Iraqi Journal of Agricultural Sciences –2026:57(1):359-367
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/k0cpgn62

THE OPTIMAL CONDITIONS FOR TOTAL SALINITY REMOVING FROM WATER USING NEW LOCAL HALOPHILIC BACTERIAL STRAINS

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ABSTRACT

This study was aimed to determine the optimal conditions for removing total salinity from an aqueous solution of known salt concentration 10% using four new bacterial strains that are tolerant to high salinity genetically diagnosed and registered in the international gene bank (NCBI). Modified nutrient broth medium (MNB) was used with the addition of 10% sodium chloride. The ability of the bacterial strains to remove total salinity was tested in terms of the decrease in total dissolved solids (TDS), where the highest removal rate was after 72 hours, at a temperature of 30°C, with an inoculum volume of 1.5% and a pH of 7. The removal rates were higher in static cultures in the same conditions compared to using the vibrating incubator at 100 rpm. The removal rate decreased when using a mixture of the cocci strain with the three other bacillus species, and the removal rates were constant when using a mixture of the bacillus strains. Total desalination rates were obtained using bacterial strains (A, B, C, and D) after applying the optimal conditions obtained from this study (94, 91, 90, and 92) %, respectively.

Key words: Contact time, Static culture, Temperature, Total dissolved soluble.

Received: 12/4/2024, Accepted: 21/7/2024, Published: 26/1/2026

INTRODUCTION

The scarcity of fresh water is considered one of the most difficult problems facing the world in light of the ongoing population increase, in addition to the scarcity of sources of that water due to climate change (which includes rising temperatures and less rainfall), and the lack of optimal use of current sources, whether through waste and/or increasing the volume of pollutants that finding its way into fresh water sources is one of the factors that complicates and increases the negative impact of this crisis on a global level.(Uma *et al.*, 2020) Researchers in this field are directed to finding appropriate solutions to the problem of securing water suitable for various human uses, including recycling wastewater and salt water after treatment, and determining appropriate methods for this according to the type of final use and the quality to be obtained. (Oren, 2010) As is known, biological treatment is considered one of the most efficient ways to get rid of various environmental pollutants, especially water pollutants (high salinity, heavy metals and other pollutants), where living cells are used, whether plant or microorganisms, because they possess properties that enable them to survive in extreme environments with reducing pollutant outputs through several mechanisms (bioaccumulation, biosorption, and biodegradation) (Vreeland, 2020). They have evolved several different molecular and cellular mechanisms to respond to the salt-stress condition. A primarily used salt-in strategy can be achieved by raising the salt concentration in the cytoplasm, and their enzymes tolerate or require high salt

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concentration which is well recognized in halophilic archaea and bacteria (Chen and Jiang 2018). As is known, bacteria have a greater ability to produce intracellular enzymes compared to fungi. The well-known mechanism is the so-called salt-out strategy or organic osmolyte mechanism allowing an osmotic adaptation by excluding salts and/or synthesizing de novo compatible solutes. This strategy is also used in Archaea and Bacteria. In addition, molecular basis of protein halotolerance and adaptation of halophilic enzymes to high salinity is by increasing a substantial number of protein charges and increased hydrophobicity (Crisler *et al.*, 2019) Halophiles have been considered for biotechnological applications. Diverse response mechanisms of halophiles under high-salinity conditions cause the production of various valuable biomolecules. It has been recognized that halophiles are also major sources of stable enzymes that function in very high salinity, an extreme condition that results in denaturation and aggregation of most conventional proteins (Thomson and Gilmore, 2024). Various studies show that some halophiles are capable of synthesizing massive amounts of compatible solutes (Hanelt and Muller, 2013). These small organic molecules are useful as stabilizers of biomolecules or stressprotective agents. Thus, the halophiles have a broad biotechnological applications ranging from agriculture to biomedical (Ali and Farahat, 2024). The ultimate goal of this study is to biologically treat salty drain water using new halophilic bacterial strains, and reuse the resulting water after treatment in agricultural crop irrigation operations to cover part of the shortage in fresh water supplies in light of the water scarcity that Iraq is suffering.

MATERIALS AND METHODS

Bacterial strains

Four new local halophilic bacterial strains were used in this study. The strains were genetically diagnosed and registered as new strains in the International Gene Bank of the NCBI, as shown in (**Table 1**). They were established in previous articles by the same researchers (under publication).

