CO_2 ADDITION TO INCREASE BIOMASS PRODUCTION AND CONTROL MICROALGAE SPECIES IN HIGH RATE ALGAL PONDS TREATING WASTEWATER

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Abstract

Challenges regarding microalgal cultivation need to be solved in order to enhance microalgae potential as a feedstock for biofuel, bioenergy, and bioproducts. The optimization of the operating strategy in high rate algal ponds treating wastewater still requires research on microalgal ecosystem response to variations in nutrients availability. For this reason, the aim of this study was to determine the effect of CO_2 addition on microalgal population diversity and wastewater treatment performance. To this end, batch and continuous experiments were carried out in an experimental plant constituted by four high rate algal ponds (500 L each) treating urban wastewater with and without pH regulation. As expected, CO_2 addition induced a significant increase in biomass concentration (between 66 and 100%). Moreover, a positive effect on microalgal biomass concentration was observed, reducing the effect of the variation in influent wastewater characteristics. Concerning the microalgal populations, the variation of inorganic carbon availability induced a shift in the dominant microalgae species. In spite of this, no variations were observed in terms of wastewater treatment efficiency. Taking together, this study highlighted the positive effect of CO_2 addition to increase biomass production and control microalgae species in high rate algal ponds treating wastewater.

Keywords: Carbon dioxide, High rate algal ponds, pH regulation, Wastewater treatment, Microalgal species, Diversity.

1. Introduction

The increasing interest on microalgae as a potential feedstock for biofuel, bioenergy and bioproducts production is recently promoting the research development in this field. Nevertheless, challenges regarding microalgal production stability still need to be solved. In fact, due to the high investing and operating cost and the low and variable productivity, microalgae production technology is still a bottleneck preventing the industrial development of microalgae biorefinery (Chisti, 2013).

High rate algal ponds (HRAPs) are open raceway ponds designed to optimize the production of microalgae. They are composed of a single or multiple loops, and most of the time are provided of a paddlewheel assuring water mixing and enhancing photosynthesis (Oswald and Gotaas, 1957). Such systems are based on oxidation pond technology, which was developed in the United States at the end of the 50's as a wastewater treatment solution attaining an efficient treatment at low costs (Oswald and Gotaas, 1957). Here, the organic matter is oxidized by heterotrophic bacteria utilizing oxygen released by microalgae, while nutrients are removed through assimilation into microalgal biomass and by volatilization (ammonia) or precipitation (phosphate).

The main advantages of HRAPs reside into the ease design, the low maintenance costs and the reduced energy requirements (Gutiérrez et al., 2016). Moreover, wastewater supplies nutrients and water requirements of microalgae, reducing biomass production costs, while microalgae and bacteria provide an effective secondary or tertiary wastewater treatment. On the other hand, major drawbacks of microalgal culture in wastewater are: 1) the presence in the culture of different microalgal strains, bacteria and pathogens hampering biomass valorization (Williams and Laurens, 2010), and 2) the low inorganic carbon content which can compromise the microalgal productivity by limiting the growth rate

(Woertz et al., 2009; Park et al., 2011). For this last reason, the addition of CO_2 to microalgal culture has been recently tested in pilot HRAPs, enhancing the biomass concentration of about 30-50% (Park et al., 2011a; Sutherland et al., 2015). Moreover, this gives an opportunity for waste gasses rich in carbon dioxide (CO_2), such as flue gas from fossil fuel burning power plants, to be used as a carbon substrate for the growth of autotrophic biomass (Van Den Hende et al., 2012). Such option, recently proposed by many authors (Chisti 2008; Lardon et al., 2009; Craggs et al., 2011; Posadas et al., 2015; Cheah et al., 2015) would increase biomass production minimizing operational costs in full-scale applications and reducing greenhouse gas emissions. While Craggs et al. (2011) focuses on CO_2 addition and the increase of biomass production, Cheah et al. (2015) presents a review on the efficiency of CO_2 biosequestration by microalgae and on factors influencing microalgal biomass productions and microalgal cultivation systems. On the other hand, Posadas et al. (2015) highlights the effect of CO_2 addition from different sources on wastewater treatment performances, biomass productivity and macroscopic (lipids, proteins, carbohydrates and ashes) biomass compositions, but did not analyze the variations of microalgae species that can be induced by CO_2 addition. In general, the experiences reported in literature mainly refer to pure cultures, while information on the influence of CO_2 addition on mixed microalgae cultures and the effect of CO_2 on the species composition of a mixed culture is still missing.

The aim of this study was to determine the effect of inorganic carbon variations induced by CO_2 addition on microalgal ecosystem characteristics (i.e. microalgae species composition) and wastewater treatment performance. To this end, batch and continuous experiments were carried out in four pilot-scale HRAPs treating urban wastewater. A better understanding of microalgal ecosystem response and wastewater treatment performance to

variations in nutrients availability could help improving the operating strategy in high rate ponds maximizing wastewater treatment as well as microalgal biomass production and valorization.

2. Materials and methods

The experiments were carried out outdoors in a pilot scale plant located at the Laboratory of Environmental Biotechnology (LBE, INRA) in Narbonne, South of France (43°11′N, 3°00′E, 13 m A.M.S.L.).

The experimental plant consisted of four identical PVC cylindrical containers with a surface area of 1.30 m^2 and a depth of 0.5 m (nominal volume 500 L). One drainage pump in each container assured continuous stirring of the liquor. The maximum pH of two HRAPs was controlled through CO_2 addition, while the other two tanks, considered as control, were not submitted to CO_2 addition. The bottom of the two HRAPs with CO_2 addition was equipped with a gas diffuser (a perforated pipe) at a flow rate of 1 L/min connected to a CO_2 bottle by means of a solenoid valve. The pH of the mixed liquor was measured every 5 seconds with a pH probe, when the measurement exceeded the pH established, the valve was opened and CO_2 was bubbled into the pond through the gas diffuser at a flow rate of 1 $L \cdot min^{-1}$. The solenoid valve was automatically closed 10 seconds after the opening.

Two experiments were carried out, the first in batch feeding conditions during 8 days and the second in continuous feeding conditions during 38 days.

2.1 Batch feeding experiments

During the batch experiments, the pH was regulated to 8 and 9 in the tanks with CO_2 addition, while it was not regulated in the control tanks.

At the beginning of the experiments, each tank was fed with 50 L of untreated urban wastewater from the wastewater treatment plant of Narbonne, filled with 400 L of tap water and inoculated with 50 L of a medium isolated from the Mèze pond (France) dominated by *Scenedesmus* sp. (about 70% of eucaryotic cells, based on microscopic observations and molecular CE-SSCP calculation). The main physicochemical characteristics of the raw wastewater were as follows: Total Suspended Solids (TSS), 108 $mg.L^{-1}$, Total Chemical Demand of Oxygen (COD), 718 $mg.L^{-1}$, ammonium nitrogen ($N\text{-}NH_4^+$), 81 $mg.L^{-1}$ and orthophosphate ($P\text{-}PO_4^{3-}$), 13 $mg.L^{-1}$. The experiments were run during 8 days until the plateau of biomass concentration was reached.

