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The effect on growth of *Chlamydomonas reinhardtii* of flue gas from a power plant based on waste combustion

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Abstract

Flue gases from a power plant based on waste combustion were tested as a carbon dioxide (CO₂) source for growing *Chlamydomonas reinhardtii*. To achieve recognition as an environmentally friendly hydrogen production method, waste gases should be used to grow this hydrogen-producing microalgae. The algae were grown in undiluted flue gas containing 11.4±0.2% CO₂ by volume, in diluted flue gas containing 6.7±0.1% or 2.5±0.0% CO₂, and in pure liquid CO₂ at a concentration of 2.7±0.2%. The NO_x concentration was 45±16 mg m⁻³, the SO₂ concentration was 36±19 mg m⁻³, the HCl concentration 4.1±1.0 mg m⁻³ and the O₂ concentration 7.9±0.2% in the undiluted flue gas. Undiluted flue gas reduced the dry weight production by around 20-25% when grown at a photon flux density (PFD) of 300 μmol m⁻² s⁻¹ artificial light and at 24 or 33°C, compared with the other treatments. A less negative effect was found at the highest flue gas concentration when the algae were grown at 75 μmol m⁻² s⁻¹ PFD. Growing the algae outdoors at a day length of 12.5 h and a temperature of around 24°C, the dry weight production was higher (about 15%) in the 2.6% CO₂ flue gas treatment compared with all other treatments. Reducing the light level by 30% through shading did not affect the dry weight production. Calculated on aerial basis the productivity reached approximately 70 g m⁻² day⁻¹ in the 300 μmol m⁻² s⁻¹ PFD treatment (corresponding to 25 mol m⁻² day⁻¹) and approximately 17 g m⁻² day⁻¹ in the 75 μmol m⁻² s⁻¹ PFD treatment (corresponding to 6.5 mol m⁻² day⁻¹). The outdoor production reached around 14 g m⁻² day⁻¹. It was concluded that the negative effect of the undiluted flue gas was attributable to the high CO₂ concentration and not to the other pollutants.

Keywords: Carbon dioxide concentration; *Chlamydomonas reinhardtii*; Flue gas; Photosynthetic active radiation

Introduction

The single-celled green alga *Chlamydomonas reinhardtii* is known to produce hydrogen when starved of sulphur under anaerobic conditions (Skjånes et al. 2007; Nguyen et al. 2011; Geier et al. 2012). At present, conventional hydrogen production is energy-intensive, and more environmentally friendly production based on biological processes is therefore of great interest (Jo et al. 2006). Today, the atmospheric CO₂ concentration of about 400 μmol mol⁻¹ strongly limits the algal growth, and additional CO₂ gas has to be supplied throughout the production phase (Geier et al. 2012). Waste CO₂ from industrial flue gases should be used in order to make the production environmentally friendly. This will also contribute to reducing

CO₂ emissions that are important to the environment (IPCC 2013). Several studies have been carried out on the effect of flue gases on the growth of microalgae (Douskova et al. 2009; Kastanek et al. 2010; Borkenstein et al. 2011). *Chlamydomonas reinhardtii* seems to have been little studied, however (see review by van den Hende et al. 2012). Flue gases contain pollutants such as NO_x and SO₂ that can reach harmful levels depending on the species (van den Hende et al. 2012). However, few studies have devoted attention to whether the harmful effects depend on environmental factors such as irradiance level and temperature. In tomato plants, it is known that susceptibility to NO_x is much higher in low-light as opposed to high-light conditions (Mortensen 1986). For microalgae, and particularly for *C. reinhardtii*, little is known about the modifying effects of climate factors. Therefore, in this work the effect of flue gas was studied on *C. reinhardtii* at

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different levels of artificial light and in outdoor conditions with and without shade, as well as at two temperature levels.

Material and methods

Chlamydomonas reinhardtii strain SAG 34.89 from SAG (Göttingen, Germany) obtained from the NIVA culture collection, Norway, was used in the experiments. The algae were stored on Petri dishes covered with TAP medium 1.5% agar (Gorman and Levine 1965). The algae were grown in the high-salt Sueoka medium (Sueoka 1960). Sodium bicarbonate was used in the medium to buffer the culture at 10 mM. The microalgae were grown in 1.0 l clear plastic bottles (80 mm inner and 82 mm outer diameter) filled with 0.85 l of growing medium (filled up to 17 cm). Tubes with these dimensions have a volume of approximately 60 l per m² surface area when placed closely together, as the bottles were in the present experiments. The light was supplied by cool white fluorescence tubes (Osram L58W/840) 24 h day⁻¹ placed about 10 cm in front of the row of bottles. The photon flux density (PFD) of the artificial light was measured by a LI-COR Model Li-250 instrument with quantum sensor (400-700 nm). The light was supplied from one side and was measured at the surface of the bottles. However, inside the culture the light level strongly decreased from the light exposed side to the opposite side of the bottles, as well as with increasing cell concentration during growth. Typically, the light level decreased by about 70% through the 8.0 cm diameter bottle at start of the experiment and by more than 99.9% at the end of the experiment, due to the increase in the algae concentration.

