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ORIGINAL PAPER

# Evaluation of Wild-Type Microalgae Species Biomass as Carbon Dioxide Sink and Renewable Energy Resource

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## Abstract

In this study, wild-type microalgae species isolated from Porsuk river (Central Anatolia, Turkey) investigated as energy production feedstock and carbon dioxide sink. The obtained experimental data have been used for energy evaluation of the whole process and size estimation of large scale microalgae plant. Growth rate, $CO_2$ mitigation rate, lipid, carbohydrate and protein content and natural settling behavior of the isolated species were investigated. The microalgae *Gleocystis ampula* had the highest growth rate equal to $0.138 \pm 0.008$ g $l^{-1}$ $d^{-1}$ which also was observed to fix carbon dioxide with the highest rate of $0.281 \pm 0.025$ g $l^{-1}$ $d^{-1}$. The highest measured lipid content of $47.32 \pm 0.40$ wt% belonged to *Scenedesmus quadricauda* (I) with an estimated lipid production rate of $51.9 \pm 0.4$ mg $l^{-1}$ $d^{-1}$. The species *Kirchneriella lunaris* showed the highest carbohydrate proportion being $72.43 \pm 6.40$ and *Micrococcus* sp. had the highest protein content of $58.11 \pm 8.5$ wt%. Promising large scale application of microalgae was concluded for biodiesel production and carbon dioxide mitigation just when efficiency of processes improved substantially. An Energy Efficiency of 1.62 was estimated following an ideally designed cultivation and dewatering approach.

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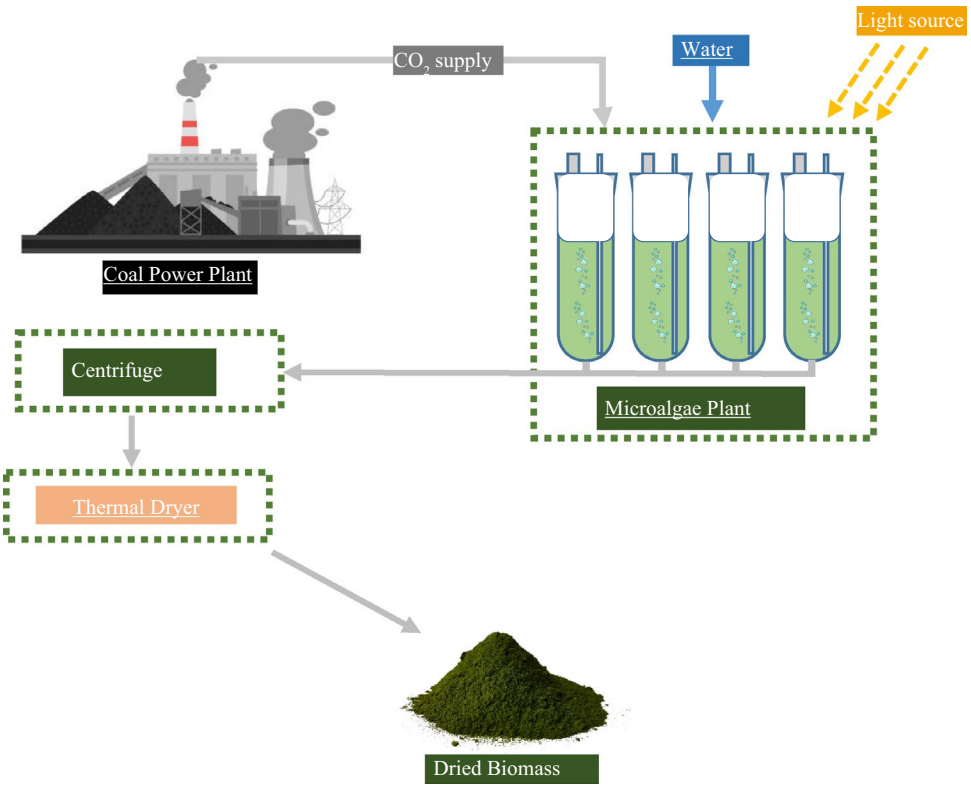
**Graphic Abstract**




**Keywords** Microalgae biomass · Biofuel · Energy efficiency · Carbon fixation · Growth rate · Life cycle assessment

## Statement of Novelty

In this paper, we report on the Wild-type microalgae species isolated from a central Anatolia (Turkey) stream and evaluated as a feed stock for biofuel production and/or carbon dioxide sink. Life Cycle Assessment of energy for microalgae photobioreactor was followed. Twenty microalgae strains have been morphologically identified and then screened based on their biochemical, their growth rate, $CO_2$ mitigation rate, lipid, carbohydrate and protein content and also their natural settling behavior were compared. The size of large scale microalgae plant for application of this technology evaluated by comparing the estimated biofuel production rate of such plants with a real conventional fossil fuel plant (Ras Tanura Refinery, KSA).

Authors believe that these are very interesting results for researchers in the field of renewable energy from microalgae biomass.

## Introduction

Microalgae are photosynthetic microorganisms able to consume carbon in mineral forms [1]. Majority of microalgae species are carbon fixing autotroph microorganisms who are able to mitigate carbon dioxide directly. They have been considered as a solution to reduce carbon dioxide content of the atmosphere [2]. They have been also studied vastly as promising species to be produced in large scale for biofuel production [3]. Photosynthetic microalgae grow fast and rely on sun light for their energy requirement. Microalgae unlike many agriculture products which are actively being used for large scale biofuel production like wheat, corn and soybean, can be cultivated in vertical reactors or pools without occupying arable lands [4]. Despite all the significant characteristics of microalgae, there is no commercially active biofuel production plant based on microalgae biomass [5, 6]. There are still bottle necks hindering the emergence of microalgae biofuel technology. In short, microalgae must be cultivated, harvested and transformed to biofuels. In cultivation step, the aim is to maximize the growth rate and at the same time the highest possible biomass concentration [7]. A variety of reactors design have been tried to optimize the rate of

biomass production and finally lowering the cost [7]. Harvesting is a challenging step because the method of harvest differ from one species to another one. Some microalgae species settle easily but for many of them they float freely in the solution and form a very stable broth [8]. Those species who settle naturally when mixing is stopped are welcomed because this will reduce the cost of harvesting significantly.

Depending on the nature of final aimed biofuel, downstream processes will differ substantially. For biodiesel, an oil extraction step following by a transesterification reaction will be needed [7, 9] whereas for bioethanol, a saccharification step i. e. the process of breaking complex carbohydrates such as starch or cellulose into its monosaccharide components, followed by a fermentation processes are required [10].

Altogether, finding species with high growth rates and also high oil or carbohydrate content will significantly affect the total cost of final product of interest. Moreover, locally isolated strains of microalgae would have higher chance of applicability because of adaptation to those local environment [11]. It may also reduce the risk of ecosystems imbalances due to unnatural interference of human by transferring foreign strains from other habitats [12, 13].

In most of the previous researches, the photobioreactors were of those configurations with low mixing efficiency that fail to maintain the same degree of homogenization in the cultivation broth [6, 14–16]. Especially, when no mechanical mixer is used, the ratio of gas line diameter, i.e. sparger, to the cross sectional area of the photobioreactor will determine the mixing efficiency [6]. This is very important to be addressed because with partial sedimentation of the biomass, the light and even dissolved carbon dioxide will no more be available for the growing cells effectively. Configurations like Roux bottles [17] and flasks [18–20] are so common but have low performance to be implemented for a screening study.

At the present work, microalgae species were isolated from a river located in central Anatolia region (Porsuk River, Eskişehir, Turkey). They were investigated for their growth rate, carbon dioxide mitigation rate, lipid, carbohydrate and protein content. They were also studied for their settling behavior in cultivation broth. These results in combination with previously published data were then used for evaluation of potential application of the technology for large scale biodiesel production and carbon dioxide mitigation.

## Materials and Methods

### Chemicals

The chemicals $NaNO_3$, $K_2HPO_4$, $MgSO_4{\cdot}7H_2O$, $CaCl_2{\cdot}2H_2O$, citric acid, ammonium ferric citrate, $EDTANa_2$, $Na_2CO_3$, $H_3BO_3$, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $Na_2MoO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, $Co(NO_3)_2{\cdot}6H_2O$ were purchased from Sigma-Aldrich, USA. Acetone, chloroform, methanol, phenol, sulfuric acid, KCl, and propanol were provided by Tekkim, Turkey. All the chemicals were of reagent grade. Nile red (9-diethylamino-5Hbenzo[a]phenoxazine-5-one) purchased from Sigma-Aldrich, USA (assay ≥ 98.0% (HPLC)). Reagent water was utilized in laboratory using a water purification unit (Thermo Scientific, Germany).

### Sampling, Culture and Isolation

Samples were taken from two points alongside Porsuk River, Eskişehir, Turkey. One of them was a deep point with very slow current adjacent to Astsubay Raif Özgür Park (39.770002, 30.498488) and the other being a shallow point with higher current and turbulences in Botanic park (39.742747, 30.460088). Previously sterilized 50 ml conical falcons were filled there and immediately transferred to lab. BG11 medium according to [21] have been used throughout the study for microalgae culture. All the cell transferring tasks were done aseptically to reduce the risk of contamination. Ten milliliters of obtained samples were transferred to 250 ml flasks containing 100 ml BG11. Triplicates of the cultures were prepared and left over a glass plate being shed with 3500 lx white light from bottom provided by 35 W fluorescent lamps for almost one month until green color was clearly observed. The light intensity was adjusted with an illuminance Meter (T-10MA Konica Minolta; Japan). Microscopic view (Olympus, Japan) of the well-grown cultures showed a mixture of multiple green and blue-green microalgae, macroalgae species, diatoms and protozoa (Fig. 1).

