Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (3) (2024), (227-237) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

Efficiency of utilizing microalgae and duckweed to remediate aquaculture effluent

Amira Abed-Elmonem[1], Reham Abd-Elhay[2], Hamed Eladel[3], Mohamed Battah[3], and Yasser T. A. Moustafa[2]
[1]Belbies Central Hospital, Belbies, Elsharqia, Egypt
[2]Limnology Dept., Central Lab. For Aquaculture Research, Agricultural Research Center.
[3]Botany and Microbiology Department, Faculty of Science, Benha University, Benha 13518, Egypt
E-mail: a01064468113@yahoo.com

Abstract

Numerous nations have experienced a shortage of water in recent years. Microalgae and duckweed have been used to clean wastewater in recent years. An in-depth knowledge of the applications of duckweed and microalgae for the removal of nutrients from aquaculture wastewater is provided by this research. The duckweed plant was taken from agricultural drainage in the Sharqia governorate of Egypt, while *Chlorella sorokiniana* was isolated from a fish culture pond. They were grown with varying amounts of aquaculture wastewater (AWW)—25%, 50%, 75%, and 100%. The most effective method for eliminating NO_3^-, NH_4^+, and TP was the 100% AWW treatment, according to the study's findings. Removal efficiencies of 92.1%, 73.2%, and 54.7% were attained using *Chlorella sorokiniana*, respectively. Although the removal efficiencies of duckweed were 83.2%, 39.3%, and 77.5%. Moreover, *C. sorokiniana*, which was cultivated in 100% aquaculture effluent, had a protein level of 24%, a carbohydrate content of 36.8%, and a lipid content of 22.8%. Duckweed grown in aquaculture effluent only had a protein level of 26.1%, a carbohydrate content of 17.6%, and a lipid content of 19.3%. To describe the fatty acid profile, the gas chromatography-mass spectrometry (GC-MS) approach was used. Fatty acid percentages for *C. sorokiniana* growing in 100% aquaculture effluent were 15.6% PUFS, 23.8 MUFAs, and 60.6% SFAs. Cis-10-pentadecylic acid and arachidic acid make up the majority of fatty acids. *C. Sorokiniana* methanolic extracts Compared to ethyl acetate extract, has more potent antibacterial activity against *Aeromonas hydrophila* and *Pseudomonas sp.*

Keywords: Aquaculture effluent, duckweed, *Chlorella sorokiniana*, remediation

1. Introduction

Egypt is ranked first in Africa and sixth internationally for fish farming, with a self-sufficiency rate of nearly 85%. It also ranks third for tilapia production. In 2023, fish output reached over 2 million tons. Fish aquaculture is a prominent industrial activity in Egypt that contributed almost 80% of the total fish production in 2017 [1], with a gross output that has been steadily rising in recent years. Fish is regarded as a crucial component of most meals meant for human consumption. It has every necessary amino acid needed for strong development and a more balanced diet. Furthermore, eating fish can stop the onset of many illnesses, including depression, heart attacks, and strokes [2]. Seafood consumption rose quickly as a result of population and economic expansion [3]. Aquaculture is the food production industry with the quickest rate of growth because of the understandable global pattern of communities and continents experiencing a constant rise in fish demand [4]. The aquaculture sector generated as much as 50% of the world's food in 2020 [5]. With an estimated value of $250 billion, the aquaculture sector outperforms the conventional fisheries approach in terms of output by 18.32 million tons [6]. With worldwide output expected to reach 87.5 million tons of aquatic animals in 2020, aquaculture production is at a record high and plays a crucial role in the provision of food and nutrition [7].

The Middle East and North Africa (MENA) areas have well-documented water scarcity issues. This region's majority of nations are semi-arid or arid. They generally have a seasonal and unpredictable distribution with limited rainfall. With up to 5% of the world's population, the MENA area has less than 1% of the world's yearly renewable freshwater resources. The world's lowest annual per capita availability in 1995 was around 1250 cubic meters, down from over 3300 cubic meters in 1960. By 2025, it is expected to have dropped another 50% to approximately 650 cubic meters [8]. The waste nutrients found in aquaculture wastewater are often high, primarily in nitrogen and phosphorus [9]. Solid waste should not accumulate within the culture system since it decomposes, may poison ecosystems receiving effluent, and can induce oxygen deprivation and ammonia toxicity [10]. Microalgae-based phytoremediation of aquaculture effluent has immense promise because of its cheap cost and excellent nutrient removal effectiveness. Algae fix carbon dioxide, release oxygen via photosynthesis, and raise the biological oxygen demand in polluted water in microalgae-based bioremediation [11]. Duckweed was shown to be able to lower phosphate and nitrogen concentrations by up to 96% and 98%, respectively [12]. Because microalgae and duckweeds are very adaptable and sensitive to changing growing environments, they have been utilized in the past to monitor and remediate wastewater [13]. It is important to note that byproducts from duckweed and microalgal treatment systems may be

used to produce biofuel and animal feed, among other things [14]. Finding microalgae and duckweed with unique growth characteristics for aquaculture wastewater was the goal of the current study. The isolates were then thoroughly evaluated for growth ability, organic tolerance, potential for nutrient removal, and production of lipids, carbohydrates, and proteins. As a result, duckweed and the optimized algal strain were cultivated in the aquaculture effluent to assess its suitability as a feedstock for biofuel and to demonstrate its practicality. It was anticipated that the study's findings would provide useful knowledge on the development of microalgae and duckweed to treat aquaculture effluent and produce biofuel as well as antibacterial applications.

2. Materials and Procedures

2.1. *Chlorella sp.* Isolation and Growth Conditions

At the Central Laboratory for Aquaculture Research (CLAR) Farm in the Sharqia Governorate of Egypt, water samples were taken from fishponds. The water samples were collected using sterilized plastic bottles and then transported to the laboratory. The dilution culture approach was followed in the separation and purification of the algae [15 & 16]. By studying the many phases of its life cycle and keeping an eye on its distinctive morpho-taxonomic traits, the algal strain (Chlorella sp.) was identified. [19].

2.2. Strain identification

Using a light microscope, the isolated species was first recognized, and 18S rDNA was used to validate its phylogenetically. Using Gene JET DNA kits (Thermo Fisher Scientific, USA), the genomic DNA was extracted from the cells. Subsequently, 18S rDNA was amplified by PCR using primers 18SF (5′GTC AGA GGTGAA ATT CTT GGA TTT A-3′) and 18SR (5′-AGG GCA GGGACG TAA TCA ACG-3′), as per the manufacturer's instructions [18].

