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Studying The Mechanism Of Cyanobacteria In Dissolved Salts Consumption And Their Effects On Its Growth Rate

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Abstract

Biodesalination, using photosynthetic species of bacteria to exclude NaCl ions from seawater could provide sustainable new saline water desalination methods associated with available methods. 90% of salts can be removed by Cyanobacteria and without pressures or external power source. This research aims to isolate and cultivate Cyanobacteria *Oscillatoriales* (O. sp.) to use in the biodesalination and reduction of total dissolved solids in seawater under the Egyptian condition and use isolated strain at an advanced level which is designing photobioreactor as a stage for biotreatment and use treated water in irrigation. Parameters presented in this paper include the growth rate of (O. sp.), Total Dissolved solids (TDS), pH, Oxidation reduction potential (ORP) and NH_4 concentration. The results showed salt reduction ranged from **3** to **4** $g.l^{-1}$, and NH_4 concentration increased up to **6.5** $mg.l^{-1}$ and more than **9.99** $mg.l^{-1}$. The ORP decreased to about **129** mv. This result is achieved from **30** to **40** g of cyanobacteria biomass.

Keywords: *Biodesalination ; desalination ; photobioreactor ; cyanobacteria ; blue-green alga.*

1- Introduction

United Nations' sustainable agenda target No. 6. is the availability of clean water, sanitation, and managing freshwater ecosystems is crucial for human health, environmental sustainability, and economic development. Therefore, it is essential to provide obtainable and sustainable management of water and hygiene for all (UN SDG's agenda)

About 2 billion people live in regions with water stress. The total renewable fresh water is the ratio of all the fresh water consumed to all renewable fresh water available. Also, about 25% of north Africa and west Asia have water stress, and 60% show a robust possibility of water scarcity. In 2012, a survey was done on water resource management at 65% of the 130 countries that responded and reported that management plans were placed at the national level [1].

Water scarcity is a critical issue; water scarcity has a direct effect on health, industry and the ecosystem. Desalination is one of the solutions for avoiding this issue. Water desalination is an elimination method for salts and different minerals from salty water. It concludes a different concept from old distillation to the very new reverse osmosis.

In Egypt, water scarcity is one of the most critical topics now and is a serious challenge. The annual water deficit has repeated in Egypt of around seven billion m3 and we may have a water shortage by

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2025, as mentioned by UNICEF.

As said by the World Health Organization [2]. The accepted total dissolved solids (TDS) level >600 $mg.l^{-1}$ is classified as good. Drinking water taste becomes noticed and unacceptable at TDS levels <1000 $mg.l^{-1}$. Practically, saline water is divided according to the level of salinity by $g.l^{-1}$ to Fresh 0 $^{-1}$, Brackish 1-5, Highly Brackish 5-15, Saline 15-30, Seawater 30-40 and Brine 40-300 $g.l^{-1}$ [3].

The salt in the ocean contained sodium and chlorine. Together they represent 85.7% of the salts dissolved in water. Also contain different major components like magnesium, sulfate, calcium and potassium. Together with NaCl, they structure 99.4% of ocean salt [4]. Water desalination methods isolate dissolved salts from water. Therefore it has the potential to produce potable water.

Using membrane techniques in salt separation requires forces such as pressure, electricity and concentration. To simulate osmotic pressures and force the water to move through the membrane. This method is highly energy consuming. The researchers are on and on trying to improve efficiency to reduce energy consumption. Desalination using membranes is one of the solutions to supply freshwater, but because of the high cost and high energy demands, it was hard to use it on a wide scale. This means the excitement of a rising need for new low-cost and sustainable desalination methods, which may help lower the effects of water scarcity. This is a more and more apparent problem because it will continue to touch the effects of climate change on a global level, so it's urgent to find a low-cost, energy-competent method to remove salinity from water [5].

The side product of desalination changes the salinity. It reduces the rate of oxygen in water at the disposal site, which causes stress and kills animals that are not used to habitats in high salt levels. In addition, chemicals like chlorine or carbon dioxide and hydrochloric acid the harm in high concentrations [6].

Biological treatment in producing drinking water happens through the biofiltration process, in which the microbial colonies are fixed on a bed material in contact with the water. Biological treatment depends on the behavior of the microbe’s community or specific microorganisms that can catalyze removing contaminants possibly present in water sources [7]. Biodesalination uses photosynthetic species of bacteria to exclude some salt ions from seawater, which could sustain other seawater desalination methods when associated to current methods if associated with current methods. In other words, Biodesalination aims to be a "sustainable way to clean drinking water and cleanness away from damaging ecosystem".