Two experiments were carried out indoor with artificial light, while a third experiment was carried out outdoor in daylight. The daylight was measured by a Delta-T Devices PAR sensor (cosine corrected within $\pm 5\%$ up to 70° incidence). The temperature was controlled by placing the bottles with the microalgae culture in water baths controlled by aquarium heaters. A circulation pump ensured a homogenous temperature in the water baths. The temperature was measured by copper-constantan thermocouples. The CO₂ concentration was measured by a Vaisala CO₂ transmitter (Type GMT221, range 0-5%). The CO₂ concentration as well as the temperatures and the daylight PAR were recorded as hourly means by a Campbell CR10X logger with an AM25T thermocouple multiplexer. In addition a Vaisala GMP instrument was used to measure the CO₂ concentrations between 0 and 20%, and the measurements were recorded as hourly means.

The flue gas

The flue gas was provided by 'Borregaard Waste to Energy' located in Sarpsborg, Norway (www.hafslund.no). This modern fuel-flexible energy recovery plant burns

approximately 80,000 tonnes of waste-based fuel and produces approximately 230 GWh per year. It has a high environmental standard. The CO₂, O₂, NO_x, NO, NO₂, SO₂, HCl, CO and TOC concentrations in the flue gas were measured at 10-minute intervals by an ABB Advance Cemas FTIR NT continuous monitoring system with extra modules for O₂ and TOC measurements (Figure 1, Table 1). NO constituted the main part of the NO_x, while NO₂ contributed only 3.4 \pm 1.4% of the total NO+NO₂ (data not presented). The mean O₂ concentration in the flue gas was 7.9 \pm 0.2%. In addition, license measurements on a series of heavy metals and dioxins in the flue gas were performed 2-4 times per year since the start of the power plant in 2010 (Table 1).

Flue gas from the chimney was sucked by pumps through two 100 l plastic tubs connected in series for condensation of water vapour. The microalgae were grown in undiluted flue gas (11.4% CO₂) or mixed with fresh air in a constant ratio using air pumps (Resun ACO-008A) to yield 6.7% and 2.5% CO₂, respectively (Figure 1, Table 1). One CO₂ concentration (2.66 \pm 0.16%) was established by mixing pure CO₂ (food quality) from bottles with fresh air. The CO₂ gas flow was determined by a capillary with a defined resistance, while the gas pressure was defined by the height of a water column. In this way, a very accurate CO₂ flow could be added to a constant rate of fresh air supplied by air pumps (Resun ACO-001, ACO-004).

The different gas mixtures were bubbled through plastic tubes with 0.3 cm inner diameter to the bottom of the bottles at a rate of approximately 100 l h⁻¹. All treatments in all experiments included three parallel bottles containing 0.85 l of culture. Three independent experiments (including a total of 60 bottles) were carried out during the same time period, all of which started with the same algae concentration of 0.20 g dry weight per litre culture. This concentration was established by adding algae from a start culture. Two of the experiments were conducted indoor with artificial lighting while the third was conducted outdoor in daylight.

Dissolved CO₂ in the growth medium

For algal growth, the concentration of dissolved CO₂ in the nutrient medium is important and not the concentration of CO₂ in the air bubbled into the culture, although a close relationship should be expected. In order to document this relationship a test with different concentrations of pure CO₂ mixed with air were bubbled through the bottles filled with nutrient medium. The concentration of dissolved CO₂ was measured using hand-held titration cells for titrimetric analysis (CHEMetrics Inc., USA, www.chemetric.com). The results showed that a progressive increase in the dissolved CO₂ concentration from about 100 to about 500 mg l⁻¹ with increasing CO₂ concentration from about 1% up to about 20% (Figure 2). Parallel to

