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Research Article

Kinetic Modeling and Evaluation of Optimal Growth, Lipid Enhancement and Harvesting Techniques of Green Microalgae *Scenedesmus* species

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Abstract

Scenedesmus species is a unique photosynthetic microalgae accumulates large quantity of lipids. In the present work, *Scenedesmus* was grown in 3.5 L photobioreactor using modified BBM under optimal conditions. Various algal harvesting techniques includes centrifugation, sedimentation, autoflocculation, chitosan flocculation and flocculation by pH adjustment were evaluated. The highest microalgal biomass yield of 0.9 g/L was observed at pH 10 when the dosage of bioflocculant chitosan of 0.1 g/L. Intracellular lipid content accumulation was evaluated using lipid extraction methods such as Folch, Bligh & Dyer and Modified Bligh & Dyer methods. The most significant increase in lipid content 58% of dry cell weight (dcw) was recorded under nitrogen deficient medium. The kinetic of the growth and lipid production of *Scenedesmus sp*., were studied under the nitrogen and phosphate deficient conditions using logistic, Luedeking–Piret and Logistic incorporated Leudeking-Piret model.

Keywords: *Scenedesmus*; Photobioreactor; Bioflocculant; Kinetics; Lipid enhancement.

Introduction

Microalgae serve as a promising feedstock to support bioenergy. Microalgae do not directly compete with food chain and possess high growth rate. And due to their increased surface to volume ratio of algal cells, they can efficiently uptake CO_2 in significantly larger amounts as compared to the terrestrial plants [1]. Also there will be no land competition in cultivating the microalgal species as they can grow efficiently for the whole year in any places includes non-arable land, photobioreactor, and waste water and can adapt to the different growing conditions [2]. It has been reported that the biomass and lipid production can be altered by using different media composition as these microalgae can manipulate their metabolism and high lipid productivity can be achieved [3].

Chlorella vulgaris lipid contents of 40% dcw was recorded when grown in low nitrogen supplemented medium and 56.6% dcw was recorded when grown under low iron-supplemented medium [4, 5]. There are some other reports includes limitations in nitrate and phosphate in culture medium appeared to be suitable stimulants for lipid accumulation in microalgae *Scenedesmus obliquus* [6].

As microalgae are grown in stable suspended state in the culture medium due to their small size (3-30 µm), low concentration (0.5-5 g/L) and negative surface charges, the recovery and processing of microalgal biomass from the culture medium is critical step in the microalgae biomass production [7, 8]. Therefore it is very important for developing a cheaperand efficient downstream process for harvesting the microalgal cells [9]. There are different harvesting strategies applied prior to various microalgal species which includes gravity sedimentation, foam fractionation [10, 11] filtration [12], centrifugation [13, 14], flocculation [15]. It has been reported that *Spirulina* can easily settled down by gravitational sedimentation efficiently due to their high cell density and increased size [16]. *Scenedesmus sp*. was one of the most promising microalgae for biodiesel production because of its high lipid content and productivity [17] and the lipid content and productivity of *Scenedesmus sp*. were 19.6–21.1% dry weight biomass and 40.8–53.9 mg/L/day, respectively [18]. Many researchers have discussed the

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method for harvesting *Scenedesmus sp*. Some of these species can be harvested using filtration, but filtering membranes used in the process will be rapidly fouled by the extracellular organic matter if the medium was filtered directly [19]. Most existing commercial system practices centrifugation for harvesting *Scenedesmus sp.* biomass [20]. Many chemicals were also been investigated as the flocculant. The flocculation potential of microalgae depends upon various properties such as cell wall compositions, extent and type of excretions, physiological conditions and, age factors [15]. Therefore, the suitable flocculation method for *Scenedesmus sp*. harvesting should be determined based on their properties.

The effective and reliable method for oil extraction from the *Scenedesmus* biomass should be employed. The choice of solvent in which microalgal lipid dissolves is of major importance for efficient lipid extraction. Traditional lipid extraction method uses methanol and chloroform as solvents. Folch *et al.* were among the first to successfully isolate lipids from animal tissues in a methanol/chloroform/ water phase [21]. The second method developed by Bligh and Dyer used the same solvents for extraction at different initial solvent to sample ratio [22]. Third method is a slightly modified method of originally proposed Bligh and Dyer to extract lipid from dry *Scenedesmus* biomass [23].

In the present study, *Scenedesmus* sp., was subjected to various media sources as well as different nitrogen and phosphate concentration for the accumulation of biomass and lipid content and different oil extraction methods was also evaluated for lipid extraction. Appropriate kinetic models has to develop in order to predict the dynamics of biomass growth and lipid production by *Scenedesmus* sp., Among various research studies and mathematical models proposed for microalgal growth and lipid production, no reports are available for kinetic modeling of *Scenedesmus* growth and lipid production under nitrogen and phosphorous deficient conditions. Therefore in the present study, the selected *Scenedesmus* sp., growth and product formation kinetics were modeled under normal as well as nitrogen and phosphorous deficient conditions using Logistic, Leudeking-Piret and Logistic incorporated Leudeking-Piret models respectively and the most suitable kinetic model for *Scenedesmus* growth and lipid production have been selected on the basis of comparison of experimental data with predicted values as a validity of the selected models..

Materials and methods

Materials

Chemicals used for the lipid extraction includes Methanol, Chloroform, Sodium chloride were purchased from Merch life science Pvt. Ltd., Mumbai. All the chemicals used were of analytical grade and used without further purification.

Microalgae strain and growth medium

Scenedesmus sp., microalgae was collected from Phycospectrum Environmental Research Centre, Chennai, India. The collected microalgae was brought to the laboratory and cultivated in modified Bold Basal Medium which was composed of (mg/L): KH_2PO_4 (160), $CaCl_2.2H_2O$ (25), $MgSO_4.7H_2O$ (75), $NaNO_3$ (250), K_2HPO_4 (75), NaCl (25), EDTA (10), KOH (6), $FeCl_2.4H_2O$ (5), H_3BO_3 (12), H_2SO_4 (conc.) (1 mL), Na_2CO_3 (1000), $MnCl_2.4H_2O$ (2.9), $ZnSO_4.7H_2O$ (0.222), $NaMoO_4.5H_20$ (0.4), $CuSO_4.5H_2O$ (0.08), $CoCl_2.6H_2O$ (0.06) and NaOH (0.1 N).

