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RESEARCH PAPER

Evaluation of Sugar Mill By-product Molasses as a Low Cost Culture Media for Microalgae

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Abstract

Microalga *Chlorella vulgaris* was cultured in different concentrations of normal molasses medium ($NMM_{0.5\ g/l}$, $NMM_{1.0\ g/l}$, and $NMM_{1.5\ g/l}$) to evaluate growth performance of the sugar mill byproduct as a low cost culture media and Bold basal medium (BBM) as control. Maximum growth of *Chlorella vulgaris* was found in $NMM_{1.0\ g/l}$ on 8th day of the culture followed by BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$. Similar findings were also observed in determining chlorophyll a content and optical density of *C. vulgaris*. Maximum cell growths 191.88 ($\times 10^5$)/mL, chlorophyll a content 10.60 (mg/l) and optical density 2.15 were recorded in $NMM_{1.0\ g/l}$. Maximum SGR of cell was determined 0.56 (mg/day) grown in $NMM_{1.0}$ g/l followed by 0.52, 0.52 and 0.48 (mg/day) in BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$, respectively. Chlorophyll a content and total biomass of *Chlorella vulgaris* followed the similar trend. Protein (46.49%) and lipid (14.18%) of *C. vulgaris* was detected significantly higher ($P<0.01$) in $NMM_{1.0\ g/l}$ than that grown in BBM and other concentrations of NMM. The growth performance of the investigating molasses medium ($NMM_{1.0\ g/l}$) indicates that the molasses may be a good low cost culture medium ingredient source for *C. vulgaris* or any other microalga species.

Introduction

Molasses are the viscous liquid by-product between pure sugar production and the wastes released from the sugar factory plant. After crushing of sugarcane the extracted sweet liquids are allowed to boil in the factory boiler. Molasses are scum discharged in large amount to produce purified sugar. Molasses is used for spirit, alcohol and foreign liquor preparation in only one sugar mill at Kero & Company, Chuadanga, Bangladesh. This molasses has some other economic and commercial importance. It is used in animal feed mostly as dairy feed and nowadays for fish feed preparation. Local people traditionally are using it to process tobacco for smoking. Indian researchers mentioned that an average of 23 Litre of molasses is produced consistent with ton of sugarcane. India produced around 2.5 million metric tons (MMT) of molasses, used for manufacturing of ethanol, commercial purposes which include alcohols and automobile gas (Rasappon et al., 2015). But ISMA 2016 reported largely higher production of 9.03 MMT molasses (Khan et al., 2006). Biofuels, especial oils, pigments and polymers are commercially important components and now microalgal biomass are considered alternative production source of these components (Perez-Garcia et al., 2011). The microalgae used for biofuels production though require less land area in comparison to cereal crops but the cultivation, harvesting and processing of algae is not less costly. Due to which still the production of microalga biomass is not

economic (Quinn & Davis, 2015). Ensuring economic price of the three major essential inputs CO_2, nutrients and water for microalga biomass it is possible to maintain significant cost reduction (Brasil et al., 2016). It indicates that the scientists are investigating to find out low cost microalga culture system. However, molasses is very cheap and available everywhere in Bangladesh. It is an important source of carbon for microalgae growth. With the expansion of aquaculture in Bangladesh, there has been an increasing trend in using chemicals in aquatic animal health management (Uddin et al., 2020). Microalgae are very much helpful for fish health. We can use microalgae rather than using chemical in aquaculture (Uddin et al., 2020). Microalgae also play an important function in oxygen further to carbon dioxide stability in the water. It acts not most effective on agro-chemical but additionally animal wastes as nicely through changing them into meals substances (Hossain et al., 2021). At some point of huge manufacturing this molasses sometimes been being stored outside of the molasses tank because of lack of boxes, brought on environmental pollution. The proper utilization of this sugar mills' by-product may prevent environmental pollution partially and may enhance its economic and commercial importance. Preserving in mind the above view, the existing study changed into undertaken to research the growth overall performance of *Chlorella vulgaris* in normal molasses medium (NMM) to evaluate its efficiency as culture medium and ultimately exploring in aquaculture system. *Chlorella* sp. is used in human health recovery against tumor, carcinogenic, viral infection, cataract, ulcer and even oxidative properties (Shibata et al., 2003) and many other prospects. By using the molasses we can save huge amount of money which required for supplying nutrient for microalgae culture. This *Chlorella vulgaris* is very health effective for fish and shrimp production because microalgae play a critical position in oxygen in addition to carbon dioxide stability in the water (Rahman et al., 2021). This research opened a new era for low cost live food production as a result fish production cost will be reduced. Fish farmers and hatchery owner will be benefited by this research outcome.

