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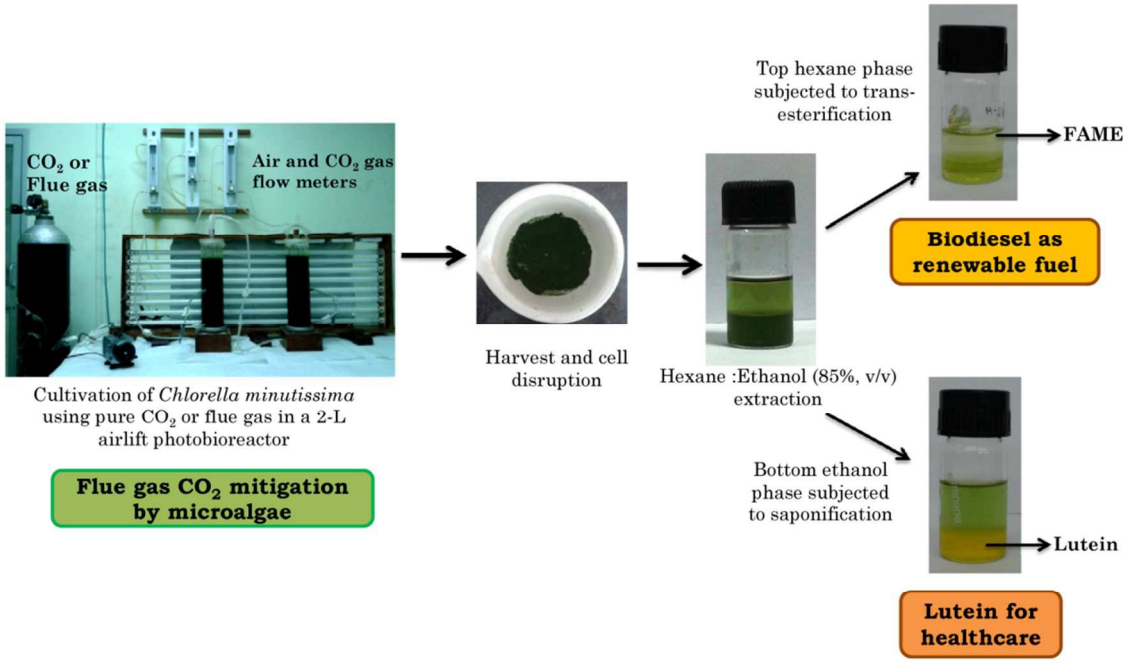
Highlights:

An integrated green microalgal biorefinery was developed for concomitant flue gas CO_2 sequestration, lutein and lipid production for potential environmental, healthcare and biofuel applications respectively.

Graphical Abstract



Process integration for microalgal lutein and biodiesel production with concomitant flue gas CO_2 sequestration: A biorefinery model for healthcare, energy and environment

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Abstract

In this study, a green microalgal feedstock based biorefinery was developed by process optimization and integration with a view to sequestering flue gas CO_2, synthesizing lutein and lipid for environmental, healthcare and biofuel applications, respectively. Out of the four microalgal cultures tested in 2–L airlift photobioreactor, *Chlorella minutissima* showed comparatively higher productivities of both lutein (2.37 ± 0.08 mg L^{-1} d^{-1}) and lipid (84.3 ± 4.1 mg L^{-1} d^{-1}). Upon optimization of the critical process parameters using artificial neural network modeling and particle swarm optimization (ANN–PSO) technique, the productivities of lutein and lipid were enhanced to 4.32 ± 0.11 mg L^{-1} d^{-1} and 142.2 ± 5.6 mg L^{-1} d^{-1} respectively, using pure CO_2 sequestered at a rate of 1.2 ± 0.03 g L^{-1} d^{-1}. One of the most interesting findings was that lutein and lipid productivities were not significantly affected by the use of toxic flue-gas, when diluted to 3.5% CO_2 with air, under the same process conditions, suggesting possible commercial usefulness of flue-gas carbon. Another major achievement is that a single step ethanol–hexane based extraction procedure, followed by parallel saponification and trans-esterification, resulted in simultaneous recovery of 94.3% lutein and 92.4% fatty acid methyl

ester. Therefore, potential industrial significance of this study lies in the development of an integrated biorefinery that may prove to be a sustainable technology platform towards addressing some contemporary challenges in healthcare, energy and environment through concomitant production of microalgal lutein as a nutraceutical and biodiesel as an alternative fuel, coupled with flue gas CO_2 sequestration.

Keywords: Microalgal biorefinery; Lutein; Lipid; Biodiesel; Flue gas; CO_2 sequestration; Process integration.

1. Introduction

Microalgae have been considered as the potent photosynthetic microorganisms, as they are envisaged to solve the challenges of food, feed and fuels production in the near future.[1] Moreover, they serve as a sustainable and potential alternative for remediating waste water, sequestering flue gas CO_2, and producing many high–value products such as carotenoids, poly unsaturated fatty acids, exo–polysaccharides, antioxidants and vitamins.[1-3] Lutein, one of the commercially important carotenoids, has gained increasing attention due to its various health care applications including prevention and amelioration of age related blindness, cataracts, certain types of cancers and atherosclerosis.[4]

A majority of works reported in the literatures have focused on directing the microalgal cultivation for one particular application; either producing a commercially important product or to sequester CO_2 from the pollutant gas. This makes the microalgal cultivation economically unattractive for commercial applications.[5, 6] Hence, it is important to have an integrated biorefinery approach that can serve three purposes. For instance, sequestration of CO_2 by microalgae, a sustainable pollution mitigation strategy, can be coupled to the simultaneous

production of two products; a high–volume, low–value product like lipid for biodiesel and a low–volume, high–value pigment like lutein for healthcare applications. However, this strategy is primarily dependent on the type of microalgal species. For example, microalgae of chlorophycean class are one of the potential strains that have been reported to accumulate significant proportion of both carotenoids (lutein) and lipid, in addition to their CO_2 sequestration potential. [7, 8]

One of the key issues in the simultaneous production of lutein and lipid is the influence of nitrogen source availability or timing of cell harvest. Generally, the accumulation of lutein reaches its maximum level near the onset of nitrogen depletion in the medium,[9] whereas, the maximum lipid accumulation is achieved upon nitrogen starved conditions.[10, 11] As a result, the maximum production of both lutein and lipid might not occur at the same cultivation time. Moreover, it is difficult to obtain the optimal process conditions for enhancing both lutein and lipid.[12] Therefore, the harvesting time and optimal conditions for improved product synthesis will be mainly dependent on which product is preferred to be produced. In the current study, lutein was considered as the primary target product as it is a growth-associated and high-value product. Accordingly, the optimal process conditions and cultivation time was prioritized for improving lutein synthesis, while lipid was also obtained as a co-product. The other critical issue in this process integration study is to extract and recover both lutein and lipid simultaneously from the biomass. It has to be noted that there are very few reports which demonstrate the feasibility of recovering two products concomitantly from the microalgal biomass.

Prommuak et al. [13] reported the simultaneous recovery of lutein and biodiesel from *Chlorella vulgaris*. They observed that the alkali catalyst used for transesterification of lipids also converted the lutein esters to free lutein at appropriate conditions. In another study, Bai et al. [14]

investigated the feasibility of concomitant separation of chlorophylls and lipids from *Chlorella pyrenoidosa*, using an appropriate solvent mixture based on solubility nature of the products. However, the novelty of our study lies in the integrated biorefinery approach that can serve three purposes at a time, namely, concomitant production of microalgal lutein and biodiesel along with CO_2 sequestration. Once the integrated biorefinery concept has been developed, the process can be scaled up by maintaining the universal scale up parameters like P/V (power consumed per unit reactor volume) and K_La (volumetric mass transfer coefficient). For instance, the optimal flow rate or superficial gas velocity that will be determined from this study can be used to calculate P/V, which is one of the critical scale-up criteria. However, the development of low-cost large scale photobioreactors, cost-effective cell separation and downstream processing techniques are essential, in order to improve the competitiveness of commercially important microalgal products and the overall process economically feasible.[5] The use of low–cost sources of CO_2, nutrients and water would likely to reduce the cost involved in microalgal biomass cultivation by more than 50% and also mitigate the pollutants considerably.[5] Hence, in the current study, flue gas was used as the viable alternative source of CO_2 for microalgal cultivation.

Thus, the present study was aimed at designing and integrating the bioprocesses for microalgae mediated flue gas CO_2 sequestration with concomitant production of lutein and biodiesel in a biorefinery model. This requires the implementation of the following strategies, namely, (i) selecting a suitable microalgal species which can yield higher productivities of both lutein and lipid, (ii) optimizing the most influential process parameters for improving the productivities of lutein as primary target product and lipid, using an advanced mathematical modeling and optimization technique, and (iii) integrating the processes of flue gas CO_2

sequestration with the concurrent recovery of lutein and biodiesel under the optimized process conditions.

2. Materials and Methods

2.1. Microalgae and culture conditions

Four green microalgal strains of chlorophycean class were used in this work and are as follows: *Chlorella minutissima* (MCC–27) from Indian Agricultural Research Institute, New Delhi; *Scenedesmus* sp. *Chlorella* sp. and *Chlorococcum* sp. were kindly provided by Institute of Bio–resources and Sustainable Development, Imphal, India. These strains show high growth rate at pH 7–9 and temperature 25–32 °C. The modified Bold's Basal medium (BBM) which was standardized earlier[15] for yielding higher lutein productivity, was used in this screening and optimization study.

2.2. Design and operation of photobioreactor

A 2–L airlift photobioreactor was appropriately designed for culturing microalgae and the design parameters are as follows: height/diameter, 3.6; illuminated surface area/volume, 0.465 cm^{-1}; area of downcomer/area of riser, 1.25 and perforated ring shaped spargers with $\Phi_{sparger}$, 5.5 cm and Φ_{pore} = 0.5 mm. The photobioreactor was equipped with cool white fluorescent lamps that were mounted on both sides of the reactor. All the screening and optimization experiments were performed in this photobioreactor in batch mode using the modified BBM with the following cultivation conditions: inoculum concentration, 50 mg L^{-1}; inoculum age, mid-log phase; pH, 7–8 and temperature, 30 ± 2 °C. The microalgal biomass was harvested near the onset of nitrogen depletion in the culture medium for all experiments.

