Effects of Inorganic Media Enriched with Sodium Acetate on the Growth Performance and Nutrient Content in The Microalga *Chlorella vulgaris*

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ABSTRACT

Inorganic nutrients are used in aquaculture to increase natural productivity. This study aimed to apply sodium acetate as a growth enhancer in inorganic media to culture the microalga *Chlorella vulgaris* in laboratory conditions. Sodium acetate-enriched Bold basal medium (BBMAC) and Urea-Phosphate-Potassium media (NPKAC1 and NPKAC2) were used as experimental treatments, while Bold basal medium (BBM) was used as a control. During eight days of culture of *C. vulgaris*, maximum cell growth of 262.35 $\times 10^5$ cells·mL^{-1} and chlorophyll *a* concentration of 12.16 mg·L^{-1} were found in BBMAC, followed by those grown in BBM, NPKAC1 and NPKAC2. Specific growth rates (SGR, μ) and chlorophyll *a* of *C. vulgaris* grown in BBMAC were significantly ($p<0.01$) higher than microalgae cultured in other media. Crude protein of *C. vulgaris* cultured in BBM as control medium was significantly ($p<0.01$) higher than that of algae grown in other media. Crude lipid content of *C. vulgaris* grown in BBMAC was significantly ($p<0.01$) higher than that of algae cultured in other media. However, there was little difference in content of crude lipids among algae grown in BBM and other inorganic media containing sodium acetate. The study indicates that media enriched with sodium acetate can enhance the cell growth and nutrient values in cultivation of *C. vulgaris*.

Keywords: Biomass, *Chlorella vulgaris*, Chlorophyll *a*, Optical density, Sodium acetate

INTRODUCTION

Microalgae are extremely diverse organisms in global aquatic environments and they play a functional role in aquatic ecosystems as primary producers. Callegari (1989) reported that about 50 microalgae species have been studied in detail with regard to their biochemistry and ecophysiology. In aquaculture systems, microalgae are considered very important in promoting live foods for raising fish and shrimp larvae. Many previous studies indicated that appropriate growth of aquatic organisms depends on the availability of essential nutrients and minerals. These essential nutrients like protein, lipid, carotene, vitamins, amino acids, polyunsaturated fatty acids and minerals come from microalgae (Geldenhuys *et al*., 1988; Vymazal, 1995; Yamaguchi, 1997; Habib *et al*., 2003).

To establish a bio-based economy, microalgae is considered as a potential source of triacylglycerides and starch to supply feedstock, and to produce fuels and chemicals (Wijffels and Barbosa, 2010; Hu *et al*., 2008; Markou *et al*., 2012). In the culture of microalgae, there is an uptake of

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CO_2 during the light period and nitrate fixation during the dark period, which enriches algal biomass production. Similarly, culture media enriched with acetate, glucose and sugar help to produce quicker and higher growth of microalgae (Lourenço *et al*., 1997; Mooij *et al*., 2013; 2016).

Several culture experiments using microalgae clearly emphasized that glucose, glycerol, sodium acetate, sucrose and waste organic materials could be used as carbon sources to produce greater amounts of microalgae (e.g. *Chlorella vulgaris*, *C. ellipsoidea*, *Selenastrum gracile*, *Spirulina platensis*, *Sp. maxima* and *Sp. major*) for protein, biomass and lipid production (Habib *et al*., 2008; Hongjin and Guangce, 2009; Estévez-Landazábal *et al*., 2013; Smith *et al*., 2015; Sharma *et al*., 2016; Silva *et al*., 2016). Interestingly, sodium acetate and glycerol have previously been used in culture media under a mixotrophic condition (Vazhappilly and Chen, 1988; Sharma *et al*., 2016; Silva *et al*., 2016; Oscar *et al*., 2017), whereby different organic carbon sources are simultaneously assimilated by respiratory and photosynthetic metabolism (Alkhamis and Qin, 2016).

The provision of live foods for aquaculture normally depends on availability of microalgae or zooplankton. Among microalgae, *Chlorella* is an important species used in aquaculture (Oscar *et al*., 2017; Muys *et al*., 2019). This alga contains high protein, lipids and long chain polyunsaturated fatty acids (PUFA). *Chlorella*, together with rotifers, is usually used as feed for marine fish larvae (Watanabe *et al*., 1983; Juario and Storch, 1984). Freshwater *Chlorella* have been produced in large quantities for food products, especially health foods (Kawaguchi, 1980; Soong, 1980), whereas during fish hatchery operation, small quantities of microalgae are required as live food for zooplankton and fish/shrimp larval rearing, and only in the production period (de Pauw *et al*., 1984).