Materials and Methods

Sample Collection and Preparation of Culture Media

Molasses changed into accrued from nearby market and normal molasses medium (NMM) were prepared in different concentrations and studied the growth performance of *Chlorella vulgaris* in these media and BBM as control. Normal molasses were measured and diluted with distilled water to make NMM $_{0.5\ g/l}$, NMM$_{1.0\ g/l}$ and NMM $_{1.5\ g/l}$ media. 260 mg/l urea was added in each of the normal molasses medium for nitrogen enrichment and autoclaved to sterilize the media at 120°C steam heat. Three remedies of NMM had been designed after a chain of laboratory trial for lifestyle of *Chlorella vulgaris* with manages as Bold Basal Medium (BBM). Chemical composition of BBM stock solutions is shown in Table 1.

For preparation of one liter BBM for lifestyle medium 10 mL of every of the inventory answers from serial no. 1-6 and 1.0 mL from every of the stock answers serial no. 7-10 (Table 1) had been pipetted to make one liter extent with distilled water in a volumetric flask.

Culture of *Chlorella vulgaris*

Chlorella vulgaris changed into cultured in 0.5 g/l, 1.0 g/l, and 1.5 g/l everyday molasses media and in BBM at Live Food Culture Laboratory, Department of Aquaculture, Bangladesh Agricultural University. *Chlorella vulgaris* had been inoculated from a stock lifestyle of six days to make a 10% suspension (optical density at 620 nm= 0.02) in all of the lifestyle remedy (Habib, 1998). A 12h:12h, light: dark system for 12 days have been maintained in the laboratory below light intensity of 2000 at 18lux/m^2/s. Non-stop aeration

Table 1. Chemical Composition (g/l) of Bold Basal Medium (BBM)

No.	Stocks of Chemicals	g/l
1.	NaNO3	25.00
2.	MgSO4. 7H2O	7.50
3.	NaCl	2.50
4.	K2HPO4	7.50
5.	KH2PO4	17.50
6.	CaCl2. 2 H2O	2.50
7.	Trace elements:	
	ZnSO4. 7 H2O	4.42
	MnCl2. 4 H2O	1.44
	MoO3	0.71
	CuSO4. 5 H2O	1.57
	Co (NO3)2. 6 H2O	0.49
8.	H3BO3	11.40
9.	EDTA-KOH solution:	
	EDTA Na2	50.00
	KOH	31.00
10.	FeSO4. 7 H2O with 1.0 mL Concentrated H2SO4	4.98

turned into additionally maintained supplying electric powered aerator linked via plastic tubes in way of culture bottles. Three replications have been taken for every way of life.

The cell count of *Chlorella vulgaris* was done in every alternate day using improved Neubuer ruling Haemacyto-meter under a light microscope. The cell number, optical density, chlorophyll-a, pH, free CO_2, dissolved oxygen, light intensity, temperature, phosphate- phosphorus and ammonia-nitrogen were measured every alternate day following standard methods (Clesceri et al., 1989).

