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Utilization efficiency of artificial carbon dioxide and corn steam liquor by *Chlorella vulgaris*

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ABSTRACT

Consumption of organic and inorganic carbon by the green alga *Chlorella vulgaris* was determined. Artificial carbon dioxide was laboratory prepared by nitric acid and calcium carbonate reaction. The exhausts carbon dioxide from algal growth container was trapped by KOH to form soluble potassium carbonate. Concerning organic carbon, corn soaking water (corn steam liquor) was used. The ex-grown alga *Chlorella vulgaris* in full BG-II media was tested under different concentrations of wastes. Growth parameters were dry weight, total chlorophyll and total carotenes. The percent of carbon dioxide capture was found to be 32.5% within 30cm culture depth. Thus, 67.5% of the injected CO_2 were drained off again to the outer media. Dry weight during indoor growth was proportionally associated with the enriched waste volume due to extra organic carbon supplementation and other required nutrients mainly nitrogen, phosphorous and potassium. The best result was obtained ($\geq$0.3g.d^{-1} of biomass) with cultures that supported by over than 10ml.l^{-1} wastes in the presence of BG-II growth media. Otherwise, alga able to grow well at 10- 30 m.l^{-1} of wastes in the absence of both BG-II growth media and carbon dioxide, but varied on their growth-reaching time. Cells reached their maximum growth after 7 days of incubation.

Key words: *Chlorella vulgaris*, corn steam liqour, organic carbon, photobioreactor

INTRODUCTION

Microalgae are expensive to produce, and different systems have been designed for the growth and handling of microalgae on a large scale (Richmond 2004; Tredici, 2004 and El-Sayed, 2007). Mineral nutrition mainly carbon represented the maximum, but also depending upon used alga, cultivation system and target of cultivation (El-Sayed, 2007). Use of commercial fertilizers as well as food industrial wastes could minimize such coast in which downed the biomass price to meet different beneficial use (El-Sayed *et al*., 2001 and El-Sayed *et al*., 2012).

Energy sources including light and/or carbon sources were early recognized as a key factor of photosynthetic process that significantly influences the whole growth and lipid accumulation of microalgae. Photoheterotrophic conditions are usually confusing to distinguish mixotrophic from photoheterotrophic growth. In photoheterotrophic cultivation, the microalgae require light as an energy source while using organic materials as the carbon source. In contrast, both organic carbon and CO_2 are

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essential carbon sources in mixotrophic growth, as a light source is also supplied. Organic carbon metabolism may exert an opposing influence on photosynthesis. Glucose can reduce the apparent affinity for CO_2 in CO_2 fixation in some algae species such as *Chlorella sp*. (Lalucat *et al*., 1984), and *Chlorella vulgaris* (Martinez and Orus, 1991).

Heterotrophically cultivated *Chlorella prototheco*ides has been shown to accumulate as much as 55% of its dry weight as oil, compared to only 14% in cells that grown photo-autotrophically (Wu and Miao, 2006); since microalgae can alter lipid metabolism in response to stress mainly the lack of bio-available nitrogen (Tornabene *et al*., 1983).

Some algal species are capable to use wastewaters as the basis of the medium, where treated sago starch effluent was used to produce animal feed grade *Spirulina* (Tanticharoen *et al*., 1993 and Bunnag *et al*., 1998). Lu *et al*., 2009 used casava starch (*Manihot esculenta* Crantz) as an alternative carbon source in batch and in 5-L fed-batch culture to produce high oil yield in *Chlorella prototheco*ides. He stated that the biomass concentration reached a maximum of 53.6 $g.l^{-1}$ after 168 h fermentation. The cell growth rate was 7.66 $g.l^{-1}.d^{-1}$, which is over nine times higher than that in shake-flask cultivation (0.82 g.l−1.d−1). Furthermore, Budiyono and Kusworo (2012) developed an integrated process of biogas production and purification from cassava starch effluent by using bio-stabilisator agent microalgae. Another implementation was achieved by Phang *et al*. (2000) on the digested effluent of sago starch that has; on average; C: N:P ratio of 24: 0.14: 1. Effluent supported growth of *Spirulina platensis* (Arthrospira) with an average specific growth rate (μ) of 0.51 d^{-1} compared with the average of 0.54 d^{-1} in the inorganic Kosaric medium.

