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The effectiveness of Oscillatoria limnetica for the removal of water high salinity under various environmental conditions

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***Abstract*---**Oscillatoria limnetica alga was tested for its extent to control water`s high salinity stresses, through sets of laboratory batch experiments during five initial salinity concentrations; 10ppt , 15ppt, 25ppt, 30ppt, and 35ppt were applied to aqueous solution under different operational conditions; water temperature and pH. Three water temperatures; 25, 30, and 35 ^{o}C, and three pH values; 5, 7, and 9, were applied interchangeably to operate for 10 successive days, at the end of each the optical density and salinity concentration were measured via the spectrophotometer and the Flame atomizer spectrophotometer successively. The main result was the gain of very good performance in the removal of salinity stress under almost all tested operational conditions. The salinity removal efficiencies ranged from 80-94%, especially at the temperature of 30 C and pH value of 7. That in addition to a very good durability to withstand high salinity stresses in almost all experimental conditions during which. Also, it was found that the salinity removal efficiency increases as the initial salinity concentration increase for almost all tested sets. The statistical examination showed relatively well normal distributions with close standard deviations in addition, the good to strong correlations for the performance measures; daily removal efficiencies vs. initial salinities, and between the daily optical densities vs. growth rates. These results of the current study may be considered to the benefit of the trustful use of oscillatoria limnatica as a very effective, low cost, environment friendly, and easy handled control mean for the water high salinity problem.

***Keywords*---**Adsorption, Cyanophyta, Oscillatoria, Phytoremediation, Salinity.

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1 Introduction

The necessity for proper management of brine waste utilizing cost-effective and innovative solutions has arisen as a result of emerging concerns connected to increasing concentrate volumes, regulatory demands, public awareness, and environmental issues. As a result, there has been a surge in interest in long-term solutions for treating saline wastewater (Sahu, 2021). Phytoremediation is a low-cost and environmentally friendly method of removing pollutants from water and soil. This treatment is a method of remediation that employs plants to reduce, extract, and retain excess nutrients and pollutants from a contaminated medium (Valipour et al., 2014). Methods of phytoremediation can be classified according to how pollutants are removed from the environment, such as degradation, extraction, containment, or a combination of two or more techniques. Plant species thought to be ideal phytoremediation agents have a high biomass, a good root system, and are fast-growing and easy to cultivate (Ali et al., 2013).

As one of very few cyanobacterial genera, Oscillatoria is known to be familiar to water quality monitoring agencies as well as part of the general biology curriculum, often being represented as one of the typical freshwater cyanobacteria (Guiry and Guiry 2018). Different Oscillatoria species have traditionally been distinguished by the width of their trichomes and the shape of their apical cells. Various organisms meet these few criteria and were thus classified as Oscillatoria due to their relatively simple morphology and lack of truly unique features (Mühlsteinová et al., 2018).

Many researchers studied the tolerance of Oscillatoria against saline stress, as NaCl is known to impose ion toxicity with a concomitant ion imbalance stress (due to the accumulation of Na- and Ci-) together with a water deficit stress (Nandagopal et al., 2021). Studies reported that salinity may negatively influences the physiology of blue-green algae and could disturb ion balance or induce nutrient deficiencies (Silveira and Odebrecht, 2019), and consequently, a decline in photosynthesis under hyperosmotic stresses could change the fine structure of the chloroplasts causing a disruption of energy transfer between the two photo systems. Shaila and Pratima (2012) reported a slight increase in the total chlorophyll contents at concentrations 0.2-0.4 M of NaCl, and decrease at higher concentrations; 0.6 and 0.8 M. In a comparable study, Wangwibulkit et al., (2008) determined that with the pH 7.5 and 9.0 and salinity concentration of 5 ppt, Oscillatoria sp. reached optimum growth with a chlorophyll-a of 4,455±435 μg/l; and there were no significant differences among the tested 0, 5 and 10 ppt groups. Chlorophyll-a biomass of Oscillatoria sp. was lower at a salinity of 15 ppt and lowest at 30 ppt.

