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Review

Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams

Stijn W.H. Van Hulle[a,b,*], Helge J.P. Vandeweyer[b], Boudewijn D. Meesschaert[c], Peter A. Vanrolleghem[a,d], Pascal Dejans[b], Ann Dumoulin[b]

[a] BIOMATH, Department of Applied Mathematics, Biometrics and Process Control, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[b] Research Group EnBiChem, Department of Industrial Engineering and Technology, University College West Flanders, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium
[c] Chemistry Department, Catholic University College of Bruges–Ostend, Zeedijk 101, B-8400 Ostend, Belgium
[d] ModelEAU, Département de génie civil, Pavillon Pouliot, Université Laval, Québec G1K 7P4, QC, Canada

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ABSTRACT

The anaerobic ammonium oxidation (Anammox) process, discovered 20 years ago, is, in combination with partial nitritation, ideally suited to treat nitrogen rich waste water streams such as digester effluent. In this review the engineering aspects and the practical application of the process are reviewed. The conventional nitrification–denitrification and nitritation–denitritation are also discussed briefly.

The environmental conditions affecting the nitrification process, free ammonia and nitrous acid concentration, temperature, pH and dissolved oxygen concentration, are discussed. These conditions can be controlled in such a way that the partial nitritation step produces an Anammox-suited influent. The Anammox reactor conditions should favour the growth of the Anammox organisms in view of their low growth rate and possible inhibition effects. Dissolved oxygen and nitrite concentrations should be kept as low as possible and biomass washout should be limited. If the partial nitritation process and the Anammox process are occuring in the same reactor, care should be taken to the dissolved oxygen concentration, the ammonium load and the nitrite concentration to obtain a sustainable co-existence between aerobic and anaerobic ammonium oxidizers.

An overview is presented of the practical implementation of autotrophic nitrogen removal. The process can be accomplished in the same reactor (1-reactor system) or by using 2 separate reactors (2-reactor system). Typically the 1-reactor system is a biofilm or granular reactor where the ammonium oxidizers are active in the outer layers of the biofilm or granule, producing a suitable amount of nitrite for the Anammox organisms that are active in the inner layers. Transport of ammonium and the produced nitrite is governed by diffusion. Finally, the different nitrogen removal processes are compared in terms of operational conditions and a direction for future work is provided.

1. Introduction

One of the elements of concern in wastewater is nitrogen, especially since the use of synthetic nitrogen fertilizer produced from atmospheric N_2 by the Haber–Bosch process has increased tenfold over the last 40 years. The human contribution to nitrogen pollution, for example in the form of urine, is ever increasing in view of the growing world population. Discharge of this nitrogen into the natural waters can lead to, amongst others, eutrophication and oxygen depletion.

In most modern wastewater treatment plants (WWTP) nitrogen, which is generally in the form of ammonium or organic nitrogen, is removed by biological nitrification/denitrification (reaction (3)). As a first step ammonium is converted to nitrate (nitrification, reaction (1)) which is then, in a second step, converted to nitrogen gas (denitrification, reaction (2)). Benefits of the process are the high potential removal efficiency, high process stability and reliability,

Abbreviations: ABF, anaerobic biological filtrated reactor; Anammox, anaerobic ammonium oxidation; CANON, Completely Autotrophic Nitrogen removal Over Nitrite; CSTR, completely stirred tank reactor; FB, fluidized bed reactor; HNO_2, nitrous acid; MBR, membrane bioreactor; MSBR, membrane sequencing batch reactor; NH_4^+, ammonium; NH_3, ammonia; NO_2^-, nitrite; OLAND, oxygen limited autotrophic nitrification denitrification; RBC, rotating biological contactor; SBR, sequencing batch reactor; SHARON, Single reactor High activity Ammonia Removal Over Nitrite; UASB, upflow anaerobic sludge blanket; UBF, upflow biofilter; UFB, upflow fixed bed.

* Corresponding author at: Research Group EnBiChem, Department of Industrial Engineering and Technology, University College West Flanders, Ghent University Association, Graaf Karel de Goedelaan 5, 8500 Kortrijk, Belgium. Tel.: +32 56 241251; fax: +32 56 241224.
E-mail address: Stijn.Van.Hulle@Howest.be (S.W.H. Van Hulle).

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Nomenclature

HRT	hydraulic residence time (d)
K_{I,HNO_2}	inhibition constant for nitrous acid concentration for ammonium oxidizers (mg HNO_2-N L^{-1})
K_{NH_3}	saturation constant for ammonia of ammonium oxidizers (mg NH_3-N L^{-1})
NLR	nitrogen loading rate (g N L^{-1} d^{-1})
NRR	nitrogen removal rate (g N L^{-1} d^{-1})
OUR	oxygen uptake rate (mg O_2 L^{-1} d^{-1})
SRT	sludge residence time (d)
TAN	total ammonium nitrogen (mg N L^{-1})
TNO_2	total nitrite nitrogen (mg N L^{-1})

relatively easy process control, low area requirement and moderate cost [1]

$$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 3H_2O + 2CO_2 \quad (1)$$

$$5C + 4NO_3^- + 2H_2O \rightarrow CO_2 + 4HCO_3^- + 2N_2 \quad (2)$$

$$4NH_4^+ + 8O_2 + 5C + 4HCO_3 \rightarrow 2N_2 + 10H_2O + 9CO_2 \quad (3)$$

Generally, the conventional biological nitrogen removal process is used for treating wastewaters with relatively low nitrogen concentrations (total nitrogen concentration less than 100 mg N L^{-1}). Some wastewater streams such as anaerobic digester effluents, landfill leachate and industrial wastewaters contain high concentrations of nitrogen [2]. One more sustainable alternative is the nitritation–denitritation process over nitrite (Eqs. (4) and (5)). This process requires less oxygen and less organic carbon in comparison with the traditional nitrification–denitrification.

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NO_2^- + 3H_2O + 2CO_2 \quad (4)$$

$$3C + 4NO_2^- + 2H_2O + CO_2 \rightarrow 4HCO_3^- + 2N_2 \quad (5)$$

The ANaerobic AMMonium OXidation (Anammox) process, which was discovered about 20 years ago [3] but was already predicted to exist 30 years ago [4], could offer another alternative for the treatment of this return stream.

In the Anammox process ammonium is oxidized under anoxic, i.e. oxygen depleted, conditions with nitrite as electron acceptor (Eq. (6)). Ammonium and nitrite are consumed on an almost equimolar basis. The Anammox process should always be combined with a partial nitritation process, such as the SHARON process [5], where half of the ammonium is oxidized to nitrite. Both autotrophic processes will increase the sustainability of wastewater treatment as the need for carbon addition (and concomitant increased sludge production) is omitted and oxygen consumption and the emission of nitrous oxide during oxidation of ammonia are largely reduced [6]. Especially since nitrous oxide has become a significant factor in the greenhouse gas footprint of the total water chain [7]

$$NH_4^+ + 01.32NO_2^- + 0.66HCO_3^- \rightarrow 1.02N_2 + 2.03H_2O + 0.66CH_2O_{1.5}N_{0.15} + 0.26NO_3^- \quad (6)$$

The combined process (partial nitritation and Anammox) was termed autotrophic nitrogen removal process and is depicted in Fig. 1.

In this contribution the engineering aspects and practical application of this autotrophic nitrogen removal process will be reviewed, with a main focus on the Anammox process.

2. Nitrogen elimination from wastewater by autotrophic nitrogen removal

For a specific application the available alternatives for nitrogen elimination need to be evaluated on a multitude of cost aspects, chemical and energy requirements, operational experience, process reliability and environmental impact. However, the selection of the best alternative is generally based on cost-effectiveness. Nitritation–denitritation produce savings in oxygen demand during nitrification, a reduction of organic matter requirements in the denitrification process and a decrease in surplus sludge production. The application of partial nitritation–Anammox goes even further in these 3 requirements as demonstrated in Table 1.

Compared to the traditional nitrogen removal process, which involves nitrification–denitrification, respectively 25% and 60% less oxygen will be consumed by the nitritation–denitritation and partial nitritation–Anammox process. Moreover, a lower (40% less) or

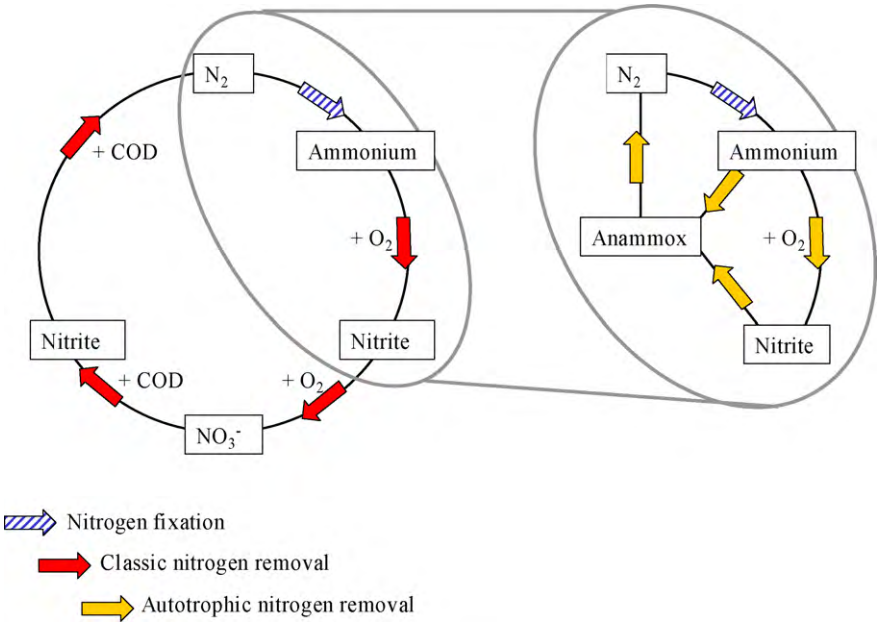

Fig. 1. The updated nitrogen cycle with autotrophic nitrogen removal.

Table 1
Comparison of various nitrogen removal processes (adjusted from [8] and [9]).

Process	Oxygen needed ($gO_2\,gN^{-1}$)	COD needed without assimilation ($gCOD\,gN^{-1}$)	COD needed with assimilation ($gCOD\,gN^{-1}$)
Nitrification–denitrification	4.57	2.86	4.0
Nitritation–denitritation	3.43	1.72	2.4
Partial nitritation–Anammox	1.72	–	–
OLAND	1.94	–	–

no organic carbon source is needed if denitritation or Anammox is used as second step [8]. Nitrate produced by the Anammox process and by the OLAND process requires also organic carbon. However, most real wastewater contain a small amount of biodegradable COD which can be used to denitrify the produced nitrate. The three processes require more or less the same buffering capacity from the treated water: 1 mol H^+ is produced per mol N converted. This implies that all processes do not require significant pH control costs in the case of a sufficient buffer capacity in the waste water stream (1 mol HCO_3^- per 1 mol NH_4^+) [10]. Mulder [11] stated that the sludge production decreased from 1.0 to 0.1 g dry weight g^{-1} N when nitrification–denitrification is compared with OLAND/partial nitritation–Anammox. As a consequence, sludge treatment and disposal costs significantly decrease. Vlaeminck [10] calculated that the application of OLAND for treating reject water could save about 85% of the operational costs in comparison with nitrification/denitrification. The cost of the autotrophic nitrogen removal process is 1 euro per kg N removed, while other conventional nitrogen removing techniques cost 2–4 euro per kg N removed. As such, 1–3 euro per kg N removed can be saved [5].

In practice the selection of either a biological or a physiochemical method for nitrogen elimination is also determined by the nitrogen concentration of the wastewater. According to Mulder [11] biological treatment by autotrophic nitrogen removal is to be preferred for concentrated wastewater streams with ammonium concentrations in the range of 100–5000 mg N L^{-1}. For this concentration range, the autotrophic nitrogen process offers a sustainable alternative to conventional techniques, such as conventional nitrification and denitrification since less energy and chemicals are needed.

