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UNIVERSIDAD DE VALLADOLID

**TRABAJO FIN DE MÁSTER**

TÍTULO: QUANTIFICATION OF $N_2O$ AND CARBON/NITROGEN REMOVAL IN AN ANOXIC – AEROBIC ALGAL – BACTERIAL PHOTOBIOREACTOR.

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FECHA: SEPTIEMBRE 2014

**RAÚL MUÑOZ TORRE, profesor contratado doctor permanente,** y **CYNTHIA ALCÁNTARA POLLO, estudiante de doctorado del Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Universidad de Valladolid,** CERTIFICAN:

Que **JESÚS MARÍA DOMÍNGUEZ NIÑO** ha realizado bajo su dirección el Trabajo Fin de Máster *Quantification of $N_2O$ and carbon/nitrogen removal in an anoxic – aerobic algal – bacterial photobioreactor*, con una duración de 4 meses (24 créditos).

Valladolid, 11 de septiembre de 2014

Reunido el Tribunal designado en Junta de Sección para la evaluación de Trabajos Fin de Máster, y después de atender a la defensa del trabajo "*Quantification of $N_2O$ and carbon/nitrogen removal in an anoxic – aerobic algal – bacterial photobioreactor*", presentado por el alumno JESÚS MARÍA DOMÍNGUEZ NIÑO, con una dedicación de 4 meses y realizado bajo la dirección del profesor contratado doctor permanente RAÚL MUÑOZ TORRE y la estudiante de doctorado CYNTHIA ALCÁNTARA POLLO, del Dpto. de Ingeniería Química, decidió otorgarle la calificación de _____.

Valladolid, 11 de septiembre de 2014

# ÍNDICE

**ABSTRACT**

The carbon and nitrogen removal potential of a novel 3.6 L anoxic-aerobic microalgal-bacterial photobioreactor configuration operated at high light intensities using both external and internal recirculations was evaluated under different synthetic wastewater feeding flow rates (900 – 1800 mL $day^{-1}$) and concentrations of inorganic carbon (≈150 and ≈250 mg $L^{-1}$) at sludge residence times of (25 ± 6 days). The removal efficiency of the organic carbon and nitrogen photobioreactor was approximately 78 ± 5 % and 55 ± 4 %, regardless of the feeding flow rate. The implementation of an external recirculation from the settler allowed to maintain biomass concentration in the anoxic and photobioreactors of 2689 ± 159 mg $L^{-1}$ and 2484 ± 297 mg $L^{-1}$, respectively, and supported the enrichment of rapidly settling algal-bacterial flocs (SVI of 61 – 69 mL $g^{-1}$) and total suspended solid concentrations if the effluent of 72 ± 16 mg TSS $L^{-1}$. $NH_4^+$ nitrification at 900 mL $day^{-1}$ in the photobioreactor was low despite the high concentration of dissolved oxygen (20 – 22 mg $O_2$ $L^{-1}$), which suggested the occurrence of a potential limitation of nitrification by inorganic carbon. The increase in inorganic carbon supply when the wastewater flow rate was increased to 1800 mL $day^{-1}$ brought a partial nitrification with N-$NO_2^-$ and N-$NO_3^-$ concentrations of 8.26 ± 0.0 mg $L^{-1}$ and 0.4 ± 0.0 mg $L^{-1}$, respectively. Wastewater treatment under this particular two-stage algal-bacterial configuration generated greenhouse gases such as carbon dioxide (despite the occurrence of an active photosynthesis in the photobioreactor), methane and nitrous oxide (mainly in the anoxic reactor).

# 1. INTRODUCTION

The synergistic relationship between microalgae and bacteria play an important role during the secondary or tertiary treatment of domestic wastewater in photobioreactors based on the current technical-economic limitations of conventional wastewater treatment technologies such as activated sludge systems or anaerobic digestion (De Godos et al., 2009; He et al., 2013). In this context, photosynthetic oxygenation together with microalgae heterotrophic metabolism can boost the degradation of organic pollutants present in wastewater, using in turn the $CO_2$ released from bacterial respiration (Muñoz and Guieysse, 2006). This $O_2$ supply significantly reduce the costs associated with conventional mechanical aeration in activated sludge systems, which can represent more than 50% of the total energy consumption of typical aerobic wastewater treatments (Tchobanoglous et al., 2003). In addition, the capacity of microalgae to simultaneously remove C, N and P via mixotrophic assimilation into biomass represents an important advantage in comparison with aerobic activated sludge or anaerobic digestion technologies in term of enhanced nutrient recovery (Craggs et al., 2004; Abreu et al., 2012; Arbib et al., 2014). Finally, the high pH, dissolved $O_2$ concentrations and solar light irradiation associated to photosynthetic processes promote pathogen deactivation and an abiotic nutrient removal via $NH_3$ stripping or phosphate precipitation (Oswald, 1988).

However, despite all these advantages, microalgal-bacterial wastewater treatment processes present technical limitations that hinder its full-scale development. The poor sedimentation capability of some microalgae species, often result in effluent suspended solid concentrations above the maximum permissible discharge limits. Likewise, the moderately short hydraulic retention times (HRTs) applied in high rate algal ponds (6-15 days) also limit the nitrification of $NH_4^+$, which is needed as a previous step to remove nitrogen via denitrification. In this context, nitrate or nitrite denitrification represents a key metabolic pathway to remove N in wastewaters with low C/Nutrient ratios when the supply of external C-$CO_2$ to remove nutrients via microalgal assimilation is not technical or economically feasible. For instance, the supply of flue gas as a source of free $CO_2$ (De Godos et al., 2010; Kao et al., 2014) is not feasible when combustion plants are far to the location of microalgal cultivation. On the other hand, the high costs associated to the supply of pure-compressed $CO_2$ in photobioreactors economically restrict its full-scale implementation as it can represent

up to 41% of the total costs of raw materials (Molina Grima et al., 2003). In addition, the environmental sustainability of microalgal-bacterial wastewater treatments have been questioned due to the potential greenhouse emissions and high water footprint associated with open algal photobioreactors (Florez-Leiva et al., 2010; Guieysse et al., 2013). In this context, microalgae decomposition or nitrogen metabolism has been shown to support significant productions of $CH_4$ and $N_2O$, two critical greenhouse gases with a global warming potential 25 and 300 times higher than $CO_2$, respectively (Ravishankara et al., 2009; Ferrón et al., 2012). Therefore, the potential emissions of $CH_4$ and $N_2O$ from microalgae cultivation systems could counteract the beneficial $CO_2$ capture during microalgae growth in a net greenhouse gas balance.

