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Application of Artificial Neural Network and Particle Swarm Optimization for modeling and optimization of CO_2 sequestration using microalgae

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Abstract: In the present study, *Chlorococcum* sp., a green alga – collected from coke-oven effluent treatment plant, was used for the sequestration of carbon dioxide at diverse initial CO_2 concentrations (5-30%), pH of the medium (7-11) and inoculum sizes (5-12.5%), respectively. The test strain showed sustainability in growth upto 30% CO_2. However, the maximum CO_2 sequestration was found to be 79.08±0.89% at 5% CO_2, pH 9 and 12.5% inoculum size for the test strain. Since phenomenological modeling is difficult to build in such systems, artificial neural network modeling technique was tried in this work to predict the CO_2 removal at dissimilar pH of the medium, diverse CO_2 concentrations and at various inoculum sizes for the test strain. Various algorithm of artificial neural network (ANN) model and lot of activation functions were tried to arrive at best ANN model for this experimental data. Developed ANN model performance was found very efficient. Optimization by particle swarm optimization (PSO) algorithm is carried out to determine the optimum value of input parameters to sequester maximum CO_2. With PSO algorithm the optimum input parameters are obtained to achieve 99% removal of CO_2. As far as known the studies on application of Artificial Neural Network and Particle Swarm Optimization for modeling and optimization of CO_2 sequestration using microalgae are very rare and here lies the novelty of the present work.

Keywords: CO_2 sequestration, Green alga, Macromolecules, FAME analysis, ANN

Introduction

CO_2 is a considerable contributor to the global warming and climate change[1]. Coal fired power plants are one of the key sources to atmospheric CO_2[2]. Flue gas releasing from power plants has a temperature of 120°C and CO_2 in the range of 10-15%[3]. Several

J. Indian Chem. Soc.
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Vol…./Issue…/pp…

techniques have been engaged so far for biofixation of CO_2, among them biological fixation using microalgae/cyanobacteria has been found as one of the efficient move towards CO_2 sequestration[4]. Cultivation of algae has been exploited as a supplementary move in treatment of flue gas, towards the lessening of CO_2 levels in the exhaust flue gas[5]. Microalgae have the advantages in biofixation of CO_2 over terrestrial plants such as CO_2 fixation in aqueous algal suspension can be achieved in a precise manner and the rate of fixation is considerably higher[6]. The microalgal biomass assemble considerable amounts of lipids, proteins and carbohydrates and some expensive compounds including vitamins and pigments, which can be utilized as an active constituent in pharmacy, feed supplements and food preservatives or in the biofuels production[5]. Further, though a considerable number of research works was done on bio-fixation of CO_2 using micro-algae, a comprehensive study encompassing detailed experimentation, data driven artificial intelligence based modeling and optimization of input parameters for maximization of CO_2 removal is very small.

Since application of artificial neural network (ANN) and particle swarm optimization technique in biological system is very sparse, in the present article, both of these techniques have been used to model and optimize the biofixation of CO_2 using a green alga of *Chlorococcum* sp. Parametric study has been done to see the effect of different parameters such pH, initial concentration of CO_2, etc. The data have been used in ANN and finally, PSO is used to get optimum values of parameters for maximum biofixation of CO_2. The studies on application of ANN and PSO for modeling and optimization of CO_2 sequestration using microalgae are very few and here lies the novelty of the present work.

Results and discussion

Growth and lethal dose study for the test strain

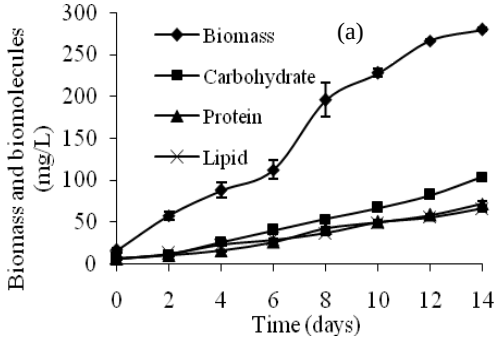

Fig. 1a. Growth of biomass and biomolecules of *Chlorococcum* sp. in BG-11 medium