For isolation and purification of the species, single cell isolation techniques was used. A droplet of microalgae culture were put on a glass slide and looked under microscope. A glass pasture pipet tip was heated over flame and pulled immediately for elongation to form a fiber like tip. The tip was then cut and looked under microscope to verify its approximate micro scale diameter. This pipet was attached to a micromanipulator and adjusted so that the pipet tip could be easily moved with a good control in the droplet. The microalgae cell of interest was pulled into the pipet with a very gentle suction from the other end of pipet. The possibly caught cell was transferred to a 10 ml tube containing 5 ml BG11. This was then distributed to 10 subcultures to maintain dilution and increase the chance of purification. These cultures left for 3 months until clear green color was observed. The purification was verified using a light microscope.

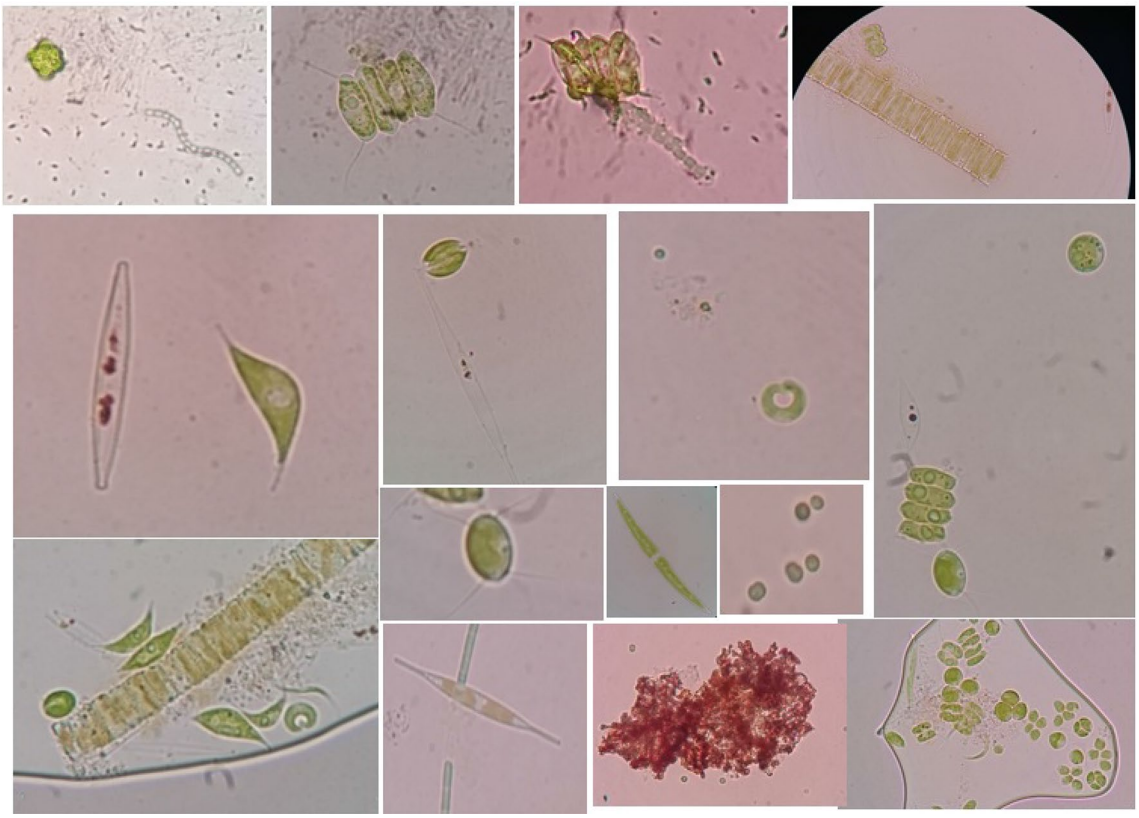


**Fig. 1** The growth of different microorganisms in mixed culture

## Calibration Curve

The next step in the research was to study the biochemical composition i.e., lipid, carbohydrate and protein content of the isolated microalgae. Throughout the study, a calibration curve was needed to convert optical density ($OD_{680nm}$, Shimadzu UV-1800 UV–Vis Spectrophotometer) to concentration in mg/l in dry basis. Cultivation of each isolated species were followed using one liter flasks as photobioreactors. They were kept under sufficient light, approximately 3500 lx, and continuously aerated with approximate equal flow rates of 1 volume of gas per volume of liquid per minuets (vvm). The air flow was passed through a 0.2 µm PTFE membrane filters (Fluoropore, Merck Millipore) to maintain sterilization. The inoculation from purified stocks were initialized at $OD_{680}$ equal to 0.5. After 20 days cultivation the solutions were then centrifuged to have a paste like microalgae biomass then two aliquots each 5 ml were transferred to weighing dishes and dried in oven for 24 h at 110 °C. The dishes were then cooled down in a desiccator and weighed again. In this way, dried mass in microalgae paste was calculated. In Parallel, 5 ml of paste was diluted serially and the $OD_{680}$ was read. In OD range were the OD vs microalgae mass concentration graph showed linear, trend line was passed through the points and the coefficient was calculated for each microalgae. The results are presented in Table 1. The observed approximate linear range for $OD_{680}$ (Table 1) showed that a range of 0.15 to 1.60 absorbance values would

**Table 1** Process specifications for energy assessment

| Process | Real case | Ideal case |
|---|---|---|
| Cultivation | | |
| Aeration | Compressor, 2.6 kW | Adiabatic compression of ideal gas |
| Substrate supply | Pump, 250 W | Potential energy to pump water to the top of PBR |
| Harvest | | |
| Thickening | Centrifuge, 125 W at 6000 rpm | Natural settling |
| Drying | 15% dw to 98% dw | 15% dw to 98% dw |

apply for all isolated microalgae. For the next steps of the study, the estimated coefficients were used to convert $OD_{680}$ to dried biomass concentration in gr/l according to:

$$\text{Biomass Con.}\,(g.l^{-1}) = C_f \times (OD_{680} - OD_b), \quad (1)$$

where $C_f$ is the conversion factor, $OD_{680}$ is the optical density at 680 nm wavelength and $OD_b$ is the optical density of blank.

## Growth Rate Determination

Bubble column type photobioreactors was used simultaneously to determine the growth rate. A sketch of the cultivation vessel with dimensions and also the real configuration is shown in Fig. 2. The cultivation vessels were identical. Air was charged into the cultivation vessels at 0.1 vvm flow rate. The light was provided at 3500 lx by 8 white fluorescent lamps. The medium was BG11 without any organic carbon. The pH was fixed at 7 while the temperature was not controlled. The ambient temperature was approximately 25 °C. Inoculation started with $OD_{680}$ equal to 0.500 ± 0.002. For the first days of cultivation 3 ml samples were taken using sterile syringes attached to 3 way luer-lock valves without need for removing caps. For the cases where OD were out of linear range (Table 1), samples were diluted as much as needed. The microalgae concentration then was estimated using conversion factors (Table 1).

## Lipid Determination

A flourometric approach was followed for lipid determination based on the work of Elsey and friends [22] with some modifications. The samples were diluted until $OD_{680}$ fell in the range of 0.1–0.3. Per sampling, 3 ml of this algal suspension was stained with 20 µl of $7.8 \times 10^{-4}$ M Nile Red dissolved in acetone and then excited at 486 nm before measuring the emission at 570 nm (Qubit 4 Fluorometer, Thermofisher, USA). Blanks were also prepared by filtering (0.22 µm) microalgae solutions and the fluorescence value was subtracted from samples value. The calculated value was used for screening of the microalgae species with regard to lipid content. Higher fluorescence corresponds to higher lipid content.