Cyanobacteria is classified as a photosynthetic species of cyanobacteria is prokaryotes that collect sunlight to produce energy and use chlorophyll a, b, and other addition pigments [8]; these pigments have a major role in elements dynamics in most aquatic environments. Cyanobacteria include more than 2000 species within 150 genera and 5 orders [9]. By means of photosynthesis, cyanobacteria can produce carbohydrates, protein and natural oils or lipids. The cyanobacteria have more than 30000 species and were previously classified morphologically into five orders as mentioned in the botanical taxonomic scheme *Chroococales, Pleurocasales, Oscillatoriales, Nostocales* and *Stigonematales* [8].

There is commercial production of photosynthetic microorganisms like the cultivation using sunlight in open systems (raceway pond), Production of photosynthetic species of microorganisms in commercial scale, as cultivation under sunlight the open systems (raceway pond). The channel or rounded type open pond is usually used for mass farming of photosynthetic cyanobacteria species; it's usually a large open canal where sunlight is the source of energy. One of its advantages is that the light source (solar radiation) is free, and its disadvantage is the limits of biomass production [10,11].

Cultivation uses sunlight in closed system, this system uses transparent walls made from plastic or glass so the vessels could also place outdoors. In the sunlight. The advantages of this type are pollution prevention. The disadvantage is the high cost of applying transparent materials and closing the system in the open air [10,11].

Cultivation uses non-natural light (artificial light) in closed systems; many designs have been developed for closed cultivation, which uses non-natural light as a source of energy; these designs are called photobioreactors. The advantages of photobioreactors enable good control and optimum producing high quality and quantity of biomass. The negatives are the cost increases in biomass

production based on photovoltaic reactors due to plastic or glass materials and the energy used for light [10,11].

Biodesalination technique using cyanobacteria, starves the cells of energy, therefore, it will stop the dynamic of ions export. Some notes that cyanobacteria can collect intracellular or extracellular osmolarity due to ionic stress [12]. Additionally, the cyanobacteria's accumulated suitable solutes exhibit a hierarchy in terms of their capacity to fend off salt stress [13]. Cyanobacteria reported growth in brine water, by forming thick mats at the wall and the bottom [14,15]. In the production process, salt-laden cells are effectively removed from desalinated water without damaging the cyanobacteria cell [6].

Therefore, the study's main objective is to examine and analyze biodesalination technique as a promising need using cyanobacteria into two minor stages: (1) Isolate, identify and characterize photosynthetic microorganisms from Egyptian seawater. (2) Desalination of artificial seawater and actual seawater with salinity between (15 -30 $g.l^{-1}$) and (30 - 40 $g.l^{-1}$) from Egypt seawater sample conditions.

2- Experiment Material

Experiments conducted at Lab of Bioengineering, Agricultural Engineering Research Institute (AEnRI)-Ministry of Agriculture and Land Reclamation (MALR). The experiments were divided into two stages; the first stage is the preliminary experiment

2.1. Seawater samples (location and picking)

Diverse samples for isolation of cyanobacteria were picked from Beer Sweir, Nuweibaa, South Sinai Governorate,(red sea), Egypt. (29.247732º, 34.734763º) at 15th August 2021 during the blooming time.

Eight samples were collected, two of which were seawater only at 1 m depth and distance, 2 m distance and 2 m depth far from shore Fig. 1a, 6 samples were including seaweed Fig. 1b to obtain cyanobacteria from them. Using a washed plastic water bottle sterilized with alcohol. Seawater samples were kept in an ice box for ~6 h (fresh seawater samples Fig. 2a) before testing for pH, TDS, ORP and ammonium. As mentioned in as should be measured directly in situ [16].

(a) (b)

Fig. 1. Seawater samples. (a) Fresh samples, (b) Samples containing seaweed after 2 weeks.

2.2. Cyanobacteria isolation media

Isolation, purification, primary screening and Identification [17,18,19]. Various culture media were necessary to isolate and cultivate different bacteria. Media BG11 and ASNIII were used as selective media.

General media for growing marine cyanobacteria included BG11 and ASNIII; BG11 is preferable for cyanobacteria growth. BG11 broth is a well-known medium for cultivating and maintaining blue-green algae (cyanobacteria) [20]. BG11 and ASNIII marine broth is a medium for marine cyanobacteria, and its composition is as the following Table No. (1):

BG11 Marine Broth is used to cultivate *Synechococcus* species and to isolate the cyanobacteria from freshwater. As well as ASNIII is a medium for the isolation from marine habitats. It's used for the cultivation of *Xenococcus* species [20].

