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Cite this: *Environ. Sci.: Water Res. Technol.*, 2017, 3, 10

## A proposed nomenclature for biological processes that remove nitrogen†

Max Weißbach,<sup>a</sup> Craig S. Criddle,<sup>b</sup> Jörg E. Drewes<sup>a</sup> and Konrad Koch<sup>\*a</sup>

For development and optimization of novel biological nutrient removal processes, factors affecting energy and greenhouse gas emissions (e.g. nitrous oxide) are increasingly important. Energy efficient pathways are exploited, such as short-cut processes for treatment of concentrated side streams. These pathways are usually well described, but a consistent nomenclature is still lacking: some functionally equivalent processes are referred to using distinct names, and some functionally distinct processes are referred to with the same name. To address this issue, we propose a standardized terminology, in which a root term designates the end product for oxidation pathways or the reactant for reduction pathways; a qualifier is used to designate the predominant oxidation–reduction regime (ORR) and an optional indicator is used to designate partial turnover rates, e.g. partial ammonia oxidation to nitrite.

Received 19th August 2016,  
Accepted 23rd November 2016

DOI: 10.1039/c6ew00216a

rsc.li/es-water

### Water impact

Recent advances in understanding biological nitrogen removal (BNR) processes have resulted in a large variety of different technologies applying short-cuts in the microbial metabolism. These developments and investigations have led to an inconsistency in the applied terminology. To overcome this issue, we proposed a transparent logic and nomenclature that enables distinct determination of every presently known catalyzed reaction in BNR.

## 1 Introduction

Nutrient removal is required to protect water quality and the environment and is thus a primary objective of wastewater treatment. The conventional activated sludge (CAS) process, an approach invented in 1914, has been optimized for removal of carbon (C), nitrogen (N), and phosphorus (P).<sup>1,2</sup> While these processes are effective, their energy requirements are high, a fact that has spurred interest in strategies that save energy<sup>3,4</sup> and enable resource recovery, avoid greenhouse gas emissions, and decrease operational costs. Wastewater treatment is at a turning point where nutrient removal will be achieved through more sustainable and integrated systems aiming at resource and energy recovery.<sup>1</sup>

Removal of organic carbon and production of methane by anaerobic digestion of organic waste streams are well established for energy recovery at municipal wastewater treatment plants. Typically, these waste streams contain high

levels of nitrogen, and a consequence is the release of high concentrations of ammonia in the digester's effluents and in the reject water from sludge dewatering, respectively. Returning these concentrated streams to the headworks imposes an additional nitrogen load on the mainstream and can potentially drive the CAS to its limits, in which case there is insufficient reducing power for denitrification. To overcome these issues, novel biological nitrogen removal (BNR) processes have been developed in the last decade. These include processes like *Stable High Ammonia Removal Over Nitrite* (SHARON®),<sup>5</sup> *Complete Autotrophic Nitrogen removal Over Nitrite* (CANON)<sup>6</sup> and *ANaerobic AMMonium OXidation* (ANAMMOX®).<sup>7</sup> All of these technologies are characterized by the use of a short-cut *via* nitrite (NO<sub>2</sub><sup>-</sup>) pathway of microbial metabolism. Such a pathway can reduce the oxygen and carbon demands for reducing power and increasing potential for energy recovery compared to CAS. Although these technical applications have primarily been applied to side stream BNR, ongoing research is presently directed to investigating mainstream implementation as well.<sup>8,9</sup> Despite their energy benefits, these processes could potentially release significant GHG emissions such as nitrous oxide (N<sub>2</sub>O), when compared to conventional BNR processes<sup>10–13</sup> (N<sub>2</sub>O has a global warming potential 310 times higher than that of CO<sub>2</sub>).<sup>14</sup> Research is on-going to

<sup>a</sup> Chair of Urban Water Systems Engineering, Technical University of Munich, Am Coulombwall 3, 85748 Garching, Germany. E-mail: k.koch@tum.de

<sup>b</sup> NSF Engineering Research Center ReNUWIt, Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305-4020, USA

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c6ew00216a



prevent greenhouse gas emissions through improved process stability and control.<sup>15</sup>