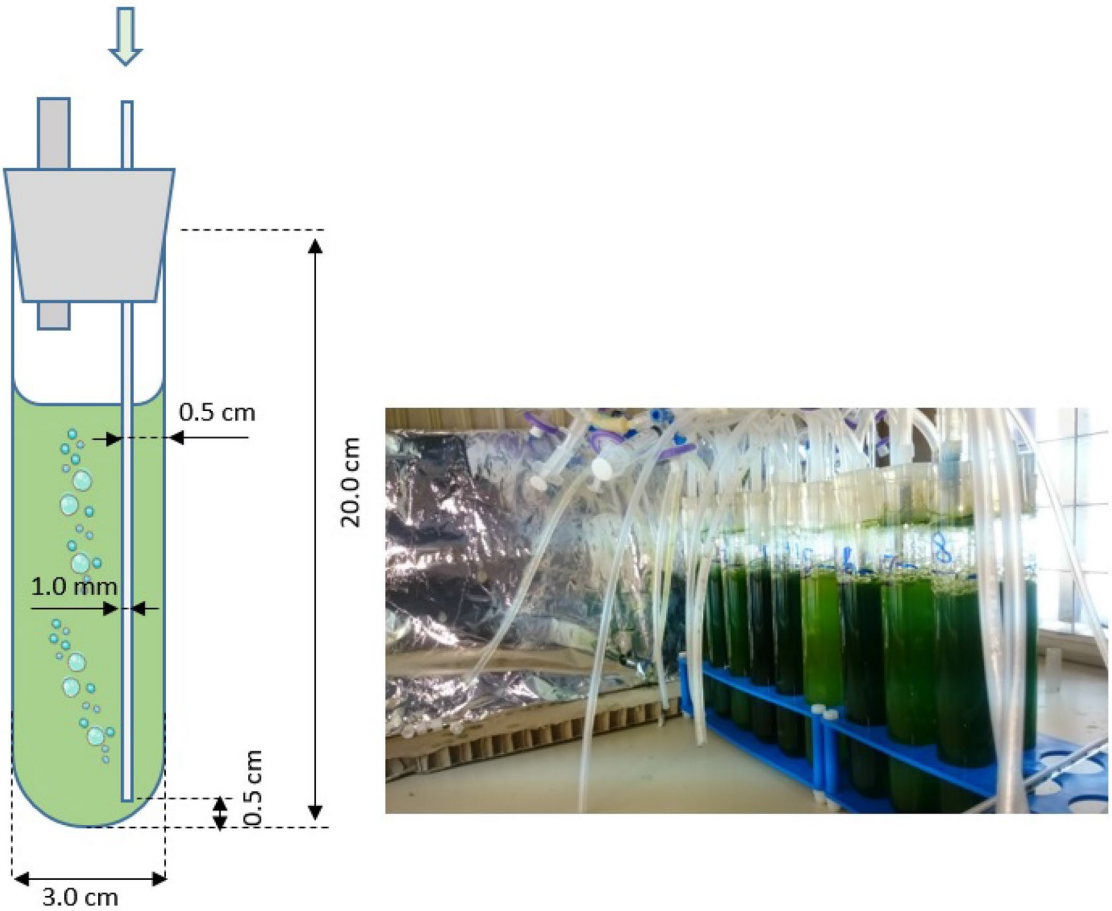



**Fig. 2** The schematic view (left) and the real view of the photobioreactors

For lipid content estimations, a calibration curve of fluorescence vs lipid content was prepared. Microalgae samples were centrifuged and the lipid was extracted and quantified according to the method of Bligh and Dyer [23].

### Carbohydrate Determination

Phenol–sulfuric acid colorimetric method was used for carbohydrate determination as was described in [24]. Dextran was used as reference sugar for standard solution preparation.

### Protein Determination

For estimation of protein a conversion factor of elemental nitrogen to protein (N-Prot factor) was used. Different studies reported ranges of N-Prot factor. In this study, the N-Prot factor equal to $4.78 \pm 0.62$ was selected based on the work of Lourenço and friends [25]. They have reported specifically on the estimation of protein content of microalgae using elemental CHN/S analysis as was used in the present work (CHN/S Elemental Analyzer, Thermoscientific, USA).

### Settling Efficiency Determination

Microalgae broth were transferred to 15 ml test tubes and left over night. The OD of the sample taken from the top layer of the broth at the start and after 24 h was read to measure the settling efficiency.

### Energy Assessment in Life Cycle of Microalgae to Dried Biomass

The energy efficiency of microalgae PBR operational energy was investigated using the experimental data and compared to the ideal process condition as summarized in Table 1. The consumed power for gas charge into PBR and centrifuge was measured using a multimeter (Fluke, USA). The light energy was excluded because in a real application the solar light is supplied.

### Large Scale Microalgae Plant Size Estimation

Two well-known concerns which microalgae technology is going to hopefully address are biodiesel production and carbon dioxide mitigation. The size of biomass production plant was estimated assuming that the plant has the same biodiesel production capacity as the Ras Tanura Refinery (Aramco, Saudi Arabiya), a petroleum refinery complex with diesel production capacity of 175,000 bpd. The growth characteristics of the microalgae species with highest lipid content was taken into account.

The plant size when used as carbon dioxide sink was estimated by considering fossil burning power plant ICDAS Biga facility (Çanakkale, Turkey) which produces 405 MWe electric power. It was assumed that a microalgae production plant to mitigate all the carbon dioxide release of the factory. The growth characteristics of the microalgae species with highest carbon dioxide fixing rate wass taken into account.

## Results and Discussions

### Identification of the Isolated Species

The purified cultures were morphologically identified with comparison to data available at [26–32]. The microalgae's microscopic view using oil immersion microscopy is provided in Fig. 3. The identified microalgae were #1: *Chroococcus disperus*, #2: *Gleocystis ampula*, #3: *Synechocystis* (I), #4: *Scenedesmus obliguus* (I), #5: *Chlorella vulgaris* (I), #6: *Phormidium uncinatum* (I), #7: *Scenedesmus quadricauda* (I) #8: *Synechocystis* (II), #9: *Phormidium uncinatum* (II), #10: *Scenedesmus dimorphus*, #11: *Microcystis aeruginosa*, #12: *Chlorella vulgaris* (II), #13: *Cyanobacterium cedrorum*, #14: *Chroococcus* sp. (I), #15: *Kirchneriella lunaris*, #16: *Scenedesmus quadricauda* (II), #17: *Chlorella vulgaris* (III), #18: *Nannochloris* sp., #19*: Chroococcus* sp. (II) and #20: *Micrococcus* sp. These species belongs to the green microalgae and cyanobacteria or blue-green microalgae subdivisions of algae which are categorized under cyanophyta and chlorophyta phylum respectively.

### Growth Rate

Growth rate is a very important factor to be determined for each of the isolated microalgae species because it determines the amount of obtainable biomass and equally the amount of fixed carbon. The rate of growth depends on multiple factors which also can be modified to maximize the rate and/or the final concentration of biomass in the solution. These factors mainly are light intensity and its duration, temperature, pH, gas flow rate, carbon dioxide concentration in the gas flow and nutritious composition [6]. Totally, 18 species out of 20 identified species were studied for their growth rate because the microalgae *Phormidium uncinatum* I&II were excluded because the photobioreactor suitable for these long chain forming microalgae would be completely different in design. A preliminary cultivation in 1 L photobioreactor with continuous bubbling for all 20 species showed that this kind of photobioreactors are not suitable for all type of microalgae. As can be seen from Fig. 3, microalgae *Phormidium uncinatum* I&II which are from *Oscillatoriaceae* family did not homogeneously dispersed in the liquid medium and either formed a single woven matt or attached to the surface. The

