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Utilization of Carbon Dioxide from Coal-Firing Flue Gas for Cultivation of *Spirulina platensis*

Oanh Thi Doan[1], Anh Kim Thi Bui[2], Kien Trung Hoang[2], Chuyen Hong Nguyen[2], Thom Thi Dang[2], Hong Diem Dang[3], Nguyet Thi Vu[2], Kim Dinh Dang[2, *]

[1]Ha Noi University of Natural Resources and Environment, Hanoi, Vietnam

[2]Institute of Environmental Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

[3]Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

Email address:

doanoanh158@gmail.com (Oanh T. D.), buianh78@yahoo.com (Anh K. T. B.), hoangkien21@gmail.com (Kien T. H.), chuyenbi@gmail.com (Chuyen H. N), thomiet@gmail.com (Thom T. D.), ddhong60vn@yahoo.com (Hong D. D.), tranvunguyet@gmail.com (Nguyet T. V.), dangkim.iet@gmail.com (Kim D. D.)

[*]Corresponding author

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Abstract: CO_2 emission from burning coal has been used as a carbon source for growing Cyanobacterium *Spirulina platensis* in order to minimize the cost of biomass production, and currently to carry out CO_2 bioremediation. This article presents the results of feeding *S. platensis* in laboratory conditions with 2 formulas including Pure CO_2 and Flue gas CO_2 upon using modified Zarrouk's medium with 1.6 g / L $NaHCO_3$ and 2g / L Na_2CO_3. Pure CO_2 with 1.2% concentrations taken from 99% vol of industrial CO_2 and CO_2 gas (1.2%) received from the flue gas through the Modular system of Exhausted Gas Treatment (MEGT). Growth of the Cyanobacterium using CO_2 - Flue gas is equivalent to CO_2 -Pure. On this basis, *S. platensis* has been cultivated outdoor in an 25 m^2 pond using CO_2 gas (1.2%) from the tunnel brick factory emissions after suitable cleaning. The experiment in an outdoor pond system of 25 m^2 indicated that the yield of biomass is of 10g/m^2d with high-protein content (62.58 ± 2.34%) and fatty acids of high nutritional value (8.72 ± 0.14%), such as Omega - 6 and Omega - 3 reaching 14.74 ± 0.42% and 26.05 ± 0.64% of total fatty acid content, respectively. The quality of *Spirulina* cultured by CO_2 gas meets the requirements for functional foods according to Vietnam national food standards. The article also presents the results of biomass productivity and chemical composition of the Cyanobacterium in different culture conditions.

Keywords: CO_2, Carbon Source, Coal – Firing, Flue Gas, Cyanobacterium, *Spirulina platensis*

1. Introduction

CO_2 – anthropogenic carbon dioxide represents the most important greenhouse gases (GHGs) that contribute to approximately 77% of the global atmospheric temperature increase [1, 2]. The increase of CO_2 concentration in the atmosphere, mainly due to burning fossil fuels like coal, oil, gas and the forest destroying raises the deep concern about the climate change, and thereby, induces great challenges to the global sustainable development [3, 4].

Burning coal generates more carbon dioxide than any other widely used fuel, including burning oil and gas that may harm the environment. Due to a rapid increase of the demand for the fossil fuels, there is a need for developing methods that allow continuous use of them through environmental friendly pathway along with reducing carbon dioxide emissions. In fact, there have been many efforts to reduce CO_2 emissions from burning fossil fuels. Overall, the current methods are focusing on CO_2 separation from emission sources and then trying to remove or capture it [5]. Some

other technologies such as chemical absorption and membrane separation, were also considered [6]. However, these methods can significantly reduce CO_2 concentration, they can not solve the problem of sustainable development [7].

