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Using an alternative medium (Molasses, yeast *Saccharomyces cerevisiae* and NPK) for cultivation of *Chlorella sp.*

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ABSTRACT

The green alga *Chlorella sp* was inoculated with different concentrations of culture media ranging from 125 to 450 mg/ml. The nutrients of the culture media (Molasses, yeast and NPK) seemed to be used by a *Chlorella sp*, it used them for its growth (increasing biomass, chlorophyll a content andcell number of *Chlorella sp*) that was weekly recorded, for a period lasted five weeks at the laboratory conditions. *Chlorella sp* gradually increased in such a way depends on the ascending concentrations of the medium, it started a growth increase after eight and four days in 125 and 250 mg/ml medium concentrations respectively. While in 350 and 450 mg/ml medium concentrations, the growth increased from the beginning of the experiment. *Chlorella sp* chlorophyll a content and biomass values in Ch 10 medium were very low in comparison with ones of *different* concentrations of the medium. No growth in control (D.W.) had occurred

Key words: *Saccharomyces cerevisiae, Chlorella, Molasses, yeast*

Introduction

Organic sources (Al-Rekabi, 2003) or waste products could affect the characteristics of media and / or natural waters as a whole that caused serious problems (Olsson *et al.*, 1995). Such wastes may change the character of an aquatic environment. One of these impacts is the fertilizing effect of some wastes that encourages the growth of plankton, algae and higher plants (Rounsfell and Everhart, 1953). This is due to the analysis of organic wastes by microorganism activities into simple compounds such as nitrate, sulfate and phosphate. According to Bennet (1970) excessive phosphate and nitrates may stimulate algal blooms to a large extent. Mulbry and Wilkie (2001) worked on the growth of benthic freshwater algae on dairy manures and found that the untreated manures gave more growth than that of treated one. Regarding the protein as a nutrition source increased for human being and became more universal essential demand, because of the highly increase in population. Thus, the scientists focused their concern to look for this sector with a lot of attention (Golucke and Oswald, 1965; Mallet *et al.*, 1998; Karjalaninen *et al.*, 1998). Algal protein had been approved as the alternative source to the human nutrition (Shelef and Soeder, 1980; Fabregas *et al.*, 1985; Al-Araji, 1996). Rezeq and James (1987) recommended the algaas the most delicious food to the Rotifers , the intermediate food chain .Espe and Lied (1999) prepared a cheapest highly qualified protein , food to supply fish food, while Ginzberg *et al.* (2000) studied the food supply for chicks from red microalgae. Laing and Utting (1980); Fabregas *et al.*

(1989); and Abdulla and Ragab (1998) studied the effect of physical and chemical parameters of media on algal growth. While Simmons (1974); Al-Asadi (1977a); Al-Asadi (1978) and Al-Asadi (2005)worked on the phosphorus and nitrogen metabolism of the green algae (*Chlorella sp*). Beijernick (1890) used inorganic materials as culture media for algal growth as a food.

The scientists started to use the organic animal stool, as food sources to the algae. Algae can be cultivated in all seasons of the year under autotrophic, mixotrophicor heterotrophic conditions. Mixotrophic and heterotrophic cultures are consideredas alternative modes of producing algae biomass and these methods can give a very high final biomass, but it is not suitable for all algae or their products (El-Sheekh, 2014). Some algae are unable to use organic substrates (Droop, 1974) either because they do not have appropriate uptake mechanisms (Nelison and Lewin, 1974), or alternatively, because they lack fully functional metabolic pathways needed for effective dissimilation of the substrate (Smith *et al*., 1967). The incapability of algae to make some metabolites in the dark is another constraint (Pauw and Persoone,1988). The ability to use organic substrate appears subject to wide variation between species and strains (Killam and Myers, 1956). For an economical biomass increase of algae it is necessary to searcha possible way of using low cost carbon sources e.g. molasses as industrial by products (Shamala *et al.*, 1982a). Molasses can be used to grow the algae either heterotrophically or mixotrophically (Becker and Venkataraman, 1979). Molasses contain 29.64 % sucrose, 24.18 % glucose, and 24.18 % fructose, raffinosein varying amounts, total nitrogen contained in molasses ranges from 0.82 % to 2.2 % (Malanowska and Labendzinski, 1969). Nitrogen is a component of various substances such as protein, amino acids, amides, ammonium salts, nitrates and nitrites (Ginterova, 1973). *Chlorella vulgaris* and *Scenedesmus obliquus* can use organic substrates under both light and dark conditions (Combres *et al.*, 1994). Molasses seems to be a promising feedstock for bioethanol production with high yield and low cost, by using the yeast *Saccharomyces cerevisiae* (Periyasamy *et al.*, 2009; Elena *et al.*, 2009; Gasmalla *et al*., 2012). Molasses analysis have also shown its richness of sulfur ash as nitrogen source represented 8% of the total mass (Zentou *et al*, 2017). Vegetable farmers mostly apply poultry manure in combination with inorganic nitrogen-based fertilizers such as Urea and NPK (Ogungbile and Olukosi, 1990), often because poultry manure alone is believed to dissolve slowly and may not meet up the yield of vegetables (Oyedeji *et al.*, 2014). Two species of *Chlorophyceae*, *Ankistrodesmus gracilis* and *Haemotococcu spluvialis*, were used to compare and evaluate the effect of sugarcane molasses as a carbon source, Sugarcane molasses may be an alternative carbon source in laboratory conditions. (Sipaúba-Tavares *et al*., 2020). The aim of this study is to investigate the effect of (Molasses, yeast and NPK) on the growth of *Chlorella sp* under laboratory conditions.