Therefore, the development of innovative operational strategies and photobioreactor configurations to simultaneously enhance both biomass harvesting and nitrogen removal is required to move towards a sustainable industrial-scale implementation of microalgal-bacterial based wastewater treatment systems. In this context, there is also a significant knowledge gap on the potential emissions of $N_2O$ and $CH_4$ from photobioreactors devoted to wastewater treatment.

In this work, the recent investigation published by De Godos et al. (2014) was used as a reference study. This study constituted the first lab-scale microalgal-bacterial aerobic-anoxic reactor configuration implemented to simultaneously promote the development of nitrifying and denitrifying communities and rapidly settling populations based on the high sludge retention time (SRT) achieved through both internal and external microalgal-bacterial biomass recirculations. Unfortunately, this study was conducted at low light intensities (far from those found outdoors) and high HRT with a limited nitrification in the photobioreactor almost during the entire experimentation, which demonstrated that the dissolved inorganic carbon concentration in the aerobic photobioreactors represents a key parameter to achieve a stable bacterial nitrification in this combined system. In addition, the lack of information about the microalgae population dynamics in this pioneer work hindered a complete characterization of the steady states achieved.

Therefore, this work constitutes a study to assess the potential of this novel anoxic-aerobic microalgal-bacterial photobioreactor process at high light intensities mimicking those found outdoor. Likewise, the influence of both inorganic carbon concentration,

the HRT, and dissolved oxygen concentration in the photobioreactor on the removal of C and N via assimilation, denitrification and/or nitrification along with the corresponding population dynamics were assessed. The sedimentation capability and $CH_4$ and $N_2O$ production potential of the biomass present in both reactors were also evaluated.

## 2. MATERIALS AND METHODS.

### 2.1. Microorganisms and culture conditions.

The anoxic and aerobic tanks were initially filled with 3.2 g TSS $L^{-1}$ of a consortium of microalgae/cyanobacteria (from now on referred to as microalgae) and aerobic activated sludge bacteria from Valladolid wastewater treatment plant (Spain). The microalgae inoculum was mainly composed of (% of cells) *Planktothrix isothrix* (72.2%), *Staurosira sp.* (9.5%), *Stigeoclonium setigerum* (5.0%), *Acutodesmus obliquus (*3.9%), *Chroococcus sp.* (3%) and *Pseudanabaena sp.* (3%). The microalgal and bacterial cultures used as inocula were initially settled (centrifugation was discarded to avoid the disruption of the flocs) and the biomass resuspended in synthetic wastewater (SWW) prior to inoculation in both reactors. The SWW was initially composed of (per L of distilled water): 500.4 mg Glucose, 1050 mg $NaHCO_3$, 458 mg $NH_4Cl$, 62 mg $KH_2PO_4$, 7 mg NaCl, 4 mg $CaCl_2{\cdot}2H_2O$, 75 mg $MgSO_4{\cdot}7H_2O$, 2.5 mg $FeSO_4$, 20 mg EDTA, 0.00125 mg $ZnSO_4$, 0.0025 mg $MnSO_4$, 0.0125 mg $H_3BO_3$, 0.0125 mg $Co(NO_3)_2$, 0.0125 mg $Na_2MoO_4$, and 6.25 x $10^{-6}$ mg $CuSO_4$. This composition resulted in 200 $mg{\cdot}L^{-1}$ of dissolved total organic carbon (TOC), 120 $mg{\cdot}L^{-1}$ of $N\text{-}NH_4^+$ and 150 $mg{\cdot}L^{-1}$ of dissolved inorganic carbon (IC). An additional buffer of 1.14 g $KH_2PO_4/L_{SSW}$ and 2.33 g $K_2HPO_4/L_{SSW}$ was also supplemented to maintain the cultivation pH at ≈ 7.5 and therefore promote bacterial nitrification and minimize N-losses by $NH_3$ stripping. The experimental set-up was operated indoors at room temperature (25 ± 1 ºC).

### 2.2. Experimental photobioreactor.

The experimental set-up consisted of two interconnected tanks (anoxic + aerobic) (Fig. 1). The aerobic tank was an enclosed jacketed 3.5 L glass tank (AFORA, Spain) with a total working volume of 2.7 L, and continuously illuminated by LED lamps (30W, Withled, Spain) arranged in a circular configuration and providing approximately 400±

51 μmol/$m^2$·s at the outer wall of the photobioreactor. The temperature and magnetic agitation of this tank were maintained constant at 24±1 ºC and 300 rpm, respectively. The anoxic reactor consisted of a 1L plastic tank with a total working volume of 0.9 L maintained in the dark and magnetically stirred at 300 rpm. The SWW, previously sterilized at 121 ºC for 20 min and maintained at 7 ºC during feeding, was fed to the anoxic tank and continuously overflowed by gravity into the aerobic photobioreactor. The algal-bacterial broth was continuously recycled from the photobioreactor to the anoxic tank in order to provide the $NO_2^-$ and $NO_3^-$ (generated in the photobioreactor via biological nitrification) as electron acceptor during denitrification process in the anoxic tank. An Imhoff cone with a volume of 1L connected to the outlet of the photobioreactor was used as settler. The algal-bacterial biomass settled was either 5 times a day pumped from the bottom of the settler into the anoxic tank and wasted 3 days a week in order to control the algal-bacterial SRT. The experiment was run for 120 days (May 2014- September 2014).

