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# Growth response of *Spirulina platensis* PCC9108 to elevated $CO_2$ levels and flue gas

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## Abstract

**Introduction**: Because their ability to capture $CO_2$, photosynthetical microorganisms have some advantages to $CO_2$ mitigation from high $CO_2$ streams such as flue gases and they can use $CO_2$ as carbon source. Recently, experts have made efforts to exploit microorganisms intended for recovering $CO_2$ from power plants.

**Materials and method**s: To achieve this purpose, we studied the growth response of the cyanobacterium *Spirulina platensis* PCC9108 under different concentrations of carbon dioxide (ranging from 0.036% to 10%) and flue gas in a bench-scale system. Preparation of different concentrations of $CO_2$ and injection into Erlenmeyer flasks was performed by a system including air compressor, $CO_2$ capsule, pressure gauge and flow meter.

**Results**: The main goal of studying this paper is a survey of organism's potential to grow by generated $CO_2$ from flue gas of power plant. It already had the potential and highest biomass production recorded at 8% $CO_2$ (v/v). Also we proved that *S.platensis* PCC9108 can be grown under flue gas, although biomass production decreased fairly. Total lipid content of algae interestingly enhanced with elevated $CO_2$ levels from ambient air to 4% and 6% which ranged from 14.5 to 15.8 and 16 dry weight (wt.) % respectively. In contrast, total protein content illustrated no difference between all treatment and its value was about 46 wt.%.

**Discussion and conclusion:** The results of present study suggested that understudied *S.platensis* PCC9108 is appropriate for mitigating $CO_2$ because of its carbon fixation ability. Also due to its high protein content, this cyanobacterium is a good candidate to produce SCP (single cell protein).

**Key words:** Biofixation of $CO_2$, Biomass production, Flue gas, Total lipid content, Total protein content, *Spirulina platensis*

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## Introduction

Considerable anthropogenic emissions caused atmospheric $CO_2$ (carbon dioxide) concentrations to get higher from an interglacial level of 280 ppm to presently 385 ppm (1). Currently, main sources of energy are fossil fuels which combustion of them is responsible for increased $CO_2$ levels (2, 3, 4). More than 170 countries have ratified the Kyoto protocol of the United Nations to make efforts for reducing greenhouse gases by 5.2% on the basis of the emissions in 1990 (5). One of the most understudied methods of $CO_2$ reduction is the use of microalgae that convert $CO_2$ from a point source into biomass (6, 7). These microorganisms have some advantages to $CO_2$ mitigation such as their ability to capture $CO_2$ from high $CO_2$ streams like flue gases (4, 8 & 9) and more efficiency for converting solar energy into biomass rather than terrestrial plants (10). Numerous studies have suggested that marine cyanobacteria are good choice to achieve this purpose (11). The anthropogenic emission of $CO_2$ from coal-fired thermoelectric plants is responsible for up to 7% of global $CO_2$ (12). Industrial exhaust gases contain up to 15% $CO_2$ providing a $CO_2$-rich source for microalgae cultivation and a potentially more efficient route for $CO_2$ biofixation (13). Although earlier it was assumed that these microorganisms are susceptible to high $CO_2$ concentration, some microalgae are now reported to grow rapidly at this condition (14, 15). Utilization of this source faced to some challenges such as high temperature, decreasing growth of some microalgae in elevated $CO_2$ and the effect of toxic gases included flue gas such as $SO_X$, $NO_X$ and CO on the growth of these organisms (8, 9).

Microalgae can fix carbon dioxide from different sources including $CO_2$ from the atmosphere, from industrial exhaust gases and in form of soluble carbonates (16). As microalgae react to concentrations of $CO_2$ differently, a bench-scale experiment is necessary for investigating response of selected microalgae to elevated $CO_2$ concentration. The aims of this study were evaluation of growth response of a cyanobacterium, *Spirulina platensis* PCC9108 to different $CO_2$ concentrations and emissive flue gas from a stove. The response was monitored in terms of changes in number of cells, dry weight of biomass and chlorophyll content. We investigated the effect of $CO_2$ and flue gas on total lipid and protein content, too.

## Materials and Methods

### Microorganisms and cultivation media

The microalgae *Spirulina platensis* PCC9108, was provided by the Pasteur Institute, France. Cells were cultivated in the modified culture medium including BG-11 and ASN-III with ratio 1:1 (v/v) (17). As the aim was investigation of different $CO_2$ concentrations effect on *S. platensis* growth, modified medium without carbon source, $NaHCO_3$, was used (18).

