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Production of Single Cell Protein (SCP) By Local Isolation of Staphylococcus lentus Hydrocarbon Degradation

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Abstract

This study included the diagnosis of Staphylococcus lentus bacteria obtained from the laboratories of the Department of Biology in the College of Education for Pure Sciences and testing the efficiency of this bacterial species for the first time in Iraq in the analysis of motor oil used as a single source of carbon and energy and production of protein-rich biomass. The liquid BHM mineral salts and submerged culture technology were used to determine the optimum conditions for biodegradation of used motor oil and (SCP) production. Different numbers were used for the pH (6, 7, 8, 9, 10) and different temperatures (25, 30, 35, 40, 45) °C, as well as different concentrations of the carbon source represented by the used motor oil (1.5, 3, 4.5, 6,7.5) ml per 100 ml BHM medium, and bacterial Inoculum was added (0.5, 1, 1.5, 2, 2.5, 3) ml / 100 ml BHM medium. We also used two nitrogen sources, ammonium sulfate $(NH_4)_2SO_4$ and urea $CO(NH_2)_2$ separately, and added Pepton to support the BHM medium. The jugs incubated for a period of (24, 72, 120, 168,216) hours, the results showed that Staph. lentus has the potential to grow in solid, liquid BHM medium with added (3%) of motor oil used as the only carbon and energy source. The amount of oil gradually faded from the BHM medium until it disappeared completely. The results showed that the best biomass production was (18.4 g / l) at pH (7), temperature (35 °C) and carbon source concentration (3%) for used motor oil and bacterial Inoculum size (1%) and by using ammonium sulfate as a source of nitrogen after incubate period (216) In an hour in a incubator shaker at a speed of 100 rpm, the crude protein ratio was (33.9%) of the dry biomass of Staph. lentus, carbohydrate (15.7%), nucleic acid ratio, DNA and RNA (2.042%) and (1.591%), respectively, the results also showed that the (SCP) product contains mineral elements such as phosphorus at (1.9831%) and copper by (0.0036%) while The percentage of potassium (0.8325%), iron (0.0271%), zinc (0.002%) and sodium (4.308%).

Keywords

Single Cell Protein, Biodegradation of Hydrocarbons, Staphylococcus lentus.

The term Single Cell Protein (SCP) refers to the biomass of dry cells from microorganisms such as bacteria, fungi and algae that grow on different carbon sources, and that the first to describe a single cell protein is Professor Carroll

Winston in 1966 to give a more comprehensive expression of microbial protein and to avoid the term Unacceptable consumption of microorganisms by consumers[1]. (SCP) contains most of the essential amino acids, but its content is low in sulfur amino acids [2]. Microscopic biology has been used since ancient times in the production of (SCP), which has been used to feed humans and animals. Certain basic characteristics must be found in the microbiology that is used to produce (SCP). One of the most important of these characteristics is that it does not cause diseases of humans, animals or plants, and that Its growth is dense in addition to being highly productive and not producing toxins, especially endotoxin internal toxins, as well as having low production costs [3]. Among the most important microorganisms that are used in the production of single-cell protein are algae, fungi, yeasts and bacteria that can grow on waste that is not consumed by the individual such as sugar cane, papyrus, and whey, fruit peels such as oranges, bananas, apples, coconuts, and dates industry waste, and all food waste as well as waste Various hydrocarbons [4]. Biological systems were used in the production of (SCP) due to the ability of these systems to carry out a wide range of biochemical reactions and their adaptation to various environmental conditions, and their ability to benefit from inexpensive and widely available carbon sources, and the importance of producing single cell proteins comes in being cheaper at the level Commercial production depends on industrial or agricultural waste, and is not affected by climate changes and natural disasters, in addition to the possibility of producing large quantities of them in a short time compared to the traditional methods of animal and vegetable protein production [5]. Hydrocarbons and petroleum products are considered one of the most important main sources of energy needed for different industries for daily life. As a result of the importance of these hydrocarbon compounds, there was a great need for them as an important source of energy. Or by negligence and human activities, these spills and leaks of petroleum products have led to environmental pollution, whether it is the land environment or the water environment with hydrocarbons, which caused extensive damage u Late organisms in those contaminated environments [6]. also resulted in industrial development, such as increasing oil production and petrochemical industries to many negative impacts on the environment [7]. Biodegradation is one of the safest ways to remove hydrocarbon wastes from the environment compared to other methods such as chemical treatment, washing and burning that give more toxic products than the hydrocarbons themselves. Biodegradation includes the use of microorganisms such as bacteria, fungi and yeasts to remove these hydrocarbon wastes through the ability of These microorganisms Degradation and dismantle hydrocarbon compounds because they have various metabolic pathways, and this technique is considered an advanced and inexpensive way to remove and Degradation many environmental pollutants, including hydrocarbon pollutants [8]. The hydrocarbon wastes resulting from the petroleum, oil refining and other industries were exploited in the field of biotechnology, as Biomass was produced using the Submerged Cultures technology and the production of single cell Protein with a high nutritional value, and according to the Food and Agriculture Organization (FAO) the percentage of Single Cell Protein ranges between 20-90% of the dry weight of the cell and contains most of the amino acids in large quantities except for the methionine amino acid as well as containing fats, carbohydrates, nucleic acids, vitamins and minerals [9].

