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Growth Characteristics of Commonly Occurring Fresh Water Chlorophycean Algae for Biodiesel Production.

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Abstract

Liquid biofuels, renewable fuels derived from biomass, are arguably one of the best transition fuels for the near-term and have made a recent resurgence in response to rising oil prices. Biodiesel can be produced from a variety of lipid feedstock, catalysts and alcohols using several possible conversion processes. Microalgae reproduce themselves using photosynthesis toconvert sun energy into chemical energy, completing an entiregrowth cycle every few days. Fresh water chlorophycean algae have great source of lipid content and proving raw material for biodiesel. This paper focuses the growth behavior of fresh water Chlorophycean algae during mass culture for biodiesel production.

Key words: Growth characteristics, biodiesel, Chlorophyceae.

INTRODUCTION

Algae are an economical choice for biodiesel production [1], because of its oil productivity and low cost. Algae can be used as renewable energy [2].The availability of carbon dioxide and the effects of pH and dissolved bicarbonate andcarbonate on it seem likely to be important. The proliferation rate of algae had been studied by Pingle and Landge [3] for better growth of algae with respect maximum production of algal oil and concluded that indigenously prepared models can play significant role in it. Carbon is a major nutrient and there isevidence that some species can use only carbon dioxide, whereas others can also directlyuse bicarbonate for photosynthesis [4]. Species like Chlorella and Scenedesmus [5] may beable to use carbonate also, though carbonate may be toxic for other specieslike Chlamydomonas and Botryococcus[6]. Data are so few that no pattern of bicarbonate utilization has yet emerged. Amongknown bicarbonate users Scenedesmusquadricauda is eutrophic [7]. Bicarbonate levels increase markedly from those in the softest oligotrophic waters tothose in the eutrophic waters of soft rock areas, and pH tends to increase with bicarbonatelevel. The availability of free CO2 (CO--H2CO3) decreases, at constant bicarbonatelevel, with increasing pH and increases, at constant pH, with increasing bicarbonate. Thecombined effect is usually an overall decrease in availability of free CO2 with increasinghardness of natural waters. Above about 1.5 m-equiv.11 alkalinity there may be a slightincrease in free CO2 concentration, since pH may then increase very little with greatlyincreasing bicarbonate concentration. Hutchinson [8] discussed the equilibrium involvedin detail.

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Materials and methods

1. Collection of Chlorophycean microalgae from water bodies

Chlorophycean algae are ubiquitous in nature though the environmental conditions affect the life forms. For present study the study area selected was north and middle part of Maharashtra (India). The localities fixed for the collection were Bhandardara dam, Nizarneshwarkitiware and Pravarariver from Ahmednagar district. Though the localities were different, the growth characteristics will be studied after isolation. The water samples were collected in plastic containers in which aeration kept possible by applying cotton plug at the mouth of container. Also glass bottles were used for carrying the water samples. The samples were collected by using plankton net of 40 mesh size. After collection these samples were brought to the laboratory on the same day for isolation of submicroscopic algae [9]

2. Isolation of Chlorophycean microalgae from water bodies:

CHU 13[3]media was selected for isolation of submicroscopic algae. The sterilized medium was poured in the petriplates (90 mm) diameter and kept for cooling overnight. For isolation of Chlorophycean algal forms, 2 ml of water samples were inoculated in sterilized petriplates having 20 ml of Chu 13 medium separately. These inoculated petriplates were incubated at $28 \pm 20C$ under 2.5 K lux fluorescent light for 2- 3 weeks under 16/ 8 hours light / dark cycles. After 20- 25 days of incubation period, the visible growth of algae appeared in the enrichment cultures. The cultures were identified by using monographs [10,11,12]

Experimental setup:

Experiments were carried out on the effects of the pH-C02-bicarbonate system on growth of the algae. Series of media with different pH values were made by addition of dilHCl /Na2CO3 to the standard medium. The major ion composition of this medium (exclusive of Na+ and HCO3-was Ca++ 6.78 mg/l (16.78 mg/l in media for selected alga for experimentation,when ammonium was supplied as a nitrogen source), Trace elements and vitamins were supplied at low microgram levels [7]and 3 mg/l TetrasodiumEthylene DiamineTetraaceticAcidwere added. Media were autoclaved at 1.05 kg/cm2 (15 lb/In2) for 15 min and allowed to stand for at least 3 days before inoculation. After this time the pH, which rose during autoclaving, had fallen to a steady value, and was measured with a Corning Model-84 pH meter to the nearest 0.01 (50.02) unit. The initial pH values given in the tables are of the media just after inoculation. Growth was measured as increase in cell or coenobium numbers over a suitable period (at least a week) of the logarithmic phase. At least three, and sometimes as many as ten, counts were made at suitable intervals and logarithmic growth curves were plotted. Results are expressed as doublings per day (l/g where g is generation time in days) [7].

The experimentswere carried out in 250 ml or 500 ml foam-stoppered flasks which were manuallyswirled once per day.

Carbonate and bicarbonate levels were determined on samples of the media, prepared,autoclaved, and allowed to stand exactly as those used in the experiments, by titrationwith standardized hydrochloric acid at 20 0C to the end point of phenolphthalein (8.46)and of a methyl red-bromocresol green indicator (4.5). Free CO2(including H2C03) was calculated from the Henderson-Hasselbalch equation:

$$pK'1 = pH - \log \frac{[HCO3-]}{[CO2 + H2CO3]}$$

Where pK'1 is –log K'1 and the dissociation constant

$$K'1 = \frac{[H+][HCO3-]}{[CO2 + H2CO3]}$$

In these calculations, pK'1= 6.38 [7]

Table 1: Effect of initial pH on growth of genus of Chlorophyceae at room temperature (2.4 Klux, 12 hrs/day)

Parameters	Growth rate (Doubling per day l/g) where g is generation period for genus				
pH	***Chlamydomonas***	***Closterium***	***Cosmarium***	***Botryococcus***	***Eudorina***
3.65	0	0	0	0	0
3.9	0	0	0	0	0
4.75	++	0	0.01	0	0
6.2	+++	0	0.16	0.49	0.5
6.3	+++	0	0.16	0.57	0.5
6.95	+++	0	0.23	0.54	0.35
7.3	+++	0	0.28	0.49	0.38
7.8	+++	0	0.26	0.51	0.51
8.1	+++	0.64	0.27	0.60	0.43
8.43	+++	0.60	0.31	0.66	0.37
9.05	+++	0.67	0.25	0.60	0.25
9.25	++	0.62	0.20	0.55	0.15
9.30	0	0.33	0.16	0.49	0
9.35	0	0.26	0	0	0
9.45	0	0	0	0	0

Table 2: Effect of Fe^{+++} level on growth of four genus of Chlorophyceae

Genus/ Conc.	Growth rate (measured as doubling per day) at different concentration of Fe^{+++} µg /L [Growth conditions 2.4 klux, 15 hrs per day at room temperature]									
	0	0.1	0.5	1	2	5	10	20	30	40	50
Botryococcus	0.62	0.51	0.60	0.57	0.59	0.62	0.60	0.56	0.61	0.61	0.64
Cosmarium	0.29	0.28	0.28	0.28	0.28	0.28	0.32	0.32	0.32	0.30	0.23
Chlamydomonas	0	0	0	0.09	0.14	0.11	0.11	0.14	0.13	0.14	0.11
Euglena	0	0	0.07	0.08	0.11	0.14	0.20	0.21	0.21	0.13	0.06