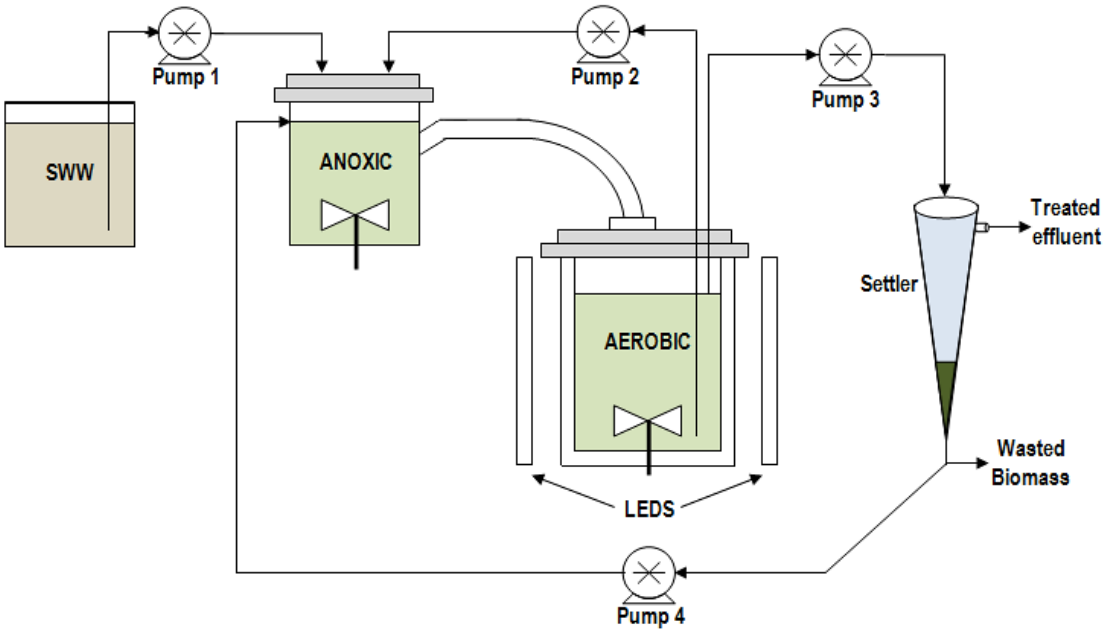


**Figure. 1** - Illustration of the anoxic - aerobic photobioreactor configuration.

### 2.3. Experimental design.

The design of the experimentation was conducted based on the hypothesis that microalgal and cyanobacterial photobioreactors for wastewater treatment can support the oxidation of $NH_4^+$ into $NO_2^-$/$NO_3^-$, which can then be easily eliminated through denitrification under anoxic conditions via internal recycling of the photobioreactor

cultivation broth. (De Godos et al., 2014). During the first 15 days (start-up stage), the process was operated at a HRT of 4 days ($HRT_{anoxic}$ = 1 days, $HRT_{aerobic}$ = 3 days), an IC concentration of 154±10 mg $L^{-1}$ and an algal-bacterial sludge retention time (SRT) of 27±4 days. The algal-bacterial cultivation broth from the photobioreactor was recycled into the anoxic tank at 3 L $day^{-1}$, while external recycling was fixed at 0.5 L $day^{-1}$. During stage I (31 days), the SRT was maintained at 27 ± 2 days and the IC concentration was increased to 247±28 mg $L^{-1}$ in order to assess the occurrence of a potential IC limitation in the process. In stage II (33 days), the inlet SWW flow was increased from 0.9 L $day^{-1}$ to 1.8 L $day^{-1}$ and the SRT was decreased to 20±7 days to evaluate the bioremediation capacity of the system (Table 1). Given the absence of nitrification in the aerobic tank, the dissolved oxygen concentration (DOC) was decreased from 22±4 mg/L to 9.3±2.9 mg/L during stage III (20 days) to rule out a photooxidation-mediated nitrifying bacteria inhibition.

**Table 1.** Operational conditions tested during the evaluation of the performance of the anoxic-aerobic photobioreactor system.

| | Stage | | | |
|---|---|---|---|---|
| **Parameter** | **Start-up** | **I** | **II** | **III** |
| **Experimental period (d)** | 15 | 31 | 33 | 20 |
| **HRT (d)** | 4 | 4 | 2 | 2 |
| **SRT (d)** | 27 ± 4 | 27 ± 2 | 20 ± 7 | 22 ± 2 |
| **Inlet flow (L $d^{-1}$)** | 0.9 ± 0.1 | 0.9 ± 0.2 | 1.8 ± 0.2 | 1.8 ± 0.5 |
| **Internal recycling (L $d^{-1}$)** | 3 | 3 | 3 | 3 |
| **External recycling (L $d^{-1}$)** | 0.5 | 0.5 | 0.5 | 0.5 |
| **IC (mg $L^{-1}$)** | 154 ± 10 | 247 ± 28 | 249 ± 10 | 214 ± 96 |
| **DOC (mg $L^{-1}$)** | 22 ± 3 | 20 ± 3 | 22 ± 4 | 9.3 ± 2.9 |

Gas samples of 100 µL were taken from anoxic and aerobic tank three times a week to record the $CO_2$, $O_2$, $CH_4$, $N_2$, and $N_2O$ headspace concentrations by GC-TCD and GC-ECD. Liquid samples of 100 ml were also drawn three times a week from the SWW tank (influent), anoxic tank, aerobic tank, purge and clarified effluent to monitor the concentration of dissolved TOC, dissolved IC, dissolved N species (TN, N-$NH_4^+$, N-$NO_2^-$ and N-$NO_3^-$) and biomass concentration as total suspended solids (TSS). The DOC, temperature and pH of the culture broth of both tanks were in situ recorded every day. The C and N content of the algal-bacterial biomass formed, along with the characterization of the populations of microalgal were also experimentally determined

in both tanks at the end of every operational stage under steady state conditions. In addition, the Sludge Volume Index (SVI) was also measured in order to determine and compare the settling characteristics of the algal-bacterial consortia established in each stage in the anoxic and aerobic tanks.

**2.4. Analytical procedures.**

The impinging irradiation was measured as PAR using a LI-250A light meter (LI-COR Biosciences, Germany) and expressed in $\mu E/m^2 \cdot s$. The pressure at the head-space of the anoxic and aerobic tanks was measured using a PN 5007 (IFM, Germany). The gas concentrations of $CO_2$, $O_2$, $CH_4$ and $N_2$ were analyzed using a gas chromatograph (Varian CP-3800, Palo Alto, CA, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15µm) and CP-Pora BOND Q (25m × 0.53 mm × 15µm) columns. The injector and detector temperatures were 150 ºC and 175 ºC, respectively. Helium was the carrier gas at 13.7 mL $min^{-1}$. The $N_2O$ gas concentration was determined using a Bruker Scion 436 gas chromatograph (Palo Alto, USA) equipped with an Electron Capture detector and a HS-Q packed column (1 m × 2 mm ID × 3.18 mm OD) (Bruker, USA). Injector, detector and oven temperatures were set at 100 ºC, 300 ºC and 40 ºC, respectively. Nitrogen was used as the carrier gas at 20 mL $min^{-1}$. External standards prepared in volumetric bulbs (Sigma–Aldrich, USA) were used for $N_2O$ quantification.

TN, TOC and IC concentrations were determined using a Shimadzu TOC-V CSH analyzer equipped with a TNM-1 module (Japan). $N\text{-}NH_4^+$ was measured through Nessler analytical method using by spectrophotometer U-200 (Hitachi, Japan) at 425 nm. $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were analyzed by HPLC-IC with a Waters 515 HPLC pump coupled with a Waters 432 ionic conductivity detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) Waters column. $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were also determined by colorimetric method according to Eaton et al. (2005). DOC was determined using an OXI 330i oximeter (WTW, Germany), while a Crison micropH 2002 (Crison instruments, Spain) was used for pH determination. The concentration of TSS and the SVI was determined according to Eaton et al. (2005). The analysis of $C_{biomass}$ and $N_{biomass}$ was conducted using a LECO CHNS-932. The identification, quantification and biometry measurements of microalgae were carried out by

microscopic examination (OLYMPUS IX70, USA) of microalgal samples (fixed with lugol acid at 5% and stored at 4 ºC prior to analysis) according to Sournia (1978).