Estimation of Chlorophyll-a Content

Optical densities of the prepared sample were analyzed at 664, 647 and 630 nm wave length operating UV-spectrophotometer (Clesceri et al., 1989). A blank in selective tube with 100% acetone was allowed to run simultaneously. Chlorophyll-a content was calculated by the following formula:

Chlorophyll-a (mg/litre) = 11.85 (OD 664) -1.54 (OD 647) - 0.8 (OD 620)

Specific growth rate (mg/day) of *C. vulgaris* on the basis of cell and chlorophyll-a content and the total biomass on the basis of chlorophyll-a content were also determined following standard methods (Clesceri et al., 1989).

Specific Growth Rate (SGR)

The specific growth rate (mg/day) of the cultured microalga was computed using following equation (Clesceri et al., 1989):

$$SGR\ (mg/day) = \ln (X_1 - X_2)/t_1 - t_2$$

Where,

X_1 = biomass concentration of the end of selected time interval;

X_2 = biomass concentration at beginning of selected time interval; and

$t_1 - t_2$ = time elapsed between the selected time in the day

Proximate Composition Analysis

The microalgae have been harvested before stationary phase and positioned in vials to centrifuge at 5000 rpm for five minutes to separate the microalgae. Then the microalgae were wiped clean with distilled water and separated with repeated centrifugation. The separated microalgae firstly saved at 0^0 C for three days after which dried inside the oven at 40^0 C. The dry samples had been preserved inside the freeze at -10^0 C for observe the proximate composition. The organized samples of *C. vulgaris* were analyzed in triplicate to estimate crude protein, lipid, moisture, crude fibre and nitrogen free extract (NFE) (within the nutrition Lab of Department of Aquaculture, Faculty of Fisheries of Bangladesh Agricultural University) following the same old techniques (Horwitz, 1984).

Analysis and Interpretation of Data

Differences within the measured parameters and the treatment means were determined using one way ANOVA and Duncan's Multiple Range Test following MSTAT statistical package (Zar, 1984).

Results

Maximum growths of *Chlorella vulgaris* were found in NMM $_{1.0\ g/l}$ on 8th day (Figure 1) of the culture followed by BBM, NMM $_{0.5\ g/l}$ and NMM $_{1.5\ g/l.}$ Similar

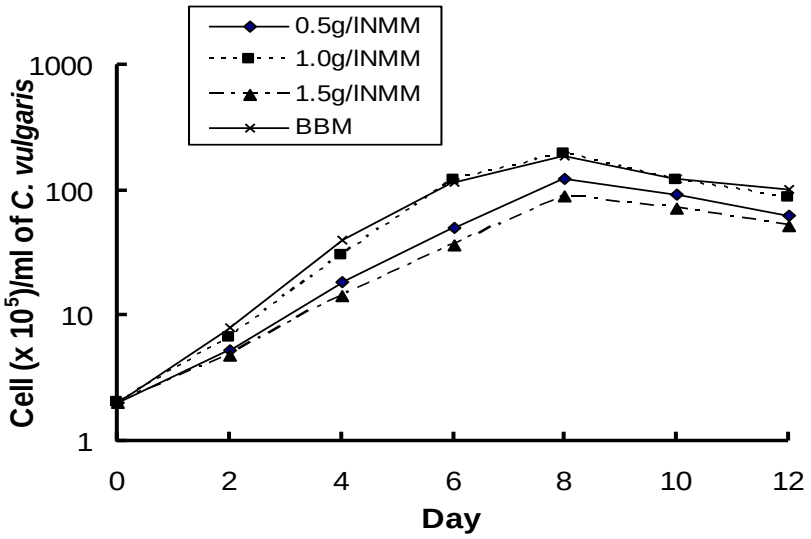

Figure 1. Semi logarithmic growth curve based on cell number ($\times 10^5$)/ml of *Chlorella vulgaris* grown in normal molasses media (NMM) and Bold basal medium (BBM) as control.

findings were also observed in determining chlorophyll a content (Figure 2) and optical density (Figure 3) and pH (Figure 4) of *C. vulgaris*. Maximum cell growths ($\times 10^5$)/mL, chlorophyll a content (mg/l) and optical density were 191.88, 10.60 and 2.15 respectively, on 8th day of the culture found in $NMM_{1.0\ g/l}$ with a higher growth performance than that grown in control (BBM).

