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DETERMINATION OF OPTIMAL CONDITIONS FOR THE PRODUCTION OF SINGLE CELL PROTEIN (SCP) BY *PSEUDOMONAS AERUGINOSA* BACTERIA USING HYDROCARBON RESIDUES (USED MOTOR OIL)

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ABSTRACT : In this study, an isolating and diagnosing of *Pseudomonas aeruginosa* from soil contaminated with hydrocarbons from the oil reservoir in Ramadi city have been done. Testing of the efficiency of this bacterial in analyzing the used motor oil as the sole source of carbon, energy and production of protein-rich biomass. Bushnell-Haas Media (BHM) broth and submerged culture techniques are used in determining the optimal conditions for biodegradation of used motor oil and (SCP) production. Different pH concentrations were used (6, 7, 8, 9, 10) with different temperatures (25, 30, 35, 40, 45°C). As well as different concentrations of the carbon source, which is represented by used motor oil (1.5, 3, 4.5, 6, 7.5 ml) per 100 mL of BHM. The bacterial inoculum was added by (0.5, 1, 1.5, 2, 2.5, 3 ml) per 100 ml to BHM medium. As a source of nitrogen, ammonium sulphate $(NH_4)_2SO_4$ and Urea $CO(NH_2)_2$ were used separately and Pepton was added to support the BHM media, incubated flasks for (24, 72, 120, 168, 216 hour). The results showed that *Ps. aeruginosa* has the potential to grow in BHM agar and BHM broth, which is added 3% of the used motor oil as the sole source of carbon and energy. The amount of oil is gradually faded from BHM until it completely disappeared and the results showed that the best production of biomass reached (19.6 g/l) at pH (8), temperature (35°C) and concentration (3%) of used motor oil.

Key words : SCP, hydrocarbon residues, *Pseudomonas aeruginosa*, used motor oil, biodegradation.

INTRODUCTION

Hydrocarbons and petroleum products are among the most important sources of energy needed for different industries and daily life. Due to the importance of these hydrocarbons, they have become an important source of energy, resulting in a large number of residues in the environment, causing many negative environmental impacts (Das and Chandran, 2011; Ahmed *et al*, 2017). The pollution of hydrocarbon residues in different environments is a problem that has been noticeable for years, resulting in the loss and destruction of biodiversity in the environment contaminated by these residues, prompting the public and especially researchers to find a solution to this problem (Kothari *et al*, 2013).

Biodegradation is one of the safest means of removing hydrocarbons from the environment compared to other methods such as chemical treatment, washing and burning, which produce more toxic products from the same hydrocarbon residues. Biodegradation includes the use of microorganisms such as bacteria, fungi and yeasts to remove these hydrocarbons through the ability of these microorganisms to analyze and dismantle hydrocarbon residues, having a variety of metabolic pathways that enable them to exploit these residues as a source of carbon and energy. This technique is a sophisticated and inexpensive way to remove and analysis of many environmental pollutants, including hydrocarbon contaminants (Medina-Bellver *et al*, 2005).

The hydrocarbon residues in the production of (SCP) are widely used through the use of microorganisms with the ability to analyze hydrocarbon residues and use it as a source of carbon and energy. Microorganisms contain a high percentage of protein and amino acids, which are the product of growth of these microorganisms after cracking hydrocarbon residues and exploiting the resulting carbon (Abduljabbar *et al*, 2009).

Pseudomonas aeruginosa is one of the most famous bacterial species in the analysis of hydrocarbon residues because it has the necessary enzymes to metabolize hydrocarbons and release them to biosurfation, which helps dissolve hydrocarbon residues and then exploit carbon as a source of carbon and energy for the growth of this species (Adegbola *et al*, 2014). As Rath *et al* (2016), Abdul-Qader *et al* (2015) point out the ability of

Pseudomonas aeruginosa bacteria in the Biodegradation of hydrocarbons, this bacterial type was widely studied for its ability to decompose hydrocarbons and produce Biosurfaction which increases the solubility of hydrocarbons and disintegrates the bonds between their atoms and thus decomposes them and uses the resulting carbon to grow.

Submerged culture was also used in the production of biomass by *Pseudomonas aeruginosa* bacteria, crude oil was used as the sole source of carbon and energy, the production rate was (0.45 g/l) of biomass after 7 days and the decomposition rate of crude oil was 55% (Subathra *et al*, 2013). Hydrocarbons were used to produce biomass by motor oil was used as the sole source of carbon and energy for the growth of *Pseudomonas* sp. Biomass production and submerged culture, these bacteria have been shown to be able to analyze motor oil and the resulting carbon consumption for growth and increase the number of cells and thereby increase biomass resulting from growth (Ramadas *et al*, 2018). The use of hydrocarbon residues in the production of (SCP) can reduce environmental pollution and increase the supply of protein with food supplements or animal feed. It is known that hydrocarbon residues is one of the most dangerous environmental pollutants, especially when left untreated. Residues in the production of (SCP) by bacteria will rid the environment of these residues and recycle them while at the same time producing biomass with high nutritionalvalue through microbial fermentation (Spalvins *et al*, 2018).

The bacterial (SCP) produced from hydrocarbon residues is highly amino acids, although there is little deficiency in some sulfuric amino acids. The bacteria content of the protein is very high up to more than 80% and the content of the nucleic acids especially the RNA sometimes about 20%, which requires some treatments to reduce before use in nutrition (Abduljabbar *et al*, 2009).

