

# Lipid Production by a CO<sub>2</sub>-Tolerant Green Microalga, *Chlorella* sp. MRA-1

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Since CO<sub>2</sub> concentrations in industrial flue gases are usually 10%–20%, one of the prerequisites for efficient CO<sub>2</sub> removal by algae is the level of tolerance of microalgal species to exposure to high concentrations of CO<sub>2</sub>. A newly isolated microalgal strain, *Chlorella* sp. MRA-1, could retain growth with high concentrations of CO<sub>2</sub> up to 15%. The highest lipid productivity for *Chlorella* sp. MRA-1 was 0.118 g/l/day with a 5% CO<sub>2</sub> concentration. Octadecenoic acid and hexadecanoic acid, the main components of biodiesel, accounted for 70% of the total fatty acids. A lipid content of 52% of dry cell weight was achieved with limited amounts of nitrogen. *Chlorella* sp. MRA-1 seems to be an ideal candidate for biodiesel production when cultured with high concentrations of CO<sub>2</sub>.

**Keywords:** Biodiesel, CO<sub>2</sub> tolerance, fatty acid composition, lipid production, microalgae

## Introduction

Biodiesel derived from soybean, windmill palm, rapeseed, and other oil plants has proved eminently suitable as a transport fuel and, because of its nontoxicity, biodegradability, and renewability, continues to generate widespread interest [20, 22]. Nevertheless, a major obstacle to increasing biodiesel production using these oil plants is their relatively high cost. In addition, growing these plants requires the allocation of large amounts of land and energy resources. Although oil plants already provide a small fraction of the liquid fuel demanded by our transportation systems, biodiesel production from oil plants still faces stiff competition from entrenched fossil fuel industries [16, 22].

Because of its higher lipid productivity and photosynthetic efficiencies in comparison with oil plants, microalgal lipids are currently regarded as the third-generation feedstock for sustainable biodiesel production [6, 7]. Furthermore, microalgae cultivation does not compete with food sources and can generate valuable co-products [31, 33]. Generally, phototrophic microalgal growth requires CO<sub>2</sub> as a carbon

source. In recent years, bioregenerative methods, particularly those using microalgae photosynthesis, have been implemented to reduce atmospheric CO<sub>2</sub> to ensure a safe and reliable living environment [11, 24]. Absorption of CO<sub>2</sub> using microalgae could convert CO<sub>2</sub> into microalgae biomass [24, 30], and producing 100 tons of algal biomass fixes roughly 183 tons of CO<sub>2</sub> [6]. At the same time, flue gases from power plants contain 10%–20% (v/v) CO<sub>2</sub>, which may provide a carbon source for large-scale microalgal cultivation [36]. Direct utilization of flue gas reduces the cost of pretreatment, but imposes extreme conditions on microalgae—such as high concentrations of CO<sub>2</sub>. Although most microalgae grow well under conditions of CO<sub>2</sub> concentrations from 1% to 5%, only a few highly CO<sub>2</sub>-tolerant species able to withstand high CO<sub>2</sub> concentrations (> 5%) have been found [1, 8, 15, 33].

For biodiesel production, the lipid content is another important factor in selecting microalgae species. Many microalgae can accumulate lipids due to excess photosynthesis, and some species can accumulate large amounts of lipids from heterotrophic or environmental stress factors, such as

nutrient deficiency [28]. In this study, the newly isolated microalgal strain *Chlorella* sp. MRA-1 was evaluated for its CO<sub>2</sub> tolerance, and the effects of CO<sub>2</sub> and nitrogen concentrations on growth and lipid accumulation were investigated.

## Materials and Methods

### Strain and Culturing Conditions

*Chlorella* sp. MRA-1 was isolated from a wastewater pond in Qingdao, China, and conserved at the China General Microbiological Culture Collection Center (CGMCC 4652; China). The strain was cultivated in a 500 ml glass bubble column (30 mm in diameter) containing 300 ml of BG-11 medium with 1.5 g/l NaNO<sub>3</sub>. The columns were maintained at 25 ± 2°C, bubbled with sterile gas composed of air supplemented with varying CO<sub>2</sub> concentrations (5%–30%). Trichromatic lamps (Y228-T5; NVC Lighting Technology Corporation, China), with spectra ranging from 380 to 780 nm, were used as light sources. Microalgal cells were grown under low illumination (50 μmol photons m<sup>-2</sup> s<sup>-1</sup>) for 3 days, and then subjected to high illumination (220 μmol photons m<sup>-2</sup> s<sup>-1</sup>) for another 10 days [19].