Cultivation of microalgae photobioreactor

The microalgal cells in exponential period were inoculated (10% v/v) and grown autotropically in 3.5 L photobioreactor containing autoclaved media. The pH was maintained at 6.8 and the temperature of 26±2°C was also maintained under 12:12 h photoperiod with a light intensity of 3500 lux provided by white fluorescent tubes. The culture was stirred two times daily to avoid sticking. The microalgae was grown for 16 days of growth period and harvested at late logarithmic phase for biodiesel production.

Harvesting strategies

Harvesting is the subsequent step right after cultivation and selection of appropriate harvesting method is of great importance for the economics of separation of algal biomass. Therefore the microalgal harvesting was done by three different methods: Centrifugation, Sedimentation and Flocculation. For all these methods, experiments were performed initially with 100 mL culture distributed in different conical flasks.

The centrifugation was done in cooling centrifuge with varying speed (2000-12000 rpm) and time (5-25 min). Once the process was completed, the pellet was dried in oven at 80°C for 3 h to collect and dry cell biomass [24]. The microalgae suspended in the media settle out of a fluid under gravity is known as settling. This is commonly applied for separation of microalgae from water [16]. The gravitational sedimentation was done by allowing the culture undisturbed for about 1 week and the sedimented algal slurry was then dried in oven at 80°C for 3 h.

Flocculation is a process in which algal particles in a medium joins together to form aggregate and settles at bottom [25]. Flocculation experiments were carried out with the effect of pH (7-11), chitosan dosage (0.02-0.12 g/L), sedimentation time (30-150 min) and pH adjustment after addition of chitosan. After sedimentation, the algal slurry was dried in oven at 80°C for 3 h.

Reuse of flocculated medium

After separation of flocs and growth medium, the pH of the growth medium was adjusted to the original value by adding 1N HCl. Fresh *Scenedesmus* culture was inoculated in the medium and the biomass yield was investigated.

Lipid extraction methods

Harvesting is followed by lipid extraction and the method used for extraction should be fast, easily scalable, effective and should not damage the extracted lipids [15]. The choice of an effective extraction method depends on the type of microalgae and cell sizes, cell wall structure and lipid and fatty acid characteristics. Thus, the extraction of lipid was carried out using the following three different methods such as Folch method [21], Bligh & Dyer method [22] and Modified Bligh & Dyer method [23] and compared.

Lipid enhancement strategies

Effect of nitrogen limitation in biomass yield and lipid accumulation

To study the effect of nitrate limitation on lipid accumulation, *Scenedesmus* sp. were allowed to grow in modified BBM media for first 16 days to attain stationary growth phase and then were transferred to fresh BBM medium with seven different concentrations of nitrate, 0 mg/L,50mg/L, 100mg/L, 150mg/L, 200mg/L, 250mg/L, 300mg/L of $NaNO_3$ and N-deficiency was achieved by substituting KNO_3 of the medium with equimolar concentrations of KCl. The microalgal culture was harvested after seven days to estimate the microalgal biomass yield and their lipid accumulations [6].

Effect of phosphorous limitation in biomass yield and lipid accumulation

For the experiments on the effects of different phosphorous concentrations, 16 days *Scenedesmus* culture grown in modified BBM media was transferred to fresh media with six different concentrations of phosphate, 10, 40, 80, 120 and 160 mg/L of KH_2PO_4 and P-deficiency was achieved by transferring the microalgal culture into the medium, where K_2HPO_4 and KH_2PO_4 were replaced by equimolar concentrations of K_2SO_4 and KCl, respectively. The culture was then harvested in seventh day for microalgal biomass estimation and lipid accumulation.

Development of kinetic modeling

Growth kinetics

Among various growth kinetics models, the appropriate model for the present study is the logistic model as it is independent of substrate consumption, facilitates exponential and endogenous metabolic phase and uses simple calculations for studying microalgal growth [26]. The *Scenedesmus* growth kinetics was quantified using logistic equation with the assumption that inhibition is proportional to x^2. The equation used for growth kinetics is given below.

$$\frac{dx}{dt} = kx\left(1 - \frac{x}{xs}\right) \qquad (2)$$

Where dx/dt is the rate of microalgal growth, x is the biomass concentration (g/L), t is the time, k is the carrying capacity (h^{-1}) and x_s is the biomass concentration at stationary phase.

Product kinetics

Lipid production kinetics was done using different unstructured models such as Leudeking-Piret and Logistic incorporated Leudeking-Piret models as these models contributes to both growth and non-growth associated phenomena for product formation. According to these models, the rate of lipid formation depends on both the instantaneous biomass concentration (x) and growth rate in al linear manner and the equation is given as,

$$r_{fp} = \alpha r_{fx} + \beta x \qquad (3)$$

where r_{fp} is the rate of product formation, r_{fx} is the rate of biomass formation and α and β are the product formation kinetic constants of the Leudeking-Piret and Logistic incorporated Leudeking-Piret model. According to Leudeking- Piret Model, the lipid production rate depends linearly upon the growth rate and microalgal cell concentration.

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \quad (4)$$

here, α and β are Leudeking-Piret kinetic constants, dp/dt is the rate of lipid production, α is the lipid formation coefficient and β a non-growth correlation coefficient. β can be evaluated as

$$\beta = \frac{\left(\frac{dp}{dt}\right)stationary\ phase}{x(s)} \quad (5)$$

where x(s) is the biomass concentration at the stationary phase.