2.3. Optimization of process conditions

The selected lutein and lipid rich microalga was considered for further improvement of lutein and lipid productivities. In some cases, the complex non-linear biological interactions cannot be completely explained by using second-order polynomial model involving response surface methodology.[16, 17] Hence, we implemented artificial neural network modeling (ANN) coupled with particle swarm optimization (PSO) technique for determining the optimum levels of critical process parameters for improved productivities of lutein (main response) and lipid.

The process parameters that critically influence the productivities of lutein and lipid were identified as light intensity, CO_2 concentration and air flow rate. These parameters also influence the performance of photobioreactor in terms of irradiance, mass transfer, mixing and hydrodynamic characteristics.[18] Indeed, the availability of nitrogen in medium affects the accumulation of lutein and lipid inversely in microalgae. However, the rate of lipid accumulation (lipid productivity) is reduced considerably for the microalgae grown under nitrogen exhausted medium.[2, 7] Moreover, the optimal nitrate concentration (13.55 mM) that was determined from our previous study[15] for enhanced lutein productivity, is corroborated with the study of Abdelaziz et al.[19] They reported that the nitrate concentration of 11.5 mM was required for maximizing lipid productivity in a green microalga *Chlorella*. Hence, nitrate concentration was not included in the experimental design, considering lutein as primary target product.

The experimental range and levels of the selected parameters are shown in Supplementary Table A.1. A central composite design (CCD) matrix was constructed for three factors (light intensity, CO_2 concentration and air flow rate) and the experimental design (Table 2) was obtained using Design Expert version 7.1.3 (Stat-Ease Inc., Minneapolis, USA). The experimental design that consists of 20 runs was carried out using 2-L airlift photobioreactor

(batch mode) in duplicates. The experimental data obtained from CCD were used for developing the neural network model and subsequently optimized by PSO technique.

ANN is applied in almost all engineering fields for the modeling of multivariate non–linear processes. Because of its robustness and ability to simulate complex biological processes more accurately, ANN has found application in process biotechnology.[17] This model can be evaluated using mean squared error (MSE) as performance index (Eq. (1)) and overall correlation coefficient (R) as precision of the model.

$$MSE = \sum \frac{(Experimentalvalue - predictedvalue)^2}{n} \quad (1)$$

PSO, a contemporary evolutionary algorithm, is basically inspired by the migration patterns of living creatures like bird flocking and fish schooling. It has recently been applied to optimize complex multivariate non–linear bioprocess, because of its properties such as faster inter–particle communication, rapid data processing and easy implementation. Owing to its ability to sort out best fitness values even after several iterations, it is believed to be superior to other computational evolutionary algorithm like genetic algorithm.[16] It updates its velocity and position at different time intervals according to Eqs. (2) and (3), respectively.

$$V_i^k = w^{k-1} + V_i^{k-1} + C_1 R_1 (L_i^{k-1} - P_i^{k-1}) + C_2 R_2 (\mathrm{G}_i^{k-1} - P_i^{k-1}) \quad (2)$$

$$\mathrm{P}_i^k = P_i^{k-1} + V_i^k \quad (3)$$

where, V_i^k and V_i^{k-1} are velocities of particle i at iteration k and k-1, respectively; $C_1$and C_2, learning factors; w^{k-1}, inertia weight; $R_1$and R_2, uniformly distributed random variables between 0 and 1; L_i^{k-1}, local best solution of particle i; G_i^{k-1}, global best solution of the group; P_i^k and P_i^{k-1}

are positions of particle i at iteration k and k–1, respectively. The working principles of ANN–PSO for optimization of bioprocesses are discussed in earlier reports.[15, 16] The computation of ANN–PSO was performed by using MATLAB version 8.0 (Mathworks Inc., Natick, USA).

2.4. Process integration

2.4.1. Flue gas generator and storage setup

An indigenously designed *in situ* flue gas generator and suction devices was used in this study. The coal required for flue gas sequestration study was kindly provided by Kolaghat Thermal Power Station (KTPS), West Bengal, India. The KTPS coal was burnt in the furnace, and water circulation via double jacketed layer was provided to cool–down the emitted gas. The flue gas, which was emitted at the chimney, was captured and passed to a filter mesh to remove the suspended particles, and then stored in cylinders through appropriate suction and compressor pumps. The composition of flue gas was measured using online flue gas analyzer (Model: FGA 53X; Make: INDUS Scientific, Mumbai, India) and they are as follows: CO_2, 12%; CO, 0.55%; O_2, 8.33%; NO_2, 63.8 ppm; SO_X, 61.9 ppm and HC, 9 ppm.

2.4.2. Microalgae mediated flue gas CO_2 sequestration process

Once the process conditions were optimized in lab conditions, the selected microalga was grown using diluted–flue gas, which corresponds to the optimal CO_2 (%) as determined by ANN–PSO technique. This experiment was carried out in batch mode at closed–outdoor conditions (near flue gas generation facility) with artificial irradiance supply (light intensity as predicted by ANN–PSO) at the temperature range between 27 °C and 33 °C.

2.4.3. Optimization of binary solvent system for simultaneous recovery of lutein and biodiesel

The solubility of lutein in different solvents was systematically studied by Craft and Soares.[20] They found that the xanthophyll lutein was sparingly soluble in hexane due to the presence of dihydroxy groups, while it exhibited comparatively higher lutein solubility in polar solvents like ethanol, methanol and 2-propanol. It is also known that the non-polar solvent like hexane can effectively separate neutral lipid fraction from the crude lipid.[21, 22] In our study, an appropriate binary solvent system involving organic and aqueous phases having different solubilities towards neutral lipids and lutein was used for the separation. To identify the suitable solvent system that can effectively separate these two products from the biomass, three binary solvent systems (each at a ratio of 1:1) namely methanol–hexane, ethanol–hexane and 2-propanol– hexane, were tested. A suitable quantity of water was added to each of these binary solvent systems to obtain two layers; lipid rich upper organic hexane phase and lutein rich bottom aqueous alcoholic phase. For effective separation of lipids and lutein, the organic and aqueous phases were extracted twice with their respective aqueous and organic phases. Finally, the pooled phases were subjected to the following reactions; the organic phase containing lipid to trans-esterification and the aqueous alcoholic phase to saponification to obtain pure lutein. The products from these two reactions were then quantified using the protocols as discussed in Section 2.5. In order to calculate the recovery efficiency of the products that obtained through this simultaneous recovery method, a known amount of biomass was also taken separately to estimate the lutein and FAME contents, as per the protocols mentioned below and were labeled as control.

2.5.Analytical procedures

The biomass concentration was estimated at $OD_{750\,nm}$ and gravimetrically. The concentration of nitrate was determined according to Ho et al.[23] The carotenoids extraction and saponification reaction to obtain pure lutein were carried out as described in Dineshkumar et al.[15] Subsequently,

lutein was quantified using reverse phase High Performance Liquid Chromatography (Agilent, USA). The total lipid content of the microalgae was measured according to Bligh and Dyer.[24] The lipid was then trans-esterified using 3-N methanolic HCl at 70° C for 5 h and extracted into hexane phase. The transesterification of lipids and quantification of fatty acid methyl ester (FAME), were performed by following the procedure of Sheng et al.[25]

The gas chromatography (Thermo Fisher Scientific–Chemito Ceres 800 plus) with BPX 70 capillary column (30 m × 0.25 mm), was used for identification and quantification of FAME. The operating conditions are as follows: injector temperature, 260 °C; detector temperature, 280 °C; injection volume, 1 µl; split ratio: 1:25 and oven temperature started at 70 °C for 1 min, increased at 5 °C/min to 180 °C for 10 min and 6 °C /min to 220 °C for 11 min. The standards such as lutein (Sigma–Aldrich) and FAME–Mix 37 component (Supelco) were used for quantification of respective products.

$$\text{FAME content} = \frac{\text{Amount of FAME obtained (mg)}}{\text{Amount of biomass taken (g)}} * 100$$

$$\text{FAME yield} = \frac{\text{Amount of FAME obtained (mg)}}{\text{Amount of } CO_2 \text{ consumed (g)}} * 100$$

The elemental composition of microalgal biomass (CHNS) was determined using a Vario MACRO Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany Make). The general empirical chemical formula of the microalgal biomass is $CH_{1.83}N_{0.11}O_{0.48}P_{0.01}$ [26]. The CO_2 fixation rate in microalgal biomass was calculated by using Eq. (4)

$$CO_2 \text{ fixation rate } (gL^{-1}d^{-1}) = \text{Carbon content}(\%) * \text{Biomassproductivity } (gL^{-1}d^{-1}) * \frac{44}{12} \quad (4)$$

The photosynthetic efficiency (P.E) was determined using (Eq. (5))

$$P.E(\%) = \frac{Biomass\ growth\ (gd^{-1}) * Enthalpyof\ biomass\ (KJ\ g^{-1})}{Irradiance\ (\mu mol\ m^{-2}\ s^{-1}) * Illuminated\ surface\ area\ (m^2) * Conversion\ factor\ (18.78\ KJs\ d^{-1})} * 100 \quad (5)$$

The total chlorophyll and carotenoids were extracted using methanol and estimated according to Welburn[27] (Eqns. (6) to (9)).

$$Chlorophylla(C_a) = 15.65A_{666} - 7.34A_{653} \quad (6)$$

$$Chlorophyllb(C_b) = 27.05A_{653} - 11.21A_{666} \quad (7)$$

$$TotalChlorophyll(mgL^{-1}) = C_a + C_b \quad (8)$$

$$TotalCarotenoids(mgL^{-1}) = \frac{1000A_{470} - 2.86C_a - 129.2C_b}{221} \quad (9)$$

