دراسة الأكتينوميسيتات المحبة للملوحة والحرارة المعزولة من تربة منطقة جدة وفعاليتها في معالجة بعض المعادن الثقيلة في المخلفات السائلة

إعداد

المحاضرة / منال جميل معتوق كيكي

ماجستير علوم (أحياء دقيقة)

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إشراف

أ.د/ إدريس منير مصطفى الترك

أستاذ الأحياء الدقيقة (جامعة طيبة _ المدينة المنورة)

كلية العلوم للبنات

جامعة الملك عبد العزيز _ جده

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Study on the Halo-Thermophilic Actinomycetes

Isolated from Jeddah Region and Their Efficiency in the Bioremediation of Some Heavy Metals in Liquid Wastes

By

Manal Jameel Matooq Kiki

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Supervised By

Prof. Dr / Idriss Muneer AL - Turk
Taiba University – Al-Madinah Al - Munawwarah

Science Faculty for Girls
King AbdulAziz University – Jeddah

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قائمة المحتويات

المستخلص

تعتبر النفايات الصناعية أحد أهم أشكال التلوث الصناعي والتي تؤدي إلى مخاطر صحية وبيئية مالم يتم إدارتها بأساليب علمية تهدف إلى إيجاد الوسائل المناسبة لتقليل مخاطرها البيئية ، كما يتم طرح العديد من المواد السامة المحتوية على كميات كبيرة من المعادن الثقيلة والأملاح ضمن مياه الصرف الصحي . وقد اتجهت الأبحاث الحديثة إلى المعالجة البيولوجية لفعاليتها وسهولة تطبيقها ، ومن جهة أخرى يعتبر موضوع معالجة المخلفات السائلة مرتفعة الملوحة من أهم النقاط التي لا تزال بحاجة إلى المزيد من الدراسة حيث أن وجود التركيزات المرتفعة من الأملاح في مياه الصرف يقلل من كفاءة المعالجة البيولوجية . ولذلك تهدف هذه الدراسة إلى عزل بعض الأكتينوميسيتات المحبة للملوحة والحرارة واستخدامها في معالجة بعض المخلفات السائلة المحتوية على المعادن الثقيلة .

وقد تناولت الدراسة ابتكار منبت جديد مخصص لعزل وتنمية الأكتينوميسيتات المحبة للملوحة أطلق علية (منبت النشا والجلوكوز ومستخلص الخميرة المدعم بمياه البحر المصنعة) . وقد تم اختبار استجابة عزلات الأكتينوميسيتات لبعض الضغوط البيئية مثل (الملوحة ، الحرارة والتلوث المعدني) ومن ثم تم اختيار (6) عزلات للدراسة المستفيضة من بين (36) عزلة من الأكتينوميسيتات المحبة للملوحة والمعزولة عند (10 ٪) كلوريد صوديوم . وقد أظهرت الدراسة ارتفاع كمية النمو بزيادة تركيز الملوحة حيث أن زيادة كلوريد الصوديوم تؤدي إلى ارتفاع النشاط الأيضي . كما وجد أن جميع عزلات الأكتينوميسيتات هي عزلات محبة للحرارة المتوسطة حيث أظهرت مقدرتها على النمو حتى عند درجة حرارة (50 م°) . وقد سجلت النتائج مقدرة العزلات على النمو في وجود تراكيز متدرجة من المعادن الثقيلة تراوحت بين (100 – 600 ملجم/ل) تضمنت الكادميوم ، الرصاص ، النحاس ، الزنك والمنجنيز ويلاحظ انخفاض معدل النمو وزيادة كمية العناصر المستهلكة مع زيادة تركيز العناصر الثقيلة ، وقد وجد أن النحاس هو أكثر العناصر المؤثرة والمثبطة للنمو في حين كان المنجنيز أقلها تأثيراً .

