Exploring the impact of *Chlamydomonas reinhardtii* Artificial Culture Medium on Ammonium and Phosphate Removal in Aquaculture Wastewater

Nguyen My Phi-Long[1,2], Le Pham Dan-Khanh[1,2], Nguyen Tran Thuy Thanh-Mai[1,2], Nguyen Duc-Hung[3] and Nguyen Thi Gia-Hang[1,2]*

1. Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, VIETNAM
2. Vietnam National University, Ho Chi Minh City, VIETNAM
3. Faculty of Natural Sciences Pedagogy, Saigon University, Ho Chi Minh City, VIETNAM
*ntghang@hcmus.edu.vn

Abstract

This study utilized Chlamydomonas reinhardtii, an algal strain isolated from shrimp aquaculture wastewater ponds in Ca Mau province to investigate its capacity for ammonium and phosphate removal from wastewater. Cultivation occurred in both Tris-Phosphate-Acetate (TAP) and Bold's Basal Medium (BBM) under controlled conditions: a temperature of 25 ± 1 ^{0}C and a 24-hour light cycle provided by light-emitting diodes (LEDs). Over 28 days, four different concentrations of ammonium and phosphate were tested.

Results demonstrated that algal biomass in the BBM medium exceeded threefold. Optimal algal biomass was observed at approximately 1.0 mg N.L^{-1} ammonium concentration and about 5.0 mg P.L^{-1} phosphate concentration. Notably, in the BBM medium, ammonium levels decreased rapidly within 7 days at a concentration of 4.0 mg N.L^{-1} whereas in the TAP medium, it required 14 days. Conversely, phosphate concentration decline commenced after 14 days in both algal culture environments.

Keywords: *Chlamydomonas reinhardtii*, Ammonium, Phosphate, TAP, BBM, Aquaculture wastewater.

Introduction

Over recent decades, water pollution has become a pressing environmental concern, mirroring the emergence as a significant environmental concern, paralleling the persistent issue of water scarcity. The discharge of nutrient-rich wastewater poses the potential for eutrophication, a serious problem particularly prevalent in enclosed water bodies[8]. The rapid growth of microalgae in nutrient-rich water can be harnessed positively in wastewater treatment to extract nutrients before discharging treated water into rivers.

Both microalgae and cyanobacteria require nitrogen (N) and phosphorus (P) to synthesize essential compounds like nucleotides, amino acids, lipids and carbohydrates, crucial for metabolic processes[5]. However, elevated levels of N and P can exacerbate eutrophication, leading to harmful blooms of microorganisms such as bacteria, cyanobacteria and microalgae. These blooms release toxins that can severely impact ecosystems and biodiversity. The increased presence of ammonium (NH_4^+) and phosphate (PO_4^{-3}) in water bodies stems from various human activities including domestic wastewater discharge, intensive fertilizer uses and agro-industrial activities[16].

The use of green microalgae in tertiary wastewater treatment was first proposed in the 1980s[29]. Since then, numerous studies have explored their efficacy, employing species like *Chlorella* sp.[2,10,35], *Scenedesmus* sp.[12,22,39], *Nannocloropsis salina*, *Spirulina major* and *Isochrysis galbana*[38]. Furthermore, the biomass generated through bioremediation procedures holds potential for various biotechnological applications such as biogas, biofuel, or biofertilizer production[1,11].

While nitrogen exists in various forms in wastewater, microalgae can only assimilate inorganic nitrogen. Despite its significance for growth, elevated nitrogen concentrations[17,42] can be toxic to microalgae cells, particularly when ammonia levels exceed 20 mg.L^{-1}. Similarly, phosphorus, essential for microalgae, primarily exists in the form of orthophosphate (PO_4^{-3}). While phosphate itself is not toxic, excessive concentrations can trigger algal and aquatic plant overgrowth[33], leading to eutrophication and oxygen depletion in water bodies. To combat this, the EPA recommends maximum phosphate concentrations of 50 µ.L^{-1} at points where phosphates enter natural water and 100 µg.L^{-1} where direct entry does not occur[14].

This study aims to compare *Chlamydomonas reinhardtii's* biomass production capabilities in artificial environments (TAP and BBM). By evaluating the algae's performance in processing ammonium and phosphate over 28 days using TAP and BBM media, it seeks to provide insights into their potential for wastewater treatment.

Material and Methods

Study sites and aquaculture wastewater: The study was conducted in Dat Moi Commune, Nam Can district, Ca Mau province (8°47'50.5" N-104°58'40.2" E), a coastal area situated within the Mekong delta region in southern Vietnam. The wastewater utilized in the study originated from an extensive shrimp farming system cultivating *Litopenaeus vannamei* Boone in 1931, commonly referred to as white-leg shrimp (Figure 1). Two primary aquaculture

methods, open and closed systems, exhibit distinct characteristics. In an open aquaculture system, water undergoes a single pass before the effluents are treated and discharged. Consequently, these systems consume significant amounts of water and the discharged effluents can have adverse environmental effects in the local area. This wastewater is directly released into the river without undergoing any mechanical or biological treatment.

The wastewater sources' characteristics were analyzed for various parameters including pH, temperature, alkalinity, total suspended solids, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD_5^{20}), Chemical Oxygen Demand (COD_5), Ammonium (NH_4^+-N), Total Nitrogen, Phosphate (PO_4^{3-}-P), Total Phosphorus (TP), Nitrite and Nitrate (Table 1). These samples were analyzed at the Microbiology laboratory, University of Science Ho Chi Minh city to identify algae strains. Subsequently, an experimental pilot study was conducted in the laboratory. The isolated samples underwent molecular biology analysis of the algae strains at the Faculty of Sciences Pedagogy, University of Saigon. Algae biomass was cultured in both Tris-Phosphate-Acetate (TAP) and Bold's basal medium (BBM).

