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Environmental Engineering for enhancing the suitability of a microalga for energy production

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Abstract - *Microalgae are considered as potential source of renewable energy generation. Algal biomass is used as key feedstock for renewable energy generation using algal resources. Accumulation of biomass mostly depends on the growth rate, which is usually regulated by pH and salinity. Present study was conducted to study the effect of variation in pH and saline ranges on growth, biomass and lipid content of a freshwater microalga Chlorella vulgaris. The growth, biomass and lipid content showed a significant difference due to the variation in pH and saline range. The growth and lipid content was maximum at pH 7.0 with compared to 6, 8 and 9. On the other hand an increase of salinity from 0.1M to 0.25 M caused decreased growth rate, whereas the lipid content showed an increasing trend.*

Key Words: *Biomass, Chlorella vulgaris, Growth, Lipids, Microalgae, pH, Salinity.*

1. INTRODUCTION

Human civilization is facing several inter connected challenges such as energy supply, food security, health, among them uninterrupted energy (electricity) supply is the major cause of concern due its imp in every aspect of human life. Global energy particularly electricity generation is mainly dependent on non-renewable resources which are depleting gradually. On the other hand global energy requirement is increasing at an alarming rate to meet the continuously growing demand of energy (electricity) supply of the word, alternate and renewable sources of energy or electricity production are being considered. Solar and wind energy are key sources of renewable power (electricity production) however a more efficient and eco-friendly way to gene rate energy which is based on biological resources is still in fancy state few countries are however successfully harnessing biological energy but extensive research are still warranted to standardized and effectively biological resources of electricity production.

The continued use of fossil fuel has now recognized as unsustainable because of depleting supplies and accumulation of CO_2 and other Greenhouse gases [1]. The combustion of fossil fuel leads to 73% of CO_2 production [2], which adversely affect the atmosphere. Therefore, the demand of biofuels is the necessity in today's scenario as it is made from non-toxic, biodegradable and renewable resources and provides environmental benefits [3]. The use of biofuel leads to decrease in harmful emission of carbon monoxide, hydrocarbons, particulate matter and to the elimination of SO_x emissions with consequent decrease in Green House effect [3]. The type of alternative sources of energy includes, hydroelectricity, energy generated from nuclear, wave and wind power and biological material [4].

Among alternative resources of energy production, biological resources have received considerable attention. Many countries are harnessing the potential of biological resources for energy production. Jatropha and other similar seed plants have been widely recognized as sources of bioenergy, but cultivation of such plants may compromise the agricultural land. Therefore, scientists are considering algae as next generation sources of biological energy [5].

Production of biodiesel using microalgal biomass appears to be viable alternative. Microalgae are diverse group of prokaryotic and eukaryotic photosynthetic organisms that grow rapidly due to their simple structure [6]. Microalgae are unicellular photosynthetic organisms, which uses sunlight and CO_2 for production of biomass they are considered having more photosynthetic efficiency than plants (7, 8, 9].

Rapid growth, large biomass and greater accumulation of lipid content in the algal cell are the important criteria for considering the algal species as potential sources of energy. In this context, the present study is aimed to enhanced the growth rate, biomass production and lipid accumulation in a common freshwater alga Chlorella vulgaris through ecological engineering e. g. manipulation of external pH and salinity.

2. MATERIAL AND METHODS

2.1 Test Organism and Growth Conditions

A locally isolated freshwater green alga *Chlorella vulgaris* was used as test organism and cultivated in BG11 medium at pH 7.0 in a culture room at 25 ± 2º C under a photoperiod of 12:12 hour at light intensity of 75 µmol photon m^{-2} s^{-1} PAR.

The composition of the BG 11 medium was as follows: 1.5g $NaNO_3$, 0.04g K_2HPO_4, 0.075g $MgSO_4.7H_2O$, 0.036g $CaCl_2.2H_2O$, 0.006g Citric Acid, 0.006g Ferric Ammonium Citrate, 0.001g EDTA (Disodium magnesium salt) and 0.02g Na_2CO_3, and 2.286mg H_3BO_3, 1.81mg $MnCl_2.4H_2O$, 0.222mg $ZnSO_4.7H_2O$, 0.039mg $Na_2MoO_4.2H_2O$, 0.079mg $CuSO_4.5H_2O$, 0.0494 mg $Co(No_3)_2.6H_2O$. pH was set between 7.0-7.5. The cultures were hand shaken two or three times daily to avoid sticking. This was referred to as control culture.