3. Results and Discussion

3.1. Comparison of lutein, lipid and biomass productivities of four chlorophycean microalgal strains

As the green microalgal species are rich in carotenoids and lipids and also exhibit high growth rate, they are generally recognized as one of the potential candidates for lutein and biodiesel production.[1, 8] In the present study, four green microalgal strains that were identified as better performing strains in our laboratory, were compared for their accumulation of both lipid and lutein. The parameters such as biomass growth and lutein and lipid production of these microalgal strains were examined at the following operating conditions: light intensity, 100 µmol m^{-2} s^{-1}; CO_2 concentration, 2 %; flow rate, 0.35 vvm (700 ml min^{-1}); temperature, 30 °C and pH, 7–8. For a better comparison, the efficiency of the production process was assessed in terms of productivity (mg L^{-1} d^{-1}), as reported by Xie et al.[9]

As discussed earlier, the four microalgal strains were harvested when the nitrogen source was about to be depleted in the medium. It was observed that the specific growth rate and the lutein productivity were higher for *Chlorella minutissima* and *Scenedesmus* sp, followed by *Chlorella* sp and *Chlorococcum* sp (Table 1). The microalga *C. minutissima* showed the highest lipid productivity (84.3 mg L^{-1} d^{-1}), followed by *Chlorococcum* sp (81.9 mg L^{-1} d^{-1}), *Scenedesmus* sp (71.8 mg L^{-1} d^{-1}) and *Chlorella* sp (56.5 mg L^{-1} d^{-1}). Among the tested microalgal species, *C. minutissima* was observed to yield higher productivities of both lutein (2.37 mg L^{-1} d^{-1}) and lipid (84.3 mg L^{-1} d^{-1}), with the specific growth rate of 1.44 d^{-1} and CO_2 fixation rate of 0.73 g L^{-1} d^{-1} (Table 1). Therefore, the strain *C. minutissima* was chosen for subsequent biorefinery study involving process optimization and integration.

3.2. Optimization of critical process parameters for improved lutein and lipid productivities

Although the cultivation of *C. minutissima* resulted in higher lutein and lipid productivities among the tested strains, these productivities was found to be relatively lower, as compared to that of reported in the relevant literature. In general, the increase in light intensity and CO_2 result in enhanced productivities of both lutein and lipid from microalgae.[4, 28] Hence, it is essential to optimize the key parameters such as light intensity, CO_2 concentration and flow rate that significantly influence the microalgal growth rate, CO_2 sequestration rate and photobioreactor performance. As discussed in Section 2.3, the optimal nitrate concentration that was previously determined for yielding higher lutein productivity was added to the medium, considering lutein as primary target product. Consequently, nitrate concentration was not included in the experimental design. Table 2 shows the productivities of lutein, lipid and biomass for different combinations of the critical process parameters.

3.2.1. Optimization of process parameters by ANN–PSO technique

The ANN model with lutein productivity as the primary objective was constructed by assigning the obtained CCD data (Table 2) as follows: training, 70%; testing, 15% and validation, 15%. The additional data points needed for training the neural network were generated using the regression equation (Supplementary Eq. A.1), as suggested by Maji et al. [29] The ANN–topology consists of three layers: input layer with 3 neurons representing the input parameters; output layer with one neuron that corresponds to the main objective (lutein productivity), and a layer between input and output layers called hidden layer, wherein the number of neurons needs to be determined for developing an efficient topology. The performance of ANN is primarily dependent on the type of training algorithm and the transfer functions employed at the hidden and output layers, while training the network. The accuracy of the ANN model was evaluated in terms of mean squared error (MSE) and overall correlation coefficient (R).

In this investigation, we found that the best performance was obtained using feed–forward back propagation training algorithm with the log–sigmoidal and linear transfer functions at the hidden and output layers respectively, in terms of low–MSE value (0.0004) and the maximum R–value of 0.995 (Fig. 1a). The use of optimal neuron number in the hidden layer is critical in achieving the best neural network architecture. Consequently, the required number of neurons was optimized as reported by Huang et al[17]. In our study, we found that the critical number of neurons in the hidden layer was 7 with respect to MSE and R- values (Supplementary Fig. A.1). Hence, 3–7–1 ANN topology (Fig. 1b) was selected. This model was validated by performing additional experiments that were different from Table 2. It was observed that the prediction error between the simulated and experimental outputs was within 3.2% (Supplementary Table A.2).

This indicated the accuracy of 3–7–1 ANN topology and thus, this developed model was used as a fitness function in PSO algorithm for predicting the optimal combinations of process parameters for enhanced lutein productivity.

The critical parameters of PSO algorithm such as population size, inertia weight and learning factors, were estimated as described in earlier reports.[16] It was observed that all the particles converged to the global optimal solution of 4.45 mg L^{-1} d^{-1} in less than 50 iterations (Fig. 1c) for the following combinations of input process parameters: light intensity, 260 µmol m^{-2} s^{-1}; CO_2 concentration, 3.5% and flow rate, 850 mL min^{-1} (0.425 vvm). Further, the efficiency of PSO technique was validated by performing the validation experiment with the above mentioned values of input parameters. The experimentally tested lutein productivity resulted in 4.32 ± 0.11 mg L^{-1} d^{-1}, which was in close agreement with the simulated output (~3% error).

Thus, the application of ANN–PSO approach for optimization of key process parameters significantly enhanced the lutein productivity from 2.37 to 4.32 mg L^{-1} d^{-1} (82% improvement). Moreover, the biomass productivity was increased from 0.407 to 0.67 g L^{-1} d^{-1} (60% enhancement) and the total lipid productivity was improved from 84.3 to 142.2 mg L^{-1} d^{-1} (69% increment). This indicated that the selected process parameters drastically influenced both lutein and lipid synthesis in *C. minutissima*. The effect of critical process parameters on lutein and lipid production is discussed in Section 3.2.2. Although the resulted lutein productivity (4.32 mg L^{-1} d^{-1}) is comparable with that of literature, the lutein content (6.37 mg g^{-1}) obtained in this study is significantly higher than that of reported in the relevant batch studies. Further, the resulted lipid productivity (142.2 mg L^{-1} d^{-1}) and content (21.2%) can be reasonably compared with the studies reported for lipid productivity obtained upon nitrogen starvation conditions.[7, 10, 28] For instance, the maximum lipid productivity of 140.35 mg L^{-1} d^{-1} (content, 22.4%) was obtained by

Scenedesmus obliquus under 5-day nitrogen starvation period.[28] Thus, these results demonstrate the usefulness of this optimization strategy for improving the productivities of lutein and lipid. In addition, it has to be noted that this microalga *C. minutissima* can be considered as a potential candidate for the production of both lutein and lipid in a biorefinery model.

3.2.2. Effect of critical process parameters on lutein, lipid and biomass productivities

The process parameters such as light intensity, CO_2 supply and aeration rate significantly influenced the synthesis of lutein and lipid in *C. minutissima*. Light acts as an important energy source for the photo-autotrophic microalgae and its intensity level strongly influences the growth rate and product accumulation.[1, 8] This is evident from our study that the increase in light intensity from 50 to 250 µmol m^{-2} s^{-1} resulted in significant enhancement in the productivities of lutein (from 1.05 to 4.13 mg L^{-1} d^{-1}), biomass (from 0.218 to 0.596 g L^{-1} d^{-1}) and lipid (from 21.1 to 126.5 mg L^{-1} d^{-1}) (Fig. 2a). The increased lutein synthesis might be attributed to the light induced rapid up-regulation of carotenoid biosynthesis genes such as phytoene synthase and phytoene desaturase.[30] Moreover, the improvements in lipid productivity and CO_2 fixation rate were mostly associated with the increase of biomass productivity, as reported by Ho et al.[28]

The increase in light intensity from 250 to 300 µmol m^{-2} s^{-1} improved the productivities of biomass and lipid moderately; however, the lutein productivity dropped slightly. The decrease in lutein accumulation at higher light intensity may be due to the size reduction of light–harvesting receptors, where the lutein is predominantly present.[23] Moreover, the photosynthetic efficiency, which is the ratio of light energy recovered by biomass to the amount of light energy supplied, was observed to decrease steadily from 10.97 to 5.7%, with the increase in light intensity from

50 to 300 µmol m^{-2} s^{-1} (Fig. 2a). Thus, the optimal light intensity of 260 µmol m^{-2} s^{-1} for enhanced product synthesis in *C. minutissima* was suitably predicted by PSO technique.

CO_2 serves as an exclusive carbon source for autotrophic microalgae. The concentration of CO_2 (%, v/v) and the aeration rate (mL min^{-1}) drastically affect the mass transfer rate and CO_2 fixation rate in microalgal biomass.[31] The amount of CO_2 present in the air (0.04%) is inadequate to achieve the high-density cultures and on the other hand, excess supply of CO_2 may inhibit the carbonic anhydrase enzyme[32], thereby reducing the biomass productivity. As shown in Fig. 2b, increasing the concentration of CO_2 in the inlet gas from 0.8 to 2.5% improved the efficiency of microalgal photosynthesis (from 4.19 to 8.05%), CO_2 fixation rate (from 0.519 to 1.01 g L^{-1} d^{-1}) and the productivities of lutein (from 2.18 to 3.41 mg L^{-1} d^{-1}) and lipid (from 69.7 to 95.1 mg L^{-1} d^{-1}). However, the further increase in CO_2 (>5%) negatively influenced the photosynthesis. This is evident from the fact that increase in CO_2 concentration up to a critical level would enhance the activity of enzymes such as carbonic anhydrase and Rubisco[33] and hence, improves the photosynthesis. A high CO_2 supply inhibits the critical enzymes involved in photosynthesis process, as a result of significant drop in pH of the medium below the critical level[32], affecting the cell growth rate. Therefore, in this study, the optimal CO_2 of 3.5% (v/v) for enhanced growth rate and product accumulation in *C. minutissima* was satisfactorily determined by PSO technique. This value is in close agreement with the optimal CO_2 (4%) reported by Nakanishi et al.[7] for the enhanced lipid productivity (169.1 mg L^{-1} d^{-1}) by *Chlamydomonas* sp. JSC4.

