Environmental Science and Pollution Research

Analysis of a photobioreactor scaling up for tertiary wastewater treatment: denitrification, phosphorus removal and microalgae production

--Manuscript Draft--

Manuscript Number:	ESPR-D-18-02643R1
Full Title:	Analysis of a photobioreactor scaling up for tertiary wastewater treatment: denitrification, phosphorus removal and microalgae production
Article Type:	Research Article
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Funding Information:	
Abstract:	The present work studies the removal of nutrients (nitrate and phosphate) from a synthetic wastewater simulating a secondary treatment effluent using the microalgae Chlorella vulgaris in autotrophic photobioreactors, together to an analysis of the critical points affecting the scaling-up process from laboratory to pilot scale. Laboratory experiments were done in open agitated 1 L photobioreactors under batch operation mode, while pilot-scale experiments were done using a 150 L closed tubular photobioreactor under continuous operation mode. In both scales, nitrate was the limiting substrate and the effect of its concentration on microalgae performance was studied. From laboratory experiments, an average microalgae productivity of 85 mgVSS L-1 d-1 and approximate maximum N-NO3- and P-PO43- removal rates of 8 mg N gVSS-1 d-1, and 2.6 mg P gVSS-1 d-1 were found. Regarding pilot scale, the average microalgae productivity slightly decreased (76 mgVSS L-1 d-1) while the approximate maximum N-NO3- and P-PO43- removal rates slightly were increased (11.7 mg N gVSS-1 d-1 and 3.04 mg P gVSS -1 d-1) with respect to the laboratory scale results. The pilot scale operation worked under lower levels of turbulence and higher dissolved oxygen concentration and light intensity than laboratory experiments; those parameters were difficult to control and they can be identified as the critical points in the differences found on both nutrient removal and microalgae production.
Response to Reviewers:	see attachment
Additional Information:	
Question	**Response**
§Are you submitting to a Special Issue?	No

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Environmental Science and Pollution Research
Editor

3-July-2018

Dear Editor:

Attached you will find the revised manuscript ESPR-D-18-02643 "*Analysis of a photobioreactor scaling up for tertiary wastewater treatment: denitrification, phosphorus removal and microalgae production*", by José Villaseñor Camacho, Carmen M. Fernández Marchante, and Luis Rodríguez Romero (corresponding author: jose.villasenor@uclm.es), in order to be reviewed for a possible publication as original research paper in *Environmental Science and Pollution research.*

The following items are included in the new submission:

- The "**Revised Manuscript**" (using MS Word).
- The "**Highlighted Revised manuscript**", that is the same revision manuscript MS Word file, using the track changes mode, where you can easily find the modifications made to the text.
- The "**Responses to reviewers**": One MS Word document containing the detailed answers to each concrete reviewer's comments. Each answer indicates the position of the modifications in the revision changes marked manuscript.
- Revised figures **3, 4 and 5**.

Yours sincerely

Dr. José Villaseñor Camacho

Revision Notes: Response to Reviewers

This document shows detailed responses to the reviewer`s comments. The responses indicate also the location (page and lines) of changes made in the highlighted revised manuscript.

Reviewers' comments:

Reviewer #1:

I have difficulty to see why nitrate is used as the sole nitrogen source in this work. It is well-known that microalgae prefer ammonium over nitrate, nitrite and urea, and ammonium is the dominate nitrogen source in secondary effluents from wastewater treatment plant. The author should provide more information to clarify the innovation of this work by investigating the nitrate removal other than ammonium removal.

RESPONSE

According to the authors 'opinion, and as it has been indicated in the introduction section, "*Nitrification is practically and successfully implemented in the aerobic step most of the classical biological treatments, but denitrification and P removal may not be considered in the plant design (old plants) or may not work properly"*. Because of it, this work is focused on nitrate discharges in secondary effluents, and this is one of the two novelty points. It is well known that most of the nitrogen removal works using microalgae are focused on ammonium removal, while the use of nitrate is not so common. This statement was already included in the manuscript (lines 73-77) and also the novelty point has been clearly indicated in the revised manuscript (lines 106-110).

Furthermore, it is a good idea to compare the performance between laboratory and pilot work and to find out the critical points affecting the scaling-up process from lab to pilot. However, if nothing could be controlled in pilot-scale, how could we compare these two systems?

From the work results, some points have been identified as to be critical, and so it has been concluded that future efforts should be made to improve control of such factors and thus to

allow studies that compare laboratory and pilot scale systems. This statement has been included in the conclusions section (revised manuscript, lines 382-385).

It is recommended to be accepted after revising or clarifying the abovementioned points.

Specific comments:

P5, line 40: add reference for BBM medium

RESPONSE

BBM is a useful medium for microalgae growth (including C. Vulgaris). A new reference has been included (revised manuscript line 120, and line 416)

P5, line 45: Why 10% CO2 was used? Is there any reference?

RESPONSE

10% CO_2 is commonly used to simulate flue gas. Two references have been included in the revised manuscript (line 122 and line 412)

P5, line 52: what is the pH in synthetic BBM medium?

RESPONSE

pH was between 6.6 and 6.8. It was indicated in the revised manuscript (line 133).

P6, line 16: Information on LED lamps (company, model and country)?

RESPONSE

Information has been included in the revised manuscript (line 138).

P7, line 48: what is the initial amount of microalgae?

RESPONSE

It was a seed, approximately 0.15 g_{vss} L^{-1}. This information has been included in the revised manuscript (line 176).

P8, line 8: flow rate instead of flowrate

RESPONSE

The change has been made.

P8, line 47: why dissolved oxygen concentration was maintained at 7.0±1.2 mg/l?

RESPONSE

It was the saturation level obtained because of the air supply using the laboratory compressor (see Figure 1, part 1). The manuscript has been modified according to this comment (line 199).

Please add error bars in the figures.

RESPONSE

Figures 3, 4 and 5 (where batch experiments were performed by triplicate) have been changed.

Reviewer #2:

This study researched the removal of nutrients (nitrate and phosphate) from a synthetic wastewater simulating a secondary treatment effluent using the microalgae Chlorella vulgaris in autotrophic photobioreactors, together to an analysis of the critical points affecting the scaling-up process from laboratory to pilot scale. Overall, this study is interested and valuable to reduce the nutrients such nitrogen and phosphorus from wastewater. However, as we know in practice, the phosphorus in wastewater is less than 10 mg L^{-1} while the input concentration of phosphorus in this study is very more higher than this value. Thus, it should be introduce the scenes where the phosphorus concentration is such high.

Regarding nutrients removal, the work has been especially focused on N removal, and nitrate has been always the limiting nutrient. The present work also studied P removal although it is true that we used always an excess P concentration (P was not limiting nutrient in our

experiments) as we used a very common growth medium for microalgae (BBM). A brief change has been included in revised manuscript (line 127) regarding this comment.

Second, there are lots of similar works using the microalgae to reduce (or accumulate) nutrients based on the results of crossrefenences. Thus, the novelty of this studying using microalgae Chlorella vulgaris to reduce nutrients concentrations should be highlighted.

