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BACTERIA-MICROALGAE INTERACTIONS DURING NITRIFICATION/DENITRIFICATION PROCESSES IN A PHOTOBIOREACTOR TREATING SWINE WASTEWATER

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ABSTRACT: The economic feasibility of photobioreactors for the production of valuable microalgae feedstock can be limited to some extent by the nitrogen (N) and phosphorus (P) fertilizers costs. In this regard, N- and P-rich residues from agribusiness have been widely considered for microalgae cultivation. Therefore, the use of photobioreactors not only constitutes a promising engineered technology to generate valuable feedstock for renewable energy but can also serve as an alternative tertiary treatment for swine nutrient-rich wastewaters. The objective of this study was to investigate the interaction between naturally occurring anaerobic biodigestion effluent wastewater bacteria and *Chlorella* sp. during nitrogen removal by nitrification/ denitrification processes within a photobioreactor. qPCR assays were used to quantify the abundance of total bacteria (16S rDNA) and denitrifiers (*nir*S). Ammonia removal coincided with the increasing growth of total bacteria (1.2×10^{10} copies μL^{-1} at 48 h) and accumulation of NO_3 and NO_2 intermediates. Low oxygen concentrations prevailed (< 0.2 mg/L) in the photobioreactor during dark periods and low microalgae biomass (up to 48 h) thus stimulating the growth of denitrifying bacteria (1.8×10^{6} copies μL^{-1} at 48 h). NO_3 accumulation coincided with denitrification inhibition by the increased dissolved oxygen-derived photosynthesis. Overall, the oxidative-reductive environment encountered in a non-sterile photobioreactor can benefit swine wastewater nutrient removal by the simultaneous enhancement of nitrification and denitrification processes. This is particularly important since the application of external oxygen sources to maintain nitrification processes can be costly and/ or insufficient to warrant adequate bacterial metabolism.
Keywords: denitrification, *nirS* gene, nitrification, photobioreactor, swine wastewater

INTRODUCTION

Swine breeding wastewaters present a global environmental concern because of the problems associated with soil acidification, water eutrophication and atmospheric ammonia emissions. These environmental problems are mostly due to the high nitrogen content from swine waste, where up to 70% of the nitrogen present in liquid manure is composed of ammonium (≅180 mM). Among several wastewater biological treatment processes used to remove nitrogen, strategies that are based on the growth of microalgae are currently been considered worldwide as alternative to produce valuable feedstock to renewable biofuels.

Photosynthetic batch reactors are able to completely remove ammonium from swine wastewater, converting 25 to 100% of nitrogen and 70 to 90% of phosphate into biomass (Gonzalez et al., 2008; Molinuevo-Salces et al, 2010; Godos et al., 2009). Nitrogen removal rates from photobioreactors inoculated with acclimated bacteria from nitrification/ denitrification sludge settler tank were comparable to conventional denitrification-nitrification activated sludge configurations (Gonzalez et al., 2008; Godos et al., 2009). During nitrification processes, ammonia (NH_4^+) is oxidized to nitrate (NO_3^-), which is the

form of nitrogen that favors nitrogen assimilation for plant growth, though microalgae (*Chlorella vulgaris*) also grow well on either NO_3^- or NO_2^- (Weathers, 1984). Nonetheless, little is known about the applicability of non-inoculated photobioreactors to remove nitrogen in carbon-limiting effluent from anaerobic swine wastewater biodigestion.

Molecular analyses have been extensively used to better understand the role of microbial-mediated biodegradation processes. Thus, targeting functional genes involved in the biological nitrogen cycle can assist to elucidate ecological interactions between bacteria and microalgae cultivation during wastewater treatment in photobioreactors. Therefore, this study addresses the potential of a mixotrophic photobioreactor to remove ammonia from swine wastewater derived from anaerobic biodigestion. Whereas, sterile conditions in photobioreactor is technically difficult to maintain, non-sterile photobioreactor was considered to better mimic conditions that are likely to prevail at field scale applications. Emphasis was placed on correlating the concentration of total bacteria (*16S* rDNA) and denitrifying bacteria (*nir*S) with nitrogen species produced over time and within the photobioreactor.

