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Influence of organic waste and inorganic nitrogen source on biomass productivity of Scenedesmus and Chlorococcum sp.

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Abstract
Algae gaining the more attention in the recent years in order to supplement the futuristic demand of fuel requirement because of its unique feature like high productivity, short duration and higher fatty acids content. However algal culturing for large-scale production is limited due to many technical and engineering challenges. One of the main constraints for large-scale biomass production is the non-availability of cost effective and affordable growth medium for open pond condition. In order to overcome this lacuna, the present study was carried out to find out the suitable cost effective growth medium using locally available resources. Farm Yard Manure an easily available organic waste yet, rich in nutrients and used for agriculture over the generations. FYM coupled with inorganic nitrogen source like urea was found to be better alternative to the synthetic growth medium, which may make wider acceptability at farmers' field for large-scale algal mass production. The present study reveals that FYM extract of 50% supplemented with 0.1% Urea was performing better for algal biomass growth in outdoor open pond condition.

Keywords: Algae, Growth medium, Open pond cultivation, Second-generation biofuel, Waste utilization.

1. Introduction

The global biodiesel market is growing at an average annual growth of 42% and estimated to reach 37.0 billion gallons by 2016 [1]. Biofuel has received considerable attention in the recent years being non-toxic, biodegradable and renewable with enormous environmental benefits since its use leads to a decrease in the harmful emissions of carbon monoxide, hydrocarbons, particulate matter and to the elimination of SO_x emissions, with a consequent decrease in the greenhouse effect in line with Kyoto Protocol agreement. Biodiesel produced from cereal grains and edible vegetable oil (first generation biofuel feedstock's) raises concerns about food security and resource allocation but use of microalgae for biodiesel production is gaining momentum worldwide due to the higher photosynthetic efficiency, higher biomass productivity and a faster growth rate than higher plants, highest CO_2 fixation and O_2 production [2, 3]. As a matter of fact, average biodiesel production yield of microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and vegetable oils [2, 4].
Algae are gaining importance as second-generation biofuel because of its promising potential over first generation biofuel feed stock sources. Algal culturing in laboratory conditions has been in practice using

various defined synthetic growth medium such as modified CHU-13 [5, 6], BBM [7], BG11 [8] and several other medium containing analytical grade salts to fulfill growth requirement of macro and micronutrients. However culturing algae outdoor in large scale for biodiesel production is limited because of non-availability of low cost affordable technology for algal culturing [9]. Use of large quantity of standardized synthetic growth medium in open pond condition for large scale culturing is non-practical and costly affair thus making algal biodiesel production not economical. Cultivation of microalgae in wastewater, dairy manure and other animal residue has been widely reported by several workers [10-17]. Addition of organic carbon though found highly stimulatory for microalgal growth but increases the cost [18]. Therefore, economically affordable and environmentally sustainable medium for mass culturing of algae biomass is necessary. In view of the above, a study was conducted to optimize affordable growth medium, which can be substituted for the synthetic growth medium in open pond condition. FYM coupled with urea as nitrogen source was used in the present study to examine its suitability as growth medium and to develop an easily available and affordable growth media for algal biomass production in open pond condition.

2. Materials and methods

2.1 Algal culture and growth condition

Chlorococcum humicola was cultured in various combination of growth medium in triplicate in the conical flask at 25 ± 1.5 °C under a photoperiod of 14:10 hr at light intensity of 1.12 Klux and no external CO_2 was administrated during the study period. The grown cultures were hand shaken 2 to 3 times daily to avoid sticking on the flask. In addition to Chlorococcum, experiments also conducted using another strain Scenedesmus bijugatus in open pond condition under glass house. No external CO_2 or light intensity was administrated in experiment 2, rather the cultures were allowed to grow under naturally prevailing environment in order to evaluate the suitability for outdoor condition. Periodical microscopic examination was performed to rule out the possibility of any fungal, bacterial and other algal contamination.

