Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Application of Bagasse Extract in Economic *Nannochloropsis oculata* Mass Production

A. B. El-Sayed[1], Nashwa A. H. Fetyan[2] , Fatma . M. Ibrahim[3]*, Sayed A. Fayed[4] and M.W. Sadik[5]

[1]*Algal Biotechnology Unit, National Research Centre, Egypt*

[2]*Soils, Water and Environment Research Institute, Agricultural Research Centre ,Egypt.*

[3]*Scholarship student - Academy of Scientific Research & Technology , Egypt.*

[4]*Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt.*

[5] *Department of Microbiology, Faculty of Agriculture, Cairo University, Egypt*

Abstract

Algae were considered as an alternative source of food and a lot of biologically active ingredient. Beside, algae nutrition seems to be the most limiting factor concerning proper growth and economy cost. The main figure in this respect is carbon nutrition. The aim of the current work was to use bagasse for carbon nutrition of *Nannochloropsis* alga beside the selection of the proper concentration.

Growth was performed using F2 growth medium for inoculum preparation and sub-culturing, while artificial growth medium was made and used to be mass culture of alga. The investigated parameters were dry weight, total chlorophyll and carotenoids. Results showed that a high nutritional composition of bagasse extract as an alternative source of organic carbon (89.9% of total cell carbon)and other nutrients N, P and K .(0.035 ppm, 7.0 ppm and 51 ppm, respectively) especially when integrated with original growth medium (F2) and bagasse extract led to extra growth enhancement. Low and medium concentrations of bagasse in cultures free growth medium led to decrease the chlorophyll content and the maximum chlorophyll content ($0.54 g.l^{-1}$). It was obtained by cultures grown with full F2 growth medium enriched by 10% of bagasse extract. By such treatment, carotene possessed the lowest ($0.14 g.l^{-1}$) outdoor zigzag shape photobioreactor led to the highest volumetric algal biomass production compare to indoor systems .Unit specification markedly affected growth characteristics and yield both of *Nannochloropsis oculata* in dry weight and oil content. Chemical composition revealed the relatively high content of carbohydrates (26.6%) and oils (11.9%) on the expense of protein content (32.8%) and the maximum figure of ash content (2%) goes back to sodium ions.

Keyword: Nannochloropsis oculata; Bagasse; Growth; Chemical composition

1. Introduction

The main consideration of alga mass production is to use the alga species with low rate of nutrient consumption parallel with high nutritive value including *Nannochloropsis oculata.*

Several obvious advantages have been indicated the algal benefits in human nutrition, human therapy, plant production, energy production and wastewater treatment [1].

Studies showed that production of algae in phytoplankton were generally higher than those found in nature. Production of algal cultures must therefore be enriched with natural nutrients in order to replace the deficiencies of nutrients in the seawater. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate. In addition, trace metals, such as, Fe, Mg, Mn, B, Mo, K, Co and Zn, are also requested. Studies showed that the nutrients used in algal biomass production can be supplied in the form of simple, easily available agricultural fertilizers [2, 3], which save costs. Carbon nutrition is the key turn parameter in algal growth and mass production since carbon was account by 50% of algal dry weight. Theoretically, about 1.85 kg of carbon dioxide is required to

*Corresponding author e-mail: fatmamohamedalhwary123@gmail.com.

Receive Date: 17 May 2020, Revise Date: 08 July 2020, Accept Date: 19 July 2020

DOI: 10.21608/EJCHEM.2020.30459.2651

produce one kilogram of dried algal biomass when all the supported gas was fully utilized within the close system. Unfortunately, under such conditions, only 15% of the fed gas was utilized [4]. Thus, use of organic carbon sources seems to be the more benefit beside the other nutrients of such sources [5, 6]. In spite of the initial carbon concentration, nutrients, agro-industrial wastes, e.g., soybean residue, cane molasses, glycerol and monosodium glutamate waste liquor (MGWL) had been increasingly applied in bioprocesses because those were excellent substrates for heterotrophic micro-organisms growth by supplying the essential nutrients [7]. Otherwise, some growth factors are present in a sufficient concentration based on the waste sources [8]. Traditional, carbon dioxide from yeast production [9] was universally used.

There are some factors could limit the algal growth especially carbon dioxide within air (0.033%). Carbon dioxide must be injected into algal cultures gas addition facilities [10]. CO_2 is expensive, so the use of it can increase the costs. In practice, air can be introduced into a deep level underwater via air stones to improve the efficiency of CO_2. Another method is to introduce CO_2-rich industrial flue gas into the cultures.

Microalgae can be classified into 2 groups according to carbon supply. The first category called autotrophs which use inorganic carbon dioxide and perform photosynthesis using light as energy source. The other category called heterotrophic which use organic carbon such as sugars. [11] reported that there are some species which can use both, organic and inorganic carbon sources are called mixotrophs.