وقد كشفت الدراسة عن مقدرة النشاط التضادي لعزلات الأكتينوميسيتات الذي أقتصر على البكتريا بالإضافة إلى مقدرتها على إنتاج العديد من الإنزيمات الخارجية . كما تم تعريف عزلات الأكتينوميسيتات بواسطة تحليل التسلسل الجزئي للجين (16SrDNA) . كما تناولت الدراسة معالجة بعض المعادن الثقيلة بواسطة عمليتي التراكم الحيوي والإدمصاص الحيوي باستخدام مياه صرف محضرة معملياً وقد كان عنصر الرصاص الأعلى إزالة في كلا الحالتين . كما تم استخدام مياه صرف صناعي خام لدراسة مقدرة العزلات على التجميع الحيوي وقد أثبتت النتائج أن إضافة الجلوكوز بنسبة (1 ٪) إلى مياه الصرف الصناعي الخام يؤدي إلى تحفيز نمو العزلات على عينات الصرف الصناعي واستهلاك المعادن بشكل أسرع ، وفي المقابل لم تسجل عملية الإدمصاص الحيوي أي تغير في تركيز المعادن الثقيلة قبل وبعد المعالجة . كما أثبتت الدراسة أن المعالجة الأولية لمخلفات الصرف الصناعي تساهم في خفض محتواها من المعادن الثقيلة مما يتيح نمو الأكتينوميسيتات بشكل أفضل وبالتالي نجاح المعالجة البيولوجية . وبتطبيق نظام المعالجة البيولوجية من خلال التراكم الحيوي لمياه الصرف الصحي مرتفعة الملوحة تمكنت عزلات الأكتينوميسيتات من النمو وإزالة كمية كبيرة من الأملاح الكلية الذائبة بنسبة تصل إلى (86.95 ٪).

4-2 التوصيات

Recommendations

1. السعي والتوجه لدراسة الكائنات الدقيقة المتطرفة يتيح المجال لاكتشاف العديد من الكائنات الدقيقة الجديدة التي تعتبر هدفاً مثالياً في تطبيقات التقنية الحيوية ، وعلى وجه الخصوص في مجال المعالجة الحيوية نتيجة خواصها الفسيولوجية المميزة .

2. إجراء الدراسات المكثفة لعزل الكائنات الدقيقة المتطرفة من البيئة المحلية بدلاً من استيرادها من مناطق أخرى ، حيث يمكن أن تفقد هذه الكائنات خواصها وفعاليتها في عمليات المعالجة نتيجة اختلاف البيئة .

3. أهمية البحث والدراسة للوصول إلى أوساط غذائية جديدة تساهم في الحصول على أكبر كمية من النمو للكائنات الدقيقة المستخدمة في مجال المعالجة البيولوجية .

4. يمكن استخدام الكائنات الدقيقة المتطرفة في معالجة المعادن الثقيلة وإزالة السمية والملوحة من المخلفات السائلة .

5. يمكن استخدام الكائنات الدقيقة المتطرفة في إزالة بعض المركبات العضوية مثل : النشا ، السليلوز ، الدهون وغيرها من مخلفات الصرف الصناعي أو الصحي .

6. يمكن القضاء على الكائنات الدقيقة الممرضة الموجودة في مخلفات الصرف الصحي من خلال المعالجة بواسطة الكائنات الدقيقة المتطرفة المنتجة لمضادات الحيوية .

7. استخدام تقنية الإدمصاص الحيوي Biosorption كوسيلة معالجة ثانوية أو نهائية لإزالة المعادن الثقيلة من موارد المخلفات السائلة أو لتعزيز كفاءة معالجة مياه الصرف .

8. تسجيل مستويات منخفضة من المعادن الثقيلة في مياه الصرف لا يعني استخدامها بشكل آمن حيث أن الاستخدام المستمر يسبب السمية مستقبلاً .

9. السماح للمخلفات الصناعية بالمرور عبر مصارف الصرف الصحي بعد المعالجة الأولية للتخلص من المعادن الثقيلة .

10. يجب دراسة إمكانية الطرح الأمن لمياه الصرف الصناعي بكافة الطرق الممكنة قبل الطرح للبيئة مثل المعالجة المسبقة عند المصدر .

11. يمكن المعالجة المشتركة لمياه الصرف الصناعي عن طريق خلط خطوط الصرف القاعدية مع الحمضية للمعادلة ، ويمكن اعتبارها خطوة معالجة تمهيدية في نفس الوقت.