2.2 Preparation of FYM extract

Well dried and decomposed Farm Yard Manure (FYM) and rain water was mixed in 1: 2.5 ratio and kept for incubation over a period of 7 days then the nutrient solution of FYM was extracted by through shaking followed by filtration through standard sieve of 150 Micron to remove debris, if any. The extracted FYM was considered as 100% and used for preparation of desired dilution. The FYM extract was not autoclaved because the aim is to optimize a growth medium, which is cost effective to allow large volume of algal culture in open pond conditions. It is not sterile but exhibited no bacterial/ fungal contamination when used for culturing algae as evident from periodical microscopic observation.

2.3 Effect of nitrogen sources of algal biomass productivity in laboratory condition

Experiment was carried out using Scenedesmus bijugatus under laboratory condition in 500 ml conical flask containing 300 ml of designated nutrient medium for 15 days to find out the suitable combination of organic and inorganic nitrogen source for mass culturing of algae. Algal cultures were subjected for the 7 treatments independently viz, T_1-CHU13 medium (Standard medium); T_2-Rain water (control); T_3-Urea @ 0.5%; T_4-Urea @ 0.1%; T_5-50% FYM extract; T_6-100% FYM extract; T_7-50% FYM extract + 0.1 % Urea. The urea concentration was optimized and found that above 0.1% is not promoting algal growth (Data not shown). A small aliquot was withdrawn periodically to monitor the algal growth by measuring OD at 540 nm.

2.4 Effect of nitrogen sources of algal biomass productivity in outdoor condition

The study was extended in open pond condition for two different algal strains (Chlorococcum humicola & Scenedesmus bijugatus) to examine the suitability of growth medium for mass culturing in outdoor conditions. Experiment comprised of 4 treatments viz, M_1-Rain water (Natural substitute of distilled water as control); M_2- 50 % FYM extract; M_3 -50 % FYM extract + 0.1 % Urea; M_4- 50 % FYM extract + 0.5 % Urea. The selected algal strains were cultured using above 4 different growth medium combinations in non-reactive tiled facility of 200 lit capacity for 30 days. Manual agitation was performed thrice a day to avoid natural settling of algae. Algal samples were harvested on 30th day and the wet algal biomass was oven dried and the dry weight was determined gravimetrically.

2.5 Measurement of algal biomass growth

Different treatments were compared for algal biomass growth using spectrophotometer (Labomad Inc.) based OD reading at 540 nm as suggested by Gouveia and Oliveira [3]. To prevent the settling and there by causing erroneous result (Turbidity), vortexing of algal sample was done to get homogenous culture before reading. Algal growth was also determined as dry weight. After harvesting the samples were centrifuged (REMI, CPR-24) at 5000 rpm for 10 min then subjected to oven drying. Dried cell mass was determined gravimetrically to express the growth as dry cell mass (g/L). The data was recorded and average of three independent experiments was plotted as graph with standard deviation as error bar.

3. Results

3.1 Effect on algal biomass growth in laboratory condition

A time course study was conducted as described in materials and methods for 15 days to study the performance of various nutrient medium and to optimize affordable growth medium for open pond culturing of Scenedesmus bijugatus. Algal biomass growth was determined by measuring OD at 540 nm periodically on 0,1,3, 8,12 and 15th day of study period. Significant increase in biomass growth was recorded at 15th day under standard growth medium for algae CHU 13 (T_1) compare to control (T_2). Medium carrying inorganic nitrogen source i.e urea (T_3, T_4) alone exhibited declining trend in biomass growth under both 0.1 and 0.5 % concentration (Figure 1A). Medium consisting of organic nutrient source i.e FYM extract exhibited beneficial effect on biomass growth compare to control (Figure 1B). Out of the two treatments, 50 % diluted FYM extract (T_5) resulted better biomass growth as against the concentrated FYM extract (T_6). Although growth medium carrying only organic nitrogen was beneficial but biomass growth was almost half of the standard medium (T_1). Algal growth medium containing 50% FYM extract and 0.1% Urea (T_7) exhibited significant increase in biomass growth compared to control (Figure 1C). This medium combination was statistically at par with the standard growth medium CHU 13.