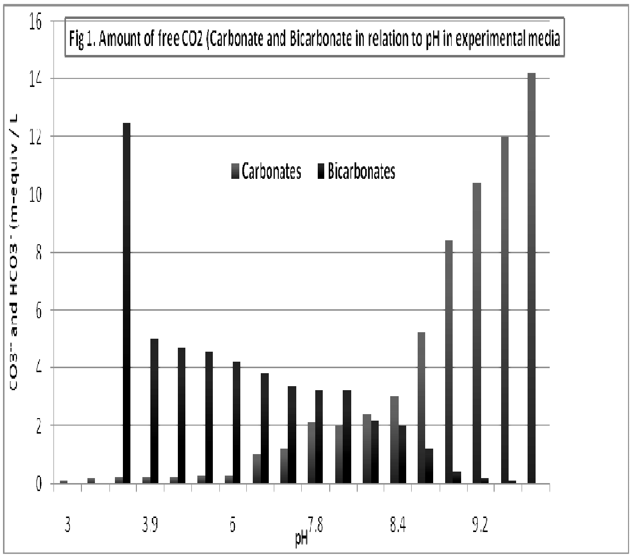
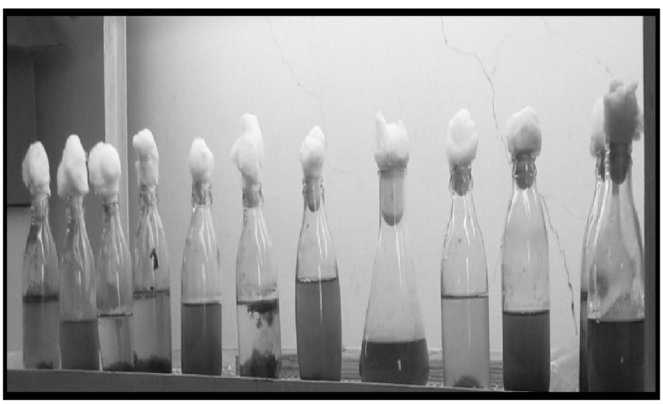


Fig. 2: Culture characteristic of chlorophycean algae at laboratory condition**.**



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Results and Discussion:

Growth characteristics if algae definitely helps in studies of taxonomy, reproduction, and wider adaptability of organisms moreover commercial production of algae. As shown in table 1 the growth rate of five species of algae in relation to pH, and Fig 1 shows the relationship of free CO2, carobonate and bicarbonate and pH Closterium did not shown any growth in acidic medium. The species showed growth pH 8.1and above. Doubling rate has observed as 0.64 l/g at pH 8.1 where as maximum 0.67 l/g at pH 9.05. This alga will show good growth pattern if pH maintained from 8.01 to 9.05, however decrease in growth rate had been observed above pH 9.20

Cosmarium showed growth rate from acidic medium to basic. Maximum growth rate was observed at pH 8.43. Overall moderate growth pattern observed at pH neutral to basic. No growth has observed at pH above 9.35. Botryococcus had shown good growth pattern over a wide range of pH. Fully grown cultures of Botryococcus if inoculated does not show any contamination. Maximum growth was observed at pH 8.43 is 0.66 l/g. observations suggest that slightly basic media is comfortable for Botryococcus.

Though Eudorina does not reported as economic of values it can be used raw materialfor fuel production. It showed good growth pattern at pH 7.8 as 0.51 l/g. slightly acidic to basic cultural media can be used for maintenance of Euglena. The alga is more comfortable with basic pH as compare to acidic. Table-2 shows effect Fe+++ concentration on growth pattern Fe+++ trigger the growth of Botryococcusfrom table it is evident that as concentration of Fe+++ increases the growth rate l/g has shown directly proportionate with ion concentration. The observations were repeated same in Cosmarium and Chlamydomonas except Euglena. Moderate concentration i.e. 20-30 µg/L of Fe+++ trigger growth of Euglena cultures.

.From fig 1 as pH increases the concentration of carbonate from media decreases and vice versa with bicarbonates. Most of the chlorophycean algae uses atmospheric CO2 for growth bat some forms like Botryococcus, Cosmarium and Eudorina uses CO2 available from media through carbonates and bicarbonates combination. The reports[6] are affirmative to the results presented here. A level of 420 mg/l NaCl decreased growth rate of M. americanaby about half and 1000 mg/l NaCl by about two-thirds. Cosmariumsp. grew less well with 420 and 1000 mg/l NaCl, though the decrease was probably insignificant, and continued to grow with 840 mg/l Na2HCO3The total ioniccontent of a hard water lake with 3 m-equiv.11 HCO3 would be about 315 mg/l and of one with 1.5 m-equiv.11 HCO3 about 158 mg/l [13]. Total ionic content was therefore unlikely to be the operative factor in preventing growth of oligotrophic species at high pH [14].

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