Starch is produced from potatoes, corn, and wheat. Corn starch fractional flow is maceration station, germ washing, starch milk dewatering, gluten thickener, glue of gluten dewatering, and chaff dehydration. The various fractions of wastewater are mainly recycled and used as processing water. The wastewater fractions that have to be treated are processing water and the condensates resulting from evaporation of the maceration water (Rosenwinkel *et al*., 2002).

The present work was performed aiming to determine the potential use of starch effluent as a source of organic carbon as well as other some nutrients including nitrogen, phosphorous and potassium; in algae production which in turn triggered the biomass accumulation and reduce the production costs.

MATERIALS AND METHODS

Alga Inoculum and growth conditions:

The green alga *Chlorella vulgaris* (NRC); was heterotrophically grown under optimum conditions of BG-II nutrient solution (Stainer *et al*., 1971) to obtain the proper inoculums. Continuous light illumination was provided from day light lamps (5x40w) reflexes from one side to give about 120μ.e of light intensity. Aeration was performed by free oil compressed air from the upper hold throughout 3mm polyethylene tube ended by compact sand distributor. Room temperature was recorded to be 27±2^{o}Cduring the whole incubation period. Incubation was employed within fully transparent polyethylene bags (75cm length x5cm diameter and 100μ thickness) containing 2.0 L of the algal broth (El-Sayed and El-Fouly, 2005). When growth reached the maximum, the biomass was collected by cooling centrifuge (RUNNE HEIDBERG model RSV-20); and washed two times to remove all of the accompanied nutrients.

Generation of carbon dioxide and autotrophic growth:

Glass burette with a tap was filled by 0.05 M of HNO_3 and dropped over quick firmed reaction vessel containing 20 g of dried $CaCO_3$ via glass connection of 3mm in diameter. The generated CO_2 was passed through a lateral glass valve to algal culture with slow aeration. The exhausting gas mixture was then trapped into 1.0M KOH solution to form soluble K_2CO_3.

Figure.1 Diagram of CO_2 generation and K_2CO_3

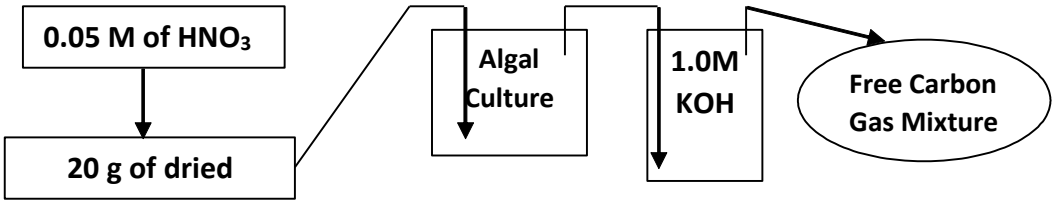


Table. 1. Elemental, chemical and biochemical analyses of starch corn wastewater

Macroelements %												
T.N	T.P	S.P	T.K	S.K	Mg^{+2}	Ca^{+2}						
2.96	0.88	0.82	1.52	1.44	0.33	0.0253						
(Microelements) ppm				O.C%	CHO %		Anions (meq.l^{-1})					
Fe	Zn	Mn	Cu		Soluble	Total	CO_3^{-2}	HCO_3^-	NO_3^-	Cl^-	SO_4^{-2}	NH_4^+
35.4	10.6	8.8	0.45	5.26	0.06	20.24	Traces	201.7	1.4	92.3	125.1	0.54

T.N= total nitrogen; T.P= total phosphorous; S.P= soluble phosphorous; T.K= total potassium; O.C= organic carbon and CHO= carbohydrates.

At the end of incubation KOH solution was titrated. Titration was performed by 0.1M HCl using two indicators including phenol phthalien (Ph.Ph) and Methyl orange (M.O) to calculate the concentration of KOH present in the solution and K_2CO_3 produced in the solution.

Heterotrophic growth and wastewater treatment:

Wastes were added to algal broth at 0.0, 5.0, 10, 15, 20, 25 and 30ml.l^{-1} of culture volume. The moderate level of waste (20ml.l^{-1}) contain the nearly amount of BG-II carbon, while the lower (8.3ml.l^{-1}) contains the same amount of nitrogen. Elemental, chemical and biochemical analyses (Table 1) were performed by the described methods of Chapman and Pratt (1984).

All treatments were performed versus the same concentration of wastes free of BG-II media to eliminate each effect. Concentrations applied and initial element content listed in Table 2.

