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Ammonia oxidizer adaptation to low dissolved oxygen concentrations for biological nutrient removal – a review on oxygen affinity, dual-substrate limitation, and decay

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Low dissolved oxygen (DO) operation in suspended growth biological nutrient removal (BNR) systems has become increasingly important for energy-efficient and intensified treatment processes. Novel strategies like continuous low DO, ammonia-based aeration control (ABAC), partial-denitrification anammox (PdNA), and partial-nitritation anammox (PNA) are becoming more commonplace and can drive processes to operate in DO regimes far below conventional norms. Yet nitrification, particularly the first step (ammonia oxidation), must be maintained. This review provides the first comprehensive overview of experimental data on ammonia oxidizer adaptation to low DO, along with analysis on how and why ammonia oxidizer oxygen kinetics respond to these low DO environments. This review examines the available evidence that stable ammonia oxidation at low DO is possible and usually corresponds to a reduction in the estimated ammonia oxidation Monod oxygen half-saturation coefficient (K_{O_2}). These changes can arise from intrinsic shifts in microbial community structure (particularly increased comammox *Nitrospira* (CMX) abundance), from extant factors like floc and microcolony size, or both. Despite evidence for these shifts, the drivers, limits, and durability of kinetic adaptation remain open questions. Also reviewed is the potential interaction of oxygen and substrate affinity, which is poorly understood and infrequently studied, especially under the non-starvation ammonia conditions common in advanced processes. This review also highlights a need to better study ammonia oxidizer decay and decay rate adaptation to low DO. Despite being critical to determining ammonia oxidizer population and thus nitrification capacity, decay kinetics under low DO remain largely unmeasured, mischaracterized, or assumed constant. Existing methods for determining decay are not well suited to capture or characterize the impacts of low DO operation. These findings suggest a necessary shift in practice: ammonia oxidation kinetic parameters, especially K_{O_2}, should not be treated as fixed parameters. SRT, DO, substrate concentration, and community structure all interact to drive nitrification performance. This review seeks to provide a comprehensive and systematic review of existing experimental results within each of these domains, with perspective on future research needs and implications for design and operation.

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Water impact

Reduction in WWRF operating DO (*i.e.* low-DO treatment) allows for major energy savings and novel treatment approaches. However, low-DO processes design is still an open question, especially with respect to nitrification. This critical review examines the evidence for stable nitrification at low DO, analyzes the drivers for nitrifier kinetic change in response to low DO, and highlights future research needs.

1 Introduction

Low dissolved oxygen (DO) biological nutrient removal (BNR) processes are the subject of increasing research interest as a tool for process intensification. Operating an activated sludge process at lower DO requires less air supply thus significantly reducing energy. Additionally, novel nitrogen removal approaches like ammonia based aeration control (ABAC), or

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shortcut nitrogen removal strategies like partial-denitrification anammox (PdNA) and partial-nitritation anammox (PNA) which often rely on maintaining a specific ratio of unoxidized ammonia and nitrite/nitrate (*i.e.* ammonia-*versus*-NO_x (AvN) control), often lead to lower bulk-liquid DO concentrations.[1–3] In all of these scenarios, maintaining aerobic ammonia oxidation capacity is critical to maintaining high quality effluent.

To avoid losing nitrification capacity when a BNR process is transitioned to low DO, the ammonia oxidizing organisms must adapt or their population must increase. While ammonia oxidizing populations are complex and contain diverse species, broadly speaking the ammonia oxidizers, or some fraction thereof, must attain a higher affinity for oxygen to operate at similar rates in a reduced oxygen environment and thus allow the treatment process to maintain nitrogen removal capacity.

Early research into nitrification processes in wastewater treatment noted the importance of dissolved oxygen,[4–6] and the relationship between DO concentration and ammonia oxidation activity was often conceptualized as a Monod function.[7,8] An example set of Monod curves for two different nitrifying populations with different maximum rates and Monod oxygen half saturation coefficients (K_{O_2}) values are shown in Fig. 1 to illustrate how these kinetics impact rates. In this example only the single Monod function of oxygen is shown, thus ammonia is assumed to be non-limiting (further sections of this paper go into detail on methods for treating multiple limitations).

Stenstrom and Poduska as late as 1980, noted that "…the reported effects of DO concentration on maximum growth rate and process dynamics are varied, and not well understood".[9] They claimed based on surveying the preceding 20 years of work from pure cultures, soil organisms, and activated sludge processes that nitrifiers were limited in their ammonia oxidation rate below DO concentrations of 1.0–2.0 mgO_2 per L, but concluded that with long enough solids retention time (SRT) nitrification can be achieved with a DO concentration of 0.5–1.0 mgO_2 per L, and that 0.3 mgO_2 per L is the lowest DO concentration at which nitrification can be considered possible. From these and other early findings, official design guidance for nitrification within BNR processes suggested operating at a DO of 2.0 mgO_2 per L to maximize nitrification efficiency without aerating unnecessarily.[10] This principle is generally recommended to this day[11] and even codified in design documents such as the Recommended Standards for Wastewater Facilities[12] and the EPA Manual on Nitrogen Control.[13]

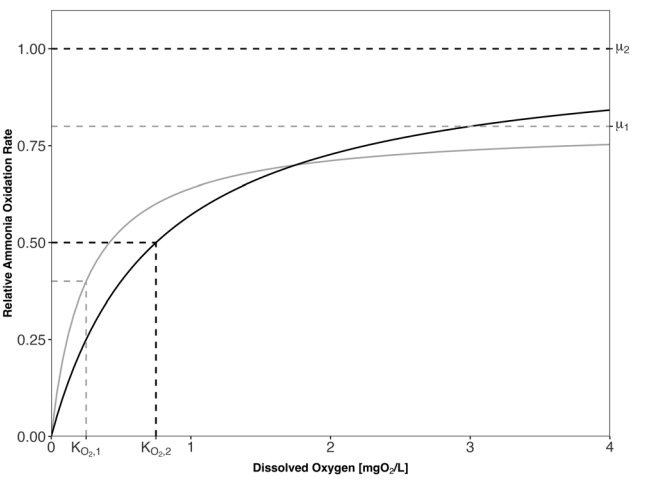

Fig. 1 Two theoretical Monod functions showing ammonia oxidation rate as a function of oxygen with different maximum rates (μ_1 and μ_2) and K_{O_2} ($K_{O_2,1}$ and $K_{O_2,2}$).

While this early literature mentions variance in nitrifier oxygen kinetics and contains studies of systems that operated at what would today be considered low DO, research was also conducted on explicitly operating nitrification at lower DO to better understand how this could be reliably achieved and understood. This research can be understood in the broader framework of variability in microbial kinetics as it is treated in wastewater processes. It has been noted by multiple researchers that kinetic parameters can vary between systems and over time, and in 1996 Grady *et al.*[14] proposed terminology to characterize the sources of parameter change. Kinetics were conceived of as "intrinsic" or "extant". Intrinsic kinetics were proposed to mean those to which the commonly used kinetic parameter symbols referred, but more particularly the kinetics of the underlying organism of interest, *i.e.* "If the kinetics truly reflect unrestricted growth they are representative only of the nature of the organisms and the substrate".[14] Extant kinetic parameters then are the microbial kinetics as they are observed in the system, or expressed by the organism given factors like the existing or recent conditions of substrate concentrations, mixing and advection, floc or biofilm structure and size, *etc.* Fig. 2 provides a graphical summary of the extant and intrinsic factors covered in this review, namely the ammonia oxidizing community makeup (intrinsic), the microcolony size (extant), and the floc size (extant).

