Water desalination of *Chlorella vulgaris*

Hau T. T. Nguyen[1], Giang T. K. Ho[1], Huong T. Nguyen[1], Sang M. Nguyen[2], Suong T. Nguyen[1], Dao N. Duy[1], & Viet B. Nguyen[1*]

[1]Department of Chemical Engineering, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Faculty of Fishery, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 23, 2022
Revised: October 01, 2022
Accepted: October 15, 2022

Keywords

Bold basal medium
C. vulgaris
Sea salt medium
Water desalination

***Corresponding author**

Nguyen Bao Viet
Email: nbviet@hcmuaf.edu.vn

ABSTRACT

Saltwater intrusion is a common phenomenon in Southern Vietnam, but salinization is becoming more serious due to the rising sea level related to climate change. Among potential methods for water desalination, the application of halophytic microalgae is gaining high interest. This study investigated the effect of *Chlorella vulgaris* (*C. vulgaris*) microalgae on reducing water salinity at different media (Bold Basal Medium and Sea Salt Medium) and in different salt concentrations (1 - 30 g/L). The results indicated that *C. vulgaris* microalgae had good growth in all mediums used and contributed to lowering the salt content from 20% to 40% after 15 days of cultivation.

Cited as: Nguyen, H. T. T., Ho, G. T. K., Nguyen, H. T., Nguyen, S. M., Nguyen, S. T., Dao, D. N., & Nguyen, V. B. (2023). Water desalination of *Chlorella vulgaris*. *The Journal of Agriculture and Development* 22(3), 39-46.

1. Introduction

Saltwater intrusion is a popular phenomenon in the Mekong River Delta, one of the most productive agricultural areas in the world. However, when the sea level rises relating to climate change, the salinization of this area is becoming more serious. During the last 5 years, saltwater has reached more than 50 km from the coast of Vietnam and threatened the agriculture of provinces along the Mekong River Delta. In general, there are not many options for desalinating water in this area. The popular method for farmers is building channels and reservoirs but this solution is not sustainable because salt will be accumulated on the land, resulting in another contamination phenomenon. Another solution is applying reverse osmosis filtration systems which consume high energy and require a high cost for membrane replacement. In considering sustainable water treatment solutions, the application of microalgae is always a potential solution (Abdel-Raouf et al., 2012). Earlier studies have reported that some microalgae not only are useful in removing a wide range of substances pollution from industrial and agricultural wastewater but also contribute to lowering salt content in water depending on its salt tolerance (Delrue et al., 2016; Gan et al., 2016).

Salt tolerance is the ability of plants to grow and complete their life cycle on substrates containing high concentrations of soluble salts (Wang et al., 2013). Survival rates were used for uncultivated and long-lived species to determine

salt tolerance (Cheeseman, 1988). The degree of growth reduction and tolerance to stress conditions varied among different plant species. In general, plants can be classified into glycophytes and halophytes. Halophytes, also known as salt-loving plants, are plants that are tolerant to the effects of salinity and possess salt-responsive genes and proteins to counteract the adverse effects of salinity (Askari et al., 2006). Meanwhile, glycophytes, also known as salinity-sensitive plants, cannot tolerate high salinity. The salinity tolerance degree of halophytes also varies between species (Yensen et al., 2016). Less tolerant halophytes reduce their growth in saline environments in response to salinity while showing better growth in non-saline soils (Zhu, 2001). Some recent studies have shown that some popular halophytic microalgae strains such as *Chlorella, Chlorococcum, Desmodesmus, Scenedesmus* and *Monoraphidium* could survive and grow in water containing high salt concentrations and remove approximately 30% chloride in water (Figler et al., 2019; Sahle-Demessie et al., 2019; Barahoei et al., 2021). However, the salt tolerance as well as water desalination effect of microalgae not only depends on the strain but also on the cultivation medium. For example, the coexistence of salts, heavy metals or non-metallic compounds often leads to complex interactions with each other and with living organisms, affecting their salt tolerance (Kumari et al., 2015). In comparison between the simpler and the complex media having a same salinity, the growth inhibition of green microalgae is often more significant in the latter (Arora et al., 2019; Sahle-Demessie et al., 2019). Unfortunately, the understanding of the effect of cultivation medium on the water desalination of microalgae is scarce and needs to be elaborated further.