Typical wastewaters with high ammonia concentrations are reject water, piggery manure, landfill leachate and some industrial waste waters (examples of these wastewaters are depicted in Table 2). Reject water originating from dewatered sludge is normally recycled to the influent of the WWTP. An analysis of the nitrogen balance of the WWTP Dokhaven in Rotterdam (The Netherlands) revealed that the reject water accounted for only a few percentages of the total flow, but 15% of the nitrogen load [5,12]. A separate treatment of this stream reduces the nitrogen load coming from the digesters and can significantly help to reduce the nitrogen concentration in the effluent of the main wastewater treatment plant. This way ever reducing discharge limits for nitrogen concentrations can be met. The landfill leachate is heavily polluted and has to be captured and treated. Often this leachate is recycled over the landfill, decreasing the COD concentration and increasing the nitrogen concentration because the landfill acts as an anaerobic bioreactor [13]. Similar to reject water this landfill leachate is characterized by a high ammonium and a low COD content [14]. Raw manure is usually separated into a thick and a thin fraction. The thick fraction can be used as soil enhancer while the thin (liquid) fraction is treated. The composition of this thin fraction can vary and depends on the separation method and the composition of the animal feed [15]. However, large concentrations of COD, nitrogen and phosphorus can be expected, which is not favourable for autotrophic nitrogen removal. Industrial processes can generate streams with high nitrogen content. Also, concentrated industrial streams with high COD content can lead to highly loaded nitrogen streams if they are first treated in an anaerobic digester. Examples can be found in the pharmaceutical industry [16], tanneries [17], slaughterhouse waste processing [18], potato processing industries, alcohol and starch production [19] and formaldehyde production [20,21]. A shortcoming of a lot of these streams is the presence of recalcitrant and/or toxic components in the streams, resulting in a high effluent COD concentration. In the latter case, despite the favourable C N^{-1} ratio there is still a need for carbon addition to allow the necessary denitrification. Carucci et al. [22] reported a minimum C N^{-1} ratio of 8 for tannery wastewater, which is much higher than the normally applied ratio of 4–6 [11]. This tannery wastewater also contains chromium, sulphide and chloride, all resulting in negative effects on the nitrification process [17,23]. Wastewater of formaldehyde production, characterized by a high organic COD content, partially inhibits both nitrification and denitrification [21] and will thus lead to a more difficult operation.

3. (Partial) nitrification

Nitrification is the aerobic oxidation of ammonium to nitrate. It consists of two sequential steps carried out by two phylogenetically unrelated groups of aerobic chemolithoautotrophic bacteria. Some heterotrophic bacteria can also oxidize ammonium to nitrate, but this is only a very small contribution to the overall ammonia oxidation. First, ammonium is oxidized to nitrite by the aerobic ammonia-oxidizing bacteria. Approximately 2 mol of protons are produced for every mole of ammonium oxidized. Ammonium oxidation is therefore an acidifying reaction. In the second step nitrite is oxidized to nitrate by the nitrite oxidizing bacteria. No single known autotrophic bacterium is capable of complete oxidation of ammonium to nitrate in a single step [24]. The key reactions of nitrification are given by Eqs. (7) and (8):

$$NH_4^+ + \tfrac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (7)$$

$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^- \quad (8)$$

In view of coupling partial nitritation with Anammox, nitrite oxidizing activity should be suppressed and ammonium should only be oxidized for about 50% to nitrite. Different influencing factors can be used to engineer a system that accomplishes this requirement, as discussed below.

The most important environmental parameters to obtain partial nitritation are the free ammonia (FA, NH_3) and free nitrous acid (FNA, HNO_2) concentration, the temperature, pH and dissolved oxygen concentration. The difference in sensitivity of ammonium and nitrite oxidizers towards these influences determines whether there will be nitrite accumulation in a nitrifying system. Indeed, nitrite oxidizers are generally more sensitive to detrimental environmental conditions than ammonium oxidizers. Hydroxylamine and chlorate inhibit irreversible nitrite oxidizers but not ammonium oxidizers. These compounds are able to inactivate the nitrite oxidizers while ammonia and nitrous acid can lead to adaptation of nitrite oxidizers. Furthermore, it is necessary to consider economic feasibility when using temperature, pH and inhibitor as regulation parameter. By applying lower oxygen concentrations, aeration will be saved and nitrite oxidizers cannot grow into the system [25].

An Anammox-suited effluent in a 2-reactor system (see further) can be produced by selection of the appropriate temperature, pH, substrate availability and ammonia and nitrous acid inhibition level in order to washout nitrite oxidizers from the system. In view of the Anammox stoichiometry, care should further be taken that only half of the ammonium is oxidized and that the partial nitritation is well controlled [26] as Anammox organisms are sensitive to substrate shocks.

Further, it should also be noted that due to adaptation of the biomass, nitrate build-up is possible, even after long-term operation [27].

3.1. Free ammonia (NH_3) and free nitrous acid (HNO_2) concentration

Free ammonia (NH_3) and free nitrous acid (HNO_2) concentration have a large influence as these uncharged nitrogen forms are the actual substrate/inhibitor for ammonium and nitrite oxidation instead of ammonium (NH_4^+) and nitrite (NO_2^-) [28,29]. This was clearly confirmed by Van Hulle et al. [30] for ammonium oxidizers active in a SHARON reactor [27].

From the regular total ammonium (TAN) and total nitrite (TNO_2) analysis which gives the sum of the ionized and unionized compounds, the free ammonia (NH_3) and nitrous acid (HNO_2) concentration can be calculated incorporating pH and temperature (°C) [29]:

$$[NH_3] = \frac{[TAN]10^{pH}}{e^{6344/(T+273)} + 10^{pH}} \quad (9)$$

$$[HNO_2] = \frac{[TNO_2]10^{-pH}}{e^{-2300/(T+273)} + 10^{-pH}} \quad (10)$$

The ratio between the charged (NH_4^+ and NO_2^-) and the uncharged form (NH_3 and HNO_2) is determined by the pH and temperature values in the reactor and can be calculated based on the acid–base equilibrium. The amount of ammonia increases with increasing pH, while the amount of nitrous acid decreases which obviously promotes ammonium oxidizers but suppresses nitrite oxidizers. Hence, nitrite oxidizers can be outcompeted in a weak alkaline environment (7.5–8) in order to produce an Anammox-suited effluent in the nitritation reactor. However, the potential of using this engineering approach seems somewhat limited since adaptation of the nitrite oxidizing bacteria has been reported [31]. Therefore, the achievement of stable partial nitritation will only occur when factors other than free ammonia and free nitrous acid are regulated [25].

Concerning inhibition it can be stated that NH_3 is the main inhibitor of nitrification at high pH (>8), whereas HNO_2 is the main inhibitor at low pH (<7.5). In literature different threshold values were proposed for nitrification inhibition [29,32], but these are very sensitive to bacteria adaptation. Anthonisen et al. [29] stated that aerobic ammonia oxidizers are inhibited at NH_3 concentrations of 8–120 mg N L^{-1} and HNO_2 concentrations of 0.2–2.8 mg N L^{-1} while inhibition of nitrite oxidation is observed at an NH_3 concentration of 0.08–0.82 mg N L^{-1} and a HNO_2 concentration of 0.06–0.83 mg N L^{-1}. Recently, Hawkins et al. [33] stated that free ammonia has only a limited impact on the inhibition of nitrite oxidation. They found that pH changes and ammonia oxidizing activity had a bigger influence on nitrite oxidizing activity.

3.2. pH

Despite a wide divergence of the reported effects of pH on nitrification, there seems to be a consensus that the optimum pH for both ammonium oxidizers and nitrite oxidizers lies between 7 and 8. A first explanation is the influence of pH on the NH_4^+/NH_3^{-1} and HNO_2/NO_2^{-1} equilibria. The preference of ammonium oxidizers for slightly alkaline environments probably is the fact that these organisms use NH_3 as substrate [28] while at certain pH values NH_3 and HNO_2 can exhibit inhibitory effects as stated above. Apart from the influence of pH on chemical equilibria in which the substrate/inhibitors are involved, direct pH effects on the activity exist [30]. Hellinga et al. [34] observed a decrease by 8 in the growth rate of nitrite oxidizers at pH 7 compared with pH 8 whereas the variation in growth rate of ammonium oxidizers at these pH values is negligible. Below pH 7, nitrification rate will decrease since carbon limitation due to CO_2 stripping will occur [35,36]. However, high nitrification rates at low pH were detected in a fluidized bed reactor with chalk as biofilm carrier [37]. In this system the chalk probably acted as a local buffer system.

3.3. Other substrates than nitrogen

As stated above, 2 mol of protons are produced for every mol ammonium oxidized. To neutralize the acidifying step, 2 mol bicarbonate are needed. In order to assure that only half of the ammonia is oxidized to nitrite 1 mol of base per mol ammonium is required [5,38]. Guisasola et al. [36] and Wett and Rauch [35] reported a reduction in ammonia oxidizing activity due to bicarbonate limitation. Moreover, Ganigué et al. [39] showed that bicarbonate is a key parameter for controlling the ammonium to nitrite molar ratio in the effluent. Long-term stable nitrite build-up in a SBR treating raw urban landfill leachate was possible with extremely high ammonium concentrations (NLR = 1.2 kg N m^{-3} d^{-1}) by controlling the bicarbonate concentration. Sludge reject water is a good waste stream for partial nitritation since a proper ammonium: alkalinity ratio of 1 is often found in these streams [5]. Nitrite oxidation might also be affected by phosphorus deficiency [40]. In a biological pre-treatment plant treating highly nitrogenous wastewaters (T > 25 °C), nitrite oxidation was substantially reduced at phosphate levels below 0.2 mg P L^{-1}. Indeed, the phosphate half-saturation coefficient for nitrite oxidizers is about one order of magnitude higher than for ammonium oxidizers (0.2 mg P L^{-1} for nitrite oxidizers and 0.03 mg P L^{-1} for ammonium oxidizers) [40]. Nitrite oxidizers are especially unable to oxidize nitrite to nitrate in the absence of phosphates, the so-called phosphate block.

3.4. Temperature

Temperature is a key parameter in the nitrification process, but the exact influence is difficult to determine because of its interaction with mass transfer, chemical equilibria and growth rate. A temperature rise creates two opposite effects: increased NH_3 inhibition and increased activity of the organisms according to the Arrhenius principle. This increased activity only holds up to a certain critical temperature above which biological activity decreases again.

Experiments with pure cultures gave an optimal temperature of 35 °C for ammonium oxidizers and 38 °C for nitrite oxidizers [41]. Van Hulle et al. [30] showed that temperatures between 35 and 45 °C are optimal for partial nitritation. However, only short-term effects were investigated. Long-term exposure to temperatures above 40 °C is expected to lead to deactivation [42]. Hellinga et al. [34] concluded that temperatures above 25 °C lead to an increase of the specific growth rate of ammonia oxidizing bacteria, which become higher than that of nitrite oxidizing bacteria. The SHARON process (Single reactor High activity Ammonia Removal Over Nitrite) is based on this principle. In this process, nitrification of ammonium to nitrite is established in a chemostat by working at high temperature (above 25 °C) and maintaining an appropriate sludge retention time (SRT) of 1–1.5 days, so that

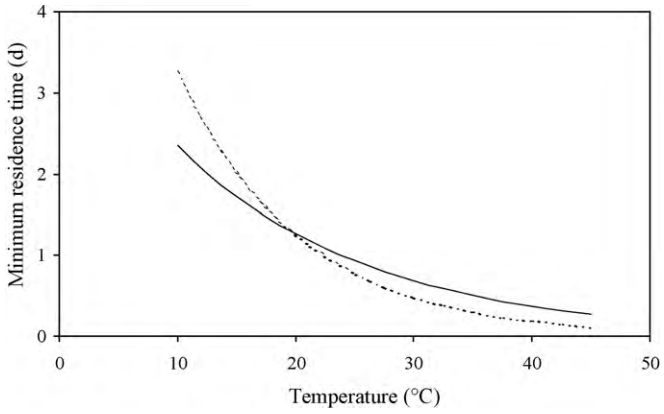

Fig. 2. Effect of temperature on the minimal required cell residence time for ammonia and nitrite oxidation. Above 25 °C it is possible to wash out the nitrite oxidizers (-) while maintaining the ammonium oxidizers (··). Calculated with parameter values described in Jetten et al. [43].

ammonium oxidizers are maintained in the reactor, while nitrite oxidizers are washed out and further nitrification of nitrite to nitrate is prevented (Fig. 2). Literature values for activation energy of ammonium and nitrite oxidizers range from 72 to 60 kJ mol^{-1} and from 43 to 47 kJ mol^{-1}, respectively (determined in studies between 7 and 30 °C) [43,44,45,46] indicating that the activity of ammonium oxidizers will increase faster than the activity of nitrite oxidizers.

The partial nitritation process was also successfully started up and maintained at lower temperature (between 15 and 30 °C) [47]. These results indicate that the application of the partial nitritation process could be not restricted to effluent with temperatures around 30 °C such as the effluent from methanogenic reactors but could be applicable to many kinds of industrial wastewater treatments. However, the performance of nitritation dramatically decrease below 15 °C.

3.5. Dissolved oxygen concentration

When it comes to nitrification, the dissolved oxygen concentration is of high importance for both ammonium oxidizers and nitrite oxidizers [48]. Ammonium oxidizers seem to be more robust against low dissolved oxygen concentration than nitrite oxidizers. Accumulation of nitrite at low dissolved oxygen is usually explained by the difference in oxygen half saturation constant (K_O) for ammonium oxidizers and nitrite oxidizers [49]. In other words, oxygen deficiency due to low dissolved oxygen concentration influences the activity of nitrite oxidizers more significantly than that of ammonium oxidizers [48]. This difference could be explained by the higher energy released per amount of oxygen consumed of ammonium oxidizers compared to nitrite oxidizers.