2.3. Algal growth

To serve as the inoculum for the next research trials, the tested microalga (*Chlorella sp.)* was cultivated in a conical flask with 500 mL of BBM and incubated under the aforementioned growth conditions. Four different concentrations of aquaculture effluent (25%, 50%, 75%, and 100%, v/v in distilled water) were used, along with the growth medium (BBM) as a control. Samples were tested five days apart, and each treatment was done in triplicate. The flasks were continuously illuminated at 30ºC with 3000 lux of light coming from daylight fluorescent bulbs.

2.4. Duckweed growth

Also referred to as aquatic plants, these plants may be rooted in sediment or can float freely on the water's surface. They can develop partially or entirely in water [19]. Random samples of duckweed were gathered from several locations within the Abassa agricultural drainage system. Duckweed samples were immersed in distilled water for half an hour after being cleaned with it. One gram of fresh duckweed was then grown in one liter of various aquaculture effluent concentrations (25, 50, 75, and 100% v/v in distilled water). Samples were tested six days apart, with each treatment being performed in triplicate. The samples were continuously illuminated at 30ºC using daylight fluorescent tubes, producing 3000 lux of light. The fresh weight of the duckweed was measured at regular intervals to assess the biomass production by sample collection.

2.5. Tracking *Chlorella sp*. Growth Rates

To assess the growth rates of *Chlorella* sp., this research assessed the optical density at 680 nm (OD680) over 48 days. Furthermore, a hemocytometer was used to count the cells, and the amount of chlorophyll (a) was calculated following [20].

2.6. Duckweed and *Chlorella sp*. biochemical analysis

Utilizing the Kjeldahl technique [21 & 22], the protein content was evaluated. The soxhlet extraction method was used to estimate the crude lipid [23 & 24]. The amount of moisture was determined by [25]. Using the incineration process, the ash content was estimated [23]. By [26], crude fiber was estimated. Samples were assessed after 7 days, and the percentage of carbohydrates was determined as follows: % of carbohydrates = 100 - (moisture + ash + protein + fat + fiber) [27].

2.7. Removal of Nutrients

The nutritional medium, microalgal & duckweed biomass, were separated from each other by centrifugation and filtration. The microalga *Chlorella sp.* and duckweed were seen to consume ammonia (NH_4^+), nitrate (NO_3^-), total phosphate (TP), and orthophosphate (PO_4) during a 5-day growth period for *Chlorella* sp. and a 6-day growth period for duckweed (the conclusion of the late exponential growth phase). (NH_4^+) The ammonia apparatus (model HI96715) was used to measure ammonia, whereas the sodium salicylate technique was employed to quantify (NO_3^-) [28&29]. Furthermore, the Ascorbic acid technique was used to quantify dissolved phosphate (PO_4) [30&31]. The technique of simultaneous digestion with persulfate was used to determine total phosphorus (TP) [32].

2.8. Fatty Acid Characterization

The dry lipid extracts were transesterified to fatty acid methyl esters (FAMEs) before gas chromatography analysis [33]. After collecting one milliliter of the crude lipid layer, 0.3 milliliters of H_2SO_4 and one milliliter of methanol were added. One milliliter of distilled water was also added, and the mixture was centrifuged for ten minutes at 4000 rpm after being vortexed for three to five minutes and incubated at 100 °C for ten minutes. Gas chromatography was used to assess FAMEs using a mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-MS direct capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The bioactive

components of *Chlorella sp*. were found by comparing the mass spectra of the compounds to those in the NIST/EPA/NIH mass spectral databases.

2.9. Algal extracts' in vitro antibacterial activity

Methanol and ethyl acetate algal extracts were evaluated for their antibacterial properties against *Aeromonas hydrophila* and *Pseudomonas sp.*

2.10 Examining Data Through Statistics

To find differences in group means at a significance level of 0.05, repeated measure analysis of variance (ANOVA) and Duncan's multiple range test were used in the statistical study. Additionally, the treatment means' standard deviation was calculated. The Statistical Analysis Systems (SAS) application was used to perform all statistics [34].

3. Results and Discussion

3.1. Isolation and identification

Chlorella sorokiniana was a unicellular, non-motile coccoid cell with an average size of 2–12 µm, and had a parietal cup-shaped chloroplast.

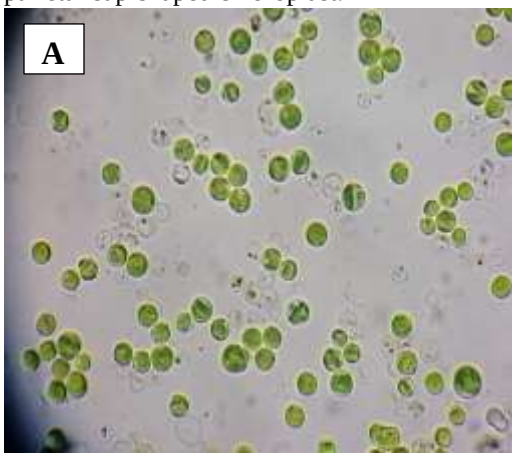

Fig. (1). (A) Light micrographs of *Chlorella sorokiniana* displaying the vegetative cells and chloroplast in detail (1000x).

Fig. (2) Duckweed aquatic plant

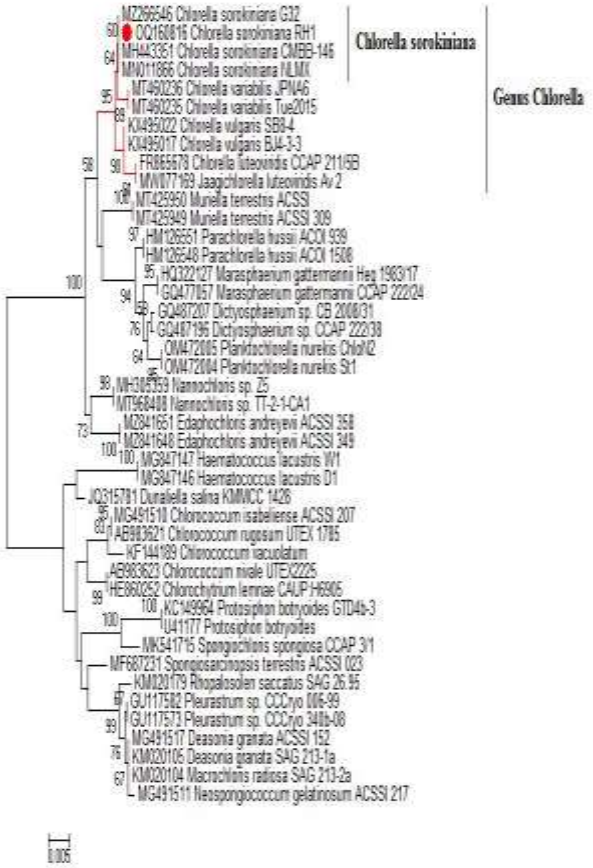

Fig. (3) Amino acid identities and divergence of theVP1 gene sequenced using Lasergene DNA Star's MegAlign module. With strains uploaded from public gene bank data, the tested sequence-isolated green alga Chlorella demonstrates an identification range of 100% *Chlorella sorokiniana.*

