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Original Article

Effects of fish silage on growth and biochemical characteristics of fresh water microalga *Scenedesmus* sp. MB 23

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ABSTRACT

Scenedesmus sp. MB 23 was cultivated in fish silage to study the effects of different concentrations on the growth and biochemical characteristics, particularly the protein, carbohydrate and lipid properties. Fish silage with 12% concentration was most effective for the growth and biomass production of *Scenedesmus* sp. The microalga reached maximum cell density (2433.89 $\times$ 10^4 cells/mL), chlorophyll-a concentration (2.766 μg/mL), specific growth rate (0.48/d) and biomass (2.73 g/L) on this medium. In mass culture, enhanced production of protein (123.87 mg/g dry weight of alga), carbohydrate (44.904 mg/g dry weight of alga) and lipid (84.21 mg/g dry weight of alga) was found using 9% fish silage. The effective reduction (up to 90%) in the concentrations of nitrate, phosphorus and ammonia in the final fish silage medium proved the removal efficiency of *Scenedesmus* sp. The enhanced production of *Scenedesmus* sp. MB 23 indicated that effective bioremediation of fish waste can be conducted using algal mass production in fish silage. The study also proved that microalgae grown in fish silage have great industrial potential and can be used as a source of feed and biofuel.

Introduction

Microalgae are the unicellular or simple multicellular microorganisms, with a higher ability to fix CO_2 than that of terrestrial plants and with higher biomass production compared to energy crops (Wang et al., 2008). In recent years, algal production has achieved much global attention due to its features such as potential growth, the formation of large amounts of biomass, not requiring arable land, high oil accumulation and feasible CO_2 sequestration, (Greenwell et al., 2010; Quinn and Davis, 2015; Ullah et al., 2015). Algal cultures have been developed as a promising sustainable source for various materials and high value products such as aquaculture feeds, human food supplements, pharmaceuticals and biofuels (Ruiz et al., 2016).

Scenedesmus sp. is the most widely used microalga in many research studies for industrial application as it is relatively easy to grow in culture and has the ability to survive extreme environments (Awasthi and Rai, 2006; Ravindran et al., 2016). It contains all the essential amino acids and good amounts of protein, lipid and macro and microelements (Cheban et al., 2015). *Scenedesmus* has been suggested as one of the most promising microalgae for biodiesel production because of its high lipid content (19.6–21.1%, dry weight biomass), productivity (40.8–53.9 mg/L/day), and high total fatty acid content (22.29 mg/g dry weight biomass) based on various reports (Mata et al., 2010; Jena et al., 2012; Prabhakaran and Ravindran, 2012).

Scenedesmus is one of the most efficiently growing algae in wastewater-derived substrates (Bohutskyi et al., 2015). Its ability to remove pollutants, such as inorganic salts of nitrogen and phosphorus and organic materials from waste water has revealed possibilities of using the alga in wastewater treatment plants (Makareviciene et al., 2011). Studies have suggested that the technologies used in the wastewater treatment sector can be used as effective solutions for the harvesting problems in biofuel production (Liam and Philip, 2010; Sahay and Braganza, 2016).

Previous studies have focused on culture of *Scenedesmus* in swine urine (Kim et al., 2007), carpet mill effluents (Chinnasamy et al., 2010), farm yard manure (Arumugam et al., 2011), landfill leachate (Edmundson and Wilkie, 2013), hydrolysate from crop residues (Sibi, 2015) and inorganic fertilizer (Nayak et al., 2016). These studies were mainly focused on enhancing the biomass production and biochemical characteristics such as the content of lipid, protein and various secondary metabolites. Some studies have also checked the capacity of *Scenedesmus* to remove inorganic

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nutrients and the accumulation of lipid from the wastewater (Makareviciene et al., 2011; Zhen-Feng et al., 2011; Hena et al., 2015).

Fish silage is a liquid product made from whole fish or parts of fish. It is liquefied by the action of fish enzymes in the presence of added formic acid which helps to prevent bacterial spoilage and is an excellent protein source having high biological activity and can be made from spoiled fish or commercial fish waste (Vidotti et al., 2003; Ghaly et al., 2013). The objective of the current study was to determine whether a new medium—fish silage—could enhance the growth and quality of microalga, *Scenedesmus* sp. MB 23, and alter the biochemical composition. Furthermore, the nutrient removal efficiency of *Scenedesmus* sp. from fish silage which is rich in inorganic nitrogen and phosphorus was investigated.

Methods

Culture conditions

Culture of *Scenedesmus* sp. MB 23 was used for the study that had been maintained in the Marine Botany Laboratory, Cochin University of Science and Technology, India. The microalga grown in Walne's medium (Walne, 1970) was used for the maintenance and cultivation. The algal strain was cultured in 500 mL media in 1 L Erlenmeyer flasks at room temperature (28–30 °C) with a light:dark period of 12:12 h, with a light intensity of 21 μmol photons/m^2/s. The cultures were shaken twice a day to avoid sticking.

Optimisation of culture conditions

Various culture conditions—media, salinity, pH, light and temperature—were optimized before culturing in fish silage medium. For each study, 10% inoculum containing $300 \pm 20.16 \times 10^4$ cells/mL was added to 100 mL media in 250 mL Erlenmeyer flasks. Triplicates were kept for each parameter. Growth was calculated by the estimation of chlorophyll-a using the method of Strickland and Parsons (1972).

The influence of four different culture media—Walne's medium, BG 11 medium, Chu #10 medium and *f*/2 medium—was studied on the growth rate of *Scenedesmus* sp.. The compositions of the different media are given in Table 1. A range in the salinity from 0 to 10 was chosen to determine the optimum salinity for growth and tolerance. Salinity was adjusted by adding sea water to fresh water and checked by using a salinometer (model 3G; Digi Auto; Yokinawa, Japan). The effect of pH (5–12) on the culture media was studied, with the pH adjusted by the addition of 1 N HCl and 1 N NaOH and checked using a portable pH meter with an accuracy of accuracy ± 0.01. To optimize the light intensity, the alga was cultured under three different light intensities (21 μmol photons/m^2/s, 39 μmol photons/m^2/s and 50.4 μmol photons/m^2/s). The light intensity was measured using a light meter (DT-1330; METRAVI; Calcutta, India) with a range of 200–50,000 lux and illumination was provided by standard, cool-white, fluorescent lamps. Different ranges of temperature (20–22 °C, 24–26 °C and 28–30 °C) were studied to determine the maximum growth of *Scenedesmus* sp.

