Bioresource Technology 100 (2009) 1100–1107

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Removal of ammonium via simultaneous nitrification–denitrification nitrite-shortcut in a single packed-bed batch reactor

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ARTICLE INFO

Article history:
Received 26 May 2008
Received in revised form 4 August 2008
Accepted 6 August 2008
Available online 14 September 2008

Keywords:
Ammonium-oxidizers
Denitrifying bacteria
Post-treatment
Ammonium removal
Packed-bed batch reactor

ABSTRACT

A polyurethane packed-bed-biofilm sequential batch reactor was fed with synthetic substrate simulating the composition of UASB reactor effluents. Two distinct ammonia nitrogen concentrations (125 and 250 mg l^{-1}) were supplied during two sequential long-term experiments of 160 days each (320 total). Cycles of 24 h under intermittent aeration for periods of 1 h were applied, and ethanol was added as a carbon source at the beginning of each anoxic period. Nitrite was the main oxidized nitrogen compound which accumulated only during the aerated phases of the batch cycle. A consistent decrease of nitrite concentration started always immediately after the interruption of oxygen supply and addition of the electron donor. Removal to below detection limits of all nitrogen soluble forms was always observed at the end of the 24 h cycles for both initial concentrations. Polyurethane packed-bed matrices and ethanol amendments conferred high process stability. Microbial investigation by cloning suggested that nitrification was carried out by *Nitrosomonas*-like species whereas denitrification was mediated by unclassified species commonly observed in denitrifying environments. The packed-bed batch bioreactor favored the simultaneous colonization of distinct microbial groups within the immobilized microbial biomass. The biofilm was capable of actively oxidizing ammonium and denitrification at high ratios in intermittent intervals within 24 h cycles.

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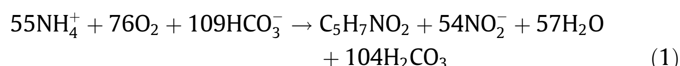
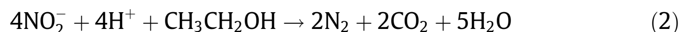
1. Introduction

Removal of nitrogenated compounds is an important aspect of post-treatment of wastewater effluents. The most common biological process requires first the oxidation of this compound to nitrogen oxides (nitrite and nitrates) and their removal with the production of N_2. Therefore, this process consists of several distinct metabolic steps. Nitrification defines the aerobic mediated step which describes the transformation of ammonia into nitrite through the activity of ammonium-oxidizing bacteria. Depending on the availability of oxygen and specific microbial populations, along with some other secondary factors, this step may be followed by further biological oxidation with the production of nitrate. This is another distinct aerobic step mediated by nitrite-oxidizing bacteria (Sinha and Annachhatre, 2007; Carrera et al., 2004). These are aerobic metabolic reactions which do not require organic substances as electron donors. Contrastingly, denitrification is a process in which nitrogen oxides (nitrites or nitrates) are reduced to nitrogen gas by replacing oxygen as the terminal electron acceptors during the anaerobic oxidation of several types of electron donors, for instance, organic matter (Shapleigh, 2006). The stoichiometric formula of chemolithotrophic ammonia oxidation into nitrite is described in Eq. (1) (US-EPA, 1975) and the hypothetical denitrification formula using ethanol as a carbon source and nitrite as electron acceptor is suggested in Eq. (2)



$$55NH_4^+ + 76O_2 + 109HCO_3^- \rightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O + 104H_2CO_3 \quad (1)$$



$$4NO_2^- + 4H^+ + CH_3CH_2OH \rightarrow 2N_2 + 2CO_2 + 5H_2O \quad (2)$$

The combination of these two processes in the same system (bioreactor) is complicated by the distinct physiological and ecological characteristics of the microbial populations involved. Several successful attempts have been previously reported (Canto et al., 2008). On the other hand, despite that full-scale applications have already been demonstrated, successful operational strategies for linking nitrification to denitrification by suppressing the participation of nitrate mediated reactions within the same packed-bed batch reactor is still not fully explored. Some authors have experimented with high inputs of ammonium concentrations, aiming to inhibit the activity of nitrite-oxidizing bacteria (Kim et al., 2006; Yun and Kim, 2003), and others achieved some success by controlling the concentration of dissolved oxygen (Pollice et al., 2002; Gee and Kim, 2004; Ruiz et al., 2005; Li et al., 2006; Antileo et al., 2006). It is clear so far, that the period of aeration is one of the main factors suppressing the production of nitrate and promoting nitrification–denitrification

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doi:10.1016/j.biortech.2008.08.003

shortcut during post-treatment (Jianlong and Ning, 2003; Katsogiannis et al., 2003; Peng et al., 2003). In addition to the periods of aeration, however, several other factors have been tested. There are experiments which tested controlled shifts in pH (Bae et al., 2002; Peng et al., 2004) temperature (Mulder et al., 2001; Jianlong and Ning, 2003), inorganic carbon concentrations (Jun et al., 2000; Hwang et al., 2000; Peng et al., 2003) and sludge retention time (Pollice et al., 2002). An integrated and controlled combination of several variables such as pH, temperature and free-ammonia concentrations have also been attempted (Kim et al., 2006). The interest in such post-treatment strategy is based on the fact that it represents a significant time-efficient and cost-effective improvement to the post-treatment process (Gupta and Gupta, 2001). An economic advantage is that a lower amount of oxygen and carbon sources are required to sustain the treatment. It has been shown that such strategy may lead to a 25% and 40% reduction in the demand for oxygen and carbon sources, respectively, in addition to a significant decrease in the production of microbial biomass within the bioreactor (Surmacz-Górska et al., 1997). Nitrification–denitrification shortcut is also time-efficient because of high specific denitrification rates which are achieved in the presence of nitrite allowing satisfactory treatment within shorter periods (Ruiz et al., 2006). According to the former authors, the rates of nitrite based denitrification may be up to 63% higher than the similar process based in the reduction of nitrate.