3.2. Growth Conditions

Using secondary processed aquaculture effluent (25%, 50%, 75%, and 100%), *Chlorella sorokiniana* and Duckweed plan were cultured. Following a 48-hour incubation period, growth parameter results (optical density, cell count, and chlorophyll a) were obtained for 8 days growth period. As seen in Figure (4&5), the 100% AWW treatment showed the greatest growth metrics when compared to the other three treatments. Duckweed and microalgae have been shown in related research to thrive quickly in aquaculture effluent. In the meantime, it may store fat or protein, which can be utilized to make biofuel and aquaculture feed [35- 37]. Numerous researches, many of which aim at biomass valorization, have concentrated on the utilization of certain microalgae strains and duckweed for the treatment of aquaculture effluents in batch assays. [38- 43]

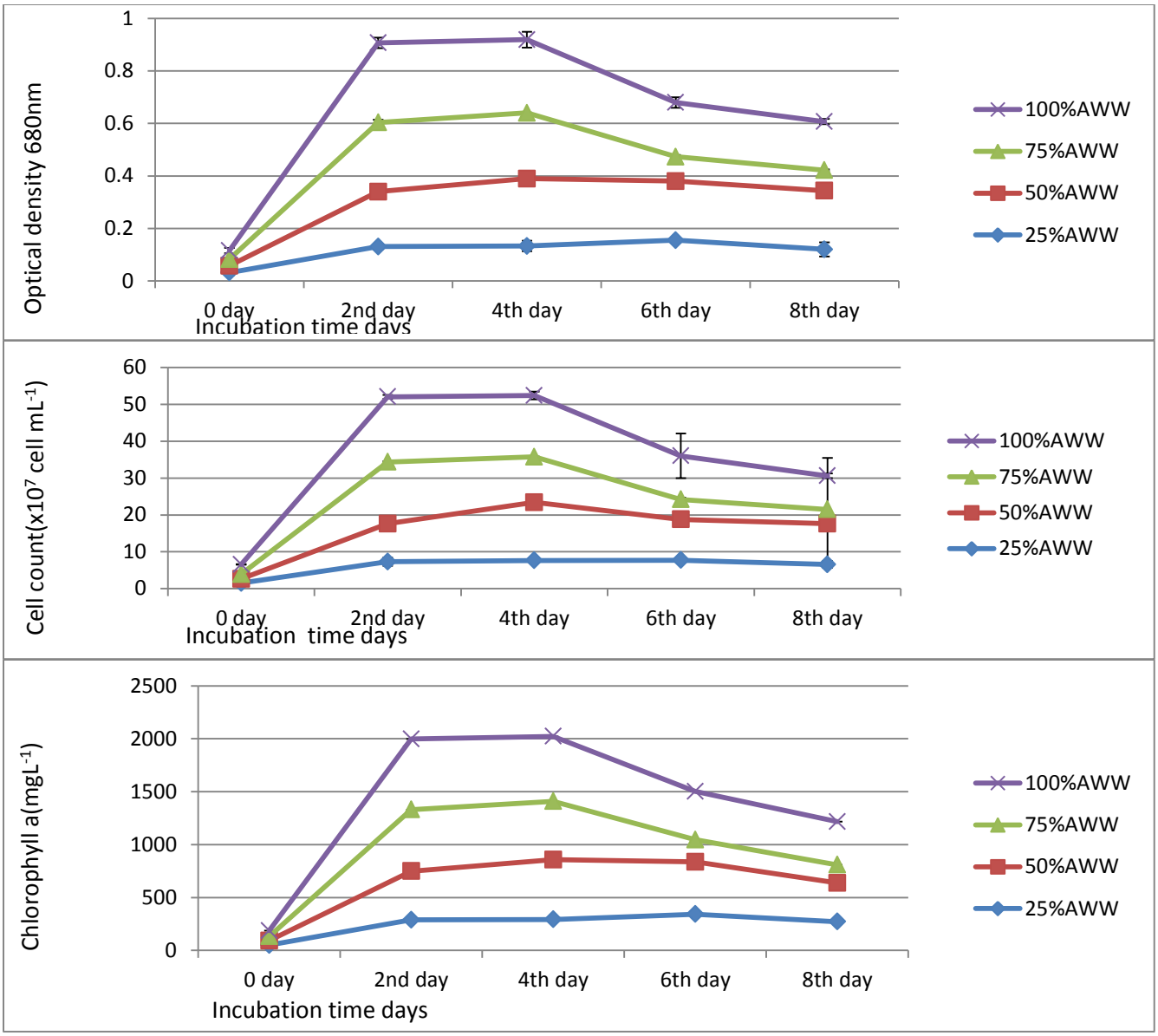

Fig. (4) *Chlorella sorokiniana* cultivated in varying concentrations of aquaculture waste water(AWW) for eight days. Optical density (OD), cell count ($\times 10^7$ cell ml^{-1}) and chlorophyll a.

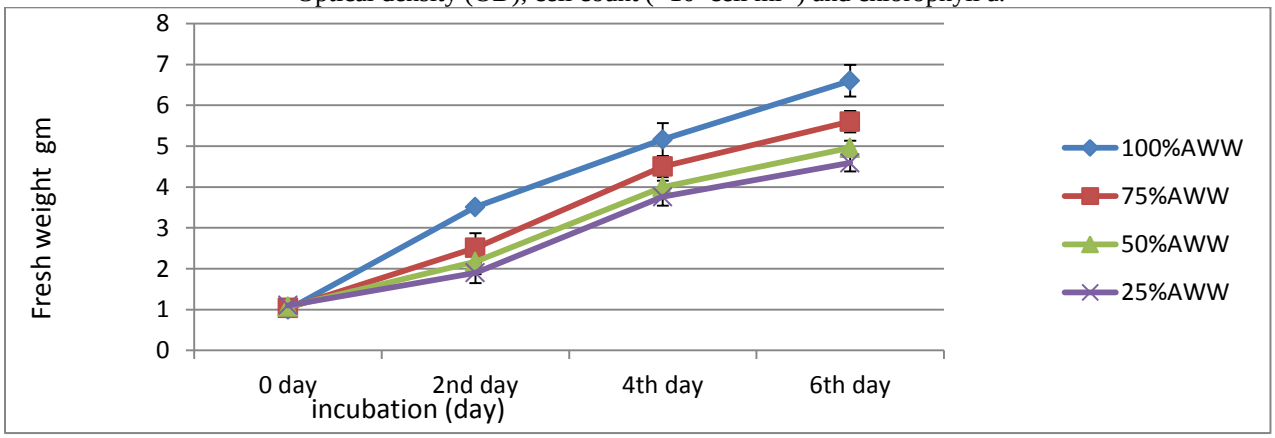

Fig. (5) The six-day development of Duckweed in varying concentrations of aquaculture wastewater (AWW) wastewater.