Specific growth rates (SGRs) of cell and chlorophyll a content of *C. vulgaris* had been found most on 8th day in $NMM_{1.0\ g/l}$ followed by BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$ (Table 2). Maximum SGR of cell was determined 0.56 (mg/day) grown in $NMM_{1.0}$ g/l followed by 0.52, 0.52 and 0.48 (mg/day) in BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$, respectively. It was supported by chlorophyll a content (g/l) of *Chlorella vulgaris* found highest 10.60 (mg/l) grown in $NMM_{1.0\ g/l}$ followed by 10.36, 6.19 and 4.67 (mg/l) cultured in BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$, respectively. Total biomass followed the similar fashion. Physico-chemical parameters were determined every alternate day and the results recorded on maximum cell growth on 8th day shown in Table 3.

Proximate composition analysis showed that maximum protein (46.49%) was found of the cultured *C. vulgaris* grown in $NMM_{1.0\ g/l}$ followed by that grown in BBM, $NMM_{0.5\ g/l}$ and $NMM_{1.5\ g/l}$ (Table 4). Crude lipid of *C. vulgaris* grown in $NMM_{1.0\ g/l}$ was detected higher followed by those cultured in $NMM_{1.5\ g/l}$, $NMM_{0.5\ g/l}$ and BBM. Maximum crude fibre of the same was determined that cultured in BBM which gradually declined those were grown in $NMM_{1.5\ g/l}$, $NMM_{0.5\ g/l}$ and $NMM_{1.0\ g/l}$. Maximum NFE of *C. vulgaris* was determined when grown in $NNM_{1.5\ g/l}$ and minimum when cultured in $NMM_{1.0\ g/l}$. Ash content of the algae was determined maximum (12.61%) grown in $NMM_{0.5\ g/l}$ and minimum (9.49%) that cultured in BBM.

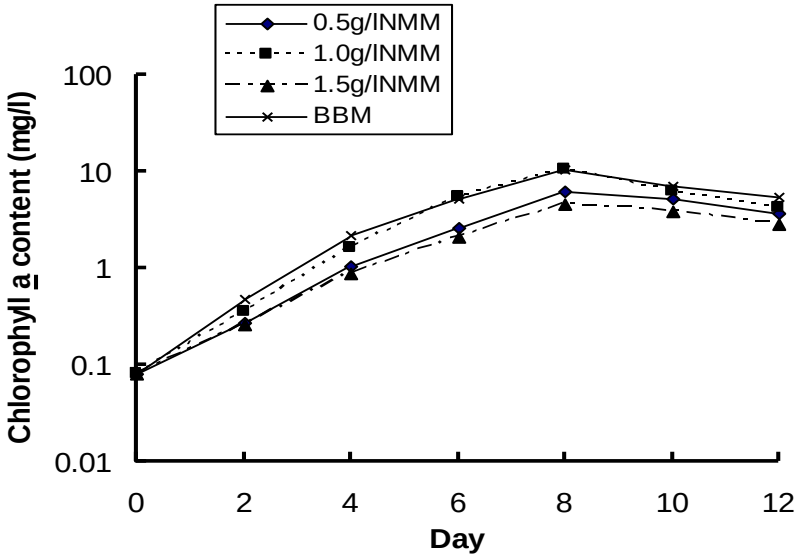

Figure 2. Semi logarithmic growth curve based on chlorophyll a content (mg/l) of *Chlorella vulgaris* grown in normal molasses media (NMM) and Bold Basal Medium (BBM) as control.