[12] reported that there are major inputs for algae growth includes sunlight, CO_2, water, nitrogen and phosphorus. Also, major nutrients such as nitrogen and phosphorous contribute to about 10–20% of algal biomass. [13] investigated that algae can grow in media within pH between 5 to 6 with the optimum range between 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture [14]. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values up to pH 9 during algal growth.

[15] reported that light is very important factor as a source of energy with specific intensity, spectral quality and photoperiod requested to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: Light intensity must be achieved 1,000 lux for indoor growth of algae within Erlenmeyer flasks. However, algae growth in large volume production need light intensity between 5,000-10,000 lux to penetrate through the culture. Light may be natural or supplied by fluorescent tubes. Too high light intensity (e.g., direct sun light, small container close to artificial light) may result in photo-inhibition. [10] investigated that marine phytoplankton are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 g/l have been found to be optimal. Too high concentration of salinity was harmful for algae growth since it might change their shape and structure due to the water pressure between media and cells.

Carbon nutrition of alga represented the maximum figure of production cost, where carbon reached 50% of algal dry weight. For this reason, it should be select the most prober carbon source, where different water sources are very poor in carbon content.

The aim of the current work is to determine the potential of bagasse extract for growth of the *Chrythophyta* alga *Nannochloropsis oculata* as an alternative carbon source and nutrients.

2. Experimental

2.1. Algae and growth conditions

Marine microalga *Nannochloropsis oculata* belonging to *Chrythophyta* was obtained from Algal Biotechnology Unit, National Research Centre, Egypt. Cultures were grown under conditions of F2 growth medium according to [16]. 2.5L air left vertical photobioreactor was used for inoculum preparation (Fig. 1a); while the other one containing 14L (Fig. 1b) was used for indoor growth and outdoor inoculum preparation. In both cases, light at 120 µ.e was provided from white fluorescent laps from the interior side. Turbulence was done using free oil compressed air. Culture maintaining and harvesting during indoor growth was achieved as described by [17].

2.2. Growth measurements

The main investigated parameters were dry weight, total chlorophyll and total carotene. For dry weight the OD of microalgal suspension was

measured at 680 nm using a spectrophotometer. Total chlorophyll and carotenes were determined according to [18].

2.3. Preparation of sugarcane bagasse aqueous extracts (SBAE)

Fresh wet bagasse was locally collected from Giza Governorate Egypt. It was subjected to acid extraction at 30°C for 24 h with 1N HNO_3, sieved over 0.2 mm and then under vacuum filtrated over Whatman 50 filter paper. Fine filtered solution was subjected to chemical analysis according to the methods described by [19].and listed in (Table 2).

2.4. Experimental design

In the current design, four different concentrations of SBAE (5, 10, 15, and 20 %) were used to grow *N. oculate* algae comparing with the standard F2 growth medium in three replicates. Growth period was 15 days in combination with10% bagasse extract (the most effective one). Scaling up was performed both in vertical tubular photobioreactor (15 tube x14L volume) during indoor stage and 1000L Zigzag-shape photobioreactor during outdoor period.

2.5. Outdoor experiment

The main effective reason on yield and productivity was found in regard with unit specification including turbulence and high exposed area and flow rate (Table 1). The sum of exposed area is 44.03 m^2 verses to 1.2 m^2 of growth volume. Thus, the ratio of exposed area to volume reached 36:1comparing with those of traditional open pond which reached 5:1.

Zigzag-Shape photobioreactor with 1000L capacity as previously mentioned structure by [20]. Was used to cultivate *N. oculata*. Prior cultivation (Fig. 2), the unit was sterilized before inoculation by hypochlorite solution overnight then washed by water till cleaning and then was filled by 1000L tap water containing 25% F2 growth medium, 10% bagasse filtrate and enriched by commercial fertilizer compounds as described by [21] in presence of sea salt to simulate the natural growth habitat (Table 1). Night illumination was provided by upper surface fluorescent lamb (6 lamb × 40 watt) along with the growth surface [22].

2.6. Harvesting and drying

By the end of induction period 14 days; as the dense culture was obtained aeration was breakdown to allow gravity sedimentation. The upper clear solution was discarded and the remainder slurry was then concentrated by centrifugation at 4000 rpm in order to reach 70% of moisture. The de-watered biomass was then refrigerated at 5^{o}C to allow cell wall cracking. One day later, the obtained biomass was then dried in 45^{o}C circulated oven, fine grinded and then subjected to oil extraction [22].

2.7. Analyses

Protein content was estimated by determining total nitrogen based on microkjeldahl methods according to [23]. Total carbohydrates were determined by phenol sulfuric acid method [24]. For lipids determination, fine algal powder was filled into 100g cellulose extraction thimbles (41x123mm); soaked overnight with solvent mixture of 3:2 (v/v) n-hexane/isopropanol in dim light at room temperature (25^{o}C) according to [25,26]. Macro and micro-nutrients were determined according to the methods adopted by [27].