2.3. The Equipment and devices used for measurement

EZ9910® Multifunctional 5 in 1 Water Quality made in China tester for ORP (Oxidation Reduction Potential) range ± 1000mV, EC range 1-19990us/cm, TDS range 1-19990ppm and pH range 0.00-14.00, Salinity Measurements (TDS, EC) with YSI High Range Salinity Device Measuring EcoSense® Conductivity Meter EC300 made in US with Accuracy: ±0.2% for salinity. The ammonium concentration was measured using a Hanna® Ammonia high-range kit made in US range of 0.0 to 50ppm ammonium.

Table 1. Marine cyanobacteria media (per liter) [20].

BG11 pH 7.1 ± 0.2 at 25°C.		ASNIII pH 7.1 ± 0.2 at 25°C.	
composition	concentration	composition	concentration
NaCl	10 g	NaCl	25 g
NaNO3	1.5000 g	MgSO4·7H2O	3.5000 g
MgSO4·7H2O	0.0750 g	MgCl2·6H2O	2 g
K2HPO4	0.0400 g	NaNO3	0.7500 g
CaCl2·2H2O	0.0360 g	K2HPO4·3H2O	0.7500 g
Na2CO3	0.0200 g	CaCl2·2H2O	0.5000 g
Citric acid	0.0060 g	KCl	0.5000 g
Ferric ammonium citrate	0.0060 g	Na2CO3	0.0200 g
EDTA disodium salt	0.0010 g	Citric acid	0.0030 g
Vitamin B12 solution	100 ml	Ferric ammonium citrate	0.0030 g
Trace metal mix A5	1 ml	Magnesium EDTA	0.0005 g
A5 trace metals	1 ml	Vitamin B12 solution	100

2.4. Incubator

A designed incubator was used to incubate the cyanobacteria under the required conditions with 4 air pumps 5W, max. Flow 3.5 $l.min^{-1}$, Fig. 2. It was placed on the higher top of the first chamber, incubator chamber was covered with a heavy Aluminum sheet for isolation to increase the efficiency of light. Moreover, it was covered from the outside with a plastic sheet to hold the temperature on cold days; it was equipped with LED (Light Emitting Diodes) with light with intensity ranging between 4000 to 5000 Lux. The chamber dimension was (25 cm Hight, 50cm width, and 28 depth) Fig. 3.

Fig. 2. Air pumps.

Fig. 3. Incubator chamber.

2.5. Halophilic cyanobacteria bulk

After isolating cyanobacteria from seawater, mass production has been made on the culture media; Some of these species can even grow at high salt concentricity, reaching NaCl saturation habitat [21]. Cyanobacteria were earlier referred to as one of the blue-green algae, which is oxygenic phototrophic species of bacteria [22,23], so the mass was made using an air pump mentioned in section 2.4 using a semi-photobioreactor. Cyanobacteria have an internal membrane system; called a photosynthetic thylakoid membrane. They have the ability to form as planktonic or make phototrophic films in sea environments. Cyanobacteria that live in water are well known for the eye-clear visible extensive blooms that form in water environments and have the presence of blue-green color biomass. Cyanobacteria were adapted to live in high salts level, as known salts are significant abiotic stress in terrestrial

ecosystems. Halophilic cyanobacteria make thick mats in salty water on the bottom of the container in the presence of sunlight; sunlight was simulated using the LED light. High salts level is an important abiotic factor that causes stress for cyanobacteria [9].

2.6. lap scale photobioreactor desalination

A small-scale photobioreactor Fig.4 has been used for the desalination technique without aeration, only using LED light to activate the photosynthetic operation. And to measure the oxygen production by the cyanobacteria as an oxidizing process measured by ORP pen. Many researchers used different microorganisms to eliminate salts from seawater [24]. Desalination by using algae to eliminate salts from salty water to produce good water to use it in different ways is a new notion; it is used and tested in studies with industrial wastewater treatment with different applications. Cyanobacterial salt ions removal process, about 90% of the salt can be removed, with no need to pressurize the water or use an external power source.

Fig.4. lap scale photobioreactor.

3. **Method**

3.1. Isolation, purification, primary screening and Identification.

Various culture media were necessary to isolate and cultivate the different kinds of bacteria [19,20,21]. Media BG11 and ASNIII were used as selective media. All the media were prepared with distilled water and sterilized in autoclave. The isolation processes started on Oct.3rd, 2021. A water sample was inoculated in (BG11 and ASNIII) agar plates and incubated at 25 °C ±2 for 8 hours light and 16 hours dark in the incubator with a light intensity of range 4000 to 5000 Lux. A critical factor for maintaining cyanobacteria growth is temperature [25]. The suitable temperature for micro algae is between 20-25°C. And about the light, cyanobacteria may consume 1/10 of the light received. More exposure to light cause faster growth. One crucial factor is keeping the water's depth level to avoid photoinhibition [26]. The pH of the medium is tuned to 7 ±2 by using H2So4 - NaOH for 1 l of each nutrient broth (BG11 and ASNIII), 1 ml of each sample was added to the medium and incubated at 25 °C ±2 for 8 hours light and 16 hours dark in the incubator with light intensity ranged between 4000 to 5000 Lux. Cyanobacteria favor an alkaline environment through the bloom, and the pH increased to < 9 [27].