Novel alternative strategies are also being developed and came to the fore recently. One approach consists of bio-electrochemical hybrid-systems to substitute the necessary amount of carbon with an electrical current to gain sufficient reducing power.<sup>16,17</sup> Another strategy is nitrogen removal coupled with energy recovery. In this respect, the *Coupled Aerobic-Anoxic Nitrous Decomposition Operation*<sup>18,19</sup> (CANDO) has been proposed for intentional N<sub>2</sub>O production and its subsequent use as an energy-yielding co-oxidant for biogas combustion. The CANDO process might also facilitate P removal and biopolymer production.<sup>19</sup>

The opportunities provided by these state-of-the-art treatment technologies are presently of high scientific relevance underscored by a growing number of peer-reviewed journal articles in this domain. As an example, publications focusing on nitrification (oxidation of ammonia (NH<sub>4</sub><sup>+</sup>) to nitrite, NH<sub>4</sub><sup>+</sup> → NO<sub>2</sub><sup>-</sup>) and denitrification (reduction of nitrite to nitrogen gas (N<sub>2</sub>), NO<sub>2</sub><sup>-</sup> → N<sub>2</sub>) peaked in 2015 with a share of ~25% within the total publication number from 2006–2015. The results were obtained from an online research in the Web of Science™ Core Collection (Fig. S1 in ESI†). These studies revealed several novel reaction mechanisms and pathways. Some of them were defined coincidentally by different authors resulting in partial ambiguity in the chosen terminology. Two expressions related to N<sub>2</sub>O emissions serve as an example of ambiguous terminology. The first is “nitrifier denitrification”, a phrase originally coined to describe unintended N<sub>2</sub>O generation when NO<sub>2</sub><sup>-</sup> is reduced *via* nitric oxide (NO).<sup>20</sup> Problems become clear when this phrase is parsed into “nitrifier” and “denitrification”. The term “nitrifier” is problematic, because it implies active nitrite-oxidizing bacteria (NOB) as a potential key-player in the production of N<sub>2</sub>O. So far, the presence of both NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> oxidation related genes in a single organism has been reported for only two *Nitrospira* species by van Kessel *et al.*,<sup>21</sup> indicating that there are organisms engaging in both pathways. However, production of N<sub>2</sub>O during the oxidation of NO<sub>2</sub><sup>-</sup> to NO<sub>3</sub><sup>-</sup>, accounting for most NOB, is not known. The use of the term “denitrification” is also problematic because it implies complete denitrification to N<sub>2</sub>. The second example is “partial denitrification”.<sup>19</sup> This phrase refers to production of N<sub>2</sub>O as the end product of denitrification or to conversion of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> and possibly also a partial conversion of NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>. Phrases such as “nitrifier denitrification” and “partial denitrification” are thus imprecise and do not adequately differentiate pathways and products. Both expressions demonstrate that denotation has become imprecise and underscore the need for a consistent terminology that differentiates pathways to avoid misunderstanding in the scientific community.

We propose here a conservative consolidation of existing terminology by including new definitions based upon a comprehensive literature review and transparent logic. The goal is to provide a combination of consistent determinants prop-

erly representing distinct reaction conditions and underlying processes. We do not claim that the approach in its present form is perfect, but we hope that our contribution could engage a discussion in the scientific community towards a standardization of the terminology facilitating communication in the future.

## 2 Scientific approach

We begin by compiling and comparing current terminology for all known BNR metabolic pathways in terms of singularity and distinctiveness. We then propose terminology that establishes a uniform nomenclature for biotechnology and process engineering. Our aim is to integrate novel elements into historically accepted terminology (such as “nitrification” and “denitrification”) so as to establish an unambiguous nomenclature. Names for oxidation sequences are formulated by identifying a root term based upon the reaction product. Oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> is therefore designated “NITRITation”; oxidation of NO<sub>2</sub><sup>-</sup> to NO<sub>3</sub><sup>-</sup> is designated “NITRATation”. Analogous historical logic is applied for denitrification, with preservation of the prefix “de” for reduction sequences. In this case, however, the focus is on the initial reactant. Reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> is designated “DENITRATation”; reduction of NO<sub>2</sub><sup>-</sup> to N<sub>2</sub> is designated “DENITRITation”. With respect to NO<sub>2</sub><sup>-</sup>, the metabolic intermediates, *i.e.* nitric oxide and nitrous oxide, require an additional indicator – the final reaction product – in order to differentiate them from nitrogen removal *via* N<sub>2</sub>.