Materials and Methods

Preparation of Bushnell-Haas Media (BHM)

Bushnell-Haas media preparation can be done by dissolving the following salts one by one in a liter of distilled water and these salts are: (1g) of KH2PO4, (1 g) of (NH4) 2SO4, (1 g) of KNO3, (0.2 g) of MgSO4.7H2O (0.05 g) of FeCl3. 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 [10].

Isolation and diagnosis of Staphylococcus lentus:

This bacterial type was isolated from soil contaminated with hydrocarbons in the fuel filling station in Habbaniyah district, and the first screening was done for samples of the contaminated soil and made ten-year dilutions and planted (1 ml) of the sixth dilution on the solid BHM medium containing (3 ml) of motor oil used as the only source of carbon energy, and secondary screening and repeated purification were carried out for the purpose of obtaining pure bacterial isolates, and these bacteria were diagnosed based on culture 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/ang2) after cooling to (30°C). It was cultured by pure colonies of Staph. lentus 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.

Staphylococcus lentus test of hydrocarbon residue analysis

The colonies of the bacterial type Staph. lentus were planted by a sterile transporter on the BHM agar in a circle with a diameter of (1 cm) in the middle of the dish, then took (3 ml) the hydrocarbon residue (used motor oil) dissolved in diethyl ether at a concentration of 5%) and sterilized by filtration and added to the medium to form A thin layer above the surface of the medium, then incubated in the incubator at a temperature of (30 ° C) for a period of (72 hours).

Determination of Optimal Conditions for Growth of Staphylococcus lentus 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 Staph. lentus (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 oven at (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 [11].

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.
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.
4 Size of Bacterial Inoculum: Bacterial inoculum is added (0.5, 1, 1.5, 2, 2.5, 3) ml/100ml BHM.
5 Type of Nitrogen Source: Two Nitrogen sources were used for of BHM, Ammonium Sulphate (NH4)2SO4 and Urea CO(NH2)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.

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 acids and 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 [12]:

$$\text{Total protein quantity} = \text{quantity of nitrogen} \times 6.25$$

Results and Discussion

Diagnosis of Staphylococcus lentus Bacteria

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

Table (1): Cultural and Microbial Characteristics of Staphylococcus lentus Bacteria.

Diagnosis adjective	Result
Shape colonies	Circular
The edges of the colonies	Irregular
Color colonies	Creamy
The strength of colonies	Mucous
High colonies	High
Shape cells	Cocci
Cell pooling	Staph
Staining by gram stain	Positive
Production of stains	Do not produce dyes

Table (2): Biochemical Tests of Staphylococcus lentus Bacteria.