Growth units:

Indoor incubation was performed using a fully transparent polyethylene bags containing 2.5 L of algal broth. Scaling up was done within 200L Plexiglass open sheet (El-Sayed, 2007). Specification of both used units listed in Table 3.

Growth measurements:

The investigated parameters were dry weight (g.l^{-1}), total chlorophyll (mg.l^{-1}) and total carotene (mg.l^{-1}). For dry weight estimation, 5ml from each replicate were separately filtered over a pre-weighted Whatman sterile membrane filters (pore size 0.45µm, 0.47 mm in diameter and white grade). After filtration, filters were left to dry for 30 minutes at 105°C circulated oven, kept over anhydrous calcium chloride till room temperature and then re-weighted. The difference between weights monitored the net dry weight of the grown alga within defined sampling time .the dry weight was calculated as g.l^{-1}.

Total chlorophyll was extracted by dimethylsulfoxide (DMSO) according to Burnison (1980). Five ml of algal suspension was centrifuged at 3500 rpm for 5 minutes. The supernatant was discarded and the residual pellet was re-suspended in 5 ml of 95% DMSO, homogenized and kept for 5 minutes at 70°C water bath. Such extract contains total chlorophyll and cell carotenoids. The extracted cells were re-centrifuged again at 3500 rpm for 5 minutes. The extracted solution was measured by reading the absorbance (A) of the pigment extract by spectrophotometer at 666m. Total chlorophyll content was calculated (mg l^{-1}) according to Seely *et al*. (1972). To recover

Table. 2. Wastes volume and initial element nutrient concentrations

Treatment	C	N	P	K	Ratios			
					C	N	P	K
T1= BG-II	0.007	0.247	0.007	0.009	1.0	35.3	1.0	1.29
T2= BG-II +5ml waste	0.270	0.359	0.051	0.085	1.0	1.33	0.19	0.31
T3= 5ml waste	0.263	0.148	0.044	0.076	1.0	0.56	0.17	0.29
T4= BG-II +10ml waste	0.533	0.543	0.095	0.161	1.0	1.01	0.19	0.30
T5=10ml waste	0.526	0.296	0.088	0.152	1.0	0.56	0.17	0.29
T6= BG-II +15ml waste	0.796	0.691	0.139	0.237	1.0	0.87	0.17	0.30
T7= 15ml waste	0.789	0.444	0.132	0.228	1.0	0.56	0.17	0.36
T8= BG-II +20ml waste	1.059	0.839	0.183	0.313	1.0	0.79	0.17	0.3
T9=20ml waste	1.052	0.592	0.176	0.304	1.0	0.56	0.17	0.29
T10= BG-II +25ml waste	1.322	0.987	0.227	0.389	1.0	0.75	0.17	0.29
T11= 25ml waste	1.315	0.74	0.22	0.38	1.0	0.56	0.17	0.29
T12= BG-II +30ml waste	1.585	1.135	0.271	0.465	1.0	0.72	0.17	0.29
T13= 30ml waste	1.578	0.888	0.264	0.456	1.0	0.56	0.17	0.29
T14= 8.3 ml waste	0.437	0.246	0.073	0.126	1.0	0.56	0.17	0.29

carotenes, saponification was performed by 5% KOH/30% MeOH and the residual was re-extracted by DMSO after the addition of 5 drops of concentrated acetic acid (Boussiba *et al.*, 1992). Carotenes absorbance was measured at 468nm and concentration was calculated ($mg.l^{-1}$) according to Davies (1976). Growth rate, doubling time, degree of multiplication (n) and percentage increase (y%) were performed using the methods adopted by Pirt (1973).

Table 3. Technical specifications photobioreactor open sheet

Item	Specification
Unit height (cm)	200
Unit width (cm)	100
Unit depth (cm)	10
Sheet thickness (mm)	9
Unit volume (L)	200
Unit surface area (cm^2)	$4x10^4$
Needed land length (m)	1.4
Needed land width (m)	0.6
Needed total area (m^2)	0.84