Organism and cultural conditions: The investigation focused on *Chlamydomonas reinhardtii* P.A. Dangeard, 1888, a small unicellular green alga, with a diameter of less than 10 µm characterized by a single eyespot[15]. This mobile organism is from a wastewater pond.

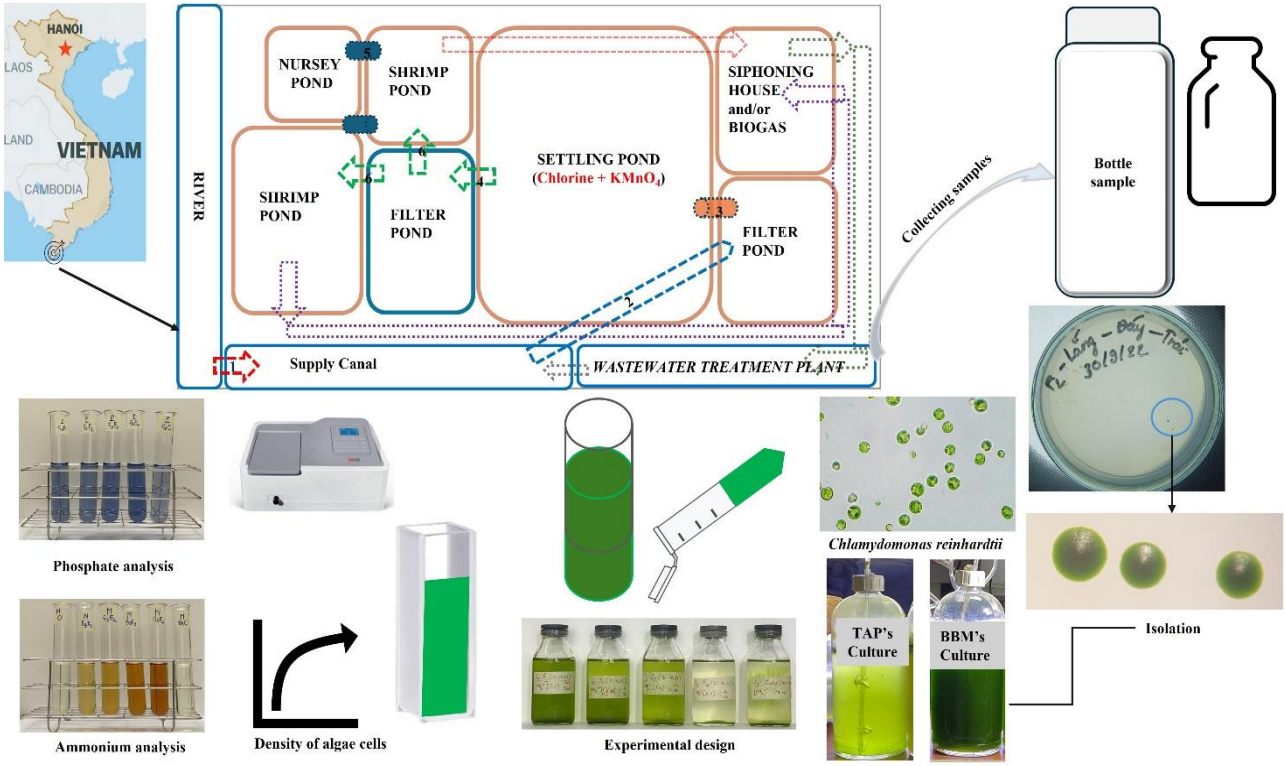

Figure 1: Diagram illustrating the sample collection location and the laboratory analysis of sample parameters.

Table 1
Wastewater source characteristics

Parameters	Unit	Value
pH		7.1
Temperature	^{0}C	30.05
Dissolved oxygen	mg/L	9.4
Alkalinity	-	104
TSS	mg/L	106.3
BOD_5	mg/L	66.0
COD_5	mg/L	136.0
Ammonium (NH_4^+-N)	mg/L	0.76
Total Nitrogen (N)	mg/L	26.1
Total Phosphor (P)	mg/L	0.65
Phosphate (PO_4^{3-} - P)	mg/L	0.48
Nitrit (NO_2^-)	mg/L	0.084
Nitrate (NO_3^-)	mg/L	7,12

Algal cells were cultured in Tris-Phosphate-Acetate (TAP) and Bold's basal medium (BBM)[9]. TAP medium supports heterotrophic or photo-heterotrophic growth of *Chlamydomonas* spp., while BBM is suitable for cultivating various green algal cultures like *Trichosarcina* spp., *Chlorococcum* spp. and *Chlorella* spp. without the requirement of soil extract or vitamins[30].

Cultivation occurred at 25 ± 1 ^{0}C under a 24-hour light cycle with LED light intensity regulated between 664 – 737 Lux. The laboratory maintained humidity at approximately 51 ± 1%. Medium preparation followed table 2 specifications. Experiments used 2,000 mL flasks with 1,200 mL of medium in a controlled environment. Glassware was cleaned with 10% HCl autoclaved at 170^{0}C.

The culture temperature was kept at 25 ± 1 °C and pH ranged from 6.4 to 6.8 for BBM and 6 to 8 for TAP to avoid growth inhibition. The initial pH was set at 7.0, monitored daily and adjusted using diluted HCl. Carbon dioxide (CO_2) supply and daily pH adjustments to 7 prevented PO_4^{3-}P precipitation and NH_4^+-N degassing. Cell growth was measured daily at 750 nm using the SP-UV 1100 UV-VIS Spectrophotometer from DLAB in Selangor, Klang, Kuala Lumpur (KL), Malaysia.

