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Influence of light intensity on bacterial nitrifying activity in algal-bacterial photobioreactors and its implications for microalgae-based wastewater treatment

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ABSTRACT

The influence of irradiance on the nitrifying activity in photobioreactors of a bacterial consortium enriched from a wastewater treatment bioreactor was assessed using independent ammonium oxidation kinetic batch tests and respirometric assays. Culture irradiance below 250 μmol m^{-2} s^{-1} did not show a significant effect on nitrification activity, while irradiance at 500 and 1250 μmol m^{-2} s^{-1} caused a decrease of 20 and 60% in the specific total ammonium nitrogen removal rates and a reduction of 26 and 71% in the specific NO_3^- production rates, respectively. However, no significant influence of irradiance on the affinity constant of NH_4^+ oxidation was observed. The increasing nitrite accumulation at higher light intensities suggested a higher light sensitivity of nitrite oxidizers. Additionally, NH_4^+ oxidation respirometric assays showed a decrease in the oxygen uptake of 14 and 50% at 500 and 1250 μmol m^{-2} s^{-1}, respectively. The experimental determination of the light extinction coefficient (λ) of the nitrifying bacterial consortium (λ = 0.0003 m^2 g^{-1}) and of *Chlorella sorokiniana* (λ = 0.1045 m^2 g^{-1}) allowed the estimation of light penetration in algal-bacterial high rate algal ponds, which showed that photoinhibition of nitrifying bacteria can be significantly mitigated in the presence of high density microalgal cultures.

1. Introduction

Microalgae mass cultivation has experienced a significant growth in the last years, boosted by the interest in third generation biofuel production. This interest on microalgal biofuels has been accompanied by economic and environmental sustainability studies, which recommended microalgae cultivation associated to either CO_2 capture from flue gases (Kesaano and Sims, 2014) or wastewater treatment in order to reduce their high operational cost and environmental impact (Park et al., 2011; Lananan et al., 2014; Kim et al., 2013). This quest for a sustainable mass production of microalgae has generated an intensive research in the development of microalgae-based technologies for the treatment of urban, industrial and livestock wastewaters (Muñoz and Guieysse, 2006). Microalgae have been used as low cost *in-situ* oxygenators for the bacterial oxidation of organic carbon and ammonium (Su et al., 2011; Lananan et al., 2014) and as a fixation tool for the removal of soluble nitrogen and phosphorous via photosynthesis during wastewater treatment (Posadas et al., 2013). In these processes, nitrification can play a key role in nitrogen management (Hernández et al., 2011). On the one hand, nitrification can prevent nitrogen losses by NH_3 volatilization (Abdel-Raouf et al., 2012), while reducing the toxic inhibitory effects of high NH_3 concentrations on microalgae growth (Collos and Harrison, 2014). In addition, nitrification can support the implementation of N removal strategies via denitrification in wastewaters with a low C/N ratio (de Godos et al., 2014).

The coexistence of nitrifying bacteria and microalgae has been

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reported in High Rate Algal Ponds (HRAP) (Park et al., 2011) and biofilm photobioreactors devoted to the domestic wastewater treatment (Muñoz and Guieysse, 2006; Posadas et al., 2013). For example, Posadas et al. (2014) observed a decrease in NH_3 stripping in an open biofilm photobioreactor treating domestic wastewater as a result of NH_4^+ nitrification and its associated pH decrease in the cultivation broth. Moreover, de Godos et al. (2014) reported removals of organic carbon and nitrogen exceeding 95% and 90%, respectively, during the treatment of wastewaters with low C/N ratios (~3) in a novel two-stage anoxic-aerobic photobioreactor. Despite the relevance of nitrification in microalgae-based wastewater treatment systems, there are few studies assessing the influence of the particular environmental conditions present in algal-bacterial photobioreactors on the performance of nitrifying communities. The particular configuration of photobioreactors, compared to the deep tanks used in activated sludge processes, entails an efficient light penetration in the algal-bacterial cultivation broth as a result of their high illuminated area to volume ratio (Merchuk et al., 2007). In this context, while early studies suggested that light can inhibit both microbial ammonium and nitrite oxidation (Alleman et al., 1987; Diab and Shilo, 1988, Guerrero and Jones, 1996a; 1996b; Hooper and Terry, 1974, Kaplan et al., 2000; Merbt et al., 2012; Müller-Neuglück and Engel, 1961; Yoshioka and Saijo, 1984), others investigations observed a light-mediated nitrification enhancement (Harris and Smith, 2009). Therefore, there is a lack of fundamental studies assessing the impact of light intensity on the microbial kinetics of NH_4^+ and NO_2^- oxidation, and its potential implications in microalgae-based wastewater treatment in photobioreactors.