Indeed numerous factors impact the observed or estimated (or apparent) K_{O_2} for a given biomass on a given substrate within a given system, such as advection, diffusion, floc size and density, temperature, inhibition, substrate and enzyme concentrations, and more,[15] as well as the dependence of the half-saturation coefficient on the maximum growth rate.[16] Many of these phenomena are difficult to measure, however, and this study focuses on the intrinsic and extant drivers for which data are available in the literature. As a consequence of this, the challenge of transferability must also be considered in any discussion on kinetic parameter estimation. Due to the number of factors that influence observed kinetics, and the differing conditions between batch-scale tests and full-scale processes, kinetics estimated *ex situ* may not perfectly capture *in situ* kinetics.[15,17] Better tools should be developed for *in situ* parameter estimation to better understand the topics covered herein.

For the sake of brevity and focus, NOB oxygen kinetics and their potential for adaptation are not within the scope of

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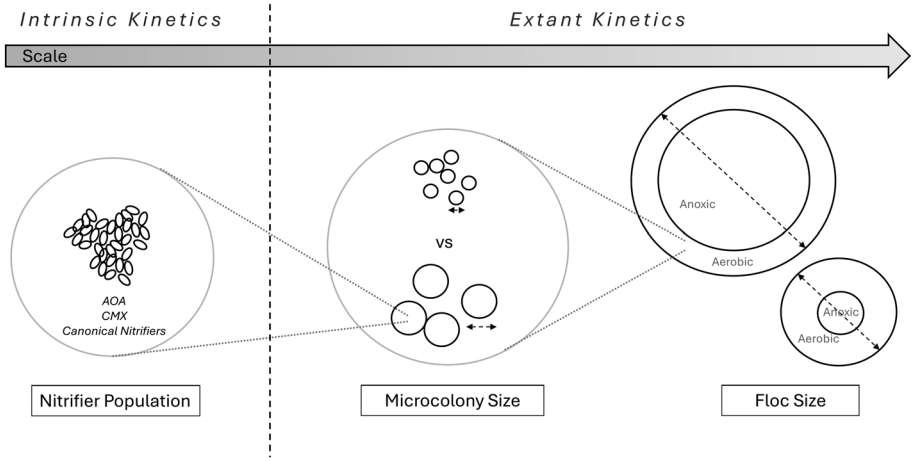

Fig. 2 Potential drivers of change in observed ammonia-oxidizer oxygen affinity K_{O_2}. Intrinsic drivers reflect shifts in the ammonia-oxidizing community (toward organisms with inherently different kinetics), while extant drivers reflect physiological/operational acclimation or phenomena. These mechanisms act across different scales, from cells to aggregates/flocs.

this paper, and studies exist comparing the observed differential response to operating DO of AOB *vs.* NOB.[18–20] The focus of the kinetic portions of this work is explicitly on the first step of nitrification, ammonia oxidation (although comammox are capable of both steps). Similarly, N_2O emission dynamics are not covered in this review. Although early studies suggested that low DO operation may increase N_2O, particularly *via* autotrophic denitrification,[21–23] recent studies appear promising (*e.g.* Jimenez *et al.*[24] found that low DO adapted biomass produced more than two-fold lower N_2O emissions than high DO adapted biomass exposed to low DO in a batch test). Further investigation is warranted on the impacts of long-term, stable, low DO operation on N_2O production.

This critical analysis of the existing literature on low DO ammonia oxidation covers.

1) A review of studies wherein low DO ammonia oxidation is observed but ammonia oxidation kinetic adaptation is not explained.

2) A review of low DO nitrification studies wherein intrinsic kinetic changes are documented (for example, a different ammonia oxidizing species or strain is present).

3) A review of low DO nitrification studies wherein extant kinetic changes are documented.

4) A review of literature and concepts on the impact of single and multiple substrate limitations with respect to ammonia and oxygen for ammonia oxidation.

5) A review of literature on the decay rate of nitrifiers and how the many unknowns about decay may impact low DO nitrification.

2 Observations of ammonia oxidation adaptation to low DO

The possibility of ammonia oxidizer adaptation to low DO conditions has been observed by researchers in the past. This section provides an overview of observations of this phenomenon, demonstrating its existence, irrespective of whether the authors of those studies attempted to justify or explain it. Subsequent sections discuss the potential intrinsic and extant causes of this phenomenon, and existing limitations in the research.

An early and influential study, Hanaki *et al.*[25] conducted experiments using lab-scale reactors and synthetic wastewater with and without organic carbon operating at a 0.5 mgO_2 per L DO and non-limiting DO. They estimated K_{O_2} by comparing activities between these two batches, thus assuming the ammonia oxidizers in both reactors had the same oxygen kinetics, and found a value on the lower end of what may be considered typical. They reported that the ammonia oxidizers in the nitrification culture that grew at low DO conditions apparently had a much higher yield, which was used to explain how the apparent rates in both batches differed little despite operating at high/low DO setpoints and having the same K_{O_2}. It seems likely in this experiment that potential variations in ammonia oxidizer kinetics may have been incorporated in the assumptions and resultant calculations for other kinetic parameters, and it should be noted the yield values and biomass values could not be calculated directly but only relative to the rate.[26] operated chemostats with nitrifiers derived from soil samples and determined that reduction in oxygen concentrations led to decreased K_{O_2} for both ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), but that reducing DO too far led to severely reduced nitrification rates. Park *et al.*[27] modified one of eight parallel trains at a full-scale UCT process to low DO operation (under 0.5 mgO_2 per L) and found that the nitrification rate in the train did not decrease and it maintained complete nitrification. Of interest in this experiment was that all of the activated sludge was recombined in the clarifiers, meaning all of the nitrifiers were continuously exposed to low and high DO conditions. Daebel *et al.*[28] operated two activated sludge pilot processes and determined that the K_{O_2} value of nitrifiers were time-dependent, providing evidence that these kinetics should not be treated as constants.

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Liu and Wang[29] ran a series of lab-scale enriched nitrifier reactors at 10, 20, and 40 day SRTs across a range of DO concentrations from 0.1 to 5.0 mgO_2 per L. They fit their process data across these operating conditions to a model that included a single value for K_{O_2} for nitrifier growth (meaning adaptation *per se* was not modelled) and a separate K_{O_2} value for nitrifier decay. It was hypothesized that the K_{O_2} for decay was high enough to offset (through increased nitrifying biomass) the decrease in rate that low DO caused, as minimal loss of nitrification was seen at the low DOs.

Fan *et al.*[30] and Wang *et al.*[31] both operated lab-scale reactors at high and low DO and found lower K_{O_2} for AOB at low DO conditions, the latter also identifying an increase in AOB yield coefficient. Microbial community analysis was performed and an increase in nitrifier abundance at low DO was noted, although the population composition of the genera tested did not appear to change appreciably at the two DO conditions.

Fig. 3 summarizes the findings of studies that specifically reported ammonia oxidizer K_{O_2} values at both high and low operating DO concentrations. Operating DO and ammonia oxidizer K_{O_2} values from each study are shown along with relative ammonia oxidation capacity contours (see the Fig. 3 caption for more detailed explanation). From these results, it is clear some researchers have found that ammonia oxidation capacity has been maintained or even increased as DO was reduced, but others have found a loss of relative ammonia oxidation capacity as a consequence of operating at low DO (due to insufficient adaptation). Despite varied experimental conditions in the studies shown in this figure, ammonia oxidation K_{O_2} adaptation occurred, establishing the possibility of such adaptation. The rest of this manuscript attempts to detail the possible drivers of this adaptation; differences in adaptation may have been due to changes in floc morphology, microcolony structure, or nitrifier population (although not all of the studies included in this figure report on each of these metrics).