ABSTRACT

Industrial waste is one of the main forms of industrial pollution that lead to health and environmental hazards if not managed by scientific appropriate methods to reduce their environmental risks. Moreover, the addition of many toxic substances containing high concentration of heavy metals and salts to the sewage water lead to increase environmental pollution. Therefore, recent research has tended to biological treatment for its effectiveness and ease application. On the other hand, the treatment of high salinity effluent is one of the most important points that still need further study, since that the presence of high concentrations of salts in the wastewater reduced the efficiency of biological treatment. Accordingly, the recent study aims to isolate some Halo-Thermphilic actinomycetes and use them in the treatment of some heavy metals in liquid waste.

The study was comprised an innovation of medium designed to isolated and growth halophilic actinomycetes which called (starch - glucose and yeast extract medium supported with artificial sea water). Also the study was observed the response of halophilic actinomycetes isolates to some environmental stresses such as (salinity, temperature and metal pollution) and then were selected (6) isolates for extensive study among (36) isolates of halophilic actinomycetes isolated at (10%) sodium chloride. The results reported a high amount of growth with increasing salt concentration, This could be due to the fact that increasing the amount of sodium chloride lead to high metabolic activity. In addition, it has found that all the isolates are Thermotolerant isolates, depending on their ability to grow until (50 C º). The results revealed the ability of the isolates to grow in the presence of gradual concentrations of heavy metals ranged between (100-600 mg/l), which contained cadmium, lead, copper, zinc and manganese, and showed a decline in the growth rate with increasing the amount of heavy metals consumption at a high concentration of heavy metals. It has been found that copper is the most inhibitory elements for growth, while manganese was the least influential.

The study observed the antibiotic activity of actinomycetes isolates which was limited towards bacteria. In addition, it was reported their ability to produce several extra cellular enzymes. The study has been identified actinomycetes isolates by partial sequence analysis of the gene (16SrDNA). The study also considered to treat some heavy metals throw bioaccumulation and biosorption by using synthetic wastewater, it was found out that lead is the highest elements removal in the both cases. Also it has been used raw industrial wastewater to study the ability of isolates in the intracellular bioaccumulation . The results reported that the addition of glucose (1%) to the raw industrial wastewater stimulated the growth of isolates and managed them for uptake heavy metals faster. On the other hand, the biosorption process did not record any change in the concentration of heavy metals before and after the treatment . Also , The study proved that the primary treatment of industrial wastewater contribute to reducing the content of heavy metals, and allowing better growth of actinomycetes and thus the success of biological treatment. On the other hand the applying of biological treatment system by halophilic actinomycetes isolates to sewage water that containing large amount of salts through bioaccumulation found out that

actinomycetes isolates can grow and remove large amount of total dissolved salts up to (86.95 %) .

Summary

Industrial waste is one of the main forms of industrial pollution that became concerned problem, which in turn leads to health and environmental risks if not managed properly by designed scientific methods to finding appropriate ways for reducing their environmental risks. In addition to that, a host of toxic substances in the sewage lead to increasing environmental pollution as they contain a large amount of heavy metals and salts.

It was proved the increasing of heavy metals concentrations in the environment due to industrial activities, where there are releasing a free heavy metals ions during industrial processes, which represent the most important inorganic contaminants in the environment and leading to the contamination of various water sources that result many health and environment warnings.

In spit of there are several methods to remove heavy metals pollution, but most of these methods are not effective in practice. Therefore, recent research has tended to biological treatment due to their effectiveness and ease of application as well as its inexpensive and environmentally friendly. On the other hand, the treatment of high salinity effluent (which produces as a result of some industrial processes) is one of the most important points that still need further study since that the presence of high salt concentrations in wastewater reduces the efficiency of biological treatment.

Although there are many studies for using different microorganisms in the biological treatment such as bacteria, yeasts and fungi , but the research for using actinomycetes in the field of bioremediation is still very limited . Worthy mentioning the fact that actinomycetes is one of the largest and most important microbial communities in s the soil and includes a wide variety of bacteria that play an important environmental role in the soil, and many of which are known with economic importance as a producer of biologically active substances such as antibiotics and vitamins.

In recent years, the researches have been looking for Extremophilic microorganisms in general, to take their advantages for many industrial and commercial applications. On the other hand the research focused on the Minor group of actinomycetes including species which have difficulty during isolation and cultivation, and these that growing under extreme conditions such as salinity. It was found that actinomycetes isolated under such conditions showed morphological and physiological differences from other species isolated under normal conditions. However, there are many environmental factors that affect their presence and activity that allowing the use of them in a wide applications in particular in the field of bioremediation of various wastes.