## 3. RESULTS AND DISCUSSION.

### 3.1. Start-up.

The removal efficiency of nitrogen, organic carbon and inorganic carbon at start up were 69 ± 12 %, 73 ± 9 % and 95 ± 1% respectively. The start-up of the anoxic bioreactor was characterized by an intensive denitrification which reduced to 0.0 ± 0 mg N $L^{-1}$ the nitrate and nitrite concentrations recirculated from the photobioreactor. However, denitrification disappeared as nitrate and nitrite concentration in the photobioreactor decreased due to the gradual limitation in inorganic carbon (57 ± 12 mg IC $L^{-1}$), which entailed an increase in the ammonium concentration to stable values of 64 ± 1 mg N-$NH_4^+$ $L^{-1}$ (Fig. 2). At this stage the pH and temperature in anoxic tank remained constant at 7.5 ± 0.1 and 25 ± 0 ºC, respectively. In this context, the fact that no IC elimination mechanism did occur in the anoxic tank suggest that the IC removal efficiency was supported by the dilution of the influent SWW with both the internal and external recyclings (Fig. 3).

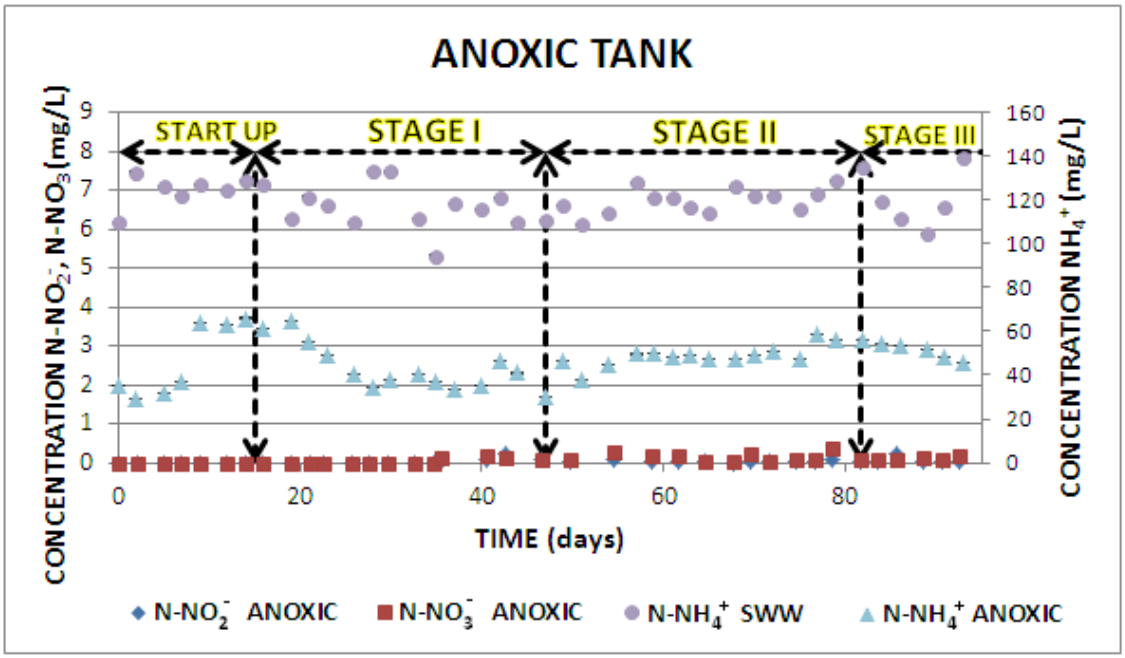


**Fig. 2-** Time course of the concentration of $NO_2^-$, $NO_3^-$ and $NH_4^+$ in the anoxic tank. Vertical bars represent the standard deviation from duplicate measurements.

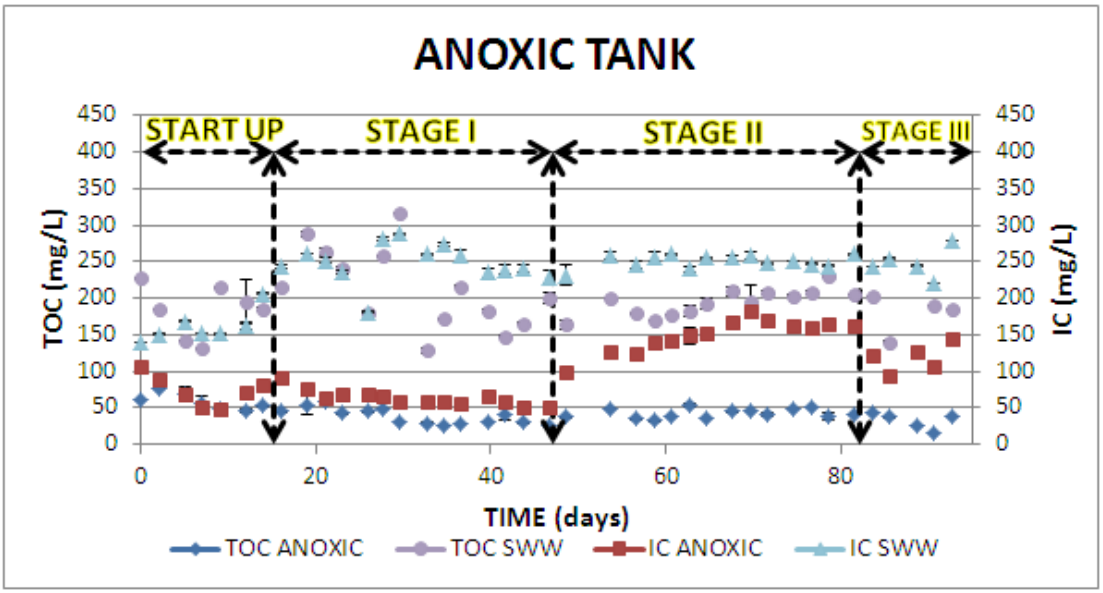


**Fig. 3-** Time course of the concentration of TOC and IC in the anoxic tank. Vertical bars represent the standard deviation from duplicate measurements.

