Sumer Journal for Pure Science (ISSN: 2790-7031)

SJPS 2025 (June), Vol. 4, No. 1, p.p.: 26-49

Production of single cell protein Using Mixed Microbial Inoculum Technology from Two Local Bacterial Isolates, *Kytococcus sedentarius*, and *Pseudomonas oleovoran*, Using Solid Medical Waste

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Abstract

This research was conducted to produce single-cell protein using a mixed inoculum of two local bacterial isolates, namely *Pseudomonas oleovorans* and *Kytococcus sedentarius*. The submerged culture technique was used in the production process using the double inoculum. After initial treatments, solid medical waste was used as the main carbon and energy source in the nutrient medium on which the two bacterial isolates were grown. Production optimization experiments were conducted to reach the best production of single-cell protein. The production optimization experiments aimed to reach the best conditions for optimal production. These experiments included studying the effect of each factor (PH, temperature, inoculum size, type and concentration of nitrogen source, and the effect of incubation duration). Optimizing the production conditions showed that the best single-cell protein production was at PH9, temperature 45°C, and inoculum size 2 ml of each of the two isolates/100 ml of the production medium. The best production was achieved using $NaNo_3$ at a medium concentration of 0.3 g/100 ml. Finally, the best production was obtained at an incubation period of 72 hours. After applying the optimum conditions, the best single-cell protein production was 1.712 g/100 ml of the production medium. The analysis and separation of the culture filtrate after single-cell protein extraction using gas chromatography (GC-MS) technique showed that this filtrate contains 15 active chemical compounds with vast and valuable applications. These compounds are Chloro-3,3-dimethyl-8--6phenyl-3,4-dihydro-1H-thiopy, Neopentane, Oxadiazole, 5-(4-tert-[1,2,4]butylphenoxymethyl)-3-(thiophen-2-yl)- and (4-Methoxyphenyl)-2-(4--2 trimethoxysilyloxy)propane and Methane, isocyanato And Silane, dimethyl(2-chloro-5-methylphenoxy)heptyloxy and Propanoic acid, anhydride and N, N'-(4-Methyl-m-phenylene)bis(p-toluenesulfonamide) and 1,3-Propanediamine and Vitamin E and Silane, dimethyl(2-chlorophenoxy)octyloxy and Vitamin E succinate and Vitamin E and Silane, dimethyl(2-chlorophenoxy)octyloxy And alpha.-Tocopherol- beta.-D-mannoside and Acetic acid, [(1,1-dimethylethyl)thio].

Keywords: SCP, *kytococcus sedentarius*, *Pseudomonas oleovoran*, mixed microbial vaccines, factors affecting SCP production.

أنتاج بروتين أحادي الخلية بأستخدام تقنية اللقاحات الميكروبية المختلطة من العزلتين البكتيريتين المحليتين *Kytococcus sedentarius* و *Pseudomonas oleovoran* بأستعمال المخلفات الطبية الصلبة

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الخلاصة

تم أجراء هذا البحث لأنتاج البروتين أحادي الخلية بأستخدام لقاح مختلط من عزلتين بكتيريتين محليتين هما بكتريا *Pseudomonas oleovoran* و *kytococcus sedentarius*. أستعملت تقنية المزارع المغمورة في عملية الإنتاج وبأستخدام اللقاح المزدوج. أستعملت المخلفات الطبية الصلبة بعد أجراء المعاملات الأولية عليها كمصدر رئيسي للكربون الطاقة في الوسط الغذائي الذي نميت عليه العزلتين البكتيريتين. أجريت تجارب تحسين الإنتاج للوصول الى أفضل إنتاج من البروتين الأحادي الخلية. أستهدفت تجارب تحسين الإنتاج الوصول الى أفضل الظروف الملائمة للحصول على الأنتاج الأمثل وشملت هذه التجارب دراسة تأثير كل من العوامل (PH، درجة الحرارة ، حجم اللقاح ، نوع وتركيز المصدر النيتروجيني وتأثير مدة الحضن). أظهرت نتائج تحسين ظروف الإنتاج أن الإنتاج الأفضل من البروتين أحادي الخلية كان عند PH9 ودرجة حرارة 45 م وحجم لقاح 2 مللتر من كل من العزلتين/100 مللتر وسط الإنتاج وكان أفضل إنتاج عند أستعمال $NaNo_3$ بتركيز 0.3 غم /100 مللتر وسط الإنتاج وأخيرا حصل على أفضل إنتاج عند مدة حضن بلغت 72 ساعة. وكان أفضل إنتاج للبروتين أحادي الخلية بعد تطبيق الظروف المثلى أعلى بمقدار 1.712 غم /100 مللتر وسط الإنتاج. بينت نتائج تحليل وفصل راشح المزرعة بعد أستخلاص البروتين أحادي الخلية وبأستعمال تقنية كروموتوغرافيا الغاز GC-MS إن هذا الراشح يحوي على 15 مركبًا كيميائيًا فعالًا ذات استعمالات واسعة ومفيدة وهذه المركبات هي 6-كلورو-3،3-ثنائي ميثيل-8-فينيل-4،3-ثنائي هيدرو-1-H ثيوبيران-4-ون و نيوبنتان و[1,2,4] أوكساديازول، 5-(4-ثالثي بوتيل فينيلوكسي ميثيل)-3-(ثيوفين-2-يل)- و ميثان، إيزوسياناتو و 2-(4-ميثوكسيفينيل)-2-(4-ثلاثي ميثوكسيليلوكسي) بروبان و سيلان، ثنائي ميثيل (2-كلورو-5-ميثيل فينوكسي) هبتيلوكسي و أنهيدريد حمض البروبانويك و N,N'- (4-ميثيل-م-فينيلين) ثنائي(p-تولوين سلفوناميد) و 1,3- ثنائي أمينو بروبان و فيتامين E و سيلان، ثنائي ميثيل (2-كلوروفينوكسي) أوكتيلوكسي و سكسينات فيتامين E و ألفا-توكوفيرول-بيتا-دي-مانوزيد و حمض الأسيتيك، [(1،1-ثنائي ميثيل إيثيل) ثيو] .

1. Introduction

Single-cell protein (SCP) is the first product of the fermentation process. It is a dried cell mass called bioprotein, microbial protein, or biomass. It is produced in large quantities by non-pathogenic microorganisms such as bacteria, algae, fungi, and yeast. However, bacteria and fungi are the main producers of this protein. This is due to their rapid development rate and high protein content in their chemical composition. In addition to proteins, SCP contains vitamins, carbohydrates, lipids, nucleic acids, amino acids, important minerals, and various other nutrients. These elements are alternatives to traditional proteins. SCP is an effective alternative to more expensive protein sources such as fish, soy products, etc. SCP can easily replace traditional protein sources in human food and animal feed without adverse effects. It has been proven to be a good protein substitute [1-3].

According to data collected by the "Food and Agriculture Organization" of the United Nations, one alarming indicator of a protein gap is that 25% of the world population lacks protein. However, conventional animal husbandry cannot provide the current population with sufficient protein-rich food. In order to meet the requirements of the rapidly increasing population Single cell protein (SCP) synthesis from microorganisms has been explored as an alternative protein source to traditional animal and plant proteins. The term "single-cell protein" was first used in 1967 by Carol Wilson to replace the terms "petroleum protein" and "microbial protein" since most of the microorganisms used are unicellular. Their protein is called single-cell protein, biomass, or important proteins isolated from pure or different strains [4].