In view of the industrial revolution that is taking place in many cities in the Kingdom, such as Yanbu and Aljubail, we are need of intensive scientific studies to get rid of industrial waste effectively in order to the positive returns can be achieved by such research on the economy, environment and health of the individual in the Kingdom.

Accordingly, the present study aims to the following points:

1. Isolation and identification of some actinomycetes isolates from the saline soils in Jeddah after the selection of Halo-Thermophilic isolates.

2. Study the response of actinomycetes isolates to some environmental pressures such as (salinity, temperature, metal pollution) and the impact of this factors on the growth and activity of the isolates.

3. Development of the research methods for the studying and isolating microorganisms , because the possibility of finding new microorganisms in the same traditional methods have been started to decrease gradually as a result for the search of easy isolation and development microorganisms.

4. Using actinomycetes isolates in the treatment of some waste such as industrial wastewater and sewage water that contain high concentrations of heavy metals and salts.

It has been observed during this study the difficulty of culturing actinomycetes isolates by using many actinomycetes media after the addition of sodium chloride in different concentrations to provide the ideal conditions of salinity . Where the problem lies in the depression of the growth rate of actinomycetes isolates, in addition to decrease the amount of growth, this could be due to the fact that necessary growth factors for the growth of most halophilic microorganisms are produced slowly and therefore required special nutrition needs for growth.

Since there are no specific media for isolated and cultivated of halophilic actinomycetes , It is attracting attention that the study innovation a new culture medium designed for isolation and cultivation halophilic actinomycetes which called (SGY) that consists of glucose , starch and yeast extract supported with artificial sea water (Starch Glucose Yeast extract Supported with Artificial Sea Water) . It has been observed a good results by using (SGY) medium in the recultivation of halophilic actinomycetes , where all isolates showed the ability to grow during short period of time with a high amount of growth significantly when compared to other culture media .

Through this study, we were obtained (36) isolates of halophilic actinomycetes that isolated at 10% sodium chloride , and then were selected (6 isolates) for extending study depending on the ability of the isolates to tolerate high concentrations of salts and heavy metals. The study revealed the ability of actinomycetes isolates to grow at different concentrations of sodium chloride, considering that the isolates (T4-U1) showed their ability to grow until (30%) . Whereas the isolates (G1-X3) can grow until (25%) , and the isolates (K2-R2) until the concentration (20%) which indicates that there are different extent appropriate for salt tolerance among the isolates.

The study has also showed a high amount of growth with increasing salt concentration in general, during the testing of the isolates to grow in the presence of gradual concentrations of sodium chloride , this could be attributed to the fact that increased the amount of sodium chloride lead to higher metabolic activity due to uprising the enzyme activity.

The study was also considered to test the ability of isolates to grow at different temperatures and the effect of temperature on the growth of actinomycetes isolates, as the needs of salinity and the ability to tolerate it based on some important conditions for development such as temperature and food components of the media . The results revealed the ability of the isolates (G1-K2-R2-U1) to grow at (50 Cº) with the limitation the highest rate of growth of all isolates at (40 Cº) with exception for the isolate (K2) which recorded the highest growth rate at (45Cº) which indicates that all of the isolates in this study are a moderate thermophiles microorganisms.

The study was show up the ability of actinomycetes isolates to resist heavy metals in the presence of gradient concentrations of heavy metals ranged between (100 - 600 mg / l), which contained cadmium, lead, copper, zinc, and manganese. The results of an estimated the amount of dry weight and consumed heavy elements by the isolates have shown generally a decline in the growth rate of the isolates as a results of an increasing concentration of heavy elements . On the other hand, there are increased in the amount of consumable heavy metals at high concentrations with different amount of consumption among the isolates.

The results of the sensitivity of the isolates to heavy metals used in this study were found that copper is the most inhibitory elements of the growth, followed by zinc , lead and cadmium, whereas the manganese is the least impact on the growth of isolates, it can be arranged as follows (Cu <Zn <Pb <Cd <Mn). The differently effect of heavy metals on the isolates may be attributed to the fact that the ability of biological oxidation of heavy highly dependent on their solubility and toxicity.