2.2 Continuous feeding experiment

In accordance with the results obtained from the batch experiment, the pH was regulated at 8 in two tanks, while it was not regulated in the control tanks. Two tanks (one with pH regulation and one control) were running for 23 days, while the other two were operated for 38 days. At day 27, the CO_2 regulation was stopped.

At the beginning of the experiment, each tank was inoculated with 50 L of a medium dominated by *Scenedesmus* sp. In this experiment, untreated urban wastewater was periodically collected from the wastewater treatment plant of Narbonne (France), diluted with tap water (1:10 v/v) and then used as nutrient complementation for microalgal growth. HRAPs are mainly used for secondary wastewater treatment (Gutierrez et al., 2016; Park et al. 2018). For this reason, we diluted untreated wastewater until reaching COD concentrations typical from secondary wastewater (200-500 mg/L). The substrate was continuously automatically fed by means of peristaltic pumps at a rate of 125 L/d in order to maintain hydraulic retention time (HRT) corresponding to 4 days.

2.3 Experimental procedure

Air temperature was gathered from a meteorological station located near the pilot plant that recorded data every 30 minutes. The average air temperature during the experiment was 24.3ºC, oscillating between 12.6ºC and 34.3ºC (Figure 1). Photosynthetically Active Radiation (PAR) was measured by a probe (Sky Instruments PAR Quantum Sensor) located on the surface of one tank recording data every five minutes. Water physical parameters (water temperature and pH) were continually measured by a pH probe (InPro 426i, Mettler Toledo, CH) and recorded every 5 sec. The pH probe was calibrated every week with standard pH solutions (4 and 7).

For microalgal growth monitoring, samples of the mixed liquor were taken from each tank three times per week at 10 am and observed with a microscope (BX 60, Olympus) and algae cells concentration was determined by means of the Thoma cell counting chamber at a 40X magnification. Moreover, the particle size distribution of each sample was determined by means of a granulometer (LS 200, Beckman coulter, USA).

Concerning wastewater treatment performance, the mixed liquor from each tank was characterized for soluble COD following the Standard Methods for Wastewater Treatment (APHA AWWA-WPCF, 2001) and for ammonium nitrogen (NH_4^+-N) determined with ion chromatograph (ICS 3000, Dionex, USA) equipped with pre-columns NGI 2 mm and CG 11 2 mm followed by separation columns CS 16 3 mm and AG 15 2 mm for cations and anions, respectively. The eluents used for this analysis were hydroxymethanesulfonic acid (25-40 mM) pumped at 0.3 $mL \cdot min^{-1}$ for cations and potassium hydroxide (10-74 mM) pumped at 0.35 $mL \cdot min^{-1}$ for anions.

An average of 160 mL of growth cultures was filtered at 3 μm and stored at -20°C for

subsequent DNA analysis. Total DNA was extracted according to Moletta et al. (2007). DNA amount and purity were assessed by spectrophotometry (Infinite NanoQuant M200, Tecan). The V7 region of the eukaryotic 18S rRNA genes were amplified with the primer pair W16 (5'-CTTAATTTGACTCAACACGG-3') and W131 (5'-6FAM-GGGCATCACAGACCTGTT-3'). 50 µl of PCR mixture were prepared using 1 µl of genomic DNA, 0.5 µM of each primer, 0.5 U of Pfu turbo DNA polymerase (Stratagene), 1x Pfu Turbo DNA polymerase buffer, 200 µM for each dNTP and ultrapure water. The PCR amplification reactions were performed in a MastercyclerEP gradient S thermal cycler (Eppendorf). After a denaturation step of 2 min at 94°C, 30 cycles (30 sec at 94°C, 30 sec a 51°C and 30 sec at 72°C) were applied followed by a 10 min step at 72°C. One microliter of PCR products was diluted and mixed with 18.8 µL of formamide and 0.2 µL of internal standard GeneScan ROX (Applied Biosystems). Samples were denatured for 5 min at 95°C and immediately cooled in ice. Capillary Electrophoresis Single-Strand Conformation Polymorphism (CE-SSCP) electrophoresis was performed using an ABI prism 3130 genetic analyzer (Applied Biosystems). The separation was performed in 50 cm long capillary tubes filled with a non-denaturing 5.6% conformation analysis polymer (Applied Biosystems). The elution was carried out at 12 kV and 32°C for 64 min. CE-SSCP raw data were analyzed using GeneScan software (Applied Biosystems) and the StatFingerprints R library (Michelland et al. 2009).

All statistical analyses, including rank correlations, were performed using R (R Core Team 2013).

3. Results

3.1 pH regulation

The effectiveness of the pH regulation during the 8 days of the batch experiments is presented in Figure 2. Ponds with pH regulation never exceeded the pH set points of 8 and 9 respectively. In contrast, pH values for control ponds reached very high values (up to 11). Similarly, Figure 3 shows the maximum daily pH in the 4 tanks during the continuous feeding experiment. Average values recorded along this period in control tanks (9.6±0.8) were higher than those detected in tanks where pH was regulated (7.8±0.8). As it can be observed in Figure 3, when CO_2 injection was stopped (day 27), the pH rapidly rose until reaching the same pH value as the control tank (10.3).

Even if pH as high as 11 can be beneficial for the pathogens disinfection, pH>10 can cause a bacterial inhibition and a consequent decrease in the pollutant removal efficiency performed by algal-bacteria ecosystems (Muñoz and Guieysse, 2006).

3.2 Cellular concentration

Results about cellular concentrations (Figures 4 and 5) indicate a clear rise along the experiments when CO_2 was supplied to the culture (increase of biomass production between 30 and 50%). This was more evident in the batch experiment (Figure 4), the higher cellular concentration in tanks with pH regulation was visible since the third day. After 6 days, the cultures only fed with wastewater reached concentrations of about 6.10^6 cells·mL^{-1}, while values were almost doubled ($1 \cdot 10^7$ and $1.2 \cdot 10^7$ cells·mL^{-1}) when CO_2 was added to the cultures (T-test ; $p<0.05$). This is in accordance with the enhancement of biomass production with CO_2 addition previously shown in various studies at different scales (Heubeck et al., 2007; Ho et al., 2010a; Park et al., 2011a, 2011b).