According to Hunik et al. [50] the half-saturation constant for dissolved oxygen is 0.16 mg O_2 L^{-1} and 0.54 for the ammonium oxidizer *Nitrosomonas europaea* and nitrite oxidizer *Nitrobacter agilis*, respectively. However, values for the half-saturation constant given in literature for activated sludge vary in the range of 0.25–0.5 mg O_2 L^{-1} and 0.34–2.5 mg O_2 L^{-1}, respectively [51]. This variation is probably due to the variation of the oxygen mass transfer efficiency in the reactors [52]. The oxygen concentration inside a sludge floc or biofilm not necessarily equals that of the water phase. The half saturation constant is therefore dependent on the biomass density, the floc size, the mixing intensity and the rate of diffusion of oxygen in the floc [53]. This was clearly demonstrated by Manser et al. [54]. They showed that the half-saturation constants for oxygen determined for sludge coming from a conventional activated sludge plant and sludge coming from a membrane bioreactor exhibited a major difference because sludge flocs in the membrane bioreactor are much smaller. Hence, diffusion resistance in these flocs can be neglected.

Peng et al. [55] and Jubany et al. [56] demonstrated that it was possible to remove nitrogen through nitrification–denitrification over nitrite by using an on-line dissolved oxygen or OUR control system. This system controls the oxygen concentration by turning aeration off at the point when ammonia oxidation had completed. This point was determined from the pH and dissolved oxygen signals. As such nitrite oxidation was prevented by limiting the oxygen supply. Aeration patterns are proposed to be an alternative parameter to control ammonium to nitrite [57]. Hyungseok et al. [58] reported that nitrate formation can effectively be prevented by frequent switching between oxic and anoxic phases. As such the aeration is switched off before all the ammonium is consumed and before nitrite can be further converted to nitrate. A prolongation of the aeration phases in a SBR lowered the stress on nitrite oxidizers resulting in an increase of nitrate [59,60]. These findings were confirmed by Sin et al. [61] who found that nitrite build-up was caused by low oxygen concentration (0.5 mg O_2 L^{-1}) and fast alternation of the aeration conditions in the system. Imposing oxygen-limiting conditions can be considered another way to outcompete nitrite oxidizers. However, it is also suggested that free hydroxylamine inhibition of nitrite oxidizing bacteria rather than a difference in oxygen affinity constants causes nitrite build-up in nitrifying systems at low dissolved oxygen concentration [62]. According to Hu [63] hydroxylamine exhibited acute and irreversible toxicity to Nitrobacter (nitrite oxidizers) and this may also cause nitrite build-up in a nitrifying system. Castignetti and Gunner [64] and Stüven et al. [65] stated also that hydroxylamine severely inhibits nitrite oxidizers.

3.6. Sludge age

Ammonium oxidizers and washout of nitrite oxidizers can be selectively accomplished by the application of an appropriate sludge retention time in suspended growth systems because of different minimum required sludge ages. The minimum doubling time for ammonium oxidizers is 7–8 h and for nitrite oxidizers 10–13 h, respectively [66]. Van Kempen et al. [67] found on the basis of full-scale experience that a maintenance of SRT between 1 and 2.5 days resulted in good performance. Selection of the AOB on the basis of different growth rates are used in the SHARON process. This process operates at a HRT (equal to SRT) of 1 day under high temperature and high oxygen concentration to favour the growth of ammonia oxidizers and to washout the nitrite oxidizing bacteria. However, successful partial nitritation were reported under longer sludge age. Pollice et al. [68,69] showed that a stable partial nitritation could be obtained under oxygen limitation independent of the sludge age of 10, 14 and 40 days while Peng and Zhu [25] also fulfilled stable nitrite accumulation under normal or even low temperature (<13 °C) with extended SRT (30 days) (Table 2).

3.7. Organic carbon and salts

It is stated that the partial nitritation process is successful for the treatment of wastewater with low C N^{-1} ratio although other streams with high organic content and high ammonium concentration such as swine wastewater [70,71] and monosodium-glutamate manufacturing streams are also used in partial nitritation processes [72].

Mosquera-Corral et al. [73] observed stimulation of the ammonia oxidation in the SHARON-process when acetate as carbon source was fed in a 0.2 g C g N^{-1} ratio leading to an effluent with nitrite to ammonia molar ratios higher than the stoichiometric

Table 2
COD, BOD, N and P concentrations (in mg L^{-1}) in waste streams with high nitrogen content.

Type wastewater	COD	BOD_5	Total nitrogen	Phosphorus	Reference
Reject water	232–12587	81–750	260–958	33–207	[179]
	390–2720	n.m.	943–1513	n.m.	[180]
	610	140	910	n.m.	[181]
Thin fraction piggery manure	n.m.	2912	707	55	[182]
	3969	1730	1700	147	[183]
	9000–13000	n.m.	3100–4300	20–40	[184]
	6456	n.m.	695	91.8	[185]
Landfill leachate	2000–5000	1500–4000	500–1000	20–50	[186]
	n.m.	45	310	n.m.	[14]
	1300–1600	n.m.	160–270	n.m.	[187]
	9660–20560	n.m.	780-1080	20–51	[188]
Tannery waste water	300–1400	n.m.	50–200	n.m.	[22]
	1940–2700	n.m	123–185	n.m	[17]
Slaughter house waste processing	1400–2400		170–200	35–55	[18][a]
Starch production	3000	990	1060	210	[189][b]
	5000–10000	2000–5000	800–1100	170–230	[19][b]
Pectine industry waste water	15000–22000	n.m	1280–2990	n.m	[190]
	8100	n.m.	1600	11	[191]

n.m.: not mentioned.
[a] After treatment in anaerobic lagune.
[b] After treatment in anaerobic digester.

ones. On the other hand, an inhibitory effect of ammonia oxidizing activity of 10% was observed when 0.3 g C g N^{-1} was brought into the reactor. Hanaki et al. [49] suggested that this inhibition was caused by a decreasing affinity of ammonia oxidizers for ammonia. One possible explanation is that the transport of ammonia from the bulk water phase to the cell of the ammonia oxidizer could be hindered by the presence of the crowded cells of heterotrophs which assimilate the ammonia and consume the oxygen before it reaches the nitrifiers. However, Hanaki et al. [49] found that for the same SRT, the ammonia oxidation efficiency decreased at higher COD concentrations but at a constant COD concentration efficiency restored again by increasing the SRT. Therefore, a moderate increase of the SRT to 2–3 days could be a possible solution to minimize the effect of heterotrophs on the ammonia oxidation.

Many industrial wastewaters rich in ammonium also contain high salt concentrations which could inhibit ammonia oxidation. However, the SHARON process still occured successfully at high NaCl concentrations of 100 mM in case of batch experiments and 427 mM in continuous operation [73]. This different behaviour was attributed to the adaptation of biomass to the saline environments.

3.8. Other influencing factors

Research by Zepeda et al. [74] showed that benzene, toluene and xylene induce a significant decrease in the values for nitrification specific rates affecting mainly the ammonia oxidation pathway. Heavy metals chromium, nickel, copper, zinc, lead and cadmium might inhibit both steps of nitrification reaction but the inhibition effects are different (Table 3; [75]).

Table 3
Inhibition to nitrification of some metal concentrations under pure culture [75].

Metal	Concentration (μg L^{-1})
Cr	0.7–785
Ni	3–860
Cu	3–5730
Zn	3–1000
Pb	0.09–1680
Cd	0.01–20

Formic, acetic, propionic and n-butyric acid all inhibited nitrite oxidation, but exhibited no significant effect on ammonium oxidation [76].

Of a dozen compounds tested by Tomlinson et al. [77], only chlorate, which is used to stop nitrite oxidation [78], cyanide, azide and hydrazine were more inhibitory to the oxidation of nitrite than to the oxidation of ammonium. Other toxic components that influence nitrite oxidation are the disinfectants bromide and chloride [79].

Light is inhibiting both ammonium oxidizers and nitrite oxidizers since cytochrome c is oxidized by light in the presence of oxygen.

4. The Anammox process

When Mulder et al. [3] observed unexplainable nitrogen losses in denitrifying fluidized bed reactors the idea was put forward that this could be attributed to ANaerobic AMMonium Oxidation (Anammox). Twenty years before, Broda [4] predicted that this process was possible on the basis of thermodynamic calculations. Van de Graaf et al. [80] showed by inhibition experiments that Anammox is a microbially mediated process and not a chemical reaction. ^{15}N labelling experiments showed that nitrite was the preferred electron acceptor instead of nitrate as first assumed [81]. Hydroxylamine and hydrazine were identified as important intermediates [43]. By making the mass balances over different Anammox enrichment cultures the overall stoichiometry of the Anammox reaction was determined as expressed in equation (11) [82]

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (11)$$

The Anammox process involves the oxidation of ammonia into dinitrogen gas in absence of oxygen [82]. This implies that the name anaerobic ammonium oxidation should actually be anoxic ammonium oxidation since nitrite is present as electron acceptor. It was found that nitrite was not only used for the oxidation of ammonium, but it was also oxidized to nitrate. This oxidation generates the reducing equivalents necessary for carbon fixation [81,83]. Since Anammox bacteria are autotrophic, the conversion

Table 4
Biodiversity of Anammox bacterial species.

Genus	Species	Source	Reference
Brocadia	*Candidatus Brocadia anammoxidans*	Wastewater	[91]
	Candidatus Brocadia fulgida	Wastewater	[192]
Kuenenia	*Candidatus Kuenenia stuttgartiensis*	Wastewater	[193]
Scalindua	*Candidatus Scalindua brodae*	Wastewater	[92]
	Candidatus Scalindua wagneri	Wastewater	[92]
	Candidatus Scalindua sorokinii	Seawater	[88]
	Candidatus Scalindua Arabica	Seawater	[194]
Others	*Candidatus Jettenia asiatica*	Not reported	[121]
	Candidatus Anammoxoglobus propionicus	Wastewater	[120]

of ammonia into dinitrogen gas can take place without addition of organic matter [84].

Since the initial discovery of Anammox activity has been reported in different wastewater treatment facilities [85], ranging from installations treating wastewater with high nitrogen load at low dissolved oxygen concentrations [86] to municipal wastewater treatment plants [87]. Further, Anammox is present in different natural environments and contributes significantly to the world's nitrogen cycle as it is found in several of the worlds seas and rivers such as the Black Sea [88] and the Thames estuary [89]. Depending on the organic load up to 70% of the N_2 production in marine sediments can be attributed to Anammox [90]. Strous et al. [91] showed that the bacteria responsible for the Anammox process are new members of the order of the Planctomycetes (Table 4). Fluorescence in situ hybridisation (FISH) probes were developed for the different Anammox species. Based on FISH analysis Schmid et al. [92] concluded that different Anammox species rarely occur in the same WWTP or enrichment culture. It seems that they all occupy their own niche and environmental conditions select for only one of the different genera [92]. However, Furukawa et al. [93] found two different genera of Anammox bacteria in a lab-scale reactor in which partial nitritation and the Anammox process are established together. Important work was performed concerning Anammox phylogeny [92], Anammox biochemistry [81,94,95,96], Anammox compartmentalisation [97,98] and Anammox unconventional membrane lipids [99].

Anammox biomass has a brown-reddish colour, which is probably due to the high cytochrome contents [43]. In last decade several techniques were developed for the detection of active Anammox organisms [85]. In Table 4 the different Anammox species are presented.

According to Schmid et al. [92], Anammox organisms have doubling time of 11 days and a biomass yield of 0.13 g dry weight per g NH_3-N oxidized. However, van der Star [100] concluded that the doubling time of Anammox bacteria is at most 5.5–7.5 days calculated on the basis of maximum conversion capacity, but possibly as low as 3 days. Researchers recently have claimed they optimized the reactor conditions to such an extent that a doubling time of 1.8 days was achieved [101]. A possible explanation for this high variation in growth rate could be the method to determine the growth rate, as Isaka et al. [101] determined the growth rate by direct counts of Anammox bacteria, while other studies rely on biomass yield and nitrogen removal rate.

This low growth rate and the difficulty in obtaining axenic cultures strongly hindered Anammox research [82,91].

4.1. Inhibition of substrates and products

The nitrite concentration is an important parameter to control since Anammox activity is inhibited by it. However, no uniformity is found about the threshold values of nitrite inhibition. Dapena-Mora et al. [102] found that 350 mg N L^{-1} nitrite correspond to 50% inhibition of the Anammox process performing activity tests. In the presence of more than 100 mg N L^{-1} nitrite, Strous et al. [103] found that the Anammox process was completely inhibited. Fux [104] showed in a long-term experiment that maintaining a nitrite concentration of 40 mg N L^{-1} over several days led to the irreversible inactivation of the Anammox organisms. This decreased activity due to nitrite inhibition can be restored by adding trace amounts of the Anammox intermediates hydroxylamine and hydrazine, even after long-term exposure to high concentrations of nitrite [103].