Materials and Methods

Phytoplankton net (20 µ mesh) was used to collect *Chlorella* sp from Shatt Al-Arab River. The collected sample was centrifuged (1000 r/m) for four minutes. The process was repeated several times. Isolation and purification of *Chlorella* sp was achieved according to Patterson (1983). A pure culture of *Chlorella* sp was obtained. Finally, a mass culture of isolated pure green alga was prepared using (Chu No. 10) media as described byAbdulla and Rajab (1998). The procedure was carried out under antiseptic conditions and fixed temperature and illumination. The stock culture was ready for further experiments. The precipitate cultured in CH 1O solution, incubated under sufficient illumination (White fluorescent tubes, 90 ö mol photon m-2 s-1) and temperature (25 °C). *Chlorella sp* was isolated from Shatt Al-Arab River. A standard initial inoculum of the isolated algae was inoculated to culture flasks. The culture flasks were supplied with various concentrations (125, 250, 350 and 480 mg/ml) of culture media that was consisted of Molasses, yeast and NPK .

Sterile technique determinations of pigment content: Chlorophyll a content were estimated in acetone extract according to Jeffrey and Humphery (1975). The content of the pigments fractions (µg chl mL^{-1} algal suspension) was then calculated under consideration of the dilution factors.

Measuring of chlorophyll and biomass of aquatic plants: Chlorophyll a and biomass were measured, by taking 5 gm of the algae (*Chlorella sp*) and crushed by porcelain mortar with 10 ml of 90% aceton and was left in refrigerator for 24 hours after surrounding the vials with aluminum foils, the filtrate was measured on 665 and750 nanometer wave lengths, using spectrophotometer (Hitachi type, 4-1500

model). After that two drops of 2N HCl were added to each sample, then the measurements of absorption were repeated using the same above mentioned wavelengths, accordingto Lorenzen,s equations (Vollenweider, 1971). The used materials; 1) 17.5 g of Yeast. 2) 6.25 g of the fertilizer N, P.K. 3) 60 ml of Molasses. 4) algae (*Chlorella sp*).

Procedure; 1) The above mentioned materials (Yeast, N.P.K. and Molasses)were added to 1.5 liter of distilled water, were left overnight. 2) A group of different volumes of the resulted mixture (125, 250, 350 and 450 ml) were used, each one of them was added to two liters of distilled water, then 10 ml of *Chlorella sp* was added to each one of the four different volumes of the above mentioned mixture and also to the Ch10 medium and the control. 3) A chemical medium (Ch 10) was used. 4) *Chlorella sp* algae 5) A control medium, only water free of nutrients.

Counting algae cells of *Chlorella sp*; this can be counted by using a Hematocytometer Chamber. Cell counts using Improved Neubauer haemocytometer Chamber (Perez; , 2006).

Algal counting: Cell number was determined using a Hematocytometer Chamber. Hemacytometer 0.1 mm deep, having improved Naubauer ruling was used. One drop of the algal suspension was pipetted on the slide,was covered and left for two minutes for algal settling. The mean counts of three replicates were taken and the data were obtained as cell mL^{-1} algal suspension.

Treatment: A standard initial inoculum of the isolated algae was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile nutrient medium (Kuhl's medium). The culture flasks were supplied with various concentrations of Molasses, yeast and N.P.K (mg/ml). The experiment lasted for one month. The growth of *Chlorella sp* increased after eight days in the flask of 125 ml solution and after four days inthe flask of 250 ml solution, while its growth started from the very beginning in 350 and 450 solutions.