RESPONSE

One of the novelty points is that most of the nitrogen removal works using microalgae are focused on ammonium removal, while the use of nitrate is not so common. Moreover, the second novelty point is focused on the scaling-up process, and the critical operating parameters to perform scale-up have been identified.

The main novelty point in the present work has been included in the revised manuscript (106-110)

Third, experiments of from lab-scale to pilot are valuable, while the running conditions are dissimilar, please give some explanations why the conditions were conducted in different pattern.

Bench-scale studies are usually performed in single-designed PBR although it is known that tubular PBR are preferentially selected for scale-up. This work considered some criteria for scale-up: maintaining the retention time, the nutrients and CO_2 concentrations, and the flow model (complete mixed system). However it is true that it was not possible for us to maintain the same high turbulence level at pilot-scale and, obviously, the weather conditions also influenced in the pilot-scale operation. Authors consider that, if possible, these factors should be better controlled and additional efforts should be made in a future in scaling-up studies. A brief change has been included in the revised manuscript regarding this comment (line 382-385).

As the authors said that "Although N and P removal rates were low compared to classical biological nutrient removal secondary processes, the use of microalgae could be considered

a more sustainable technology for wastewater treatment. ", If I am the coauthor, I will estimate the cost and the running fee, to compare the classical biological nutrient removal secondary process, to check which one is the feasible at economical level.

RESPONSE

Under the authors' opinion is quite difficult to propose a broad calculation of cost and running fee to compare PBR vs activated sludge BNR systems, The works previously reported and cited in the manuscript (Judd et al 2015; Marbelia et al 2014) give details about the economic and environmental advantages/disadvantages of both systems. Some details have been included in the revised manuscript in order to improve the las paragraph in discussion section: According to Judd et al (2014), because of the lower nutrient removal rates and the high surface (low depth) necessary for microalgae, PBR systems may be 15 times slower than classical activated sludge BNR systems, and overall there is two order of magnitude difference in footprint between them. Against this, the biomass yield is quite lower in PBR and microalgae is considered a valuable product. Additionally, Marbelia et al (2014) describe different scenarios to combine classical wastewater treatment plants with microalgae nutrient removal and they propose approximate values of power consumption and operation costs (lines 355-365).

Analysis of a photobioreactor scaling up for tertiary wastewater treatment: denitrification, phosphorus removal and microalgae production

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Abstract

The present work studies the removal of nutrients (nitrate and phosphate) from a synthetic wastewater simulating a secondary treatment effluent using the microalgae *Chlorella vulgaris* in autotrophic photobioreactors, together to an analysis of the critical points affecting the scaling-up process from laboratory to pilot scale. Laboratory experiments were done in open agitated 1 L photobioreactors under batch operation mode, while pilot-scale experiments were done using a 150 L closed tubular photobioreactor under continuous operation mode. In both scales, nitrate was the limiting substrate and the effect of its concentration on microalgae performance was studied. From laboratory experiments, an average microalgae productivity of 85 mg_{VSS} L^{-1} d^{-1} and approximate maximum N-NO_3^- and P-PO_4^{3-} removal rates of 8 mg N g_{VSS}^{-1} d^{-1}, and 2.6 mg P g_{VSS}^{-1} d^{-1} were found. Regarding pilot scale, the average microalgae productivity slightly decreased (76 mg_{VSS} L^{-1} d^{-1}) while the approximate maximum N-NO_3^- and P-PO_4^{3-} removal rates slightly were increased (11.7 mg N g_{VSS}^{-1} d^{-1} and 3.04 mg P g_{VSS}^{-1} d^{-1}) with respect to the laboratory scale results. The pilot scale operation worked under lower levels of turbulence and higher dissolved oxygen concentration and light intensity than laboratory experiments; those parameters were difficult to control and they can be identified as the critical points in the differences found on both nutrient removal and microalgae production.

Keywords

Photobioreactor, microalgae, nitrate removal, phosphorus removal, scale-up, *Chlorella vulgaris*.

1. Introduction

Nutrient removal in modern domestic wastewater treatment plants is usually included in the secondary biological step and it is achieved by means of well-known processes such as nitrification, denitrification and biological phosphorus removal (Ekama 2015), the whole of them is called as Biological Nutrient Removal (BNR). Nitrification is practically and successfully implemented in the aerobic step most of the classical biological treatments, but denitrification and P removal may not be considered in the plant design (old plants) or may not work properly in modern plants because of the operation costs or the lack of pre-hydrolized easily biodegradable organic matter in the wastewater, which is an important requisite (Zheng et al. 2015). Thus, it is usual that nitrogen removal is not satisfactory and it is present mainly as $N\text{-}NO_3^-$ in the effluent, and also the P concentration may exceed the discharge limit, which caused adverse environmental impacts.

Because of this problem there are currently different tertiary systems for the removal of nitrate and phosphate in secondary effluents. Some of them are based on physicochemical fundamentals but they are associated with significant costs due to the consumption of chemical reagents. An alternative to such physicochemical methods may be the biological nutrient removal using microalgae and photobioreactors. There is currently a lot of information in the scientific literature about the use of microalgae for N and P removal in wastewater (Cai et al. 2013). It can be achieved through an autotrophic photosynthetic process capable to treat a secondary effluent with no biodegradable organic matter. Microalgae may be also used in mixotropic processes, a combination of heterotrophic and autotrophic biological treatment in which bacteria and microalgae simultaneously remove organic carbon, carbon dioxide from heterotrophic respiration, nitrogen and phosphorus

(Zheng et al. 2015). Since microalgae prefer ammonium rather than oxidized forms of nitrogen, the number of scientific papers focused on ammonium removal is higher than those about nitrate removal, despite the fact that nitrate is the main nitrogen form in secondary effluents from wastewater treatment plants (Cai et al. 2013). Moreover, the potential of nitrate-accumulating microalgae for nutrient recovery has not been adequately investigated to date (Coppens et al. 2014).

In addition to nutrients capture, photosynthetic autotrophic processes using microalgae have been widely used for carbon dioxide removal in exhaust combustion gas and for microalgae biomass production because of its multiple uses. Unlike sewage sludge from wastewater treatment plants, microalgae biomass is considered as a valuable raw material instead of a biowaste. Many applications of microalgae biomass have been proposed and investigated (Odjadjare et al. 2017): (i) transformation into valuable bioproducts such as lipids, oil, fatty acids, pigments, vitamins and proteins, (ii) transformation into energy sources, e.g. biofuels, biogas or biohydrogen, and (iii) animal food manufacture. The cost of such biomass production could be significantly reduced by using treated sewage as inorganic nutrients source (Cabanelas et al. 2013).

However, most of the research work regarding microalgae nutrient removal have been made under laboratory scale, while the research focused on scale-up to pilot or full scale photobioreactors did not receive so much attention. It is very important to study the differences that could appear in the scale-up step because some parameters such as temperature, light intensity or turbulence are easily controlled under lab scale but not under pilot scale (Acién Fernández et al. 2013). Several authors reported scale-up investigations where both nutrient removal efficiency (Van den Hende et al. 2014) and microalgae productivity (Lam et al. 2015) decreased in pilot scale photobioreactors.