Remarkable is also the difference in tolerance for nitrite between the different Anammox genera. The inhibition experiments conducted by Strous et al. [103] were performed with *Candidatus Brocadia anammoxidans*. Experiments of Egli et al. [105] with *Candidatus Kuenenia stuttgartiensis* showed that the Anammox process was only inhibited at nitrite concentrations higher than 182 mg N L^{-1}. Furthermore, experiments by Strous et al. [103] showed that increasing the nitrite concentration changed the stoichiometry of ammonium and nitrite consumption from 1.3 g nitrite per gram ammonium at 0.14 g N L^{-1} nitrite to almost 4 g nitrite per gram ammonium at 0.7 g N L^{-1} nitrite. From the distorted stoichiometry at high nitrite concentrations, it can be concluded that the microorganisms under these conditions did not only use ammonium as the electron donor but also must have generated an internal electron donor to reduce the nitrite. This changing stoichiometry was also noticed at higher temperatures. Dosta et al. [106] observed an nitrite:ammonium consumption ratio of 1.38:1 at a working temperature of 30 °C but this ratio decreased to 1.05:1 when the reactor was operated at 18 °C.

The Anammox process is not inhibited by ammonium or by the by-product nitrate up to concentrations of at least 1 g N L^{-1} [103]. Dapena-Mora et al. [102] observed a 50% activity loss with high concentrations of ammonium and nitrate (770 and 630 mg N L^{-1}, respectively).

It is known that the chemolithoautotrophs mainly utilize inorganic carbon as carbon source. Therefore, the influent bicarbonate concentration is an important factor to affect the Anammox enrichment. Dexiang et al. [107] observed low Anammox activity at low bicarbonate: ammonium ratios of 2.3:1. At these conditions a limitation of the activity could occur since not enough CO_2 is present. On the other hand, high bicarbonate concentrations (bicarbonate:ammonium ratio of 4.7:1) lead also to inhibition. A possible explanation could be the formation of a high amount of free ammonia since the pH in reactor reached 8.1.

4.2. Phosphate and sulphide

Similarly to nitrite inhibition a difference in tolerance for phosphate exists between different Anammox species. van de Graaf et al. [83] experienced a loss of activity for *C. Brocadia anammoxidans* at phosphate concentrations above 155 mg P L^{-1}, while Egli et al. [105] did not see any inhibitory effect of phosphate when a culture of *C. Kuenenia stuttgartiensis* was supplied with up to 620 mg P L^{-1}. Dapena-Mora et al. [102] observed at the same phosphate level of 620 mg P L^{-1} 50% inhibition of Anammox activity. In batch tests using sludge from a highly loaded lab-scale rotating biological contactor containing *C. Kuenenia stuttgartiensis*, phosphate was shown to partially inhibit the Anammox process [108]. Anammox activity decreased to 63% of the normal activity at 55 mg P L^{-1} and further to 20% at 110 mg P L^{-1}. At 285 mg P L^{-1} no further decrease was observed (80% inhibition).

The effect of sulphide on the activity was also tested since SO_4^{2-} reduction often takes place in anaerobic digestion mainly transformed into H_2S. In anaerobic conditions, sulphate reducing bacteria produce sulphide with organic carbon as electron donor. Wastewaters such as seafood processing, leather tanning, oil refining and alcohol fermentation not only contain organic carbon and nitrogen but also sulphur compounds. Dapena-Mora et al. 2007 [102] showed an Anammox inhibition of 50% at low sulphide concentration of 9.6 mg S L^{-1} while van de Graaf et al. [83] showed a resistance of Anammox to at least 64 mg S L^{-1} in continuous and batch experiments. This large difference in sulphide inhibition could be explained by the addition of nitrate as electron donor for the Anammox biomass in van de Graaf et al. [83] since sulphide could reduce nitrate to nitrite, which is the preferable electron donor of the process. Recently, simultaneous removal of ammonium and sulphate by Anammox have been reported by Yang et al. [109].

4.3. Oxygen

Anammox bacteria are strictly anaerobic and are inhibited by dissolved oxygen. Inhibition caused by low concentration of oxygen was demonstrated however to be reversible. Egli et al. [105] stated that oxygen inhibits Anammox metabolism reversibly at low oxygen levels (air saturation of 0.25–2%) but probably irreversibly at high levels (>18% air saturation). Strous et al. [110] concluded from experiments with intermittent oxygen supply that the Anammox process was reversible inhibited by oxygen, making partial nitritation and Anammox possible in one reactor [110].

4.4. Organic carbon

Landfill leachate and wastewaters from digested animal waste contain high nitrogen concentration but also high organic carbon levels. Still, there are considered to be good influent streams for Anammox reactor. During anaerobic digestion fast biodegradable organic content is converted to biogas. As such, only slow biodegradable organic matter will be present in these wastewaters. Ruscalleda et al. [111] found that Anammox and denitrifiers could co-exist and play an important role in treating streams with high quantities of slowly biodegradable organic carbon such as digested liquor and landfill leachate. In such streams, heterotrophic denitrifying growth is limited by the low availability of easily biodegradable organic carbon. As consequence denitrifiers are not able to dominate in these systems and could not outcompete Anammox organisms. Undigested animal streams contain high nitrogen concentration but also high organic carbon levels. Since most of the fast degradable organic carbon content is oxidized in the proceeding partial nitritation step, the content of organics would be low enough so that denitrifiers does not outcompete Anammox.

Several other studies reported that presence of organic matter has a negative impact on Anammox growth [43,71,112–116]. In presence of certain amounts of organic carbon, Anammox organisms are not longer able to compete for nitrite with denitrifiers. This could be due to the fact that the growth rate of denitrifiers is higher than Anammox bacteria [91]. Moreover, the denitrification reaction is thermodynamically more favourable than anaerobic ammonium oxidation (the Gibbs free energy of Anammox bacteria is −355 kJmol^{-1} [43]), while the Gibbs free energy of denitrifying bacteria is −427 kJmol^{-1} [117]). Therefore, heterotrophic denitrifiers would grow faster when organic carbon is present in combination with ammonium and nitrite eliminating place for Anammox organisms. The threshold concentration for organic carbon in which denitrifiers outcompete Anammox bacteria differs from report to report. Güven et al. [115] stated that Anammox bacteria are not longer able to compete with heterotrophic denitrifying bacteria at C N^{-1} ratio above 1 while Chamchoi et al. [114] stated that an organic matter concentration above 300 mg COD L^{-1} or COD to N ratio of over 2.0 inactivate Anammox organisms in a UASB reactor fed with fat milk as organic carbon source. Milenuevo et al. [71] observed a complete inhibition of the Anammox process at COD concentrations up to 292 mg L^{-1} while Tang et al. [113] stated that denitrifiers became dominant at high influent COD:NO_2^- N ratio of 2.9:1.

Anammox removes only 90% of the incoming nitrogen as ammonia/nitrite and leaves 10% of nitrogen as nitrate in the effluent. A co-existence of Anammox and denitrification in one reactor would aid to reduce the nitrate concentration in the reactor. Under anoxic conditions nitrate can be reduced by denitrifiers to nitrite as intermediate which can be utilized by Anammox for the oxidation of ammonium [118].

Anammox activity is completely and irreversible inhibited by low concentrations of methanol (15 mg L^{-1}) and ethanol [115]. This aspect must be taken in account since methanol is often used to remove nitrate in a post-denitrification step. A possible explanation for the methanol inhibition is the formation of formaldehyde by the Anammox enzyme hydroxylamine oxidoreductase [119].

Recent studies observed that some organic carbon sources do not have an inhibition effect on the Anammox acitivity. Kartal et al. [120] reported that Candidatus *Brocadia fulgida* and Candidatus *Anammoxoglobus propionicus* are able to oxidize acetate and propionate, respectively. Experiments by Güven et al. [115] with propionate as carbon source showed that Anammox organisms oxidized propionate with nitrate and/or nitrite as electron acceptor and simultaneously converted ammonia anoxically. The amount of Anammox bacteria and denitrifiers did not change over time, suggesting that Anammox organisms are indeed able to compete with heterotrophic denitrifiers for propionate. Awata et al. [122] used batch test to investigate the ammonium removal and the carbon incorporation by the Anammox bacteria in the presence of short chain fatty acids present in digestor liquor such as acetate, formate and propionate. They found that propionate did not influence the ammonium removal activity but decreased the incorporation of inorganic carbon. Acetate showed no inhibition in ammonium removal and inorganic carbon incorporation while formate inhibited the Anammox process in the two aspects. It is not yet known whether the Anammox bacteria incorporate acetate directly or indirectly. It could be possible that the CO_2 used by Anammox was derived from denitrification with organic matter such as acetate. Experiments with Anammox cultures in batch experiments by van de Graaf et al. [83] showed that carbon sources such as acetate and glucose had a positive effect on Anammox activity. The continuous experiments however, fed with acetate, glucose and formate showed a negative effect on Anammox activity [83]. Dapena-Mora et al. [102] used batch tests to observe the effect of inhibition effects on the Anammox performance. They found that concentrations of 50 mM acetate resulted in 70% inhibition of the Anammox process while a concentration up to 10 mM did not decrease the activity significantly [102].

Adaptation of Anammox bacteria to streams with toxic components are reported. Toh and Osbolt [123] and Toh et al. [124] described an acclimation of the Anammox organisms to synthetic coke-oven wastewater which contain not only high concentration of organics (2000–2500 mg L^{-1} COD) but also some toxic chemicals such as phenol (300–800 mg L^{-1}), cyanides (10–90 mg L^{-1}) and thiocyanates (300–500 mg L^{-1}). The initial attempt to enrich the bacteria first failed but stepwise addition of phenol 50–500 mg L^{-1} aided to acclimate the Anammox. After a culture of 15 months, the ammonium removal rate peaked to 0.062 kg N m^{-3} d^{-1}.

4.5. Salts

In natural saline ecosystems only anammox species belonging to Scalindua genus have been detected [90]. The other genera are known to inhabit freshwater ecosystems [125].

Dapena-Mora et al. [102] found that NaCl concentrations below 150 mM did not affect the Anammox activity while KCl and Na_2SO_4 had affect at concentrations higher than 100 and 50 mM, respectively. They stated that the different inhibitory effect of NaCl and Na_2SO_4 was attributed to the ion sodium at the tested concentration. Hence, the inhibitory effect of Na_2SO_4 could be related to the higher concentration of Na^+ ions in the medium compared to its concentration when NaCl was added at the same molarities. Van de Graaf et al. [83] observed no effect of KCl on the activity at concentrations of 50 mM. Kartal et al. [125] reported the adaptability of a freshwater Anammox biomass, i.e. *C. Kuenenia stuttgartiensis*, to salt concentrations as high as 30 g L^{-1} in a lab-scale investigation provided that salt concentrations was gradually increased. The nitrogen removal efficiency and maximum Anammox activity of the salt adapted sludge was very similar to the reference freshwater sludge. Windey et al. [126] operated an OLAND under saline conditions and came to the same conclusions as Kartal et al. [125].

4.6. Temperature and pH

Several authors found that the optimum temperature for the operation of Anammox bacteria was around 30–40 °C [103,105]. Dosta et al. [106] used batch tests to observe the short-term effect of temperature on Anammox activity. They found that the maximum activity of non-adapted Anammox biomass ranged between 35 and 40 °C, while a temperature of 45 °C caused an irreversible decrease of the Anammox activity due to biomass lysis. Small differences in optimal temperature were found for *K. stuttgartiensis* and *B. anammoxidans*. *B. anammoxidans* showed highest activity at 40 °C [103] while the highest activity of *K. stuttgartiensis* was observed at 37 °C [105] at an optimal pH of 8.

However, Cema et al. [127] and Isaka et al. [128] proved that the Anammox process in a RBC and anaerobic biological filtrated reactor, respectively could be successfully operated at a low temperature of 20 °C. The slow adaptation of the Anammox sludge seems a key factor in order to operate an Anammox reactor at low temperatures since a drastic change in the operational conditions could lead to a destabilization of the biological system [129]. During the operation of this reactor at low temperature, neither changes on the physical properties of sludge nor qualitative changes on the bacterial populations were found. However, a strong decrease in the nitrogen conversion rate was observed. Isaka et al. [128] solved this problem and reached a high nitrogen conversion rate of 8.1 kg N m^{-3} d^{-1} by decreasing HRT and by using an appropriate non-inhibiting nitrite concentration in the influent.

As stated above, an advisable start up strategy is needed to operate a Anammox system at low temperatures. First, the required amount of biomass must be produced in a separate reactor at a temperature close to the optimum temperature. Then, the biomass can be gradually adapted to low temperatures in the same reactor and finally the low-temperature adapted biomass can be inoculated in the low-temperature reactor [106]. Moreover, research performed with marine Anammox samples reported measurable activities at low temperatures. Rysgaard et al. [130] found Anammox activity in Arctic sediments between −1.3 and 30 °C, the optimum temperature being 12 °C. Similar results were found by Dalsgaard and Thamdrup [90] giving an optimal temperature of 15 °C for marine sediments from the Baltic North Sea. In both cases, a strong decrease of the nitrogen conversion rate was observed.