3.3. Biochemical Composition

Table (1) displays the measured protein, carbohydrate, and lipid contents of *C. sorokiniana* grown in 100% aquaculture wastewater and BBM. The protein content was found to be (23.96% & 18.8%), carbohydrate content to be (36.96% & 45.74%), and lipid content to be (22.78% & 20.06%). Our findings concurred with the findings of related research. About the biomass composition, lipids (15.82 ± 0.15%), carbohydrates (48.64 ± 0.83%), and protein content (17.93 ± 1.21%), [44] found that *Chlorella vulgaris* was.

Table 2 displays the measured protein, carbohydrate, and lipid contents of Duckweed grown in agricultural drainage canals and 100% aquaculture wastewater. The protein contents were 26.1% and 18.96, the carbohydrate content was 17.6% and 24.8, and the lipid content was 19.3% and 19.87. Our findings almost exactly matched the findings of related research. In a similar vein, [45] investigated the possibilities of integrated multitrophic aquaculture based on duckweed in real-world settings. Duckweed yields substantial biomass with a protein value of 21.84 ± 2.45%. The leftover biomass may be used as a source of carbohydrates for the synthesis of further biofuels once the lipids have been extracted.

Table (1) *Chlorella sorokiniana* incubated for 8 days; the impact of aquaculture wastewater and control (BBM) on the biochemical makeup of the organism.

parameters	Aquaculture Wastewater Yield %	Control (BBM) Yield %
Lipid	22.78	20.06
Protein	23.96	18.8
Carbohydrate	36.96	45.7
Ash	7.5	6.6
Moisture	6.8	6.7
fibre	2	2.1

Table (2) The impact of agricultural drainage canals and aquaculture wastewater on the biochemical makeup of duckweed after a 6-day incubation period.

parameters	Aquaculture Wastewater Yield (%)	agricultural drainage canal Yield (%)
Lipid	19.3	19.87
Protein	26.1	18.96
Carbohydrate	17.6	24.8
Ash	16.8	15.7
Moisture	7.8	7.9
fiber	12.4	12.8

3.4. Removal of Nutrients

As shown in Figure (6) and Table (3), After five days, *Chlorella sorokiniana* was able to remove 92.07% of the nitrate-N from aquaculture effluent, according to the study's findings. These findings are consistent with earlier research that showed that aquaculture effluent was treated by *Nannochloropsis oceanica, Cyclotella atomus, and Conticribra weissflogii* to remove 90% of nitrate-N in five days and 99% of it in seven [46].

According to our findings, *Chlorella sorokiniana* was able to remove 73.2% of the total ammonium from aquaculture effluent after five days. This finding is consistent with other studies that have used algae to decrease ammonium nitrogen from wastewater by around 67%–81%, and some algal species have been shown to substantially remove nutrients from wastewater [48, 49, 50, 51& 52].

After five days of incubation, our findings show that the orthophosphate and total phosphorus removal efficiency after *Chlorella sorokiniana* was added to aquaculture effluent were 54.1% and 54.7%, respectively. It has been previously documented that adsorption on the cell surface plays a substantial role in the removal of phosphorus from wastewater, which might account for the decrease in phosphate content [53]. These findings are consistent with those of earlier research, such as Su [54], which showed that batch cultures of filamentous blue-green algae could remove phosphate with efficiencies ranging from 56% to 73% after eight days of incubation. Using cultures of *Chlorella vulgaris* in synthetic wastewater (10 days of treatment) results in a phosphorus removal effectiveness of 78% [55]. When it came to extracting phosphate from combinations of municipal and refinery wastes, *Chlorella* and *Scenedesmus* were the most successful algal strains [56].

This research aimed to determine if Duckweed could be utilized to remove phosphate and nitrogen from aquaculture effluent, as shown in Table (4) and Figure (7). Duckweed grows well in aquaculture effluent, and plants easily absorb NO_3-N and NH_4-N. Specifically, NO_3-N concentrations dropped quite quickly. The NO3-N and NH_4-N removal efficiencies that were determined to be the greatest were 83.2% and 39.5 percent, respectively. This study found that Duckweed absorbed nitrate more than total ammonium. This finding is consistent with previous research that suggested *Lemna minor* might be utilized to extract nitrogen from aquaculture effluent. *Lemna minor* grew well in aquaculture effluent, and plants easily absorbed NO_3-N and NH_4^+-N. According to calculations, the greatest removal rates per square meter of water surface for NH_4^+-N and NO_3-N were 206 and 158 mg·m-2·day-1, respectively [57].

The current study demonstrated that Duckweed could grow well in aquaculture effluent and that plants could easily absorb TP and PO_4. The maximum removal efficiency of TP and PO_4^- from aquaculture effluent was 77.5% and 48.7%, respectively. In a similar vein, several studies found that duckweed systems are capable of eliminating 63–99% of the total phosphorus present in home wastewater covered with duckweed [57-59].

Table (3) *Chlorella sorokiniana* cultured for 5 days in synthetic (BBM) and varied concentrations of aquaculture waste; nutrient removal rate (RR, mg L^{-1} day^{-1}).

	T. Ammonia	NO_3	PO_4	TP
Control	0.1c ± 0.01	0.18c± 0.01	0.05a± 0.001	0.13b ± 0.001
100%AWW	0.31a ± 0.002	0.49a± 0.01	0.03b± 0.002	0.18a ± 0.02
75%AWW	0.24b ± 0.01	0.32b± 0.02	0.02c± 0.003	0.12b ± 0.01
50%AWW	0.13c ± 0.003	0.2c± 0.003	0.01d ± 0.001	0.07c ± 0.002
25%AWW	0.06d ± 0.004	0.09d± 0.02	0.008e ± 0.0004	0.03d ± 0.003

Table (4) shows the nutrient removal rate (RR, mg L^{-1} day^{-1}) of duckweed cultivated for six days in various aquaculture effluent concentrations.