Experimental design: The experiment began with harvesting algal cells in the log phase using centrifugation (Hettich, model EBA 20, Germany). These cells were then suspended in filtered sterile distilled water three times to eliminate any remaining TAP and BBM nutrients. Trials were conducted in 100 mL glass bottles, each containing the culture medium and a stock solution of either ammonium or phosphate at four varying concentrations, totaling 100 mL. The experiment lasted 28 days (or 4 weeks), with cultures maintained in the laboratory at a temperature range of 24-26 °C and a light intensity of 700 Lux, measured on the surface of the cultures using a standard digital illuminance (Light) meter ST-1308. All experiments were conducted under continuous illumination.

Table 2
Comparison of chemical ingredients in TAP and BBM growth media, highlighting the inclusion of Hunter's trace element in TAP. The TAP Trace elements and EDTA listed are from Hunter's trace element solution, adjusted to 6.5 with KOH pellets before formulating TAP. Both media maintain a pH range of 7-7.2.

Chemical Component	Final concentration in 1 liter TAP Tris-Phosphate-Acetate	Final concentration in 1 liter BBM Bold Basal Medium
$NaNO_3$		2.94 mN
$CaCl_2$•$2H_2O$	0.35 mM	0.17 mM
$MgSO_4$•$7H_2O$	0.4 mM; TAP salt	0.3 mM
K_2HPO_4	0.6 mM; phosphate solution	0.43 mM
KH_2PO_4	0.4 mM; phosphate solution	1.29 mM
NaCl	-	0.43 mM
EDTA Stock		~ 8.5 µM
Iron Stock		~ 0.9 µM
Borron Stock		~ 9 µM
Bold Trace Stock		
H_2SO_4 conc.		
$ZnSO_4$•$7H_2O$	0.077 mM; trace element	1.50 µM
$MnCl_4$•H_2O	0.026 mM; trace element	0.36 µM
MoO_3		0.26 µM
$CuSO_4$•$5H_2O$	0.005 mM; trace element	0.31 µM
$Co(NO_3)_2$•$6H_2O$		0.084 µM
NH_4Cl	7.5 mM; TAP salt	
Na_2HPO_4	-	
Hunter's Trace Stock		
Na_2EDTA•$2H_2O$	0.134 mM	
H_3BO_3	0.184 mM; trace element	
$FeSO_4$•$7H_2O$	0.018 mM; trace element	
$CoCl_2$•$6H_2O$	0.007 mM; trace element	
$(NH_4)_6Mo_7O_{24}$•$4H_2O$	0.0008 mM; trace element	
Tris Acetate Stock		
Trisma Base	0.02 M (or 19.97 mM)	
Glacial Acetic Acid	0.017 M (Carbon source) (or 0.1%)	

The average concentrations of NH_4^+-N stock solution (10 mg N/mL) in the experimental cultures were 0.5, 1.0, 2.0 and 4. mgN.L^{-1} respectively, after adding the culture medium (TAP or BBM) to 100 mL bottle samples. Similarly, for experimental phosphate, the average concentrations of PO_4^{3-}-P stock solution (50 mg P/mL) were 5.0, 10.0, 15.0 and 20.0 mgP.L^{-1}, with the culture medium added to achieve a total volume of 100 mL. Additionally, a control sample without the stock solution of ammonium or phosphate, containing only algal cells with either TAP or BBM medium, was prepared and was maintained under identical conditions to the experimental samples.

Collected data: Weekly samples were taken from each treatment bottle to measure NH_4^+-N and PO_4^{3-}-P concentrations. NH_4^+-N concentrations were determined using the Nessler method while PO_4^{3-}-P concentrations were measured using the acid ascorbic method, following procedures outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Concurrently, controls were assessed to detect any potential loss of NH_4^+-N due to outgassing or PO_4^{3-}-P due to precipitation. Cell dry weight determination occurred at the experiment's conclusion. Each sample underwent filtration through a 0.45 µm pore size pre-weighted glass fiber filter (Whatmann, Maidstone, UK) and subsequent drying at 105°C for two hours. Following this, samples were centrifuged at 6,000 rpm for five minutes to separate the algal cells.[3]

The mean productivity of each batch test, Q_x (mg.L^{-1}), was calculated as equation 1[3]:

$$\frac{X_1 - X_0}{t_1 - t_0} \quad (1)$$

where X_1 (mg.L^{-1}) is the dry weight measured at the time t_1 (day) and X_0 (mg.L^{-1}) is the dry weight at the time t_0 (day). The growth rate of algae was calculated according to the equation 2[18]:

$$\frac{\ln\frac{N_2}{N_1}}{t_2 - t_1} \quad (2)$$

where N_2 and N_1 are the number of cells at times t_2 and t_1, respectively.

The total amount of NH_4^+-N removal during the experiment, Q_N (mg.L^{-1}), was calculated as per eq. 3[3]:

$$Q_N = N_0 - N_m \quad (3)$$

where N_0 (mg.L^{-1}) is the initial NH_4^+-N concentration. N_m (mg.L^{-1}) is the NH_4^+ -N concentration remaining in the medium at the end of the experiment.

The total amount of PO_4^{3-} -P removal during the experiment (mg.L^{-1}) was calculated as per eq. 4[3]:

$$Q_P = P_0 - P_m \quad (4)$$

where P_0 (mg.L^{-1}) is the initial PO_4^3-P concentration and P_m (mg.L^{-1}) is the PO_4^3-P concentration remaining in the medium at the end of the experiment.

Statistical analysis: A two-way analysis of variance (ANOVA) was employed to compare the variables of ammonium and phosphate concentrations across different periods and concentrations. Student's t-test was used to compare ammonium (or phosphate) concentrations between the TAP and BBM media. Regression testing was conducted to assess the relationship between algae density and ammonium (or phosphate) concentration, with significance set at p-values < alpha = 0.05. All analyses were executed using the R statistical software version 4.3.1.