3. Results and discussion

3.1. Bagasse chemical composition

Comparing of F2 growth medium (Table 2) in concern chemical composition with the initial content of bagasse extract (BE) showed the high nutritional load of bagasse extract as an alternative source of organic carbon and other nutrients. As presented in table 2; except nitrogen; most of the initial nutrients of F2 were received from the filtered sea water (ASW), which contributes 95% of the net media volume and also poor of carbon source. Thus, the lowest carbon source of sea water obligates the use of external carbon source. Organic carbon seems to be the most effective one to avoid the high losses of food or industrial grade carbon dioxide [4,28]. Using of organic wastes were universally used for massive algal biomass production and such technique ultimately reduces the production costs [29]. Bagasse as well as organic wastes not only rich in carbon and other nutrients, but it is also rich in other growth promoters including vitamins and phytohormones [9].

Besides, the osmo regulator effect, chelating effect and buffering action against the rising of media reaction to alkaline side were considered as the main

reasons of bio-stimulating effect. Comparing with other-used organic carbon wastes such as citrate [5]. Bagasse extract seems to be rich in simple sugars mainly sucrose which are easily utilized by microorganisms as a carbon source [9].

3.2. Effect of BE concentration on N.oculata growth

Dry weight accumulation of bagasse grown algal cultures possessed different growth patterns (Fig. 1). Bagasse extract grown cultures exhibited the lowest growth dry weight compared with other treatments (F2 medium with bagasse extract). This effect could be attributed to the nitrogen deficient of bagasse waste which accounted by 0.035 ppm of the whole BE. Nitrogen deficiency is the main reason concerning dry weight failure of algal biomass. Under such condition, at least improving of chlorophyll biosynthesis is severely acute as well as protein percentage. Sever nitrogen depletion led to chlorophyll and protein decomposition with a rise in carotenoids and oils when other conditions saved algal cell against injury [30].

In parallel, integration of algal growth medium (F2) and bagasse waste led to extra growth enhancement due to the presence of all required nutrients. High concentrations of BE might lead to growth delay in accordance to trophic mode under continuous light regime. At the end of experiment, the highest biomass productivity (1.718 $g.l^{-1}$) was obtained in full medium enriched by 10 % BE followed by full medium and 20 % BE (1.608 $g.l^{-1}$) (Fig. 3). The initial nutrients of bagasse extract (Table 2) showed the promising future in algae production using it as a major or sole carbon source beside the accompanied minerals and safe cost.

In spite of the initial mineral content of bagasse extract, the main reason in concern to dry weight accumulation enhancement could be attributed to the presence of a high quantity of organic carbon. Physiological and nutritional aspects of algae nutrition claimed that about 50% of algal cell composition is carbon and one kilogram of dried algal biomass required about 1.8 kg of carbon regardless the carbon source [31]. Carbon dioxide was considered as the most traditional carbon source, however the utilization rate by algal cells in closed system is very low and such value is minimized when used in open air production [32]. Thus, with high losing rate and the rise of carbon dioxide cost, using of organic carbon seems to be more benefit. A lot of organic sources in algae production were successfully used even in mass production purposes such as citrate, corn, okra, potato peels, canola, cassava, olive …. etc. [33].

Nannochloropsis is a promising source of commercially valuable pigments [34]. Its pigment composition was mostly Chlorophyll(a) with a small amount of carotenoids under favourable conditions. The adjustment of pigment composition was a mechanism for microalgae to adapt to environmental stresses [35]. Factors affecting the bio-accumulation of such pigments were fully understood. Carotenoids accumulation by microalgae depends on both nutritional status [36,37]. Also, shifting of photosynthetic metabolism to carotenoids accumulation by lipid biosynthesis should be considered [38].

A series of agricultural and food industrial-wastes were already used in algal mass production mainly as unconventional carbon sources such as corn steam liqueur [4,39]; citrate wastes [5,6].and okra [40].

3.3. Chlorophyll

A slight difference on chlorophyll content was occurred regarding growth under different bagasse extract concentrations as compared with those found in dry weight accumulation by *Nannochloropsis* alga. Low and medium concentrations of bagasse extract in cultures free growth medium led to decrease the chlorophyll content which could be ascribed to the high organic carbon content and chlorophyll seems to be functionless as compared with control cultures or growth medium.

Cultures grown under 10% of bagasse extract enriched growth medium. Increasing of bagasse content might accompanied by high nutritional load in concern macro and micro-elements which required more carbon skeleton for further cell metabolism. This finding could be confirmed by the results obtained when such cultures were incubated by bagasse extract and enriched by different growth medium. Accordingly, 10% of bagasse waste enriched growth medium seems to be the most proper artificial growth medium mixture for both dry weight and chlorophyll accumulation (Fig. 4).

