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Artificial seawater biodesalination and biodiesel production using some microalgal species.

Hassan, M. K. [(1)]*; Abou Elkheir, Wafaa [(2)]; Hashem, A. I. [(3)]; El Malky. M. Gh. [(4)]; Amr M. Helal [(5)] and Abdelkader, S. A. [(6)]

1) Faculty of Environmental Studies and Research, Ain Shams University
2) Women's College for Science, Arts and Education, Ain Shams University
3) Faculty of Science, Ain Shams University
4) Faculty of Environmental Studies and Research, Ain Shams University
5) National Institute of Oceanography and Fisheries. NIOF
6) Women's College for Science, Arts and Education, Ain Shams University

***Corresponding Author: megale_85@yahoo.com**

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ABSTRACT

Existing water resources cannot satisfy human needs due to an increase in population, the growth of urbanization, and the scarcity of freshwater sources worldwide. Utilizing plant species, microbes, algae, or a mix of these can be used to effectively desalinate seawater through biological processes. The objective of this study was to investigate the possibility of seawater desalination by using green microalgae (*Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Dunaliella salina)* and the potential for combining it with the manufacture of biodiesel. The results showed that *Chlorella vulgaris* and *Scenedesmus quadricauda* were significant for the desalination of seawater. The TDS removal percent reached 82% and 79% during the culturing of *Chlorella vulgaris* and *Scenedesmus quadricauda* (respectively), without adding any nutrients while the removal percent reached 82% and 80% (respectively), during culturing with adding nutrients. The results of *Dunaliella salina* showed that there is no significant difference in the desalination of seawater. Characterization of the algal biodiesel obtained from *Chlorella vulgaris* and *Scenedesmus quadricauda* revealed that it fits the international specifications.

INTRODUCTION

As the population expands and the environment becomes further influenced by climate change, access to fresh and clean drinking water is shrinking. Over 785 million people in the world lack access to clean drinking water (**Liu and Bridget, 2020**). Because of the increase in global population and the limited availability of freshwater reserves, desalination from seawater has gained importance **(Nagy *et al.*, 2017)**. The expansion of industries and agriculture in Egypt has led to an unmatched demand for fresh water resources. 69.4% of Egypt's total water supply comes from the River Nile,

with the remaining 30.6% coming from rain, heavy downpours, groundwater, recycled agricultural and sanitary drainage water, and desalinated seawater (**Abdelzaher and Awad, 2022).**

Given the importance of seawater desalination to meet the demands of earth's inhabitants, this research examines how this process might be made less challenging to utilize as a real solution, not simply a transient opportunity **(Ayaz *et al.,* 2022)**. A novel and affordable idea is the use of algae to remove salt from saline water and produce water for a number of uses. This novel approach has the potential to be successful in resolving the desalination issue at the lowest possible cost **(Kesaano and Sims, 2014)**

It was discovered that halophilic algae (*Scenedesmus* sp., *Chlorella vulgaris*) grown in a photobioreactor (PBR) successfully sequestered salt from brackish water and seawater under carefully controlled conditions, allowing their potential use in biological desalination **(Abomohra *et al.,* 2017)**, which was a very interesting study on the isolation of halophilic microalgae. The microalgae have a greater capacity to lower the salinity of the water since they are more likely to absorb water soluble nutrients than they require for nutrition. It is possible to desalinate or lessen the salinity of water by using several types of macrophyte (aquatic plants), microphyte (algae), and microorganisms or their combinations, as well as biological processes (**El Sergany *et al.,* 2019**).

Biodesalination involves the two processes of salt removal from a saline environment, biosorption and bioaccumulation **(Zafar *et al.,* 2021)**. Bioadsorption is the process of rapidly attaching ions or molecules to a wet or dry algal surface **(Kumar *et al.,* 2021)**. It is a metabolically independent and reversible process that occurs on the surface due to adherent surface properties with no energy cost **(Kumar *et al.,* 2021)**. Bioaccumulation is a slow energy-dependent biological process that happens in living cells via salt uptake or accumulation **(Kumar *et al.,* 2021)**.

The utilization of algae to reduce salinity and provide algal growth has a substantially lower energy requirement when the algae are harvested for biofuel generation **(Gautam and Kapoor, 2022)**. Biodesalination requires the separation of algae cells from water in a cost-effective and efficient manner. The efficient utilization of biodesalination technology will provide a reliable, low-cost supply of drinking and agricultural water while also producing valuable bio-based fuel as an energy byproduct for desalination **(Sahle-Demessie *et al.,* 2019)**. Algae contain 20%-80% oil, which can be successfully turned into various fuels such as kerosene oil and biodiesel. Furthermore, the techno-economic feasibility of algae-based biodiesel manufacturing has been shown. (**Chisti, 2007 and Murthy & Kumar, 2021**).

MATERIALS AND METHODS

1- Algal species selectivity and cultivation

In this study, *Chlorella vulgaris, Scenedesmus quadricauda* and *Dunaliella salina* were obtained from the National Institute of Oceanography and Fishers located in El-Kanater El-Khiria, Qalyubia, Egypt. The microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* were cultured in BG-11 media according to **Stanier *et al.* (1971),** while *Dunaliella salina* was cultivated using a Johnson's medium according to **Johnson and Richardson (1968).** For 14 days, all microalgae were vaccination at a rate of 50 mg/L in 4 L bottles **Figure (1)**. The culture media were autoclaved at 121 °C for 20 minutes before inoculation using an autoclave (STERIF0W-1341) and continuously mixed by an aerator (Hei-mix S, Heidolph, Germany) at a rate of 0.5 L/min., the necessary illumination was supplied by sunlight, the photoperiod was 16/8 h of day/night cycle, a temperature of 30±2 °C and pH was adjusted at 7.5.