Table 1
Culture media compositions used for growth of *Scenedesmus* sp. MB 23.

Constituent (mg/L dH_2O)	BG 11	f/2	CHU#10	Walne's
$NaNO_3$	15	750		1000
$Ca(NO_3)_2$			400	
$K_2HPO_4 \cdot 3H_2O$	400		50	
$NaH_2PO_4 \cdot H_20$		50		200
Na_2CO_3	200		200	
$MgSO_4 \cdot 7H_2O$	750		250	
$CaCl_2 \cdot 2H_2O$	360			
$MgNa_2EDTA \cdot H_20$	10	436		450
$FeCl_3 \cdot 6\ H_2O$		31.5	8	13
Ferric Ammonium Citrate	60			
Fe Citrate solution	10 μL			
Citric acid	60			
$CoCl_2 \cdot 6H_2O$		100		200
H_3BO_3	28.60			336
$MnCl_2 \cdot 4H_2O$	18.1	1800		3.6
$ZnSO_4 \cdot 7H_2O$	2.2	220		
$ZnCl_2$				210
$CuSo_4 \cdot 5H_2O$	790	98		200
$Na_2MoO_4 \cdot 2H_2O$	3.91	63		
$Co(NO_3)_2 \cdot 6\ H_2O$	494			
$(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$				90
Thiamine HCl (VitaminB1)		2		10
Biotin (Vitamin H)		10		
Cyanocobalamin		10		0.50

Collection and preparation of fish silage media

Fish silage is a common organic manure available along the Kerala coast, India and is produced from fish waste. The soluble nutrients in fish silage are given in Table 2. The fish silage used in this study was obtained from The 'Jaivashree' activity group, Pallipuram Panchayath, Ernakulam, Kerala, India. Suitable amounts of fish silage were weighed and diluted in sterile water to obtain the stock solution. The media were sterilized in an autoclave at 121 °C for 20 min and cooled to room temperature prior to use. The fish silage stock solution was diluted with sterilized fresh water to give 3%, 6%, 9%, 12%, 15% and 18% fish silage media. Walne's medium (Walne, 1970) was kept as the control medium to compare the growth and biochemical characteristics. All cultivations were done in 250 mL Erlenmeyer flasks at room temperature and placed under white fluorescent light (light intensity of 36 μmol photons/m^2/s) with a light:dark cycle of 12:12 h. An amount of 10% inoculum containing 300.4×10^4 cells/mL was added to 100 mL of media. Triplicate cultures were prepared for every medium.

For mass culture, 12 L fibre tanks were filled with 10 L of media and were inoculated with 10% inoculum containing 301.9×10^4 cells/mL. The culture was maintained under room temperature and illuminated with a light intensity of 39 μmol photons/m^2/s provided by standard, cool-white, fluorescent lamps for a light:dark period of 16:8 h. The culture tanks were aerated using an electric aerator fitted with an air filter to avoid contamination.

Analytical methods

The growth was calculated by the estimation of chlorophyll-a (Strickland and Parsons, 1972), and the cell concentration of each sample was measured by counting the cell number every third day. The biomass content in 100 mL cultures was calculated at the end of cultivation. After 30 d of cultivation, nitrate (NO_3–N) was estimated using the resorcinol method (Zhang and Fischer, 2006), nitrite (NO_2–N), phosphorous (PO_4–P) using the method of

Table 2
Composition of fish silage.

Component (soluble)	Amount (%)
Nitrogen	2.5–4.5
Phosphorous	1.5–3.0
Potassium	1.5–2.5
Ash having micronutrients	15.0–18.0
Moisture	10.0–12.0

Strickland and Parsons (1972) and ammonia (NH_3–N) using the method of Emmet (1968).

For the analysis of various biochemical factors such as protein, carbohydrate and lipid, 100 mL algal sample from each concentration of mass-cultured alga was centrifuged at 10,000 rpm for 10 min at 4 °C. The residue was again centrifuged with distilled water and the content was filtered through 1 μm pore size GF/F filter paper. The total protein of the sample was analyzed using Lowry's method (Lowry et al., 1951), total carbohydrate was estimated using the phenol sulfuric acid method (Dubois et al., 1956) and lipid was estimated using the modified Barnes and Blackstock (1973) method. Algal growth was expressed in terms of the specific growth rate in microns/day which was calculated from increase of active chlorophyll per unit time as per the modified method of Pirt (1975) and shown in Equation (1):

$$\text{Specific growth rate} = \ln [(N_1 - N_0)/(t_1 - t_0)] \quad (1)$$

where N_0 and N_1 are the quantitative expression of the active chlorophyll of cells at the beginning (t_0) and the end (t_1) of the selected time interval during incubation.

Statistical analysis

The software programs Excel (version 2013; Microsoft; Redmond WA, USA), and IBM SPSS (version 22.0; IBM Corp.; Armonk, NY, USA) were used to analyze the results obtained from various studies and for testing the presence of significant differences ($p > 0.05$) among different parameters.

Results and discussion

Optimal conditions for the growth of Scenedesmus sp. MB 23

The maximum growth rate (0.208/d) of *Scenedesmus* was obtained in the Walne's medium with maximum chlorophyll-a. Even though Walne's was a marine culture medium, it had a higher growth rate over BG 11 (the common freshwater medium) perhaps because of the high nitrate content (Fig. 1A). *Scenedesmus* sp. showed good salinity tolerance from 0 to 8 parts per trillion (ppt) but the maximum chlorophyll-a concentration and specific growth rate (0.260/d) was obtained at 0 ppt salinity (Fig. 1B). Studies by Makareviciene et al. (2011) and Kaewkannetra (2012) have reported the salt tolerance of *Scenedesmus* and they observed a decreased biomass with increased salinity.