Test	Result
Oxidase	+ve
Catalase	+ve
Indol	-ve
Urease	-ve
Citrate	-ve
Motility	-ve
Methyl red	+ve
Voges - Proskauer (VP)	-ve
Gelatin decomposition	-ve
Hemolysis	γ
Growth on MacConky	+ve

Staphylococcus lentus Efficiency Test in Hydrocarbon Residues Analysis:

The clear zone was formed around the developing colonies of Staph. lentus on BHM agar with (3%) of the used 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 (96 hours) the hydrocarbon residues from agar media completely disappeared as a result of their consumption by selected bacterial isolates as shown in Figure 1.

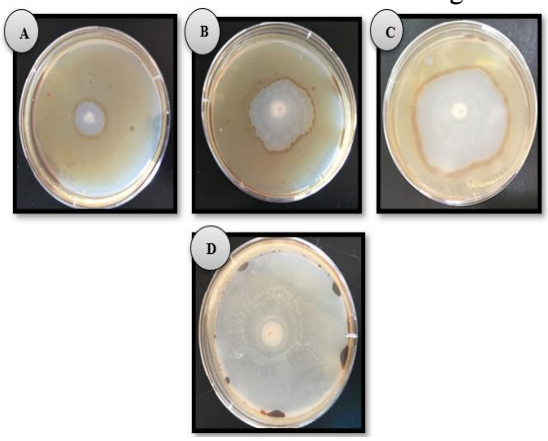

Figure (1): The transparent area is formed around the colonies of Staphylococcus lentus Bacteria grown on BHM agar with added motor oil used as the only carbon and power source at 30 ° C and pH 7, (A) After 24 hours of incubation. (B) After 48 hours of incubation. (C) After 72 hours of incubation. (D) After 96 hours of incubation.

The results showed the ability of Staph. lentus bacteria to Degradation the hydrocarbon wastes that were represented by the used motor oil and with high efficiency, and this is due to the ability of bacteria to use these wastes as a single source of carbon and energy for growth, the bacteria continued to Degradation the waste until it disappeared from the dish completely due to its adaptation to the carbon source and its continuous need He has to build its cellular parts to ensure their continued division [13]. Staphylococcus sp. was isolated from benzene-contaminated soil in Karachi by [14] it was noted that Staphylococcus sp. had the ability to Degradation Polycyclic Aromatic Hydrocarbons (PAHs) of naphthalene, Phenanthrene and xylene when adding them to BHM after 1-week, at a rate of (1%), From cuddling with a temperature of (37)°C.

Staphylococcus haemolyticus was identified as a hydrocarbon-degrading bacterium as a study was conducted by [15] during which it was found that this bacterial species had the ability to Degradation crude oil residues as a single source of carbon and energy.

Determination of Optimal Conditions for Growth of Staphylococcus lentus and Single Cell Protein(SCP) Production:

1 pH

Figure (0) shows the average dry weight of the biomass of Staph. lentus bacteria at different numbers of pH, and it was found that the highest rate of dry weight of the biomass was (1.175 g / 100 ml) at pH (7) and the dry weight of the biomass was raised when the pH was raised or lower than (7).

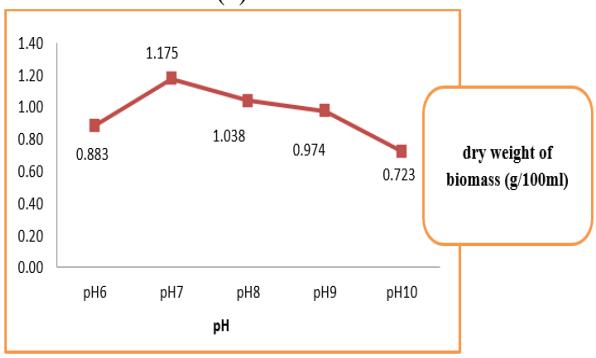

Figure (2): Dry weight of biomass resulting from the decomposition of used motor oil by Staph. lentus growing on BHM with (3%) of used motor oil after (120 h) incubation at (30°C) at different pH numbers (6-7-8-9-10).