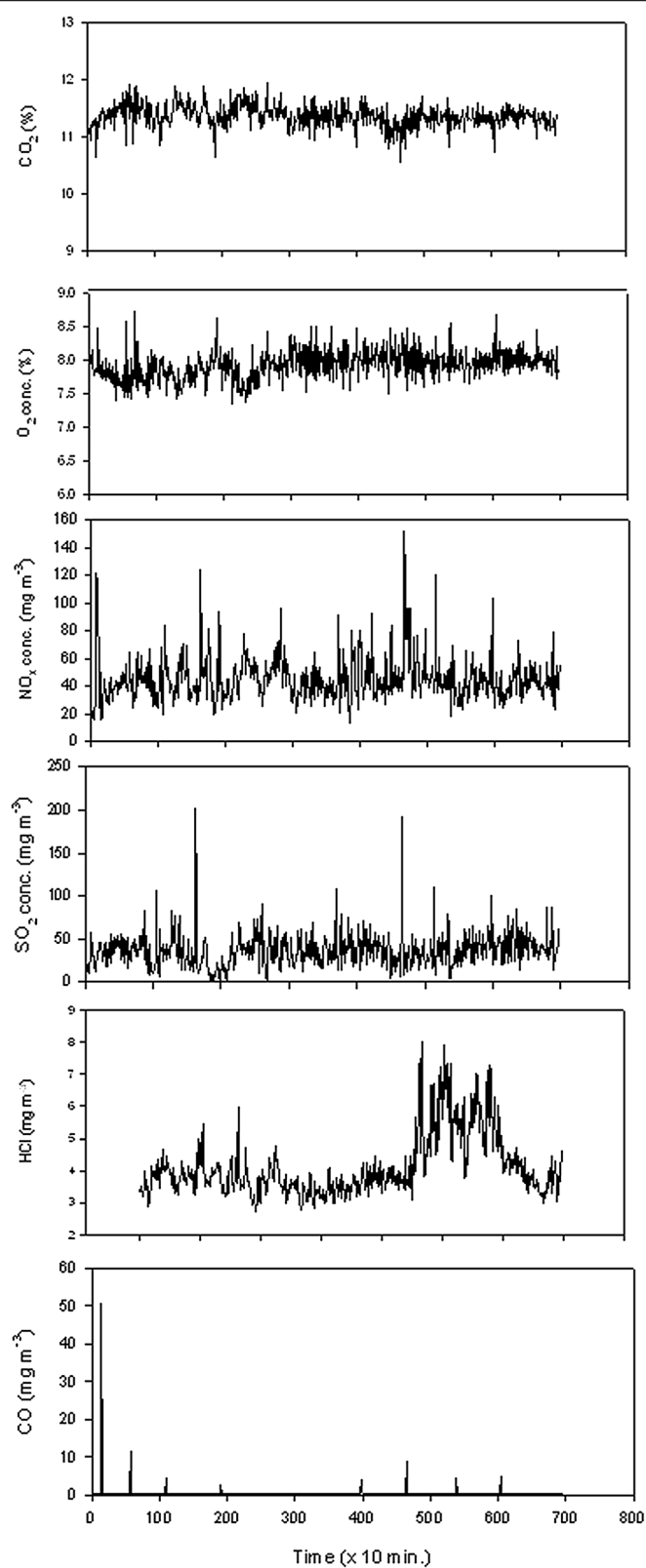


Figure 1 The concentration of different gases in undiluted flue gas.

Table 1 Mean concentrations (\pm SD) of different pollutants as measured in the different flue gas concentrations

	CO ₂ conc. (%)		
	11.37 \pm 0.19	6.71 \pm 0.11	2.50 \pm 0.04
NO _x (mg m ⁻³)	45.0 \pm 15.8	26.6 \pm 9.3	9.9 \pm 3.5
SO ₂ (mg m ⁻³)	36.1 \pm 19.0	21.3 \pm 11.2	7.9 \pm 4.2
HCl (mg m ⁻³)	4.11 \pm 0.95	2.43 \pm 0.56	0.90 \pm 0.2
CO (mg m ⁻³)	0.45 \pm 2.00	0.27 \pm 1.18	0.10 \pm 0.4
TOC (mg m ⁻³)	0.714 \pm 0.436	0.421 \pm 0.257	0.157 \pm 0.096
*Hg (μ g m ⁻³)	0.28 \pm 0.35	0.17 \pm 0.21	0.062 \pm 0.046
*HF (mg m ⁻³)	0.063 \pm 0.020	0.037 \pm 0.012	0.014 \pm 0.004
*Dioxins (ng m ⁻³)	0.00151 \pm 0.00134	0.00089 \pm 0.00079	0.00033 \pm 0.00029
*As+Co+Cr+Cu+Mn	0.0211 \pm 0.0545	0.0124 \pm 0.0322	0.0046 \pm 0.0120
+Ni+Pb+Sb+V (mg m ⁻³)			

The concentrations were measured continuously in the undiluted flue gas (11.37%) and the concentrations in the diluted flue gases were reduced to the same extent as the CO₂ concentration. *These concentrations were measured 2-4 times per year in the period 2010-2013 (n=10, \pm SD).

this increase the pH decreased from 7.6 to about 6.5. The measurements were done at 23°C. Dissolved CO₂ as measured at 7.0% CO₂ in the air was 311 \pm 12, 297 \pm 12 and 297 \pm 12 mg l⁻¹ (n=3, \pm SE) at 23, 28 and 33°C, respectively.

The experiments

Experiment 1

The microalgae were grown at the three flue gas concentrations and one concentration with pure CO₂ from bottles (Figure 1, Table 1). Two photon flux densities (PFD) were continuously applied, 75 and 300 μ mol m⁻² s⁻¹, corresponding to 6.5 and 25.9 mol m⁻² day⁻¹ PAR, respectively. Two rows of twelve bottles with algae culture were placed closely adjacent to each other in a water bath. One row along one side of the water bath was exposed to 300 μ mol m⁻² s⁻¹ PFD, and the other row along the opposite side was exposed to 75 μ mol m⁻² s⁻¹ PFD. A black sheet across the water bath eliminated any light pollution between the two light treatments. The water bath was made of transparent plexiglass, and one and four fluorescent tubes placed 10-15 cm from the bottles (outside the water bath) produced the low and high PFD, respectively. The temperature was 33 \pm 2°C. The dry weight (mg l⁻¹ culture), pH and O₂ concentration in the culture were measured after three and five days, and the production per m² and day was calculated using the vertical projected area of the bottles.

Experiment 2

The same flue gas and pure CO₂ gas treatments were applied in this experiment as in Experiment 1. In this experiment a PFD of 300 μ mol m⁻² s⁻¹ given continuously was used. The temperature was 19 \pm 2°C during the first day, and was thereafter increased to 24 \pm 2°C. The

temperature was controlled as in Experiment 1. Twelve bottles were included in the experiment, and the dry weight concentration and pH were measured four and five days after the start.