For determination of reaction pathways, mechanisms and dominant organisms, the oxidation–reduction regime (ORR) is used as a qualifier. We initially considered ranges of dissolved oxygen (DO) concentration for each ORR, but, to the best of our knowledge, specific threshold values have yet to be defined, except for the fully anoxic case where DO is zero. Further research is needed to elucidate the DO ranges for oxidoreductase activities and DO profiles within biofilms so as to differentiate such processes from the mean DO values commonly applied to the bulk mixed liquor. For now, we define just two ranges within the DO constrained ORRs: (1) low (sub-oxic) and (2) high DO (aerobic). Finally, “partial” is applied as an optional second determinant to designate incomplete conversion of reactants. An example would be “partial nitrification” for the case where <100% of the initial NH<sub>4</sub><sup>+</sup> load is converted to NO<sub>2</sub><sup>-</sup>. Based on these results, the logical combination of our terminology consists of two highly recommended and one optional quantitative indicator:

Oxidation: quantitative indicator\* + ORR + reaction pathway (product specific)

Reduction: quantitative indicator\* + ORR + reaction pathway (educt specific)

\*only if necessary

This approach represents a flexible nomenclature for complete integration of the presently relevant processes and to potentially derive the terms for novel treatment steps in the future.



### 3 Proposed terminology

#### 3.1 Pathways of conventional BNR processes

Mainstream nitrification/denitrification (N/DN) processes are still the most widely applied techniques for nitrogen removal in conventional biological wastewater treatment. The underlying pathways are complete oxidation of  $\text{NH}_4^+$  during nitrification and reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  *via* denitrification (Fig. 1) resulting in  $\text{NO}_3^-$  and  $\text{N}_2$  as desired products, respectively. The underlying pathways are well understood and have been the basis for design and operation of conventional BNR processes for decades:<sup>22</sup>



Within N/DN, some metabolites are shared intermediates for oxidation and reduction pathways. The major intermediates are nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) (eqn (1) and (2)). During nitrification,  $\text{N}_2\text{O}$  is both a stable by-product of chemical oxidation *via* hydroxylamine ( $\text{NH}_2\text{OH}$ )<sup>20</sup> and a product of microbial denitrification pathways. Fig. 1 provides an overview of the discussed microbial pathways together with the proposed nomenclature. Nitric oxide (NO) can also be generated chemically by disproportionation of nitrous acid ( $\text{HNO}_2$ ) in wastewater treatment systems, however, appreciable quantities are only released under acidic conditions<sup>23</sup> and such conditions are uncommon in BNR systems.

#### 3.2 Role of the ORR

The key process parameter commonly manipulated to control reaction pathways in engineered BNR processes is the DO, a parameter that sets the predominant ORR.<sup>24–26</sup> The ORR in BNR processes can typically be characterized as aerobic (oxidation pathways), anoxic (reduction pathways), and sub-oxic (reduction and oxidation pathways occur simultaneously<sup>26</sup>). These categories capture the predominant biochemical dynamics within a particular process. The ORR also constrains and defines the structure and function of the microbial community and dominance of key organisms. Ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are predominant under aerobic conditions; denitrifying bacteria (DB) are predominant under anoxic conditions. Under sub-oxic conditions, a process phase quite typical for contemporary treatment technologies, such as the SHARON or CANON processes, AOB are the dominant organisms<sup>27,28</sup> and can catalyze N reduction reactions.<sup>20,29</sup>

#### 3.3 Pathways of novel BNR processes

In contrast to the terminology used for conventional nitrification/denitrification, the terminology that is used to describe metabolic pathways in contemporary BNR processes is diverse and inconsistent. The typical first step is the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$ . Nitrite is the precursor for subsequent reduction in several well-established processes (*e.g.*, denitrification, SHARON®, CANON, DEMON® and ANITAMOX®). Nitrogen elimination as  $\text{N}_2$  is carried out by anaerobic ammonia oxidizing (so-called Anammox) bacteria<sup>8</sup> (*planctomycetes*) or by DB.<sup>30</sup> Frequently, these treatments are carried out simultaneously in single-stage systems.<sup>31,32</sup> From a biochemical