Nowadays, in order to meet the demand for sustainable industrial development, it is highly desirable that exhaust emissions are treated thoroughly and sustainably through CO_2 recovery for use in photosynthesis. The recovery of CO_2 for microalgae culture is a novel pathway that has been studied and delivered in the reports. Lopes et al., 2008 indicated that as much as 40% of the carbon dioxide on the Earth can be absorbed by photosynthesis, in which microalgae or cyanobacteria make a great contribution with high species diversity and wide distribution in the ecological system [8]. So, photosynthesis by microalgae is an effective way to utilize CO_2 sources [9].

The selected culture strains have a considerable impact on biological fixation of CO_2 by level of temperature, SO_x, NO_x and CO_2 from flue gas [10]. Richmond mentioned that, cellular contents of *Spirulina platensis* were not changed by varying environmental conditions, compared with eukaryotic microalgae [11]. This alga is an excellent candidate for producing single cell protein due to its high protein content and nutritional value. Study on *S. platensis* due to potential of biomass production under high CO_2 concentration in flue gas is a good solution for CO_2 biofixation and for decreasing atmospheric CO_2 [12, 13].

Our objective is to assess the possibility of using CO_2 from coal combustion emissions for the growth of *Spirulina platensis.*

2. Materials and Methods

2.1. CO_2 Source

Pure CO_2 with 1.2% concentrations taken from 99% vol of industrial CO_2.

CO_2 received from the flue gas through the Modular system of Exhausted Gas Treatment (MEGT) described is the page [14].

2.2. Cyanobacterium and Cultivation Medium

Spirulina platensis strain, classified as *Arthrospira* (*Spirulina*) *platensis* used for the experiments, was supplied from the Collection of Microalgae and Cyanobacteria of Institute of Environmental Technology, Vietnam Academy of Science and Technology.

Culture medium: The medium for the microalgal growth is Zarrouk's medium modified by reducing $NaHCO_3$ to 1,36 g/L and by adding Na_2CO_3 to 2g/L [15].

2.3. Experimental Design

Laboratory Cultivation: Spirulina platensis cyanobacteria is cultivated in glass columns with a volume of 1 liter (inner diameter of 60 mm, height of 412 mm) which are maintained at a temperature of 27-32°C and illuminated by cold fluorescent light with intensity of 5,000 lux, and lighting time of 8 hours/day (Fig. 1.a). The liquid columns of Cyanobacterium are continuously bubbled with CO_2 - Flue gas (1.2 vol% CO_2) or CO_2 - Pure as control experiments (1.2 vol.% CO_2), at a rate of 50 L/min regulated by various valves. The pH of the medium is continuously controlled over time by pH equipment. Distilled water is added daily to eliminate evaporation effects during incubation.

(a) (b)

Fig. 1. *Spirulina platensis culture system at laboratory conditions (a) and at outdoor conditions (b).*

Spirulina platensis pond parameters have an area of 25 m^2 with culture depth of 0.25 m. This pond used 1.2% CO_2 from coal-fired flue gas which was cleaned via MEGT [14]. The experiments were carried out in 5 months (Fig. 1.b). The conditions for the Cyanobacterium culture: the average outdoor light intensity was about 25,000 lux, the temperature was in the range of 27 – 32°C.

The pH of the suspension is maintained at 8.5 - 9.5 and water is also added daily to eliminate evaporation effects. The pond was aerated by the paddle wheel system [16] in order to maintain moving speed of the suspension of about 18 cms^{-1}. The samples containing the *Spirulina* suspension were collected every two days for OD measurement at wavelength of 445nm using spectrophotometer UV-Vis 2450, Shimadzu,

Japan. Each month, the fresh biomass of the *Spirulina* was collected for quality analysis.

2.4. Sampling and Analysis

Samples were collected for biomass growth analysis (OD) and biomass quality analysis (lipids, fatty acids, total protein, fiber (%), carbohydrates, polysaccharide, ash, moisture and some important elements).

