain On the fluidity of the cellular membrane (Degreif *et al.* , 2017) . The results of this study are consistent with those of Rasouli *et al.* (2021), which received the highest production of single-cell oil at a temperature of 30 m using *Kocuria sp bacteria.* . as consistent with zainuddin *et al.* (2022) which obtained the best oil production through the development of *yeast Yarrowia lipolytica* at a temperature of 30 M. The temperature of the brood is a determinant of the growth of the microorganism and oil production, as there is a thermal range at which oil is produced, depending on the microorganism produced and the sources of access and the middle of production.

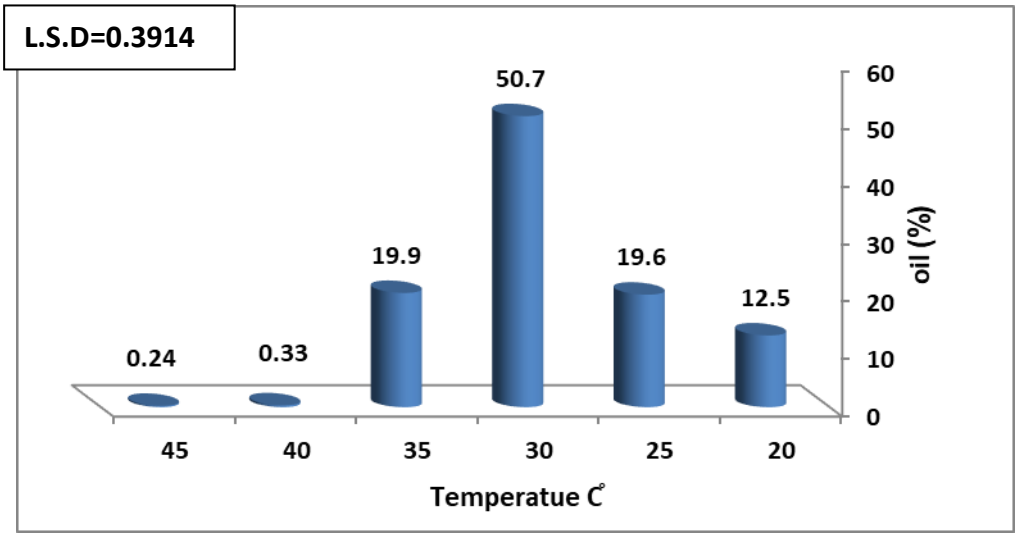

Figure (7) Temperature effect in the production of single-cell oil

Carbon source concentration:

The results shown in figure 8 showed that the best production of single-cell oil was at 2% of corn coals elected as the best carbon source of oil production, oil production was 50.7% after 48 hours and at pH 10 and temperature of 30 m. While the percentage of oil produced at other ratios decreased and the oil production was 11.4% at 5% of the carbon source. These results are due to the compatibility between the The carbon source (corn coalt) with the density of microbiology used in production, in order to suit the nutritional requirements and to obtain the energy sources of the producing microorganism. While when adding high percentages of corn coefficient, this acts as a inhibitor for the production of oil from microbiology, because this leads to the preoccupation of all effective sites of enzymes produced with proteins and amino acids and the surplus of the base material remains not available sufficient amount of active sites in the enzyme Izadi *et al.,* 2020).

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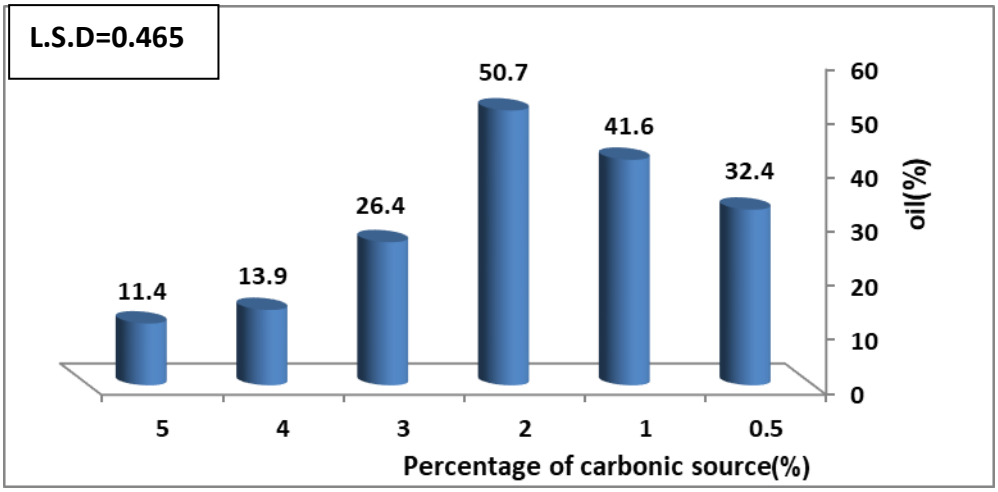

Figure 8 Effect of carbon source ratio in single-cell oil production

Nitrogen source type:

The results shown in table 9 showed that the bacteria used *Pseudomonas fluorescens* gave the highest oil production of 50.7% using pepton as a nitrogen source, while oil productivity decreased when using an inorganic source such as ammonium sulfate to reach 38.3% oil, oil productivity decreased further when using nitrogen-sourced urea to 32.2% and was 24.6% less productive when using sodium nitrate as a nitrogen source . (2020) The pepton is an important source of nitrogen that it used to obtain the highest oil production when developing *Trichosporon mycotoxinivorans* yeast , because it provides organic growth factors that bacteria need for better growth in addition to being the main source of nitrogen.