3.4. Carotenes

Back to the main carotenoids function in plant and algae as a light filter absorbed high light irradiation and deliver it to chlorophyll. Thus, under optimum conditions carotenoid content be lower as much as 1.0% of algal dry weight. Unfavorable conditions led to a massive increase of carotenoids content as a

protective function and cells tended to fix carbon via fatty acids via organic carbon.

The carotenogenesis and carotenoids production by algae strategies are based on the enhancement of vegetative growth to enter such period with sufficient amount of protein and enzymes. Cells entered carotenogenesis with low initial nitrogenous compounds and organic matter represented the sever failure of dry weight accumulation with low carotenoids content. Here, addition of bagasse extracts to control medium increases the organic carbon load which might altered the chlorophyll and carbon fixation processes and the carotenoids are expected to increase. The variable response to the given concentrations might be associated to the dry weight accumulation. Generally, control medium enriched by 5 and 15% of Bagasse extract resulted the maximum yield of carotenoids (Fig. 5)

3.5. Outdoor cultivation and yield

Outdoor was progressed using Zigzag shape photobioreactor. Unit specification markedly affected growth characteristics and yield both in dry weight and oil content. Unit structure and specification markedly affected growth performance and net biomass. The current growth unit was characterized by extra light exposed area vice the net volume by the same location (NRC);

Growth kinetics in concern volumetric biomass productivity expressed as growth rate resulted 0.119451g.l^{-1}.d^{-1} with doubling time of 5.8h. In addition, the percentage increase and degree of multiplication were found to reach 83.3% and 77.82%; respectively indicating the proper growth of *Nannochloropsis* under the ambient conditions (Table 3).

3.6. Chemical composition of outdoor grown N. oculata

Chemical composition of outdoor *Nannochloropsis* grown algae as listed below showed the relatively high protein content (32.8%); however high salt grown algae were considered as low as nitrogen and protein content. The increasing of protein content under the given conditions could be described to the initial composition of bagasse in concern to organic carbon and other accompanied nutrients beside the growth unit specification. Widely, the main component of marine algae as well as the saline water grown algae it oils and carbohydrates. So, the given results were 26.6 and 11.9% of total carbohydrates and oil content, respectively. Such results confirmed the hypothesis determine the closely relationship between salinity and cell metabolites (Table 4).

Effect of salinity (15, 25, 35, 45, and 55%) on growth, biochemical composition, and lipid productivity of *N.oculata* was investigated. The results demonstrated that the dry biomass of *N.oculata* was the highest at a salinity of 25% among the treatments in the first 10days cultivation ($P<0.05$). During days 14–19 (stage III), the dry biomass productivity was the highest at a salinity of 35% ($P<0.05$).

The algae had the highest chlorophyll a content (26.47 mg g^{-1}) at 25% salinity in stage I, and it decreased continuously at stage III. Protein content (as% of dry biomass) of algae reached the highest value of 42.25± 2.10% at 15%, and the lipid content was the highest of 32.11± 1.30% of dry biomass at 25%. However, the lipid productivity of these algae was the highest at 35% (64.71 mg l^{-1} d^{-1}; $P<0.001$). C16 series content was the highest among the total fatty acid methyl esters (FAME), and Eicosapentaenoic acid C20:5n-3 (EPA) content was high at the low salinity. Fatty acid profiles of *N. oculata* varied significantly under different salinities [41].

[42] applied NaCl induction with the optimal salt concentration at the late-exponential growth phase and found that the algae *Monoraphidium dybowskii* could increase total lipid content to 41.7% in a day. *Nannochloropsis oculata* showed a good adaptation to changes in salinity within the range 0 - 40 gl^{-1}[43].

4. Conclusions

Carbon nutrition could be serving as the most economically limiting factor in algae mass production. Organic carbon seems to be the most proper source reducing production costs and enhancing growth potential due to the accompanied macro and micro-nutrients.

5. Acknowledgments

This research work has been carried out as a part of activities of Algal Biotechnology Unit, National Research Centre, Dokki, Cairo, Egypt, (Prof. Dr. Abo El-Khair B. El-Sayed). The authors express thanks to all other stuff members. The Academy of Scientific Research & Technology.