3 Ammonia oxidizer adaptation to low DO due to change in intrinsic kinetics

Changes to intrinsic ammonia oxidizer kinetics have been investigated primarily by looking at microbial community composition and attempting to identify shifts in the strains or species that make up the ammonia oxidizing community at high and low DO concentrations. Early work noted overall shifts in ammonia oxidizing populations that included canonical ammonia oxidizers and explored the sublineages present. Ammonia oxidizing archaea (AOA) were also the subject of investigation as they were found in multiple low DO systems. More recently, comammox were discovered and garnered significant attention due to their heretofore unknown but widespread presence in wastewater treatment processes; multiple researchers finding significant presence of these nitrifiers in low DO systems. Each of these types of population shifts (and the attendant changes in intrinsic kinetics) have been observed at low DO, but the understanding of how, why,

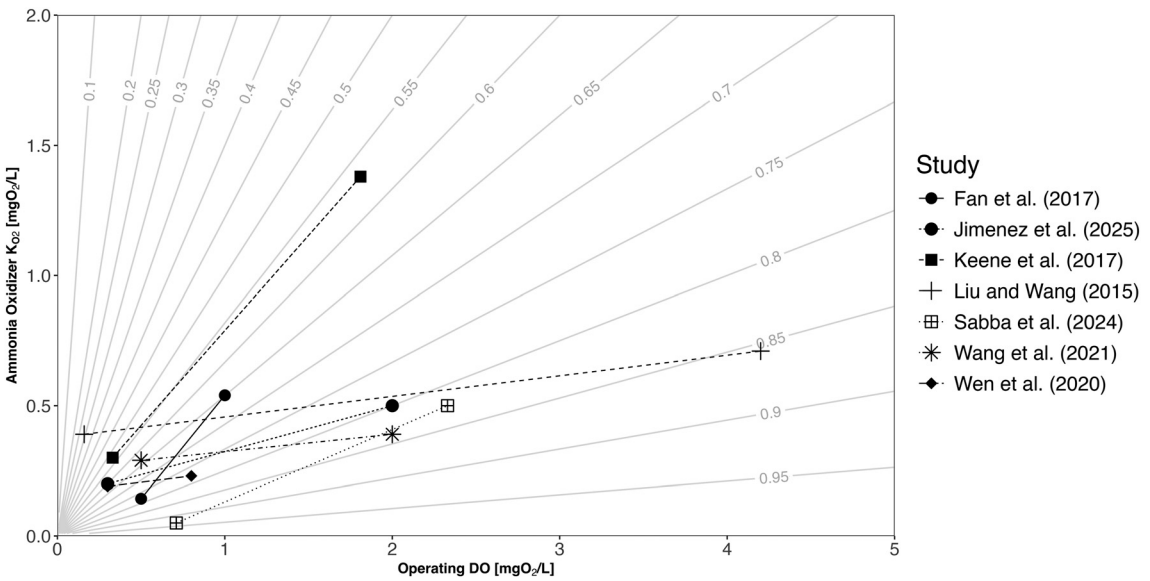

Fig. 3 Ammonia oxidation capacity contour plot showing relative ammonia oxidation rate for different values of operating DO and ammonia oxidizer K_{O_2}. Relative ammonia oxidation rate assumes all else is held equal and is calculated *via* the Monod function ($S_{O_2}/(K_{O_2} + S_{O_2})$) where S_{O_2} is the operating DO. Gray lines indicate equal-rate contours, *i.e.* all combinations of operating DO and ammonia oxidizer K_{O_2} that fall along a contour will give the same relative ammonia oxidation rate. When operating DO is reduced, ammonia oxidizer K_{O_2} must also reduce along a slope parallel the nearest line, or ammonia oxidation capacity will be different at the lower operating DO. Experimental findings where estimates of ammonia oxidizer K_{O_2} was reported at different operating DO are shown on the plot. Slopes less than the slope of surrounding contours indicate a loss of capacity, greater slopes a gain of capacity (the more significant the deviation, the greater the relative loss or gain in capacity). Studies included in this figure are only those that specifically reported both high and low operating DO values and ammonia oxidizer K_{O_2}.

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and to what extent these population shifts will occur in any given low DO process is still an area of active research.

3.1. Canonical ammonia oxidizing bacteria

Ammonia oxidizing bacteria (AOB) are a broad set of chemolithotrophic bacteria capable of oxidizing ammonia to nitrite; known for over a century.[32] Numerous researchers have investigated their potential to perform ammonia oxidation at low DO concentrations. Park and Noguera[33] ran two chemostats with enriched nitrifier cultures (10 day SRT) at high and low DO concentrations. It was determined that the reactors grew nitrifiers that were phylogenetically distinguishable, but both belonged to the *Nitrosomonas europaea* sublineage. The researchers claim that the modelled K_{O_2} values for the two enrichments differed, but the differences appeared quite small and the confidence intervals of the estimates overlapped. Interestingly, the determined maximum specific growth rate for the low DO culture was significantly higher than for the high DO, but this is not commented on in the work. This work also documents a full-scale experiment in which two parallel activated sludge treatment trains were maintained, one with high DO and one with low DO for 6 months. At the end of the experiment, the AOB identified in the low DO process mostly belonged to the *Nitrosomonas europaea* sublineage, while the high DO train AOB were primarily associated with *N. oligotropha*. Park and Noguera[34] also found that by isolating and kinetically testing two strains of AOB found in low DO nitrifying reactors that one had a high affinity for oxygen and a low affinity for ammonia (0.24 mgO_2 per L and 1.62 mgN per L respectively) and the other had a low affinity for oxygen and a high affinity for ammonia (1.22 mgO_2 per L and 0.48 mgN per L respectively). Bellucci *et al.*[35] ran two sets of high and low (0.5 mgO_2 per L) DO reactors on synthetic wastewater with the same SRT to demonstrate the ability of nitrifiers to grow and fully nitrify in low DO conditions. The ammonia oxidizers that grew in the low DO reactor were capable of complete ammonia removal and were estimated to have an unusually high yield. The yield was inferred from calculations where the AOB population (X_{AOB}) was determined using a combination of biomass measures assuming a specific cell size and density and cell counts from qPCR. Metagenomic techniques were also employed to determine that the ammonia oxidizing community in the low DO reactor was a subset of and contained fewer species than the ammonia oxidizing community in the high DO reactor.

3.2. Ammonia oxidizing archaea

Numerous researchers identified the potential for AOA to play a role in low DO nitrification. AOA have been noted to be successful ammonia oxidizers at extremely low DO concentrations (<0.1 mgO_2 per L).[36,37] Measurements of intrinsic AOA K_{O_2} have been performed by multiple researchers; reported values range from 0.038–0.125 mgO_2 per L (see Table 1). These K_{O_2} values are lower than those typically estimated for canonical or mixed nitrifier communities, which range from 0.25 to >1.0 mgO_2 per L under typical operating DO.[20] Researchers have also found AOA present in full-scale activated sludge plants.[38–40]

Park *et al.*[46] used PCR to measure the presence of archaeal amoA in sludge samples from five water resource recovery facilities (WRRFs) operating at long SRTs and low DO. Their results provided evidence that AOA may be common in low DO nitrifying reactors. Wells *et al.*[47] analyzed the abundance of different organisms in a full-scale WRRF and found the abundance of AOB amoA genes were strongly negatively correlated with operating DO and no correlation was found between DO and AOA amoA gene abundance. However, the plant was only operated at high DO concentrations. This was proposed as evidence that specific AOB may adapt to low DO conditions better than others (in this case *Nitrosospira*), and others have identified this possibility as well.[48] Giraldo *et al.*[49] observed that in a full-scale membrane bioreactor operated at low DO, the biomass had a very low K_{O_2} value and using qPCR identified AOA as the dominant nitrifier. Liu *et al.*[50] operated a 40 day SRT lab-scale membrane activated sludge reactor fed with synthetic wastewater at high DO (2.0 mgO_2 per L) and low DO (0.4 mgO_2 per L) and found that AOB abundance decreased while AOA abundance increased dramatically with lowering DO. The reported ratio of AOA/AOB amoA in the low DO condition was >19×. Jimenez *et al.*[24] conducted a survey of 13 full-scale low DO facilities and found that for plants operated below 0.5 mgO_2 per L, AOA abundance was 20% on average. Data were also provided for a single plant that transitioned from an operating DO of 2.0 mgO_2 per L to 0.3 mgO_2 per L, and AOA abundance was found to increase significantly from 9% (±6.2%) to 31% (±8.4%).