The large deficit of protein sources in the global market is a problem that could expand in the next few decades. The expected increase in population to 9 billion by 2050 could have a negative impact on this situation. This would lead to an overall increase in food demand. Furthermore, the future of fish and animal husbandry and protein crop production may be disrupted by the continued loss of water and agricultural land, reducing the primary sources of protein used in the human diet [5]. Consumer awareness and willingness to eat healthy food have increased significantly in recent years. In order to meet these needs, scientists are looking for innovative ways to produce food. Single-cell protein is an easily digestible source with a balanced amino acid composition. Biomass (single-cell protein SCP) is an available bioproduct obtained using many inexpensive wastes, including agricultural and industrial wastes, as a culture medium [6]. Since the early 1950s, studies have been conducted on new and unconventional protein sources that can replace traditional animal and plant proteins. As a result, a new protein production technology called single-cell proteins has been developed. This technology uses microbes in the production process. This innovative approach offers new possibilities for protein production and has received significant attention in recent years. The protein extract or biomass from a single culture or mixed culture of microorganisms is called SCP [7]. Single-cell proteins may be very useful in solving these problems and are considered a protein-rich food supplement and a component of human and animal nutrition originating from microorganisms such as bacteria, algae, fungi, and yeast [8].

It has been recently reported that various bacteria can efficiently treat many pollutants. According to recent studies, microorganisms are the ones that enzymatically degrade pollutants. Then, converting them into useful and non-toxic compounds is the basic process of bioremediation. Since bioremediation is only effective when environmental conditions allow microbes to multiply and become active. Implementing bioremediation requires modifying

environmental factors to promote faster microbial growth, decomposition, and conversion of pollutants. Microbial growth is influenced by several factors, such as pH, temperature, adequate nutrient content, and other factors [9].

Many processes, such as the production of various enzymes, proteins, and many types of fermented foods, use mixed inoculums, in which the microbial inoculum consists of two or more organisms. Mixed culture fermentations can be more beneficial than monocultures in several ways, such as increased productivity, improved utilization of substrates, increased adaptability to changing conditions, and increased resistance to contamination by undesirable microorganisms [10]. In nature, many different species of microorganisms naturally coexist and interact with each other. Many of them work best when combined with other species of microorganisms. It has been shown that using a single strain in microbial fermentations can hinder many important biochemical processes. However, using a variety of strains gives the best results because many strains of bacteria, fungi, and yeast can grow synergistically with each other. They are widely used as mixed cultures to improve production processes [11].

1.1 Research objectives

1.1.1 Primary Objective:

To produce single-cell protein (SCP) using a mixed microbial inoculum of *Kytococcus sedentarius* and *Pseudomonas oleovorans* by utilizing solid medical waste as the primary carbon and energy source.

1.1.2 Secondary Objectives:

To optimize key parameters for SCP production, including pH, temperature, nitrogen source type and concentration, inoculum size, and incubation time, to achieve maximum SCP yield.

To identify and characterize the active chemical compounds in the culture filtrate using gas chromatography-mass spectrometry (GC-MS).

To evaluate the potential of solid medical waste as a sustainable and effective substrate for SCP production in the context of bioremediation and waste management.

2. Materials and Methods

2.1 Isolation of single-cell protein Producing Bacteria

In order to isolate bacteria capable of degrading solid medical waste and using them to produce single-cell protein, 30 soil samples were collected from different areas of Anbar Governorate, 1 gram was taken from each soil sample after purification from contaminants and decimal dilutions were made with distilled water in test tubes. Then, 1 ml of each of the fourth and fifth dilutions of each soil sample was placed in Petri dishes. Then, sterile PVC agar medium was added, which consisted of (0.5g of K_2HPO_4, 0.4g of KH_2PO_4, 0.1g of NaCL, 0.02g of $CaCL_2$, 0.2g of $(NH_4)_2SO_4$, 0.02g of $MgSO_4$, 0.012g of $FeSO_4$, 0.1g of $MnSO_4$, and 3g of (PVC) Polyvinyl chloride with the addition of 15g of the agar-agar hardener in 1000 ml of distilled water). The plate was moved in a circular motion clockwise and counterclockwise to ensure homogeneous distribution of the sample. The plates were left until the medium solidified and incubated in an incubator at 30 ℃ for 6 days in an upside-down manner. The isolates were then purified by sub-culturing on the surface of PVC agar medium using a flame-

sterilized loop to obtain single pure colonies. The purified isolates were then screened (primary and secondary screening) on the same medium by culturing them in a circle with a diameter of (1 cm) in the center of the dish. They were incubated for 6 days inverted at 30 °C. This medium was used to test the ability of bacteria to degrade PVC by observing growth in the center of the dish. The medium's ability to degrade was detected by observing growth and measuring the diameter of the colony or the transparent area formed in the center of the dish.

2.2 Testing of selected isolates on solid medical waste agar medium

The selected bacterial isolates were cultured after primary and secondary screening on solid medical waste medium, the same PVC agar medium prepared previously. However, polyvinyl chloride was replaced by solid medical waste. The selected isolates were cultured in a circle with a diameter of (1 cm) in the center of the dish. They were incubated for 6 days at 30°C. This medium was used to test the ability of bacteria to decompose solid medical waste by observing growth in the center of the dish. The property of decomposing solid medical waste was detected by observing growth and measuring the diameter of the colony or the transparent zone in the center of the dish. Based on this test, three efficient isolates were selected and used in the subsequent stages of the study.

2.3 Testing the efficiency of selected isolates on the analysis of solid PVC medium in synergy

In order to test the ability of selected bacterial isolates to grow on solid PVC medium together and analyze the medium in synergy, the plates containing the medium were inoculated with each of the three isolates in the form of a circle with a diameter of (1 cm) at the ends of the plate. The plates were incubated inverted at 30 °C for 6 days. These isolates' growth and analysis on solid PVC medium were observed, and the two best isolates that grew in synergy were selected.

2.4 Identification of bacterial isolates

The selected bacterial isolates were identified based on cultural characteristics, microscopic properties, and biochemical tests. The diagnosis was confirmed using the Vitek 2 Compact device.

2.5 Preparation of bacterial Inoculum

The two most efficient bacterial isolates were selected to analyze the solid medical waste medium to prepare the bacterial inoculum. Then, after growing the two selected isolates on the solid Nutrient agar medium, a portion of the colony was taken from each of the two isolates. Each was cultured in conical flasks containing the sterile liquid Nutrient Broth medium. The flasks were shaken well and incubated at 30°C for 24 hours to obtain the liquid inoculum for each of the two isolates. This process was repeated at each stage of the subsequent study.

2.6 Estimation of single-cell protein SCP

To estimate the single-cell protein produced, the following steps were followed:

The two selected bacterial isolates were grown as a mixed inoculum on the production medium (medical, solid waste medium) in 250 ml conical flasks with 100 ml of medium for each flask. The pH of the medium was adjusted to 7. These flasks were inoculated with a double inoculum of both bacterial isolates, with 1 ml of each inoculum. The bacterial culture was

incubated at 30°C for 72 hours. After the incubation period, the bacterial culture was filtered using gauze. The biomass was separated from the bacterial culture filtrate. Then, the bacterial culture filtrate was centrifuged at 3000 rpm for 10 minutes. The precipitate was added to the separated biomass. The biomass was washed with distilled water several times. Then, the biomass (single-cell protein) was placed in an electric oven at 65°C for 48 hours for drying. Then, the biomass (a single-cell protein produced) was weighed and calculated after drying.

3. Optimization of the optimal conditions for the growth of selected bacterial isolates producing single-cell protein:

The liquid medical waste medium was used. The medium consisted of (0.2g $NaNo_3$, 0.05g KH_2PO_4, 0.02g $MgSO_4.7H_2O$, 0.002g $CaCl_2.2H_2O$, 0.002g $MnSO_4.H_2O$, 0.002g $FeSO_4.7H_2O$, 0.02g Yeast extract, 0.02g Peptone, and 2 g of solid medical waste) in 100 ml of distilled water to determine the factors affecting the production of single-cell protein. These included (pH, temperature, types of nitrogen sources, concentration of the selected nitrogen source, inoculum size, and incubation duration). The medium was placed in 250 ml conical flasks, with 100 ml of liquid medium containing solid medical waste per flask. The media were sterilized by autoclaving. Then, the media in the flasks were inoculated with the two isolates. The two bacteria were used. The cultures were incubated in the incubator at 30°C for 72 hours. After the incubation period, the biomass was weighed after washing and drying to determine its SCP content.