The third influencing factor is aeration rate and it plays crucial roles such as minimizing photo–limitation or shelf-shading in high-density cultures, distributing the nutrients homogeneously in the culture medium, and maximizing CO_2 dissolution and O_2 evolution.[33] In the current study, the cultivation of *C. minutissima* in customized airlift photobioreactor for

aeration rates in the range of 395 to 600 mL min^{-1} resulted in poor mixing and gas-liquid mass transfer. Thus, low yields of biomass, lipid and lutein were obtained (Fig. 2c). When the aeration rate was increased to 900 mL min^{-1}, remarkable improvements were observed for photosynthetic efficiency (from 4.17 to 7.78%), CO_2 fixation rate (from 0.52 to 0.96 g L^{-1} d^{-1}), lutein productivity (from 2.27 to 3.57 mg L^{-1} d^{-1}) and lipid productivity (39.6 to 97.8 mg L^{-1} d^{-1}) (Fig. 2c). However, when the flow rate was further increased (≥ 1200 mL min^{-1}), the productivities of biomass, lipid and lutein were found to be reduced, which may be due to shear stress to the cells. The another probable reason is that the higher flow rates tend to reduce the retention time of gas bubbles and thereby decreasing the utilization of CO_2 by the microalgal cells.[31] Hence, the optimum aeration rate of 850 mL min^{-1} (0.425 vvm) for improved product synthesis in *C. minutissima* was adequately determined by PSO technique.

3.3. Process integration for the development of microalgal biorefinery model

3.3.1. Microalgae mediated flue gas CO_2 mitigation

In this study, the microalga *C. minutissima* was grown using flue gas under the optimized conditions, as determined by ANN–PSO technique. The concentration of CO_2 present in the flue gas (12% CO_2) was appropriately diluted to 3.5% (optimal CO_2 %, v/v) with inlet air using suitable gas flow meters. Although *C. minutissima* could be grown effectively using undiluted flue gas, it was presumed that the loss of CO_2 from the photobioreactor could be significantly reduced by sparging diluted-flue gas. This experiment was performed near the flue gas generation facility (closed–outdoor conditions) and artificial illumination of 260 µmol m^{-2} s^{-1} (optimal light intensity) was continuously supplied. However, the temperature was left uncontrolled and it was found to vary between 27 °C and 33 °C.

The experimental setup of CO_2 sequestration by *C. minutissima* under the optimized process conditions is shown in Supplementary Fig.A.2. Fig. 3a and b illustrates the time-course profiles for biomass production, CO_2 fixation rate, productivities of lutein and lipid, and nitrate uptake under pure CO_2 and flue gas cultivation conditions. The microalga *C. minutissima* was observed to utilize the flue gas CO_2 as the carbon source effectively. Moreover, the diluted flue gas with reduced amounts of NO_X and SO_X did not significantly affect the growth rate of microalga. This is in accordance with the study of Kao et al.[34], which reported that the dilution of flue gas with air is essential to maximizing the efficiency of CO_2 removal and the productivities of biomass and lipid in *Chlorella* sp. MTF-15.

In the present study, the maximum biomass, lutein and lipid productivities of flue gas aerated cultures were found to be 0.64 g L^{-1} d^{-1}, 4.15 mg L^{-1} d^{-1} and 139.3 mg L^{-1} d^{-1}, respectively (Table 3). These productivities were found to be almost consistent with that of pure CO_2 (3.5%) sparged cultures of *C. minutissima* (Fig.3a and b). To the best of our knowledge, this is the first report that investigates the production of lutein from flue gas grown microalgae. Hence, the further characterization of the product lutein from flue gas grown biomass needs to be carried out. The similar lipid contents obtained for CO_2 and flue gas sparged cultures, can be substantiated with the studies of Chiu et al.[35] and Kumar et al.[36] However, the FAME profiles of CO_2 and flue gas aerated cultures showed significant variations, as discussed in Section 3.3.3. It was observed that there were no statistically significant differences in CO_2 fixation rate, photosynthetic efficiency, contents of chlorophylls and total carotenoids between CO_2 and flue gas sparged cultures of *C. minutissima* (Table 3). Therefore, these results demonstrate that the microalga *C. minutissima* can serve as a potential candidate for the remediation of flue gas and production of lutein and lipid. The process flow diagram for microalgal biorefinery model for the production of biofuels

and lutein with simultaneous flue gas carbon sequestration is shown in Fig.4. It is also shown that the defatted and depigmented biomass can further be subjected for carbohydrate extraction and subsequent bioethanol production by fermentation process.

3.3.2. Simultaneous recovery of lutein and biodiesel

The next step in the development of an integrated biorefinery model is to achieve the maximum possible recovery of both lutein and biodiesel simultaneously from the biomass. The scheme for the concurrent recovery of lutein and biodiesel (FAME) is shown in Fig. 5. Once the products of biomass were extracted using different binary solvent systems, the amount of water needed for proper phase separation was tested. The quantity of water required for methanol, ethanol and 2-propanol containing systems was found to be 10%, 15% and 30% (v/v), respectively. Table 4 shows the simultaneous recovery of lutein and FAME obtained by different solvent systems used. It was observed that different binary solvent mixtures showed almost similar recoveries of FAME ranging between 91.5% and 93.2%. This indicates the efficiency of hexane in extracting the lipids from aqueous alcoholic mixtures. It has to be noted that the polar solvents can easily penetrate the cell walls and thereby facilitate the non-polar solvent hexane for effective extraction of neutral lipids.[22] The maximum lutein recovery in aqueous phase was obtained in ethanol/hexane mixture of 94.3%, followed by methanol/hexane (90.5%) and 2-propanol/hexane systems (87.8%). The comparatively low lutein recovery by 2-propanol/hexane system might be attributed to higher water content (30%, v/v) in its aqueous phase than other solvent systems. Among different binary solvent mixtures tested for maximum recovery of both products from *C. minutissima*, ethanol/hexane system was found to give higher lutein and FAME contents (Table 4). This may be due to the existence of optimal intermolecular attractions and

relative solubility differences between the solvent molecules and intracellular products during extraction.[37]

The present findings are in agreement with that of Bai et al.[14], which reported that the use of methanol/hexane system resulted in simultaneous recovery of 98% lipid and 90% chlorophyll from *Chlorella pyrenoidosa*. Despite the fact that the supercritical CO_2 extraction offers various advantages over classic solvent extraction methods, the study using such an expensive method for fractionation of lipids and pigments[38] resulted in a recovery of only 70% of the pigments along with the total extracted lipids. The pigments that were recovered during supercritical fluid extraction include astaxanthin, zeaxanthin/lutein, canthaxanthin and β-carotene. The lower recovery of pigments may be due to the entrainment of pigments by the lipids and subsequent reduction in the solubility of carotenoids towards the supercritical solvent.[38] In another study, Prommauk et al.[13] achieved almost complete recovery of lutein and biodiesel by performing simultaneous saponification and trans-esterification using alkali catalyst under appropriate conditions. However, a slightly higher concentration of alkali catalyst resulted in partial saponification of FAME that reduced the product yields. In addition, the complex product separation process may require additional equipments for the evaporation and recovery of solvents used. Hence, the method developed in the present study can be considered as simple and effective for the single-step extraction and separation of products using appropriate solvent mixture. Moreover, this study resulted in satisfactory yields of both the products and all the solvents used in the process can be recycled as shown in Fig. 5.

Thus, the current study rationally demonstrated the integration of biorefinery strategy involving concurrent production of lutein and biodiesel with flue gas CO_2 mitigation. This approach may effectively improve the competitiveness of commercially important microalgal

products. The preliminary economic assessment by Prommauk et al.[13] suggested that the process for concomitant production of lutein and biodiesel may be economically feasible. Further, sensitivity and economic analyses indicated that a maximum of 95 USD worth of lutein could be produced per kilogram of biodiesel. However, the detailed techno-economic assessment including the costs for capital, biomass production and subsequent downstream processes should be performed for commercial realization of lutein and biodiesel production, which is the focus of our future study.

3.3.3. FAME composition analysis

The predominant fatty acids of CO_2 and flue gas sparged cultures of *C. minutissima*, were identified as follows: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) (Fig. 6a and b). These fatty acids have been reported to be more appropriate for biodiesel[25, 39]. Table 5 shows the relative percentage composition of FAME of CO_2 and flue gas sparged cultures of *C. minutissima*. The total saturated fatty acid content of flue gas aerated cultures (67.4%) was found to be slightly higher than that of pure CO_2 sparged cultures (61.1%). On the others hand, the total unsaturated fatty acids of flue gas grown cultures (32.6%) was moderately decreased, as compared to pure CO_2 grown biomass (38.9%). A similar trend was also observed by Chiu et al.[35], which reported that the levels of saturated fatty acid was increased from 48.6% to 62.3%, when the microalgae *Chlorella* sp. MTF-7 was grown using flue gas. It has to be noted that a higher level of saturated fatty acids may increase the stability of biodiesel, as the unsaturated fatty acids lack oxidative stability.[34] The presence of small amounts of unsaturated fatty acids such as C20:1 (1.1%) and C20:3 (2.7%), was also observed in this study, when *C. minutissima* was grown using flue gas. This may be due to the stress imposed by flue gas components, as demonstrated by Kumar et al.[36] Thus, the fatty acids with this obtained

composition may satisfactorily meet the desirable requirements of fuel properties such as cetane number, cold flow properties and oxidative stability.

4. Conclusion

The present study convincingly demonstrated the development of an integrated biorefinery for microalgae based flue gas carbon sequestration and simultaneous production of commercially important microalgal products, namely, lutein and biodiesel. The application of ANN–PSO strategy for optimizing the critical process parameters resulted in significant enhancement in the productivities of lutein and lipid. The microalga *Chlorella minutissima,* when grown under the optimized conditions, could efficiently capture CO_2 from flue gas at a considerably higher fixation rate. Subsequent to this, the microalgae mediated flue gas CO_2 bioremediation process was satisfactorily integrated with the concurrent production of lutein and biodiesel. This study is expected to positively contribute to the contemporary scientific literature and it is supposedly the first report on process optimization and integration for the flue gas CO_2 sequestration with concomitant production of algal biomass, lutein and biodiesel.