According to Kong et al. (2010) the optimal pH of many freshwater microalgae is about 8, and higher or lower pH could decrease microalgae productivity due to nutrient availability (pH equilibrium) or direct toxicity. For instance, it has been showed that pH rising from 8 to 9 reduced *Chlorella* sp. and *Chaetoceros* sp. productivity by 22% (Weissman and Goebel, 1988). In line with this, in the batch experiment presented in this study the pH regulation to 8 lead to a microalgal production 17% higher than the production obtained at pH 9. The fact was less evident in the continuous experiment due to the high variation of cellular concentration along the experiment and the shift in the dominant species showed in Figure 7 and discussed later in this manuscript.

Indeed, a more complex pattern was found in the continuous feeding experiment (Figure 5). In this case, higher fluctuations in biomass concentrations were detected. Average cellular concentrations of the control tanks ranged between $8 \cdot 10^5$ cell·mL^{-1} and $5 \cdot 10^6$ cell·mL^{-1}. After a period characterized by almost constant concentrations (around $4 \cdot 10^6$ cell·mL^{-1}), a sharp decrease of the feeding nutrients concentration (days 15-17) induced a drop of cellular concentrations (from $4 \cdot 10^6$ to $1 \cdot 10^6$ cells·mL^{-1}) followed by a new rise of cellular concentration following ammonium concentrations. In tanks with pH regulation, the culture was more stable, with slighter oscillations (from $3 \cdot 10^6$ to $9 \cdot 10^6$ cells·mL^{-1}). In spite of the fluctuation in nutrients concentrations, cell concentrations were almost constant until the 20th day of the experiment, when a slight decrease was detected (from $6 \cdot 10^6$ to $3 \cdot 10^6$ cells·mL^{-1}). After the shutdown of the CO_2 injection (day 28), a sharper drop (from $3 \cdot 10^6$ to $9.5 \cdot 10^5$ cell·mL^{-1}) was followed by a new increase of the cell concentrations (from $9.5 \cdot 10^5$ to $1.6 \cdot 10^6$ cell·mL^{-1}).

Such oscillations of cell concentrations are partially due to the variations of nutrient concentrations in the feeding medium attributed to the use of real urban wastewater.

Indeed, the ammonium concentration brought to the culture was in general low (about 6 $mg \cdot L^{-1}$ in average) all over the experiment, with even lower values (1-2 $mg \cdot L^{-1}$) between the 10^{th} and the 20^{th} day. The influence of the nutrients seems to affect mainly the control cultures, while lower and delayed effects were observed in the cultures supplemented with CO_2. The Spearman's rank correlation coefficient indicated a stronger correlation between feeding ammonium concentrations and cellular concentrations in control tanks (0.606, P-value=0.002) than in tanks with CO_2 addition (-0.301, P-value=0.152). In other words, if CO_2 is added, the cellular concentration is more stable and less dependent on the feeding nutrients concentrations.

This means that the addition of inorganic carbon to a microalgae culture is a key trigger for increasing biomass productivity and stability, this is mainly due to the importance of inorganic carbon availability for photosynthesis and microalgae nutrition.

The benefit of CO_2 addition could be allocated to a) the increasing of carbon availability and b) the regulation of an optimal pH for microalgal growth. Moreover, in the case of low ammonium availability, the limitation of high values of pH could increase nutrients availability for microalgae by mediating nutrient removal processes such as ammonia volatilization and phosphate precipitation, which may occur at pH>8 (Heubeck et al., 2007). For example, in the batch experiment (Figure 4) even if all the nutrients were consumed in all the tanks, the maximal biomass concentration reached was lower when pH was not regulated (pH>10). This means that not all nutrients were converted to microalgal biomass, but part of them was certainly stripped or precipitated. At the same time, CO_2 addition could prevent the pH increase to basic values that could shift the equilibrium to higher free ammonia concentrations and inhibit microalgal growth (Heubeck et al., 2007, Uggetti et al. 2014).

3.3 Microalgae species

The daily microscopic observations showed singular patterns in microalgal populations. In the batch experiment, since the 6th day of the experiment, microalgae species with different size and shape appeared in the cultures with CO_2 addition. The new rod-shape microalgae were smaller (<2 µm) than the dominant *Scenedesmus* sp. inoculated at the beginning of the experiment. This shift of populations was supported by the granulometry data (Figure 6). The tanks with pH regulation were characterized by specific profiles of size particles as compared to the control tanks. Indeed, tanks with pH regulation presented a higher proportion of cells with a diameter around 2 µm (6.1% and 7.6% in tanks with pH regulation) than tanks control (from 2.1% to 2.9%). This shift was greater when the pH was regulated at 8, in correspondence with the higher quantity of CO_2 added.

In the continuous feeding experiment, a shift in the dominance of microalgae population was also observed when CO_2 was added to the culture, reproducing exactly the observations found in the batch experiments. The molecular fingerprinting patterns (CE-SSCP analyses) supported the microscopic observations. Figure 7 reports the relative abundance of peaks corresponding to different microalgae species. Figure 7a indicates the relative proportion of the microalgae species present in the inoculum (*Scenedesmus* sp.), its constant relative abundance along the experiment clearly showed a stable presence of *Scenedesmus* sp. in control tanks. Nevertheless, the *Scenedesmus* sp. abundance in tanks with pH regulation decreased after 3-5 days of experiment reaching an almost non-detectable value, but increased again in abundance when the pH regulation was stopped (day 28).

On the other hand, Figure 7b illustrates the relative abundance of spherical microalgae, observed at the microscope in tanks with pH regulation few days after the beginning of the

278 experiment. There was a clear correlation between the abundance of this new species and
279 the pH regulation. In fact, such peak only appeared when CO_2 was injected in the medium
280 and disappeared when the CO_2 injection was stopped (day 28). This phenomenon could
281 explain the greater oscillations of cellular concentrations detected in Figure 5. Indeed, the
282 sudden drop of cellular concentration observed around the day 6 corresponded to the
283 decrease of *Scenedesmus* sp. abundance. The second decrease of cellular concentration
284 (day 23) followed by a new increase (day 30) indicated the drop of the spherical microalgae
285 population and the regrowth of the *Scenedesmus* sp.

286 According to the results found in this study, the response of the control ecosystem to the
287 drop of influent ammonium concentration was a reduction in cellular concentration (Figure
288 5) with a consequent reduction of microalgae biomass production. However, in tanks
289 supplied with CO_2, the cell concentration was more stable and the ecosystem responded
290 with a shift in the dominant species (Figure 7). The maintenance of a diverse and flexible
291 microalgal community allowed the ecosystem to cope with nutrient variations (Yachi and
292 Loreau 1999). This was observed either in the batch feeding conditions or in the continuous
293 feeding conditions. The shift of the dominant population can be explained by fluctuations
294 in the C/N ratio. Indeed, the Redfield ratio (Redfield, 1958) assumes that $C/N<6.6$ indicates
295 C-limitation, while $C/N>6.6$ designates N-limitation. However, such value is species
296 dependent. For example, Chen and Johns (1991) found that C/N ratio of approximately 20
297 indicated a change from carbon to nitrogen limitation for *Chlorella* sp. Thus, change of the
298 C/N ratio can influence the dominant species of a microalgal culture. In this sense, the CO_2
299 injection can increase the C/N ratio of the medium and consequently alter the dominant
300 microalgae species. Note that the higher C/N value for *Chlorella* found by Chen and Johns
301 (1991) would explain the shift found in this experiment to *Chlorella* sp. when CO_2 was

added to the culture. Similarly, the literature suggests that the number and type of algae dominating lakes, streams, estuaries, and oceans are influenced by the relative supplies of nitrogen and phosphorus (Elser et al., 2007).