In this study, the halophytic algae *Chlorella vulgaris* (*C. vulgaris*) was cultivated in a traditional medium (Bold Basal Medium) and a simulated Sea Salt medium at different sodium chloride contents using a Photobioreactor system in carefully controlled conditions (light density and air flow rate). Subsequently, the growth rate of microalgae and their salt absorption rate were continuously measured during 15 cultivation days to investigate the salt tolerance as well as the water desalination capacity of *C. vulgaris* in these media.

2. Materials and Methods

2.1. Materials

C. vulgaris was provided by the Laboratory of Microalgae (Nong Lam University - Ho Chi Minh City, Vietnam). Chemical substances and ocean salt (hw-Marinemix professional) were purchased from Xilong Scientific (China) and Wiegandt (Germany), respectively. The chemical composition of culture media was summarized in Table 1.

Table 1. Cultivation media composition

Bold Basal Medium (BBM)	
Chemical substances	Composition content
$NaNO_3$	25000 mg/L
$MgSO_4.7H_2O$	7500 mg/L
K_2HPO_4	7500 mg/L
KH_2PO_4	17500 mg/L
$CaCl_2.2H_2O$	2500 mg/L
H_3BO_3	114 mg/L
$EDTANa_2$	500 mg/L
KOH	310 mg/L
$FeSO_4.7H_2O$	498 mg/L
H_2SO_4	98 mg/L
Sea Salt Medium (SSM) at 30 mg/L	
Chemical substances	Composition content
Ca^{2+}	440 mg/L
Mg^{2+}	1320 mg/L
Na^{+}	10000 mg/L
Cl^{-}	20000 mg/L
$NaNO_3$	25000 mg/L
K_2HPO_4	7500 mg/L
KH_2PO_4	17500 mg/L

Source: Andersen (2004).

2.2. Sample preparation & experimental design

Firstly, the mother culture was grown in the Bold Basal Medium at 25°C for 15 days to achieve a microalgae density of 60×10^6 cells/mL. Then, the culture was transferred to photobioreactors (Figure 1) to investigate water desalination. The initial density of *C. vulgaris* for all the bioreactors was 10^6 cell/mL. For BBM, sodium chloride was added to the solution to reach the salt concentrations in the range at 1,000 to 15,000 ppm. For SSM, sea salt was diluted in water to adjust the concentrations in a range of 1,000 to 30,000 ppm. The photobioreactors were operated with a

light intensity of 4,000 - 8,000 Lux and an aeration rate of 4 L/min.

Figure 1. The photobioreactor system.

2.3. Microalgae growth measurement

The density of microalgae was estimated based on the combination of light microscopy and optical density (Barahoei et al., 2021). Firstly, microalgae were filled into a counting chamber (Marienfield, Germany) and observed under a light microscope (DM2500, Leica, Switzerland) equipped with a digital camera (DFC450C, Leica, Switzerland). Besides, the optical density (OD) of *C. vulgaris* at various concentrations was determined at 680 nm using a spectrophotometer (UV1100, MRC Lab, Israel) to build the growth curves for microalgae.

2.4. Salinity reduction measurement

Microalgae were collected on days 3, 6, 9, 12 & 15. For pre-treatment, samples were precipitated by using a benchtop centrifuge (Rotofix 32A, Hettich, Germany) at 4,000 rpm. Then the water salinity reduction was estimated using a conductivity meter (HI98318, Hanna, Rumani) and a salinity refractometer (SLI-10, China).

2.5. Statistical analysis

All measurements were triplicate performed. Analysis of variance (ANOVA) and Least Significant Difference (LSD) Test with 95% confidence was applied using the Statgraphics software (version 14) to investigate the effect of cultivation time and salt concentrations on the salt tolerance and the water desalination capacity of microalgae.