A pH level of 7–10 was found to be good for cell growth and chlorophyll-a concentration. However, the maximum specific growth rate (0.309/d) was obtained at pH 10 (Fig. 1C). It has been reported that *Scenedesmus* sp. can grow well at different pH levels due to some physiological metabolism by which the alga can adjust the pH value to achieve proper growth (Xiao et al., 2011). In the optimization of light intensity, it was observed that the algal specific growth rate (0.282757/d) was highest at 39 μmol photons/m^2/s (Fig. 1D). At a lower intensity of light, (21 μmol photons/m^2/s) the lag phase was delayed and at a higher intensity (50.4 μmol photons/m^2/s), the alga attained an early lag phase but the growth and cell size were comparatively low. An increase in the light intensity can lead to photo-oxidation which leads to decreased photosynthesis and productivity (Richmond, 2004).

Statistical analysis showed that there were no significant differences in the growth of *Scenedesmus* sp. MB 23 under different temperature ranges but the maximum specific growth rate (0.1895/d) was by cells grown in 24–26 °C (Fig. 1E). Xin et al. (2011) obtained similar results in a study conducted on *Scenedesmus* sp. LX1.

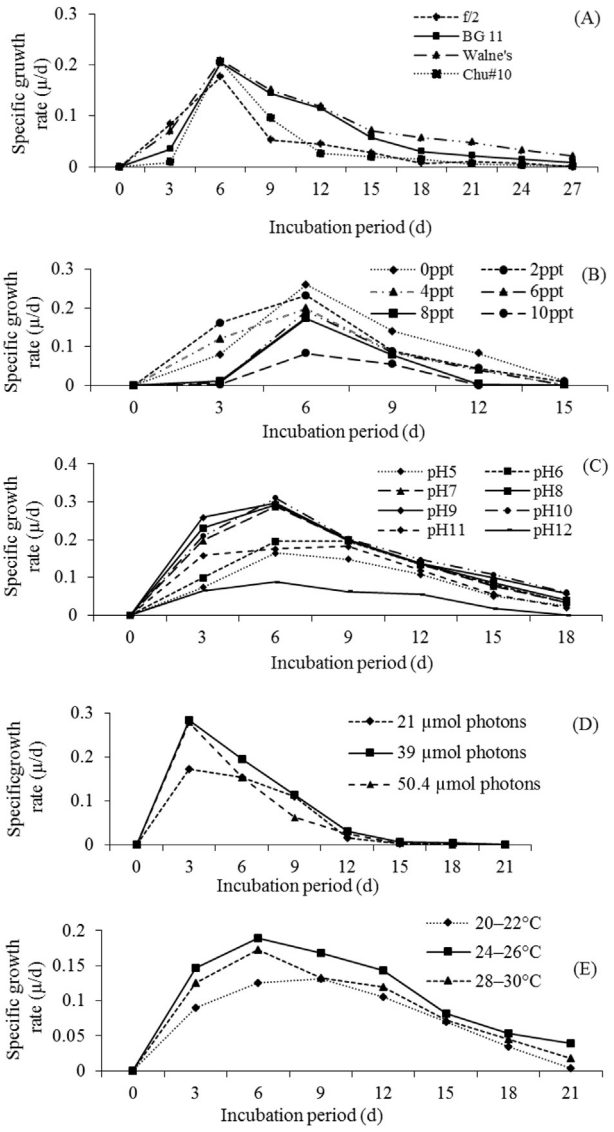

Fig. 1. Optimization of culture conditions. Specific growth rate in terms of chlorophyll-a were plotted for: (A) optimization of media; (B) salinity in parts per trillion (ppt); (C) pH; (D) light; (E) temperature.

Growth rates of Scenedesmus sp. MB 23 in fish silage medium

In recent years, the biofuel production from algal biomass commercially cultivated in inorganic fertilizers, by-products of anaerobic digestion, artificial drainage waters, manure containing a high nutrient content (such as pig and poultry manures) has gained wide attention (Fenton, 2012). The current study used a novel approach to cultivate *Scenedesmus* sp. in fish silage to attempt to use this as a medium for micro-algal cultivation. The growth of *Scenedesmus* sp. in different concentrations of fish silage media (3%, 6%, 9%, 12%, 15%, 18%) was checked against Walne's medium as the control.

In 100 mL cultures, the cell density (2433.89×10^4/mL) was found to be higher in media with 12% fish silage concentration (Fig. 2A). At 3% and 6% fish silage concentrations, cell numbers were less than in the Walne's medium and the least cell density was observed in the 18% fish silage medium. The maximum chlorophyll-a concentration (2.766 μg/mL) was obtained in 12% fish silage medium on day 9 (Fig. 2B). The highest two specific growth rates were observed in 12% (0.48/d) and 9% (0.46/d) silage which were