In this context, the present work shows the results of an experimental study in which *Chlorella vulgaris* microalgae was used to remove nitrate and phosphate from a synthetic wastewater simulating a secondary treatment effluent. The study was performed both at laboratory and pilot scales. Laboratory experiments were done in open agitated photobioreactors under batch operation mode and using different nitrate concentrations, while pilot-scale experiments were carried out in a closed tubular photobioreactor (150 L reaction volume) under continuous operation mode. One of the novelty points is that most of the nitrogen removal works using microalgae are focused on ammonium removal, while the use of nitrate is not so common. Moreover, the second novelty point is focused on the scaling-up process, and the critical operating parameters to perform scale-up have been identified. Thus, the objectives of the present work were: (i) to assess the $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ removal rates and biomass yields both under laboratory and pilot scale, (ii) to study the relationship between the differences found in those parameters and the main operating variables for both scales, and (iii) to identify the most critical operating parameters in the scale-up process.

2. Materials and Methods

2.1. Microalgae and growth medium

Chlorella vulgaris was obtained from the Culture Collection of Algae in the University of Las Palmas de Gran Canaria (Spain). The microalgae culture was incubated in closed flasks using Bold's Basal Medium, BBM, as synthetic culture medium (Frumento et al. 2016), weekly growth cycles at ambient temperature (approximately 21ºC) and day/night alternation. Air with 10% CO_2 simulating flue gas was bubbled during light periods (Judd et al 2015; Duarte et al 2016).

BBM was chosen as a synthetic medium to simulate the secondary effluent as it contains nitrate and phosphate as the main inorganic nutrients, although P concentrations used in BBM were higher than in the usual secondary effluent levels. BBM also presents buffer capacity for pH control, and it was the liquid medium used both in the laboratory and pilot-scale experiments. Its composition was the following (mg L^{-1}): $NaNO_3$, 250.0; $CaCl_2 \cdot 2H_2O$, 25.0; $MgSO_4 \cdot 7H_2O$, 75.0; K_2HPO_4, 175.0; KH_2PO_4, 75.0; NaCl, 25.0; EDTA, 50.0; KOH, 31.0; H_3BO_3, 11.5; $FeSO_4 \cdot 7H_2O$, 5.0; $ZnSO_4 \cdot 7H_2O$, 8.8; $MnCl_2 \cdot 4H_2O$, 1.8; $CuSO_4 \cdot 5H_2O$, 1.6. pH was between 6.6 and 6.8.

2.2. Experimental installations

Figure 1 shows a scheme of the experimental installation used for the lab-scale experiments. It consisted of a photobioreactor with the following parts: (1) a system for atmospheric air feeding enriched in CO_2 (2), in order to simulate a combustion exhaust gas; (3) a thermostated closed chamber with adjustable temperature; (4) UEETK (USA) 28cm LED lamps for artificial lighting and a timer (5) to adjust the duration of light/dark cycles; (6) a multiple magnetic stirring system and several 1 L glass bottles (7) which acted as completely mixed batch reactors that contained the microalgae suspensions and the liquid growth medium, receiving the air/CO_2 mixture flow through bubbling (8).

Figure 2 shows a scheme (a) and a photograph (b) of the pilot-scale installation. It consisted of a tubular photobioreactor that contained the following parts: (1) a system for atmospheric air feeding enriched in CO_2 similar to the one used in laboratory; (2) a CO_2 absorption tank (100 L) with a mechanical stirring system that contained the liquid growth medium; (3) the feeding system of the liquid growth medium saturated in CO_2 consisting of a peristaltic pump; (4) a 150 L tubular photobioreactor composed by consecutive tubes of 1.5 m long and 9 cm diameter; (5) a degasification unit; (6) a peristaltic pump for liquid

flow recirculation; (7) an effluent outlet; and (8) an atmospheric air compressor to improve the turbulence. It was also equipped with temperature and lighting sensors. The system worked under continuous operation mode as a completely mixed biological reactor without biomass recirculation. The whole pilot-scale installation was located into a greenhouse (Figure 2b) which allowed temperature control by an air-conditioning device, and maximum light intensity control by a manual adjustable solar radiation mitigation system. The greenhouse was located next to the Institute of Chemical and Environmental Technology of the University of Castilla-La Mancha, Ciudad Real (Spain).

2.3. Experimental procedure

Lab-scale nutrient (nitrate and phosphate) removal experiments were performed under batch operation mode. Glass bottles were filled with the growth liquid medium. Depending on the experiments, the growth medium contained different nitrate concentration. The same initial amount of microalgae was inoculated in all bottles and then magnetic mixing and light/darkness cycles were connected during 10 days. Air containing 10% (v/v) CO_2 was bubbled only during light cycles. Temperature was 21ºC. Mixing rate was 10 s^{-1} (Approximate Reynolds number of $25 \cdot 10^3$). Light/darkness cycles were 12h/12h and light intensity was 100 μmol m^{-2} s^{-1}. Three experiments were performed: N1, N2 and N3 that contained initial concentrations of 14.6, 28.2 and 40.8 mg $N\text{-}NO_3^-$ L^{-1}, respectively. Experiments were made by triplicate. The liquid samples were taken every 10 hours from start to day 3, and then every 24 hours until the end.

Pilot-scale nutrient removal experiments were made under continuous operation mode during 7 months. The system worked as a tubular completely-mixed biological reactor without biomass recirculation, that is, the hydraulic retention time was the same as the

cell (microalgae) retention time. The photobioreactor was filled with the BBM liquid growth medium and inoculated with an initial amount of microalgae culture (approximately 0.15 g_{VSS} L^{-1}) from the laboratory. A batch operation was applied during the first week, as acclimation step in order to reach enough microalgae concentration, and then a continuous flow of CO_2 saturated liquid growth medium containing 40.8 mg N-NO_3^- L^{-1} was used (as in the N3 laboratory experiment). According to the results previously found in the laboratory experiments, hydraulic retention time were varied throughout the experiment: 5.5 d in the first month, 6.0 d in the second month and 6.5 d in months 3 to 7 (which was the main stationary period to compare with the laboratory results), corresponding to mean flow rates of 27.3, 25.0 and 23.1 L d^{-1}, respectively. Atmospheric air was supplied by a compressor to favour liquid flow and mixing and degasification of excess dissolved oxygen (Acién Fernández at al. 2013).

2.4. Analytical methods

All analytical methods followed Standard Methods (A.P.H.A., 1998). Microalgae concentration in the liquid samples was measured as volatile suspended solids (VSS) by weight loss after ignition at 550ºC. Nitrogen (N-NO_3^-) and phosphorus (P-PO_4^{3-}) concentrations were measured by colorimetric methods using a DR2700 Hach portable spectrophotometer (Colorado, USA). Dissolved inorganic carbon (IC) was measured by a TOC analyser (Shimadzu TOC-VCSH, Columbia, USA). The pH was measured by a pH-meter (*PCE-228M*). Dissolved Oxygen was measured using a *YSI 5000* dissolved oxygen probe. Light intensity was measured by a *Collihigh* illuminometer.

3. Results and Discussion

3.1. Evolution and control of experimental conditions

Experimental conditions were easily controlled in the laboratory experiments. Temperature was maintained in 21.0 ± 2ºC, pH was 6.5 ± 0.5 and saturation dissolved oxygen concentration was 7.0 ± 1.2 mg L^{-1}. Light intensity was kept constant at 100 μmol $m^{-2}s^{-1}$.

