Waste Biomass Valor (2018) 9:2015–2019
DOI 10.1007/s12649-017-9987-9

ORIGINAL PAPER

Potential of *Chlorella vulgaris* to Abate Flue Gas

Rafael García-Cubero[1] · José Moreno-Fernández[1] · Mercedes García-González[1]

Received: 17 October 2016 / Accepted: 12 June 2017 / Published online: 21 June 2017

Abstract The likely use of continuous cultures of *Chlorella vulgaris* for flue gas abatement was studied. Firstly, no pH-controlled photo-bioreactors were operated in order to understand the tolerance of this microalgae to high CO_2 concentrations in the airstream. Thus, the effect in biomass productivity, CO_2 fixation and biochemical composition of different percentage of pure CO_2 (ranging 1–12%) in the air supply was investigated. When the inlet CO_2 concentration varied from 1 to 10%, no statistical differences were found *(ANOVA, $p < 0.05$)* in the rate of carbon assimilation (0.6–0.8 g L^{-1} d). In all the cases, biomass presented a high content both proteins and lipids (40 and 25% respectively). However, when cultures were supplied with 12% of pure CO_2 in the airstream, pH drastically dropped and cultures were not viable. Next, the potential use of CO_2 contained in a simulated flue gas as a unique source of carbon was evaluated. Thus a mix of gases mimicking those presented in an exhausted stream of a power plant was used to aerate constantly the cultures. In this condition, cultures were only viable either when the simulated flue gas stream was diluted twelve times with air (resulting a constant supply of 1% CO_2 in the airstream) or no diluted but being used by pulse to control the pH of the culture. In both cases, cultures achieved a steady state, rendering 0.7 and 0.9 g CO_2 assimilated L^{-1} d^{-1} respectively. Biomass presented high content of proteins and lipids (40% respectively) in both conditions. These results suggest that the use of exhausted gases can make more economically feasible the production of microalgae and generate a valuable biomass rich in proteins and lipids.

Keywords Flue gas abatement · CO_2 fixation · Continuous culture · *Chlorella vulgaris*

Introduction

Global warming is reaching alarming level due to the increasing concentration of anthropogenic carbon dioxide in the atmosphere. Industries related to electricity generation, natural gas processing, cement, iron and steel manufacturing and combustion of municipal solid waste are the major contributors of atmospheric CO_2 increase [1]. United States Environmental Protection Agency (USEPA) has stated that energy production and consumption, mainly from transportation, contributed 71% of greenhouse gas emission world-wide in 2010 [2]. Since industrial revolution, atmospheric level of CO_2 has risen until 400 ppm determined in 2015 [3]. In order to reduce the CO_2 levels in the atmosphere, different abiotic (physical) methods have been evaluated, including either injection into geological formations or in deep oceans and the utilization of absorbent materials [4]. However, these energy-consuming methods require significant space of storage, associated with elevated costs of monitoring, operation and maintenance, raising serious concerns about potential CO_2 leakage over time [5]. CO_2 might be considered as a raw material for biomass production and not only as a waste. Thus, biologic abatement can be considered a promising alternative to those abiotic methods. Specifically, microalgae have received attention in the last years. These photosynthetic microorganisms can grow faster than plants, do not require

✉ Rafael García-Cubero
rafael.cubero@wur.nl

[1] Instituto de Bioquímica Vegetal y Fotosíntesis (IBVF), Universidad de Sevilla-Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Bioquímica Vegetal y Fotosíntesis Avda., Américo Vespucio 49, 41092, Seville, Spain

fertile land or potable water, reach efficiencies in the solar energy utilization up to 5%, and are able to use direct flue gases as their carbon source [6]. On average, 1.8 tn of CO_2 are necessary to produce 1 tn of microalgae biomass, which can be rich in valuable products such as carotenoids, amino acids, TAGs and starch, or even to be used in bioremediation processes [7].

CO_2 requirement increases the price of microalgae biomass. Thus alternatives to reduce production costs, like the use of flue gases, have been proposed [8]. The flue gas composition is variable and depends on what is being burned. Usually it consists of nitrogen (typically more than 66%) derived from the combustion air, carbon dioxide and water vapour as well as excess of oxygen (also derived from the combustion air). Additionally, it contains a small percentage of a number of pollutants, such as particulate matter (like soot), carbon monoxide, nitrogen oxides (NO_x) and sulphur oxides (SO_2) [9].