**Fig. 1** Schematic representation of the proposed terminology. The continuously lined arrows indicate microbially catalyzed reaction pathways, the dashed lined arrows chemical reactions, reaction pathways are indicated by dash-dotted lines. In yellow: The aerobic and sub-oxic reaction pathways summarized in the “aerobic” regime. In green: Reductive pathways including the intermediate steps in nitrite reduction to  $\text{N}_2\text{O}$ . Denitrification is included for both aerobic and anoxic regimes with respect to the mitigation of  $\text{N}_2\text{O}$  emissions. In blue: The deammonification pathway.



point-of-view, however, the seemingly “simultaneous” reactions are a chain of oxidation and reduction reactions at different micro-scale ORRs.

**3.3.1 Aerobic ORR.** In contemporary practice of aerobic and sub-oxic systems, the adjective “full” refers to processes that are designed to achieve essentially 100% conversion of reactants (e.g.  $\text{NH}_4^+$ ) to products (e.g.  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ). In contrast, the adjective “partial”<sup>33–35</sup> is used to refer to processes designed to achieve less than 100% conversion<sup>30,36,37</sup> e.g. “partial nitrification” or is used to differentiate short-cut technologies from conventional treatment e.g. “partial nitrification” ( $\text{NH}_4^+ \rightarrow \text{NO}_2^-$  instead of  $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ) (Table 1, eqn (3.a) and (3.b)). Frequently, the intended use is not specified and has to be assessed by the context of the particular publication. In order to enable a distinctive definition, we propose use of the word “partial” to designate all processes that bring about partial oxidation or reduction of educts, respectively.

Oxidation of nitrite to nitrate (Table 1, eqn (4)), a typical side reaction in nitrification processes,<sup>38,39</sup> is carried out by

nitrite oxidizing bacteria (NOB) *via* “nitrification”.<sup>40</sup> This terminology is in accordance with the proposed scheme and is adopted as is. In contrast, generation of  $\text{N}_2\text{O}$  *via*  $\text{NH}_2\text{OH}$  under aerobic and sub-oxic conditions, an important reaction for greenhouse gas emissions at wastewater treatment plants<sup>11,14,41</sup> and energy recovery options,<sup>18,19</sup> does not follow an agreed-upon terminology (Table 1, eqn (5)). Processes that generate  $\text{N}_2\text{O}$  clearly need better definition. Investigations by Daelman *et al.*<sup>11</sup> revealed that the share of  $\text{N}_2\text{O}$  within the total carbon footprint can be as high as 78%. However,  $\text{N}_2\text{O}$  is also a potential energy recovery option as co-oxidant in biogas combustion processes.<sup>18</sup> To systematize the nomenclature, we propose the term “nitrousation” for its aerobic microbial generation of  $\text{N}_2\text{O}$  (Fig. 1).

**3.3.2 Anoxic ORR.** For the reductive pathways, historically the prefix “de-” has been commonly used (e.g., “Denitrification”). To be conservative, we propose to generally maintain the logic, *i.e.* initial nitrogen compound plus prefix “de-”. This will result in two terms, “denitration” (Table 1,

**Table 1** Literature compilation of BNR steps, according to ORR (A: aerobic, S: sub-oxic, An: anoxic), change in the oxidation state of nitrogen (O: oxidation, R: reduction) and currently used terminology with qualitative indicators. The same background color represents ambiguity; italicized letters represent imprecise terms; and adopted terms are listed in bold green font)