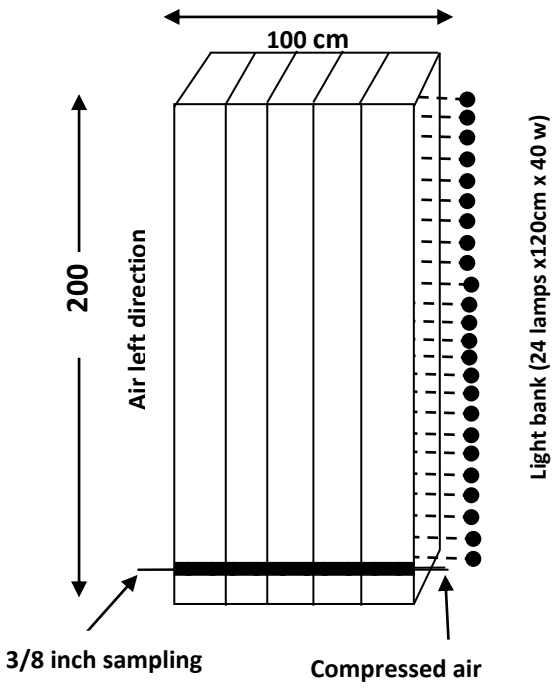
RESULTS AND DISCUSSION

Utilization rate of generated carbon dioxide and autotrophic growth:

Addition of HNO_3 over 20 g of $CaCO_3$ generates carbon dioxide according to the common equation as:

Thus, the generated carbon dioxide was gravelly self-injected to algal growth vessel according to the ambient slow gas pressure that used for turbulence. The amount of generated carbon dioxide was calculated as 8.8 per each 20 g of calcium carbonate. The excess of carbon dioxide drained out of algal vessel and trapped into a concentrated solution of potassium hydroxide to form potassium carbonate.

Figure.2. Open sheet photobioreactor



The concentration of KOH used was 1.0 M and the remainder after reaction with CO_2 was 0.335 M meaning that 18.63 g of K_2CO_3 (0.135 M) was formed due to trapping of 5.94 g of CO_2. Accordingly, 2.86 g of CO_2 was utilized by alga during such period. The obtained biomass was 0.6 $g.l^{-1}$ (three days later).

100 unit $CaCO_3$ + HNO3 ⟶ $Ca(NO_3)_2$ + H_2O + 44 unit CO_2

20 g $CaCO_3$ + HNO_3⟶$Ca(NO_3)_2$ + H_2O + 8.8 g CO_2

Thus, one liter of algal broth consumed 1.43 g of CO_2 producing 0.6 $g.l^{-1}$ of algal biomass meaning that about 2383 g of carbon dioxide are required for the production of one kg of dried algal biomass. the actual growth was 0.14 g utilizing 1.43 g of CO_2 since the culture volume was 2.0L. By this result about 1430 g of carbon dioxide is required for the production of 1.0 kg of dried algal biomass. By this result, the percent of capture carbon dioxide was found to be 32.5% within 30cm culture depth. Thus, 67.5% of the injected CO_2 were drained again increasing the green house effect. In spite the obtained biomass, the amount of carbon dioxide fed to routine algal cultures (1.5%) must use in care through the modifying of growth unit used (El-Sayed, 2011). In addition, the formed potassium carbonate and calcium nitrate could be use in algal nutrition as a source of carbon, nitrogen, potassium and calcium.

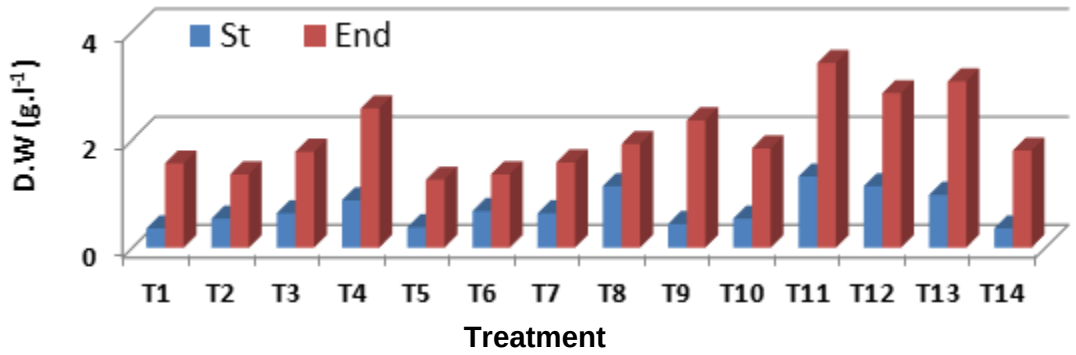
44 unit CO_2 + KOH ⟶ 138 unit K_2CO_3
5.94 g CO_2 + KOH ⟶ 18.63 g K_2CO_3 + H_2O