### Cultivation conditions and aeration system

All runs were carried out using a working volume of 400 ml in 500ml Erlenmeyer flasks and initial biomass concentration was 0.1 $gL^{-1}$ with OD ca. 0.13 at 670 nm (19, 20). Discontinuous light with 16h light:8h dark photoperiod was provided by means of four 40W-fluorescent lamps and periodically confirmed by an illuminance meter, model LEYBOLD 666 230, providing a light intensity of 3200 lux (lx) at the vessel's surface (21). In order to reduce the long lag phase, cultures cultivated in enriched air with 1% $CO_2$ for a week before inoculation (12, 21).

Preparation of different concentrations of $CO_2$ and injection into Erlenmeyer flasks performed by a system including air compressor, $CO_2$ capsule, pressure gauge

and flow meter. The pressure of injected air was formerly adjusted to 100 mbar by microregulator -composed of several pressure regulators stabilizing the pressure step by step- and lastly flowmeter set up flow rate of the input air to culture medium (Fig. 1). Finally, projected $CO_2$-enriched air was monitored by $CO_2$ analyzer (model Guardian D-600). Using above system, growth of *S.platensis* investigated under five concentrations of $CO_2$ (v/v) (ambient air as control (0.036%), 4%, 6%, 8% and 10%). In order to study flue gas effect on *S. platensis*, effluent way of a working stove connected to suction gate of air compressor (Fig. 2). Flue gas components was determined by flue gas analyzer (Testo model t350 XL). Table 1 lists some average characteristics of the flue gas during the sampling interval. All runs were aerated at a rate of 0.25 volume of intended air per volume of media per minute (v/vm) (7).

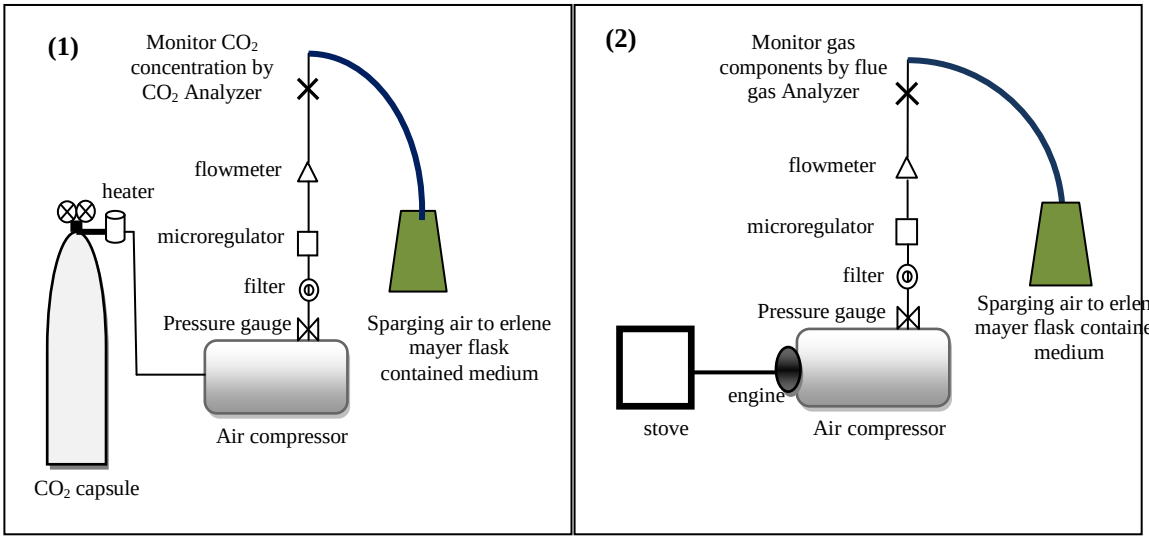



Fig. 1, 2- Schematic diagram of aeration system, (1) for preparing different concentrations of $CO_2$ and injects it into microalgal culture media, (2) for injection of flue gas from stove to microalgal culture media.