Production of microalgae biomass is relatively expensive due to high costs of the culture medium. Accordingly, alternative low-cost media are currently being developed to produce microalgae with comparable nutritional values. Previous studies indicated that the microalgal growth performance in inorganic fertilizer (NPK, 20-5-20) media was similar or greater than in commercial medium (Ashraf *et al*., 2011; Brito *et al*., 2013; Sipaúba-Tavares *et al*., 2011; 2017). Additionally, previous studies on the cost-effective ratio of agricultural fertilizer media showed that inorganic fertilizer medium was eight times cheaper than conventional ones (Ashraf *et al*., 2011; El Nabris, 2012; Brito *et al*., 2013). Despite this evidence, such commercial uses of microalgae have not been developed in Bangladesh. However, the freshwater microalga *Chlorella vulgaris* may yield great benefits when used in nutrition products as well as in live food development for aquaculture. The present study was undertaken to produce fresh and healthy cells of *C. vulgaris* for live food production in aquaculture systems using sodium acetate as a growth enhancer in inorganic media.

MATERIALS AND METHODS

Sample collection and preparation of media

Chlorella vulgaris was inoculated from pure laboratory stock culture. The experiment was conducted in the Live Food Culture Laboratory, Department of Aquaculture, Faculty of Fisheries, BAU, Mymensingh. Three different inorganic media were prepared for algae cultivation in the laboratory: (1) Bold basal medium (BBM) with 0.10% (w:v) sodium acetate (BBMAC) (Thompson *et al*., 1988) (Table 1), (2) N:P:K=1.0:1.0:0.50 with 0.10% (w:v) sodium acetate (NPKAC1) and (3) N:P:K=1.0:1.50:1.0 with 0.10% (w:v) sodium acetate (NPKAC2), based on modification of Geldenhuys *et al*. (1988) (Table 2). BBM medium was used as a control. The prepared media were kept overnight to reach ambient temperature and then kept for 72 h to check for any microbial growth prior to inoculation with *C. vulgaris*. Clear solution in flasks indicated no microbial growth.

Culture experiment of Chlorella vulgaris

Chlorella vulgaris (six days old, optical density, OD_{620}=0.20) was inoculated in 1.0 L of BBMAC, NPKAC1, NPKAC2 and BBM to produce algal cultures, with three replications of

each medium. Filtered aeration and 80 W fluorescent light at 12 h:12 h L:D cycle was maintained for 12 days and all cultured algae were harvested before the stationary phase.

The microalgae were harvested at the beginning of the experiment (day 0) and every subsequent two days by withdrawing a 20-mL sample from each flask. The samples were filtered using Whatman microfilter paper (0.45 µm mesh size) through high velocity vacuum pump. Filter papers were carefully collected, folded (keeping algae inside), cut into small pieces and put in a plastic centrifuge tube (20 mL capacity). Then, 10 mL acetone was added to the plastic tube, and the filter paper with algae was ground using a glass rod. Finally, chlorophyll *a* of prepared samples was calculated using the formula: 11.85×(OD 664 nm) – 1.54×(OD 647 nm) – 0.08×(OD 630 nm) following Clesceri *et al*. (1989).

Table 1. Composition of inorganic nutrients of Bold basal medium (BBM) with 0.10% sodium acetate (BBMAC).

No.	Chemicals/Compounds	Concentration in stock solution ($g \cdot L^{-1}$)	Inoculation in culture medium ($mL \cdot L^{-1}$)
1.	$NaNO_3$	25.00	10.0
2.	$MgSO_4 \cdot 7H_2O$	7.50	10.0
3.	NaCl	2.50	10.0
4.	K_2HPO_4	7.50	10.0
5.	KH_2PO_4	17.50	10.0
6.	$CaCl_2 \cdot 2H_2O$	2.50	10.0
7.	Trace elements:		1.0
	$ZnSO_4 \cdot 7H_2O$	8.82	(All trace elements same
	$MnCl_2 \cdot 4H_2O$	1.44	as in stock solution)
	MoO_3	0.71	
	$CuSO_4 \cdot 5H_2O$	1.57	
	$Co(NO_3)_2 \cdot 6H_2O$	0.49	
8.	H_3BO_3	11.40	1.0
9.	EDTA-KOH solution:		1.0
	EDTA Na_2	50.00	
	KOH	31.00	
10.	$FeSO_4 \cdot 7H_2O$	4.98	1.0
	Conc. H_2SO_4	1.0 mL per 1.0 L	

Table 2. Composition of inorganic fertilizers ($mg \cdot L^{-1}$) containing sodium acetate.