2.2 Optimization of Medium on Different pH and Salinity Ranges

The one set of triplicates of cultures for evaluation were grown in 150 ml Erlenmeyer flasks containing 50 ml BG11 medium on different pH ranges (6.0–9.0 at an interval of 1.0), and another set of triplicates for different salinity ranges (0.1 M - 0.25 M at an interval of 0.05, for saline cultures the pH was set at 7.0) in a culture room at 25 ± 2º C under a photoperiod of 14:10 hat light intensity of 75 µmol photon m^{-2} s^{-1} PAR without sparging with air or CO_2.

2.3 Growth Measurement

The growth of the algae was determined by measuring absorbance at 663 nm algal growth was measured on regular intervals (7, 14, 21 and 28 day respectively) using a spectrophotometer.

2.4 Measurement of Dry Weight

Dry weight (dcw) was determined gravimetrically. A known volume of algal culture was centrifuged at 5000 rpm for 15 min and the harvested biomass was dried at 80°C to constant weight and the weight of the dried biomass was recorded manually.

2.5 Extraction and Estimation of Lipid from Algal Biomass

Extraction of lipid was performed following the protocol of Bligh and Dyer [10]. To a 15 ml glass vial containing a known amount of algal biomass, 2 ml methanol and 1 ml chloroform were added and kept for 24 h at room temperature. The mixture was agitated in a vortex for 2 min, and 1 ml of chloroform was again added and the mixture shaken vigorously for 1 min; 1.8 ml of distilled water was added and the mixture was agitated in a vortex again for 2 min. The layers were separated by centrifugation for 10 min at 2000 rpm. The lower layer was filtered through Whatman No. 1 filter paper into a previously weighed clean vial (*W*1). Evaporation was carried on in a water bath and the residue was further dried a 104ºC for 30 min. The weight of the vial was again recorded (*W*2). Lipid content was calculated by subtracting *W*1 from *W*2, and was expressed as % dcw.

2.6 Statistical Analysis

All the experiments were performed in triplicates, the results were analyzed statistically by SPSS for two-way ANOVA.

3. RESULT AND DISCUSSION

3.1 Effect of Varied pH on Growth and Biomass

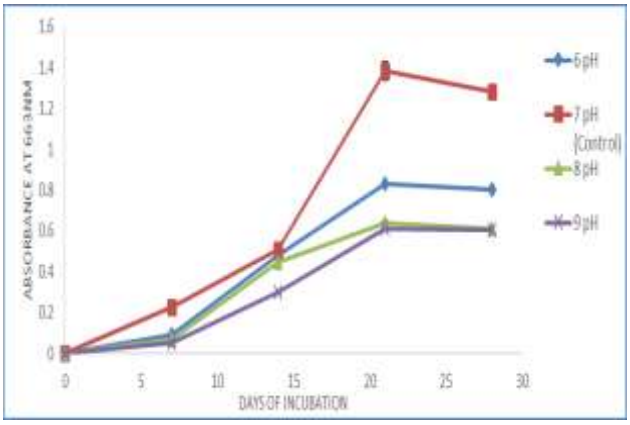

Figure 1: Growth Absorbance of *Chlorella vulgaris* with reference to the days of incubation

The Growth curve of *C. vulgaris* is shown in figure 1, the maximum algal density was statistically significant ($P < 0.05$) in control culture (pH 7) compared to pH 6, 8 and 9. The algal density was increased in all pH with passage of time. But control culture with pH 7 showed approximately, 40.0, 53.0 and 56% increased growth compared to pH 6.0; 8.0 and 9.0, respectively, on day 21. In control culture (pH 7) algal density exhibited increase of 83% from 7day to 21 day.

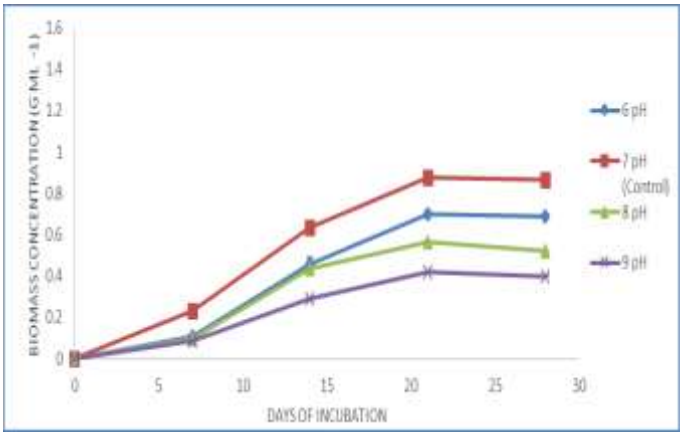

Figure 2: Biomass concentration of *Chlorella vulgaris* at variable pH ranges with reference to the days of incubation

Experiments were performed in triplicates under optimized conditions to determine the biomass yield, presented in figure 2, the most significant increase in biomass yield to 87.0 % was obtained in control culture on day 21 compared to pH 6, 8 and 9. pH 7 and pH 6 showed difference of 53.7%, whereas pH 8 and pH 9 showed 57.4% and 62.8% difference respectively on day 7 in comparison to control culture. The biomass yield was continuously increasing till day 21st after that no further increase was observed.