Species from genus Chlorella have been considered as promising microorganisms for CO_2 abatement [10, 11]. The aim of this study was to maximize the CO_2 fixation and biomass productivity by the green microalga *Chlorella vulgaris* sparged in different concentrations of pure CO_2 or flue gas in the airstream as well as to evaluate the effect of both in the biochemical composition of the biomass produced for a future valorisation focusing in the starch content for a possible use for bioethanol production.

Materials and Methods

Microalga and Culture Conditions

Chlorella vulgaris UAM 9–88 was grown photo-autotrophically in the medium Arnon [12] supplied with $NaNO_3$ as to reach 20 mM.

Continuous cultures were performed in 2.0 L capacity in a jacketed sterilized bubble column photo-bioreactor (0.07 m diameter, 0.50 m height), containing 1.8 L of cell suspension and continuously sparged with air (33 L (L culture^{-1}) h^{-1}). Temperature was maintained at 20 °C and pH at 7.5 by injection on demand of pure CO_2 or flue gas into the air stream. In experiments with no pH-control, either pure CO_2 or flue gas were diluted into the airstream in order to reach a constant supply of 1, 3, 6, 8, 10 and 12% of carbon dioxide. The photo-bioreactor was illuminated by six Phillips PL-32 W/840/4p white-light lamps. Light intensity followed a sine cycle of 12 h light/12 h dark, providing 3000 μmol_{PAR} m^{-2} s^{-1} as maximal incident irradiance on the photo-bioreactor surface. Initially, the photo-bioreactors were inoculated with batch-grown cells and operated in batch mode until stationary phase was attained. Then, it was switched to operate in continuous mode; fresh medium was continuously fed during the light period (12 h) at a dilution rate of 0.5 d^{-1}, harvesting simultaneously the same volume of culture. Once steady state was achieved, analytical determinations were performed. A steady state was considered after 4–8 constant determinations of dry weight. All experiments were done in triplicate.

Analytical Determinations

Biomass was harvested by centrifugation for 10 min 1500×*g*, lyophilized (Virtis Sentry) and stored at −20 °C for future analysis. All the analytical measurements were made in triplicate.

Microalgae biomass concentration was determined by dry cell weight (DCW) measurement and total organic carbon (TOC) concentration in culture samples using a TOC analyser (Shimadzu V-CPH) [13, 14]. The amount of fixed CO_2 was calculated from TOC values, taking into account that 1 g of total organic carbon corresponds to 3.66 g of fixed CO_2 [14]. An elemental analyser CHNS-O Thermo (Flash-EA 1112) was used to determine the carbon content in the dry biomass. Protein content was measured using the Lowry method [15], the lipid content was determined as described by Kochert [16] and total carbohydrates content was measured using phenol–sulfuric method [17]. Starch content was analysed by a modification of the spectrophotometric protocol described by Lin [18]. 5 mg of lyophilized samples were rinsed in 1 ml solution of chloroform:methanol (2:1 v/v) as solvent and mechanically disrupted by a bead beater (0.5 mm diameter glass beads) in order to remove all the pigments. After centrifugation (4500 rpm, 5 min), pellet contained starch, was solubilized (KOH 0.2 M; 100 °C; 30 min), and pH readjusted (pH 5) using 1 N acetic acid. Free glucose residues were obtained by α-amylases and amyloglycosidases treatment (0.2 U μl^{-1} in CH_3COONa 0.1 M pH 4.5 and 0.03 U μl^{-1} in CH_3COONa 0.1 M pH 4.5 respectively). Free glucose was determined as increase of absorbance to 340 nm by the NADH generation, with hexokinase (1 U μl^{-1} in HEPES 100 mM pH 7.7) and glucose 6-phosphate dehydrogenase (2.5 U μl^{-1} in HEPES 100 mM, pH 7.7). The content was referred to a standard curve of starch treated as the same way.

Numerical Methods

The organic carbon in the biomass was determined according to Eq. 1:

$$C_{\text{organic biomass}}\left(g\,L^{-1}\right) = TOC_{\text{culture}}\left(g\,L^{-1}\right) - TOC_{\text{supernatant}}\left(g\,L^{-1}\right) \quad (1)$$

Biomass concentration was determined according to Eq. 2:

$$\text{Biomass concentration}\left(g\,L^{-1}\right) = C_{\text{organic biomass}}\left(g\,L^{-1}\right) \times \%C_{\text{elemental analysis}} \quad (2)$$