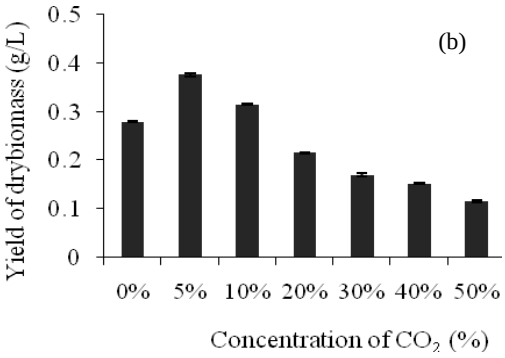

Fig. 1b. Lethal dose analysis of *Chlorococcum* sp. in BG-11 medium

Growth of the isolated green algal strain has been studied in BG-11 medium in terms of biomass yield and thereafter, for the production of lipid, protein and carbohydrate (Fig. 1a). The concentrations of biomass, lipid, protein and carbohydrate have been increased 4.9, 5.4, 7.4 and 9 fold with respect to those concentrations on day 2 for *Chlorococcum* sp. One of the probable reasons for the proliferation may be due to the presence of high concentration of nitrogen (nitrate) and phosphorous in the BG-11 medium[7]. Researchers reported that phosphorous (P) is one of the crucial nutrients in proliferation and metabolism of microalgae[8]. *Chlorococcum* sp. has shown growth upto 50% CO_2 concentration, however, the concentration of biomass is higher (0.315±0.0014 g/L) upto 10% CO_2 than that of the control (0.279±0.002

g/L) (Fig. 1b). The biomass concentration decreases (0.215±0.001 g/L) at and beyond 20% CO_2. The biomass concentration at 5 and 10% CO_2 is 1.34 and 1.13 times higher than that of control. Anjos et al.[6] reported the biomass growth inhibition at higher CO_2 concentrations.

Optimization of input parameters during CO_2 sequestration

Effect of pH on sequestration of CO_2

Results reveal that with increase in pH from 7 to 9, CO_2 sequestration increases. The sequestration of CO_2 has been found to be 45.79±0.89%, 73.28±1.34% and 50.81±0.45% for *Chlorococcum* sp. (green alga) at 5% CO_2, 10% inoculum size at pH 7, 9 and 11, respectively, after 14 days of incubation (Fig. 2a).

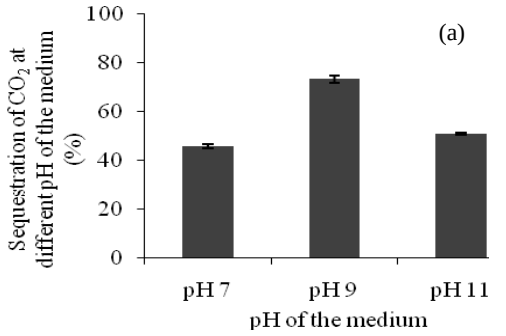

Fig. 2a. Sequestration of CO_2 using *Chlorococcum* sp. at different initial pH of the medium

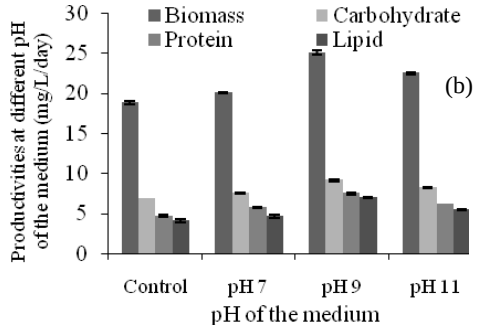

Fig. 2b. Productivities of biomass and biomolecules of *Chlorococcum* sp. at different initial pH of the medium

The maximum productivities of biomass and macromolecules such as lipid, protein and carbohydrate during optimization of input parameters have been found as 25.07±0.25, 6.99±0.12, 7.5±0.15 and 9.16±0.12 mg/L/day for green algae at pH 9 and 10% inoculum size (Fig. 2b). With further raise in pH to 11 the productivities are decreased. It has also been observed that the productivities obtained at dissimilar pH conditions supplemented with CO_2 are found to be maximum than that of control. pH of the medium plays a significant role in growth of the microalgae species, as it has significant effect on the activity of diverse enzymes.