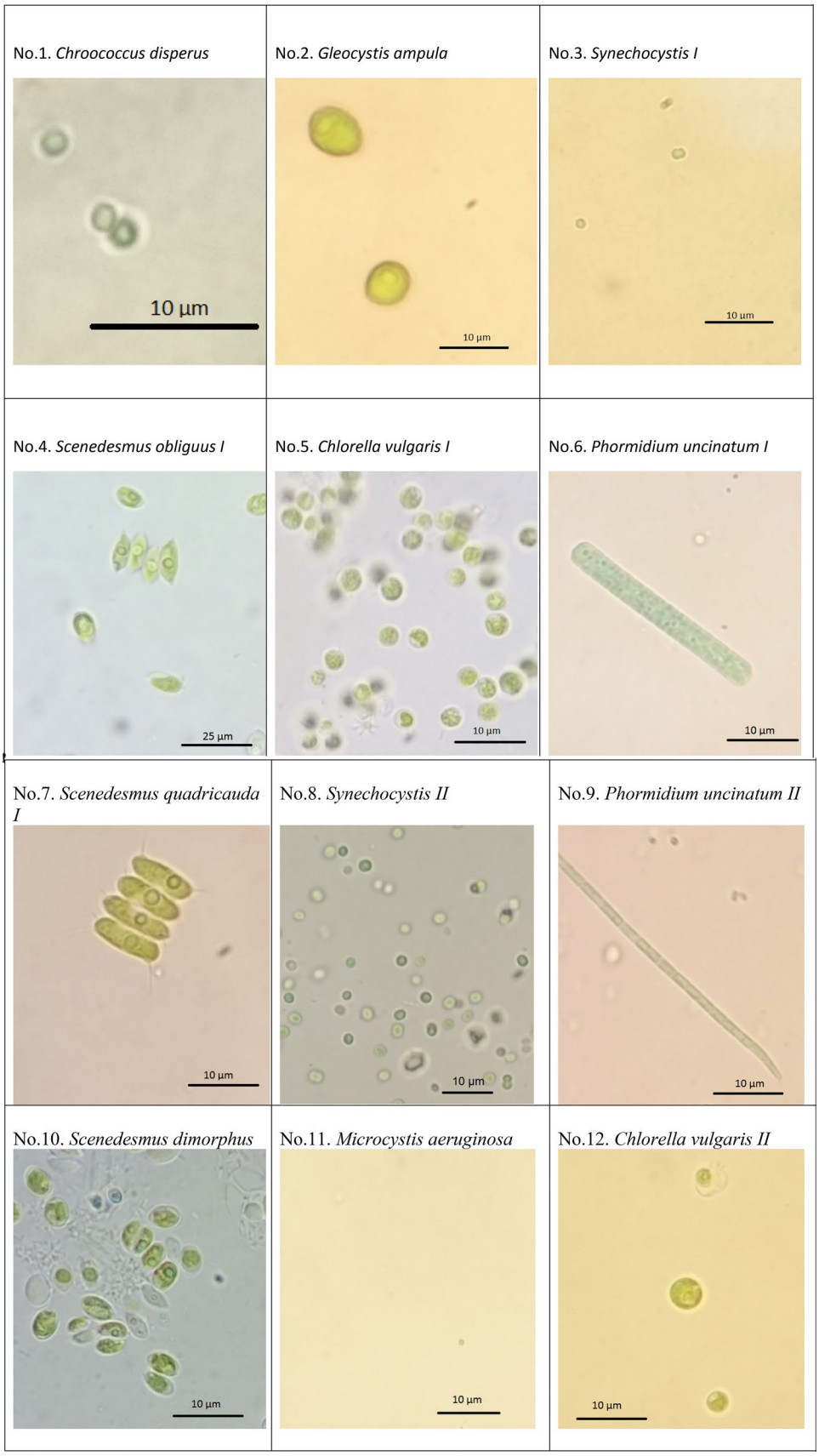
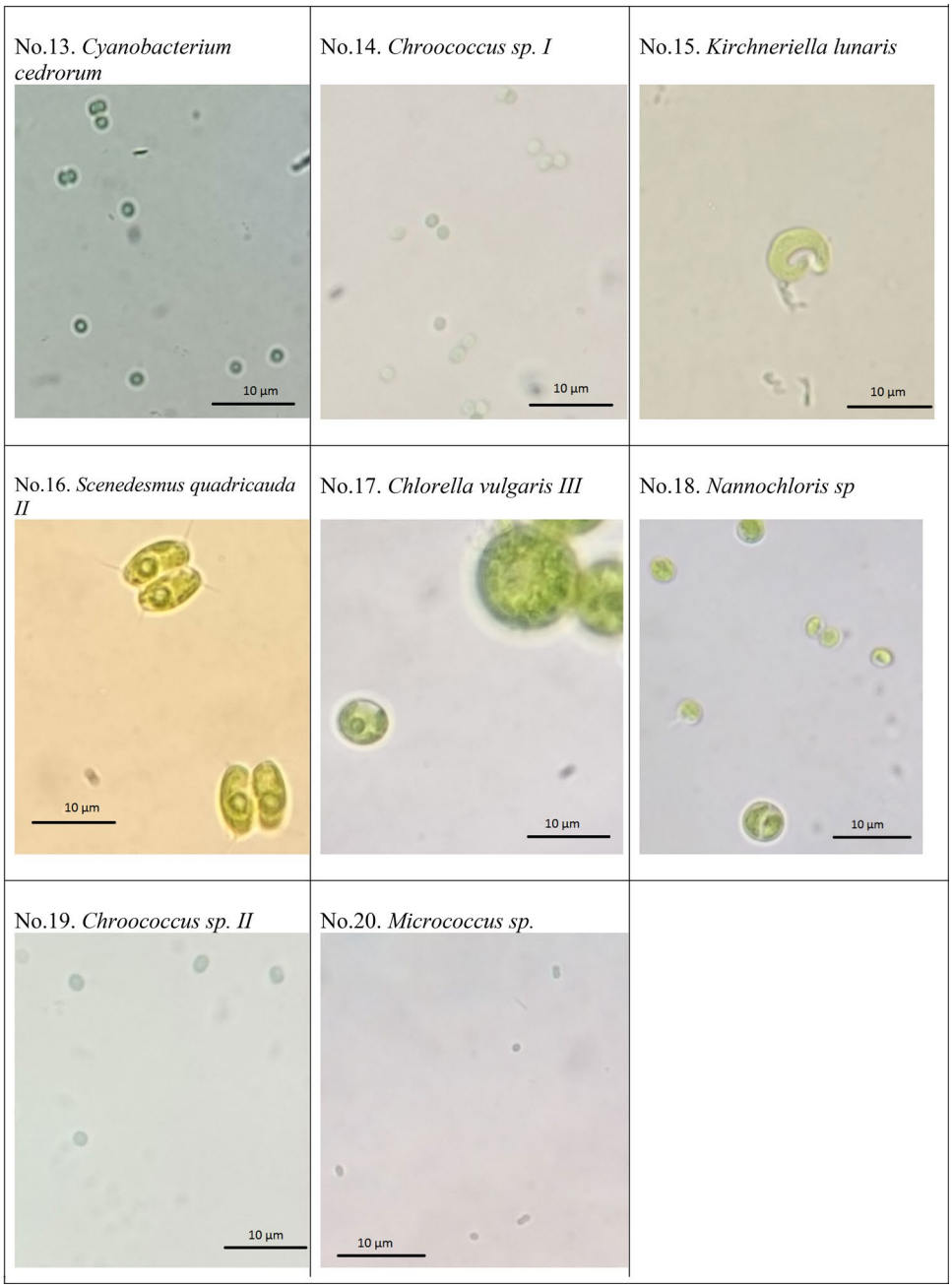



**Fig. 3** The microscopic view (oil immersion) of the isolated microalgae species

**Fig. 3** (continued)




obtained growth curves are shown in Fig. 4. The slope of the linear phase of the growth was calculated as the growth rate (Table 3). The microalgae *Gleocystis ampula, Scenedesmus dimorphus* and *Chlorella vulgaris* (II) showed the highest growth rate with 0.138 ± 0.008, 0.137 ± 0.010 and 0.133 ± 0.008 g $l^{-1}$ $d^{-1}$ respectively. In opposite, microalgae *Kirchneriella lunaris*, *Microcystis aeruginosa* and *Chlorella vulgaris* (I) were the slowest growing species with growth rates of 0.042 ± 0.001, 0.056 ± 0.004 and 0.079 ± 0.005 g $l^{-1}$ $d^{-1}$ in order. The highest recorded concentration during 35 days of cultivation is also reported (Table 3) where microalgae *Scenedesmus quadricauda* (I), *Gleocysis ampula* and *Scenedesmus quadricauda* (II) reached the highest concentration with 2.831 ± 0.061, 2.625 ± 0.023 and 2.496 ± 0.025 g $l^{-1}$ in dried basis. Many studies have reported growth rate as specific growth rate μ (day $^{-1}$) where for production applications is not a simply comparable factor which is based on cell number rather than cell mass [33, 34]. As can be seen from Fig. 2, microalgae cells are very different in cell size. Some species have varying cell size during their growth

which is to say a mass based growth rate report is a more practical approach. These values when compared to the optimized growth rate values in previous studies are promising for example Arbib et al. [35] has reported 0.424 g $l^{-1}$ $d^{-1}$ for a strain of *Scenedesmus*.

## Elemental Analysis and the Estimation of Carbon fixation Rate

The elemental analysis for carbon, hydrogen and nitrogen is presented in Table 2. The carbon content of microorganism is an important factor since it determines the potential for carbon fixation [36–38]. Carbon content is also a determining factor for fuel characteristics where higher value are interested [39, 40]. The microalgae *Chlorella vulgaris* (I) showed the highest elemental carbon content being 57.52 ± 0.10%. With a lesser amount *Chroococcus disperus* and *Chlorella vulgaris* (II) respectively were composed of 56.48 ± 3.27 and 56.37 ± 0.92% elemental carbon. The least value was 43.77 ± 0.80% obtained for *Phormidium uncinatum* (II). As can be seen, there is a meaningful difference for carbon content between isolated species. From the data obtained for growth rate and the biomass elemental carbon, the rate of carbon dioxide fixation was estimated (Table 3). It was observed that the highest rate of carbon dioxide mitigation was 0.281 ± 0.025 g $l^{-1}$ $d^{-1}$ for *Gleocystis ampula*. These results showed that carbon content in dried biomass of microalgae changes significantly among species therefore the approximated formula for biomass molecular structure as in [41] should not be used for total carbon dioxide fixation potential of different species.

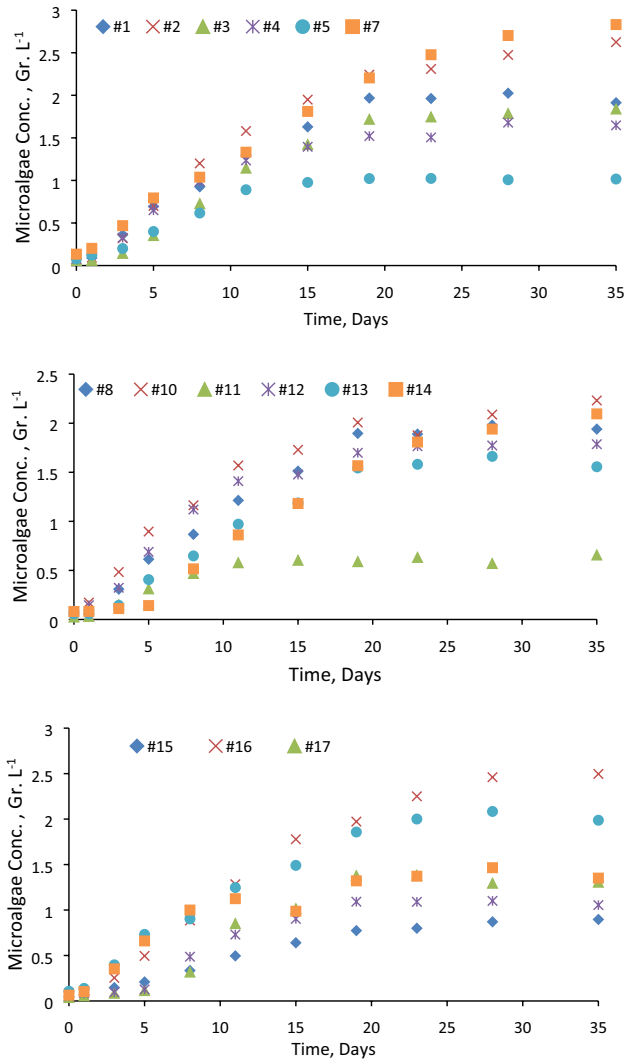



**Fig. 4** The growth curve for isolated microalgae

## Biochemical Composition

Lipids, carbohydrates and proteins are the main biochemical macromolecule composition of microalgae. These are important to be quantified because these are determining factors for final product of interest. The microalgae with higher lipid content is more favorable for biodiesel production while the carbohydrate rich species are suitable for bioalchohols production via fermentation [42]. On the other hand, a protein rich species may be suitable to be used as feed ingredient for cattle or fishery industries. Although in this study, well-known procedures for determining these biochemicals have been used, the estimated results are not absolute values because the conversion coefficients for protein estimation is not species-specific coefficients for analyzed microalgae or the carbohydrate has been estimated using standards prepared by dextran as a reference sugar which may not be an exact indicator for isolated microalgae.