Fig. (1):- Culture of microalgae

The harvested biomass was allowed to precipitate before being filtered using 0.45 mm pore size Whatman GF/C filter paper to get concentrated algae paste **(Hamid *et al.*, 2016)**. The concentrated algae paste was then resuspended in artificial seawater to start the desalination process.

2- Preparation of Artificial seawater for culturing microalgae

Artificial seawater was prepared according to **ASTM (2021) D1141-98R21** method.

3- Algal Bioassay Procedures for Biodesalination

The desalination process applied on artificial seawater using each of the three microalgae grown previously on BG-11 medium (*Chlorella vulgaris and Scenedesmus quadricauda***)** and Johnson's medium (*Dunaliella salina)* as follows:

3-1- Biodesalination of artificial seawater without adding any nutrients:

The previously mentioned microalgae were grown separately in three 20-liter transparent polyethylene bottles of artificial seawater without adding nutrients according to **Moayedi *et al.*, (2019)** at 30±2 °C, pH 7.5, mixing continuously by an aerator (Hei-mix S, Heidolph, Germany) at a rate of 0.5 L/min., under the effect of photoperiod 16/8 h

of day/night cycle, for 18 days divided into 3 stages, each stage consisting of six days, and in each stage, the daily change in the salinity of industrial sea water was monitored by measuring total dissolved solids (TDS), electrical conductivity (EC) and microalga growth rate (GR).

3-2- Biodesalination of artificial seawater with adding nutrients:

The tow algal species *Chlorella vulgaris and Scenedesmus quadricauda* were grown separately in tow 20-liter transparent polyethylene bottles of artificial seawater and then feeded by algal nutrients from the modified BG-11 culture medium to promote algal growth according to **Barahoei *et al.* (2021)** at 30±2 °C, pH 7.5, mixing continuously by an aerator (Hei-mix S, Heidolph, Germany) at a rate of 0.5 L/min., under the effect of photoperiod 16/8 h of day/night cycle, for 18 days divided into 3 stages, each stage consisting of six days, and in each stage, the daily change in the salinity of industrial sea water was monitored by measuring total dissolved solids (TDS), electrical conductivity (EC) and microalga growth rate (GR).

4- Parameters of measuring desalination artificial seawater:

4-1- TDS and EC

TDS and EC were assessed daily during the study period to detect salinity and salts in the artificial seawater. TDS was measured using the evaporation method described in **APHA (1995)**, and EC was recorded using a conductivity YSI model 33 S.C.T. meter.

4-2- Optical Density (OD)

The growth pattern was measured in terms of optical density (OD) at 680 nm wavelength using UV–visible spectrophotometer Optima sp-300, in every day of the experimental period. The optical density was plotted in biomass to make regression between optical density and biomass.

The specific growth rate (μ) of the microalgae is calculated using the equation $\mu = \ln(N_2/N_1)/(t_2 - t_1)$, where μ is the specific growth rate, and N_1 and N_2 are the biomass at time 1 (t_1) and time 2 (t_2), respectively.

5- Biomass collection: -

After centrifugation at 8000 rpm for 30 minutes using a bench-top centrifuge (Centrifuge Universal1200, Germany), the harvested biomass was rinsed with double-distilled water and dried in air temperature to release water **(Castro *et al.*, 2015)**.

6- Oil extraction and biodiesel production: -

The dried algal samples were mixed with 20 ml chloroform and 20 ml methanol to extract algal oil; biodiesel was produced by transesterification of the produced oil; 50 ml of the oil was mixed with 10 ml methanol and 0.5 g sodium hydroxide in a round bottomed flask fitted with a mechanical stirrer and a condenser, and the reaction mixture was heated at 70^{o}C with stirring for 3 hours. The flask contents were then transferred to a

separating funnel, and the methyl ester was produced as the upper layer **(Chisti, 2007; Hossain *et al.*, 2011)**.

7- Biodiesel Characterization

The purified product of oil esterification was tested for fuel properties estimation and evaluation using the American standard specification for biodiesel **(ASTM, 2008) D6751**. The tests included Kinematic viscosity at 40 ºC (D445), Density (D4052), Flash point (D93), Cetane number (D631), Cloud point (D5773), and Pour point (D97).

8- Gas chromatography (GC) analysis of algae biodiesel: -

Gas chromatographic analysis was used to assess the fatty acid content of algal fatty acids methyl esters using an Agilent 6890 gas chromatograph unit fitted with a flame ionization detector (FID) **(Firemichael *et al.*, 2020).**

The study was carried out in the Atomic Energy Commission's central laboratories, Nasr City Branch, Cairo, Arab Republic of Egypt, using an Agilent 6890 plus equipped with an HP-50 capillary column (0.53 mm x30 m, 0.5 lm film) and a FID. Pure Nitrogen (1.5 ml/min) was utilized as the carrier gas at 260oC injector temperature, 275^{o}C detector temperature, split ratio (1:50), sample size 1 L, and the temperature program was 80-240^{o}C at a fixed rate of 5o C/min. The fatty acid esters were detected using chromatography and a prepared reference mixture of fatty acid methyl esters (**Liang *et al.*, 2005**).