Effect of CO_2 concentration

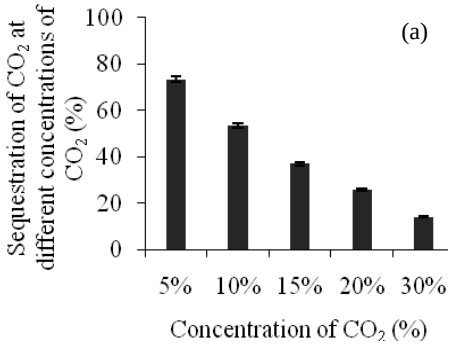

Fig. 3a. Sequestration of CO_2 using *Chlorococcum* sp. at different CO_2 concentrations

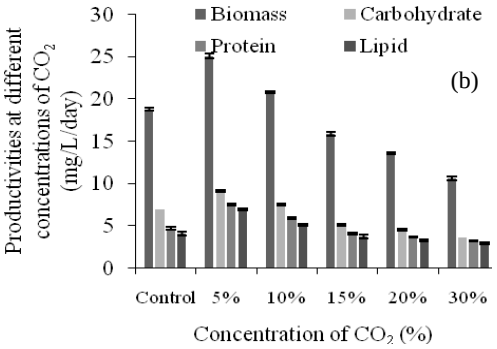

Fig. 3b. Productivities of biomass and biomolecules of *Chlorococcum* sp. at different CO_2 concentrations

The maximum sequestration of CO_2 has been found to be 73.27±1.34%, 53.32±0.89%, 36.87±0.60%, 25.71±0.34% and 14.01±0.17% for *Chlorococcum* sp. for the initial CO_2 concentrations of 5%, 10%, 15%,

J. Indian Chem. Soc.
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Vol…./Issue…/pp…

20% and 30%, respectively, after 14 days under laboratory conditions (Fig. 3a). All other parameters have been maintained constant at pH 9 and 10% inoculum size. It is obvious that higher sequestrations are expected at lower concentrations of CO_2 for the test strain.

The productivities of biomass and macromolecules at dissimilar concentrations of CO_2 have been found to be higher than control upto 10% CO_2 for the green algae (Fig. 3b). The maximum productivities of biomass (25.07±0.25 mg/L/day), lipid (6.99±0.12 mg/L/day), protein (7.5±0.15 mg/L/day) and carbohydrate (9.16±0.12 mg/L/day) have been found at 5% CO_2, 10% inoculum size and pH 9 and it is comparable with the green alga *Chlorococcum littorale*[9]. Also, the productivities of macromolecules at 15, 20 and 30% CO_2 are less than that of control. Though the sequestration of CO_2 is maximum at 5% CO_2, the increase in productivities of biomass, lipid, protein and carbohydrate are 2.06, 2.01, 1.47 and 1.49 fold at 10% CO_2. Since the amount of carbonate present was less (1.3 g/L) at 5% CO_2, lower values of productivities can be expected. Lam et al.[8] stated that the growth rate of *Chlorella* sp. was inhibited above 2% (v/v) CO_2. Researchers reported that the microalgal cultivation above 5% (v/v) CO_2 is considered as deadly to the growth of microalgae[10].

Effect of inoculum size

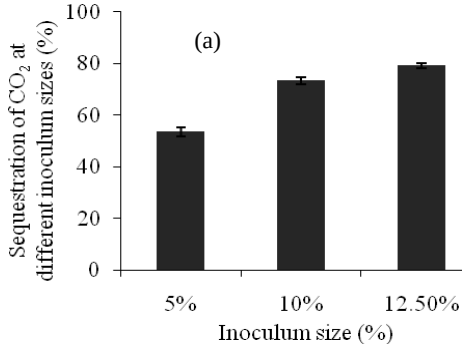

Fig. 4a. Sequestration of CO_2 using *Chlorococcum* sp. at different inoculum sizes

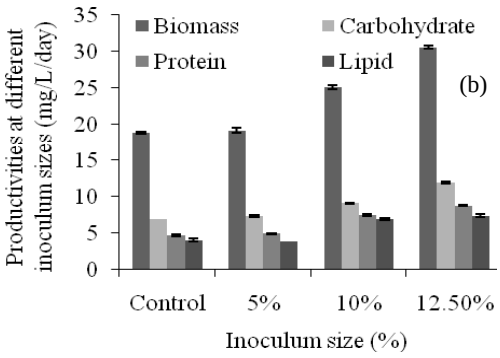

Fig. 4b. Productivities of biomass and biomolecules of *Chlorococcum* sp. at different inoculum sizes