Reaction #	BNR step	ORR applied			O/R	Applied terminology	Ref.
		A	S	An			
3.a	$\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^-$				O	Full partial nitrification	Pijuan <i>et al.</i> <sup>12</sup>
						<b>Nitrification</b>	Zeng <i>et al.</i> <sup>28</sup> Regmi <i>et al.</i> <sup>30</sup>
						<i>Partial nitrification</i>	Chen <i>et al.</i> <sup>36</sup> Liu <i>et al.</i> <sup>57</sup> Rodríguez-Caballero and Pijuan <sup>58</sup>
3.b	$\text{NH}_4^+ \rightarrow 0.5 \text{NH}_4^+ + 0.5 \text{NO}_2^-$				O	<b>Nitrification</b>	Kampschreur <i>et al.</i> <sup>59</sup> Joss <i>et al.</i> <sup>32</sup> Lackner <i>et al.</i> <sup>25</sup>
						<b>Partial nitrification</b>	Ganigué <i>et al.</i> <sup>60</sup> Lackner <i>et al.</i> <sup>7</sup> Hu <i>et al.</i> <sup>61</sup>
						<i>Partial nitrification</i>	Okabe <i>et al.</i> <sup>34</sup> Abzazou <i>et al.</i> <sup>62</sup> Daverey <i>et al.</i> <sup>35</sup>
4	$\text{NO}_2^- \rightarrow \text{NO}_3^-$				O	<b>Nitrification</b>	Mauret <i>et al.</i> <sup>63</sup> Kim <i>et al.</i> <sup>64</sup> Courtens <i>et al.</i> <sup>65</sup>
5	$\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow 0.5 \text{N}_2\text{O}$				O	—	Tallec <i>et al.</i> <sup>66</sup> Ahn <i>et al.</i> <sup>67</sup> Schreiber <i>et al.</i> <sup>23</sup>
6	$\text{NO}_3^- \rightarrow \text{NO}_2^-$				R	<b>Denitrification</b>	Bourrel <i>et al.</i> <sup>68</sup> Malovany <i>et al.</i> <sup>69</sup> Mano and Santana <sup>70</sup>
7	$\text{NO}_2^- \rightarrow \text{NO} \rightarrow 0.5 \text{N}_2\text{O} \rightarrow 0.5 \text{N}_2$				R	<b>Denitrification</b>	Zeng <i>et al.</i> <sup>28</sup> Regmi <i>et al.</i> <sup>30</sup> Kulikowska and Bernat <sup>71</sup>
8	$\text{NO}_2^- \rightarrow \text{NO} \rightarrow 0.5 \text{N}_2\text{O}$				R	<i>Partial denitrification</i>	Scherson <i>et al.</i> <sup>18</sup> Scherson <i>et al.</i> <sup>19</sup>
9	$\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2$				O/R	<b>Deammonification</b>	Daigger <sup>44</sup> Jenicek <i>et al.</i> <sup>42</sup> Bilyk <i>et al.</i> <sup>43</sup>
						Anaerobic ammonium oxidation/Anammox®	Liu and Horn <sup>57</sup> Hu <i>et al.</i> <sup>61</sup> Marina <i>et al.</i> <sup>72</sup>
10	$\text{NO}_2^- \rightarrow \text{NO} \rightarrow 0.5 \text{N}_2\text{O}$				R	<i>Nitrifier denitrification</i>	Wrage <i>et al.</i> <sup>20</sup> Law <i>et al.</i> <sup>73</sup> Wunderlin <i>et al.</i> <sup>74</sup>
11	$\text{NO}_3^- \rightarrow \text{NO}_2^-$				R	<i>Partial denitrification</i>	Gong <i>et al.</i> <sup>56</sup>



eqn (6)) and “denitrification” (Table 1, eqn (7)), which have already been applied in this context and are adopted for the novel terminology framework.

In studies by Scherson *et al.*,<sup>18,19</sup> an anoxic carbon-limited biochemical reaction pathway for N<sub>2</sub>O production (Table 1, eqn (8)) has been referred to as “partial denitrification”. Considering the historical interpretation of “denitrification”, the expression is misleading as it relates to the production of N<sub>2</sub>. However, applying the scheme for reductive pathways to the CANDO process causes the problem that “denitrification” already refers to nitrogen removal *via* N<sub>2</sub>. As a qualitative indicator for a clear product specification, we propose “nitrous” in addition – resulting in the expression “nitrous denitrification” (Fig. 1).