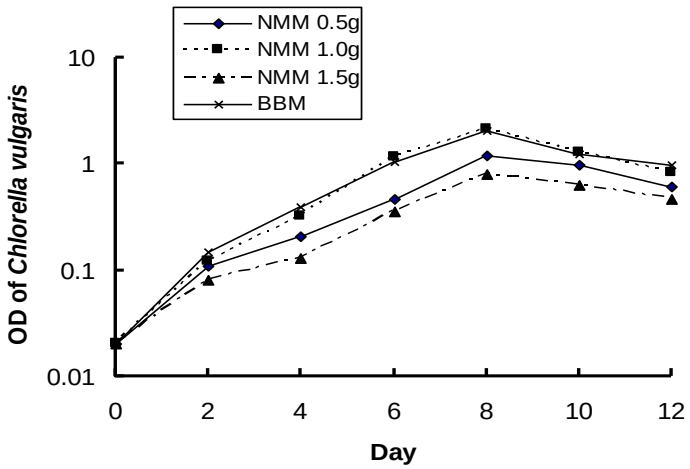

Figure 3. Semi logarithmic growth curve based on optical density at 620 nm of media contained *Chlorella vulgaris* grown in normal molasses media (NMM) and Bold Basal Medium (BBM) as control.

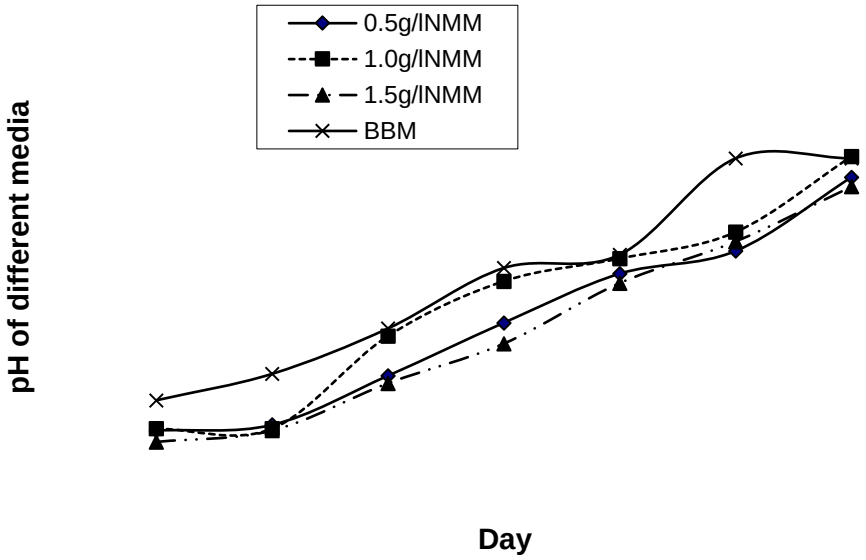

Figure 4. Hydrogen ion concentration (pH) of different concentrations of normal molasses media (NMM) and Bold Basal Medium (BBM) contained *Chlorella vulgaris*.

Table 2. Specific growth rate (μ/day) of cell, chlorophyll a (chlo-a) and total biomass of *Chlorella vulgaris* grown in different concentration of normal molasses media (NMM) and Bold Basal Medium (BBM)

Parameters	$NMM_{0.5\ g/l}$	$NMM_{1.0\ g/l}$	$NMM_{1.5\ g/l}$	BBM
SGR of cell	$0.51^{b} \pm 0.02$	$0.56^{a} \pm 0.01$	$0.48^{c} \pm 0.02$	$0.52^{b} \pm 0.03$
SGR of chlo-a	$0.51^{b} \pm 0.01$	$0.56^{a} \pm 0.03$	$0.48^{c} \pm 0.03$	$0.52^{b} \pm 0.04$
Total biomass	414.73^{b}	709.98^{a}	212.89^{c}	694.34^{a}
(Chlo-a × 67)*	± 14.07	± 20.78	± 17.76	± 18.72

Means (±SD) with different superscripts in each row indicate significant differences (P<0.01) *mg/l

Table 3. Mean (±SD) Cell no. ($\times 10^5$)/mL and physico-chemical parameters obtained during maximum growth of *Chlorella vulgaris* in different concentrations of normal molasses medium (NMM) and BBM as control on 8th day