3.1 Effect of pH

A set of conical flasks containing the production medium were prepared. The pH of the medium in this study was adjusted to the numbers (6-7-8-9-10) to identify the optimal pH for the production of single-cell protein in the liquid medium.

3.2 Effect of temperature

A set of conical flasks containing the production medium was prepared. The temperature was set at different degrees (25-30-35-40-45 C) to determine the optimum temperature for producing single-cell protein in the liquid medium.

3.3 Effect of inoculum size

The production medium was inoculated with the mixed inoculum and different inoculum sizes (2-4-6-8) ml for each mixed inoculum/100 ml to determine the optimum inoculum size for single-cell protein production in the liquid medium.

3.4 Effect of nitrogen sources

Three different nitrogen sources (urea, ammonium sulphate, sodium nitrate) were used to select the best nitrogen source for single-cell protein production in a liquid medium.

3.5 Effect of nitrogen source concentration

Several concentrations of the selected nitrogen source sodium nitrate (0.1/0.2/0.3/0.4/0.5) g/100 ml were used to determine the optimal concentration for single-cell protein production in a liquid medium.

3.6 Effect of incubation time

This experiment was conducted to obtain the best incubation time to obtain the best production. The liquid cultures were incubated in the fixed incubator for different incubation times (24-48-72-96-120) hours to determine the appropriate incubation time for single-cell protein production in a liquid medium.

3.7 Identification of active compounds using GC-MS

To identify the chemical compounds resulting from the biological activities of bacteria during their growth and production of single-cell protein present in the bacterial culture filtrate, gas chromatography was used using a GC-MS device. Using gas chromatography to identify the chemical components, GC/MS analysis was performed using Trace GC UItra / ISQ Single, Scientific thermos TG-5MS, Quadrupole M5 fused silica capillary column (30 m 0.251 mm, 0.1 mm). The gas chromatography/mass spectrometry study used helium gas as the carrier gas at a constant flow rate of 1 ml/min. An electronic ionization system was used with an ionization energy of 70 eV. The temperature of the injector and the MS transfer line were set at 280 °C. The oven temperature was programmed at an initial temperature of 50 °C (hold for 2 min) to 150 °C at an incremental rate of 7 °C min and then to 270 °C at an incremental rate of 5 °C min (hold for 2 min), then to 310 °C as a final temperature at an incremental rate of 3.5 °C min (hold for 10 min). Quantification of all identified components was investigated using the relative peak area ratio. Tentative identification of compounds was made based on a comparison of relative retention time and mass spectra with those of NIST and WILLY library data for GC/Ms [12].

4. Results and discussion

4.1 Initial isolation on pure PVC agar medium

After conducting the initial isolation process on 30 different soil samples brought to the laboratory from different areas in Anbar Governorate, 72 different bacterial isolates were obtained. These isolates can grow and be analyzed on a sterile PVC agar medium. These isolates varied in their ability to analyze the medium based on the diameters of the colonies growing on PVC medium, which was used as the sole source of energy and carbon at a pH of 7.0, a temperature of 30 C, and an incubation period of 72 hours. After that, the purification process was carried out for the first and second time for the growing bacterial colonies by replanting them by planning on the same medium until obtaining single and pure bacterial colonies by observing their growth in the plates to use them in the subsequent study. The variation in the analysis of the PVC medium may be due to either the ability of the bacterial isolates to consume the nutrient medium or the source of these isolates. [13] obtained 6 different bacterial strains belonging to the genus Bacillus spp from soil with the ability to degrade low-density polyethene (LDPE) based on the observed growth of colonies on LDPE-containing agar medium. The isolated bacterial strains include *Bacillus coagulans*, *Bacillus sporothermodurans*, *Bacillus carboniphilus*, *Bacillus neidei*, *Bacillus smithii*, *and Bacillus megaterium*.

4.1.1 Primary screening of bacterial isolates producing single-cell protein SCP

The results of re-purifying bacterial isolates that analyzed PVC medium showed the selection of 35 bacterial isolates that can grow on PVC agar medium with high efficiency based on colony diameters to conduct secondary screening. There was a variation in the average

diameters of the colonies growing on the medium, as shown in Table (1). The diameters of the growing colonies ranged from (1-5 cm). The variation in the diameters of the bacterial isolate colonies may be either the growth requirements or the source or nature of the selected bacterial isolates. The colonies growing in the plates after primary screening showed their ability to analyze the medium clearly, as shown in Table (1). [14] obtained 4 important bacterial strains: *AK-1 (MW898426)*, *AK-2 (MW898428)*, *AK-3 (MW898430)*, and *AK-4 (MW898432)*. These four strains belong to different genera: *Stenotropicomonas .sp*, *Serratia .sp*, and *Pseudomonas .sp*, respectively. It was found that these strains can degrade plastics, including polyethene and polystyrene.

Table 1- Selected bacterial isolates from the initial screening

N	Isolation symbol	Soil type	Region	Diameter (cm)
1	A1	wheat crop soil	Garma	3
2	A2	wheat crop soil	Garma	2
3	A3	wheat crop soil	Garma	2
4	A4	wheat crop soil	Garma	2
5	A5	wheat crop soil	Garma	2
6	A6	wheat crop soil	Garma	2
7	B1	Generator soil	Alkhaldia	2
8	C1	Alfalfa crop soil	Eastern Heseba	2.5
9	C2	Alfalfa crop soil	Eastern Heseba	2.5
10	C3	Alfalfa crop soil	Eastern Heseba	2
11	D1	Okra crop soil	Eastern Heseba	1.5
12	D2	Okra crop soil	Eastern Heseba	2
13	E1	Vineyard soil	Garma	2
14	E2	Vineyard soil	Garma	2
15	E3	Vineyard soil	Garma	2.5
16	E4	Vineyard soil	Garma	1.5
17	F1	Soil contaminated with household waste	Alsofia	2.5
18	G1	Alfalfa crop soil	Garma	2.5
19	G2	Alfalfa crop soil	Garma	1
20	G3	Alfalfa crop soil	Garma	2
21	H1	Soil contaminated with household waste	Alsofia	2
22	H2	Soil contaminated with household waste	Alsofia	1
23	I1	Generator soil	Alsydkia	2
24	I2	Generator soil	Alsydkia	2.5
25	J1	swamp soil	Almadeq	1.5

26	K1	Apple and orange orchard soil	Eastern Heseba	2
27	K2	Apple and orange orchard soil	Eastern Heseba	2.5
28	L1	Sandy soil	Garma	2
29	L2	Sandy soil	Garma	2
30	L3	Sandy soil	Garma	5
31	M1	Blacksmith soil	Alkafaat	1.5
32	N1	home garden soil	Alfalluja	3
33	N2	home garden soil	Alfalluja	2.5
34	N3	home garden soil	Alfalluja	3
35	N4	home garden soil	Alfalluja	2

4.1.2 Secondary screening of bacterial isolates producing single-cell protein SCP

After completing the primary screening, the secondary screening process was carried out. The 35 selected bacterial isolates were planted on PVC medium in a circle with a diameter of (1 cm) in the middle of the dish to select the most efficient isolates in analyzing the solid PVC medium. After incubating them for 6 days, the ability of these bacteria to analyze the PVC medium was detected. In light of the results shown in Table (2), the 4 most efficient bacterial isolates that could analyze the PVC medium were selected by measuring the lysis diameters of these isolates. The efficiency of the isolates in analyzing the PVC medium varied. The highest rates of the diameters of the growing colonies were for the isolates with the symbols (A1, L1, N1, N2) (3, 5, 3, 3) cm, respectively. [14] used nylon bags as the sole energy source and carbon in liquid nylon bag medium to grow *Kytococcus sedentarius* bacteria isolated from landfill soil in Anbar Governorate. The degradation rate of nylon bags used in the study was 43.2% when applying the optimum conditions.