Chemicals used	NPKAC1	NPKAC2
Urea	260 mg (119.60 mg N)	260 mg (119.60 mg N)
Triple super phosphate	1.05 g (119.60 mg P)	1.58 g (179.40 mg P)
Muriate of potash	154.64 mg (59.80 mg K)	154.64 mg (59.80 mg K)
Sodium acetate	1.0 g	1.0 g

All of the microalgal samples were collected before reaching the stationary phase. Final experiment was conducted after performing two preliminary growth trials. Growth parameters and proximate composition of microalgae were analyzed following Clesceri *et al*. (1989) and Habib (1998).

Measurement of growth rate, chlorophyll a and nutrient content

During the study, pH, cell density, cell weight, specific growth rate (SGR, μ) and chlorophyll *a* concentration of the microalgal cultures were determined according to Clesceri *et al*. (1989). The cell numbers and optical density of algal cultures were recorded every two days using haemocytometer with microscope and UV-spectrophotometer (Mittion Roy, Spectronic 1001 plus) at 620 nm, respectively. The collected microalgae on filter paper was separated through repeated centrifugation at 3,000 rpm for 10 min and washed at least three times. Filter papers were incubated overnight at 45 °C, and then the dried papers were put in desiccators for at least 30 min and weighed. The separated algae were preserved in the –10 °C deep chamber of a freezer (LG Electronics Model No: GR-T312GE) for later use. For determination of phosphate, nitrate, nitrite and ammonia, HACH DR/4000 UV Spectrophotometer (Expotech USA Inc.) and HACH, DR/2010 Portable dial logging spectrophotometer were used. Light intensity was measured by Digital Lux Meter (Lutron LX-1). Dissolved oxygen and temperature were measured by dissolved oxygen and temperature meter (Lutron Model No. DO-5009); pH was measured using digital pH meter (YSI Model No. 60/10 FT). Proximate composition such as moisture, crude protein, crude lipids, ash, and nitrogen-free extract of microalgae was analyzed following methods of Horwitz (1984).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare the means of SGR of cells, total biomass, crude protein and crude lipid of microalgae grown in different media. Tukey test was applied to test for significant difference between treatment means (Zar, 1984).

RESULTS AND DISCUSSION

Our experiments showed that the maximum mean cell number ($\times 10^5 \cdot mL^{-1}$) of *Chlorella vulgaris* was found in BBMAC on day 8 of culture (262.35), followed by BBM (208.48), NPKAC1 (182.26) and NPKAC2 (168.19) (Figure 1). Similarly, the highest chlorophyll *a* concentration and optical density of media were observed in BBMAC (12.18 $mg \cdot L^{-1}$ and 2.48, respectively) on day 8 (Figures 2–3). Specific growth rates (SGR, μ) of both algal cell numbers and chlorophyll *a* concentrations of *C. vulgaris* grown in BBMAC and BBM were significantly ($p<0.01$) higher than for microalgae cultured in NPKAC1 and NPKAC2 (Table 3). Total biomass of this microalga grown in BBMAC was significantly ($p<0.01$) higher than that of microalgae cultured in BBM, followed by BBMAC2 and BBMAC1 (Table 3).

Table 3. Specific growth rates (SGR, μ) based on number of cells ($x10^5 \cdot mL^{-1}$) and chlorophyll *a* concentration (Chl*a*, $mg \cdot L^{-1}$) and total biomass of *Chlorella vulgaris* grown in BBMAC, NPKAC1, NPKAC2 and BBM media.