MATERIALS AND METHODS

Preparation of Bushnell-Haas Media (BHM)

Bushnell-Haas media preparation can be doneby dissolving the following salts one by one in a liter of distilled water and these salts are: (1g) of KH_2PO_4, (1 g) of $(NH_4)_2SO_4$, (1 g) of KNO_3, (0.2 g) of $MgSO_4.7H_2O$ (0.05 g) of $FeCl_3$. This medium was used to determine the susceptibility of bacterial isolates to the consumption of hydrocarbons after adding hydrocarbon compounds to the media as a single source of carbon and energy (Malatova, 2005).

Isolation and Diagnosis of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa bacterial type was isolated from the soil contaminated with hydrocarbons in the oil reservoir in Ramadi. The primary screening of contaminated soil samples was carried out and decimal dilutions have been made and culture (1 ml) of the sixth dilution on the BHM containing (3 ml) of the used motor oil as the single source of carbon and energy.Secondary screening and purification were conducted to obtain efficient bacterial isolates in the analysis of hydrocarbon residues. These bacteria were identified based on cultural characteristics and biochemical tests.

Preparation of Bacterial Inoculum

A (100 ml) of Nutrient Broth was incubated in a (250ml) flask and adjust pH to (7) and sterilize for (15) minutes at (121°C) and Pressure (15 pound/ang^2) after cooling to (30°C). It was cultured by pure colonies of *Pseudomonas aeruginosa* by sterile loop and incubated for (24 hours), the growth is observed through the center-formed turbidity and then stored in the refrigerator at a temperature of 4°C for use in future experiments.

Pseudomonas aeruginosa Efficiency test in Hydrocarbon Residues analysis

Colonies of the bacterial type *Pseudomonas aeruginosa* was cultured bya sterile looponBHM agar in a circle (1 cm) in the middle of the Petri dish, then taken the (3ml) hydrocarbon residues (used motor oil) dissolved in the diethyl ether with a concentration of (5%) sterilized by filtration was added to the BHM agar to form a thin layer above the media surface and incubate the dishes in the incubatorat (30°C) for (72 hours).

Determination of optimal conditions for growth of *Pseudomonas aeruginosa* and (SCP) production

A (100 ml) of BHM broth has been added in (250 ml) flasks. Then a 3 ml of hydrocarbon residues (used motor oil) added which sterilize by filtration and solvent in diethyl ether at a concentration of (5%) as a single source of carbon and energy. The bacterial inoculum of *Ps. aeruginosa* (1 ml), incubate the flasks in the shaking incubator at (100 rpm) and at (30°C) for 120 hours. Determine the dry weight of the biomass after centrifugation by using centrifuge at (4000 rpm) for 15 min. The media has Filtered with filtration papers and the precipitate has taken and dry in ovenat (60°C) for (24 h). The biomass is weighed by a sensitive balance to estimate the dry weight of the biomass, which represents the final product of single cell protein (Aboud *et al*, 2017).

1. **pH :** Several grades of pH (6, 7, 8, 9, 10) were used with a single degree difference using NaOH and HCL diluted to modify the pH. The dry weight of biomass was measured as mentioned above.

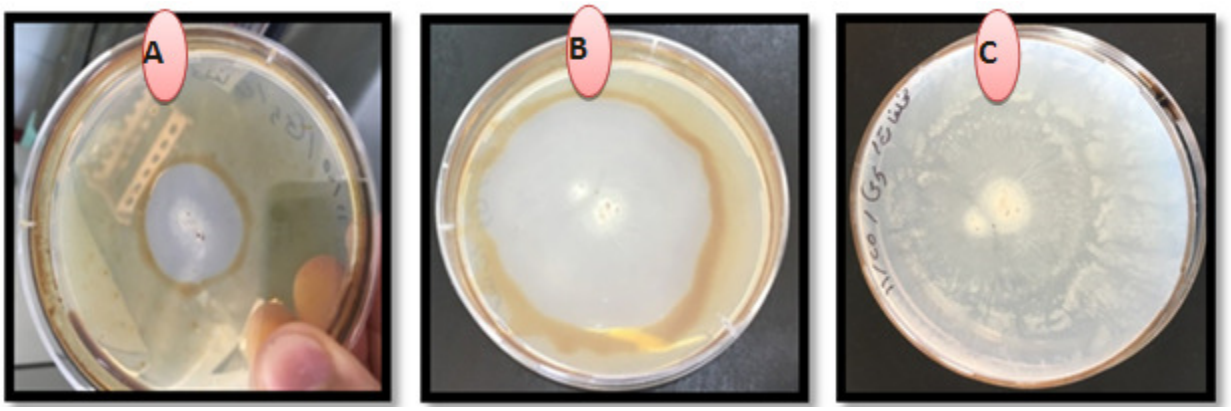

Photo 1 : Formation of Clear Zone around colonies *of Pseudomonas aeruginosa* colonies planted on the center of BHM Agar with addition of used motor oil as the single source of carbon and energy at (30°C) and pH (7). (A) After (24) hours of incubation. (B)After (48) hours of incubation. (C) After (72) hours of incubation.