Results and Discussion

Comparison of Ammonium concentrations (mgN.L^{-1}) between TAP and BBM Media: After a 28-day evaluation of *Chlamydomonas reinhardtii* strains cultured in TAP and BBM mediums supplemented with NH_4^+-N stock solutions at concentrations of 0.5, 1.0, 2.0 and 4.0 mgN.L^{-1}, alongside a controlled trial without NH_4^+-N supplementation, no statistically significant differences were observed in ammonium concentrations ($p > 0.05$) (Table 3).

However, when examining NH_4^+-N processing capability at each stage (a stage lasting 7 days) under similar environmental conditions of light, temperature, pH and humidity, NH_4^+-N concentration began to gradually decrease from the 2nd week (after 14 days). At a test concentration of 4.0 mgN.L^{-1} in the TAP medium, the initial NH_4^+-N concentration of 3,254 mgN.L^{-1} had reduced to 2,704 mgN.L^{-1} by the 14th day, indicating an approximate 83% reduction.

In contrast, the BBM culture medium exhibited a decrease in ammonium concentration after 7 days of testing. The initial concentration of 4,887 mgN.L^{-1} sharply decreased to 3,194 mgN.L^{-1} (approximately 65%). Previous studies utilized TAP medium for culturing Chlamydomonas sp. in the laboratory due to its ability to support heterotrophic or photo-heterotrophic growth at room temperature, while BBM medium is rich in nutrients. The results of ammonium concentration in TAP and BBM media at 0.5, 1.0 and 2.0 mgN.L^{-1} showed minimal change compared to the initial concentration (Figure 2).

Chlamydomonas preferentially utilizes ammonium as inorganic nitrogen for growth. When ammonium is limited, other inorganic or organic nitrogen sources can be assimilated after their transformation into ammonium, subsequently incorporated into carbon skeletons by the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle. The oxidized nitrogen forms nitrate and nitrite constitutes inorganic sources efficiently assimilated after reduction to ammonium.

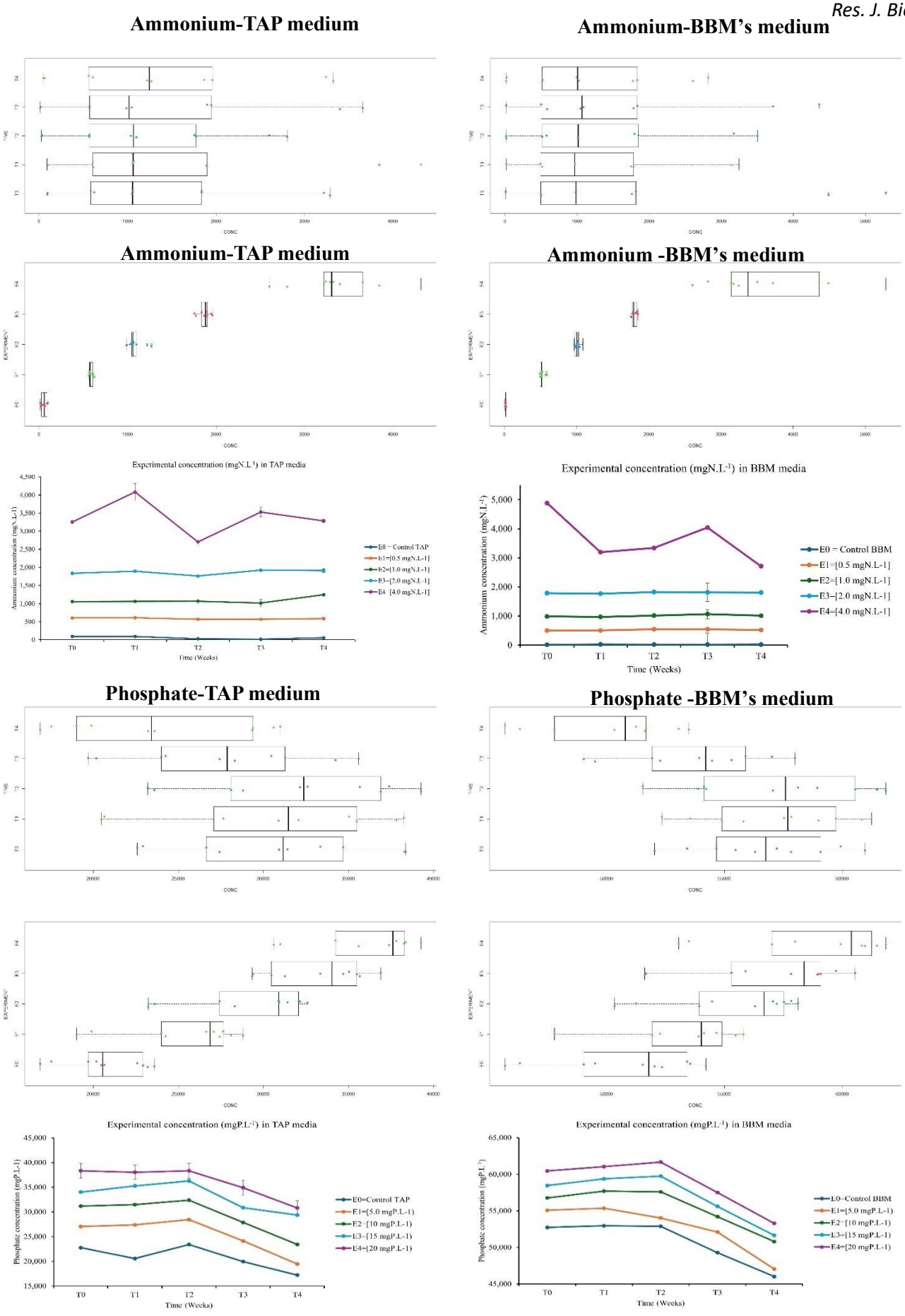

Figure 2: Comparison of Ammonium Concentration (mgN.L^{-1}) and Phosphate Concentration (mgP.L^{-1}) between TAP and BBM Media