The change of dominant population associated with CO_2 addition revealed an interesting property of the ecosystem studied that warrant stability of performance. Indeed, two functionally redundant species with overlapping ecological niches may be interchangeable in response to a shift in environmental conditions, in light of the insurance hypothesis (Yachi and Loreau 1999). The ecosystem demonstrated a high elasticity, which allowed the microalgae population to persist in spite of the perturbation imposed to the culture ($pH \leq 8$, no pH regulation). In both cases in which CO_2 was added, the dominant population changed after about 6 days. Moreover, once the CO_2 addition was stopped, the ecosystem was able to adapt to the new carbon availability by changing again the dominant microalgae species.

3.3 Wastewater treatment performance

In the batch experiments, COD and NH_4^+-N were completely removed in all the tanks after 1 day of the experiment. Concerning the continuous feeding experiment, Figure 8 shows the concentrations of organic matter (COD) and nutrients (NH_4^+-N and PO_4^{3-}-P) in the influent and within the culture. As it can be observed, in spite of the increasing COD concentrations in the influent due to the variable characteristics of real wastewater, the heterotrophic bacteria present in all the systems were able to remove almost all the influent COD already after 5 days (Figure 8a). Average removal rates of 93% were achieved in tanks with and without CO_2 addition, reaching concentrations below 125 mg O_2/L, which is the maximum COD concentrations for discharge in a natural medium sets by the European urban wastewater treatment 91/271/EEC Directive (Council Directive, 1991). A

similar pattern was observed for NH_4^+-N (Figure 8b), reaching concentrations below 1 mg/L. This was also due to the low ammonium concentrations resulting from the wastewater dilution.

In general, the results suggest that CO_2 addition and the consequent change in microalgal abundance and species would not alter the property of the system in terms of wastewater treatment.

5. Conclusions

This study deals with the effect of inorganic carbon variations induced by CO_2 addition on microalgal ecosystems and wastewater treatment performances. A pilot plant simulating 4 high rate algal ponds treating diluted untreated urban wastewater was studied during more than one month under batch and continuous feeding conditions. The following conclusions can be drawn:

- CO_2 addition to microalgae culture resulted in an increase of biomass production by 66-100%.
- CO_2 addition had a positive effect on microalgal biomass concentration, reducing the effect of ammonium concentrations variability in the influent wastewater.
- The variation of inorganic carbon availability induced a change of the dominant microalgae species without affecting wastewater treatment performance.
- Maintaining ecosystem diversity may serve as an insurance for ecosystem service that allows coping with fluctuations of the environment.

Overall, this study demonstrated that CO_2 addition promotes the control of the microalgae species and improves biomass production, leading to a culture less dependent on the wastewater nutrients concentrations.

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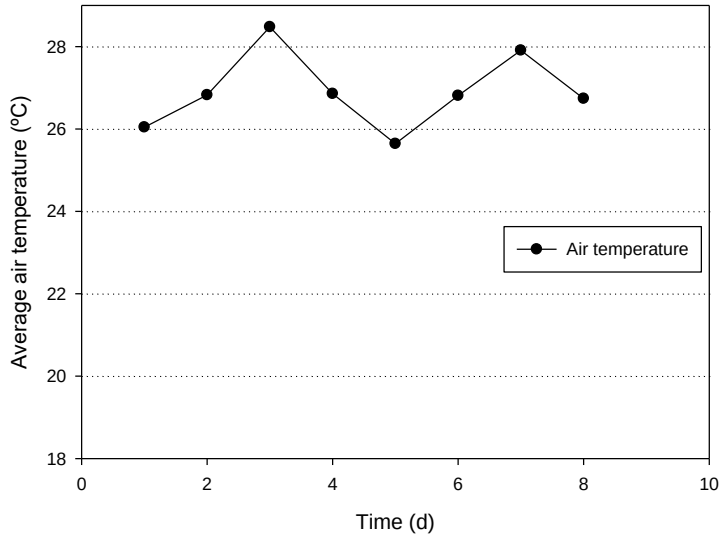
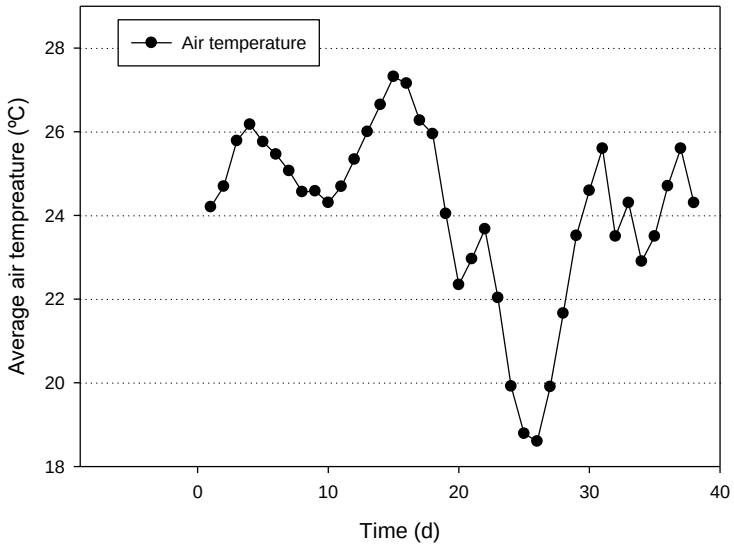
Figures



a)



b)

Figure 1. Average air temperature recorded during the experiment a) batch feeding and b) continuous feeding.