In this study, the influence of irradiance on the nitrifying bacterial activity was evaluated through kinetics and respirometric assays. In addition, the light extinction coefficients of nitrifying bacteria and *Chlorella sorokiniana* were experimentally determined and used to estimate the potential impact of light penetration on the global nitrification process in wastewater-treating HRAPs.

2. Materials and methods

2.1. Microorganisms and cultivation media

The bacterial photoinhibition assays were inoculated with a nitrifying bacterial community obtained from a sequencing batch rotating disk bioreactor treating synthetic wastewater for 2 years using NH_4^+ as the sole energy and nitrogen source. The inoculum was prepared by acclimating the nitrifying community for 6 months in a modified Nakos and Wolcott mineral salt medium (MSM) (Elbanna et al., 2012), in the absence of light, at a constant pH of 7.5 ± 0.2 and dissolved oxygen concentrations over 5 mg L^{-1}. Every 4 weeks, the culture was centrifuged and resuspended in fresh MSM to maintain an active inoculum. The MSM contained (in g L^{-1}): 0.3 $(NH_4)_2SO_4$; 0.136 $CaCl_2$; 0.175 $MgSO_4 \cdot 7\ H_2O$; 0.5 $NaHCO_3$; 13.5 Na_2HPO_4; 0.7 KH_2PO_4; 0.005 $FeSO_4 \cdot 7\ H_2O$ and 0.00375 $NaMoO_4$. The MSM was autoclaved at 121 °C for 20 min, prior pH adjustment to 8.2 to avoid salt precipitation. This enrichment procedure ensured the selective enrichment of a community composed by nitrifiers.

The microalga *Chlorella sorokiniana* CCAP 211/8k was supplied by University of Huelva (Spain). The microalga was cultivated in modified M-8a medium containing (in g L^{-1}): 0.74 KH_2PO4; 0.26 $Na_2HPO_4 \cdot 2H_2O$; 0.4 $MgSO_4 \cdot 2H_2O$; 0.013 $CaCl_2 \times 2H_2O$; 1.15 NH_4Cl; 0.116 $C_{10}H_{12}N_2NaFeO_8$; 0.0372 $NA_2EDTA \cdot 2H_2O$; 6.18×10^{-5} H_3BO_3; 1.3×10^{-2} $MnCl_2 \cdot 4H_2O$; 3.2×10^{-3} $ZnSO_4 \cdot 7H_2O$; 1.83×10^{-3} $CuSO_4 \cdot 5H_2O$. The pH of the medium was adjusted to 7.0 and autoclaved at 121 °C for 20 min. The inoculum was maintained at 25 ± 2 °C under continuous illumination at 150 μmol m^{-2} s^{-1} provided by fluorescent lamps.