Biomass productivity was calculated according to Eq. 3:

$$\text{Biomass productivity}\left(\text{g L}^{-1}d^{-1}\right) = \text{Biomass}\,(g) \times \text{Volume of culture}\left(\text{L}^{-1}\right) \times \text{Time}\left(\text{d}^{-1}\right) \quad (3)$$

CO_2 fixation rate was calculated according to Eq. 4:

$$CO_2\text{fixation rate}\left(\text{g L}^{-1}\text{d}^{-1}\right) = \Delta C_{\text{organic biomass}}\left(\text{g L}^{-1}\text{d}^{-1}\right) \times 44/12 \quad (4)$$

Flue Gas Composition

The composition of the gases used to simulate an exhausted stream of a power plant was: 12% (v/v) CO_2, 0.06% (v/v) SO_2, 0.08% (v/v) NO_2, 5% (v/v) O_2 and the rest N_2.

Statistical Analysis

Statistical analysis of data was performed by using software 7.0 Statgraphics. One-way ANOVA test was performed to evaluate the difference among treatments. The Tukey test was used as post hoc analysis to compare means.

Results

Effect of Different Concentration of Pure CO_2 Supplied into the Airstream

In order to define a technology for CO_2 abatement based on *C. vulgaris* is necessary to check its tolerance to different CO_2 concentrations. Therefore, cultures were aerated with a constant supply of different percentage of pure CO_2 (1, 3, 6, 8, 10 and 12%) into the airstream.

No significant differences were observed when CO_2 supply ranged from 1 to 10% (ANOVA, $p<0.05$). In all the cases, a steady state was achieved and cultures rendered 0.4–0.5 g biomass per litre and day (resulting 0.8–1 g CO_2 assimilated L^{-1} d^{-1}). Nevertheless, when higher CO_2 concentrations were assayed (12%), cultures were not viable (Fig. 1).

The biomass presented high contents in proteins (≥40%) and lipids (≥25%). In addition, carbohydrates and starch content were constant (approx. 20 and 5% respectively) in all the conditions (Fig. 2).

Effect of Different Concentration of Flue Gas Supplied into the Airstream

After it was evaluated the tolerance of *C. vulgaris* to different concentrations of pure CO_2, next was to investigate its viability to be cultivated using flue gas. Thus, to determine the optimal ratio of air:flue gas, the synthetic mixture of flue gases was injected into the airstream either pure

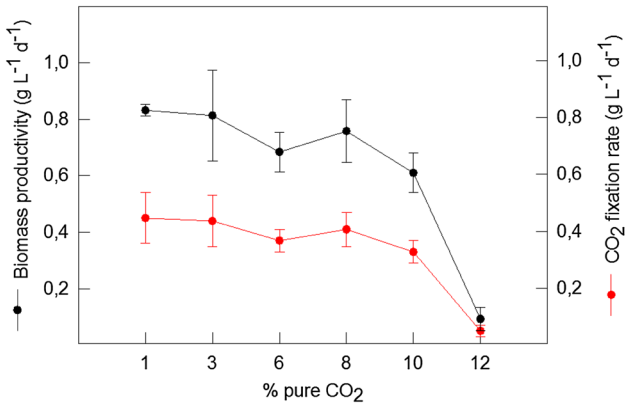

Fig. 1 Biomass productivity and CO_2 fixation rate of *C. vulgaris* continuous cultures with different concentrations of pure CO_2 injected into the airstream. Culture conditions: Temperature 20 °C, Dilution rate 0.5 d^{-1}, I_{max} 3000 μmol_{PAR} m^{-2} s^{-1}

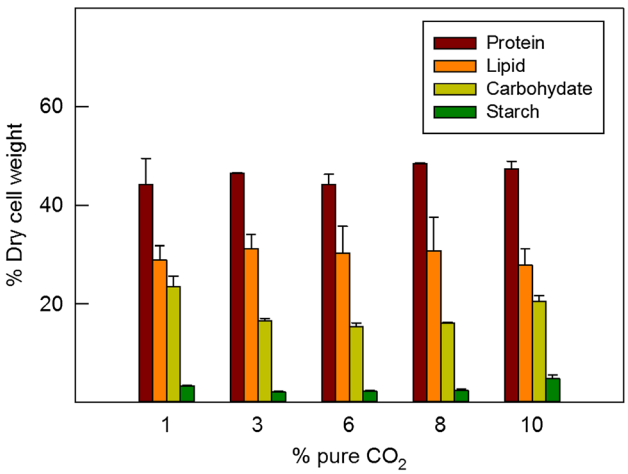

Fig. 2 Dry cell weight composition of *C. vulgaris* in the culture conditions described in Fig. 1

(resulting in a constant supply of 12% CO_2) or diluted with air (5/6, 2/3, 1/2, 1/4 and 1/12 times, resulting in a constant supply of 10, 8, 6, 3 and 1% CO_2 respectively).