	T. Ammonia	NO_3	PO_4	TP
100%AWW	0.07 ± 0.006	0.412± 0.008	0.01± 0.0002	0.12 ± 0.008
75%AWW	0.035±0.003	0.23± 0.01	0.006± 0.001	0.081± 0.005
50%AWW	0.03 ± 0.003	0.14± 0.01	0.0035± 0.001	0.05 ± 0.001
25%AWW	0.013 ± 0.001	0.05± 0.0003	0.002± 0.0003	0.0135 ± 0.003

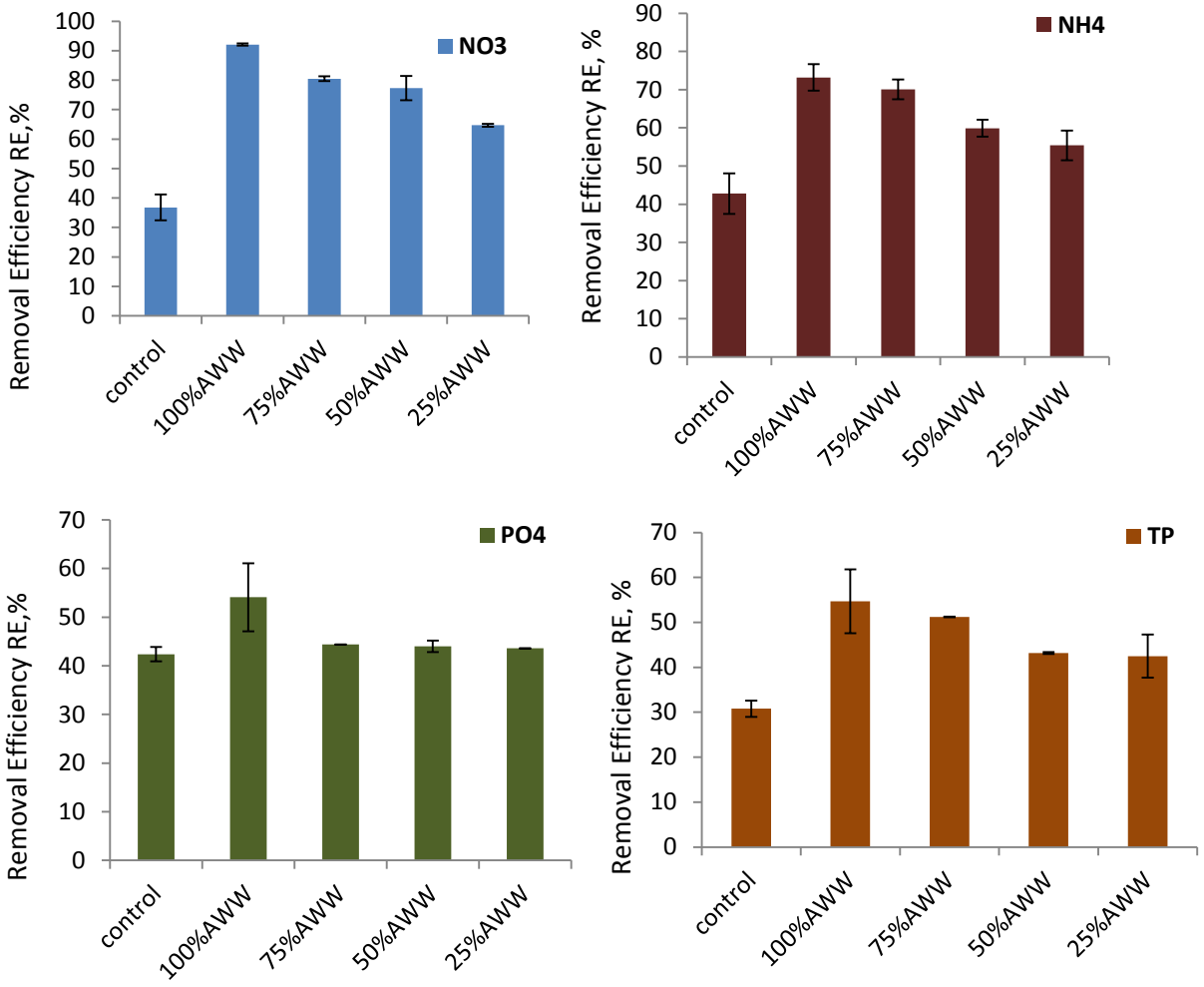

Fig. (6) *Chlorella sorokiniana* cultured for 5 days in varying amounts of synthetic (BBM) and aquaculture wastewater (AWW) nutrient removal efficiency (RE, %).