PH is one of the main environmental factors that influence the bioavailability of pollutants, the availability of other nutrients, the activity of biological processes and hence the overall biological treatment method of contaminated hydrocarbons for soil and water [16]. [17] indicated that pH affects the biodegradation process of hydrocarbons through its

effect on the chemical composition of hydrocarbon compounds. Thus increasing their solubility and consequently their consumption by microorganisms, and that changing the pH value from the optimal rate of bacterial cell growth leads to bacterial cell stress which It leads to its energy consumption, which in turn leads to a decrease in its rate of growth and production of biomass [18].

2- Temperature

Figure (3) shows the average dry weight of the Staph. lentus bacteria at different temperatures, and the results show that the average dry weight of the biomass reached a maximum (1.227 g /100 ml) at a temperature of (35)°C and the dry weight of the biomass decreased when the temperature was raised or lowered than (35)°C.

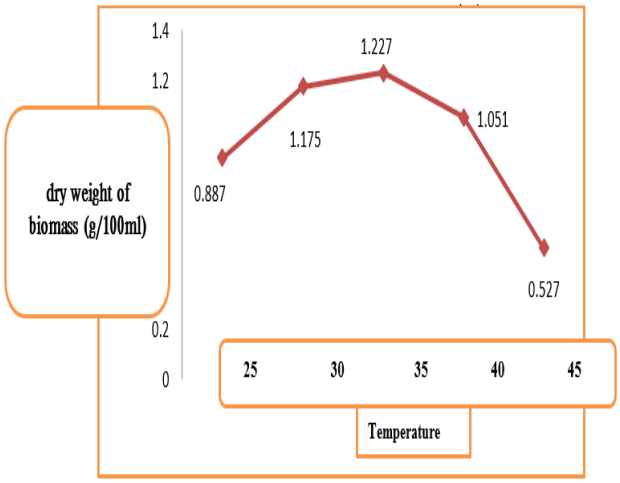

Figure (3): Dry weight of biomass resulting from the decomposition of used motor oil by Staph. lentus growing on BHM supplemented with (3%) of used motor oil after (120 h) incubation at a different temperature (25-30-35-40-45) °C and pH (7).

The results show that for Staph. lentus bacteria susceptibility to high temperatures up to (45)°C, but the best growth was in a temperature range between (30 - 40)°C, while the maximum growth was at a temperature of (35)°C where the highest amount of dry weight of the biomass was recorded.

[19] indicated that better biodegradation of hydrocarbons by Staphylococcus spp. at a wide range of temperatures it falls between (32-37) °C, as the bacteria consume hydrocarbons, which leads to an increase in their growth and an increase in the concentration of their cells.

3- Concentration of the carbon source:

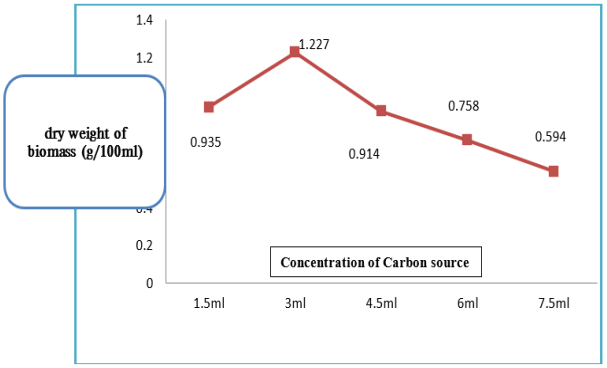

Figure (4): Dry weight of the biomass derived from the decomposition of used motor oil by Staph. lentus developing on BHM with different concentrations of used motor oil (1.5-3-4.5-6-7.5) ml after (120 h) incubation at (35°C) and pH (7).

