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Nutrient consumption of green microalgae, *Chlorella* sp. during the bioremediation of shrimp aquaculture wastewater

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ABSTRACT

Aquaculture products are among the biggest contributor to food supplies to meet the global food demands of the growing population over these past few years. For aquaculture to continue developing, an effective wastewater treatment is required to lessen the environmental effects. This study examined the potential of *Chlorella* sp. to reduce nutrients in shrimp aquaculture wastewater and correlate with the growth kinetics of the algae during the bioremediation process. Six different *Chlorella* sp. inoculation dosages ranging from 0 to 60 % (v/v) were used in this study. Marine water wastewater (MW) and Freshwater wastewater (FW) where the two types of shrimp wastewater were employed. Results indicated that the 30 % (v/v) and 40 % (v/v) were the optimum dosage for MW and FW. During the treatment, microalgae cell density increased more than tenfold compared to the initial value. Moreover, batch culture resulted in the specific growth rate concentration of 0.18 k day^{-1} and 0.15 k day^{-1}, respectively. Those dosage also resulting the highest removal efficiencies with removal of ammonia, nitrite and orthophosphate of 96.77 %, 82.07 %, 75.96 % and 90.10 %, 87.09 %, 95.60 %, respectively. The application of FTIR spectroscopy was employed in this study to analyze the functional group in the microalgae biomass. The results of the scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy Analysis (EDS) also included to further illustrate how microalgae biomass was affected by the treatment in this study. Therefore, the research from this study could be used in design novel microalgae treatments that offer a thorough and environmentally beneficial method of treating shrimp aquaculture wastewater.

1. Introduction

The rapid growth of the human population has led to the fast expansion of aquaculture industries to support the global demand. Aquaculture effluent discharge has increased dramatically over the world. Approximately 82 m^3/kg production/year estimation of wastewater generated from aquaculture industries [1]. Wastewater from the aquaculture industry has a large amount of chemical, microbial pollutants, suspended solids and nitrogenous compounds [2]. With concern to the pollution generated by aquaculture, the pollutants discharged from aquaculture industries could destroy the receiving aquatic environment such as eutrophication and deterioration towards the natural ecosystem [3]. Many technologies have been created and applied to minimize the water pollution and one of those technologies that are being developed bioremediation.

Bioremediation uses naturally existing microorganisms and alternative aspects of the natural environment to treat discharged water of its nutrients. It has been demonstrated that bioremediation is more affordable than other technologies for the cleanup of hazardous waste [4]. Algae are used in phytoremediation, a sort of bioremediation, to enhance the water quality. Bioremediation has utilized plant-based remediation such as macro and microalgae. It has been found that microalgae effectively use the nitrogen and phosphorus in wastewater for cell development. Microalgae can take up these chemicals and transform them into biomass that can be used.

Microalgae biomass has become a very promising feedstock in recent

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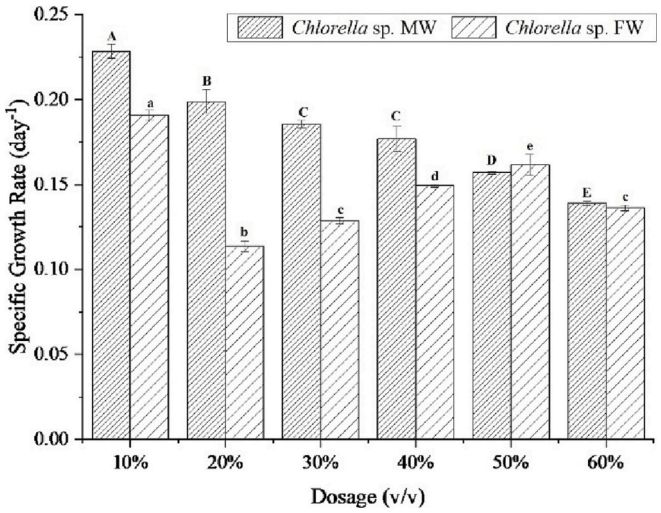

Fig. 1. Specific growth rate of *Chlorella* sp. at exponential phase during bioremediation.
Different capital letters (A-B-C-D-E) indicate significant difference of SGR among used dosage for MW while different lowercase (a-b-c-d-e) indicate significant difference of SGR among used dosage for FW.

years for sustainable biofuels such as biodiesel, bioethanol and biogas [5,6]. The increased cost required for microalgae cultivation is one of the difficulties. This is due to the continued usage of expensive chemicals like Conway or Walne fertilizers to replenish nutrients in growth media [6,7]. The production of microalgae biomass and its nutritional will be significantly influenced by the total nutrient composition and suitable nutrient concentration. Therefore, to meet the suit nutritional needs of microalgae during culture, it is necessary to replace the culture media with macronutrients and micronutrients. One viable substitute for culture media is wastewater.

Microalgae have been recognized as promising agents for improving wastewater quality while collecting nutrients from wastewater at low cost and in an environmentally friendly manner [8–10]. Additionally, heavy metal compounds and pesticides produced by industrial and agricultural wastewaters can be removed using microalgae [11]. Utilizing nutrient-rich of aquaculture wastewater as a growth medium for the development of microalgae could reduce the reliance on chemical pesticides. However, there is currently little research on the simultaneous production of microalgae and bioremediation of aquaculture wastewater. It is also yet to be determined how nutrient uptake and microalgal growth differ between fresh and marine aquaculture wastewater.

This study aims to determine the biomass yield and nutrient uptake by the *Chlorella* sp. microalgae species in aquaculture effluent during bioremediation. The ratio of microalgae and wastewater also considered as an important factor affecting the algae growth and the bioremediation performance. In addition, FTIR spectroscopy was used to examine the functional groups in the biomass of the microalgae. The organic chemical groups -OH, -COOH, NH_2, and C═O were detected in the microalgae biomass by FTIR analysis. SEM was used to characterize the shape of the microalgae cell and EDS was used to examine the chemical characterization of the nitrogen and phosphorus content in the microalgae biomass. The results of this research could enhance microalgae capacity to remove nutrients from different aquaculture effluent. Technologies based on microalgae offer a promising alternative for treating aquaculture wastewater. The success or failure of aquaculture output depends on how well water quality is maintained.