3.2 Effect of varied pH on Lipid Accumulation

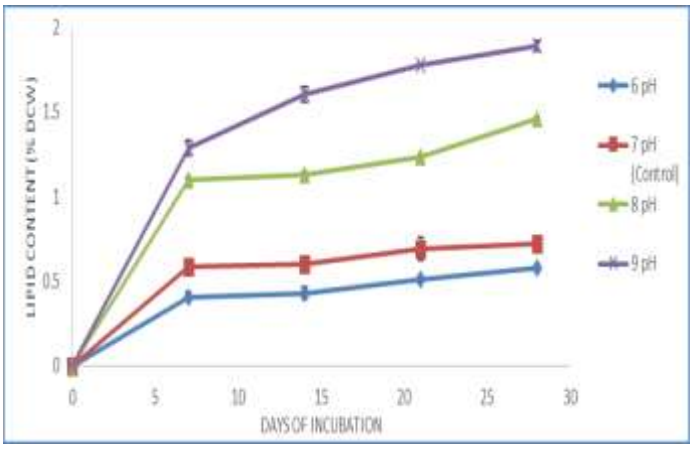

Figure 3: Relationship between pH of the culture medium of *Chlorella vulgaris* and lipid accumulation with reference to the days of incubation

Experiments conducted at varied pH to study lipid accumulation in *C. vulgaris* resulted in increase/decrease in lipid yield with respect to control (pH 7) shown in Figure 3. pH 6 showed decrease in lipid pool compared to pH 7. Lipid yield showed increase of 124 folds and 178 folds respectively, in pH 8 and pH 9 on day 21 and decline was observed on day 28.

3.3 Effect of Salinity on Growth

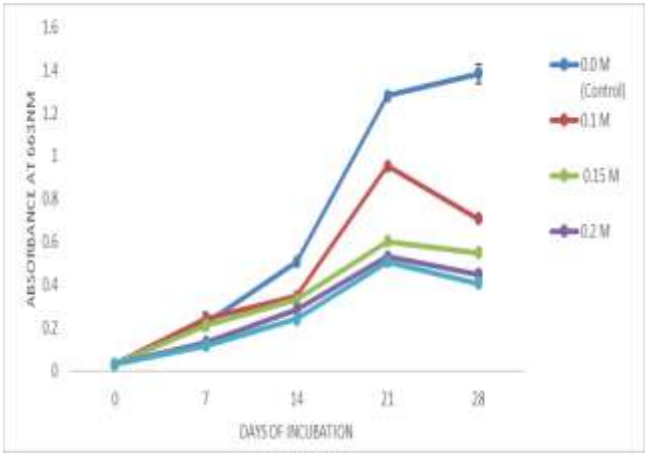

Figure 4: Growth of *Chlorella vulgaris* in medium supplemented with variable salinity ranges

The growth curve of *C. vulgaris* with variable salinity is shown in figure 4. Statistical analysis conclude that growth differs significantly ($P < 0.05$) with variable salinity. Tukey's post-hoc analysis suggests that control culture showed maximum growth and it decreased as salinity in medium was increased from 0.1M to 0.25M. Approximately, 46.0 fold increase was observed on day 21 in the control culture compared to zero day. The increasing trend of growth was observed from zero day to 21st day in all concentrations of salinity after, which decline was observed on 28th day. Lowest growth was observed in culture with 0.25M salinity, an increase of 16.9 fold was observed with respect to zero day on day 21st.