Lipids and fatty acids were analyzed according to the methods of Bligh and Dyer 1959 [17]. Total protein was determined by Kjeldahl method, multiplying by 6.25. Fiber, carbohydrates, ash, moisture were determined by the method of analysis AOAC 2000 [18]. Arsenic, Cd, Pb and Hg concentrations in *Spirulina* samples were measured using a Atomic Absorption Spectroscopy AA-6800, Shimadzu, Japan [19].

2.5. Data Analysis

All the data in mean and standard deviation were performed using Microsoft excel for Windows.

3. Results and Discussion

3.1. Growth of S. platensis in Two Formulas: Pure - CO_2 and Flue Gas - CO_2 at the Laboratory Scale

In the growth process, *Spirulina platensis* can use inorganic C sources under forms of CO_2, $NaHCO_3$ or Na_2CO_3 but the primary and most appropriate source is still HCO_3^- [13]. The supplementation of CO_2 to the algae culture medium does not only provide C source but also control pH of the suspension. Fig. 2 presents the results of *Spirulina platensis* growth rate in 2 different formulas at the laboratory scale.

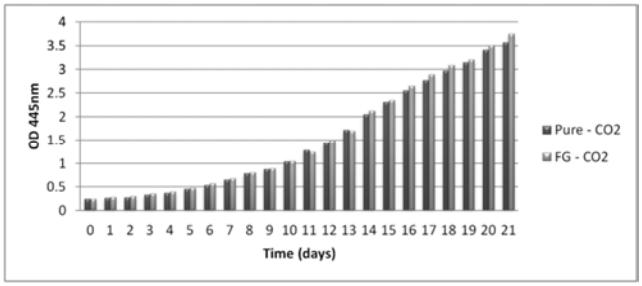

Fig. 2. *The growth of Spirulina platensis with Pure CO_2 and Flue gas CO_2 at the laboratory scale.*

After 21 days of experiment, *Spirulina platensis* increased biomass both in the 2 experimental formulas. Difference in *Spirulina platensis* biomass between two formulas (Pure CO_2 and FG CO_2) is negligible. However, in the last week of the experiment, the growth of *Spirulina platensis* in FG- CO_2 formula was slightly better than in Pure-CO_2 formula. The achieved results may be explained that there is a small amount of NO_x as nutrient for algae besides CO_2 in the coal burning emissions [20].

In addition to assessing efficiency of the above 2 sources of CO_2 on the growth of *S. platensis*, we also analyzed the nutritional composition of the biomass of this Cyanobacterium (Table 1).

Table 1. *Chemical composition of fresh biomass of S. platensis cultured in different experimental formulas (per 100 g dry weight ± 5.43 g).*

Parameters	Unit	Pure – CO_2	FG – CO_2	Parameters	Unit	Pure – CO_2	FG – CO_2
Moisture	g	2.51 ± 0.04	2.46 ± 0.03	Lead (Pb)	ppm	0.54 ± 0.02	0.57 ± 0.03
Protein	g	61.32 ± 1.48	61.21 ± 1. 34	Cadmium (Cd)	ppm	< 0.01	< 0.01
Fat (Lipids)	g	8.63 ± 0. 19	8.68 ± 0.12	Arsenic (As)	ppm	0.13 ± 0.01	0.06 ± 0.01
Fibre	g	0.4 ± 0.05	0.39 ± 0.06	Mercury (Hg)	ppm	< 0.01	< 0.01
Ash	g	8.52 ± 0.35	8.64 ± 0.27	Others	g	18.62 ± 1.22	18.62 ± 1.08
Carotenoids	mg	121 ± 5.26	149 ± 6.62				

The research results presented in Table 1 show that there is no difference in chemical composition of the biomass between two formulas and the use of CO_2 from coal-fired emissions for *Spirulina platensis* cultivation has been proved to be advantageous, and could be applied in large scale.