2 Experimental setup

Five flasks are used to contain the media, inoculum, and each of five different concentrations of salt; 10, 15, 25, 30, and 35 ppt. the setup procedure starts by the addition of the media, then the flasks are to be sterilized using an autoclave with a temperature of 121 C and pressure of 15 bar for 10 minutes and cooled down to one of the understudy testing temperatures; 25 ^{o}C, 30 ^{o}C, and 35 ^{o}C. following the setting of the required temperature, and the required pH; 5, 7, and

9, the inoculums are to be added; one of the algae understudy, for each of the aforementioned initial salinity to each flask. After each of 10 days period's aftermath, 30 ml should be drawn and filtered to measure the NaCl via a flame atomizer spectrophotometer, and 50 ml form the unfiltered sample are drawn to measure the optical density OD using the ultraviolet spectrophotometer. This procedure would be repeated for each combination of the aforementioned variables; initial salinity, temperature, and pH. The measurements of the OD of the oscillatoria limnatica suspension are to be via Spectrophotometer at an absorbance wave length of 683 nm, using culture medium; BG11, as a blank (El-Sheekh et al., 2013). The growth rate (K) and doubling time (G) were calculated according to the following equations (Liu et al., 2021);

$$\boldsymbol{K = 3.322 * \frac{\log OD_t - \log OD_0}{t}} \tag{1}$$

$$\boldsymbol{G = \frac{0.301}{K}} \tag{2}$$

Where; t: time (days), ODt: optical density at time (t) time, OD0: optical density at zero time.

The Salinity removal efficiency at different times of each of the various test conditions is determined according to the following formula;

$$\boldsymbol{Removal\ efficiency = \frac{C_t - C_0}{C_0} x\ 100} \tag{3}$$

Where;

Ct: Salinity concentration at time t (ppt), C0: Initial salinity concentration (ppt).

2.1. Statistical analysis

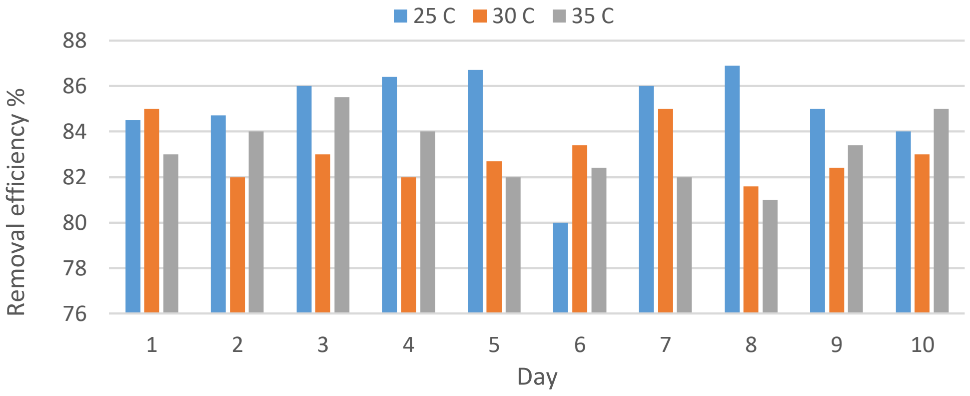
Excel 2013 statistical analysis software was used to determine the various statistical indices for the various data sets; the average (Av.) and the Correlation coefficient (R) that assess the existence of relationships between those sets of variables, if any. Also, this software was used to process the one-way ANOVA to examine the existence of differences between the various data sets and hence decide their statistical significance.