The relationship between mode of product formation and microorganism growth was classified as follows. Class 1:α = 0 and β ≠ 0, the relationship between product formation and microbial growth was unrelated. Class 2: α ≠ 0 and β ≠ 0, the relationship between product formation and microbial growth was partially connected and Class 3:α≠ 0 and β = 0, the product formation is linearly proportional to the microalgal growth [27]. The error percentage for cell growth and product formation was calculated using eq. (6).

$$\%\ error = \frac{(experimental - predicted)}{experimental} \times 100 \quad (6)$$

The growth and lipid accumulation in *Scenedesmus* sp., increases in nitrogen and phosphorous deficient media as a result of different combinations of multiple pathways [28-30]. Therefore constants of Leudeking-Piret and Logistic incorporated Leudeking-Piret model were determined through regression analysis by different values of lipid production rate and specific growth rate of microalgal biomass.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was done at SAIF, IITM. The GC-MS uses the principle of separation technique, sample was analyzed in JEOL GCMATE II Gas Chromatography Mass Spectrometry (GC-MS) system equipped with a straight deactivated 2 mm direct injector liner, 15m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness) and a detector with triple-axis detection using helium as carrier gas. The flow rate of carrier gas is 1.50 ml/min; injector and column oven temperature was programmed at 280° C and 80° C and injector mode split ratio of 20:1.

Results and discussions

Scenedesmus biomass harvesting by centrifugation

Based on the microalgal particle size and density differences, the centrifugal acceleration enhances the algal concentration and the supernatant can be easily discharged or reused. One of the most important parameters for the harvesting of *Scenedesmus* was centrifugation rate. Series of experiments were made at different centrifugation rates varying from 2000 to 12000 rpm for 20 min. Fig. 1(a) shows that the biomass yield was sharply increased up to centrifugation rate of 10000 rpm, a slow increase was observed up to 12000 rpm and then remained almost constant. Thus, the optimal centrifugation rate of 10000 rpm was selected and the maximum yield of 0.86 g/L of *Scenedesmus* biomass was obtained. Another important parameter is the centrifugation time. In order to get the best centrifugation time, the experiments were performed in the range of 5-25 min at 10000 rpm. The results Fig. 1(b) showed that the centrifugation time had a significant influence on the biomass yield. Maximum biomass yield of 0.86 g/L was obtained at 20 min of centrifugation time and then remained constant. And thus the optimum time for *Scenedesmus* harvesting was chosen as 20 min.

Scenedesmus harvesting by sedimentation

The rate of sedimentation was mainly affected by ageing of the cells, particle size and time. Experiments were performed by varying time in the range of 2-10 days. The result demonstrated that the *Scenedesmus* biomass yield increases with increase in sedimentation time. Fig. 1(c) shows that 0.85 g/L of biomass yield was obtained at day 8 and then remained constant and therefore the optimal sedimentation time was selected as around one week. Sedimentation rate can be further enhanced by addition of flocculants to the system.

Scenedesmus harvesting by flocculation

Autoflocculation by adjusting pH

At elevated pH, there will be spontaneous aggregation of particles, resulting in sedimentation of *Scenedesmus*. Fig. 2(a) shows

the effect of pH ranging from 7 to 11 on the flocculation and biomass yield for harvesting *Scenedesmus*. The original pH of the culture medium was 9.3. In this pH, around 50 % of microalgal cells were settled down in 3 h. The efficiency was increased when pH was adjusted upto 10.

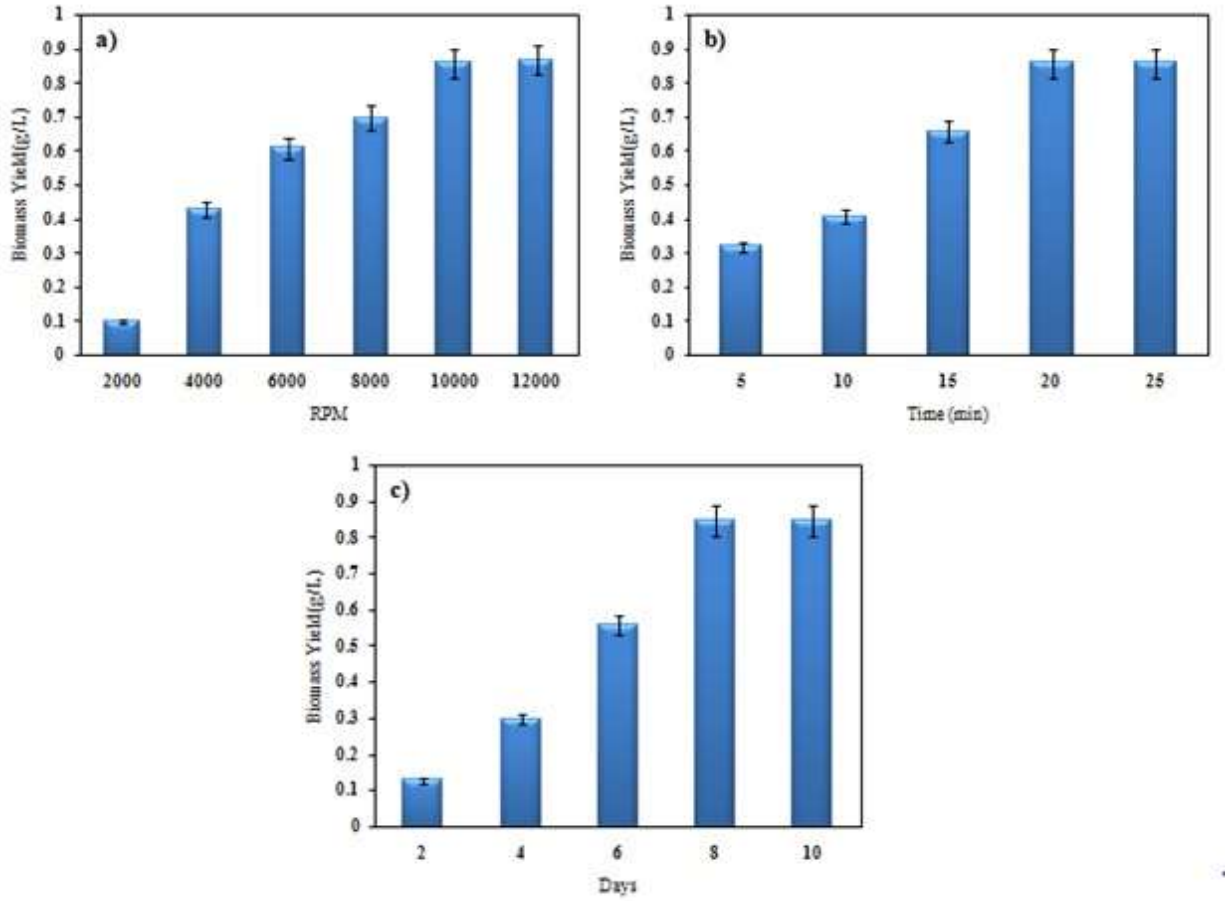

Fig.1. (a) Effect of RPM on *Scenedesmus* biomass yield, (b) Effect of centrifugation time on *Scenedesmus* biomass yield, (c) Effect of time in sedimentation