Only when the flue gas was diluted 12 times (1% CO_2), cultures achieved a steady state. In the rest of conditions assayed, pH dropped drastically. In the viable condition, cultures rendered 0.4 g biomass L^{-1} d^{-1}, assimilating 0.7 g CO_2 L^{-1} d^{-1}. In this case, biochemical composition was high both in proteins and lipids (close to 40% respectively) but carbohydrates and starch represented barely 15 and 3% respectively (Fig. 3).

Effect of Flue Gas for pH Control

A set of experiments was performed to assess whether it was possible to use non-diluted flue gas (12% CO_2) to

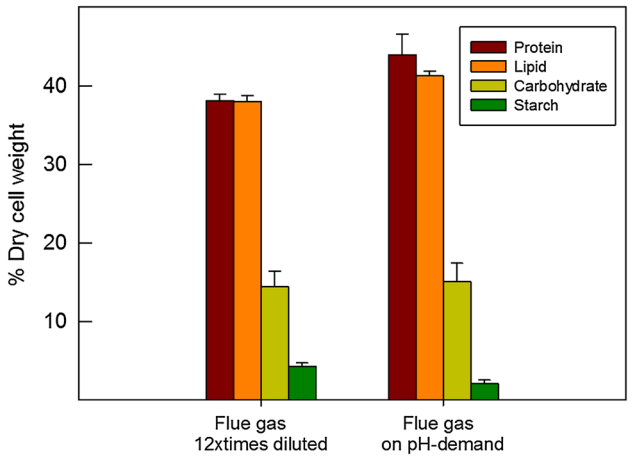

Fig. 3 Dry cell weight composition of *C. vulgaris* in continuous cultures when supplied with 12 times diluted flue gas (inlet 1% CO_2) (*left*) or non-diluted but injected on pH-demand (*right*)

control the pH of culture by injection on pH-demand. Cultures reached a steady state, rendering a biomass productivity of 0.5 g L^{-1} d^{-1} and CO_2 fixation rate of 0.9 g L^{-1} d^{-1}. Biomass presented a biochemical profile similar to those obtained when flue gas was diluted 12 times, with a high protein and lipid content (both ≥40%) and a reduction in carbohydrates and starch (15 and 3% respectively) (Fig. 3).

Discussion

To increase the productivity of microalgae it is necessary to supply the cultures with a carbon source preferably as CO_2 [19]. Nevertheless, atmospheric CO_2 concentration is low and typically represents, alongside low mass transfer in bioreactors, the main bottleneck to achieve high biomass densities inside of bio-reactors [1]. Hence, CO_2 supply signifies one of the major OPEX (operational expenditures) in microalgae biotechnology [20]. Consequently, in recent years, methods to reduce costs have been proposed, focusing mainly in the use of flue gases from waste incineration, coal, kerosene, gas or fuel–oil power plants [21–26]. Generally, exhausted gases contain high CO_2 concentrations (most of cases higher than 15%), making them suitable for production of microalgal biomass [27]. To date, most of the studies have focused on the microalgae tolerance to high carbon dioxide concentrations but few to evaluate possible applications of the biomass generated [28]. Thus, it is necessary to evaluate the biochemical composition of the biomass generated when flue gas is used.

First step to establish systems for flue gas abatement based on *C. vulgaris* is to understand its tolerance to high CO_2 concentrations. Generally, microalgae growth is affected and decreased at high CO_2 concentrations [29]. In this work, *C. vulgaris* exhibited a broad tolerance to different concentrations of pure carbon dioxide, (1–10% CO_2). These results confirm data published by other authors [5, 30, 31].

Aside from tolerating high CO_2 concentrations, it is important to consider the effect in the culture of SO_x and NO_x present in flue gas. The direct use of flue gas to cultivate species from genus Chlorella have been studied broadly [32], evidencing that *C. vulgaris* is a suitable organism for bio-mitigation. Nevertheless, culture conditions and NO_x and SO_x content in the flue gases are very heterogeneous and can affect the carbon assimilation [28, 33] Our results also agree with those presented by Keffer [10] ensuring that NO_x and SO_x do not affect the Chlorella growth when flue gas is used by pulse.