3.2. Selection and biomass production

The selected samples were 2 samples No.1 and No. 2, were inoculated in ASNIII medium and incubated at 25 ±2 °C for eight hours in the artificial light. Sixteen hours in the dark in the incubator with light intensity ranging between 4000 to 5000 Lux, as illustrated in Fig.10a and Fig.10b. Identification of cyanobacteria has been made by microscopic observation by spreading isolated culture on a glass slide with glass cover. Observed by the objective lens of multiple light microscopes under 10X, 45X and oil 100X.

3.2.1. Growth rate calculations

Cyanobacteria has five phases to grow in batch cultures: lag phase, exponential phase, declining phase, stationary phase and finally, death phase. The culture is most effective when it is followed by the constant exponential growth [28]. Growth rates were observed by wet weight method to time in a fixed volume, after inoculating a .01 g of the sample in 1 l of fresh media and the weight every 15 days. The time intervals were every 15 days. Cyanobacteria advanced a mechanism to adjust a wide range of different environmental factors [29]. Under appropriate growth conditions, lab cultures of cyanobacteria have growth rates of 0.21 and 0.99 per

day; in other words, 0.3 to 1.4 doublings per day, respectively [30].

3.3. *Using The Biomass in desalination*

The biomass was harvested and then added to artificial seawater to test the reduction of salts in the water. Some researchers reported that microorganisms could develop two tactics for the osmotic process for the salt ions changing concentrations by its cytoplasm: "salt-in & salt-out tactics [9,31]. The use of the salt-in tactic gathers a considerable amount of inorganic ions as KCl within the cytoplasm to guarantee the absorption of water and turgor pressure [32,33]. Thajuddin and Subramanian (1994) reported fifty different species within nineteen genera at salty pans; these pans' salinity is greater than 50 ppt [34]. So, the salt concentration was too high, 50 $g.l^{-1}$, to start the desalination technique. Cyanobacteria is divided into euryhaline, which can live in a wide range of salinities and stenohaline, which can live only in a narrow range of salinities. Types that live in brine are known as hypersaline, and types that live in brackish are known as hyposaline [35]. Cyanobacteria that are salt tolerant can make a scale of osmolytes, which means solutes can keep high inside osmolarity and turgor pressure without harming proteins or cellular constituents [8].

3.4. *Measuring salinity reduction and ORP*

Salinity reduction was measured most simply by measuring the TDS level and EC and ORP as indicators of the presence of high O2 produced by the cyanobacteria and exchange related to active oxidant. Using cyanobacteria in salt ions exchanging, and use it in removing Na^+ and Cl^- ions from seawater. Cyanobacteria can change remarkably in appearance, depending on the environmental conditions [7, 27]. The most critical abiotic parameters requirements for cyanobacteria are nutrients and water [36]., Carbon-dioxide, minerals, light, pH and temperature. Every kind of algae species has its requirements. This reaction the standard reaction for the autotrophic growth [26].

$$H_2O + CO_2 + light \rightarrow C_6H_{12}O_6 + O2 + H_2O \qquad (1)$$

4. Results and discussion

For the 8 different samples collected from seawater to isolate cyanobacteria, the analysis of fresh seawater samples (6 hours sample's old) is illustrated in Table (2) and Table (3). This sample's temperature was 17 °C, its salinity was 39.9 ppt & the concentration of TDS was 42.4 $g.l^{-1}$.

Table.2. Analysis of fresh seawater without any seaweed sample.

Test	Result
Salinity, ppt	39.90
TDS, $g.l^{-1}$	42.42
EC, ms	48.42
pH	6.93
ORP, mv	277
Temperature, °C	17.30
Ammonium concentration, $mg.l^{-1}$	1.40

For sample 5, no cyanobacteria appeared in any experiment, they changed in physical and chemical properties and due to water turbidity, it wasn't easy to measure the ammonia level in samples 5 and 3. Sample No. 3 and 5 showed a different strain of red cyanobacteria in Fig. 5 and a phosphorous odor on ASNIII media. Under anaerobic conditions. Therefore, these samples were carried out from the experiment. Sample No. 4 showed red bacterial formation (slimy red algae) under anaerobic conditions and blue-green algae under standard conditions (without aeration or isolation) Fig. (5).