Parameters	Different concentrations of NMM (mg/l)			
	$NMM_{0.5}$	$NMM_{1.0}$	$NMM_{1.5}$	BBM
Cell no. ($\times 10^5$)/mL	123.36±5.18	191.88±6.60	89.41±5.80	185.63±7.18
Light intensity (lux/m^2/s)	1890	1900	1920	1890
P^H	7.94±0.03	8.02±0.04	7.89±0.04	8.54±0.04
Temperature (°C)	28.11±0.02	28.10±0.02	28.11±0.01	28.11±0.02
DO (mg/l)	3.99±0.03	4.15±0.04	3.93±0.05	4.57±0.06
PO_4-P (mg/l)	1.04±0.04	1.16±0.05	1.42±0.06	3.13±0.06
NH_3- N (mg/l)	0.52±0.06	0.57±0.05	0.62±0.05	0.13±0.02
NO_3- N (mg/l)	0.99±0.05	1.19±0.06	1.28±0.05	7.65±0.19
NO_2- N (mg/l)	0.06±0.01	0.06±0.01	0.07±0.01	0.06±0.02
Alkalinity (mg/l)	71.1±9.87	79.8±9.87	85.5±17.1	119.7±17.1
CO_2 mg/l	28.33±2.89	30.0±5.0	30.0±5.0	30.0±5.0

Table 4. Proximate composition (amount % dry matter) of *Chlorella vulgaris* grown in different concentrations of normal molasses media (NMM) and Bold Basal Medium (BBM)

Composition	NMM 0.5 g/l	NMM 1.0 g/l	NMM 1.5 g/l	BBM
Moisture	8.66^{b}±0.08	8.37^{b}±0.09	9.30^{a}±0.08	6.21^{c}±0.09
Crude protein	44.15^{ab}±0.10	46.49^{a}±0.18	42.47^{c}±0.13	45.44^{ab}±0.11
Crude lipid	12.81^{b}±0.06	14.18^{a}±0.09	12.88^{b}±0.08	10.47^{c}±0.12
Crude fiber	7.75^{b}±0.10	6.48^{c}±0.09	8.27^{b}±0.08	10.43^{a}±0.12
NFE	22.68^{b}±0.08	21.44^{b}±0.08	24.23^{a}±0.21	23.17^{a}±0.22
Ash	12.61^{a}±0.19	11.42^{b}±0.08	12.16^{a}±0.08	10.49^{b}±0.12

Mean (±SD) with different superscripts in each raw indicates significant differences (P<0.01).

Discussion

Cell number, specific growth rates (SGRs) of the cell and chlorophyll a content and total biomass of *Chlorella vulgaris* grown in $NMM_{1.0\ g/l}$ were determined significantly higher ($P<0.01$) than those grown in the control and other concentrations of the NMM (Table 2). The molasses medium ($NMM_{1.0\ g/L}$) might have contains plenty of nutrients for microalgae growth and the color of the medium allowed to permit adequate light to accumulate chlorophyll-a content and optimum cell growth. Continuous aeration and heterotrophic nature of the media also having influence on producing CO_2 and O_2 balance for proper growth performances (Habib et al., 1998; Habib, 1998; Chui, 1993; Anton et al., 1994). Crude protein and crude lipid of *Chlorella vulgaris* grown in $NMM_{1.0\ g/l}$ were higher than those cultured in other media.

