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The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants

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ABSTRACT

Nitrite is known to accumulate in wastewater treatment plants (WWTPs) under certain environmental conditions. The protonated form of nitrite, free nitrous acid (FNA), has been found to cause severe inhibition to numerous bioprocesses at WWTPs. However, this inhibitory effect of FNA may possibly be gainfully exploited, such as repressing nitrite oxidizing bacteria (NOB) growth to achieve N removal via the nitrite shortcut. However, the inhibition threshold of FNA to repress NOB (~0.02 mg HNO_2-N/L) may also inhibit other bioprocesses. This paper reviews the inhibitory effects of FNA on nitrifiers, denitrifiers, anammox bacteria, phosphorus accumulating organisms (PAO), methanogens, and other microorganisms in populations used in WWTPs. The possible inhibition mechanisms of FNA on microorganisms are discussed and compared. It is concluded that a single inhibition mechanism is not sufficient to explain the negative impacts of FNA on microbial metabolisms and that multiple inhibitory effects can be generated from FNA. The review would suggest further research is necessary before the FNA inhibition mechanisms can be more effectively used to optimize WWTP bioprocesses. Perspectives on research directions, how the outcomes may be used to manipulate bioprocesses and the overall implications of FNA on WWTPs are also discussed.

1. Introduction

Biological nutrient removal (BNR) is by far the most economic and environmentally friendly way to achieve nitrogen and phosphorus removal from wastewater (Tchobanoglous et al., 2003). Compared to chemical treatment methods, BNR reduces chemical consumption and cost, reduces the production of waste solids, and has lower energy requirement. In the nitrification process, ammonium is converted to nitrite by ammonium oxidizing bacteria (AOB, nitritation) and nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB, nitratation). Almost all nitrifying bacteria are autotrophic. For each carbon-atom fixed, autotrophic bacteria consume 80% of the energy generated from substrate oxidation, which results in a very low growth yield (Kelly, 1978). Denitrification is the process of nitrate reduction into nitrite and then into molecular nitrogen, which is performed by a functional group of heterotrophs that use oxidized nitrogen (NO_3^-, NO_2^-, NO and

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N_2O) as the electron acceptor in respiration. This is usually coupled with oxidation of organic compounds as the electron donor for energy generation.

Nitrite oxidation is typically considered to be the rate limiting step under normal operating conditions for nitrification. However, many environmental conditions can cause ammonia oxidation rates to exceed those of nitrite oxidation, leading to nitrite accumulation. For example, high temperature will cause AOB to out-compete NOB because of their greater growth rate at temperatures higher than 25 ° C (Hellinga et al., 1998). NOB also has been shown to have lower affinity for oxygen than AOB (Ciudad et al., 2006). Hence, a low dissolved oxygen (DO) conditions will favour nitrite accumulation. Nitrite accumulation can also occur under anoxic conditions, due to differences in the denitrification kinetics between nitrate and nitrite reduction (Glass and Silverstein, 1998). Imbalanced reduction rates can be attributed to many environmental factors such as, high temperature, the presence of high DO anoxically, low pH, types of carbon source (e.g. volatile fatty acids, and methanol) and low carbon to nitrogen ratios. It can also be due to the absence of certain denitrifying species or enzymes in the sludge (Meng et al., 2010). Further, it has been reported that the presence of phosphate (can depress nitrite reduction markedly), heavy metals, sunlight (NOB is more sensitive to sunlight than AOB) and reactor operation (e.g. length of sludge retention time (SRT) and hydraulic retention time (HRT)) can also cause nitrite accumulation (Philips et al., 2002).

The presence of a high concentration of nitrite has been reported to be a severe inhibitor on a wide range of microorganisms. Depending on the concentration and operating pH and temperature, this inhibition can slow, or even completely cease microbial activities and reconfigure the microbial community structure. While nitrite accumulation can be viewed as an unfavourable occurrence in conventional wastewater treatment plants (WWTP), there are advanced processes which exploit the use of nitrite as an intermediate to achieve "shortcut" nitrogen removal (Hellinga et al., 1998; Kuai and Verstraete, 1998; Strous et al., 1999). Such processes have a number of potential advantages including: lower carbon source requirements in denitrification, lower consumption of oxygen in nitrification, reduction of reactor volumes due to lower HRT requirements, higher denitrification rates and smaller sludge production (Turk and Mavinic, 1986). These advantages are even more notable where a wastewater contains high ammonium or low organic carbon contents.

