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Optimization of Various Growth Media to Freshwater Microalgae for Biomass Production

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Abstract: In recent decades, microalgae have acquired attention from pharmaceuticals to biofuels. The growth and total chorophyll content of three economically important microalgae (*Chlorella* sp. NTAI01, *Monoraphidium* sp. NTAI02 and *Scenedesmus* sp. NTAI03) isolated from fresh water body in five selected culture media on different days of incubation was studied for biomass production. Biomass feedstock has reviewed great interest to be used as an alternative and renewable source of energy. All the three organisms showed varied growth pattern and total chlorophyll content in different culture media. However the growth and total chlorophyll content of *Chlorella* sp. NTAI01 and *Monoraphidium* sp. NTAI02 was optimum in Half strength Chu 10 medium. In case of *Scenedesmus* sp. NTAI03 the growth and total chlorophyll content was found to be significant in Bold's Basal medium. The Acidified Bold's Basal medium and BG-11 medium fairly supports the growth of all the three microalgae whereas the Modified Hoagland's medium does not support the microalgal growth. The optimized growth medium will be used for biomass production for biofuel application.

Key words: Microalgae, growth medium, chlorophyll, biomass, biofuel

INTRODUCTION

Microalgae are a diverse group of eukaryotic photosynthetic microorganisms that can grow rapidly due to their simple structure which colonizes both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass. Each species of microalga produces different ratio of lipids, carbohydrates and proteins in the account of the biomass. These cellular components were used for human welfare.

Chlorella can be used as a food additive owing to the taste and flavour-adjusting actions of its coloring agent (Yamaguchi, 1997; Gouveia *et al*., 1996). *Dunaliella salina* is exploited for its β-carotene content that can reach 14% of dry weight (Metting, 1996). *Monoraphidium* sp. GK12 is exploited for astaxanthin content which is widely used as a valuable food additive (Fujii *et al*., 2008). Many oleaginous microalgae like *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp., *Botryococcus braunii* were exploited for biodiesel production (Chisti, 2007). Some microalgal species are established in the skin care market, the main one being *Chlorella* (Stolz and Obermayer, 2005).

Recently, microalgae have also garnered interest for production of valuable molecules ranging from therapeutic proteins to biofuels. The biofuel production have been reported as first generation and second generation based on development and the production of biofuels using biomass as a sustainable biological resources have been discussed earlier (Abdeshahian *et al*., 2010). To produce high quality biomass, attention must be paid to culture status. Large-scale production of microalgal biomass depends on many factors, the most important of which are nutrient availability, temperature and light (Shay *et al*., 1987). These factors influence the growth of microalgae and the composition of the biomass produced by causing changes in metabolism.

In the development of microalgal product, one of the major target is to select suitable nutrient medium (Montserrat *et al*., 1993; Gong and Chen, 1997). The choice of medium mainly depends on several factors that include chemical composition of the medium (Borowitzka, 2005). Several media are available for the cultivation of microalgae, even it is essential to know which medium is

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optimum for the maximum growth of microalgae. Considering these facts, the present study focused to evaluate the suitable medium among Bold's Basal medium, Half strength Chu 10 medium, Acidified Bold's Basal medium, BG-11 medium and Modified Hoagland's medium for culturing the most economically important *Chlorella* sp., *Monoraphidium* sp. and *Scenedesmus* sp. The main objective of the present investigation is to analyse the growth and total chlorophyll of the above said microalgae in order to find out best defined inorganic medium for cultivation.

MATERIALS AND METHODS

Samples: The microalgal samples were collected from stagnant fresh water body in Bharathidasan University campus, Tiruchirappalli, India.

Isolation and identification: The collected samples were enriched initially in BG-11 broth in conical flasks (250 mL) at 24±2°C under 37.5 $\mu mol^{-1}m^2 sec^{-1}$ intensity with 16:8 h photoperiod for 10 days. Then the enriched culture samples were spread on BG-11 agar plates and incubated at the above said conditions. After incubation, individual colonies were picked and transferred to the same media for purification in 250 mL conical flask. The culture broth was shaken manually for five to six times a day. The purity of the culture was monitored by regular observation under microscope. The isolated microalgae were identified microscopically using light microscope with standard manual for algae (Kant and Gupta, 1998).

