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OPTIMIZATION OF FERTILIZER BASED MEDIA FOR THE CULTIVATION OF *SCENDESMUS* SPECIES.

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ABSTRACT

Microalgae can efficiently utilize CO_2 from atmosphere and are responsible for more than 50% of the total global carbon fixation. The algal biomass is a good source of various commercially important compounds. Physiochemical parameters such as light intensity, pH, and temperature are known to influence the growth rate and biochemical composition of the algae. Nutrient composition like Nitrogen and phosphorus of the culture media also influences biomass production. It is not economically viable to use analytical grade nutrients for mass cultivation of microalgae. This investigation was conducted with the aim of providing a simple and inexpensive medium to decrease the cost of large scale production of *Scendesmus* sp. Various fertilizer based media such as Nitrogen, Phosphorous, and Potash (Potassium) (NPK),Di Ammonium Phosphate(DAP),Urea Superphosphate Trace metals(UST) NPK, DAP, UST have been used for biomass production. As UST medium was found to support maximum biomass productivity, further optimizations of media components of UST medium and physical factors that influence the microalgal growth were optimized. optimized. The concentration of Urea and Phosphate at 522mg/L and 28 mg/L respectively, and the trace metals at the concentration of 1m L L^{-1} at the of PH 8 and the light intensity of 1700 Lux at room temperature were observed as optimum for the growth of algae. In UST medium, the biomass productivity and the specific growth was found to be 0.4 $gL^{-1}d^{-1}$ and 0.3(μ) (d^{-1}) respectively and in BG11 medium, the biomass productivity and the specific growth was 0.12 $gL^{-1}d^{-1}$ and 0.09(μ) (d^{-1}) respectively. A twofold increase in biomass productivity in UST medium compared to BG 11 medium was observed. Thus UST medium can serve as an economically viable medium for the mass production of *Scendesmus* sp.

KEY WORDS: **Physico chemical parameters, nutrition, Biomass production, optimization and specific growth rate**

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INTRODUCTION

Microalgae are photoautotrophic microorganisms found both in fresh water and marine environment that can convert solar energy into chemical energy through photosynthesis.[1] During the last few years, microalgae have gained interest due to its rapid growth rate and ability to fix carbon dioxide in the atmosphere; a major greenhouse gas causing global warming. Algae can efficiently fix Co_2 in the atmosphere where more than 50% of the Co_2 in the atmosphere is fixed during photosynthesis.[2] Microalgae can produce a wide range of products such as protein, lipid, carbohydrate, carotenoids and also recognized as potential source of biodiesel because of high oil content. The growth rate, photosynthesis, activity of cellular metabolism and composition of algae is influenced by providing ideal growth factors such as temperature, light, pH, and nutrients.[3,4,5] .Temperature is one of the essential key factors for influencing the Cell size, nutrient requirement and biochemical components [6].The pH has a significant impact on solubility and availability of Co_2, a carbon source so maintenance of ideal pH is essential for algal cultivation.[7]The optimum light intensity is associated with photosynthesis and cellular composition of the microalgae.[8] During photosynthesis, algae utilize light but it cannot be stored, so the light should be supplied sustainable manner.[9] In mass production of microalgae, nutrients play a major role for the growth and productivity.[10, 11] Among nutrients, Nitrogen plays an important role in the formation of structural and functional proteins, whereas Phosphorus, acts as an important component required for normal growth and development of algal cells.[12] The biochemical composition of protein, carbohydrate, fatty acids, lipids, Chlorophyll and carotenoids of microalgae is influenced by the concentration of Nitrogen and Phosphorus[13].Trace metals are essential for phycophysiology, even though they present in small quantities in algal cells[14]. The production of microalgae with reduced costs is necessary while considering for large-scale cultivation for commercial purposes. The nutrients supplied in media routinely employed for the cultivation of algae are of analytical grade and represent the most expensive constituents of the culture media. The media usually prepared from premixed stock solutions and the preparation also time consuming process.[15] To overcome the aforementioned problems, media based on agricultural fertilizers such as Urea, SSP (Single super phosphate) and NPK which are inexpensive and simple to prepare can be employed for algal biomass production. To achieve higher yield in Biomass productivity, optimization of nutrients concentration and cultural conditions could be employed.[16] Therefore, the present study was undertaken to evaluate the growth performance of *Scenedesmus* sp in different fertilizer medium, screening of potent medium based on Biomass productivity and optimization of nutrients and cultural conditions for maximum yield and to compare the growth performance with conventional BG 11 medium.