2. **Temperature :** Different temperatures (25, 30, 35, 40, 45°C) were used, after which the amount of biomass produced was estimated.
3. **Concentration of carbon source :** The carbon source is added at different concentrations (1.5, 3, 4.5, 6, 7.5 ml) from the carbon source (used motor oil) to different flasks each containing (100 ml) of BHM medium.
4. **Size of Bacterial Inoculum :** Bacterial inoculum is added (0.5, 1, 1.5, 2, 2.5, 3 ml/100ml) BHM media.
5. **Type of Nitrogen source :** Two nitrogen sources were used for of BHM media, Ammonium Sulphate $(NH_4)_2SO_4$ and Urea $CO(NH_2)_2$ separately and peptone additive to support the medium.
6. **Incubation time :** Incubated the flasks for (24, 72, 120, 168, 216 hours), estimated the dry weight of the biomass, which represents the final product of single cell protein.

Statistical analysis

The experiments were designed as single-factor experiments using the completely randomization design (CRD) and statistically analyzed by the adoption of the statistical program (SPSS). The statistical analysis included computation of the arithmetic mean and a comparison between two averages using a test of the least significant difference (LSD) ($P < .05$). Morality was tested for the studied factors.

Production (SCP) and study of protein components product

A two liter flask of BHM was used for the purpose of preparing (1 liter) of in the production of SCP. The optimum production conditions identified in the previous paragraph were applied. The dry weight of the biomass (g/l) was estimated to be Fat, Carbohydrate, Metal elements, Amino acidsand Nucleic acid (DNA and RNA), as well as the total protein value using the Kjeldahl method and by estimating the amount of nitrogen in dry biomass and according to the following equation (AOAC, 2000):

Total protein quantity = Quantity of nitrogen × 6.25

RESULTS AND DISCUSSION

Diagnosis of *Pseudomonas aeruginosa* bacteria

Pseudomonas aeruginosa was identified based on culture characteristics, physiological traits and biochemical tests according to Tables 1 and 2.

Pseudomonas aeruginosa efficiency test in hydrocarbon residues analysis

The clearzone was formed around the developing colonies of *Pseudomonas aeruginosa* on BHM agar with (3%) of theused motor oil used as a single source of carbon and energy. During the first (24 hours) of incubation, the clear zone diameter increased after (48 hours). After 72 hours, the hydrocarbon residues from agar media completely disappeared as a result of their consumption by selected bacterial isolates as shown in Fig. 1.

The results showed the ability of *Pseudomonas aeruginosa* to analyze hydrocarbon residues, which was the used motor oil with high efficiency. This is due to the ability of the bacteria to exploit these residues as a single source of carbon and energy to grow. The bacteria continued to analyze the residues until they completely disappeared from the dish due to their adaptation to carbon source and their need its continuous construction of its cellular parts to ensure its continued division (Abioye *et al*, 2012). Subathra *et al* (2013) observed that maximum clear zone was formed around the bacterial species *Pseudomonas aeruginosa* when growing on the

Table 1 : Cultural and Microbial Characteristics of *Pseudomonas aeruginosa* Bacteria.

Diagnosis adjective	Result
Shape colonies	Circular
The edges of the colonies	Irregular
Color colonies	Creamy
The strength of colonies	Dry
High colonies	High
Shape cells	Strenuous
Cell pooling	Single
Stainingby gram stain	Negative
Production of stains	Pyocyanin produces green dye

Table 2 : Biochemical tests of *Pseudomonas aeruginosa* Bacteria.

Test	Result
Oxidase	+ve
Catalase	+ve
Indol	-ve
Urease	+ve
Citrate	+ve
Motility	+ve
Methyl red	-ve
Voges -Proskauer (VP)	-ve
KIA	+ve / CO_2/acid
Gelatin decomposition	+ve
Growth on Citramide	+ve
Growth on MacConky	+ve

BHM agar media with (1%) Hydrocarbon residues (used motor oil) added as the singlesource of carbon and energy, about (11 mm) after (7) days of incubation at (25°C) (Ramadass *et al*, 2018). Referred to the role of *Pseudomonas aeruginosa* in the Biodegradation of used motor oil and showed that this bacterial species had the ability to consume used motor oil as a single source of carbon and energy to possess the enzymes necessary for the metabolism of hydrocarbons.

Determination of Optimal Conditions for growth of *Pseudomonas aeruginosa* and Single Cell Protein (SCP) Production

pH

Fig. 1 shows the rate dry weight of the biomass of *Ps. aeruginosa* at different pH numbers. It was found that the highest rate dry weight of biomass was (1.212 g/ 100ml) at pH (8) and the dry weight of the biomass was reduced when pH was increased or decreased about (8).

The results of the statistical analysis at a significant level ($P < 0.05$) showed significant differences between the SCP values at pH (6-7), (7-8) and (9-10). There were no significant differences between (SCP) At pH values (6-10) and (8-9).

pH is an important factor affecting the environment and growth of microorganisms as it can grow in a different set of pH values depending on the characteristics of different microbes. pH can affect the biological activity of microbial products that can affect the use of more metabolic products. Thus pH values affect the degradation of crude oil hydrocarbons and the production of biomass (AL-Dulimy, 2015). Changing the pH value from the optimal rate of growth of bacterial cells leads to bacterial cell stress, leading to energy consumption. This in turn, leads to lower growth rates and production of biomass (Al-Hawash *et al*, 2018).

Temperature

Fig. 2 shows the rate dry weight of the biomass of *Ps. aeruginosa* at different temperatures, and the results show that the rate dry weight of the biomass has reached a maximum of (1.614 g/100ml) at (35°C) and the dry weight of the biomass when lifting or lowering the temperature is lessthan (35°C).