Experiment 3

In this experiment the microalgae were grown outdoors during four days under the different CO₂ treatments in full daylight and in 70% daylight by shading with white plastic (Figure 3). The bottles were closely placed adjacent to each other in water baths in rows with six bottles facing to the south. In the forefront row the culture received full daylight while the shade was given on the back row placed about 30 cm behind. Two water baths were needed for the 24 bottles including four CO₂ and two light treatments. The temperature was as a mean 24°C, varying from a peak of around 30°C at midday down to around 22°C during the night. The experiment was carried out in mid-September and the day length was 12.5 h (06.50 – 19.30 h). The building of the power station was located a few meters north of the experiment. The PFD varied from 0 to a maximum of about 1600 μ mol m⁻² s⁻¹ in full daylight and up to about 1100 μ mol m⁻² s⁻¹ in shaded conditions (Figure 2). The mean PAR was 17.1 and 12.0 mol m⁻² day⁻¹ in full daylight and in shaded conditions, respectively. At the Meteorological station 5 km from the experimental site (Østad, Sarpsborg, 59°N, 11°E) the corresponding daylight was measured to 19.7 mol m⁻² day⁻¹ when converted from global radiation to PAR (www.bioforsk.no, Agricultural Meteorological service). The higher measured value here was probably due to the light sensor with 180° view (Kipp & Zonen, CM11 pyranometer) and more diffuse light from the north since the building shaded for the light from this direction in the experiment. Mean effective PFD in the experiment was calculated by assuming that PFD above different threshold values (100, 200 μ mol m⁻² s⁻¹ etc.) has no effect on the growth (has reached the light saturation level) of the algae (Figure 4).

The dry weight was measured by vacuum filtering 10 or 20 ml of culture through a 90 mm filter (Whatman GF/B, cat. No. 1821-090) and drying it in an oven for four hours at 100°C. No pore size of this filter is given, however, all algal cells remained on the filter since no colouration of the filtered water was observed. The data were analysed using the SAS-GLM procedure (SAS institute Inc., Cary, USA) based on the bottles as replicates (n=3).

Results

Experiment 1

From an initial concentration of 0.2 g l⁻¹, the dry weight reached its maximum level after three days at the highest PFD, since no further increase was found on the fifth

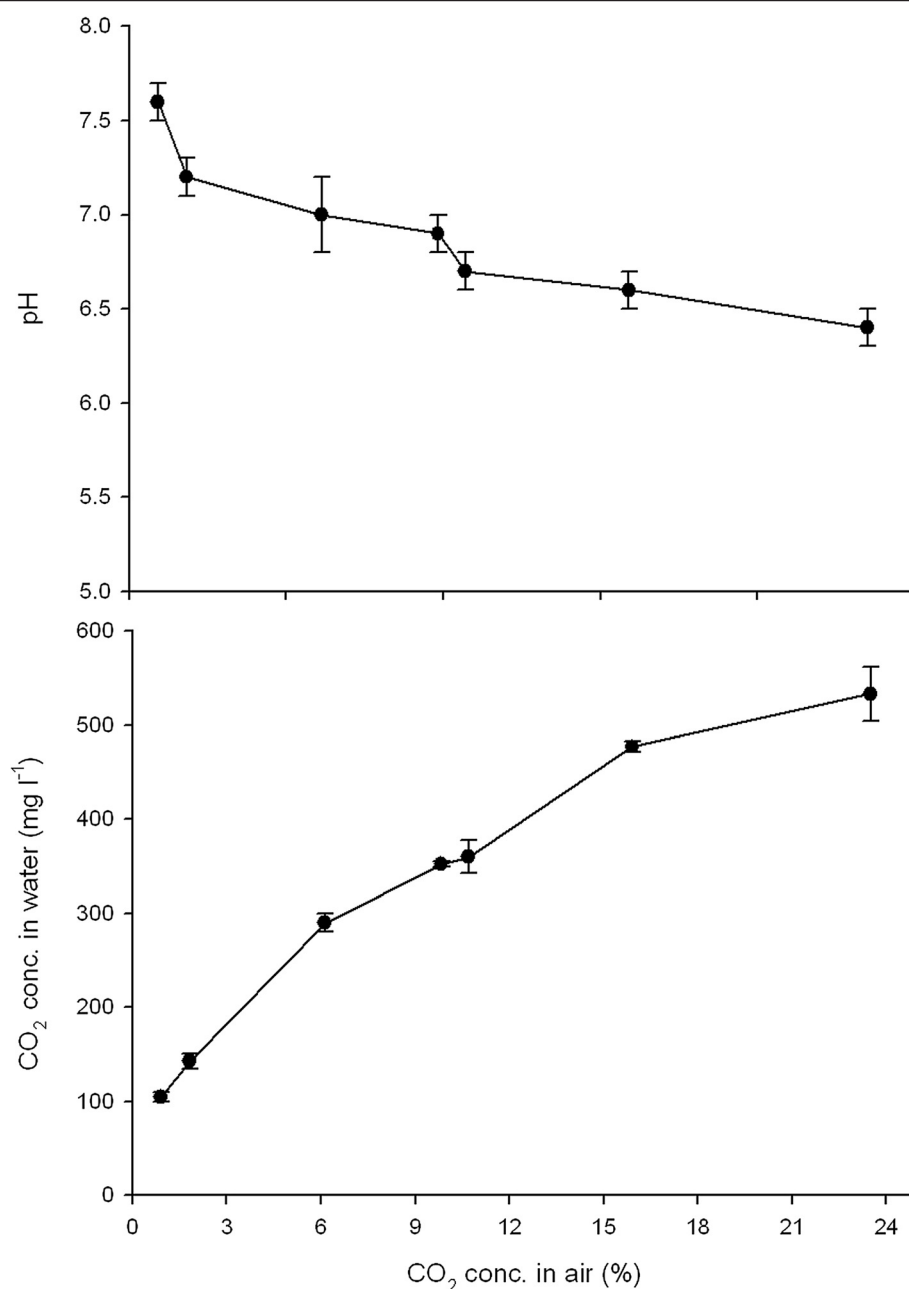


Figure 2 The concentration of CO₂ dissolved in the culture and pH of the growth medium as influenced by the CO₂ concentration in the air bubbled through the bottles (means, \pm SE).

