Biospectra : Vol.9(2), September, 2014. pp.89-94

An International Biannual Refereed Journal of Life Sciences

Plant Sciences

ISSN : 0973-7057

Quantitative estimation of carbohydrates in indigenous blue green algae from the wetlands of Bihar.

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Received , 29th June, 2014; Revised: 21st July, 2014

Abstract: Concern over energy, the environment and natural resources, is increasing across the globe. Production of bio fuels from less exploited resources is now emerging as a topic of intensive research. Biomass is an attractive feedstock, as it is a renewable resource that could be sustainably developed in the future. Algal biomass viewed as a simple, fast growing biomass has the potential to act as an excellent source of bio fuels. Bihar being rich in algal biodiversity, it is holding a massive potential of these resources for energy production. Utilization of algal biomass for bio ethanol production is an eco-friendly and sustainable approach for bio-fuel production. The present investigation exhibits the age dependent quantitative estimation of carbohydrates in four genera of blue green algae, viz; Eucapsis minuta, Anabaena laxa, Arthrospira platensis and Scytonema coactile, collected from wetlands of Muzaffarpur (Bihar).It was found that the amount of total intracellular carbohydrates have shown increasing tendency in all the four genera under investigation, during their exponential phase i;e up to 30 days in E.minuta and up to 25 days in A.laxa, A.platensis and S.coactile. The amount of carbohydrates gradually decreased in decline growth phase in all the four genera of blue green algae under investigation. The increase in the amount of carbohydrates in the exponential phase might be due to gradual accumulation of polysaccharides and decrease in the amount of carbohydrates in their decline growth phase might be the age influenced photosynthetic efficiency of organisms or might be due to cell autolysis.

Key words: Ethanol, catbohydrates, Eucapis minuta, Arthrospira platensis, Scytonema coactile, Anabaena laxa.

INTRODUCTION

The inevitable role of energy in economic progress of a country can hardly be avoided. India is also facing formidable challenge in meeting its energy demands in providing adequate energy of desired quality in various forms. Photosynthetic biological sources have the potential to provide an environment friendly sustainable energy system for the society besides mitigating CO_2 (Alonso *et al* 2002). Numerous crops have been proposed or being used for the commercial production of bio-fuel like (i) sucrose containing feed stocks (e.g sugar cane, sugar beet etc.) (ii) Starchy material (e.g corn, jowar, wheat etc.) (iii) Lignocelluloses bio-mass (e.g grasses, straw etc.).However, these feed stocks are too expensive for ethanol fermentation as they compete with food security and farm economy (FAO., 2008). Hence, algal bio-mass considered as a simple, fast growing biomass superior to any other biomass for bio-fuel production. Due to their environmental and economic sustainability, security of supply, absence of lignin, high photosynthetic efficiency, fast growing rate and role in reduction of greenhouse gas emissions (Chisti, 2007,Dismukes et al; 2008). The state of Bihar present in the Northern region of India has an ambient of harboring rich diversity of fresh water microalgae because of its continental

monsoon type of climate. Bihar has around 4416 wetlands (3241 natural and 1175 man-made) and total area associated with wetlands is estimated 403209 ha that is

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around 4.4% of the geographical area (National wetland inventory and assessment, NWIA ,2005). Bihar has massive potential for microalgae cultivation with its abundant sunlight and can effectively exploit the potential of these resources for energy production (Shay, 1993; Ginzburg, 1993; Dote *et al.*, 1994; Minowa *et al*., 1995; Ratledge, 2004). Certain algae are rich in carbohydrates of various forms such as starch, cellulose, glucose and other sugars and polysaccharides (Templeton *et al*., 2002)**,** they provide an economically viable feed stock for fermentation of ethanol (Hirano, *et al.,* 1997). Utilization of algal biomass for bio-ethanol production is an eco-friendly and sustainable approach for bio-fuel production·

The present study made by author aims at the determination of age dependent quantitative variability in carbohydrates in four blue-green algae under investigation viz., Anabaena laxa, Scytonima coactile, Arthrospira platensis, and Eucapsis minuta so that the optimal utilization of this bioactive product can be done. The following four blue green algal taxa are quit common in Muzaffarpur. Due to their faster rate of growth and easy availability in this area, they were selected for the present study and collected from different places of Muzaffarpur and proper identification was done before study. Out of the four taxa under taken, two of them are non-heterocystous whereas other two are heterocystous forms.