Parameters	BBMAC	NPKAC1	NPKAC2	BBM
SGR of cells	0.51^{a}±0.02	0.42^{b}±0.01	0.41^{b}±0.02	0.49^{a}±0.01
SGR of Chl*a*	0.51^{a}±0.01	0.43^{b}±0.02	0.41^{b}±0.02	0.50^{a}±0.00
Total biomass (Chl*a* × 67)*	815.49^{a}±17.44	565.26^{c}±13.3	536.67^{c}±12.41	674.91^{b}±12.02

Note: Means (±SD) with different superscripts in each row are significantly different ($p<0.01$). *$mg \cdot L^{-1}$, calculation based on Vonshak and Richmond (1988).

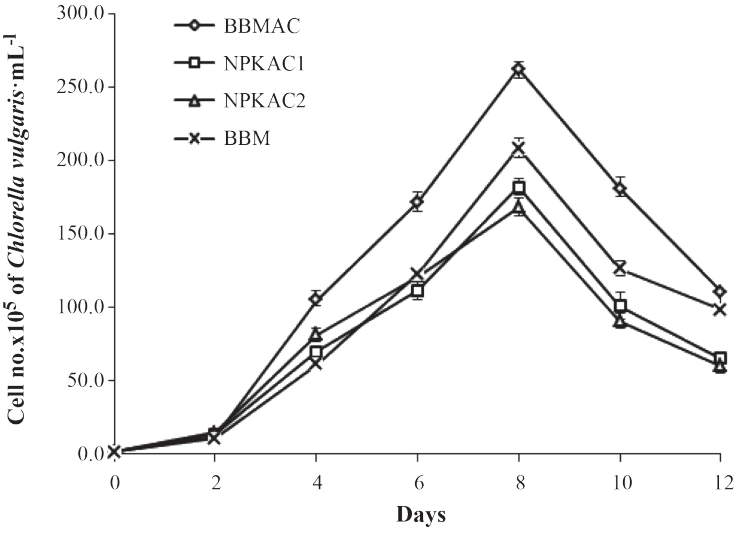

Figure 1. Growth curve based on cell number ($x10^5 \cdot mL^{-1}$) of *Chlorella vulgaris* cultured in BBMAC, NPKAC1, NPKAC2 and BBM media.

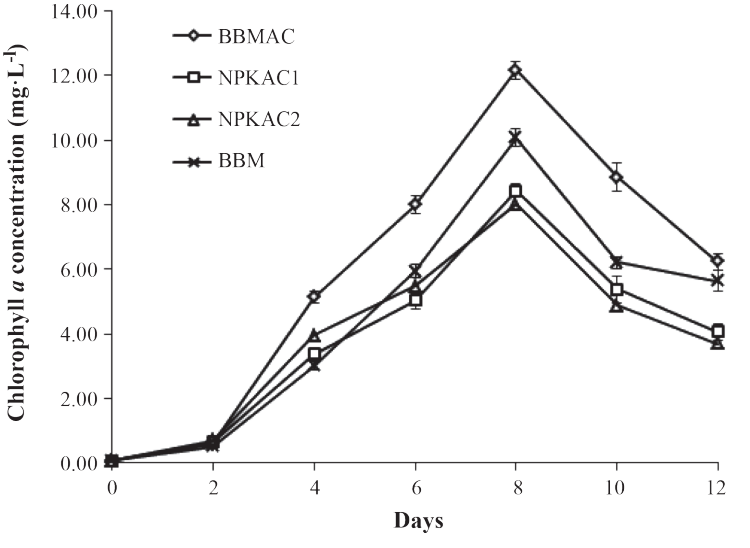

Figure 2. Growth curve based on chlorophyll *a* concentration ($mg \cdot L^{-1}$) of *Chlorella vulgaris* cultured in BBMAC, NPKAC1, NPKAC2 and BBM media.

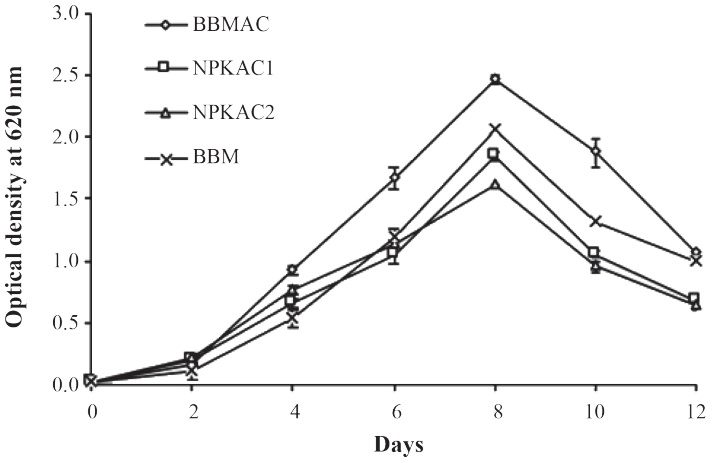

Figure 3. Optical density of BBMAC, NPKAC1, NPKAC2 and BBM media during *Chlorella vulgaris* culture.