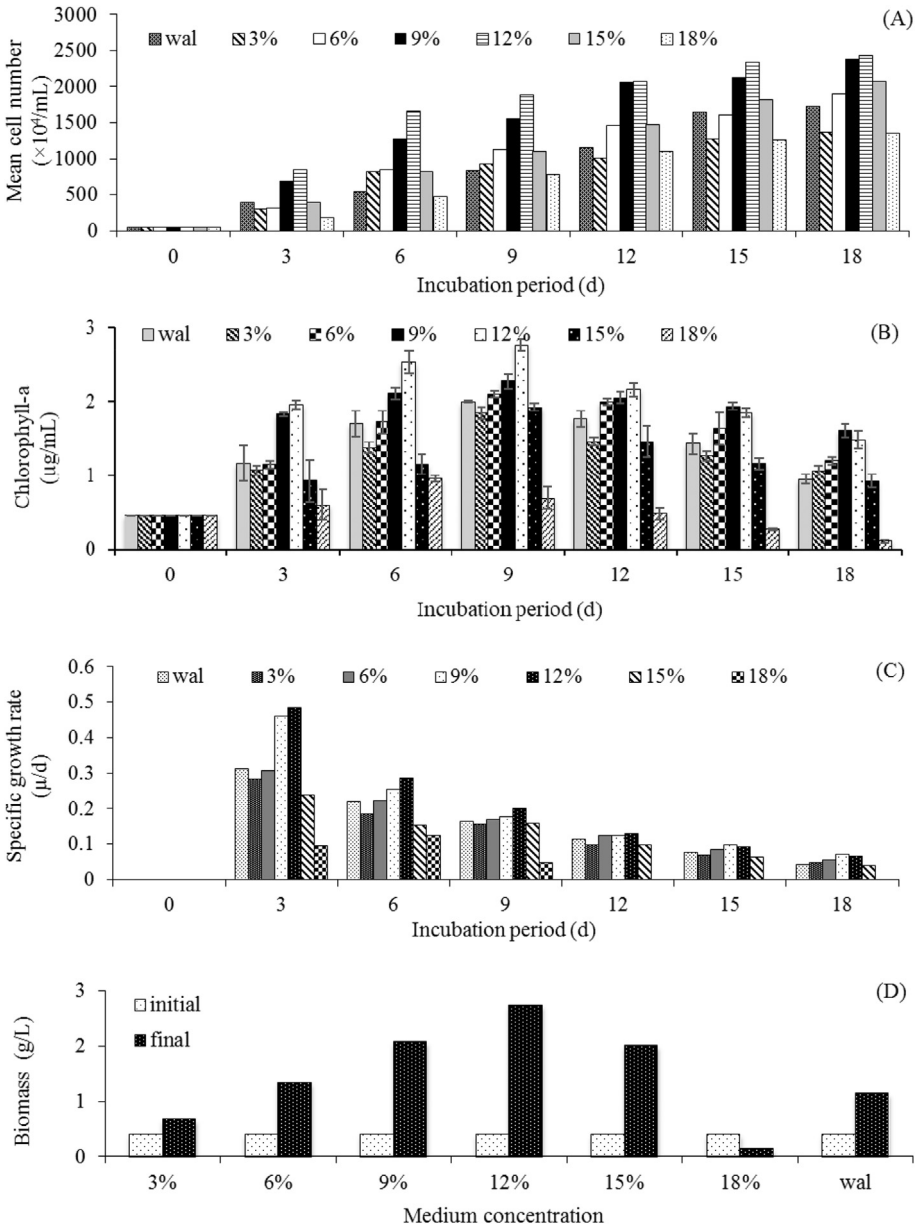

Fig. 2. Growth rate of *Scenedesmus* sp. MB 23 cultured on 100 mL different concentrations of fish silage and Walne's medium (Wal) as the control: (A) mean cell number; (B) chlorophyll-a value (mean ± SD); (C) specific growth rate in terms of chlorophyll-a; (D) biomass.

comparatively higher than that obtained using Walne's media (0.31/d). The 3% silage medium (0.28/d) and 15% silage medium (0.23/d) had lower growth rates, but with 6%, the rate was almost the same as the growth in Walne's medium (Fig. 2C). The results proved that a lower concentration of fish silage was well suited to culturing *Scenedesmus*, and at higher concentrations of silage, the alga was killed, perhaps due to the color development which resisted light penetration and thus photosynthesis was inhibited (Hena et al., 2015). The biomass after 30 d cultivation showed a sevenfold increase in the 12% concentration (2.73 g/L), but there was only a threefold increase (1.33 g/L) in Walne's medium, (Fig. 2D). It was noted that the cultures remained active for a period of 4 mth without any subculturing.

In large-scale culture (Fig. 3A–C), 3% silage concentration and Walne's medium had similar growth, but growth was lagged in the 6% and 9% concentrations. In the 12%, 15% and 18% concentrations of fish silage, there was no growth until day 9. However, the cell density increased on day 12 as the alga became acclimatized to the high silage concentrations. After day 15, the *Scenedesmus* sp. in the 9% fish silage had a higher chlorophyll-a content (1.540 µg/mL) than in the control medium (Fig. 3B). The cell count was also higher in the 9% silage medium (494.893 × 10^4 cells/mL). Then after 15 d of cultivation, it was noted that there was an increase in the growth of the alga with the higher silage concentration. Statistical analysis showed that there was a significant difference in the growth of *Scenedesmus* sp. at the different concentrations of fish silage media compared to the Walne's medium. The variation in the results compared to those in 100 mL cultures might have been be due to settling of sediments at the higher fish silage concentrations when used in large scale cultivation. Thus, a pre-fermentation treatment could be effective to synchronize the growth at various concentrations (Kim et al., 2007).

