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Wastewater Opportunities for Denitrifying Anaerobic Methane Oxidation

Yali Wang,[1,2]
Dongbo Wang,[1,2,*]
Qi Yang,[1,2,*]
Guangming Zeng,[1,2] and
Xiaoming Li[1,2]

Denitrifying anaerobic methane oxidation (DAMO) can concurrently reduce methane emissions and nitrogen levels in aquatic environments, but how useful is this process? We propose the use of DAMO-based technology as a tool for sustainably operating wastewater treatment plants (WWTPs).

Manipulating WWTPs Can Mitigate Global Warming and Water Eutrophication

Global warming and water eutrophication (the bloom of blue-green algae caused by an excessive supply of nutrients) are widely recognized as serious environmental problems worldwide. Methane, with a global warming potential 20-fold higher than that of carbon dioxide, is an important greenhouse gas that accounts for ~20% of the global temperature increase. Methane can be emitted from many natural environments, such as freshwater sediments and agricultural origins, and from several engineered systems such as waste landfills and WWTPs. The eutrophication of freshwater environments, which is partly attributed to nitrogen from rainfall, agricultural discharges, and WWTP effluents, has become another serious global water management issue. Among these sources, WWTPs are the only engineering source that significantly contributes to both global warming and water eutrophication. Engineers therefore have opportunities to mitigate these two global environmental issues.

Currently, two main issues faced by WWTPs are the shortage of carbon sources in wastewaters and their substantial emission of greenhouse gases. With the increasing human population worldwide, more energy and resources are required, and sustainably addressing the two issues is therefore a big challenge. The recently described DAMO process might be the most promising solution if this microbial process can be utilized and integrated with other technologies in WWTPs at full scale.

DAMO: An Important Microbial Process for Nitrogen and Carbon Cycles

The microbial process of DAMO, where methane is oxidized anaerobically to provide electrons for denitrification, creates an important link between the nitrogen and carbon cycles [1]. The microorganisms responsible reported to date include '*Candidatus* Methylomirabilis oxyfera' (M. oxyfera), a bacterial group affiliated with the candidate division NC10 (a group of methane-oxidizing microorganisms), and '*Candidatus* Methanoperedens nitroreducens' (M. nitroreducens), an archaeal group related to anaerobic methanotrophic archaea [1–3]. The DAMO archaea convert nitrate to nitrite using electrons derived from methane, while the DAMO bacteria reduce nitrite to nitric oxide and then bioconvert nitric oxide to nitrogen and oxygen via the inter-aerobic denitrification pathway (Figure 1).

Three categories of mathematical models have so far been proposed to better understand the DAMO process (Figure 1). The models are developed based on the enrichment of DAMO bacteria (model A); DAMO bacteria and ANAMMOX (anaerobic ammonium oxidation) bacteria (model B); or DAMO bacteria–DAMO archaea–ANAMMOX bacteria (model C); these model structures thus contain different microbial pathways. The definitions of each of these kinetic and stoichiometric matrices are detailed in the literature [4–7].

Substrate concentrations (e.g., methane and nitrite) and environmental parameters (e.g., O_2, temperature, and pH) affect the DAMO process. For example, the nitrite depletion rate increases as the methane partial pressure increases from 1.8 kPa to 8.9 kPa [8]. Overloading nitrite had a toxic effect on the activity of M. oxyfera; the highest nitrite reduction rate of M. oxyfera was measured at 2.29 mmol NO_2^--N day^{-1} [9]. ANAMMOX bacteria compete with M. oxyfera for available nitrite, and therefore excess ammonium would risk washing out M. oxyfera because ANAMMOX bacteria have a higher affinity for nitrite than the DAMO bacteria [6]. Methane oxidation was not affected by oxygen exposure, but denitrification in the DAMO process was significantly affected as a result of suppression of nitrite reductase and nitrate reductase [10]. Oxygen exposure could suppress the activity of M. oxyfera by affecting protein/nucleic acid synthesis and the cell division process.

Can We Apply DAMO in WWTPs?

DAMO could remove methane and nitrogen concurrently without requiring expensive electron donors such as acetate and ethanol, and it therefore seems to be a promising solution to the two issues faced by WWTPs mentioned previously. However, can we employ it in WWTPs? Although DAMO is still a developing technique without any full-scale applications to date, several efforts have been made at the bench scale. For example, Kampman *et al.* incorporated a DAMO process into a UASB (upflow anaerobic sludge blanket)