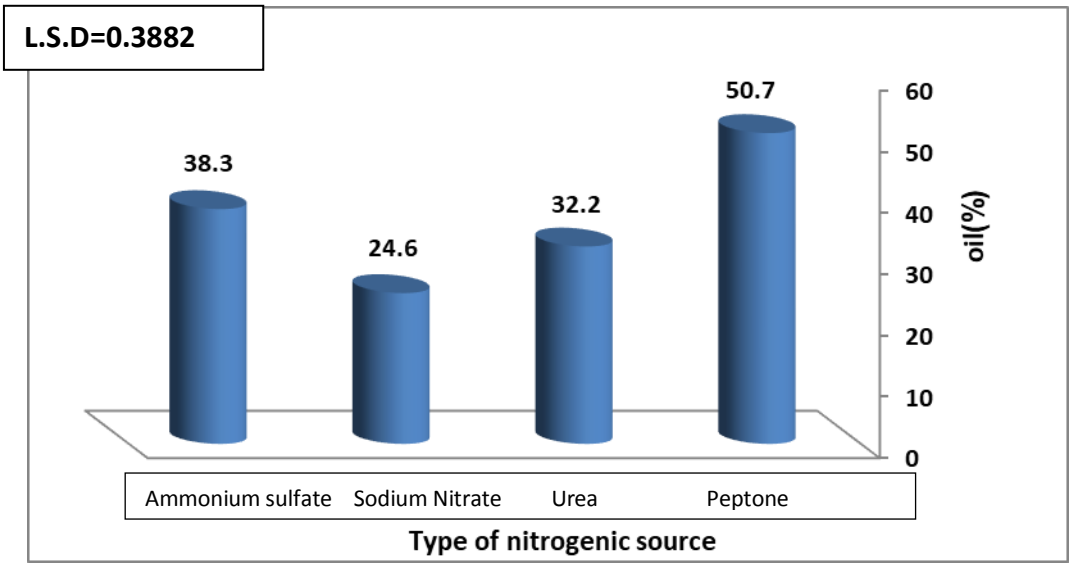

Figure (9) The effect of nitrogen source type in the production of single-cell oil

Incubation duration:

Among the figure (10) of bacterial isolation *Pseudomonas fluorescens* that the best production of single-cell oil was after a period of 48 hours if the oil production rate reached 50.7% at 30 m temperature and at pH 10 while the percentage of oil production decreased when the duration of the lap increased. The decrease in oil production when the incubation period increases is due to the depletion of nutrients and the secretion of toxins in the middle, and the increase in the duration of the incubation leads to environmental changes in the middle such as pH change and izmosian pressure, as well as the possibility of self-decomposition of cells and the accompanying release of metabolic substances that adversely affect the production of oil Villarreal(Soto *et al).* , 2018)). These results are consistent with what Röttig *et al*. (2016)

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The best production of single-celled oil from *bacillus subtilis* bacteria was after 48 hours of incubation . (2021), which explained that the best oil production was after a 48-hour lap by *kocuria sp bacteria.* .

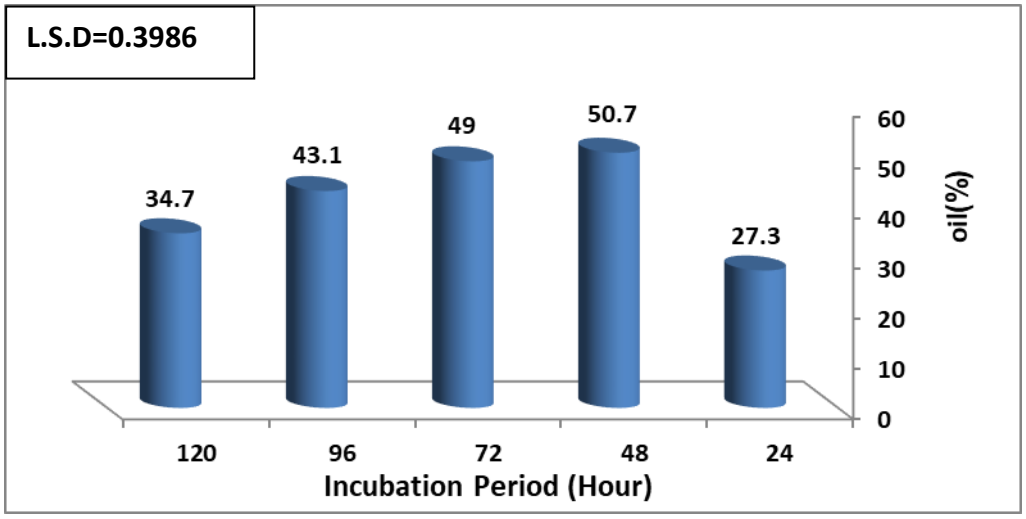

Figure (10) Effect of incubation duration (hour) in the production of single-cell oil

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