Test medium: In order to optimize the appropriate culture medium for the selected isolates, they were subjected to five different media of varying nutrient composition, as stated, (i) Modified Hoagland's Medium (MHM), (ii) Bold's Basal Medium (BBM), (iii) Acidified Bold's Basal Medium (ABBM), (iv) Half strength Chu 10 medium(HC10) and (v) BG 11 Medium (BGM). The individual compositions of the five medium were listed in Table 1 (Andersen, 2005).

The growth medium was prepared based on their compositions, transferred into 100 mL conical flasks and sterilized at 121°C for 15 min at 15 lbs. The selected isolates were inoculated in these five medium and used for further experimentation. The inoculated cultures were gently shaken in order to accelerate the algal growth. All the tests were carried out in triplets.

Growth analysis: The growth of the algal biomass was assessed by means of optical density with five days intervals from 5th day upto 25th day at 540 nm (Miron *et al*., 2003) using UV-Vis spectrophotometer (OPTIZEN 3220).

Estimation of chlorophyll: Pigments were extracted at regular intervals of five days from 5th day upto 25th day. Cells were harvested by means of centrifugation. Subsequently, total chlorophyll was extracted in 80% acetone (Arnon, 1949).

RESULTS

Three morphologically different strains were isolated from stagnant fresh water body, Bharathidasan University of Tiruchirappalli district, Tamil Nadu, India. The morphology of the isolated strains NTAI01, NTAI02 and NTAI03 were studied under light microscope (OPTIC X) and were tentatively identified as *Chlorella* sp. measuring about 6.7-7 µm in diameter, *Monoraphidium* sp. 16.75-17.0 µm in length and 10.05-10.25 µm in width, *Scenedesmus* sp. about 30.15 µm in length and 6.67 µm in width (Fig. 1).

Table 1: Medium composition of MHM, ABB, BBM, HC10 and BG-11

Modified Hoagland's medium	Acidified Bold's Basal medium	Bold's Basal medium	Half strength Chu 10 medium	BG 11 medium
$(NH_4)_2NO_3$-0.115 g	$(NH_4)_2SO_4$-0.25 g	$NaNO_3$-0.25 g	$Ca(NO_3)_2$-2 g	$NaNO_3$-1.5 g
H_3BO_3-0.003 g	$NaNO_3$-0.75 g	$MgSO_4.7H_2O$-0.075 g	K_2HPO_4-0.25 g	K_2HPO_4-0.04 g
$Ca(NO_3)_2$-0.656 g	$CaCl_2.2H_2O$-0.025 g	NaCl-0.025 g	$MgSO_4.7H_2O$-1.25 g	$MgSO_4.7H_2O$-0.075 g
$CuSO_4$-0.08 mg	$MgSO_4.7H_2O$-0.075 g	K_2HPO_4-0.075 g	Na_2CO_3 - 1 g	$CaCl_2.2H_2O$-0.036 g
$Fe_2(C_4H_4O_6)_3$-0.005 g	$K_2HPO_4.3H_2O$-0.075 g	KH_2PO_4-0.175 g	Na_2SiO_3-1.25 g	$C_6H_8O_7$-0.006 g
$MgCl_2$-0.24 g	KH_2PO_4-0.175 g	$CaCl_2.2H_2O$-0.025 g	$FeCl_3$-0.04 g	$Fe(NH_4)_3(C_6H_5O_7)_2$,
$MnCl_2$-0.016 mg	NaCl-0.025 g	$ZnSO_4.7H_2O$-8.82 mg	H_3BO_3-0.248 mg	Na_2CO_3-0.02 g
KNO_3-0.3 g	Na_2EDTA-0.005 g	$MnCl_2.4H_2O$-0.44 mg	$MnSO_4.H_2O$-0.147 mg	EDTA-0.001 g
$ZnSO_4$-0.22 mg	$FeCl_3.6H_2O$-0.0006 g	MoO_3-0.71 mg	$ZnSO_4.7H_2O$-0.023 mg	Trace metal mix
---	$MnCl_2.4H_2O$-0.0002 g	$CuSO_4.5H_2O$-1.57 mg	$CuSO_4.5H_2O$-0.010 mg	H_3BO_3-2.86 g
--	$ZnCl_2.6H_2O$-0.00003 g	$Co(NO_3)_2.6H_2O$-0.49 mg	$(HN_4)_6Mo7O24.4H_2O$-0.007 mg	$MnCl_2.4H_2O$-1.81 g
--	$CoCl_2.6H_2O$-0.00001	H_2BO_3-11.42 mg	$Co(NO_3)_2.6H_2O$-0.014 mg	$ZnSO_4.7H_2O$-0.222 g
--	$Na_2MoO_4.2H_2O$-0.00002 g	EDTA-50 mg	Vit B1-5 mg	$Na_2MoO_4.2H_2O$-0.39 g
--	Vit B1-0.012 g Vit B12-10 µg	KOH-31 mg	Vit B7-2.5 mg	$CuSO_4.5H_2O$-0.079 g
--	--	$FeSO_4.7H_2O$-4.98 mg	Vit B12-2.5 mg	$Co(NO_3)2.6H_2O$-0.0494 g
--	--	H_2SO_4-1 µL	--	
--	--	Vit B1-10 µg	--	
--	--	Vit B12-10 µg	--	

