Engineering biological nitrogen removal in wastewater treatment via the control of nitrite oxidising bacteria using free nitrous acid

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Nitrogen compounds need to be removed or captured from wastewater streams before disposal to protect our aquatic environments from eutrophication. Particular bacteria facilitating the biological removal of nitrogen during wastewater treatment include ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB), denitrifiers, as well as anaerobic ammonium oxidising (Anammox) bacteria. Manipulating these microbial communities can improve efficiency in nitrogen removal. Bypassing nitrate production by selectively inhibiting NOB reduces the need for oxygen and the addition of external carbon for the nitrogen removal. Various approaches to selectively inhibit NOB in the nitrification process are available. Here we present an approach using the biocide, free nitrous acid (FNA) to selectively suppress NOB growth thereby improving the efficiency of the nitrogen removal process.

Improving efficiency of nitrogen removal

The principal forms of nitrogen species in wastewater are ammonium, nitrite and nitrate as well as organic nitrogen. Removal from wastewater is essential before release into aquatic environments to prevent nitrogen build up that may lead to eutrophication and endanger aquatic life[1]. For wastewater treatment, biological nitrogen removal is favoured over physical-chemical processes due to efficiency and cost benefits[2].

Conventional biological nitrogen removal in wastewater treatment (WWT) plants involves a 2 step biological process: autotrophic nitrification followed by heterotrophic denitrification. These steps result in nitrogen gas being released from the system (Figure 1*a*). The nitrification step includes the oxidation of ammonia to nitrite via ammonia-oxidising bacteria (AOB) and then oxidation to nitrate through the activity of nitrite oxidising bacteria (NOB). Nitrification is then followed by heterotrophic denitrification where nitrate is reduced to nitrite and finally to nitrogen gas[3]. Here, the inhibition of NOB activity can be beneficial for achieving lowered operational costs for WWT.

Recently, a novel autotrophic nitrogen removal process, i.e. deammonification, has been developed. This consists of a partial nitrification or nitritation, in which approximately half the ammonium is converted to nitrite by AOB. This is then followed by an anaerobic ammonium oxidation (anammox) process, governed by anammox bacteria, wherein the remaining ammonium and nitrite is converted to Nitrogen (N_2) (Figure 1*b*)[4]. The anammox process has gained much research traction and has been applied extensively in Europe. It requires less energy through reduced aeration and requires no input of organic carbon compared to the conventional nitrification-denitrification WWT process[5–7]. Nitritation, the partial conversion of ammonium to a 50 : 50 mixture of ammonium and nitrite, is favoured as a feed for anammox (Figure 1*b*). Thus, a reduction in the activity of NOB is necessary to achieve this favoured feed and obtain energy efficiency and reduced costs.

Hence inhibiting NOB is beneficial for achieving a more cost and energy efficient WWT process. This applies to both the conventional nitrification-denitrification and the anammox process (Figure 1).

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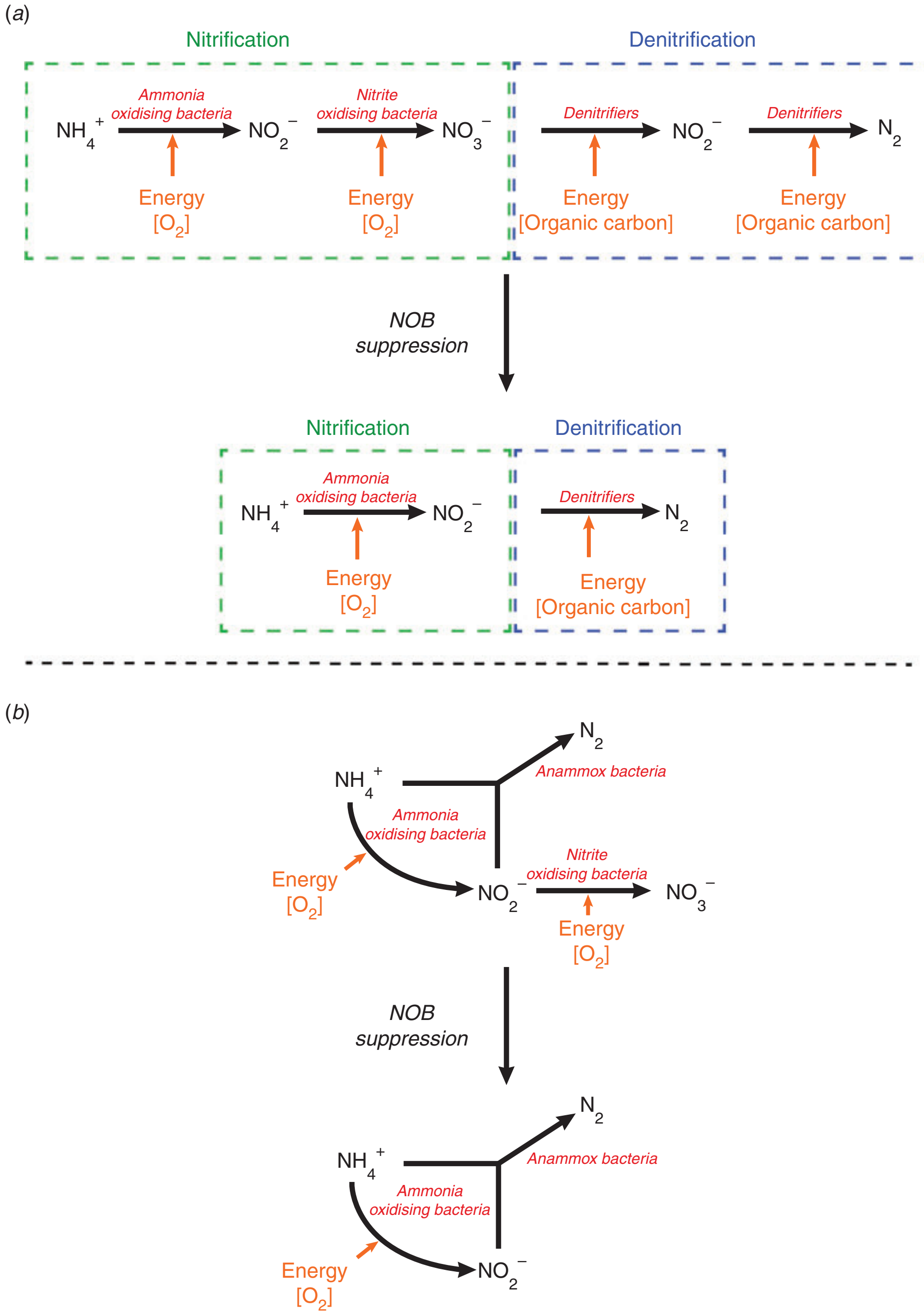