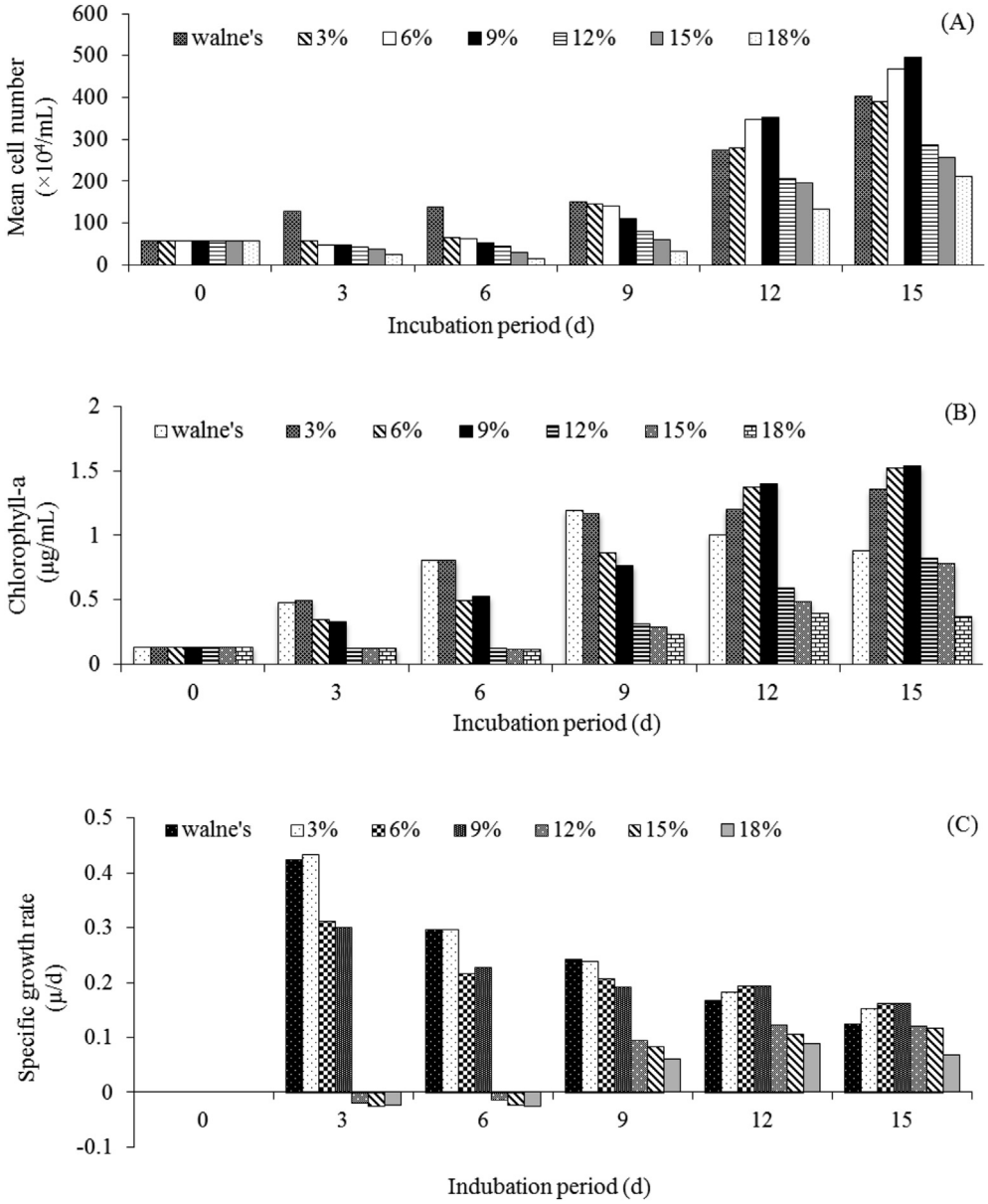

Fig. 3. Growth rates of *Scenedesmus* sp. MB 23 in mass culture at different fish silage concentrations and Walne's medium (Wal) as the control: (A) algal growth in terms of cell density; (B) chlorophyll-a; (C) specific growth rate in terms of chlorophyll-a.

The specific growth rate varied from 0.30/d to 0.48/d at different fish silage concentrations (Fig. 2C), which was found to be higher than that reported by Nayak et al. (2016) in inorganic fertilizer media, while Zhou et al. (2011) reported a higher growth rate (0.498/d) for *Scenedesmus* in municipal wastewater. The final biomass obtained using fish silage was comparatively greater than all the other findings. The 12% fish silage, had a sixfold increase (2.73 g/L) in the initial biomass (0.4 g/L) compared to the control (1.13 g/L). Mandal and Mallick (2011) reported biomass levels of 2.0, 2.2 and 2.3 g/L from cultivating in N-11 medium supplemented with fishpond discharge, municipal tank discharge and poultry litter, respectively.

Kim et al. (2007) reported that the optimal growth period of *Scenedesmus* for commercial cultivation was approximately 40 d. In the 100 mL cultures, it was observed that the cells grown in the control medium (Walne's medium) changed color to yellow after 2 mth while the cells at all concentrations of fish silage were active for more than 4 mth. These results were similar to those from the study by Kim et al. (2007), where the cells grown in a newly introduced swine urine medium (KEP1 medium) retained a green color for about 5 mth.

Nutrient removal in the culture media

The current study may have been the first attempt to address the possible industrial application of fish silage as a culture medium and thereby reducing the negative effects of fish waste causing eutrophication. The concentrations of different nutrients such as nitrate, nitrite, ammonia and phosphate were analyzed in the media before and after the growth of alga, to test the ability of the alga to absorb and accumulate these nutrients in the cell. Since fish silage is composed of fish waste, the medium is rich in nutrients. The percentage of nutrients decreased considerably after 30 d of growth (Fig. 4A and B). The level of nitrate was significantly