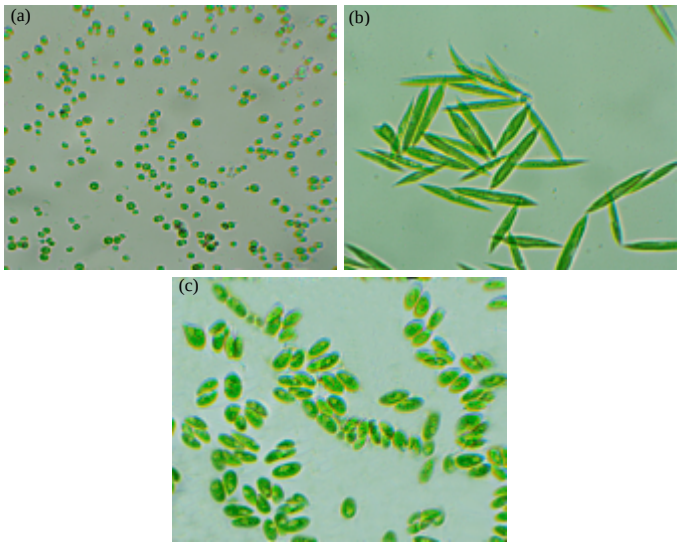

Fig. 1(a-c): Microphotograph of (a) *Chlorella* sp. NTAI01, (b) *Monoraphidium* sp. NTAI02 and (c) *Scenedesmus* sp. NTAI03

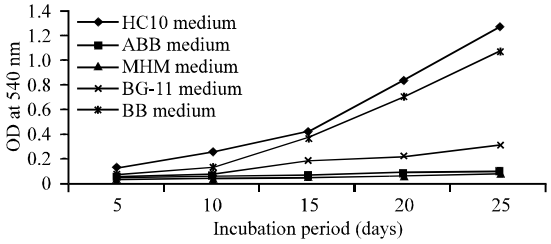

Fig. 2: Growth curve of *Chlorella* sp. NTAI01 from 5th day till 25th day

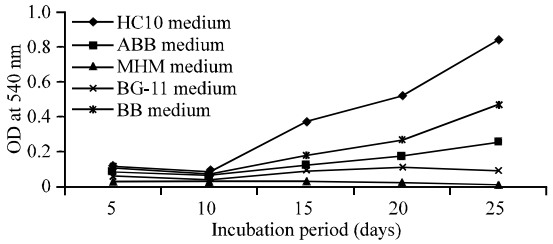

Fig. 3: Growth curve of *Monoraphidium* sp. NTAI02 from 5th day till 25th day

Five inorganic medium with varying chemical composition were used in the present investigation. The organisms used in this study showed variations in their growth pattern and chlorophyll content. Among the five medium, Half strength Chu 10 medium and Bold's Basal medium were found to enhance the growth of three microalgae.