3 Results and discussions

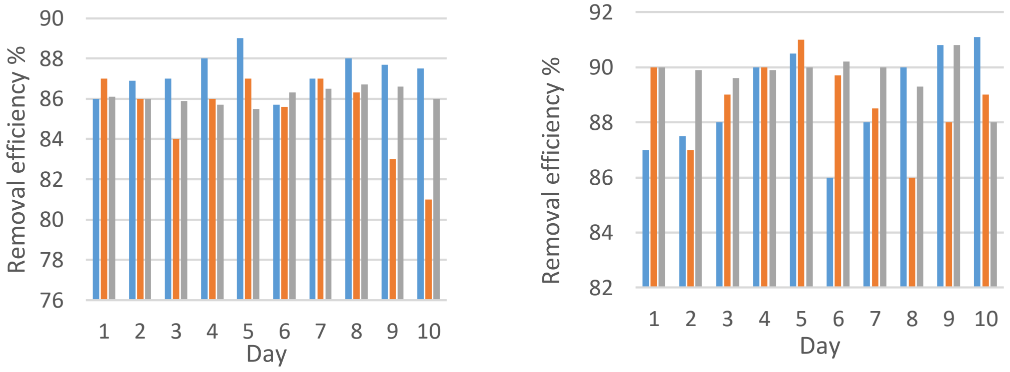
3.1. Salinity removal

The role of the temperature in the salinity removal process is analyzed through the classification of the aforementioned experimental results in groups for each pH value. In general, all these results showed the tendency toward higher salinity removal efficiencies as the initial salinity increases, and this is attributed to the good acclimation of the Oscillatoria limnetica algae with the salinity environment in an agreement with the findings of Melero-Jiménez et al. (2022).

The major indication from Fig. 2 suggests that the temperature of 25 °C dominates other temperatures in gaining higher salinity removal efficiency at pH=5. This is with a nearly stable trend for the majority of the tested initial salinity concentrations. The removal efficiency at the temperature of 30 °C showed significant ups and downs while treating the low concentrations, but it showed a proportional stable and higher manner with the salinity concentrations of 30 ppt and 35 ppt. A similar argument can describe the case with the temperature of 35 °C, with the difference that it showed middle final removal efficiencies between the higher at 25°C and the lower at 30 °C temperatures.