The results of (Antibiotic Activity) for actinomycetes isolates were reported the ability of all isolates to produced antibiotics with the exception of isolate (U1) . The antibiotic Activity of all the isolates was limited towards bacteria (antibacterial activity), while there are no any activity towards fungi (Antifungal Activity). In addition, the results also observed the ability of actinomycetes isolates on the production of several extra cellular enzymes. Where it was reported the analysis of starch by all the isolates and the cellulose by the isolates (G1-T4-U1), it was also recorded fat, protein degradation by the isolates (G1-X3-T4-U1). Since that the halophilic microorganisms is the primary source of enzymes that are resistant to salinity (Extremely Halophilic Enzymes), that called (Haloenzymes) which are characterized by their stability at high salinity.

The study was also considered to test the ability of actinomycetes isolates for consuming (9) different sources of carbon, where all the isolates assimilated the glucose, fructose and starch efficiently. As for the other carbon sources it has been recorded significant variation between the isolates in their ability for consuming .

The study has been identified actinomycetes isolates which selected for the study by using molecular sequence analysis of the gene 16SrDNA (500Pb 16SrDNA Gene Sequence Analysis), as it was quick and effective way for identifying actinomycetes accurately and within a short period where it was rely directly on sequence databases.

According to (NCIMB) laboratory report from the United Kingdom were searched through the EMBL public database. The closest EMBL database matches to the sequences of the isolates (G1-X3) were to the

Species *Nocardiopsis halophila* at (99.5%) for the isolate (G1) and percentage (99.6%) for the isolate (X3) , as for the isolates (K2-R2) were to the species *Nocardiopsis rosea* at (99.1%) for both isolates.

On the other hand the closest EMBL database matches sequences of the isolate (T4) was to unpublished sequences of the species *Actinopolyspora salina at* (99.4%) . as to the isolates (U1) ,The closest EMBL database matches sequences of this isolate was to unpublished sequences of the species *Actinopolyspora mortivallis* at (98.2 %) , but this is not considered high enough to be a species level match ,worthy mentioning it has been considered as a new species of the genus *Actinopolyspora* .

Although the study was observed the ability of actinomycetes isolates to growing and resisting heavy metals which are used in the study by adding them individually to the media used in the development . When we used the synthetic wastewater containing a mixture of heavy metals (Cd-Cu-Mn-Pb-Zn) the isolates did not able to grow during incubation period extended for 8 weeks , whereas the addition of mixture heavy metals has usually more strong effect on the microorganisms.

It was observed clearly that the adding of glucose (1%) to the synthetic wastewater was stimulated the growth of actinomycetes isolates within 4 weeks of incubation , then managed the actinomycetes isolates for uptake heavy metals faster . This could be attributed to the fact that actinomycetes were able to removing the adverse impact of heavy metals through active participation in the breaking down of organic materials, where the process of organic compounds oxidation is associated with heavy metals reduced .

The results of estimating the amount of heavy metals remaining in the medium showed that lead is the highest consumed element, followed by copper, zinc, manganese and finally the cadmium. Where microorganisms interact with metals through a number of mechanisms, and active processes to remove metals by microorganisms due to the phenomenon of heavy metals bioaccumulation within the cells (Intracellular bioaccumulation).

The results of removal heavy metals from synthetic wastewater by using biosorption through the dead biomass of actinomycetes isolates revealed that the lead is the most absorbed elements by all the isolates (G1-K2-R2), where the percentage of biosorption (96.2, 100, 100%) of this isolates respectively, followed by both copper and zinc, manganese and finally the cadmium . Considering that the driving force for the biosorption process was resulting from the cell wall of this biomass that contain anionic groups which have high adsorbent ability towards ions. Moreover, the cell wall of actinomycetes has a high adsorption capacity due to the high content of peptidoglecan and teicoic acid in their cell wall.

The results of comparing the quantity of heavy metals removed from the synthetic wastewater that processed by bioaccumulation and biosorbtion revealed that lead is the highest elements removal in both cases . Followed by copper, zinc, cadmium and finally the manganese with different amount among the isolates , although there was no evidence of an association between the microbial resistance of heavy metals and the biosorption dynamic.

The study was also used raw industrial wastewater samples (A-B-C-D) to study the ability of actinomycetes isolates to grow and remove heavy metals from the samples. However all the isolates can not grow on the samples (A-C-D), While the isolates (G1-X3-K2-R2) were able to grow on the sample (B) that containing zinc and lead at concentration (12.82, 29.84 mg/l) for each of them respectively. Considering that the percentage removal of lead and zinc (100%) by the isolates (G1-X3-R2) in spite of the long time incubation period, which extended for 8 weeks.