Pilot scale conditions were more difficult to control than in laboratory. Temperature, pH, dissolved oxygen concentration and lighting values monitored throughout the experimental period have been included as supplementary material (online resource, Figure S1). Temperature values varied between 15 and 28ºC during the first 100 d, although it were better controlled and maintained (around 24ºC) during the rest of the experiment; pH varied between 6.5 and 8.0 by means of the buffer capacity of the BBM growth medium; dissolved oxygen concentration varied between 6 and 12 mg L^{-1} approximately, and, finally, light intensity reached higher values than those of lab-scale tests (in the range 100-300 μmol m^{-2} s^{-1}) during the first 100 days, although it was better controlled (in values around 90 μmol m^{-2} s^{-1}) throughout the rest of the experimental period. Observed variations in temperature and lighting were mainly due to changes in external climatic conditions, while pH changes may be attributed to variations in carbon availability throughout the experiment (Acién Fernández et al. 2013). Therefore, it seems clear that, in spite of the control systems implemented in the pilot plant used, the achievement of precise control of the operating conditions at the pilot plant scale is a complex issue that needs to be adequately addressed in future experiments.

3.2. Nutrient removal and microalgae production in laboratory

Figure 3(a) shows the growth of microalgae during the laboratory batch experiments using different values of the initial nitrate concentration (mean values from 3 replicates). As previously indicated, batch experiments were performed under excess concentrations

of N and P, being $N\text{-}NO_3^-$ the limiting nutrient at the end of the experiments (as discussed in Figure 4). Microalgae growth profiles were straight lines indicatives of first order growth kinetics, without a clear influence of the initial nitrate concentration. The maximum growth rate began to decline from day 8. An average microalgae productivity value of 85 $mg_{VSS}\ L^{-1}\ d^{-1}$ was calculated during this period. Taking into account also the average biomass concentration, an approximate maximum specific growth rate of 0.18 d^{-1} may be calculated, which corresponds to an approximate hydraulic retention time of 5.5 d in a hypothetical continuous operation mode.

Figure 3(b) shows the dissolved inorganic carbon (IC) consumption measured in the closed batch tests carried out in the laboratory (average values from three replicates). The gas mixture air/CO_2 (10% v/v) was bubbled into the microalgae suspension (0.25 $g_{VSS}\ L^{-1}$) in BBM medium until it was saturated with CO_2. Then, the air/CO_2 flow was stopped and the dissolved inorganic carbon (IC) concentration consumption was measured during several hours. It can be observed a constant maximum carbon consumption rate during the first 9 h approximately. It means that an excess of inorganic carbon, as well as nitrogen and phosphorus, was kept during the first 9 h and therefore it was not the limiting substrate for microalgae growth. Under such conditions, the maximum microalgae IC consumption rate in the laboratory photobioreactor may be calculated, obtaining a value of 118 mg C $g_{VSS}^{-1}\ d^{-1}$. Taking into account the microalgae productivity value previously calculated, an approximate biomass yield of 1.2 $g_{VSS}\ g_C^{-1}$ was obtained.

Figure 4(a) shows the $N\text{-}NO_3^-$ concentration profiles in the three laboratory batch experiments performed with different initial nitrate concentrations. Figure 4(b) shows the values of the biomass specific $N\text{-}NO_3^-$ removal rates (mg N $g_{VSS}^{-1}\ d^{-1}$). Assuming the typical variability of the experimental results in this type of biological processes (see

dashed lines in Figure 4b), it is possible to observe a Monod-type trend in the removal rates and, thus, it may be approximately established the range in which the specific N removal rate is maximum. Maximum N removal rate was approximately 8 mg N g_{VSS}^{-1} d^{-1}, and it began to decrease from N concentrations of approximately 18 mg L^{-1} (that is, approximately from day 5 in N3 experiment) and, from that concentration, it can be assumed that N became the limiting nutrient as the liquid medium was always saturated in CO_2 and P concentration was quite high (Figure 5). N removal rates during the last days were very low and some additional days would be necessary to the complete N depletion.

Figure 5(a) shows the P-PO_4^{3-} concentration profiles in the laboratory batch experiments while Figure 5(b) shows the specific P removal rates (mg P g_{VSS}^{-1} d^{-1}). The average biomass specific P removal rate observed during the first days, that is, in the period where the maximum N removal rate was kept (no N limitations), was approximately 2.6 mg P g_{VSS}^{-1} d^{-1}. As it was previously indicated, P was not a limiting nutrient in the present work. Moreover, the buffer capacity of the BBM growth medium kept pH between 6.0 and 7.0 avoiding P precipitation (Cai et al. 2013).

From the approximate values of the nutrient removal rates previously calculated, a mass stoichiometric removal ratio IC/N/P of 100/6.8/2.2 was found. It would indicate a mass removal N/P ratio of approximately 3.1, which can be considered as a low value compared to previously reported values (N/P = 7) regarding N and P removal by microalgae (Acién Fernández et al. 2013; Ruiz et al. 2013); nevertheless, these works usually refer to ammonium nitrogen capture instead of nitrate.

3.3. Nutrient removal and microalgae production in the pilot-scale photobioreactor

Figure 6 shows (a) the microalgae and (b) the dissolved inorganic carbon (IC) concentrations, in the effluent of the pilot scale photobioreactor during the 7 months of continuous operation. According to the results obtained in the laboratory experiments, the hydraulic retention time (HRT) used in the first month of operation was 5.5 d. However, as the removal of the limiting nutrient (nitrate) was not completed (see later discussion, Figure 7), HRT was increased to 6.0 d in the second month and, finally, to 6.5 d during the rest of the continuous operation period. It can be observed in Figure 6a that the effluent microalgae concentration was approximately stabilized in 0.5 g_{VSS} L^{-1} (horizontal line), which means an average microalgae productivity of 76 mg_{VSS} L^{-1} d^{-1}, that is approximately 12% lower than the productivity value found in laboratory (Section 3.2).

The IC effluent concentration (Figure 6b) showed significant fluctuations but an approximate average value of 85 mg L^{-1} (horizontal line) may be considered; it corresponded to an approximate removal efficiency of 82% with respect to the IC in the saturated liquid stream flowing into the photobioreactor from the absorption tank. Thus, it was calculated a IC removal rate value of 121 mg C g_{VSS}^{-1} d^{-1} and a biomass yield of 1.26 g_{VSS} g_C^{-1}, which are similar values to those found in the laboratory experiments.

The microalgae productivity values obtained in the present work could be compared to other previously reported values. We have selected previous works regarding nitrogen (mainly ammonium) and phosphate removal in secondary effluents using *Chlorella vulgaris*. For instance, Ruiz et al. (2013) reported values between 40 and 170 g L^{-1} d^{-1}; Gao et al. (2014) reported 10.3 g L^{-1} d^{-1}; and Marbelia et al. (2014) reported 33 g L^{-1} d^{-1} using 5 d as HRT. Honda et al. (2012) reviewed microalgae productivity values between 48 and 1500 mg L^{-1} d^{-1} under different experimental conditions. Finally, Arbib et al.