MATERIAL AND METHODS

A non-sterile 9-L glass bottle reactor was utilized as a photobioreactor. The swine wastewater was collected from an upflow anaerobic sludge blanket (UASB) effluent reactor with the following characteristics (g L^{-1}): pH 7.9, 3–8 TSS, 1.5–6.5 TOC, 2.5–4.5 BOD_5, 5–8 $CaCO_3$ alkalinity, 1.5–2 TN, 0.900–1.5 NH_3-N. Diluted wastewater (2:5 tap water) was inoculated with 30% v/v microalgae (10 g L^{-1} dry weight of *Chlorella vulgaris*). The reactor was continuously stirred and maintained at room temperature (21±1°C) with a photoperiod of 12h. Samples were collected daily and analyzed for pH, dissolved oxygen (DO), chlorophyll (Porra et al., 1989), NO_2, NO_3, and NH_3 (APHA, 2005).

Total bacteria (*16S* rDNA) and denitrifying (*nirS*) bacteria were estimated by real-time quantitative PCR (qPCR) analysis with primers and conditions described by Chon et al (2011). DNA was extracted according to MoBio UltraClean Microbial DNA kit following manufacturer's instructions. Standard curves were prepared (10^9 to 10^1 gene copies mL^{-1} of *nirS* or 16S gene copies) by amplification of *nirS* fragments and insertion into pCR® 2.1-TOPO® vector (Invitrogen, USA) then further transformed into DH5α *Escherichia coli* competent cells (Sambrook et al., 2001). Clones were grown in Luria-Bertani medium plates supplemented with ampicillin (50 mg/ml). Colonies were chosen and the plasmidial DNA was extracted by alkaline method (Sambrook et al., 2001). The presence of the inserted sequence in plasmid DNA was confirmed by conventional PCR as described by Chon et al (2011). SYBR green kit was used to quantify DNA with qPCR temperature conditions for targeting *16S* and *nirS* as previously demonstrated by Chon et al. (2011).

RESULTS AND DISCUSSION

Chlorella vulgaris was able to grow in the photobioreactor fed diluted swine wastewater from anaerobic biodigestion effluent with a specific exponential growth rate (μ) of 0.06 day^{-1} (Figure 1).

NH_3-N concentration steadily decreased from 430 mg L^{-1} to 235 mg L^{-1} reaching a removal efficiency of 45% after 96 h of treatment (Figure 2). The increasing bacteria (*16S* rDNA) concentration (from 1.5×10^4 at 0 h to 1.2×10^{10} at 48 h) provided circumstantial evidence to support nitrification/denitrification processes. NO_3^- (30 mg L^{-1}) and NO_2^- (50 mg L^{-1}) accumulation served to further support the occurrence of nitrification during the initial stages of the biodegradation process (up to 48h). These results support the notion that photobioreactor inoculation with acclimated bacteria from nitrification/ denitrification activated sludge may not be necessary in order to accomplish satisfactory nitrogen

removal. pH decreased from 7 to 6 during the first 48 h of the experiment (data not shown) and was likely associated with acidification caused by nitrification when ammonia is used as a nitrogen source by either bacteria or microalgae (Godos et al., 2009; Gonzalez et al, 2008; Molinuevo-Sales et al. 2010). Low DO values (< 0.2 mg L^{-1} up to 48h) observed during dark periods and low microalgae biomass (Figure 1) served to stimulate the growth of denitrifying *nir*S-harboring bacteria (Figure 2). After 48h, the high microalgae biomass associated with higher DO-derived photosynthesis (above O_2 solubility levels >8 mg L^{-1}) inhibited denitrification resulting NO_3^- accumulation (Figure 2).