2. Materials and methods

2.1. Wastewater collection

Aquaculture wastewater was collected from the hatchery pond of shrimp, *Penaeus vannamei* for marinewater bioremediation (MW) and *Macrobrachium rosenbergii* for freshwater bioremediation (FW) at Universiti Malaysia Terengganu (UMT), Malaysia. Filtered sterile wastewater was prepared by autoclaved for 20 min at 120 °C. This method was used to ensure unnecessary species were killed. As a result, it remove other microorganisms from samples while preventing changes of the nutrient content in wastewater such as undergo the nitrification process before it was employed in the bioremediation process.

2.2. Microalgae cultivation

Pure cultivation of green microalgae genus *Chlorella* was obtained from Live feed Laboratory, Institute of Tropical Aquaculture Hatchery UMT. It was grown in Guillard's F/2 media for marine species, *Chlorella* sp. UMT LF2 and Bold's Basal Medium (BBM) for freshwater species, *Chlorella* sp. UMT LF1 under sterile conditions. Microalgae were cultures with an initial concentration of 1.0×10^5 cell·mL^{-1} algal cells. Cell density was calculated every two days using an improved Neubauer Haemocytometer. The specific growth rates (μ) of microalgae were determined during the exponential growth phase by the Eq. (1):

$$\mu = \left[\frac{ln N_2 - ln N_1}{t_2 - t_1}\right] \quad (1)$$

where μ is the specific growth rate, and N_1 and N_2 are the biomass at time 1 (t_1) and time 2 (t_2), respectively [12].

2.3. Bioremediation process

Green microalgae genus *Chlorella* was used for the bioremediation of shrimp aquaculture wastewater due to its simple cell cycle, high growth rate and having photosynthetic and metabolic pathways similar to higher plants [13]. *Chlorella* sp. were cultured until early exponential growth phase, Day 4 to Day 6 of the cultivation period. The batch bioremediation process was conducted using six different inoculation dosages: 0, 10, 20, 30, 40, 50, 60 % (v/v) with a total experiment volume of 1.5 L. The growth performance and nutrient analysis of microalgae (removal efficiency) were monitored every 2 days. The removal efficiency (%) of nutrients were determined by the Eq. (2):

$$R\ (\%) = \frac{C_o - C_t}{C_t} \times 100 \quad (2)$$

where R (%) is the removal efficiency of nutrients, C_0 (mg L^{-1}) is the initial concentration of the nutrient, and C_t (mg L^{-1}) is the final concentration of the nutrient at time t.

Aquaculture wastewater can be a major source of food requirements for microalgae cultivation and contributes to the reduction of nutrient [14]. Therefore, the uptake of nutrients, ammonia, nitrite and orthophosphate in batch bioremediation under sterile condition was studied. The initial NH_3^+, NO_2^- and PO_4^- concentrations for all seven different treatment were approximately 2.80 ± 0.05 mg L^{-1}, 1.5 ± 0.05 mg L^{-1} and 4.1 ± 0.05 mg L^{-1}, respectively for MW and 3.6 ± 0.05 mg L^{-1}, 1.75 ± 0.05 mg L^{-1} and 4.1 ± 0.05 mg L^{-1} respectively for FW before inoculated with *Chlorella* sp.

2.4. Water quality monitoring

The water quality analyses were carried out with the collection of 50 mL of the water sample from each treatment and control at 2-day interval until 14 days treatment period. The water samples were clarified by centrifugation (Hettich Zentrifugen Universal 1200, Germany) at

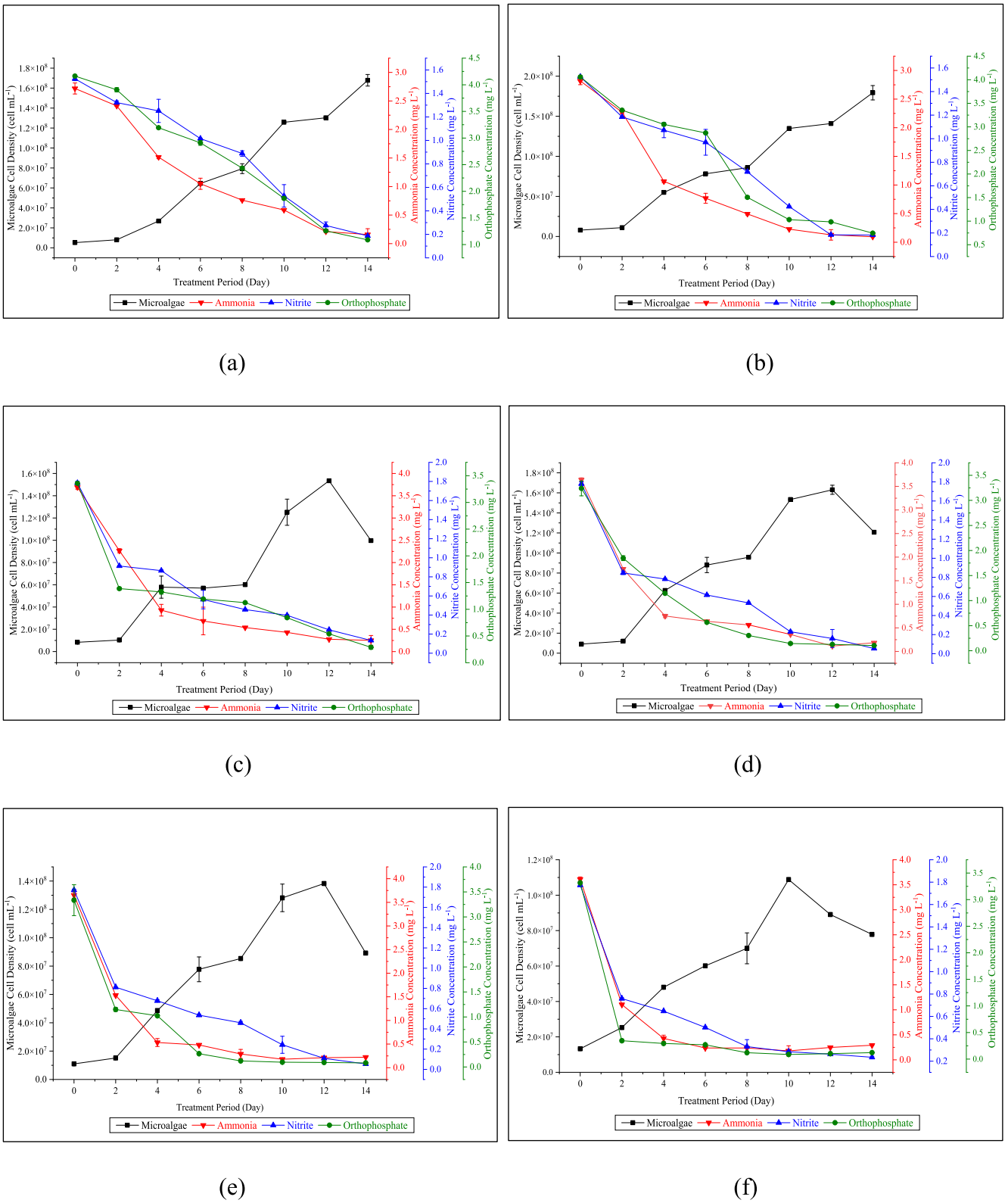

Fig. 2a. Bioremediation performance at (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, and (f) 60 % (v/v) microalgae *Chlorella* sp. inoculation dosages throughout 14-days treatment period for Marine water Treatment (MW).