3. Results and Discussion

3.1. *Chlorella vulgaris* growth in different media

The growth of *C. vulgaris* in BBM and SSM at different salt concentrations after 15 cultivation days is depicted in Figure 2. In general, *C. vulgaris* showed a good growth at salt concentrations of 1 - 15 g/L. When the salt concentrations were greater than 20 g/L, the growth of *C. vulgaris* seemed to be inhibited and the medium color changed from green to yellow. A similar result was reported in a recent study of the salt tolerance of *C. vulgaris* (Sahle-Demessie et al., 2019). The authors suggested that at the high salt concentration (>10 g/L), nutrient deficiencies could occur and therefore microalgae must spend more time for synthesizing new chlorophyll molecules as well as binding new proteins.

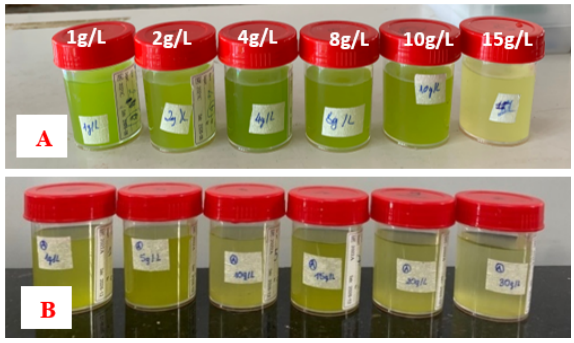

Figure 2. Microalgae at different salt concentrations. Bold basal medium (A), sea salt medium (B).

To estimate their growth curves, microalgae images were observed under a microscope (20 x) and the cell density was determined by combining an improved Neubauer counting chamber with ImageJ software (Figure 3). Meanwhile, the optical density of microalgae was also measured by a spectrophotometer at 680 nm (Barahoei et al., 2021). Obtained results revealed that *C. vulgaris* growth during the cultivation could be quickly estimated with a high reliability ($R^2 > 0.99$; Figure 4) using the optical density value based on the following equations:

$$\text{BBM: } y = 4.10^{-8}x + 0.0284$$
$$\text{SSM: } y = 3.10^{-8}x + 0.0652$$

with y being the absorbance value measured at 680 nM and x being the microalgae cell density.

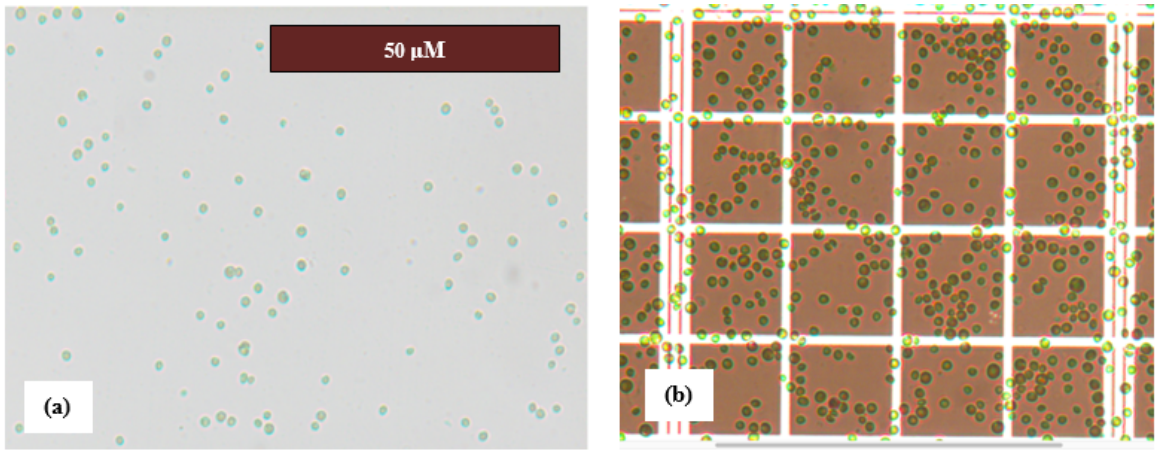

Figure 3. Microalgae images under the microscope with scale bar 50 µM (a) and the improved Neubauer counting (b) chamber.