## Lipid

The estimated percentage of lipid, carbohydrate and protein is presented in Table 3. A graphic presentation is also provided in Fig. 5 for ease of comparison between different species. As can be seen (Fig. 6) *Scenedesmus quadricauda* (I), *Microcystis aeruginosa* and *Nannochloris* sp. had the highest lipid content being 47.32 ± 0.40, 39.7 ± 0.31 and 33.95 ± 1.9% respectively. In a previous screening study for high lipid content, a *scenedesmus* sp. was found to have 40.0% lipid content [19]. Because of high lipid content of these species, they were promising candidates as biomass producers for oil based biofuels like biodiesel. From a process point of view, the rate of lipid production in a real plant may be more important than the lipid percentage in the biomass. Therefore by taking the data for growth rate into account, the rate of lipid production for isolated species were estimated as presented in Table 3 and graphically shown in Fig. 7. In this case, the highest estimated lipid production

**Table 2** The $OD_{680}$ conversion factors to dried biomass concentration and elemental analysis of isolated microalgae

| No | Microalgae type | Family b: blue-green g: green | Coefficient factor for OD to concentration | | | CHN analysis | | |
|---|---|---|---|---|---|---|---|---|
| | | | Linear range | $C_f$ | $R^2$ | %C | %N | %H |
| 1 | *Chroococcus disperus* | b | 0.3–2.50 | 0.153 | 0.994 | 56.48 ± 3.27 | 10.97 ± 0.75 | 7.50 ± 0.01 |
| 2 | *Gleocystis ampula* | g | 0.2–2.10 | 0.310 | 0.999 | 55.51 ± 3.86 | 7.53 ± 0.49 | 7.40 ± 0.13 |
| 3 | *Synechocystis* (I) | b | 0.17–1.85 | 0.154 | 0.998 | 53.32 ± 7.38 | 11.52 ± 0.10 | 7.77 ± 0.20 |
| 4 | *Scenedesmus obliguus* (I) | g | 0.15–2.20 | 0.250 | 0.998 | 50.40 ± 0.62 | 7.52 ± 0.13 | 7.33 ± 0.00 |
| 5 | *Chlorella vulgaris* (I) | g | 0.15–1.88 | 0.194 | 0.998 | 57.52 ± 0.10 | 7.11 ± 0.15 | 7.83 ± 0.02 |
| 6 | *Phormidium uncinatum* (I) | b | – | – | | 46.74 ± 0.17 | 8.97 ± 0.03 | 6.87 ± 0.02 |
| 7 | *Scenedesmus quadricauda* (I) | g | 0.18–2.87 | 0.432 | 0.999 | 48.81 ± 2.70 | 9.08 ± 0.50 | 7.62 ± 0.15 |
| 8 | *Synechocystis* (II) | b | 0.44–2.12 | 0.171 | 0.997 | 49.82 ± 0.09 | 10.83 ± 0.11 | 7.35 ± 0.01 |
| 9 | *Phormidium uncinatum* (II) | b | – | – | | 43.77 ± 0.80 | 9.84 ± 0.26 | 6.73 ± 0.08 |
| 10 | *Scenedesmus dimorphus* | g | 0.20–2.10 | 0.249 | 0.998 | 51.45 ± 0.62 | 7.21 ± 0.05 | 7.58 ± 0.07 |
| 11 | *Microcystis aeruginosa* | b | 0.2–1.90 | 0.087 | 0.999 | 51.30 ± 0.18 | 11.50 ± 0.22 | 7.45 ± 0.07 |
| 12 | *Chlorella vulgaris* (II) | g | 0.20–1.79 | 0.207 | 0.992 | 56.37 ± 0.92 | 7.28 ± 0.12 | 7.81 ± 0.04 |
| 13 | *Cyanobacterium cedrorum* | b | 0.18–2.24 | 0.133 | 0.990 | 52.21 ± 0.07 | 10.81 ± 0.08 | 7.19 ± 0.10 |
| 14 | *Chroococcus* sp. (I) | b | 0.25–2.53 | 0.171 | 0.990 | 51.33 ± 4.67 | 10.80 ± 0.27 | 7.63 ± 0.07 |
| 15 | *Kirchneriella lunaris* | g | 0.23–1.96 | 0.151 | 0.997 | 51.29 ± 0.83 | 4.99 ± 0.01 | 7.66 ± 0.06 |
| 16 | *Scenedesmus quadricauda* (II) | g | 0.22–1.85 | 0.359 | 0.999 | 51.77 ± 0.49 | 7.37 ± 0.25 | 7.32 ± 0.01 |
| 17 | *Chlorella vulgaris* (III) | g | 0.20–2.65 | 0.249 | 0.993 | 49.16 ± 1.79 | 6.54 ± 0.05 | 7.41 ± 0.11 |
| 18 | *Nannochloris* sp. | g | 0.17–2.00 | 0.198 | 0.990 | 53.70 ± 1.99 | 6.73 ± 0.07 | 7.64 ± 0.15 |
| 19 | *Chroococcus* sp. (II) | b | 0.25–2.40 | 0.162 | 0.990 | 48.32 ± 1.44 | 10.49 ± 0.93 | 7.13 ± 0.25 |
| 20 | *Micrococcus* sp. | b | 0.21–2.40 | 0.176 | 0.999 | 54.96 ± 2.79 | 12.16 ± 0.82 | 8.05 ± 0.08 |

**Table 3** Growth characteristics and biochemical compositions of isolated microalgae

| No. | Growth rate ($g\ l^{-1}\ d^{-1}$) | Max conc. ($g\ l^{-1}$) | $CO_2$ fixing rate ($g\ l^{-1}\ d^{-1}$) | Lipid (%) | CHO (%) | PRO (%) | Lip pro. rate ($mg\ l^{-1}\ d^{-1}$) | Settling efficiency after 24 h (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.109 ± 0.006 | 2.024 ± 0.011 | 0.226 ± 0.018 | 6.73 ± 2.29 | 19.68 ± 1.58 | 52.45 ± 7.70 | 7.3 ± 2.5 | 0.35 |
| 2 | 0.138 ± 0.008 | 2.625 ± 0.023 | 0.281 ± 0.025 | 21.71 ± 3.68 | 31.26 ± 5.69 | 35.98 ± 5.22 | 30.0 ± 5.1 | 96.04 |
| 3 | 0.101 ± 0.007 | 1.838 ± 0.002 | 0.196 ± 0.030 | 4.23 ± 1.21 | 26.62 ± 0.74 | 55.07 ± 7.16 | 4.3 ± 1.2 | 2.53 |
| 4 | 0.110 ± 0.005 | 1.679 ± 0.001 | 0.202 ± 0.010 | 17.49 ± 0.20 | 41.67 ± 6.69 | 35.94 ± 4.70 | 19.2 ± 0.2 | 92.92 |
| 5 | 0.079 ± 0.005 | 1.023 ± 0.006 | 0.166 ± 0.011 | 31.13 ± 0.32 | 52.39 ± 6.12 | 33.97 ± 4.47 | 24.5 ± 0.3 | 53.52 |
| 7 | 0.110 ± 0.003 | 2.831 ± 0.061 | 0.196 ± 0.012 | 47.32 ± 0.40 | 41.04 ± 2.58 | 43.42 ± 6.12 | 51.9 ± 0.4 | 89.43 |
| 8 | 0.103 ± 0.005 | 1.980 ± 0.016 | 0.187 ± 0.009 | 24.58 ± 0.93 | 28.98 ± 0.91 | 51.79 ± 6.74 | 25.2 ± 1.0 | 2.00 |
| 10 | 0.137 ± 0.010 | 2.232 ± 0.001 | 0.259 ± 0.019 | 26.97 ± 4.44 | 55.02 ± 6.01 | 34.46 ± 4.48 | 37.0 ± 6.1 | 92.82 |
| 11 | 0.056 ± 0.004 | 0.657 ± 0.001 | 0.105 ± 0.008 | 39.70 ± 0.31 | 17.75 ± 1.61 | 54.99 ± 7.21 | 22.1 ± 0.2 | 1.19 |
| 12 | 0.133 ± 0.008 | 1.786 ± 0.001 | 0.274 ± 0.017 | 30.88 ± 3.00 | 55.45 ± 3.93 | 34.79 ± 4.55 | 40.9 ± 4.0 | 33.54 |
| 13 | 0.087 ± 0.007 | 1.662 ± 0.002 | 0.167 ± 0.013 | 16.60 ± 5.02 | 32.83 ± 4.28 | 51.68 ± 6.71 | 14.5 ± 4.4 | 1.76 |
| 14 | 0.100 ± 0.005 | 2.096 ± 0.006 | 0.188 ± 0.020 | 19.19 ± 3.19 | 27.80 ± 2.80 | 51.62 ± 6.82 | 19.2 ± 3.2 | 49.70 |
| 15 | 0.042 ± 0.001 | 0.896 ± 0.002 | 0.079 ± 0.002 | 24.55 ± 7.18 | 72.43 ± 6.40 | 23.84 ± 3.09 | 10.3 ± 3.0 | 14.03 |
| 16 | 0.128 ± 0.001 | 2.496 ± 0.025 | 0.243 ± 0.003 | 26.42 ± 2.77 | 38.24 ± 0.25 | 35.24 ± 4.73 | 33.8 ± 3.5 | 94.88 |
| 17 | 0.091 ± 0.010 | 1.382 ± 0.001 | 0.164 ± 0.019 | 29.78 ± 2.23 | 66.71 ± 14.50 | 31.26 ± 4.06 | 27.2 ± 2.0 | 65.52 |
| 18 | 0.077 ± 0.012 | 1.101 ± 0.001 | 0.151 ± 0.024 | 33.95 ± 1.90 | 67.69 ± 3.74 | 32.15 ± 4.18 | 26.0 ± 1.5 | 71.98 |
| 19 | 0.096 ± 0.008 | 2.084 ± 0.021 | 0.170 ± 0.015 | 24.30 ± 2.71 | 24.99 ± 1.04 | 50.12 ± 7.86 | 23.3 ± 2.6 | 0.03 |
| 20 | 0.106 ± 0.012 | 1.465 ± 0.003 | 0.213 ± 0.026 | 11.56 ± 4.70 | 27.19 ± 1.99 | 58.11 ± 8.50 | 12.2 ± 5.0 | 2.15 |

