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Nitrogen removal by simultaneous partial nitrification, anammox and denitrification (SNAD) in a structured-bed reactor treating animal feed processing wastewater: Inhibitory effects and bacterial community

Ricardo Gabriel Bandeira de Almeida[a], Carla Eloísa Diniz dos Santos[a,*], Taíssa Colucio Lüders[a], Valéria Del Nery[a], Cintia Dutra Leal[b], Alyne Duarte Pereira[b], Juliana Calábria Araújo[b], Russel J. Davenport[c], Ana Cláudia Barana[d], Deize Dias Lopes[e], Márcia Helena Rissato Zamariolli Damianovic[a]

[a] Laboratory of Biological Processes, Centre for Research, Development and Innovations in Environmental Engineering, São Carlos School of Engineering, University of São Paulo, Av. João Dagnone 1100, 13563-120, São Carlos, SP, Brazil

[b] Department of Sanitary and Environmental Engineering, Federal University of Minas Gerais (UFMG), Av. Antonio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil

[c] School of Civil Engineering & Geosciences, Newcastle University, NE1 7RU Newcastle Upon Tyne, UK

[d] Department of Food Engineering, State University of Ponta Grossa, Av. Gal. Carlos Cavalcanti, 4748, 84030-900, Ponta Grossa, PR, Brazil

[e] Department of Civil Engineering, State University of Londrina, Rod. Celso Garcia Cid, Km 380, 86051-991, Londrina, PR, Brazil

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ABSTRACT

The aim of this study was to investigate the post-treatment of UASB effluent by treating animal feed production wastewater using simultaneous partial nitrification, anammox and denitrification (SNAD) in a structured-bed reactor subjected to low aeration and recirculation. The average nitrogen loads applied were 0.307, 0.249 and 0.149 $kgN\,m^{-3}d^{-1}$, correlated to COD/N ratios of 0.28, 0.41 and 0.26 (Phases 1, 2 and 3, respectively). The best mean values for removal efficiencies of total-N and COD were obtained in Phase 1 with 48 ± 24% and 63 ± 20%, respectively, reaching a maximum total-N removal efficiency of 79%. The anammox process was the main pathway of nitrogen removal, as pointed out in the nitrogen mass balance. High free ammonia (FA) concentrations in Phases 2 and 3, associated to the limitation of oxygen supply, caused inhibition of nitrite oxidizing bacteria (NOB) activity, leading to NO_2 accumulation, having an impact on the denitrifying activity. At the end of the operational period, sequencing analysis detected sequences related to heterotrophic denitrifiers (22.5%), anammox bacteria, *Candidatus Anammoximicrobium* (2%) and ammonia oxidizing bacteria (AOB) belonging to *Nitrosomonadales* and *Nitrosomonas* (0.6%). These results demonstrated that nitritation, denitrification and anammox were likely in the processes involved in nitrogen removal in this reactor.

1. Introduction

Pet food industries play an important role in preserving the environment and valuing agro-industrial waste. These companies use effluent produced in poultry and cattle slaughterhouses as raw material. If this waste was not reused, it would be disposed of in the environment (Jayathilakan et al., 2012; Wosiack et al., 2015). Effluent from poultry and cattle slaughterhouses has a high concentration of oils and grease, nitrogenous and sulfurous compounds derived from the protein breakdown (Jeganathan et al., 2006; Liu et al., 2004; Wosiack et al., 2015). This composition makes biological treatments difficult due to the complexity and high pollutant loading. According to Jeganathan et al. (2006), in aerobic treatment processes, oil and grease considerably affect oxygen mass transfer. In anaerobic processes, poor activity sludges can be developed and foam can accumulate on the surface of the water (Jeganathan et al., 2006). These factors result in biomass losses with effluent, which can significantly affect a system's efficiency (Jeganathan et al., 2006). Actions to mitigate operational problems related to high organic loads, which are commonly generated in processing industries, have been successfully developed (Bustillo-Lecompte and Mehrvar, 2015). In this context, diverse process combinations such as anaerobic, aerobic and facultative lagoons, activated sludge and

* Corresponding author.
E-mail addresses: rgbalmeida@gmail.com (R.G.B.d. Almeida), carladiniz@sc.usp.br (C.E.D.d. Santos), tacoluders@gmail.com (T.C. Lüders), vdelnery@gmail.com (V. Del Nery), cintia@desa.ufmg.br (C.D. Leal), alyneduartep@gmail.com (A.D. Pereira), juliana@desa.ufmg.br (J.C. Araújo), russell.davenport@ncl.ac.uk (R.J. Davenport), acbarana@uepg.br (A.C. Barana), dilopes@uel.br (D.D. Lopes), mdamianovic@sc.usp.br (M.H.R.Z. Damianovic).