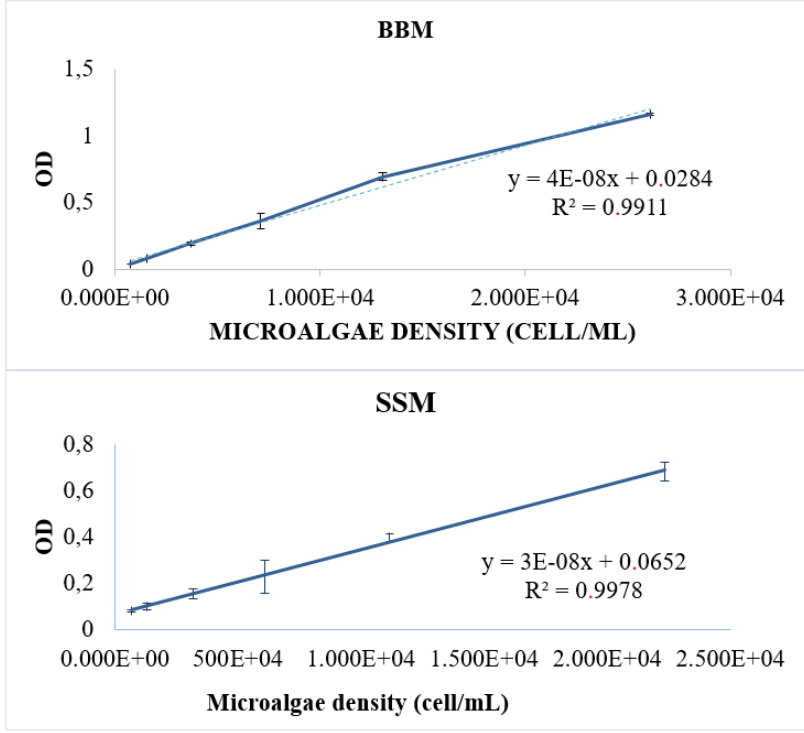

Figure 4. Standard curve for determination of algae density in bold basal medium (BBM) & sea salt medium (SSM) using spectrophotometer method.

The development of *C. vulgaris* cell density during 15 days of cultivation in BBM and SSM with different salt concentration was summarized in Figure 5. Similar to previous studies (Sahle-Demessie et al., 2019; Barahoei et al., 2021), our results revealed that microalgae showed good growth in BBM at most salt concentrations and reached 40 - 60 million cells/mL after 6 days of cultivation. Although the growth rate of microalgae decreased in the following stage, the cells were still alive in media having less than 15 g NaCl/L. As described in a previous section, the inhibition of *C. vulgaris* occurred when the salt content in BBM was over 15 g/L and a significant drop was recognized in the growth curve of microalgae.

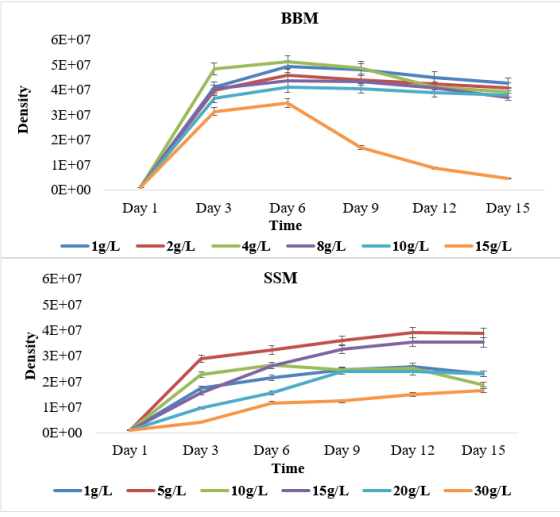

Figure 5. The growth curve of *C. vulgaris* in different media (bold basal medium-BBM & sea salt medium-SSM) over 15 days of cultivation.