3.2. Growth and Productivity of Spirulina platensis in Culture Conditions at Dan Phuong, Hanoi

Optical density (OD) measurement for *Spirulina platensis* growth was applied in our study. The OD_{445nm} and dry biomass of the *Spirulina platensis* were determined every two days. The results in Fig. 3 demonstrated the variation of OD_{445nm} of the culture suspension. On the first two days *S. platensis* grew slowly, the OD_{445nm} value increased from 0.21 to 0.35. After ten days, *S. platensis* grew rapidly from 0.34 - 0.35 to 1.09 - 1.11. The highest level of OD_{445nm} in this experimental process reached up to 1.67 – 1.73 when the biomass harvest was performed for maintaining the algal OD relatively constant.

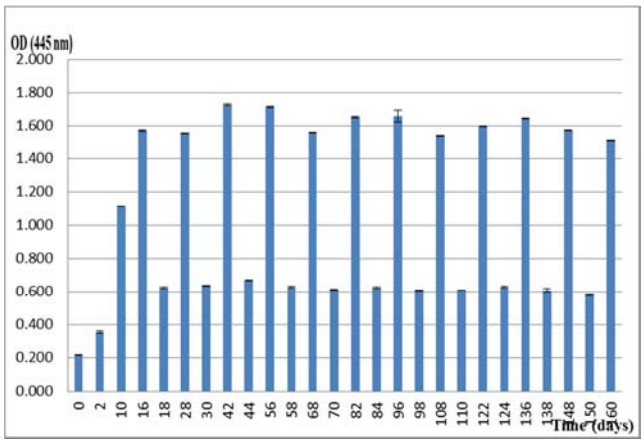

Fig. 3. *Spirulina platensis' growth at outdoor conditions.*

Carbon dioxide is well adsorbed inside the *S. platensis* culture medium with pH> 8.5. During the photosynthesis, alkaline medium is normally created through the metabolic processes by phototrophic microorganisms participating in the transport of hydroxide ion (OH^-) outwards its cell through catalytic reaction by carbohydrate anhydrase. As a result, the medium with phototrophic organisms as *Spirulina platensis* displays a strong alkaline property that helps them adsorb CO_2 with high efficiency [21]. Therefore, there have been a lot of studies taking into account the microalgae using CO_2 for nutritive biomass production. Cheng *et al*., (2006) has cultured *Chlorella vulgaris* in photobioreactor presenting that its growth rate is good in the medium with 1% CO_2 [22]. *Aphanothece microscopica* Nägeli (RSMan92) was cultured in tubular photobioreactors with different concentration of the carbon dioxide (3, 15, 25, 50 and 62%), light intensity (960, 3000, 6000, 9000 and 11000 lux), and temperature (21.5, 25, 30, 35 and 38.5°C) in order to determine the optimum condition, the highest CO_2 absorption processes for this microalgae strain [8]. In the study of Sydney et al. (2010), *Botryococcus braunii* presented the highest CO_2 fixation rate, followed by *Spirulina platensis, Dunaliella tertiolecta, Chlorella vulgaris* (as 496.98, 318.61, 272.4, and 251.64 mg l^{-1} d^{-1}, respectively) [23].

In this research, flue-gas emissions from coal combustion contained CO_2 with the amount around of 1.2%. Using such a gas source for the algae production pond with the aeration time of 6 to 8 hr d^{-1} made the pH of their medium not to raise sharply, and keeps the pH unchanged in the range of 8.5-9.6. Additionally, using a modified Zarrouk's medium (the content of bicarbonate reduced to 1.6 gl^{-1} $NaHCO_3$ and adding 2 gl^{-1} carbonate - Na_2CO_3) in which CO_2 extracted from coal-fired gas gave good results in *Spirulina* growth during the 180-day experiment.

For assessing the growth and productivity of *S. platensis* using CO_2 in the outdoor conditions, the analysis of chemical composition in the Cyanobacterium biomass was also carried out (Table 2 and 3). The obtained results presented in Table 2 indicated that *S. platensis* was rich in protein, reaching up to 62.69% dry weight while the lipid content did not exceed 9%.