2.2. Photoinhibition assays in batch ammonium oxidation kinetic tests

The influence of irradiance on nitrifying activity was assessed in eight e-flasks (250 mL) initially filled with nitrifying bacterial inoculum (12 mL) and 148 mL of MSM. Concentrated NH_4^+ solution was also added in order to achieve initial total ammonium nitrogen (TAN) and biomass concentrations of 118 ± 4 mg-N L^{-1} and 0.34 ± 0.05 g-VSS L^{-1}, respectively. The cultures were incubated at 0, 250, 500, 1250 μmol m^{-2} s^{-1} in the absence or presence of LED lamps (0, 5, 7 and 10 W) under magnetic stirring for 15 days at 27 ± 2 °C. Each light condition was tested in duplicate. The spectrum of the commercial LED lamps used in this study presented 85% similarity to sunlight irradiation. The experimental set-up is shown in Fig. 1. The dissolved oxygen concentration was maintained above 5 mg-O_2 L^{-1} via aeration of the nitrifying cultivation broths. At this point it should be highlighted that the average Monod half saturation constant for O_2 in nitrifying bacterial communities typically ranges from 0.4 to 0.6 mg-O_2 L^{-1}, which suggest that dissolved oxygen was not a limiting factor during this experimentation (Metcalf and Eddy, 2003). The pH was maintained constant in the range 6.5–7.5 by adding $NaHCO_3$ from a 40 g L^{-1} stock solution. TAN, NO_2^- and NO_3^- concentrations were monitored every 2 days by sampling 10 mL of cultivation broth. The samples were centrifuged at 6000 rpm for 5 min, filtered through 0.22 μm mixed cellulose ester membrane filters and stored at −4 °C prior to analysis. Aliquots (5 mL) were also drawn every 6 days to record biomass concentration as total suspended solid (TSS) concentration. Kinetic parameters of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) were estimated by direct parameter fitting using the Solver function of Excel (Microsoft), integral and differential methods (Supplementary Materials). In the case of AOB, the integrated form of the Monod equation was linearized and data from ammonia consumption curves were used to estimate the maximum consumption rate and affinity constant values. Data obtained from the nitrite concentration profiles were not enough to provide a good fit to the Monod model and, therefore, nitrate concentration data were used to calculate the maximum consumption rate of NOB.

2.3. Photoinhibition assays using respirometric tests

Additional respirometric tests were also performed for 6 days in order to assess the influence of irradiance on nitrifying bacterial activity. Four e-flasks (250 mL) were initially filled with nitrifying bacterial inoculum (12 mL) and 148 mL of MSM. Concentrated NH_4^+ solution was also added in order to provide initial TAN and biomass concentrations of 150 ± 4 mg-N L^{-1} and 0.73 ± 0.05 g-VSS L^{-1}, respectively. A higher biomass concentration compared to that applied in section 2.2, was used to record significant O_2 consumptions.

The flasks were exposed to four irradiance levels (0, 250, 500, 1250 μmol m^{-2} s^{-1}) under the same incubation conditions described above. The bacterial oxygen uptake rate (OUR) was monitored at cultivation days 2, 4 and 6, by transferring 70 mL of cultivation broth to a respirometer illuminated under the same irradiance used for cultivation. The bacterial broth was initially air-saturated and dissolved oxygen (DO) was recorded on-line for approximately 30 min in triplicate assays. Aliquots (15 mL) were drawn at days 2, 4 and 6 of cultivation to determine the TSS and TAN concentrations. Control respirometric assays in the absence of ammonia were carried out to determine the endogenous bacterial respiration. For this purpose, biomass samples were taken from the

