

# **A critical review of the future trends and perspectives for the implementation of Anammox in the main line of municipal WWTPs**

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## **Abstract**

The use of technologies based on partial nitrification (PN) and anaerobic ammonium oxidation (Anammox) is a cost efficient and sustainable alternative for nitrogen removal from wastewaters with low COD/N ratios. These technologies allow savings in biodegradable organic matter consumption; oxygen requirements (about 40% lower compared to the conventional process of nitrification/denitrification, because only 50% of the ammonium needs to be oxidized to nitrite); and sludge management costs. Despite the advantages of the PN/Anammox process, it also has some limitations which led to a full scale application relatively restricted to wastewaters from mesophilic anaerobic digesters, especially in the reject line of the wastewater treatment plants (WWTPs).

However, the PN/Anammox process also opens the interesting possibility of transforming most of the biodegradable organic matter arriving to the WWTP into biogas (i.e. methanisation), because that biodegradable COD is not anymore necessary to denitrify. This can lead to an energetically self-sufficient WWTP, which means important cost savings. The main limitations and challenges arising from this application would be: the treatment of low strength wastewater; the possible effects of the biodegradable COD reaching the PN/Anammox system; the operation at low temperatures; the effective retention of the microbial populations of interest; the use of one-step or two-steps systems; and the effective control of the process during the start-up and the stable operation

Some of the proposed solutions which will be detailed along this paper are: the use of biofilm biomass (with or without carrier); advanced on line control systems usually based on the continuous measurement of ammonium and/or NO<sub>x</sub>; performing the start-up close to optimum conditions and later acclimation at low temperature and low strength wastewater.

Keywords: Anammox, biological nutrient removal, energetic self-sufficient, municipal wastewater, sustainability.

## **Introduction**

The use of technologies based on anaerobic ammonium oxidation (Anammox) is a cost efficient and sustainable alternative for nitrogen removal from wastewaters with low COD/N ratios [1, 2]. The Anammox bacteria are autotrophic, thus biodegradable organic matter is not necessary. Besides, since the substrates of Anammox sludge are nitrite and ammonium, the requirements of oxygen are about 40% lower compared to the conventional process (i.e. nitrification/denitrification) because only about 50% of the ammonium needs to be oxidized to nitrite. The production of sludge is also much lower compared to the conventional process due to the small biomass yield of the autotrophic Anammox organisms [3].

Despite the advantages of the Anammox process, it also has some limitations [2], e.g. inhibition by substrates and exogenous compounds including biodegradable COD [4], optimum temperature in the mesophilic range [5], slow start-up [6]... Thus, its full scale application is still relatively restricted to wastewaters from mesophilic anaerobic digesters, especially in the reject line of the wastewater treatment plants (WWTPs). In fact, Lackner et al. [7] reported that about 75% of the full-scale PN/Anammox reactors (as of 2014) were operated for side-stream treatment of

municipal wastewater. Furthermore, these authors [7] reported that the majority of the full scale PN/Anammox systems, both at municipal or industrial WWTPs, are treating high strength wastewaters, with ammonium concentrations around 1 g N/L, which are far higher compared to the usual ammonium concentrations of the municipal wastewaters.

However, the Anammox process also opens the interesting possibility of transforming most of the biodegradable organic matter arriving to the WWTP into biogas (i.e. methanisation), because that biodegradable COD is not anymore necessary to denitrify [8]. This can lead to an energetically self-sufficient WWTP, which means important cost savings [9, 10]. This application has already been proposed by Jetten et al. [11] as early as almost two decades ago. However, it has been barely implemented for the moment [12, 13]. Therefore, the main focus of this work is reviewing the latest advances on the application of the Anammox process in the main line and the existing limitations. The most important issues which will be discussed are: The management of high COD/N ratio (pretreatment); the treatment of low strength wastewater; the operation at low temperatures; the effective retention of the microbial populations of interest; the use of one-step or two-steps systems; and the effective control of the process during the start-up and the stable operation [8]. Some of the proposed solutions which will be detailed are: Physicochemical or biological methods to concentrate the biodegradable COD followed by anaerobic digestion; the use of biofilm biomass (with or without carrier); and advanced on line control systems usually based on the continuous measurement of nitrogen species.