The results of the statistical analysis at a significant level ($P < 0.05$) showed significant differences between the rates of production (SCP) at temperatures (25°C-30°C), (30°C-35°C), (35°C-40°C). There was nosignificant difference between the production rates (SCP) at temperatures (24-40°C).

These results show that the bacteria *Ps. aeruginosa* was able to withstand the high temperatures up to (45°C). However, the best growth was in a thermal range between (30 – 40) while the maximum growth was at (35°C), which recorded the highest dry weight of the biomass. Temperature affects the biodegradation of hydrocarbons by affecting the physical nature and chemical structure of hydrocarbons, also affects the rate of hydrocarbon metabolism by microorganisms and the formation of biomass (Das and Chandran, 2011). Zhang *et al* (2012) noted that temperatures have an effect on the growth of *Ps. aeruginosa* bacteria and its consumption of hydrocarbons and their production of emulsifiers and biomass. This study showed that raising or lowering the temperature of (35°C) leads to a decrease in the level of growth in cells and thus lower the rate of production of biomass.

Concentration of carbon source

Fig. 3 shows the rates dry weight of the biomass of *Ps. aeruginosa* when adding different concentrations of the carbon source represented by usedmotor oil, showing that the highestrate dry weight of the biomass of isolation *Ps. aeruginosa* (1.614 g/100ml) at (3%) concentration of carbon source and decrease the dry weight of biomass when increasing or decreasing

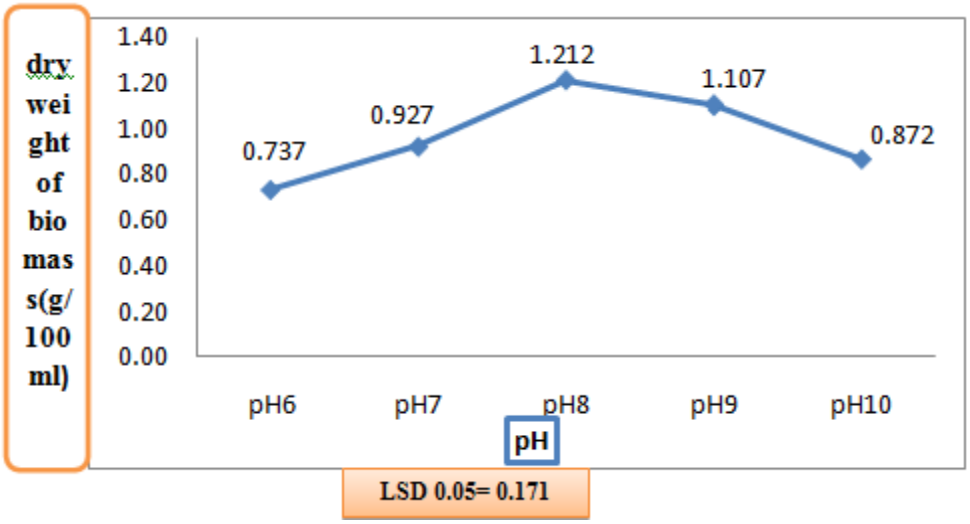

Fig. 1 : Dry weight of biomass resulting from the decomposition of used motor oil by *Ps. aeruginosa* growing on BHM with (3%) of usedmotor oil after (120 h) incubation at (30°C) at different pH numbers (6-7-8-9-10).

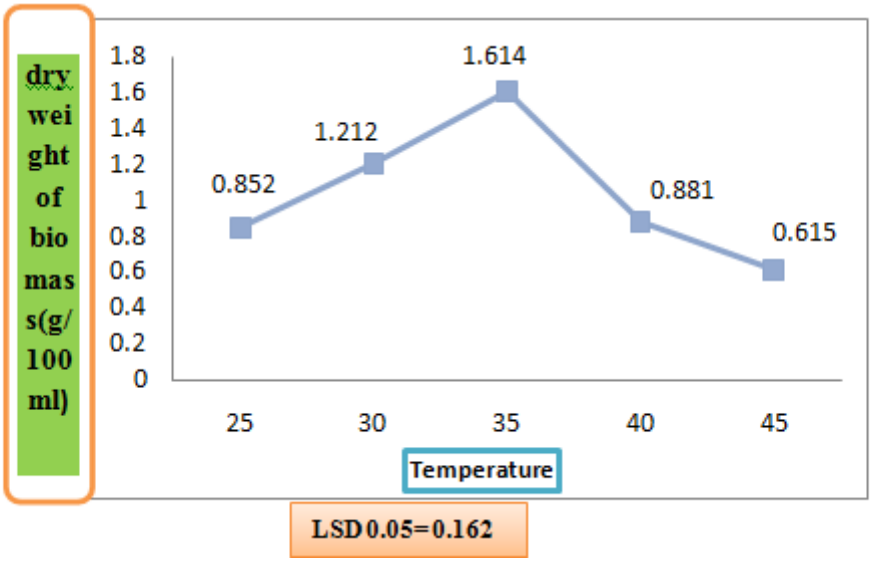

Fig. 2 : Dry weight of biomass resulting from the decomposition of used motor oil by *Ps. aeruginosa* growing on BHM supplemented with (3%) of usedmotor oil after (120 h) incubation at a different temperature (25-30-35-40-45°C) and pH (8).

concentration of (3%).

The results of the statistical analysis at a significant level (P <0.05) showed significant differences between the production rates (SCP) at the concentrations (1.5-3) ml and (3-7.5) ml and (6-7) ml and there was no significant difference between the production rates (SCP) at concentrations (1.5-6 ml).