Since the development of methods to detect AOA, not all researchers experimenting with low DO nitrification have found them. Liu and Wang[51] operated two bench-scale

Table 1 Estimated AOA Monod oxygen-half saturation values from the literature. Values in parenthesis are reported standard deviations

Study	Reported K_{O_2} (mgO_2 per L)	Organism	Enrichment
Martens-Habbena *et al.*[41]	0.125 (0.018)	Marine AO (*Ca. Nitrosopumilus maritimus* strain SCM1)	Pure culture
Park *et al.*[42]	0.064 (0.014)	Marine sediment AOA (strain AR)	Enrichment culture
Qin *et al.*[43]	0.109 (0.00042)	Marine AO (*Ca. Nitrosopumilus maritimus* strain SCM1)	Pure culture
Qin *et al.*[43]	0.038 (0.00048)	Marine AOA (strain PS0)	Pure culture
Straka *et al.*[44]	0.093 (0.016)	Freshwater AOA (strain DW and AC2)	Enrichment culture
Straka *et al.*[44]	0.090 (0.045)	Soil AOA (*Nitrososphaera viennensis*)	Pure culture
Xie *et al.*[45]	0.131 (0.08)	Activated sludge + soil AOA (*Nitrosocosmicus* sp.)	Enrichment culture

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activated sludge reactors fed synthetic wastewater at 10 day and 40 day SRTs. Both reactors were operated in periods with high and low DO and no AOA were detected in either phase. Canonical AOB remained the dominant ammonia oxidizer and it was hypothesized this was due to the greatly reduced nitrifier decay rate at low DO which allowed the total population size of the nitrifiers to increase, not due to a change in K_{O_2}, yield, or growth rate. Arnaldos *et al.*[52] ran two enriched culture sequencing-batch-reactors (SBRs) at high and low DO. AOA were not detected in either reactor but the same lineage of AOB was predominant in both; the low DO ammonia oxidizers had a lower K_{O_2}. Interestingly, the low DO nitrifying community developed enhanced expression of a heme protein within the cells, which was hypothesized to decrease the intracellular DO concentrations thus increasing the diffusive force of oxygen into the cell from the bulk liquid. Fitzgerald *et al.*[53] ran two lab-scale, enriched culture reactors at 10 day SRTs at DO concentrations of 0.3 mgO_2 per L or less. One of the reactors was seeded with sludge from a WRRF operating at high DO, the other from a WRRF operating at low DO. Almost no AOA were detected in either seed sludge or at the end of the experiment, demonstrating that AOA were not necessary for successful low DO nitrifier adaptation. The reactor seeded with low DO acclimated sludge began nitrifying at low DO quickly (<25 days) while the reactor seeded with high DO acclimated sludge took over 80 days to adapt and nitrify fully.

The state of the current literature on AOA as major factors in low DO nitrification is not clear, although there is no doubt remaining that AOA are capable of ammonia oxidation at very low DO concentrations and are present in some wastewater treatment plants. The earliest reports on the phenomena found both the presence[46] and absence[35] of AOA in low DO nitrifying reactors. Over a decade later, researchers of low DO nitrification still find conflicting results.[50,53] The literature exploring this issue comes primarily from bench-scale reactors fed with synthetic wastewater at conditions that may differ from full-scale processes or from comparisons between samples taken from different wastewater treatment plants. At the very least, it would appear unwise from an engineering design perspective to rely upon the growth of AOA for low DO nitrification, and caution would be advised in reading too much into the measured kinetic parameters of different AOA strains as indicative of what would be expected in a full-scale WRRF. The exact conditions that cause the presence or absence of AOA in low DO processes are still not fully understood, and additional kinetic factors such as ammonia affinity and differential growth rates between and within species undoubtedly play a role.

3.3. Comammox

In 2015, a *Nitrospira* species was discovered that was capable of complete nitrification of ammonia to nitrate.[54,55] This organism is referred to as comammox (complete ammonia oxidation) and has been of interest to researchers given its potential favorable kinetics for low DO, lower N_2O emissions,[56] and prevalence within nitrification processes despite being previously unrecognized. Different comammox (CMX) strains and clades have been identified, and they have been found in a diversity of activated sludge processes[57–62] and even drinking water systems[63] around the world. It has been suggested that CMX have low maximum ammonia rate and high growth yield.[64] This is particularly interesting because for the past 30 years some researchers reported that nitrifiers had high yield when adapted to low DO (see Section 3.1). Recently attempts have been made to characterize the intrinsic K_{O_2} of these organisms (Table 2); both studies found CMX to have extremely high oxygen affinity (K_{O_2} < 0.02 mgO_2 per L).

Camejo *et al.*[58] operated an 80 day SRT SBR fed synthetic wastewater and seeded with biomass from a full-scale process. Oxygen concentrations were kept low (<0.5 mgO_2 per L) and significant CMX abundance was noted (very little AOA were identified). During a second phase of the experiment, still at low DO, CMX abundance dropped to near zero but full nitrification was maintained; no other known AOB or AOA were detected in this second phase and the authors speculated the presence of still-unrecognized ammonia oxidizing organisms. Roots *et al.*[67] operated an intermittently aerated SBR using real wastewater for influent. DO setpoints were kept at 1.0 mgO_2 per L or lower and complete nitrification was achieved; the process performed better than the full-scale high DO nitrification reactor at the facility from which the raw influent was taken, which was used as a control. After significant time operating at reduced DO, CMX composed >90% of ammonia oxidizers (measured by amoA) in the reactor. It should be noted the reactor was run with a very long SRT (99 days). Beach and Noguera[68] pointed out some potential issues with qPCR primers in use at the time with detecting CMX and designed three new primers for some candidate CMX species; they found CMX present in the majority of samples analyzed from low DO systems, none in the high DO systems, and noted that the largest abundance was found in systems with long SRTs. How *et al.*[60] operated a lab-scale nitrifying SBR fed real wastewater at a DO concentration of 1.7 mgO_2 per L and then 0.9 mgO_2 per L with a 20 day SRT and consistently achieved full nitrification. CMX were found to be the dominant nitrifiers throughout the experiment. Wen *et al.*[20] reported on a full-scale membrane bioreactor (MBR) process that transitioned from relatively low

Table 2 Estimated CMX Monod oxygen-half saturation values from the extant literature. Values in parenthesis are reported 95% confidence intervals

Study	Reported K_{O_2} (mgO_2 per L)	Organism and enrichment
Hou *et al.*[65]	0.006 (0.001)	*Ca. Nitrospira nitrosa*-dominant enriched sludge
Zhu *et al.*[66]	0.017 (0.002)	"*Nitrospira*-dominant" sludge (comammox enrichment)

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(0.8 mgO_2 per L) to even lower (0.5 mgO_2 per L) operation at an 18 day SRT. Complete nitrification was maintained at both DO setpoints, and the already relatively low determined K_{O_2} of AOB (0.23 mgO_2 per L) decreased further to 0.19 mgO_2 per L (although the error of these parameter estimates is not reported in the study, and it is possible these two values are not significantly different). *Nitrospira* were detected as the main nitrifying organism during the study, and it was speculated that these could be comammox or another unknown AOB. Li *et al.*[69] operated two bench-scale MBRs at high and low DO, 2.0 mgO_2 per L and 0.5 mgO_2 per L respectively, and found that while both were capable of full nitrification, the high DO reactor ammonia removal was more consistent. CMX was found to be the dominant nitrifier in both reactors, particularly in the low DO reactor (the CMX/AOB amoA ratio was >17). Jimenez *et al.*[24] found in their low DO survey that for full-scale plants operated below 0.5 mgO_2 per L, CMX abundance was 66% on average. In the same study, a single plant was monitored before and after transition from an operating DO of 2.0 mgO_2 per L to 0.3 mgO_2 per L; CMX abundance was found to increase significantly from 11% (±4.7%) to 60.5% (±10.9%). Two recent studies have attempted comammox enrichment for the purpose of kinetic estimation. Hou *et al.*[65] attempted to enrich *Candidatus Nitrospira nitrosa* under low DO conditions and succeed in increasing its prevalence 26.5 fold; the results of their subsequent kinetic measurements are shown in Table 2. Zhu *et al.*[66] comparatively enriched *Nitrospira*-dominant (comammox) and *Nitrosomonas*-dominant floccular sludges and also reported a lower apparent oxygen affinity constant for the *Nitrospira*-dominant sludge, which are reported in Table 2.