rates (Fig. 7) were 51.9 ± 0.4, 40.9 ± 4.0 and 37.0 ± 6.1 mg. $l^{-1}$. $d^{-1}$ respectively for *Scenedesmus quadricauda* (I), *Chlorella vulgaris* (II) and *Scenedesmus dimorphus*. The distribution of data into two categories of blue-green and green microalgae in Fig. 5 represented a haphazard distribution of the acquired data between two groups which presented lack of correlation. Additionally, the calculated correlation factor of 0.501 revealed no relation between the microalgae phylum and lipid content.

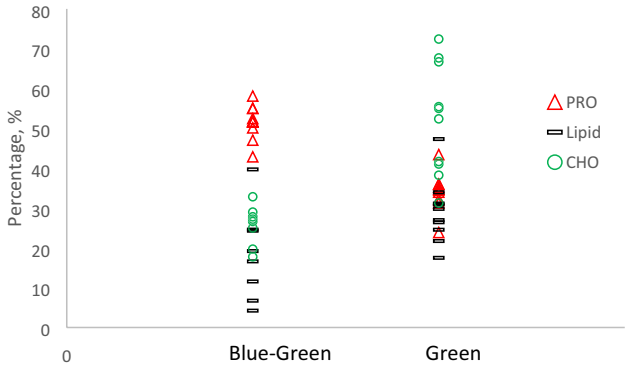



**Fig. 5** Distribution of lipid, carbohydrate and protein content in Blue-Green and Green microalgae

## Carbohydrate

The results for carbohydrate contents are presented in Table 3 and as bar charts in Fig. 5. The highest amount was recorded for *Kirchneriella lunaris* with 72.43 ± 6.40% of the dried mass where Shady et al. reported 75.0% for total carbohydrates of this microalgae [43]. At the second and third ranks were *Nannochloris* and *Chlorella vulgaris* (III) sp. respectively with 67.69 and 66.71% carbohydrate contents. All of these three species belonged to green microalgae family. The distribution of data into two categories of blue-green and green microalgae in Fig. 5 clearly shows that the green microalgae species had obviously higher carbohydrate content. The calculated correlation factor was 0.787 which revealed the significant correlation of microalgae type and carbohydrate content. The growth rate for these species were 0.042 ± 0.001, 0.077 ± 0.012 and 0.091 ± 0.010 g $l^{-1}$ $d^{-1}$ respectively. The growth rate could be optimized for each of the species to increase the production rate but with the same growth condition for all the species in this research, the daily carbohydrate production rate was estimated as 31 ± 3, 52 ± 8 and 61 ± 14 mg per liter of cultivation.

## Protein

As can be seen from Fig. 6 and Table 3, *Micrococcus*, *Synechocystis* (I) and *Microcystis aeruginosa* species