During the study, pH of media in all treatments remained below 9.0; pH ranged from 7.21±0.08 to 8.50±0.04 in BBMAC, the medium in which maximum growth of *Chlorella* was recorded (Table 4 and Figure 4). The lowest pH recorded was 6.9 and the highest was 8.9, both for cultures grown in NPKAC2 (Figure 4). Temperature was stable and below 29 °C in all treatments. The minimum temperature recorded was 27 °C in the culture with BBMAC medium (Table 4). Maximum light intensity recorded was 2100±20 lux (35.28± 0.04 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 4). The highest dissolved oxygen (DO) was recorded on day 8 in BBMAC medium (4.85±0.03 $mg \cdot L^{-1}$) and the lowest was found on day 12 in NPKAC1 medium (2.96±0.03 $mg \cdot L^{-1}$). Maximum orthophosphate was recorded in NPKAC2 (172.17±5.53 $mg \cdot L^{-1}$) and minimum in BBM (3.02±0.06 $mg \cdot L^{-1}$) (Table 4). Highest ammonia-nitrogen (NH_3-N) was found on day 12 in NPKAC2 (2.52±0.02 $mg \cdot L^{-1}$) and the lowest was in BBMAC (0.02±0.02 $mg \cdot L^{-1}$) (Table 4). Maximum nitrate-nitrogen (NO_3-N) was found in NPKAC2 (21.69±1.69 $mg \cdot L^{-1}$), followed by NPKAC1 (21.36 ±2.11 $mg \cdot L^{-1}$), BBM (15.04±2.66 $mg \cdot L^{-1}$) and BBMAC (13.47±1.79 $mg \cdot L^{-1}$) (Table 4). Maximum nitrite-nitrogen (NO_2-N) was recorded on day 12 in NPKAC1 (0.18±0.02 $mg \cdot L^{-1}$) and NPKAC2 (0.18±0.02 $mg \cdot L^{-1}$) and minimum in BBMAC (0.02±0.01 $mg \cdot L^{-1}$) and BBM (0.02±0.01 $mg \cdot L^{-1}$) on day 0 (Table 4).

Crude protein (% dry weight) of *Chlorella vulgaris* cultured in BBM (control) was significantly ($p<0.01$) higher than that grown in BBMAC, while treatments with NPKAC2 and NPKAC1 were lowest (Table5). Muys *et al*. (2019) reported that *Chlorella* spp., including *C. vulgaris* contained 43 % crude protein, similar to the present findings. Crude lipids of *C. vulgaris* grown in BBMAC was significantly ($p<0.01$) higher than that of microalgae cultured in NPKAC2 and NPKAC1, and all experimental treatments were higher than the control (BBM). Meanwhile, NFE (nitrogen-free extract) of the microalgae cultured in BBMAC was significantly ($p<0.01$) lower than in other media. The ash content of the microalgae grown in the three experimental treatments (BBMAC, NPKAC1 and NPKAC2) was significantly higher ($p<0.01$) than the control (Table 5). Muys *et al*. (2019) found that ash content (total minerals) in commercially available *Chlorella* spp. was within the regulated limits, which agrees with the present results.

The present study clearly indicated that BBM with the additional micronutrient sodium acetate (BBMAC) showed maximum growth performance with respect to cell growth, chlorophyll *a* concentration with an enhanced SGR, and total biomass of *Chlorella vulgaris* (Table 3). Our findings agree with those from the previous study of Habib (1998) on production of *Chlorella vulgaris* and

Table 4. Mean ranges of physico-chemical parameters recorded during *Chlorella vulgaris* culture in different media.