For the SSM at most salt concentrations, *C. vulgaris* needed more time for growing and achieved the maximum cell density after 9 - 12 cultivation days. In comparison with BBM, the cell densities (number of cell/mL) of *C. vulgaris* cultivated in SSM are lower at the same salt concentration. This phenomenon is mainly related to the responses and adaptations of microalgae to environmental conditions (Hiremath & Mathad, 2010). According to many studies (Hasegawa, 2000; Hoque, 2007), the adaptation of eukaryotic organisms such as microalgae to adverse environmental conditions often results in changes in metabolism, synthesis or accumulation of some organic substances or osmosis behavior. When comparing BBM and SSM compositions, the latter had a lower content of nutritional compounds, and this could contribute to alleviating the growth rate of microalgae. On the other hand, BBM had higher ratios of Na^+/Ca^{2+} and Na^+/K^+ in comparison to SSM which could negatively affect the stress tolerance of plants with the change in environmental conditions. Therefore, the survival of *C. vulgaris* in SSM was still recognized at the very high salt concentration (20 - 30 g/L) while a significant decrease of microalgae was observed in BBM containing over 15 g NaCl /L.

3.2. Water desalination capacity of *C. vulgaris*

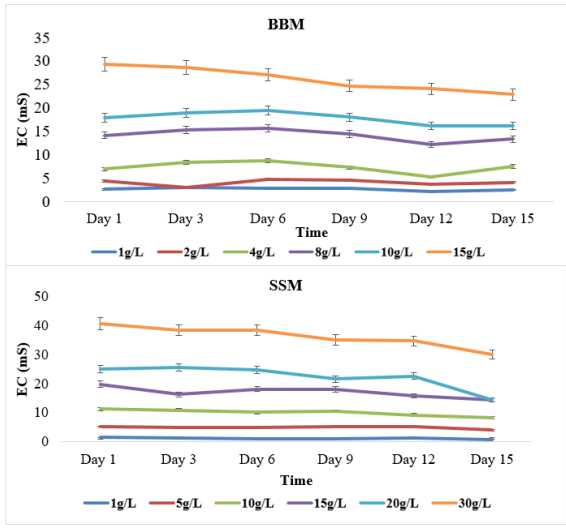

Figure 6. Electric conductivity (EC) changes in bold basal medium (BBM) & sea salt medium (SSM) over 15 days.

To estimate the water desalination, the electric conductivity and the salinity are the most popular indicators. These parameters were measured during the cultivation and the results were summarized in Figure 6 & 7. As expected, obtained results demonstrated that the salt content in water tended to decrease with the growth of microalgae. For instance, the salinity of BBM was reduced by 5% to 10% while the conductivity was reduced by about 2% to 20%. The 2 - way ANOVA analysis (Tables 2 & 3) also confirmed that salt concentration and water treatment duration were factors that significantly influenced the ability to reduce water salinity when using microalgae *C. vulgaris* ($P < 0.05$). Based on LSD

Table 2. Analysis of variance for electric conductivity

Bold basal medium					
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Main effects					
A:Concentration	2464.89	5	492.979	277.67	0.0000
B:Time	38.8759	5	7.77518	4.38	0.0053
Residual	44.385	25	1.7754		
Total (corrected)	2548.15	35			
Sea salt medium					
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Main effects					
A:Concentration	4967.18	5	993.435	294.44	0.0000
B:Time	102.136	5	20.4272	6.05	0.0008
Residual	84.3499	25	3.374		
Total (corrected)	5153.66	35			

Table 3. Analysis of variance for salinity

Bold basal medium					
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Main effects					
A:Concentration	699.591	5	139.918	474.85	0.0000
B:Time	2.6721	5	0.53442	1.81	0.1466
Residual	7.3665	25	0.29466		
Total (corrected)	709.629	35			
Sea salt medium					
Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Main effects					
A:Concentration	2075.97	5	415.194	179.34	0.0000
B:Time	62.5487	5	12.5097	5.40	0.0017
Residual	57.8776	25	2.3151		
Total (corrected)	2196.4	35			

comparison, the desalination effect was more considerable in the medium containing a higher initial salt concentration ($P < 0.05$).