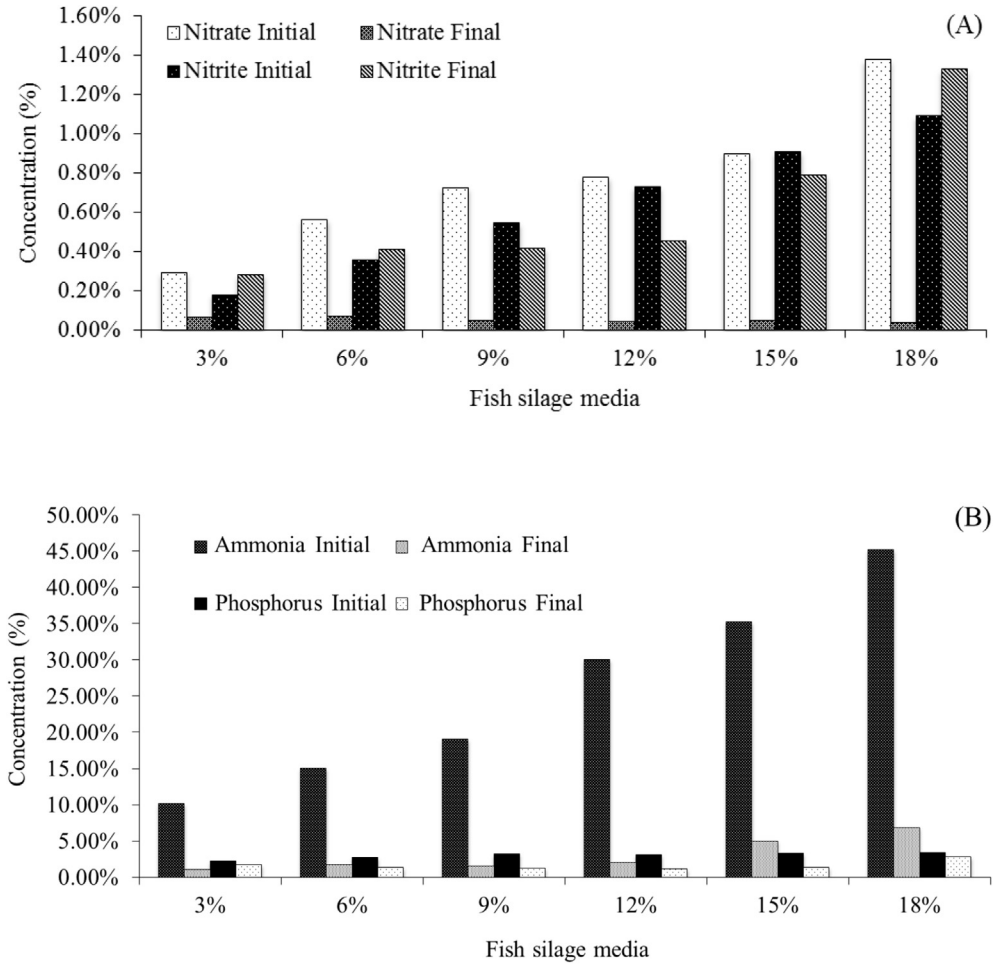

Fig. 4. Nutrient analysis of fish silage after 30 d growth: (A) nitrite and nitrate concentrations; (B) phosphorus and ammonia concentrations.

reduced in all media. However, the level of nitrite increased in the 3% and 18% media. Ammonia was found to be present in high concentrations in all the silage media. Nonetheless, after 30 d of algal growth, the level of ammonia had significantly decreased at all concentrations except for the 18% and 15% fish silage.

The level of phosphorus also showed a reduction, but in the 18% and 3% concentrations the reduction was not significant (a decrease from 2.21% to 1.73% at the 3% silage concentration and from 3.38% to 2.84% at the 18% silage concentration). The nitrate, phosphorus and ammonia concentrations in the fish silage were effectively reduced (up to 90%) in the final medium when compared with the initial medium composition. Makareviciene et al. (2011); Hena et al. (2015) and Gupta et al. (2016) also reported the same trends. However, in the present study, a significant increase was noted in the percentage of nitrite and this could have been due to the conversion of ammonia to nitrite and this nitrite could not be effectively converted to nitrate by the alga since nitrate was readily available. The results agreed with the findings of Park et al. (2010), that *Scenedesmus* does not differentiate between ammonium and nitrate as a nitrogen source, so the alga can be effectively utilized for treatment of ammonia-rich wastewater.

The initial and final pH levels increased from 6.7 to 9.7. Jena et al. (2012) reported that the pH increased from an initial value of 6.8–11.5 due to the utilization of CO_2 which led to the accumulation of free OH^- ions in *Scenedesmus* sp. An elevated pH level can also enhance the removal of ammonia by volatilization and of phosphate by precipitation (Garcia et al., 2000; Craggs, 2005). Xin et al. (2010) also reported that in a pH above 7 *Scenedesmus* sp. grew faster with ammonium as a nitrogen source than with urea or nitrate.

Biochemical enhancement in fish silage

The biochemical analysis in the large scale cultivation of *Scenedesmus* sp. that was done every 2 d showed that there were no significant differences in the biochemical composition of cells grown in Walne's media and at lower fish silage concentrations. However, significant differences were observed in the biochemical composition between higher and lower fish silage concentrations. The 9% fish silage had the highest protein concentration (123.87 mg/g) and the highest carbohydrate concentration (44.43 mg/g) on day 14 of culture (Fig. 5A and B). The control medium and lower concentrations of fish silage (3%, 6%) produced higher concentrations of protein in the log phase and lower concentrations in the stationary phase but the carbohydrate level was lower in the log phase and higher in the stationary phase. Cheban et al. (2015) reported 23% total protein in recirculating aquaculture system wastewater and Zuliani et al. (2016) reported 31.1% in sewage sludge.

The total lipids in the log and stationary phases did not vary much in Walne's medium and in 3% fish silage. However, in the 6% and 9% silage concentrations, the highest lipid contents (84.00 mg/g and 84.21 mg/g, respectively) were observed on day 14 (Fig. 5C). The lipid content at higher concentrations of silage increased from day 12 onward. Despite the lower growth rate at these concentrations, the algal lipid content was higher, perhaps because of the