Parameter	BBMAC	NPKAC1	NPKAC2	BBM
Light intensity ($lux \cdot m^{-2}s^{-1}$)	1660-2030	1580-2100	1800-2070	1830-2040
pH	7.21±0.08- 8.50±0.04	7.25±0.04- 8.55±0.07	6.89±0.04- 8.86±0.05	7.02±0.06- 8.54±0.05
Temperature (°C)	27.07±0.04- 28.09±0.03	27.09±0.03- 28.69±0.02	27.09±0.02- 28.70±0.02	27.08±0.03- 28.70±0.02
DO ($mg \cdot L^{-1}$)	3.45±0.04- 4.85±0.03	2.96±0.03- 4.16±0.06	2.98±0.02- 3.96±0.02	3.41±0.03- 3.44±0.04
PO_4-P ($mg \cdot L^{-1}$)	3.61±0.05- 5.48±0.06	117.66±1.24- 53.17±1.63	172.17±5.53- 103.70±1.47	5.39±0.06- 3.02±0.06
NH_3-N ($mg \cdot L^{-1}$)	0.02±0.02- 0.90±0.03	0.09±0.01- 2.34±0.07	0.11±0.02- 2.52±0.02	0.02±0.01- 0.95±0.03
NO_3-N ($mg \cdot L^{-1}$)	13.47±1.79- 2.01±0.21	21.36±2.11- 2.23±0.11	21.69±1.69- 2.29±0.02	15.04±2.66- 1.95±0.15
NO_2-N ($mg \cdot L^{-1}$)	0.02±0.01- 0.15±0.01	0.03±0.03- 0.18±0.02	0.03±0.01- 0.18±0.02	0.02±0.01- 0.15±0.01

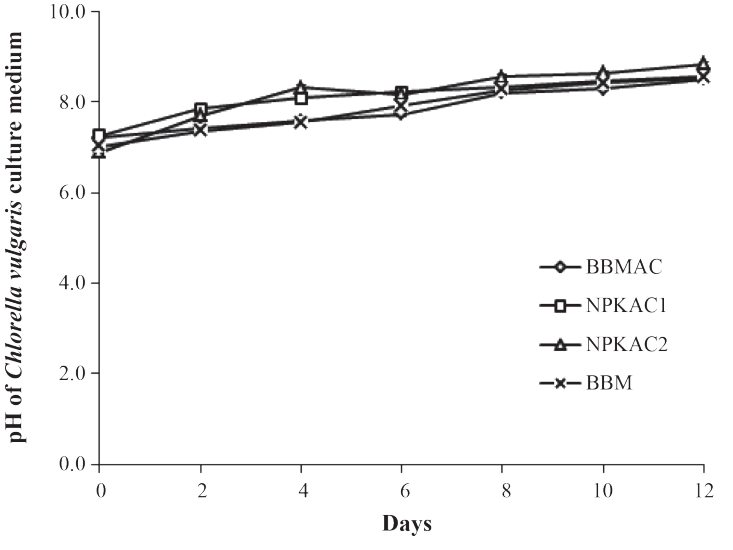

Figure 4. Mean pH of BBMAC, NPKAC1, NPKAC2 and BBM media during *Chlorella vulgaris* culture.

Table 5. Proximate composition (% dry matter) of *Chlorella vulgaris* grown in BBMAC, NPKAC1, NPKAC2 and BBM.

Components	BBMAC	NPKAC1	NPKAC2	BBM
Moisture	7.51^{a}±0.13	6.76^{b}±0.09	6.66^{b}±0.08	6.21^{c}±0.09
Crude protein	43.04^{b}±0.16	41.32^{c}±0.10	41.45^{c}±0.08	45.44^{a}±0.11
Crude lipids	19.70^{a}±0.31	17.45^{b}±0.21	17.87^{b}±0.08	10.49^{c}±0.02
Crude fiber	4.33^{c}±0.04	3.97^{d}±0.11	5.91^{b}±0.09	10.43^{a}±0.12
NFE*	13.06^{c}±0.12	18.75^{a}±0.17	15.22^{ab}±0.18	16.80^{a}±0.22
Ash	12.31^{a}±0.11	11.69^{a}±0.08	12.75^{a}±0.10	10.49^{b}±0.12

Note: *NFE was calculated by adding percentage values of moisture, crude protein, crude lipids, crude fiber and ash (% dry basis) and subtracting from 100 %. Means (±SD) with different superscripts in each row indicate significant differences ($p< 0.01$).