To assess the effects of different nitrogen concentrations on the growth and lipid accumulation of microalgae, we prepared four samples with an initial NaNO<sub>3</sub> concentration of 0.375, 2.5, 5, and 10 g/l; and blowing air supplemented with a 5% CO<sub>2</sub> concentration. The growth of microalgae was monitored using a UV/VIS spectrophotometer at 750 nm (UNICO 2600, Shanghai, China), and the final biomass was determined by measuring the dry cell weight (DCW). After 13 days, a 5 ml microalgae sample was taken and filtered through preweighted 0.8 μm microporous filter paper. The filter paper was oven-dried for 6 h at 105°C. The difference between the final weight and the weight of the paper before filtration was taken as the DCW.

### Lipid Extraction

Microalgal cells were harvested by centrifugation at 3,000 rpm for 10 min. A freeze-drying procedure was performed using a vacuum freeze dryer (Alpha1-2LD plus; Martin Christ GmbH, Osterode, Germany) to get dry algal powder. The lipids were extracted as previously described [3], with modifications. Four milliliters of chloroform and 2 ml of methanol were added to a glass tube containing 50 mg of dry algal powder. The mixture was shaken at 180 rpm at 30°C for 12 h, and then centrifuged at 3,000 rpm for 10 min. The supernatant was transferred to a new glass tube, and 2 ml of methanol and 3.6 ml of water were added to make the final ratio of chloroform:methanol:water to be 10:10:9 (v/v/v). Further centrifugation of this mixture at 3,000 rpm for 10 min produced two distinct layers. The chloroform layer was transferred into a preweighted glass tube and removed at 60°C under the protection of N<sub>2</sub>. The lipid was dried in a vacuum-drying oven (Liantian DZF-6050, Hangzhou, China) at 0.09 Mpa, 60°C, for 2 h. The final

weight was measured using an electronic balance (0.01 mg; XS105 DualRange; Mettler Toledo, Zurich, Switzerland). Extraction was performed in duplicate for each sample.

### Analysis of Fatty Acid Composition

Transesterification was performed by incubating the total lipids sample in 2 ml of methanol containing 2% (v/v) H<sub>2</sub>SO<sub>4</sub> for 2.5 h at 85°C. After cooling to room temperature, 1 ml of HPLC-grade hexane and 1 ml of saturated NaCl solution were added. The mixture was centrifuged for 10 min at 3,000 rpm, and the upper layer containing fatty acid methyl esters (FAMES) was obtained for further analysis. FAME analysis was performed with a gas chromatograph (Varian 450-GC; Varian Inc., USA) using a fused CP-WAX 58 column (25 m × 0.25 mm). The carried gas was helium, and 1 μl of methyl ester sample solution was injected for analysis. The split ratio was 1:30. The temperature program was as follows: column temperature was maintained at 100°C for 2 min and then elevated to 250°C at a rate of 10°C/min and maintained for a further 3 min. The injector temperature was set at 250°C. The flame-ionization detector temperature was set at 280°C. Fatty acids were identified by comparing the retention time obtained with the analytical standard FAME mixture C8–C24 (Sigma-Aldrich, Shanghai, China).

### Elemental Analysis

The elemental composition (C, H, N, and S) of microalgae biomass under different conditions was analyzed using Vario EL (Elementar Analysensysteme GmbH, Hanau, Germany) with helium as the carrier gas. The combustion temperature and oxygen pressure were 1,150°C and 0.25 MPa, respectively.

### Carbon Dioxide Fixation Rate

The carbon dioxide fixation rate of microalgae can be calculated by the following equation [35]:

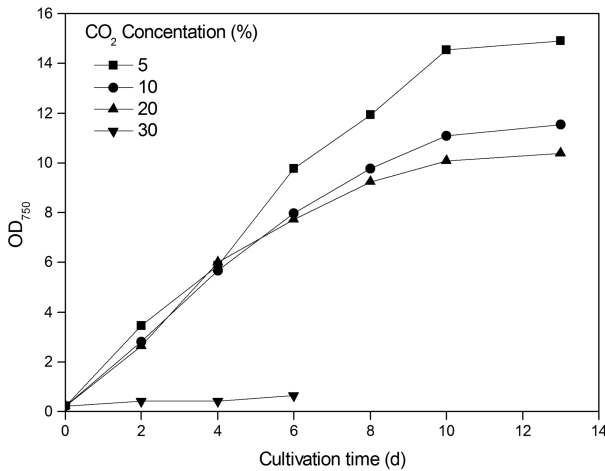
$$\text{Carbon dioxide fixation rate} = \frac{X_{\max} - X_0}{t} \times C \times \frac{M_{\text{CO}_2}}{M_C} \quad (1)$$

where  $X_{\max}$  and  $X_0$  represent maximum dry cell weight and initial inoculated dry cell weight (mg/l);  $t$  stands for the time required to reach the maximum dry cell weight; and  $C$  is the carbon content of the dry cell biomass per the element analyzer found in Table 2.  $M_{\text{CO}_2}$  and  $M_C$  represent the molecular weight of CO<sub>2</sub> and elemental carbon, respectively.

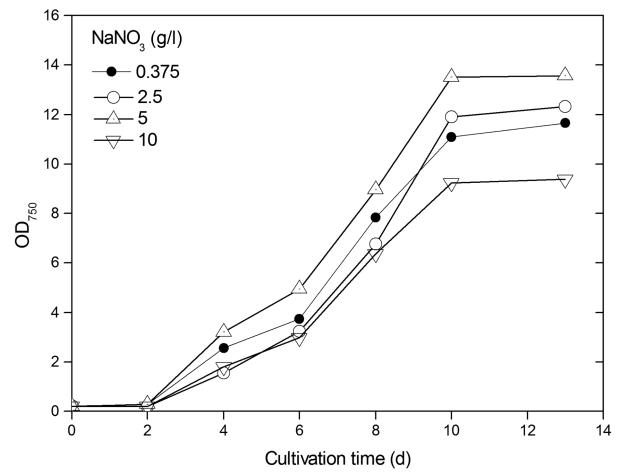
## Results

### Cultivation and Lipid Production of *Chlorella* sp. MRA-1 with Different Concentrations of CO<sub>2</sub>

*Chlorella* sp. MRA-1 could grow in the presence of 5% to 20% CO<sub>2</sub> and reached a stationary stage after 10 days (Fig. 1). The maximum biomass concentration of 4.48 ± 0.30 g/l was achieved with 5% CO<sub>2</sub>. With 10% and 20%



**Fig. 1.** Growth of *Chlorella* sp. MRA-1 under different CO<sub>2</sub> concentrations in BG-11 medium with 1.5 g/l NaNO<sub>3</sub>.



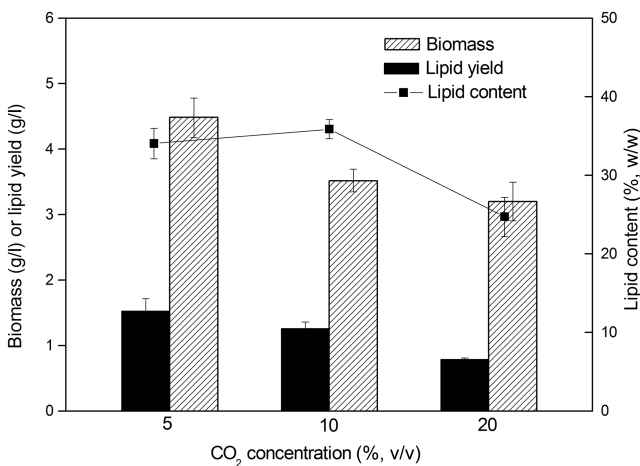
**Fig. 3.** Growth of *Chlorella* sp. MRA-1 with different nitrogen concentrations in BG-11 medium with 5% CO<sub>2</sub>.

CO<sub>2</sub>, biomasses of 3.52 ± 0.17 and 3.20 ± 0.29 g/l were obtained, respectively. After 13 days, the lipid contents and lipid yields of strain MRA-1 (grown with different CO<sub>2</sub> concentrations) were compared (Fig. 2). The highest lipid yield of 1.53 g/l was obtained when cultured with 5% CO<sub>2</sub>. When the CO<sub>2</sub> concentration was increased, the lipid yield decreased greatly (Fig. 2). Lipid yields of the cells grown with CO<sub>2</sub> concentrations of 10% and 20% were 1.26 and 0.79 g/l, respectively.