Table 2- Selected bacterial isolates from secondary screening

N	Isolate	code	Colony diameter (cm)
1	Wheat crop soil – Garma	A1	3
2	sandy soil – Garma	L1	5
3	Home Garden Soil – Fallujah	N1	3
4	Home Garden Soil – Fallujah	N2	3

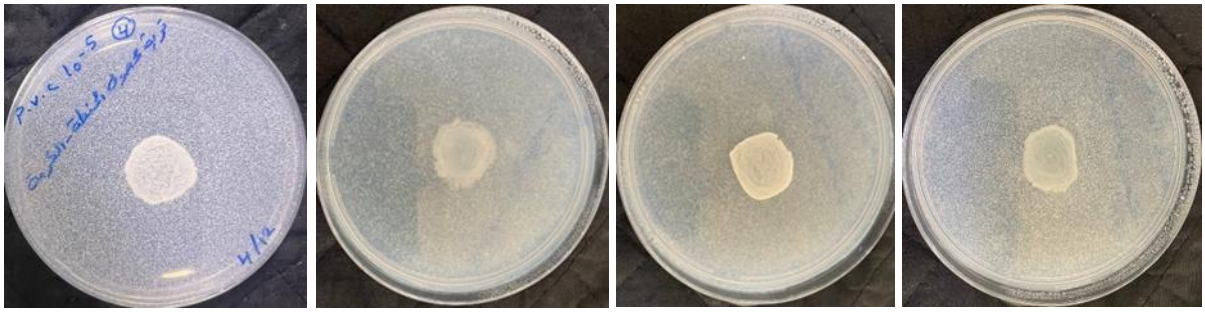

Figure -1 shows the growth of bacterial isolates selected from secondary screening.

4.1.3 Testing the ability of selected bacterial isolates to decompose solid medical waste medium

The four selected isolates in the previous paragraph were cultured again in the middle of the dish under the same previous conditions, except that the PVC material in the culture medium was replaced with solid medical waste. After incubation, the diameters of the bacterial colonies growing on the medium were measured. The isolates with the largest colony diameter were selected. Three bacterial isolates were selected that could decompose solid medical waste efficiently, and their diameters were (4, 4, 3) cm, as shown in Table (3). This indicates that the selected bacterial isolates can decompose and utilize solid medical waste as a source of energy and carbon.

Table 3- Selected bacterial isolates on solid medical waste medium

N	Isolate	code	Colony diameter (cm)
1	Wheat crop soil - Garma	A1	4
2	Fallujah home garden soil	N1	4
3	Fallujah home garden soil	N2	3

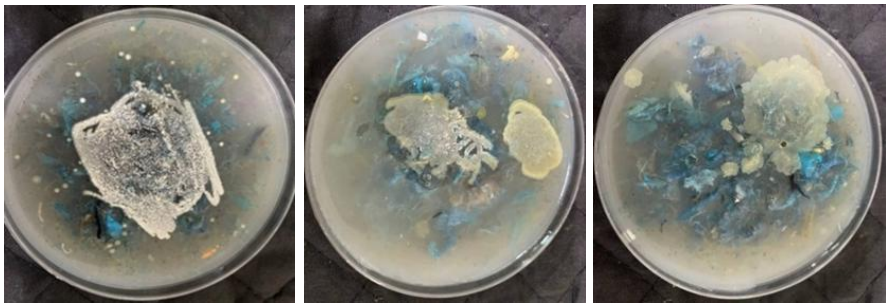

Figure -2 shows the growth of selected isolates on a solid medical waste medium.

4.1.4 Testing the ability of selected bacterial isolates to grow synergistically on PVC medium

The results of testing the ability of selected bacterial isolates to grow synergistically with each other showed that the two isolates with the local codes (N1, A1) were the best in their ability to grow synergistically and analyze PVC medium, as shown in Figure (3).

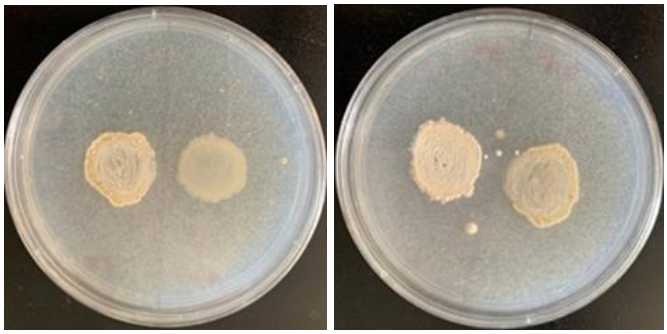

Figure -3 shows the growth of synergistic bacterial isolates.

4.2 Diagnosis of selected isolates:

The results of the cultural, microscopic, biochemical and 2 Vitek system tests for the two selected bacterial isolates with codes (A1, N1) isolated from the soil showed that: The first bacterial isolate with the local code (A1) belongs to the species *kytococcus sedentarius* which belongs to the genus that carries the characteristics of *Kytococcuc* and which previously belonged to *Micrococcus*. However, it was recently changed based on the evolutionary and chemical taxonomic characteristics [16-18]. The diagnosis was confirmed with the Vitek 2 compact device. The second bacterial isolate with the local code (N1) belongs to *Pseudomonas oleovoran*. The diagnosis was also confirmed with the vitek 2 compact device [19]. It was first described by [20]. From a taxonomic point of view, this bacterium belongs to the 16 rRNA S group (*P. aeruginosa*). Table (4) shows the diagnostic characteristics, and Table (5) shows the biochemical tests.

Table 4- Cultural and microscopic characteristics of selected bacterial isolates

N	Diagnostic features	*kytococcus sedentarius A1*	*Pseudomonas oleovoran N1*
1	shape	circle	Curly circle
2	color	white	white
3	The texture	solid	dry
4	Transparency	dark	dark
5	edge	Round	circle
6	Cell shape	circle	cirle
7	high	Hight	hight
8	Gram stain	positive	negative
9	Cell aggregation	Pairs or quartets	Individual
10	Production of dyes	not productive	not productive

Table 5- Biochemical tests of selected isolates

N	Test type	*Pseudomonas oleovoran N1*	*kytococcus sedentarius A1*
1	Gram stain	-	+
2	Oxidase	+	-
3	Catalase	+	+
4	Indole	-	-
5	Methyl red	-	-
6	Voges-Proskauer	-	-
7	Citrate utilization	-	-
8	Motility	+	-
9	Urease	+	+
10	Gelatin hydrolysis	-	-
11	Arginine	-	+

negative test (-) positive test (+)

4.3 Factors affecting the production of single-cell protein SCP

4.3.1 Effect of pH

The results shown in Figure (4) showed an increase in the rate of single-cell protein production with an increase in pH up to 9. The average weight of biomass reached 0.9675 g/100 ml. Then, the productivity decreased with the increase in pH to 0.9437 g/100 ml at pH 10.