MATERIALS AND METHODS

Algal culture

The green algae, *Scenedesmus* sp used for the present study was isolated from Sulur pond, (Latitude:11.03ºN Longitude:77.13ºE) a freshwater pond situated in Sulur town Panchayat, 25km east of Coimbatore city, Tamil Nadu, India.

Semi-continuous cultivation of Scenedesmus sp

The strain was grown and maintained in BG 11 medium. Ten millilitre of the culture was inoculated into 90 ml of BG-11medium (Nutrients (g/L) $NaNO_3$-1.5, K_2HPO_4-0.04, $MgSO_4.7H_2O$ -0.075, $CaCl_2.2H_2O$ -0.036, Citric acid -0.006, Ferric ammonium citrate-0.006, EDTA (Na_2 Salt) and Na_2CO_3-0.02.Trace metals (mg/L) H_3BO_3-2.86, $MnCl_2.4H_2O$-1.81, $ZnSO_4.7H_2O$ -0.22, $Na_2MoO_4.2H_2O$-0.39, $CuSO_4.5H_2O$-0.079, Co $(NO_3)_2.6H_2O$ -0.049). Initial pH of the prepared medium was adjusted to 7 using 1N NaOH and 1N HCl as required and incubated at room temperature by providing artificial light through Philips 40W/TLD tube lights at the intensity of 1500 Lux and Light cycle followed was 16:8h (light : dark) photoperiod followed by sub culture for every 15 days.In order to obtain an algal biomass at the same phase of growth, semi continuous mode of cultivation was carried out. Mass cultivation of the isolate in a semi continuous mode was done in a Teflon fabricated photo bioreactor of 25L capacity with the height of 44cm and width of 97cm. The bioreactor was attached with a mixer and aerator. The pre culture was started as a batch culture. The photo bioreactor was inoculated with 10% initial inoculum corresponding to Optical density of 0.32 at 680nm into BG11 medium prepared using sterilized water at the pH of 7. and incubated at room temperature with a light intensity of 1500 Lux using four daylight fluorescent CFL lamps (Havells CFL 11W LED) for 16:8h (light : dark) photoperiod.[17] Artificial aeration was supplied with an aquarium air pump fitted with a regulator which pumps air at a rate of 120 bubbles per minute. When the cell density reached about an optical density value > 1 at 680nm, two litre of culture broth was replaced with fresh BG11 medium every 48 hours. The growth of the algae was monitored by observing the optical density every 48 hours at 680 nm. The density of the cell before and after dilution of growth medium at every 48 hours was also measured. The growth rate in the media was observed until the culture reaches stationary phase. Biomass productivity (Pmax) in BG 11 medium and the specific growth rate was calculated. All the experiments were performed in triplicate.

Assessment of quality of water used for media preparation

The water used for medium preparation was subjected to the assessment of various physico chemical parameters like Nitrate, Phosphate, Ammonium, Total Dissolved solids and were estimated by standard procedures.[18]

Biomass estimation & Growth kinetics

The biomass concentration was determined every 48 hours by measuring optical density at 680 nm in a UV-Visible spectrophotometer (DMB-PC Based UV Spectrophotometer-Systronics 2202). Micro algal dry weight per litre (gL^{-1}) was also determined by filtering the cells through Whatmann No.1 filter paper. The paper

was dried until reaching a constant weight .[19]The biomass concentration was calculated with a calibration curve relating optical density with dry weight of biomass. Biomass concentration was calculated from the regression equation obtained by plotting optical density values versus dry weight of the biomass. Biomass productivity (Pmax) was calculated by using the formula $P_{max} = (W_f-W_o)/(t- t_0)$ $gL^{-1}day^{-1}$,Where W_f and W_0 were the final and initial biomass concentration, respectively and t is final cultivation time in days and t_0 initial time in days.[20]

Growth kinetics

Specific Growth rate ((μ) (d^{-1})) is a measure of number of generations (the number of doublings) that occur per unit of time in an exponentially growing culture. The exponential (straight line) phase of growth was carefully determined and specific growth rate was obtained by the following equation.[21] The specific growth rate of the microalgae was calculated using the equation ,$\mu\ d^{-1} = \ln (N_2/N_1)/ (t_2 - t_1)$, where μ is the specific growth rate, and N_1 and N_2 are the biomass at time 1 (t_1) and time 2 (t_2), respectively.