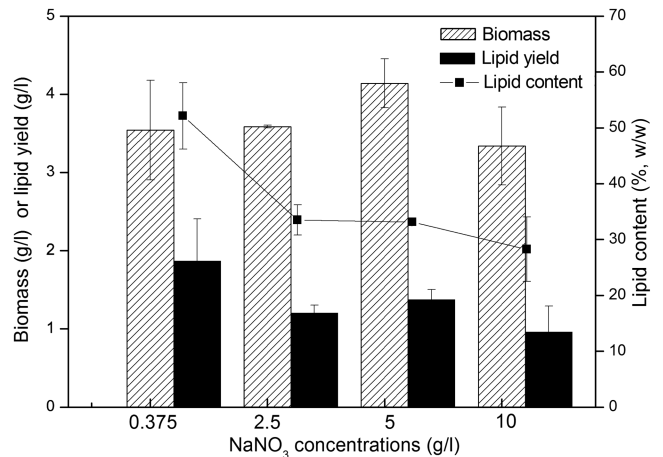
**Effects of Different Nitrogen Concentrations on the Growth and Lipid Accumulation of *Chlorella* sp. MRA-1**

To improve the lipid productivity, the effects of different

nitrogen concentrations on the growth and lipid accumulation of *Chlorella* sp. MRA-1 were investigated (Fig. 3). The maximum biomass concentration of 4.14 ± 0.31 g/l was achieved in 13 days when the alga was grown in BG-11 medium with 5 g/l NaNO<sub>3</sub>. Microalgal cells were collected, and extractable lipid contents (% DCW) were determined by the gravimetric method. The lipid contents and productivities of *Chlorella* sp. MRA-1, grown with different concentrations of NaNO<sub>3</sub>, are shown in Fig. 4. Strain MRA-1 showed the highest lipid content of 52.1% DCW with 0.375 g/l NaNO<sub>3</sub>. The lipid contents of the cells grown with 2.5, 5, and 10 g/l NaNO<sub>3</sub> were significantly lower than with 0.375 g/l NaNO<sub>3</sub>. As has been shown, nitrogen limitation



**Fig. 2.** Algae biomass, lipid yield, and lipid content of *Chlorella* sp. MRA-1 in BG-11 medium with 1.5 g/l NaNO<sub>3</sub> under different CO<sub>2</sub> concentrations.



**Fig. 4.** Algae biomass, lipid yield, and lipid content of *Chlorella* sp. MRA-1 grown in BG-11 medium with different concentrations of NaNO<sub>3</sub> with 5% CO<sub>2</sub>.

**Table 1.** Fatty acid composition of *Chlorella* sp. MRA-1 grown with different CO<sub>2</sub> and NaNO<sub>3</sub> concentrations.

| Fatty acid                  | NaNO <sub>3</sub> concentration (g/l) |       |       |       | CO <sub>2</sub> concentration (%) |       |       |
|-----------------------------|---------------------------------------|-------|-------|-------|-----------------------------------|-------|-------|
|                             | 0.375                                 | 2.5   | 5     | 10    | 5                                 | 10    | 20    |
| Saturated fatty acids       |                                       |       |       |       |                                   |       |       |
| C16:0                       | 18.36                                 | 18.14 | 19.83 | 17.43 | 16.09                             | 18.57 | 17.96 |
| C18:0                       | 2.01                                  | 2.29  | 3.07  | 0.99  | 0.77                              | 1.97  | 1.54  |
| C20:0                       | 0.10                                  | 0.22  | 0.25  | 0.12  | 0.16                              | 0.11  | 0.08  |
| C22:0                       | 0.18                                  | 0.17  | 0.24  | 0.09  | 0.23                              | 0.19  | 0.17  |
| C24:0                       | 0.83                                  | 0.51  | 1.12  | 0.91  | 0.37                              | 0.85  | 0.70  |
| Sum                         | 21.48                                 | 21.32 | 24.51 | 19.53 | 17.61                             | 21.69 | 20.45 |
| Monounsaturated fatty acids |                                       |       |       |       |                                   |       |       |
| C16:1                       | 0.79                                  | 0.82  | 0.79  | 3.25  | 1.07                              | 0.82  | 0.83  |
| C18:1                       | 52.04                                 | 51.91 | 53.49 | 56.98 | 57.77                             | 54.31 | 55.46 |
| C20:1                       | 0.22                                  | 0.09  | 0.20  | 0.21  | 0.22                              | 0.14  | 0.12  |
| Sum                         | 53.04                                 | 52.82 | 54.48 | 60.43 | 59.07                             | 55.27 | 56.41 |
| Polyunsaturated fatty acids |                                       |       |       |       |                                   |       |       |
| C16:2                       | 3.63                                  | 3.99  | 3.04  | 2.95  | 2.47                              | 2.20  | 2.26  |
| C16:3                       | 3.08                                  | 2.97  | 2.42  | 1.00  | 4.12                              | 3.99  | 3.82  |
| C18:2                       | 9.87                                  | 10.44 | 8.37  | 7.80  | 5.82                              | 6.66  | 7.20  |
| C18:3                       | 8.83                                  | 8.39  | 7.13  | 8.20  | 10.86                             | 10.13 | 9.87  |
| C18:4                       | 0.07                                  | 0.07  | 0.05  | 0.07  | 0.05                              | 0.07  | 0.00  |
| Sum                         | 25.48                                 | 25.86 | 21.01 | 20.03 | 23.31                             | 23.05 | 23.14 |