CONCLUSIONS

This work was conducted to demonstrate the interaction between bacteria-microalgae within a mixotrophic photobioreactor simulating ammonia bioremediation from swine wastewater. Microalgae growth promoted the establishment of simultaneous oxidative-reductive environments and nitrification and denitrification processes. The role of microalgae was particularly important to aerobic ammonia removal contributing with adequate levels of oxygen needed to warrant complete bacterial nitrification. In this regards, engineered photobioreactors can be built to minimize the costs and technical difficulties associated with the implementation of external oxygen supplies. In a nutshell, naturally-occurring bacteria-microalgae interactions in photobioreactors can provide an attractive polishing step to effectively remove ammonia (and perhaps other nutrients such as phosphorus) from swine wastewater previously treated by anaerobic digestion.

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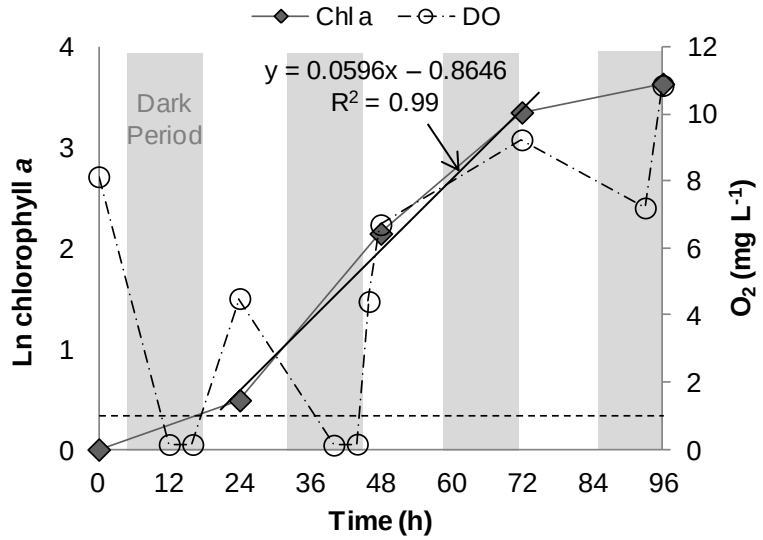

Figure 1. Chlorophyll a and dissolved oxygen concentrations profile. Dashed line represents minimum oxygen concentration required to support nitrification. Denitrification occurs at DO<0.2 mg L^{-1}. Linear regression represents microalgae growth rate (μ).

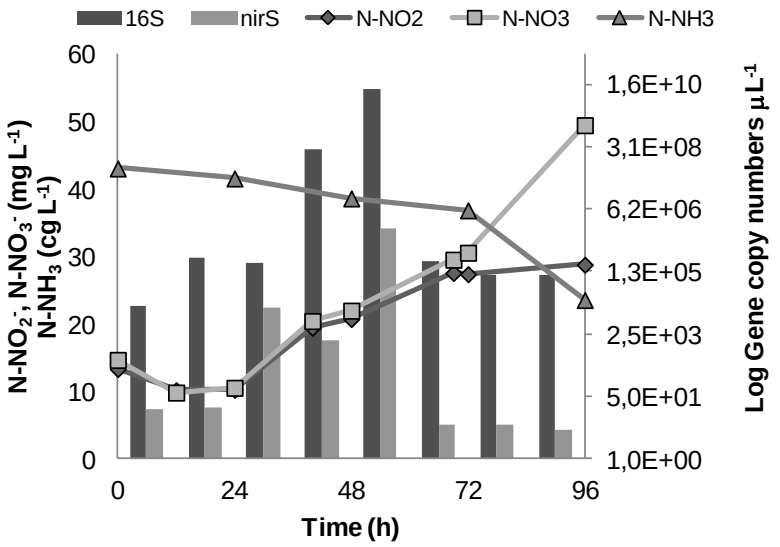

Figure 2. Average ammonia, nitrate, nitrite, total bacteria (*16S* rDNA) and denitrifying (*nirS*) bacteria concentration profile within the photobioreactor.