9- Statistical Analysis

The statistical analyses were carried out using SPSS software **(SPSS, 2012)**. Data obtained were analyzed statistically to determine the degree of significance using one-way analysis of variance (ANOVA) at probability level $P \leq 0.05$. A post-hoc test was applied according to Duncan's test, when differences are significant.

RESULTS

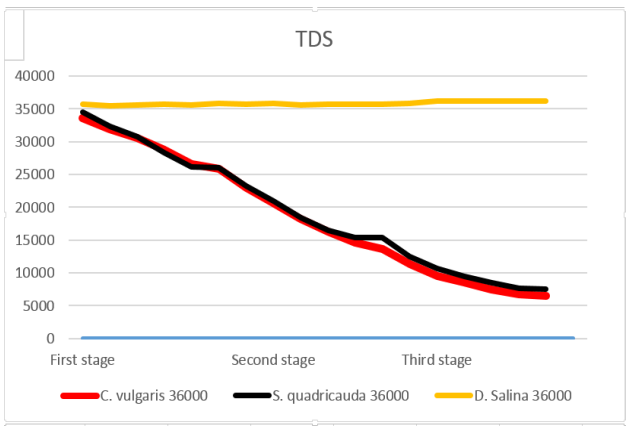
Desalination of artificial sea water without adding any nutrients:

The data in **Table 1 and figure 2** showed that the initial values of TDS and EC for the artificial seawater during the culturing of each *Chlorella vulgaris, Scenedesmus quadricauda* and *Dunaliella salina* without nutrients were **36000** mg/L. and **56160** µs/cm. respectively at the beginning of the experiment. There was a marked gradual decline in TDS and EC values during the culture period and a gradual increase in the growth rate of the microalgae especially *Chlorella vulgaris* and *Scenedesmus quadricauda*. *Chlorella vulgaris* and *Scenedesmus quadricauda* cultures recorded the TDS values of **6500** and **7557** mg/L respectively, at the end of the experiment, while *Dunaliella salina* culture recorded **36200** mg/L. The EC values of **10140, 11788** and **56300** µs/cm were recorded for *Chlorella vulgaris*, *Scenedesmus quadricauda*, and

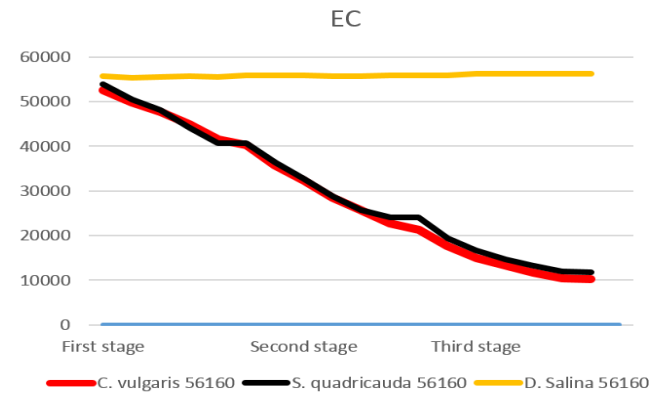
Dunaliella salina respectively. The growth rate values showed a gradual increase during the investigation period for *Chlorella vulgaris* and *Scenedesmus quadricauda*, while showed fixed values for *Dunaliella salina.* The desalination of artificial seawater reached **82% and 79%** during the culturing of *Chlorella vulgaris* and *Scenedesmus quadricauda* respectively, while reached **0%** during the culturing of *Dunaliella salina.*

Table 1: TDS, EC and Growth rate values during the culturing of *C. vulgaris, S. quadricauda* and *D. salina* without nutrients.