9000 rpm for 5 min to separate microalgae biomass for producing clear water to perform water quality analysis. The Ammonia (NH_3^+), Nitrite (NO_2^-) and Orthophosphate (PO_4^{3-}) determination were carried out using standard methods, Phenate Method (4500-NH_3.F), Colorimetric Method (4500-NO_2^-.B) and Ascorbic Acid Method (4500-P.E) adopted from APHA (2012). The Dual-Beam UV–Vis Spectrophotometer (Shimadzu UV-1800, Japan) was used to analyze nutrients concentration.

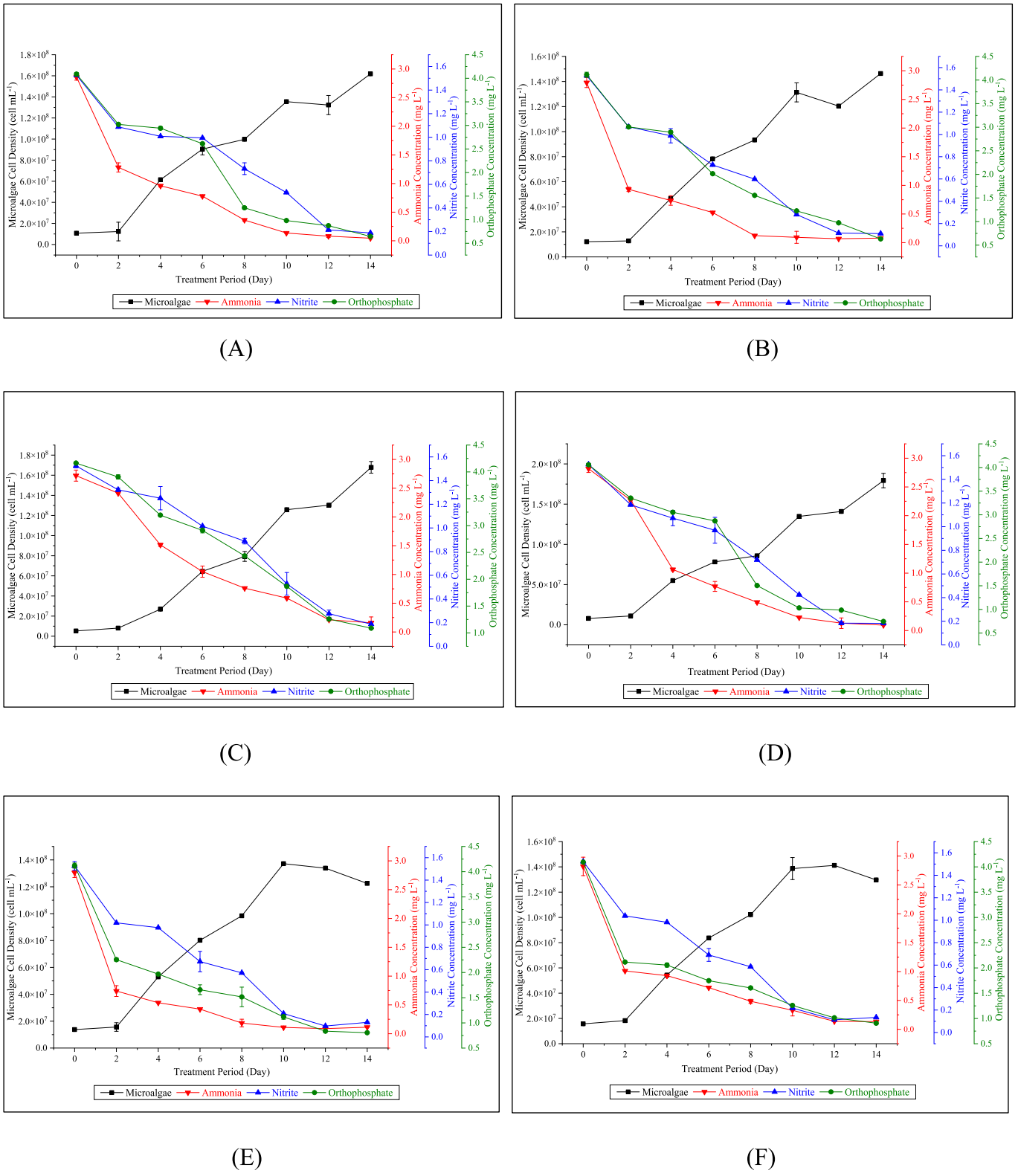

Fig. 2b. Bioremediation performance at (A) 10, (B) 20, (C) 30, (D) 40, (E) 50, and (F) 60 % (v/v) microalgae *Chlorella* sp. inoculation dosages throughout 14-days treatment period for Freshwater Treatment (FW).

2.5. Fourier Transform Infrared (FTIR) analysis

Microalgae cultures were cultured and harvested during late log phase for FTIR analysis. 100 mL of grown microalgae culture was centrifuged at 10,000 rpm for 10 min and the pellet was dried. Both the microalgae cultures, *Chlorella* sp. in media and treatment were processed. Microalgae biomass from marine cultures were rinsed with 0.5 M ammonium formate prior to centrifugation to remove salt from the biomass. The wet microalgae pellets were dried in freeze-dryer using Freezon 4.5 L −50 °C Benchtop Freeze Dryer (USA) for 24 h to form the

Table 1
The removal efficiency (%) of ammonia, nitrite, and orthophosphate for MW and FW at Day 10.