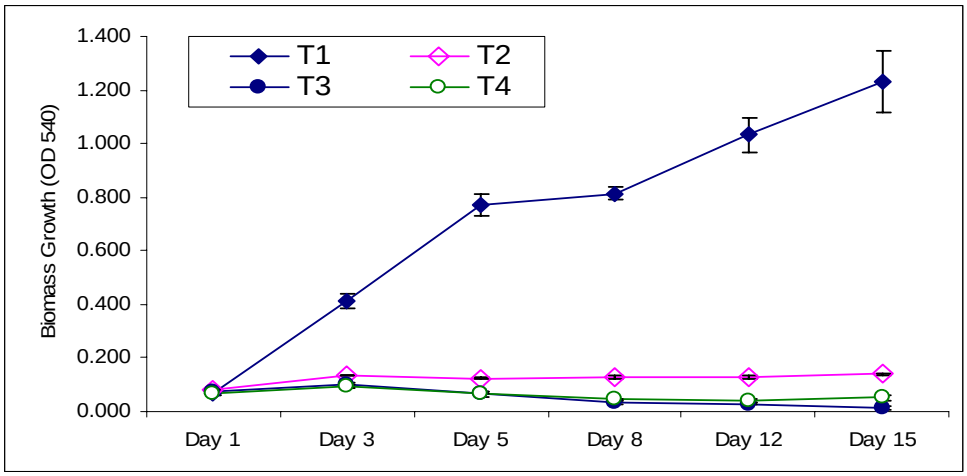
3.2 Effect on algal biomass growth in outdoor open pond conditions

Performance of growth medium T_7 on algal biomass productivity in outdoor condition was also validated using different algal strains. In this experiment the algal biomass productivity was examined by supplementing 0.1 and 0.5% urea with 50% FYM extract against control (M_1). Effect of medium combinations on algal biomass productivity in outdoor condition was significant in two different strains namely Scenedesmus and Chlorococcum. Dry biomass recovery was found two fold higher than the control (M_1) for both the strains in M3 medium comprising 50% FYM extract supplemented with 0.1% urea. Further increase in nitrogen concentration i.e 0.5 % urea (M_4) could not exhibit beneficial effect on algal biomass productivity. Irrespective of medium composition, algal biomass yield as dry weight was higher for Scenedesmus (ranged from 0.45 to 1.10 g / lit) compared to Chlorococcum yielding a dry mass of 0.08 to 0.99 g / lit.

4. Discussion

Majority of synthetic growth medium comprises of mineral nutrients like nitrogen, phosphorus and potassium and salts like chlorides and sulphates etc, which facilitates the algal growth. In the present study, Farm Yard Manure (FYM) was tested for its suitability as algal growth medium along with inorganic nitrogen supplement. FYM is also a rich source of organic acids and its quite common for microalgae to take up and convert reduced organic compounds such as sugars or organic acids from the growth medium as revealed by Mussgnug [19]. In the present study, treatments comprising either urea or FYM alone could not exhibit satisfactory algal biomass growth, which may be attributed to the imbalance and non-availability of essential nutrients. Rather, treatments comprising urea alone shown a deleterious effect on algal growth as also suggested by earlier workers on growth and lipid content of B. braunii and Neochloris oleoabundans, respectively [20, 21]. Good performance of algae in T_7 may be attributed to the fact that farmyard manure consists of N, P and K, several macro and micronutrients (Ca, Mg, Fe, Mn, Zn, Cu) with a C: N ratio near to 30 [22, 23]. Addition of urea 0.1% has lowered the C: N ratio facilitates availability of nitrogen quickly for algae growth. In general broad C: N ratio make nitrogen unavailable due to immobilization and addition of urea has been beneficial in decreasing the C:N ratio and thus making the nitrogen quickly available for growth [24-26]. It is reported that the nitrogen source in various forms are important for the growth of algae [20]. Increase in nitrogen concentration from 0.1 to 0.5% urea could not exhibit beneficial effect on biomass productivity of

Scenedesmus and Chlorococcum. Similar trend of Growth inhibition was also observed at higher nitrogen concentration (500-1000 mg/lit) for Chlorella vulgaris in photobioreactor system [27] and B. bruanii [20].