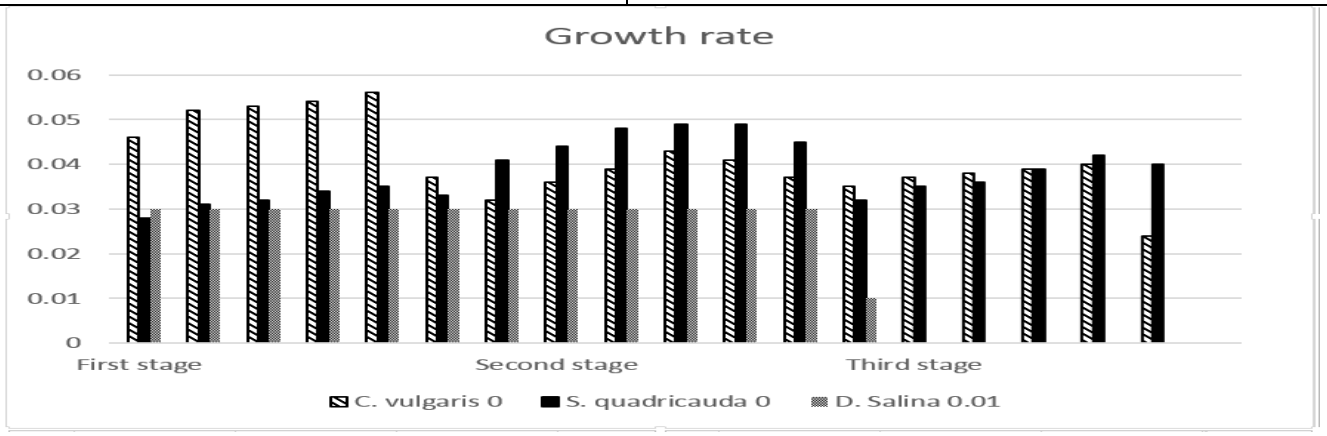
		TDS (mg/L)			**EC (**µs/cm)			**Growth rate Absorbance (nm)**		
		C. vulgaris	*S. quadricauda*	*D. Salina*	*C. vulgaris*	*S. quadricauda*	*D. Salina*	*C. vulgaris*	*S. quadricauda*	*D. Salina*
First stage	**Day 0**	36000	36000	36000	56160	56160	56160	0	0	0.01
	Day 1	33610	34547	35700	52431	53892	55692	0.046	0.028	0.03
	Day 2	31950	32330	35500	49842	50434	55380	0.052	0.031	0.03
	Day 3	30577	30777	35550	47699	48011	55458	0.053	0.032	0.03
	Day 4	28783	28320	35700	44902	44179	55692	0.054	0.034	0.03
	Day 5	26610	26127	35600	41511	40757	55536	0.056	0.035	0.03
	Day 6	25820	26043	35800	40279	40627	55848	0.037	0.033	0.03
Second stage	**Day 1**	22917	23250	35750	35750	36270	55770	0.032	0.041	0.03
	Day 2	20653	20980	35800	32219	32728	55848	0.036	0.044	0.03
	Day 3	18283	18390	35650	28522	28688	55614	0.039	0.048	0.03
	Day 4	16347	16517	35700	25500	25766	55692	0.043	0.049	0.03
	Day 5	14587	15403	35750	22755	24029	55770	0.041	0.049	0.03
	Day 6	13670	15390	35750	21325	24008	55770	0.037	0.045	0.03
Third stage	**Day 1**	11333	12463	35800	17680	19442	55848	0.035	0.032	0.01
	Day 2	9533	10630	36200	14872	16582	56300	0.037	0.035	0
	Day 3	8537	9417	36200	13317	14690	56300	0.038	0.036	0
	Day 4	7450	8490	36200	11622	13244	56300	0.039	0.039	0
	Day 5	6690	7617	36200	10436	11882	56300	0.040	0.042	0
	Day 6	6500	7557	36200	10140	11788	56300	0.024	0.040	0



(a) TDS



(b) EC



(c) Growth rate

Fig. 1. (a) TDS, (b) EC and (c) Growth rate values during the culturing of *C. vulgaris*, *S. quadricauda* and *D. salina* without nutrients.

Desalination of artificial seawater with adding nutrients:

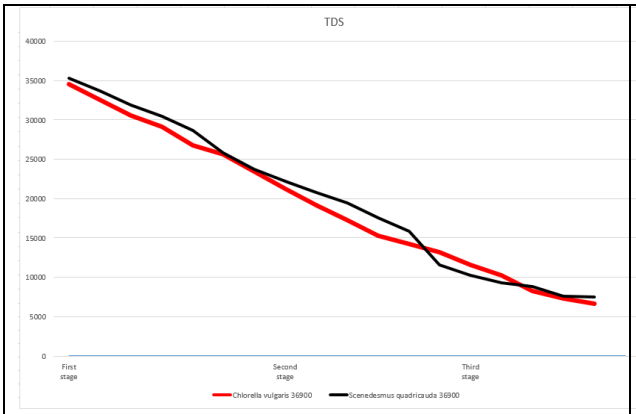
The data in **Table 2 and Figure 3** showed that the initial values of TDS and EC for the artificial seawater with adding nutrients during the culturing of each *Chlorella vulgaris* and *Scenedesmus quadricauda* were **36900** mg/L. and **57560** µs/cm. respectively at the beginning of the experiment.

There was a marked gradual decline in TDS and EC values during the culture period and a gradual increase in the growth rate of the microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda*. *Chlorella vulgaris* and *Scenedesmus quadricauda* cultures recorded the TDS values of **6597** and **7510** mg/L respectively, at the end of the experiment. The EC values of **10291,** and **11716** µs/cm were recorded for *Chlorella*

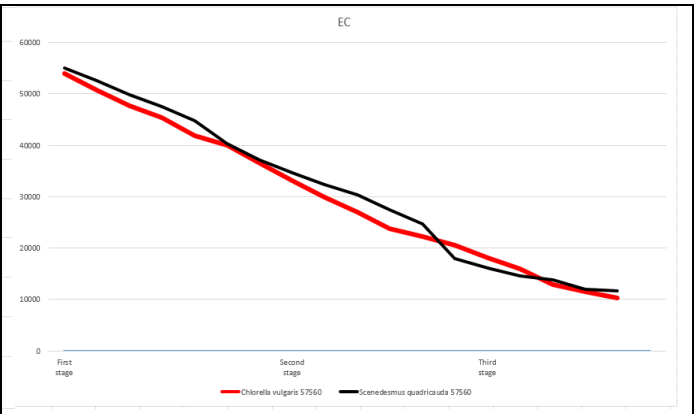
vulgaris and *Scenedesmus quadricauda respectively.* The growth rate values showed a gradual increase during the investigation period for *Chlorella vulgaris* and *Scenedesmus quadricauda.* The desalination of artificial seawater reached **82%** and **80%** during the culturing of *Chlorella vulgaris* and *Scenedesmus quadricauda* respectively.

Table 2: TDS, EC and Growth rate values during the culturing of *C. vulgaris and S. quadricauda* with adding nutrients.