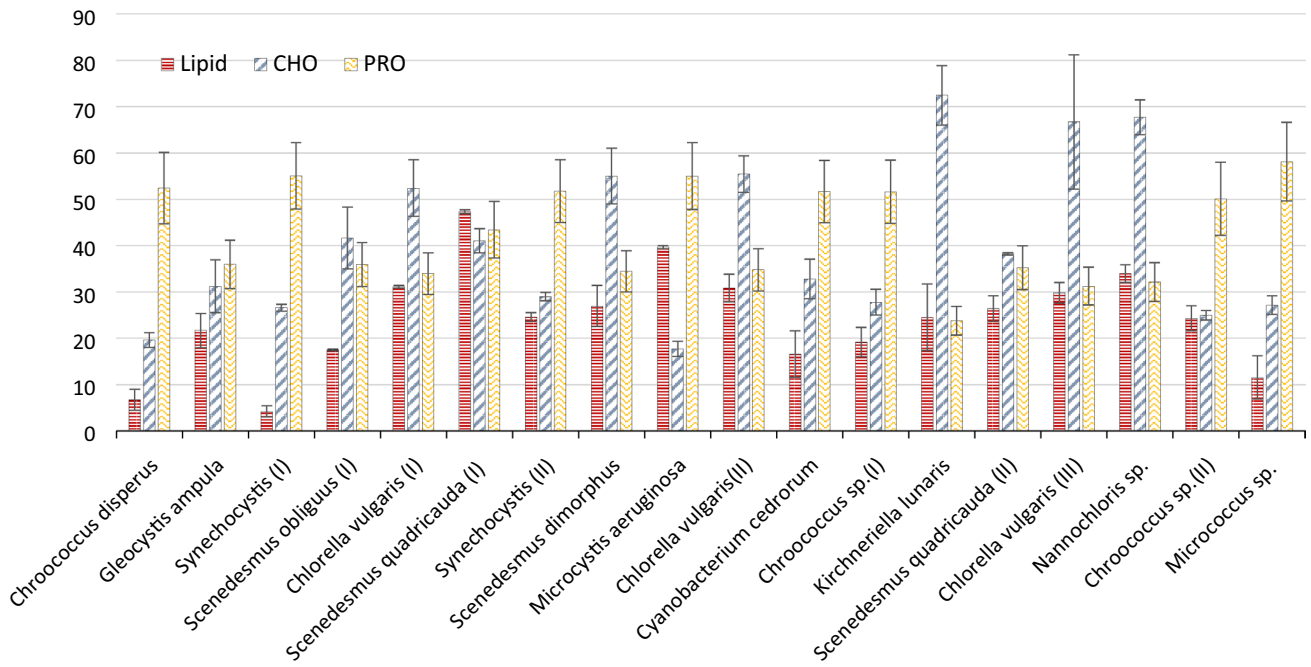



**Fig. 6** Lipid, carbohydrate and protein content of purified microalgae species

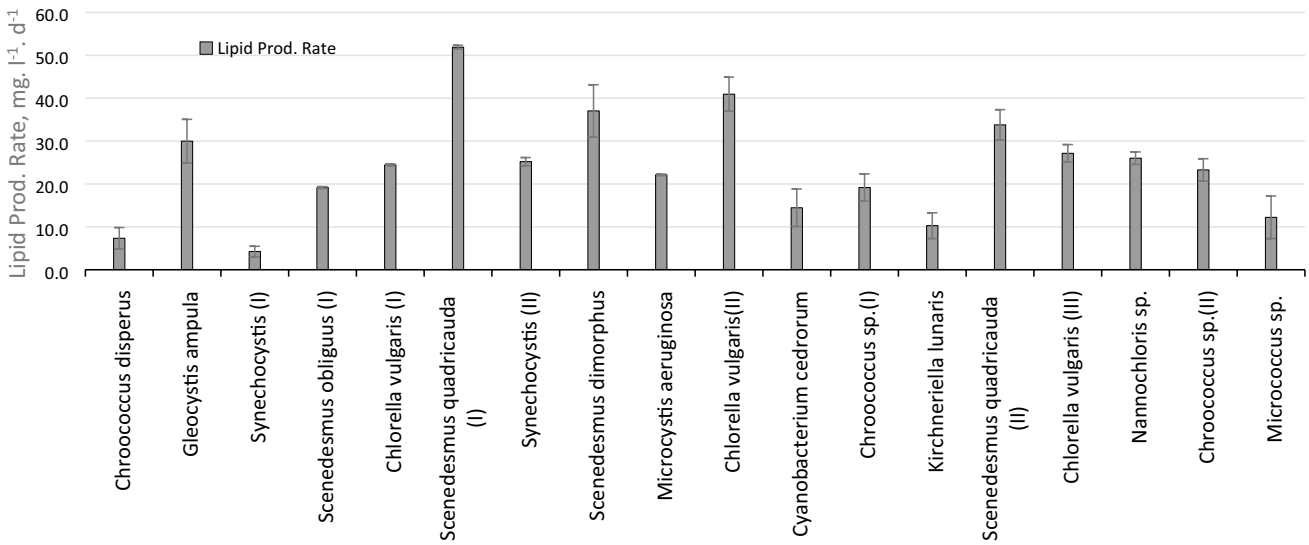



**Fig. 7** Lipid production rate of purified microalgae species

had the highest protein contents which in order were 58.11 ± 8.50, 55.07 ± 7.16 and 54.99 ± 7.21% of the dried mass. Interestingly they were all cyanobacteria i. e. blue-green microalgae. The averaged data on protein content for all the isolated blue-green species was 51.57 ± 2.24% while for green microalgae was 34.11 ± 1.46%. The distribution of data into two categories of blue-green and green microalgae in Fig. 5 clearly shows that the blue-green microalgae species had a significantly higher protein content where the correlation factor in this case was − 0.928 which showed a very strong relation between microalgae family and their protein content. The results for protein content is considerably high and can be potentially used as supplementary feed material for animals or aquaculture breeding industries [44] or even direct consumption by human [45].

## Microalgae Natural Settling behavior

Microalgae species settling behavior are different mainly because of their size, motion ability and their floating capabilities. From an application point of view this was important to be studied because the species which settle easily can be easily harvested without extra costs for processes such as centrifuge and chemical coagulation. The photograph in Fig. 8 shows that for some species the settling is almost complete like for example the microalgae Gleocystis ampula, *Scenedesmus quadricauda* (II) and *Scenedesmus obliquus* (I). The settling efficiency for mentioned species were respectively 96.04, 98.88 and 92.92%. For the Microalgae *Scenedesmus quadricauda* (I) which was the most suitable one for lipid production, the settling efficiency was 89.43% which was very significant. Contrarily, some species formed a very stable solution with almost no settling like for instance microalgae *Chroococcus* sp. (II), *Chroococcus disperus* and *Microcystis aeruginosa*. The analysis of correlation between estimated cell size and the settling efficiency showed a value of 0.75 which reveals a positive correlation of cell size and settling efficiency. The graph for scattered data in Fig. 9 showed that only one species i.e. *Kirchneriella lunaris* falls apart from these positive correlation. When this point was excluded, the correlation value even increased to 0.84. The calculated average of settling efficiency for blue-green and green species were 70.47 and 7.47% respectively which showed that blue-green microalgae form much more stable solutions than green microalgae. These results showed that green microalgae can be harvested more easily and perhaps less costly.

## Energy Analysis in Life Cycle of Microalgae to Dried Biomass

The obtained experimental data was used to perform an overall energy balance. As can be seen from Fig. 10 the energy consuming tasks includes microalgae plant operations like carbon dioxide/air supply, light and substrate supply, harvesting processes including dewatering using centrifuge and drying. Similar approaches for energy assessment have been followed in [46–51].

The energy efficiency (EE) was calculated as the ratio of obtainable energy via embedded energy in biomass as HHV over the total consumed energy;

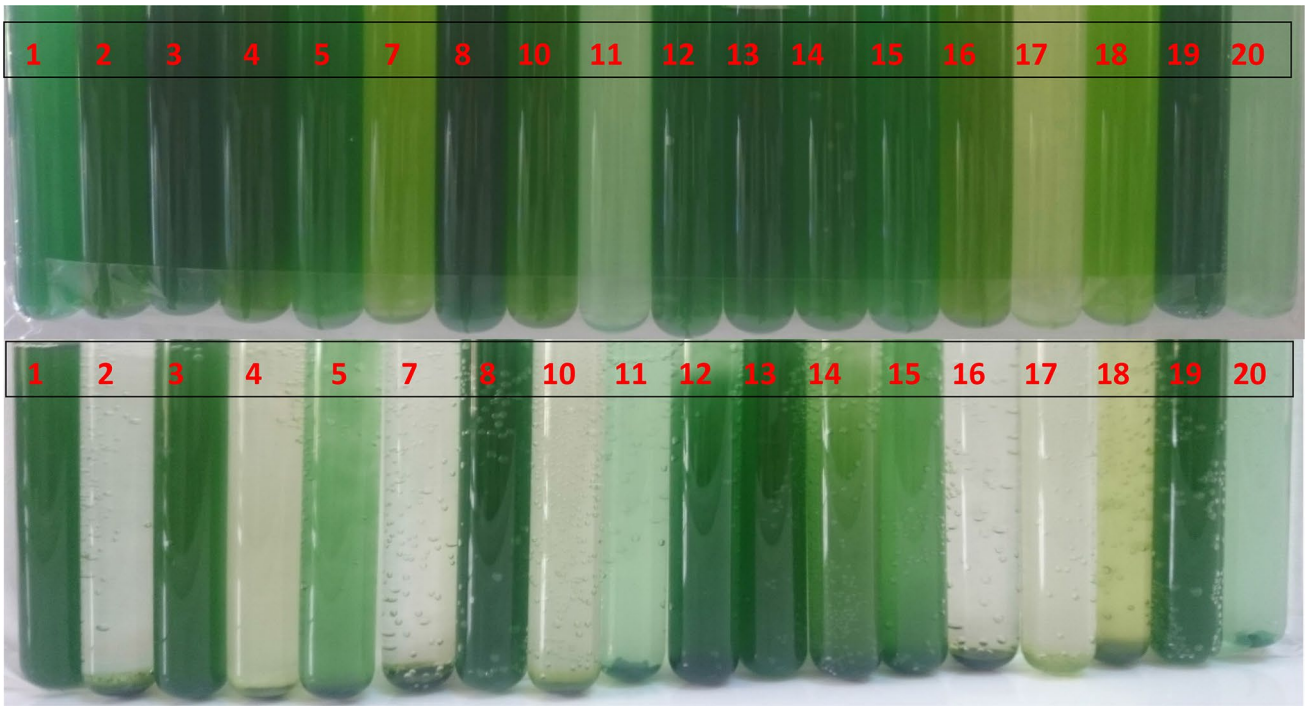



**Fig. 8** The natural settling of microalgae cells in the solution; Up: homogenous solutions at the beginning, Down: Settled solutions after 24 h for 1: *Chroococcus disperus*, 2: *Gleocystis ampula*, 3: *Synechocystis* (I), 4: *Scenedesmus obliguus* (I), 5: *Chlorella vulgaris* (I), 7: *Scenedesmus quadricauda* (I) 8: *Synechocystis* (II), 10: *Scenedesmus dimorphus*, 11: *Microcystis aeruginosa*, 12: *Chlorella vulgaris* (II), 13: *Cyanobacterium cedrorum*, 14: *Chroococcus* sp. (I), 15: *Kirchneriella lunaris*, 16: *Scenedesmus quadricauda* (II), 17: *Chlorella vulgaris* (III), 18: *Nannochloris* sp., 19: *Chroococcus* sp. (II) and 20: *Micrococcus* sp

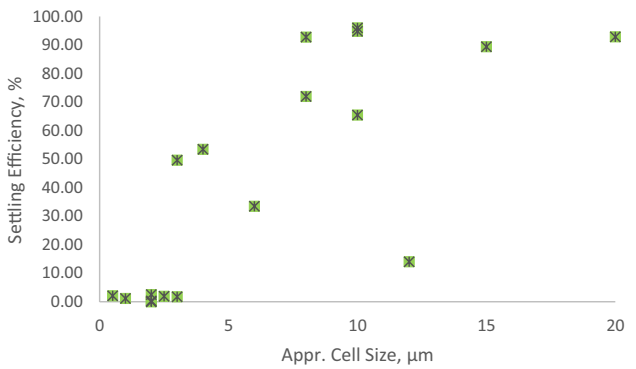



**Fig. 9** Distribution of natural settling efficiency with approximate cell size of isolated microalgae

$$NEE = HHV_{Pr}/Consumed\ Energy \quad (2)$$

The embedded energy in biomass was calculated using highest heating value (HHV) as was described in [6] using elemental analysis data. The calorific value of biomass more strongly depends on the carbon content of the biomass where considering biochemical composition, a higher lipid content is always desirable for its higher carbon content comparing to protein and carbohydrates [52, 53].

## Microalgae PBR Operational Energy Requirements

For a bubble column PBR, substrate along with microalgae inoculation charges into PBR and following the provision of light and carbon dioxide the operation starts. Type of PBR and its geometry significantly affects operational cost since in a low height PBR like for example in open raceway pond type, lesser energy will be required comparing to a bubble column where gas must be compressed and substrate to be pumped. In the present work, bubble column PBRs were used where the aeration rate was 0.1 vvm. Microalgae *Gleocystis ampula* with the highest GR of 0.138 g/l d and HHV of 27.185 kJ/g was selected for this evaluations.