can stimulate lipid accumulation in *Chlorella* sp. MRA-1. In this study, the maximum lipid yield of 1.87 g/l was achieved with a nitrogen limitation of 0.375 g/l NaNO<sub>3</sub>.

#### Fatty Acid Composition of *Chlorella* sp. MRA-1 Grown with Different CO<sub>2</sub> and NaNO<sub>3</sub> Concentrations

The fatty acid composition of *Chlorella* sp. MRA-1 grown with different CO<sub>2</sub> and NaNO<sub>3</sub> concentrations was analyzed using GC (Table 1). The major fatty acid components for all cells grown under different conditions were octadecenoic acid and hexadecanoic acid, constituting 70% of the total

fatty acid content. The fatty acid profiles of total lipids were similar for *Chlorella* sp. MRA-1 grown with different concentrations of NaNO<sub>3</sub>. However, the proportion of monounsaturated fatty acids that included C16:1, C18:1, and C20:1 reached 60.43% of the total lipid composition when strain MRA-1 was cultured with 10 g/l NaNO<sub>3</sub>. This amount was 11%–14% higher than for the cells grown with 0.375, 2.5, and 5 g/l NaNO<sub>3</sub>.

#### Element Analysis and Carbon Dioxide Fixation Rate

The levels of carbon, nitrogen, hydrogen, and sulfur in

**Table 2.** Elemental analysis of *Chlorella* sp. MRA-1 grown with different CO<sub>2</sub> and NaNO<sub>3</sub> concentrations.

| Element | NaNO <sub>3</sub> concentration (g/l) |              |              |              | CO <sub>2</sub> concentration (%) |              |              |
|---------|---------------------------------------|--------------|--------------|--------------|-----------------------------------|--------------|--------------|
|         | 0.375                                 | 2.5          | 5            | 10           | 5                                 | 10           | 20           |
| N%      | 2.48 ± 0.14                           | 6.03 ± 1.69  | 6.23 ± 0.13  | 6.02 ± 0.41  | 6.00 ± 0.18                       | 5.82 ± 0.12  | 4.98 ± 0.05  |
| C%      | 53.72 ± 1.30                          | 51.57 ± 1.72 | 51.48 ± 1.17 | 50.04 ± 1.67 | 52.18 ± 2.80                      | 56.11 ± 4.13 | 56.69 ± 5.65 |
| H%      | 7.46 ± 0.14                           | 7.22 ± 0.25  | 7.03 ± 0.15  | 6.94 ± 0.17  | 7.32 ± 0.29                       | 7.27 ± 0.25  | 7.13 ± 0.34  |
| S%      | 2.33 ± 1.00                           | 1.31 ± 0.23  | 1.09 ± 0.16  | 1.21 ± 0.20  | 1.20 ± 0.29                       | 1.18 ± 0.23  | 0.98 ± 0.18  |
| C/N     | 21.78 ± 1.51                          | 9.74 ± 5.12  | 8.28 ± 0.36  | 8.36 ± 0.78  | 8.71 ± 0.59                       | 9.64 ± 0.74  | 11.39 ± 1.22 |
| C/H     | 7.20 ± 0.07                           | 7.14 ± 0.08  | 7.32 ± 0.04  | 7.21 ± 0.11  | 7.12 ± 0.13                       | 7.71 ± 0.35  | 7.94 ± 0.46  |

Data are reported as means ± standard deviation of triplicates.