The high irradiation of the photobioreactor (400 ± 51µmol/$m^2$·s) resulted an effective utilization of the inorganic carbon for biomass formation supported by both photosynthesis and nitrification by nitrifying bacteria, which resulted in a final concentration of 8.1 ± 1.7 mg IC $L^{-1}$ at the effluent (Fig.5). The ammonium concentration in the photobioreactor increased along the start-up stage up to values of 54 ± 1 mg N-$NH_4^+$ $L^{-1}$ concomitantly with a deterioration in nitrification, which resulted in a decrease in nitrate concentrations from 29 ± 0 mg N-$NO_3$- $L^{-1}$ by day 0 to 0.0± 0 mg N-$NO_3$ $L^{-1}$ by day 15 (Fig. 4). The inlet organic carbon (204 ± 47 mg TOC $L^{-1}$) was consumed to 45 ± 5 mg TOC $L^{-1}$ by heterotrophic and mixotrophic biomass to growth while the removal of IC supported dissolved oxygen concentrations of 23 ± 3 mg $O_2$ $L^{-1}$ with a carbon yield of 1 g C assimilated/g C SWW (Fig.5). The removal of nitrogen by assimilation during this start-up phase accounted for 0.20 g N assimilated/g N SWW (Fig. 4). The pH and temperature in aerobic tank were 8.0 ± 0.1 and 25 ± 0 ºC, respectively.

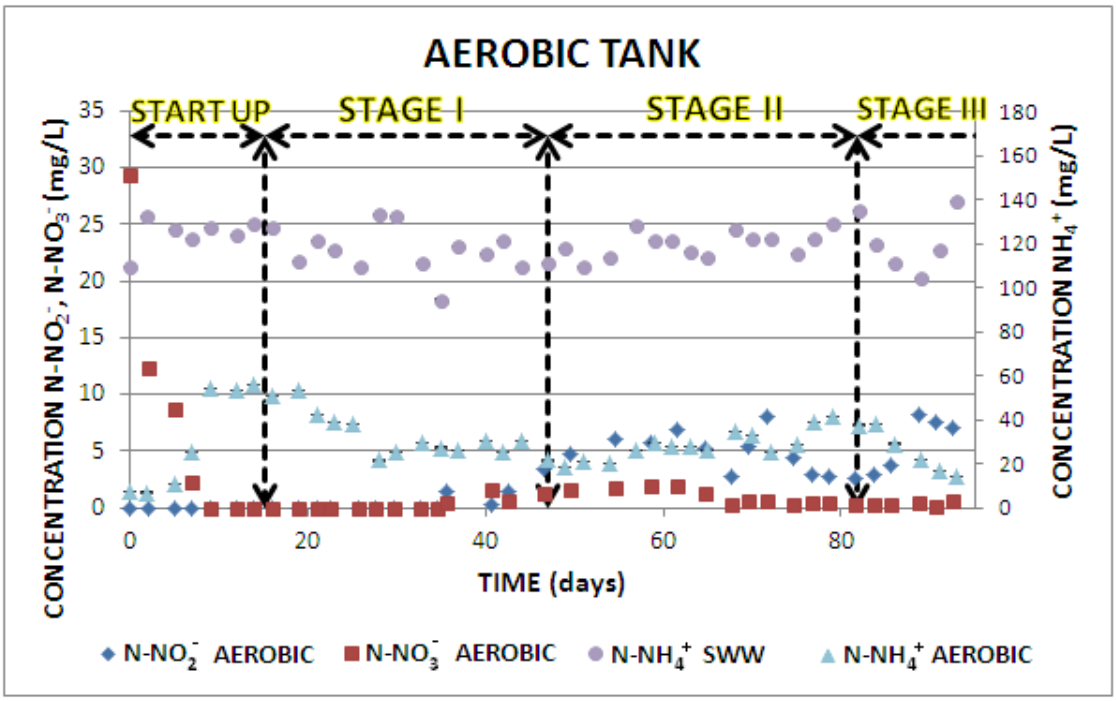


Fig. 4- Time course of the concentration of $NO_2^-$, $NO_3^-$ and $NH_4^+$ in the photobioreactor. Vertical bars represent the standard deviation from duplicate measurements.

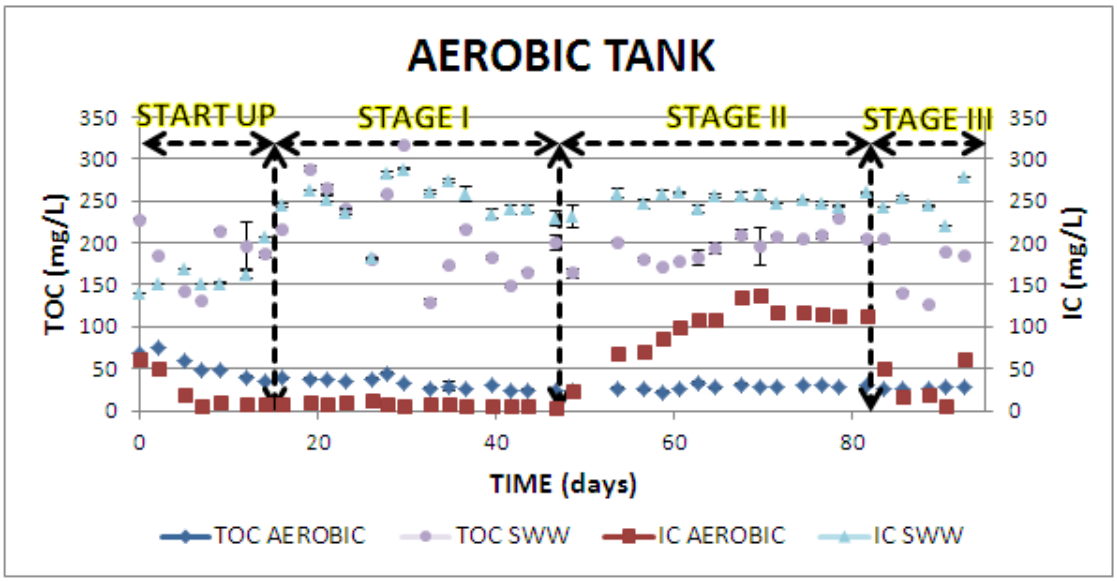


**Fig. 5**- Time course of the concentration of TOC and IC in the photobioreactor. Vertical bars represent the standard deviation from duplicate measurements.

### 3.2. Stage I.

At stage I, the removal efficiency of nitrogen was the same that in start-up stage (69 ± 3 %) while removal efficiency of organic and inorganic carbon increased to 85 ± 4 % and 97 ± 0 %, respectively. At this stage, in anoxic tank, the consumption of organic carbon

by microalgae-bacterial consortium was high with a concentration of 31 ± 5 mg TOC $L^{-1}$ along 31 days of stage I. These low organic carbon concentrations were supported by the dilution of the SWW with the internal and external recirculations and by assimilation into mixotrophic and heterotrophic biomass. The inorganic carbon concentration remained at 58 ± 5 mg IC $L^{-1}$ due to the dilution caused by the recirculations (Fig. 3), while a negligible denitrification occurred as a result of the negligible $NO_2^-$ and $NO_3^-$ concentrations provided by the internal recirculation from the photobioreactor (Fig. 4). The pH and temperature remained constant and similar to the previous stage with values of 7.6 ± 0.1 and 25ºC, respectively.