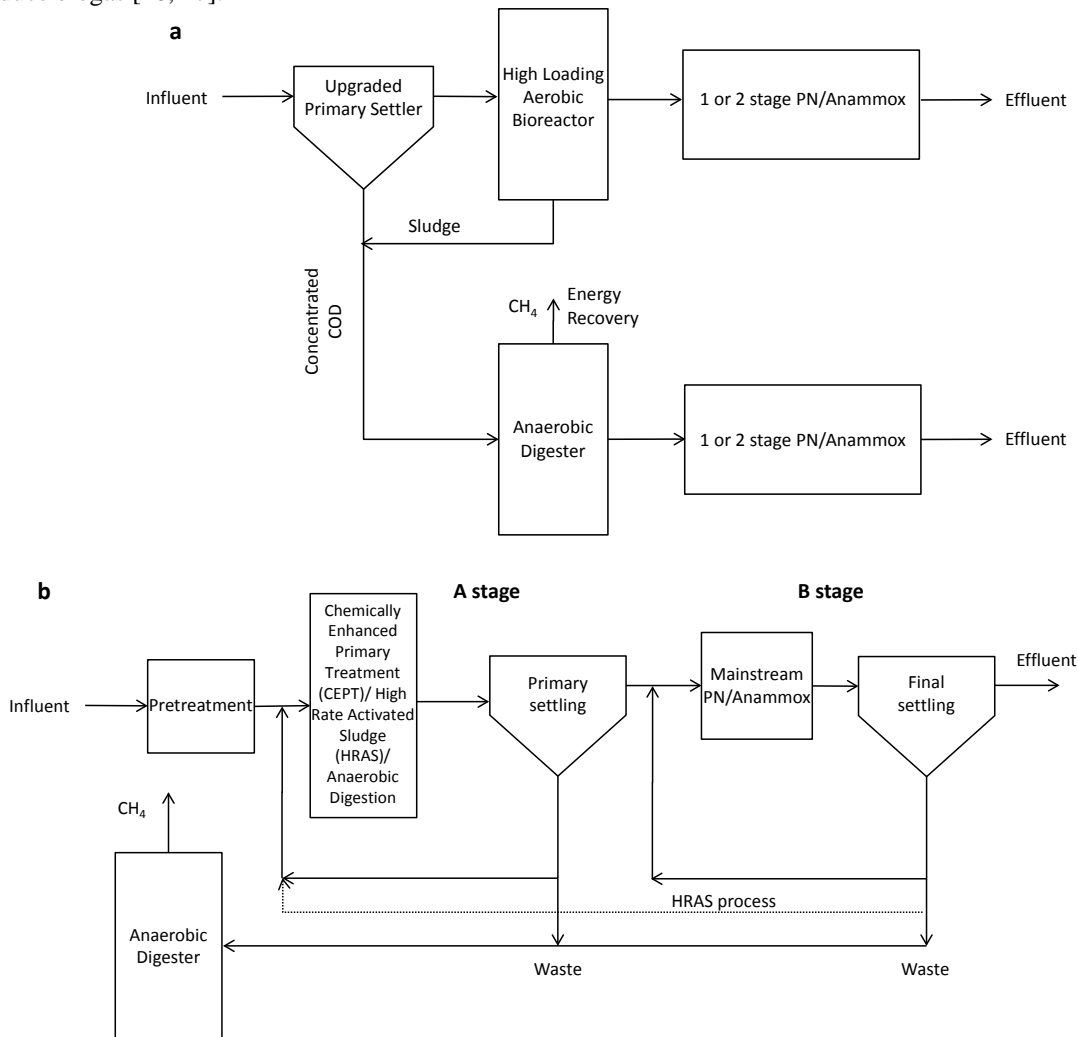
#### **High COD/N ratio management: Strategies for maximize C energy recovery**

Municipal wastewater is a potential source of chemical energy in form of organic carbon [13]. Besides, the COD/N ratio of this type of wastewater (typical ratios around 10-12 [14]) is usually significantly higher than the optimum desirable for a PN/Anammox treatment (<2-5, according to Lackner et al. [15], or even <0.5, according to Daigger [16]). Firstly, because biodegradable COD in the presence of oxidized forms of nitrogen promotes growth of denitrifying bacteria, which are able to outcompete Anammox populations [15, 17]. Secondly, biodegradable organic matter is inhibitory for the Anammox biomass [4]. Therefore, biodegradable COD should be removed not only as a way to recover energy but also to prevent Anammox inhibition and/or suppression by competition.