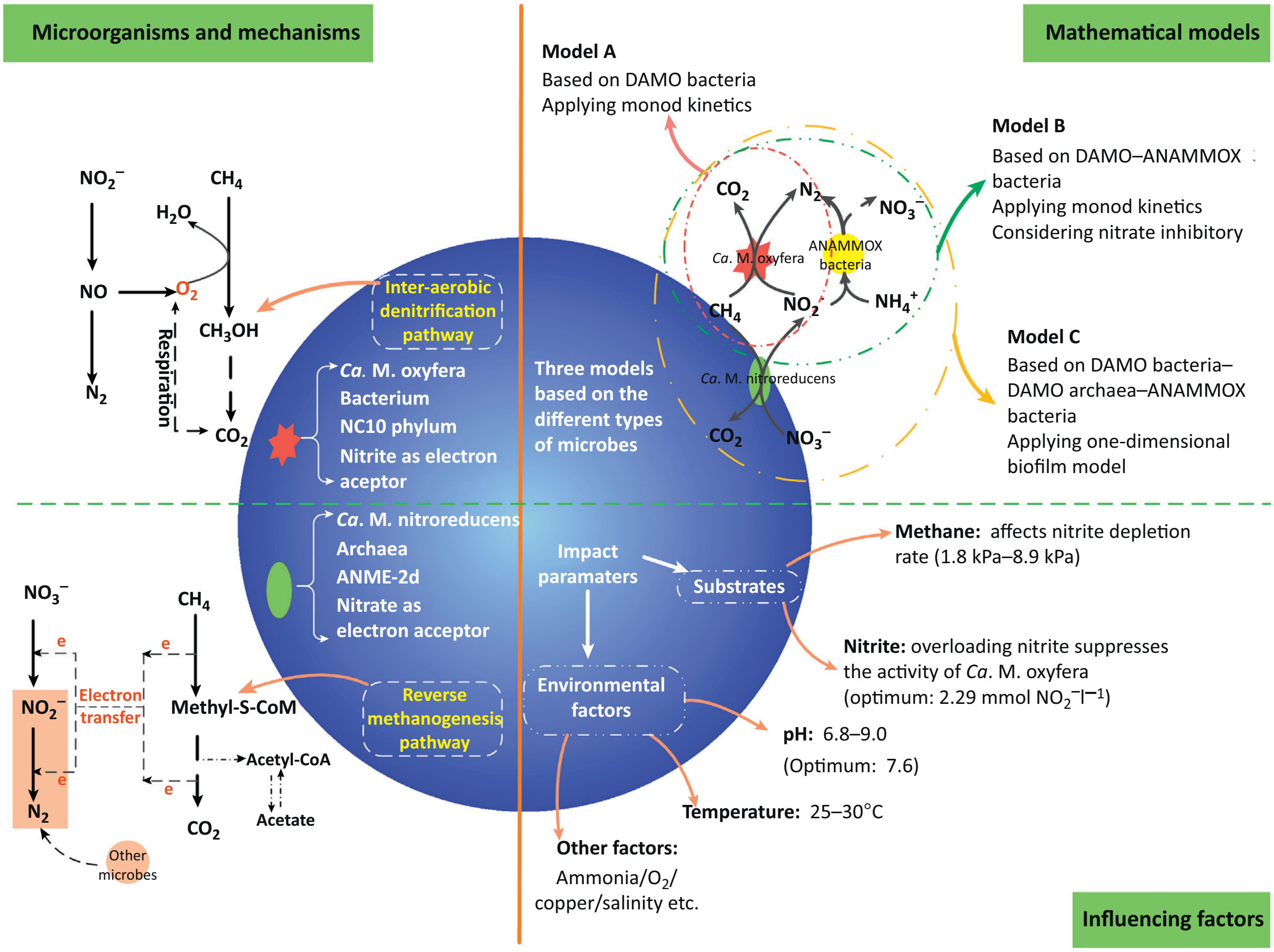

Figure 1. Information about the Denitrifying Anaerobic Methane Oxidation (DAMO) Process, Including the Microorganisms Responsible, Relevant Mechanisms, Mathematical Models, and Influencing Factors.

digester system and a nitritation reactor to treat sewage. The UASB digester converted organic substrates to methane and produced ammonium via ammonification, while the nitritation reactor supplied the nitrite required by the DAMO bacteria. In steady-state operation, this process obtained a maximum nitrogen removal rate of 37.8 mg N l^{-1} day^{-1} [11]. Zhu *et al.* enriched ANAMMOX and DAMO bacteria in a laboratory-scale reactor with the seed sludge taken from a full-scale ANAMMOX bioreactor [12]. Furthermore, Shi *et al.* designed a membrane biofilm reactor to enrich for both ANAMMOX and DAMO microorganisms [13]. Methane was delivered from the interior of hollow fibers and an ANAMMOX–DAMO biofilm grew on outer wall of the fiber. This bench-scale reactor enriched the microbe population to 20–30% DAMO bacteria, 20–30% DAMO archaea, and 20–30% ANAMMOX bacteria, and achieved nitrate and ammonium removal rates of ~190 mg N l^{-1} day^{-1} and ~60 mg N l^{-1} day^{-1}, respectively [13]. All these attempts indicate that DAMO has potential application in WWTPs.