Selenastrum gracile, as well as Gladue and Maxey (1994) on production of *Ankistrodesmus convolutus* using different inorganic media enriched with sodium acetate. Combres *et al*. (1994) also reported that the acetate group enhanced the growth of the microalgae *Scenedesmus obliquus*. Furthermore, Rahardini *et al*. (2018) also recorded similar cell growth of *Chlorella* sp. using inorganic fertilizer media with different combinations of ammonium sulfate (ZA), urea and triple superphosphate.

Sharma *et al*. (2016) cultured five species of *Chlorella* in various nutrient media with different organic carbon sources like glucose, glycerol, sodium acetate, and sucrose under mixotrophic conditions, and found that *Chlorella vulgaris* achieved the highest lipid productivity (by 3.5 fold) in glycerol-supplemented culture compared to the control medium and compared to all other species. Hongjin and Guangce (2009) reported that growth rates of *Chlorella sorokiniana* cultured in glucose and sodium acetate (NaAc) increased in stationary phase after 48 h at 30 °C. They also indicated that additional carbon sources remarkably stimulated lipid accumulation in the microalgae.

In the present study, the highest amount of lipids in *Chlorella vulgaris* was recorded in the culture with BBMAC (Table 5), and this was probably due to a slight reduction of crude protein and carbohydrate (NFE) in cells (Sukenik and Carrneli, 1990; Tan and Johns, 1991; Chu *et al*., 1995). Microalgae can easily absorb acetate, glucose and sugar molecules because of their highercell permeability and active enzyme capability (Tsuzuki and Miyachi, 1989; Tan and Johns, 1991; Laliberte and de la Noüe, 1993; Chu *et al*., 1995; Lourenço *et al*., 1997). Laliberte and de la Noüe (1993) reported that the acetate group is usually channeled into protein and carbon compounds. However, this study showed that microalgae grown in acetate-supplemented media possessed comparatively lower carbohydrate than that of BBM. Similar trends were also observed in the cases of protein and crude fiber (Habib, 1998). This might be due to lipid accumulation, which was higher when NaAc-supplied treatments provided adequate nutrients in mixotrophic condition. In addition to inorganic carbon, *C. vulgaris* cultured in BBMAC showed higher cell growth and lipid accumulation than those of BBM. Microalgae grown in other inorganic media using NaAc also showed higher lipid accumulation than the control, although the cell growth was higher in BBM (Table 5). The microalgae might have benefitted from the additional inorganic carbon source of NaAc in different culture media provided its mixotrophic growth condition. Here the cultured algae might have obtained the advantages of both phototrophic and heterotrophic culture (Li *et al*., 2014) and consumed energy from organic and inorganic carbon sources, as well as light provided in the culture system.

Maximum cell growth of *Chlorella vulgaris* was found on day 8 of culture with a pH value ranging from 8.2-8.6. A hydrogen ion concentration (pH) level that is too high or too low could slow the algal growth by disrupting cellular processes.

The favorable pH range for most of the microalgal species cultured falls between 7 and 9. The optimum range, furthermore, was reported to be 8.2–8.7 (Ukeles, 1971). Tadashi *et al*. (2018) recorded pH levels of 7.1–7.7 for microalgae cultivation. Maximum number of *Chlorella* cells were produced in BBMAC on day 8, with a temperature of 28 ºC. Most commonly cultured microalgae have a temperature tolerance range from 16 to 27 ºC, but the optimum range may be considered to be 20 to 24 ºC (Hoff and Snell, 1989). The researchers also reported that temperatures lower than 16 ºC will slow algal growth, while temperatures higher than 35 ºC are lethal for a number of species (Hoff and Snell, 1989). Ukeles (1976) studied the effect of temperature on growth of *Chlorella* sp. and indicated that maximum growth was between 14–29 ºC, which was equal to control at 20.5 ºC, and found no growth at 8–9 ºC or at 32–35 ºC. Physicochemical conditions of culture media such as temperature, light, pH and nutrients affect not only chlorophyll absorption and algal growth but also influence the activity of cell metabolism and nutrient values (Huang *et al*., 2013).