Figure 1. A schematic overview of the selective suppression of NOB in both (*a*) the conventional nitrification-denitrification process and in the (*b*) anammox process, which would allow for improved energy saving during the biological nitrogen removal in WWT.

Achieving selective NOB suppression

Several approaches have been applied for controlling the NOB activity in both conventional nitrogen removal and the anammox process. A challenge for these processes is the selective inhibition of NOB activity and/or growth while retaining the presence of AOB (Figure 1). Both these groups have similar slow growth kinetics but require large substrate turnover and respiratory rates[5]. Various physical and chemical methods are proposed for suppression of NOB growth in bioreactors and include running bioreactors at limiting dissolved oxygen (DO) concentrations, increasing the temperature in combination with low solid retention times, and the use of intermittent aeration. However the relatively low nitrogen concentrations and low temperature in mainstream wastewater make it difficult to selectively inhibit NOB while allowing AOB to grow[5]. Interestingly, the addition of free nitrous acid (FNA) is successfully shown to selectively suppress NOB activity and growth in conditions otherwise favourable for nitrification[5].

Free Nitrous Acid (FNA) suppression of NOB growth

The protonated form of nitrite, FNA is a biocide that is bacteriostatic in parts per billion and bacteriocidal in parts per million levels[8].

However, different types of microorganisms exhibit varying tolerance to FNA[8]. It turns out that AOB have substantially higher tolerance to FNA compared to NOB, and this provides opportunity to selectively inhibit NOB[9,10]. Indeed, it is seen that a FNA treatment of sludge, combined with low oxygen levels, selectively inhibit NOB and partially inhibit AOB. This treatment achieved partial nitritation and produced a water composition ideal for anammox nitrogen removal[5].

Biocidal mechanisms of FNA action on various bacteria

Improved control of such bacterial populations will be achieved through understanding the antimicrobial effects of FNA and by determining the counteracting responses of the different organisms. Transcriptomic responses recently studied in *Pseudomonas aeruginosa* PAO1 and *Desulfovibrio vulgaris* reveal that FNA causes severe disruption to the bacterial energy conserving mechanisms[9,11]. Additionally, *Desulfovibrio vulgaris* shows multiple responses to oxidative stress during FNA exposure.

The higher tolerance of AOB to FNA is intriguing given that NOB have additional metabolic pathways to deal with high levels of nitrite compared to AOB. Both AOB and NOB have the nitrite detoxifying protein nitrite reductase *(nirK)* that converts nitrite to nitric oxide (NO)[12]. However, NOB have additional nitrite reductase *(nirBD)* and nitrite oxidoreductase *(norA/B)* genes, and thus possess the potential to convert nitrite to ammonia and nitrate, which could better alleviate toxic nitrite levels. Recently, a metaproteomic investigation revealed that FNA induced oxidative stress upon a nitrifying community. However, AOB was able to tolerate elevated levels of FNA compared to NOB due to a superior oxidative stress response[13].

Concluding remarks

Determining the mechanisms of FNA action in both AOB and NOB is important to establish a deeper understanding of the difference in tolerance of these 2 groups of highly relevant nitrogen removal bacteria. Such understanding is significant for optimising strategies for improved reactor performance and for reducing the operational costs involved in the nitrogen removal WWT process.

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