Table1-data from analysis of flue gas injected into *S. platensis* media.

| Flue gas component | NO | $NO_2$ | CO | $CO_2$ | $O_2$ | Temperature |
|---|---|---|---|---|---|---|
| Values | 24 ppm | 4 ppm | 7 ppm | 4% | 14.4% | 140.5°C |

**Analytic determinations**

Triplicate samples of culture media were collected aseptically every 24h and pH was also controlled by digital pH meter (CONSORT) at the same time. The biomass concentration (X, $gL^{-1}$) was estimated spectrophotometrically by measuring the optical density at 670 nm using a spectrophotometer (model SECOMAM XS5 Uvi light), and converted using a calibration curve to the dry weight (22):

$C_b$ ($gL^{-1}$) = 0.460 ×$OD_{670}$ + 0.050 $R^2$= 0.991, where $C_b$, is the biomass concentration. A same calibration curve was also provided to measuring number of cells as another growth parameter to verify biomass concentration that was (22):

$N = [71.67 \times OD_{670} - 1.329] \times 10^3$ $R^2$ = 0.99, where N is the number of cells per ml of culture media.

**Biomass analysis**

For extracting chlorophyll, 1ml of sample centrifuged for 5min at 13000 rpm and pellet were washed two times. One ml ethanol was added and incubated at 70°C in water bath for 20 min. Samples were centrifuged again (13000 rpm) and finally measured absorbance of supernatant at 650

and 665 nm (5). Chlorophyll content was measured with under equation(17):

Chlorophyll a (mg $L^{-1}$) = 16.5 ($A_{665}$) – 8.3 ($A_{650}$)

For lipid extraction, the microalgal cells were collected by centrifugation of a 30-mL sample of culture at 3000 g for 15 min. The cells were washed twice by dissolving pellet in deionized water and centrifuging again. Sample was precipitated in methanol/chloroform (2/1, v/v) and homogenized for 15 min to disrupt cells. Chloroform and 1% NaCl were added to mixture to obtain a ratio of methanol, chloroform, and water of 2:2:1. Chloroform phase was collected by centrifuging. Chloroform was evaporated under vacuum in a rotary evaporator to remove organic solvent. The residual substances from evaporation was weighted as lipid and divided to dry weight of sample (23).

Total protein was determined according to Bradford assay (1976) using bovine serum albumen standards. For cell disruption, 5 ml lysis buffer (including 2% v/v Triton x-100, 1% w/v SDS, 100mM NaCl, 10mM Tris-HCl and 1mM EDTA) was added to the sample, vortex with glass bead for 10 min and at last incubated at 90°C in water bath for 10 min. Protein precipitation was followed using two proportions of cold 25% and 10% trichloroacetic acid (TCA). Precipitated protein was suspended in 0.5 mL 1.0 N NaOH for the Bradford assays (24). All samples for lipid and protein content analysis harvested at the end of linear phase.

**Kinetic parameters**

The number of cells per ml was used to calculate the maximum specific growth rate ($\mu_{max}$, $d^{-1}$) during the logarithmic phase (17):

$$\mu_{max} = (\ln N_2 - \ln N_1) / t_2 - t_1$$

where $N$ is the number of cells and $t$ represents the time in which samples were taken.

Productivity ($P$, g $L^{-1}$ $d^{-1}$) was obtained using the below equation (17):

$$P = (X_t - X_0) / (t - t_0)$$

where $X_t$ is the biomass concentration (g $L^{-1}$) at $t$ (d) and $X_0$ is the biomass concentration at inoculation ($t_0$). Maximum productivity during cultivation was designated $P_{max}$ (g $L^{-1}$ $d^{-1}$).

**Statistical analysis**

The experimental results were evaluated by comparison of the growth curves and analysis of variance (ANOVA) of the kinetic parameters, significance being tested by the Duncan test at Sig<0.05. Repeated measures analysis of variance tests were used for comparison of growth parameters in different treatments during 7 days.

## Results

**Growth response to elevated CO2 levels**

The highest chlorophyll a content (21.86 µg $mL^{-1}$), dry weight (1.75 mg $mL^{-1}$) and number of cells ($294\times10^3$ per ml) of *S. platensis*, which were 8, 4 and 3 times more than that of alga under ambient $CO_2$, respectively. Maximum chlorophyll a content was recorded at 6% CO2, although maximum cell number and dry weight were observed at 8% $CO_2$ level (Fig. 3-5). Interestingly, understudied *S. platensis* showed a sharp increase in chlorophyll synthesis and biomass production when the $CO_2$ concentration was raised from ambient concentration to 8%. However, growth of algae and chlorophyll synthesis inhibited under treatment by 10% $CO_2$. The highest maximum biomass concentration ($X_{max}$) and maximum productivity ($P$) values for *S. platensis* observed at 8% $CO_2$, but about highest maximum specific growth rate ($\mu_{max}$), belonged to 6% $CO_2$. Values for $X_{max}$, $\mu_{max}$ and $P$ of algae in the presence of

different concentrations of carbon dioxide and flue gas are shown in Table 2.