The maximum CO_2 sequestration has been observed as 53.52±1.78%, 73.27±1.34% and 79.08±0.89% for the green alga when the inoculum sizes are at 5%, 10% and 12.5%, respectively (Fig. 4a). Other parameters have been set as pH 9, 5% CO_2. It is obvious that with raise in inoculum size, the number of microalgal cells also increases which eventually leads to the high sequestration of CO_2. Therefore, the productivities of biomass and macromolecules are maximum when the inoculum size is maximum (12.5%). The utmost productivities of biomass (30.54±0.20 mg/L/day), lipid (7.4±0.18 mg/L/day), protein (8.84±0.05 mg/L/day) and carbohydrate (11.95±0.10 mg/L/day) have been found at 12.5% inoculum size, pH 9 and 5% CO_2 for the test strain (Fig. 4b). These productivities are higher than the productivities of *Chlorococcum* sp. when grown in CHU-10 medium[11]. Researchers reported that the sequestration of CO_2, biomass and macromolecules concentration increased when the inoculum size was varied from 5 to 12.5%[12]. Though the sequestration of CO_2 and productivities were found to be maximum at 12.5% inoculum size, effective inoculum size was selected as 10%, since the productivities at 10% and 12.5% inoculum size were comparatively same.

Characterization of the test strain

FTIR study of *Chlorococcum* sp. before and after CO_2 mitigation was performed (Figure not shown). The functional groups at 1056, 1385, 1543, 1648, 2933 and 3408 cm^{-1} for the green algae when grown in BG-11 medium are responsible for C-O, C-H (bend), N-O, C=O, C-H (stretch) and N-H (amide) bonds. There is no much change in the functional groups of the biomass of green algae after CO_2 uptake.

Time variation of CO_2 sequestration using microalgae

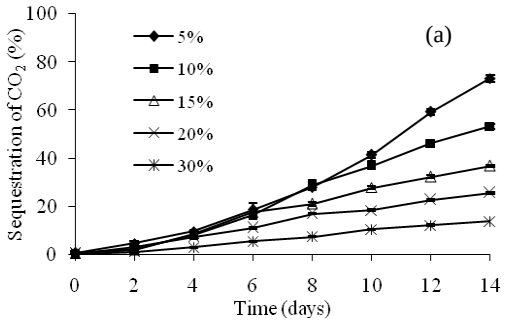

Fig. 5a Sequestration of CO_2 using *Chlorococcum* sp. at different CO_2 concentrations

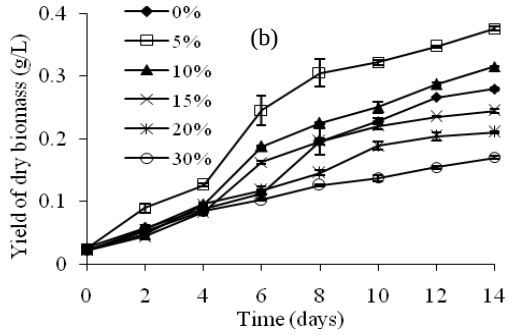

Fig. 5b. Yield of dry biomass of *Chlorococcum* sp. at different CO_2 concentrations

Time variation study of CO_2 sequestration has been done at different CO_2 concentration (5-30%) while, pH and inoculum size have been set as 9 and 10% for 14 days. The CO_2 sequestration decreases from 73.27±1.34 to 14.01±0.17% for change of CO_2 concentration from 5 to 30% (Fig. 5a). Since the amount of carbonate present was less (1.3 g/L) at 5% CO_2, higher sequestration could be expected.

The concentration of biomass has been increased from 0.28±0.002 to 0.315±0.001 g/L with variation in concentration of CO_2 from 0 to 10% (Fig. 5b). The biomass concentration has been decreased to 0.17±0.003 g/L with further increase in CO_2 concentration to 30%. However, the maximum concentration of biomass (0.376±0.0014 g/L) has been observed at 5% CO_2. The reduction in biomass growth has been observed for the test strain supplemented at elevated CO_2 concentrations (15%), may be allied due to some changes in the photosynthetic characteristics of the microalgal strain used. Researchers reported that the CO_2 sequestration and microalgal growth was inhibited at high concentrations of CO_2[5]. When microalgae proliferated at high concentrations of CO_2 (15% or more), several microalgae showed lower uptake of CO_2, higher photosynthetic warmth to O_2 and lesser activity of CA, the enzyme which is responsible for photosynthetic exploitation of inorganic carbon source[13]. As per previous reports the maximum biomass production of *Chlorella* sp. was found to be 0.099±0.001 g/L when grown at 15% CO_2 under high density inoculum[14]. However, in the present study, the green alga has shown 0.25±0.003 g/L of biomass at 15% CO_2. The CO_2 bio-fixation and biomass production vary markedly depending on the uniqueness of microalgal species and cultivation conditions[15].