The stability of the flocs was monitored with time. The maximum biomass yield of 0.85 g/L was obtained at pH 10. After 2 h of sedimentation, the flocculation efficiency could still keep higher when the pH was adjusted to 11 but some microalgal cells at this pH in the medium might die after 24 h. Hence, the medium with pH 10 was more suitable for effective flocculation. The effects of pH in increasing the flocculation efficiency for harvesting *Scenedesmus* sp. was also previously investigated [8]. They found the efficiency was greatly raised when the pH was increased to 10.6. The result was similar to the conclusion in this work but as both the microalgae cultivated were under different conditions, the suitable pH for flocculation in the two cases was not the same. The reason behind the autoflocculation is as pH increases, it enhances the formation of positive superficial charged precipitate due to the presence of metal cations in the medium that would absorb the negatively charged microalgal cells and hence flocculates [8].

Effect of dosage of chitosan in flocculation

Cationic polyelectrolyte will give better flocculation and thus chitosan is chosen as organic bioflocculant as they are biodegradable and do not contaminate microalgal biomass and also exhibited higher flocculation efficiency at relatively short time [31]. The flocculant dosage has been recognized as a critical parameter in flocculation process as it influences both the extent and rate of flocculation reaction by producing stable flocs at shorter time. Therefore, preliminary experiments were undertaken to determine the optimal bioflocculant dosage and sedimentation time for flocculating the microalgal cells. The dosage of chitosan was varied from 0.02-0.12 g/L to study its influence in the rate of flocculation. Fig. 2(b) shows maximum biomass yield of 0.8 g/L was obtained at the bioflocculant dosage of 0.1 g/L of chitosan.

Optimization of flocculation time

The effect of flocculation time was conducted at optimized dosage of bioflocculant by varying time between 30 and 150 minutes. From the Fig. 2(c), the maximum biomass yield of 0.8 g/L was obtained at 120 min at the chitosan dosage of 0.1 g/L and then remained constant and therefore the optimal flocculation time was selected as 2 h.

Effect of flocculation with pH adjustment after addition of chitosan

The importance of pH adjustment in flocculation process has been reported by many researchers [9, 32]. The changes in flocculation efficiency at different pH was adjusted with 1 M sodium hydroxide and 1 N hydrochloric acid followed by addition of chitosan. As pH affects the zeta potential of charged particles, it interferes with flocculation after adding flocculants. The change in flocculation was shown in Fig. 2(d) for chitosan at different pH between 7 and 11. Chitosan's molecular structure can be influenced by pH and due to its acidic characteristic; the pH of the cultured medium was reduced from 9 to 7 after addition of the bioflocculant. The highest biomass yield of 0.9 g/L was obtained at pH 10 when the dosage of chitosan was 0.1 g/L.