How to Apply DAMO in WWTPs

We propose here two possible strategies incorporating DAMO into either side-stream or main-stream wastewater treatment for the future operation of WWTPs (Figure 2). In the side-stream concept (Figure 2A), wastewaters in the main stream are treated by an activated sludge-based bioreactor (e.g., an oxidation ditch) in which most of the nitrogen, phosphorus, and organic matter in the wastewaters are removed. The excess sludge is digested in an anaerobic digester to stabilize the properties of the sludge, reduce the volume of the sludge, and produce the energy gas methane. The digestion liquid usually contains 1000–1500 mg/l ammonium, constituting 10–20% of the WWTP total nitrogen. To reduce the nitrogen level in the main-stream line, we propose incorporating a combined nitritation–DAMO–ANAMMOX system into the side-stream line before the digestion liquid is recirculated into

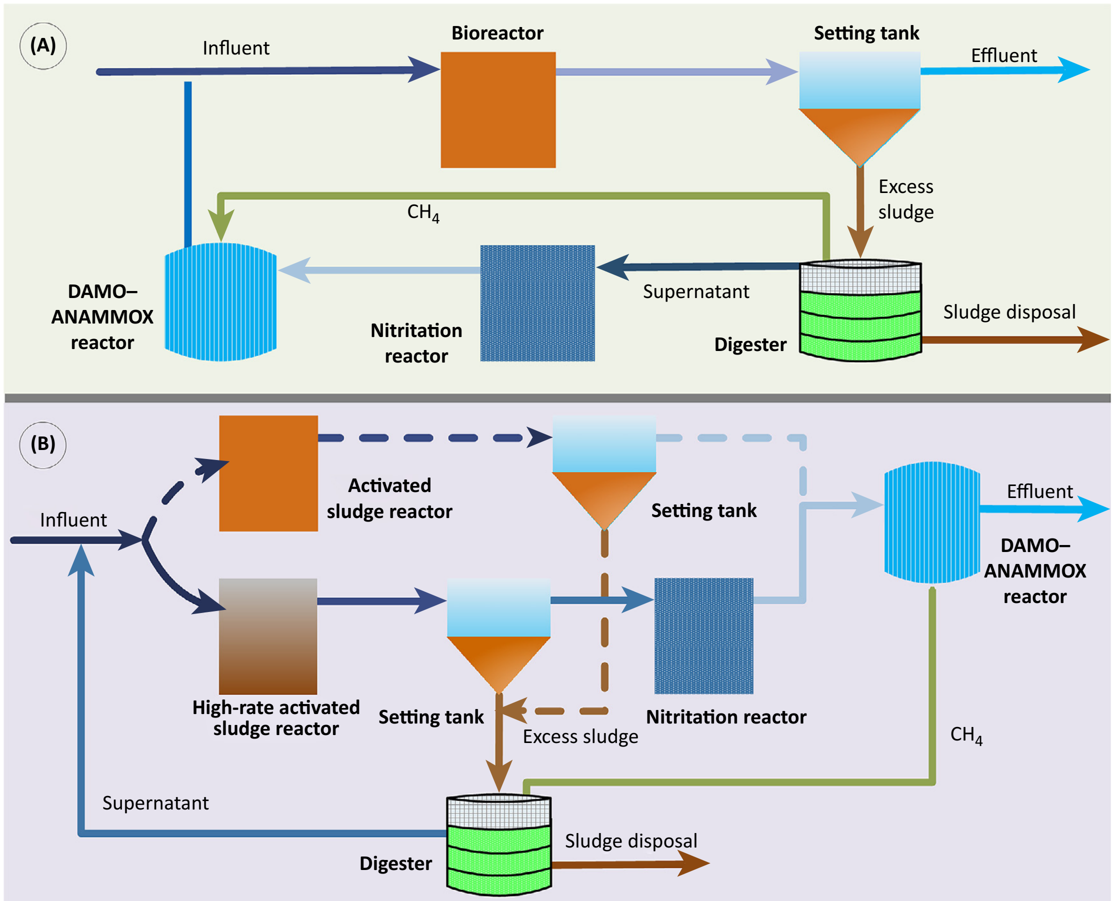

Figure 2. Two Proposed Strategies for Incorporating DAMO into Wastewater Treatment for the Future Operation of WWTPs. (A) Incorporating the DAMO process in the side stream; (B) incorporating the DAMO process in the main stream with either the traditional 'activated sludge' process (dashed line) or advanced wastewater treatment technologies (solid line). The methane required in the DAMO–ANAMMOX reactor would be produced from the anaerobic digestion of sewage sludge. We estimate that this production accounts for <10% of the total methane generated in the side-stream line, while it accounts for either <10% (dashed line) or <50% (solid line) of the total methane generated in the main-stream line. The remainder methane can be utilized to generate electricity. In the main-stream line, the DAMO process would be dominant in a DAMO–ANAMMOX reactor if the effluent from the traditional activated sludge process contains little ammonium. Abbreviations: ANAMMOX, anaerobic ammonium oxidation; DAMO, denitrifying anaerobic methane oxidation; WWTP, wastewater treatment plant.