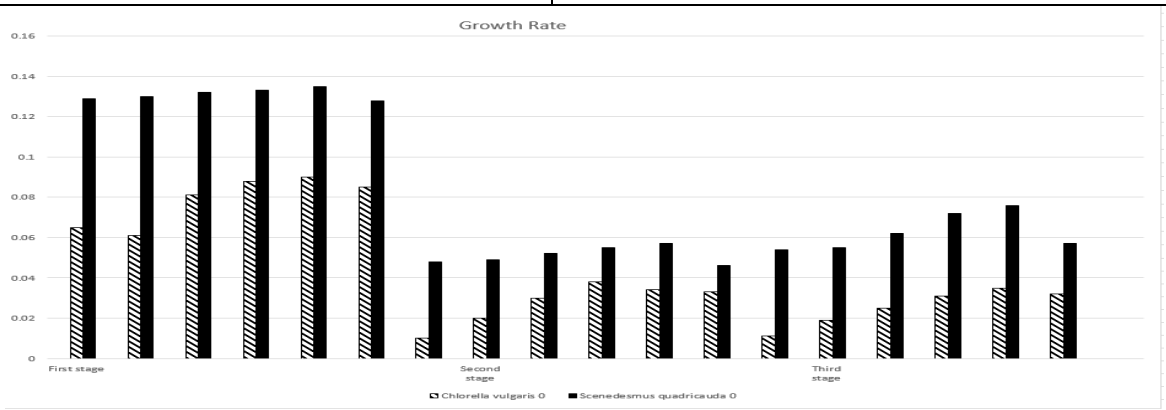
		TDS (mg/L)		**EC (**µs/cm)		**Growth rate Absorbance (nm)**	
		C. vulgaris	*S. quadricauda*	*C. vulgaris*	*S. quadricauda*	*C. vulgaris*	*S. quadricauda*
First stage	**Day 0**	36900	36900	57560	57560	0.00	0.00
	Day 1	34533	35277	53872	55031.6	0.065	0.129
	Day 2	32560	33717	50794	52598	0.061	0.13
	Day 3	30547	31903	47653	49769	0.081	0.132
	Day 4	29090	30470	45380	47533	0.088	0.133
	Day 5	26777	28680	41772	44741	0.09	0.135
	Day 6	25597	25837	39931	40305	0.085	0.128
Second stage	**Day 1**	23403	23773	36509	37086	0.01	0.048
	Day 2	21220	22200	33103	34632	0.02	0.049
	Day 3	19150	20737	29874	32349	0.03	0.052
	Day 4	17250	19480	26910	30389	0.038	0.055
	Day 5	15240	17580	23774	27425	0.034	0.057
	Day 6	14253	15817	22235	24674	0.033	0.046
Third stage	**Day 1**	13173	11533	20550	17992	0.011	0.054
	Day 2	11593	10280	18086	16037	0.019	0.055
	Day 3	10247	9296	15985	14502	0.025	0.062
	Day 4	8217	8840	12818	13790	0.031	0.072
	Day 5	7323	7633	11424	11908	0.035	0.076
	Day 6	6597	7510	10291	11716	0.032	0.057



(a) TDS



(b) EC



(c) Growth rate

Fig. 3. (a) TDS, (b) EC and (c) Growth rate values during the culturing of *C. vulgaris* and *S. quadricauda* with adding nutrients.

Algal biodiesel Characterization:

Table 3 data demonstrate that the physical and chemical characteristics of algal biodiesel fractions, such as viscosity, density, flash point, cetane number, Cloud point, and pour point values, were comparable to petro- diesel **ASTM (2021)** standards. The kinematic viscosity of 4.82 mm2/s, density of 0.882 kg/m3, flashpoint of 189°C, cetane number of 47, cloud point of 0 °C, and pour point of -10 °C of the biodiesel generated from *Chlorella vulgaris* oil were all determined. The kinematic viscosity of 5.0 mm2/s, density of 0.88 kg/m3, flashpoint of 140°C, cetane number of 60, cloud point of 0 °C, and pour point of -11 °C of the biodiesel derived from *Scenedesmus quadricauda* oil were all measured. The characteristics of *Chlorella vulgaris* and *Scenedesmus quadricauda* biodiesel were compared to ASTM standards, and high-quality biodiesel was identified.

Table 3: The physico-chemical properties of algal biodiesel

Properties/ method	**Algal biodiesel**		**Limit (ASTM) Std.**
	S. quadricauda	***C. vulgaris***	
Kinematic viscosity at 40 ºC (mm^2/s) **(D445)**	5.0	4.82	1.9 – 6.2
Density kg/m^3 **(D4052)**	0.88	0.882	0.86 – 0.89
Flash point (ºC) **(D93)**	140	189	130 max
Cetane number **(D631)**	60	47	47
Cloud point (ºC) **(D5773)**	0	0	-3 to 12
Pour point (ºC) **(D97)**	-11	-10	-15 to 10

Gas chromatography mass spectrometry (GC-MS) analysis:

The biodiesel derived from *Chlorella vulgaris* and *Scenedesmus quadricauda* were analyzed to determine the percent fatty acids composition. Identified peaks of the FID and their relative percentages are summarized in **Table 4** and **Figures 4 & 5.** The results showed that *Chlorella vulgaris* and *Scenedesmus quadricauda* lipids consisted of fatty acids with 10 to 24 carbon chain lengths, with lauric, stearic, and lignoceric acid having the highest concentrations.