Dosage, % (v/v) / Nutrients	Ammonia (%)		Nitrite (%)		Orthophosphate (%)	
	MW	FW	MW	FW	MW	FW
0	0.77[a]	−3.05[a]	−0.11[a]	−0.17[a]	−0.01[a]	1.05[a]
10	78.36[b]	86.14[b]	65.41[b]	76.89[b]	55.19[b]	56.19[b]
20	91.94[c,d]	88.95[c]	65.23[b]	78.44[c]	74.59[c]	73.49[c]
30	96.77[c]	88.34[d]	82.07[b]	77.76[b]	75.96[c]	74.93[c]
40	95.65[c]	91.93[e]	81.63[c]	87.09[d]	70.19[d]	95.60[d]
50	96.14[c]	93.13[e]	86.42[c]	86.30[e]	72.89[e]	97.14[d]
60	88.32[d]	90.01[d]	85.72[c]	83.96[e]	69.31[d]	97.39[d]

Superscripts letter (a, b, c, d, e) refer to means for group in homogenous subset.

Table 2
The removal rate (k day^{-1}) of ammonia, nitrite, and orthophosphate for MW and FW at Day 10.

Dosage, % (v/v) / Nutrients	Ammonia (%)		Nitrite (%)		Orthophosphate (%)	
	MW	FW	MW	FW	MW	FW
0	0.0008	0.0013	0.0004	0.0013	0.0004	0.002
10	0.21	0.197	0.15	0.182	0.1	0.084
20	0.258	0.237	0.162	0.206	0.131	0.119
30	0.296	0.19	0.149	0.162	0.138	0.139
40	0.273	0.22	0.2	0.218	0.129	0.256
50	0.231	0.199	0.204	0.225	0.108	0.278
60	0.208	0.162	0.195	0.133	0.094	0.201

dried algae powders. The samples were analyzed using FTIR Spectrometer Thermofisher Scientific Nicolet™ iS™ 10 (USA). For this study, a view from the microscope was chosen from the transmission region between 4000 and 400 cm^{-1} wave number range, 4 cm^{-1} resolution and aperture of 20 × 20 μm square aperture, placed over a clear field (background) and 32 scans were taken as spectra.

2.6. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) analysis

The surface morphology of the microalgae was obtained using scanning electron microscopy analysis scan-brand Floor Top Scanning Electron Microscope (SEM) TESCAN/VEGA, CZECH REPUBLIC. The SEM was equipped with EDX BRUKER (Silicon Drift Energy Dispersive Spectrometer model Quantax Compact with XFlash 600Mini). Before the experiment, microalgae biomass was processed using the mentioned technique in FTIR analysis. To perform the analysis SEM, part of the microalgae biomass was bonded to stub with a tape of black carbon and coated with fine thin layer gold, Au to protect the sample and increase the conductivity.

2.7. Data analysis

All experiment data were analyzed in triplicate and graphical analyses were plotted using Origin 2022 software (Origin Lab Corp., USA) for the determination of interactions between factors. Statistical analyses were performed through IBM SPSS ver. 23.0. Normality and homogeneity of variances of the data were satisfied via Shapiro-Wilk test and Levene's test, respectively. Specific growth rate (SGR) of *Chlorella* sp. and removal efficiency of nutrient (%) in different concentrations (10 %, 20 %, 30 %, 40 %, 50 %, and 60 % (v/v)) inoculation in aquaculture wastewater was analyzed by One-Way Analysis of Variance (ANOVA), followed by Tukey HSD test. Results were considered as statistically significant at $p < 0.05$ in this experiment [15].

3. Results and discussion

3.1. Growth performance of microalgae

The performance of microalgae growth primarily governed by nutrients and yields of algae also can be boosted when the nutrients such as nitrogen and phosphorus are readily available in the growth medium [5]. Besides, the growth patterns of *Chlorella* sp. have depicted similar growth pattern at different dosages concentration. The growth kinetics of *Chlorella* sp. throughout bioremediation process suited with microbial growth kinetics by the growth phases of lag, exponential, stationary and declining phases [16]. Fig. 1 illustrated the growth performance of microalgae, *Chlorella* sp. by determining the specific growth rate (SGR) throughout the aquaculture wastewater bioremediation within 14 days treatment period.

The specific growth rate (SGR) of *Chlorella* sp. in this study was determined at exponential phase (Day 10). Fig. 2a and 2b shows that the specific growth rate for *Chlorella* sp. in both treatment, MW and FW. All the different treatment for MW consistently yielded the highest SGR than FW. For MW, the highest SGR (0.228 day^{-1}) was found at 10 % inoculation and lowest SGR (0.139 day^{-1}) was found at 60 % inoculation of microalgae. While for FW, the highest SGR (0.191 day^{-1}) was found at 10 % inoculation and lowest SGR (0.114 day^{-1}) was found at 20 % inoculation of microalgae. SGR for 60 % (v/v) was decreased due to the high competition between microalgae cell for limited available nutrient thus inhibiting effective absorption of nutrient into *Chlorella* sp. biomass [17].

SGR values at various inoculation concentrations (10 %, 20 %, 30 %, 40 %, 50 %, and 60 % (v/v)) were significantly different for FW with ($F = 175.029$, $p < 0.05$), while similar SGR was discovered between the inoculum concentrations of 30 % (v/v) and 40 % (v/v) via Post hoc Tukey's HSD test and Bonferroni test ($p > 0.05$). While for MW ($F = 131.133$, $p < 0.05$) and the SGR were found similar between inoculum concentration, 20 %, 30 % and 40 %. This implies that the microalgae cell growth rate was significantly affected by the amount of cell density that was inoculated [18].

3.2. Effect of microalgae concentration on nutrient consumption

The findings demostrate that microalgae can assimilate the nitrogen from a variety sources, including ammonium, nitrate, nitrite and urea [19]. Additionally, ammonia is the most energy-efficient nitrogen source since less energy is needed for its uptake. Table 1 tabulate the removal efficiency and nutrient availability for different dosages of microalgae for Day 10. According to the analysis, the ammonia concentration were significantly reduced for all different dosages both MW and FW except 0 % (v/v). However, the bioremediation performance of ammonia concentration in MV more effective than FW since the dosage of 20 % of MW already had produced 90 % removal as opposed to 40 % for FW. Generally, ammonium was the predominat nitrogen component in aquaculture, but this study also found that nitrite was present in significant amounts.