Similarly, the salinity of SSM decreased from 22% to 44% while the conductivity was reduced by about 23% to 43%. This result is in line with the finding in a recent study on the salt tolerance of *C. vulgaris* (Barahoei et al., 2021). The author reported that *C. vulgaris* microalgae could reduce about 45% of salt content in the sea salt culture medium with an initial concentration of 1 g/L - 5 g/L. The mechanism for salt adsorption in marine microalgae is not completely clear. It can be assumed that the cell wall of microalgae is a double enveloped membrane composed of phospholipids and plays an important role in exchanging metabolites and ions (Safi et al., 2013). When the salt concentration in aqueous solution is too high, anion Cl- tends to accumulate on the surface of the microalgae membrane and then attracts Ca^{2+}, Na^{+} & K^{+}. This phenomenon could contribute to accelerating the transportation of these ions through the cell membrane of microalgae resulting in a salinity reduction (Amezaga, 2014). Therefore, with a higher initial salt concentration in the cultivation medium, the salinity reduction should be more considerable. According to Figure 6 & 7, this trend was recognized for both media during the cultivation.

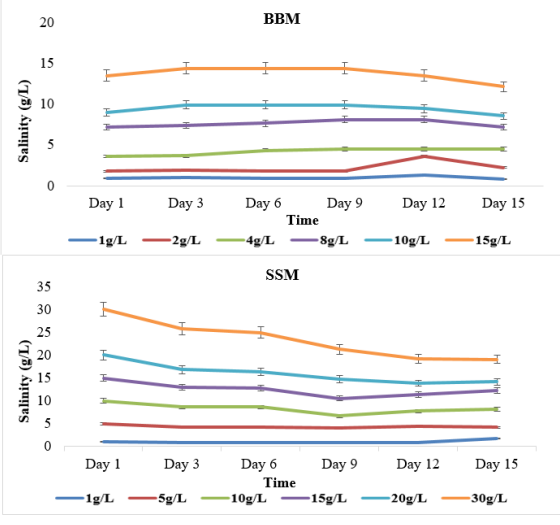

Figure 7. Salinity changes in bold basal medium (BBM) & sea salt medium (SSM) over 15 days

4. Conclusions

This study investigated the salt tolerance and water desalination capacity of the microalgae *C. vulgaris* in the traditional medium and the stimulated ocean medium. As expected, *C. vulgaris* showed good growth in both media and achieved 40 - 60 million cells/mL after 15 days of cultivation. For the salt tolerance, obtained results revealed that halophytic microalgae such as *C. vulgaris* could survive and develop in the sea salt medium with a salt content of up to 30 g/L. Based on electrical conductivity and salinity measurement, the growth of *C. vulgaris* contributed to the reduction of NaCl content in a range of 2 - 22% (for BBM) and 22 - 42% (for SSM). However, the study was conducted at the laboratory scale using small photobioreactors. Further studies, therefore, should focus on investigating and optimizing the water desalination capacity of *C. vulgaris* at the pilot scale using stirred tank reactors or tubular flow reactors.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

This study was financially supported by Nong Lam University (Project No. CS-SV21-CNHHTP-01). The authors also acknowledge to Assoc. Prof. Dr. Vinh Truong (NLU) for his support of microalgae strain.

References

Abdel-Raouf, N., Al-Homaidan, A. A., & Ibraheem, I. B. M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences* 19(3), 257-275. https://doi.org/10.1016/j.sjbs.2012.04.005.

Amezaga, J. M., Amtmann, A., Biggs, C. A., Bond, T., Gandy, C. J., Honsbein, A., Karunakaran, E., Lawton, L., Madsen, M. A., Minas, K., & Templeton, M. R. (2014). Biodesalination: A case study for applications of photosynthetic bacteria in water treatment. *Plant Physiology* 164(4), 1661-1676. https://doi.org/10.1104/pp.113.233973.

