

The effectiveness of filamentous bacteria *Streptomyces* spp in the treatment of organic pollutants

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Abstract

This study, conducted from 2022 to 2023, investigated the role of *Streptomyces* bacteria in the bioremediation of soils contaminated with organic pollutants in Sharqat District and Qayyarah, Salah al-Din Governorate. A total of 15 *Streptomyces* isolates were obtained from polluted and garden soils. All isolates were Gram-positive and showed the ability to produce catalase and amylase enzymes, in addition to distinct morphological characteristics such as chalky, circular colonies and variable hyphae colors. These bacteria are known for their slow growth and the production of volatile compounds responsible for their characteristic odor. The isolates were used in soil treatment experiments under controlled conditions. Prior to treatment, the contaminated soils showed high levels of organic matter (6.899%), electrical conductivity (33,407 $\mu\text{S}/\text{cm}$), and a slightly alkaline pH (7.9). After treatment with *Streptomyces*, the soil's organic matter content dropped to 3.381%, electrical conductivity to 4,208 $\mu\text{S}/\text{cm}$, and the pH increased to 10.04, indicating active microbial degradation of pollutants. Similarly, under natural conditions, the organic matter decreased to 3.10%, and electrical conductivity to 4,439 $\mu\text{S}/\text{cm}$, with a rise in pH, confirming the bacteria's resilience and efficiency in pollutant breakdown. These findings highlight the significant potential of *Streptomyces* in bioremediation, as they can adapt to harsh environments and actively degrade organic contaminants, thus improving soil quality and reducing pollution levels.

Introduction

Persistent organic pollutants have been raised POPs, synthetic organic chemicals, produced intentionally or unintentionally, have attracted widespread public concern in recent years[1]. These chemicals are toxic and pose a major environmental concern due to their persistence, long-distance transport, bioaccumulation, and potential adverse effects on living organisms[2]. Uncontrolled inputs combined with poor environmental management often result in elevated levels of POPs in affected estuaries. Since the adoption of the Stockholm Convention on Persistent Organic Pollutants, various technologies have been widely developed[3]. The main focus has revealed the need for low-cost methods that can be easily implemented in developing countries, such as electrochemical techniques. POPs are known to be resistant to conventional treatment methods such as agglomeration, coagulation, filtration, and oxidative chemical treatment[4]. However, several advanced wastewater treatment technologies, such as activated carbon adsorption, biodegradation using membrane bioreactors, and advanced oxidation processes (AOPs), have been applied to treat POPs[5]. Bioremediation is a process that uses living organisms to remove or reprocess an environmental contaminant through metabolic processes and plants to eliminate hazardous contaminants and restore the ecosystem to its original state [6]. These strategies include natural mitigation, biomagnification, or biostimulation, which involve microorganisms such as fungi, algae, bacteria, and filamentous bacteria[7]. Microorganisms are found in a variety of a wael, Thrives in soil, water, plants, animals, deep water, and ice, bioremediation technology is widely used and is currently growing rapidly [8]. The presence of bacteria is affected Streptomyces is a genus belonging to the filamentous bacteria Actinomycetes in the soil with available organic materials, as it depends on the decomposition of organic materials by bacteria and fungi to be ready to be taken by filamentous bacteria. The growth of Streptomyces is affected by the pH, as its decrease to less than 5 leads to the cessation of vital activities. It also increases in the surface layer of the soil and decreases as the depth increases from 2. and the appropriate pH for these bacteria is (8-6.5)[9, 10]. Therefore, this study aimed to observe the effectiveness of this bacteria and its essential role in treating organic pollutants.

Materials and methods

Collected 15 samples of soil contaminated with organic waste (hydrocarbons)And also from garden soil from different intersections in Salah al-Din Governorate, Sharqat District, as well as the Ramana area affiliated with the Qayyarah District, samples were taken at a depth of 10-15 cm after scraping 1 cm from the soil surface .Samples were placed in sterile polyethylene bags or glass bottles and labeled with the name Sample Date collected and other information Then the samples were transferred to the laboratory, where decimal dilutions were made, and the first three dilutions were planted on medium. Starch casein agar to obtain a large number of isolates of Streptomyces bacteria were placed in the incubator at 25°C for 5-7 days to obtain successful isolates containing spore chains ,The phenotypic diagnosis of the aerial hyphae was carried out in terms of growth, color and dyes soluble in the medium. Smears of these isolates were prepared and stained with Gram stain to determine their reaction to the dye and to know the shapes of the cells and spore chains.

Biochemical tests.

Biochemical tests performed to diagnose filamentous bacteria include the oxidase test, catalase test, urease test, and gelatinase test.

