

# Application of anammox based processes in urban WWTPs: Are we on the right track?

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## SUPPLEMENTARY MATERIALS

### A. Calculation of the effect of NH<sub>3</sub> presence on the effluent quality of an anaerobic sludge digester

Methanogenesis was considered the limiting step of the anaerobic digestion since sludge was thermally hydrolyzed. To calculate the biodegradable organic matter present in the effluent S (g COD<sub>biodegradable</sub>/L) at different solid retention times (SRT (d)) and NH<sub>3</sub> concentrations (mg NH<sub>3</sub>/L), Equation 1 derived from the balance of the microorganisms to an anaerobic digester and modified according to the inhibitory effect of free ammonia on methanogenic bacteria described by Li et al. [1] was used. Values of the kinetic parameters were taken from Metcalf and Eddy [2] and Li et al. [1] (Table S1).

$$S = \frac{K_s \cdot (1 + b \cdot SRT)}{SRT \cdot \left( \mu_{max} \frac{K_i}{K_i + NH_3} - b \right) - 1} \quad (\text{Equation 1})$$

**Table S1.** Kinetic parameters used to quantify the effect of NH<sub>3</sub> on the effluent quality of an anaerobic sludge digester.

Parameter		Value	Reference
K <sub>s</sub>	affinity constant for organic matter	0.36 g COD/L	[2]
b	decay coefficient	0.03 d <sup>-1</sup>	[2]
μ <sub>max</sub>	maximum growth rate	0.25 d <sup>-1</sup>	[2]
K <sub>i</sub>	inhibitory constant for NH <sub>3</sub>	847 mg NH <sub>3</sub> /L	[1]

### B. Calculation of treatment costs to remove both organic matter and ammonia from the supernatant of anaerobic sludge digesters

A supernatant with an ammonium concentration of 1,800 mg NH<sub>4</sub><sup>+</sup>-N/L and a COD<sub>biodegradable</sub>/N ratio of 1 g/g was considered to calculate the treatment costs. The operating costs were calculated taking into account oxygen consumption, sludge production and methane production. In the case where no pretreatment or aerobic pretreatment was applied, it was assumed that organic matter

was totally removed and the supernatant from the sludge anaerobic digesters, once treated, was returned to the mainstream. Therefore, a total decay of heterotrophic biomass generated under aerobic conditions with an associated extra oxygen consumption was considered. Overall oxygen consumption related to organic matter removal was calculated according to Equation 2:

$$\text{Oxygen consumption (kg O}_2\text{/m}^3\text{)} = \text{COD} \cdot (1 - 1.42 \cdot Y_H \cdot f_b) \quad (\text{Equation 2})$$

where COD: biodegradable COD concentration (kg O<sub>2</sub>/m<sup>3</sup>); 1.42: ratio of kg COD/kg VSS<sub>biomass</sub>; Y<sub>H</sub>: yield coefficient of heterotrophic biomass (0.43 kg VSS/kg COD [2]); f<sub>b</sub>: the fraction of biodegradable COD contained in the biomass (X<sub>p</sub>) (0.15).

In the case of an anaerobic pretreatment, the methane production was calculated taking into account that all the organic matter present in the supernatant was removed and anaerobic biomass generated would decay under anaerobic conditions, generating extra methane (Equation 3):

$$\text{Methane generation (m}^3\text{CH}_4\text{/m}^3\text{)} = 0.35 \cdot \text{COD} \cdot (1 - 1.42 \cdot Y_A \cdot f_b) \quad (\text{Equation 3})$$

Where 0.35: ratio of m<sup>3</sup> CH<sub>4</sub>/kg COD; Y<sub>A</sub>: yield coefficient of anaerobic biomass (0.08 kg VSS/kg COD [2]).

Oxygen consumption associated with nitrogen removal was calculated using stoichiometry (1.8 kg O<sub>2</sub>/kg NH<sub>4</sub><sup>+</sup>-N [3]). Since 11% of the ammonia is converted to nitrate during its removal through the PN/A processes, which is returned to the mainstream where it can be used as an electron acceptor by denitrifying bacteria, a saving of 2.86 kg O<sub>2</sub>/kg NO<sub>3</sub><sup>-</sup>-N was considered [2].