day (Table 2). At low PFD, the algae concentration continued to grow until the fifth day. The dry weight production during the first three days was significantly lower in the 11.4% CO₂ flue gas treatment (about 20%) than in the 6.7% and 2.5% CO₂ flue gas treatments at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, while the decrease was less (5-10%) in the low PFD treatment. The dry weights at the two lower flue gas concentrations were slightly higher compared with the 2.6% control CO₂ treatment using

pure liquid gas. The dry weight production per day was about four times higher at 300 than at 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD, reaching about 70 $\text{g m}^{-2} \text{day}^{-1}$. The dry weight produced per mol photosynthetic active photons was the same at both PFD levels. Increasing the flue gas concentration slightly decreased the O₂ content and decreased the pH in the algae culture. Increasing the light level slightly increased the O₂ content and decreased the pH.

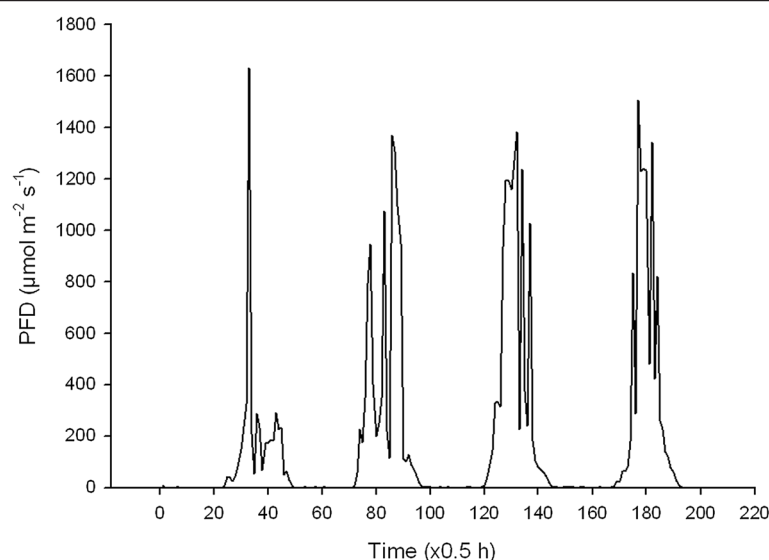


Figure 3 The photon flux density (PFD) of daylight during the experimental period.

Experiment 2

The dry weight concentration increased significantly from the fourth to the fifth day in this experiment when the algae were grown at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD and $24 \pm 2^\circ\text{C}$ (Table 3). The dry weight production was significantly lower (about 25%) at the highest flue gas concentration compared with the other treatments. The increase in algae concentration from 1.6 to 2.9 g l^{-1} from the fourth to the fifth day resulted in an algal production of around $80 \text{ g m}^{-2} \text{ day}^{-1}$ in the different treatments, except in the 11.4%

CO_2 flue gas treatment, where the production was around $60 \text{ g m}^{-2} \text{ day}^{-1}$. At the end of the experiment, the pH decreased from 6.8 to 6.0 when the flue gas concentration was increased from the lowest to the highest level.

Experiment 3

Reducing daylight by 30% shade had no significant effect on the growth of the algae (Table 4). The dry weight production was $12\text{--}14 \text{ g m}^{-2} \text{ day}^{-1}$ as a mean during four days. In this experiment, the dry weight production was

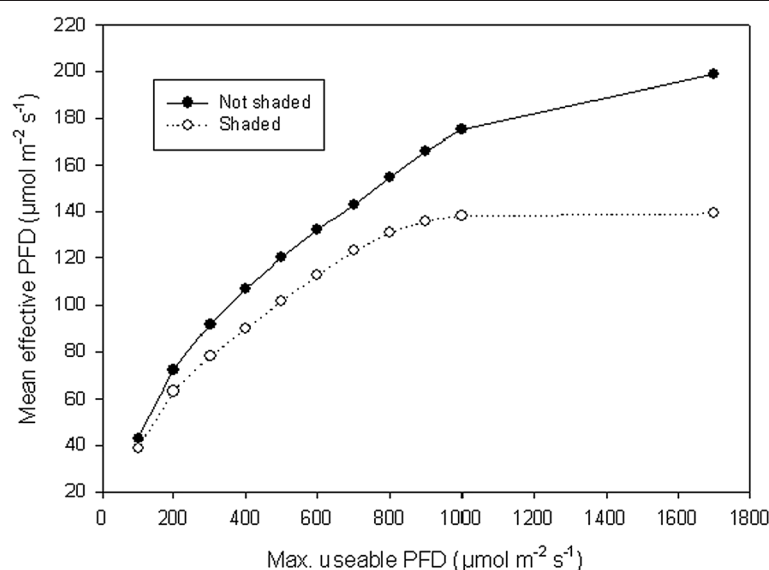


Figure 4 Mean effective PFD at different threshold values of daylight useable for the growth of the microalgae. PFD values above the threshold value were set to the threshold value, i.e. if PFD values above $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ are recognised as having no effect, the effective PFD was set to $300 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 2 The effect of different CO₂ concentrations supplied by flue gas (FI) and one concentration supplied by pure liquid CO₂ gas (C) on pH, O₂ concentration in the culture and dry weight concentration (n=3, ±SE) after 3 and 5 days of *C. reinhardtii* grown at 75 and 300 μmol m⁻² s⁻¹ PFD