(2015) reported values between 600 and 800 g L^{-1} d^{-1} using again 5d as HRT. All these reported results show great variability depending on the specific experimental conditions.

Figure 7 shows the effluent nutrient concentrations ($N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$) during the operation of the pilot scale photobioreactor (data points) together with the inlet N and P concentrations (horizontal lines). As previously indicated, phosphorus was always in excess, therefore being nitrate the limiting nutrient and the parameter used to decide about the HRT conditions. Effluent $N\text{-}NO_3^-$ concentrations were in the range 0-25 mg L^{-1} throughout the first month of operation (HRT 5.5 d), which indicated that this nutrient was not completely used. On the contrary, after increasing HRT to 6.5 d, N was almost completely consumed and thus N became as limiting nutrient. According to the high P inlet concentration and the lower P capture capacity of microalgae, P removal efficiency was quite lower than N removal efficiency.

From Figure 7, average effluent N and P concentrations were estimated, being 2.1 and 38.3 mg L^{-1}, respectively. They corresponded to nutrient removal rates of 5.9 mg N $L^{-1}d^{-1}$ and 1.5 mg P $L^{-1}d^{-1}$, respectively, and biomass specific removal rates of 11.7 mg N g_{VSS}^{-1} d^{-1} and 3.04 mg P g_{VSS}^{-1} d^{-1}. According to the IC, N and P removal rates at the final stationary period, the mass stoichiometric removal ratio IC/N/P was calculated as 100/9.8/2.5. It means a N/P ratio of 3.9 which is higher than that obtained in the laboratory experiments (3.1) but still lower than those formerly reported in the literature (Acién Fernández et al. 2013; Ruiz et al. 2013).

As previously stated, nitrate removal studies by microalgae are not as common as ammonium removal studies. Honda et al (2012) reported lower growth rate values for *C. vulgaris* using nitrate compared to those using ammonium. Gao et al. (2014) reported that

C. vulgaris did not seem to use nitrate as substrate while, in contrast, Aslan and Kapdan (2006) reported that *C. vulgaris* removed 510 mg $N\text{-}NO_3^-$ and 29 mg $P\text{-}PO_4^{3-}$ in a 6 d batch period. Finally, Coppens et al. (2014) showed some results for different nitrate-accumulating microalgae (but not for *C. vulgaris*).

3.4. Scaling up implications and comparison with classical BNR techniques

The scaling up has been carried out in the present work using as main criteria to use a similar HRT in lab and pilot plant experiments. Additionally, air was fed in the same conditions, an excess of IC and P concentrations was kept and we tried to maintain similar values of T, pH and lighting. However, different results were obtained under both scales. The main differences observed in the pilot-scale test with respect to laboratory were a slight decrease in microalgae productivity (which caused that higher HRT were necessary) and a slight increase in N and P removal rates. So, in general, it cannot be said that scaling up of our process caused a clear efficiency decrease as reported in previous works (Ruiz et al. 2013; Van den Hende et al. 2014).

The main differences observed in the operating conditions between the two processes (laboratory and pilot scales) were the liquid flow mode and turbulence levels, the dissolved oxygen concentrations and the lighting level. High dissolved oxygen concentrations in the pilot scale process could have influenced negatively the microalgae performance as inhibition could appear at oxygen concentrations from 7 mg L^{-1} (Acién Fernández et al. 2013). An air compressor was necessary to avoid dead flow zones in the horizontal tubes which would cause even higher oxygen accumulation. Moreover, the movement induced to microalgae is quite different in both systems reaching much lower turbulence levels in the pilot scale; in fact, Reynolds number was $25 \cdot 10^3$ in laboratory test while it was lower than 10 in the pilot plant. Arbib et al. (2013) reported that high

turbulence is necessary to avoid biofouling and excessive dissolved oxygen levels. Biofouling (microalgae accumulation in the internal reactor surface) was detected only in the pilot scale operation in the present work, which was related to the low turbulence and the high reactor specific surface (m^2 m^{-3}). Biofouling in the pilot scale caused organic waste accumulation that could eventually produce problems such as reported by Grobbelaar (2012), i.e. predators, pathogens and alien microalgae invasion. Regarding the light intensity, since the higher specific surface of the pilot scale photobioreactor, it could receive more light than the lab-scale reactor; in fact, light intensity was only correctly controlled in the pilot plant during the last stationary 100 days. In general, it can be said that all abovementioned factors were difficult to control and would cause the differences found between the results from laboratory and pilot scales.

Regarding the nutrient removal applications, on one hand, the current microalgae technology is clearly slower and less effective than classical BNR secondary processes but, on the other hand, it could be a more sustainable technology. Judd et al. (2015) reported the advantages and disadvantages of using microalgae instead of the classical BNR processes. According to these authors, because of the lower nutrient removal rates and the high surface (low depth) necessary for microalgae, PBR systems may be 15 times slower than classical activated sludge BNR systems, and overall there is two order of magnitude difference in footprint between them. Against this, the biomass yield is quite lower in PBR and microalgae is considered a valuable product, and they also conclude that microalgae nutrient removal is less effective but involves lower operation costs and, additionally, allows CO_2 capture. Additionally, Marbelia et al. (2014) describe different scenarios to combine classical wastewater treatment plants with microalgae nutrient removal and they propose approximate values of power consumption and operation costs,

and they reported that conventional BNR are energy-intensive and involve extra equipment instruments which may cover 60-80% of the total energy consumption in the treatment process.

4. Conclusions

The results showed in this work showed that the microalgae *Chlorella.vulgaris* is capable to effectively remove nitrate and phosphate from a synthetic secondary effluent, with no need of organic carbon and rendering a valuable waste material. Although N and P removal rates were low compared to classical biological nutrient removal secondary processes, the use of microalgae could be considered a more sustainable technology for wastewater treatment.

Microalgae productivity values found here were similar to previous reported works. The work in a 150 L pilot plant showed the difficulties to keep an adequate control of the process variables. Nevertheless, although microalgae production was lower those that of the laboratory tests, N and P removal rates were slightly increased in the pilot plant. On summary, it can be said that scaling up of the process caused some differences with respect to the laboratory results being them mainly attributed to the differences in turbulence, dissolved oxygen concentrations and lighting levels. These points have been identified as to be critical, and so it is considered that future efforts should be made to improve control of such factors and thus to allow studies that compare laboratory and pilot scale systems.

Acknowledgments

The author would like to thank the company C.T. Ingenieros for funding this research and the students Rosa M. Sánchez, Pedro Capilla, Antonio J. Expósito and Juan L. Lillo for their assistant in carrying out the experiments.

Conflicts of interest: The authors declare that they have no conflict of interest.