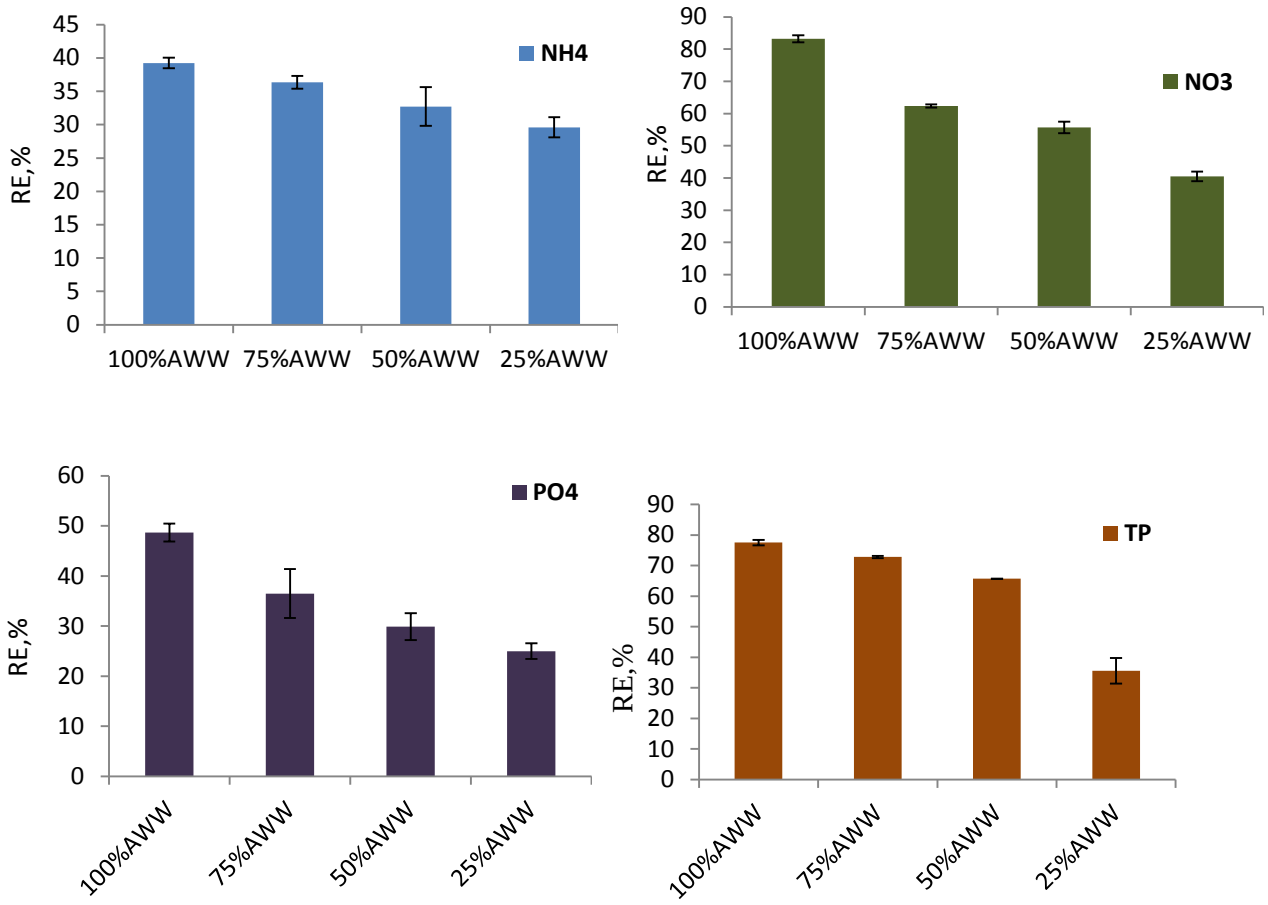

Fig. (7) Duckweed growing for six days in varying concentrations of aquaculture wastewater (AWW) and its nutrient removal efficiency (RE, %).

3.5. Antibacterial activity of isolated microalgal extracts

Table 5 displays our findings, which indicate that *Chlorella sorokiniana* ethyl acetate extract has more potent antibacterial activity against *Aeromonas hydrophila and Pseudomonas sp.* than methanolic extracts.

Various investigations have shown that a multitude of chlorophytes, including *Chlorella variabilis*, have demonstrated potential for antibacterial and antioxidant properties [60]. Three microalgae, *Isochrysis galbana*, *Scenedesmus sp., and Chlorella sp.*, were shown to have dominating chemicals by Alsenani [61]. These compounds included oleic acid, DHA, EPA, and linoleic acid, and the extracts of these fatty acids were shown to be able to suppress the development of gram-positive bacteria.

Table (5) *Aeromonas hydrophila* and *Pseudomonas sp.* are the targets of *Chlorella sorokiniana's* antimicrobial activity (ethyl acetate & methanolic extracts) (inhibition zone estimated as mm).

	Algal methanolic extract (30µl & 35 µl)		**Control (Methanol solvent only)**	
Bacterial species	***Chlorella sorokiniana* 30µl**	***Chlorella sorokiniana* 35µl**	**30µl**	**35µl**
1-*Aeromonas hydrophila*	10 ± 1.4	12a ± 1.4	10NS ± 1.4	10ab ± 1.4
2- *Pseudomonas* sp.	0 ± 0	0 ± 0	0NS ± 0	0NS ± 0

Table (5)

Bacterial species	Algal Ethyl acetate extract (30µl & 35 µl)		Control (Ethyl acetate solvent only)	
	Chlorella sorokiniana 30µl	*Chlorella sorokiniana* 35µl	30µl	35µl
1-*Aeromonas hydrophila*	15a ± 1	20a ± 1	0b ± 1	10b ± 1
2- *Pseudomonas* sp.	10a ± 1.2	15a ± 1.2	0b ± 1.2	10b ± 1.2

Fig. (8) Picture of a Petri plate demonstrating how *Aeromonas hydrophila* grows in response to *Chlorella sorokiniana's* ethyl acetate extract

3.6. Fatty Acid Composition

In According to our findings, the overall percentages of SFAs, MUFAs, and PUFAs in *C. sorokiniana* cultivated in aquaculture wastewater (AWW) were 54.01, 34.69, and 11.29%, respectively. The proportion of total fatty acids in Control (BBM) was 60.64% for SFAs, 23.81 for MUFAs, and 15.55% for PUFS. Arachidic acid C20:0 SFA was (36.88 and 47.44%) for BBM and 100%AWW, respectively, and is prominent in microalgae lipids. as shown in figure (9), and Cis-10-Pentadecylic acid C15:1MUFA was 12.03 and 23.14%) for BBM and 100%AWW, respectively.

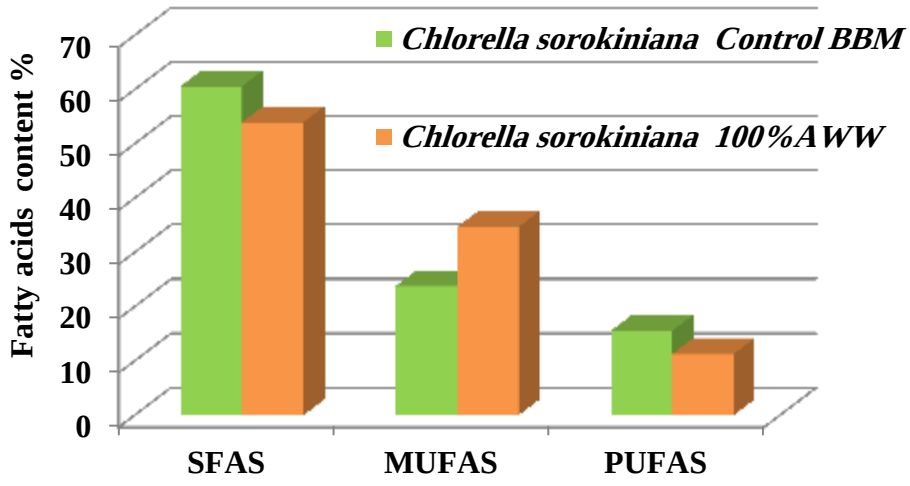

Fig. (9) Fatty acid groups of *Chlorella sorokiniana* cultured in (BBM) and 100% aquaculture wastewater (AWW), saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs).