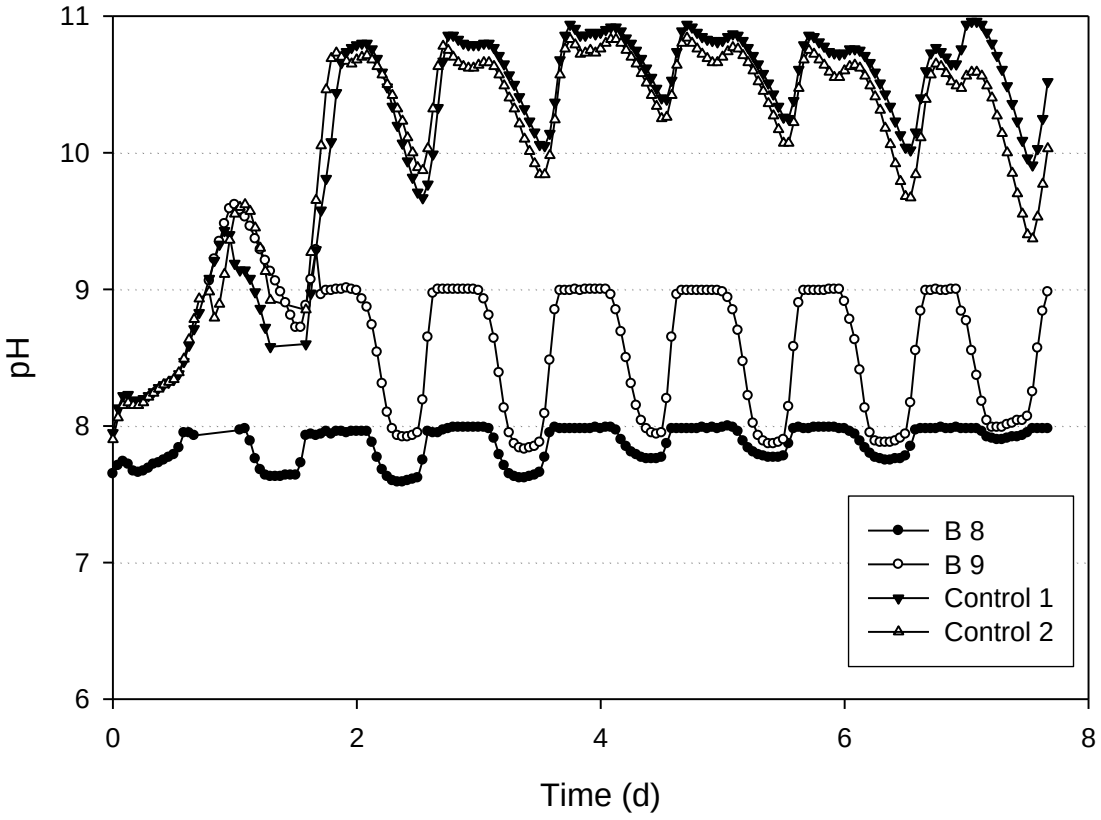


Figure 2. pH pattern recorded in the four tanks along the batch experiments. pH regulation was obtained by CO_2 addition after day 1.

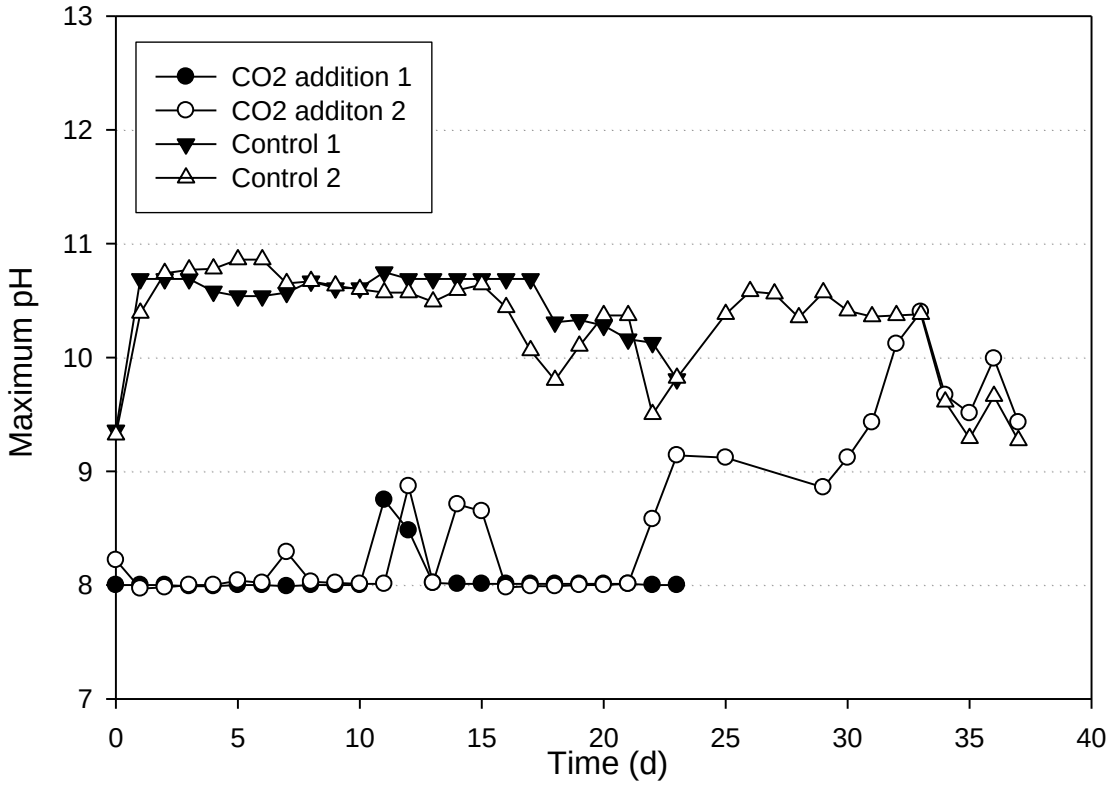
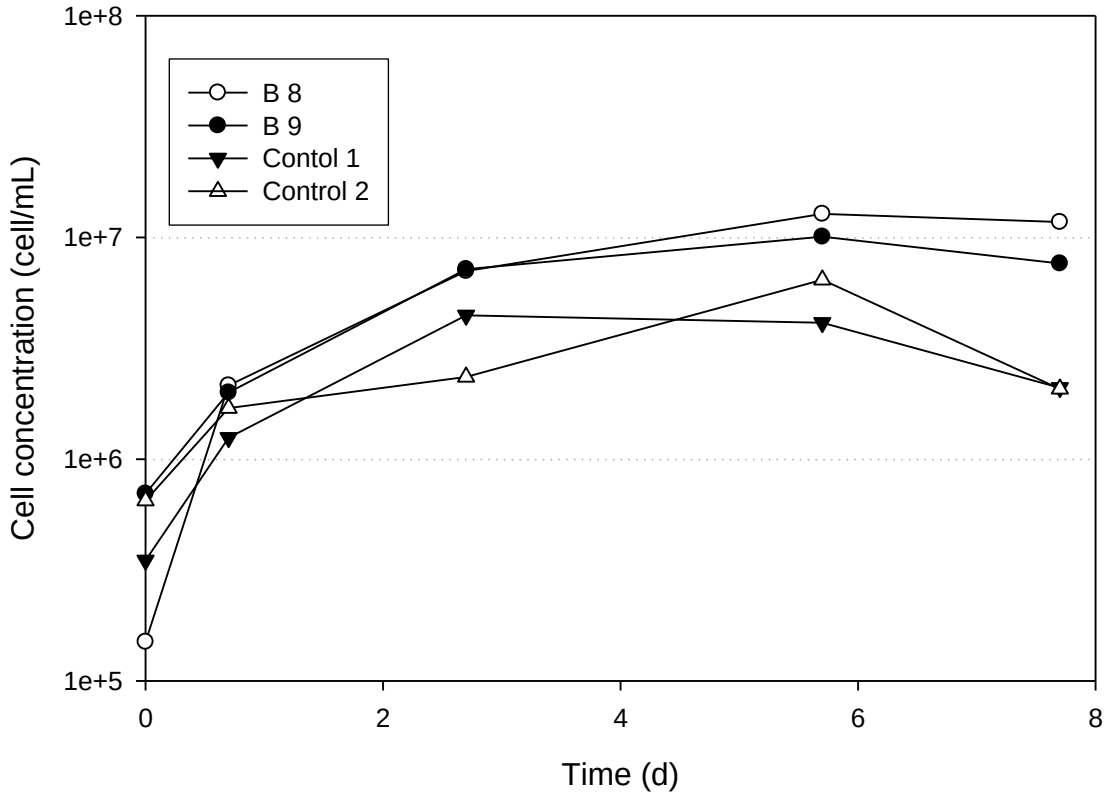