Comparing this result with the growth of alga under the ambient waste revealed that the initial content of organic carbon 5.26% and according to the above equations, one liter of algal broth require 1.43 g of carbon dioxide equal to 0.39 g carbon. Waste contains 5.26% of organic carbon that will provide algal growth media by 0.0526 g of carbon. So, to reach the same quantity of carbon dioxide (0.39g carbon); 7.414 ml of waste will expect to the optimum. Over concentration of carbon dioxide were early tested by several studies, but the common used concentration was 1.5% of air mixture. Even under such concentration, most of carbon dioxide gas drained off the surrounded atmosphere and also increased the acidity of growth media due to the forming of carbonic acid and carbonate; however it exhibited useful effect as the acidic reaction of healthy growth media almost shifted to alkaline reaction. The aforementioned hypothesis was taken as the base of the next treatments of wastes and also explained whether the optimum waste concentration used.

As early described by Garbisu *et al*. (1992) nitrate removal by immobilized *Phormidium laminosum* is strictly dependent on light and CO_2 availability. The cost of CO_2 addition to the culture is very high. Zaborsky (1985) reported that carbonte; the carbon source of blue green alga *Spirulina* represented about 75% of nutritional costs. Morever, carbon nutrition is a growth unit depending, where, closed system seems to be more efficient in carbon utilization (El-Sayed, 2011). In open systems at normal pH (< 9) the transfer efficiency of CO_2 is low, and it is doubtful whether the increased productivity achieved through the addition of CO_2 offsets the high cost of the CO_2 required (Borowitzka, 1992).

Carbon concentrations and C:N ratios were rarely considered. Many media have a bicarbonate concentration of about 2 mM and nitrogen (nitrate) of about 500 mM or higher, which yields a C:N ratio of about 4:1. According to the Redfield ratio, the chemical composition of the average phytoplankton is 106C:16N:1P, or 6.7C:1N. Therefore, most media are nitrogen-rich relative to carbon, and carbon could become limiting, depending on the growth rate of the phytoplankton and the surface area of the medium through which atmospheric CO_2 can diffuse (Riebesell *et al*., 1993). Using of urea at 0.53 $g.l^{-1}$ that equal to 17.6mMN provided algal culture by C:N ratio of 0.106:0.0247. The shortage of carbon content could be provided from atmospheric carbon dioxide during open door mass culture of *Scenedesmus* and *Chlorella* in Egypt (El-Sayed *et al*., 2001).

Figure.3. Growth dry weight of Chlorella vulgaris grown under different volume of starch effluent.



T1= BG-II, T2= BG-II +5ml waste, T3= 5ml waste, T4= BG-II +10ml waste, T5=10ml waste, T6= BG-II +15ml, T7= 15ml waste, T8= BG-II +20ml waste, T9=20ml waste, T10= BG-II +25ml waste, T11= 25ml waste, T12= BG-II +30ml waste, T13= 30ml waste and T14= 8.3 ml starch effluent.

Heterotrophy and waste effect on growth dry weight

Indoor cultivation:

Preliminarily observation suggested the growth failure was observed with cultures that incubated with starch effluent over concentrations of 35 ml.l^{-1} wastes. Growth failure could be ascribed to the presence of extra nutrients mainly phosphorous as compared with such concentration of BG-II content. BG-II contains phosphorous as K_2HPO_4 (0.04g.l^{-1}) provided growth media by 7.12 ppm at a final concentration. One ml of wastes provided the growth media by the nearest phosphorous quantities (8.8 ppm). Thus, 308 ppm of phosphorous reached the hyper concentration from 35ml of wastes. Other hypothesis might explain the losses of light saturation under the higher concentrations due to the increasing of solution turbidity. Growth as dry weight was found in proportional relationship to the supplied carbon. Consequently, excess of carbon supplementation is expectedly affecting the yield. Maximum carbon content was found in the highest (1.6%) with T12 (BG-II +30ml waste) and T13 (30ml waste); however T12 resulted in the higher dry weight might be goes back to the lower concentration of nitrogen versus to carbon content, although they slightly differed in phosphorous and potassium content.