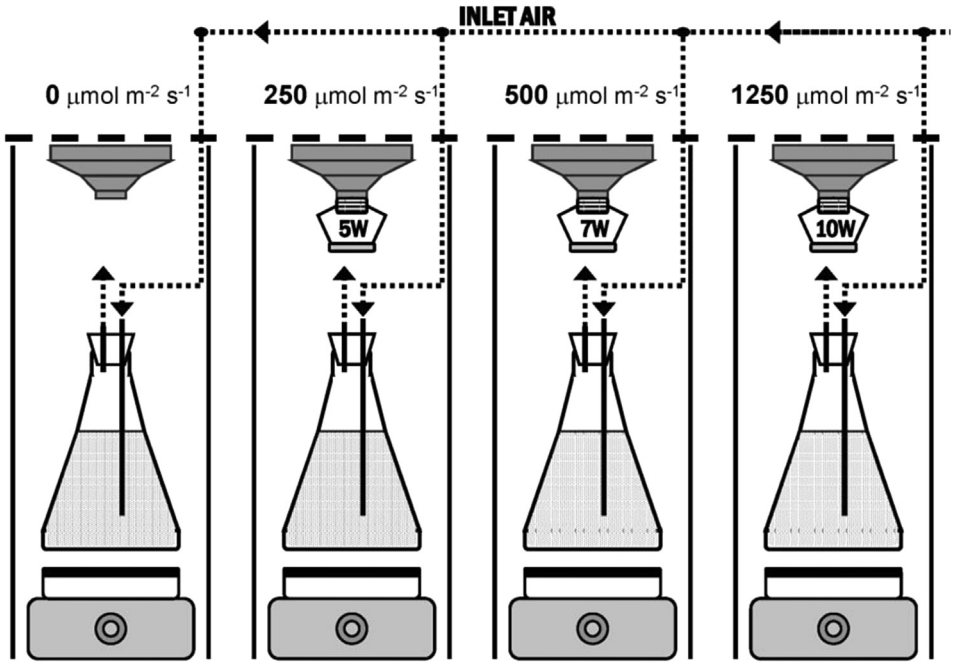

Fig. 1. Schematic of the set-up used in the batch kinetic tests of ammonium oxidation.

cultivation flasks, centrifuged and resuspended in MSM twice before performing the determination of the endogenous bacterial respiration. The values of the endogenous bacterial respiration were subtracted from the volumetric O_2 uptake rates recorded in the NH_4^+ supplemented assays.

2.4. Determination of the light extinction coefficients of nitrifying bacterial community and C. sorokiniana

Exponential growth cultures of *C. sorokiniana* and the nitrifying bacterial consortium were concentrated by centrifugation (6000 rpm for 5 min), washed twice in phosphate buffer solution (PBS) and resuspended in sterile water. Ten serial dilutions in distillated water of the microbial suspensions were prepared (from 1 to 1:20 for *C. sorokiniana* and from 1 to 1:10 for bacterial cultures). Light transmittance (T) through the microbial suspensions was measured in a 120 mL glass cell (7 cm long × 2.5 cm wide × 7 cm deep), illuminated with a LED lamp (Ekoline mod. IP-65 15W, China) from one side at an irradiance of 580 μmol m^{-2} s^{-1}. Light transmittance was determined measuring irradiance on the other side of the glass cell using a quantum light measurement system (LI-250A light meter with sensor LI-190, LI-COR, USA). Light transmittance tests with distilled water were used as a reference transmittance value. The extinction coefficients were obtained in independent sets of tests with either microalgae or bacteria. The extinction coefficients of the bacterial consortium and *C. sorokiniana* were determined from the linearization of the Beer-Lambert equation:

$$\ln\left(\frac{1}{T}\right) = \lambda \cdot X \cdot L \quad (1)$$

Where λ represents the microbial extinction coefficient (m^2 g^{-1}), X the microbial cell concentration (g m^{-3}) and L the light path (m).

2.5. Analytical procedures

TAN concentration was determined using Hach test kits (26069-45). NO_2^- and NO_3^- concentrations were determined by ion chromatography (Metrohm 882 Compact IC Plus, Switzerland). Dissolved oxygen concentration and pH were monitored using a Star3 DO electrode (Thermo Orion, USA) and a WD 35802-00 electrode (Thermo Orion Star A121, USA). TSS and VSS concentrations were analyzed according to APHA Standard Methods. Irradiance was measured with a quantum light measurement system (LI-250A light meter with sensor LI-190, LI-COR, USA).

2.6. Statistics

All analytical determinations were performed in duplicate unless otherwise specified. The results are given as the average with its corresponding standard deviation and analyzed for significance using an analysis of variance and Tukey's multiple comparison test at $\alpha = 0.05$.