Adaptation of Scenedesmus sp in different fertilizer based medium

To develop an economical fertilizer based medium, media were supplemented with Di ammonium phosphate (DAP), Nitrogen Phosphorus Potassium (NPK) 20:20:20 and Urea and Superphosphate (UST) were examined separately with trace metals.. Similar to the algae cultivation using BG 11 medium, Semi continuous mode of cultivation is employed in these media. In NPK 20:20:20 medium, the concentration of NPK is added at a concentration of 522 mg/L and superphosphate at a concentration of 28 mg/L along with trace metals solution at a concentration of 1ml/L were added. Likewise in DAP medium, DAP is added at a concentration of 522 mg/L along with trace metals solution at a concentration of 1ml/L were added. In UST medium, Urea is added at a concentration of 522 mg/L and superphosphate at a concentration of 28 mg/L along with trace metals solution at a concentration of 10ml/L were added. These concentrations maintained in the above mentioned media is same as the concentration of Sodium nitrate and Di potassium Hydrogen phosphate as Nitrogen and Phosphorus respectively in BG 11 medium . These media were used for the growth of *Scenedesmus* sp by providing the same inoculum volume and cultural conditions maintained for the algal growth in BG 11 medium. The growth rate in different media was observed until it reaches the stationary phase and compared with the growth rate of *Scenedesmus* sp in BG 11 medium.

Screening and optimization of fertilizer based medium and physiochemical parameters

Among different type of media used for screening based on the biomass production of *Scenedesmus* sp, a medium is subjected to optimization of nutrients and cultural growth conditions.

Urea

To optimise the concentration of urea required for the growth of *Scenedesmus* sp, UST medium supplemented with different concentration of urea was prepared using sterile water. Concentration of urea supplemented in the medium was 500 mg/L, 522 mg/L and 544 mg/L and maintaining the phosphate and trace metals constant (phosphate 28 mg/L and Trace Metal Solution 1.0 mL L^{-1}). This is followed by providing same cultural conditions for the growth of algae similar to BG 11 medium for semi continuous mode of cultivation until it reaches the stationary phase followed by the determination of Biomass productivity.

Super Phosphate

To optimise the concentration of phosphate required for the growth of *Scenedesmus* sp, UST medium supplemented with different concentration of phosphate was prepared using sterile water. Concentration of phosphate supplemented in the medium was 26 mg/L, 28 mg/L and 30 mg/L and maintaining the urea and trace metals constant (urea 522mg/L and trace metal Solution1.0 mL L^{-1}). This is followed by providing same cultural conditions for the growth of algae similar to BG 11 medium for semi continuous mode of cultivation until it reaches the stationary phase followed by the determination of Biomass productivity.

Trace Metals

To optimise the concentration of Trace Metal solution (TMS) required for the growth of *Scenedesmus* sp, UST medium supplemented with different concentration of TMS was prepared using sterile water. Concentration of TMS supplemented in the medium was 0.5 mL L^{-1}, 1 mL L^{-1} and 1.5 mL L^{-1} maintaining the urea and phosphate (urea 522 mg/L and phosphate 28 mg/L). This is followed by providing same cultural conditions for the growth of algae similar to BG 11 medium for semi continuous mode of cultivation until it reaches the stationary phase followed by the determination of Biomass productivity.

pH

To optimise the pH required for the growth of *Scendesmus* sp, three set of UST medium supplemented with urea 522mg/L phosphate 28 mg/L and trace metal Solution 1.0 mL L^{-1} was prepared using sterile water. The pH of the medium in the flasks was adjusted to 7, 7.5 and 8 using 1N HCL and 1N NaOH. This is followed by providing same cultural conditions for the growth of algae similar to BG 11 medium for semi continuous mode of cultivation until it reaches the stationary phase followed by the determination of Biomass productivity.