Modified nutrient broth (M N B)

The MNB media was prepared as described by the manufacturing company. The prepared media was modified by adding 10% NaCl (Santhaseelan *et al.*, 2022). All culture media used in the study were sterilized with an autoclave at a temperature of 120°C and a pressure of 15 pounds/inch^2 for 15 minutes.

Inoculum preparation

The number of cells (per ml) for the bacterial inoculums used in this study was estimated using the McFarland method (9), where decimal dilutions containing (10^8CFU) were chosen for all experiments in this study.

Contact time

The flasks containing the modified medium (MNB) and inoculated with 1% of the bacterial cultures were incubated at 37°C for different periods including (48, 72, 96 and 120 hours), and the results were recorded.

Optimum temperature

To determine the optimal temperature, the same steps were repeated in the above experiment, and the flasks were incubated at (10, 20, 30, 40, and 50) °C, taking into account the contact time resulting from the previous experiment.

Inoculum volume

The flasks prepared by the previous method were inoculated with different inoculum sizes of bacterial cultures (0.5, 1, 1.5, 2 and 2.5%), taking into account the results obtained from previous experiments.(Youn and Seo, 2022).

Optimum pH

Hydrochloric acid and sodium hydroxide solutions were prepared at a concentration of 1 M each to adjust the pH of the culture media used in the experiment**.** The initial pH of the culture media used was adjusted to (5, 5.5, 6,

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6.5, 7, 7.5, and 8), and the same steps were repeated in the previous experiments, taking into account the results obtained from the above experiments.

Ventilation : The flasks were prepared in the same way as the previous experiments, adopting the results of the optimal conditions obtained from those experiments, and incubated in a shaking incubator at a speed of (100 rpm), and the results were recorded.=

Efficiency of mixing strains in the bioremediation process

The experiment was conducted by inoculating flasks by mixing two strains together with equal inoculum sizes (1% for each strain) for the strains (A+B),(A+C),(A+D),(B+C),(B+D) and (C+D) used in this study (Table 1), in order to study their synergistic effect on the desalination process, taking into account the results of previous experiments.

Calculate total salinity consumption

Total salinity consumption was calculated as a function of the decrease in total dissolved solids (TDS) by the bacterial strains used in the experiments of this study by the following equation:

Removal rate %=(TDS1-TDS2)/TDS1 *100

TDS1=before removal (ppm).

TDS2= after removal (ppm).

The (HANNA HI 98192) device was used to conduct all experiments.

30 ml were withdrawn from the flasks and placed in sterile tubes. They were

Centrifuged at 6000 rpm for 10 minutes. The clear solution was taken and readings were taken with the device mentioned above for all experiments.

Statistical analysis

The statistical analysis system- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means (ANOVA/ one way) in this study.*(P≤0.05) significant difference.

Bacterial strains

Table 1. Bacterial strains used in this study

Strain code	Strain name	NCBI code
A	***Salinicoccus sp. Strain Salman***	(OQ825941.1)
B	***Oceanobacillus sp. Strain Elham A***	(OQ978219.1)
C	***Oceanobacillus sp. Strain Thana***	(OQ825943.1)
D	***Oceanobacillus sp. Strain Mostafa***	(OQ825942.1)

RESULTS AND DISCUSSION

Optimum conditions

In order to reach the highest efficiency in consuming the materials to be removed (total salinity) from the medium, it is necessary to study some of the optimal conditions affecting this process (Yadav *et al.*, 2021). In fact, the greatest amount of salt is consumed when the microorganism (halophilic bacteria) reaches the highest growth rates, and therefore the amounts of salts present in the growth medium can decrease because those salts are among the basic factors determining the growth and reproduction of these types of microorganisms (Liu *et al.*,2019). Hens, the optimal conditions for removal are the same as the optimal conditions for the growth of the microorganisms.