The optimal pH interval for Anammox is 6.7–8.3 with an optimum of 8.0 [103].

4.7. Biomass concentration

Biomass concentration plays a crucial role for the Anammox activity. Strous et al. [91] found that Anammox is only active when cell concentrations are higher than 10^{10}-10^{11} cells ml^{-1}, even in purified cultures. This could be explained by the need for intercellular communication for activity [34]. Another possible explanation is that hydrazine diffuses relatively easy to the outside of the cell and a minimum internal concentration is necessary for Anammox activity. Sinninghe Damsté et al. [99] however showed that the cellular membranes are less permeable than normal linear membrane lipids.

Perhaps the presence of contaminating cells, 1 on 200–500, is necessary to sustain long term growth, because these cells can guarantee vitamin supply and the removal of toxic components [91,95]. Pynaert et al. [131] put forward the hypothesis that the presence of ammonium oxidizers is necessary for the re-activation of Anammox organisms after disturbance of the system. By the production or accumulation of hydroxylamine or hydrazine by ammonium oxidizers, Anammox organisms can re-activate their metabolism. Once the process is re-established, the ammonium oxidizers are not supposed to significantly participate in the Anammox process. This "sparking" was also described by Strous [132] because it was found that the addition of the intermediates hydroxylamine or hydrazine was necessary to restart Anammox activity after inhibition. On the other hand, Dapena-Mora et al. [102] did not observe a notable effect on the activity at different initial biomass concentration of 0.25–2.0 g VSS L^{-1}.

4.8. Suspended solids

Flocculants are often used to remove colloidal organic and inorganic substances from wastewater previous to the Anammox process. Therefore, the effect of these flocculants on the Anammox process are tested in batch tests by Dapena-Mora et al. [102]. Concentrations up to 1 g L^{-1} polymeric positively charged compound used as flocculant did not cause a detrimental effect on the Anammox activity. In the study of Yamamoto et al. [133], a large amount of influent suspended solids present in the partial nitrified digested liquor attached to the nonwoven materials covering the anammox biomass growing on the carries. This caused a decrease in Anammox activity and became the main reason responsible for the unsatisfactory performance of the Anammox process. The use of a flocculant improved the settle ability of the influent suspended solids and reduced their accumulation inside the reactor but the flocculant itself attached also on the surface of the nonwoven carriers and hence reducing Anammox activity. An unstable operation of the Anammox reactor is also reported due to precipitation of phosphate salts. Trigo et al. [134] operated an Anammox MSBR in which the membrane acted as a barrier that retained the inorganic precipitation salts causing an accumulation of non-suspended volatile solids in the biomass. Precipitation of these salts on the biomass surface interfered with microbial activity causing a decrease of nitrogen removal from 100 to 10 mg L^{-1} per day.

4.9. Other influencing factors

Anammox activity was also found to be sensitive to visible light. A decrease in activity of 30 to 50% was observed by van de Graaf et al. [83]. As a result the equipment for further experiments by these researchers was covered with black plastic and paper to eliminate this light effect. Arrojo et al. [135] showed the effect of shear stress on the Anammox process in a SBR. They stated that stirring speeds up to 180 rpm had no negative effect on the performance of the Anammox process. Anammox activity and the average diameter decreased to 40% and 45%, respectively while

nitrite accumulated in the reactor when a rotating speed of 250 rpm was tested.

5. Practical implementation of autotrophic nitrogen removal

An Anammox step has to be preceded by a partial nitritation step. This can be accomplished in the same reactor (1-reactor system) or by using 2 separate reactors (2-reactor system).

The use of a single reactor has some advantages with respect to the partial nitritation–Anammox configuration. Single-stage processes generally have higher volumetric nitrogen removal rate and lower capital costs than 2-stage systems since no additional nitritation reactor volume is required for ammonium oxidation without nitrogen removal [136]. However, Hao et al. [137] and Nielsen et al. [138] reported difficulties in dissolved oxygen regulation and incomplete nitrogen removal treating high loaded wastewaters. With a 2-reactor system nitrititation and Anammox are separated in space allowing flexibility and a more stable process performance since both steps can be controlled separately [136,139]. In a first reactor half of the ammonium is converted to nitrite, while in a second reactor Anammox is active. It is important that the influent of the Anammox reactor has a constant composition in view of the nitrite toxicity, independent of the strategy used to obtain this Anammox-suited influent. The application of the two units configuration would be appropriated when toxic or organic biodegradable compounds are present since these compounds will be degraded in the proceeding nitritation step avoiding its entrance to the Anammox reactor [140,141]. Hao et al. [137] and Nielsen et al. [138] stated that for high loaded waste streams the relatively high investment costs for a partial nitritation–Anammox process will be compensated by lower operational costs and efficient nitrogen removal performance.

A major disadvantage of these autotrophic nitrogen removal processes is the low growth rate of AOB and Anammox bacteria. The performance of reactors involving slow growing bacteria can be enhanced by applying high sludge retention time. This could be achieved by applying carrier materials to develop biofilms or by self-aggregation in granules [206].

An overview of different reactors described in literature is presented here. From this overview it becomes clear that a lot of the experimental knowledge on autotrophic nitrogen removal are described in lab scale reactors although in recent years full-scale reactors are brought into operation.

5.1. Partial nitritation and anammox in one reactor (1-reactor system)

In a 1-reactor system, a co-culture of aerobic and anaerobic ammonium-oxidizing bacteria is established under microaerobic conditions to avoid inhibition of Anammox bacteria by oxygen and to achieve appropriated conditions to obtain partial nitritation [110]. In those system, the growth of NOB (and subsequent nitrate production) is prevented due to their lower affinity to oxygen compared to AOB and for nitrite compared to Anammox bacteria [49]. Possible inhibition of nitrite oxidizers by free ammonium has also been suggested [19].

Different kind of systems such as SBR, gas-lift, RBC and moving bed reactors were used to obtain the microaerobic conditions for the 1-step process. In biofilm or granule reactors the ammonium oxidizers are active in the outer layers of the biofilm (or granule), producing a suitable amount of nitrite for the Anammox organisms that are active in the inner layers. This way the Anammox organisms are protected from oxygen, which is consumed in the outer layers [136]. A variation on the classic biofilm reactor is the membrane aerated biofilm reactor (MABR [142]). In MABR systems hydrophobic, gas-permeable membranes are used for bubbleless oxygen transfer. In the oxygen rich region near the membranes ammonium oxidizers are converting ammonium to nitrite, while in the ammonium rich region near the water phase Anammox organisms are active.

When these biofilms and granular systems are used to perform the process, mass transfer resistance uses to be the limiting step. As long as ammonium concentration outside the biofilm is much higher than the oxygen or nitrite concentration, ammonium diffusion into the biofilm will not limit the process rate. If the nitrite produced in the outer layer is mainly consumed in the inner layer, oxygen is the main limiting factor controlling the overall rate. Sliekers et al. [143] and Szatkowska et al. [144] reported that oxygen transfer was indicated as the limiting factor for a lab-scale air-lift and a pilot-scale moving bed reactor, respectively. This oxygen limitation can be attributed to the slow diffusion into the biofilm/granule or from a not-efficient gas–liquid transfer.

Nitrite concentration is a strong inhibitor of the Anammox process. If nitrite is consumed at about the same rate as it is produced this inhibition effect is not of significance. No negative effect of nitrite was observed by Vazquez-Padin et al. [140] although during the first 100 days of operation a mean nitrite concentration of 25 mg N L^{-1} was registered. Probably a concentration gradients inside the granules resulted in a low nitrite concentration at the location of the Anammox bacteria.

Various names are used to describe the 1-reactor systems [104]: the OLAND-process (Oxygen Limited Autotrophic Nitrification and Denitrification) [145], the CANON process (Completely Autotrophic Nitrogen removal Over Nitrite) [146], aerobic/anoxic deammonification or DEMON [147,148] and the SNAP process (Single-stage Nitrogen removal using Anammox and Partial nitritation) [93]. The difference lies in the organisms that were originally assumed to be responsible for anaerobic ammonium oxidation. In both the OLAND-process and the aerobic/anoxic deammonification process nitrifiers were assumed to perform this ammonium oxidation under microaerobic conditions [145,149]. In the CANON process Anammox bacteria were assumed to be responsible. Studies [108,150] with FISH analyses confirmed that anaerobic ammonium oxidation in all reactors was performed by Anammox organisms, although Pynaert et al. [108] did not exclude a specific role for the aerobic ammonium oxidizers.

Most CANON systems reported in literature were operated at 30–35 °C with a maximal nitrogen removal rate of 0.075–1.5 kg N m^{-3} d^{-1} [143,151]. At these temperatures AOB grow faster than NOB and also the growth of Anammox bacteria is stimulated since this temperature range lies close to their optimal temperature. However, in an air pulsating SBR operated at 20–24 °C a similar maximal nitrogen removal of 0.5 kg N m^{-3} d^{-1} [140] are reported while only a slightly activity of NOB was observed. The feasibility of achieving a quick start-up and high nitrogen removal rates in autrophic nitrogen removing systems at temperature around 20 °C was already reported by Isaka et al. [128] and Dosta et al. [106] in a two stage system and Pynaert et al. [131] in one stage system.

The efficient retention of biomass in a SBR makes it possible to cultivate slowly growing bacteria. However, higher nitrogen removal rates were obtained in a RBC [108,131] and in an air lift reactor [143].

Model simulations indicated that the maximum nitrogen removal rate was achieved only when the dissolved oxygen concentration kept pace with the ammonium surface load [137]. For fluctuating ammonium loading rates in engineering dissolved oxygen can be regulated through online feed-back control [138]. With a simulation study Hao et al. [137] showed that the optimal bulk oxygen concentration for a CANON biofilm reactor is about 1 mg O_2 L^{-1},

although this optimum depends on the biofilm thickness and density, boundary layer thickness, the COD content of the influent and the temperature. Oxygen control of these CANON systems is therefore necessary.

Kuai and Verstraete [145] first introduced the term OLAND describing lab-scale research with a SBR reactor fed with synthetic influent in which only 0.050 kg N m^{-3} d^{-1} ammonium was removed. Sliekers et al. [151] conducted experiments in lab-scale completely mixed reactors using a specific start-up pattern consisting of anoxic inoculation with Anammox biomass followed by oxygen supply to develop nitrifying microorganisms. Ammonia was mostly converted to nitrogen gas (85%) while the remainder was recovered as nitrate. However, the nitrogen removal rate in the SBR [151] was still low with only 0.064 kg N m^{-3} d^{-1}. FISH analysis confirmed the absence of nitrite oxidizers and the presence of aerobic ammonia oxidizers (45%) and anaerobic ammonium oxidizers (40%) in the CANON biomass [151]. Recently, De Clippeleir et al. [152] and Vazquez Padin [140,206] observed high nitrogen removal rates in a SBR provided that granulation occurred. The operation of these granular sludge reactors is very similar to biofilm reactors. In the outer oxic layer ammonium is converted to nitrite, while Anammox is active in the centre of the granule.

Pynaert et al. [108,131] constructed, operated and characterized an OLAND RBC system fed with both synthetic and actual waste water and a fixed oxygen concentration in which high removal rates could be achieved. Within 100 days after inoculation of a granular anaerobic sludge a maximum ammonium removal of 1.80 kg N m^{-3} d^{-1} was achieved. In Sliekers et al. [143] a gas lift reactor with high conversion rate of up to 1.5 kg N m^{-3} d^{-1} was easily maintained. Recently also artificial wetlands were used as autotrophic nitrogen removing systems resulting in 50–60% nitrogen removal [153,154].

The term aerobic/anoxic deammonification or DEMON was first used when significant losses of inorganic nitrogen of up to 90% were observed in the nitrification step of a rotating biological contactor (RBC) treating ammonium-rich landfill leachate under low oxygen conditions [147]. Extended nitrogen loss was also observed in other RBCs in Switzerland and the UK [86,155]. None of the plants were specifically built for deammonification, but nitrogen elimination was established over time. In the Swiss RBC about 50% of the bacteria population in the biofilm consisted of Anammox. Next to RBC's continuous flow moving-bed pilot plants were run as well. Optimal ammonium elimination was achieved at a bulk oxygen concentration of 0.7 mg O_2 L^{-1}. The end product is always N_2, although Gaul et al. [156] reported up to 12% N_2O production caused by incomplete heterotrophic denitrification under anoxic or oxygen-limited conditions. The first full-scale application with deliberate deammonification in a moving bed reactor using Kaldnes® carriers was put into operation in April 2001 [157] at the WWTP of Hattingen (Germany). Two identical reactors had a volume of 67 m^3 and an effective biofilm surface area of 13,400 m^2. The oxygen concentration was kept below 1 mg O_2 L^{-1}. First results are given in Cornelius and Rosewinkel [158]. Currently full-scale plants are in operation in for example Strass (Austria) and Zurich (Switzerland). The plant in Strass treats the wastewater of 200,000 population equivalents, and is equipped with a 500 m^3 sequencing batch reactor (SBR) for deammonification of reject-water originating from digested-sludge dewatering [159]. In Zurich a 1400 m^3 reactor is operational treating over 500 g N m^{-3} d^{-1} with conversions over 90% [160].