**Table 3.** Tolerable concentration of CO<sub>2</sub> and biomass yield by microalgae.

| Species                               | CO <sub>2</sub> concentration (%) | Biomass yield (g/l/day) | Lipid productivity (mg/l/day) | Cultivation condition               | References    |
|---------------------------------------|-----------------------------------|-------------------------|-------------------------------|-------------------------------------|---------------|
| <i>Botryococcus braunii</i> SAG-30.81 | 5                                 | 0.207                   | 30.81                         | BioFlo Fermentor                    | [27]          |
| <i>Botryococcus raunii</i>            | 10                                | 0.026                   | 5.51                          | Bioreactor                          | [30]          |
| <i>Chlorella</i> sp.                  | 9–10                              | 0.15                    | NA                            | Erlenmeyer flasks                   | [18]          |
| <i>Chlorella vulgaris</i> LEB-104     | 5                                 | 0.31                    | 11.54                         | BioFlo Fermentor                    | [27]          |
| <i>Spirulina platensis</i> LEB-52     | 5                                 | 0.73                    | 14.3                          | BioFlo Fermentor                    | [26]          |
| <i>Chlorella kessleri</i>             | 6                                 | 0.206                   | NA                            | Vertical tubular photobioreactors   | [9]           |
| <i>Chlorella</i> sp.                  | 6–8                               | 0.323–0.38              | NA                            | Thin-layer photobioreactor          | [10]          |
| <i>Chlorella</i> sp. MRA-1            | 5                                 | 0.344                   | 117.6                         | Column                              | This research |
|                                       | 10                                | 0.271                   | 96.92                         | Column                              | This research |
|                                       | 20                                | 0.246                   | 60.76                         | Column                              | This research |
| <i>Scenedesmus</i> sp.                | 10                                | 0.217                   | 39.44                         | Bioreactor                          | [30]          |
| <i>Scenedesmus obliquus</i>           | 12                                | 0.106                   | NA                            | Vertical tubular photobioreactors   | [9]           |
| <i>Synechocystis aquatilis</i>        | 10                                | 0.15                    | NA                            | Vertical flat-plate photobioreactor | [37]          |

NA: not available.

*Chlorella* sp. MRA-1 grown with different CO<sub>2</sub> and NaNO<sub>3</sub> concentrations were analyzed (Table 2). The carbon content was 53.72% ± 1.30% when grown with 0.375 g/l NaNO<sub>3</sub>, which was higher than that found in cells grown with sufficient nitrogen. Cultivation with high concentrations of CO<sub>2</sub> (10% and 20%) also led to microalgae cells exhibiting a carbon content greater than 56%. However, the highest C/N ratio of 21.78 ± 1.51 was obtained when the cells were cultured with 0.375 g/l NaNO<sub>3</sub>. This indicated that nitrogen deficiency could induce *Chlorella* sp. MRA-1 to produce more lipid than CO<sub>2</sub> stress.

The carbon dioxide fixation rate was compared when this strain was cultured with different CO<sub>2</sub> concentrations (Table 4). The maximum CO<sub>2</sub> fixation rate of *Chlorella* sp. MRA-1 was 710.7 mg/l/day when cultured with 5% CO<sub>2</sub>. The CO<sub>2</sub> fixation rate declined when higher levels of CO<sub>2</sub> were supplied.

## Discussion

Waste gases from thermal power stations contain 10%–

20% (v/v) CO<sub>2</sub>, which may provide a carbon source for microalgae cultivation. Several microalgal species have been tested with high CO<sub>2</sub> concentrations (Table 3). *Chlorococcum littorale* could grow with 60% CO<sub>2</sub> using the stepwise adaptation technique [5]. Another high CO<sub>2</sub>-tolerant species is *Euglena gracilis* whose growth was enhanced under 5%–45% of CO<sub>2</sub>. *Scenedesmus* sp. could grow with 80% CO<sub>2</sub>, but maximum cell mass was observed with 10%–20% CO<sub>2</sub> [13]. It is also reported that some species such as *Chlorella* sp. and *Cyanidium* sp. could even grow with 100% CO<sub>2</sub> [2, 21].