Contact time

The contact time is considered one of the important factors in biological treatment processes, as it provides time for living cells to reach the highest growth rates and thus increases the rates of consumption of materials removed. **(Table 2)** shows the consumption rates for the four bacterial isolates through different times (48, 72, 96, and 120) hours. The highest rates of consumption appeared after 72 hours for isolates (A, B, C, and D), which were (84%, 83%, 80, and 82%),

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respectively, and began to decrease over the time, reaching 120 hours, as the growth rate for bacterial cells reaches to the peak after 72 hours and began to decline after the growth curve reached a stationary phase. It decreased further at 120 hours, which is the beginning of the cell death phase (Puspaningum and Titah, 2020).It was also recorded that it has a relatively long lag phase compared to other types of bacteria (Mesa-Marin *et al.*, 2019). In some related studies, many species of salt-tolerant bacteria have reported that the optimal incubation period is 72 hours, meaning that it may be longer than other species of bacteria because of their reliance on growth mechanisms that are different from what is found in other species due to the presence on relatively higher salt concentrations in their growth environments. (Oakes *et al.*, 2025).

Table 2. Effect of Optimum contact time in total dissolved solids TDS consumption

Strain	48 hrs.	72 hrs.	96 hrs.	120 hrs.	LSD
A	62%	84%	70%	68%	6.95 *
B	60%	83%	67%	65%	6.29 *
C	58%	80%	65%	62%	7.61 *
D	61%	82%	68%	64%	7.08 *
				* (P≤0.05)	

*A ***Salinicoccus sp. Strain Salman***
* B ***Oceanobacillus sp. Strain Elham A***
*C ***Oceanobacillus sp. Strain Thana***
*D ***Oceanobacillus sp. Strain Mostafa***

Optimum temperature

(Table3) shows the effect of different temperature on the consumption of TDS by the bacterial strains (10, 20, 30, 40, and 50) °C. This range represents the average temperature in Iraq over the year in the case of conducting biodesalination experiments in the field. Removal rates were relatively high between (20-40°C) and reached the highest at (30°C), where the consumption rates for the four bacterial strains (A, B, C and D) were (90%, 88%, 85% and 89%), respectively. Halophilic bacteria grow at a temperature slightly higher than room temperature (30°C) (Al Zamzami *et al.*, 2025). The optimal temperature for the growth of these microorganisms may be affected by the amount of salts present in the growth environment (Yu *et al.*, 2022).

Table 3. Effect of optimum temperature in total dissolved solids (TDS) consumption.

Strain	10°C	20°C	30°C	40°C	50°C	LSD
A	58%	78%	90%	83%	45%	8.71 *
B	55%	75%	88%	81%	43%	8.55 *
C	52%	71%	85%	80%	40%	7.96 *
D	55%	76%	89%	82%	42%	8.37 *
				* (P≤0.05)		

A Salinicoccus sp. Strain Salman
**** B Oceanobacillus sp. Strain Elham A***
****C Oceanobacillus sp. Strain Thana***
****D Oceanobacillus sp. Strain Mostafa***

Inoculum volume

The effect of different inoculum volumes (0.5%, 1%, 1.5%, 2% and 2.5%) ml/ml (each ml contains 10^8 CFU) on the total desalination rates by experimental bacteria strains (A, B, C and D) was, the inoculum size 1.5% has the highest consumption rates (92, 90, 89 and 91)% respectively was shown in **(Table 4).** The initial inoculum volume is considered an important factor in the microorganism reaching ideal growth rates in a particular environment. Starting with small inoculum volume, led to insufficient or weak growth of the microorganism in its environment. (Sedrah *et al.*, 2021). Additionally, starting with inoculum volume higher than the optimal level in a medium with limited nutrients and growth

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P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
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factors, the result will have an adverse effect on the final growth outcome of the bacteria due to competition for nutrients and the accumulation of waste in the medium itself (Mohamed and Al-Shamary, 2022).

Table 4. Effect of Optimum inoculum volume in total dissolved solids (TDS) Consumption.

Strain	0.5%	1%	1.5%	2%	2.5%	LSD
A	70%	84%	92%	88%	78%	7.93 *
B	66%	83%	90%	85%	75%	8.21 *
C	60%	80%	89%	80%	70%	7.05 *
D	65%	82%	91%	83%	75%	9.02 *
					* ($P \leq 0.05$)	

A *Salinicoccus sp. Strain Salman
*** B *Oceanobacillus sp. Strain Elham A***
C *Oceanobacillus sp. Strain Thana
D *Oceanobacillus sp. Strain Mostafa

Optimum pH

The results showed that the highest rates of total desalination ranged at pH close to neutral between 6.5 and 7.5, and the highest rates were at pH 7, where they reached (94, 91, 90 and 92)% respectively, for the four bacterial strains used as it is shown in **(Table 5).**

Most halophilic and halotolerant microorganisms prefer neutral environments .It grows best in an environment with a pH 6.8 to 7.5 (John *et al.*, 2020). Extreme pH beyond neutrality towards acidic or basic leads to obstructing microbial growth by increasing positive and negative ions in both directions. Thus, it leads to an increase in ionic interactions and thus affects the functional composition of bacterial cells (Olaleye *et al.*, 2025). It is noteworthy that the average pH of the water of general downstream drain in Iraq ranges between (7-8) throughout the year, which means the possibility of using biodesalination treatment using the bacterial strains, which is the main goal to be achieved next.