The present study deals with the evaluation of growth (OD value), total chlorophyll in *Chlorella* sp. NTAI01, *Monoraphidium* sp. NTAI02 and *Scenedesmus* sp. NTAI03 which showed variation in their growth pattern and pigment in all the five medium. In general, Half strength Chu 10 medium and Bold's Basal medium were found to greatly influence the growth of the selected microalgae followed by Acidified Bold's Basal medium and BG-11 medium whereas minimum growth was examined in Modified Hoagland's medium. Both *Chlorella* sp. NTAI01 and *Monoraphidium* sp. NTAI02 exhibited maximum growth in HC10 medium whereas *Scenedesmus* sp. NTAI03 showed maximum growth in Bold's Basal medium. The growth of *Chlorella* sp. NTAI01, *Monoraphidium* sp. NTAI02 and *Scenedesmus* sp. NTAI03 in different culture media was assessed by OD value and chlorophyll content.

Based on the optical density measurement, it was found that at 25th day, growth of *Chlorella* sp. NTAI01 has been highly favoured by Half strength Chu10 medium followed by Bold's Basal medium (Fig. 2). From the 5th day onwards, the OD value of *Chlorella* sp. NTAI01 gradually increased and reached maximum value at 25th day, in the Half strength Chu 10 medium. Moreover at 25th day, the OD value has been increased by 10 fold with that of 5th day. In the meantime, the growth rate was fairly recorded in BG-11 medium followed by Acidified Bold's Basal medium and Modified Hoagland Medium. In case of *Monoraphidium* sp. NTAI02, the growth was greatly influenced by Half strength Chu 10 medium (Fig. 3) followed by Bold's Basal Medium and it was

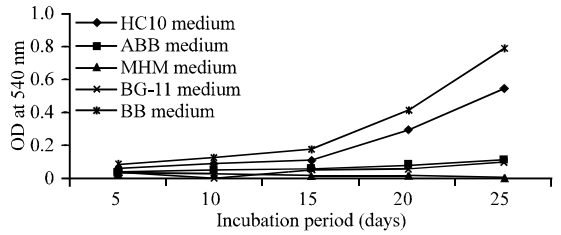

Fig. 4: Growth curve of *Scenedesmus* sp. NTAI03 from 5th day till 25th day

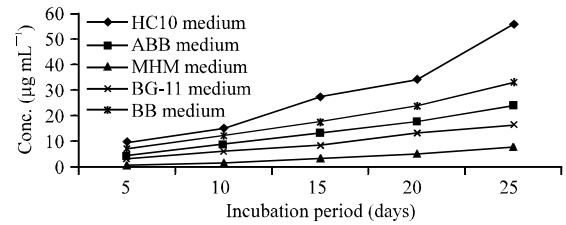

Fig. 5: Total chlorophyll content of *Chlorella* sp. NTAI01

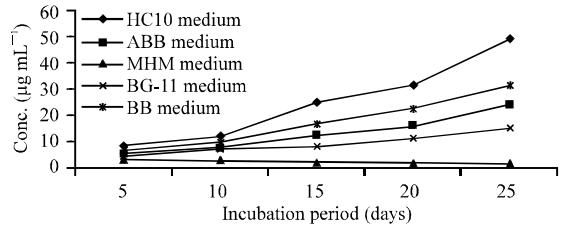

Fig. 6: Total chlorophyll content of *Monoraphidium* sp. NTAI02

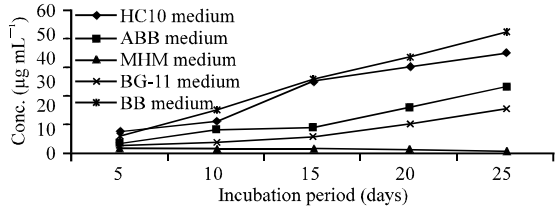

Fig. 7: Total chlorophyll content of *Scenedesmus* sp. NTAI03

evaluated by means of its increased OD value. It was observed that there was 9.60 fold increases with that of 5th day. Next to that, growth was supported fairly by Acidified Bold's Basal medium followed by BG-11 medium. The OD value was gradually decreased in Modified Hoagland's Medium and it showed that the medium did not favour the growth of this isolate. In case of *Scenedesmus* sp. NTAI03, growth was favoured well in Bold's Basal medium (Fig. 4) followed by Half strength Chu 10 medium, Acidified Bold's Basal medium, BG-11 medium, whereas Modified Hoagland's medium did not support the growth of the same.