To carry out the energy balance, energy consumption of 1 kW·h/kg O<sub>2</sub> and energy production of 3.5 kW·h/m<sup>3</sup> CH<sub>4</sub> were considered. Pumping energy consumption was calculated basis on an energy efficiency of the pump of 0.7 and a reactor height of 5 m in the case of the aerobic pretreatment and nitrogen removal units and 10 m in the case of the anaerobic pretreatment unit. The overall sludge production was calculated considering only the inert fraction of the biomass initially generated (X<sub>p</sub>) and a VSS/TSS ratio of 0.75. An energy cost of 0.1 euro/kW·h, a sludge dryness of 25% and a management cost of 50 Euro/Ton sludge were used to determine the operating costs.

Capital costs related to the implementation of different configurations to remove nitrogen from the thermal hydrolysis pretreated (THP) sludge anaerobic digester (THP/AD) dewatering liquors were estimated. The studied configurations were: a) single-stage nitrogen removal system operated at a nitrogen loading rate (NLR) of 0.6 kg N/(m<sup>3</sup>·d) [4] without pretreatment; b) single-stage nitrogen removal system operated at a NLR of 0.6 kg N/(m<sup>3</sup>·d) with aerobic pretreatment; c) single-stage nitrogen removal system operated at a NLR of 1.7 kg N/(m<sup>3</sup>·d) [5] with aerobic pretreatment; d) single-stage nitrogen removal system operated at a NLR of 0.6 kg N/(m<sup>3</sup>·d) with anaerobic pretreatment; e) single-stage nitrogen removal system operated at a NLR of 1.7 kg N/m<sup>3</sup>·d with anaerobic pretreatment. The second case has been considered in order to guarantee the stable performance of the nitrogen removal system during possible unstable operational periods of the sludge anaerobic digesters. The use of two sequential batch reactors (SBR), operated in parallel, has been considered for implementing the single-stage nitrogen removal and biological aerobic pretreatment while carrying out the anaerobic pretreatment, a UASB type reactor has been considered. Capital costs were determined for wastewater treatment plants (WWTPs) of different sizes (expressed in terms of inhabitants equivalent). Nitrogen load

associated with THP/AD dewatering liquor was calculated as 20% of the N load entering the WWTP, estimated based on a flowrate of 250 L/(inhabitant equivalent-d) incoming nitrogen concentration of 50 mg N/L. The flow rate of the THP/AD dewatering liquors stream was determined considering its ammonium concentration (1,800 mg  $\text{NH}_4^+\text{-N/L}$ ). The volume of the biological aerobic pretreatment reactor was estimated based on a hydraulic retention time (HRT) value of 10 h [5], while an inlet organic loading rate of 10 kg COD/( $\text{m}^3\cdot\text{d}$ ) was used as a criterion to determine the volume of the UASB type reactor [2]. Equipment costs were adapted from US EPA [6] and Danish Energy Agency [7] since the calculated reactor volumes were within the range of reactor volumes used to obtain these cost correlations. A lifetime of 20 years for facilities was considered. Prices were updated taking into account the evolution of the Chemical Engineering Plant Cost Index (CEPCI) [8].

### C. Calculation of the maximum ammonia inlet concentration to fulfill the discharge nitrogen limit

In the case that ammonia was only removed by a PN/A system implemented in the sludge line, equation 3 can be used to relate the ammonium concentration at the inlet of the WWTP ( $\text{NH}_4^{+in}$  (mg  $\text{NH}_4^+\text{-N/L}$ )) with the nitrogen concentration at the outlet ( $N_{eff}$  (mg  $\text{NH}_4^+\text{-N/L}$ )) depending on the fraction of the nitrogen load diverted to the sludge line ( $f_{Nsl}$ ). This equation was derived considering the nitrogen mass balance of both mainstream and sludge line and the stoichiometry of the anammox process and a value of 10 mg N/L was considered for  $N_{eff}$  according to the European discharge requirements for sensitive areas.

$$\text{NH}_4^{+in} = \frac{N_{eff}}{(1 - f_{Nsl}) + 0.11 \cdot f_{Nsl}} \quad (\text{Equation 3})$$

If all the ammonium present in the sludge line was oxidized to nitrite and then this nitrite was removed by the anammox process, the ammonium concentration at the entrance to the WWTP ( $N_{in}$  (mg  $\text{NH}_4^+\text{-N/L}$ )) could be related to the nitrogen concentration at the outlet ( $N_{eff}$  (mg N/L)) by means of equation 4. This equation considers that the sludge line initially contains 20% of the inlet WWTP nitrogen load and this load can be increased in a fraction of  $f_{Nosl}$  by supplying extra sludge from other WWTPs to the sludge digesters.