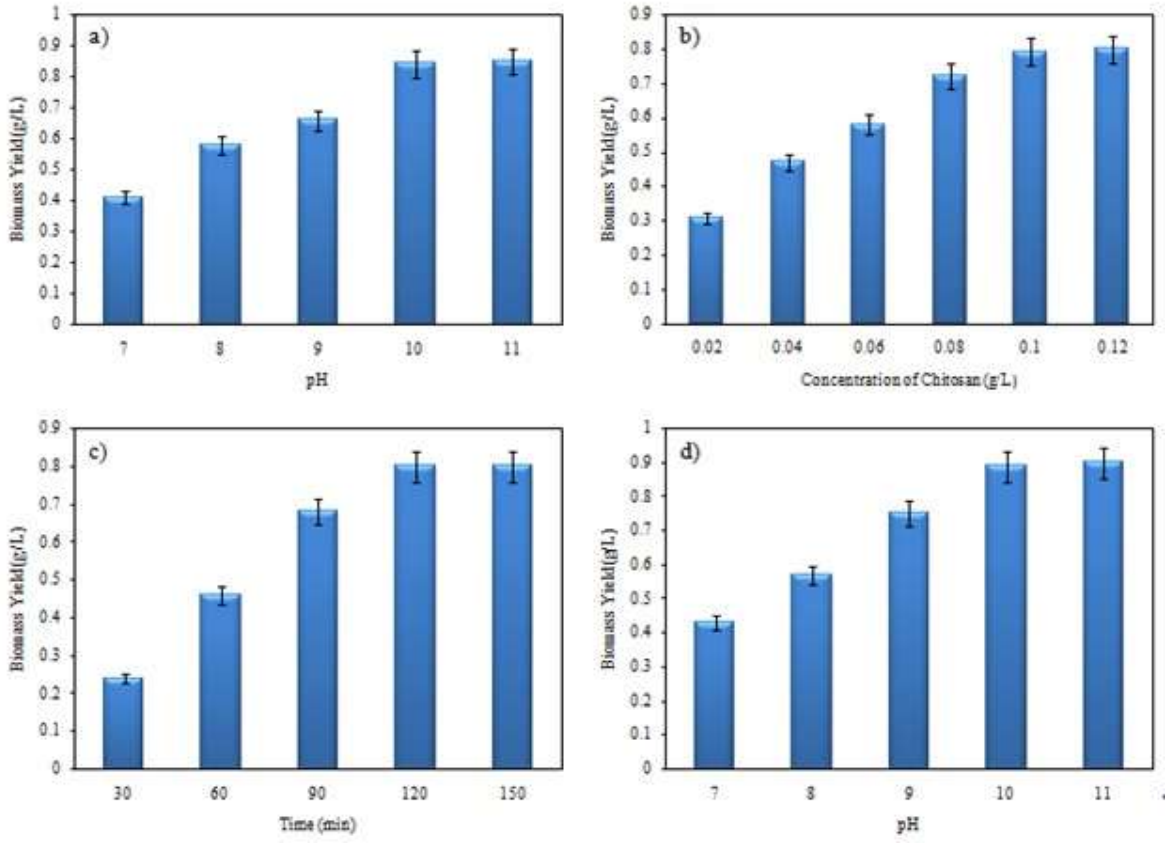

Fig. 2. (a) Effect of pH adjustment in autoflocculation on *Scenedesmus* biomass yield, (b) Effect of different dosages of chitosan on *Scenedesmus* biomass yield, (c) Effect of time after addition of chitosan on *Scenedesmus* biomass yield, (d) Effect of flocculation at different ph adjustment after addition of chitosan on *Scenedesmus* biomass yield

Reuse of flocculated medium for cultivation

For many other flocculants, the used growth medium after flocculation was usually disused as the medium was contaminant with flocculant. However for the microalgae harvested by pH adjustment after addition of chitosan, since chitosan would not contaminate the growth medium and thus the growth medium after flocculation might be reused by neutralizing pH. The biomass of *Scenedesmus* sp. cultivated in the reused media was close to that of fresh media indicating the culture solution could be reused with significant yield of biomass.

Lipid estimation in Scenedemus biomass

In this study, we did a comparative analysis of three different lipid extraction methods for effective lipid recovery namely Folch method, Bligh & Dyer method and Modified Bligh & Dyer method. For

Scenedesmus sp. the percent lipid contents were as follows: 28, 30 and 33% total lipid/g dry weight with Folch, Bligh & Dyer and Modified Bligh & Dyer method. The highest lipid extraction was obtained in Modified Bligh & Dyer method as it was the only method that employs ultrasonication assisted for disruption of microalgal cell wall in extraction of lipids and therefore this method was selected as most efficient method for lipid extraction in *Scenedesmus* sp.

Effect of nitrogen limitation in biomass yield and lipid accumulation

The time course of growth of *Scenedesmus* sp. in normal and N-deficient media is shown in Fig. 3(a). Growth of *Scendesmus* increased steadily normal media but in N-deficient media the biomass yield increased upto 7 days and then decreases due to nitrogen starvation. This is due to the fact that nitrogen serves as an essential component in protein synthesis and thus at starvation condition, it affects the overall growth and impairs the cell division process. At initial stages, the prior protein and chlorophyll content in the microalgal cells were sufficient for photosynthesis but in course of time the overall cell growth would get affected. But the limitation of nitrate was appeared to be a suitable stimulant for lipid accumulation (Fig. 3(b)) in *Scenedesmus* sp. The most effective lipid enhancement of upto 58% dcw was observed when the stationary phase culture was subjected to the nitrate media deficient for 7 days.

Effect of phosphorous limitation in biomass yield and lipid accumulation

Fig. 3(c) shows the growth of *Scenedesmus* sp. in normal as well as P-deficient media. Biomass yield under P-deficiency media showed a negative trend than normal media as phosphates acts as a constituent element for ATP synthesis and essential for photophosphorylation relevant to microalgal cell growth and metabolism. In contrast, there was a modest increase in the lipid accumulation upto 47% dcw shown in Fig. 3(d) when the stationary phase culture was transferred to the P-deficient media for 7 days.