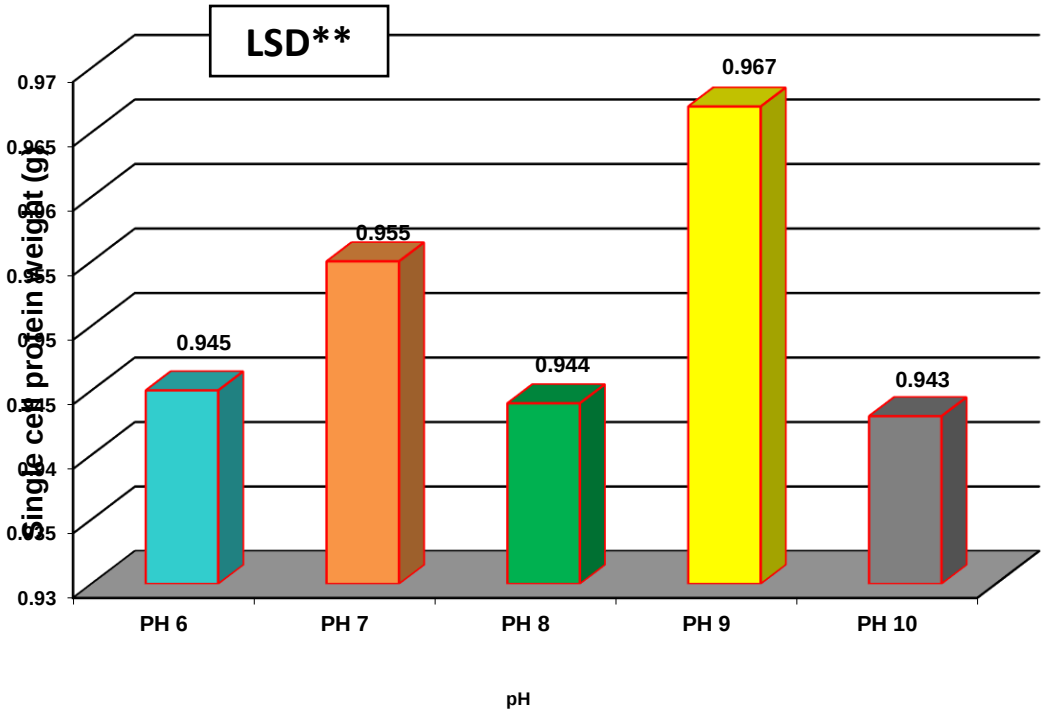

Figure-4 Effect of pH on single-cell protein production

pH is one of the essential factors in SCP production. It affects enzymatic pathways and hydrolysis rates [21-22]. pH affects the growth of microbial cells by influencing metabolic processes. It also affects the solubility of nutrients in the nutrient environment [23]. Changing the pH value beyond the optimum for bacterial cell growth stresses the bacterial cells and leads to their stress. This leads to energy consumption. This, in turn, leads to decreased growth rates and biomass production. Moreover, the acidity of the external medium is likely to affect the structure and permeability of the cell membrane [24]. Some studies indicate that the pH range of any microbial strain may vary depending on the composition of the medium. Both dry weight and protein content have been shown to decrease gradually with pH values that are higher or lower than the optimum pH [25]. Higher pH leads to a gradual decrease in protein concentration due to changes in cell membrane permeability and solubility of some medium components [26]. Some studies have suggested that the optimum pH for SCP fermentations has different values since microbial inoculums have diverse physiological requirements. The reason for this is the diversity of microbial strains used. However, the pH range of 3.5-7.0 is used in the majority of SCP fermentation research [27]. However, when used in SCP production, bacteria are generally more likely to be active at alkaline pH values [28]. Our results are inconsistent

with those of [29], who obtained a maximum biomass production of 6.2 g/L with 53.5% protein content at pH 8.0.

4.3.2 Effect of Temperature

Figure (5) shows the study results indicate increased biomass production with increasing temperature to peak at 45°C. The average weight of biomass reached 1.6058 g/100 ml of medium. At the same time, the lowest biomass production was obtained at 35°C, at 1.504 g/100 ml of medium.

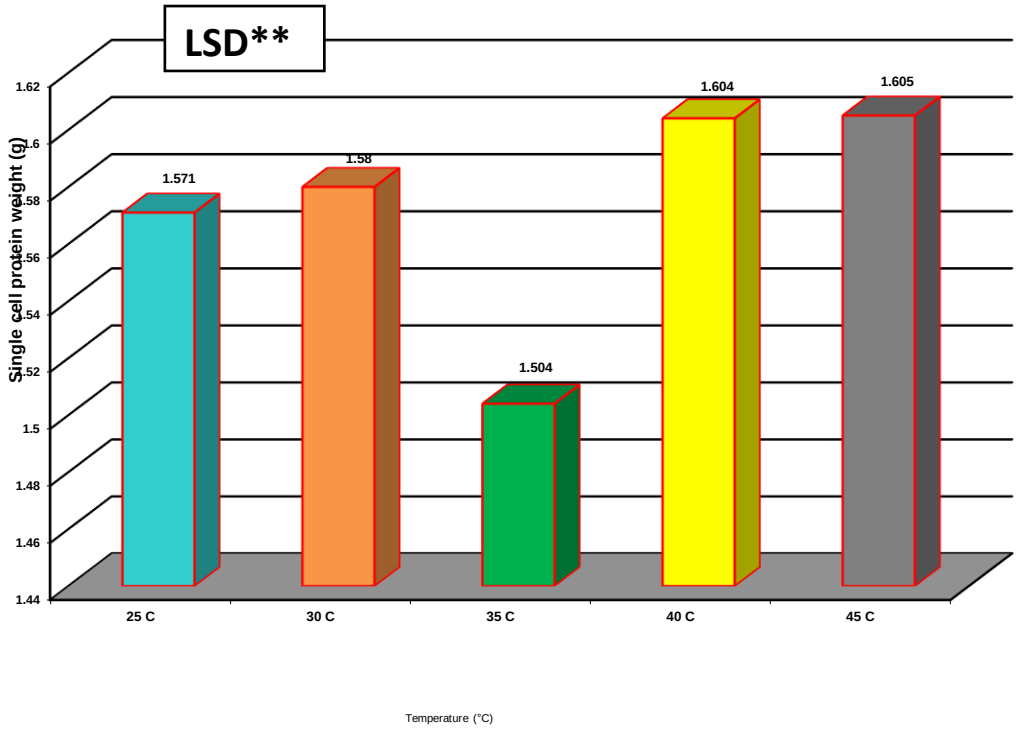

Figure-5 Effect of temperature on single-cell protein production

Temperature is one of the most variable factors affecting SCP production in liquid cultures. Since it affects the growth and activity of microorganisms and metabolic pathways that target SCP production and feedstock utilization [30-31]. High temperatures have the potential to inactivate enzymes of the metabolic pathway. Low temperatures may hinder the passage of nutrients across the cell membrane. Some studies indicate that raising or lowering the temperature above the optimum level leads to a decrease in the growth level of cells and, thus, a decrease in the rate of biomass production [32-33]. In a study conducted on single-cell protein production, [34] obtained the best biomass production at 40 °C. It affected the growth, production and synthesis of SCP by the isolated *Bacillus subtillis*.

4.3.3 Effect of inoculum size

Figure (6) shows the effect of inoculum size on single-cell protein production. The highest biomass production was obtained at a weight rate of 1.6410 g/100 ml at an inoculum size of 2 ml/100 ml of mixed inoculum. Then, the production decreased with increasing inoculum size. The lowest single-cell protein production was obtained at an inoculum size of 8 ml/100 ml medium. The biomass weight was 1.537 g/100 ml medium. The decrease in production may be

because the concentration of both *K. sedentarius* and *P. oleovoravs* increases with increasing inoculum size. Thus, the competition between microorganisms for the production medium increases.