Samples number 1 and 2 under the microscope were filamentous green bacterial cells gained after the isolation of pure culture, Fig.6. Micrographs of cyanobacteria isolations appeared as filamentous blue-green algae by comparing the morphology structure with previous studies, and the spots were identified as class *Oscillatoriales* of cyanobacteria, Fig.7 in abundance. The biomass of BG11 had more chlorophyll content than that of ASNIII cyanobacteria.[37] .

Table.3. Analysis of fresh seawater sample that contains different unclassified seaweeds.

Test	Result					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Salinity, ppt	29.10	29.10	41.30	39.00	32.10	42.70
TDS, $g.l^{-1}$	35.70	37.70	51.20	48.50	40.50	54.00
EC, ms	37.90	46.70	49.60	46.70	49.10	51.90
pH	7.10	7.00	7.00	7.00	7.10	7.00
ORP, mv	278.60	216.60	218.60	227.30	219.60	227.30
Temperature, °C	16.00	16.50	15.20	15.20	16.50	15.40
Ammonium concentration, $mg.l^{-1}$	3.00	3.00	ND	2.80	ND	2.90

Fig.5. Red bacteria (red slim algae).

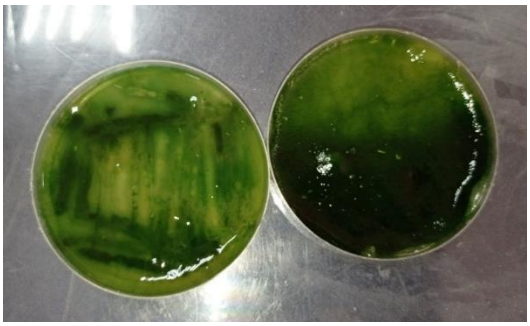


Fig.6. Cyanobacteria formation.

Fig.7. Morphology of *Oscillatoriales* class of cyanobacteria [37].

In sample No.1, the chlorophyll had a strong appearance, as shown in Fig 6. Still, sample No. 2 has a weak saturation of green chlorophyll color, which refers to the amount and shape of green macro algae in the seawater sample and the LED color (white light showed the greenish one, and yellow showed the light green one). Graft was taken from this isolated sample and planted in 2 different broth media to test the salts reduction in both media, Fig.8.

Fig.8. Sample inoculation. (a).1 mm of sample.

Fig.8. Sample inoculation. (b).1 mm sample inoculated in 1l medium.

Then the growth rate was measured by wet weight method by filtering the cyanobacteria from its broth medium and weight the bulk from the first day to the end of the experiment using the following equations [38,39]:

Growth rate w\time (K)

$$\mathbf{K} = \frac{\mathbf{Ln\,(N2\,/\,N1)1}}{\mathbf{(t2-t1)}} \qquad (2)$$

$$\text{Divisions.day}^{-1} = \frac{\mathbf{K\prime}}{\mathbf{Ln2}} \qquad (3)$$

$$\text{Generation time} = \frac{\mathbf{1}}{\mathbf{Div/day}} \qquad (4)$$

Where:

N1 biomass at time1(start time) (t1), $g.day^{-1}$

N2 biomass at time2 the second time (t2), $g.day^{-1}$

Table.4. Growth, division rate and the generation time for 2 selected samples with 2 media.

Parameter	BG11		ASNIII	
	Sample 1	Sample 2	Sample 1	Sample 2
Growth rate (K) g.day^{-1}	0.974	0.672	0.670	0.669
Divisions per day	1.400	0.970	0.960	0.960
Generation time (day)	0.710	1.030	1.033	1.030

In the BG11 media for sample one, the growth rate was higher than in the ASNIII, even though sample one showed a strong appearance and chlorophyll. However, sample 2 showed a similar growth rate even with the salinity difference in the ASNIII medium. In sample No. 2, the cyanobacteria formation starts to appear strongly after 15 days.

The wet weight of the cyanobacteria harvested from the flasks after 10 days. It was as follows (BG11 No.1= 2.81, BG11 No.2 = 2.38, ASNIII No.1= 2.93, ASNIII No.2 = 2.72) in the middle of the experiment on day 30th.

Table.5. Salinity reduction (TDS) g.l^{-1}.

Intervals (days)	BG11		ASNIII	
	Sample 1	Sample 2	Sample 1	Sample 2
1	26.00	26.00	35.00	35.00
10	25.30	25.75	34.10	34.40
20	24.60	25.10	33.50	33.80
30	24.00	24.00	32.70	33.20
40	23.20	24.90	32.00	33.00
50	23.40	24.10	31.80	31.90
60	23.70	24.30	32.00	32.10

The salinity reduced by 2 to 4 g.l^{-1} in both media BG11 and ASNIII, Fig. (9 and 10), Demessie et al. [40] mentioned that they had a reduction ranging between 3.58 and −7.68 per day. And ions (Na^+, K^+, Cl^-) can enter to the cell as a response to a sudden increase in medium salinity.[41].