As shown in Figure-3, variable responses on dry weight accumulation were observed as *Chlorella vulgaris* cultures incubated with the recommended BG-II media in the presence of different volumes of starch effluent. In spite of the biomass accumulation, cultures were mainly differed in the request day to reach the maximum growth dry weight (retention time); and consequently the rate of biomass production which could be serve as economically harvesting time. Control culture which received BG-II reached the maximum (1.2g.l^{-1}) after 9 days of incubation and producing; on the average; 0.13g.d^{-1}. When alga was incubated by the same amount of nitrogen 17.6mM (the original BG-II media according to Stainer *et al*., 1971) from starch effluent (8.3ml.l^{-1} waste); approximately the same pattern on yield, time and rate of productivity comparing with those of BG-II grown culture.

The slight increase (0.13-0.16 g.l^{-1}) might be goes back to the accompanied nutrients received from starch effluent mainly organic carbon. All other treatments surpass the control both as effluent plus/or free BG-II growth media. The maximum growth ($\geq$0.3g.d^{-1}) was obtained by the cultures that received BG-II+10ml wastes; 20ml wastes; 25ml wastes; BG-II+30ml wastes and 30ml wastes (Table 4).

Table 4. Treatments, biomass, daily yield and biomass retention time of Chlorella vulgaris grown under different volumes of starch effluent.

T	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14
S	0.4	0.54	0.6	0.88	0.4	0.7	0.64	1.14	0.44	0.54	1.32	1.14	0.98	0.36
E	1.6	1.36	1.8	2.58	1.3	1.4	1.58	1.92	2.36	1.84	3.42	2.86	3.08	1.8
Y	1.2	0.82	1.2	1.7	0.9	0.7	0.94	0.78	1.92	1.3	2.1	1.72	2.1	1.44
Y.D	0.13	0.14	0.24	0.24	0.3	0.23	0.16	0.11	0.32	0.22	0.35	0.34	0.3	0.16
D	9th	6th	5th	7th	3rd	3rd	6th	7th	6th	6th	6th	5th	7th	9th

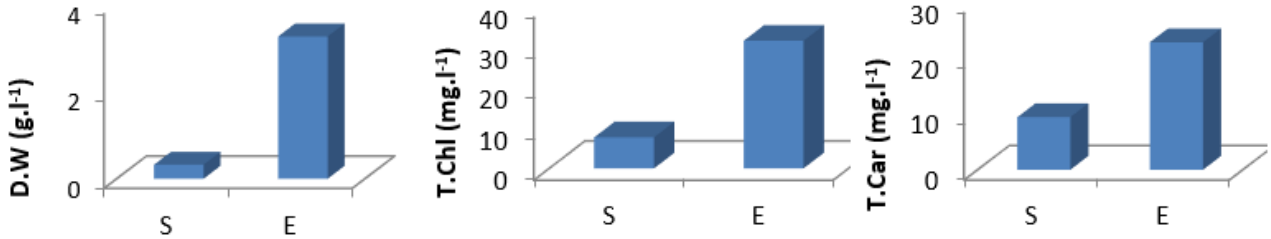
T1= BG-II, T2= BG-II +5ml waste, T3= 5ml waste, T4= BG-II +10ml waste, T5=10ml waste, T6= BG-II +15ml, T7= 15ml waste, T8= BG-II +20ml waste, T9=20ml waste, T10= BG-II +25ml waste, T11= 25ml waste, T12= BG-II +30ml waste, T13= 30ml waste and T14= 8.3 ml starch effluent.

Heterotrophic production has been successfully used for algal biomass and metabolites (Chen *et al*., 1996 and Miao and Wu, 2006). In this process, microalgae are grown on organic carbon substrates such as glucose in stirred tank bioreactors or fermenters. Algae growth is independent of light energy, which allows for much simpler scale-up possibilities since smaller reactor surface to volume ratios may be used (Eriksen, 2008). These systems provide a high degree of growth control and also lower harvesting costs due to the higher cell densities achieved (Chen and Chen, 2006). The set-up costs are minimal, although the system uses more energy than the production of photosynthetic microalgae because the process cycle includes the initial production of organic carbon sources via the photosynthesis process (Chisti, 2007).

Chlamydomonas reinhardtii in the presence of externally provided organic carbon (acetate), grows well, albeit with rates slower than those of the wild type, accumulates chlorophyll, protein and starch, and otherwise assembles the photosynthetic apparatus (White and Melis, 2006).