During the maximum *Chlorella vulgaris* cell growth, the light intensity was 2000–2070±20 lux (35.28 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Hoff and Snell (1989) suggested a range of light intensity for culturing microalgae from 1000–10,000 lux depending on volume and density of the culture, and an optimum range of 2,500–5,000. Dissolved oxygen ranged from 3.96±0.02 to 4.86±0.02 $mg \cdot L^{-1}$ during maximum cell growth of *C. vulgaris*. Alam *et al*. (2003) found maximum dissolved oxygen on the tenth day of culture in inorganic media, ranging from 4.93 to 5.46 $mg \cdot L^{-1}$. During microalgae culture in the laboratory, continuous aeration was pumped through aerators, which helped to remove non-volatile gases through bubbling air, and provided easier respiration in light and dark hours. Due to photosynthesis and respiration of microalgae, a balance of CO_2 and O_2 occurred naturally in the culture system.

Very high amounts of orthophosphate were detected in NPKAC2 and NPKAC1, which was supported by Alam *et al*. (2003), but phosphate-phosphorous ranged from only 3.02±0.06 to 5.48± 0.06 $mg \cdot L^{-1}$ in BBM and BBMAC. Besides carbon, nitrogen and phosphorous are principal nutrients required by phytoplankton. Redfield (1958) studied varieties of sea water samples of plankton and other organisms. After analyzing he found that the atoms of phosphorus, nitrogen and carbon were existed in an average ratio of 1:16:106. Redfield ratio of 16N:1P was found optimum N:P atomic ratio in respect to nutrient requirement and growth of phytoplankton (Liu *et al.*, 2011). They studied microalgae *Microcystis aeruginosa* in five resources of different ratios of N:P of 1, N:P of 16, N:P of 40, N:P of 100 and N:P of 200. Finally, they concluded that the uptake of nutrients and growth of *Microcystis aeruginosa* was maximum in the resource of atomic ratio N:P of 16 which was investigated and optimized earlier by Redfield (1958). Microalgae species grown in greater N:P molar concentration (high N and low P), more actively remediate ammonium and phosphate than those species cultured in lower N:P internal composition of waste water media (Whitton *et al*., 2016). They studied five freshwater species of microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenesdesmus obliquus*, *Stigeoclonium* sp. and *Microcystis aeruginosa* under mono culture system with an N:P molar ratio of approximately 2:1 for *M. aeruginosa* and 6:1 for early four species. Nitrogen exists in nature in its various forms (ammonia, nitrate and nitrite) and is cycled within the environment. Alam *et al*. (2003) reported high ammonia-nitrogen, ranging from 9.59 to 27.33 $mg \cdot L^{-1}$, in different inorganic media. In this study, nitrite-N ranged from 0.02±0.06 to 0.18±0.02 $mg \cdot L^{-1}$ among the different culture media (Table 4). Nitrate-N was found to range from 1.95±0.15 to 21.69±1.69 $mg \cdot L^{-1}$, with maximum on day 0 and gradually falling to a minimum on day 12 of the study. On day 8, the nitrate levels ranged from7.96±0.63 to 9.43±2.18 $mg \cdot L^{-1}$ among treatments. A similar trend was reported in sugar mill and sweetmeat waste media used for culture of *Chlorella* and *Scenedesmus* species (Khan *et al*.,2006; 2018; Toyub *et al*., 2008). Alam *et al*. (2003) determined a high range of nitrate (39.08 to 70.83 $mg \cdot L^{-1}$) with a maximum on the first day and minimum on the tenth day of culture. Approximately 90 % of nitrate-N was removed from media due to algal uptake and assimilation. Similar findings were reported for *C. vulgaris* cultures containing 40-80 mg $N \cdot L^{-1}$ (Tam and Wong, 1996).

CONCLUSION

Bold basal medium (BBM) is a proven-quality medium for microalgae culture, but it is very costly, as is sodium acetate-enriched BBM (BBMAC). This BBMAC showed 22.89 % higher growth of *Chlorella vulgaris* than that of BBM due to the use of the growth enhancer. Media containing sodium acetate-enriched inorganic fertilizer (NPKAC1 and NPKAC2) also showed promising results in terms of cell growth and other nutrient values such as protein, lipid and other contents. In comparison to BBM, these two media showed 13.41 % and 21.38 % lower cell growth, respectively. For mass culture, we need to develop growth media from low-cost inorganic fertilizer or low-cost agroindustrial wastes (e.g., sugarmill wastes) for live food production in aquaculture.

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