CO ₂ treatment	PFD	Day 3			Day 5		Mean dry weight production		
		pH	O ₂ (mg l ⁻¹)	Dry w. (mg l ⁻¹)	pH	Dry w. (mg l ⁻¹)	Day 0 - 3		
							mg l ⁻¹ day ⁻¹	g m ⁻² day ⁻¹	g mol ⁻¹
2.6% C	75	7.1±0.0	7.5±0.2	798±27	6.9±0.1	1360±149	266±9	16.2±0.6	2.50±0.09
2.6% C	300	7.0±0.1	8.3±0.1	3297±46	6.8±0.1	3040±94	1099±15	67.0±0.9	2.59±0.04
2.5% FI	75	7.3±0.1	7.8±0.4	877±84	7.1±0.1	1338±88	292±28	17.8±1.7	2.74±0.26
2.5% FI	300	7.0±0.0	8.5±0.1	3527±48	6.8±0.1	3423±91	1175±16	71.7±0.9	2.77±0.04
6.7% FI	75	7.0±0.1	7.0±0.3	845±36	6.6±0.1	1343±70	282±12	17.2±0.8	2.64±0.12
6.7% FI	300	6.7±0.0	7.5±0.2	3663±124	6.4±0.2	3343±91	1221±41	74.5±2.5	2.89±0.10
11.4% FI	75	6.8±0.1	6.4±0.2	777±14	6.5±0.1	1147±23	259±5	15.8±0.3	2.43±0.04
11.4% FI	300	6.7±0.1	7.3±0.2	2933±36	6.5±0.1	2843±45	978±12	59.6±0.7	2.30±0.03
F-value and significance level:									
CO ₂		64.5***	50.9***	17.6***	25.6***	3.54*	17.6***	17.6***	5.73**
PFD		91.1***	77.4***	3512***	10.7**	403***	3511***	3511***	0.51
CO ₂ x PFD		3.79*	0.59	10.9***	1.32	1.25	10.9***	10.9***	0.89

The productivity during the first three days was calculated as the increase in culture concentration, in g dry weight production per m² and day, as well as in g dry weight produced per mol of photosynthetic active radiation. F-values and significance levels are stated as follows: *p<0.05; **p<0.01; ***p<0.001.

10-20% higher in the 2.5% CO₂ flue gas treatment than in the other treatments. The production per mol photons was increased 40-50% by 30% shading.

Discussion

The undiluted flue gas containing 11.4% CO₂ caused a decrease in the dry weight production compared with lower flue gas concentrations (2.5 and 6.7%). This was particularly the case when the dry weight production was very high (up to 70-80 g m⁻² day⁻¹), obtained at 300 μmol m⁻² s⁻¹ PFD continuously applied (25.9 mol m⁻² day⁻¹ PAR). In low-light conditions, (continuously 75 μmol m⁻² s⁻¹ PFD or 6.5 mol m⁻² day⁻¹ PAR) or in sunny daylight with a day length of 12.5 h (17.2 mol m⁻² day⁻¹ PAR) when the growth rate was much lower, less or no negative effect was found of the undiluted flue gas. The

question was whether the negative effect was related to the high CO₂ concentration itself or to the accompanying air pollutants. Separate measurements indicated that the dissolved CO₂ concentration in the culture with undiluted flue gas might be about 400 mg l⁻¹ as compared with about 150 mg l⁻¹ in diluted flue gas with a concentration of 2.5% CO₂. This is far below the saturating level of CO₂ in water that is about 1500 mg l⁻¹ at 23°C and 1200 mg l⁻¹ at 33°C. The present pollutant levels of NO_x and SO₂ below about 50 mg m⁻³ in the flue gas seldom seem to cause growth reduction in microalgae (Matsumoto et al. 1997; Douskova et al. 2010; van den Hende et al. 2012; Farrelly et al. 2013; Jiang et al. 2013). Other flue gas compounds such as CO, HCl, HF and heavy metals such as Hg have received little attention so far (van den Hende et al. 2012). Probably the concentrations

Table 3 The effect of different CO₂ concentrations supplied by flue gas (FI) and one concentration supplied by pure liquid CO₂ gas (C) on pH and dry weight concentration (n=3, ±SE) after 4 and 5 days of *C. reinhardtii* grown at 300 μmol m⁻² s⁻¹ PFD

	Day 4		Day 5		Dry weight increase		
	pH	Dry w. (mg l ⁻¹)	pH	Dry w. (mg l ⁻¹)	(mg l ⁻¹ day ⁻¹)	g m ⁻² day ⁻¹	g mol ⁻¹
2.6% C	7.1±0.1	1457±56	6.9±0.1	2810±141	1353±92	82.6±5.6	3.19±0.22
2.5% FI	7.0±0.0	1622±21	6.8±0.1	2943±48	1321±51	80.6±3.1	3.11±0.12
6.7 FI	6.5±0.1	1635±21	6.3±0.1	2917±100	1282±79	78.2±4.9	3.02±0.19
11.4% FI	6.4±0.1	1377±42	6.0±0.3	2380±81	1003±54	61.2±3.3	2.36±0.13
F-value and significance level:							
CO ₂	49.8***	17.6***	20.4***	7.31*	5.24*	5.24*	5.24*

The productivity from day four to five was calculated as the increase in culture concentration, in g dry weight production per m² and day, as well as in g dry weight produced per mol of photosynthetic active radiation. For significance levels see Table 2 footnote.