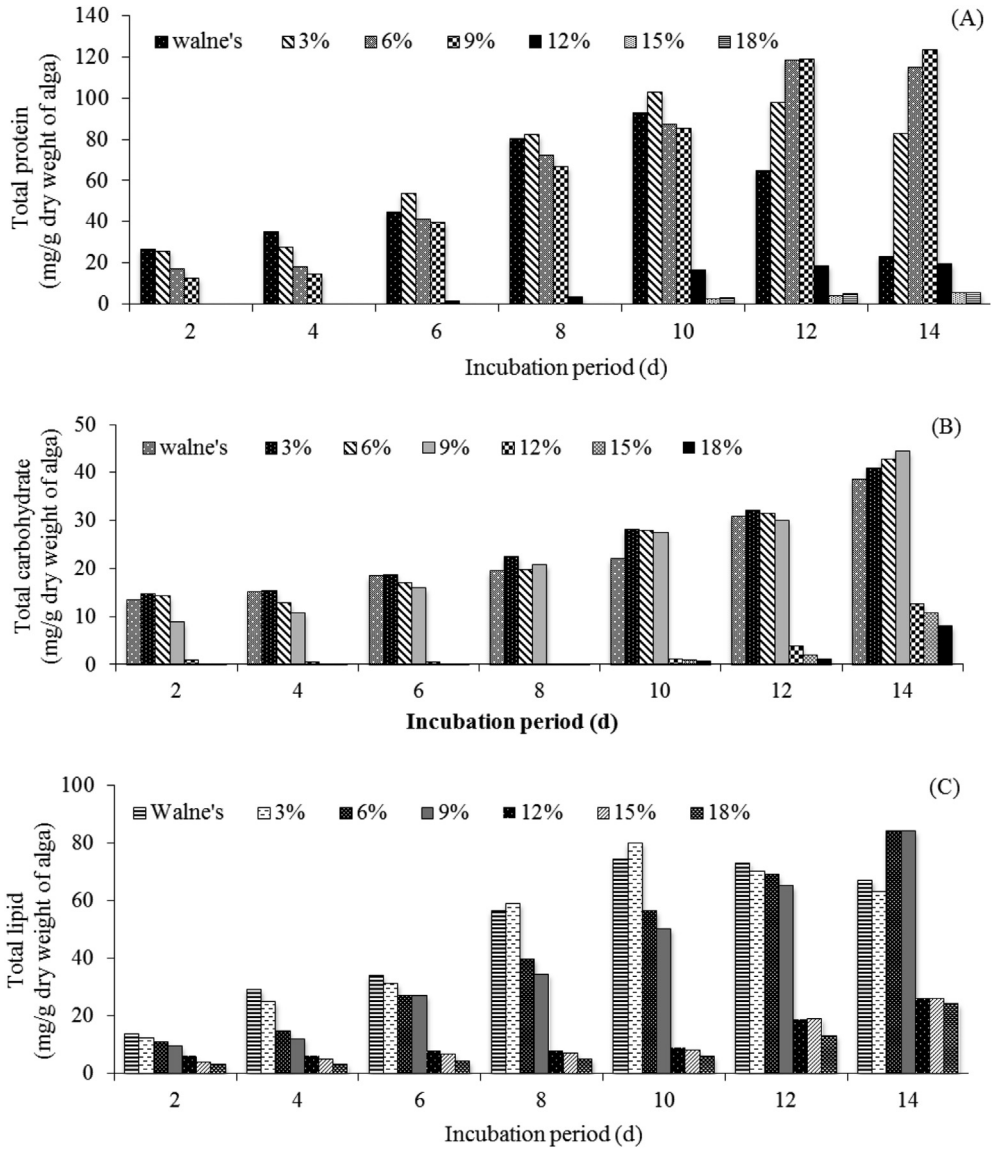

Fig. 5. Analysis of biochemical production by *Scenedesmus* sp. MB 23 at different concentrations of fish silage and Walne's medium (Wal) as the control: (A) protein; (B) carbohydrate; (C) lipid.

presence of large-sized single cells of *Scenedesmus* in these media. Minhas et al. (2016) reported that high physiological stress led to high lipid accumulation. Many scientists have reported the lipid productivity of *Scenedesmus* when cultured in wastewater. For example, Sacristan de Alva et al. (2013) reported lipid accumulation of 280 mg/L when *S. acutus* was cultured in treated wastewater, while Gupta et al. (2016) reported 23.26 ± 3.95% weight per weight of lipid in wastewater and Nayak et al. (2016) obtained 28.55% lipid accumulation in inorganic fertilizer medium. Even though the current study was conducted for only 15 d, a high yield of lipid was produced, which would definitely increase during further cultivation. This proves fish silage as a promising medium to grow *Scenedesmus* for high lipid production.

The study attempted to utilize a low cost but nutritionally rich culture medium and to enhance the biochemical characteristics of *Scenedesmus* sp. MB 23. The study introduced fish silage as an efficient culturing medium for microalgal cultivation with a high biomass, chlorophyll-a content, cell number and specific growth rate. The culture was maintained for many months in fish silage without any addition of culturing medium and enhanced the biochemical properties of the alga, including the lipid content (296.62 mg/g dry weight of alga), which suggested fish silage as a low-cost medium for biofuel production. As silage is composed of fish waste, the media could ameliorate environmental damage through cultivating *Scenedesmus* sp., (the most popular alga in the phyco remediation process). The enhanced production of algal biomass in the current study indicated that the alga grown in fish silage has great industrial potential and can be used as a source of feed and biofuel.

Conflicts of interest

None declared.

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References

Arumugam, M., Agarwal, A., Arya, M.C., Ahmed, Z., 2011. Influence of organic waste and inorganic nitrogen source on the productivity of *Scenedesmus* and *Chlorococcum* sp. Int. J. Energy Environ. 2, 1125–1132.

Awasthi, M., Rai, L.C., 2006. Interactions between zinc and cadmium uptake by free and immobilized cells of *Scenedesmus quadricauda* (Turp.) Berb. Acta Hydrochim. Hydrobiol. 34, 20–26.

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilin method for total lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.

Bohutskyi, P., Liu, K., Nasr, L.K., Byers, N., Rosenberg, J.N., Oyler, G.A., Betenbaugh, M.J., Bouwer, E.J., 2015. Bioprospecting of microalgae for integrated biomass production and phytoremediation of unsterilized wastewater and anaerobic digestion centrate. Appl. Microbiol. Biotechnol. 99, 6139–6154.

Cheban, L., Malischuk, I., Marchenko, M., 2015. Cultivating *Desmodesmus armatus* (Chod.) Hegew. in recirculating aquaculture systems (RAS) wastewater. Arch. Pol. Fish. 23, 155–162.

Chinnasamy, S., Bhatnagar, A., Hunt, R.W., Das, K.C., 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour. Technol. 101, 3097–3105.

Craggs, R.J., 2005. Advanced integrated wastewater ponds. In: Shilton, A. (Ed.), Pond Treatment Technology. IWA Scientific and Technical Report Series. IWA, London, UK, pp. 282–310.

Dubois, N.K., Gilles, A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method of determination of sugars and related substances. Anal. Chem. 18, 350–356.

Edmundson, S.J., Wilkie, A.C., 2013. Landfill leachate: a water and nutrient resource for algae-based biofuels. Environ. Technol. 34, 1849–1857.

Emmet, R.T., 1968. Direct Spectrophotometric Analysis of Ammonia in Natural Water by the Phenol-hypochlorite Reaction. Naval Ship Research and Development Center, Washington. D.C., p. 2570. Rep.

Fenton, O., 2012. Agricultural nutrient surpluses as potential input sources to grow third generation biomass (microalgae): a review. Algal Res. 1, 49–56.

Garcia, J., Mujeriego, R., Hernandez-Marine, M., 2000. High rate algal pond operating strategies for urban wastewater nitrogen removal. J. Appl. Phycol. 12, 331–339.

Ghaly, A.E., Ramakrishnan, V.V., Brooks, M.S., Budge, S.M., Dave, D., 2013. Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. J. Microb. Biochem. Technol. 5, 107–129.

Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2010. Placing microalgae on the biofuels priority list: a review of the technological challenges. J. R. Soc. Interface 7, 703–726.

Gupta, S.K., Ansari, F.A., Shriwastav, A., Sahoo, N.K., Rawat, I., Bux, F., 2016. Dual role of *Chlorella sorokiniana* and *Scenedesmus obliquus* for comprehensive wastewater treatment and biomass production for biofuels. J. Clean. Product. 115, 255–264.