Independent from N/DN, the oxidation reduction reaction carried out under anoxic conditions by *planctomycetes* is known as “deammonification”<sup>42–44</sup> as well as “anaerobic ammonium oxidation”<sup>13,33,45–48</sup> or “ANAMMOX®” (Table 1, eqn (9)). The process is based on an alternative pathway<sup>49</sup> in the biological nitrogen metabolism and decoupled from the reaction chains in N/DN. In contrast to the potential bi-directional reaction pathways in N/DN, deammonification is uni-directional (Fig. 1). In contrast to nitrification or denitrification, deammonification is an intracellular reaction pathway without any transition links within the metabolic web. Due to its isolation, the one-directional redox-reaction and lack of available intermediates, the process is integrated independently (Fig. 1). However, since ANAMMOX® is a licensed process by Paques (EL Balk, NL), we continue using the term “deammonification” in order to distinguish between the technical process as a registered trademark and the underlying metabolic pathway.

**3.3.3 Sub-oxic ORR.** During sub-oxic conditions, AOB catalyze reduction and oxidation reactions “simultaneously”.<sup>50</sup> Depending upon reactor conditions, different products can be produced under reductive conditions (*i.e.*, NO, N<sub>2</sub>O, N<sub>2</sub>). The production of N<sub>2</sub>O in this respect represents the same reaction pathway as in the second stage of the CANDO process (Table 1, eqn (10)), but the mechanism is related to the sensitivity of nitrous oxide reductase to oxygen<sup>51–53</sup> instead of carbon limitation. A commonly used phrase in this context is “nitrifier denitrification”,<sup>20,29,54,55</sup> but with respect to the microbial community as well the terminal reduction step, this expression is misleading. The term “nitrifier” implies the participation of nitrite oxidizing microorganisms, and denitrification historically implies N<sub>2</sub> as the final product.<sup>22</sup> In order to specify this particular reaction pathway, the term “nitrous denitrification” is proposed. To distinguish between the CANDO process and this reaction, a proper definition of the ORR is necessary. According to the previous explanations, the determinant “sub-oxic” is adequate resulting in “sub-oxic nitrous denitrification”.

A comparison of Gong *et al.*<sup>56</sup> and Scherson *et al.*<sup>18,19</sup> elucidates another ambiguous expression. Both authors use the phrase “partial denitrification”, but very different processes are described: Scherson *et al.*<sup>18,19</sup> refer to N<sub>2</sub>O production,

while Gong *et al.*<sup>56</sup> refer to sub-oxic reduction of NO<sub>3</sub><sup>−</sup> to NO<sub>2</sub><sup>−</sup> (Table 1, eqn (11)). According to our proposed scheme, the pathway of Gong *et al.* can be distinctly described as “sub-oxic denitrification”. Moreover, because the dominant organisms (*i.e.* AOB and NOB) under sub-oxic conditions are the same as under “aerobic” conditions, it is clear that the reduction reaction mechanisms are related to “aerobic” organisms and the “sub-oxic” determinant can thus be omitted from the terminology.

## 4 Conclusion

This work has shown that the structure of different combinations of metabolic pathways applied in BNR processes has become more complex and inconsistent, leading to ambiguity and confusion within the research community and industry. For future communication and development of BNR processes, we therefore propose to apply a consolidated terminology that enables a more exact definition of the metabolic pathways.

The presented approach enables a distinct identification of every possible step that is important for BNR technologies (Fig. 1). Because of its flexibility it can also be adapted for novel derivatives of state-of-the-art BNR technologies. For a process removing nitrogen as nitric oxide, for example, application of the proposed nomenclature would give the phrase “nitric denitrification”. For the reduction of NO<sub>3</sub><sup>−</sup> to N<sub>2</sub>O “nitrous denitrification” can be derived. While such processes have not been technically applied yet, it illustrates the flexibility of the terminology. Additionally, presently emerging bioelectrochemical technologies also fit into the scheme, since their catalyzed oxidation reduction reactions are the same as in biological systems. Hence, with the adoption of our presented approach a more exact communication about all known biological and bioelectrochemical technologies can be facilitated and will potentially avoid further misunderstanding. However, we do not claim that the presented approach is perfect, but it hopefully triggers further discussion within the community to agree on a commonly accepted terminology.

## Acknowledgements

The International Graduation School of Science and Engineering (IGSSE) of TUM is gratefully acknowledged for financial support (Project Water 06, PANOWA). Furthermore, we appreciate the support of the Bavaria California Technology Center (BaCaTeC) for supporting the scientific exchange between our two institutions (No. 22 [2013-2]).

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