Within the first five days of the treatment period, *Chlorella* sp. bioremediation indicated low removal of nitrite and orthophosphate. However, the concentration of nitrite dramatically decreased throughout the treatment and efficiently removed >80 % when the dosages increased from 30 % (v/v) and 40 % (v/v) for MW and FW, respectively. It was noticed that the removal efficiencies were lower at lower dosages concentrations, below than 20 % (v/v). The concentration of nitrite was maintained in this study because the use of sterile microalgae culture and wastewater, without the effects of a complex microbiome that can convert the N and P concentrations. The process of nitrification was suggesting negligible throughout the treatment process. The nitrification process is the process involved the oxidation of ammonia to nitrite by ammonia-oxidizing bacteria, and nitrite to nitrate by nitrite-oxidizing bacteria.

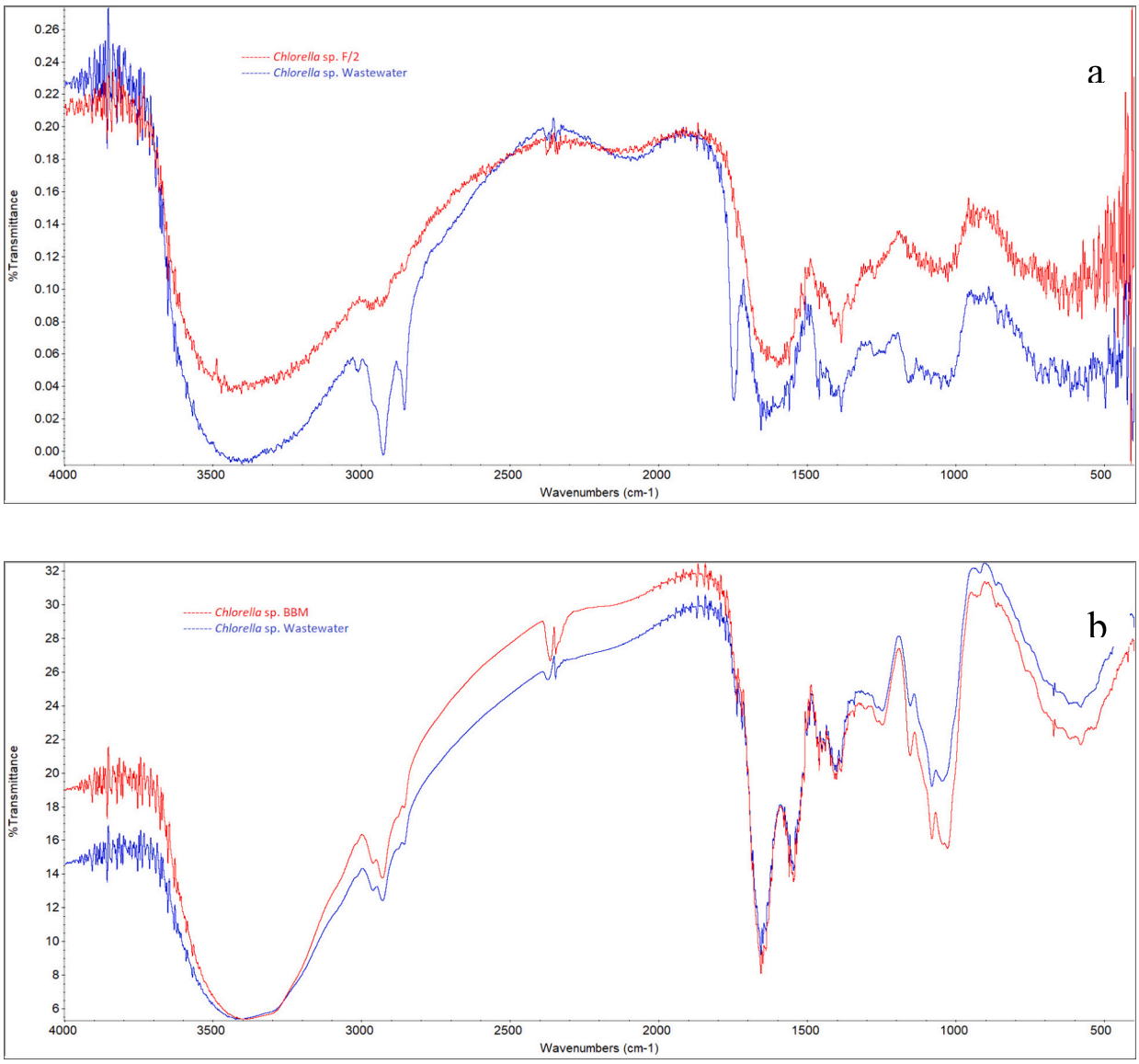

Fig. 3. FTIR Spectral image of *Chlorella* sp. culture in medium (red line) and aquaculture wastewater (blue line). a refer to marine water, Image b refer to fresh water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3a
Functional group /Assignment of *Chlorella* sp. of Marine Water (MW). References adopted from [28].

Band	Main peak (cm^{-1})	Wave number range (cm^{-1})	Typical band assignment from literature
1.	3854.3	3900–3800	-NH_2 stretching vibration
2.	3401.50	3700–3100	Water v(O—H) stretching Protein v (N—H) stretching (amide A)
3.	2926.58/ 2853.91*	3000–2800	Lipid – carbohydrate Mainly vas(CH_2) and vs(CH_2) stretching
4.	1744.87*	1800–1700	Cellulose–Fatty Acids v(C=O) stretching of esters
5.	1637.09	1700–1600	Protein amide I band Mainly
6.	1458.25	1500–1400	Protein δas(CH_2) and δas(CH_3) bending of methyl, Lipid δas(CH_2) bending of methyl
7.	1155.91	1200–900	Carbohydrate v(C-O-C) of Polysaccharides

(*) Refer to peak present at Bioremediation Process only.

Table 3b
Functional group /Assignment of *Chlorella* sp. of Fresh Water (FW). References adopted from [28].