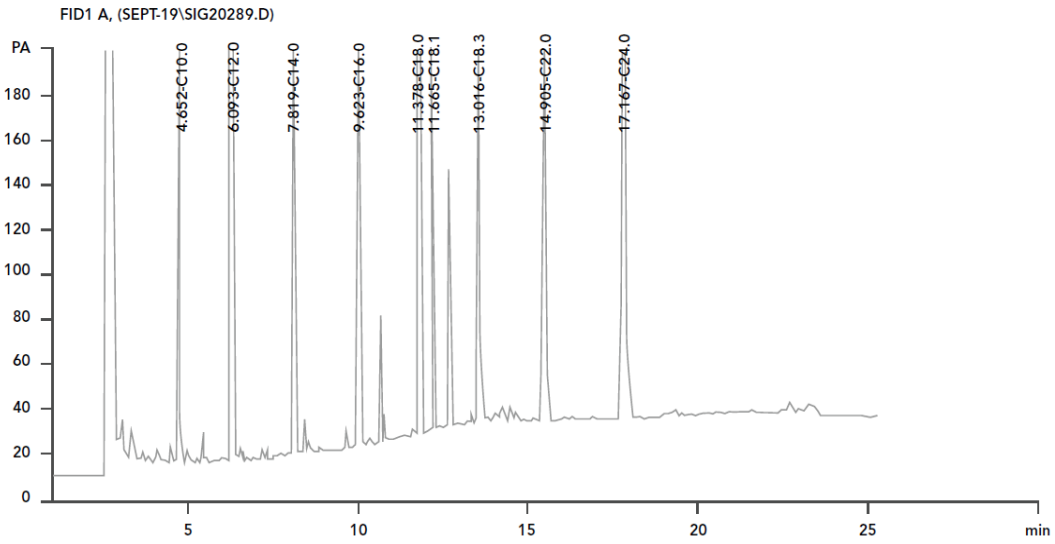


Fig. 4. GC-MS Spectra of *C. vulgaris* biodiesel

Table 4: Fatty acids composition in *C. vulgaris* and *S. quadricauda* biodiesel

Peaks no.	*C. vulgaris*		*S. quadricauda*		Component name	Molecular formula	Molecular weight (g/mol)
	RT (min)	Area %	RT (min)	Area %			
1	4.576	6.04	4.652	6.2	Capric acid	$C_{10}H_{20}O_2$	172.268
2	6.093	39.19	6.182	41.6	Lauric acid	$C_{12}H_{24}O_2$	200.322
3	7.819	5.48	7.933	5.78	Myristic acid	$C_{14}H_{28}O_2$	228.37
4	9.623	5.32	9.746	5.29	Palmitic acid	$C_{16}H_{32}O_2$	256.43
5	11.378	13.43	11.493	13.76	Stearic acid	$C_{18}H_{36}O_2$	284.48
6	11.665	4.5	11.787	5.44	Oleic acid	$C_{18}H_{34}O_2$	282.47
7	12.189	2.19	12.326	0.00	Linoleic acid	$C_{18}H_{32}O_2$	280.4472
8	13.016	6.22	13.163	6.19	Others		
9	14.905	6.83	15.096	6.47	Behenic acid	$C_{22}H_{44}O_2$	340.592
10	17.167	9.79	17.387	9.16	Lignoceric acid	$C_{24}H_{48}O_2$	368.63

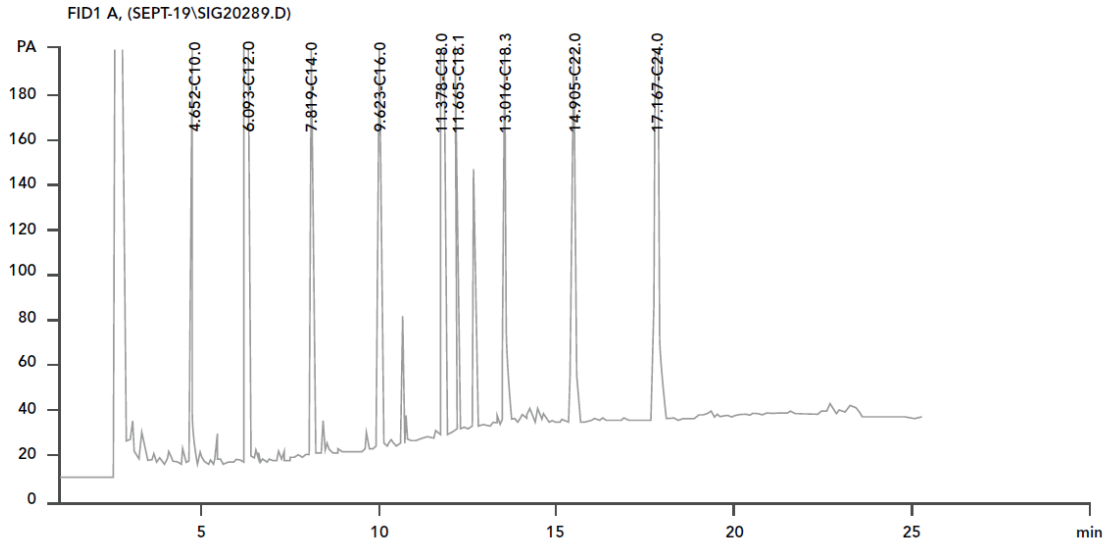


Fig. 5. GC-MS Spectra of *S. quadricauda* biodiesel

6. Statistical Analysis:

This study aimed to compare the impact of some microalgal species (*Chlorella vulgaris and Scenedesmus quadricauda*) in the desalination of artificial seawater. Hence, the multiple comparison (Two-way ANOVA) was used to show whether there was any significant difference between the types of microalgae cultured in artificial seawater either alone or supplemented with nutrients using the rates of EC, TDS and growth rate values of these microalgae applied as parameters for measuring the salinity percentage.