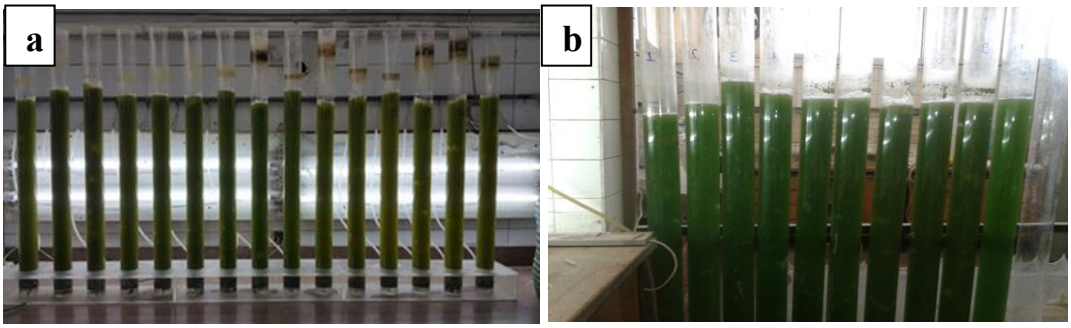

Fig. 1(a) 2.5 L vertical tubular photobioreactor and (b) Vertical tubular photobioreactor (14L)

Table 1 General specification of Zigzag-shape photobioreactor

Item	Specification
Tube length (cm)	200
Circle diameter (mm)	110
Circle radius (mm)	55
Tube thickness (mm)	5
Circle circumference (cm)	34.56
Tubes volume (L)	760
Unit volume (L) $_{approx.}$	1000
Cylinder surface area (cm^2)	$2.76x10^5$

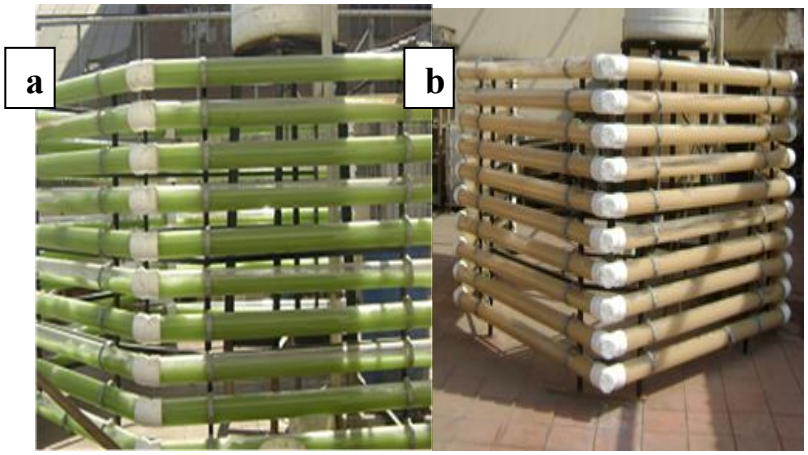

Fig. 2 Zigzag-Shape photobioreactor with 1000L (a) Vegetative (b)Stress

Table 2 Some chemical analyses of bagasse extract (BE); F2 growth medium and artificial sea water (ASW).

Analysis	O.C	N	P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu
Ppm											
BE	98.9	0.035	7.0	51	44	33.75	32.5	1.15	1.02	0.42	0.03
F2	ND	12.35	1.25	ND	ND	ND	9298	ND	ND	ND	ND
ASW	ND	124	2.0	97	141	3	8469	45	2	4	1.2

ND. Not detected

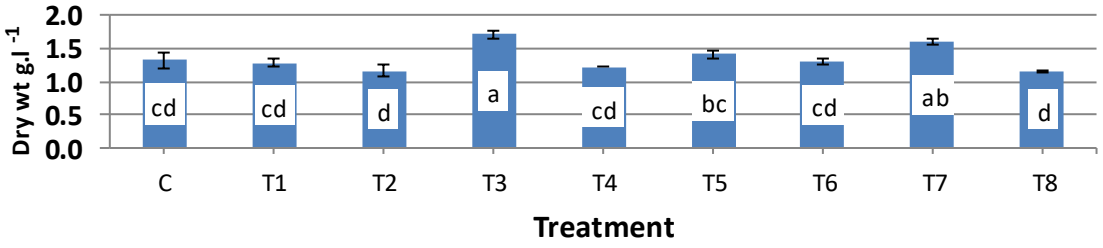

Fig. 3 Dry weight (g.l^{-1}) of *N. oculata* grown under different BE concentrations extract combined with some nutrient concentrations. C=control (medium),T1=medium +bagasse extract 5% ,T2=bagasse extract 5% ,T3=medium +bagasse extract 10% ,T4=bagasse extract 10% ,T5=medium +bagasse extract 15% ,T6=bagasse extract 15%, T7=medium +bagasse extract 20%,T8=bagasse extract 20%