The symbiosis between microalgae and bacteria in stage I in the photobioreactor resulted in an efficient organic and inorganic carbon removal, with residual concentrations of 27 ± 3 mg TOC $L^{-1}$ and 6.4 ± 1.1 mg IC $L^{-1}$ at the effluent (Fig. 5). The nitrification in this stage was negligible (0.9 ± 0.5 mg $N\text{-}NO_3^-$ $L^{-1}$) likely due to the intensive photosynthetic process, which boost that most of the ammonium and inorganic carbon were consumed by assimilation into biomass. This N removal into biomass entailed a production of 0.20 g N assimilated/g N SWW, which resulted in an effluent concentration of 27 ± 3 mg $N\text{-}NH_4^+$ $L^{-1}$ (Fig. 4). TSS of effluent at this stage were 54 ± 15 mg TSS $L^{-1}$ being under the maximum permissible discharge limit in EU legislation which is 60 mg SST $L^{-1}$ to 2000 -100000 IE (Inhabitants Equivalent) (European Directive 91/271/CEE on discharge of domestic waters). The pH and DOC were 8.0 ± 0.2 and 20 ± 3 mg $O_2$ $L^{-1}$, respectively, while the temperature was maintained at 25 ºC during stage I.

The microalgal population evolution in the anoxic and aerobic reactor was similar during the entire experiment (Fig. 6) and was composed by *Planktothrix isothrix* (32%), *Pseudanabaena sp* (42%) and *Stigeodonium setigerum* (11%). The populations that distinguished the reactors were on one hand *Scenedesmus ecornis* whose was found in higher proportion in anoxic reactor (67%) and on the other hand *Scenedesmus obtusus* whose was the main population in aerobic tank (48%) (Fig. 6). The SVI of the consortium biomass from the anoxic tank and photobioreactor were 69 mL $g^{-1}$ and 67 mL $g^{-1}$, respectively. According to Parker et al., 2001, a SVI values below 100 mL $g^{-1}$ are considered a good settling sludge, while SVI values above 150 are typically associated with filamentous growth. Therefore, the microalgal-bacterial biomass herein studied presented a good sedimentation capacity.

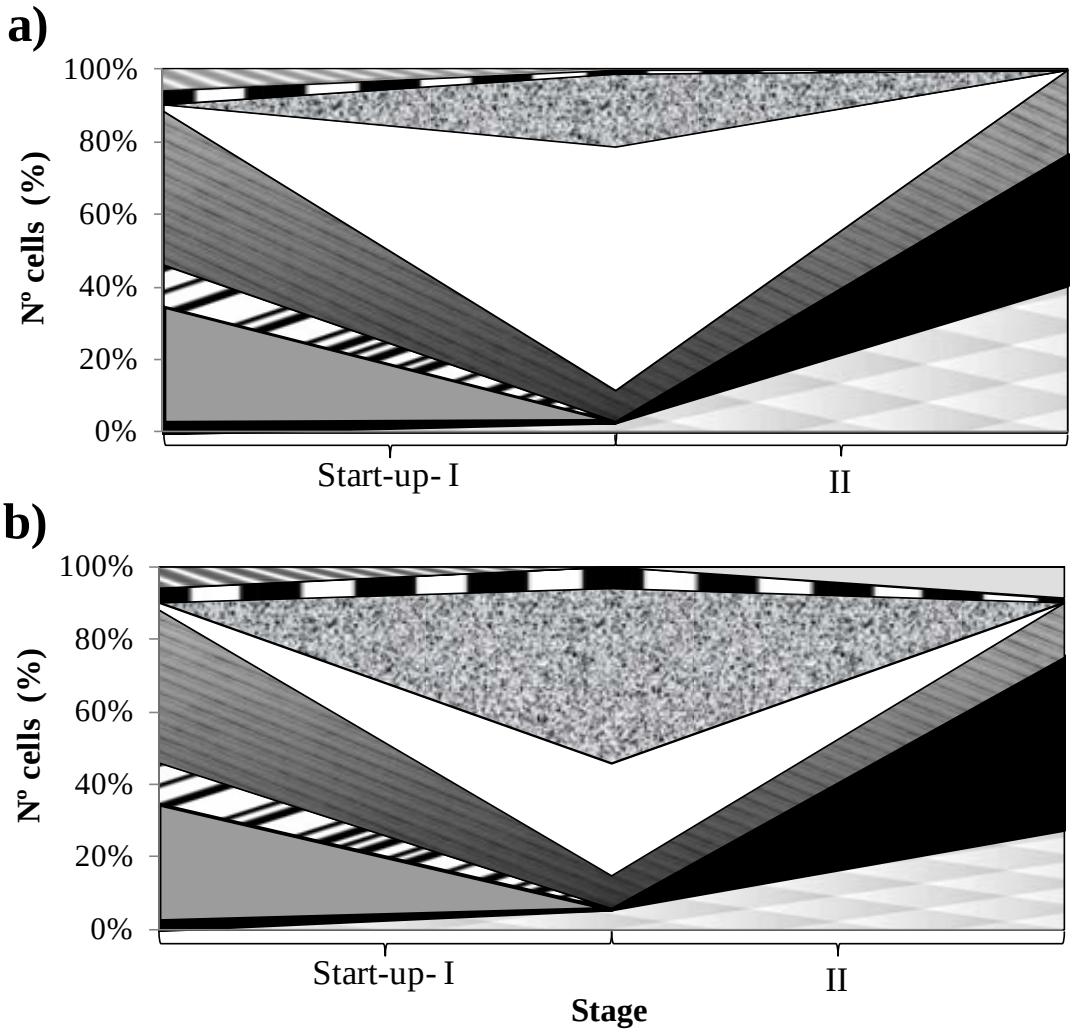


**Fig. 6**- Time course of the microalgae population structure in the anoxic (a) and aerobic (b) tanks during stage I and II. *Scenedesmus ecornis*, *Pseudanabaena sp.*, *Acutodesmus obliquus*, *Chlorella sp.*, *Scenedesmus obtusus*, *Stigeoclonium setigerum* *Planktothrix isothrix*, *Phormidium sp*, *Staurosira sp.* and Others.