Evaluation of macromolecules content

For the present strain, the lipid concentration increases from 65.71±3.3 to 75.7±1.7 mg/L when the CO_2 concentration varies from 0 to 10% and decreases to 45.72±1.6 mg/L with further increase in CO_2 concentration to 30%. However, the maximum concentration of lipid has been found as

104.28± mg/L at 5% CO_2, pH 9 and 10% inoculum size (Fig. 5c).

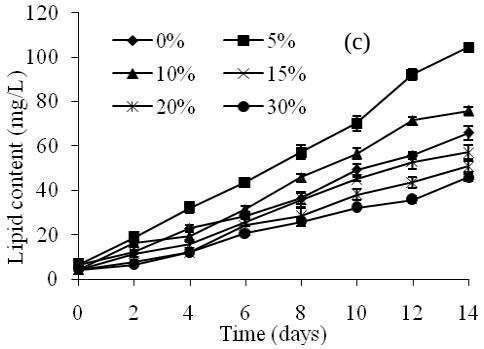

Fig. 5c. Lipid content of *Chlorococcum* sp. at different CO_2 concentrations

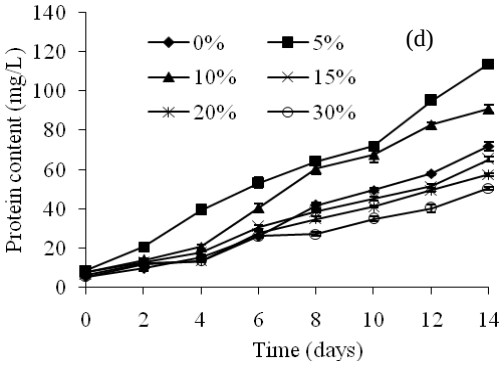

Fig. 5d. Protein content of *Chlorococcum* sp. at different CO_2 concentrations

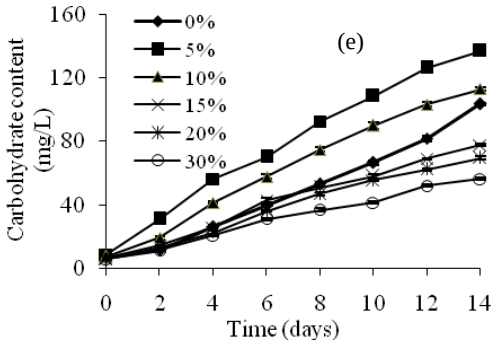

Fig. 5e. Carbohydrate content of *Chlorococcum* sp. at different CO_2 concentrations

The carbohydrate and protein concentrations follow the same trend as that of biomass and lipid content under identical condition. The protein content increases from 71.87±2.2 to 113.75±2.2 mg/L with increase in CO_2 concentration from 0 to 5% for green alga (Fig. 5d). The concentration of protein decreases to 50.63±0.72 with further increase in CO_2 to 30% for the test strain. The maximum concentration of carbohydrate has been found as 137±1.7 mg/L at 5% CO_2 for green alga (Fig. 5e) after 14 days. With further increase in CO_2 concentration 15 to 30%, the carbohydrate content decreases.

FAME analysis

The fatty acid contents suitable for the production of biodiesel (C16:0) have been found as 43.26% and 46.64% for *Chlorococcum* sp. before and after sequestration of CO_2, respectively (Figures not shown). These amounts have been found to be higher than that of *Scenedesmus* sp. at 15% CO_2[16]. The fatty acid contents (C16:0) for *C. vulgaris*, *B. terribilis* and *B. braunii* were found to be 34.82%, 35.46% and 30.39%, respectively, which were lower than that of the present test strain under diverse CO_2 concentrations[17].

Development of a Suitable ANN Model and Optimization using PSO Model

ANN optimization

Typical input-output data has been used in ANN training of CO_2 sequestration for the model performance (Data not shown). The performance of the developed model is as follows; total number of data point: 133, optimum number of node: 15, activation function: Sigmoidal (Hidden layer) and linear (output), training algorithm: Levenberg-Marquardt and prediction accuracy of R^2 (0.999) and very low MSE (1.18). These values suggest the good fitting of experimental data with the ANN model predicted ones. Thus, the suitability of proposed ANN is established.