Two studies have discussed the potential for only certain clades of CMX to be preferentially selected by low DO. Zheng *et al.*[70] cultivated activated sludge samples in lab-scale reactors fed only ammonia with different DO concentrations ranging from 0.5–17 mgO_2 per L. CMX were found to be the majority nitrifiers in each reactor and in the lowest oxygen reactor were enriched significantly beyond the predominance found in the seed sludge. AOB and AOA were also detected, but at much lower levels (making up 10% or less of the nitrifying population as measured by 16 s rRNA gene abundance). The majority of CMX in the low DO reactors was made up of a CMX strain from clade A, although they noted a different clade A strain appeared to be more prevalent at higher DO. Sabba *et al.*[62] noted increased comammox abundance in a low DO process (10–12 day SRT) and a high DO process (12–14 day SRT), and found no comammox at another high DO facility (8.5 day SRT). The low DO facility had a very low estimated K_{O_2} for nitrifiers (0.05 mgO_2 per L) but this estimate was made using DO measurements that only went down to 0.25 mgO_2 per L and CMX clade A and clade B were present. Both high DO facilities had higher estimated K_{O_2} for nitrifiers (0.5 mgO_2 per L at both) and one had CMX clade A present. Thus, it was suggested that the ability of CMX to grow at low DO and provide complete nitrification may not be a feature of the species, but only of specific strains.

Two early studies on CMX in nitrification processes operated reactors at quite long SRTs,[58,67] and other researchers have noted that CMX may be more prevalent or require long SRT systems.[59] However, more recent full-scale low DO studies have found CMX in abundance in relatively shorter (<15 day) SRT systems,[62] including its consistent presence in over a dozen full-scale low DO facilities with SRTs ranging from 4.5–18.2 days.[24] It appears, then, that long SRT is not a requirement for low DO CMX. Much is also left to learn about potential differences within CMX strains, and why some experiments appear to show CMX predominance at both low and high DO concentrations. As CMX may not be particular to low DO, the understanding of their role and presence in engineered systems is currently not enough to design around.

It does not appear that any low DO activated sludge studies, since the discovery of CMX and development of primers to detect it, have failed to detect CMX (although Liu *et al.*[50] found it to be only a very small fraction); this may in fact be evidence against its particular significance to engineering design. If the organism is so ubiquitous, especially at low DO, then no special steps may be necessary to grow it. If it can be shown to have measurably different in-process kinetics, this still may not necessitate a focus on the organism itself for design (unlike the case of other novel organisms like anammox), only that those different kinetics are used for design purposes.

To provide a clear picture of the current low DO knowledge landscape, studies where both high and low DO operation were documented, and estimated ammonia oxidizer K_{O_2} values were provided for both, are shown in Table 3. The nitrifier population information, if provided, is also summarized therein.

4 Ammonia oxidizer adaptation to low DO due to change in extant kinetics

The second category of changes to the kinetics relevant to ammonia oxidizer low DO adaptation is change in the extant, or observed, kinetics. Extant kinetics may include anything not considered in the intrinsic kinetics of the organisms; for nitrifiers this has often been advection and diffusion limiting mechanisms, such as the floc size (in a suspended growth system) and more recently the microcolony size and number (within the floc). Both of these factors have been found relevant to nitrifier oxygen kinetics, and are thus of interest when considering adaptation to low DO.

4.1. Floc size

Based on a general understanding of advection and diffusion, floc size (and the related field of biofilm formation/thickness) have long been of interest to wastewater process researchers. Early on Beccari *et al.*[72] demonstrated an empirical relationship between K_{NH} and measured floc size. Beccari *et al.*[73] then developed an early model to demonstrate that floc size

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Table 3 Overview of ammonia oxidizer kinetic outcomes from studies that specifically reported both operating DO and estimated ammonia oxidizer K_{O_2} values for high and low operating DO values. Standard deviations are reported, where present, as provided by original study authors

Study	Processes	Operating DO (mgO_2 per L)		Estimated K_{O_2} (mgO_2 per L)		Same biomass[a]	Comment
		High	Low	High	Low		
Sabba *et al.*[62]	Full-scale BNR	5.00	0.71	0.50	0.05	N	No AOA detected. CMX clade A found at high DO, CMX clade A + clade B found at low DO
Wang *et al.*[31]	Lab-scale BNR	2.00	0.50	0.39 ± 0.05	0.29 ± 0.04	Y	Slightly higher canonical AOB and *Nitrospira* abundance at low DO
Wen *et al.*[20]	Full-scale MBR	0.80	0.30	0.23	0.19	Y	No AOA detected. *Nitrospira* was the only known nitrifier detected
Keene *et al.*[71]	Full-scale BNR + pilot BNR	1.80	0.33	1.38 ± 0.23	0.30 ± 0.14	N	No AOA detected. Overall nitrifier population diverged between high and low DO processes
Fan *et al.*[30]	Lab-scale BNR	1.00	0.50	0.54 ± 0.09	0.14 ± 0.02	Y	—
Liu and Wang[29]	Lab-scale BNR	4.2	0.16	0.71	0.39	Y	—
Jimenez *et al.*[24]	Full-scale BNR (A/O)	2.0	0.3	0.5	0.2	Y	Increase in AOA and CMX abundance, details provided in text

[a] "Same biomass" indicates whether a study compared the behavior of a single biomass in a process at different operating conditions (Y), or two different biomasses from two different processes (N), where operating conditions, process configurations, and influent characteristics may differ.

significantly impacted nitrifier oxygen kinetics, and validated this model with experimental data. Their steady-state model assumed uniform biomass distribution with fixed intrinsic kinetic parameters in a spherical floc with negligible external diffusional resistance (essentially no boundary layer). Experimental data to validate the model came from batch tests run with non-limiting ammonia concentrations and low TSS concentrations (very high F/M) wherein DO was increased to non-limiting conditions and modelled as it dropped to zero; floc sizes were measured with a particle size analyzer. They found that at particle size diameters ≤40 μm the internal diffusion resistances can be neglected, but at larger diameters (common in wastewater treatment) and especially at low DO the impact of floc size becomes pronounced: larger internal diffusion resistance will lead to higher apparent measurements of K_{O_2}.