The ability of mixotrophs to process organic substrates means that cell growth is not strictly dependent on photosynthesis; therefore light energy is not an absolutely limiting factor for growth (Andrade and Costa, 2007**),** as either light or organic carbon substrates can support the growth. Examples of microalgae that display mixotrophic metabolism processes for growth are the cyanobacteria *Spirulina platensis*, and the green alga *Chlamydomonas reinhardtii* (Chen, 1996). The photosynthetic metabolism utilizes light for growth while aerobic respiration uses an organic carbon source (Zhang *et al*., 1999). Chojnacka and Noworyta (2004) found that mixotrophic cultures of *Spirulina* sp reduced photoinhibition and improved growth rates over both autotrophic and heterotrophic cultures. Successful production of mixotrophic algae allows the integration of both photosynthetic and heterotrophic components during the diurnal

Figure.4. a) Dry weight (g.l-1), b) total chlorophyll (mg.l-1) and c) carotenes (mg.l-1) of 200 L photobioreactor Chlorella vulgaris with 10ml.l-1 of starch effluent. S= biomass at start time, E=biomass at the end time



T1= BG-II, T2= BG-II +5ml waste, T3= 5ml waste, T4= BG-II +10ml waste, T5=10ml waste, T6= BG-II +15ml, T7= 15ml waste, T8= BG-II +20ml waste, T9=20ml waste, T10= BG-II +25ml waste, T11= 25ml waste, T12= BG-II +30ml waste, T13= 30ml waste and T14= 8.3 ml starch effluent.

cycle. This reduces the impact of biomass loss during dark respiration and decreases the amount of organic substances utilized during growth. These features infer that that mixotrophic production can be an important part of the microalgae-to-biofuels process.

Some algae species can grow autotrophically in the light using carbon dioxide and heterotrophically in the dark using organic compounds as energy and carbon-source. For instance, the green alga, *Haematococcus pluvialis* was able to grow heterotrophically as well as mixotrophically on acetate as well as autotrophically in the presence of carbon dioxide (Kobayashi *et al*., 1992).

Growth is influenced by the media supplement with glucose during the light and dark phases; hence, there is less biomass loss during the dark phase (Andrade and Costa, 2007). Growth rates of mixotrophic algae are higher in closed photobioreactors than for open pond cultivation, but are considerably lower than for heterotrophic production (Brennan and Owende, 2010).

Among the different organic carbon sources, glucose is the most widely used source of organic carbon and it is relatively inexpensive; however, acetate, citrate, and other organics have been used (Blanch and Clark, 1997, Humphrey, 1998 and Kuhlmann *et al*., 1998). It is obligated to use such wastes to meet the beneficial use and minimize production costs that save the demand of biofuel production from algae. It must be mentioned that many algal organisms are capable of using either metabolism process (autotrophic or heterotrophic) for growth, meaning that they are able to photosynthesize as well as ingest prey or organic materials (Zhang *et al*., 1999 and Graham *et al*., 2009).

Concerning other nutrients, as shown in Table 2, CNPK ratio affected growth of *Chlorella vulgaris*, however the net effect slightly returns to the media osmosis. It was observed that BG-II growth media supports algal culture by CNPK nutrients on a ratio of 1.0C:35.3N:1.0P:1.29K and wide differences between all treatments and the original media. No treatment was meted the aforementioned ratio of BG-II media. Addition of starch effluent reduces the ratio, but sharply increases the organic carbon in growth media (treatments) and increasing of waste volumes led to the growth enhancements. Treatments which produced $\geq 0.3 g.d^{-1}$ (T5, 9, 11, 12 and 13) were characterized by high carbon and nitrogen ratios ranged from 0.56 to 0.79 versus to carbon ratio. Concerning phosphorous, such ratio was found to be 0.17, while potassium ratio was 0.29. Economically, T5 (10ml.l-1 of starch effluent seems to be the best growth media for mass production of the green alga *Chlorella vulgaris*.

Outdoor cultivation:

Scaling up of the obtained results as the examined alga *Chlorella vulgaris* that grown in 200L photobioreactor at a final volume 10 $ml.l^{-1}$ of starch waste resulted in a linear growth curve and the growth decline was observed by the 8th day of incubation. By such time 2.34 $g.l^{-1}$ of algal biomass was obtained. Growth decline could be ascribed to the unit specification due to the losses of light efficiency. By other word the exposed area ($4m^2$) became very small to algal growth at the higher algal concentration due to self shading and competition. Concerning growth rate, $0.26g.d^{-1}$ was calculated by the end of incubation (7 days). Extension growth time (14 days) was resulted in $1.5g.l^{-1}$.