C. reinhardtii can use most extracellular amino acids as a sole nitrogen source; however, their assimilation is strongly dependent on a supply of acetate as a carbon source. Only ammonium enters the cell readily and is subsequently incorporated; the derived oxoacids cannot be used in acetate-containing media.[32]

Chlamydomonas efficiently utilizes the inorganic nitrogen forms ammonium, nitrate and nitrite for growth. Ammonium is preferred over the oxidized compounds since its assimilation has a lower energetic cost and many genes involved in nitrate/nitrite assimilation are repressed in the presence of ammonium. Despite this, Chlamydomonas can utilize oxidized forms in the presence of ammonium under natural CO_2 conditions (air). In other words, ammonium repression is more efficient than nitrate induction at high CO_2, but nitrate can induce the assimilation route for oxidized nitrogen at low CO_2, even in the presence of ammonium. In *Chlamydomonas*, as in plants, nitrate is not only a major nutrient but also confers strong regulatory effects on both metabolic and developmental pathways and in *Chlamydomonas*, gametogenesis.[34]

Ammonia (NH_3-N) concentrations, though typically lower than ammonium (NH_4^+-N), are highly toxic and should be closely monitored. High concentrations of ammonia/ammonium stimulate plankton communities' primary production, leading to rapid algae growth and water eutrophication, which can severely impact aquatic ecosystems. The subsequent decline in algae populations increases dissolved organic matter, promoting microbial respiration and reducing dissolved oxygen levels.

Anoxic waters increase marine fish and vertebrates' sensitivity to ammonia toxicity. Ammonia and its salts are also toxic to humans, fish and crustaceans, with a disproportionately greater impact on aquatic organisms' growth and infancy. Accumulation of ammonia in fish and invertebrate larvae can lead to population extinction, threatening ecosystems and fisheries worldwide[4, 21]. Additionally, ammonia can convert into nitrite and nitrate via nitrification under certain conditions, both of which have toxic effects[7, 28]. Given the importance of ammonia nitrogen in production, life and environmental ecology, it is crucial to detect, sense and monitor ammonia nitrogen levels in various water bodies.

Furthermore, ammonia nitrogen (NH_3-N) is a vital analyte in agricultural, biotechnological and clinical industries[37], encompassing ammonia and free ammonium ions (NH_4^+-N), with composition dependent on water pH. At pH less than 8.75, NH_4^+-N predominates, while at pH greater than 9.75, ammonia nitrogen exists primarily as NH_3[24]. High ammonia concentrations can significantly impact the environment with ammonium concentrations above 20 mg NH_4^+-N per liter posing toxicity risks[20]. Apart from ammonia, urea NH_2CONH_2 and nitrite (NO_2^-) serve as nitrogen sources, although nitrite's high toxicity at elevated concentrations makes it less desirable[19].

Table 3
Comparison of ammonium concentrations (mgN.L^{-1}) and phosphate concentrations (mgP.L^{-1}) between BBM and TAP culture mediums across the 5 experimental designs over time (weeks).

Culture medium	**BBM (N=50)**	**TAP (N=50)**	**Overall (N=100)**	***p.*overall**
Ammonium (mgN.L^{-1})				
Mean (SD)	1395 (1328)	1394 (1186)	1390 (1250)	0.998
Phosphate (mgP.L^{-1})				
Mean (SD)	54957 (4116)	28905 (6255)	41900 (14100)	**< 0.001*****

Time (Weeks)	**T0 (N=20)**	**T1 (N=20)**	**T2 (N=20)**	**T3 (N=20)**	**T4 (N=20)**	**Overall (N=100)**	***p.* overall**
Ammonium							
Mean (SD)	1500 (1500)	1419 (1306)	1287 (1084)	1453 (1360)	1314 (1076)	1390 (1250)	0.984
Phosphate							
Mean (SD)	43674 (14026)	43905 (14581)	44467 (13840)	40636 (14116)	36971 (13954)	41900 (14100)	0.414

Experimental design	**E0 (N=20)**	**E1 (N=20)**	**E2 (N=20)**	**E3 (N=20)**	**E4 (N=20)**	**Overall (N=100)**	***p.* overall**
Ammonium							
Mean (SD)	36.1 (32.2)	553 (44.1)	1059 (78.0)	1832 (62.8)	3503 (682)	1390 (1250)	**< 0.001*****
Phosphate							
Mean (SD)	35764 (15613)	39071 (14492)	42331 (13765)	45057 (12566)	47431 (12063)	41900 (14100)	0.066

Comparison of Phosphate Concentration (mgP.L^{-1}) between TAP and BBM Media: Similar to the ammonium test, *Chlamydomonas reinhardtii* strains were exposed to identical temperature, light, pH and humidity conditions. Four different concentrations of phosphate stock solutions (5.0, 10.0, 15.0 and 20.0 mgP.L^{-1} respectively) were utilized, along with a control trial without phosphate stock solution supplementation. The experiment spanned 28 days, employing both TAP and BBM media. Results revealed a significant statistical difference between the two culture environments ($p < 0.001$) (Table 3). By week 2 (after 14 days), most tests at varying concentrations exhibited a decline. In the TAP medium, at a test concentration of 15.0 mgP.L^{-1}, signs of an increase were observed in the third week. In contrast, the BBM medium showed a consistent decrease in phosphate concentration at all tested concentrations over time (Figure 2).