Movement test

The test was performed by preparing the tubes containing sterile semi-solid agar medium were prepared by adding 8 g agar per liter of liquid nutrient broth medium and were inoculated by the stabbing method and then incubated for 25 for 24 hours, observe the formation of a hazy area around the stabbing area as evidence of a positive test.

Technique implantation on-chip

This test is for filamentous bacteria to detect their ability to aerial and terrestrial circulation of bacteria according to the following steps: I took a square piece or rectangular from the middle, sterile starch casein agar was poured into plates and placed aseptically on the surface of a glass slide inside a sterile dish. Then it was inoculated from the opposite sides by pricking using a pricking needle. Then place the clean and sterile glass slide cover. Incubate the slide culture at room temperature. 25m for a period of 4 days, and the glass slide was then examined under a microscope using special forces. X4 then X40-X10 [5].

Testing the ability of bacteria to bioremediate organic pollutants

The test was conducted by preparing a culture medium specifically designed to detect the ability of bacteria to bioremediate organic pollutants. The medium consisted of: The 1000 g of soil, 20-15ml of Gas oil, 20-15ml of fuel oil, 15-20ml of engine oil and 15ml of engine oil (cherries)

Measurement chemical characteristic

pH measurements

The pH was measured using a Professional Benchtop pH meter, model BP3001. The device was pre-calibrated before measurement using solutions with a pH of (9-7-4) and tested immediately within the same day's sampling in the laboratory[11].

Organic materials measurements

The percentage of organic matter in the soil was determined using the dry method adopted by [12], which is based on the principle of weight loss resulting from the combustion of organic matter. Initially, 5 grams of soil sample were taken using a sensitive balance. The sample was then placed inside a heat-resistant ceramic pellet dish, and the combined weight of the dish and sample was recorded before any treatment. The sample was then dried in an oven at 70°C for 24 hours to remove only moisture without affecting the organic matter. The sample was then reweighed to determine its dry weight (M1). Subsequently, the dried sample was subjected to muffle furnace combustion at 450°C for 2 hours, a temperature sufficient to decompose and burn all organic matter. After the combustion was complete, the final weight of the sample and dish was measured together (M2). To calculate the ash weight.

$$\text{TOM} = \frac{\text{MO} - \text{M2}}{\text{MO} - \text{M1}} \times 100$$

Ash weight = Weight of the pellet with the sample after incineration - Weight of the empty pellet.

Mo: Weight of the empty pellet

M1: Weight of the pellet with the sample upon drying

M2: Weight of the pellet with the sample after burning

Measurement physical characteristic

Electrical conductivity measurements

The electrical conductivity of the soil was measured using the suspension method (1:5) after preparing the suspension and after filtration. The electrical conductivity of the filter was read using an electrical conductivity device at a rate of three readings and the average of those readings. The error rate was searched for after calibrating the device with its standard solutions[13]. As for the water samples, the ability of the water to conduct electricity was examined by immersing the electrode in water after placing it in the special glass bottles for a minute until the reading was fixed. It was then recorded in $\mu\text{s}/\text{cm}$. The final conductivity value was then extracted.

Preparation of Sterile Soil Medium

It was prepared by sifting the agricultural soil using a 2 mm sieve. It was then placed in an electric oven at 100°C for 24 hours to ensure it was free of microorganisms. It was then mixed with the above-mentioned ingredients to ensure homogeneity. The electrical conductivity, pH, and organic matter content of the medium were then checked, and the medium was used for processing.

Results and discussion

Isolation of filamentous bacteria Streptomyces

Isolation results showed 7 isolates of filamentous bacteria, Streptomyces. Isolated from soils contaminated with organic pollutants and 8 isolates from garden soil. The results of this study are consistent with what was mentioned[14]. Soil rich in organic matter is the natural environment for the spread of the bacteria Streptomyces. This study is also consistent with previous studies indicating the isolation of bacteria Streptomyces from the soil [15]. It is the main bacteria in the soil.

Diagnosis Bacterial Streptomyces Depending on morphological and biochemical characteristics

All isolates showed a positive result on Gram stain and when examined microscopically at high magnification.10x It appeared in a branched form. The results of the phenotypic description also showed that the isolates varied in their phenotypic characteristics. Some of them appeared in a creamy pink color on the medium starch casein agar. All isolates showed positive detection of amylase, catalase, and gelatin enzymes. After that, all isolates whose phenotypic and biochemical characteristics did not match those of the excluded bacteria, Streptomyces. The colonies on the culture medium take different shapes, including chalky, wrinkled, flat, circular, or semi-circular, The shapes of the hyphae inside the culture medium also vary between olive yellow, orange, and light, as Figure (1) shows. Some bacterial isolates are also distinguished by the fact that they produce a smell similar to the smell of wet soil after rain, due to the production of volatile compounds, which are: geosmin The gene responsible for its production is one of the fixed genes within the dependent species,

and these results are consistent with the results of previous studies [16]. In addition, bacteria, *Streptomyces*, are distinguished Slow growth on culture medium [17].