Light

To optimise the light intensity required for the growth of *Scenedesmus*sp, three set of UST medium supplemented with urea 522 mg/L phosphate 28 mg/L and trace metal Solution 1.0 mL L^{-1} was prepared using sterile water. Each of the three flasks were then subjected to different light intensity of 1500 Lux, 1600 Lux, 1700Lux and Light cycle followed was 16:8h (light : dark) photoperiod. Throughout the study the intensity of the light was measured using Digital Lux meter (MODEL 1: LX -1010B). This is followed by providing same cultural conditions for the growth of algae similar to BG 11 medium for semi continuous mode of cultivation until

it reaches the stationary phase followed by the determination of Biomass productivity.

RESULT AND DISCUSSION

The micro algal culture *Scenedesmus* sp grown in BG11 medium reaches stationary phase on 11th day. The Micrograph of the isolate, *Scenedesmus* sp has been shown in the figure1.

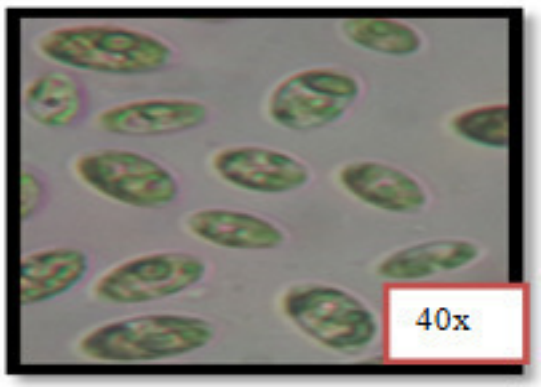


Figure 1
Monograph of Scenedesmus sp

The image of *Scenedesmus* sp viewed under Light Microscope. (Olympus MODEL CH20i BIMF) at the Magnification of 40X. The water used for the media preparation for the cultivation of *Scenedesmus* sp has been subjected to the analysis of various physico chemical parameters are shown in the table 1.

Table 1
Analysis of Physico chemical parameters of the water used for media preparation

S.No	Parameters	Water
1.	Temperature	30^{0}C
2.	pH	8.2
3.	Nitrite(mg/L)	0.65
4.	Nitrate(mg/L)	0.01
5.	Phosphate(mg/L)	0.18
6.	Silicate(mg/L)	0.2
7.	Ammonia(mg/L)	0.010
8.	Salinity(mg/L)	0.23
9.	Alkalinity(mg/L)	0.07
10.	TDS(Total Dissolved Solids) (mg/L)	200
11	TSS(Total Suspended Solids) (mg/L)	10
12.	Dissolved Oxygen(mg/L)	5.008

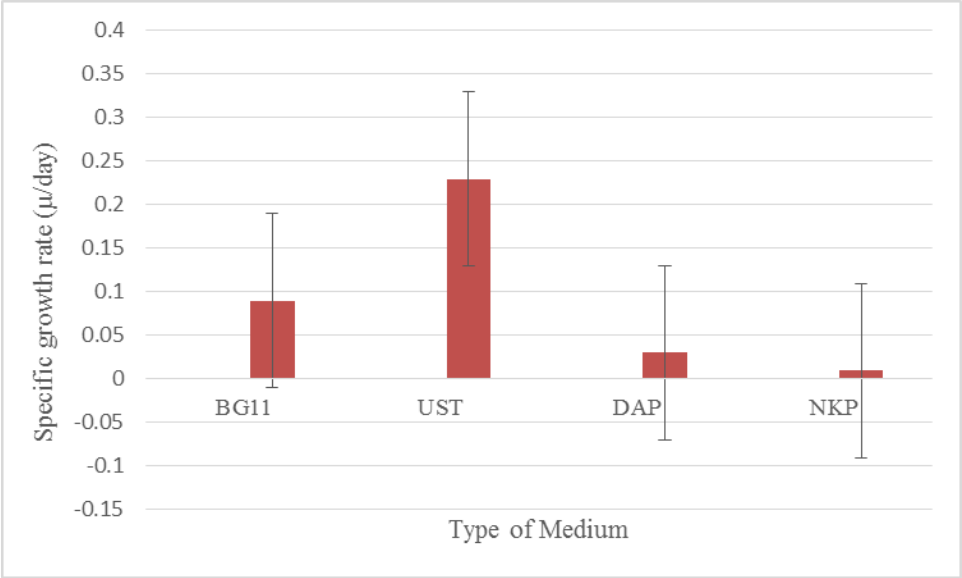
The micro algal culture, *Scenedesmus* sp grown in different fertilizer based media exhibits better growth in UST medium when compared with other two media such as DAP,NPK and also with BG 11 medium. The Biomass productivity and the saturation day of *Scenedesmus* sp grown in different media are shown in the table 2. The specific growth of BG 11 and different type of fertilizer based media are shown in the graph 1. Since the biomass productivity of *Scenedesmus* sp is observed to be higher in in UST media which which has been subjected to further optimization study.