Phosphorus is another essential macronutrient for growth, taken up by algae as inorganic orthophosphate (PO_4^{3-}). The uptake of orthophosphate is an energy-dependent process. Microalgae can assimilate excess phosphorus, storing it within cells as polyphosphate granules. These reserves sustain prolonged growth in the absence of available phosphorus. Unlike immediate responses to temperature and light, an alga's growth rate may not immediately respond to changes in external phosphorus concentration[16,41]. Since 1987, Mostert and Grobbelaar[25] found that phosphorus concentration in cells varied with supply concentration from a maximum of 1,170 mg dry mass per mg P at a supply concentration of 0.1 mgP.L^{-1} to as low as 10 mg dry mass per mg P at supplies of 5 mg P.L^{-1} and greater.

Natural water becomes eutrophic when phosphate concentrations range from 0.035 to 0.1 ppm. To control eutrophication, the Environmental Protection Agency (EPA) regulates the maximum phosphate concentration in streams not directly entering natural water at 100 µg.L^{-1}. Therefore, routine analysis of phosphate content in both wastewater and natural waters is essential.

The growth and development of microorganisms, soil-dwelling algae like *Chlamydomonas* and plants in natural environments and agricultural settings are often limited by phosphorus availability. To optimize crop yields, PO_4^{3-} is included as a significant component of commercial fertilizers. Much of this supplemental PO_4^{3-} leaches from soil and flows into nearby lakes and rivers inducing algal blooms that generate anoxic conditions and cause massive fish kills[40]. The sustainability of agricultural yields and the quality of aquatic ecosystems will benefit from more efficient use of PO_4^{3-} by crop plants, reducing farmers' dependence on rock PO_4^{3-} reserves, which can have serious economic and ecological consequences[34].

Algae are autotrophs, synthesizing organic molecules from inorganic nutrients. The stoichiometric formula for the most common elements in an average algal cell is $C_{106}H_{181}O_{45}N_{16}P$ and these elements should be present in these proportions in the medium for optimal growth[34]. High nitrogen-to-phosphorus ratio, about 30:1, suggests phosphorus limitation, whereas low ratio, about 5:1, suggests nitrogen limitation[42]. However, since wastewater often exposes algae to nutrient concentrations up to three orders of magnitude higher than under natural conditions, growth is more likely limited by carbon and light[27]. The rate at which an algal cell uptake a specific nutrient, depends on the concentration gradient and diffusion rates through the cell wall. The thickness of the unstirred water layer outside the cell wall also affects diffusion rates, with thicker layers resulting in slower diffusion rates. Turbulence in water is essential to minimize such thick boundary layers and enhance mass transfer rates of nutrients and metabolites[25, 42].

Comparison of Cell density in TAP and BBM Culture Media: To collect *Chlamydomonas reinhardtii* biomass for our experiment, we cultured it in both TAP and BBM media. Cell density was measured at the optimal 750 nm wavelength and a standard curve was used to calculate algae biomass. Biomass increased from the first to the twelfth day and then decreased from the thirteenth day onward (Figure 3). Therefore, biomass collection began on the twelfth day, maintaining equal cell density in both TAP and BBM media. The initial algae density in the TAP medium for the ammonium experiment was 2.2 x 10^7 cells. L^{-1} while in the BBM medium, it was 6.1 x 10^7 cells. L^{-1}. Results indicated significantly higher biomass productivity in the BBM medium. Most microalgae are fast-growing and produce valuable biomass suitable for various industrial applications, boasting high protein and lipid content, rendering it highly energy-efficient.

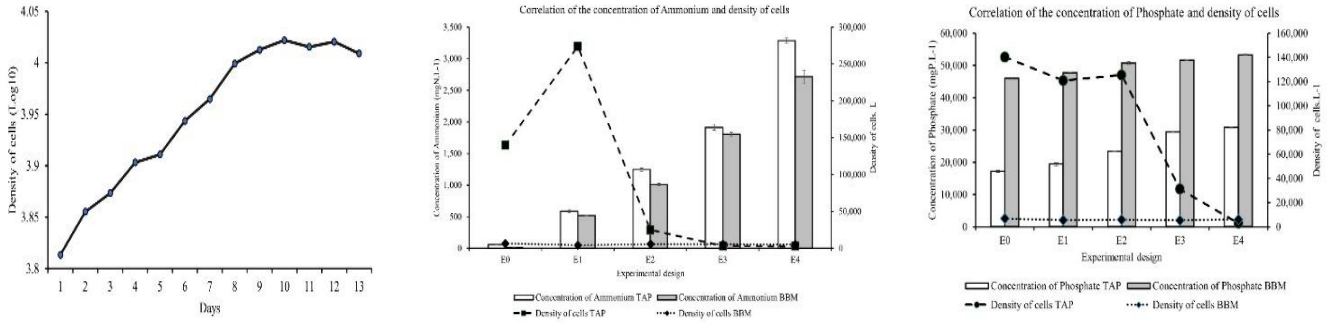

Figure 3: Biomass of algae and comparison of cell density in TAP and BBM culture media

After assessing the effects of different ammonium concentrations on *C. reinhardtii* algae growth, it was observed that at approximately 0.5 mgN.L^{-1}, algal cell density exhibited robust growth in TAP medium (2.7 x 10^5 cells. L^{-1}). However, algae density gradually decreased at 1.0 mgN.L^{-1} (0.2 x 10^5 cells. L^{-1}) and sharply decreased at 2.0 and 4.0 mgN.L^{-1}, reaching 0.3 x 10^4 and 0.2 x 10^4 cells. In contrast, algal cell density in the BBM medium did not show the same level of growth as in the TAP medium, developing weakly and slowly, ranging only from 4,000 to 5,000 cells L^{-1}. Furthermore, culturing them in an environment without ammonium supplementation also revealed that algae density in the TAP medium continued to grow stronger than in the BBM medium (density in TAP: 1.3 x 10^5; density in BBM: 0.6 x 10^4 cells. L^{-1}).