$$\text{NH}_4^{+in} = \frac{N_{eff}}{0.8 - \frac{0.2 \cdot (1 + f_{Nosl})}{1.32} + 0.11 \cdot \frac{2.32}{1.32} \cdot 0.2 (1 + f_{Nosl})} \quad (\text{Equation 4})$$

To determine the operating conditions (dissolved oxygen level, SRT and temperature) that limit nitrate production in the mainstream to values lower than 1 mg  $\text{NO}_3^-\text{-N/L}$ , nitrite oxidizing bacteria (NOB) mass balance under steady-state conditions was applied to the reactor located in the mainstream that removes the organic matter and oxidizes the ammonium to nitrite (Equation 5).

$$Q_{inr} \cdot X_{inNOB} - Q_p \cdot X_{pNOB} - Q_{ef} \cdot X_{efNOB} + \mu_{NOB} \cdot X_{rNOB} \cdot V_r = 0 \quad (\text{Equation 5})$$

Where  $Q_{inr}$ : inlet flowrate (L/d);  $X_{inNOB}$ : concentration of NOB in the influent (mg VSS/L);  $Q_p$ : purge sludge flowrate (L/d);  $X_{pNOB}$ : NOB concentration in the purge stream (mg VSS/L);  $Q_{ef}$ : effluent flowrate (L/d);  $X_{efNOB}$ : NOB concentration in the effluent (mg VSS/L);  $\mu_{NOB}$ : specific growth rate of NOB ( $\text{d}^{-1}$ );  $X_{rNOB}$ : NOB concentration inside the reactor (mg VSS/L);  $V_r$ : volume of the reactor (L).

By rearranging Equation 5, Equation 6 can be obtained. For known values of both solids retention

time in the mainstream ( $SRT_{ms}$  (d)) and temperature ( $T$  ( $^{\circ}C$ )), the dissolved oxygen level (DO (mg  $O_2/L$ )) limiting the nitrate concentration in the effluent to 1 mg  $NO_3^-$ -N/L was calculated by solving Equation 6 using Solver (Excel). For this, an inlet NOB concentration ( $X_{inNOB}$ ) of 0.004 mg VSS/L was considered (adapted from Duan et al. [9]). As the inlet nitrite concentration ( $NO_{2r}$  (mg  $NO_2^-$ -N/L)) depends on the amount of  $NO_2^-$  supplied to the reactor from the sludge line, a high concentration of nitrite was considered (100 mg  $NO_2^-$ -N/L) in order to expect the worst possible scenario where the Monod term respect to  $NO_2^-$  was 1. Since the maximum nitrate concentration in the effluent should be 1 mg  $NO_3^-$ -N/L, the nitrite concentration inside the reactor ( $NO_{2r}$ ) was taken as 99 mg  $NO_2^-$ -N/L. To consider the effect of temperature on both the NOB decay coefficient ( $b_{NOB(T)}$ ,  $d^{-1}$ ) and the NOB specific growth rate ( $\mu_{NOB(T)}$ ,  $d^{-1}$ ), the model proposed by West Models Guide [10] was considered. Stoichiometric and kinetic parameters used to solve Equation 6 are described in Table S2.

$$\frac{X_{inNOB}}{\left( X_{inNOB} \cdot (1 - b_{NOB(T)} \cdot SRT_{ms}) + \frac{Y_{NOB} \cdot (NO_{2o}^- - NO_{2r}^-)}{(1 + b_{NOB(T)} \cdot SRT_{ms})} \cdot SRT_{ms} \right)} - \frac{1}{SRT_{ms}} + \mu_{maxNOB(T)} \cdot \frac{DO}{K_{SDONOB} + DO} \cdot \frac{NO_{2r}^-}{K_{SNNOB} + NO_{2r}^-} - b_{NOB(T)} = 0 \quad (\text{Equation 6})$$

**Table S2.** Kinetic and stoichiometric parameters of NOB used to determine operating conditions that limit nitrate concentration in a mainstream reactor to 1 mg  $NO_3^-$ -N/L.