Reference

[1] Gafrd (General Authority for Fishery Resources Development), 2017. Year-Book of fishery statistics in Egypt (2008-2017), Cairo.

[2] Ryu B, Shin K-H, Kim S-K (2021), Muscle protein hydrolysates and amino acid composition in fish. Mar Drugs 19:377.

[3] Naylor RL, Kishore A, Sumaila UR et al (2021), Blue food demand across geographic and temporal scales. Nat Commun 12:5413. https://doi.org/10.1038/s41467-021-25516-4.

[4] Tran N, Chu L, Chan CY et al (2019), Fish supply and demand for food security in Sub-Saharan Africa: an analysis of the Zambian fish sector. Mar Policy 99:343–350. https://doi.org/10.1016/j.marpol.2018.11.009.

[5] Hawrot-Paw, M.; Koniuszy, A.; Gałczyn´ska, M.; Zaja¸c, G.& Szyszlak-Bargłowicz, J. (2020). Production of microalgal biomass using aquaculture wastewater as growth medium. Water 2020, 12, 106.

[6] Tacon AGJ (2020), Trends in global aquaculture and aquafeed production: 2000–2017. Rev Fish Sci Aquac 28:43–56. https://doi.org/10.1080/23308249.2019.1649634

[7] FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. 10.4060/cc0461en.

[8] Abdel-Raouf a, A.A. Al-Homaidan b,*, I.B.M. Ibraheem b,c. (2012) Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 19, 257–275.

[9] Mook, W. T., Chakrabarti, M. H., Aroua M. K., Khan, G. M. A., Ali, B. S., Islam M. S., and Hassan, M. A. (2012). Removal of total ammonia nitrogen (TAN), nitrate and total 93 organic carbon (TOC) from aquaculture wastewater using electrochemical technology: a review. Desalination, 285: 1-13.

[10] Xin L., H. Hong-ying, G. Ke & S. Ying-xue, (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp., Bioresour. Technol. 101 (2010) 5494–5500.

[11] Abubakar F. B. , Sani Ibrahim & Olatunji Moruf (2023). Phytoremediation of Aquaculture Wastewater: A Review of Microalgae Bioremediation Science World Aslan S, Kapdan IK. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol. Eng. 28:64–70. doi: 10.1016/j.ecoleng.

[12] Zhou Y., Guimin Chen, Anton Peterson, Xiaomeng Zha, Jingwen Cheng, Sijia Li, Dandan Cui, Haotian Zhu, Olena Kishchenko, Nikolai Borisjuk. (2018) Biodiversity of Duckweeds in Eastern China and Their Potential for Bioremediation of Municipal and Industrial Wastewater. DOI: 10.4236/gep.2018.63010 PDF HTML XML 1, 467 Downloads 2,945 Views.

[13] Silveira, E.O., Lutterbeck, C.A., Machado, Ê.L., Rodrigues, L.R., Rieger, A., Beckenkamp, F., Lobo, E.A., 2019. Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands. Sci. Total Environ. 705, 135864.

[14] Mehrabadi, A., Craggs, R., Farid, M. M., 2015. Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. Bioresource Technol. 184, 202–214.

[15] Venkataraman G. S. (1969). The cultivation of algae, by G.S. Venkataraman. Indian Council of Agricultural Research, [New Delhi], 9-319.

[16] El-Ayouty, E.Y. and Ayyad, M. A. (1972). Studies on blue green algae of the Nile Delta. 1-Description of some species in a wheat field. Egypt. J. Bot., 15:283-321.

[17] D. S., Sri Astuti & Siti Alaa (2019). The growth of microalgae Chlorococcum sp. isolated from Ampenan estuary of Lombok Island in Walne's medium. AIP Conference Proceedings 2199(1):050003. DOI:10.1063/1.514130.

[18] Liu C, Huang X, Wang X, Zhang X, Li G (2006) Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. Phycologia 45:190–19820.

[19] Wersal, R. M. and Madsen, J. D. (2012). Aquatic plants, their uses and risks. Revised. http://www.ippc:int/large files/2012/ippc-IRSS Aquatic plant-study-2012-final pdf.

[20] Mckinney, G. (1941). Absorption of light by chlorophyll solutions. J. Biol. Chem. 140, 315-322.

[21] Crampton EW, Harris LE. (1969). Applied Animal Nutrition. Printed by W.H. Freeman and company, 1969, 49.

[22] Pearson T, Galfre G, Ziegler A, Milstein C. (1977), A myeloma hybrid-producing antibody specific for an allotypic determinant on IgD-like molecules of the mouse. Eur J Immunol. 1977; 7: 684-690.

[23] Maynard J. (1970), Methods of Food Analysis, Academic Press, New York, NY, 1970, 163.

[24] Jacobs MB. (1973). The Chemical Analysis of Food Products. 3ed. New York: Robert Krieger, Publishing Co, 1973, 80.

[25] AOAC. 1995. (Association of Official Analytical Chemicals). Official Method of Analysis, 12th ed, Association of official Analytical Chemists, Washington DC, 1995, 832.

[26] AOAC., 1980. Official Methods of Analysis. 13th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

[27] Bhuiyan R.R., Md. Simul Bhuyan, Tahmina Sharif Anika, Mohammad Nurul Azim Sikder and Hossain

Zamal (2016). Determination of proximate composition of fish feed ingredients locally available in Narsingdi region, Bangladesh, International Journal of Fisheries and Aquatic Studies 2016; 4(3): 695-699.

[28] Scheiner. D. 1974. A modified version of the sodium salicylate method for analysis of wastewater nitrates. Water Research, 8 (10): 835-840.

[29] Water Quality. (2000). Determination of Nitrate. Part 3. Spectrometric Method Using Sulphosalicylic Acid. SR ISO 7890-3 (2000).

[30] APHA (American Public Health Association) (1985). Standard methods for the examination of water and wastewater. 16th ed., Washington, D.C. 1268pp.

[31] Boyd, C.E. and Tucker, C.S. (1992). Water quality and pond soil analysis for aquaculture. Alabama agricultural experimental station. Auburn. Uni.183.

[32] Ross, A and Boyd, C.E. (1998). Digestion Procedure for the Simultaneous Determination of Total Nitrogen and Total Phosphorus in Pond Water. Journal of the world aquaculture society, 29(3):300-303.