As well as, the ammonium concentration was increasing during mass production phases (60 days) as shown in Fig. (11 and 12). Also, Oxidation reduction potential was reduced, Fig. (12).

Ammonium concentration increased to more than 10 mg.l^{-1}, which will be good when using this amount in agriculture but not in aquaculture. At the end of the experiment, the high concentration of ammonium in the media caused cell death. S.Canizales *et al.* [42] mentioned that the high presence, mainly in the form of ammonium, can inhibit microalgae growth due to ammonia toxicity. Plus, the increase in the salts after day 40 referred to the release of the salt accumulated in and out of the cyanobacteria cell.

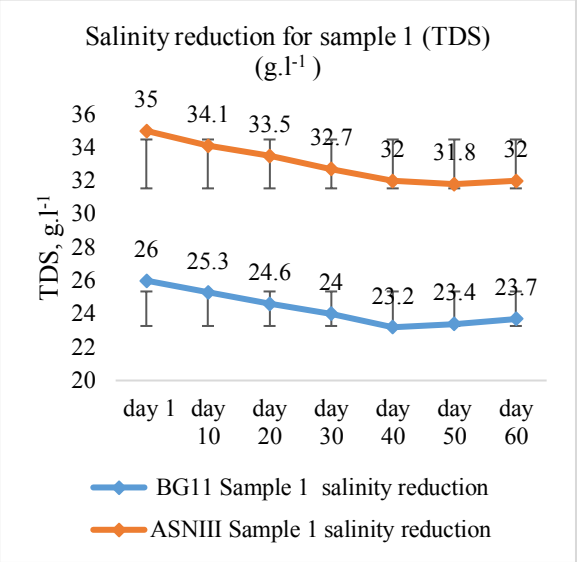


Fig.9. Salinity reduction sample 1 (TDS) (g.l^{-1}).

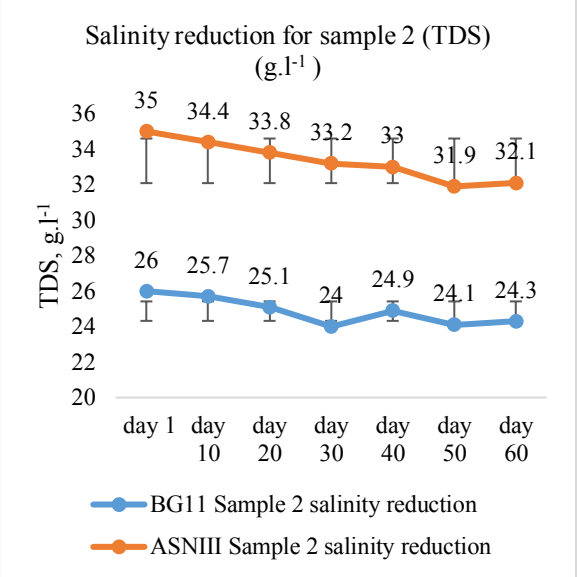


Fig.10. Salinity reduction sample 2 (TDS) (g.l^{-1}).

Table.6.Ammonium concentration mg.l^{-1}

Intervals (days)	BG11		ASNIII	
	Sample 1	Sample 2	Sample 1	Sample 2
1	0.00	0.00	0.00	0.00
15	2.40	2.90	3.00	2.90
30	3.90	4.50	6.60	5.20
45	5.10	6.30	8.80	6.92
60	6.47	8.80	>9.99	8.88

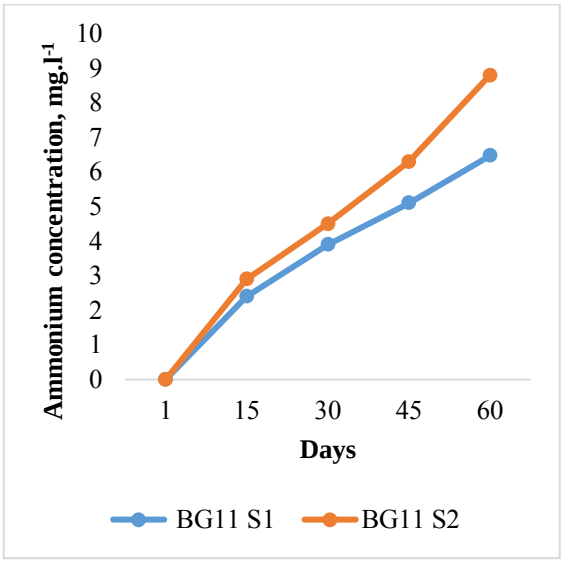


Fig.11. Ammonium concentrations in BG11 (mg.l^{-1}).