For the ideal case (Table 1) it was supposed that the available gas mixture is in normal ambient temperature 298 K and 1 at pressure. The work of compressor was calculated by assuming an adiabatic compression. The measured and calculated data are presented in Table 4. The energy efficiency of 0.05 in the real application is very low which shows that the obtainable energy in the biomass is much lesser than the consumed energy. In comparison, the EE equal to 1.63 was estimated for an ideal process assuming that the compressor works ideally and the microalgae could settle naturally without the help of centrifuge. This is still a promising result because instead of bubble column PBRs, raceway ponds

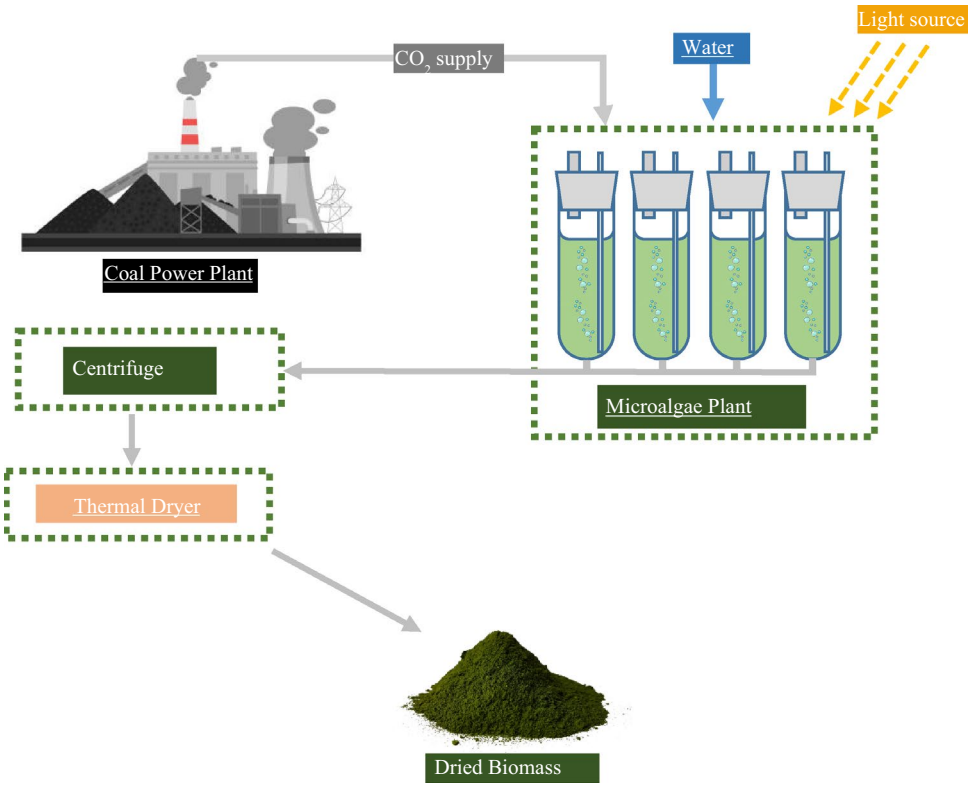



**Fig. 10** The schematic of microalgae dried biomass production line

might be used to avoid any compression energy which is the bottle neck of the cultivation step. The experimental settling behavior in Sect. 3.5 clearly shows that for some species the centrifuge step can be avoided practically. The other energy intensive part was the drying step with the calculated value of 14.6 kJ/g. This value also might be reduced substantially using advanced solar dryers [54].

Drying is the most energy consuming step which may constitute 70–75% of total harvesting cost [55]. There are different drying methods like solar drying as cheapest method, convective air drying, spray drying and freeze drying [56, 57]. Every methods comes with cons and pros like for example freeze drying is the best in preserving the quality of biomass while spray dryers are fast and more applicable in large scale but still very high energy consuming process [57]. Drying with solar systems or substantially efficient technologies must be taken into account to avoid this much of huge energy uptake [57]. Using solar drying systems demands large land area while dryness up to 90% dw is even achievable based on the season and geographical location of the site [54]. There is also chance of evaluating the heat of the flue gas to increase evaporation rate or to reduce the size of drying facility when applied along with a solar system.

## Land Use of Microalgae Plant When Applied for $CO_2$ Capture

A coal burning power plant like ICDAS Biga facility (Çanakkale, Turkey) which produces 405 MWe electric power was taken as source of carbon dioxide. The aim was to estimate the size of such microalgae plant with the capacity for mitigation of all the $CO_2$ release of a fossil based power plant. It is assumed that all the required energy for microalgae plant is supplied by the power plant and therefore the produced $CO_2$ equivalent to the consumed energy is already considered in the feed $CO_2$ line to the microalgae plant. With an approximate emission factor of 762 kg $CO_2$/MWh of electric energy [58] for such plant, almost 7407 ton/day $CO_2$ is being released to the atmosphere. Based on the carbon

**Table 4** Energy assessment in life cycle of microalgae

| | Aeration (kJ/g) | Water supply (kJ/g) | Centrifuge (kJ/g) | Drying (15% to 98% dw) (kJ/g) | EE |
|---|---|---|---|---|---|
| Real case | 474.518 | 0.821 | 40.570 | 14.657 | 0.05 |
| Ideal case | 2.012 | 0.014 | na[a] | 14.657 | 1.63 |

[a]Natural settling with 97% efficiency was assumed

dioxide fixation rate equal to 0.281 ± 0.025 g $l^{-1}$ $d^{-1}$ for *Gleocystis ampula,* a microalgae photobioreactor facility with a rough size of 26.3 million $m^3$ will be needed to consume that amount of carbon dioxide. Assuming that for every cubic meter of photobioreactor, one square meter of land would be occupied, almost 2630 ha of area will be occupied for such facility. Hopefully, with optimization of process to double the growth rate and by efficient design of photobioreactors which occupy less land by half, the estimated 2630 ha would reduce to 657.5 ha which equals to the area of a 2560 m by 2560 m square which is very reasonable.

### Land Use of Microalgae Plant When Applied for Biodiesel Production

To have a more realistic understanding of the results about biochemical composition of microalgae for a biofuel production purpose, the estimated biodiesel rate with regard to the highest lipid production rate of this study was compared to the diesel production rate of an existing large capacity refinery. This method was introduced in [6] where microalgae plant size (MAPS) was estimated using Eq. (2).

$$MAPS\left(m^3\right) = \frac{D_{pr} \times \rho_d \times 0.159}{GR \times L \times C_f} \quad (3)$$

where $D_{pr}$ is diesel production rate of a fossil diesel producing factory (bpd), $\rho_d$ is biodiesel density (kg/$m^3$), GR is the growth rate of microalgae (g/l d), L is the lipid content of biomass (between 0–1.0), $C_f$ is the conversion factor of lipid to biodiesel (between 0–1.0) and 159 is units conversion coefficient. Ras Tanura Refinery (Aramco, Saudi Arabiya), a petroleum refinery complex with diesel production capacity of 175,000 bpd (By 2017) [59] was selected as reference. According to Wahlen et al. [60], the microalgae oil was converted to FAME with an efficiency of almost 77 percent for some green microalgae ($C_f$ = 0.77). Assuming an average density equal to 880 kg/$m^3$ (EN 14214) for biodiesel, GR = 0.110 g/l d, lipid content of 47% (L = 0.47) the size of photobioreactor facility to produce the same amount of diesel as in Ras Tanura Refinery, would roughly be 615 million $m^3$. This estimation is for biomass production without any optimization on growth condition or other downstream processes. In case where every cubic meter of photobioreactor would occupy one square meter then 61,500 ha of land will be required which is a very unfeasible size. Any modification which would result in doubling the rate will reduce the size of such facility by half. A previous research on optimizing growth rate of microalgae [6] showed that the growth rate could increase to 0.525 g/l d for microalgae Scenedesmus sp. It also possible to increase the lipid content using genetic engineering techniques [61] and even nutrient starving approaches to as high as 60%. Microalgae oil conversion to biodiesel with high yields of 90% is also reported in literature [62–64]. Recalculating MAPS with these conditions results in 86.4 million $m^3$. Optimistically assuming that 2 $m^3$ of PBR volume per each square meter of land could be constructed, then still 4320 ha will be occupied. This is equal to the area of square shaped land with 6.5 km each side. Although this is still a very large land area, it is not beyond possibility.

## Conclusion

The findings of the present research on the isolated microalgae species from central Anatolia region showed that there are promising species with potential use as biofuel feedstock. Some of them, especially from green microalgae, had considerable lipid content which makes them a potential biomass producer for oil based biofuel applications. Interestingly, their quiet high growth rate, which was actually achieved without optimization of the growth conditions, sounded promising for commercial scale biofuel projects.

Amongst the isolated species, there were species which naturally settle down in the solution almost completely when mixing were stopped. This is important because the harvesting costs is a bottle neck in microalgae biotechnology.

Life cycle assessment of microalgae to produce dried biomass for the real case of the present study and in parallel an ideal case showed that with some achievable improvements in the cultivation and drying step, application of microalgae technology as a biofuel source is not far beyond realization.

Microalgae plant size estimations showed that after improving the process efficiency including microalgae growth rate, lipid content, oil to biodiesel conversion and occupied land per unit volume of PBR, reasonable amount of land area would be occupied for large scale application of the technology for both $CO_2$ capture and/or biodiesel production in an scale comparable to the production capacity of fossil fuel production or burning plants.

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