Table 2
Biomass productivity and time taken for growth saturation of Scenedesmus sp in different media

Type of Medium	Day of saturation	Biomass productivity (P_{max}) ($gL^{-1}d^{-1}$)
BG 11 medium	11	0.12±0.02
UST	12	0.31±0.06
DAP	7	0.057±0.02
NPK	3	0.023±0.01

In Table 2 the data are expressed as mean ± SD n=3 for biomass productivity.

Graph 1
Growth pattern of Scenedesmus sp in BG 11 medium and in various fertilizer based media



In graph 1 the data are expressed as mean ± SD n=3 for different media

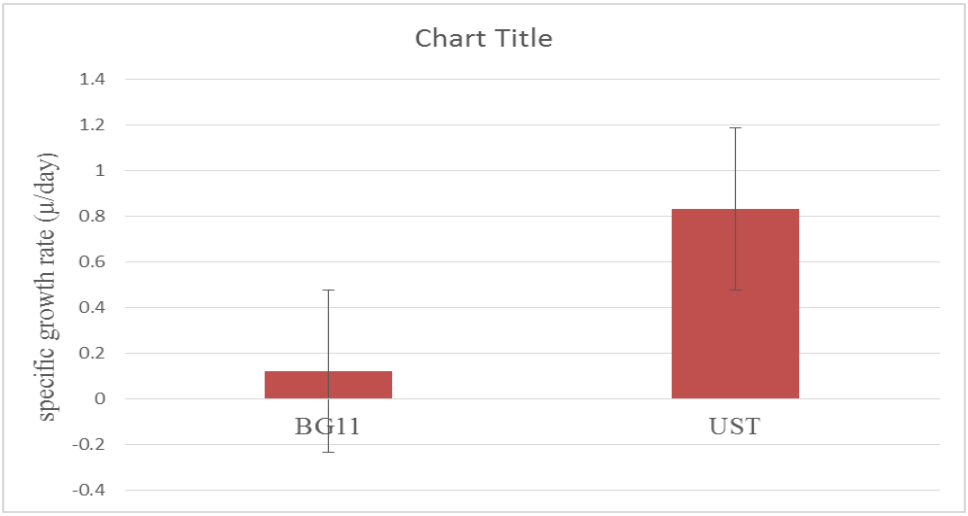
As a result of optimization of nutrients concentration and cultural conditions, *Scenedesmus* sp could yield higher rate of biomass when urea urea is supplemented at a concentration of 522 mg/L mg^{-l},, phosphate at 28 mg/L and trace metal solution solution solution at 1.0 ML L^{-1} at the pH pH of 8 with the light intensity of 1700 Lux in room temperature. The biomass productivity and specific growth rate of *Scenedesmus* sp in UST medium were 0.4 $gL^{-1}d^{-1}$0.4 g/l/day and 0.83 /day 0.83(µ) (d^{-1}) whereas in BG 11 medium, the biomass production is 0.12 $gL^{-1}d^{-1}$and specific growth rate is 0.25(µ) (d^{-1}) . The biomass productivity and the specific growth of the algal culture in different media at different parameters considered for optimization were shown in the table 3 and the comparison of specific growth rate between BG 11 and UST medium of *Scenedesmus* sp was shown in the Graph 2.