According to the data obtained in **Tables 5** and **6,** there is no significant difference between the two microalgal species in different two nutritional media (either sea water only or with adding nutrients) regarding TDS and EC in the cultivated three stages. Also, on performing Pearson Correlation, it was observed that a significant positive correlation between TDS and conductivity / TDS and growth curve ($P<0.005$).

Table 5: Mean ± Standard deviation (SD) of TDS values in artificial seawater during the three cultivated stages.

TDS			
Algal cultures	**Stage I**	**Stage II**	**Stage III**
C. vulgaris in artificial seawater	29550±2870	17740±3360	8340±1750
C. vulgaris in artificial seawater + nutrients	29850±3190	18410 ±3310	9520±2420
S. quadricauda in artificial seawater	29690±3240	18320±3020	9360±1800
S. quadricauda in artificial seawater + nutrients	30980±3220	19930 ±2760	9840±2150
P-Value	NS	NS	NS

*Data was expressed as Mean ±Standard deviation, NS: Non-Significant

Table 6: Mean± Standard deviation (SD) of EC values in artificial seawater during the three cultivated stages.

EC			
Algal cultures	**Stage I**	**Stage II**	**Stage III**
C. vulgaris in artificial seawater	40.6± 0.51	20.9± 0.47	10.46± 0.28
C. vulgaris in artificial seawater + nutrients	40.8±0.50	30.1±0.43	10.53± 0.28
S. quadricauda in artificial seawater	40.6±0.45	20.8±0.52	10.30± 0.28
S. quadricauda in artificial seawater + nutrients	40.7±0.50	20.9±0.52	10.49± 0.78
P-Value	NS	NS	NS

*Data was expressed as Mean ±Standard deviation, NS: Non-Significant

Table 7: Correlation between the EC, TDS, and Growth rates of the Microalgae.

Correlations			
		EC	**Growth rate**
TDS	**Pearson Correlation**	1.000**	0.144*
	P-Value	0.000	0.038

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The experiments conducted in this study were to investigate the feasibility of using some species of microalgae for artificial seawater desalination, whether nutrients were added to it or not, as well as the selection of algal species that can survive in artificial seawater. The rate of microalgal growth was recorded using turbidity changes over a period of 18 days. **Tables 1 and 2** demonstrate the growth rate of grown microalgae as well as the daily rate of change in EC and TDS values as indicators of salinity percentage.

The ability of *Chlorella vulgaris* and *Scenedesmus quadricauda* to desalinate artificial seawater:

C. vulgaris and *S. quadricauda* showed growth successfully in artificial seawater, these results agree with those of **Louye *et al.* (2019)** who used the freshwater algae *C. vulgaris* in desalination of different concentrations of saltwater starting from artificial seawater (38 ppt) going through brackish water (20 ppt) and ending with low salt water (5 ppt). The author reported that *C. vulgaris* succeeded in removing salts from all specimens and salt removal was greater at higher salinities which agrees also with the studies of **El Nadi *et al.* (2014)** and **Gan *et al.* (2016)** (both of which have used *Scenedesmus obliqus* for desalination). Several studies supported this finding as **Kumar *et al.* (2015) and Wei *et al.* (2020)** they observed that the salt removal mechanism in microalgae is similar to heavy metal removal, which involves both bio adsorption and bioaccumulation or bio absorption processes.

Kumar *et al.* (2021) noted that the bio desalination mechanism of algae is explained by the rapid bonding of ions or molecules on the wet or dried algal surface. It is a metabolically independent and reversible process that occurs on the surface due to the surface characteristics of the adherends with no energy expenditure.

The present study agrees with **Nie *et al.* (2020)** who mentioned that microalgae absorb the salts, using them in their metabolism. They are the most active microalgae because they can survive in a wide range of salinity in their habitat. *Chlorella vulgaris* and *Scenedesmus sp*. have been widely used for wastewater treatment due to their natural colonization of ponds, fast growth rates, and high nutrient uptake capabilities.

These results approved with **Figler *et al.* (2019)** who studied the salinity tolerance, salinity, and nutrient reducing ability of nine common freshwater microalgal species from the genera *Chlorella, Chlorococcum, Desmodesmus, Scenedesmus,* and *Monoraphidium,* and they reported that, the studied green microalgal species are halotolerant ones, which can proliferate in environments with high salt concentrations.

By following the TDS and EC values shown in **Table 1 and Figure 1**, through which it is possible to infer the percentage of salinity, it was found that *C. vulgaris* and *S. quadricauda* were significant for biodesalination of artificial seawater. TDS and EC removal percent reached **82% and 79%** for *C. vulgaris* and *S. quadricauda* respectively during culturing without adding nutrient, which reduced salinity percentage by absorbing salts into the biomass. The present study results agreed with those of **Moayedi *et al.* (2019)** who noted that *Chlorella vulgaris* and *Scenedesmus sp.* can desalinate seawater without using food elements, agreed also with **Louye *et al.* (2019)** who reported that *C. vulgaris* could reduce TDS ranging from 3 ppt to 1 ppt every hour for all specimens.

The results in **Table 2 and Figure 2** showed that TDS and EC removal percent reached **82% and 80%** for *C. vulgaris* and *S. quadricauda* respectively during culturing with adding nutrients, these results in harmony with that of **Barahoei *et al.* (2021)** who use *C. vulgaris* for brackish water desalination. The results showed that adding nutrients improved the growth of microalgae and salt removal efficiency even more.