the main-stream line. In the nitritation reactor, the ammonium in the digestion liquid is partially converted to nitrite, and the effluent is then further treated in the DAMO–ANAMMOX co-culture reactor. In such a reactor, DAMO archaea reduce nitrate to nitrite while DAMO and the ANAMMOX bacteria jointly reduce nitrite to nitrogen gas. The maximal nitrogen removal efficiency of nitritation–ANAMMOX is only ~70% because nitrate (which constitutes 11% of the total nitrogen) is an end-product of the ANAMMOX reaction, and residual ammonium and/or nitrite may remain in effluent when the ideal molar ratio of 1.32 to 1 of nitrite to ammonium is not produced by the partial nitritation process. A combined nitritation–DAMO–ANAMMOX system might overcome the drawbacks of nitritation–ANAMMOX.

In the main-stream concept, the DAMO–ANAMMOX reactor could be utilized either as a post-treatment unit or even as the core unit for sustainable WWTP operation, depending on the treatment process used (Figure 2B). For conventional WWTPs using the traditional 'activated sludge' process, the DAMO–ANAMMOX reactor could be used as a post-treatment unit without retrofitting existing activated sludge installations (Figure 2B, dashed line). The effluent of the traditional 'activated sludge' process generally contains relatively high levels of nitrate and some residual ammonium, and both could be removed in the added DAMO–ANAMMOX reactor without either extra oxygen input or additional expensive electron donors such as acetate or methanol.

There is growing understanding that WWTP operation should be shifted toward sustainable paradigms that either are energy-neutral or output energy, and therefore the main-stream deammonification-based process is the more promising technology. The DAMO–ANAMMOX reactor would be at the heart of main-stream deammonification (Figure 2B, solid line). First, most of the organic matter (~80%) and phosphorus (~90%) in wastewater is absorbed and stored in the high-rate activated sludge-based bioreactor [14]. The ammonium-rich effluent is then treated in a nitritation reactor to partially convert ammonium to nitrite [15]. Finally, the nitritation effluent is further treated in the DAMO–ANAMMOX reactor. Using this approach, the DAMO-based technologies have significant potential for the sustainable operation of WWTPs.

The Way Forward

To make DAMO suitable for field application, the following issues should be addressed in the future.

(i) Further understanding the microbial behaviors of DAMO microorganisms. To accelerate the cultivation period of DAMO microorganisms and increase reaction rates, efforts will be necessary to further elucidate the physiology, mechanisms, and kinetics of the known M. oxyfera and M. nitroreducens species, identify other microbes potentially carrying out DAMO process, and explore the interaction between DAMO microorganisms and other microbial groups.

(ii) Scaling up DAMO to a practical level. The biggest challenge is how to concurrently scale up the system size to a real-world level while achieving practically useful reaction rates and performances. In addition, the gas generated by the digester also contains other components such as CO_2 and H_2S, and thus their impact on DAMO needs to be considered in field situations.

(iii) Better controlling the DAMO hybrid process. In engineered systems, reasonable coordination between DAMO and other units such as activated sludge unit and nitritation is crucial for the successful application of such emerging technology. Such a combined system requires delicate optimization of both system design and process operation, and mathematical modeling and advanced monitoring/control strategies may be useful in this regard. However, this could increase the construction investment, a factor that will need to be evaluated in the future.

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[1]College of Environmental Science and Engineering, Hunan University, Changsha 410082, China
[2]Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, China

*Correspondence:
w.dongbo@yahoo.com (D. Wang) and
yangqi@hnu.edu.cn (Q. Yang).

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Ethnophytotechnology: Harnessing the Power of Ethnobotany with Biotechnology

John de la Parra[1] and Cassandra L. Quave[2,3,*,@]

Ethnobotany (the scientific study of traditional plant knowledge) has aided the discovery of important medicines. However, as single-molecule drugs or synergistic mixtures, these remedies have faced obstacles in production and analysis. Now, advances in bioreactor technology, metabolic engineering, and analytical instrumentation are improving the production, manipulation, and scientific understanding of such remedies.