A summary of several of the lab-scale experimental studies described in literature is given in Table 5. In Table 6 results from full-scale experiences is presented.

Two strategies are possible to start-up a one-reactor autotrophic nitrogen removal system. The first method is the inoculation of nitrifying biomass into a well performing Anammox reactor and supplying air into the reactor to maintain microaerobic conditions.

Table 5
Summary of several lab-scale experimental studies on 1 reactor systems for autotrophic nitrogen removal described in literature.

Reactor type	Volume (L)	Influent type	Inocula	HRT (d)	SRT (d)	DO (mg L^{-1})	pH	Temperature (°C)	Removal rate (kg N m^{-3} d^{-1})	N removal (%)	Reference
Air lift	1.8	Synthetic	Anammox + nitrifying sludge	0.42	–	0.5	7.5	30	1.5	42	[143]
RBC	44	Synthetic	RBC biomass		–	0.6		29	1.05	89	[108]
	50	Synthetic	RBC biomass	1	–	0.3	7.85	30	1.80	88	[131]
	50	Sludge liquor	RBC biomass	1	–	1.0	7.85	14	0.42	42	[131]
SBR	1.5	Reject water diluted with tapwater 1:1[a]	Nitrifying granular + 'Anammox'	0.5	30–110	0.5	7.5–7.9	21	0.5	78	[140]
	2.0	Synthetic	'Anammox'	1	–	<0.1	7.8	30	0.06	50	[151]
	2.5	Synthetic	OLAND	–	–	0.3–0.7	>7.4	32–34	1.1	–	[152]
	10	Sludge liquor			–	0.6		30	0.06	76	[8]
Upflow granular bed reactor	50	Sludge liquor	Nitrifying:denitrifying activated sludge		–	1.8		30	0.36	60	[156]
Moving bed biofilm reactor	4	Synthetic	Nitrifying biofilm + Anammox		–	0.5		35	0.77	89	[142]

Note: Some of the characteristics of the reactors were not reported.
[a] To reach an ammonium concentration of 0.15–0.35 g N L^{-1}.

Table 6
Overview of the operational conditions and nitrogen removal performance of several pilot and full scale 1-reactor autotrophic nitrogen removal systems.

Reactor type	Volume (m^3)	Influent type	pH	T (°C)	DO (mg O_2 L^{-1})	Removal rate (kg N m^{-3} d^{-1})	Removal efficiency (%)	Reference
SBR	500	Sludge liquor	7.05–7.10	25–30	0.3	0.6	84	[195]
	400	Sludge liquor	7.05–7.10	25–30	0.3	0.4	90	[196]
	4.1	Sludge liquor	7.4–7.6	25	0.5–1.0	0.65	90	[197]
Upflow reactor	600	Sludge liquor	8.0	30–35	2.0–3.0	1.3	75–80	[197]
MBR	Full scale	Landfill leachate	–	–	0.5	0.33	73	[198]
	Full scale	Landfill leachate	6.9	–	0.3	0.33	84	[199]
Moving bed	0.04	Sludge liquor	8.0–8.5	27	<1.0	0.5	60–70	[200]
	0.04	Sludge liquor	8–8.1	28–29	0.8–2.0	0.12–0.22	75–71	[155]
	21	Sludge liquor	7.6–8	23–27	1.2–2.6	0.38	62	[144]
	Full scale	Sludge liquor	7.8	30	3	0.35	64	[201]
	Full scale	Sludge liquor	8	27	–	0.21	72	[201]
RBC	265	Landfill leachate	8.3 (7.4–8.7)	28 (27–30)	0.7–1.0	0.15–0.26	40–70	[147]
	33	Landfill leachate	7.3	16	1.0–2.0	0.25–0.57	30–70	[86]
	240	Landfill leachate	8.1 (7.2–8.8)	14 (10–28)	0.8–1.2	1.7	30–70	[92]

Note: Some of the characteristics of the reactors were not reported.

Otherwise, a partial nitritation reactor can be operated under oxygen limited conditions obtaining an ammonium:nitrite ratio of 1:1 before Anammox biomass is inoculated into the reactor [131,142]. The second strategy seems to be more appropriated since an important decrease of Anammox activity will be observed when the first method is applied [143,151,161]. This high nitrifying activity can protect the Anammox bacteria from oxygen and provides them enough nitrite. The inoculation of Anammox enriched biomass in a partial nitritation reactor accelerates the start-up and allows to increase the ANR after 1 or 2 months instead of the several months or even years without inoculation [140,208]. Moreover, only a limited amount of Anammox biomass is necessary to start-up the CANON process with this second strategy.

5.2. Partial nitritation and anammox in separate reactors (2-reactor systems)

5.2.1. Partial nitritation

The challenge for the first reactor in a 2-reactor system is to obtain a stable, Anammox-suited effluent, i.e. with a molar ammonium:nitrite ratio of 1:1.32 according to the stoichiometry proposed by Strous et al. [82]. In practice, however, this ratio will be closer to 1:1 in view of the desire to prevent nitrite inhibition, i.e. by providing an excess of ammonium. Up to now three types of reactors were used to achieve this: completely stirred tank reactors (CSTR), membrane bioreactors (MBR) and sequencing batch reactors (SBR). In the MBR and SBR reactor high sludge retention times are obtained (50–75 days) [162]. In the MBR the SRT is difficult to manipulate unlike in suspended growth systems which brings difficulty to suppress nitrite oxidizers even under oxygen-limited concentrations [163]. Fux et al. [164] also stated that a long-term nitrite production without nitrate accumulation can be unreliable in biofilm systems since the control of the sludge age is difficult. None of the selection criteria applied such as high free ammonia, low oxygen concentration or high ammonium loading rate led to selective suppression of nitrite oxidation in a long-term laboratory and pilot scale moving-bed biofilm reactor. For full scale applications, a CSTR or a SBR with suspended biomass is recommended.

Further, the footprint of an MBR systems is reduced due to the absence of settling tanks and the reduction in bioreactor volume due to the higher biomass concentration [163].

The possibility to obtain an Anammox-suited effluent by SHARON process was tested by van Dongen et al. [5] and Mosquera-Corral et al. [73] in a CSTR with reject water as influent at a temperature of 35 °C and a HRT and SRT of 1 day. The ammonium was for 53% oxidized to nitrite without pH control resulting in a nitrite: ammonium ratio of 1.13:1. In the subsequent Anammox reactor nitrite was therefore the limiting component. Van Hulle et al. [27] described the start-up and operation of a lab-scale SHARON reactor operated at 35 °C without pH-control. An Anammox-suited influent was obtained with synthetic influent containing an ammonium loading rate up to 1.5 kg N m^{-3} d^{-1}. Udert et al. [165] described also good SHARON performance with urine as influent. In the CSTR an ammonium:nitrite ratio of 1:1 was obtained at a HRT of 4.8 days and a pH of 9.2.

The SHARON technology is nowadays successfully used at full scale to treat effluents from sludge digesters. Full-scale SHARON reactors are currently in operation at the sludge treatment site Sluisjesdijk of the WWTP of Rotterdam and Utrecht (The Netherlands) [12]. Fux et al. [38] also operated a 2.1 m^3 CSTR reactor in Zurich at a HRT of 1.1 days and a temperature of 30 °C without pH control. Digester effluent from two different WWTPs was tested obtaining an Anammox-suited ammonium:nitrite ratio of 1:1.32 at a pH between 6.6 and 7.2.

Although the SHARON process is successfully started up at full scale, there are still some disadvantages connected to this process. Sludge digesters operate at high HRT values guaranteeing a stable composition of its effluents for the subsequent SHARON process (low biodegradable organic matter and a bicarbonate to ammonia molar ratio of 1). When the HRTs in the digesters are lower than usual or when industrial wastewaters are used, fluctuations of the influent composition into the SHARON reactor will occur. Therefore, operational parameters such as DO or pH must be controlled in the preceding SHARON process to obtain an optimal nitrite:ammonium ratio [26]. Another disadvantage is the limited maximum volumetric loading rate of SHARON reactor, as sludge is constantly withdrawn. To assure stable operation, the minimum HRT of a chemostat is limited to 1–1.2 days. In MBR, SBR or biofilm systems biomass is retained giving the advantage that HRT can be uncoupled from SRT and HRT lower than 1 day is possible resulting in much higher loading rates (i.e. smaller reactors with similar treatment capacity [136]). Protozoa can cause problems in the operation of a SHARON reactor mainly if real wastewater is used [5]. A possible solution is to lower the reactor pH to 6 for 2 h or to incorporate non-aerated periods. A pH-lowering can be obtained by reducing the influent flow under constant aeration. Non-aerated periods, however, clearly have a negative effect on the nitrogen conversion by nitrifiers and the SHARON reactor has to be 30% larger to maintain good nitrite formation [5]. Moreover, the required performance temperature of SHARON is higher. When the effluent of the treated stream is lower than 24 °C the maximal growth rate of AOB turns lower than that of

nitrite oxidizers and ammonium is fully oxidized into nitrate [38]. Therefore, to achieve partial nitritation at temperature lower than 24 °C other strategies such as inhibition of NOB by ammonia and nitrous acid or operation at low oxygen concentrations should be applied.

Wyffels et al. [166] used a MBR as a first step of the autotrophic nitrogen removal process at low dissolved oxygen concentrations (<0.1 mg O_2 L^{-1}). The membrane had to be regularly cleaned to prevent clogging. The pH was controlled at 7.9 and the temperature was set to 35 °C, although an experiment at room temperature was conducted as well. Lowering the temperature had no significant effect on the obtained nitrite:ammonium ratio. Similarly, lowering the NH_3 concentration, and possibly lowering the NH_3 inhibition on nitrite oxidizers, had no significant effect on the obtained nitrite:ammonium ratio. This indicates that oxygen limitation is the most important operational factor. Feng et al. [167] and Xue et al. [163] also used the MBR to obtain good partial nitritation performance at low dissolved oxygen concentration. Feng et al. [167] stated that alkalinity also played an important factor to achieve a nitrite: ammonium ratio of 1.3:1 while Xue et al. [163] reported that free ammonia inhibited the nitrite oxidizers.

Ganigué et al. [168] showed that the SBR is a feasible technology to achieve stable influent for an Anammox reactor when urban landfill leachate is treated. At low pH values biological activity decreased due to an inhibitory effect by free nitrous acid and a lack of bicarbonate. On the other hand, high pH values indicated a decrease in oxygen uptake rate caused by free ammonia inhibition. As such, pH is considered to be an important factor. Udert et al. [165] used a SBR to treat urine at a temperature of 24.5 °C while varying the oxygen concentration between 2 and 4.5 mg O_2 L^{-1}. The pH at the start of the reaction cycle was 8.8 and gradually decreased to a minimum of 6 as ammonium conversion continued. At this pH ammonium conversion stopped probably because NH_3 limitation and HNO_2 inhibition obtaining an ammonium:nitrite ratio of 1. Another possible explanation is the inhibition of nitrite oxidizers by the intermediaire hydroxylamine. Yamamoto et al. [133] applied the partial nitritation and Anammox process to treat swine wastewater digester liquor. They observed that a stable conversion of ammonia into nitrite of 58% could be reached in a biofilm reactor due to inhibition of free ammonia and free nitrous acid. The inhibition of free ammonia was also brought forworth by Liang et al. [169] and Qiao et al. [170] who treated landfill leachate and digested liquid manure, respectively in a biofilm reactor achieving a nitrite: ammonia molar ratio near 1.3.

In the different experiments described above, different conditions were used to favour the growth of ammonium oxidizers over nitrite oxidizers in order to produce an Anammox-suited-influent. Four principles can be distinguished: the operation of the reactor at low dissolved oxygen concentration (<0.5 mg O_2 L^{-1}), the operation of the reactor at high pH (7.5–8.5), which increases the ammonia availability and decreases the nitrous acid availability, the operation of the reactor at high temperature (>25 °C), a limited nitrification time which stops ammonium oxidation before its depletion and the presence of a bicarbonate limitation which stops nitrification. Table 7 summarizes several experiments described in literature.