Part of the research done to date has been focused on the use of microalgae for flue gas mitigation, without taking into account the composition of biomass produced. One of the scopes of our research was to valorize the microalgal biomass. Our results indicate that independently of the origin of carbon source (pure CO_2 or from flue gas), the biomass present a high content in proteins and lipids making it attractive for cattle and poultry feed [34]. Likewise, the starch content in the produced biomass can use for energy production whether principles of bio-refinery are applied [35, 36].

Conclusions

The results of this study show that *C. vulgaris* UAM 9–88 can be used for flue gas bio-mitigation with an efficient use of CO_2 and tolerate the presence of NO_x and SO_x. The generated biomass exhibited high content in protein and lipid as well as to present fermentable carbohydrates as starch, representing a feasible way to produce microalgal biomass for a future green-based economy.

Acknowledgements This work was financially supported by SOST-CO_2 CENIT-project in collaboration with Inabensa S.A.

References

1. Kumar, K., Dasgupta, C.N., Nayak, B., Lindblad, P., Das, D.: Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour. Technol. **102**(8), 4945–4953 (2011). doi:10.1016/j.biortech.2011.01.054
2. Wai Yan Cheah, Pau Loke Show, Jo-Shu Chang, Tau Chuan Ling, Juan, J.C: Biosequestration of atmospheric CO_2 and flue gas-containing CO_2 by microalgae. Bioresour. Technol. **184**, 190–201 (2015). doi:10.1016/j.biortech.2014.11.026
3. Qafoku, N.P.: Climate-change effects on soils: accelerated weathering, soil carbon, and elemental cycling. In: Advances