MATERIAL AND METHODS

• All the four taxa under investigation were followings:

• Eucapsis minuta, Frich.

• Anabaena laxa.

• Arthrospira pletensis, var. tenuis (Rao, C.B.) Comb. Nov.

Scytonema coatile, Motagne ex Born. Flah.

Out of these four blue-green algae Eucapsis minuta belongs to the order Chroococcales and Anabaena laxa, Arthrospira platensis, and Scytonema coactile to the order Nostocales

Isolation and Purification of Algae under Investigation

The methods suggested by Stein (1973) were adopted for isolation and purification of blue-green algae under investigation. After obtaining uni-algal culture of all the four taxa, they were cultured properly in "Hughes medium" under laboratory conditions. It is important to mention here that out of four taxa under investigation two of them Eucapsis minuta and Arthrospira platensis grew properly in Hughes (+) medium where as the other two Anabaena laxa and Scytonema coactile grew properly in Hughes (—) medium and were regularly exposed to 3300 lux light in a daily cycle of 18 hours light and 6 hours darkness by fluorescent tubes fitted at the distance of 20" from culture vessels. Proper temperature and pH were also ascertained.

Growth phase of Organisms

The determination of growth phase of organisms is the prerequisite for present study, as it aims at, to determine the quantitative variation of carbohydrate at different growth phases of organisms. The growth of unicellular form Eucapsis minuta was determined by measuring optical density of its homogenous suspension in culture medium at a wave length of 660nm by UV Visible recording spectrophotometer against the blank of growth medium. Determination of growth phases of filamentous forms Arthrospira pletensis, Anabaena laxa, and Scytonema coactile were done by dry weight method.

Estimation of intracellular carbohydrates

The quantitative estimation of intra cellular carbohydrates was done by methods suggested by Hewitt (1958) and Lewin (1956).

(a) Total intracellular carbohydrates : Forthe estimation of total intracellular carbohydrate, 100 mg of dried algal mass was homogenized in 100 ml distilled water. 1 ml of this homogenized algal mass was then treated with 10 ml of 0.2% anthrone reagent to develop characteristic blue green color. The absorbance of this treated algal sample was measured at 620 nm with the help of UV-visible light spectrophotometer. Starch was used as a standard.

(1) Intra cellular free carbohydrates – Intra cellular free carbohydrate was extracted by thoroughly crushing 100 mg dry algal mass in 100 ml 80% ethyl alcohol. The alcoholic extract of free sugar was separated by centrifugation; this extracted free carbohydrate was estimated by the anthrone method mentioned above (cf. Chaykin

Sterling, 1970).

(2)Intracellular bound carbohydrates- Intracellular bound sugar was estimated by deducting intracellular free sugar from total intracellular sugar.