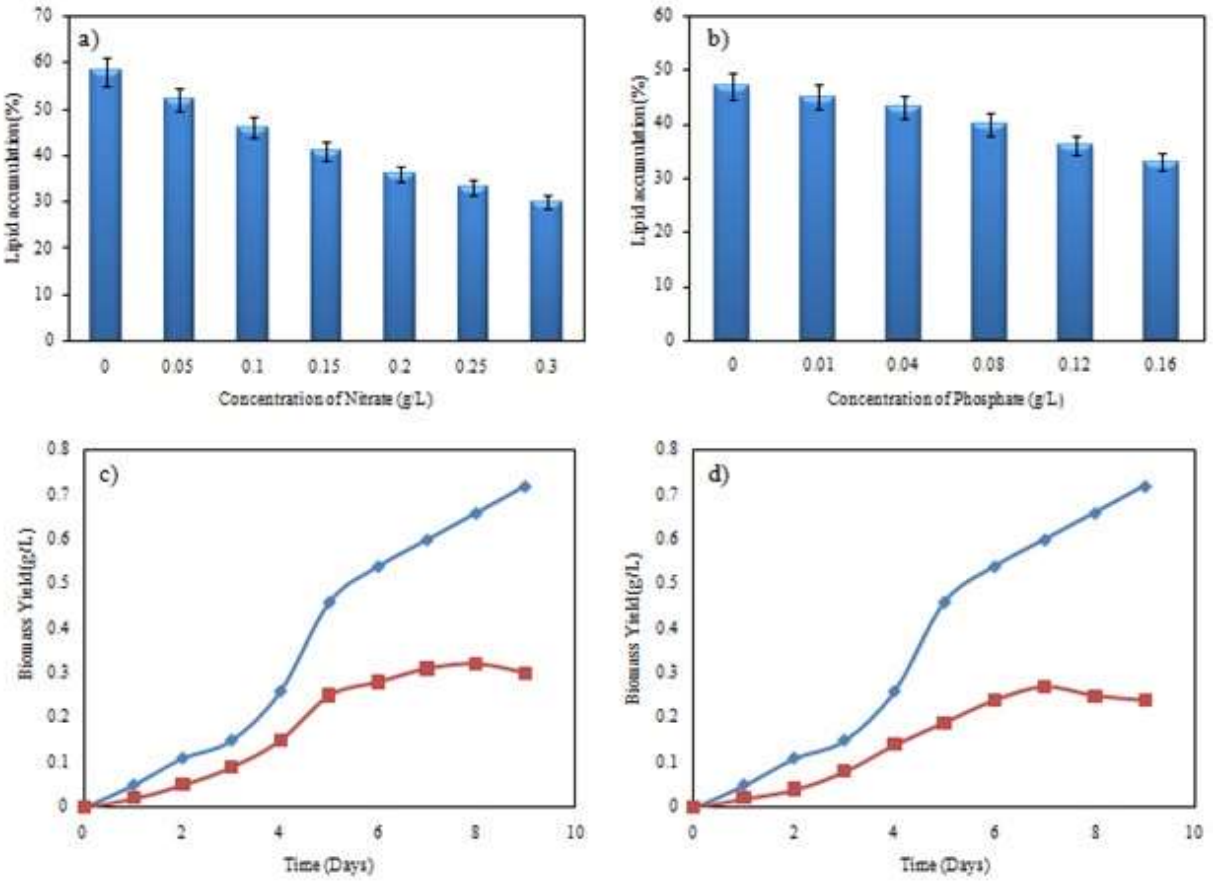

Fig. 3. (a) Effect of different concentration of nitrate for lipid accumulation in *Scenedesmus* sp., (b) Growth of *Scenedesmus* Sp. in normal and N-deficient media, (c) Effect of different concentration of phosphate for lipid accumulation in *Scenedesmus* sp., (d) Growth of *Scenedesmus* in normal and P-deficient media

Kinetic studies

The kinetics of *Scenedesmus* cell growth and lipid production grown in normal condition, nitrogen and phosphorous deficient conditions were analyzed and simulated with respect to the obtained experimental values. The kinetic modeling consists of two steps namely evaluation of parameters of Logistic (K) and Leudeking-Piret as well as Logistic incorporated Leudeking-Piret models (α and β). The relationship between actual versus predicted values is explained briefly using regression coefficient, R^2 values for biomass growth and lipid accumulation profiles observed in different media. This regression coefficient measures the strength of linear relationship between the experimental (actual) and predicted values obtained using the above mentioned three models.

The high R^2 and prediction values denote that logistic model is the most suitable for *Scenedesmus* growth with a minimum error of 2.35% at normal, 3.09% at N-deficient condition and 4.2% at P-deficient condition. Fig.4 (a),(b),(c) shows the experimental and model predicted data of *Scenedesmus* biomass growth in Normal, N-deficient and P-deficient media. In Fig. 4(a), the growth of cells increases till around 12^{th} day as the modified BBM media supplies all the essential nutrients for the microalgal growth and correspondingly the logistic model parameters of K and μ_{max} were estimated as 0.404 h^{-1} and 0.9 h^{-1}. Fig. 4 (b) and (c) showed the negative trend in the microalgal biomass growth compared to the growth profile of *Scenedesmus* in normal media.

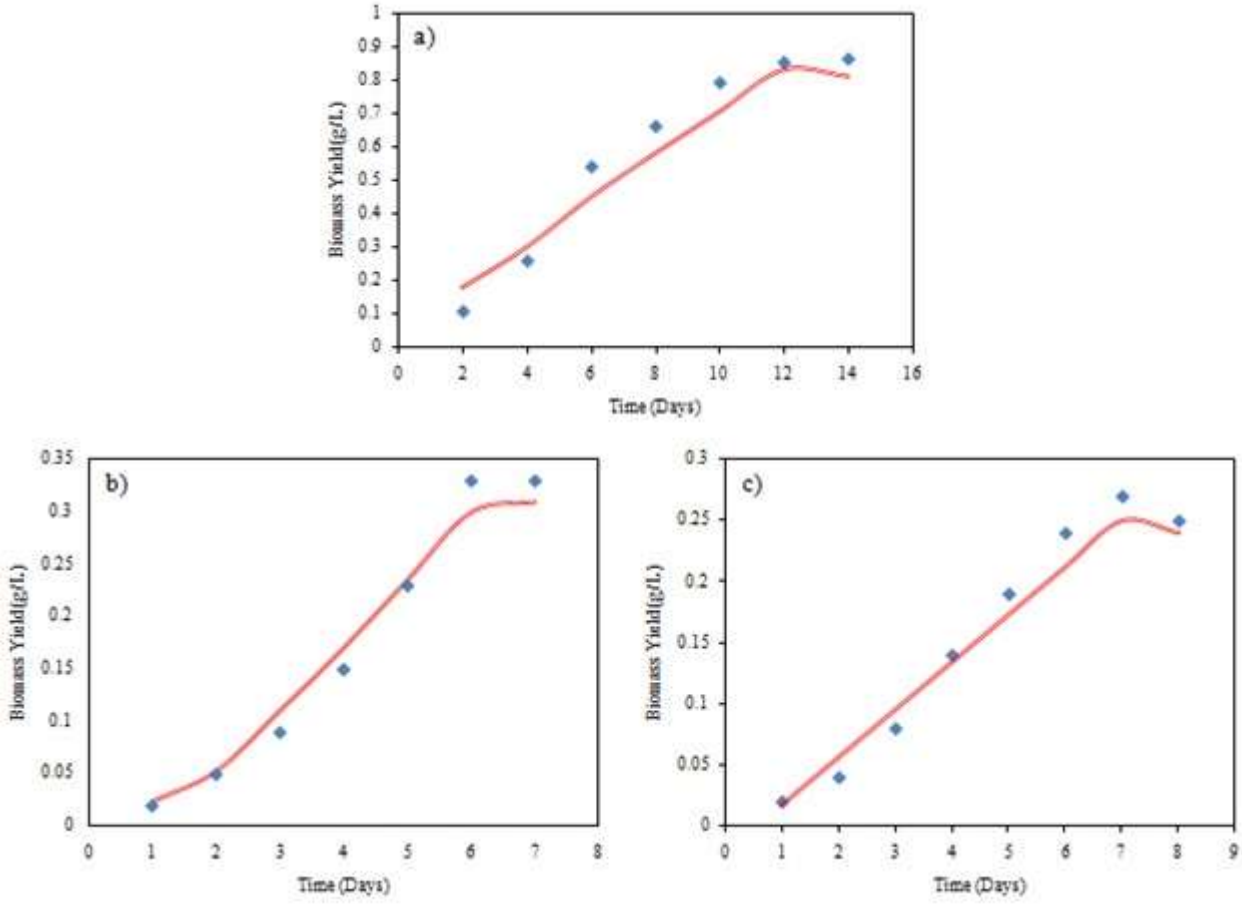

Fig. 4. (a) Experimental data (♦) and model prediction (-) of *scenedesmus* biomass growth in normal media, (b) Experimental data (♦) and model prediction (-) of *scenedesmus* biomass growth in N-deficient media and (c) Experimental data (♦) and model prediction (-) of *scenedesmus* biomass growth in P-deficient media