Hena, S., Fatmah, S., Tabassum, S., 2015. Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water. Resour. Ind. 10, 1–14.

Jena, J., Nayak, M., Panda, H.S., Pradhan, N., Sarika, C., Panda, P.K., Rao, B.V.S.K., Prasad, R.B.N., Sukla, L.B., 2012. Microalgae of Odisha coast as a potential source for biodiesel production. World. Environ. 2, 11–16.

Kaewkannetra, P., 2012. The effect of CO_2 and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production. Biotechnol. Bioproc. Eng. 17, 591–597.

Kim, M.K., Park, J.W., Park, C.S., Kim, S.J., Jeume, K.H., Chang, M.U., Acerman, J., 2007. Enhanced production of *Scenedesmus* sp. (green microalgae) using a new medium containing fermented swine wastewater. Bioresour. Technol. 98, 2220–2228.

Liam, B., Philip, O., 2010. Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sustain. Energy Rev. 14, 557–577.

Lowry, O.H., Rosenbrough, N.J., Farr, A., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Makareviciene, V., Andruleviciute, V., Skoruoskaite, V., Kasperoviciene, J., 2011. Cultivation of microalgae *Chlorella* sp. and *Scenedesmus* sp. as a potential biofuel feedstock. Environ. Res. Eng. Manag. 3, 21–27.

Mandal, S., Mallick, N., 2011. Waste utilization and biodiesel production by the green microalga *Scenedesmus obliquus*. Appl. Environ. Microbiol. 77, 374–377.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sustain. Energy Rev. 14, 217–232.

Minhas, A.K., Hodgson, P., Barrow, C.J., Adholeya, A., 2016. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Front. Microbiol. 7, 1–19.

Nayak, M., Thirunavoukkarasu, M., Mohanty, R.C., 2016. Cultivation of fresh water microalgae *Scenedesmus* sp. using low cost inorganic fertilizer for enhanced biomass and lipid yield. J. Gen. Appl. Microbiol. 62, 7–13.

Park, J., Jin, H.F., Lim, B.R., Park, K.Y., Lee, K., 2010. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. Bioresour. Technol. 101, 8649–8657.

Prabhakaran, P., Ravindran, A.D., 2012. *Scenedesmus* as a potential source of biodiesel among selected microalgae. Curr. Sci. 102, 616–620.

Quinn, J.C., Davis, R., 2015. The potential and challenges of algae based biofuels: a review of the techno-economic, life cycle, and resource assessment modeling. Bioresour. Technol. 184, 444–452.

Ravindran, B., Gupta, S.K., Cho, W.M., Kim, J.K., Lee, S.R., Jeong, K.H., Lee, D.J., Choi, H.C., 2016. Microalgae potential and multiple roles- current progress and future prospects: an overview. Sustainability 8, 1215–1230.

Richmond, A., 2004. Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia 512, 33–37.

Ruiz, J., Olivieri, G., Vree, J.D., Bosma, R., Willems, P., Reith, J.H., Eppnik, M.H.M., Kleinegris, D.M.M., Wijffels, R.H., Barbosa, M.J., 2016. Towards industrial products from microalgae. Energy Environ. Sci. 9, 3036–3043.

Sacristan de Alva, M., Luna-Pabello, V.M., Cadena, E., Ortíz, E., 2013. Green microalgae *Scenedesmus acutus* grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. Bioresour. Technol. 146, 744–748.

Sahay, S., Braganza, V.J., 2016. Microalgae-based biodiesel production: current and future scenario. J. Exp. Sci. 7, 31–35.

Sibi, G., 2015. Low cost carbon and nitrogen sources for higher microalgal biomass and lipid production using agricultural wastes. J. Environ. Sci. Technol. 8, 113–121.

Strickland, J.D.H., Parsons, T.R., 1972. A practical hand book of sea water analysis. Can. Bull. Fish. Aquat. Sci. 167, 310 pp.

Ullah, K., Ahmad, M., Sofia Sharma, V.K., Lu, P., Harvey, A., Zafar, M., Sultana, S., 2015. Assessing the potential of algal biomass opportunities for bioenergy industry: a review. Fuel 143, 414–423.

Vidotti, R.M., Viegas, E.M.M., Carneiro, D.J., 2003. Amino acid composition of processed fish silage using different raw materials. Anim. Feed. Sci. Technol. 105, 199–204.

Walne, P.R., 1970. Studies on food values of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus*. Fish. Invest. Land. Ser. 26, 1–64.

Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO_2 bio-mitigation using microalgae. Appl. Microbiol. Biotechnol. 79, 707–718.

Xiao, R., Chen, R., Zhang, Y.H., Li, H., 2011. Microalgae *Scenedesmus quadricauda* grown in digested wastewater for simultaneous CO_2 fixation and nutrient removal. J. Biobased Mater. Bioenergy 5, 234–240.

Xin, L., Hong-ying, H., Ke, G., Jia, Y., 2010. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. Ecol. Eng. 36, 379–381.

Xin, L., Hong-ying, H., Yu-ping, Z., 2011. Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. Bioresour. Technol. 102, 3098–3102.

Zhang, Z.J., Fischer, C.J., 2006. A simplified method for direct spectrophotometric determination of nitrate in sea water. Mar. Chem. 99, 220–222.

Zhen-Feng, S., Xin, L., Hong-Ying, H., Yin-Hu, W., Tsutomu, N., 2011. Culture of *Scenedesmus* sp. LX1 in the modified effluent of a wastewater treatment plant of an electric factory by photo membrane bioreactor. Bioresour. Technol. 102, 7627–7632.

Zhou, W., Li, Y., Min, M., Hu, B., Chen, P., Ruan, R., 2011. Local bioprospecting for high lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production. Bioresour. Technol. 102, 6909–6919.

Zuliani, L., Frison, N., Jelic, A., Fatone, F., Bolzonella, D., Ballottari, M., 2016. Microalgae cultivation on anaerobic digestate of municipal wastewater, sewage sludge and agro-waste. Int. J. Mol. Sci. 17, 1692–1709.