3. Results

3.1. Photoinhibition assays in batch ammonium oxidation kinetic tests

The kinetic tests illuminated at 0, 250, 500 and 1250 μmol m^{-2} s^{-1} showed a linear ammonium removal within the first 4, 4, 6 and 14 days of cultivation, respectively (Fig. 2). The initial specific ammonium removal rates at 0 and 250 μmol m^{-2} s^{-1} were not significantly different (0.101 ± 0.003 mg-TAN mg-VSS^{-1} d^{-1}), while a reduction of 20% and 64% was observed at 500 and 1250 μmol m^{-2} s^{-1}, respectively (Table 1). Likewise, while the specific nitrate production rates were comparable at 0 and 250 μmol m^{-2} s^{-1} (0.090 ± 0.003 mg-NO_3^- mg-VSS^{-1} d^{-1}), a reduction of 26% and 71% was recorded at 500 and 1250 μmol m^{-2} s^{-1}, respectively (Table 1). On the other hand, nitrite concentration gradually increased up to 15.7 ± 1.5 mg-N L^{-1} at day 4 in the cultures illuminated at 0 and 250 μmol m^{-2} s^{-1}. However, nitrite was completely depleted at day 10 (Fig. 2). In the tests illuminated at 500 μmol m^{-2} s^{-1}, maximum nitrite concentrations

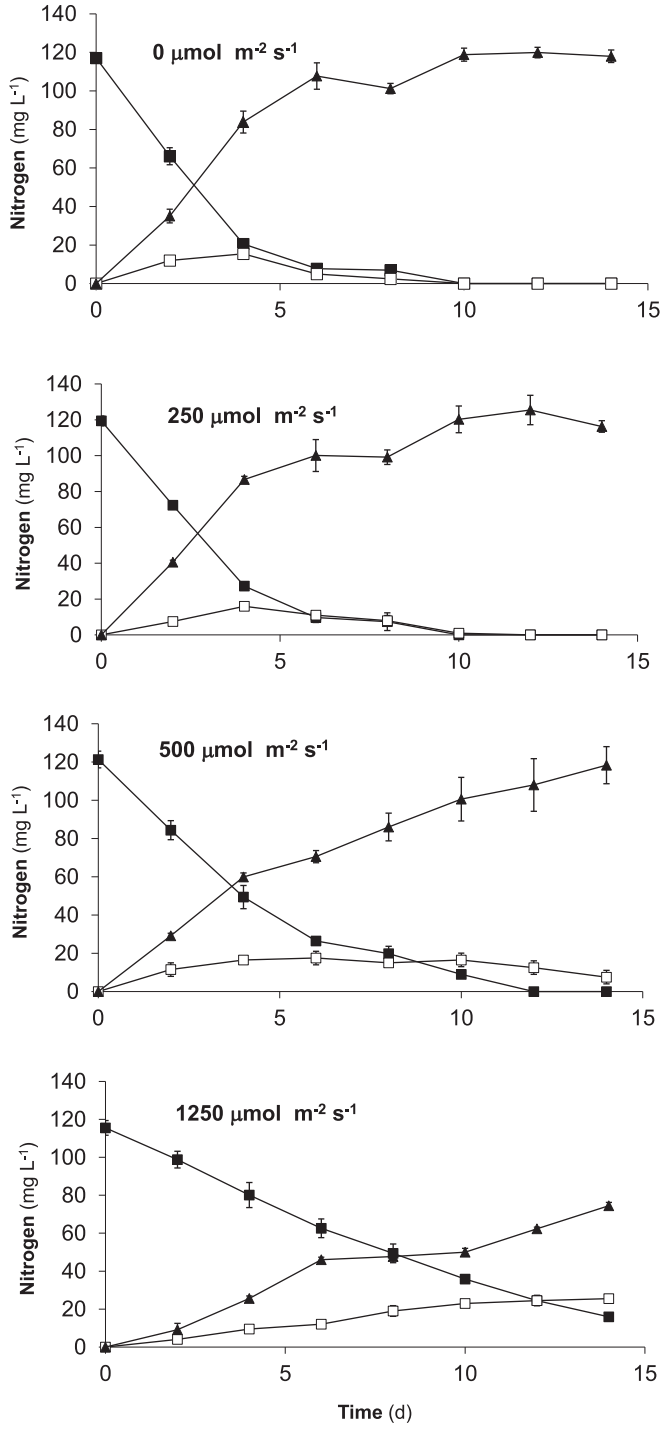

Fig. 2. Time course of (■) TAN, (▲) $N{-}NO_2^-$ (□) and $N{-}NO_3^-$ concentration in the photoinhibition kinetic batch assays under different light intensities. Vertical bars represent standard deviation.