RESULT AND DISCUSSION

The quantitative estimation of intracellular carbohydrates has been done for both free and bound carbohydrate at the interval of every 5 days, starting from 10 days old culture up to 45 days old culture in each of the four algae under investigation. The result obtained after the quantitative determination have been presented in the Table-1 to Table-4. It is clear from the Tables that amount of total intra cellular carbohydrates increased gradually up to the 25th day in Arthrospira platensis, Scytonema coactile and Anabaena laxa but in Eucapsis minuta this increase continues up to the 30th day (I;e during exponential phase). During static phase the amount remained almost unchanged. A gradual decreasing tendency in the amount of total carbohydrates, thereafter, was noticed during decline phase of all the four organisms under investigation. The amount of free and bound intracellular carbohydrates also exhibited similar increasing and decreasing pattern (Table-1toTable-4). Some of the references regarding the age dependent variability have been reported by following workers: Kosenko (1975), has reported an initial decrease in the carbohydrate contents of Anabaena variabilis and Amorphonostoc paludosium at their lag phase followed by further increase along with age tillit reaches its constant value at the stationary phase. Kumar (1986) and Shukla (1989) has also reported such age dependent variability in the amount of algal polysaccharides. Out of four organisms under investigation the highest amount of carbohydrates was found in Scytonema coactile (20.14%) on 25th day and the lowest amount in Eucapsis minuta (12.10%) on the same day. The increase in the amount of carbohydrates in the exponential phase might be due to gradual accumulation of polysaccharides and decrease in the amount of carbohydrates in their decline growth phase might be the age influenced photosynthetic efficiency of organisms or might be due to cell autolysis.

Table-1: Intracellular carbohydrates at different growth phase (in % dry wt.) in E. minuta

Age of culture (in days)	Free	Bound	Total
10	2.90±0.005	3.85±0.014	6.75 ±0.009
15	3.20±0.017	4.05±0.012	7.25 ±0.014
20	3.35±0.050	5.60 ±0.018	8.95 ±0.034
25	4.20±0.011	7.90 ±0.024	12.10 ±0.017
30	4.20±0.007	8.45 ±0.020	12.65 ±0.013
35	3.90±0.015	8.23 ±0.009	12.13 ±0.012
40	3.62±0.006	8.05 ±0.008	11.67 ±0.007
45	3.45±0.008	7.85 ±0.011	11.30 ±0.009

Table-2: Intracellular carbohydrates at different growth phase (in % dry wt.) in A.platensis

Age of culture (in days)	Free	Bound	Total
10	5.05 ±0.012	7.20 ±0.040	12.25 ±0.260
15	5.23 ±0.004	7.45 ±0.012	12.68 ±0.008
20	5.69 ±0.020	8.52 ±0.022	14.21 ±0.021
25	6.03 ±0.012	9.92 ±0.020	15.95 ±0.016
30	5.92 ±0.040	9.63 ±0.014	15.55 ±0.027
35	5.60 ±0.019	9.42 ±0.009	15.02 ±0.014
40	5.29 ±0.030	9.31 ±0.030	14.60 ±0.030
45	5.15 ±0.011	9.17 ±0.017	14.32 ±0.014

Table-3: Intracellular carbohydrates at different growth phase (in % dry wt.) in A.laxa

Age of culture (in days)	Free	Bound	Total
10	4.20 ±0.011	6.90 ±0.030	11.10 ±0.020
15	4.38 ±0.003	7.60 ±0.040	11.98 ±0.021
20	4.65 ±0.007	9.60 ±0.050	14.25 ±0.028
25	5.27 ±0.006	9.82 ±0.020	15.09 ±0.013
30	5.23 ±0.021	9.74 ±0.030	14.97 ±0.025
35	4.90 ±0.012	9.02 ±0.040	13.92 ±0.026
40	4.60 ±0.011	8.70 ±0.030	13.30 ±0.020
45	4.43 ±0.030	8.50 ±0.019	12.93 ±0.024

Table-4: Intracellular carbohydrates at different growth phase (in % dry wt.) in S.coactile

Age of culture (in days)	Free	Bound	Total
10	4.80 ±0.005	10.23 ±0.02	15.03 ±0.012
15	4.92 ±0.008	11.40 ±0.03	16.32 ±0.019
20	5.30 ±0.009	12.32 ±0.04	17.62 ±0.024
25	5.62 ±0.012	14.52 ±0.02	20.14 ±0.016
30	5.60 ±0.011	14.48 ±0.03	20.08 ±0.020
35	5.20 ±0.012	14.29 ±0.04	19.49 ±0.026
40	4.82 ±0.015	13.70 ±0.06	18.52 ±0.037
45	4.73 ±0.016	13.42 ±0.05	18.15 ±0.033

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