The estimated kinetic parameter values obtained from these models are mentioned in Table 1. The determination coefficient (R^2) values of the experimental and predicted values were analyzed to find out the best fit model for lipid accumulation. The Logistic incorporated Leudeking-Piret model showed comparatively better R^2values among all tested models with a minimum error of 2.06% at normal, 2.4% at N-deficient condition and 3.9% at P-deficient

condition and therefore this model could properly describe the relationship between biomass and lipid accumulation in different nutrient composition.

Fig. 5 (a-c) shows the proposed mathematical model fitting of lipid production in *Scenedesmus* sp., in normal, N-deficient and P-deficient media. The maximum lipid accumulation in *Scenedesmus* was observed in N-deficient conditions and this was found to be in agreement with the report of Packer et al., 2011 [33]. It was also reported that microalgae counter to the nutrient deficient condition by degrading nitrogen-containing macromolecules and accumulate carbon reserve compounds such as lipid for the maintenance of cells. The product kinetic constants, α and β are growth and non-growth associated parameters during lipid accumulation and it was observed that α>β in normal (Table 1), N-deficient and P-deficient conditions predicted that lipid accumulation is more growth associated.

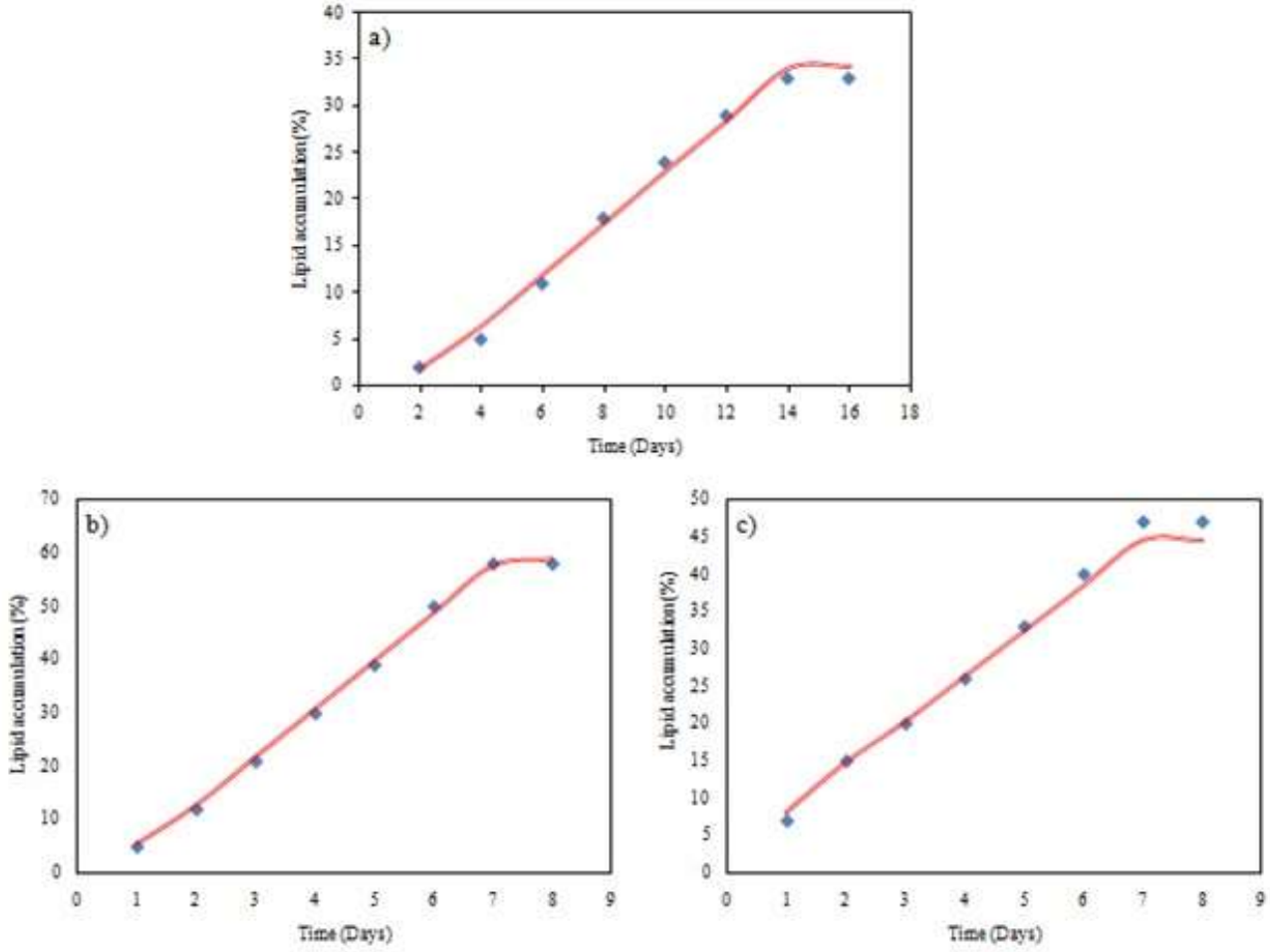

Fig. 5. (a) Experimental data (♦) and model prediction (-) of lipid accumulation in *scenedesmus* innormal media, (b) Experimental data (♦) and model prediction (-) of lipid accumulation in *scenedesmus* inN-deficient media and (c) Experimental data (♦) and model prediction (-) of lipid accumulation in *scenedesmus* inP-deficient media