Growth determined as total chlorophyll exhibited the same manner and growth rate was dropped from 0.285 at the 8th day to be 0.007 by the 14^{th} day of incubation. This observation could confirm the abovementioned concept of growth decline due to the losses of light penetration and competition. Other confirmation could be also observed due to the slight increase of carotene content during all of the incubation period. It is expected that growth of algae within Photobioreactor almost increase carotenes content due to the increases on light exposure efficiency. In addition, lower chlorophyll content seems to be a primary indicator of unfavorable growth conditions mainly high light intensity beside nutrients depletion.

Regardless the enhancing effect of the used waste, growth unit also plays an important role on algal growth. The simplest approach is to blend CO_2 with air, for example, 0.2 to 5.0% of the total gas flow (Lee and Pirt 1984, Merchuk *et al*., 2000, Morita *et al*., 2001, Babcock *et al*., 2002). With an open system, most of the CO_2 will exit off the culture. Use CO_2 to control the pH of the culture (Lee and Pirt 1984, Delente *et al*., 1992, Babcock *et al*., 2002). It was also documented that photobioreactors can be bubbled with air, but the low CO_2 concentration in air (0.033%) will often limit phototrophic growth. With an airflow of 1 $L \cdot min^{-1}$, assuming all carbon dioxide is used and the biomass is 50% carbon, there is enough carbon to support 3.54 $x10^{-4}$ grams $biomass \cdot min^{-1}$; this is a very low productivity (Behrens, 2005 and Gitajnjali et al, 2015).

It may be concluded that under stress conditions, mainly nitrogen deficient or starvation as well as salinization of algal growth media, the media reaction shifted to alkaline and the presence of carbon dioxide is functionless. Under such conditions cells tended to increase its secondary metabolites mainly lipids and carotenoids through the carotenes metabolism of di-carbon fragments like organic acids.

As early observed by Droop (1954) and Borowitzka *et al*. (1991), acetate- at small quantities; appeared to be an important as a carbon source; enhancing both growth and carotenogensis, however, the effect of acetate was concentration dependent. Higher concentrations inhibiting growth, but markedly increasing astaxanthin content per cell. Acetate addition in excess may generate a relative shortage of nitrogen inducing cyst formation and astaxanthin accumulation triggered by a high carbon/nitrogen (C/N) The algal cells seem to reduce their nitrogen uptake and begin to use the cellular nitrogen as in typical N-deficiency, although nitrogen exists in the culture medium ratio (Kakizono *et al*., 1992).

El-Sayed (2010) found that citrate wastes at high concentration (50 $ml.l^{-1}$) support growth media by high organic carbon and other organic acids which stimulate both vegetative growth and carotenoids accumulation. It is important to note that such wastes contain 0.47% of organic carbon and the C:N ratio was 0.73:1.0. As mentioned before, in the absence of essential nutrients including nitrogen and phosphorous, increasing of acetate concentrations also rise the salinity potential of the growth.

The changes of photosynthetic pigments under mixotrophic culture vary from strain to strain. For example, there was significant loss of both chlorophyll a and b in *Scenedesmus acutus* (Ogawa and Aiba, 1981) and *Platymonas subcordiformis* (Xie *et al*., 2001) in mixotrophic growth with acetate. There are some microalgae that the content of photosynthetic pigments increased a little, such as *Chlamydomonas humicola* (Laliberté and de-la-Noüe, 1993). There are also several microalgae that only show small changes in pigment composition in response to organic carbon supplementation such as *Spirulina platensis* (Marquez *et al*., 1993). In addition, the composition of photosynthetic pigments varies with the difference of the organic carbon sources. For example, under mixotrophic culture of *Phaeodactylum tricornutum* UTEX-640, starch, glycerol, and lactate stimulated the content of chlorophyll a, and lactate also enhanced the content of carotenoids. Starch, glycerol, and glycine had the opposite effect on the carotenoids (Liu *et al*., 2009).

CONCLUSION

Organic carbon could be serving as the best solution of algae nutrition instead of carbon dioxide. Organic carbon not only provided algal growth media by energy source, but also rich in different nutrients based on waste sources. The main reason obligate the use of organic waste is to reduce the exhausting carbon dioxide since up to 80% of the used gas drained again to the outer media. In the present case, 10 $ml.l^{-1}$ of starch effluent save the normal algal production.

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