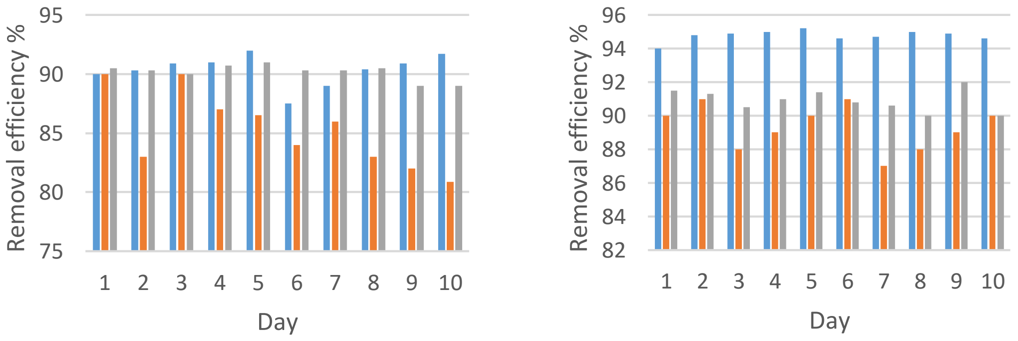


a. Salinity conc.=10, ppt



b. Salinity conc.=15, ppt

c. Salinity conc.=25, ppt

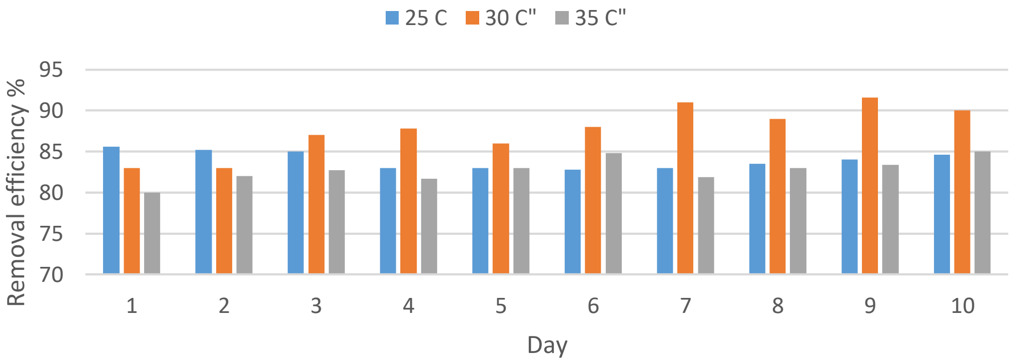


d. Salinity conc.=30, ppt

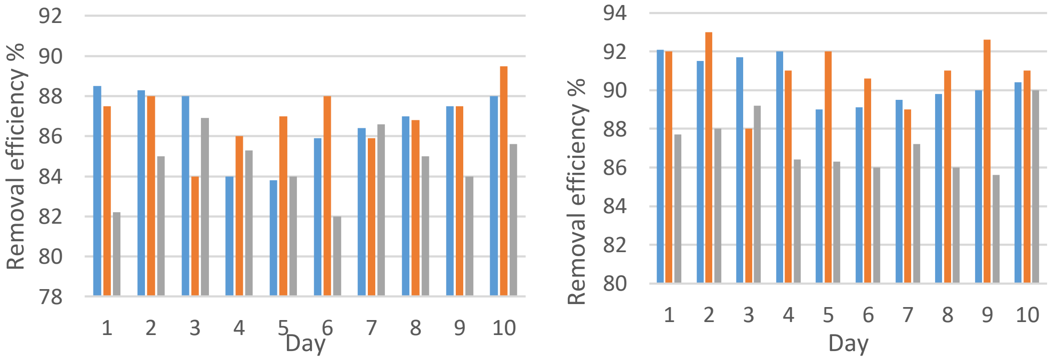
e. Salinity conc.=35, ppt

Figure 1 variation of the removal efficiency for various initial salinity concentrations, temperatures, and pH=5.

The case at pH= 7 somewhat differed as the removal efficiencies with sample temperatures of 30 ºC and 35 ºC slightly dominated these at the temperature of 25 ºC in handling lower salinity concentrations; 10 – 25 ppt, as shown in Fig. 3.11. The temperature of 25 ºC took the place of the temperature of 35 ºC in that regard while handling higher salinity concentrations.