Results and Discussion

It is apparent from Table 1, that the best results are achieved in the concentration of molasses, yeast and N.P.K. on the number of algal cells (*Chlorella sp*) per ml with the effect of Ch 10 and the value of control. *Chlorella sp*/ml at the concentrations of 250 and 450 which reached 1115000 and 010000 cell/ ml, respectively at the fourth measurement. However, the Ch 10 gives only 220000 cell/ml, with the value at the control was 20000 cell/ml.

Table 1. Impact of Molasses, yeast *Saccharomyces cerevisiae* and N.P.K on the number of algal cells (*Chlorella sp*) per ml.

Molasses, yeast And NPK (ml/l)	Number of algal cells / ml					
	First measurement	Second measurement	Third measurement	Fourth measurement	Fifth measurement	Fifth measurement
125	310000	590000	955000	750000	855000	855000
250	90000	675000	530000	1115000	1105000	1105000
350	850000	450000	730000	955000	765000	765000
450	460000	570000	730000	1010000	1430000	1430000
Ch 10					220000	20000
DW						20000

Table 2. Impact of Molasses, yeast *Saccharomyces cerevisiae* and N.P.K. on chlorophyll a content of algal cell *(Chlorella sp)* per ml, with the effect of Ch 10 and the value of control.

(Chlorophyll a) Molasses, yeast and N.P.K (mg/ml)	Chlorophyll a content of algal cells (mg/ml)			
	First measurement	Second measurement	Third measurement	Fourth measurement
125	5.82	18.85	24.65	15.63
250	4.89	16.93	15.11	22.61
350	16.78	13.29	19.89	21.68
450	4.22	16.74	17.99	22.7
Ch 10	–	–	–	9.83
Distilled water	–	–	–	0.7

Table 3. Impact of Molasses, yeast *Saccharomyces cerevisiae* and N.P.K on the biomass of algal cells (*Chlorella sp*) with the effect of C h 10 and the control value.

Period Molasses, yeast and N.P.K (mg/ml)	The biomass of algal cells / ml			
	First measurement	Second measurement	Third measurement	Fourth measurement
125	389.94	1262.95	1651.55	1047.21
250	327.63	1134.31	1012.37	1514.87
350	1124.26	890.43	1332.63	1452.56
450	282.74	1121.58	1205.33	1520.9
Ch 10	———	———	———	658.61
Distilled water	———	———	———	46.9

The effects of molasses, yeast and N.P.K. on the Chlorophyll a content of *Chlorella* sp were best shown by the 250,350 and 450 by by the 250, 350 and 450 concentrations in which the results were 22.61, 21.68 and 22.7 mg/ ml, Table 2, respectively, (Table 2). While at the Ch 10, the algal concentration was 9.83 mg/ml, and at the control the value was 0.7 mg/ml.

The impact of molasses, yeast *Saccharomyces cerevisiae* and N.P.K. on the biomass of *Chlorella sp* were best shown at the 250 and 450 mg/ml which were 1514.87 and 1520.9 cells/ml and to a lesser extent by the 350 mg/ml (1452.56 cell/ml) (Table 3). Meanwhile, the algal biomass at the Ch 10 was 658.61, and at the control was 46.9 cell/ml.

Molasses was used in the experiment because it is cheap and as carbon source (Sipaúba-Tavares *et al.*, 2020). The addition of different concentrations of the medium (125, 250, 350 and 450 mg/ml) that was consisted of Molasses, yeast *Saccharomyces cerevisiae* and

N.P.K. (mg/ml) produced an increase in number, the Chlorophyll content a and biomass of algal cells (*Chlorella sp*). *Chlorella* sp gradually increased in such a way depends on the ascending concentrations of the medium. The growth rate icreased with increasing concentrations of molasses (El-Sheekh, 2014).

Chlorella sp started a growth increase after eight and four days in 125 and 250 mg/ml medium concentrations respectively. While in 350 and 450 mg/ml medium concentrations, the growth increased from the beginning of the experiment. At the end of the experiment *Chlorella* sp' s chlorophyll a content and biomass values in Ch 10 medium were very low in comparison with ones of different concentrations (125, 250, 350 and 450 ml/l) of the medium (Molasses, yeast and N.P.K). The growths in Ch 10 medium and control (D.W.) medium did not permit the follow up of them so the comparisons were at the end of the experiment. The yeast and *Chlorella sp* form between them a symbiotic relationship (Naidoo *et al.*, 2019). Using the fertilizer NPK as considered a cheap nutrient medium in growth of *Chlorella sp* by comparison with (Chu.10 medium), the experimentali data showed that the use of the NPK fertilizer as cultivation medium in *Chlorella sp* had given a growth rate of microalgae more than that the one produced by Chu.10 (Al-Mashhadani and Khudhair, 2017).

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