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Fig.1 Lab-scale photobioreactor

Fig. 2 Pilot-scale photobioreactor. (a) Scheme. (b) Photograph

Fig. 3 (a) Microalgae growth and (b) dissolved inorganic carbon consumption in the laboratory batch experiments

Fig. 4 (a) $N\text{-}NO_3^-$ concentration profiles and (b) biomass specific $N\text{-}NO_3^-$ removal rates in the laboratory batch experiments

Fig. 5 (a) $P\text{-}PO_4^{3-}$ concentration profiles and (b) biomass specific $P\text{-}PO_4^{3-}$ removal rates in the laboratory batch experiments

Fig. 6 Pilot scale operation: (a) effluent microalgae concentration and (b) effluent dissolved inorganic carbon (IC) concentration

Fig. 7 Nutrients effluent concentrations ($N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$) during the operation of the pilot scale photobioreactor

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Figure 1

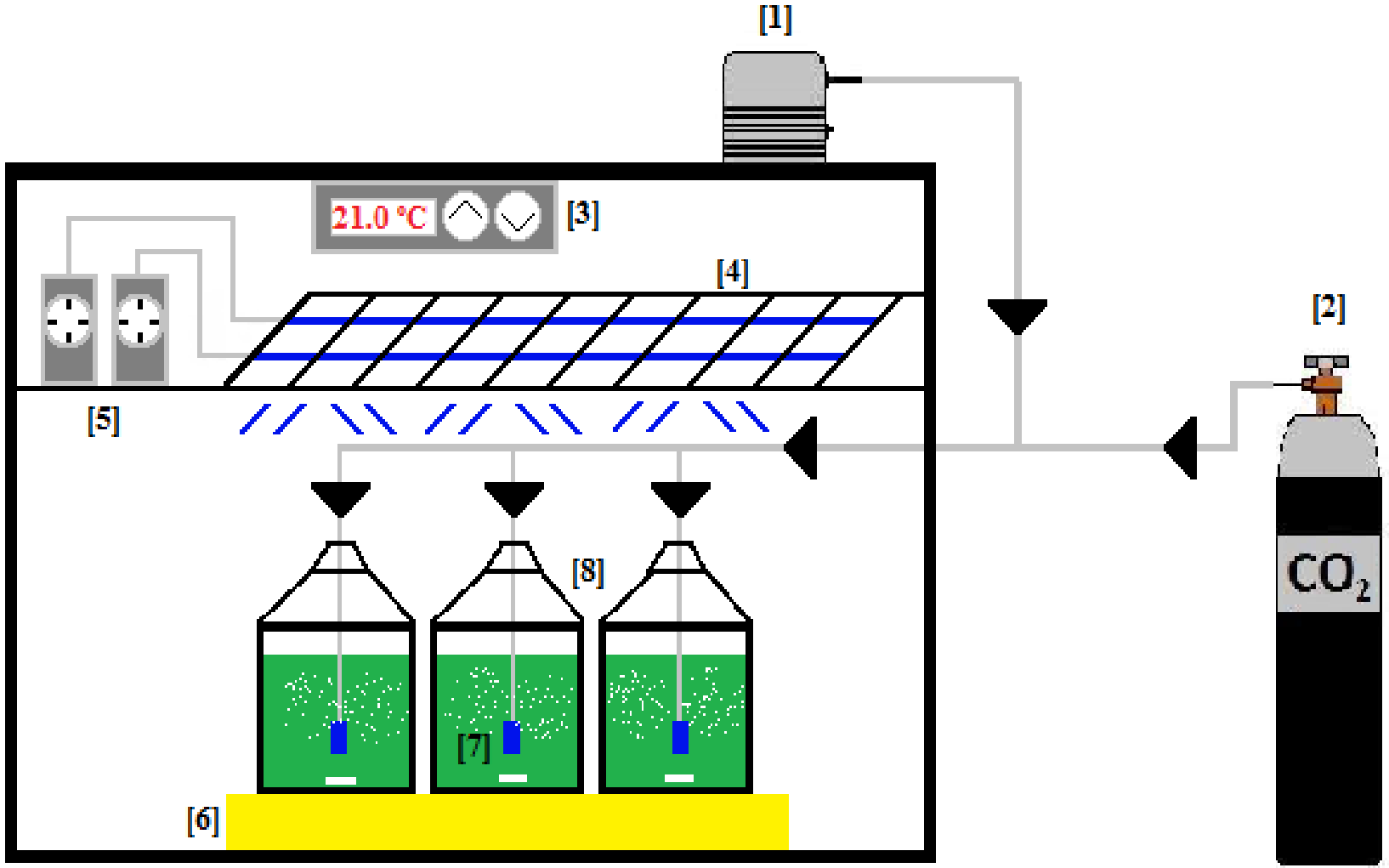
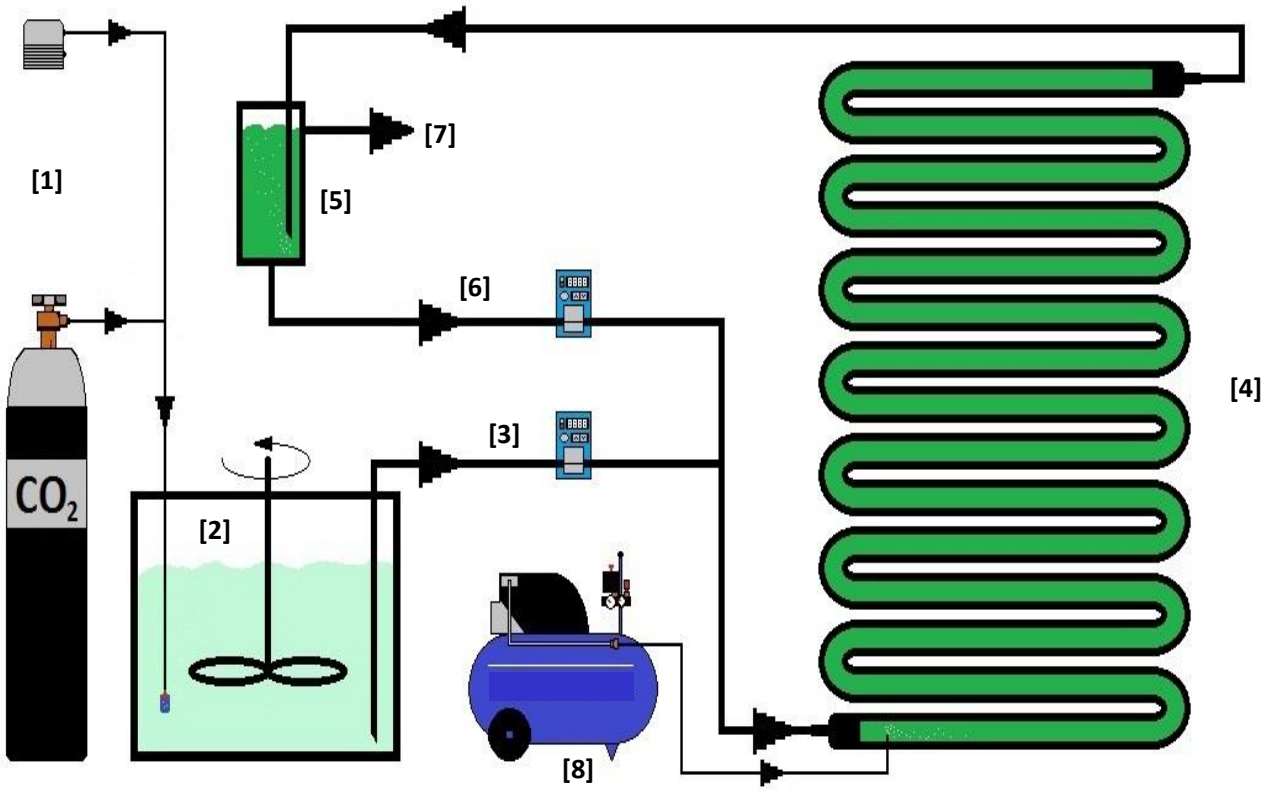