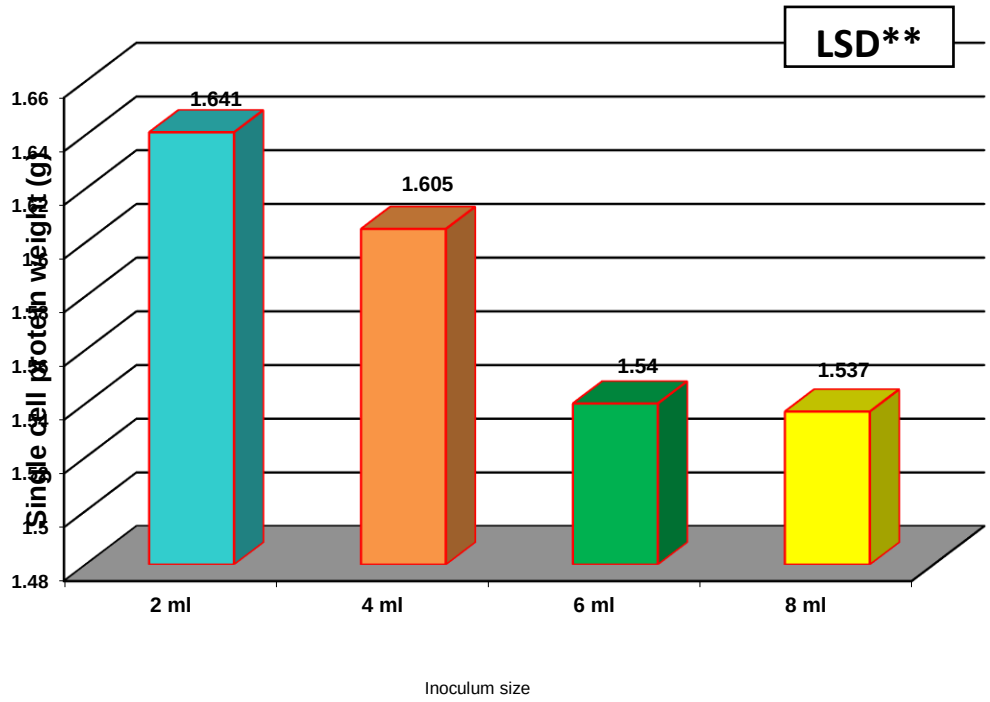

Figure -6 Effect of vaccine size on single-cell protein production

Inoculum levels affect the growth of microorganisms. The inoculum size affects SCP's fermentation process and protein content [27]. When the inoculum size is small, it causes a decrease in productivity due to insufficient biomass. That is, microorganisms cannot reach the state of growth and reproduction quickly and sufficiently to consume the nutrient medium. In contrast, large inoculum sizes lead to the formation of microbial crowding. This leads to an imbalance in the nutrient medium. It also leads to the rapid depletion of nutrients before they are physiologically ready to start the production process due to the competition of microorganisms with each other for nutrients. Thus, it limits the growth of microbes. As well as the synthesis of microbial protein, but when the inoculum size is sufficient, nutrients and oxygen will be provided for growth without competition between microbes for the nutrients available in the medium, and productivity will be at its best [35-36]. In a study conducted by [37] single-cell protein was produced at an optimal inoculation concentration of 6% from Candida utilis isolate. The researchers observed that the inoculation concentration was 6% and that dissolved oxygen and consumed oxygen were in equilibrium. In another study by [34] single-cell protein production was improved using an inoculum volume of 1.5 ml of Bacillus subtillis isolate using environmentally available paper waste.

4.3.4 Effect of nitrogen sources

The results shown in Figure (7) show the effect of the type of nitrogen source on the production of single-cell protein. The maximum production was obtained using the nitrogen source sodium nitrate ($NaNO_3$). The productivity reached 1.6707 g/100 ml. While the

productivity decreased when using urea and ammonium sulfate to reach (1.662 and 1.662 g/100 ml medium) respectively.

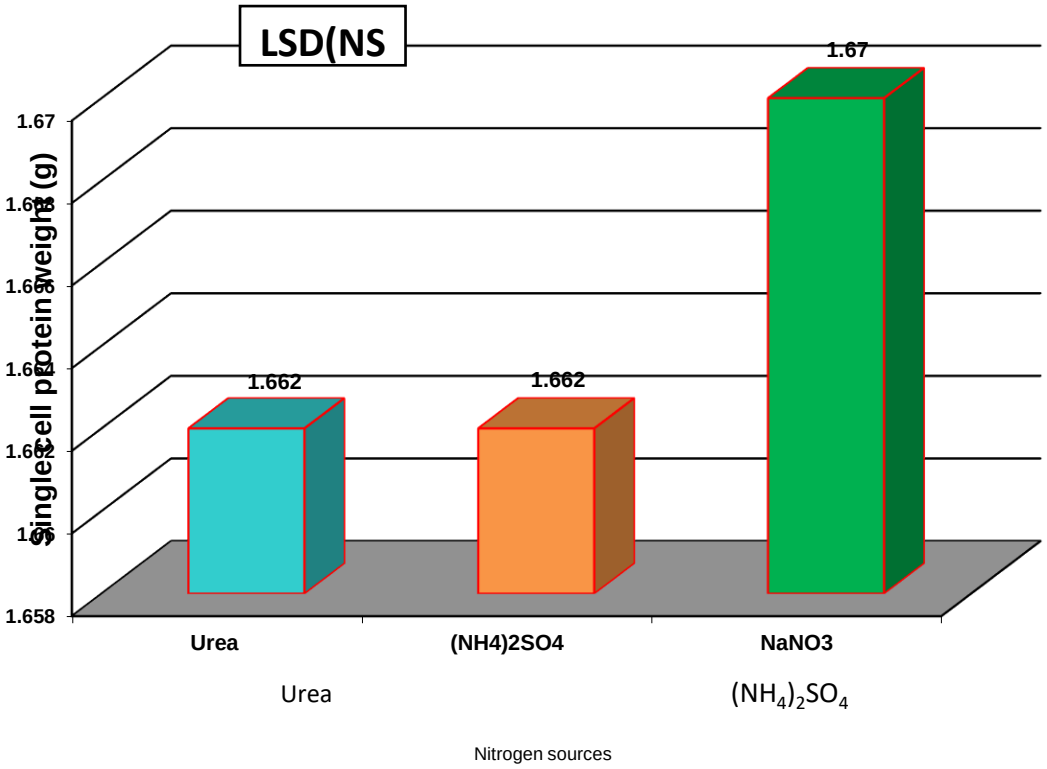

Figure -7 Effect of nitrogen source type on single-cell protein production

During protein synthesis, nitrogen is an important factor due to the structural properties of proteins [6]. Nitrogen sources are added in liquid fermentation to produce microbial biomass because nitrogen plays an important role in protein synthesis. It is also the main limiting factor for biomass concentration. It also synthesizes biomolecules involved in biomass production [38]. Microbial growth and activity are affected by nitrogen. Generally, microorganisms require a supply of nitrogen in all its forms as a medium for growth. Nitrogen metabolism, whether organic or inorganic, produces proteins, amino acids, nucleic acids, and cell wall components. It is evident that microorganisms exhibit slight differences in growth patterns in the presence of different nitrogen sources due to the relative solubility of these sources [39]. Microbes can utilize various nitrogen sources to synthesize the cell's structural and functional nitrogenous components. Nitrogen deficiency slows down the growth of microbes due to the inhibition of transport proteins that help move sugars across the cell membrane into the cell. It has been observed that an adequate supply of nitrogen sources enhances the growth of microbes [26]. Nitrogen is an important component of all amino acids that build the protein molecule. The effect of the nitrogen source on the medium reveals that maximum biomass (4.56 g/L) with a protein content of 27.9% was obtained using ammonium sulphate as a nitrogen source [40].

4.3.5 Effect of nitrogen source concentration

The results in Figure (8) show the effect of the nitrogen source concentration selected in the previous experiment as the best nitrogen source in the production of single-cell protein.

Protein productivity increased with increasing nitrogen source concentration until reaching the maximum protein production at a weight rate of 1.7126 g when using a concentration of 0.3 g/100 ml of sodium nitrate. Production was stable with increasing concentration above 0.3 g/100 ml. In contrast, productivity decreased with decreasing this concentration. It was less productive when using a concentration of 0.1 g/100 ml, reaching 1.668 g/100 ml of medium.