This test underscores the clear dependence of cell density on ammonium concentration in the environment, following the equation:

Density of cells = (0.9 x 10^5) – 33.94 x Concentration of ammonium ($p = 0.06 < \alpha = 0.1$).

Conversely, in the phosphate testing environment, algal cell density sharply decreased at a concentration of 15 mgP.L^{-1} (0.3 x 10^5 cell.L^{-1}) and 20 mg P.L^{-1} in TAP medium (0.2 x 10^5 cells. L^{-1}) with the equation:

Density of cells = (2.03 x 10^5) – 3.9 x Concentration of phosphate ($p = 5.5 \times 10^{-8} < \alpha = 0.001$).

Microalgae biomass has been successfully cultivated in large-scale reactors in an artificial broth medium for commercial purposes. Algae biomass production in wastewater treatment offers a cost-effective way to recycle nutrients and recover energy, as the needed nutrients are abundantly present in wastewater[13, 31]. However, wastewater is typically not utilized for commercial microalgae biomass production[26]. Using microalgae in wastewater treatment can significantly enhance biomass quantity, making it cost-effective for various industrial applications, particularly for biogas and biofuel production. Nevertheless, algal biomass grown on wastewater may not be suitable for some industries, such as food processing, or even for use as fertilizer, if the wastewater contains high levels of heavy metals and other contaminants.

Conclusion

After a 4-week experiment utilizing *Chlamydomonas reinhardtii* algae to remediate environments with elevated nutrient concentrations (ammonium and phosphate), significant findings emerged. It was noted that in the BBM culture medium, algal biomass reached three times higher levels compared to the TAP culture medium. Particularly, the highest algal biomass was observed in a culture with approximately 1.0 mgN.L^{-1} ammonium concentration and 5 mgP.L^{-1} phosphate concentration, suggesting potential applications in biomass production and aquatic animal feed. Furthermore, the efficiency of ammonium treatment in water environments using this *Chlamydomonas* strain was notably superior in the BBM medium compared to the TAP medium. At 4.0 mgN.L^{-1} concentration, ammonium levels decreased rapidly within 7 days in the BBM medium whereas it took 14 days in the TAP medium for a similar reduction.

In contrast, phosphate concentrations began declining after 14 days in both algae culture environments, across almost all tested concentrations. These findings imply the feasibility of utilizing natural nutrient sources in aquatic wastewater ponds. However, further exploration into the activities of symbiotic microbial groups is warranted, as they could significantly enhance processing efficiency.

Acknowledgement

This research is funded by the University of Science, VNU-HCM under the grant number T2023-42.

References

1. Ansari A.A., Khoja A.H., Nawar A., Qayyum M. and Ali E., Wastewater treatment by local microalgae strains for CO_2 sequestration and biofuel production, *Applied Water Science*, **7(7)**, 4151-4158 **(2017)**

2. Aslan S. and Kapdan I.K., Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae, *Ecological Engineering*, **28(1)**, 64-70 **(2006)**

3. Baird R.B., Eaton A.D. and Rice E.W., Standard Methods For The Examination Of Water And Wastewater 23th, American Public Health Association **(2020)**

4. Boardman G.D., Starbuck A.M., Hudgins D.B., Li X. and Kuhn D.D., Toxicity of ammonia to three marine fish and three marine invertebrates, *Environmental Toxicology*, **19(2)**, 134-42 **(2004)**

5. Borowitzka M.A., Beardall J. and Raven J.A., The Physiology of Microalgae, *Developments in Applied Phycology*, **6**, 673 **(2016)**

6. Brown N. and Shilton A., Luxury uptake of phosphorus by microalgae in waste stabilization ponds: current understanding and future direction, *Reviews in Environmental Science and Bio/ Technology*, **13(3)**, 321-328 **(2014)**

7. Camargo J.A., Alonso A. and Salamanca A., Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates, *Chemosphere*, **58(9)**, 1255-1267 **(2005)**

8. Chislock M.F., Doster E., Zitomer R. and Wilson A.E., Eutrophication: Causes, Consequences and Controls in Aquatic Ecosystems, *Nature Education on Knowledge*, **4(4)**, 10 **(2013)**

9. Culture collection of algae-UTEX, Available from: https://utex.org **(2024)**

10. de-Bashan L.E. and Bashan Y., Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003), *Water Research*, **38(19)**, 4222-4246 **(2004)**

11. Diniz G.S., Silva A.F., Araújo O.Q.F. and Chaloub R.M., The potential of microalgal biomass production for biotechnological

purposes using wastewater resources, *Journal of Applied Phycology*, **29(2)**, 821-832 **(2016)**

12. González L., Cañizares R. and Baena S., Efficiency of ammonia and phosphorus removal from Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*, *Bioresource Technology*, **60**, 259-262 **(1997)**

13. Gutzeit G., Lorch D., Weber A., Engels M. and Neis U., Bioflocculent algal-bacterial biomass improves low-cost wastewater treatment, *Water Science Technologies*, **52(12)**, 9-18 **(2005)**

14. Habibah N., Dhyanaputri I.G.A.S., Karta I.W., Sundari C.D.W.H. and Hadi M.C., A simple spectrophotometric method for the quantitative analysis of phosphate in the water samples, *Journal Science of Technology*, **7(2)**, 7 **(2018)**

15. Harris E.H., The Chlamydomonas Sourcebook-Introduction to Chlamydomonas and Its Laboratory Use, Elservier, 483 **(2008)**