Band	Main peak (cm^{-1})	Wave number range (cm^{-1})	Typical band assignment from literature
1.	3854.31	3900–3800	-NH_2 stretching vibration
2.	3448.02	3700–3100	Water v(O—H) stretching Protein v (N—H) stretching (amide A)
3.	2927.34	3000–2800	Lipid – carbohydrate Mainly vas(CH_2) and vs(CH_2) stretching
4.	1654.28	1800–1600	Protein amide I band Mainly v(C=O) stretching
5.	1559.70	1600–1500	Protein amide II band mainly δ(N—H) bending and v(C—N) stretching
6.	1458.38	1500–1400	Protein δas(CH_2) and δas(CH3) bending of methyl, Lipid δas(CH2) bending of methyl
7.	1079.73	1200–900	Carbohydrate v(C-O-C) of polysaccharides Nucleic Acid (and other phosphate-containing compounds) vs (>P=O) stretching of phosphodiesters

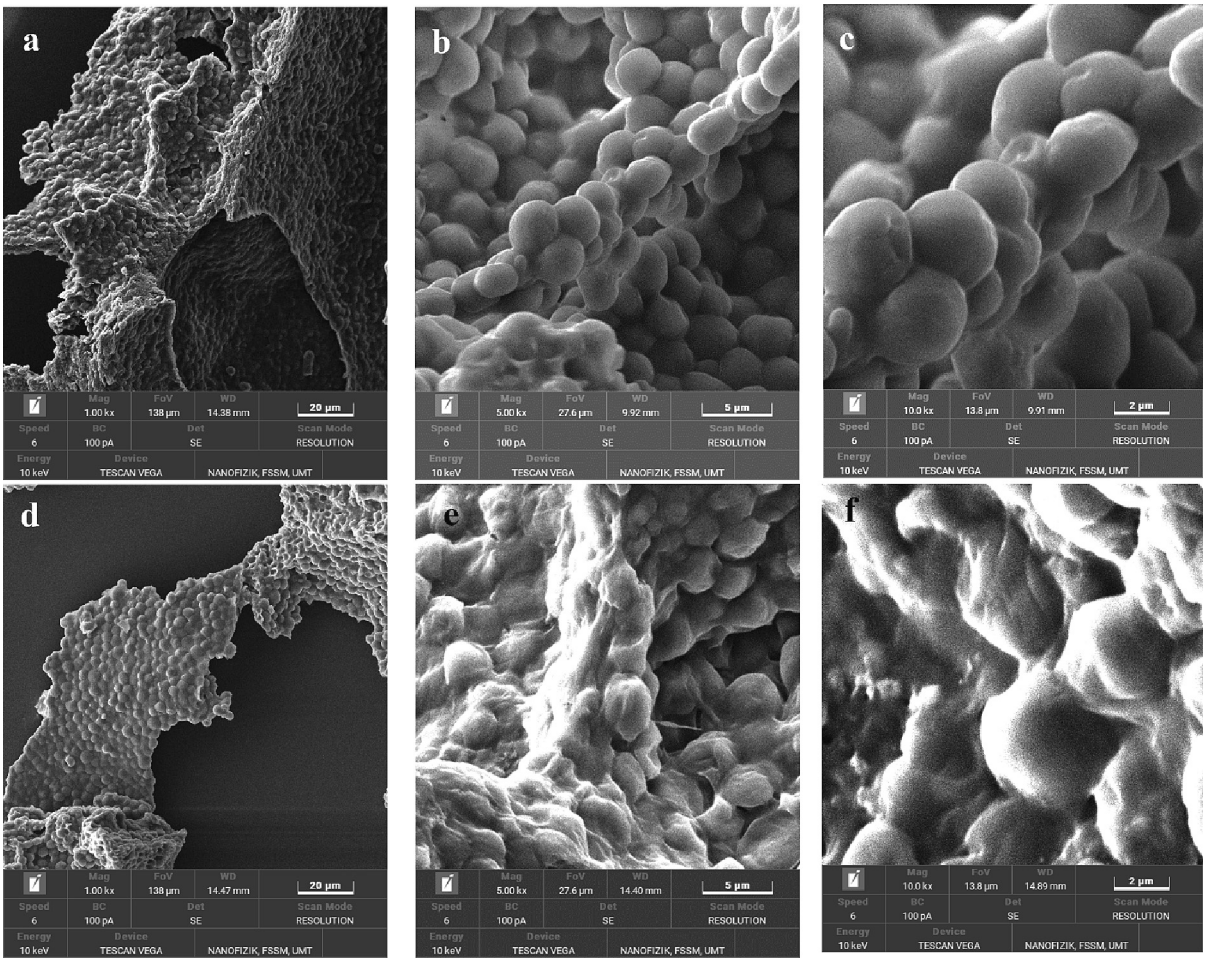

Fig. 4. SEM Image for microalgae magnification x1000, x5000, x10,000. Different letters refer to the genus *Chlorella* culture in different condition at different magnification, (a-b-c) refer to the genus *Chlorella* culture in Guillard's F/2 Media, (d-e-f) refer to the genus *Chlorella* culture in MW, (g-h-i) refer to the genus *Chlorella* culture in BBM, and (j-k-l) refer to the genus *Chlorella* culture in FW.

Phosphorus also crucial component for the growth of microalgae and frequently a major limiting factor for algal growth [20]. Typically, only orthophosphate that is assimilated by phytoplankton and can be utilize for cell development [21]. The absorbed phosphorus is usually retained as polyphosphate granules and will be useful to algae during their growth cycle. A study [22] mentioned that nutrient in the form of orthophosphate was reduced due to absorption by *Chlorella* sp. and stored as polyphosphates within the cells. Additionally, the overall findings show that phosphorus concentration in the form of orthophosphate, PO_4^{3-} for MW was eliminated with a lower removal efficiency <80 %, whereas in FW completely removed from the wastewater. This is postulated due to the green algae species like *Chlorella vulgaris* are capable of absorbing phosphorus only to a limited extent. Similarly, PO_4^{3-} removal in all treatments was higher was compared against the control.

Apart from that, the removal efficiency and removal rate of dosages 0 % (v/v) were the lowest, as could be seen in Fig. 1 and Table 1, and there were no significant differences among the other dosages for all nutrients because it was used as a control treatment and run without a microalgae inoculum.

The removal rate was positively affected by the dosage of microalgae (Table 2). The highest value of removal rate was observed different depending on the nutrients and dosages. The apparent removal rate (k. day^{-1}) at 30 % (v/v) was 0.296 k day^{-1} which is in accordance with the removal efficiency thats suggested as the maximum ammonia removal efficiency for MW. For FW, the removal rate for the 30 % (v/v) is 0.19 k day^{-1} and among the lowest compare to the other dosages. The dosage 20 % (v/v) in FW had achieved the faster rate of ammonia removal at Day 10, 0.237 k day^{-1} which remove about 88.95 % from the water sample.