Nowadays, the most practical and widely implemented way to transform biodegradable COD into recoverable energy is the anaerobic digestion process. Taking into account the stoichiometry of the methanogenesis, if the conversion is complete, the maximum production of CH<sub>4</sub> from biodegradable COD is about 0.35 Nm<sup>3</sup> CH<sub>4</sub>/kg COD [13]. However, municipal wastewater is usually more diluted than the typical influent treated by anaerobic digestion and, consequently, its chemical energy is more difficult to harvest. The direct application of anaerobic digestion is not only hampered by this low strength but also by the moderate to low temperature of the water [13]. Except in tropical or subtropical regions, municipal wastewater would usually be at 10-15 °C [18]. Besides, at these moderate temperatures a significant part of the produced CH<sub>4</sub>, up to 40% [12], can be dissolved in the effluent, being useless in terms of energy recovery and also posing a risk of environmental release. Anyway, despite these drawbacks, Gao et al. [19] have successfully applied an Up-flow Anaerobic sludge Fixed-Bed (UAFB) reactor to directly treat a municipal influent prior

to a PN/Anammox system. Anyway, as expected, the average COD removal was barely over 40 % at 17 °C. Still, this direct anaerobic treatment might be an alternative when the COD removal optimization techniques are not available.

There are some different strategies to maximize the biodegradable COD recovery and its conversion into energy [10, 13]. Probably, the easiest alternative to be implemented in wastewater treatment plants consists in the up-concentration of the organic carbon and the maximization of the anaerobic digestion [10, 13]. This up-concentration can be performed by several techniques [12]: maximised/upgraded primary settling (Figure 1a [10]), sieving, dynamic sand filtration (DSF), dissolved air flotation (DAF) or bio-flocculation/High Rate Aerobic System (HRAS) (including aerobic granulation). This last technique will aim to convert dissolved biodegradable carbon into biomass (e.g. activated sludge or aerobic granules) in high-rate/low-HRT reactors. The objective will be the maximum conversion of C into biomass, with relatively low C mineralisation (conversion into CO<sub>2</sub>) and N removal in this reactor [17]. Several authors use the name A-B stage process for this treatment strategy, being the “A process” the biodegradable COD concentration and methanisation, and the “B process” the mainstream PN/Anammox (Figure 1b [17]). Then the purged activated or granular sludge [20], together with the particulate COD, will be separated (usually by settling) and digested in order to produce biogas [10, 17].



**Fig. 1. a)** Adapted flow scheme of the WWTP proposed by Mendez et al. [10], **b)** Flow scheme of the “A-B stage” WWTP adapted from Xu et al. [17]

### **Low strength and low temperature wastewaters**

The treatment of low strength and low temperature wastewaters, produced in such treatments like the ones discussed in the previous section to transform biodegradable COD into energy, adds difficulties not only for the Anammox step but also for the PN step. The effective selection and growth of the Ammonium Oxidizing Bacteria (AOB), outcompeting the Nitrite Oxidizing Bacteria (NOB), in order to obtain the oxidation to  $\text{NO}_2^-$  of about 50% of the  $\text{NH}_4^+$ , can be much more difficult when treating these types of wastewaters [17]. Two of the selection driving forces commonly used are based on high ammonium concentration (i.e. NOB selective inhibition by free ammonia [21, 22]) and on the wash-out of NOB due to the faster growth kinetics of AOB at the mesophilic range of temperature (e.g. SHARON process [23]). In this case however, the wastewaters to be treated will be at ambient temperature, which, unless in hot/tropical climates, will be significantly lower than the mesophilic range of temperatures. Besides, the low ammonium concentration, usually around or under 50 mg/L [19, 24], will make the inhibition by free ammonia virtually negligible [17]. In fact, Al-Omari et al. [25] reported that the out-selection of NOB can be the most challenging issue to be addressed for the effective worldwide implementation of mainstream shortcut nitrogen removal processes.