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References

1. G. Markou and E. Nerantzis, *Biotechnol. Adv.,* 2013, 31, 1532-1542.
2. M. K. Lam and K. T. Lee, *Biotechnol. Adv.,* 2012, 30, 673-690.
3. Y. F. Shen, *Rsc Adv*, 2014, 4, 49672-49722.
4. J. M. Fernandez-Sevilla, F. G. Acien Fernandez and E. Molina Grima, *Appl. Microbiol. Biotechnol.,* 2010, 86, 27-40.
5. R. Slade and A. Bauen, *Biomass Bioenerg.,* 2013, 53, 29-38.
6. N. H. Norsker, M. J. Barbosa, M. H. Vermue and R. H. Wijffels, *Biotechnol. Adv.,* 2011, 29, 24-27.
7. A. Nakanishi, S. Aikawa, S. H. Ho, C. Y. Chen, J. S. Chang, T. Hasunuma and A. Kondo, *Bioresour. Technol.,* 2014, 152, 247-252.
8. L. Brennan and P. Owende, *Renewable and Sustainable Energy Reviews*, 2010, 14, 557-577.
9. Y. Xie, S. H. Ho, C. N. Chen, C. Y. Chen, I. S. Ng, K. J. Jing, J. S. Chang and Y. Lu, *Bioresour. Technol.,* 2013, 144, 435-444.
10. E. J. Olguin, *Biotechnol. Adv.,* 2012, 30, 1031-1046.
11. Y. Huang, J. Cheng, H. X. Lu, R. Huang, J. H. Zhou and K. F. Cen, *Rsc Adv*, 2015, 5, 50851-50858.
12. I. Urreta, Z. Ikaran, I. Janices, E. Ibanez, M. Castro-Puyana, S. Castanon and S. Suarez-Alvarez, *Algal Res*, 2014, 5, 16-22.
13. C. Prommuak, P. Pavasant, A. T. Quitain, M. Goto and A. Shotipruk, *Chem. Eng. Technol.,* 2013, 36, 733-739.

14. M.-D. Bai, C.-H. Cheng, H.-M. Wan and Y.-H. Lin, *J. Taiwan Inst. Chem. Eng.,* 2011, 42, 783-786.
15. R. Dineshkumar, G. Dhanarajan, S. K. Dash and R. Sen, *Algal Res.,* 2015, 7, 24-32.
16. G. Dhanarajan, M. Mandal and R. Sen, *Biochem Eng J*, 2014, 84, 59-65.
17. J. Huang, L. H. Mei and J. Xia, *Biotechnol. Bioeng.,* 2007, 96, 924-931.
18. C. Posten, *Eng. Life Sci.,* 2009, 9, 165-177.
19. A. E. Abdelaziz, D. Ghosh and P. C. Hallenbeck, *Bioresour. Technol.,* 2014, 156, 20-28.
20. N. E. Craft and J. H. Soares, *J Agr Food Chem*, 1992, 40, 431-434.
21. F. Yang, C. Cheng, L. Long, Q. Hu, Q. Jia, H. Wu and W. Xiang, *Energy & Fuels*, 2015, 29, 2380-2386.
22. Y. Li, F. G. Naghdi, S. Garg, T. C. Adarme-Vega, K. J. Thurecht, W. A. Ghafor, S. Tannock and P. M. Schenk, *Microb Cell Fact*, 2014, 13.
23. S. H. Ho, M. C. Chan, C. C. Liu, C. Y. Chen, W. L. Lee, D. J. Lee and J. S. Chang, *Bioresour. Technol.,* 2014, 152, 275-282.
24. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.,* 1959, 37, 911-917.
25. J. Sheng, R. Vannela and B. E. Rittmann, *Bioresour. Technol.,* 2011, 102, 1697-1703.
26. Y. Chisti, *Biotechnol. Adv.,* 2007, 25, 294-306.
27. A. R. Wellburn, *J. plant physiol.,* 1994, 144, 307-313.
28. S. H. Ho, C. Y. Chen and J. S. Chang, *Bioresour. Technol.,* 2012, 113, 244-252.
29. K. Maji, D. K. Pratihar and A. K. Nath, *Opt Laser Eng*, 2014, 53, 31-42.
30. F. Bohne and H. Linden, *Biochim. Biophys. Acta.*, 2002, 1579, 26-34.
31. L. H. Fan, Y. T. Zhang, L. H. Cheng, L. Zhang, D. S. Tang and H. L. Chen, *Chem. Eng. Technol.,* 2007, 30, 1094-1099.

32. L. Cheng, L. Zhang, H. Chen and C. Gao, *Sep. Purif. Technol.,* 2006, 50, 324-329.
33. M. Anjos, B. D. Fernandes, A. A. Vicente, J. A. Teixeira and G. Dragone, *Bioresour. Technol.,* 2013, 139, 149-154.
34. C. Y. Kao, T. Y. Chen, Y. B. Chang, T. W. Chiu, H. Y. Lin, C. D. Chen, J. S. Chang and C. S. Lin, *Bioresour. Technol.,* 2014, 166, 485-493.
35. S. Y. Chiu, C. Y. Kao, T. T. Huang, C. J. Lin, S. C. Ong, C. D. Chen, J. S. Chang and C. S. Lin, *Bioresour. Technol.,* 2011, 102, 9135-9142.
36. K. Kumar, D. Banerjee and D. Das, *Bioresour. Technol.,* 2014, 152, 225-233.
37. K. Ramluckan, K. G. Moodley and F. Bux, *Fuel*, 2014, 116, 103-108.
38. B. P. Nobre, F. Villalobos, B. E. Barragan, A. C. Oliveira, A. P. Batista, P. A. Marques, R. L. Mendes, H. Sovova, A. F. Palavra and L. Gouveia, *Bioresour. Technol.,* 2013, 135, 128-136.
39. G. De Bhowmick, G. Subramanian, S. Mishra and R. Sen, *Algal Res.,* 2014, 6, 201-209.

Table 1

Comparison of biomass, lutein and lipid productivities of chlorophycean microalgal strains

Microalgal strains	Biomass productivity ($g\ L^{-1}\ d^{-1}$)	Lutein productivity ($mg\ L^{-1}\ d^{-1}$)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	Specific growth rate (μ, d^{-1})
Scenedesmus sp.	0.381 ± 0.012	2.05 ± 0.05	71.8 ± 3.5	1.36 ± 0.01
Chlorella minutissima	0.407 ± 0.015	2.37 ± 0.08	84.3 ± 4.1	1.44 ± 0.03
Chlorococcum sp.	0.314 ± 0.011	1.18 ± 0.06	81.9 ± 3.2	1.19 ± 0.02
Chlorella sp.	0.350 ± 0.014	1.49 ± 0.05	56.5 ± 2.7	1.27 ± 0.01

Data shown are the average of two experiments ± S.D.

Table 2

Central composite design for critical process parameters as independent process variables with lutein productivity (mg $L^{-1}d^{-1}$), lipid productivity (mg L^{-1} d^{-1}) and biomass productivity (g L^{-1} d^{-1}) as the responses

Run order	Light intensity (µmol m^{-2} s^{-1})	CO_2 (%)	Flow rate (mL min^{-1})	Lutein productivity (mg L^{-1} d^{-1})	Lipid Productivity (mg L^{-1} d^{-1})	Biomass productivity (g L^{-1} d^{-1})
1	175	5	900	3.58	98.6	0.541
2	250	7.5	600	2.34	91.3	0.410
3	300	5	900	4.05	139.4	0.680
4	250	2.5	1200	4.11	131.4	0.568
5	100	7.5	600	1.12	72.6	0.335
6	250	2.5	600	3.77	112.5	0.445
7	175	5	1404	2.74	75.2	0.351
8	175	5	900	3.58	98.4	0.535
9	50	5	900	1.05	21.1	0.218
10	175	5	900	3.57	98.3	0.527
11	100	2.5	600	1.31	78.4	0.381
12	175	5	900	3.59	97.1	0.542
13	175	5	900	3.57	99.2	0.541
14	175	5	395	2.27	39.8	0.290
15	175	9.2	900	1.73	78.4	0.374
16	175	0.8	900	2.18	69.7	0.291
17	100	7.5	1200	1.4	73.5	0.330
18	250	7.5	1200	3.21	109.4	0.522
19	100	2.5	1200	1.36	81.2	0.473
20	175	5	900	3.59	97.8	0.541

Data shown are the average of two experiments

Table 3

Biomass growth characteristics and biochemical composition of *C. minutissima* cultivated using CO_2 and flue gas.

Parameters	Units	pure CO_2 sparged	Flue gas CO_2 sparged
Biomass productivity	g L^{-1} d^{-1}	0.67 ± 0.018	0.64 ± 0.013
Lutein productivity	mg L^{-1} d^{-1}	4.32 ± 0.11	4.15 ± 0.09
Total lipid Productivity	mg L^{-1} d^{-1}	142.2 ± 5.6	139.3 ± 4.8
Specific growth rate	d^{-1}	1.69 ± 0.02	1.58 ± 0.03
Total chlorophyll content	mg g^{-1}	62.51 ± 3.4	59.64 ± 2.8
Total carotenoid content	mg g^{-1}	8.58 ± 0.24	8.31 ± 0.19
Saturated fatty acid content	%	61.1 ± 2.3	67.4 ± 2.5
Unsaturated fatty acid content	%	38.9 ± 1.4	32.6 ± 1.1
Elemental analysis			
Carbon	%	48.59	48.98
Hydrogen		7.60	7.63
Nitrogen		8.41	9.73
Sulfur		0.74	1.13
CO_2 fixation rate	g L^{-1} d^{-1}	1.19 ± 0.03	1.15 ± 0.02
Photosynthetic efficiency	%	6.49	6.21

Table 4

Simultaneous recovery of lutein and fatty acid methyl ester (FAME) from flue gas sparged cultures of *C. minutissima* by different solvent systems.