**Growth response to flue gas**

Although, growth of *Spirulina platensis* PCC9108 under flue gas decreased somewhat, but in the highest chlorophyll a content was ca. 7 times more than that of alga under ambient $CO_2$ (Fig. 3). Also, number of algal cells for treatment with 4%, 6% and 8% $CO_2$ and flue gas were in same statistical level (Fig. 5). However, obtained biomass (dry weight) is lower than that recorded for 8% $CO_2$ (Fig. 4). Productivity and specific growth rate of algae were lower than that gained for all runs. Values for $X_{max}$, $\mu_{max}$ and $P$ of algae under treatment with flue gas are listed in Table 2.

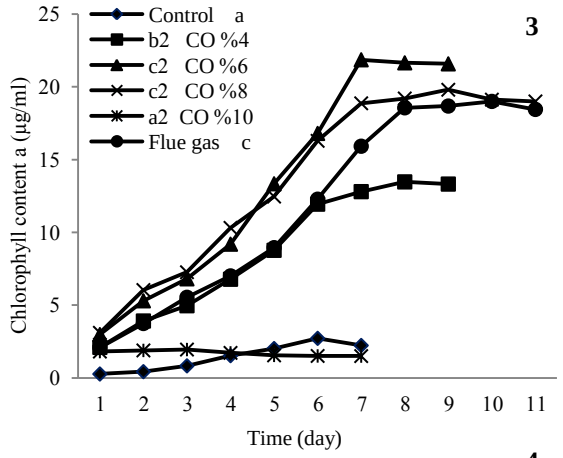
Table 2- Effect of elevated $CO_2$ and flue gas on growth parameters of *S. platensis*. All parameters calculated for logarithmic growth phase.

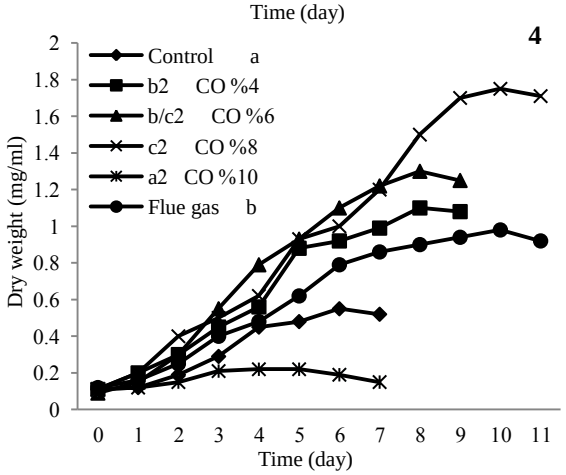
| $CO_2$ Level (%,v/v) | $X_{max}$ (mg/ml) | $\mu_{max}$ ($day^{-1}$) | $P$ ($gl^{-1}day^{-1}$) |
|---|---|---|---|
| Ambient air | 0.55 b | 0.27 a | 0.073 a |
| 4% $CO_2$ | 1.16 d | 0.35 b | 0.123 b |
| 6% $CO_2$ | 1.30 e | 0.41 c | 0.151 c |
| 8% $CO_2$ | 1.75 f | 0.27 a | 0.163 c |
| 10% $CO_2$ | 0.22 a | ----- | ----- |
| Flue gas | 0.98 c | 0.28 a | 0.086 a |

Each value is the mean of three samples. For each parameter, different lowercase letters across a row indicate significant difference between means, while the same letters show insignificant difference (ANOVA, Duncan's test, $p<0.05$).

**Effect of increasing concentrations of CO2 and flue gas on total lipid and protein content**

From the data obtained in this work, total lipid content in the *S. platensis* ranged from 13.8 to 16 wt.% and reached to highest amount at 6% $CO_2$. Generally, total lipid content of understudied *S. platensis* was not changed with increasing $CO_2$ concentration or under flue gas (Fig. 6). Also, as shown in Fig. 6, there was not any difference in total protein content between treatments. However, results have shown that this cyanobacterium has high total protein content ranging from 45.8 to 47.5 wt.%.