stabilized at 16.4 ± 1.0 mg-N L^{-1} from day 4–10 and steadily decreased to 7.5 ± 2.5 mg-N L^{-1} at day 14. A steady increase in nitrite concentration up to 25.5 ± 1.5 mg-N L^{-1} was recorded at 1250 μmol m^{-2} s^{-1} throughout the 14 days of cultivation. A negligible NH_3 stripping was observed regardless of the illumination, as shown by the final average TAN nitrification ratios of 1.01 ± 0.04 g($N{-}NO_2^- + N{-}NO_3^-$) g($N\text{-}TAN_{removed}$)$^{-1}$. No significant variation of the biomass concentration of the nitrifying population (0.34 ± 0.05 g-VSS L^{-1}) was recorded over the 14 days of experiment.

3.2. Photoinhibition assays using respirometric tests

Fig. 3 shows the influence of irradiance on OUR at days, 2, 4 and 6. The average OUR of the nitrifying bacterial culture (0.239 ± 0.009 mg-O_2 L^{-1} min^{-1}) did not exhibit significant differences in the range 0–500 μmol m^{-2} s^{-1} in respirometric tests performed at day 2, while a decrease of 36% in the OUR was recorded in the culture exposed to 1250 μmol m^{-2} s^{-1}. At days 4 and 6 no significant differences were observed in the OUR (0.268 ± 0.013 mg L^{-1} min^{-1}) at the two lower irradiance levels, while a decrease of 14% and 49% was recorded at 500 and 1250 μmol m^{-2} s^{-1}, respectively.

3.3. Determination of the light extinction coefficients of nitrifying bacterial community and C. sorokiniana

The light extinction coefficients of the nitrifying bacterial community and *C. sorokiniana* were estimated in the concentration ranges of 0.4–1.8 and 0.04–0.81 g L^{-1}, respectively. The microalgal and bacterial extinction coefficients were 1.045 L g^{-1} cm^{-1} and 0.003 L g^{-1} cm^{-1}, respectively.

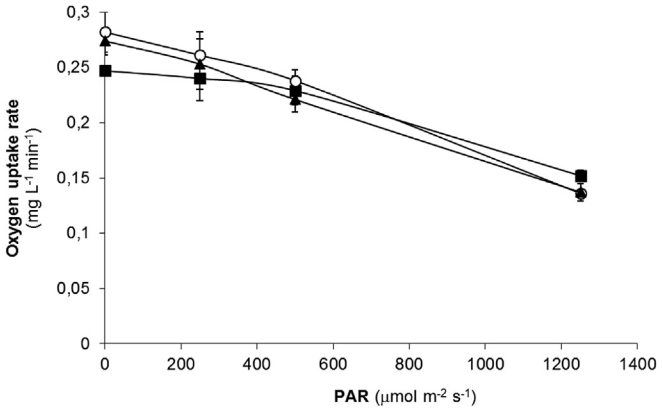

Fig. 3. Influence of irradiance on the O_2 uptake rates of the nitrifying culture at days (■) 2, (○) 4 and (▲) 6. Vertical bars represent the standard deviation.

Table 1
Specific initial TAN removal and nitrate production rates during the photoinhibition kinetic batch assays at different light intensities.