Hu *et al.* [14] summarized the total lipid contents of oleaginous green algae reported in the literature, which showed an average total lipid content of 25.5% DCW. Mata *et al.* [23] suggested *Chlorella* sp. to be a good option for biodiesel production, according to a wide range of literature. In a previous study reported by Demorais and Costa [9], *Chlorella kessleri* isolated from a coal-fired thermoelectric power plant showed a maximum biomass productivity of 0.087 g/l/day with 6% CO<sub>2</sub>, even growing in a medium containing up to 18% CO<sub>2</sub>. Tang *et al.* [29] reported that a

**Table 4.** Carbon dioxide fixation rate of *Chlorella* sp. MRA-1 in this work.

| CO <sub>2</sub> concentration (%) | Carbon dioxide fixation rate (mg CO <sub>2</sub> /l/day) | Biomass yield (mg/l/day) | Culture medium |
|-----------------------------------|--|--------------------------|----------------|
| 5                                 | 710.7  | 344                      | BG-11          |
| 10                                | 599.7  | 271                      | BG-11          |
| 20                                | 548.6  | 246                      | BG-11          |



lipid content of 20.65% DCW was achieved by a CO<sub>2</sub>-tolerant microalga, *Chlorella sorokiniana* CS-01, when cultured with 5% CO<sub>2</sub> [29]. The lipid content of *Chlorella vulgaris* grown with 10% CO<sub>2</sub> was found to be less than 11.92% DCW [32]. In the present study, it was shown that *Chlorella* sp. MRA-1 could grow with 5%–20% CO<sub>2</sub>, with the best growth (0.34 g/l/day) achieved at 5% CO<sub>2</sub>. The highest CO<sub>2</sub> fixation rate was achieved when the strain was grown with 5% CO<sub>2</sub>. Lipid content and lipid productivity of *Chlorella* sp. MRA-1, when cultured with 5% CO<sub>2</sub>, were 34.1% DCW and 116 mg/l/day, respectively.

A high content of algal lipid increases the process yield coefficient and reduces the cost of extraction and purification per unit product [4]. Algal lipids respond to nitrogen deprivation stress and CO<sub>2</sub> enrichment in a heterogeneous fashion. Pratt and Johnson [25] proposed that lipid synthesis was promoted at high CO<sub>2</sub> levels in *Chlorella*. In this study, we also found that a higher lipid content was achieved with higher CO<sub>2</sub> concentrations. On the other hand, it is known that nitrogen deprivation promotes lipid accumulation [17]. In the present study, *Chlorella* sp. MRA-1 accumulated more lipid through nitrogen limitation; the highest lipid content of 52.1% DCW was obtained when cells were grown in BG-11 medium with 0.375 g/l NaNO<sub>3</sub>—one-fourth the normal nitrogen concentration.

The unsaturation degree of fatty acids in *Chlorella vulgaris* was inversely related to the CO<sub>2</sub> concentration. Tsuzuki et al. [30] reported that the unsaturation levels of fatty acids in *C. vulgaris* were greater in low-CO<sub>2</sub> cells than in high-CO<sub>2</sub> cells, indicating a shift of the relative content of saturated and unsaturated fatty acids with a change in CO<sub>2</sub> concentrations. In this study, we also found that the contents of unsaturated fatty acid (monounsaturated fatty acids and polyunsaturated fatty acids) were greater in low-CO<sub>2</sub> cells than in high-CO<sub>2</sub> cells in *Chlorella* sp. MRA-1. Moreover, the contents of monounsaturated fatty acids in lipids were lower under nitrogen starvation conditions, which is similar to *Tetraselmis subcordiformis* in previous reports where the major increase in fatty acids under nitrogen deprivation conditions was of the monounsaturated type [26]. An additional characteristic of oleaginous microalgae is the suitability of lipids for biodiesel in terms of the type and amount produced by a microalgal species (e.g., chain length, degree of saturation) [12]. This study confirms that the major components of the lipids from *Chlorella* sp. MRA-1 are suitable for biodiesel production.

In conclusion, the newly isolated strain *Chlorella* sp. MRA-1, with its tolerance to high concentrations of CO<sub>2</sub>

and high lipid productivity, could efficiently exploit flue gases for biodiesel production. We also found that nitrogen deficiency effectively induced the strain to produce more lipid than CO<sub>2</sub> stress. *Chlorella* sp. MRA-1 seems to be an ideal candidate for biodiesel production when cultured with high concentrations of CO<sub>2</sub>.

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