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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