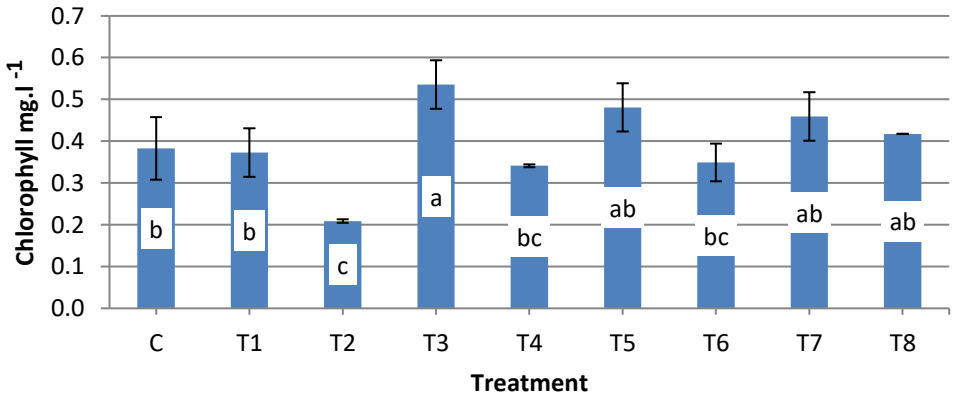

Fig. 4 Total chlorophyll (mg.l^{-1})of *Nannochloropsis oculata* grown under different BE concentrations combined with same nutrient concentrations. C=control (medium) ,T1=medium+bagasse extract 5% ,T2=bagasse extract 5% ,T3=medium +bagasse extract 10% ,T4=bagasse extract 10% ,T5=medium +bagasse extract 15% ,T6=bagasse extract 15%, T7=medium+bagasse extract 20%,T8=bagasse extract 20%

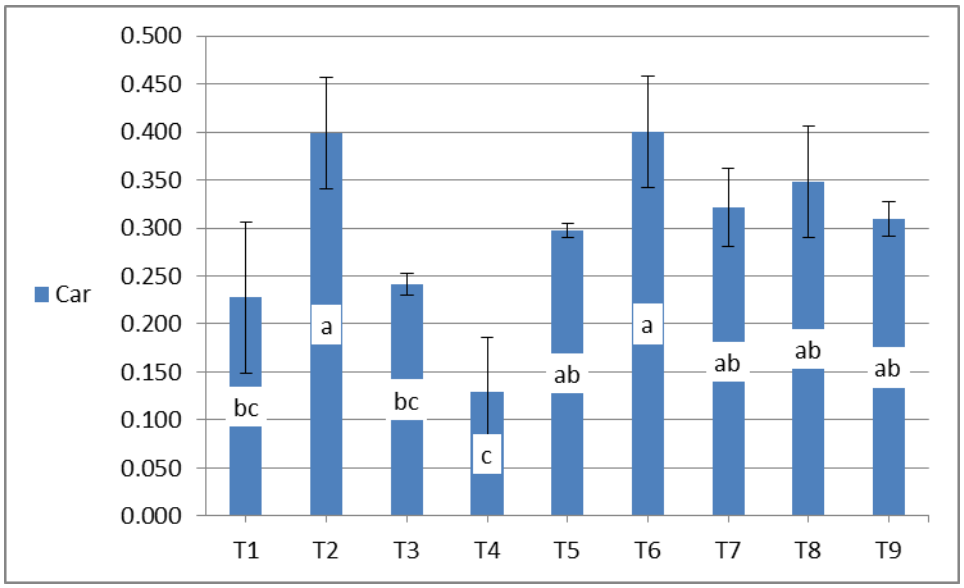

Fig. 5. Carotene (mgl-1)of *Nannochloropsis oculata* grown under different BE concentrations combined with same nutrient concentrations

C=control (medium),T1=medium+bagasse extract 5% ,T2=bagasse extract 5% ,T3=mediium+bagasse extract 10% ,T4=bagasse extract 10% ,T5=medium +bagasse extract 15% ,T6=bagasse extract 15%, T7=medium+bagasse extract 20%,T8=bagasse extract 20%.

Table 3 Growth kinetics of outdoor grown *N.oculata* with bagasse extract BE).

Growth rate (μ)	Doubling Time	Degree of multiplications (n)	Percentage increase
0.12	5.80	2.58	77.82

Table 4 Chemical composition of outdoor produced *Nannochloropsis oculata*

Biochemical analysis %									
Crude Protein	Total Carbohydrate		Lipid	Total Chlorophyll	Carotene	Ash	Fiber	Moisture	
32.8	26.6		11.9	1.19	2.2	9.8	8.75	6.76	
Ash analysis									
%						ppm			
N	P	K	Na	Ca	Mg	Fe	Zn	Mn	Cu
5.2	0.55	0.43	2.0	1.38	0.23	0.45	0.26	0.14	0.04

CP=crude protein, T.Car= Total carotenoids, T.Chlorophyl, Car= Carotenoides

References

[1] Craggs R.J., Heubeck S., Lundquist T. J. and Benemann J. R. Algal biofuels from wastewater treatment high rate algal ponds. *Water Science Technology* ,63 (4), 660-665 (2011).

[2] Anonymous. The design and operation of live feeds production systems.In: Rotifer and micro-algae culture systems, Proceedings of a US-Asia Hawaii. *The Oceanic Institute*, 3-52 (1991).