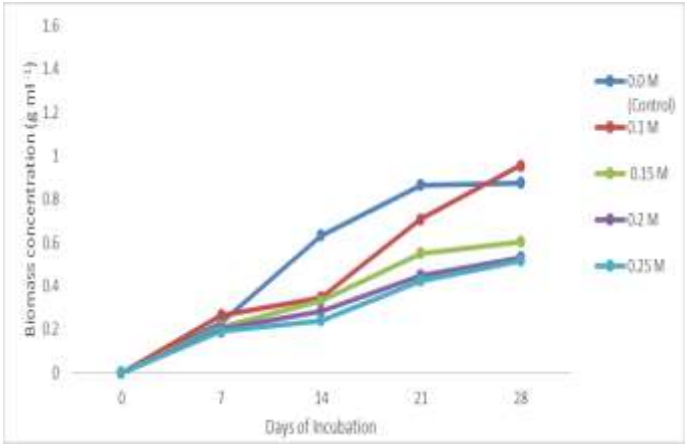

Fig. 5: Biomass of *Chlorella vulgaris* in medium supplemented with variable salinity ranges

The biomass also statistically significant ($P < 0.05$) showed similar trend as shown by the growth *i.e* highest biomass was observed in control culture and minimum in culture growing in 0.25 M saline medium. The increasing trend was observed from zero days to 28th day. Highest 87 fold increase was observed in control culture on day 28th. 0.25 M saline culture showed only 52 fold increase with respect to zero days.

3.4 Effect of Salinity on Lipid Content

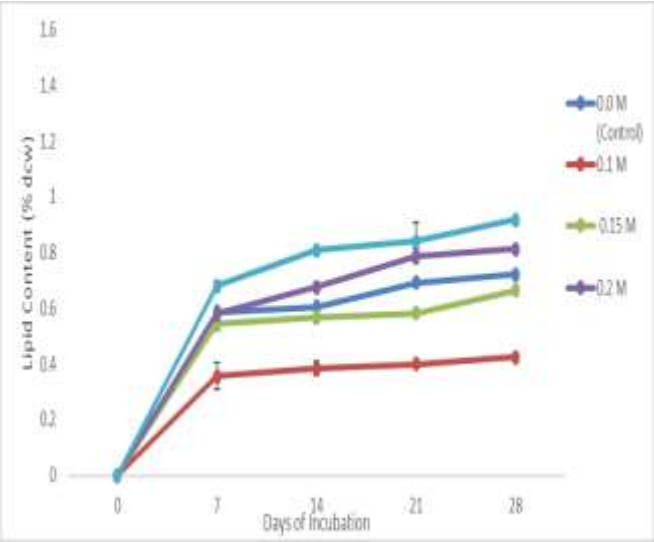

Fig. 6: Lipid accumulation in C*hlorella vulgaris* at different concentration of NaCl

The lipid accumulation in C. vulgaris at different saline ranges was studied in triplicates under optimized conditions and it differed significantly ($P < 0.05$). The highest lipid yield was observed in culture growing in 0.25 M saline medium and lowest was observed in control culture (0.0M saline medium).

The yield increased from zero days to 28th day. On 2th day 91 fold increase was observed in 0.25 M saline culture with respect to 81 fold, 72 fold, 66 fold and 42 fold increase in 0.2M, 0.15 M, 0.1 M and control culture i.e 0.0 M respectively.

4. CONCLUSIONS

The sudden change in growth conditions and stresses like deficiency of nutrients or high salinity in some algae has significant impact on growth biomass and lipid yield.

The experiments conducted to study growth of *C. vulgaris* with variable pH showed that maximum growth occurs at control pH 7 as the pH was increased to 8 and 9 the decrease in algal density was observed which can be correlated to the fact that higher pH limits carbon availability from CO_2, hence algal growth is suppressed (11, 12, 13). The biomass extracted from all variable pH ranges also showed highest biomass at control pH7. This is directly proportional to algal growth. Decreased algal growth observed at alkaline pH is due to the fact that the mother cell, cell wall flexibility increases which in turn prevents its rupture and inhibits autospore release thus increasing the time for cell cycle completion [13, 14]. In terms of days both growth and biomass had shown increasing trend from 0 to 21 day after which the declining trend was observed.

The lipid accumulation at pH 9 was highest and as the pH was decreased the lipid accumulation also showed decrease as stated by [13, 14] alkaline pH indirectly results in increase in triglyceride accumulation.

Exposing algae to lower or higher salinity levels than their natural levels can change growth rate and alter composition. In our experiments the growth rate and biomass decreased as the NaCl concentration in the growth medium increased and hence highest growth was observed in 0.0M culture and lowest was observed in culture grown in 0.25 M salinity. This could be associated with the fact that *C.vulgaris* was unable to adapt at higher saline ranges. Cultivation with different salinity ranges although showed similar time course i.e growth increased from 0 to 21st day and declined on day 28. Higher lipid content was however observed in the medium with highest NaCl concentration ie at 0.25 M.

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