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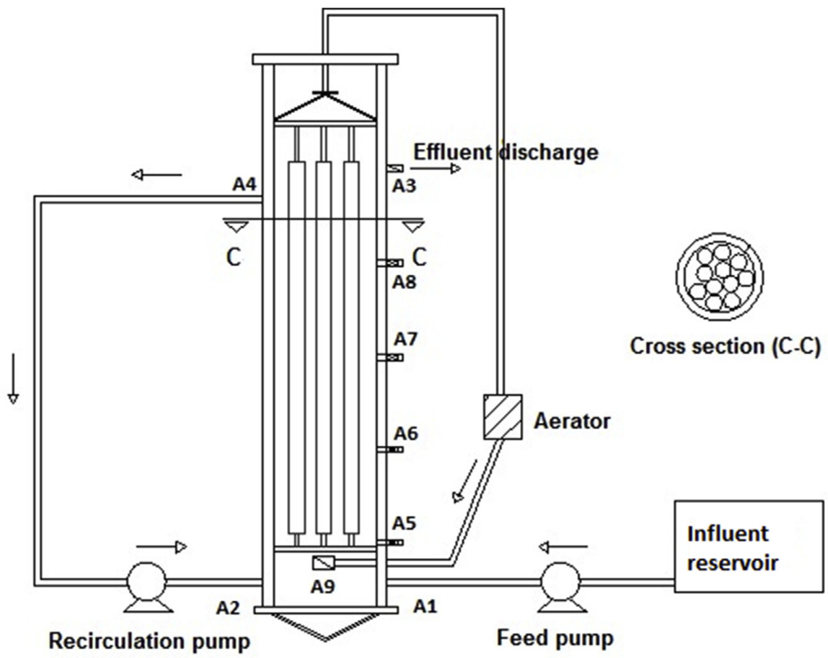

Fig. 1. Schematic diagram of the experimental apparatus (A1: feeding entrance; A2: recirculation entrance; A3: effluent discharge; A4: recirculation exit; A5: air diffusion) (Adapted from Moura et al., 2012).

trickling filters can be used (Bustillo-Lecompte and Mehrvar, 2015; Massé and Masse, 2000). However, the residual nitrogen content should be removed in post-treatment units at wastewater treatment plants (WWTP). In this context, it is essential to develop technologies which can metabolize the residual organic matter and nitrogen from this type of wastewater in order to satisfy the discharge standards.

Biological treatment systems that use immobilized biomass demonstrate greater robustness to tolerate toxic and high organic loadings, minimizing biomass washout (Mijaylova-Nacheva and Canul-Chuil, 2006) despite the increased flow velocities (Mockaitis et al., 2014). Biomass immobilization has been successfully used to promote the simultaneous nitrification denitrification (SND) process (Moura et al., 2012; Reboleiro-Rivas et al., 2015; Santos et al., 2016; Lin et al., 2016). SND systems have gained attention due to their potential of achieving nitrogen removal rates similar to those values observed in conventional two-stage reactors. They are also cost-effective in terms of construction and operation (Moura et al., 2012; Pochana and Keller, 1999; Yoo et al., 1999). Studies showing nitrogen and organic matter removal efficiencies between 93% and 95%, respectively, were published for different types of wastewater in operating lab-scale SND reactors (Barana et al., 2013; Liu et al., 2010; Moura et al., 2012; Santos et al., 2016; Wosiack et al., 2015). In cases where there are low C/N ratios, anammox (anaerobic oxidation of ammonia) bacteria can also coexist as a complementary pathway for nitrogen removal, as observed by Barana et al. (2013) and Santos et al. (2016).

Based on the fact that SND occurs due to the maintenance of a dissolved oxygen (DO) gradient in the biofilm, systems that provide a suitable DO concentration for nitrification and resistance to oxygen transfer in the biofilm interior are the most suitable (Pochana and Keller, 1999). The occurrence of the SND process by the shortened pathway has attracted considerable attention as an effective approach to reduce energy consumption (Yoo et al., 1999). This is possible by limiting the oxygen supply, aiming to minimize the nitrite (NO_2-N) oxidation (nitratation) rate, while maximizing the ammonium (NH_4-N) oxidation rate (nitritation) (Park and Bae, 2009).

Recently, the simultaneous partial nitrification, anammox and denitrification (SNAD) process under limiting DO concentration was investigated (Chen et al., 2009). Firstly, NH_4^+-N was oxidized to NO_2^--N by ammonia-oxidizing bacteria (AOB). The available NO_2^--N was utilized by the anammox bacteria to oxidize the remaining NH_4^+-N to N_2, releasing NO_3^--N, which will be reduced by heterotropic denitrifiers by using the residual organic matter as an electron donor (Chen et al., 2009, 2017; Wang et al., 2010). Therefore, in SNAD systems, anammox takes place as a primary process for nitrogen removal and is responsible for NO_3^--N production and consequently correlates with the denitrification occurrence. Recent studies have demonstrated that COD/N ratio is an important parameter to ensure SNAD stability (Daverey et al., 2013; Chen et al., 2017). In addition, it was proved that this bioprocess is favored in attached systems, as maintaining a long sludge retention time (SRT) can develop an anammox community (Chen et al., 2009; Liang et al., 2014), as this group has a low specific growth rate (Strous et al., 1998).

Nonetheless, the interruption of nitratation is not easily achieved (Yang and Yang, 2011; Yoo et al., 1999). Previous studies have concluded that other operational parameters also affect the ammonium/nitrite oxidation rates such as pH, temperature, SRT and substrate loads (Anthonisen et al., 1976; Chung et al., 2007). When extreme changes in pH occur, the effects of substrate concentration become considerably important because of their inhibitory effects when unionized (Park and Bae, 2009). Anthonisen et al. (1976) observed that in basic conditions, unionized free ammonia (FA; NH_3) concentrations of 0.1–1.0 and 10 to 150 mgFA L^{-1} inhibited both ammonium and nitrite oxidation, respectively.

The main aim of this study was to investigate the simultaneous process of partial nitrification, anammox and denitrification for post-treatment of animal feed processing wastewater in a structured-bed reactor subjected to low aeration. In this context, this study offers some important insights into the mechanisms associated to inhibition effects on nitratation and denitrification and also the microbial characterization developed in the bioreactor, which are essential for understanding the system's performance.