The above results show that *Ps. aeruginosa* bacteria can withstand high concentrations of hydrocarbon residues as a single source of carbon and energy up to (7.5%). However, the best growth was at a concentration of (3%). The bacterial cells were able to adapt to this concentration and invest it as a single source of carbon and energy and the production of emulsifiers that helped the cells to break down fat and consume it. The increase in the concentration of hydrocarbon residues from 3% leads to a decrease in the level of cell division due to its high toxicity (Abdelkader *et al*, 2015). Also the amount of production at the concentration (1.5%) of the used motor oil is due to the lack of carbon source which negatively affects the growing of bacterial isolates and thus decreases concentration and decreases biomass resulting amount (Pi *et al*, 2017).

The size of the bacterial inoculum

The results showed in Fig. 4 that the size of the bacterial inoculum (1 ml) for *Ps. aeruginosa* is the best, with a rate dry weight of biomass (1.614 g/100 ml).

The results of the statistical analysis at a significant level (P <0.05) showed significant differences between the (SCP) production rates at inoculum sizes (0.5-1), (1-1.5), (1.5-2)ml and (2.2.5) ml and there were no significant

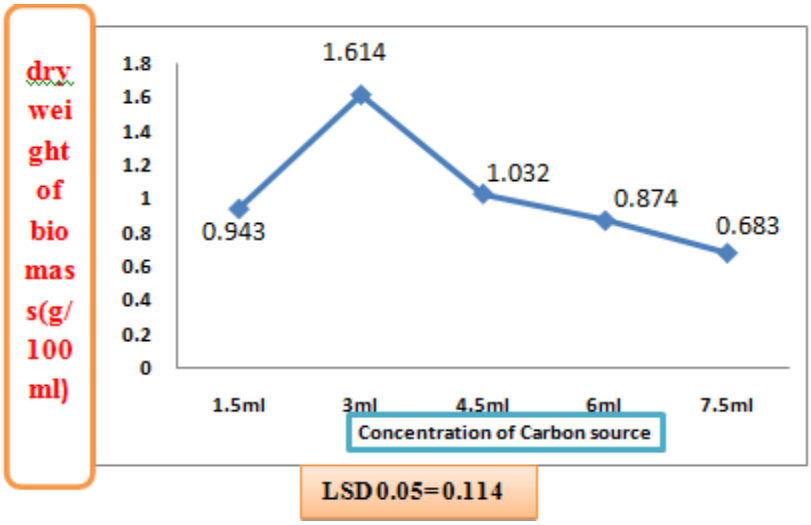

Fig. 3 : Dry weight of the biomass derived from the decomposition of used motor oil by *Ps. aeruginosa* developing on BHM with different concentrations of used motor oil (1.5-3-4.5-6-7.5) ml after (120 h) incubation at (35°C) and pH (8).

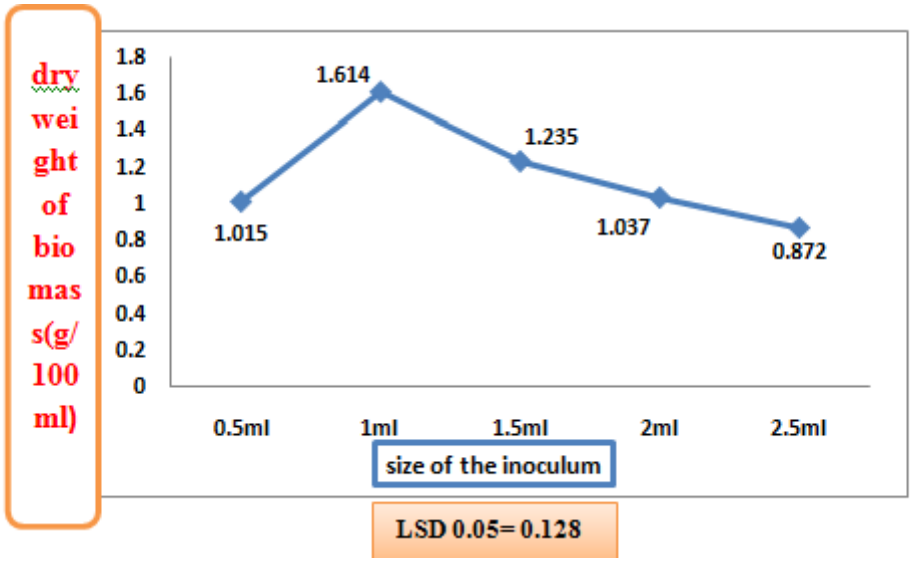

Fig. 4 : Dry weight of biomass resulting from the decomposition of used motor oil by *Ps. aeruginosa* growing on BHM with 3% of used motor oil after (120 h) incubation at a different temperature of (35°C) and pH (8) and with different inoculum volume (0.5, 1, 1.5, 2, 2.5) ml.