Table 4 The effect of different CO₂ concentrations supplied by flue gas (FI) and one concentration supplied by pure liquid CO₂ gas (C) on pH and dry weight concentration (n=3, ±SE) after four days of *C. reinhardtii* grown in daylight or 70% daylight (shaded)

CO ₂ treatment	Light	pH	Dry w. (mg l ⁻¹)	Dry weight increase	
				g m ⁻² day ⁻¹	g mol ⁻¹
2.6% C	Shaded	7.19±0.1	815±45	11.6±0.6	1.00±0.06
2.6% C	Daylight	7.1±0.0	762±43	12.4±0.7	0.72±0.04
2.5% FI	Shaded	7.1±0.1	960±18	14.3±0.7	1.23±0.06
2.5% FI	Daylight	7.1±0.1	935±45	14.6±0.3	0.85±0.02
6.7% FI	Shaded	6.8±0.1	867±83	11.9±0.7	1.03±0.06
6.7% FI	Daylight	6.7±0.1	780±46	13.2±1.3	0.76±0.07
11.4% FI	Shaded	6.5±0.1	792±32	12.1±0.6	1.05±0.05
11.4% FI	Daylight	6.4±0.2	797±41	12.1±0.5	0.70±0.03
F-value and significance level:					
CO ₂		110***	5.14*	5.14*	5.31**
Light		3.00	1.49	1.49	79.9***
CO ₂ x Light		1.00	0.36	0.36	0.63

The mean productivity during the four days of the experimental period was calculated as g dry weight production per m² and day and as g dry weight produced per mol of photosynthetic active radiation.

For significance levels see Table 2 footnote.

in the present flue gas were so low that they would have no effect on the growth. However, microalgae possess very high metal uptake capacities and accumulation in the cells will therefore take place (de-Bashan and Bashan 2010). High CO₂ concentrations (18-19%) from pure liquid CO₂ gas, however, have recently been found to decrease the dry weight production in the same *C. reinhardtii* strain (Mortensen and Gislerød 2014). Fischer et al. (2006) showed that cells of the same species were more susceptible to high-light stress under high CO₂ concentrations than under low concentrations. In the present study, however, the negative effect of the high concentrations seemed to be more related to a high growth rate than to high-light conditions. It can also be noted that the maximum dry weight concentration reached in the algae culture in the flue gas decreased to the same extent (in percentage) as the dry weight production, indicating higher respiration or lower photosynthetic activity in the algae. The negative effect of the 11.4% flue gas in the present experiment was in contrast to the stimulating effect of flue gas, probably due to lower O₂ content, found in some studies on microalgae (Vance and Spalding 2005; Douskova et al. 2009; Kliphuis et al. 2011). Growing *Chlorella* sp. at 2-20% CO₂ (v/v) simulating flue gas from biogas gave the same effect as growing the algae in food grade CO₂ at the same concentrations (Douskova et al. 2010). The environmental conditions could play a role here, and they might also be the reason for the positive effect of the moderate flue gas concentration with 2.5% CO₂ in the present experiment in daylight.

The production at low-level light 24 h day⁻¹ (6.5 mol m⁻² day⁻¹ PAR) was at the same level (around 14 g m⁻² da⁻¹) as at about a three times higher PAR in daylight, which

demonstrates the limitation of the algae as regards utilising the high irradiance level. The productivity in daylight was typical of outdoor production systems and the high productivity was typical of controlled environmental conditions in laboratories (Grobbelaar 2012). The light use efficiency in the present study was found to be the same in the range 75-300 μmol m⁻² s⁻¹ PFD. If we assume that all daylight above 300 μmol m⁻² s⁻¹ PFD has a value of 300 μmol m⁻² s⁻¹, the mean PFD of the daylight will decrease from 199 to about 90 μmol m⁻² s⁻¹ or 7.8 mol m⁻² day⁻¹ PAR. This level is comparable to the low-light level with artificial light applied 24 h day⁻¹. In addition to the constraint caused by light saturation, the presence of a dark period is known to decrease algae growth much more than would be expected from the reduction in PAR (Jacob-Lopez et al. 2009). This means that long day lengths and lower maximum irradiance levels at high latitudes would be beneficial for algae production during the summer months. However, short days and low PAR during large parts of the year make the production of algae impractical in such locations. Growing *C. reinhardtii* with the aim of using it to produce hydrogen should be based on using daylight in combination with flue gas in order to ensure a positive energy balance (Lam et al. 2012). However, large-scale systems that can utilise the high irradiance levels of daylight much better than today (Slegers et al. 2013) are a prerequisite for future energy-efficient hydrogen production using microalgae. Flue gas is an important CO₂ source. However, while care should be taken to ensure a CO₂ concentration that is optimal, the presence of pollutants in the flue gas in today's industrial emissions seems to be less of a problem in relation to the growth of the algae.