Parameter		Value	Reference
$b_{NOB(T)}$	decay coefficient	$0.033 \cdot e^{(0.061 \cdot (T-20))}$ $d^{-1}$	[10]
$Y_{NOB}$	yield coefficient	0.05 mg VSS/mg $NO_2^-$ -N	[11]
$\mu_{maxNOB(T)}$	maximum growth rate	$0.79 \cdot e^{(0.061 \cdot (T-20))}$ $d^{-1}$	[10]
$K_{SDONOB}$	affinity constant for DO	0.5 mg $O_2/L$	[12]
$K_{SNNOB}$	affinity constant for nitrite	0.2 mg $NO_2^-$ -N/L	[11]

To determine the effluent quality obtained when bioaugmentation of ammonium oxidizing bacteria (AOB) from the sludge line was applied according to the WWTP configuration described in Figure 3c. First, an AOB mass balance was applied under steady-state conditions to the mainstream reactor (which removes organic matter and oxidizes ammonium to nitrite) in order to calculate the ammonium effluent concentration as a function of the operating conditions (dissolved oxygen level, SRT and temperature) (Equation 7).

$$Q_{inr} \cdot X_{inAOB} + Q_{sl} \cdot X_{slAOB} - Q_p \cdot X_{pAOB} - Q_{ef} \cdot X_{efAOB} + \mu_{AOB} \cdot X_{rAOB} \cdot V_r = 0 \quad (\text{Equation 7})$$

Where  $X_{inAOB}$ : concentration of AOB in the influent (mg VSS/L);  $Q_{sl}$ : flowrate coming from the sludge line (L/d);  $X_{slAOB}$ : AOB concentration in the sludge line (mg VSS/L);  $X_{efAOB}$ : AOB concentration in the effluent (mg VSS/L);  $\mu_{AOB}$ : specific growth rate of AOB ( $d^{-1}$ );  $X_{rAOB}$ : AOB concentration inside the reactor (mg VSS/L).

The flow rate coming from the sludge line was calculated taking into account that the nitrogen load of the sludge line supposes 20% of the nitrogen load that enters the WWTP (Equation 8):

$$Q_{sl} = \frac{0.2 \cdot (1 + f_{Nosl}) \cdot Q_{inT} \cdot NH_{4in}^+}{NH_{4sl}^+} \quad (\text{Equation 8})$$

Where  $Q_{inT}$ : flowrate entering to the WWTP (L/d);  $NH_{4in}^+$ : ammonium concentration that enters the WWTP (mg  $NH_4^+$ -N/L);  $NH_{4sl}^+$ : ammonia concentration in the sludge line (mg  $NH_4^+$ -N/L).

The concentration of AOB in the sludge can be calculated taking into account its observed yield coefficient and the ammonium concentration in the sludge line (Equation 9):

$$X_{slAOB} = \frac{Y_{AOB} \cdot NH_{4sl}^+}{1 + b_{AOB30^\circ C} \cdot SRT_{sl}} \quad (\text{Equation 9})$$

Where  $Y_{AOB}$ : yield coefficient of AOB (mg VSS/mg  $NH_4^+$ -N);  $b_{AOB30^\circ C}$ : decay coefficient of AOB at 30 °C ( $d^{-1}$ );  $SRT_{sl}$ : SRT of AOB in the sludge line (d). For the latter parameter, a value of 1.5 d was chosen [13].

By rearranging Equation 7 and combining it with Equations 8 and 9, Equation 10 can be obtained. The dilution effect of the flowrate from the sludge line on the mainstream fraction subjected to ammonium oxidation was negligible (less than 2%). This equation was solved using Solver (Excel) to calculate the remaining ammonium concentration value in the reactor ( $NH_{4r}^+$  (mg  $NH_4^+$ -N/L)) depending on its SRT, DO concentration and temperature. This concentration allows determining the maximum ammonium concentration entering the WWTP ( $NH_{4in}^+$  (mg  $NH_4^+$ -N/L)) that would allow fulfilling a discharge limit ( $N_{ef}$  (mg N/L)) of 10 mg N/L. (Equation 12). For a given temperature, the pair of SRT and DO values obtained by Equation 6, which limit nitrate concentration inside the reactor to 1 mg  $NO_3^-$ -N/L, was used to calculate the remaining ammonium concentration in the reactor (Equation 10), except when the DO value provided by Equation 6 for a given SRT and temperature values exceeded the value of 2 mg  $O_2$ /L. In these cases, the DO concentration was limited to 2 mg  $O_2$ /L for economic reasons. The calculation of the maximum ammonium concentration at the entrance to the WWTP that would allow compliance with a discharge limit of 10 mg N/L was carried out through an iteration process using both Equations 10 and 12. For this, an inlet NOB concentration ( $X_{inNOB}$ ) of 0.16 mg VSS/L was considered (adapted from Duan et al. [9]). To take into account the effect of temperature on both AOB decay coefficient ( $b_{AOB(T)}$ ,  $d^{-1}$ ) and AOB specific growth rate ( $\mu_{AOB(T)}$ ,  $d^{-1}$ ) the model proposed by West Models Guide [10] was considered. Stoichiometric and kinetic parameters used to solve Equation 9 are described in Table S3. For Equation 12, the value of  $NO_{3r}^-$  was set at 1 mg  $NO_3^-$ -N/L.