Despite the intense research in the area and the fact that some full-scale reactors may at some degree successfully sustain such a process; there are several factors that have not yet been explored. The effect of biomass immobilization as means to improve long-term nitrification–denitrification shortcut has not yet been comprehensively tested. In addition, very little is known about the microbial communities which may evolve in such conditions and their potential role to sustain high rate reactions. There is very little information about the identity and proportion of microbial species responsible to sustain such a process of post-treatment. Therefore, the approach of using polyurethane foam as a support material for microbial adhesion to promote nitrification–denitrification shortcut is a novelty and it may show promising applications from long-term post-treatment strategies. An advantage of using polyurethane foam as a physical substrate is that attached biofilm is known to select and sustain microbial populations of slow growing organisms (Hirl and Irvine, 1996). Ammonium-oxidizing organisms are often out competed by other organisms because of their slow growing rates (Liu and Tay, 2001) and this may affect high nitrification rates performances in post-treatment systems. It has been hypothesized that in mixed cultures microbial colonization of polyurethane foam matrices may favor spatially heterogeneous aerobic and anaerobic niches with the emergent effect of significantly promoting nitrification–denitrification shortcut process. Therefore, to the best of our knowledge few studies have been carried out to test if attached biofilms and polyurethane biomass immobilization strategy would improve long-term stability of ammonium-oxidizing bacteria as well as promoting denitrification in the same reactor with ethanol as a viable carbon source, and one of the goals of this study is to address this shortfall. Therefore, the first aim of our study was to explore the strategy of combining ammonium, ethanol, and biomass immobilization in polyurethane matrices to test distinct short periods of aeration/non-aeration in order to promote nitrogen removal by sustainable nitrite-shortcut process within a single packed-bed batch reactor. The second goal of our study was to carry out a microbial characterization of the biofilm aiming to specifically identify the potential organisms involved in the distinct steps of nitrification–denitrification short-cut process with some indication of their proportional distribution within the nitrifying–denitrifying active biofilm.

2. Methods

2.1. Experimental apparatus

The experimental apparatus consisted of an adapted sequential batch reactor for supporting microbial immobilized biomass in polyurethane cubic matrices as described by Cubas et al. (2004). The reactor was operated at 24 h cycles divided by intermittent aeration/non-aeration periods of 1 h each. The 6 l cylinder reactor was constructed with Plexiglas in which a stainless steel basket was used as a support apparatus for the polyurethane matrices containing the immobilized microbial biomass. The reactor was installed inside a controlled temperature chamber at 30 °C. Liquid homogenization and optimization of mass transference between the liquid phase and the microbial biomass were provided by mechanical impellers installed in the central space of the reactor's main body. Operating parameters such as mechanical agitation, periods of aeration, feeding, the addition of carbon sources and the effluent discharge were automated and controlled by microcomputer software developed on DELPHI 3 and operated on MS-Windows.

2.2. Substrate composition and operational conditions

Sludge from a full-scale domestic wastewater treatment plant (Flores WWTP, at Rio Claro City, Brazil) was used as a microbial biomass inoculum for seeding the polyurethane foam cubic matrices according to the procedures proposed by Zaiat et al. (1994). Synthetic wastewater was prepared with 13.4 mg/l $CaCl_2 \cdot 2H_2O$; 200 mg/l $MgSO_4 \cdot 7H_2O$; 139 mg/l K_2HPO_4; 204 mg/l KH_2PO_4; quelant iron and trace elements for supporting the growth of nitrifying organisms were added according to Shmidt and Belzer (1984). Sodium bicarbonate was used as a buffer and also as potential inorganic carbon source for the nitrification process. Ethanol was used as an electron donor for the denitrification phase at final carbon to nitrogen ratios of 3:1. Synthetic wastewater was prepared daily and kept in a refrigerator to avoid degradation. Ammonium was added to the synthetic wastewater in two distinct trials of 160 days each, first at 125 mg/l (0–160 days) and later at 250 mg/l (160–320 days). The reactor was filled with such synthetic wastewater within a period of 10 min and a sample of the reactor's liquor was obtained before starting the 24 h cycles for monitoring purposes. The first period of 1 h aeration started soon after 10 min of influent recharge. This phase was followed by 1 h without aeration. Sequences of aerated and non-aerated periods were repeated up to the end of 24 h cycle. Each cycle was completed with the discharge of the liquid after the mechanical agitation had been interrupted. In order to keep the ethanol concentration close to a COD/N ratio of 3:1 during the denitrification phase (non-aeration period), a fraction of the ethanol solution was added at the beginning of each anoxic period. Ethanol was not added during the periods of aeration. Dissolved oxygen (DO) concentrations were kept within the range of 2.0–2.7 mg/l by opening a valve installed in the reactor's air pipeline. These operations were automatically controlled by a microcomputer equipped with oxygen meter equipment (1401, Orion) constantly monitoring shifts in DO.

2.3. Water quality analysis

Nitrogen removal efficiency was measured during 160 days. Inorganic nitrogen profiles, NO_2^-/NO_t (the sum of the nitrite and nitrate) and DO/NH_3 ratios, free-nitrous acid ($N–HNO_2$/l) and free-ammonia ($N–NH_3$/l) were measured during the 24 h cycles. All the analyses were performed according to the standard methods (APHA, 1998). Ammonium ions were determined by distillation

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