Table 3
Optimization of physicochemical parameters for the cultivation of Scenedesmus sp

Parameters	Day of saturation	Biomass productivity (P_{max}) ($gL^{-1}d^{-1}$)	Specific growth rate (µ) (d^{-1})
Urea (500) mg/l	9	0.27±0.07	0.56±0.01
Urea (522) mg/l	12	0.31±0.02	0.64±0.07
Urea (544) mg/l	10	0.27±0.01	0.56±0.01
Phosphate (26) mg/l	9	0.22±0.06	0.45±0.04
Phosphate (28) mg/l	11	0.30±0.04	0.62±0.03
Phosphate (30) mg/l	7	0.12±0.03	0.25±0.02
Trace Metals(0.5) ml/l	10	0.22±0.01	0.45±0.04
Trace Metals (1) ml/l	12	0.37±0.01	0.77±0.06
Trace Metals (1.5) ml/l	11	0.30±0.06	0.62±0.05
pH (7)	9	0.29±0.08	0.60±0.01
pH (7.5)	11	0.32±0.05	0.66±0.04
pH (8)	12	0.34±0.02	0.70±0.04
Light (1500) Lux	10	0.22±0.03	0.45±0.07
Light (1600) Lux	11	0.29±0.07	0.60±0.04
Light (1700) Lux	13	0.33±0.06	0.68±0.01
Optimized UST Media	**14**	**0.4**±0.07	**0.83**±0.01

In Table 3 the data are expressed as mean ± SD n=3 of Biomass productivity and specific growth rate.

Graph 2
Specific growth rate of Scenedesmus sp in BG 11 and UST medium



In Graph 2 the data are expressed as mean ± SD n=3 which shows the specific growth rate between BG 11 and UST media.

Optimum Temperature plays a major role for algal growth because if the temperature is beyond the optimum level the protein synthesis is reduced followed by decreased growth rate.[22] Temperature also plays a major role in determining the biochemical composition and fatty acid profile of the cell.[23] It is reported that *Scenedesmus sp* can able to grow in a wide range of temperature (10-30^{0}C).24In this study, room temperature is observed to be ideal for the effective growth of algae. In optimization of Light, at the light intensity of 1700 Lux the cell density is being increased. In another study, 2500 lux is maintained for the growth of *Scenedesmus sp* for significant biodiesel production.[25] However the differences in the metabolic path in different algae may be related to the difference in response to environmental conditions. The optimum pH range is 8.2 to 8.7 for preferred for algal growth since higher or lower pH than ideal range may decrease the photosynthetic activity. In this study, the rate biomass in *Scenedesmus sp* was high at the pH of 8 at room temperature. In a Literature, the specific growth rate of *Scenedesmus sp* was found to be 0.63 d^{-1} maintaining the temperature at 25 ± 0.5^{0}C with the pH of 8.[26]A positive influence of urea as nitrogenous source in the growth performance of *Spirulina* has been reported. Among all other organic nitrogenous sources, urea gained much importance in large scale algal cultivation, due to its cheap cost than others.[27,28] The biomass productivity is higher in optimized UST medium which may be considered urea urea is the the best source of nitrogen nitrogen for this strain *Scenedesmus* sp. The specific growth rate of *Scenedesmus dimorphus* and *Scenedesmus quadricauda* was found to be 0.54 /day and 0.392 /day respectively when 0.1g/L urea is supplemented.[29] Similar observations were reported previously for the cultivation of *Chlorella.* [30] The superphosphate, phosphorus source is also an important factor for growth since it reduces nitrogen losses from the liquid to the gaseous state.[31]

CONCLUSION

In this study, screening of fertilizer based media and optimization of cultural conditions of potent media for enhanced biomass production were analyzed. Among various media, UST medium in optimized serve as potential source for the mass production of *Scenedesmus* sp. The results suggest that, it is possible to employ urea urea, superphosphate ssuperphosphate and trace trace elements in optimized concentration to scale up biomass production of *Scenedesmus* sp. The results of the present study agree with most of the literature concerning the use of agricultural fertilizer on the fact that the commercial fertilizers formulations can be use be used as effective analytical grade reagent for algae cultivation.

CONFLICT OF INTEREST

Conflict of interest declared none.

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