Table 5. Effect of Optimum pH in total disolved solids(TDS) consumption.

Strain	5	5.5	6	6.5	7	7.5	8	LSD
A	34%	51%	80%	91%	94%	89%	72%	9.84 *
B	30%	48%	77%	88%	91%	85%	68%	8.37 *
C	30%	45%	75%	86%	90%	82%	65%	9.41 *
D	32%	48%	78%	90%	92%	82%	68%	9.17 *
							* ($P \leq 0.05$)	

A *Salinicoccus sp. Strain Salman
*** B *Oceanobacillus sp. Strain Elham A***
C *Oceanobacillus sp. Strain Thana
D *Oceanobacillus sp. Strain Mostafa

Ventilation

By applying all the optimal conditions obtained from previous experiments, the effect of ventilation on the total desalination process was studied using bacterial strains (A, B, C, and D). The shaking incubator was used at (100 rpm), it was observed that the removal rates decreased by (72, 67, 65 and 70) % respectively, while the static culture gave higher removal rates (94, 91, 90, and 92) %, respectively as shown in **(Figure 1).** Stirring prevents salt molecules from penetrating through bacterial cell membranes sufficiently compared to the static culture in environments with high salt concentrations, as most natural environments for bacteria that are tolerant of high salinity are considered immobile or relatively static, which leads to weak bacterial growth when stirred(Yoo *et al.*, 2023). The solubility of oxygen drops down when salinity increases, as it decreases by half at a concentration of 10% NaCl and to more than 80% at a concentration of 30% NaCl. Therefore, the natural environments of halophilic bacteria are with a relatively weak presence of oxygen (Nosalova *et al.*, 2022).

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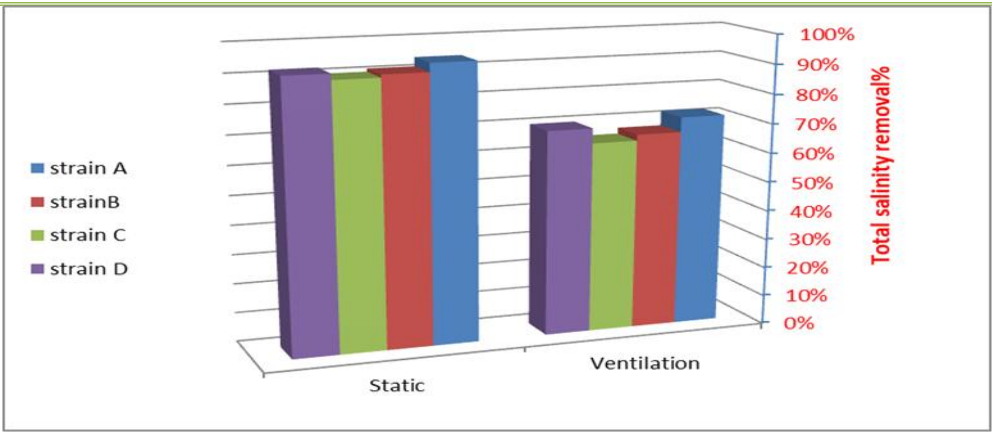

Figure 1. Effect of ventilation using *A *Salinicoccus sp. Strain Salman* * B *Oceanobacillus sp. Strain Elham A* *C *Oceanobacillus sp. Strain Thana* *D *Oceanobacillus sp. Strain Mostafa* ion using on removal of total salinity