	Irradiance (μmol m^{-2} s^{-1})			
	0	250	500	1250
TAN removal rate (mg-N mg-VSS^{-1} d^{-1})	0.101 ± 0.003	0.098 ± 0.009	0.08 ± 0.005	0.036 ± 0.002
Nitrate production rate (mg-$N\text{-}NO_3$ mg-VSS^{-1} d^{-1})	0.088 ± 0.008	0.092 ± 0.007	0.066 ± 0.000	0.026 ± 0.002

4. Discussion

This study confirmed that ammonium and nitrite oxidizers can be photoinhibited at the high irradiances typically encountered under outdoors conditions, which can achieve 2000–3000 μmol m^{-2} s^{-1} during peak hour (Wang et al., 2008). Culture irradiance below 250 μmol m^{-2} s^{-1} did not influence the activity of nitrifying bacterial cultures in terms of specific ammonium removal rate and nitrate production rates. However, light intensities of 1250 μmol m^{-2} s^{-1} reduced nitrifying activity by 64% and 71% when evaluating the initial specific ammonium removal rate and initial specific nitrate production rate in the absence of light supply, respectively (Table 1). The conducted respirometric assays (Fig. 3) also confirmed the absence of photoinhibition at low light intensities and a 47 ± 7% reduction in nitrifying activity at 1250 μmol m^{-2} s^{-1}. These results were comparable with those published by Guerrero and Jones (1996a, 1996b) for *Nitrosomonas cryotolerans* and *Nitrosococcus oceanus*, which lost up to 90% of their NH_4^+ oxidizing activity after 2 h of sunlight exposure at 1900 μmol m^{-2} s^{-1}. Likewise, Hooper and Terry (1973) found that an irradiance of 900 μmol m^{-2} s^{-1} reduced by 50% ammonium oxidation in *Nitrosomonas europaea*. In a recent study, a complete inhibition of the NH_4^+ oxidizers *N. europaea* and *Nitrospira multiformis* at 500 μmol m^{-2} s^{-1} was reported using culture irradiation with a wavelength spectrum of 400–680 nm (Merbt et al., 2012). Nitrite accumulation in the cultivation broth at increasing light intensities suggests that nitrite oxidizers were significantly more affected by light than ammonium oxidizers. Based on the occurrence of aerobic conditions in the assays and the final average TAN nitrification ratios of 1.01 ± 0.04 g($N-NO_2^-$ + $N-NO_3^-$) g($N\text{-}TAN_{removed}$) recorded, the decrease in nitrite concentration observed was likely due to nitrite oxidation to nitrate (Fig. 2). Similar results were obtained by Kaplan et al. (2000) in a study conducted in a natural water body, where light was identified as a major factor inhibiting nitrification and causing the accumulation of nitrite during late spring and summer. Earlier studies with soil (Müller-Neuglück and Engel, 1961; Schon and Engel, 1962) and aquatic nitrifiers (Diab and Shilo, 1988; Yoshioka and Saijo, 1984) also observed that NO_2^- oxidizers were more light-sensitive than NH_4^+ oxidizers. However, Guerrero and Jones (1996b) concluded that the NH_4^+ oxidizers *Nitrosomonas cryotolerans* and *Nitrosococcus oceanus* exhibited a higher light sensitivity than the nitrite oxidizers *Nitrococcus mobilis* and *Nitrobacter* sp. This suggests that photosensitivity seems to be species-dependent. Likewise, a differential and integral linearization of the maximum TAN uptake rates in our study showed no significant influence of irradiance on the affinity constants (K_S = 25 ± 6 mg-N L^{-1}), which suggests that light inhibition over ammonium oxidation could be represented by a non-competitive model. At this point, it must be stressed that the high Ks here reported might be explained by the previous acclimation of the nitrifying bacteria to high ammonium concentrations (Khin and Annachhatre, 2004).