### 3.3. Stage II.

The elimination of TOC during stage II was higher than in stage I, with a removal efficiency of 86 ± 1 %, while nitrogen and inorganic carbon removals were lower with values of 64 ± 3 % and 52 ± 4%, respectively. Concentration of organic carbon in anoxic tank was higher than stage I with a value of 44 ± 6 mg TOC $L^{-1}$. However, most of this removal was mediated by a dilution effect created by the internal and external recirculations. The inorganic carbon concentration at this stage was 166 ± 10 mg IC $L^{-1}$ (pH = 7.5 ± 0) (Fig. 3). The average concentration of nitrates generated in the

photobioreactor at stage II was lower than stage I (0.5 ± 0.1 mg $N\text{-}NO_3^-$ $L^{-1}$), due to the increase of ammonium, whose concentration was 34 ± 6 mg $N\text{-}NH_4^+$ $L^{-1}$. Therefore, the biomass grown in the anoxic tank corresponded with the heterotrophic bacteria that use the $NO_3^-$ or the residual dissolved oxygen coming from the photobioreactor as electron acceptors to oxidize the organic carbon and form new biomass, with an assimilation of 0.52 g N assimilation / g N SWW (Fig. 2).

On one hand the ammonium of aerobic tank was used to perform the nitrification (with concentrations of nitrite and nitrate of 4.3 ± 2.1 mg $N\text{-}NO_2$ $L^{-1}$ and 0.5 ± 0.1 mg $N\text{-}NO_3$ $L^{-1}$, respectively), on the other hand, the remaining of the ammonium removed was consumed by biomass to build its own structure (Fig. 4). The concentration of inorganic carbon in aerobic tank was 120 ± 11 mg IC $L^{-1}$ during steady state, which allowed nitrification to occur and overcome the above referred IC limitation. The concentration of organic carbon remained stable at 29 ± 2 mg TOC $L^{-1}$ resulting in a removal efficiency of 86 ± 1 % in the global system, and was likely consumed by the microalgal-bacterial symbiosis. Despite the increase in organic loading rate as a result of the decrease in HRT, the system was able to maintain stable DOC of 22 ± 4 mg $O_2$ $L^{-1}$ (Fig. 5). The pH and temperature in this stage remained constant at 7.7 ± 0.1 and 24 ± 1 ºC, respectively.

Stage II was characterized by an increase in the population of *Chorella sp.* (36% at the anoxic reactor and 42% at the photobioreactor) and *Acutodesmus obliquus* (41% at the anoxic reactor and 28% at the photobioreactor). The contribution of *Scenedesmus obtusus* and *Scenedesmus ecornis* decrease over time. Others species such as *Planktothrix isothrix* from the sludge disappeared in the second stage. Anoxic tank and photobioreactor had a SVI in stage II of 63 mL $g^{-1}$ and 61 mL $g^{-1}$ respectively. The decrease in the HRT from 4 days to 2 days in this stage likely promoted the decrease in SVI as consequently the dilution rate in the system increase from 0.25 $d^{-1}$ to 0.5 $d^{-1}$. Thus, the free living microalgae species with a µmax (maximum specific growth rate) lower than 0.5 $d^{-1}$ were wash out from the system, increasing the sedimentation capacity in the system.

### 3.4. Stage III.

The removal efficiency of nitrogen and organic carbon at stage III were lower than previous stage (59 ± 13 % and 84 ± 3 %, respectively), while inorganic carbon was higher than stage II (83 ± 15 %).The concentration of organic carbon and inorganic carbon in anoxic reactor was 34 ± 11 mg TOC $L^{-1}$ and 126 ± 25 mg IC $L^{-1}$, respectively (Fig. 3). Denitrification was also an efficient process with nitrite and nitrate concentrations of 0.07 ± 0.09 mg N-$NO_2^-$ and 0.10 ± 0.04 mg N-$NO_3^-$, respectively (Fig. 2). At this stage the nitrogen and carbon assimilation accounted for 0.11 g N assimilated / g N SWW and 0.47 g C assimilated / g C SWW, respectively.

The third stage was characterized by the introduction of air diffusion in the photobioreactor at a flow of 10 mL $min^{-1}$, which supported an efficient $O_2$ stripping and stabilized the concentration of dissolved oxygen at 9.3 ± 2.9 mg $O_2$ $L^{-1}$. The concentration of inorganic carbon in the aerobic reactor decreased to 44 ± 39 mg IC $L^{-1}$ likely due to either an enhanced photosynthesis mediated by the mitigation of a potential DOC mediated inhibition or by an enhanced IC stripping (Fig. 5). $NH_4^+$ nitrification slightly improved, with concentrations of nitrite increasing from 2.9 mg N-$NO_2^-$ to 7.1 mg N-$NO_2^-$ and of nitrate from 0.23 mg N-$NO_3^-$ $L^{-1}$ to 0.52 mg N-$NO_3^-$ (Fig. 4). The organic carbon concentration in photobioreactor was 27 ± 2 mg TOC $L^{-1}$, this organic carbon being aerobically consumed by heterotrophic biomass (Fig. 5). The temperature and pH remained constant at an average values of 24 ± 1 ºC and 7.5 ± 0.0, respectively.

### 3.5. Greenhouse gases generated.

Wastewater treatment using algal-bacterial symbiosis in the anoxic and aerobic reactors generated greenhouse gases such as nitrous oxide, and methane to a lesser extent in the anoxic reactor. The anoxic reactor mainly generated nitrous oxide at the highest concentration in stage II with an average value of 17 ± 17 mg $N_2O$ $L^{-1}$, while during stage III the average $N_2O$ headspace concentration was 0.5 ± 0.3 mg $N_2O$ $L^{-1}$. The concentrations of methane were 6.5% and 1.7% at second and third stage, respectively, and were likely mediated by the high residence time of the wastewater in the anoxic tank (Fig. 7). Carbon dioxide was another greenhouse gas that anoxic tank generated, with values of 2.2 ± 1.1 % that was constant along throughout the experiment. Also

there were gases not involved in the greenhouse effect such as oxygen and nitrogen, whose concentrations were similar for the 95 days of study with average values of 2.8 ± 1.1% and 92 ± 1 %, respectively.