[3] Vonshak, A. Laboratory techniques for the cultivation of microalgae. In: CRC Handbook of microalgal mass culture. Richmond A. (Ed.). CRC Press, Inc., *Boca Raton*, 117-145 (1986).

[4] El-Sayed, A.B., Battah, M. G. and El-Sayed, E.W. Utilization efficiency of artificial carbon dioxide and corn steam liquor by Chlorella vulgaris. *Biolife*, 3(2),391-403 (2015).

[5] El-Sayed, A.B. Carotenoids accumulation in the green alga Scenedesmus sp. incubated with industrial citrate waste and different inductions stress. *Nature and Science*, 8(10), 34-40 (2010).

[6] El-Sayed, A.B., Hoballah, E.M. and Khalafallah, M.A. Utilization of Citrate Wastes by Scenedesmus sp. I- Enhancement of VegetativeGrowth. *Journal of Applied Sciences Research*, 8(2), 739-745 (2012).

[7] Pandey, A., Soccol, C.R, Nigam, P. and Vanete,T . Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. *BioresourceTechnology*,74, 69-80 (2000).

[8] Gujarathi N.P., Haney B.J and Linden J.C. phytoremediation potential of Myriophyllumaquaticum and Pistiastratiotes to modify antibiotic growth promoters, tetracycline, and oxytetracycline, in aqueous wastewater systems.*International Journal of Phytoremediation* ,7(2),99-112 (2005).

[9] Smallegange, R. C., Schmied, W. H., van Roey, K. J., Verhulst, N. O., Spitzen, J., Mukabana, W. R., and Takken, W. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito Anopheles gambiae. *Malaria Journal*, 9 (2) (2010).

[10] Mata, T.M.; Martins, A. A. and Caetano, N.S. Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews*,14(1),217–232 (2010).

[11] Dahiya, A., Biomass to Biofuel. *Bioenergy*, 1st edition, 219-238(2014).

[12] Benemann, J.R and Oswald, WJ. Systems and economic analysis of microalgae ponds for conversion of carbon dioxide to biomass, Pittsburgh Energy Technology Centre, Pittsburgh, PA, US *Department of Energy's Aquatic Species Program* (1996).

[13] Zeng, X.; Danquah, M.; Chen, X. and Lu, Y. Microalgae bioengineering: From CO2 fixation to biofuel production. Renewable and

Sustainable *Energy Reviews*, 15(6): 3252-3260(2011).

[14] Lam, M. K. and Lee, K. T. Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnology Advances,* 30 (3), 673-690 (2012).

[15] Metsoviti, M. N., Papapolymerou, G., Karapanagiotidis, I. T. and Katsoulas, N. Effect of Light Intensity and Quality on Growth Rate and Composition of *Chlorella vulgaris*. *Plants*, 9(1), 31 (2020)

[16] Guillard, R.R.L. Culture of Phytoplankton for Feeding Marine Invertebrates. In: Smith, M.L. and Chanley, M.H., Eds., Culture of Marine Invertebrates Animals. *Plenum Press*, 29-60 (1975).

[17] Aboulthana, W.M., El-Feky, A.M., Ibrahim, N.E., Sahu, R.K. and El-Sayed A.B. Evaluation of the pancreatoprotective effect of *Nannochloropsis oculata* extract against streptozotocin-induced diabetes in rats. *Journal of Applied Pharmaceutical Science*,8(6), 046-058 (2018).

[18] Boussiba, S.; Fan, L. and Vonshak, A. Enhancement and determination of astaxanthin accumulation in green alga Haematococcuspluvialis. Methods in Enzymology, 213, Carotenoids Part A, Lester Packer (ed.), *Academic Press*, 386-371(1992).

[19] Chpman, H.D. and Pratt, P.F. Methods for Soils. Plants and Waters. Berkeley: university of California, *Division of Agricultural Sciences, USA* (1974).

[20] Ayoub, H.F., Abdelghany, M.F. and El-Sayed, A.B. Effects of Diatoms *Amphora* coffeaeformis on growth parameters, non specific immunity and protection of the Nile tilapia (Oreochromis niloticus) to Aeromonas hydrophila infection. *Egyptian Journal of Aquatic Biology and Fisheries,* 23(1),413- 426 (2019).

[21] El-Sayed, A.B. Abdalla, F. E. and Abdel-Maguid, A. A. Use of Some Commercial Fertilizer Compounds for *Scenedesmus* Cultivation. *Egyptian Journal of Phycology*, 2, 9-16 (2001).

[22] El-Sayed AE-K and El-Sheekh M. Outdoor Cultivation of *Spirulina platensis* for Mass Production . *NotulaScientiaBiologicae,* 10(1),38-44,(2018).