As the 14 days treatment period, the dosages 30 % (v/v) was selected as the highest performance of nutrient consumption for marine wastewater treatment (MW) based on removal efficiency, with NH_3^+ and NO_2^- were 0.135 mg L^{-1} and 0.274 mg L^{-1} of nutrient availability and the removal efficiency were 96.77 % and 82.07 %, respectively. On the other hand, for the freshwater treatment (FW), the dosage 40 % was choosed as the best dosage, resulting the highest removal efficiency as compared to other dosages which were 90.10 %. 87.09 % and 95.6 % for ammonia, nitrite and orthophosphate, respectively.

For further investigation on the effect of *Chlorella* sp. inoculation concentrations on nutrients removal, the correlation analysis between growth and nutrient were performed individually on Day 10 treatment period for both MW and FW. The findings demonstrated that the positive correlation exists when nutrient concentrations decrease exponentially proportional throughout the treatment as the growth cell density rises and it's complied with the First Order Kinetic Model. This study was confirmed with the assumption make previously that the growth of microalgae was influenced by the reduction of nutrient content in wastewater.

Figs. 2a and 2b showed that the decreasing of nutrients (ammonia,

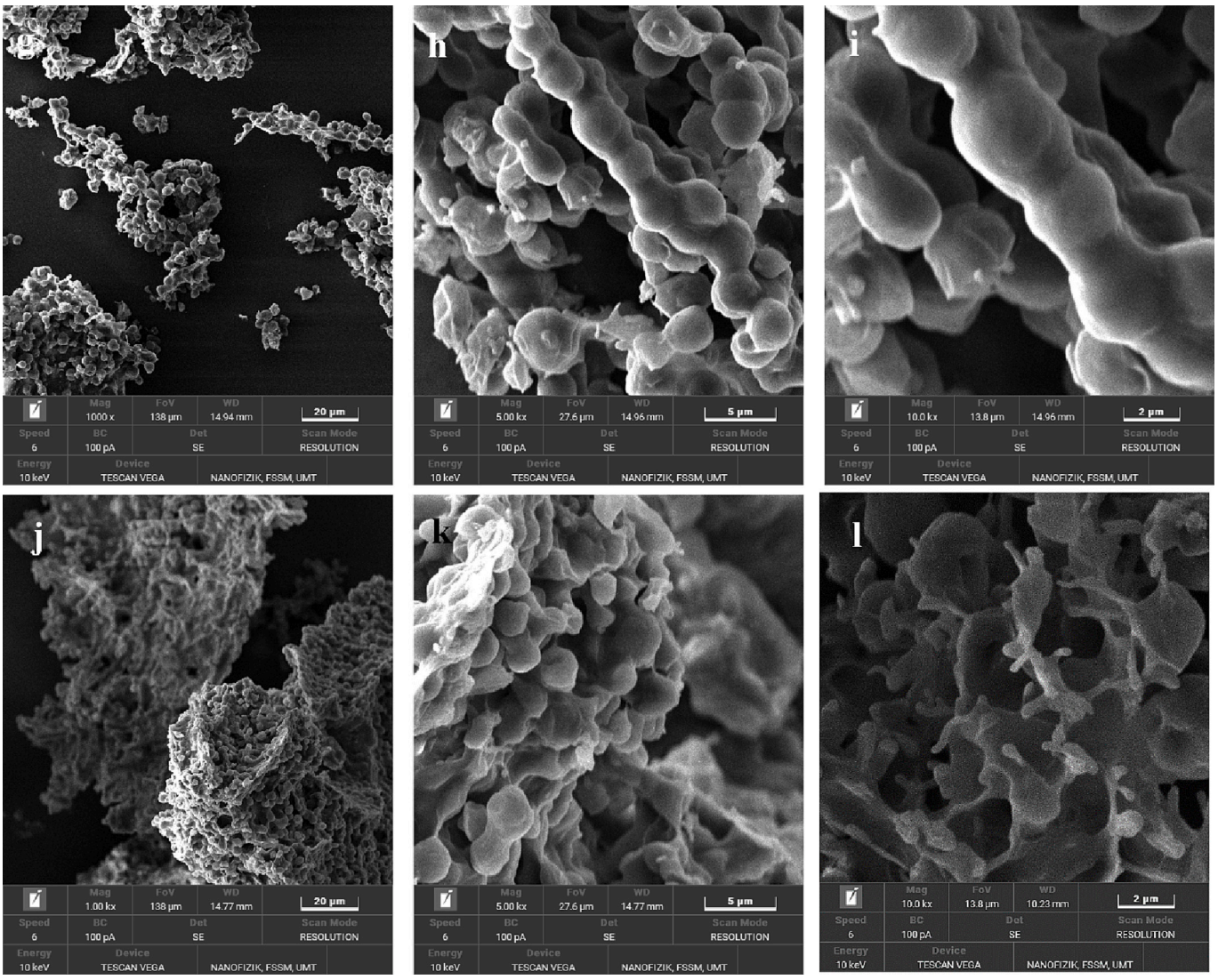

Fig. 4. (*continued*).

Table 4
Elemental identification by EDS.

Biomass sample	Contents of element by weight (%)			
	Chlorella sp. LF-2 Guillard's F/2 Media	*Chlorella* sp. LF-2 MW	*Chlorella* sp. LF-1 BBM	*Chlorella* sp. LF-1 FW
Carbon, C	73.56	58.26	53.27	57.75
Nitrogen, N	4.69	17.39	12.63	6.47
Oxygen, O	9.80	20.16	31.94	18.34
Phosphorus, P	5.87	2.94	0.97	12.18
Sulphur, S	6.08	1.24	1.2	5.17

nitrite, and ortophosphate) was in accordance with the microalgal biomass growth, suggesting the conversion of nutrient into biomass for both marine water (MW) and freshwater (FW) treatment. Similar growth pattern were depicted in the *Chlorella* sp. growth in different treatment with relatively short lag phase in the first two days and followed the exponential phase in the six to eight days. It was observed that the death phase began on Day 12 towards the end of the treatment period except 10 % and 20 % (v/v). As illustrated in Figs. 2a and 2b, the microalgae displayed a brief lag phase of one to two days when the cell concentration were increased about two-folds from the initial biomass density. Therefore, short lag phase revealed that the microalgae had excellent adaption characteristics to the aquaculture wastewater.