5.2.2. Anammox

The practical application of the Anammox process is still limited by its long start-up periods (up to 1 year) due to the very low growth rate and low cell yield of Anammox organisms. Loss of a fraction of the sludge due to wash-out with the effluent could further augment the start-up period. Hence, it is essential to use a reactor with high biomass retention. The cultivation of slow-growing microorganisms relies mostly on the ability of biomass to form biofilms or aggregates such as flocs or granules [100]. So far a large range of bioreactor types have been evaluated for the enrichment of Anammox bacteria: fixed bed reactors, fluidized-bed-reactors, UASB-reactors, SBR, gas-lift reactors [136,171]. Among them, the SBR was accepted for Anammox enrichment for its simplicity, efficient biomass retention, homogeneity of mixture in the reactor, stability and reliability for a long period of operation, stability under substrate-limiting conditions and high nitrogen conversions [5,43,82]. The SBR reached a biomass retention of 90% which was 1.4 times more than in a fluidized bed reactor [172]. Strous et al. [172] started up the Anammox process in a fixed-bed and fluidized bed reactor with glass and sand particles as carriers but could not prevent biomass loss due to floating sludge caused by entrapped gas bubbles. The same situation occurred in the gas lift reactor at increased nitrogen removal rate [173]. Dapena-Mora et al. [173] stated that mechanical stirring in a SBR could be more effective to eliminate the gas entrapped in the granules compared to the less abrasive stress in a gas-lift. Further, also the application of non-woven fibers can incease the biomass retention as several experiments with nonwoven fibers demonstrated a short start-up time and high nitrogen removal rates [98,101].

An alternative for obtaining full biomass retention in Anammox systems might be the use of membrane bioreactors (MBR). Unlike the reactors with granular biomass, the MBR enables cultivation of slow growing bacteria with biomass retention and without a selection on settling ability. van der Star [100] pointed out that the MBR reactor is a more powerful tool for Anammox research as high production of almost pure suspended anammox cells could be obtained avoiding the diffusion limitations within flocs or granules. A membrane SBR which is a combination of a SBR and a biofilm system was applied by Trigo et al. [134] achieving a high nitrogen removal rate. Wang et al. [176] used a stirrer in the MBR to make the Anammox bacteria suspended as free cells and a more homogeneous distribution of substrates and biomass can be achieved. However, for full-scale applications biofilm- or granular-based bioreactors are preferable over MBRs since anammox bacteria easily form sludge granules or biofilms obtaining a high biomass concentration in the reactor on a simple and economical way. Further, fouling of the membrane system could occur. The operation costs due to backwashing (high energy consumption) or external cleaning with chemicals are inevitable in engineering practice [134,177]. Moreover, wastewater always contains a certain amount of solids which are also retained in a MBR reactor. This accumulation of solids could decrease the activity in a full-scale MBR-based anammox process [133].

A summary of the experimental studies described in literature is given in Table 8. From these studies the potential of the Anammox process can be seen since total nitrogen removal rates up to 26 kg N m^{-3} d^{-1} in a fixed bed reactor fed with synthetic wastewater [174]. In contrast, the nitrogen removal rate is not so high in engineering. A possible explanation of the lower nitrogen removal rates in pilot plants is the limited availability of substrate in real waste waters. The efficiency of biomass retention is another factor which determines the maximum conversion while in biofilm reactors, nitrite fux to the biofilm is another potential limitation. Isaka et al. [128] stated that HRT has an influence on the nitrogen removal rates. Under appropriate nitrite and ammonium concentrations nitrogen conversion rates can be increased by decreasing the HRT. Wyffels et al. [136] stated that the maximum nitrogen removal rate of Anammox organisms is limited by the growth rate of ammonium oxidizers in the SHARON process since a minimum HRT of 1 day is needed.

The concentration of nitrite during the start-up is of crucial importance for growth: a too low amount will result in substrate limitation and thus slower growth, while concentration above 20 mg N L^{-1} can already lead to inhibition. As such, nitrite

Table 7
Summary of several experimental studies concerning partial nitritation in view of coupling with an Anammox reactor described in literature.

Process	Reactor type	Volume (L)	Influent type	pH	Temperature (°C)	DO (mg L^{-1})	SRT (d)	HRT (d)	N load (kg N m^{-3} d^{-1})	NO_2^-:NH_4^+ ratio	Nitrate in the effluent (%)	Reference
SHARON	CSTR	2100	Sludge digester effluent from WWTP	6.6–7.2	29	2.7	1.05–1.18	1.05–1.181	0.56	1.4	Negligible	[38]
SHARON	CSTR	2	Synthetic	7.1	35	–	1–1.5	1.54	1.5	1	–	[27]
SHARON	CSTR	10	Sludge digester effluent from WWTP	6.7	35	–	1	1	1.2	0.74	113	[5]
SHARON	CSTR	2.8	Urine	9.2	30	2.5–4	4.8	4.8	1.580	1	Negligible	[165]
SHARON	CSTR	3.2	Digested effluent of fish canning	7.5	35	>2	1	1	0.1	1	No nitrate	[73]
Partial nitritation	SBR	7.5	Urine	6–8.8	24.5	2–4.5	>30	4	0.560	1	Negligible	[165]
Partial nitritation	SBR	20	Landfill leachate	6.8–7.1	36	2	3–7	1.5	1.5	0.6–1.5	<5	[168]
Partial nitritation	Biofilm	10.8 + 2.5	Pre-filtered digested liquor of swine water[a]	–	25	5	13	1	1.0	1.38	<5[b]	[133]
Partial nitritation	Upflow fixed bed biofilm reactor	11	Landfill leachate	8.4	30	0.8–2.3	–	Varying	0.27–1.2	58.3		[169]
Partial nitritation	Column with PEG carrier	8.0	Digested liquid manure	7.5–8	30	2.5–6.5	–	–	3.8	1.22	2.3	[170]
Partial nitritation	MBR	1	Synthetic	8	35	<0.6	35	0.23	–	1.30	Trace	[167]
Partial nitritation	MBR	14	Synthetic	8	35	0.3–0.5	–	0.67	0.450	1	–	[163]
Partial nitritation	MBR	1.5	Pre-filtered Sludge digester effluent of WWTP	7.9	30	<0.2	Varying	0.58–1	0.73–1.45	1.13	–	[166]

Note: Some characteristics of the reactors were not reported.
[a] Diluted with tap water to achieve a NLR of 1 kg N m^{-3} d^{-1}.
[b] Conversion efficiency of ammonia in nitrate.

Table 8
Overview of operational parameters and nitrogen removal of a selection of studies on Anammox.

Reactor type	Volume (L)	Influent type	Inoccula	HRT (d)	pH	T (°C)	NLR ($g\,N\,L^{-1}\,d^{-1}$)	NRR ($g\,N\,L^{-1}\,d^{-1}$)	SNR ($g\,N\,g\,VSS^{-1}\,d^{-1}$)	N removal (%)	Reaction N ratio (NH_4^+:NO_2^-:NO_3^-)	Reference
SBR	20	Anaerobic digester centrate	Anammox	–	7.5–7.8	35	0.380	0.2[a]	–	85	1:1.20:0.13	[202]
SBR	1600	Digester effluent from WWTP	Anammox biomass	–	7.45–7.59	30.4–31.8	0.650[a] (2.6)	0.56–0.64 (2.4)	0.075	85–99	1:1.38:0.32	[38]
SBR	1	Anaerobic digester supernatant	Anammox biomass	1	7.5	19–21	0.28	0.08	0.13	69	–	[206]
SBR	1.2	Synthetic	Digested sludge of WWTP	0.1	7.5–8	30	2.7	2.0	–	–	1:1.08:–	[203]
SBR	1	Synthetic	Anammox granular sludge	0.625	7.8–8	35	1.0	0.7	0.65	78	1:1.11:0.2	[173,204]
SBR	1	Synthetic	Activated sludge	1	7.8	35–36	0.6	0.6	0.21	–	–	[205]
SBR	10	Digester liquor	Anammox	1	7–8	30–37	1.0	0.55–0.95	0.18	–	1:1.03:–	[5]
SBR	3	Effluent from a fish-canning industry pre-digested	Anammox sludge	1.8	7.5–8.2	35	0.34–0.67	0.3	0.44	40–80	–	[140]
Air lift	70,000	Digester effluent from WWTP	Activated sludge + Anammox biomass	–	7–8	30–40	9.5	9	–	–	1:1.32:0.25	[175]
Airlift	7 + 3	Synthetic	Anammox granular sludge	1	7.9–8.1	30	2.3	2.0	1.15	88	1:1.28:0.26	[173]
Airlift	1.8	Synthetic		0.28	7.5	30	10.7	8.9	–	–	–	[143]
MBR	1.5	Digester effluent from WWTP	Anammox biomass	0.75–1.1		20–30	0.65–1.1	0.55	–	82	1:1.05:0,20	[136]
MBR	5	Synthetic	Anammox granular sludge	1	8	35	0.74	0.71	0.45	73.6	1:1.22:0.22	[134]
MBR	4.8	Synthetic	Nitrifying and denitrifying Activated sludge	2	8	35	–	–	0.35	90	1:1.15:–	[176]
CSTR	0.73	Liquid manure digestor liquor	Anammox granular sludge	0.2	7.5	30	3.73	2.60	–	77	1:1.20:0.22	[170]
Fixed bed	0.8	Synthetic	Activated WTP-sludge	0.06–0.3	7.0–7.5	37	0.1–9.4	6.2	–	–		
	0.8	Synthetic	Concentrated activated WWTP-sludge	0.01–0.3	7.0–7.5	37	0.1–58.5	26.0	1.6	–	1:1.2:0.33	[174]
UF	36	Landfill leachate	Activated sludge + Anammox sludge		7.5–8	30	–	0.11	–	62	1:1.09:0.07	[169]
UF	2.85	Liquid manure	Anammox sludge	1	7.2–7.6	35	0.39	0.22		55	1:1.67:0.53	[133]
UF	2.85	Synthetic	Anammox sludge	1	7.2–7.6	35	0.67	0.5		73	1:1.26:0.33	[133]
FB	2.5	Synthetic	Denitrifying sludge	0.9–1.75	8	36	2.0	1.8	0.18	–	1:1.5:–	[172]
FB	2.5	Effluent of digested WWT sludge	Denitrifying sludge	0.14–11	8	36	2.5	1.5	0.15	–	1:0.55:–	[172]
Continuous flow	19.4	Digested effluent from WWTP	Anammox biomass	–	7.5–7.8	35	0.14–0.38	0.33	–	85–91	1:1.21:0.13	[178]
ABF	0.2	Synthetic	Anammox biomass	0.03	7.2	20–22	12	8.1	–	–	–	[128]
ABF	0.2	Synthetic	Anammox biomass	0.06	7.2	37	19.1	11,5	–	–	–	[128]
UBF	200	Effluent of partial nitritation		0.38	7.5	30	7.0	6.4				[207]
UBF	1.2	Synthetic	Digested sludge of WWTP	0.12	7.5–8	30	2.5	2.0	–	–	1:1.37:–	[203]
UASB	1 + 0.5	Piggery waste	Granular sludge	5	8.2–8.5	35	1.02[b]	0.66	0.08	80	1:1.65:0	[70]
UASB	1 + 0.5	Piggery waste	Granular sludge	5	8.2–8.5	35	0.84[b]	0.59	0.06	82	1:1.13:0	[70]

[a] A nitrite surplus in the effluent of the nitritation reactor is balanced by adding raw digester effluent.
[b] With addition of synthetic nitrite.

levels could increase even more leading to complete process failure. Start-up of Anammox reactors is often characterized by a gradual increase of nitrite concentration in the influent. The nitrite:ammonium ratio in the influent reaches 1 although often an excess of ammonium is used allowing a lower overall nitrogen removal efficiency but guaranteeing a more stable process. Since the Anammox process is anaerobic, the absence of oxygen is an essential step especially during the start-up of reactors [173]. Further, the impact of variability in real streams on the performance of Anammox in full-scale reactors is not well understood [178] yet.

To fasten up the start-up period, Anammox biomass is often used as inocula of Anammox reactors. The fast start-up time of 14 days in a SBR reactor by Sliekers et al. [143] was due to the inoculation of the reactor with fully active Anammox sludge. For the other reactors start-up time was significantly higher. Sequential addition of the pre-enriched Anammox sludge was also selected as a strategy for the engineering practice in the Netherlands [175]. The 10 L lab scale reactor was directly scaled up to a full scale reactor of 70 m^3 reactor. This reactor was initiated in Rotterdam in 2002 and the start-up took nearly 3.5 years. Now stable operation reached a nitrogen removal rate of 9.5 $kg\,N\,m^{-3}\,d^{-1}$ [175] (Table 8).

6. The future of autotrophic nitrogen removal: Towards full-scale applications with low start-up times

Autotrophic nitrogen removal offers a useful and sustainable alternative for treating highly loaded nitrogen streams with an unfavourable carbon to nitrogen ($C\,N^{-1}$) ratio. The process has already been studied extensively on lab-scale and pilot-scale by research groups around the world. The resulting engineering aspects and practical implementation of these studies were reviewed in this contribution. An Anammox-suited effluent can be produced by selection of the substrate availability and the appropriate temperature, pH and oxygen conditions in the partial nitritation reactor. Careful control of this reactor as well as the limitation of inhibiting factors in the Anammox reactor are essential for the successful operation of the combined process.

These efforts on lab-scale resulted in a growing number of full-scale applications. For the further development of autotrophic nitrogen removing processes, research should be conducted towards fast start-up strategies and sustainable control of the process. Further research into the fundamental Anammox behaviour will certainly help to improve this operational control.