There was highly significant difference in the growth rate of *C. vulgaris* and *S. quadricauda* during the culturing period, these results agree with those of **Demetriou *et al*. (2007) and Zhang *et al*. (2015)** who explained that TDS are taken up by algal cells and absorbed as minerals and nutrients to support their physiological functions and metabolism, decreasing TDS in the water.

The ability of *Dunaliella salina* to desalinate artificial seawater:

The results in **Table 1 and Figure 1** showed that there was no systematic increase or shortage of TDS and EC values for artificial seawater treated with *Dunaliella salina* without nutrients. Also, there was no significant difference in the growth rate during the culturing period, so *D. salina* was not significant for biodesalination of artificial seawater. The present results can be explained by the fact that the seawater used to grow *Dunaliella salina* was not have salinity enough, and that *D. salina* needs more salts to grow. This explains what the authors **Kageyama *et al.* (2017)** who reported that *D. salina* also can grow in high-salt concentration water bodies and uptakes carotenoids at higher sodium chloride concentrations, also it is the richest source of β-carotene, with an optimum β-carotene output of approximately 24 percent NaCl, these results are not in harmony with those of **Moayedi *et al.* (2019)** who noted that reduced salt absorption in algae is caused by the usage of salt in the metabolism, development, and proliferation of algae. Their

study's absorption procedure revealed that the catch of *D. salina* has a good ability to remove salt and can be used as a suitable proposal for salt removal from saline water.

Algal biodiesel Characterization:

Table 3 results demonstrated the physical and chemical properties of algal biodiesel corresponded to ASTM standards. Because of inadequate atomization, more viscous gasoline is often inappropriate for use in diesel engines. *Chlorella vulgaris* and *Scenedesmus quadricauda* biodiesel has a higher flashpoint than ASTM requirements. This makes biodiesel fuel safer to handle and store. The cetane number of the biodiesel obtained in this study is higher than ASTM standards values. This reflects the higher quality of the biodiesel obtained here, these results agree with those of **Murthy and Kumar (2021).**

The results in **Table 4** and **Figs. 3 & 4** showed that *C. vulgaris* and *S. quadricauda* lipids consisted of fatty acids between 10 and 24 carbon chain length and the higher concentration fatty acids were lauric, stearic and lignoceric acid. According to **Bartley *et al.* (2013)** when salt stress is applied to saline microalgae, the fatty acids in the membrane begin to denature. Microalgae raise lipid synthesis in response to unfavorable conditions. Many studies have been conducted to investigate the possibility of lipid formation to protect salt pressure. Furthermore, some researches show that increasing salt has an effect on the composition of intracellular lipids. Saturated and monounsaturated fatty acid concentrations (palmitic acids) have increased, however polyunsaturated fatty acid concentrations (linoleic acid) have decreased due to excessive salt stress (**Takagi and Yoshida, 2006).** This agrees also with those of **Sharma *et al*. (2012)** who found that most halophytic microalgae, including *C. vulgaris*, *Dunaliella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp., showed promising lipid accumulation under salt stress. Salt stress is an excellent method for increasing algal lipid content (**Gautam and Kapoor, 2022).**

The presence of these three saturated fatty acids in algal biodiesel can improve the cold-flow properties of the fuel, as well as its oxidative stability. This makes algal biodiesel a promising alternative to petroleum-based diesel fuel. In addition to these three fatty acids, algal biodiesel also contains other saturated fatty acids, such as myristic acid and palmitoleic acid. It also contains monounsaturated fatty acids like oleic acid as well as polyunsaturated fatty acids like linoleic acid. The fatty acid composition of algal biodiesel varies depending on the algal species utilized, the growth factors, and the extraction procedure **(Yu, 2013).**

Economic feasibility:

The initial economic analysis for 1 L of the biodiesel produced from *C. vulgaris* and *S. quadricauda* as well as the pricing comparison in **Table 8** demonstrated the affordability and competitiveness of our biodiesel product. These observations in agreement with that of **Tyagi and Bhardawaj (2016)** who reported that algae are an economical choice for biodiesel production, because of its availability and low cost.

In addition, the advantage of using seawater as a potential source of cultivation media instead of fresh water increase the economic value of biodesalinaiotn of seawater and production of biodiesel which in agreement with **Sahle-Demessie *et al.*, 2019** who noted that the economical supply of drinking and agricultural water will be made available through the effective application of biodesalination technology, which will also result in the production of valuable biofuel as an energy byproduct for desalination.

Table (8):-Price comparison

Initial cost of the biodiesel obtained from		**Biodiesel prices in different countries**		**Bench mark of production cost from egypt**		**Petro diesel**
S. quadricauda	*C. vulgaris*	USA	India	from	To	Egypt
12.2	**10.7**	**25.1**	**14.72**	**10**	**12**	**8.25**

CONCLUSION

In general, the results of this study indicate that the use of *Chlorella vulgaris* and *Scenedesmus quadricauda* will be effective in reducing salinity while the use of *Dunaliella Salina* will not. Bio-desalination has several advantages over traditional desalination methods. It is more sustainable, as it does not require the use of energy-intensive processes such as distillation. It is also more scalable, as it can be used to produce large amounts of water and biodiesel. The combination of bio-desalination and biodiesel production could provide a sustainable solution to the world's water and energy challenges. By using algae to both desalinate seawater and produce biodiesel, we can create a closed-loop system that is both environmentally friendly and economically viable.

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