Figure 1

(a)



(b)

Figure 2

Figure 3

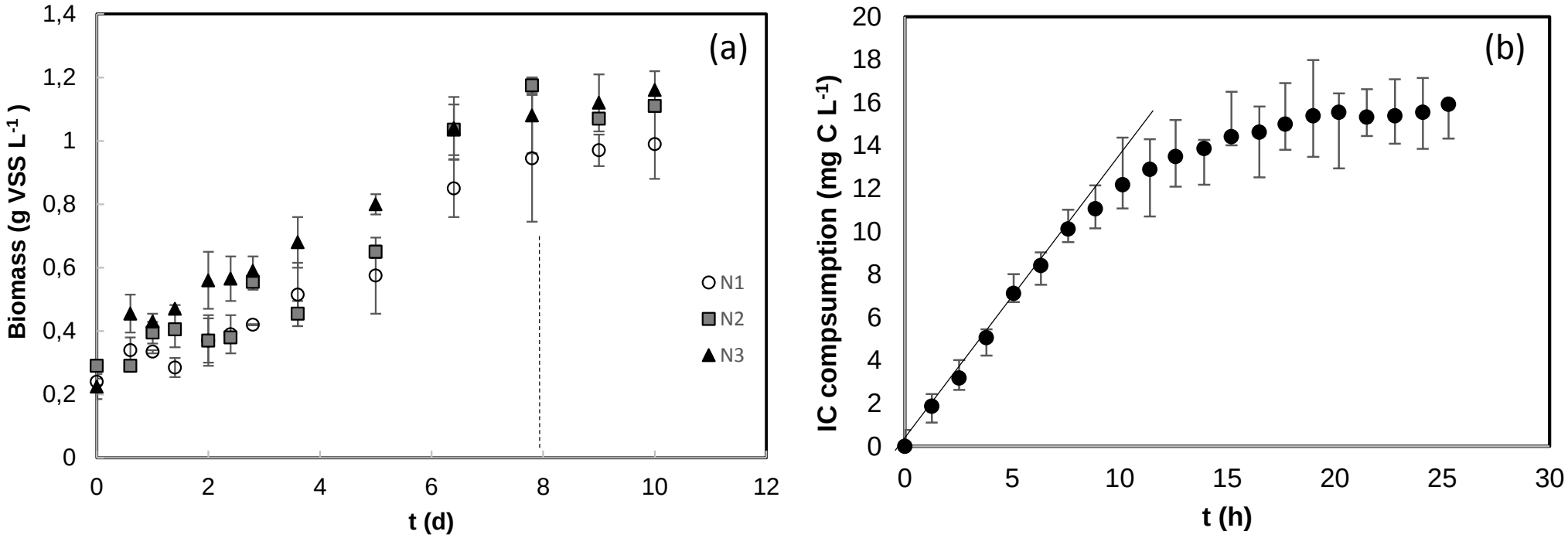


Figure 3

Figure 4

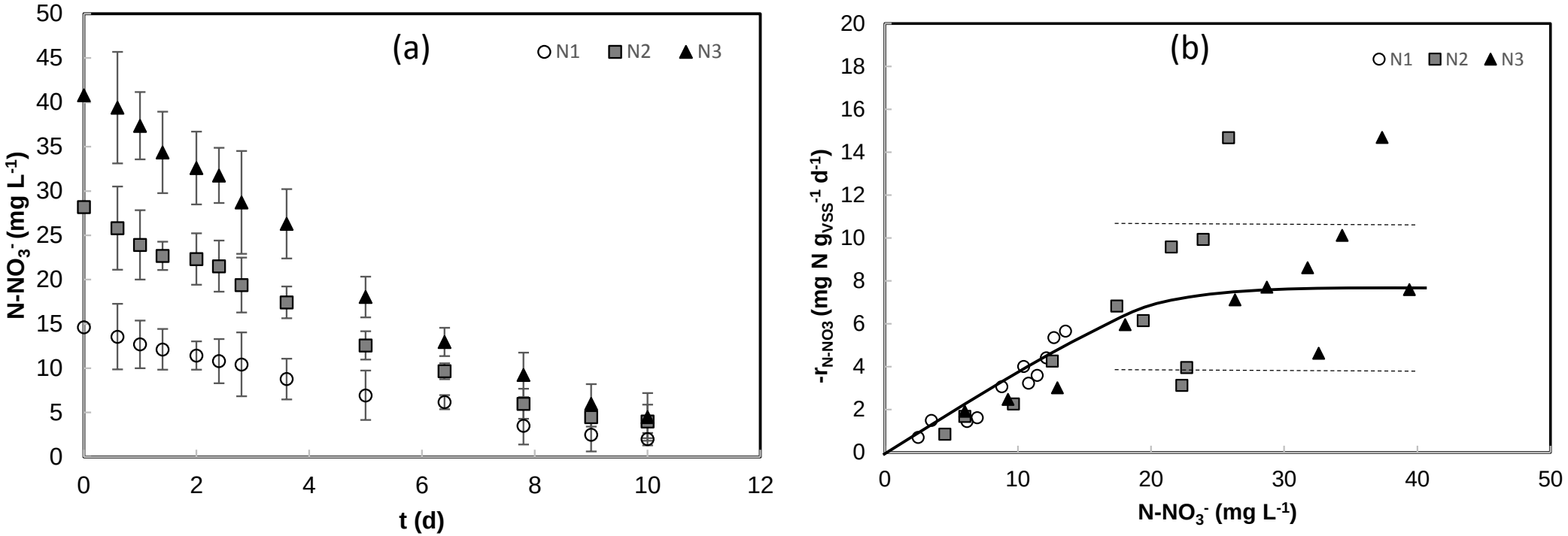


Figure 4

Figure 5