[23] Ma, T.S. and Zauzag, C., Micro kejeldahl determination of nitrogen. A new indicator and an improved rapid method. *Industrial and Engineering Chemistry Analytical*,14(3),280-282(1942).

[24] Dubois ,M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and F. Smith. Colorimetric method for determination of sugars and Related substances. *Analytical Chemistry*, 28, 350-356 (1956).

[25] Hara, A. and Radin, N. Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, 90(1),420-426 (1978).

[26] Radin, N. S. Extraction of tissue lipids with a solvent of low toxicity. *Methods in enzymology*, 72, 5-7(1981).

[27] Chpman, H.D. and Pratt, P.F. Methods for Soils, Plants and Waters. Berkeley: university of California, *Division of Agricultural Sciences, USA* (1974).

[28] El-Sayed, A.B., Abdalla, F. E. and Abdel-Maguid, A. A. Use of Some Commercial Fertilizer Compounds for *Scenedesmus* Cultivation. *Egyptian Journal of Phycology*, 2, 9-16 (2001).

[29] El-Sayed A.B., El-Fouly, M.M. and El-Sayed, A.A. Utilization efficiency of elevated nitrogen, phosphorous and potassium concentrations by the green algae *Scenedesmus sp.* The 17th International Symposium of CIEC Plant Nutrient Managment under Stress Conditions. NRC, Cairo, Egypt.(2008).

[30] El-Sayed, A.B. Some Physiological Studies on Green Algae. Ph.D. Thesis, Plant Physiology Departmennt, Faculty of Agriculture Cairo University, Egypt (1999).

[31] Rodolfi, L., Chini Zittelli, G., Bassi. N., Padovani, G., Biondi, N., Bonini, G. and Tredici. M.R. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. . *Biotechnology and Bioengineering*,102(1),100-112(2008).

[32] Chisti, Y.Biodiesel from microalgae. *Biotechnology Advances*,25, 294-306(2007).

[33] Dang, N. M.and Lee , K.,Decolorization of organic fertilizer using advanced oxidation process and its application for microalgae cultivation. *Journal of Industrial and Engineering Chemistry*,59,297-303 (2018).

[34] Lubián, M. L.; Montero, O.; Moreno-Garrido, I.; Huertas, E.; Sobrino, C.; del Valle, M.G. and Parés, G. (2000). *Nannochloropsis (Eustigmatophyceae*) as source of commercially pigments. *Journal of Applied Phycology* ,12 ,249-255 (2000).

[35] Shtaida, N.; Khozin-Goldberg, I. and Boussiba, S. The role of pyruvate hub enzymes in supplying carbon precursors for fatty acid synthesis in photosynthetic microalgae. Photosynthesis Research, 125, 407–422 (2015).

[36] Cero'n, M.C., Sa'nchez, A., Ferna'ndez, J.M., Molina, E. and Garcı'a, F. Mixotrophic growth of the microalga Phaeodactylumtricornutum Influence of different nitrogen and organic carbon source on productivity and biomass

composition. *Process Biochemistry*,40(1), 297–305(2005).

[37] Tittel, J., Bissinger, V., Gaedke, U. and Kamjunke, N. Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from an acidic mining lake. *Protist*, 156, 63–75(2005).

[38] El-Shafey, Y. H., El-Fouly, M. M., Khalil, M. M., Abdallah, F. E. and El-Sayed, A. B. Secondary carotenoids accumulation by some green algae species. The First Congress on the Recent Technologies in Agriculture, Faculty of Agriculture , Cairo University, Egypt(1999).

[39] Battah, M.G., El-Ayoty, Y.M., Esmael, A.E. and Abd El-Ghany, S.E. Effect of different concentrations of sodium nitrate, sodium chloride, and ferrous sulphate on the growth and lipid content of *Chlorella vulgaris*. *International Journal of Agricultural Technology*,10(2),339-353(2014).

[40] Sheraz, M. K., El-Sayed, A.B., Hassan, A.A., El-Shazly, H. A.M. and Ibrahim, M.T. use of okara waste for algae nutrition. *Journal of Agriculture Science*, 25(1),271-279 (2017).

[41] Gu, N., Gang, Q. L., Tan, L. Y., Huang, L. and Lin, J. Effect of salinity on growth, biochemical composition, and lipid productivity of *NannochloropsisoculataCS 179. Engineering in Life Science*, 12(6), 631–637 (2012).

[42] Yang, H., He, Q., Rong, J., Xia, L. and Hu, C. Rapid neutral lipid accumulation of the alkali-resistant oleaginous Mono raphidiumdybowskii LB50 by NaCl induction. *Bioresource Technology*, 172,131–137 (2014).

[43] Mohammady, N.G. and Fakhry, E.M. Compromising Between Growth and Oil Production of *Nannochloropsisoculata* Cultivated Under Halo-Stress. *Life Science* 11(5) ,193-197 (2014).