Figure 3. Maximum daily pH value recorded along the continuous experiments in the four tanks. The dotted vertical line indicates the stop of the pH regulation (day 27).



462

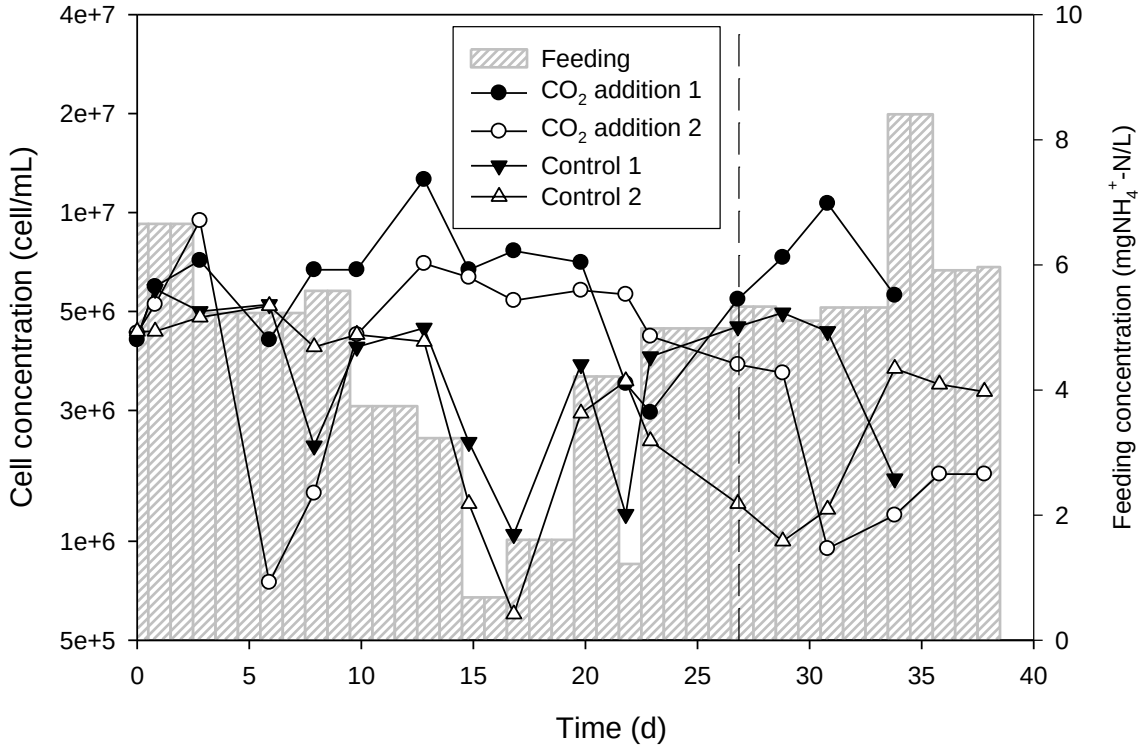
463 Figure 4. Cellular concentration determined in the four tanks by microscopic observation

464 along the batch experiment.

465

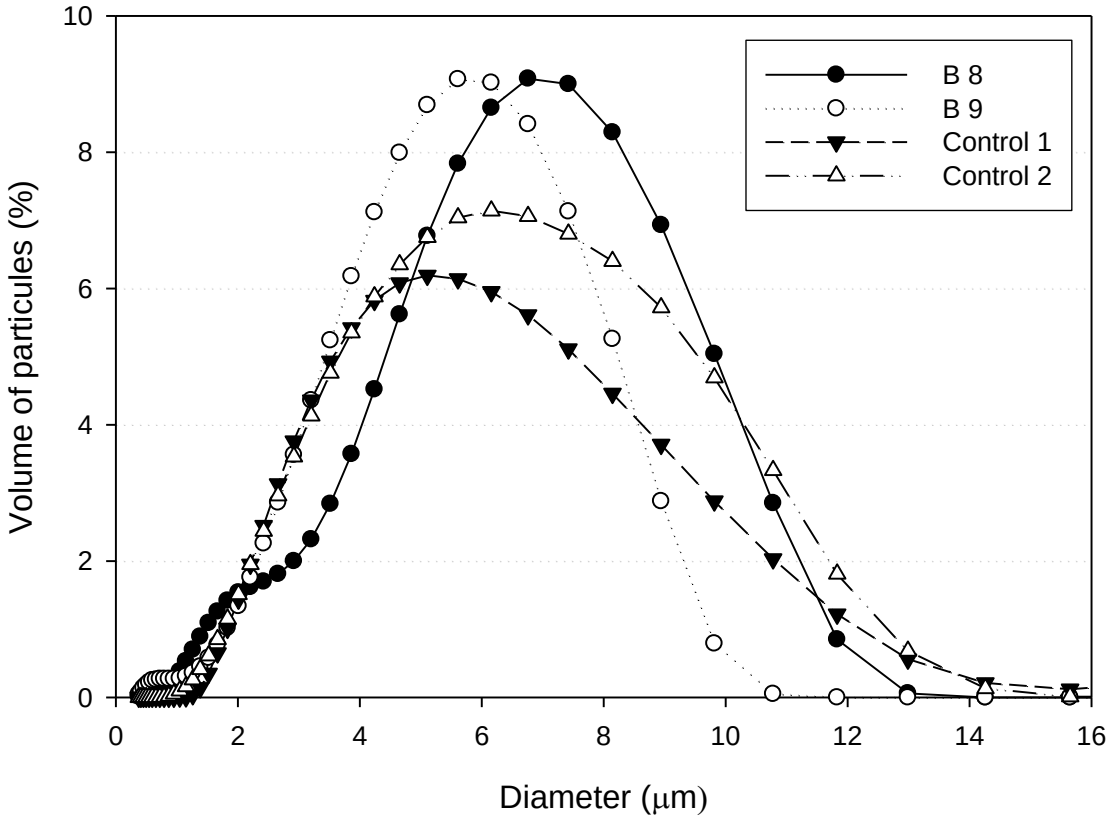
466

467



468

469 Figure 5. Cellular concentrations (determined by microscopic observation) together with
470 the ammonium feeding concentration (in grey) along the continuous experiment. The
471 dotted vertical line indicates the stop of the pH regulation (day 27).