Andersen, R. A. (2004). *Algal culturing techniques* (1st ed.). New York, USA: Elsevier Academic Press.

Arora, N., Laurens, L. M. L., Sweeney, N., Pruthi, V., Poluri, K. M., & Pienkos, P. T. (2019). Elucidating the unique physiological responses of halotolerant *Scenedesmus* sp. cultivated in sea water

for biofuel production. *Algal Research* 37, 260-268. https://doi.org/10.1016/j.algal.2018.12.003.

Askari, H., Edqvist, J., Hajheidari, M., Kafi, M., & Salekdeh, G. H. (2006). Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves. *Proteomics* 6(8), 2542-2554. https://doi.org/10.1002/pmic.200500328.

Barahoei, M., Hatamipour, M. S., & Afsharzadeh, S. (2021). Direct brackish water desalination using *Chlorella vulgaris* microalgae. *Process Safety and Environmental Protection* 148, 237-248. https://doi.org/10.1016/j.psep.2020.10.006.

Cheeseman, J. M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiology* 87(3), 547-550. https://doi.org/10.1104/pp.87.3.547.

Delrue, F., Álvarez-Díaz, P. D., Fon-Sing, S., Fleury, G., & Sassi, J. F. (2016). The environmental biorefinery: Using microalgae to remediate wastewater, a win-win paradigm. *Energies* 9(3), 132. https://doi.org/10.3390/en9030132.

Figler, A., B-Béres, V., Dobronoki, D., Márton, K., Nagy, S. A., & Bácsi, I. (2019). Salt tolerance and desalination abilities of nine common green microalgae isolates. *Water* 11 (12), 2527. https://doi.org/10.3390/w11122527.

Gan, X., Shen, G., Xin, B., & Li, M. (2016). Simultaneous biological desalination and lipid production by Scenedesmus obliquus cultured with brackish water. *Desalination* 400, 1-6. https://doi.org/10.1016/j.desal.2016.09.012.

Hiremath, S., & Mathad, P. (2010). Impact of salinity on the physiological and biochemical traits of *Chlorella vulgaris* beijerinck. *Journal of Algal Biomass Utilization* 1(2), 51-59.

Hoque, M. A., Okuma, E., Banu, M. N. A., Nakamura, Y., Shimoishi, Y., & Murata, Y. (2007). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *Journal Plant Physiology* 164(5), 553-561. https://doi.org/10.1016/j.jplph.2006.03.010.

Kumari, A., Das, P., Parida, A. K., & Agarwal, P. K. (2015). Proteomics, metabolomics, andionomics perspectives of salinity tolerance in halophytes. *Frontiers in Plant Science* 6, 537. https://doi.org/10.3389/fpls.2015.00537.

Safi, C., Zebib, B., Merah, O., Pontalier, P., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable and Sustainable Energy Review* 35, 265-278. https://doi.org/10.1016/j.rser.2014.04.007.

Sahle-Demessie, E., Hassan, A. A., & Badawy, A. E. (2019). Bio-desalination of brackish and seawater using halophytic algae. *Desalination* 465, 104-113. https://doi.org/10.1016/j.desal.2019.05.002.

Wang, X. C., Fan, P., Song, H., Chen, X., Li, X., & Li, Y. (2013). Comparative proteomic analysis of differentially expressed proteins in shoots of Salicornia europaea under different salinity. *Journal of Proteome Research* 8(7), 3331-3345. https://doi.org/10.1021/pr801083a.

Yensen, N. P. (2006). Halophyte uses for the twenty-first century. In Khan, M. A., & Weber, D. J. (Eds.). *Ecophysiology of high salinity tolerant plants. Tasks for vegetation science* 40, 367-396. https://doi.org/10.1007/1-4020-4018-0_23.

Zhu, J. K. (2001). Plant salt tolerance. *Trend in Plant Science* 6(2), 66-71. https://doi.org/10.1016/S1360-1385(00)01838-0.