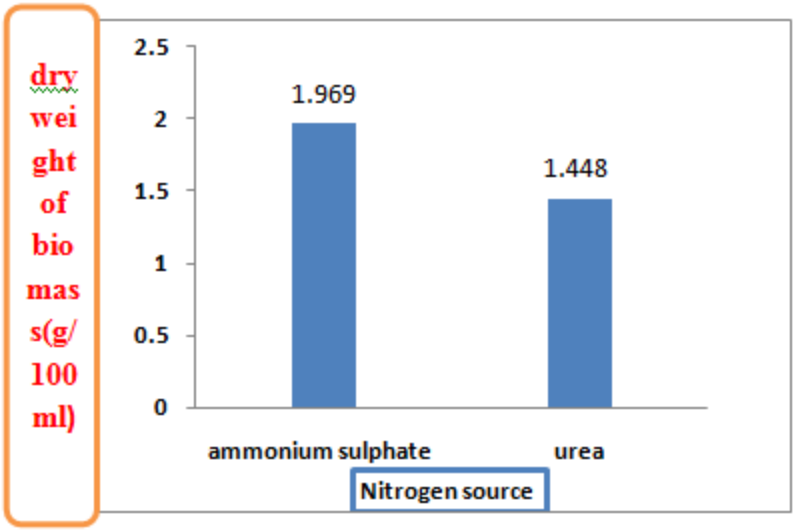

Fig. 5 : Effect of nitrogen source in biomass production by *Ps. aeruginosa* growing BHM with 3% of used motor oil as the single source of carbon and energy incubating for (120 h) at (100 rpm) and at (35°C).

Table 3 : Optimal conditions for the production process (SCP) by Bacterial isolation *Ps. aeruginosa.*

Conditions under study	Optimal production conditions
pH	8
Temperature	35°C
Concentration of carbon source (used motor oil)	3%
Size of bacterial inoculum	1%
Type of nitrogen source	Ammonium sulphate
Incubation time	216 hours

Table 4 : Ingredients (SCP) produced by Bacterial Isolation *Ps. aeruginosa*.

Compound	Percentage%
Crude protein	27.3
Carbohydrates	19.87
Fat	10
DNA	1.764
RNA	1.182
DNA+RNA	2.946

differences between (SCP) production rates at inoculum sizes (0.5-2) ml.

The increase in the volume of the inoculum from (1 ml) increases the demand for the carbon source, so that the remaining carbon begins to decrease. The lack of nutrients leads to slower growth of the bacteria and consequently the decrease of its concentration, which affects the fermentation process. Thus, the low (SCP) (Aboud *et al*, 2017; Naveen *et al*, 2010). The size of the inoculum is one of the important signs that should be taken care of to improve the production of the (SCP) and the effect of the size of the inoculum in the Lag phase, as well as that the size of the inoculum used should be proportional to the amount of media prepared for fermentation will lead to a reduction in the process of production and initiation of the production process (Akindele and Fagade, 2015).

Type of Nitrogen source

The results in Fig. 5 shows that the use of ammonium sulphate as a nitrogen source gave a better result than urea. The rate dry weight of biomass was the highest when adding ammonium sulphate. The dry weight of the biomass was 1.969 g/100 ml.

The results of the statistical analysis at a significant level ($P < 0.05$) showed significant differences between the SCP production rates at the used nitrogen sources.

It is clear from the above results that the use of Ammonium Sulphate gave better production than the use

Table 5 : Concentration of mineral elements in (SCP) produced by Bacterial isolation *Ps. aeruginosa.*

Element	Percentage%
P	1.8315
Cu	0.0457
K	0.7852
Fe	0.0159
Zn	0.0063
Na	2.813

Table 6 : Concentration of amino acids in (SCP) produced by Bacterial Isolation *Ps. aeruginosa* compared with amino acids in Eggs.

Essential amino acids	Concentration (mg/g)	
	SCP	Eggs
Leucien	1.87	7.6
Phenylalanine	2.74	5.4
Cysteine	3.92	2.2
Arginine	4.97	3.3
Alanine	4.80	7.4
Serine	3.59	9.7
Tryptophan	1.56	1.7
Non-essential amino acids	**Concentration (mg / g)**	
	SCP	Eggs
Aspartic	2.59	9.1
Glycine	3.12	3.1
Proline	4.71	2.9
Total concentration (mg/g)	33.87	

of urea as a source of nitrogen, due to the nature of chemical composition of the compound used as well as the ability of bacteria to dismantle the compound and the exploitation of nitrogen resulting in the protein production of the bacterial cell as well as the construction of the bases of nitrogen and nuclear acids and is sometimes suggested toadd some support to the culture media such as peptone to restore deficiency of certain nutrients whose concentration is insufficient to support growth (Reihani and Khosravi-Darani, 2019).

Incubation time

Fig. 6 shows the effect of incubation time in the production of biomass by *Ps. aeruginosa* showed that as the incubation time increased, the dry weight of the biomass increased to (2.015 g/100 ml) after (9 days).

The results of the statistical analysis at a significant level ($P < 0.05$) showed significant differences between (SCP) and incubation rates (24-72)hours and (27-120)hours, There were no significant differences between (SCP) production rates at incubation time (120-168) hours