Chu *et al.*[74] conducted measurements of heterotrophic K_{O_2} in a respirometer at various mixing energies (mean energy dissipation rate). They found that decreased mixing energies led to larger floc diameters and much higher K_{O_2} value below a minimal threshold. It should be noted, however, that mixing energy can affect both floc size and advection, and this study does not attempt to differentiate these impacts separately. Manser *et al.*[75] operated an MBR and a conventional activated sludge (CAS) pilot plant to develop and measure the oxygen kinetics of small (80 μm) and large (300–500 μm) floc systems respectively (both at 20 day SRT with non-limiting DO concentrations). Floc size was measured using a laser light scattering device. A model was developed similar to Beccari *et al.*[73] except that a boundary layer was included and the floc biomass was heterogenous – nitrifiers were assumed to be concentrated in the interior of the floc and heterotrophs were distributed uniformly throughout. The experimental and modelling results demonstrated that AOB, NOB, and OHO K_{O_2} values were estimated to be 2–3 times higher in the process with larger floc sizes, and this difference could be reasonably well explained with their floc model. Daigger *et al.*[76] used DO microprobes to actually measure the DO concentration within individual flocs, providing direct evidence of what had previously been speculated and modelled to occur. Wu *et al.*[77] used sonication to break apart flocs and achieve different floc sizes. They found that the measured K_{O_2} of AOB increased from ~0.20 mgO_2 per L at 50 μm average floc diameter to ~1.25 mgO_2 per L at 450 μm average floc diameter. However, the NOB K_{O_2} was found to be less sensitive to floc diameter. The lower NOB K_{O_2} observed in this study was explained by their 1-D diffusion model, but the authors speculate microcolony structure (discussed in the next section) plays an important role as well. Keene *et al.*[71] demonstrated that their low DO pilot process (<0.33 mgO_2 per L) had smaller floc sizes compared with the control high DO full-scale process (152 μm *vs.* 117 μm, measured *via* image analysis). Ammonia oxidizers in the low DO pilot had a lower measured K_{O_2} as well (0.30 mgO_2 per L *vs.* 1.38 mgO_2 per L). Based on the previously published models and experimental data, Keene *et al.*[71] finding such a large difference in K_{O_2} with such a small difference mean floc diameter may well be evidence against floc size necessarily being a major driver of K_{O_2} in these systems. Yu *et al.*[78] explored AOB/NOB kinetic competition but in the process of doing so reported estimated AOB K_{O_2} values at varying floc sizes. Data from these studies, as well as those on the impact of microcolony size on nitrifier K_{O_2}, are summarized in Fig. 4. This figure shows the overall positive correlation, and even similar slopes found amongst some researchers, between floc size and nitrifier K_{O_2} across a wide range of particle sizes.

Floc size may significantly influence ammonia oxidizer K_{O_2}, and is itself influenced by process operational conditions. SRT in particular has been observed to play a role in floc size. Hocaoglu *et al.*[79] found a reduction in floc size at

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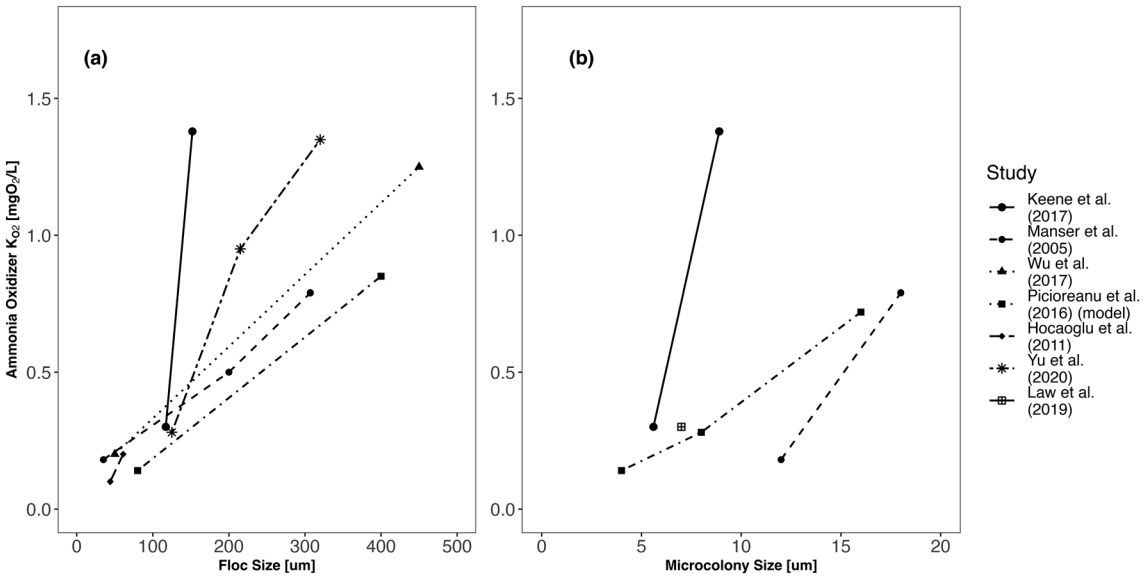

Fig. 4 Reported ammonia oxidizer K_{O_2} values for differing floc sizes and microcolony sizes as reported in the literature.

reduced SRT (60 days to 20 days) in an MBR along with lower model-estimated K_{O_2} values. Li and Stenstrom[80] also documented a positive relationship between SRT and floc size in a lab-scale MLE, lab-scale IFAS, as well as with full-scale data.

4.2. Microcolonies

In addition to floc size, what occurs within the floc may determine observed K_{O_2} just as much, if not more. While this field of research is much newer, there has been significant research interest in the impacts of nitrifier microcolonies, or cell clusters, within flocs. Manser *et al.*[81] ran an MBR and CAS pilot in parallel and found using metagenomic techniques no significant difference in nitrifying population. They found that the MBR system not only lead to smaller floc sizes, but to smaller AOB microcolony sizes as well. Picioreanu *et al.*[82] constructed a 3D model of AOB and NOB microcolonies within flocs at different sizes and used this to analyze the data previously collected by Manser *et al.*[81] They demonstrated with this model that larger microcolonies, like larger flocs, lead to higher observed K_{O_2} values. They claim the impact of colony size is more significant than the impact of floc size, but this significance is itself contingent upon numerous additional variables (biomass concentration, number of colonies, uniformity of colony size, *etc.*). Picioreanu *et al.*[82] also claimed that in general longer SRT could lead to larger microcolonies, mirroring the relationship with floc size. Thus, longer SRTs may lead to higher extant K_{O_2} values, if microcolony size is in-fact a dominant driving force in the phenomena.

Keene *et al.*[71] observed, along with the reduction in floc size mentioned previously, a reduction in AOB colony size at low DO. This reduction (40%) was larger than the noted reduction in floc size (20%). Either or both of these reductions are posited to have contributed to the lower observed K_{O_2} of nitrifiers at low DO. Law *et al.*[19] in a study on AOB *vs.* NOB kinetic selection *via* DO concentration changes, demonstrated that AOB and NOB expressed very different microcolony structures. They suggest, among other things, that apparent half-saturation values may be a function of microcolony shape and porosity, not merely microcolony diameter, adding an additional layer of complexity to the claims of Picioreanu *et al.*[82]

5 Single and multiple substrate limitations in the context of ammonia and oxygen limitation of ammonia oxidation

The majority of work on low DO nitrification processes has been done on systems that achieve, or seek to achieve, complete ammonia oxidation. However, novel processes such as ammonia based aeration control (ABAC), partial-denitrification anammox (PdNA), and partial-nitritation anammox (PNA) and their various implementations are designed to perform incomplete aerobic nitrification, leaving an appreciable ammonia concentration throughout the nitrification process. For the anammox processes, ammonia is not oxidized completely in the aerobic process so that anaerobic ammonia oxidation may occur. These processes have been an area of intense research for the past few years, and full-scale implementations have been achieved.[83,84] The drivers for PdNA and PNA are reduced external carbon demand for denitrification, reduced oxygen demand for aerobic ammonia oxidation, and reduced supplemental alkalinity design. These processes can also realize significant capacity savings, as maximum available aerobic SRT for nitrification generally determines the capacity of a BNR

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process, and anammox allows shifting the burden of ammonia oxidation from aerobic to anaerobic (often biofilm) processes.[85]

Little-to-no work has been done to analyze how this residual ammonia concentration (non-starvation conditions) intersects with low DO operations. As documented by Arnaldos *et al.*[15] with respect to other organisms, differing substrate concentrations and conditions may lead to differing substrate degradation pathways. In the numerous experiments discussed so far, some processes had no measurable effluent ammonia, meaning some portion of the process must be substrate limited (starvation), while others had 1–2 mgNH_x–N per L or more; little discussion or attention is given to this by study authors. Of course, residual ammonia concentration is also dependent on the SRT of the system[9] so the two factors, substrate limitation and SRT, cannot be easily disentangled.

