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Trade-off between mesophilic and thermophilic denitrification: Rates vs. sludge production, settleability and stability

Emilie N.P. Courtens [a], Siegfried E. Vlaeminck [a], Ramiro Vilchez-Vargas [a], Arne Verliefde [b], Ruy Jauregui [c], Dietmar H. Pieper [c], Nico Boon [a,*]

[a] *Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, 9000 Gent, Belgium*

[b] *The Particle and Interfacial Technology Group (PaInT), Ghent University, Coupure Links 653, 9000 Gent, Belgium*

[c] *Microbial Interactions and Processes Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany*

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ABSTRACT

The development of thermophilic nitrogen removal strategies will facilitate sustainable biological treatment of warm nitrogenous wastewaters. Thermophilic denitrification was extensively compared to mesophilic denitrification for the first time in this study. Two sequential batch reactors (SBR) at 34 °C and 55 °C were inoculated with mesophilic activated sludge (26 °C), fed with synthetic influent in a first phase. Subsequently, the carbon source was switched from acetate to molasses, whereas in a third phase, the nitrate source was fertilizer industry wastewater. The denitrifying sludge maintained its activity at 55 °C, resulting in an immediate process start-up, obtaining nitrogen removal rates higher than 500 mg N g^{-1} VSS d^{-1} in less than one week. Although the mesophilic SBR showed twice as high specific nitrogen removal rates, the maximum thermophilic denitrifying activity in this study was nearly 10 times higher than the activities reported thus far. The thermophilic SBR moreover had a 73% lower sludge volume index, a 45% lower sludge production and a higher resilience towards a change in carbon source compared with the mesophilic SBR. The higher resilience was potentially related to a higher microbial diversity and evenness of the thermophilic community at the end of the synthetic feeding period. The thermophilic microbial community showed a higher similarity over the different feeding periods implying a more stable community. Overall, this study showed the capability of mesophilic denitrifiers to maintain their activity after a large temperature increase. Existing mesophilic process systems with cooling for the treatment of warm wastewaters could thus efficiently be converted to thermophilic systems with low sludge production and good settling properties.

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* *Corresponding author*. Tel.: +32 9 2645976; fax: +32 9 2646248.
E-mail address: Nico.Boon@UGent.be (N. Boon).

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1. Introduction

Thermophilic aerobic wastewater treatment is an attractive process for the treatment of warm wastewaters (>50 °C). Conventional mesophilic biological treatment of e.g. hot industrial wastewaters, wastewater with high seasonal temperatures or supernatant from thermophilic anaerobic digesters requires cooling by the use of heat exchangers and cooling towers. Although some energy can be recovered, these cooling requirements are associated with higher capital and operating costs, and an environmental impact due to greater water consumption and a potential increase in energy consumption and carbon dioxide emissions (Saidura et al., 2010). Moreover, besides the elimination of the cooling requirements, thermophilic aerobic processes are also known to be more stable, to achieve higher specific rates (smaller bioreactors can be used), to produce less biological sludge and to achieve better hygienization (Lapara and Alleman, 1999).

Biological nitrogen removal typically consists of an oxidative and a consecutive reductive step, i.e. conventionally nitrification/denitrification. Thermophilic aerobic (oxidative) processes have been extensively studied, focusing on the oxidation of organic material, while the fate of nitrogen was mainly unclear. Until now, the main nitrogen removal mechanisms in these thermophilic aerobic systems are assumed to be ammonia (NH_3) stripping and nitrogen assimilation into biomass (Abeynayaka and Visvanathan, 2011; Kurian et al., 2005). The recent discovery of thermophilic ammonia and nitrite oxidizers such as *Candidatus Nitrosocaldus yellowstonii* (de la Torre et al., 2008) and *Nitrospira calida* (Lebedeva et al., 2011), have stimulated the development of thermophilic nitrification for wastewater treatment, resulting in a few recent lab-scale studies (Courtens et al., 2014; Lopez-Vazquez et al., 2013; Shore et al., 2012). As NH_3 stripping shifts the nitrogen problem to the gas phase, and the COD/N ratio in high-strength nitrogenous wastewaters is too low to obtain sufficient nitrogen removal based on assimilation, the development of thermophilic nitrification is necessary.