In the case of *Chlorella* sp., this species able to utilize both ammonium and nitrite for the syntesize of glutamine and glutamate with the involvement of glutamine synthetase (to gather energy from the breakdown of ammonium) and glutamate synthetase (to produce glutamate using nitrite) [23]. In addition to sufficient nitrogen, P also benefit the lipid content in *Chlorella* sp. followed by the increasing accumulation of poly-P inside cells [24]. The high uptake of ammonia, nitrite, and orthophosphate indicate a good utilization of nutrients by *Chlorella* sp. to support the cell growth [25]. According to the graph, the concentration of ammonia started to increase for all treatments between Days 12 and 14, especially for the 50 % and 60 % (v/v) treatments. Given that microalgae begin to enter the death phase on this day, it might be related to the microalgae's growth phase. As reported from my previous study, bioremediation using *Claries gariepinus* wastewater, this phonemonen happened due to release of absorbed nutrient from microalgae biomass as it experienced early death phase. During this growth phase, the *Chlorella* sp. biomass started to autolyze and degrade.

3.3. Characterization of microalgae morphology

3.3.1. FTIR analysis

FTIR spectroscopy played a crucial role for the characterization of the biochemical composition of phytoplankton [26]. In general, all chemical bonds have a number of bending and stretching vibrations with varying energies, which produce the various absorption bands. In addition, the composition and molecular functional groups can be determined by analysing the position, width, and intensity of infrared light absorption. The results of FTIR transmittance of microalgae biomass from wave number range of 4000–400 cm^{-1} indicates the presence of organic component groups of amine, alcohol, aromatic, alkyne, alkene, acid, ether and alkyl halide groups as well as organic contents such as carbohydrates, proteins, and lipids in *Chlorella* sp. The spectral absorption bands were identified in accordance with information that has been published.

Fig.3 shows the results of FTIR transmittance of four distinct microalgae biomass. Examinations of the infrared spectra of all biomass revealed the presence of the seven uniques bands at 3900–3800 cm^{-1}, 3700–3100 cm^{-1}, 3000–2800 cm^{-1}, 1800–1700 cm^{-1}, 1700–1600 cm^{-1} (MW only), 1600–1500 cm^{-1} (MW only), and 1200–900 cm^{-1}. This indicates that there are variances in the composition of the microlgae biomass despite the fact that they have comprable organic groups. The FT-IR spectrum of *Chlorella* sp. used in this study is similar reported by Ferreira et al. [27].

The typical band assignment from literature is summarized in Tables 3a and 3b. The band contributions were postulated from residual water (band 2), lipids (bands 3 and 6), cellulose (band 4), proteins (bands 5 and 6, 4, 5 and 6), and carbohydrate (band 7). The peaks located at 2853.91 and 1744.87 correspond to the lipid – carbohydrate and cellulose–fatty acids only obtained in bioremediation process of MW, suggesting the presence of additional constituents in nutrients from actual wastewater.

3.3.2. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy Analysis (EDS)

Advanced microscopy, such as scanning electron microscopy (SEM), is necessary to characterize microalgae [29]. After 14 days of treatment with aquaculture wastewater, the *Chlorella* sp. biomass cells were examined visually using light microscopy, SEM, as well as energy dispersive spectroscopy (EDS) to determine their elemental composition (EDS). In this investigation, SEM microscopy was employed to assess the surface features and morphological changes in the cell wall composition and shape of microalgae biomass after the bioremediation process.

Fig. 4 (a-b-c), scanning electron microscopy (SEM) visualization for all microalgae biomass revealed that cells were attached to each other. According to the findings, the sphericity and surface smoothness of microalgae particles were consistently observed throughout culture in widely used media, Guillard's F/2 Media. In contrasts, the irregular nonporous morphology with cavities on the surface of cells were discovered when subjected to bioremediation process. The *Chlorella* sp. LF1 might be associated with the component presenting in the aquaculture wastewater and created the cell-wall bound substance. This hypothesis was supported by the development of a new peak, which is demonstrable by previously findings, in FTIR analysis.

This result is further confirmed by [30] that the the surface of *Chlorella* sp. had irregular nonporous morphology with cavities on the surface after the treatment. By studying the structure of the particle, the results could serve as a foundation for understanding that the bioremediation using microalgae have affect's the cells of microalgae. The SEM analysis also revealed significant changes in the morphology of the investigated microalgae.

Characterization the chemical composition on cell surface microalgae was analysis using the combination of SEM accomplished with X-ray (EDX) (Table 4). EDS analysis is important to study since its enable to provide valuable information regarding the composition adsorbent surface for a sample. It should be highlighted that SEM provides only a qualitative evaluation of the surface structure and not able to specify the internal structure of cell [31]. When SEM is combined with EDX technique, it can provide valuable input in determining the distribution of various elements on the microalgae biomass. Tables 3a and 3b represented the result of elemental analysis of microalgae biomass. The data in terms of atomic percentages demonstrated the presence of C, N, O, P and S, which are the main components of cellular macromolecules [32].

After the bioremediation process, the percentages of N and O on the surface of the microalgae biomass showed a higher accumulation in MW, whereas C, P, and S were the lowest when compared to microalgae cultivated in Guillard's F/2 Media. In contrast to the microalgae biomass in FW, N and O were less abundant than C, P, and *S. Maximum* absorption peaks in the spectral region of lipids and carbohydrates were also produced by the greater oxygen accumulation in MW [33].

4. Conclusions

As conclusion, the results suggest that different wastewater types require different inoculation dosages for optimal bioremediation efficiency. For MW, the highest bioremediation efficiency was achieved at 30 % inoculation dosage and for FW, the highest bioremediation efficiency was achieved at 40 % inoculation dosage. This study also demonstrated that the varying concentrations of microalgae have significant impact on the growth performance of microalgae. Additionally, microalgae acknowledged able to transform nutrients; nitrogen and phosphorus from wastewater into biomass and bioproducts to boost the sustainability of wastewater treatment. Overall, the successful application of bioremediation approach was accomplished using microalgae-based by nutrient consumption from aquaculture wastewater and is relevant for future application in the aquaculture industry.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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CRediT authorship contribution statement

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Nurfarahana Mohd Nasir: performed the experiment and manuscript writing, Ahmad Jusoh: design, supervise the research project and comments on the critical part of manuscript, Nik Nor Liyana Nik Ibrahim: formatting, conduct on final revision of manuscript comments on the critical manuscript writing, Razif Harun: formatting, conduct on final revision of manuscript comments on the critical manuscript writing, Nazaitulshila Rasit: formatting, conduct on final revision of manuscript comments on the critical manuscript writing, Wan Azlina Wan Abdul Karim Ghani: supervise of the research project and comments on the critical manuscript writing. Setyo Budi Kurniawan: reviewed drafts of the paper.

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