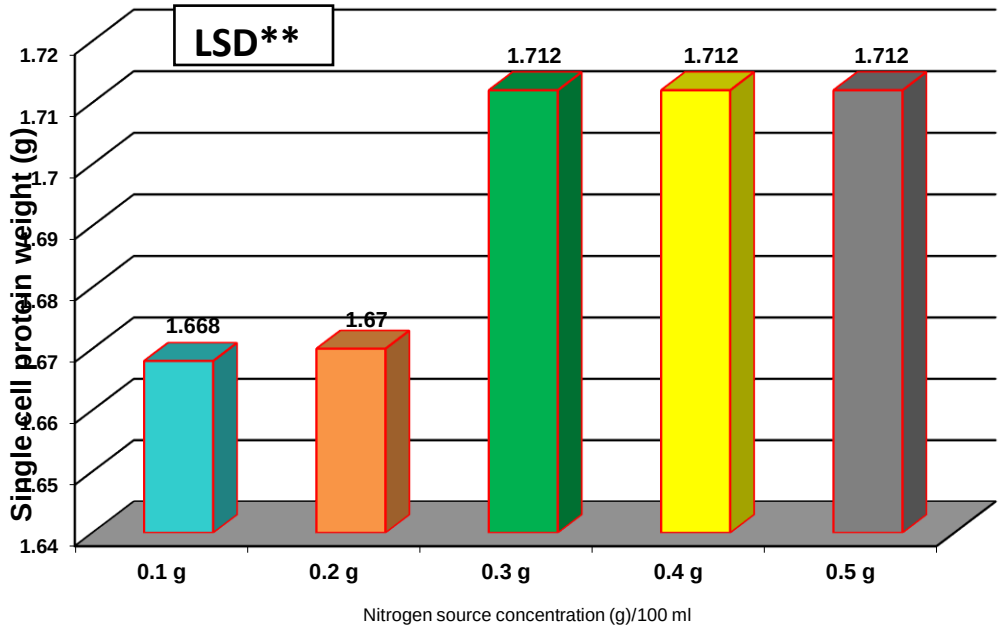

Figure -8 Effect of nitrogen source concentration on single-cell protein production

Nitrogen source concentration is a significant factor influencing SCP production. Nitrogen sources may have inhibitory or stimulatory effects on protein production by microorganisms [41]. Nitrogen source is the main component of proteins and nucleic acids. In its absence, microorganisms cannot survive [42]. Studies have shown that adding sodium nitrate to the culture medium increases SCP production [43]. However, it is also important to control the source and concentration of nitrogen because high concentrations of nitrogen sources negatively affect the growth of microorganisms and their tolerance to high temperatures. This may require replacing nitrogen sources [44]. Whereas when nitrogen concentration is low, the growth of microorganisms decreases. In the extreme case of nitrogen depletion, cells stop growing even when all other nutrients are available [45]. The medium containing 0.4% peptone was an ideal nitrogen source for the isolate Candida lipolytica 10 DSM 70561, SM-0301, to produce the highest single-cell protein content of 11.24 g/L [46].

4.3.6 Effect of Incubation period

Figure (9) shows the effect of the incubation period on single-cell protein production. The best production was after an incubation period of 72 hours, and the average dry weight was 1.7126 g/100 ml. There was a decrease in protein production when the incubation period was increased or decreased from 72 hours. Using bacteria that reach their maximum density during the logarithmic phase may be the reason for this. The number of cells in the bacterial culture will be negatively affected. The amount of biomass generated will decrease if it grows after this phase.

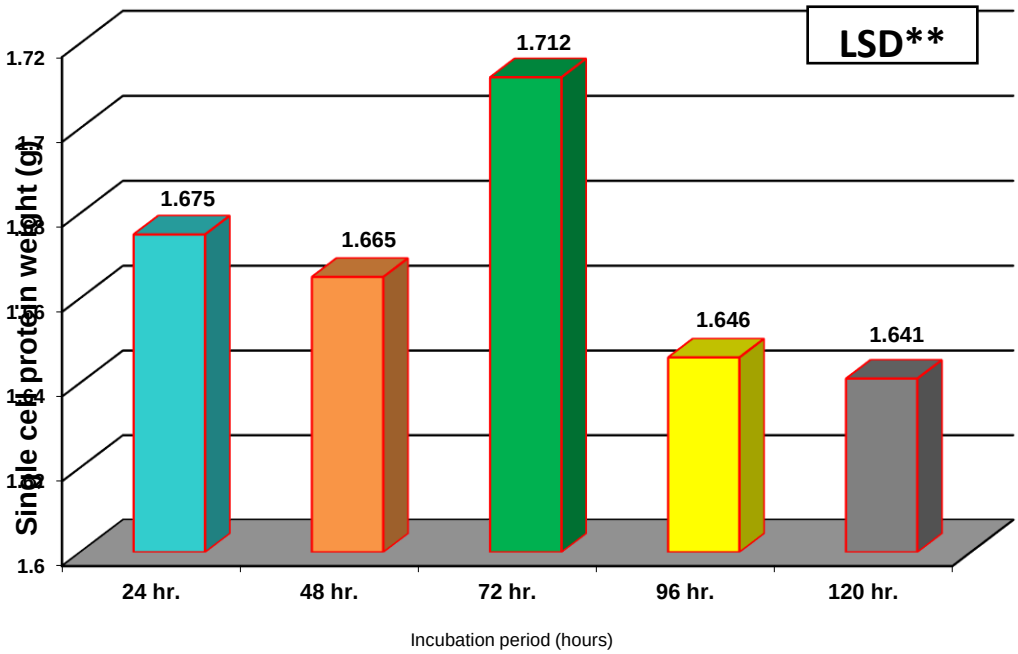

Figure -9 Effect of incubation duration (hour) on single-cell protein production

Fermentation duration can negatively affect the growth of microorganisms [47]. Prolonging the incubation duration beyond 72 h may reduce the protein concentration due to the consumption of nutrients during the long fermentation process and the limited availability of nutrients. Increasing the incubation duration may have led to the consumption of the produced protein by microorganisms to maintain their viability and metabolic activity. As a result, protein content decreased when the incubation duration exceeded 72 h [48]. Several variables, including (selected isolate type, inoculum size, temperature, pH, and production medium) affect the incubation duration. Microorganisms can proliferate and generate biomass by increasing the incubation duration. This increases productivity and depends on the specific growth conditions and nutrient availability. Due to nutrient depletion and changes in (pH, cell autolysis and toxin release), productivity can decrease with increasing incubation duration [49-50]. Also, the decrease in SCP biomass due to prolonged incubation duration may be due to cell lysis with the release of secondary metabolites during logarithmic growth [51]. The result is consistent with the study of [52], who indicated that the best biomass production was obtained after 72 h of incubation.

4.4 Qualitative analysis of chemical compounds using (GC-mass) technology GasChromatography-Mass Spectrometer

The analysis and separation of the bacterial culture filtrate produced in this study showed that it contained many active chemical compounds. This was done by separating these compounds using the gas chromatography (GC-mass) technique. As shown in Table (6), the sources show the importance of many of these compounds.

Table 6- shows some of the compounds that were identified using GC-mass technology.