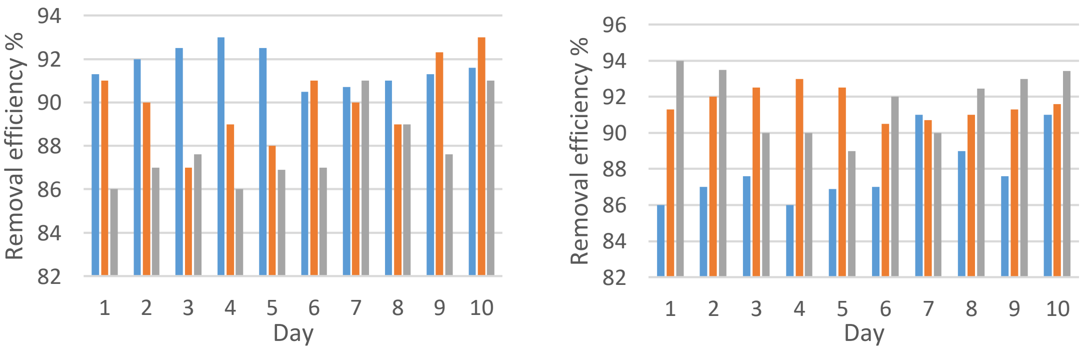


a. Salinity conc.=10 ppt



b. Salinity conc.=15, ppt

c. Salinity conc.=25ppt

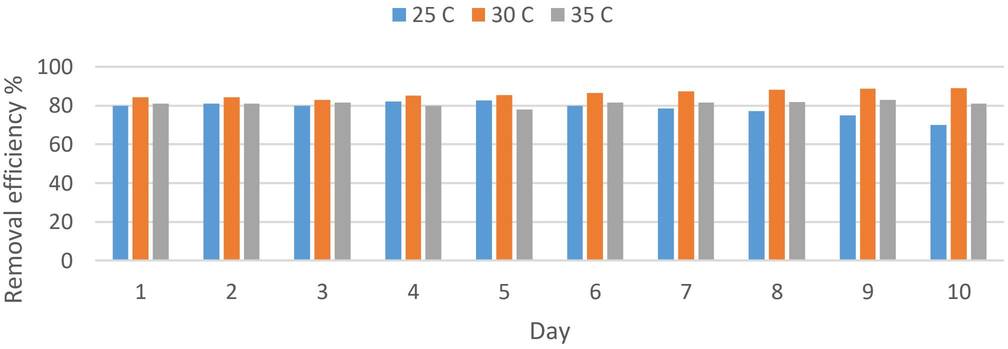


d. Salinity conc.=30ppt

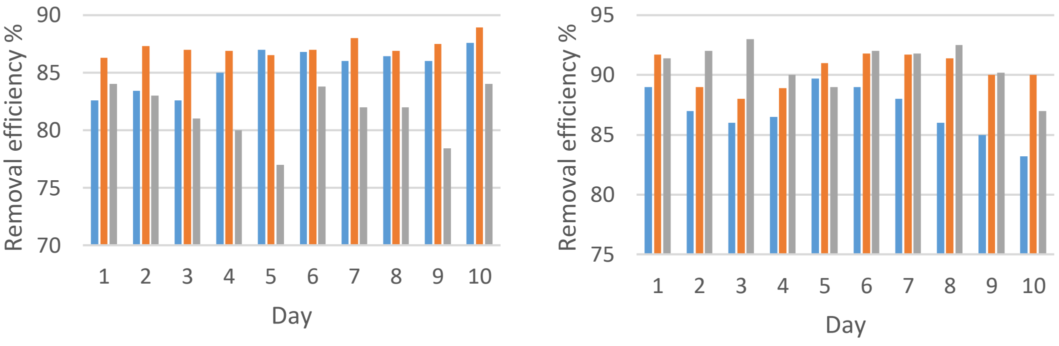
e. Salinity conc.=35 ppt

Figure 3. variations of the removal efficiency for various initial salinity concentrations, temperatures, and pH=7

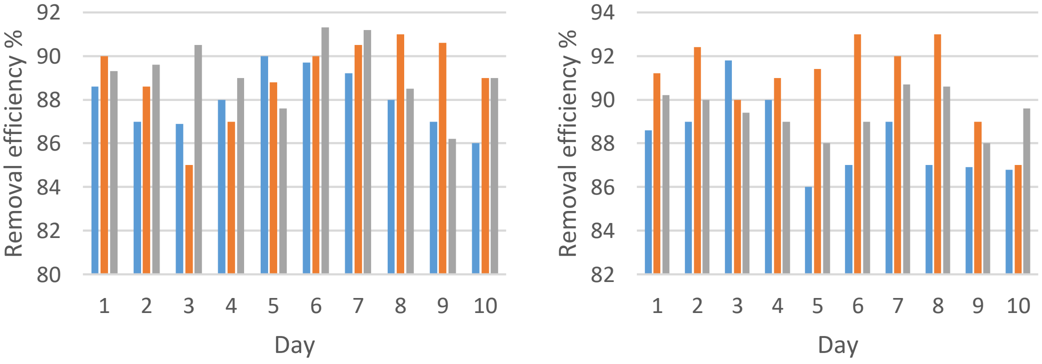
As the pH was raised to 9, the experiments showed that the average removal efficiencies related to the temperature of 30 ᵒC, dominate these related to the other two temperatures; 25 ᵒC and 35 ᵒC, as shown in Fig. 4. In general, the removal efficiencies are less than their values at pH value of 7 and relatively close to those at pH=5.



a. Salinity conc.=10 ppt



b. Salinity conc.=15 ppt c. Salinity conc.=25 ppt



d. Salinity conc.=30 ppt e. Salinity conc.=35 ppt

Figure 4. Variations of the removal efficiency for various initial salinity concentrations, temperatures, and pH=9.