Efficiency of mixing strains in the bioremediation process : In order to improve the total salinity consumption efficiency, a mixtures of bacterial strains were used in pairs (A+B),(A+C),(A+D),(B+C),(B+D) and (C+D) and compared to the consumption rates of the individual strain. **(Table 6)** showed that the desalination percentages for (A + B), (A + C) and (A + D) were (78,75 and 77)% respectively, while (B+C), (B+D), and (C+D) were (90, 88, and 90)% respectively, compared to the efficiency of the single strains (A, B, C and D), which were (94, 91, 90 and 92)% respectively. It is noted that the total desalination results for (A+B), (A+C) and (A+D) decreased compared to the results of the individual strains in terms of removal rates. This can be attributed to the different spices of strains used, as strain A is cocci while the rest of the strains are bacilli. Which may result from their presence in the same environment in a relationship of (antagonistic growth). This type of growth relationship in each species of microorganism present in an environment competes with other species to obtain nutrients and other growth requirements in order to become dominant (Kapadia *et al.*, 2022). For example, by producing antibodies or secreting bacteriocins and other metabolic compounds that are considered unsuitable for the growth of other species (Neagu and Stancu, 2025), and thus the decrease in the rate of biological removal occurred for these reasons. It has been noticed that the desalination rates for (B+C), (B+D) and (C+D) were close to the rates of the individual strains, as all strains are of the same bacillus species. The approximate consistency in the results shows that the growth relationship among these bacterial strains is of the symbiotic type, that is, the growth of one may or may not affect the other, which leads to growth rates remaining approximately the same in the case of the presence of individual strains in the same medium(Tourova *et al.*, 2022**).**

Table 6. Comparison among mixes and individual strains in biodesalination

Strains mix		Individual	
(A+B)	78%	A 94%	B 91%
(A+C)	75%	A 94%	C 90%
(A+D)	77%	A 94%	D 92%
(B+C)	90%	B 91%	C 90%
(B+D)	88%	B 91%	D 92%
(C+D)	90%	C 90%	D 92%

A *Salinicoccus sp. Strain Salman
*** B *Oceanobacillus sp. Strain Elham A***
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CONCLUSION

The bacterial strains showed a high ability to remove total salinity as indicated by the total

P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/k0cpgn62

dissolved solids in vitro when applying the optimal conditions (contact time, temperature, pH, inoculum size, aeration) obtained from this study. Synergistic growth between bacterial strains did not play a role in increasing the overall salinity removal rates, compared to their individual growth.

ACKNOWLEDGEMENT

The authors wish to express their thanks and appreciation to the University of Baghdad\ College of Agricultural Engineering Sciences for providing the necessary facilities to this work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

JOURNAL DECLARATION

The Second author (**Elham I. AL-Shamary**) serves as an editor for Iraqi Journal of Agricultural Sciences but was not involved in the peer review process of this manuscript beyond their role as an author. The authors declare no other conflict of interest.

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Iraqi Journal of Agricultural Sciences –2026:57(1):359-367
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/k0cpgn62

الظروف المثلى لإزالة الملوحة الكلية من الماء باستعمال سلالات بكتيرية محلية جديدة متحملة للملوحة العالية

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المستخلص

هدفت هذه الدراسة الى تحديد الظروف المثلى لإزالة الملوحة الكلية من محلول مائي معلوم التراكيز الملحية باستعمال اربعة سلالات بكتيرية جديدة متحملة للملوحة العالية مشخصة جينيا ومسجلة في بنك الجينات العالمي(NCBI), تم استخدام الوسط المغذي السائل المحور بإضافة 10% كلوريد الصوديوم , تم اختبار قابلية السلالات البكتيرية على الازالة بدلالة انخفاض المواد الصلبة الذائبة الكلية حيث كانت اعلى نسبة ازالة بعد مرور 72 ساعة وتحت درجة حرارة 30م وبحجم لقاح 1.5% وبرقم هيدروجيني بلغ 7. وكانت نسب الازالة اعلى في الحالة الساكنة مقارنة مع استخدام الحاضنة الهزازة 100 دورة بالدقيقة. انخفضت نسبة الازالة عند استخدام مزيج من السلالة الكروية مع السلالات الثلاث الاخرى العصوية , وكانت نسب الازالة ثابتة عند استخدام مزيج السلالات العصوية فيما بينها. تم الحصول على نسب ازالة للملوحة الكلية باستعمال السلالات البكتيرية (A,B,C,D) بعد تطبيق الظروف المثلى المستحصل عليها من هذه الدراسة(94,91,90,92)% على التوالي.

الكلمات المفتاحية: وقت التماس , مزرعة الحالة الساكنة ,درجة الحرارة, المواد الصلبة الذائبة الكلية.