Figure (4) shows the dry weight ratios for the Staph. lentus bacteria when adding different concentrations from the carbon source represented by the used motor oil, it was found that the highest average weight of the biomass is dry (1.227 g /100 ml) at a concentration of (3%) of the carbon source and the amount of the dry weight of the biomass is reduced when the concentration is increased or less than (3%).

From the above results it is clear that Staph. lentus bacteria can withstand high concentrations of hydrocarbon wastes as a single source of carbon and energy up to 7.5%, but the best growth was at a concentration of 3%. Bacterial cells were able to acclimate to this concentration and invest it as a single source of carbon and energy and produce bioemulsions that helped cells break down fat and consume it from Before bacteria, increasing the concentration of hydrocarbon wastes from 3% leads to a decrease in the level of cell division due to its high toxicity [20]. The amount of production at concentration (1.5%) of the used motor oil was reduced due to a source deficiency Carbon, which negatively affects the growth of isolates Bacterial thus decreases its concentration and the resulting biomass decreases [21].

4- The Size of the Bacterial Inoculum:

The results showed in Figure (5) that the size of the bacterial Inoculum (1 ml) for Staph. lentus was the best, with average dry weight for isolation biomass (1.227 g /100 ml).

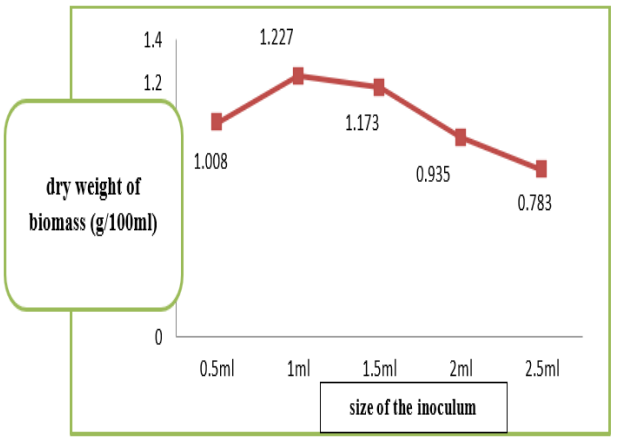

Figure (5): Dry weight of biomass resulting from the decomposition of used motor oil by Staph. lentus growing on BHM with 3% of used motor oil after (120 h) incubation at a different temperature of (35°C) and pH (7) and with different inoculum volume (0.5, 1, 1.5, 2, 2.5) ml.

It is clear from the results above that the size of the Inoculum (1%) is the optimum for degrading motor oil used as a single source of carbon and energy by Staph. lentus bacteria thus increases its growth and increases the biomass resulting from the division of bacterial cells. The increase in the size of the Inoculum by (1 ml) increases the demand for the carbon source, so the remaining carbon begins to decrease as a result of competition between cells on carbon, and the lack of essential nutrients such as carbon, nitrogen, phosphorous and others leads to The growth of bacteria slows and thus their concentration decreases, which affects the fermentation process and consequently the resulting (SCP) decreases [22].

5- Type of nitrogen source:

The results show in Figure (6) that the use of ammonium sulfate as a source of nitrogen gave a better result than urea and the average weight of the dry weight of the biomass reached its maximum when adding ammonium sulfate, as the dry weight of the biomass reached (1.364 g / 100 ml).

The results indicated that the use of urea as a source of nitrogen has a negative effect on the growth of Staph. lentus bacteria and its release of bioemulsifiers that enhance the used motor oil metabolism and consequently reduced the biomass resulting from it being non-resolving for urea and not producing urease, while adding ammonium sulfate had a positive effect on the growth of bacterial isolate Staph. lentus and the dismantling of the used motor oil and thus the concentration of cells increased in the BHM-supported peptide medium which led to increased biomass production [23].