Compound name	% SI	The importance of the compound
Methane, isocyanato	98	Methyl isocyanate is a valuable commercial intermediate in producing carbamate pesticides, rubber, adhesives, and plastics. The most well-known application of methyl isocyanate and other isocyanate derivatives is manufacturing polyurethane foams. This is an industry worth approximately $65 billion worldwide and growing annually. Additionally, methyl isocyanate has been detected in space. It is believed to be a critical chemical building block for forming peptide bonds [53].
Neopentane	98	Neopentane is the simplest of the alkanes and is a low-boiling liquid. It is an ideal model fuel for studying the kinetics and behavior of straight-chain alkanes. Its kinetic and thermochemical principles can be applied to larger fuel molecules [54].
Acetic acid, [(1,1-dimethyl ethyl)thio]	97	It is an organic compound of importance in organic chemistry consisting of acetic acid linked to a thiol group that bears a tert-bu group. This compound combines acetic acid's acidic properties with the thiol group's sulfuric properties. This makes it a highly reactive compound with many potential uses. It is used as an intermediate in the synthesis of other organic compounds. It is used in biological and chemical studies as a reagent or indicator.
Propanoic acid, anhydride	97	Propionic anhydride is an organic chemical compound in the acid anhydride class. It is characterized by an oxygen bond linking two acyl groups derived from propionic acid. It is used in chemical reactions as an acyl agent. It can add an acyl group to other molecules and is used to manufacture polymers, pharmaceuticals and active materials. It is also used in chemical laboratories as a chemical reagent in many reactions.
1,3-Propanediamine	97	Propanediamine is an organic compound that belongs to the diamine class, which are organic compounds that contain two amino groups (-NH_2) attached to a carbon chain. In the case of 1,3-propanediamine, the two amino groups are located on carbon atoms number 1 and 3 in the propane chain. It is used to produce many polymers, such as polyurethane and polyamide. It is also used as one of the raw materials in manufacturing many other chemical compounds, such as medicines and cleaning agents. It is also used in chemical reactions as a catalyst and water treatment process.
alpha.-Tocopherol-.beta.-D-mannoside	96	It is a complex organic compound consisting of the union of two important molecules: alpha-tocopherol and mannose. When the two molecules are combined, a new compound called alpha-tocopherol-beta-D-mannoside is produced. This compound combines alpha-tocopherol's antioxidant properties and mannose's sugar properties. It is believed to have many potential health benefits, including improving the absorption of vitamin E in the body, strengthening the immune system, protecting cells from oxidative damage, and improving skin health.

Vitamin E succinate	93	Vitamin E succinate is a modified form of vitamin E. A new compound called vitamin E succinate is formed when vitamin E is bound to succinic acid. It is an antioxidant that is vital in protecting cells from damage. It may have a stronger antioxidant effect than regular vitamin E. This helps protect cells from damage caused by free radicals. It is also used as an anti-inflammatory in the body and protects the heart and blood vessels from disease. It is also used in nutritional supplements and skin care products.
Vitamin E	92	Vitamin E is a fat-soluble vitamin crucial to maintaining a healthy body. It has many properties, including being a powerful antioxidant. This means it helps protect cells from damage caused by free radicals. Skin Health It helps maintain skin moisture and elasticity, reducing the appearance of wrinkles. Cardiovascular Health It helps prevent blood clots and protects blood vessels from damage. Immune System Health It supports the function of the immune system and strengthens its ability to fight disease. Eye Health It helps protect the eyes from damage caused by blue light and ultraviolet rays.
N,N'-(4-Methyl-m-phenylene)bis(p-toluene sulfonamide)	90	It is a complex organic compound with a cyclic structure that is important in many industrial applications. It is used in the polymer industry as a catalyst or inhibitor and in the dye industry. It is also used as an intermediate in manufacturing other chemical compounds. It is important in scientific research as it studies the properties of new materials and compounds.
Vitamin E aceta	89	Vitamin E acetate is a modified form of vitamin E when vitamin E is combined with acetic acid. A new compound (vitamin E acetate) is produced. It is one of the most important antioxidants that protect the body's cells from damage. Immune system health Supports the function of the immune system and strengthens its ability to fight diseases. Cardiovascular health Helps prevent blood clots and protects blood vessels from damage. Eye health Helps protect the eyes from damage caused by blue light and ultraviolet rays. Skin health Contributes to maintaining the moisture and elasticity of the skin and reduces the appearance of wrinkles.
Silane, dimethyl(2-chlorophenoxy)octyloxy	82	It is a complex organo-silicon compound and is considered a derivative of silane. This compound consists of a central silicon atom linked to two methyl groups, a phenoxy group containing a chlorine atom, and an octyloxy group. It can be used to manufacture silicone polymers, which are used in many applications such as lubricants, electrical insulators, and adhesives. It can be used in the manufacture of chemicals. It can be used to manufacture protective coatings that have heat and water resistance properties. It may be used as a special lubricant due to its viscous properties.
Silane, dimethyl(2-chloro-5-methylphenoxy)heptyloxy	81	This compound consists of a central silicon atom bonded to two methyl groups. A phenoxy group substituted with a chlorine atom, a methyl group, and a heptyloxy group. It may be relatively stable under normal conditions. However, it may react with strong acids and bases. In general, this compound is an important raw material

		in many industries. However, it should be handled with care due to its chemical properties. It is used to manufacture silicone polymers, which are used in many applications, such as lubricants. Electrical insulators and adhesives manufacture chemicals, lubricants, and protective coatings.
2-(4-Methoxyphenyl)-2-(4-trimethoxysilyloxy)propane	77	This compound consists of a propane chain linked to a benzene ring on one side and a silane group (containing three methoxy groups) on the other. The presence of the methoxy group on the benzene ring increases the polarity of the compound and its ability to interact with other materials. It dissolves in many organic solvents, such as acetone, toluene, and ethanol. However, it is insoluble in water. This compound works by forming silicon-oxygen bonds with surfaces. These bonds are powerful and give the materials to which this compound is applied properties such as adhesion to many surfaces, resistance to high temperatures, water resistance (prevents water leakage), and electrical insulation (prevents the passage of electric current). This compound is considered an important raw material in many industries. It plays a significant role in developing new materials with distinct properties.
Oxadiazole, 5-(4-tert-[1,2,4] butylphenoxymethyl)-3-(thiophen-2-yl)-	75	It is a complex heterocyclic organic compound (containing a heterocyclic ring, i.e. containing atoms other than carbon). It is used in many applications, especially medicine and the pharmaceutical industry. This compound is believed to work by interacting with specific proteins or enzymes in the cell, which leads to the inhibition of their vital functions. This compound is used in the manufacture of medicines. It has promising biological properties such as antibacterial and antifungal activity. It is used as an intermediate in the manufacture of other chemical compounds and is also used in scientific research.
6-Chloro-3,3-dimethyl-8-phenyl-3,4-dihydro-1H-thiopy	73	It is a complex organic compound containing a heterocyclic ring (a ring containing a sulfur atom instead of carbon) called thioB. Its density is expected to be higher than water's, so it is soluble in nonpolar organic solvents such as benzene and toluene. It is less soluble in polar solvents such as water. It has many potential uses, including pharmaceutical, chemical, and scientific research. This organic compound generally represents a starting point for many potential applications.

5. Conclusion

This study aimed to recycle solid medical waste and utilize it in the production of single-cell protein and other compounds of practical importance, the number of which reached 15 compounds. Two local bacterial isolates were obtained, namely *Kytococcus sedentarius* and *Pseudomonas oleovoran*, which have the ability to consume and decompose these wastes. A mixed sample of the two bacterial isolates was prepared and used in this study. It was shown that solid medical waste obtained from local hospitals can be utilized to obtain products of practical importance at low economic costs.

6. Recommendations

Continue conducting other studies to isolate new types of bacteria and explore their potential in analyzing solid medical waste and confirming their role in enhancing the production of single-cell protein. Conduct an analysis process to determine the type of amino acids entering into the composition of single-cell protein and the proportions of each. The possibility of using microbial vaccines consisting of more than two microorganisms (bacteria, fungi, yeast) to work together synergistically to increase production processes. Use other types of waste existing and renewable in the local environment and benefit from them to obtain products of economic importance.

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