472

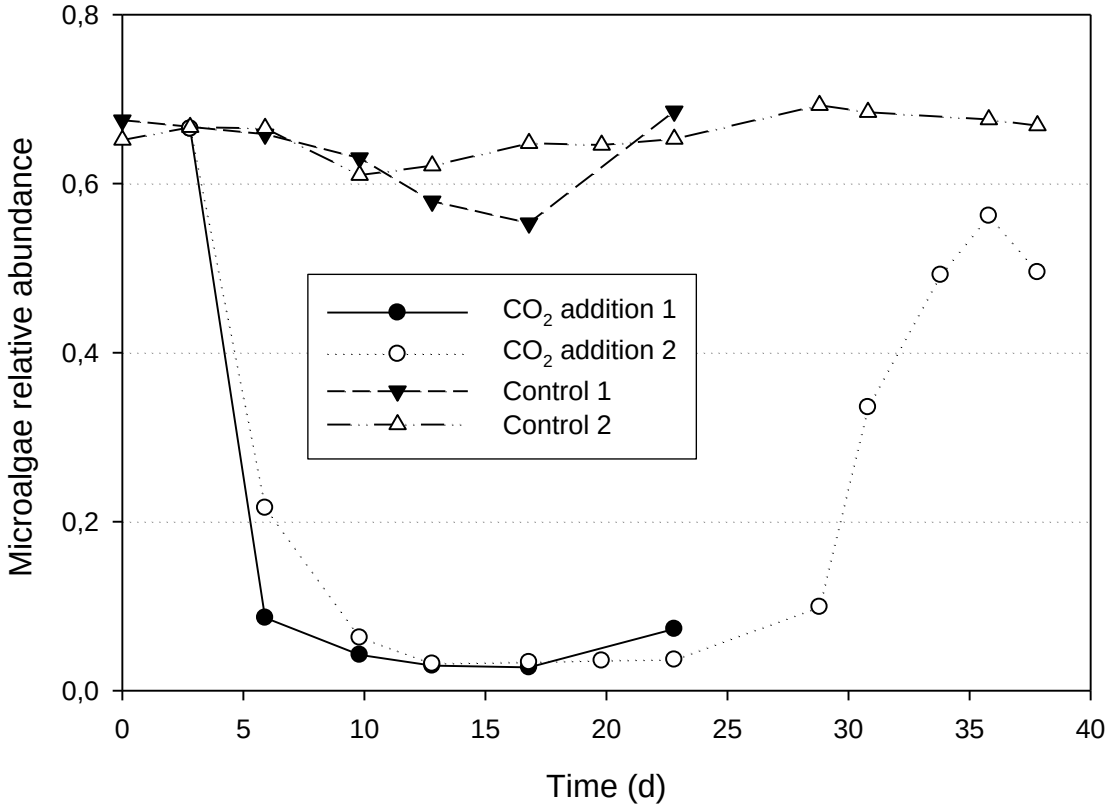
473

474 Figure 6. Size distribution of particles in the four tanks along the batch experiment.

475

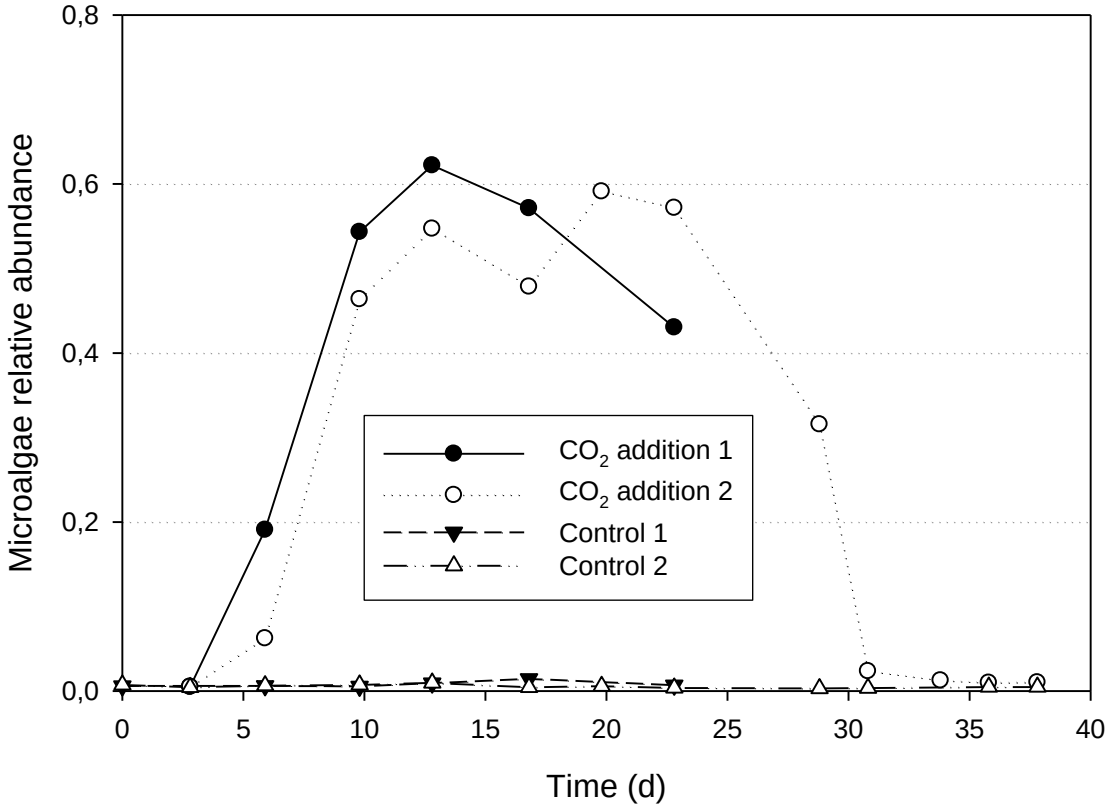
476

477 a)


0,8
0,6
0,4
0,2
0,0
Microalgae relative abundance
0
5
10
15
20
25
30
35
40
Time (d)
CO2 addition 1
CO2 addition 2
Control 1
Control 2