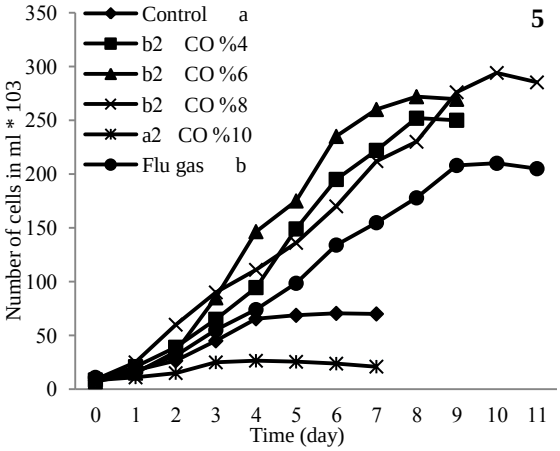



Fig. 3-5-Chlorophyll a content (3), Dry weight (4) and Number of cells (5) of *S. platensis* under different concentrations of $CO_2$ (v/v) (ambient air as control (0.036%), 4%, 6%, 8% and 10%) and flue gas. Each value is the mean of three repeats. Different letters in front of symbols indicate significant difference between means, while the same letters show insignificant difference (ANOVA, Duncan's test, $p<0.05$).

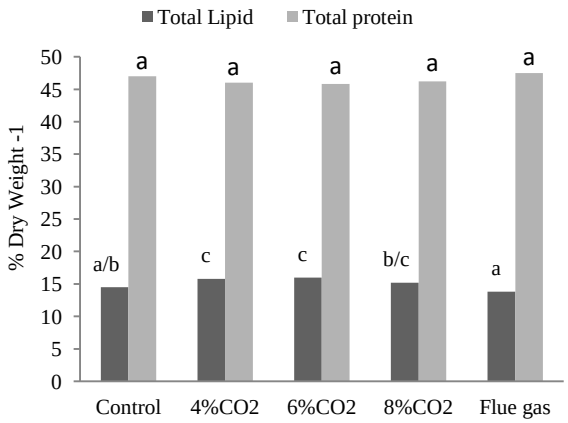



Fig. 6- Effect of elevated $CO_2$ and flue gas on total lipid and protein of *S. platensis*. Each value is the mean of three repeats. Different letters indicate significant difference between means (ANOVA, Duncan's test, $p<0.05$).

## Discussion and conclusion

Algae play a vital role in the biogeochemical cycling of carbon. Although many algae consume different sources of organic carbon, $CO_2$ is the main source of carbon for the majority of them. Growth response of *Spirulina platensis* PCC9108 in terms of dry weight, number of cells and chlorophyll a showed similar pattern and all suggested best growth observed at 8% $CO_2$ treatment. Morais and colleagues reported best growth of *S. platensis* at level of 6% $CO_2$ in air, while Andrews and coworkers observed best growth of *S. platensis* at levels of 6% and 12% $CO_2$ (21, 25). Repeated measures analysis showed that growth rate of alga at first day for control are more than other treatments. However, from second days its growth rate at 8% and 6% $CO_2$ were uppermost. These results suggested that growth of alga inhibited under effect of $CO_2$ – elevated levels at first 24h, but then adapted and grew progressively.

Previously, inhibition of microalgal growth at high $CO_2$ levels reported, but it's reason is not well illustrated. The general notion is that, the toxicity of a very high $CO_2$ concentration is due to the lowering of pH and increase in the algal performance at higher levels of $CO_2$ may be because of the enhanced availability of dissolved $CO_2$ (5). Interestingly, some researchers have reported that there was no inhibition at elevated concentrations of $CO_2$. For instance findings by Geoghegan reveal that the growth rates of *C. vulgaris* were similar whether the algae were grown in 5% $CO_2$ or high $CO_2$ concentrations such as 20% $CO_2$ in air. Also, work by Morais and Costa (21) shows that *C. vulgaris* grown in vertical tubular photobioreactor actually grows better in 12% $CO_2$ than in 6% $CO_2$ in air.

As results shown, *S. platensis* used in this study grew well under flue gas. Partially inhibition in growth possibly is due to toxic effect of CO, $SO_x$ and $NO_x$ or high temperature. $SO_x$ and $NO_x$ can have a significant effect on the growth rates and health of the microalgae. Of greatest concern is the effect of them on the pH of the microalgal growth medium (26). For algae to utilize $CO_2$ from power plants, it needs to tolerate concentrations of about 15 to 20% $CO_2$ in air (27). Most authors found that at these $CO_2$ concentrations the growth

of microalgae is completely or partially inhibited (7). However it has been shown that the inhibition can be alleviated by adaptation to $CO_2$. The inoculums of alga adapted to 5% $CO_2$ resulted in enhanced algal growth in 15% $CO_2$ compared with culture grown by ambient air. Also, algae can be adapted to high $CO_2$ concentration by gradual increases of $CO_2$ concentration (28).