In absence of inhibition factors to select AOB and wash-out NOB, the population selection in the PN step will have to rely on fine-tuning the concentrations of the involved species, i.e. oxygen and nitrogen species [26] and, eventually, on the use of biofilms [27]. A previous work by Bartoli et al. [28] has already focused on the measurement and control of the residual ammonium concentration in the reactor and the ratio between that ammonium and the dissolved oxygen (DO) concentrations in order to obtain nitrification only to nitrite. However, they worked with high strength wastewaters, so that strategy is not directly applicable in the case of municipal wastewater. More recently, Wett et al. [29] reported that transient anoxia (i.e. intermittent aeration) is an efficient way to control PN of municipal wastewater and repress NOB activity. They also reported that AOB bioaugmentation was beneficial for the process. De Clippelair et al. [27], however, achieved the stable operation of a one-step PN/Anammox with rotating biodisc technology treating a municipal-like synthetic influent (55-60 mg  $\text{NH}_4^+\text{-N/L}$ ) at 15 °C. They needed relatively high DO concentrations (3-4 mg/L) and nitrite accumulation in the reactor (up to an average of 31% of the  $\text{NH}_4^+$  consumed) to outcompete NOB versus AOB and Anammox. In these conditions, the reactor produced significant amounts of the undesirable nitrogen oxides. Regmi et al. [26], based on previous works on affinity for nitrogen substrates and DO [30-33] agreed on the strategy followed by De Clippelair et al. [27] and postulated that operating at non-limiting concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and DO, together with transient anoxia and short (or even “aggressive”) Sludge Retention Time (SRT) operation, was the best strategy to effectively control a PN system to treat municipal wastewater. Regmi et al. [26] proved their strategy by operating a pilot scale (340 L) plant under the mentioned conditions, obtaining relatively high nitrogen removal (57% on average) without carbon and alkalinity addition and at a low HRT. In order to implement the control system, the use of online sensors for nitrogen species was one of the keys of the process [26] and they are expected to help further to obtain even a more efficient and stable operation in future pilot or full scale applications [25]. Actually, Al-Omari et al. [25] conducted simulation studies and concluded that the use of “ammonia versus NOx” control was the best for mainstream nitrification. Despite all these successful works on advanced control

systems, this is expected to continue being a research hotspot and the first full-scale implementations are expected in the near future.

Other aspect to be considered is that the application of PN to side-streams coming from anaerobic digestion of sludge is favoured by the fact that these types of wastewaters very often have alkalinity/ammonium molar ratios about 1-1.2 [34]. Taking into account that the oxidation of ammonium to nitrite produces two equivalents of protons per equivalent of ammonium converted, about 50-60% of the ammonium will be oxidised to nitrite when the alkalinity is totally consumed [34]. At that point the value of pH will decrease enough to stop the PN, thus the alkalinity content of digested side-streams is helping to control the PN. The alkalinity of the municipal wastewater is relatively low and alkalinity/ammonium molar ratios can easily be lower than 0.5 and, even for municipal wastewater with high alkalinity, that ratio can barely reach 0.8-0.9 [35]. This fact implies that careful control of pH is essential to perform PN process and some consumption of reagents may be necessary. However, it is also important to remark that, since the Anammox reaction consumes protons [3], when PN and Anammox are carried out in the same single-stage reactor, the alkalinity consumption caused by PN will be partially compensated by the Anammox process.

Regarding the application of the process at relatively low temperature and apart to maintaining the control/stability of the PN process, the biological activity of the Anammox population will be much lower than when relatively hot digested side-streams are treated. Despite Anammox has been proved feasible at temperatures below 20 °C [5, 18, 35], its activity will be significantly lower (roughly 2-4 times [36]) than that observed at its optimum temperature. Even when some degree of acclimation to low temperatures is possible [5, 36], the lower specific activity implies the need for high retention of Anammox biomass, topic that is discussed in the next section.

### **Effective retention of Anammox biomass**

Anammox bacteria grow very slow [37, 38]. There is still not a big consensus about their exact doubling time, probably in part due to the lack of a pure Anammox culture to this date. Anyway, in lab-scale reactors and in conditions close to the optimum, it ranges from a minimum of around 6 d [39] to a more typically reported value of about 10-12 d [6, 38]. At pilot/full scale and, even more, at low temperatures the observed doubling times can go up to 25-30 d [34,40]. Therefore, it is clear that optimum Anammox biomass retention is essential in its application to municipal wastewater, especially at temperatures below 20-25 °C.