$$\begin{aligned} & \frac{X_{inAOB} + \frac{0.2 \cdot (1 + f_{NO_2}) \cdot NH_{4in}^+ \cdot Y_{AOB}}{f_{NO_2} \cdot (1 + b_{AOB30^\circ C} \cdot SRT_{sl})}}{\left( \left( X_{inAOB} + \frac{0.2 \cdot (1 + f_{NO_2}) \cdot NH_{4in}^+ \cdot Y_{AOB}}{f_{NO_2} \cdot (1 + b_{AOB30^\circ C} \cdot SRT_{sl})} \right) \cdot (1 - b_{AOB(T)} \cdot SRT_{ms}) + \frac{Y_{AOB} \cdot (NH_{4in}^+ - NH_{4r}^+)}{(1 + b_{AOB(T)} \cdot SRT_{ms})} \right) \cdot SRT_{ms}} - \frac{1}{SRT_{ms}} \\ & + \mu_{maxAOB(T)} \cdot \frac{DO}{K_{sDOAOB} + DO} \cdot \frac{NH_{4r}^+}{K_{sNAOB} + NH_{4r}^+} - b_{AOB(T)} = 0 \end{aligned} \quad (\text{Equation 10})$$

Where  $f_{NO_2}$ : fraction of the flowrate entering to the WWTP that is diverted to the reactor where organic matter is removed and ammonium is oxidized to nitrite, this fraction can be calculated considering that the inlet stream of the anammox reactor should have a  $NH_4^+/NO_2^-$  molar ratio of 1/1.32 (Equation 11).

**Table S3.** Kinetic and stoichiometric parameters of AOB used to determine operating conditions that limit nitrogen concentration in the WWTP effluent to 10 mg N/L.

Parameter		Value	Reference
$b_{AOB(T)}$	decay coefficient	$0.05 \cdot e^{(0.094 \cdot (T-20))} d^{-1}$	[10]
$Y_{AOB}$	yield coefficient	0.15 mg VSS/mg $NH_4^+$ -N	[11]
$\mu_{maxAOB(T)}$	maximum growth rate	$0.8 \cdot e^{(0.094 \cdot (T-20))} d^{-1}$	[10]
$K_{sDOAOB}$	affinity constant for DO	0.6 mg $O_2$ /L	[10]
$K_{sNAOB}$	affinity constant for ammonium	0.75 mg $NH_4^+$ -N/L	[10]

$$\frac{0.8 \cdot (1 - f_{FNO_2^-})}{0.8 \cdot f_{FNO_2^-} + 0.2 \cdot (1 + f_{Nosl})} = \frac{1}{1.32} \quad (\text{Equation 11})$$

$$N_{in} = \frac{N_{ef} - f_{FNO_2^-} \cdot \frac{2.32}{1.32} \cdot (NH_{4r}^+ + NO_{3r}^-)}{0.11 \cdot (1 + f_{Nosl})} \quad (\text{Equation 12})$$

### List of abbreviations:

AD:	Anaerobic sludge digesters
Anammox:	Anaerobic ammonia oxidation
AOB:	Ammonia-oxidizing bacteria
COD:	Chemical oxygen demand
Comammox:	Complete ammonia-oxidizing bacteria
Damo:	Denitrifying anaerobic methane oxidation
DO:	Dissolved oxygen
NOB:	Nitrite-oxidizing bacteria
OFMSW:	Organic fraction of municipal solid waste
SRT:	Solid retention time
THP:	Thermal hydrolysis pretreatment
VFA:	Volatile fatty acid
VSS:	Volatile suspended solids
WWTP:	Wastewater treatment plant

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