16. Harrison P.J., Algal Cultures and Phytoplankton Ecology, *Phycologia*, **15(3-4)**, 453 **(2019)**

17. Johansen M.N., Microalgae - Biotechnology, Microbiology and Energy, Nova Science, 491 **(2011)**

18. Krzemínska I., Pawlik-Skowrónska B., Trzcínska M. and Tys J., Influence of photoperiods on the growth rate and biomass productivity of green microalgae, *Bioprocess and Biosystem Engineering*, **37(4)**, 735-741 **(2014)**

19. Li D., Xu X., Li Z. and Wang T., Detection methods of ammonia nitrogen in water: A review, *Trends in Analytical Chemistry*, **127**, 16 **(2020)**

20. Liu X., Xu Q., Guo Y., Yu X. and Deng T., Ammonia Nitrogen Speciation Analysis in Aquatic Environments, *Advances in Biological Sciences Research*, **3**, 339-341 **(2016)**

21. Mader H.S. and Wolfbeis O.S., Optical ammonia sensor based on upconverting luminescent nanoparticles, *Analytical Chemistry*, **82**, 5002-5004 **(2010)**

22. Martínez M.E., Sa1nchez S., Jiménez J.M. and Yousfi F.E., Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*, *Bioresource Technology*, **73**, 263-272 **(2000)**

23. Mitra M., A green micro-algal growth media modified for use as a stringent minimal media for bacteria, University of West Georgia, 5 **(2020)**

24. Molins-Legua C., Meseguer-Lloret S., Moliner-Martinez Y. and Campíns-Falcó P., A guide for selecting the most appropriate method for ammonium determination in water analysis, *Trends in Analytical Chemistry*, **25(3)**, 282-290 **(2006)**

25. Mostert E.S. and Grobbelaar J.U., The influence of nitrogen and phosphorus on algal growth and quality in outdoor mass algal cultures, *Biomass*, **13(4)**, 219-233 **(1987)**

26. Munoz R. and Guieysse B., Algal-bacterial processes for the treatment of hazardous contaminants: a review, *Water Research*, **40(15)**, 2799-2815 **(2006)**

27. Noue de la J., Laliberte G. and Proulx D., Algae and wastewater, *Journal of Applied Phycology*, **4**, 247-254 **(1992)**

28. Nuñez L., Cetó X., Pividori M.I., Znoni M.V.B. and del Valle M., Development and application of an electronic tongue for detection and monitoring of nitrate, nitrite and ammonium levels in waters, *Microchemical Journal*, **110**, 273-279 **(2013)**

29. Oswald W., The role of microalgae in liquid waste treatment and reclamation, In Algae and human affairs, Lembi C. and Waaland J., editors, Cambridge University Press, 255-281 **(1989)**

30. PhytoTech Laboratories, Bold's Basal Medium (BBM), Available from: https://phytotechlab.com **(2024)**

31. Rawat I., Kumar R.R., Mutanda T. and Bux F., Dual role of microalgae: Phytoremediation of domestic wastewater and biomass production for sustainable biofuels production, *Applied Energy*, **88(10)**, 3411-3424 **(2011)**

32. Rudin M.C. and Benavides A.M.S., Ammonium and phosphate removal using batch laboratory cultures by microalgae and cyanobacteria isolated from Costa Rica water bodies, *Revista de Biología Tropical*, **66**, S83-S91 **(2018)**

33. Shyla B., Mahadevaiah V. and Nagendrappa G., A simple spectrophotometric method for the determination of phosphate in soil, detergents, water, bone and food samples through the formation of phosphomolybdate complex followed by its reduction with thiourea, *Spectrochim Acta A Mol Biomol Spectrosc*, **78(1)**, 497-502 **(2011)**

34. Stern D.B., The *Chlamydomonas* Sourcebook-Organellar and Metabolic Processes, Academic Press, 1119 **(2008)**

35. Tam N. and Wong Y., Effect of ammonia concentrations on the growth of *Chlorella vulgaris* and nitrogen removal from media, *Bioresource Technology*, **57**, 45-50 **(1996)**

36. Taranger G.L., Karlsen Ø., Bannister R.J., Glover K.A., Husa V., Karlsbakk E., Kvamme B.O., Boxaspen K.K., Bjørn P.A., Finstad B., Madhun A.S., Morton H.C. and Svåsand T., Risk assessment of the environmental impact of Norwegian Atlantic salmon farming, *ICES Journal of Marine Science*, **72(3)**, 997-1021 **(2015)**

37. Timmer B., Olthuis W. and Berg V.D.A., Ammonia sensors and their applications-a review, *Sensors and Actuators B: Chemical*, **107(2)**, 666-677 **(2005)**

38. Viegas C., Nobre C., Correia R., Gouveia L. and Gonçalves M., Optimization of Biochar Production by Co-Torrefaction of Microalgae and Lignocellulosic Biomass Using Response Surface Methodology, *Energies*, **14**, 24 **(2021)**

39. Voltolina D., Gomez-Villa H. and Correa G., Nitrogen removal and recycling by Scenedesmus obliquus in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle, *Bioresource Technology*, **96(3)**, 359-362 **(2005)**

40. Wang L., Min M., Chen P., Chen Y., Liu Y., Wang Y. and Ruan R., Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant, *Applied Biochemistry and Biotechnology*, **162(4)**, 1174-1186 **(2010)**

41. Whitton B.A. and Potts M., Ecology of Cyanobacteria II_ Their Diversity in Space and Time, Springer Netherlands, 752 **(2012)**

42. Wong Y.S. and Tam N.F.Y., Wastewater treatment with algae, New York, Springer-Verlag Berlin Heidelberg, 241 (**1998)**.

(Received 05th March 2024, accepted 07th May 2024)