The most widely studied and implemented mechanisms to obtain high SRT Anammox reactors rely on the formation of biofilms (either autoaggregation in form of granules or growing on support materials) [41], whose high density allows very high retention of biomass in the reaction systems. Membrane bioreactors have also been used at lab-scale [39] and as a research tool, but they have been barely applied at full scale applications. Actually, some of the recent works towards municipal wastewater treatment by PN/Anammox rely on biofilm technology [19, 27, 42]. Another technology, which has actually been used for Anammox reactors since its discovery [38], is the Sequencing Batch Reactor (SBR). It can be used or not in combination with biofilm systems and allows very high SRTs, close to total retention of biomass [41]. Some of the last years' efforts on municipal water treatment were also successfully

employing SBRs [18, 26] and they are expected to continue being a popular Anammox technology in the future, not only because of the good biomass retention, but also due to their versatility and operational flexibility.

### **One-step vs two-steps systems**

In the case that the organic C recovery pretreatment is able to remove most of the biodegradable organic matter of the wastewater, then the one-step PN/Anammox process would be appropriate to carry out the subsequent ammonium removal. In fact, such a system has been proved at temperatures around 20 °C removing loading rates up to 0.45 kg  $\text{NH}_4^+\text{-N}/(\text{m}^3 \text{ d})$  [43]. One of the main advantages of using one-step systems would be significantly lowering the investment costs [44], because of the savings on area occupation (and eventually purchase), civil work and equipment. Besides, as it was commented before, the consumption and production of protons of PN and Anammox can be (partially) compensated.

If the soluble COD is not completely removed or if the variability of the influent is expected to be high, a two-steps systems might be more interesting. For example, Gao et al. [19] chose that type of system and it was able to cope with the relatively low COD removal of the previous treatment step. Anyway, Lotti et al. [42] reported the efficient operation of an one-step Anammox reactor treating a municipal-like effluent and receiving about 60 mg COD/L. Nevertheless, taking into account the relatively difficult control of the PN when treating this type of wastewaters, the use of two steps allows the optimisation of each process. Some of the constrains and optimum values for PN and Anammox would be difficult or even impossible to meet simultaneously, so with this strategy each process can be optimised independently. This is the case of the advanced PN control system proposed by Regmi et al. [26], which had to be implemented in a two-steps system in order to achieve a highly efficient and stable conversion of about half the ammonium to nitrite.

Table 1 summarizes some of the most significant examples of application of PN/Anammox to municipal wastewaters, both employing one and two-steps technologies.

Type of process	Aeration type	COD/N ratio	Temperature (°C)	NRR (kg N/m <sup>3</sup> d)	Ref.
3 steps for AD and PN/Anammox: UAFB, PN-SBR, UFBR Anammox	Aeration time controlled PN	5 (before AD)	12-27	0.83	[19]
1 step: RBC	Not controlled, intermittent in space	2	14-15	0.53	[27]
1 step: SBR	Not controlled, continuous supply	0	12	0.02	[18]
2 steps: PN-CSTR, MBBR Anammox (not operated)	Intermittent, controlled by NH <sub>4</sub> <sup>+</sup> /NO <sub>x</sub> ratio	6.7	25	0.15	[26]
2 steps: PN (not operated), UFGSB Anammox	-	0.6-1	10-20	1.85 (20 °C) 0.34 (10 °C)	[42]
1 step: Pilot scale plug flow granular reactor	Intermittent	0.67 (BOD/N)	19	0.16-0.19	[45]

AD: Anaerobic Digestion; PN: Partial Nitrification; UAFB: Up-flow Anaerobic sludge Fixed Bed; UFBR: Up-flow Fixed-bed Biofilm Reactor; SBR: Sequencing Batch Reactor; RBC: Rotating Biological Contactor; CSTR: Continuously Stirred Tank Reactor; MBBR: Moving Bed BioReactor; UFGSB: ; BOD: Biological Oxygen Demand; NRR: Nitrogen Removal Rate.

**Table 1.** Summary of significant experimental works on PN/Anammox treating municipal mainstream effluents.

## Conclusions

The application of the PN/Anammox process to the main stream of the municipal WWTPs opens the possibility for the self sufficient or energy generating treatment plant. This highly desirable objective, both in terms of environmental sustainability and cost savings, has driven the research towards the main line implementation of the PN/Anammox. Significant advances have been obtained to overcome the main limitations of the process, focusing on COD removal and C energy recovery, advanced control systems, improved biomass retention and other issues. These aspects are discussed in the present review, taking into account that, despite all this research effort, the application of PN/Anammox to municipal wastewater is still not a mature technology, so it will continue being a hot research topic in the future.

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