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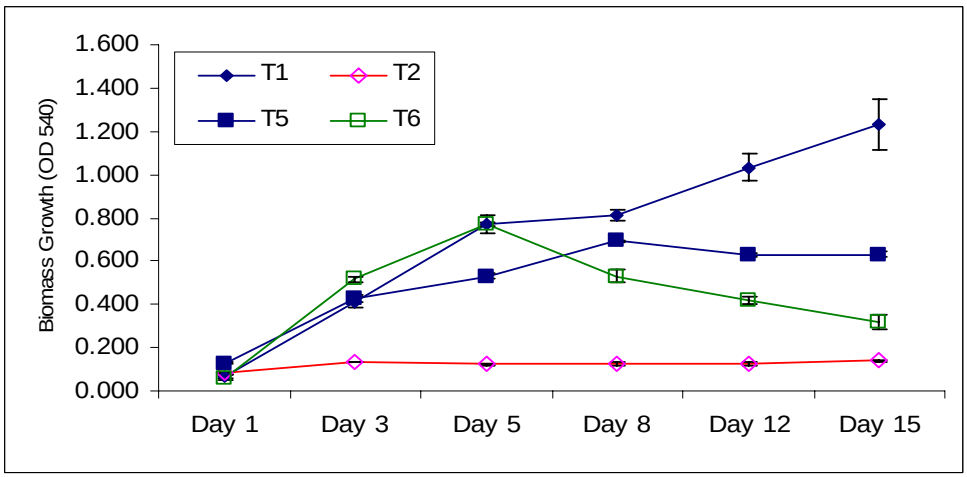

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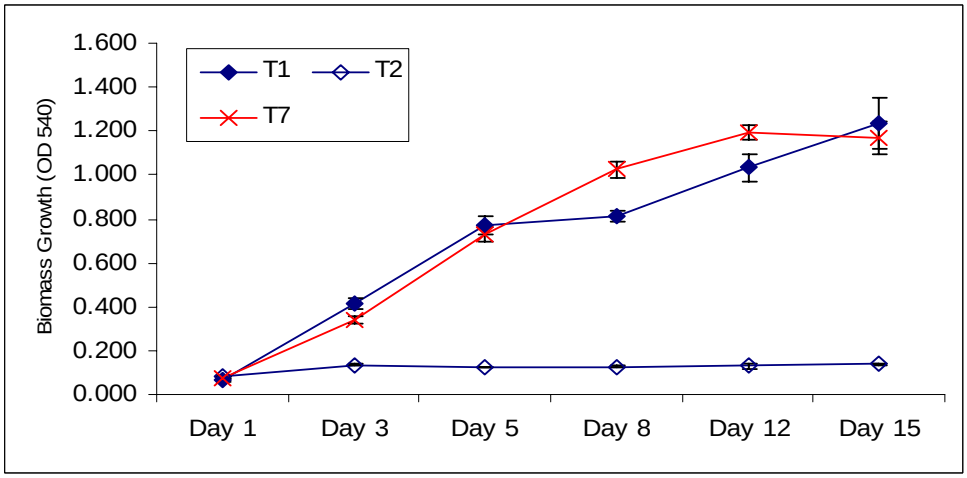

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Figure 1. Effect of growth medium on algal biomass growth in laboratory condition. T_1-CHU-13 (♦), T2- Rain water (◊) T3- Rain water + 0.1% Urea (●) and T4 Rain water + 0.5% Urea (○), T5- 50% FYM extract (■), T6 50% FYM extract (□) T7 50% FYM extract + 0.1% Urea (×). The experiment was conducted with three replications; Standard deviation was calculated and expressed as error bar