Concerning the reductive denitrification step, multiple thermophilic denitrifying bacteria have been isolated from different environments e.g. *Bacillus thermodenitrificans*, *Geobacillus* sp. and *Anoxybacillus pushchinensis*, isolated from soil, mud and manure amended soil, respectively (Mishima et al., 2009; Mora et al., 1998; Yamamoto et al., 2006). Besides these spore-forming bacteria, also archaea (Cabello et al., 2004) and non-spore forming bacteria such as *Thermus thermophilus* are known to denitrify at thermophilic temperatures (Bricio et al., 2011). Despite the widespread occurrence of thermophilic denitrifying micro-organisms in natural ecosystems, to our knowledge, only one study was focused on the development of a thermophilic denitrifying reactor for wastewater treatment. Laurino and Sineriz (1991) investigated denitrification in a lab-scale upflow sludge blanket (USB) reactor at 55 °C fed with ethanol as carbon and energy source. The USB reactor was inoculated with thermal mud originating from a hot spring and started-up in batch mode for 15 days. After switching to continuous mode, a maximum nitrogen removal rate of 1317 mg N L^{-1} d^{-1} with a nitrate removal efficiency of 78.4% was observed, resulting in a maximal specific removal rate of 51 mg N g^{-1} VS d^{-1}.

The current study investigated whether a non-thermophilic inoculum, i.e. mesophilic denitrifying sludge (26 °C), can be used for the start-up of a thermophilic (55 °C) sequential batch reactor (SBR). A parallel mesophilic control SBR (34 °C) was inoculated with the same sludge enabling an extensive comparison between mesophilic and thermophilic denitrification. Both functional aspects such as maximal specific nitrate removal rate, sludge production, sludge settleability and nitrous oxide production and phylogenetic diversity of the microbial community were compared for different substrate complexities ranging from synthetic influent to real waste streams.

2. Materials and methods

2.1. *Set-up and operation of the denitrifying reactors*

Two parallel sequential batch reactors (SBR) had an effective liquid volume of 2 L and an inner diameter of 12 cm. Operational temperatures were chosen from an application point of view, representing the typical temperatures of mesophilic (34 °C) and thermophilic (55 °C) digestates, for the mesophilic (control) and thermophilic SBR, respectively. The reactor vessels were jacketed, and the temperature was controlled with a circulating thermostatic water bath. The 2-h cycle consisted of a 10 min feeding period and a 80 min reaction period, both including stirring (60 rpm), followed by a 15 min settling period and a 15 min decanting period. The reactors were inoculated with nitrifying/denitrifying (N/DN) sludge, originated from a landfill leachate wastewater treatment plant with an average temperature of 26.3 ± 3.6 °C, at an initial biomass concentration of 4.0 ± 0.2 g volatile suspended solids (VSS) L^{-1}. During start-up of the reactors, considerable amounts of sludge washed out. After stabilization, sludge was wasted in order to keep the sludge concentration around 2 g VSS L^{-1}. For the mesophilic SBR 1.3 ± 0.7 g VSS was wasted on a daily basis, while practically no sludge was wasted in the thermophilic SBR.

An identical feeding strategy was applied for both reactors, whereby different wastewater matrices and COD types were used ranging from sodium nitrate containing tap water to industrial wastewater (WW) from the fertilizer industry, sodium acetate (NaAc) and diluted molasses (Table S1). Other than NO_3^- and COD, the influent also contained $(NH_4)_2SO_4$ (0.05 g N g^{-1} NO_3^-–N) and KH_2PO_4 (10 mg P L^{-1}) and was acidified throughout the whole experiment with HCl resulting in an influent pH of 2.5 ± 0.4 in order to indirectly control the pH in the reactor. Three main feeding periods were distinguished according to the COD and NO_3^- source: the synthetic period (COD_{NaAc}/N_{NO3}), the real wastestream/synthetic period ($COD_{Molasses}/N_{NO3}$) and the real wastestream (WS) period ($COD_{Molasses}/N_{Fertilizer\ WW}$), respectively, each including different phases, depending on the NO_3^- loading rate (Table 1). The transitions between the three feeding periods occurred after at least 5 times the sludge retention time (SRT of 2.3 ± 0.5 and 4.9 ± 0.8 days for the mesophilic and thermophilic SBR) to ensure a 'stable' microbial community and at a same loading