3.2. Growth rate

The scheme of daily variation of growth rate for almost all testing sets showed, in general, two phases; a phase of logarithmic decrease followed by a stagnant or mild decrease phase in a good agreement with the findings of many past researchers such as Saeid et al. (2018), Nadi and Hosseiny (2019). Table 1 summarizes the mean values of growth rate for each experimental setup.

Table 1
Mean values of growth rate and doubling time for the various test combinations

Test combination	Initial salinity ppt									
	10, 000		15,000		25,000		30,000		35,000	
	K d-1	G d	K d-1	G d	K d-1	G d	K d-1	G d	K d-1	G d
25C&pH=5	0.273	1.51	0.274	1.45	0.326	1.34	0.098	3.21	0.205	1.94
25C&pH=7	0.171	2.22	0.201	1.76	0.302	1.3	0.207	1.65	0.217	1.51
25C&pH=9	0.137	2.27	0.200	1.9	0.040	10.14	0.064	9.88	0.084	4.59
30C&pH=5	0.094	3.3	0.060	5.78	0.143	3.09	0.233	1.6	0.176	1.81
30C&pH=7	0.274	1.56	0.239	1.66	0.256	1.52	0.274	1.46	0.348	1.23
30C&pH=9	0.228	1.39	0.225	1.46	0.248	1.46	0.292	1.28	0.225	1.53
35C&pH=5	0.558	0.78	0.564	0.7	0.296	1.32	0.368	0.96	0.119	3.25
35C&pH=7	0.380	1.07	0.261	1.29	0.236	1.36	0.163	2.21	0.226	1.43
35C&pH=9	0.506	0.88	0.234	1.31	0.271	1.34	0.177	1.88	0.162	2.02

Aside from some tiny disturbance along the first few days when the logarithmic decrease in growth rate was determined sharp in an immediate response to the salinity during the acclimation phase, the calculated growth rates at the temperature of 25 ^{o}C and pH values of 5, and 7 showed good durability during the most of the experimental days and the second half of the testing period for the pH=9. This was expected to imply good removal efficiencies, as will be discussed later. Another indication from these results was that the higher the initial salinity, the higher, although slightly, the relevant growth rate, in agreement with the findings of Nurul Salma et al., (2013) who worked on Chaetoceros calcitrans and Chlorella sp. under similar conditions to these of this study.

The set of temperature 30 ^{o}C showed higher growth rates against higher salinity concentrations at the pH value of 5 in a different attitude from the samples of pH values of 7 and 9 where almost comparable daily growth rates were determined against the various initial salinity concentrations. This would explain the comparable final salinity removal efficiencies during these experimental sets. Also, the tendency of growth rates to show almost negligible changes during the last few experimental days may indicate that the new cell generation was close to the cells lost leading to some comparable salinity removal values, in an agreement with the results of Endalkachew et al. (2019).

The case of the temperature of 35 ^{o}C showed some tiny higher growth rates, although similar trends, at pH values of 5 and 7, as compared to the above two sets. The increase of pH value from 5 to 7 appeared to enhance the salinity removal efficiency and hence resulted in a somewhat increase in the algae growth rates especially under high initial salinity stresses, as compared to the other experimental sets. This may refer to better adaptation to neutral mediums. The set of pH=9 showed some sudden low growth rates at the beginning incubation days especially against higher salinities, followed by smoothly constant values for the rest of the period that are comparably between these at pH values of 5 and 7. This is a sign of the increase in the number of dead cells at the start of the experiment while treating high salinities at the temperature of 35 °C and pH=9, as it was recorded by Aguilera et al. (2021).