The possibility of multiple limiting substrates (in this case, oxygen and ammonia) has long been discussed in the activated sludge literature. The earliest assumptions were that only a single substrate could be limiting.[9] If there were potential for multiple limiting substrate it was taken to be non-interactive, meaning only one substrate could be limiting at a time (the most limiting one). Double Monod (interactive) functions, where two Monod limitation functions are multiplied together, were suggested[86] but also dismissed by other researchers.[87] Doubts were expressed both ways, whether it would be right to dismiss the complexity of an engineered process with many substrates or how it could make sense to multiply together ever-more limiting mathematical terms.[9,88,89] Numerically, the multiplicative Monod functions are simpler to solve than the non-interactive single limiting substrate functions.

Additional interacting models of varying complexity were introduced over time. It is not clear exactly if or how this issue was resolved, but most activated sludge modelling software available today appears to rely primarily, but not solely, on multiplicative Monod functions. Stewart *et al.*[90] provide a thorough overview and analytical comparison between some single and multiple Monod models. There does not appear to be a simple answer to how to apply these models to oxygen and ammonia limitations for nitrifier rates, but most of the research already surveyed herein appears to use the multiplicative model.

Beyond the question of how to model the potential interaction of multiple Monod terms, there appear open questions about what occurs at the biological level with multiple limiting substrates. Some research suggests that different ammonia concentrations can select for different types of AOB through different substrate affinity, and a similar phenomenon applies to oxygen as well.[91] Park and Noguera[34] documented two strains of AOB which had either high affinity for DO and low affinity for ammonia, or *vice versa*. However, low DO operation has been shown to shift NOB communities towards populations that have low half-saturation values for both oxygen and ammonia.[92] Microcolony or cluster density has also been suggested as a cause for the large range of K_{NH} and K_{O_2} observed in AOB, even within the same lineages,[91] but this is unfortunately one of the more limited areas of the literature currently. Very low ammonia concentrations may drive AOA growth, as they have been reported to have K_{NH} values for ammonia far below those of AOB.[93,94] Liu *et al.*[50] hypothesized this kinetic difference was responsible for the shift of nitrifier population to AOA at low DO in their experiment. CMX have similarly been reported to have significantly higher affinity for ammonia.[61]

6 Nitrifier decay rate and low DO

Multiple studies of microbial cultures have shown that AOB survive starvation well, and have a good ability to generate energy as soon as substrates are available again.[91] It has been well documented that nitrifier decay rates are much higher, an order of magnitude larger or more, in aerobic conditions *versus* anoxic conditions.[95,96] Low DO conditions could therefore impact not only growth conditions, but decay conditions as well, and there is very limited evidence that ammonia concentrations could play a role in decay also. Little literature exists comparing oxygen or ammonia limitation impacts on nitrifier decay, but some pure culture work shows that ammonia starvation drives a 5-fold higher decay rate than oxygen starvation,[97] though these results have been met with skepticism and have not been replicated.[91] The role of ammonia starvation on decay is not reflected in any current models, perhaps with good reason as ammonia is available throughout the decay process (thus also available for regrowth) from the death and lysis of other organisms (namely heterotrophs), as will be discussed further on.

Most of the low DO work herein surveyed has paid little attention to decay rates: pure and enriched culture studies rarely measure it distinctly and the original ASM models do not include any kinetic modifiers to nitrifier decay but instead treat it as constant.[98] Munz *et al.*[99] provides a good overview of how other researchers have proposed implementing decay dynamics in ASM models as well as proposing their own implementation. The authors recognize that aerobic, anoxic, and anaerobic decay rates of nitrifiers differ and various kinetic switches between these rates are discussed and analyzed. The kinetics can become quite complex and difficult to measure, depending on whether the K_{O_2} values for decay and growth are the same or distinct (both approaches are used in the literature). If independent values for K_{O_2} for decay are used, they appear to typically be drawn from decay rate measurements at various high DO concentrations[99] or extrapolation between highly aerobic and anoxic decay rates.[95,96,100]

Two studies (Liu and Wang[29,51]) focused on decay as a driver for low DO nitrification specifically and determined the decreased decay rate at low DO conditions was the primary contributor to why nitrification rates did not decrease at low DO. Liu and Wang[51] used metagenomic evidence to bolster this claim. Liu and Wang[29] modelled

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steady state operation across a range of DO setpoints and fit a model to the reactors' effluent NH_x *vs.* DO. This model assumed two separate, but constant, K_{O_2} for growth rate and decay rate (each a single Monod function). This model form, in disallowing for adaptation (different K_{O_2} at different DO setpoint), forces the variability across the DO operating conditions to be a function of decay difference, and the model fits are very dependent on very small differences in effluent ammonia. Further, this work also suggested that the nitrifiers do adapt and different K_{O_2} values were measured using batch-tests from the highest and lowest DO reactors. However, the authors do not attempt to reconcile these two methods, and no attempt is made to separately batch-test measure the decay rate or K_{O_2} for the decay rate.

Mehrani *et al.*[101] proposed a lower value for comammox decay rate than conventional nitrifiers but did not provide a literature source for this value. As far as can be determined, no explicit considerations for decay rate have been proposed for AOA.

Methods used to measure decay generally follow the same format: a reactor of sludge is aerated for many days and samples are taken from this reactor at intervals and the maximum nitrification rate is measured.[102] The decline in this maximum nitrification rate over time is an indicator of the decay rate. It should be noted, then, that this method to measure decay rate is subject to the problems of transferability as well, and here to a large degree as the conditions in the decay reactor will differ widely from those the organisms ever experience in the treatment process. There are no commonly accepted alternatives to measure this parameter. There are shorter-duration, but still *ex situ*, methods for measuring maximum specific growth rate and decay simultaneously, but these require assumptions about one or the other value and do not get around the transferability problem.

Melcer *et al.*[103] performed a comprehensive evaluation of many kinetic estimation methods including nitrifier decay rate. They note that estimated decay rates in the literature have not only varied widely, but varied with time as different methods for measuring decay were popular during different decades. Experimental methods for determining maximum specific growth rate will be more or less sensitive to decay rates depending on the SRT of the experiment. A new modelling approach to estimate decay was developed by this group, taking into account the ammonia released by heterotrophic death–lysis–hydrolysis in the decay reactor. This method requires regularly measuring the nitrate concentration in the decay reactor but can impact the calculated nitrifier decay value by almost 50%. If this is the case, this would call into question many of the nitrifier decay rate estimates published before this paper, as this method was novel when introduced. Pruden *et al.*[104] provide the most comprehensive recent analysis of nitrifier decay rate measurements in which decay rate measurements were performed using typical methods and DNA measurement methods. Using the method of Melcer *et al.*[103] they found similar values of nitrifier decay rates. However, it was noted in their work that considering the ammonia production in the decay reactor from heterotrophic decay made almost no difference to the estimated decay rate, in stark contrast with Melcer *et al.*[103] They also noted considered variability in nitrifier activity measurements (more so AOB than NOB) across hundreds of tests at various times and temperatures, noting that many outlier values had to be discarded (and different decay values were estimated, depending on which values were taken to be outliers). Importantly, the AOB decay rate estimated using DNA measures was 50% less than the AOB decay rate estimated by loss of nitrification activity.