After the addition of (1%) glucose to wastewater samples , the actinomycetes isolates (G1-X3-K2-R2) were able to grow on the sample (C) that containing aluminum, chromium and zinc at concentration (38.37, 17.20, 7.05 mg / l) for this elements respectively . The results have been detected the removal of chromium at (100%) by the isolates (K2-R2), and the zinc were removed at (100%) by the isolate (X3).

The study of heavy metals biosorption from crude industrial wastewater has observed that there are no change in the concentration of heavy metals before and after the treatment for all the samples (A-B-C-D) which indicates that there is no occurrence of biosorption process . This could be attributed to the overlap between the biosorption and the high concentrations presence of organic matter. It can also be an overlap between the other ions with the important biosorption heavy metals to be absorbed, where there is a competition towards absorption sites leading to reducing heavy metals binding . Therefore, the lucrative marketing opportunities for biosorption appear to be used as a secondary or (Polish Treatment) of wastewater or when it has been used to remove metals from liquid diluted waste.

Primary treatment (physio-Chemical) has been performed in this study through raising the hydrogen ion concentration, where heavy metals are precipitated in the form of metal hydroxides. After the deposition of metals from industrial wastewater samples , sludge was separated and estimated the heavy metals in the sediment . The results observed the presence of heavy metals in large quantities when compared to primary concentrations in the samples before the treatment , which indicate that the process of precipitation is leading to concentrating metals in the solutions.

The aluminum was the highest elements in the sediment; followed by the zinc and finally the chromium. Also it was observed that there are some elements which give unread results before the treatment, such as (Ba, Cd, Cu, Ni, Pb, Mn) which indicates the presence of these elements mainly at low concentrations in the samples before the treatment.

The results of heavy metals analysis after primary treatment of wastewater revealed that there is a significant reduction in the concentration of heavy metals, whereas the sample (B) gave unread results for all heavy metals. As for other samples it was detected unread results for all elements except of (Al, Cr, Zn) for the sample (A) and (Cr, Zn) for the samples (C-D).

The study carried out the cultivation of actinomycetes isolates on wastewater samples (A-C-D) to test the ability of the isolates to accumulate heavy metals throw bioaccumulation after heavy metals precipitation . The results revealed the ability of the isolates (K2-R2) to grow on the sample (A) and they can removed the zinc at (100%) when

its concentration (9.06 mg / l) , they were also able to remove chromium at (71.78 - 74.18%) for both isolates respectively when the concentration its (69.03 mg/l) , and the aluminum at (84.32 - 85.10%) when the concentration is (12.62 mg /l).

Also, all the isolates were able to grow on the sample (C) that containing chromium only at (11.39 mg / l) , in which the isolates(G1-X3-K2-R2) were able to remove it at (71.11 - 71.64 -69.79 -37.74%) of the mentioned isolates respectively. As for the sample (D) that containing chromium and zinc at (146-2.82 mg /l) , all the isolates were able to remove the zinc at (100%). While chromium removal is varied among the isolates (G1-X3-K2-R2) where the removal percentage (75.68 - 83.90 - 74.45 - 78.35%) of the mentioned isolates respectively.

The results of treatment sewage water that containing large amount of salts (17900 mg / l) by halophilic actinomycetes isolates revealed that the isolates (G1-X3-K2-R2) were able to grow on the sample. As well as the results of estimated the remaining of total dissolved salts (TDS) in the samples after actinomycetes culturing , revealed that isolate (K2) is the highest consumption of salt among the isolates by (86.95%) .

Finally, there is clear need for more basic research on the Waste Management of high salinity liquid waste since that high concentrations of salinity in the wastewater, reduced the effectiveness of biological treatment. Moreover the research on using halophilic microorganisms in waste treatment is limited and there is a little on the treatment of wastewater at high concentrations of salinity. Considering that halophilic

bacteria is characterized by several attributes that enable the treatment of Wastewater . Where they can metabolism a wide range of materials as they can use many of them as electron receptors . In addition to the production of some materials outside the cells and accumulate stored materials inside the cells beside the means of adaptation to living in extreme saline environments.

(لايوجد ملخص عربي)