Our results shown total lipid and protein content of understudied *S. platensis*, not changed so much under effect of diverse $CO_2$ concentration and flue gas. Work by Chiu et al. (2009) shows an increase in the biomass production and lipid accumulation with a $CO_2$ concentration increase in the aeration of *Nannochloropsis oculata* cultures. Likely, increase of lipid content affected by $CO_2$ levels is a strains specific character (29). Richmond mentioned that, cellular contents of cyanobacteria (eg. *Spirulina platensis*) were not changed by varying environmental conditions, compared with eukaryotic microalgae (17). However, this alga is an excellent candidate for producing single cell protein (SCP) due to its high protein content. Also, understudied *S. platensis* due to potential of biomass production under high $CO_2$ concentration and flue gas is a good candidate for $CO_2$ biofixation and decreasing atmospheric $CO_2$. Morovere, produced biomass could be utilized for the marketable production of several chemicals which are either unique or found at relatively high concentrations, such as phycocyanin, myxoxanthophyl and zeaxanthin (30).

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# پاسخ اسپیرولینا پلاتنسیس به رشد در سطوح افزایش یافته دی‌اکسیدکربن و گاز خروجی

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## چکیده

**مقدمه:** میکروارگانیسم‌های فتوسنتز کننده به دلیل توانایی‌شان در به دام انداختن دی اکسید کربن، پتانسیل خوبی در تثبیت دی اکسید کربن حاصل از منابع مختلف، از جمله گاز خروجی کارخانه‌های صنعتی، به عنوان منبع کربن دارند. در سال‌های اخیر با افزایش روز افزون گازهای گلخانه ای به ویژه دی اکسید کربن تلاش‌های زیادی برای بررسی امکان کاهش تولید دی اکسید کربن از کارخانجات صنعتی با استفاده از میکروارگانیسم‌ها انجام شده است.

**مواد و روش‌ها:** به منظور دستیابی به این هدف، پاسخ رشد سیانوباکتری اسپیرولینا تحت تیمار با غلظت‌های مختلف دی اکسید کربن (۰/۰۳۶ تا ۱۰ درصد) و نیز گاز خروجی از دودکش در مقیاس آزمایشگاهی مطالعه شد. برای ایجاد مقادیر مورد نظر دی اکسید کربن از کمپرسور هوا و کپسول دی اکسید کربن استفاده شد.

**نتایج:** بالاترین غلظت تولید بیومس در غلظت ۸ درصد گزارش شد. همچنین، رشد این سیانوباکتری تحت تیمار با گاز خروجی از دودکش نیز مشاهده شد، اگرچه میزان تولید بیومس در این تیمار تا حدی کاهش یافت. شایان ذکر است محتوی لیپید تام آن با افزایش غلظت دی اکسید کربن از میزان موجود در جو تا ۴ و۶ درصد به ترتیب ۱۴/۵، ۱۵/۸ و ۱۶ درصد وزن خشک محاسبه شد. در مقابل محتوی پروتئین تام آن با وجودی که مقدار قابل توجهی به دست آمد، حدود ۴۶ درصد وزن خشک، ولی در تیمارهای مختلف تغییر قابل ملاحظه‌ای مشاهده نشد.

**بحث و نتیجه‌گیری:** هدف از انجام این مطالعه، بررسی امکان رشد این میکروارگانیسم با استفاده از دی‌اکسید کربن موجود در گاز خروجی کارخانجات صنعتی بود و نتایج بدست آمده از آن نشان داد که ارگانیسم تحت بررسی پتانسیل خوبی برای تثبیت زیستی دی اکسید کربن از منابع مختلف، به ویژه گاز حاصل از احتراق، داشته و نیز به واسطه محتوی پروتئینی بالا به عنوان کاندید خوبی برای تولید پروتئین تک یاخته مطرح می‌باشد.

**واژه‌های کلیدی:** تثبیت زیستی دی اکسید کربن، تولید بیومس، گاز دودکش، اسپیرولینا پلاتنسیس، پروتئین تام، لیپید تام

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* نویسنده مسؤول مکاتبات

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