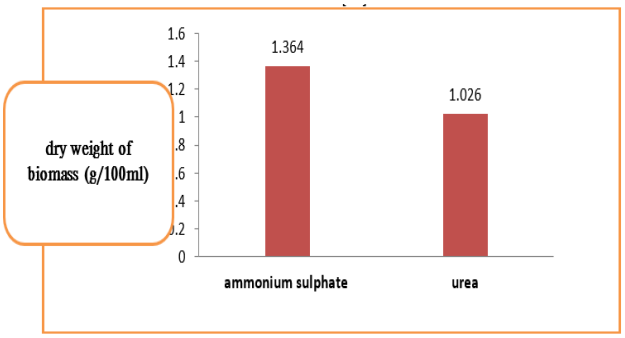

Figure (6): Effect of nitrogen source in biomass production by Staph. lentus growing BHM with 3% of used motor oil as the single source of carbon and energy incubating for (120 h) at (100 rpm) and at (35°C) and pH (7).

6-Incubation Time

Figure (5) shows the effect of incubation time in the production of biomass by Staph. lentus bacteria, showed that as the incubation time increased, the dry weight of the biomass increased to (1.847 g / 100 ml) after (216 hours).

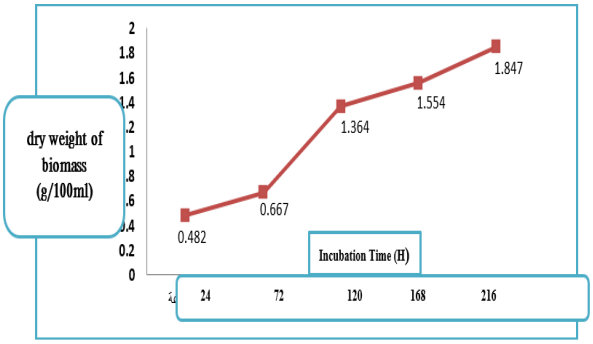

Figure (7): Production of biomass by Staph. lentus developing on BHM with 3% of used motor oil as a single source of carbon and energy after (24, 72, 120, 168, 216) hours lap time in the shaking incubator (100 rpm) and (35°C) and pH (7).

The results indicated an increase in the amount of dry weight of the biomass over time, and this coincides with the complete decomposition of the added hydrocarbon wastes to the medium, due to the acclimatization of the bacteria with the medium contaminated with the hydrocarbon wastes and the ability of the bacteria to disassemble those wastes and produce biomulsions that helped dissolve the waste in the

medium. They can survive because they are able to metabolize using their vital systems. Pollutants are often energy sources for microorganisms and it turns out that they have a wide range of enzymes that enable them to consume many pollutants [24].

(SCP) Production and Study of Protein Ingredients:

The optimum conditions were applied as shown in Table(3) to produce the unicellular protein Staph. lentus bacteria and after the end of the incubation period, a dry weight of the biomass was obtained, amounting to (18.4 g / l). The product (SCP) components as in Table (4) were estimated, as the results showed that the percentage of crude protein in the SCP produced was (33.9%), Table (5) shows the mineral elements in the (SCP).

Table (3) Optimal conditions for the production process (SCP) by Bacterial Isolation Staph. lentus.

Conditions under study	Optimal production conditions
pH	7
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 Staph. lentus.

Compound	percentage%
Crude protein	33.9
Carbohydrates	15.7
Fat	7.3
DNA	2.042
RNA	1.591
DNA+RNA	3.633

Table (5) Concentration of mineral elements in (SCP) produced by Bacterial Isolation Staph. lentus.

Element	percentage%
P	/,761/
Cu	. ,. . 14
K	. ,6103
Fe	. ,. 05/
Zn	. ,. . 0
Na	2,1. 6

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