The major finding regarding the doubling times was that at the temperature of 35 °C shorter average times were recorded for almost all pH values as compared to the other two temperatures; 30 °C and 25 °C. The average doubling time at a temperature of 35 C varied from 0.7 days to 2.0 days in a comparable scheme to that recorded by Endalkachew et al. (2019) varied in the range of 0.63 to 1.81 days for Scenedesmus species and 3.1 to 5.9 for Chlorella vulgaris. These doubling times suggest good recovery for the Oscillatoria limnetica cells to withstand the salinity stress and hence act more effectively in the salinity removal task.

3.3. Statistical analysis

The relationship between the daily optical density and the testing time was tested via the determination of the correlation coefficient for each set as summarized in tables 2. The majority of these results pointed out good to very strong statically significant relationship (- 0.5> R> 0.5). The low correlation coefficient sets are noticed either at the lowest initial salinity concentration or at the highest ones where the removal efficiencies and the algal growth rates have shown some significant fluctuations as mentioned before.

Table 2
The correlation coefficients between the measured OD and the testing time

Experiment set	Initial salinity concentration ppt				
	10	15	25	30	35
	Correlation coefficient r				
T=25 °C , pH=5	0.94	0.90	0.85	0.93	0.94
T=25 °C , pH=7	0.82	0.88	0.79	0.96	0.89
T=25 °C, pH=9	0.91	0.94	0.80	0.92	0.94
T=30 °C, pH=5	0.96	0.95	0.88	0.90	0.95
T=30 °C, pH=7	0.81	0.88	0.86	0.88	0.84
T=30 °C, pH=9	0.97	0.97	0.97	0.96	0.98
T=35 °C, pH=5	0.67	0.92	0.80	0.97	0.91
T=35 °C, pH=7	0.89	0.92	0.94	0.89	0.94
T=35 °C, pH=9	0.72	0.98	0.94	0.94	0.93

The significance of testing various salinity concentrations on the salinity removal efficiencies for each experiment set was assessed via the application of ANOVA tests for a confidence value of 95%. The results of this step are summarized in table 3. The P-value of all experiment sets were less than 0.05 and hence the null hypothesis is rejected and the calculated efficiencies are statistically significant. That in addition to the other evidence of F-value; F > Fcritical. The correlation coefficients between the initial salinity concentration and the removal efficiency for each of the sample`s pH and temperature values are also demonstrated. Although the number of data values was small, that would not result in high precision for the calculated correlation coefficients, the majority of these results were near their maximum value; 1, which is a good sign of the strong relationship between the initial salinity concentration and the removal efficiency under the used experimental conditions.

Table 3
A summary of ANOVA tests for the salinity removal efficiency vs. various initial salinity concentration

Temperature °C	pH	R	F	P-value	F critical
25	5	0.859	66.22206	2.83E-18	2.57874
	7	0.840	75.65474	2.17E-19	2.57874
	9	0.789	29.45579	4.69E-12	2.57874
30	5	0.955	18.0182	6.74E-09	2.57874
	7	0.914	7.757384	7.64E-05	2.57874
	9	0.938	15.35304	5.44E-08	2.57874
35	5	0.841	147.9823	2.79E-25	2.57874
	7	0.958	27.2365	1.62E-11	2.57874
	9	0.608	78.47998	1.06E-19	2.57874

4 Conclusions

The study confirmed the ability of Oscillatoria limnetica to result salinity removal efficiencies in the range between 80-94%. High proportion of salinity removal efficiencies were recorded after the first 2-3 days of incubation, while the additional removal efficiencies during the rest of the ten days were less than 10% at most in almost all the tested samples. It was found that the Oscillatoria limnetica was more effective neutral to alkali mediums (7 ≤ pH ≤ 9) at water temperature of 30 °C, with very good ability to withstand the high salinity stresses during the test period of ten days, during which they pursued relatively acceptable growth rates that allowed them to act as salinity removal mediums. The ANOVA statistical analysis results confirmed the credibility of the research experiments where excellent confidence level of 95% for the significance in the tested sets were proved, and fair to strong relationships between the data sets; -0.5 > R > 0.5, were determined.

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