Method	Lutein content (mg per gram biomass)	% Lutein recovery	FAME yield (mg per gram CO_2 consumed)	FAME content (mg per gram biomass)	% FAME recovery
Control*	6.37 ± 0.11	-	56.97 ± 0.85	101.4 ± 1.5	-
Methanol: Hexane	5.76 ± 0.07	90.5	53.09 ± 0.66	94.5 ± 1.1	93.2
Ethanol: Hexane	6.01 ± 0.09	94.3	52.64 ± 0.47	93.7 ± 0.8	92.4
2-propoanol: Hexane	5.59 ± 0.06	87.8	52.13 ± 0.41	92.8 ± 0.7	91.5

Data shown are the average of three experiments ± S.D.

*Control: Lutein and FAME contents were extracted and analyzed separately using a known amount of biomass.

$$\%\ \text{Product recovery} = \frac{\text{Product content obtained from simultaneous recovery}}{\text{Amount of product obtained from control method}}$$

Table 5

Comparison of relative percentage composition of fatty acid methyl ester (FAME) of *C. minutissima* grown using CO_2 and flue gas

FAME	CO_2 sparged culture	Flue gas aerated culture
Capric (C10:0)	2.4	2.7
Lauric (C12:0)	6.8	6.6
Tridecanoic (C13:0)	2.2	0.8
Myristic (C14:0)	1.1	1.9
cis-10-Pentadecanoic (C15:1)	2.1	3.9
Palmitic (C16:0)	30.6	39.2
Palmitoleic (C16:1)	2.1	0.7
Heptadecanoic (C17:0)	0.7	0.6
cis-10-Heptadecanoic (C17:1)	1.9	1.8
Stearic (C18:0)	16.5	14.8
Oleic (C18:1n9c)	9.6	10.2
Linoleic (C18:2n6c)	8.7	6.5
α-linolenic ((C18:3n3)	14.5	5.7
Arachidic (C20:0)	0.8	0.8
cis-11-Eicosenoic (C20:1n9)	–	1.1
cis-11,14,17-Eicosatrienoic (C20:3n3)	–	2.7
Saturated FA (%)	61.1	67.4
Unsaturated FA (%)	38.9	32.6

Figure Captions

Fig.1. (a) Regression plot of experimental and ANN predicted values. (b) Schematic diagram of optimized ANN topology consists of an input layer, a hidden layer with log-sigmoidal transfer function, and an output layer with pure linear transfer function. (c) Evolution of best fitness by PSO.

Fig.2. The characteristic profiles of photosynthetic efficiency, CO_2 fixation rate, and the productivities of lutein and lipid as a function of (a) light intensity, where CO_2 and flow rate were kept at their zero levels (b) CO_2 concentration, where light intensity and flow rate were held at their zero levels and (c) air flow rate, where light intensity and CO_2 were kept at their zero levels. (Zero levels: light intensity, 175 μmol m^{-2} s^{-1}; CO_2, 5% and air flow rate: 900 mL min^{-1}).

Fig.3. Time-course profiles of biomass production, CO_2 fixation rate, productivities of lutein and lipid, and nitrate uptake of *C. minutissima* cultivated using (a) pure CO_2 and (b) flue gas CO_2 under the optimized conditions.

Fig.4. Microalgal biorefinery model for the production of lutein and biofuels with concomitant flue gas CO_2 sequestration.

Fig.5. Schematic diagram for the concurrent recovery of lutein and biodiesel from *C. minutissima* that was grown using flue gas CO_2 under standardized process conditions

Fig.6. FAME profiles of *C. minutissima* cultivated using (a) pure CO_2 and (b) flue gas CO_2

Figure 1

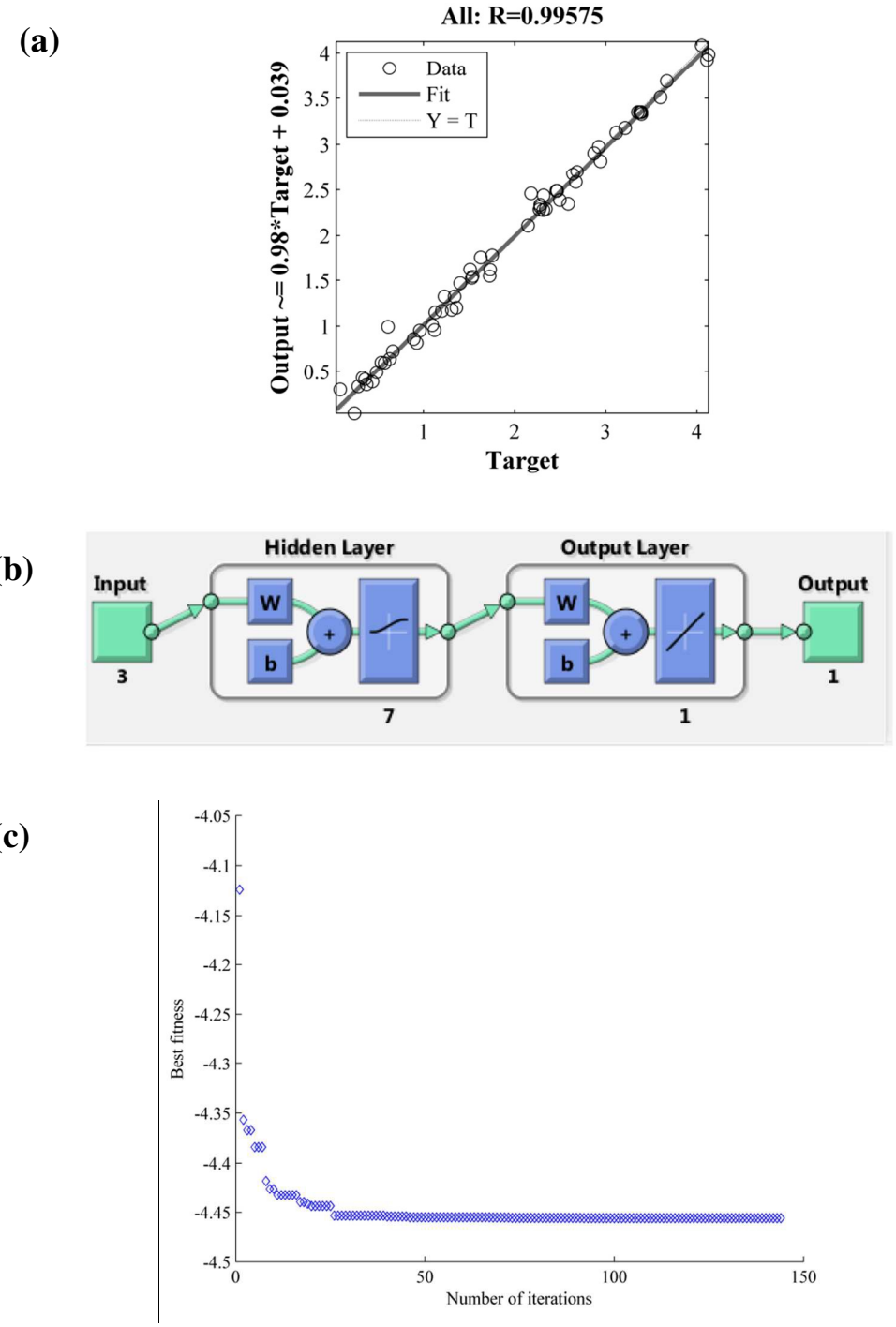
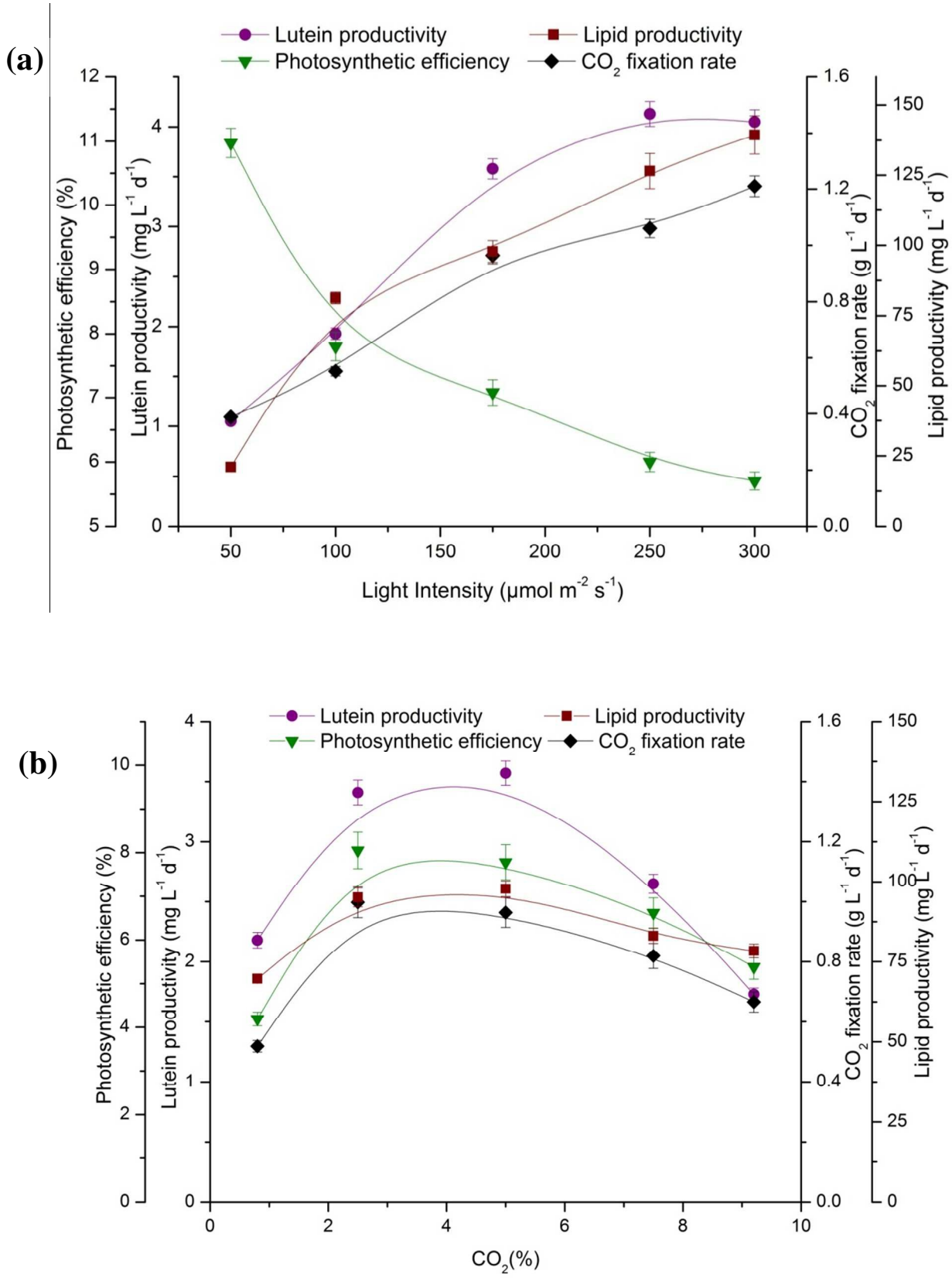