During present study light intensity below 2000 lux/m^2/s were maintained with a photoperiod of 12:12 hours, light : dark for the algal culture. In the study *C. vulgaris* growth was found maximum at pH 8.02 which has similarity with the findings of Alam et al., (2003); Khan et al., (2006); and Anaga & Abu, (1996). Toyub et al., (2008) found a pH range 8.03-8.07 which is very close to this finding. The average temperature was recorded around 28.50 ± 0.40°C in the media during the present study. Alam et al., (2003) recorded almost similar temperature range 26.8-30°C in microalgae culture with maximum cell growth on 10th day at 28.0°C. Many other investigators (Thinh, 1994; Khan et al*.*, 2018; Miah et al., 1999) also found almost similar temperatures in microalgae culture. Mayo (1997) reported maximum cell growth of *Chlorella vulgaris* at an optimum temperature of 32.4°C. Dissolved oxygen (DO) level 3.01-4.57 mg/l was more or less steady throughout the culture period. Maximum DO was recorded in the control medium during the peak period. The DO levels in other treatments also showed similar trends. This might be due to normal photosynthetic activity of the microalgae in light hours and continuous aeration. Khan et al., (2018) recorded similar trends before inoculation of *Chlorella vulgaris*. Other researchers (Miah et al., 1999; Alam et al., 2003) found maximum DO level in culture of *Chlorella* sp. in different inorganic media were 4.49 mg/l and 5.46 mg/l, respectively.

Phosphate-phosphorus (PO_4-P) was determined maximum 5.42 mg/l at the beginning in BBM and the minimum1.04 mg/l at the peak time on 8th day in the $NMM_{0.5\ g/l}$. In all the treatments maximum PO_4 level were recorded at the beginning and minimum were recorded at the peak time before stationary phase. Similar findings were recorded by many researchers (Hussain, 2001; Alam et al., 2003; Khan et al., 2006; Toyub et al., 2008) in culture of *Chlorella* sp. Ammonia-nitrogen (NH_4-N) and nitrite-nitrogen (NO_2-N) were determined minimum during inoculation of *Chlorella vulgaris* and maximum at the death phase. Similar trends were observed (Khan et al., 2006) during *Chlorella vulgaris* cultured in sugarmill effluent media. In case of nitrate nitrogen, it was found maximum at the beginning of the culture and minimum at the stationary phase. Almost inverse trend was observed in the cases of ammonia-nitrogen and nitrite-nitrogen level at stationary phase. Hussain (2001) found maximum 0.85 mg/l ammonia-nitrogen on 10th day and minimum 0.09 mg/l at the beginning of the culture of *Chlorella ellipsoidea* in different concentrations of jackfruit seed powder media (JSPM) and BBM.

In the present study, Carbon dioxide (CO_2) ranged from 20 mg/l to 40 mg/l throughout the culture period. Carbon dioxide was recorded 30.0 mg/l during maximum cell growth on 8th day of the culture and the lowest amount 20.0 mg/l was recorded on 12th day at death phase of the culture. CO_2 is a soul of carbon in the culture system which also provides essential pH stabilization (Ukeles, 1971).

Conclusion

Sugar is produced approximately in one hundred fifteen countries within the global. Brazil and India are holding the primary and 2nd in sugarcane manufacturing nations, respectively (Poddar & Sahu, 2017). India is the second one biggest manufacturer of sugar within the world. Now it can be imagined how vast volume of molasses and other wastes are releasing sugar producing countries and causing environmental problem including Bangladesh. On the other hand, having variety of uses of these industrial wastes and specially, molasses as a low cost ingredient and carbon source may be considered suitable for growth of microalgae. In this study the cell growth of *Chlorella vulgaris* in $NMM_{1.0g/l}$ showed it a promising culture media. It may enhance the interest of researchers to come forward for commercial production of microalgae (Strop, 2014; Rajesh et al., 2015; Rasappon et al., 2015; Perez-Garcia et al., 2011) using molasses and it may be a good environment friendly solution through aquaculture.

Ethical Statement

Not applicable.

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Author Contribution

First Author: Data analysis and interpretation; Second Author: Data Curation, Investigation, Methodology, Visualization and Writing -original draft; Third Author: Funding Acquisition, Project Administration, Resources, Writing -review and editing;

and Fourth Author: Supervision, Writing - review and editing and also provided final approval of the version to publish the workand he is the corresponding author of this research.

Conflict of Interest

The author declares that he has no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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