Fig. 1. shows the shapes of colonies with Gram stain for bacteria *Streptomyces*

Activity Bacterial *Streptomyces* in the treatment of organic pollutants

The results showed that the physical and chemical properties of soil contaminated with organic pollutants prior to the bioremediation process using *Streptomyces* bacteria showed a significant decrease in soil pH, reaching 7.9 in the contaminated soil. This decrease in pH can be attributed to the presence of hydrocarbon contaminants, which produce organic acids upon microbial or chemical decomposition[18]. These acids accumulate in the soil matrix, lowering the overall pH compared to uncontaminated soil samples or control soil samples, which typically exhibit higher values (≥ 8.0). This observation is consistent with previous findings in [19] and is consistent with the general concept that hydrocarbon-contaminated soils often exhibit increased acidity due to the formation of low-molecular-weight organic acids during decomposition.

In addition to the pH changes, a significant increase in electrical conductivity (EC) was recorded, reaching values of 33,407 $\mu\text{s}/\text{cm}$, indicating the accumulation of dissolved ions, likely due to the decomposition of organic compounds[20]. Furthermore, the organic matter content also increased, reaching 6.899%, which is consistent with scientific studies indicating that the accumulation of organic residues in contaminated soil contributes to high electrical conductivity values [19].

Following the bioremediation treatment using *Streptomyces* bacteria, the results revealed notable improvements in the physicochemical properties of the contaminated soil, highlighting the positive and effective role of microbial remediation. Under controlled incubation conditions and optimal temperature, the *Streptomyces* bacteria demonstrated a high capacity to utilize the existing organic pollutants—primarily hydrocarbons—as a nutrient source.

This microbial activity resulted in a significant reduction in organic matter content, which decreased from 6.899% to 3.381%, confirming the degradation and removal of organic pollutants by the bacterial action. Additionally, the electrical conductivity (EC) of the soil also dropped markedly, reaching 4,208 $\mu\text{S}/\text{cm}$, indicating a reduction in the concentration of soluble ions previously released by pollutant breakdown[21].

Concurrently, a notable increase in pH was recorded, reaching 10.04, reflecting a shift toward alkalinity. This pH elevation is associated with the microbial metabolism and degradation of acidic organic compounds, leading to a less acidic environment. The increase in pH, accompanied by the reduction in EC and organic matter, supports the successful colonization and proliferation of *Streptomyces* in the contaminated medium.

The observed high bacterial biomass and effective degradation of hydrocarbons serve as clear evidence of the efficacy of *Streptomyces* in bioremediation. These findings are in line with those reported by [22], who also documented similar improvements in soil properties post-treatment using actinomycetes. Figure 2 illustrates the key changes before and after the bioremediation process.

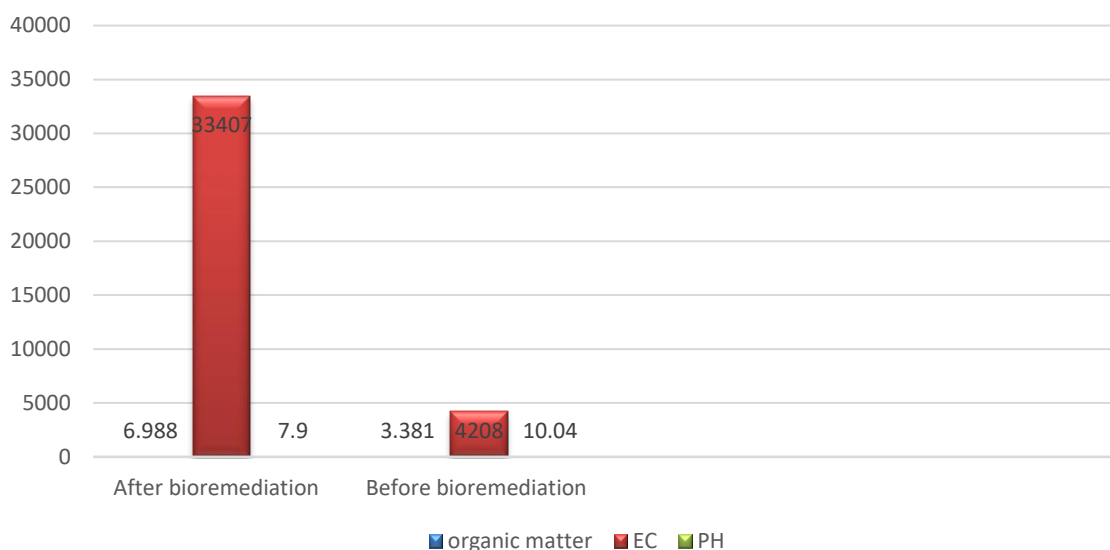


Fig. 2 Bioremediation of organic pollutants under controlled conditions

The result that appeared is consistent with many studies, such as the studies conducted by [12]. It was shown that the variation in environmental conditions between seasons and harsh conditions did not have a significant impact on the ability of the bacteria *Streptomyces*. On the decomposition of organic pollutants and evidence of the success of the experiment under these conditions, Figure (3) illustrates this. evidence of the success of the experiment under these conditions, Figure (3) illustrates this.