The total chlorophyll content of *Chlorella* sp. NTAI01 at 5th day was found to be 9.7 µg mL^{-1} and it was increased to 45.58 µg mL^{-1} at 25th day (Fig. 5) in Half strength Chu 10 medium. In *Monoraphidium* sp. NTAI02, the total chlorophyll was found to be 8.587 and 42.91 µg mL^{-1} on 5 and 25th day respectively (Fig. 6). In case of *Scenedesmus* sp. NTAI03, the total chlorophyll content was found to be 6.02 and 42.18 µg mL^{-1} at 5 and 25th day respectively in Bold's Basal medium (Fig. 7).

DISCUSSION

Many researchers utilized unialgal cultures of *Chlorella* sp., *Monoraphidium* sp. and *Scenedesmus* sp. for various applications starting from therapeutics to biofuels production so it is necessary to find the optimal medium for their biomass accumulation.

It was stated that the elements like N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn are essential for algal growth and these elements are added in the form of salts (Oh-Hama and Miyachi, 1988; Kaplan *et al*., 1986). The concentration of these inorganic elements may vary from one medium to the other.

The nitrogen source, $CaNo_3$ in Half strength Chu 10 medium is responsible for the protein biosynthesis, thereby it accounts for algal growth. Similarly the phosphate source, K_2HPO_4 present in Half strength Chu 10 medium, enhances algal growth. Earlier reports suggested that K_2HPO_4 enhanced dark reaction in *Selenastrum* sp. that leads to its rapid growth (Turpin, 1986). $MgSO_4$ was the magnesium source which induces the chlorophyll production thereby overall biomass was tend to be increased thus magnesium concentration positively affected the chlorophyll content which was similar to previous study on *Chlorella vulgaris* (Mandalam and Palsson, 1998).

In the present study, Na_2CO_3 and Na_2SiO_3 were the sources of carbon and silica, higher OD value and maximum chlorophyll content illustrated that these nutrients also supported the growth of microalgae. Moreover, these two inorganic nutrients maintain the alkaline buffering in the medium. Previous exploration on *Chlorella vulgaris* showed that sodium carbonate and silicate in modified Chu 10 medium was responsible for higher growth and chlorophyll content (Sharma *et al*., 2011).

$FeCl_3$ was the iron source in Half strength Chu 10 medium resulted in higher chlorophyll content when compared to other medium. It was reported that iron was

one of the element which is vital in algal growth, whereas iron deficiency leads to growth retardation and decrease in chlorophyll content (Wiesnner, 1962). The presence of iron was directly proportional to the overall productivity.

In addition to macronutrients, micronutriens like trace metals and vitamins are known to induce the growth of microalgae. Mn, Cu, B, Zn and Co in trace amounts are capable of inducing the growth of microalgae, at the same time higher the concentration of these micronutrients leads to retard the growth of microalgae. The Half strength Chu 10 medium have optimal amount of these micronutrients which resulted in maximum growth and chlorophyll content. Moreover, vitamins like B_{12}, B_1 present in the medium induces growth of *Chlorella* sp. NTAI01 and *Monoraphidium* sp. NTAI02. It was supported by previous works on utilization of vitamins by members of chlorophyta (Croft *et al*., 2006).

CONCLUSION

The present work investigated the effect of five different medium namely Half strength Chu 10 medium, Bold's Basal medium, Acidified Bold's Basal medium, BG-11 medium and Modified Hoagland medium on growth and total chlorophyll content of *Chlorella* sp. NTAI01, *Monoraphidium* sp. NTAI02 and *Scenedesmus* sp. NTAI03. Present experiments clearly showed that the Half strength Chu 10 medium favours the growth of *Chlorella* sp. NTAI01 and *Monoraphidium* sp. NTAI02, whereas the Bold's Basal medium supports the growth of *Scenedesmus* sp. NTAI03. The cultivation of these microalgae offers many new opportunities in microalgal product development.

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