PSO optimization

Maximum and minimum values of input parameter are fixed as follows: pH of the

medium [11, 7], initial CO_2 concentration [30%, 5%], inoculum size [12.5%, 5%] and time [14 days, 2 days], respectively. The values of four input parameters will be assumed in these ranges and PSO passes these values to ANN trained model to get optimum output.

PSO algorithm keeps on changing until percentage CO_2 removal is maximized[18]. Five sets of optimum input parameters are obtained using PSO algorithm to achieve 100% removal (Table 1). The advantage of PSO algorithm lies in the fact that one can get optimum values by performing minimum number of experimentations.

Experimental

Growth and lethal dose study for the test strain

The growth of the test strain was studied in BG-11 medium ($NaNO_3$: 1.5 g/L, K_2HPO_4: 0.04 g/L, $MgSO_4.7H_2O$: 0.075 g/L, $CaCl_2.2H_2O$: 0.036 g/L, Citric Acid: 0.006 g/L, Ferric ammonium citrate: 0.006 g/L, Na_2CO_3: 0.02 g/L, EDTA: 0.001 g/L, Trace metal: 1 mL/L)[19]. The test strain was cultivated in different conical (Erlenmeyer) flasks. Finally, the flasks were set aside in algal incubator under laboratory conditions such as temperature: 25ºC, light intensity: 2400 Lux, dark/light cycle ratio: 8 h/16 h. The samples were withdrawn at regular time intervals and the biomass was collected from broth by centrifugation at 5000 rpm for 15 min (ELTEK TC8100F, India). The collected microalgal biomass was dried out in an air oven at 60°C for 12 h. The produced biomass was used for the production of lipid, protein and carbohydrate using standard methods[19]. The lethal dose analysis was performed to find out the sustainability of the test strain under CO_2 stress condition by growing it in dissimilar CO_2 concentrations in BG-11 medium. The specific CO_2 concentrations were prepared by dissolving the sodium carbonate salt in BG-11 equivalently using stoichiometric relations[12].

$$\log [HCO_3^-] = pH - \log [CO_2] - 6.30 \quad (1)$$

$$\log [CO_3^{2-}] = pH - \log [HCO_3^-] - 10.09 \quad (2)$$

At pH 8, the values of sodium carbonate concentrations equivalent to 5%, 10%, 20%, 30%, 40% and 50% CO_2 concentrations in the BG-11 medium are 10.9 g/L, 21.8 g/L, 43.6 g/L, 65.5 g/L, 87.3 g/L and 109.2 g/L, respectively. The microalgal strain was grown at different CO_2 concentrations separately. The microalgal growth was investigated for two weeks (14 days), the desired concentrations of CO_2 for the test strain were found based on the biomass growth.

Optimization of input parameters during CO_2 sequestration

Biofixation of CO_2 was done by one factor at a time analysis (OFAT) as described by Upendar et al. (2018)[20]. Initially, pH was varied from 7-11 and all other parameters such as initial CO_2 concentration and inoculum size were set invariable at 5% and 10%, respectively. Initial CO_2 concentration was varied later from 5-30% keeping other variables at their corresponding values. Finally, inoculum size was varied from 5-12.5% keeping other variables at their adequate values. The solutions prepared were cultivated with the test strain under laboratory conditions and were grown for two weeks. The cultured samples were drawn after 14 days and the supernatant was collected by centrifugation. The collected supernatant was analysed for carbonate concentration by titration using 0.1 N Hydrochloric acid. All the adequate values of initial CO_2 concentration, inoculum size and pH of the medium were found on the basis of biofixation of CO_2 and biomass growth. The biomass produced after CO_2 biofixation was utilised for the production of macromolecules. The biomass

productivities were calculated using Equation 3: [21]

$$P = \frac{(x_1 - x_o)}{(\theta_1 - \theta_0)} \quad (3)$$

Where, θ_0 =0th day (days), θ_1 =beginning of stationary phase (days), x_0 =Biomass and biomolecules concentration on 0th day (mg/L), x_1 = Biomass and biomolecules concentration after log phase (mg/L).