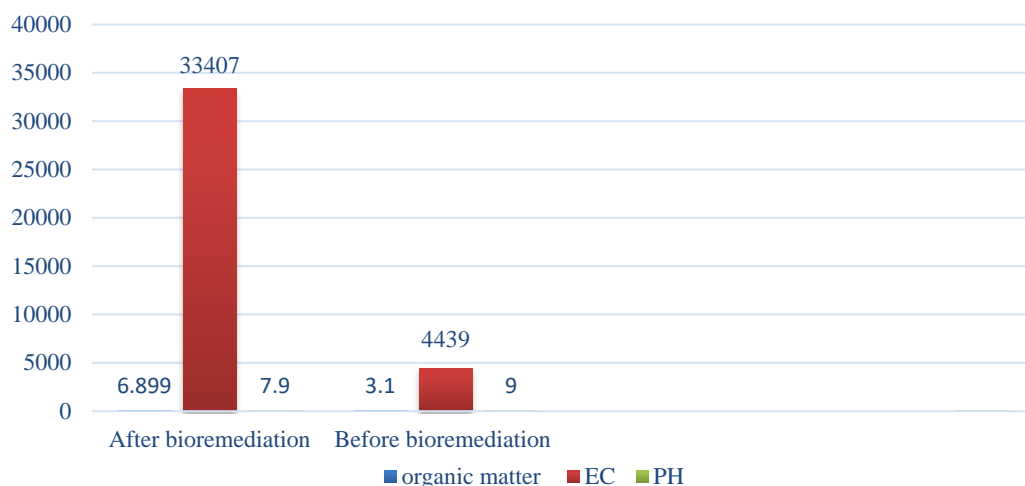


Fig.3 Bioremediation of organic pollutants under natural conditions.

Conclusion

Streptomyces spp has a large efficiency in bioremediation process which had a benefit in ecosystem. It has a high effect in uncontrolled environmental condition(temperature) which lead to decrease the concentration of organic contamination and decrease the electrical conductivity and increase the pH to more alkaline pH.

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فعالية البكتريا الخيطية *Streptomyces* في معالجة الملوثات العضوية

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الخلاصة:

أجريت هذه الدراسة خلال الفترة من عام 2022 إلى 2023، وهدفت إلى تقييم كفاءة بكتيريا *Streptomyces* في المعالجة الحيوية للتربة الملوثة بالملوثات العضوية في منطقتي قضاء الشرقاط وقضاء القيارة بمحافظة صلاح الدين. تم عزل 15 سلالة من بكتيريا الخيطية *Streptomyces* من تربة ملوثة وأخرى من حدائق سكنية، حيث أظهرت جميع السلالات صفات إيجابية لصبغة غرام، وقدرتها على إنتاج إنزيمي الكاتاليز والأميليز، فضلاً عن امتلاكها خصائص مورفولوجية مميزة مثل المستعمرات الطباشيرية والدائرية واختلاف في ألوان وأشكال الهيافات. أجريت المعالجة الحيوية باستخدام هذه السلالات تحت ظروف مختبرية مسيطرة. قبل المعالجة، أظهرت التربة الملوثة نسبة عالية من المادة العضوية بلغت 6.899%، والتوصيلية الكهربائية وصلت إلى 33407 مايكروسيمنز/سم، وكان الأس الهيدروجيني pH بحدود 7.9. بعد المعالجة، انخفضت المادة العضوية إلى 3.381%، وانخفضت التوصيلية الكهربائية إلى 4208 مايكروسيمنز/سم، مع ارتفاع في قيمة pH إلى 10.04، مما يشير إلى فعالية البكتيريا في تحلل الملوثات العضوية. وبالمثل، تحت الظروف الطبيعية، تراجعت نسبة المادة العضوية إلى 3.10%، والتوصيلية الكهربائية إلى 4439 مايكروسيمنز/سم، مع ارتفاع إضافي في قيمة pH، مما يدل على قدرة *Streptomyces* على العمل في البيئات القاسية وتحلل الملوثات العضوية بكفاءة. تُظهر النتائج إمكانية واعدة لاستخدام هذه البكتيريا كوسيلة فعالة وآمنة لمعالجة التربة الملوثة.

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