Fig.1. (a) Regression plot of experimental and ANN predicted values. (b) Schematic diagram of optimized ANN topology consists of an input layer, a hidden layer with log-sigmoidal transfer function, and an output layer with pure linear transfer function. (c) Evolution of best fitness by PSO.

Figure 2



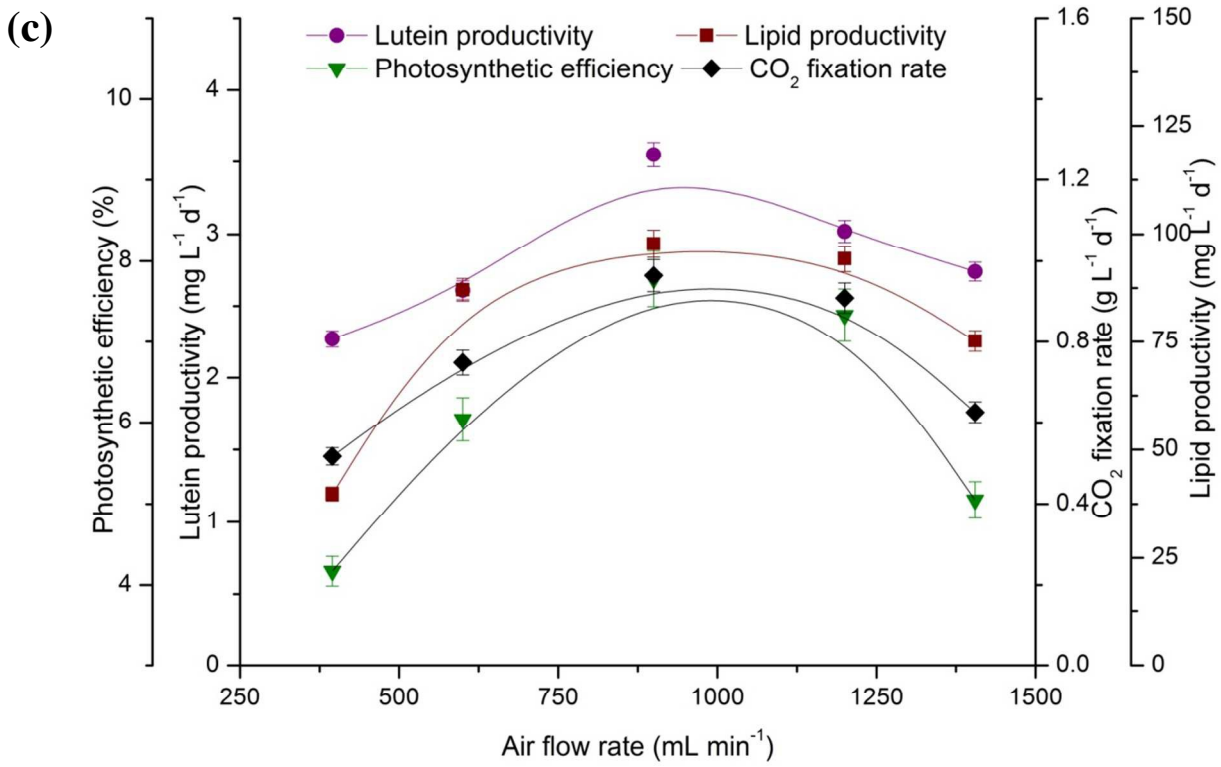


Fig.2. The characteristic profiles of photosynthetic efficiency, CO_2 fixation rate, and the productivities of lutein and lipid as a function of (a) light intensity, where CO_2 and flow rate were kept at their zero levels (b) CO_2 concentration, where light intensity and flow rate were held at their zero levels and (c) air flow rate, where light intensity and CO_2 were kept at their zero levels. (Zero levels: light intensity, 175 µmol m^{-2} s^{-1}; CO_2, 5% and air flow rate: 900 mL min^{-1}).

Figure 3

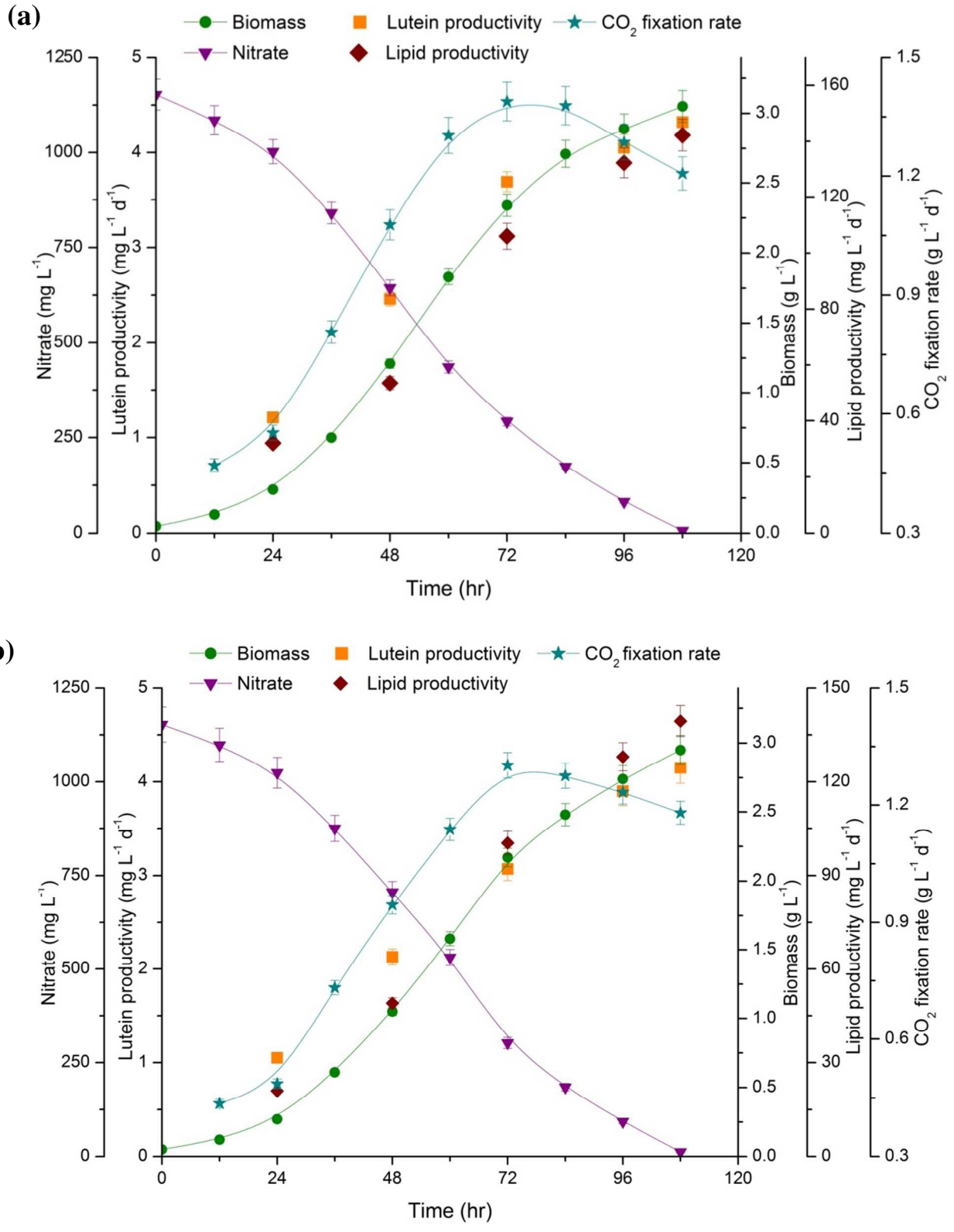


Fig.3. Time-course profiles of biomass production, CO_2 fixation rate, productivities of lutein and lipid, and nitrate uptake of *C. minutissima* cultivated using (a) pure CO_2 and (b) flue gas CO_2 under the optimized conditions.