Characterization of cellular biomass

Fourier Transform Infrared (FTIR) study of cellular biomass was done for the test strain to get the availability of organic groups on the surface of cellular biomass before and after biofixation of CO_2. For the FTIR study, two types of samples were prepared. At first, test strain was grown in BG-11 with 10% inoculum, pH 9 and 25ºC temperature (control), subsequently the test strain was grown in BG-11 medium supplemented with CO_2 (5%). The samples were grown for two weeks in incubator under laboratory conditions. After two weeks the biomass was collected using centrifugation and dried in hot air oven and thereafter used for the FTIR examination.

Time variation of CO_2 biofixation using test strain

Time variation of biofixation of CO_2 was studied at various carbon dioxide concentrations (5-30%). All other variables like pH, inoculum size and temperature were set at their most effective conditions i.e., the conditions at which the sequestration of CO_2 and concentration of biomass and biomolecules were found to be maximum. The solutions were inoculated using the test strain and were placed in incubator under laboratory condition for 14 days. The biomass generated was utilised for the production of macromolecules using standard methods as mentioned in 'Growth and lethal dose study for the test strain' subsection.

FAME analysis

Fatty acid methyl esters (FAMEs) are the molecules that are principally present in biodiesel. FAME analysis reveals that the fraction of lipid content of the test strain can directly be used as biodiesel production. Total lipid content was extracted from dry biomass before and after biofixation of CO_2. The total lipid extracted and its fractions such as phospholipids (PL), glycolipids (GL) and neutral lipids (NL) were transesterified into FAMEs as described by Mistry et al.[11].

Development of a suitable ANN model and optimization using PSO model

Development of ANN model

Experiments were carried out to validate CO_2 sequestration using test strain. The following four parameters were considered which have large effect on biofixation of CO_2 using algal strains: (i) time of contact, (ii) initial CO_2 concentration, (iii) pH, (iv) inoculum size. Percentage of CO_2 removal is taken as target. For development of ANN model, the algorithm as discussed by Upendar et al.[18] was followed. The topology consists of four input nodes and one output node. Best ANN algorithm was selected during building time.

The aptness of ANN model is examined by MSE and coefficient of determination (R^2). Less than one hour was required for calculation of this large number of ANN models using intel i-5 processor.

Development of a suitable PSO model

Once an accurate and reliable ANN model was built up, the next optimization algorithm is tried to optimize the input parameters namely time, pH, initial concentration, inoculum size and so that the percentage removal of CO_2 is maximized.

Since the ANN equations are complicated and comprises of various exponential terms and weights, normal optimization algorithm is difficult to apply on ANN model. For development of PSO algorithm the steps of Upendar et al.[18] were followed to maximize CO_2 sequestration.

Conclusion

CO_2 mitigation using *Chlorococcum* sp. has been found to be a promising option for CO_2 fixation and higher biomass yield. The maximum biofixation of CO_2 has been observed as 79.08±0.89% at 5% CO_2, 12.5% inoculum size and at pH 9, respectively. The strain could grow well upto 30% CO_2. The higher productivities of lipid, protein and carbohydrate indicate the possibility of usage of such strain for able CO_2 biofixation and value-added production. In this study, various ANN model with different algorithms have been evaluated to predict CO_2 sequestration. Very low RMSE and high R^2 value of the final ANN model indicates that developed model has successfully captured the nonlinear relationship between CO_2 fixation and initial concentration, inoculum size, pH of the medium and time. The PSO algorithm is then applied on ANN model to optimize the input parameters, so that CO_2 removal is maximized. The results of PSO optimization is very encouraging as it shows that more than 99% CO_2 removal is possible by optimizing the initial concentration, inoculum size and pH of the medium. The advantage of PSO optimization is to determine the optimum value of input parameters automatically and thus, it can avoid lots of experimentation. Such ANN modeling and PSO optimization technique can be extended to any other complex system where phenomenology is fully understood.

Conflict of interest

On behalf of all the authors, I declare that there is no conflict of interest.

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Table 1. Multiple optimum responses and corresponding operating parameter obtained by Particle Swarm Optimization (PSO) for the removal of CO_2 using *Chlorococcum* sp.

S. No	Time (days)	Initial concentration (%)	Inoculum size (%)	pH	Sequestration of CO_2 (%)
1	13.99	5.04	12.50	11.00	100.00
2	13.99	5.00	12.50	11.00	100.00
3	14	5.00	12.50	10.99	100.00
4	13.99	5.00	12.50	10.99	100.00
5	14	5.004	12.50	11.00	100.00