478

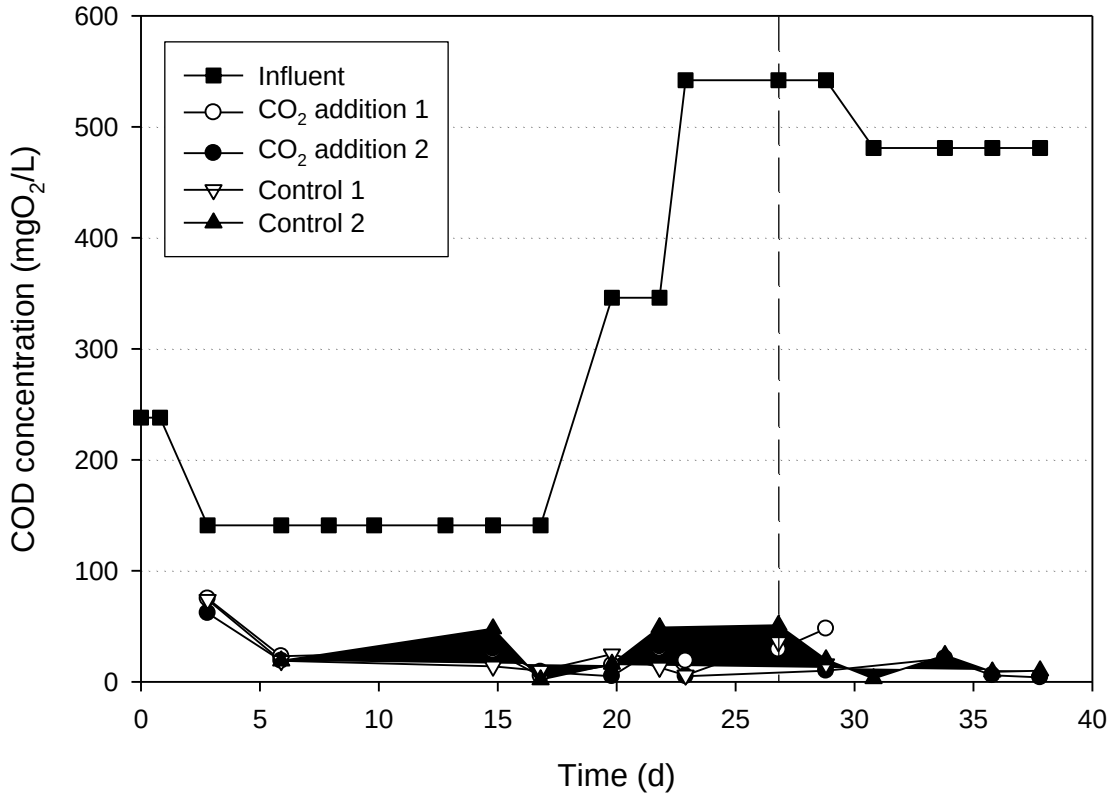
479 b)



480

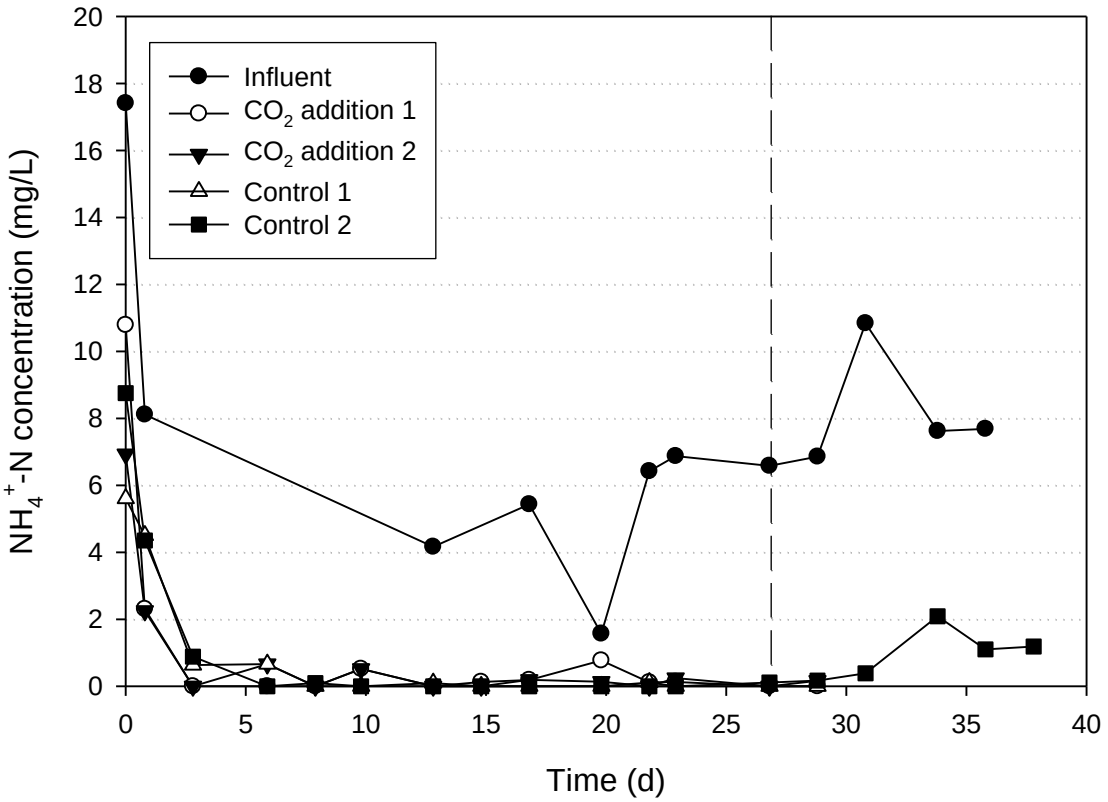
481 Figure 7. Microalgae relative abundance resulting from molecular fingerprinting analysis
482 along the continuous experiment. a) Relative abundance of Peaks 163 and 235 indicating
483 microalgae visually identified as *Scenedesmus* sp. b). Relative abundance of Peak 96
484 indicating a small spherical microalgae visually identified as *Chlorella* sp. The dotted
485 vertical line indicates the stop of the pH regulation (day 27).

486


Influent
CO2 addition 1
CO2 addition 2
Control 1
Control 2
COD concentration (mgO2/L)
Time (d)
0
100
200
300
400
500
600
0
5
10
15
20
25
30
35
40

487

488 b)



489

490 Figure 8. Chemical Oxygen Demand (COD) (a) and ammonium nitrogen (NH_4^+-N) (b)
491 concentrations in the four tanks along the continuous experiment. The dotted vertical line
492 indicates the stop of the pH regulation (day 27).

493