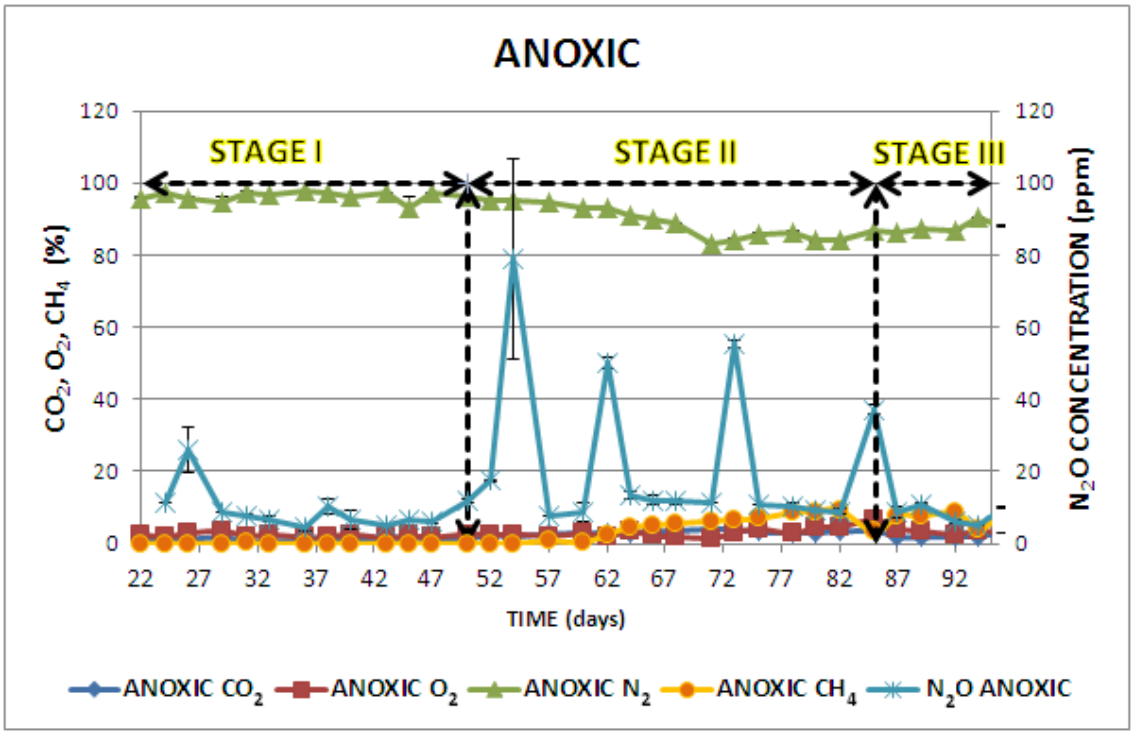


**Fig. 7-** Time course of gas phase concentration of in the headspace of the anoxic reactor. Vertical bars represent the standard deviation from duplicate measurements.

The average concentration of nitrous oxide in stage II and stage III in the headspace of the photobioreactor was 60 ± 17 mg $N_2O$ $L^{-1}$ and 0.52 ± 0.30 mg $N_2O$ $L^{-1}$, respectively. The values in third stage were significantly lower because the air diffused displaced and diluted the $N_2O$ accumulated in the headspace during the previous stages. The headspace methane concentration in the aerobic reactor was very low as a result of the high DOC recorded during stage III. Oxygen and nitrogen were other gases generated during the wastewater treatment in aerobic tank, whose composition was maintained constant along 3 stages of study, with average values of 26% ± 4 and 74 ± 4%, respectively (Fig. 8).

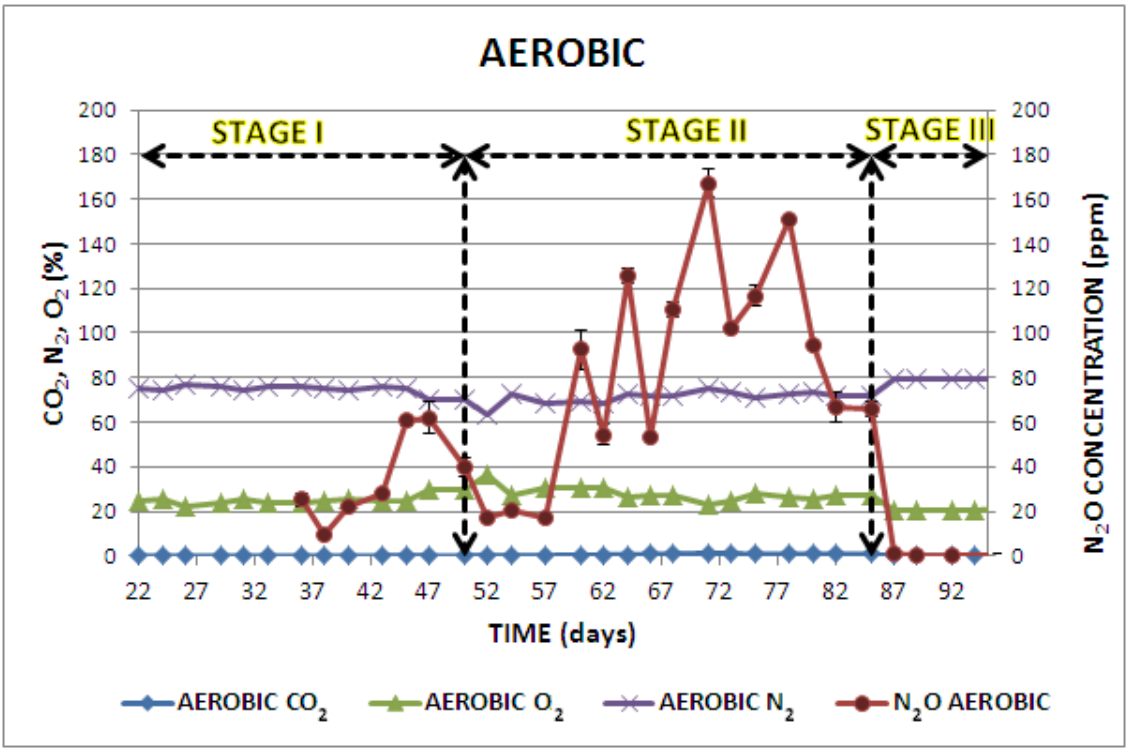


**Fig. 8-** Time course of gas phase concentration of  in headspace of the aerobic reactor. Vertical bars represent the standard deviation from duplicate measurements.

## 4. CONCLUSIONS

This experimental work assessed the wastewater treatment in an innovative anoxic-aerobic algal-bacterial photobioreactor operated at high light intensities, which enabled an efficient removal of organic carbon and nitrogen. The recirculation of the algal-bacterial biomass flocs from the bottom of the settler to the anoxic reactor provided a low SVI in both the anoxic tank and the photobioreactor. The removal efficiency of the settler during process operation at a HRT of 2 days accounted for 97 ± 1 %, but was not sufficient to provide effluent TSS below the maximum E.U discharge limits. $NH_4^+$ nitrification in the photobioreactor at a HRT of 4 days was low despite the high concentration of dissolved oxygen (20-22 mg $O_2$ $L^{-1}$) and $NH_4^+$, which suggested the occurrence of a potential limitation of nitrification by inorganic carbon. The increase in inorganic carbon supply when the HRT was decreased to 2 days brought about an increase in $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations, which allowed process operation under a nitrification-denitrification configuration. Finally, the treatment of wastewater generated greenhouse gases such as carbon dioxide, methane and oxide nitrous, which can jeopardize the environmental sustainability of the process.

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