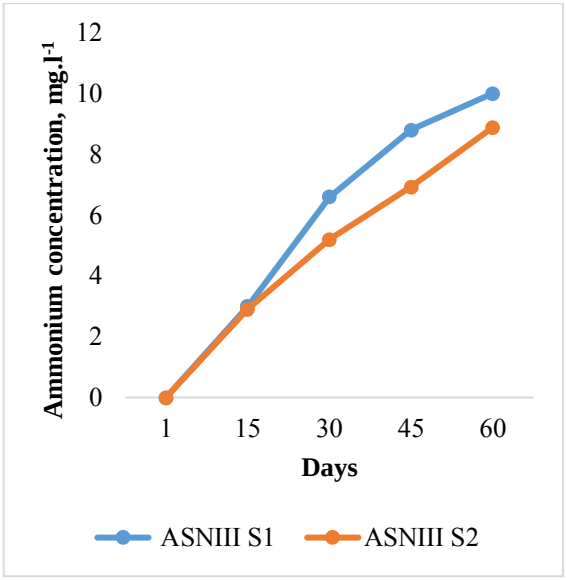


Fig.12. Ammonium concentrations in ASNIII (mg.l^{-1}).

Table.7. Oxidation-Reduction Potential (ORP), mv.

Intervals (days)	BG11 mv		ASNIII mv	
	Sample 1	Sample 2	Sample 1	Sample 2
1	378	360	288	299
15	350	351	272	281
30	333	336	260	270
45	325	320	256	261
60	318	316	249	257

The Redox Potential was measured as indicator of O_2 present, the tests showed that at the beginning of the experiment, the Redox was high because of the aeration that was attached to accelerate the cyanobacteria division; starting from day 15, the air pumps removed, and the ORP measured without any aeration source. ORP can detect anaerobic microbial activity in the environment, such as in the water column or sediment [43].

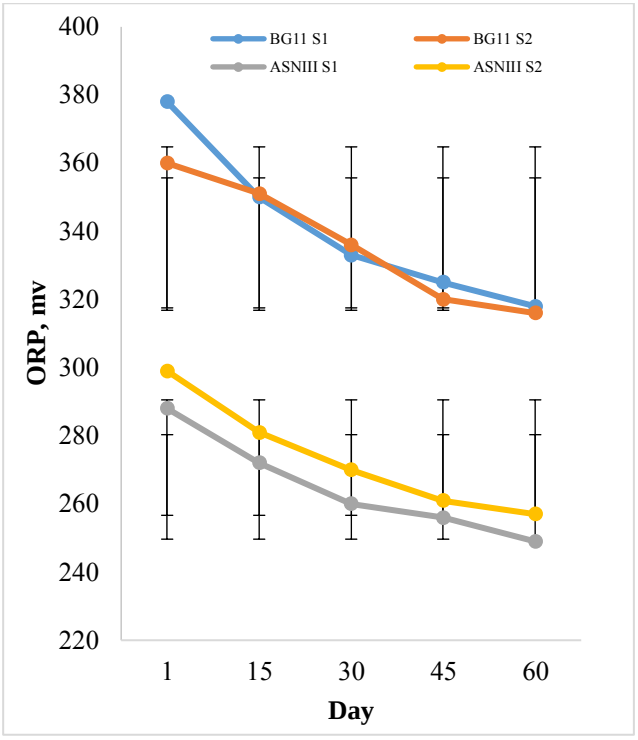
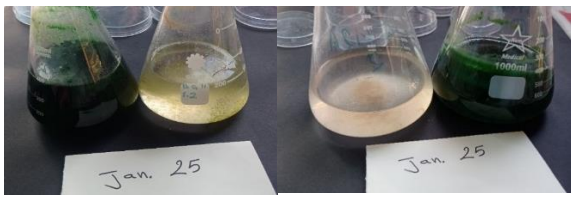


Fig.13. Oxidation-Reduction Potential Reduction, (mv).

The preferred media is the higher in salinity which is ASNIII and the cyanobacteria showed a reasonable growth rate in this halophilic medium. Sample 1 Fig.(14a) has a growth rate higher than sample 2 Fig.(14b). Starting from day 45, the flask started to change in colour because of dead cells Fig. (15).



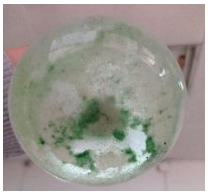
(a) (b)

Fig.14.Sample (1 and 2) growth rate in BG11 and ASNIII medium. (a) BG11 sample (1 and 2), (b) ASNIII sample (1 and 2).

Fig.15.Chlorophyl in live cells and light-yellow dead cells.