Three mechanisms have been hypothesized to explain the inhibitory effect of light on nitrifying bacterial activity. Hooper and Terry (1974) showed that ammonia oxidation was irreversibly inactivated by visible light in cultures of *Nitrosomonas europaea* and hypothesized that photoinactivation could have been mediated by a photooxidative damage caused by 400–430 nm photons to either the enzyme ammonium oxygenase or its associated porphyrins cofactors. These authors also found that when oxygen was absent or at very low concentrations in the presence of light, cells were completely protected from photoinactivation, suggesting that inactivation involved a not fully understood light-induced reaction with oxygen. In this context, Hyman and Arp (1992) concluded that high light intensities can affect the synthesis of polypeptides involved in the ammonia monooxygenase system. On the other hand, Bock (1965) and Guerrero and Jones (1997) suggested that a light mediated damage in cytocrome-c (a protein involved in the electron transport chain) could be also responsible for the decrease in nitrifying activity at high light intensities, based on specific light absorption studies on porphyrins from nitrifying microorganisms. Finally, other authors hypothesized that a prolonged exposure to high light intensities over 300 nm could also mediate a significant damage on key proteins, chromophores and nucleic acids, although the specific light dosage and macromolecules affected were not identified (Eisenstark, 1971; Guerrero and Jones, 1997; Jagger, 1983).

In order to quantitatively assess the impact of light on the overall bacterial ammonium oxidation in HRAPs treating wastewater under outdoor conditions, a simulation of light penetration in an algal-nitrifying bacterial culture in a 20 cm deep HRAP was performed. Algal-bacterial cultures containing 95% *C. sorokiniana* (λ = 0.1045 m^2 g^{-1}) and 5% of nitrifying bacteria (λ = 0.0003 m^2 g^{-1}) at concentrations of 500, 1000, 2000 and 3000 g-TSS m^{-3} were selected as model cultivation broths. These algal-bacterial concentrations are typical in HRAPs implementing biomass settling and recycling during the treatment of centrifuged digestates (de Godos et al., 2014). The assessment considered an irradiance of 1500 μmol m^{-2} s^{-1} at the HRAP surface (typically found at peak hours in a sunny day) and a critical photoinhibition threshold of 500 μmol m^{-2} s^{-1}. The results showed that the presence of microalgae in high density cultures in HRAPs can mitigate the photoinhibition of nitrifying bacteria. A reduction in nitrifying activity of 11.0, 5.5, 2.7, 1.9% might be expected at 500, 1000, 2000 and 3000 mg-TSS L^{-1}, respectively, based on the fraction of cultivation broth exposed above the light inhibition threshold experimentally determined in this study (Fig. 4). These results revealed that, despite the high photosensitivity of nitrifying cultures, sunlight is not expected to cause a significant inhibition on the nitrification capacity of algal-bacterial photobioreactors during wastewater treatment.

5. Conclusions

This study showed a significant inhibitory effect of irradiance on the nitrifying activity of NH_4^+ and NO_2^- oxidizers above 500 μmol m^{-2} s^{-1}. Nitrite oxidizers exhibited a lower phototolerance at the light intensities typically found under outdoors conditions. However, this inhibitory effect can be mitigated under photobioreactor operation due to the high extinction coefficient of

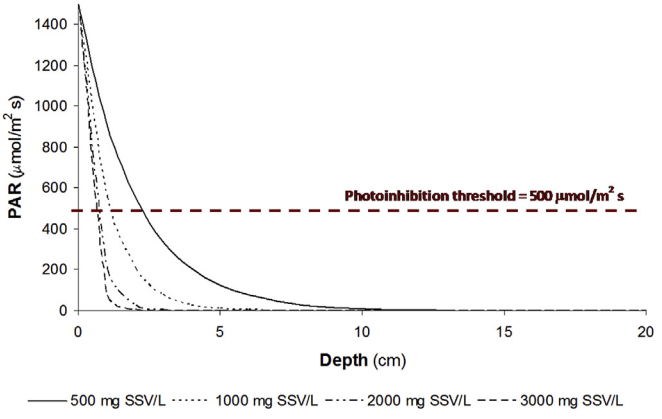

Fig. 4. Light penetration in a 20 cm HRAP at different algal-bacterial biomass concentrations (assuming a microbial population composed of 95% *C. sorokiniana* and 5% nitrifying bacteria) at an impinging irradiance of 1500 μmol m^{-2} s^{-1}.

the microalgae that symbiotically coexist with nitrifying bacteria.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2016.06.006.

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