[33] Arif, M.; Wang, L.; Salama, E.S.; Hussain, M.S.; Li, X.; Jalalah, M.; Liu, P. Microalgae isolation for nutrient removal assessment and biodiesel production. BioEnergy Res. 2020, 13, 1247–1259.

[34] Statistical Analyses Systems (SAS) (2024). SAS Program ver. 9.4, SAS institute incorporation, Cary, NC 27513 USA.

[35] Ferdoushi, Z.; F. Haque; S. Khan and M. Haque, 2008. The Effects of two Aquatic Floating Macrophytes (Lemna and Azolla) as Biofilters of Nitrogen and Phosphate in Fish Ponds.Turkish Journal of Fisheries and Aquatic Sciences, 8: 253-258.

[36] Beer LL, Boyd ES, Peters JW 2009. Engineering algae for biohydrogen and biofuel production. Curr. Opin. Biotech. 2009; 20:264–271. doi: 10.1016/j.copbio.2009.06.002.

[37] Bellou S, Baeshen MN, Elazzazy AM 2014. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol. Adv. 2014; 32:1476–1493. doi: 10.1016/j.biotechadv.2014.10.003.

[38] Sirakov, I.; K. Velichkova; S. Stoyanova; D. Dinev and Y. Staykov, 2015. Application of naturalzeolites and macrophytes for water treatment in recirculation aquaculture systems. Bulg. J. Agric. Sci., Supplement1, 21: 147–153.

[39] Ansari F. A., Singh P., Guldhe A., Bux F. (2017). Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal. Res. 21, 169–177. doi: 10.1016/j.algal.2016.11.015.

[40] Guldhe A., F.A. Ansari, P. Singh, F. Bux (2017). Heterotrophic cultivation of microalgae using aquaculture wastewater: A biorefinery concept for biomass production and nutrient remediation Ecol. Eng., 99 (2017), pp. 47-53, 10.1016/j.ecoleng.2016.11.013.

[41] Guo Z., Y. Liu, H. Guo, S. Yan, J. Mu (2013), Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production J. Environ. Sci., 25 (2013), pp. S85-S88, 10.1016/S1001-0742(14)60632-X.

[42] Khatoon, H. Banerjee, S., Syahiran, M., Noordin, M., Bolong, A. and Endut, A. (2016). Re-use of aquaculture wastewater in cultivating microalgae as live feed for aquaculture organisms. Desalination and Water Treatment. 57: 29295-29302.

[43] Liu Y., Junping Lv, Jia Feng, Qi Liu, Fangru Nan, Shulian Xie (2019). Treatment of real aquaculture wastewater from a fishery utilizing phytoremediation with microalgae. https://doi.org/10.1002/jctb.5837 Citations: 55.

[44] Ana T. Couto 1a, Martim Cardador 1a, Sergio Santorio, Luz Arregui, Benedetto Sicuro, Anuska Mosquera-Corral, Paula M.L. Castro 1, Catarina L. Amorim 2022. Cultivable microalgae diversity from a freshwater aquaculture filtering system and its potential for polishing aquaculture derived water streams Microalgae from aquaculture for bioremediation

[45] Paolacci S., Vlastimil Stejskal & Marcel A. K. Jansen &Damien Toner (2022). Wastewater valorisation in an integrated multitrophic aquaculture system; assessing nutrient removal and biomass production by duckweed species.

[46] Zhang L.; Jichang Han ; Shuonan Ma ;Yuanbo Zhang ;Yumeng Wang & Jilin Xu (2023). Comprehensive evaluation of growth characteristics, nitrogen removal capacity, and nutritional properties of three diet microalgae. Sec. Marine Fisheries, Aquaculture and Living Resources Volume 10 - 2023 | https://doi.org/10.3389/fmars.2023.1117043.

[47] Garcia, J., Green, B.F., Lundquist, T., Mujeriego, R., Hernandez- Marine, M., and Oswald, W.J. (2006). Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresource technology, 97, 1709-1715.

[48] Khan, M. and Yoshida, N. (2008). Effect of L-glumatic acid on the growth and ammonium removal from ammonium solution and natural wastewater by Chlorella vulgaris NTM06 aquaculture organisms. Desalination and Water Treatment. 57: 29295-29302.

[49] Powell, N., Shilton, A., Chisti, Y., and Pratt, S. (2009). Towards a luxury uptake process via microalgae defining the polyphosphate dynamics. Water Res., vol. 43, No. 17, pp. 4207-4213.

[50] Park, J.B.K. and Craggs, R. J. (2010). Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Sci. Technol 61:633-639.

[51] Park, J.B.K., Cragg, R. J. and Shilton, A.N. (2011). Wastewater treatment with high rate algal ponds for biofuel production. Bioresource technology, vol. 102, No. 1, pp. 35-42.

[52] Martinez, M.E., Sanchez, S., Jimenez, J.M., Yousfi, F., and Munoz, L. (2000). Nitrogen and phosphorus removal from urban wastewater by the microalgae Scendesmus obliquus. Bioresour technol 73:263-272.

[53] Su, Y., Mennerich, A., and Urban, B. (2011). Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture, water Res. 45 3351-3358.

[54] Aslan, S. & Kapdan, I.K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol. Eng. 2006, 28, 64–70.

[55] Granter, M., Gajin, S., and Dalmacija, B. (1984). The possibility of phosphate elimination by the use of algae in the process of wastewáter purification, Microbiología, Vol.21, No. 1, pp. 63-73.

[56] Paolacci S., Vlastimil Stejskal & Marcel A. K. Jansen (2021). Estimation of the potential of Lemna minor for effluent remediation in integrated multi-trophic aquaculture using newly developed synthetic aquaculture wastewater. Aquaculture International 29:2101–2118.

[57] Körner, S., and Vermaat, J. E. 1998. The relative importance of Lemna gibba L., bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic wastewater. Wat. Res. 32(12): 3651-3661.

[58] Gupta, C. and D. Prakash, 2014. Duckweed: an effective tool for phyto-remediation. Toxicological & Environmental Chemistry, pages1-11, DOI: 10.1080/02772248.2013.879309.

[59] N. L. , Kit Yinn Teh 2, Su Shiung Lam 3, Anne Marie Kaben 4, Thye San Cha 5(2015). Optimization of cell disruption methods for efficient recovery of bioactive metabolites via NMR of three freshwater microalgae (Chlorophyta(Bioresource Technology).

[60] Alsenani, F., Tupally, K. R., Chua, E. T., Eltanahy, E., Alsufyani, H., Parekh, H. S., & Schenk, P. M. (2020). Evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds. Saudi Pharmaceutical Journal, 28(12), 1834-1841.