After cell death, it precipitates, and the salt accumulates in the bottom of the flask, the level of salinity in the bottom was higher than the surface, and that's because of the cell death; It forms as dry layer (looks like the dry leaf) even in the exitance of water and the salt rereleased in water, that refers to the absence of the colloidal layer that covers the Bactria, which works as salts ions adsorbent. Sample 2 before the cyanobacteria appeared to be observed by the eye formed a yellow suspension. Then the cyanobacteria start to form, as cleared in Fig. (16). Stages of cyanobacteria from formation to death are illustrated in Fig. (16).

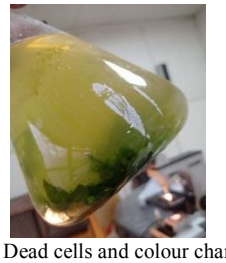
St. (1) Yellow suspension



St. (2) Cyanobacteria start formation

St. (3) Cyanobacteria growth

St.(4) Cyanobacteria biomass



St. (5) Dead cells and colour changing

Fig.16. Cyanobactria stages from formation to death.

The two samples under the microscope appear as a filamentous cyanobacterium; when classified the Bactria morphological from reference [37], it shows that the strain is *Oscillatoria* (9 strains are contained within this genus) PCC 7105 Fig. (17).

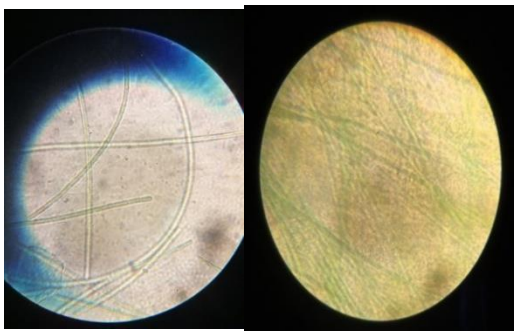


Fig.17. Cyanobacteria under microscope.

The variation of the chlorophyll saturation was because of the white and yellow LED used in the incubation phase, which will be taken into the stage of designing the PBR.

Cyanobacteria cultivation has many ways. One is using open water resources, and the other is building a photobioreactor. You cannot use the sea to cultivate cyanobacteria because the algae requirements are hard to fulfill in the sea. So providing light, carbon dioxide, and stable temperature is impossible in the sea or opens huge areas [44].

Closed photobioreactors (PBRs) are preferable to minimize water evaporation and contamination. They are suitable to control the factors that increase microorganism growth, photosynthesis efficiency, light intensity level, temperature degree, and CO_2 that will be precise in PBRs.

The retention time in the PBR does not exceed more than 45 days. In the PBR design, preferably, there is an outlet for dead cells and an inlet for fresh seawater to desalinate.

The aeration caused water splashing and salt accumulation in the flask walls, the acceleration of cyanobacteria division and a high level of ORP. Due to water quality laws and greater usage of O_3 for drinking water conduct, the biological treatment to produce drinking water is currently getting more attention. And it is an effective strategy [45]. And No aeration showed the presented results; with aeration, the growth rate was doubled.

Cyanobacteria is photosynthetic prokaryotes it need sunlight to produce energy by chlorophyll and several different pigments [8]. Cyanobacteria with chlorophyll D could bloom in different surroundings with low visible light; therefore, micro-environmental aspects are significant in knowing the Specialization of specific microorganisms [46]. The *Oscillatoriales* showed a reduction in salinity with a small amount of wet weight; in the case of doubling

the used amount, the reduction will increase. Using *Oscillatoriales* as an economic phase in water biodesalination is necessary for facing water scarcity. Water filters were used to improve taste or to eliminate any undesired matters. But the cost is still unsatisfactory, and many products are imported, which further add to the cost.[47]. suppose the *Oscillatoriales* used in the brackish water of healthy water with high salinity will show a good result. In that case, this will be the next stage of this work to do some experiments on lower salinity water.

The level of ammonium concentration in rejected water after filtration will be effective for agriculture, and this is the main target of finding a novel source of water that can be used in agriculture.

5. Conclusion

Produce a higher biomass (double the biomass) and add it to the same amount of seawater and measure the desalination over time.

No aeration, no shaking needed, but also it should be in the normal condition of air pressure; in the presence of pressure, a barophilic bacteria formed than the cyanobacteria.

Temperature of 24 to 28 ºC increased the growth rate; the low temperature affected the growth rate.

Making the rRNS16 Identification to proceed using this strain which showed a good adaptation and carried to the high salinity in some cases, the salt amount was about 70 g.l-1, and the cyanobacteria survived.

Design a photobioreactor that offers a suitable environment for cyanobacteria and has a chopper to double the yield of cyanobacteria.

Design a photobioreactor with a pull device to scrape the mate of cyanobacteria from the photobioreactor walls.

6. Conflicts of interest

The authors declare that they do not have any conflict of interest.

7. Formatting of funding sources

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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