Due to the inhibitory effects nitrite can impose on biomass, a relevant current challenge is to understand when such inhibition can occur, which in turn can contribute to the optimisation of advanced nitrogen removal processes. This review analyzes nitrite inhibitory effects on commonly found microbial communities in WWTPs, to yield better understanding of the inhibitory mechanisms involved. Strategies to reduce these inhibitory effects will also be discussed.

2. Free nitrous acid (FNA) – the true inhibitor on bacteria in BNR systems

FNA, the protonated form of nitrite, has been shown in numerous cases to be the cause of inhibition for bacteria in WWTPs, rather than the nitrite anion itself. FNA can be determined through the nitrite concentration, pH and temperature, by the formula $S_{N-NO_2}/(K_a \times 10^{pH})$, where $K_a = e^{-2300/(273+T(^\circ C))}$ (Anthonisen et al., 1976). The studies that have investigated the factors affecting this inhibition and its severity at different FNA levels on the primary organisms responsible for BNR are summarised below.

2.1. FNA inhibition on nitrifiers

Anthonisen et al. (1976) had first reported inhibition in nitrification processes is a function of nitrite concentration and pH. As nitrite is produced, pH decreases due to the release of hydrogen ions. The nitrite produced thus will exist in equilibrium with the unionized form (FNA). They had concluded that the inhibition on nitrification is related to the concentration of unionized nitrous acid (FNA) rather than the nitrite anion concentration, and the inhibition on nitrification will be initiated at an FNA concentration of 0.22–2.8 mg HNO_2-N/L.

Later studies have found variable inhibitory threshold levels, where an FNA concentration range of 0.42–1.72 mg HNO_2-N/L has resulted in a 50% reduction in AOB activity (Anthonisen et al., 1976; Stein and Arp, 1998; Hellinga et al., 1999; Fux and Siegrist, 2004; Vadivelu et al., 2006a; Tan et al., 2008; Tora et al., 2010). A combination of process factors and the microbial populations within the sludge of these different studies could have been responsible for this wide range of inhibitory threshold levels. Tora et al. (2010) found that situations of inorganic carbon limitations enhanced the inhibitory effect of FNA in their AOB enriched culture, however, this finding contrasted with the study of Vadivelu et al. (2006a). It is noteworthy that the dominant organism in the case of Tora et al. (2010) bound to the more general Nso190 probe (81%), while in the case of Vadivelu et al. (2006a), the sludge was dominated by organisms binding the NEU probe (82%), and no binding to the Nso190 probe was observed. This difference in microbial community could explain the contrasting FNA inhibition effects. Moreover, different species and strains within a genus could possess distinct tolerances towards FNA. The genome of *Nitrosomonas europaea* was suggested to have some different metabolic properties as compared to *Nitrosomonas eutropha*, which notably includes abilities to use nitrite as an electron acceptor anaerobically (Zart and Bock, 1998; Chain et al., 2003; Stein et al., 2007). This suggested possibility of a difference in their capacity to tolerate FNA. FNA also affects multiple metabolic pathways in AOBs. By decoupling their energy generation and growth processes, Vadivelu et al. (2006a) demonstrated that FNA has different inhibitory effects on *Nitrosomonas*' anabolic and catabolic activities, with the biosynthesis process being the most sensitive. It should be noted that FNA inhibition on nitritation can be reversible; Yang et al. (2003) found that the recovery period of nitritation from FNA inhibition lasted about 12 days.

The range of FNA concentrations affecting NOB activity has been found to start from 0.011–0.07 mg HNO_2-N/L, where complete inhibition was observed at 0.026–0.22 mg HNO_2-N/L (Prakasam and Loehr, 1972; Anthonisen et al., 1976; Vadivelu et al., 2006b; Zhang et al., 2010). *Nitrobacter* was found to be the dominant community in most of these studies, and indeed have been commonly found in laboratory scale reactors,