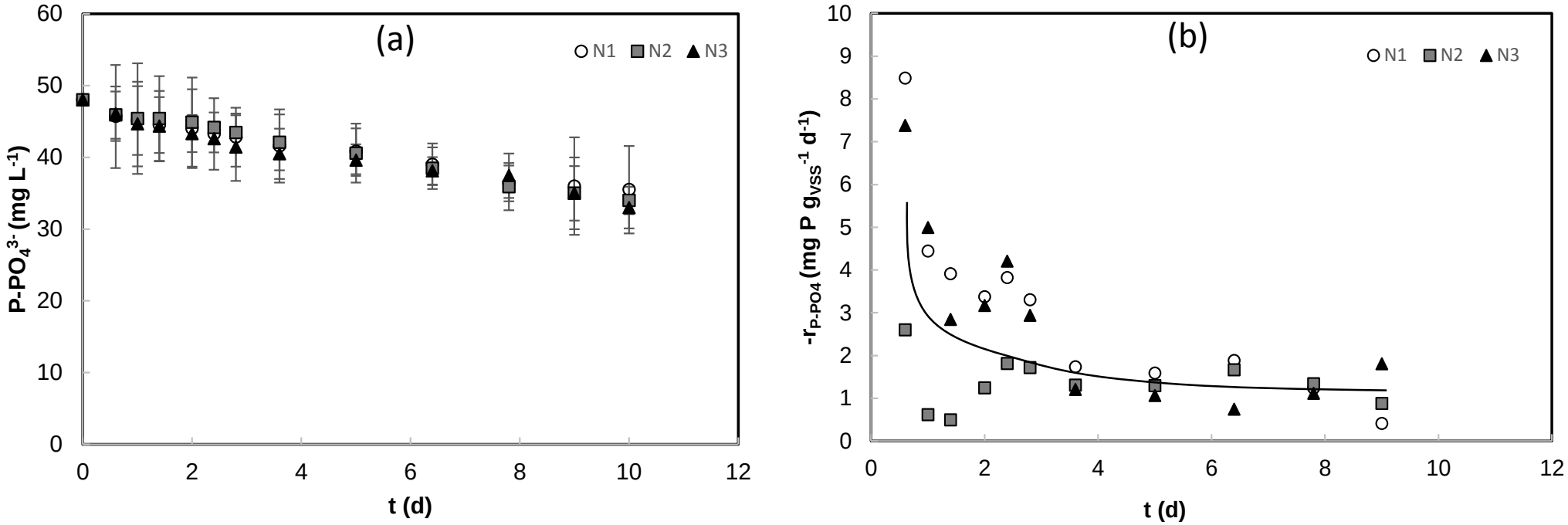


Figure 5

Figure 6

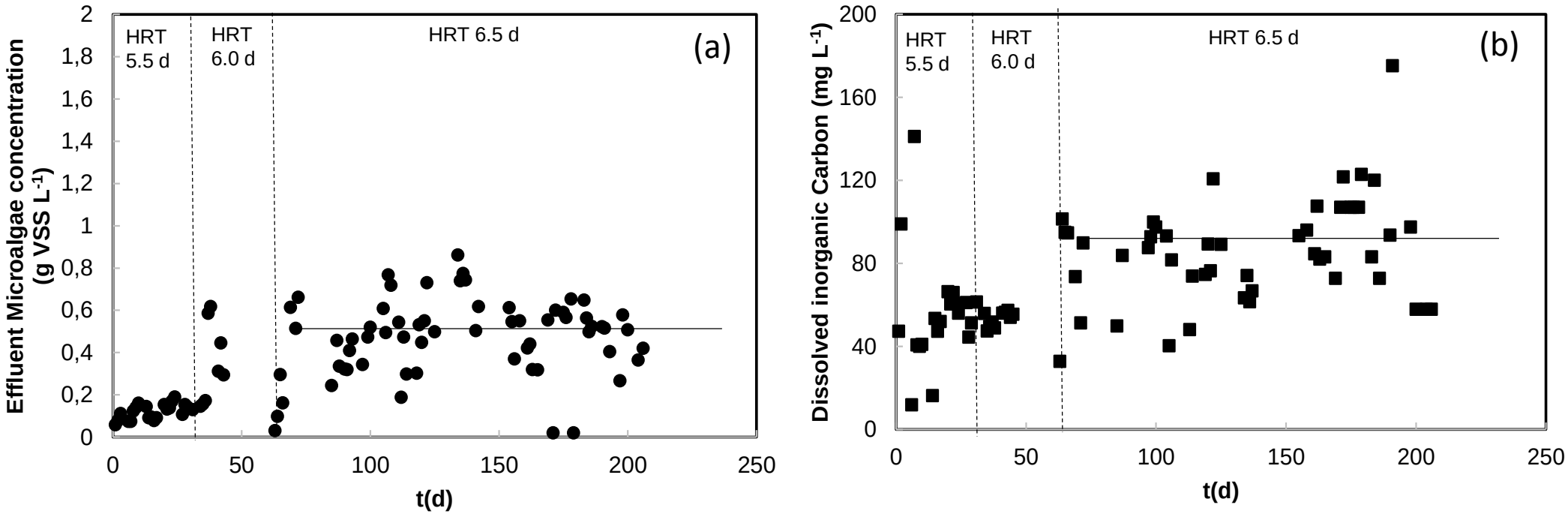


Figure 6

Figure 7

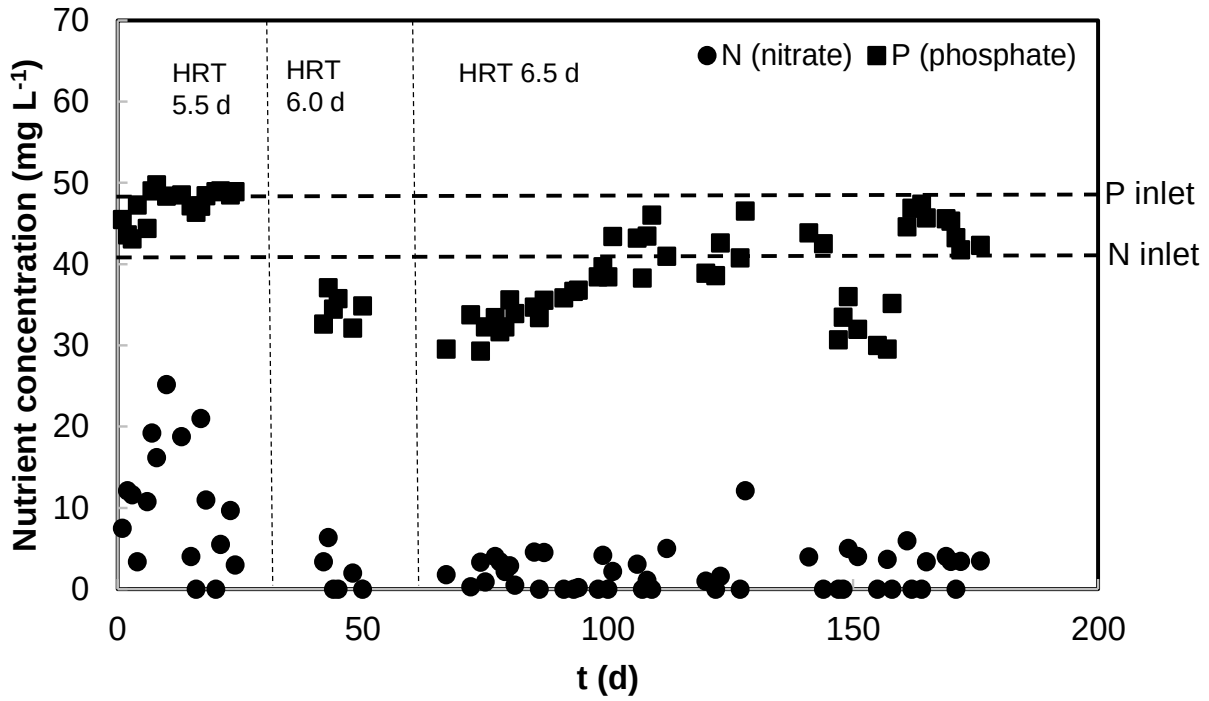


Figure 7

Highlighted Revised manuscript