in Agronomy, vol. 131. pp. 111–172. (2015). doi:10.1016/bs.agron.2014.12.002
4. Kumar, K., Banerjee, D., Das, D.: Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. Bioresour. Technol. **152**, 225–233 (2014). doi:10.1016/j.biortech.2013.10.098
5. Anjos, M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., Dragone, G.: Optimization of CO_2 bio-mitigation by *Chlorella vulgaris*. Bioresour. Technol. **139**, 149–154 (2013). doi:10.1016/j.biortech.2013.04.032
6. Chisti, Y.: Biodiesel from microalgae. Biotechnol. Adv. **25**, 294–306 (2007). doi:10.1016/j.biotechadv.2007.02.001
7. Acién, F.G., Gonzalez-Lopez, C.V., Fernandez-Sevilla, J.M., Molina-Grima, E.: Conversion of CO_2 into biomass by microalgae: how realistic a contribution may it be to significant CO_2 removal? Appl. Microbiol. Biotechnol. **96**(3), 577–586 (2012). doi:10.1007/s00253-012-4362-z
8. Acién, F.G., Fernández, J.M., Magán, J.J., Molina, E.: Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol. Adv. **30**, 1344–1353 (2012). doi:10.1016/j.biotechadv.2012.02.005
9. Kandimalla, P., Desi, S., Vurimindi, H.: Mixotrophic cultivation of microalgae using industrial flue gases for biodiesel production. Environ. Sci. Pollut. Res. (2015). doi:10.1007/s11356-015-5264-2
10. Keffer, J.E., Kleinheinz, G.T.: Use of *Chlorella vulgaris* for CO_2 mitigation in a photobioreactor. J. Ind. Microbiol. Biotechnol. **29**(5), 275–280 (2002). doi:10.1038/sj.jim.7000313
11. Chien-Ya, K., Tsai-Yu, C., Yu-Bin, C., Tzai-Wen, C., Hsiun-Yu, L., Chun-Da, C., Jo-Shu, C., Chih-Sheng, L.: Utilization of carbon dioxide in industrial flue gases for the cultivation of microalga *Chlorella* sp. Bioresour. Technol. **166**, 485–493 (2014). doi:10.1016/j.biortech.2014.05.094
12. Arnon, D.I., McSwain, B.D., Tsujimoto, H.Y., Wada, K.: Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll a and removal of phycocyanin. BBA-Bioenerg. **357**(2), 231–245 (1974). doi:10.1016/0005-2728(74)90063-2
13. Del Río, E., Armendáriz, A., García-Gómez, E., García-González, M., Guerrero, M.G.: Continuous culture methodology for the screening of microalgae for oil. J. Biotechnol. **195**, 103–107 (2015). doi:10.1016/j.jbiotec.2014.12.024
14. Clares, M.E., Moreno, J., Guerrero, M.G., García-González, M.: Assessment of the CO_2 fixation capacity of *Anabaena* sp. ATCC 33047 outdoor cultures in vertical flat-panel reactors. J. Biotechnol. **187**, 51–55 (2014). doi:10.1016/j.jbiotec.2014.07.014
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**(1), 265–275 (1951)
16. Kochert, G.: Quantitation of the macromolecular components of microalgae. In: Hellebust, J.A., Craigie J.S., Handbook of Physcological Methods. (eds.), Cambridge University Press, Cambridge, 189–195 (1978)
17. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**(3), 350–356 (1956)
18. Lin, T.-P., Caspar, T., Somerville, C., Preiss, J.: Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* (L.) Heynh lacking ADPglucose pyrophosphorylase activity. Plant Physiol. **86**(4), 1131–1135 (1988). doi:10.1104/pp.86.4.1131
19. Benemann, J.R.: Utilization of carbon dioxide from fossil fuel-burning power plants with biological systems. Energy Convers. Manage. **34**, 9–11 (1993). doi:10.1016/0196-8904(93)90047-e
20. Kadam, K.L.: Plant flue gas as a source of CO_2 for microalgae cultivation. Economic impact of different process options. Energy Convers Manage. **38**, 505–510 (1997)
21. Maeda, K.: CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Convers. Manage. **36**, 717–720 (1995). doi:10.1016/0196-8904(95)00105-M
22. Kadam, K.L.: Environmental implications of power generation via coal-microalgae cofiring. Energy **27**(10), 905–922 (2002)
23. Chae, S.R., Kang, S.T., Watanabe, Y., Shin, H.S.: Development of an innovative vertical submerged membrane bioreactor (VSMBR) for simultaneous removal of organic matter and nutrients. Water Res. **40**(11), 2161–2167 (2006). doi:10.1016/j.watres.2005.10.043
24. Doucha, J., Straka, F., Livansky, K.: Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin layer photobioreactor. J. Appl. Phycol. **17**, 403–412 (2005). doi:10.1007/s10811-005-8701-7
25. Brown, L.M.: Uptaken of carbon dioxide from flue gas by microalgae. Energy Convers. Manag. **37**(6–8), 1363–1367 (1996). doi:10.1016/0196-8904(95)00347-9
26. Wang, B., Li, Y., Wu, N., Lan, C.Q.: CO_2 bio-mitigation using microalgae. Appl. Microbiol. Biotechnol. **79**(5), 707–718 (2008). doi:10.1007/s00253-008-1518-y
27. de Morais, M.G., Costa, J.A.V.: Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers. Manag. **48**(7), 2169–2173 (2007). doi:10.1016/j.enconman.2006.12.011
28. Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder, V., Vitova, M.: Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl. Microbiol. Biotechnol. **82**(1), 179–185 (2009). doi:10.1007/s00253-008-1811-9
29. Solovchenko, A., Khozin-Goldberg, I.: High-CO_2 tolerance in microalgae: possible mechanisms and implications for biotechnology and bioremediation. Biotechnol. Lett. **35**, 1745–1752 (2013). doi: 10.1007/s10529-013-1274-7
30. Nielsen, E.S.: Carbon dioxide as carbon source and narcotic in photosynthesis and growth of *Chlorella pyrenoidosa*. Physiol. Plant. **8**(2), 317–335 (1995)
31. Mortensen, L.M., Gislerød, H.R.: The growth of *Chlorella sorokiniana* as influenced by CO_2, light, and flue gases. J. Appl. Phycol. (2015). doi:10.1007/s10811-015-0649-7
32. Van Den Hende, S., Vervaeren, H., Boon, N.: Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. Biotechnol. Adv. **30**(6), 1405–1424 (2012). doi:10.1016/j.biotechadv.2012.02.015
33. Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., Simões, M.: Carbon dioxide capture from flue gases using microalgae: engineering aspects and biorefinery concept. Renew. Sustain. Energy Rev. **16**(5), 3043–3053 (2012). doi:10.1016/j.rser.2012.02.055
34. Yakoob, Z., Ali, E., Zainal, A., Mohamad, M., Takriff, M.S.: An overview: biomolecules from microalgae for animal feed and aquaculture. J. Biol. Res.-Thessalon. **21**(1), (2014). doi:10.1186/2241-5793-21-6
35. Norsker, N.H., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H.: Microalgal production—a close look at the economics. Biotechnol. Adv. **29**, 24–27 (2011). doi:10.1016/j.biortech.2014.11.026
36. Wijffels, R.H., Barbosa, M.J.: An outlook on microalgal biofuels. Science **329**, 796–799 (2010). doi:10.1126/science.1189003