Competing interests

The authors declare that they have no competing interests.

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References

- Borkenstein CG, Knobelechner J, Frühwirth H, Schagert M (2011) Cultivation of *Chlorella emersonii* with flue gas derived from cement plant. *J Appl Phycol* 23:131–135
- de-Bashan LE, Bashan Y (2010) Immobilized microalgae for removing pollutants. Review of practical aspects. *Bioresour Technol* 101:1611–1627
- Douskova I, Doucha J, Livansky K, Machat J, Novak P, Umysova D, Zachleder V, Vitova M (2009) Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. *Appl Microbiol Biotechnol* 82:179–185
- Douskova I, Kastanek F, Maletkova Y, Kastanek P, Doucha ZV (2010) Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalga-products. *Energy Conversion Management* 51:606–611
- Farrelly DJ, Everard CD, Fagan CC, McDonnell KP (2013) Carbon sequestration and the role of biological carbon mitigation: a review. *Renew Sust Energ Rev* 21:712–727
- Fischer BB, Wiesendanger M, Eggen RIL (2006) Growth condition-dependent sensitivity, photodamage and stress response of *Chlamydomonas reinhardtii* exposed to high light conditions. *Plant Cell Physiol* 47:1135–1145
- Geier SC, Huyer S, Praebst K, Husmann M, Walter C, Buchholz R (2012) Outdoor cultivation of *Chlamydomonas reinhardtii* for photobiological hydrogen production. *J Appl Phycol* 24:319–327
- Gorman DS, Levine RP (1965) Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 54(6):1665–1669
- Grobbelaar JU (2012) Microalgae mass culture: the constraints of scaling-up. *J Appl Phycol* 24:315–318
- IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, www.climatechange2013.org
- Jacob-Lopez AJ, Scoparo CHG, Lacerda LMCF, Franco TT (2009) Effect of light cycles (night/day) on CO₂ fixation and biomass production by microalgae in photobioreactors. *Chem Eng Process* 48:306–310
- Jiang Y, Zhang W, Wang J, Chen Y, Shen S, Liu T (2013) Utilization of simulated flue gas for cultivation of *Scenedesmus dimorphus*. *Bioresour Technol* 128:359–364
- Jo HJ, Lee DS, Park JM (2006) Modeling and optimization of photosynthetic hydrogen gas Production by green alga *Chlamydomonas reinhardtii* in sulphur-deprived circumstance. *Biotechnol Prog* 22:431–437
- Kastanek F, Sabata S, Solcova O, Maletkova Y, Kastanek P, Branyikova I, Kuthan K, Zachleder V (2010) In-field experimental verification of cultivation of microalgae *Chlorella* sp. using flue gas from cogeneration unit as a source of carbon dioxide. *Waste Management and Research* 28(Kastanek F, Sabata S, Solcova O, Maletkova Y, Kastanek P, Branyikova I, Kuthan K, Zachleder V):961–966
- Kliphuis AMJ, Martens DE, Janssen M, Wijffels RH (2011) Effect of O₂:CO₂ ratio on the primary metabolism of *Chlamydomonas reinhardtii*. *Biotechnol And Bioengineering* 108:2390–2402
- Lam MK, Lee KT, Mohamed AR (2012) Current status and challenges on microalgae-based capture. *Int J Greenhouse Gas Contr* 10:456–469
- Matsumoto H, Hamasaki A, Sioji Nikuta Y (1997) Influence of CO₂, SO₂ and NO in flue gas on microalgae productivity. *J Chem Eng Japan* 30:620–624
- Mortensen LM (1986) Nitrogen oxides produced during CO₂ enrichment III. Effects on tomato at different photon flux densities. *New Phytol* 104:653–660
- Mortensen LM, Gislerød HR (2014) The growth of *Chlamydomonas reinhardtii* as influenced by high CO₂ and low O₂ in flue gas from a silicomanganese smelter. *J Appl Phycol* (in press)
- Nguyen AV, Toepel J, Burgess S, Uhmeyer A, Bilfernez O, Doebe A, Hankamer B, Nixon P, Wobbe L, Kruse O (2011) Time-course global expression profiles of *Chlamydomonas reinhardtii* during photo-biological H₂ production. *PLoS ONE* 6(12):e29364, doi:10.1371
- Skjånes K, Lindblad P, Muller J (2007) BioCO₂ – a multidisciplinary, biological approach using solar energy to capture CO₂ while producing H₂ and high value products. *Biomol Eng* 24:405–413
- Slegers PM, van Beveren PJM, Wijffels RH, van Straten G, Boxtel AJB (2013) Scenario analysis of large scale algae production in tubular photobioreactors. *Appl Energy* 105:395–406
- Sueoka N (1960) Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 46:83–91
- van den Hende S, Vervaeren H, Boon N (2012) Flue gas compounds and microalgae: (Bio-) chemical interactions leading to biotechnological opportunities. *Biotechnol Advances* 30:1405–1424
- Vance P, Spalding MH (2005) Growth, photosynthesis, and gene expression in *Chlamydomonas* over a range of CO₂ concentrations and CO₂/O₂ ratios: CO₂ regulates multiple acclimation states. *Can J Botany* 83:796–809

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