The potential for nitrifier dormancy rather than true decay should also be considered. Because decay is typically inferred from a loss of measured nitrification activity, it can be difficult to distinguish inactivity from biomass loss. Dormancy is generally viewed as reversible, but the timescale for reactivation is not well defined. Comparative measurements of dormancy *versus* decay are also limited. For example, AOB can maintain abundance while showing significantly reduced amoA expression during cold-temperature nitrification failure.[105] Johnston *et al.*[106] found that AOB and AOA could be revived after two months of starvation, long after AOB amoA dropped below detection limits. Pruden *et al.*[104] note that molecular detection does not confirm viability, while culture-based counts can underestimate viable cells (*e.g.*, sublethal injury can yield nonculturable but still viable cells). Friedrich *et al.*[107] also suggested that differential DNA marker *versus* activity decay rates may be due to dormancy.

For consideration in design, it is likely that decay rates and their kinetics play a role in low DO nitrification and adaptation, but to what extent is not easily demonstrated. Decay rate is a critical kinetic as it determines the population (X_{NITO}) of nitrifiers in a process at a given load and SRT, and the nitrifier population and maximum specific growth rate together determine the maximum ammonia uptake rate of the process, which governs capacity. It is also well established that nitrifier decay rates depend on oxygen concentrations (at the very least, they are quite different between anoxic and aerobic conditions). Given a model structure where decay rates vary as a function of DO it would be difficult to determine whether nitrifiers at low DO have lower decay rates *per se* (intrinsic kinetics) or they are merely kinetically limited due to operating DO (extant kinetics) and would be harder still to determine if this decay kinetic factor is itself altered by operating DO concentration (K_{O_2}-decay is itself adapting). A discussion of extant and intrinsic decay kinetics and decay kinetic adaptation with respect to nitrifiers and oxygen appears to be absent from the literature.

Current decay rate values, and their associated oxygen kinetics, despite being one of the most important factors affecting nitrifier population and therefore nitrification capacity, should be met with some skepticism as:

1) It is not clear whether decay oxygen kinetics should share the same K_{O_2} value as growth rate, and if not, how it should be measured.

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3) No attempt has been made to understand decay adaptation to low DO and what proportion is extant or intrinsic.

3) No explicit measures of decay rates at low DO exist, only extrapolations from high DO decay or anoxic decay.

4) Decay rates in the existing literature may have been measured without taking into account ammonia release from heterotroph decay, but reports vary significantly on whether this matters.

5) AOB decay rate is much more variable than NOB decay rate even when measured using the best methods available, but it is not clear why.

6) AOB decay does not align with death (measured *via* DNA destruction) implying some kind of dormancy may occur, and measured decay rates may only reflect an immediate effect on nitrification capacity.

7) Current measures of decay are *ex situ* methods that differ drastically from extant, in-process conditions.

7 Conclusions

This critical review has surveyed the literature on low dissolved oxygen ammonia oxidation with regard to understanding the mechanisms by which ammonia oxidizer communities adapt to low dissolved oxygen conditions. The evidence demonstrates that stable ammonia oxidation at low DO is achievable in activated sludge systems and consistently correlates with reduced apparent ammonia oxidizer K_{O_2} values. Studies spanning laboratory reactors to full-scale wastewater treatment plants have documented successful ammonia oxidation at DO concentrations well below the conventional 2.0 mgO_2 per L threshold.

Although early studies noted the possibility for ammonia oxidizer adaptation to low DO conditions, in the intervening decades enough data has been gathered to lay out a more concrete understanding of this phenomenon. The mechanisms underlying this adaptation can be characterized as belonging to two different pathways, although they lie along a spectrum: intrinsic and extant changes; the available data on these mechanisms has been presented in this study.

Intrinsic kinetic adaptation involves shifts in the ammonia-oxidizing community toward organisms with higher oxygen affinity. Both AOA and CMX have been shown to have very high oxygen affinity, and both have been documented in low DO systems. In fact, every low DO nitrification study that has looked for CMX has found them. Work on improving CMX detection method accuracy is ongoing; further information on CMX primer development and specificity for detecting clade A and clade B can be found in.[108–110] The predominance of these organisms with lower intrinsic K_{O_2} would suggest lower observed K_{O_2} in these systems, which the published research demonstrates.

Extant kinetic adaptation encompasses a broad category of physical and structural changes that affect the apparent oxygen affinity of the biomass without necessarily changing the underlying microbial community. Significant data with respect to ammonia oxidation and operating DO has been gathered for two of these factors: floc size and microcolony size. Multiple studies have demonstrated a link between floc size and apparent K_{O_2} as well as microcolony size and K_{O_2}. Increased floc size leads to higher apparent K_{O_2}, most likely through an increase of diffusional resistance against DO entering the floc.

It remains unclear how intrinsic (community) shifts and extant (mass-transfer/structure) effects interact *in situ*, largely because almost no studies reporting reduced apparent K_{O_2} concurrently quantified floc or microcolony structure. The limited available evidence (one study) suggests that intrinsic shifts can be the dominant driver. In the only study that paired oxygen-kinetics shifts with detailed community characterization, a substantial decrease in apparent K_{O_2} coincided with a marked nitrifier community shift (including increased CMX) while floc size changed little, implying that extant changes were not necessary to explain the observed kinetic improvement.[71] More broadly, even if unmeasured floc or microcolony changes occurred in other studies, the consistent magnitude and direction of apparent K_{O_2} reductions across systems indicates that any concurrent extant effects were not sufficient to overwhelm the net shift toward higher apparent oxygen affinity observed under sustained low-DO operation.

The pathway to low DO ammonia oxidation, and thus increased potential for process intensification is then: changing operating conditions (lower DO) places enough selective pressure on ammonia oxidizers to generate a community shift over time, which eventually lowers the apparent K_{O_2} and allows for maintaining a substantial fraction of ammonia removal capacity at the reduced operating DO. The timeline for implementing this is not well established; Jimenez *et al.*,[24] surveying over a dozen full-scale low DO facilities, reported that approximately 20 days was needed for full acclimation. It was also reported that the facilities in the study also did not need to increase their SRT to accommodate reduced ammonia removal rates, meaning capacity was maintained as well.

This critical review also surveyed the literature on additional important considerations for ammonia oxidation at low DO. 1) Higher available substrate (ammonia) concentration, a feature in advanced processes like ABAC or those with downstream anammox like PNA or PdNA, may have a significant impact on apparent ammonia oxidizer kinetics, but little research has addressed this explicitly. 2) Significant open questions remain for the impact of oxygen on ammonia oxidizer decay rates, which are critical for process design and kinetic estimation or modelling. 3) Ammonia oxidizer decay rates are likely impacted by operation at low DO and are critical for predicting nitrification capacity, but they are difficult to measure and kinetically parameterize due to problems of methodological accuracy and transferability; numerous open questions remain.

For designers, owners, and operators, process intensification is an important and attainable goal. But these novel processes typically demand operation at DOs outside the typical ranges, and the limits of current knowledge and process design are

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tested. This is compounded as these operating conditions significantly impact and interact with each other, but the state-of-knowledge must be advanced, as these processes need to be designed, built, and operated. Kinetic adaptation is real, although questions remain about the durability of these shifts, but it is not sufficient to assume static ammonia oxidation kinetics, especially with respect to oxygen. Delineating the intrinsic and extant nature of these shifts provides some evidence about this, but significant work remains to be done. Future work must also resolve outstanding questions around decay kinetics and substrate interactions, especially in systems with residual ammonia concentrations, to better predict system capacity and resilience under low DO conditions.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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