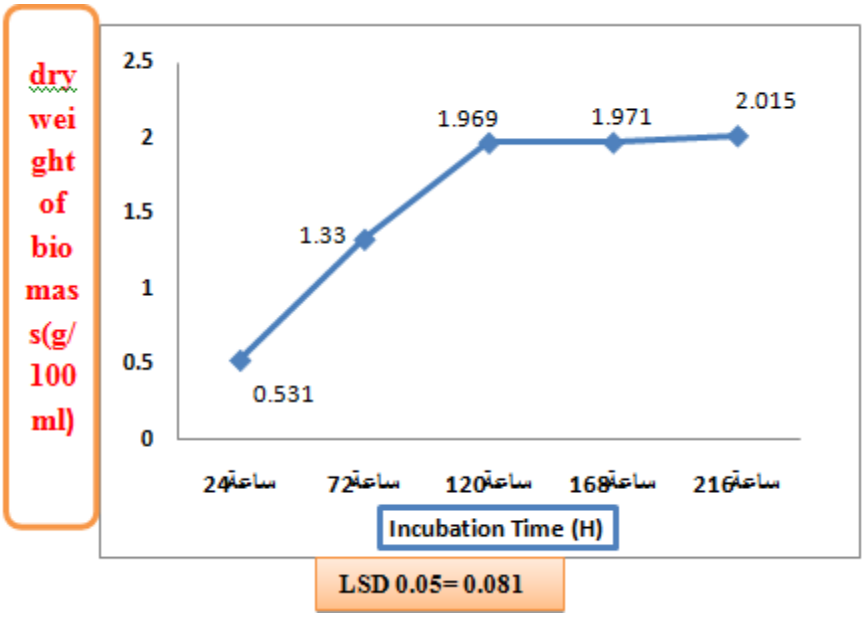

Fig. 6 : Production of biomass by *Ps. aeruginosa* developing on BHM media with 3% of used motor oil as a single source of carbon and energy after (24, 72, 120, 168, 216) hours lap time in the shaking incubator (100 rpm) and (35°C).

and (168-216) hours.

The results showed an increase in the dry weight of the biomass over time. This coincides with the complete decomposition of the hydrocarbons added to the medium. This is due to the adaptation of the bacteria to the polluted medium with the hydrocarbon residues and the ability of the bacteria to disassemble the residues and the production of biological emulsions that helped dissolve the residues in the medium. They can survive because they are able to metabolize their biological systems. Contaminants are often sources of energy for microorganisms and they have a wide range of enzymes that enable them to consume many pollutants (Naveen *et al*, 2010).

Production (SCP) and study of protein components

Optimal conditions were used as shown in Table 3 to produce (SCP) by *Ps. aeruginosa* and after incubation period obtained a dry weight of biomass (19.6 g/l). The components of (SCP) were estimated as in Table 4. The results showed that the crude protein content in (SCP) was (27.3%). Al-Hadithi *et al* (2018) obtained crude protein (24.4%) when growing *Raoutella ornithinolytica* bacteria on potato residues. The percentage of Nucleic acids in (SCP) produced (2.946%) is less than the permissible limit (5-7%) for the use of (SCP) as animal feed and does not require the removal of these nucleic acids. The problem with the use of (SCP) as animal feed is the presence of nucleic acidshigher than the permissible (5-7%) (Iumphg and Oshumy, 2018).

Researchers found that the rise in the percentage of nucleic acids over the permissible limits leads to a high proportion of purine bases that are nucleic acids and which are converted into the body during metabolism to uric acid (Nasseri *et al*, 2011). The percentage of carbohydrates in (SCP) was (19.87%) and the fat content was (10%) higher than that of Aboud *et al* (2017). The percentage of carbohydrates (17%) and fat (2.46%) the development of bacteria *Bacillus cereus* and *Fusarium solani* on the remnants of the *Cynodon dactylon* as a single source of carbon and energy. Due to the high proportion of fat to contain the center of a high rate of fat and transmitted to the biomass as well as metabolic reactions that result in large amounts of fat (Aboud *et al*, 2017).

The results also show that (SCP) contains metal elements with varying percentages as shown in Table 5. Minerals are of great importance and are important in supporting the growth of (SCP) producing microorganisms and thus increasing the nutritional value of (SCP) produced either for human use or as animal feed (Al-Hadithi *et al*, 2018). It also contains essential and non-essential amino acids as shown in Table 6. The results showed that the concentration of essential amino acids in the (SCP), such as Cysteine, Arginine, Glycine and Proline was higher than in the eggs. The other amino acids were less concentrated than the eggs in the (SCP). The product in this study is free of amino acid methionine and sulfuric acid. Raya (2014) noted that (SCP) has a high content of amino acids other than methionine and that the presence of amino acids in the (SCP) is economically important since it is used as additives to animal feed, especially fish and poultry.

CONCLUSION

The size of bacterial inoculum (1%) and Ammonium Sulphate as a nitrogen source after (216) hours in the shaking incubator at 100 rpm. The percentage of crude protein (27.3%) of the dry biomass of *Ps. aeruginosa*, Carbohydrate (19.87%), DNA and RNA (1.764%) (1.182%) respectively. also showed that (SCP) contains mineral elements such as: P (1.8315%), Cu (0.0457%), K (0.7852%), Fe (0.0159%), Zn (0.0063%) and Na (2,813%), (SCP) contains essential amino acids such as Leucien (1.87 mg/100g), Phenylalanine (2.74 mg/100g), Cysteine (3.92mg/100g), Arginine (4.97mg/100g), Alanine (4.80 mg/100g (Serine (3.59 mg/100g) and Tryptophan (1.56 mg/100g). The non-essential amino acids found in (SCP) are Aspartic (2.59 mg/100g), Glycine (3.12 mg/100g) and Proline (4.71mg/100g).

REFERENCES

Abduljabbar A H, Raouf S R and ALhelu J (2009) Petroleum Single Cell Protein Production. *Engineering & technology Journal* **27**(3), 468-472.

Abdul-Qader D F, Arif H H and Hammad A A (2015) Test the efficiency of hydrocarbon degradation by isolated bacteria from soil contaminated with oil derivatives. *Anbar University Journal of Pure Sciences* **9**(3).