Characterization of Scenedesmus oil

The extracted *Scenedesmus* oil was further analyzed by GC-MS in scan mode to identify the fatty acid profile with the help of NIST library. The peaks in the chromatogram (Fig. 6) reveal the abundance of fatty acids where the maximum peak represents the presence of 10- Octadecenoic acid, methyl ester (C 18:2) with the retention time at 17.6 min. It is also reported that the molecular characteristic of the fatty acids, such as carbon chain length and degree of unsaturation will highly influence the properties of microalgal oil [34]. At stressed conditions, 10- Octadecenoic acid, methyl ester (C 18:2), a non-polar lipid which serves as storage lipid in *Scenedesmus* sp was induced highly for easy catabolising to provide energy. These lipids were synthesized in the light, and reutilized for polar lipid synthesis in the dark conditions that accumulates highly during N and P depleted conditions [35].

Table 1. Estimated Kinetic Parameters for *Scenedesmus* growth and lipid accumulation

Model	Parameters	Normal Media	N-Deficient Media	P-Deficient Media
Logistic	X_0 (g/L)	0.41	0.2	0.27
	μ (h^{-1})	0.306	0.057	0.037
	μ_{max} (h^{-1})	0.9	0.4	0.27
	K (h^{-1})	0.404	0.325	0.29
Leudeking Piret	R^2	0.957	0.953	0.939
	α	2.456	0.421	0.409
	β	0.072	0.17	0.15
Logistic incorporated Leudeking Piret	R^2	0.995	0.969	0.999
	α	0.33	0.445	0.635
	β	0.16	0.35	0.15
Inference		$\alpha>\beta$	$\alpha>\beta$	$\alpha>\beta$

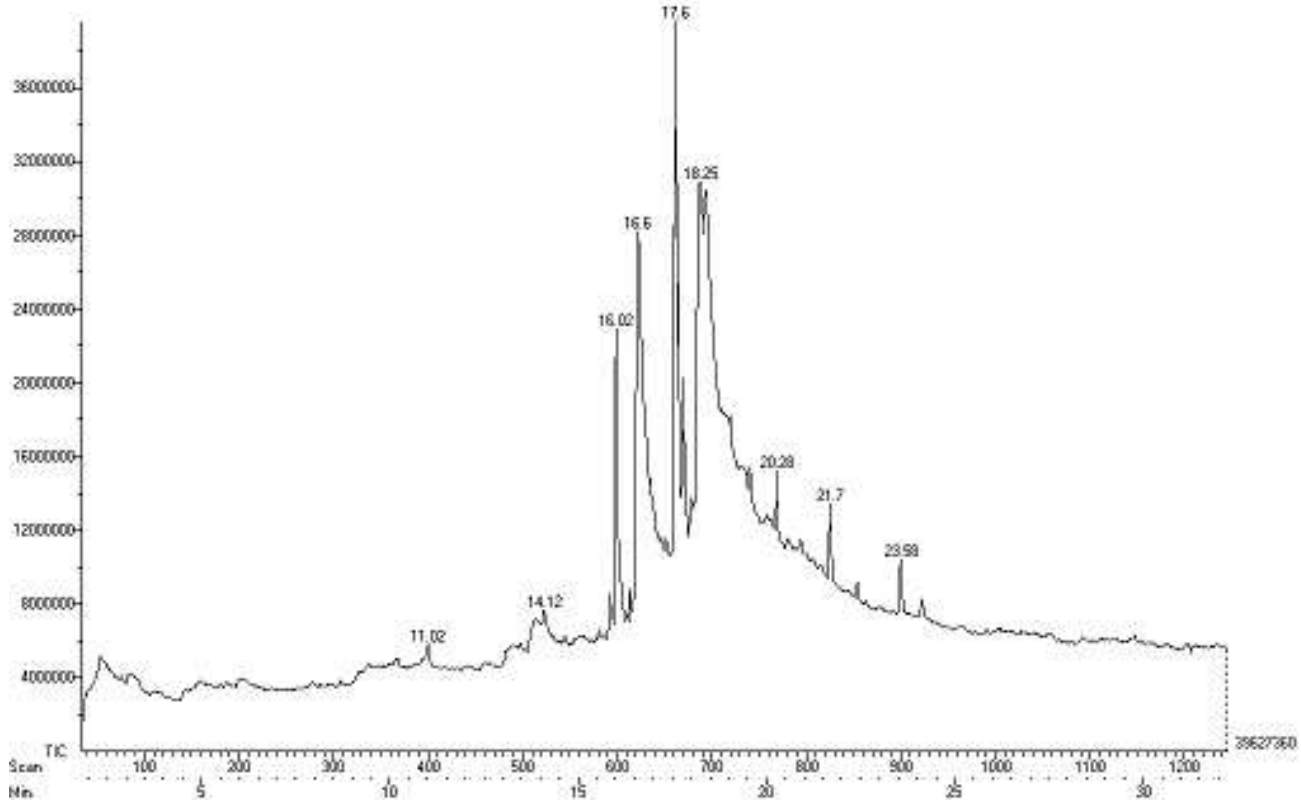

Fig. 6. Characterization of *Scenedesmus* oil by GC-MS

Conclusions

Among the various *Scenedesmus* biomass harvesting techniques investigated, the highest microalgal biomass yield of 0.9 g/L was obtained using bioflocculent dosage of chitosan is 0.1 g/Lat pH 10. The flocculated media was reused for one more time after pH neutralization. The highest lipid extraction of about 33% total lipid/g dry weight was obtained in Modified Bligh & Dyer method. Moreover *Scenedesmus* sp. depicted a profound increase in lipid yield to 58% (dcw) and 47% (dcw) under nitrate and phosphate deficient media respectively and the extracted lipid was characterized. The growth and lipid production kinetic results revealed that the lipid production in *Scenedesmus* sp. is growth associated.

Conflicts of interest

The authors declare no conflict of interest.

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