To validate the beneficial effect of FYM and urea combination on cell growth, experiment was conducted at large scale in glasshouse in 200 lit capacity pond facility. Results of this experiment presented in Figure 2, revealed that M_3 (Farm Yard Manure extract 50% + urea @ 0.1%) exhibited higher algal biomass growth for both the algal strains used in the experiment on final day (30^{th} day) of harvest. Both algal strains exhibited similar trends for biomass growth in all the growth medium combinations, which indicates the wider applicability of growth medium under study. The results also revealed the incremental trend in biomass growth for both the strains, when urea is being supplemented with FYM extract as evident from Figure 2. The average cell biomass growth of 0.9 g/lit for Scenedesmus obliquus under laboratory conditions has also been reported [3]. A biomass yield of 2.0, 2.2 and 2.3 g/lit was obtained under growth medium (N-11) supplemented with fishpond discharge, municipal tank discharge and poultry litter respectively for Scenedesmus obliquus showing suitability of these waste discharges as medium for growing algae [18]. Suitability of Scenedesmus bijugatus at Pithoragarh condition with good biomass yield has also been recorded in our previous work [28]. Poor biomass growth in M2 (FYM extract alone), which may be attributed to the broad C: N ratio due to the presence of FYM extract only as no readily available nitrogen source was supplemented. Thus based on the findings of both the experiments, growth medium comprising 50% FYM extract + 0.1% Urea exhibited its potential as good low cost alternative, which can be substituted for the synthetic growth medium in open pond condition. It is noteworthy to mention that since the growth of algae was measured by spectrophotometer, therefore, periodical microscopic examination was performed to examine the presence of specific strain and to rule out the possibility of other contamination like bacterial, fungal and algal strain, which may cause erroneous results in the reading. No contamination was observed throughout the study period, which may be attributed to the fact that during photosynthesis algae often leads to elevated levels of dissolved oxygen and pH in growing medium, which reduces the contamination level [29-31]. Thus, the results implies that 50% FYM extract supplemented with 0.1% urea could be used as affordable and suitable media for mass culturing of algae for biodiesel production.

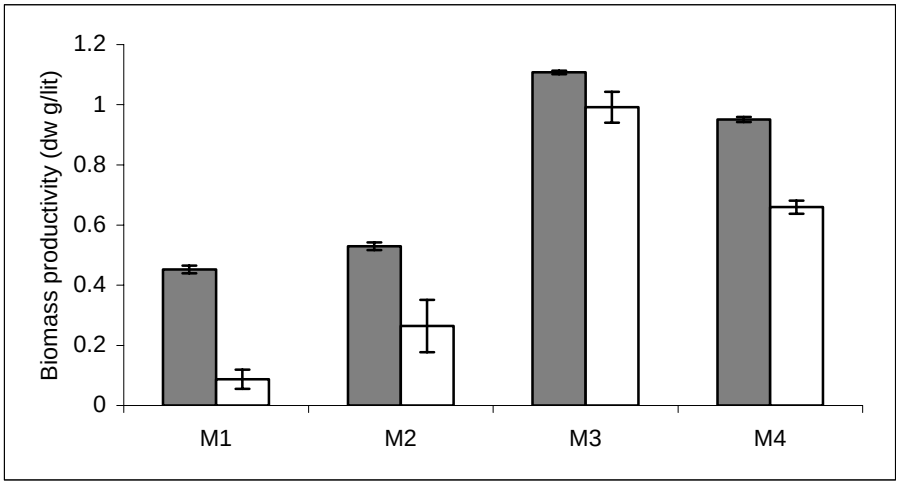

Figure 2. Effect of growth medium on algal biomass (dry weight) in open pond conditions. Filled bar represents the Scenedesmus bijugatus and unfilled bar indicates the Chlorococcum humicola. M_1-Rain water (Natural substitute of distilled water as control); M_2- 50 % FYM extract; M_3 -50 % FYM extract + 0.1 % Urea; M_4- 50 % FYM extract + 0.5 % Urea. The experiment was conducted with three replications; Standard deviation was calculated and expressed as error bar

5. Conclusion

The experiments were conducted to investigate the effect of various nutrient combination on algal biomass growth and also to standardize a low cost growth medium for algal biomass growth in open pond because analytical grade chemicals are costly and required in large quantity for large-scale algal biomass culture. The findings of these experiments revealed that FYM extract (50%) along with urea (0.10%) could be a good cost effective alternative for satisfactory algal biomass growth in open pond condition. Algae grown using this eco-friendly growth medium can be used for various applications like biodiesl production, values added nutraceutical products, pigments and animal feeds. The growth media

as evident from experimental result can overcome the limitation of algal biomass production in large scale.

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