Table 2. *Biomass quality of S. platensis cultivated outdoor after spray drying (per 100 g dry weight ± 5.02g).*

Parameters	Unit	CO_2 from flue gas via MEGT	Parameters	Unit	CO_2 from flue gas via MEGT
Moisture	g	2.39 ± 0.04	Lead (Pb)	ppm	0.14 ± 0.01
Protein	g	62.58 ± 2.34	Cadmium (Cd)	ppm	< 0.01
Fat (Lipids)	g	8.72 ± 0.14	Arsenic (As)	ppm	0.04 ± 0.01
Fibre	g	0.43 ± 0.03	Mercury (Hg)	ppm	< 0.01
Ash	g	9.83 ± 0.06	Others	g	16.05 ± 0.97
Carotenoids	mg	44 ± 3.44			

Moreover, the *Spirulina* also contained fatty acids having high nutritional value, such as Omega - 6 and Omega - 3 which reached 14.74% and 26.05% of total fatty acid content, respectively (Table 3).

Table 3. *Composition of fatty acids in biomass after spray drying.*

Fatty acids	Scientific name	Quantity (%)
14:0	Pentadecanoci acid	ND
16:0	Hexadecanoic acid	45.48 ± 1.24
16:1n-7	9-Hexadecenoic acid	4.43 ± 0.12
17:0	Heptadecanoic acid	ND
17:1n-5	Heptadecenoic acid	ND
18:0	Octadecanoic acid	ND
18:1n-6	Octadecenoic acid	3.59 ± 0.08
18:3n-3	9,12,15-octadecatrienoic acid	26.05 ± 0.64
18:3n-6	6,9,12-octadecatrienoic acid	14.74 ± 0.42
20:0	Eicosanoic acid	5.71 ± 0.09
20:3n-6	11,14,17-eicosatrienoic acid	ND
20:4n-6	5,8,11,14-eicosatetraenoic acid	ND

ND: non detection

As shown in Fig. 3, during the experimental production of the *Spirulina* at the outdoor conditions, the Cyanobacterium was harvested when OD_{445} values achieved about 1.6 with the productivity of about 10g/m^2d. This showed that the using CO_2 from coal-fired flue gas for culturing *Spirulina platensis* is feasible for mass culture.

The quality of *Spirulina* cultured by CO_2 gas from coal-fired flue gas of the Tuynel Brick Factory is good and equivalent to that of Siam Algae Company (SAC) assessed by Japan Food Researcher Laboratories [24]. Heavy metal concentrations, including Pb, Cd, As, Hg, and the others (Table 2), of the *Spirulina* remained within acceptable limits for functional foods according to the Decision No. 46/2007/QD-BYT and VNNTR 8-2:2011/BYT (Vietnam) [25, 26]. This is an important basis for using *Spirulina* as a nutritive food or functional food source for humans. In the context of global climate change, *Spirulina platensis* not only makes a positive contribution to reducing greenhouse gases – CO_2 but could be also good biomass for different purposes.

4. Conclusion

At the laboratory scale, biomass growth and quality of *Spirulina platensis* in 2 formulas (Pure CO_2 and Flue gas CO_2) are equivalent. The experiment in an outdoor pond system of 25 m^2 indicated that the yield of biomass is of 10g/m^2d with high-protein content of 62.58 ± 2.34% and fatty acids of high nutritional value (8.72 ± 0.14%), such as Omega - 6 and Omega - 3 reaching 14.74% ± 0.42 and 26.05 ± 0.64% of total fatty acid content, respectively. The obtained results allowed evaluating the potential of using CO_2 from coal combustion emissions for *S. platensis* culture with cost effective way for carbon sources and also for environment protection.

Nomenclature

GHGs: Greenhouse Gases
MEGT: Modular system of Exhausted Gas Treatment
OD: Optical Density
VNNTR: Viet Nam National technical regulation

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