Figure 4

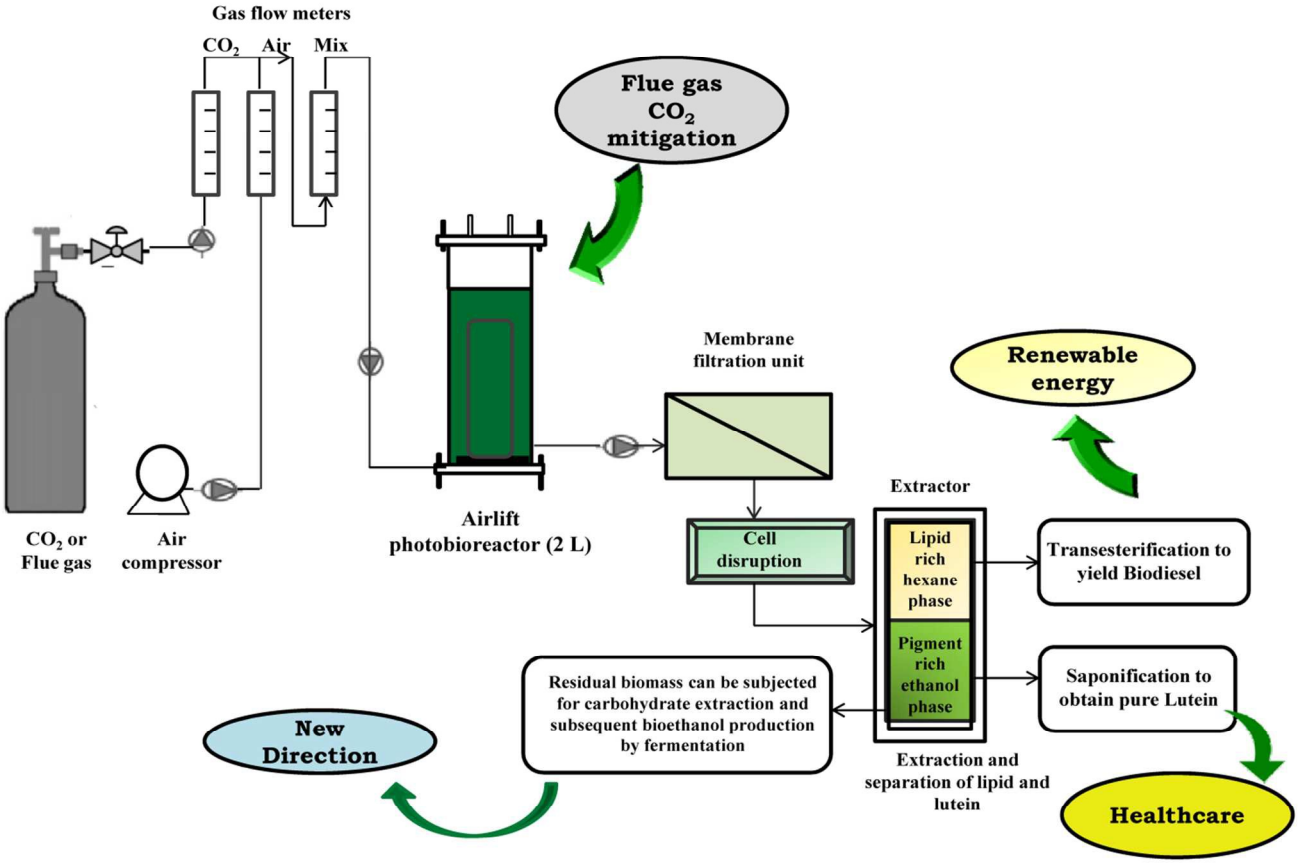


Fig.4. Microalgal biorefinery model for the production of lutein and biofuels with concomitant flue gas CO_2 sequestration

Figure 5

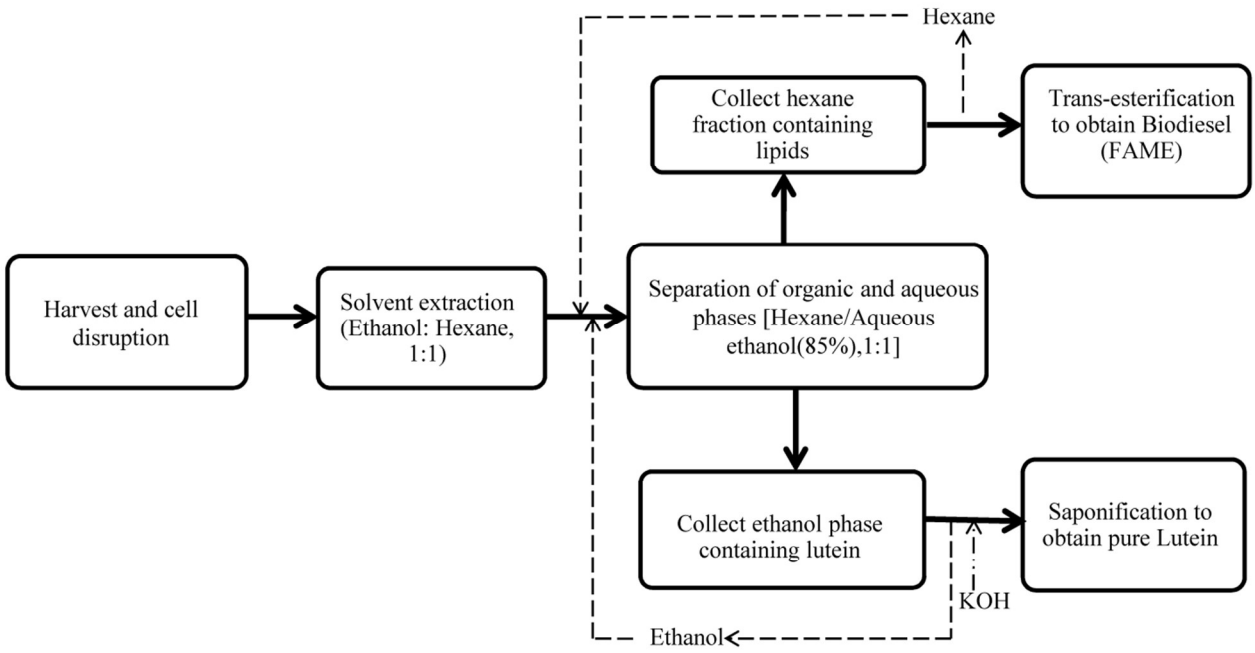


Fig.5. Schematic diagram for the concurrent recovery of lutein and biodiesel from *C. minutissima* that was grown using flue gas CO_2 under standardized process conditions

Figure 6

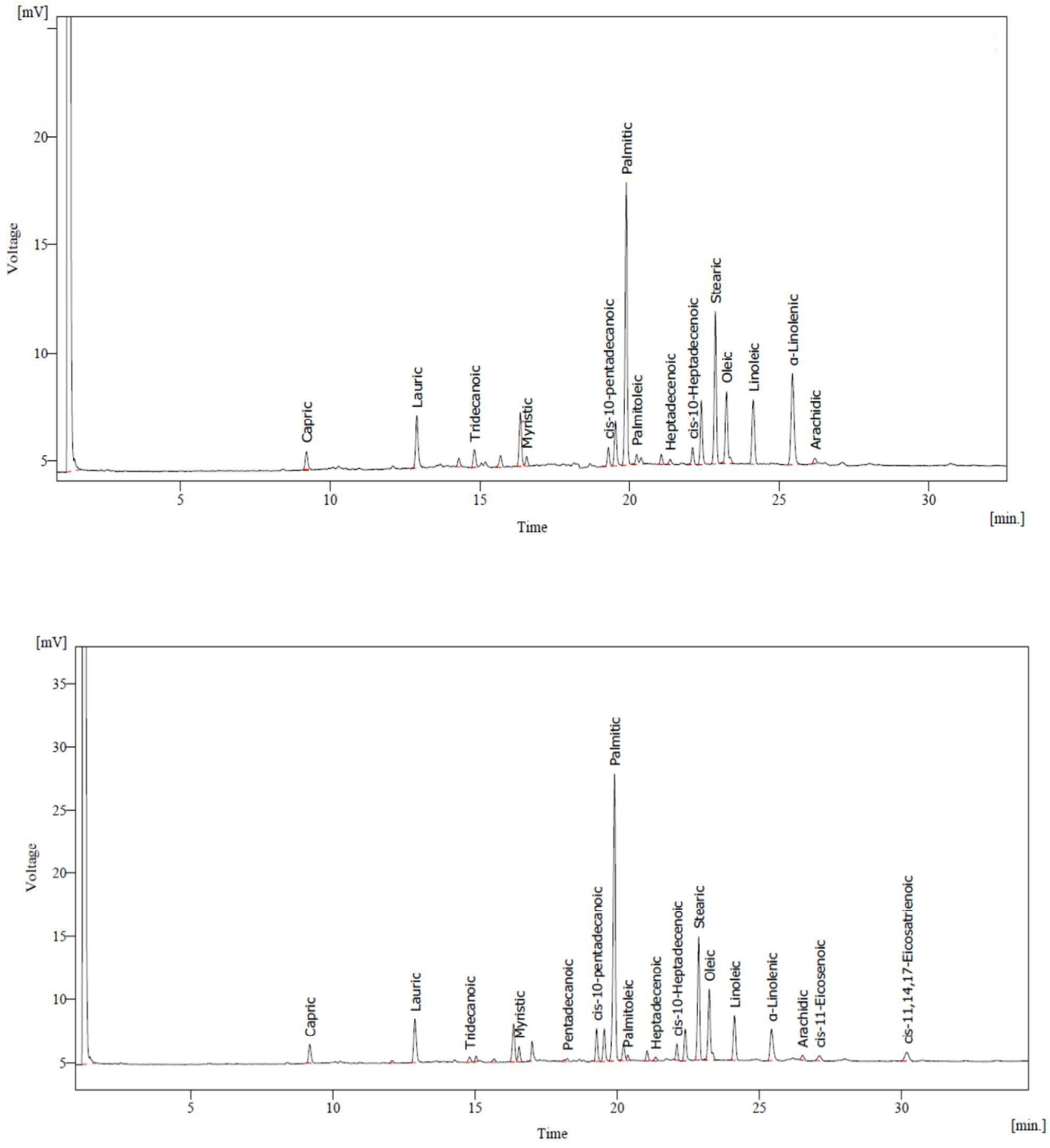


Fig.6. FAME profiles of *C. minutissima* cultivated using (a) pure CO_2 and (b) flue gas CO_2