Abioye O P, Agamuthu P and Abdul-Aziz R A (2012) Biodegradation of used motor oil using organic residues amendment.Hindawi Publishing Corporation. *Biotechnology Research International* Volume 2012, Article ID 587041, 8 pages doi:10.1155/587041.

Aboud M F, Al-Rawi D F and Hamza H M (2017) Production of singl cell protein from *L. cynodondactylon* with the use of *Bacillus cereus* and *Fusarium solni*. *Journal of the Center for Biotechnology Research* **11**(2), 14-21.

Adegbola G M, Eniola K I T and Opasola O A (2014) Isolation and identification of indigenous hydrocarbon tolerant bacteria from soil contaminated with used engine oil in Ogbomoso, Nigeria. *Pelagia Research Library Advances in Applied Science Research* **5**(3), 420-422.

Ahmed S M E, Amin M A and Shebl R I (2017) White-Rot Fungi in Biodegradation of Selected Polycyclic Aromatic Hydrocarbon Model. Department of Microbiology and Immunology Faculty of Pharmacy, Cairo University.

Akindele S T and Fagade O E (2015) Enhancing the Nutritive Values of Agroresiduess for Animal Feed Production Using Selected Bacterial Strains. *Journal of Environmental Science, Toxicology and Food Technology* **9**(11) Ver. II, 96-100.

AL-Dulimy A A H (2015) Efficiency of Hydrocarbon by Bacteria Isolated from Oil Derivatives Contaminated Soils, *M.Sc. Thesis* University of Anbar, Iraq.

Al-Hadithi O A H, Al-Rawi D F and AlAni M Q A (2018) Single cell protein production by soil *Raoutella ornithinolytica* incubated on residues potato, paper and corn cob media. *Onl J Vet Res.* **22** (12):1137-1144.

Al-Hawash A B, Dragh M A, Li S, Alhujaily A, Abbood H A, Zhang X and Ma F (2018) Principles of microbial degradation of petroleum hydrocarbons in the environment. *The Egyptian Journal of Aquatic Research* **44**(2), 71–76.

AOAC (2000) *Official Methods of Analysis*. 17th ed. Washington, DC : Association of official Analytical Chemists.

Das N and Chandran P (2011) Microbial Degradation of Petroleum Hydrocarbon Contaminants. Environmental Biotechnology Division, School of Biosciences and technology, VIT University, Vellor, Tamil Nadu 632014, India.

Iumphg D W and Oshumy H P (2018) Functional properties of phosphorylated yeast protein: Solubility, water-holding capacity, and viscosity. *Journal of Agricultural and Food Chemistry* **34**, 670-674.

Kothari V, Panchal M and Srivastava N (2013) *Microbial Degradation of Hydrocarbons*. Institutr of Science, Nirma University, Ahmedabad, India.

Malatova K (2005) Isolation and characterization of hydrocarbon degrading bacteria from environmental habitats in Western New York State. Thesis Rochester Institute of Technology, Accessed from.

Medina-Bellver J I, Marin P, Delgado A, Rodríguez-Sánchez A, Reyes E, Ramos J L and Marqués S (2005) Evidence forin situ crude oil biodegradation after the Prestige oil spill. *Environmental Microbiology* **7**(6), 773–779.

Nasseri A T, Rasoul-Amini S, Morowvat M H and Ghasemi M H (2011) Single Cell Protein: Production and process. *American Journal of Food Technology* **6**, 103-116.

Naveen K S, Manoharan N, Ganesan S, Manivannan S P and Velsamy G (2010) Isolation,screening in vitri mutational assessment of indigenous soil bacteria for enhanced capability in petroleum degradation. *Int. J. Environ. Sci.* **1**(4), 0976 - 4402.

Pi Y, Chen B, Bao M, Fan F, Cai Q, Ze L and Zhang B (2017) Microbial degradation of four crude oil by biosurfactant producing strain *Rhodococcu* ssp. *Bioresource Technology* **232**, 263–269.

Ramadass K, Megharaj M, Venkateswarlu K and Naidub R (2018) Bioavailability of weathered hydrocarbons in engine oil-contaminated soil: Impact of bioaugmentation mediated by *Pseudomonas* spp. on bioremediation, *Science of the Total Environment* **636,** 968–974.

Rath K, Singh A B, Chandan S and Vatsala R S (2016) Isolation and characterization of biosurfactant producing strain *Pseudomonas aeruginosa* SMVIT 1 from oil contaminated soil. *J SciInd Res.* **75**, 681–686.

Raya R R (2014) The Use Of Biotechnology to treatment of coastal Remains of Marine Organisms Aim of producing Biomass to be used as fodder, High Institute of marine Researches, Depatment of marine Biology, Syria.

Reihani S F S and Khosravi-Darani K (2019) Influencing factors on single cell protein production by submerged fermentation: A review. *Electron J Biotechnol*. **37**, 34-40.

Spalvins K, Zihare L and Blumberga D (2018) Single cell protein production from residues biomass: comparison of various industrial by-products. *Energy Procedia* **147**, 409-418.

Subathra M K, Immanuel G and Suresh A H (2013) Isolation and Identification of hydrocarbon degrading bacteria from Ennore creek. *Bioinformation* **9**(3), 150–157.

Zhang X S, Xu D J, Zhu C Y, Tserennyam L and Scherr K E (2012) Isolation and identification of biosurfactant producing and crude oil degrading *Pseudomonas aeruginosa* strains. *Chem. Eng. J*. **209**, 138–146.