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References

[1] Metcalf & Eddy, Inc., Revised by G. Tchobananoglous, F.L. Burton, Wastewater engineering: treatment, disposal and reuse. McGraw-Hill, McGraw-Hill series in water resources and environmental engineering, New York, USA, 2003.
[2] U. Wiesmann, Biological nitrogen removal from wastewater, Advances in Biochemical Engineering/Biotechnology 51 (1994) 113–154.
[3] A. Mulder, A.A. van de Graaf, L.A. Robertson, J.G. Kuenen, Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor, FEMS Microbiology Ecology 16 (1995) 177–184.
[4] E. Broda, Two kinds of lithotrophs missing in nature, Zeitschrift fur Allgemeine Mikrobiologie 17 (1977) 491–493.
[5] U. van Dongen, M.S.M. Jetten, M.C.M. van Loosdrecht, The SHARON®-Anammox® process for treatment of ammonium rich wastewater, Water Science & Technology 44 (1) (2001) 153–160.
[6] M.S.M. Jetten, S.J. Horn, M.C.M. van Loosdrecht, Towards a more sustainable wastewater treatment system, Water Science & Technology 35 (9) (1997) 171–180.
[7] M.J. Kampschreur, H. Temmink, R. Kleerebezem, M. Jetten, M.C.M. van Loosdrecht, Nitrous oxide emission during wastewater treatment, Water Research 43 (2009) 4093–4103.
[8] Y.-H. Ahn, Sustainable nitrogen elimination biotechnologies: a review, Process Biochemistry 41 (2006) 1709–1721.
[9] E. Plaza, J. Trela, L. Gut, M. Löwén, B. Szatkowska, Deammonification process for treatment of ammonium rich wastewater. In: Integration and optimisation of urban sanitation systems, Joint Polish-Swedish Reports, No. 10. TRITA-LWR REPORT 3004, pp. 77–87, Gdansk, Poland, March 23–25, 2003.
[10] S.E. Vlaeminck, Biofilm and granule applications for one-stage autotrophic nitrogen removal, Phd Thesis, Ghent University, Belgium, 2009.
[11] A. Mulder, The quest for sustainable nitrogen removal technologies, Water Science & Technology 48 (1) (2003) 67–75.
[12] J.W. Mulder, M.C.M. van Loosdrecht, C. Hellinga, R. van Kempen, Full-scale application of the Sharon process for the treatment of rejection water of digested sludge dewatering, Water Science & Technology 43 (11) (2001) 27–134.
[13] M.M. Clabaugh, Nitrification of landfill leachate by biofilm columns, MSc Thesis, Virginia Polytechnic Institute and State University, USA, 2001, 43 p.
[14] P. Ilies, D. Mavinic, The effect of decreased ambient temperature on the biological nitrification and denitrification of a high ammonia landfill leachate, Water Research 35 (2001) 2065–2072.
[15] T. Feyaerts, D. Huybrechts, R. Dijkmans, 2002, Best beschikbare technieken voor mestverwerking, Technical report, Vlaams Instituut voor Technologisch Onderzoek VITO (in Dutch).
[16] J. Carrera, J. Baeza, T. Vicent, J. Lafuente, Biological nitrogen removal of high-strength ammonium industrial wastewater with two-sludge system, Water Research 37 (2003) 4211–4221.
[17] S. Murat, G. Insel, N. Artan, D. Orhon, Peformance evaluation of SBR treatment for nitrogen removal from tannery wastewater, in: IWA Speciality Symposium on Strong Nitrogenous and Agro-Wastewater, vol. 2, Seoul, Korea, June 11–13, 2003, pp. 598–605.
[18] J. Keller, K. Subramaniam, J. Gösswein, P. Greenfield, Nutrient removal from industrial wastewater using single tank sequencing batch reactors, Water Science & Technology 35 (6) (1997) 137–144.
[19] U. Abeling, C.F. Seyfried, Anaerobic-aerobic treatment of potato-starch wastewater, Water Science & Technology 28 (2) (1993) 165–176.
[20] J. Campos, A. Sanchez, A. Mosquera-Corral, R. Mendez, J. Lema, Coupled BAS and anoxic USB system to remove urea and formaldehyde from wastewater, Water Research 37 (2003) 3445–3451.
[21] J. Garrido, R. Mendez, J. Lema, Simultaneous urea hydrolysis, formaldehyde removal and denitrification in a multifed upflow filter under anoxic and anaerobic conditions, Water Research 35 (2001) 691–698.
[22] A. Carucci, A. Chiavola, M. Majone, E. Rolle, Treatment of tannery wastewater in a sequencing batch reactor, Water Science & Technology 40 (1) (1999) 253–259.
[23] M. Ros, A. Gantar, Possibilities of reduction of recipient loading of tannery wastewater in Slovenia, Water Science & Technology 37 (8) (1998) 145–152.
[24] A. Abeliovich, Transformations of ammonia and the environmental impact of nitrifying bacteria, Biodegradation 3 (1992) 255–264.
[25] Y. Peng, G. Zhu, Biological nitrogen removal with nitrification and denitrification via nitrite pathway, Applied Microbiology and Biotechnology 73 (2006) 15–26.
[26] E.I.P. Volcke, M.C.M. van Loosdrecht, P.A. Vanrolleghem, Controlling the nitrite:ammonium ratio in a SHARON reactor in view of its coupling with an Anammox process, Water Science & Technology 53 (4–5) (2006) 45–54.
[27] S.W.H. Van Hulle, S. Van Den Broeck, J. Maertens, K. Villez, B.M.R. Donckels, G. Schelstraete, E.I.P. Volcke, P.A. Vanrolleghem, Construction, start-up and operation of a continuously aerated laboratory-scale SHARON reactor in view of coupling with an Anammox reactor, Water SA 31 (2005) 327–334.
[28] I. Suzuki, U. Dular, S.C. Kwok, Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas europaea* cells and extracts, Journal of Bacteriology 120 (1974) 556–558.
[29] A.C. Anthonisen, R.C. Loehr, T.B.S. Prakasam, E.G. Srinath, Inhibition of nitrification by ammonia and nitrous acid, Journal of Water Pollution Control Federation 48 (1976) 835–852.
[30] S.W.H. Van Hulle, E.I.P. Volcke, J. López Teruel, B. Donckels, M.C.M. van Loosdrecht, P.A. Vanrolleghem, Influence of temperature and pH on the kinetics of the SHARON nitritation process, Journal of Chemical Technology & Biotechnology 82 (2007) 471–480.
[31] O. Turk, D.S. Mavinic, Maintaining nitrite buildup in a system acclimated to free ammonia, Water Research 23 (1989) 1383–1388.
[32] T.B.S. Prakasam, R.C. Loehr, Microbial nitrification and denitrification in concentrated wastes, Water Research 6 (1972) 859–869.

[33] S. Hawkins, K. Robinson, A. Layton, G. Sayler, Limited impact of free ammonia on *Nitrobacter* spp. Inhibition assessed by chemical and molecular techniques, Bioresource Technology 101 (2010) 4513–4519.
[34] C. Hellinga, A. Schellen, J.W. Mulder, M.C.M. van Loosdrecht, J.J. Heijnen, The SHARON process: an innovative method for nitrogen removal from ammonium-rich wastewater, Water Science & Technology 37 (1998) 135–142.
[35] B. Wett, W. Rauch, The role of inorganic carbon limitation in biological removal of extremely ammonia concentrated wastewater, Water Research **37** (2002) 1100–1110.
[36] A. Guisasola, S. Petzet, J.A. Baeza, J. Carrera, J. Lafuente, Inorganic carbon limitations on nitrification: Experimental assessment and modelling, Water Research 41 (2007) 277–286.
[37] S. Tarre, M. Beliavski, N. Denekamp, A. Gieseke, D. de Beer, M. Green, High nitrification rate at low pH in a fluidized bed reactor with chalk as the biofilm carrier, Water Science & Technology 49 (11–12) (2004) 99–105.
[38] C. Fux, M. Boehler, P. Huber, I. Brunner, H. Siegrist, Biological treatment of ammonium-rich wastewater by partial nitrification and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant, Journal of Biotechnology 99 (2002) 295–306.
[39] R. Ganigué, J. Gabarro, A. Sànchez-Melsió, M. Ruscalleda, H. López, X. Vila, J. Colprim, M.D. Balaguer, Long-term operation of a partial nitritation pilot plant treating leachate with extremely high ammonium concentration prior to an anammox process, Bioresource Technology 100 (2009) 5624–5632.
[40] O. Nowak, K. Svardal, H. Kroiss, The impact of phosphorus deficiency on nitrification—case study of a biological pretreatment plant for rendering plant effluent, Water Science & Technology 34 (1–2) (1996) 229–236.
[41] C. Grunditz, G. Dalhammar, Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*, Water Research 35 (2001) 433–440.
[42] C. Hellinga, M.C.M. van Loosdrecht, J.J. Heijnen, Model based design of a novel process for nitrogen removal from concentrated flows, Mathematical and Computer Modelling of Dynamical Systems 5 (1999) 351–371.
[43] M.S.M. Jetten, M. Strous, K.T. van de Pas-Schoonen, J. Schalk, U.G.J.M. van Dongen, A.A. Van De Graaf, S. Logemann, G. Muyzer, M.C.M. van Loosdrecht, J.G. Kuenen, The anaerobic oxidation of ammonium, FEMS Microbiology Reviews 22 (1999) 421–437.
[44] W. Helder, R.T.P. De Vries, Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard estuary), Netherlands Journal of Sea Research 17 (1983) 1–18.
[45] G. Knowles, A.L. Downing, M.J. Barrett, Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of electronic computer, Journal of General Microbiology 38 (1965) 263–278.
[46] F.E. Stratton, P.L. Mc Carty, Microbiological aspects of ammonia oxidation of swine waste, Canadian Journal of Microbiology 37 (1967) 918–923.
[47] T. Yamamoto, K. Takaki, T. Koyama, K. Furukawa, Novel partial nitritation treatment for anaerobic digestion liquor of swine wastewater using swim-bed technology, Journal of Bioscience and Bioengineering 102 (6) (2006) 497–503.
[48] S. Philips, H.J. Laanbroek, W. Verstraete, Origin, causes and effects of increased nitrite concentrations in aquatic environments, Re/Views in Environmental Science and Bio/Technology 1 (2002) 115–141.
[49] K. Hanaki, C. Wantawin, S. Ohgaki, Nitrification at low-levels of dissolved-oxygen with and without organic loading in a suspended-growth reactor, Water Research 24 (1990) 297–302.
[50] J.H. Hunik, J. Tramper, R.H. Wijffels, A strategy to scale-up nitrification processes with immobilized cells of *Nitrosomonas europaea* and *Nitrobacter agilis*, Bioprocess Engineering 11 (1994) 73–82.
[51] D. Barnes, P.J. Bliss, Biological Control of Nitrogen in Wastewater Treatment, E.&F.N. Spon, London, UK, 1983, p. 365.
[52] G. Ciudad, O. Rubilar, P. Munoz, G. Ruiz, R. Chamy, C. Vergara, D. Jeison, Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process, Process Biochemistry 40 (2005) 1715–1719.
[53] E.V. Münch, P. Lant, J. Keller, Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors, Water Research 30 (1996) 277–284.
[54] R. Manser, W. Gujer, H. Siegrist, Consequences of mass transfer effects on the kinetics of nitrifiers, Water Research 39 (2005) 4633–4642.
[55] Y. Peng, Y. Chen, S. Wang, C. Peng, M. Liu, X. Song, Y. Cui, Nitrite accumulation by aeration controlled in sequencing batch reactors treating domestic wastewater, Water Science & Technology 50 (10) (2004) 35–43.
[56] I. Jubany, J. Lafuente, J.A. Baeza, J. Carrer, Total and stable washout of nitrite oxidizing bacteria from a nitrifying continuous activated sludge system using automatic control based on Oxygen Uptake Rate measurements, Water Research 43 (2009) 2761–2772.
[57] T. Hidaka, H. Yamada, M. Kawamura, H. Tsuno, Effect of dissolved oxygen conditions on nitrogen removal in continuously fed intermittent-aeration process with two tanks, Water Science & Technology 45 (2002) 181–188.
[58] Y. Hyungseok, A. Kyu-Hong, L. Kwang-Hwan, K. Youn-Ung, S. Kyung-Guen, Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in a intermittently-aerated reactor, Water Research 33 (1999) 145–154.
[59] A. Katsogiannis, M. Kornaros, G. Lyberatos, Enhanced nitrogen removal in SBRs by bypassing nitrate generation accomplished by multiple aerobic/anoxic phase pairs, Water Science & Technology 47 (11) (2003) 53–59.
[60] R. Blackburne, Z. Yuan, J. Keller, Demonstration of nitrogen removal via nitrite in a sequencing batch reactor treating domestic wastewater, Water Research 42 (2008) 2166–2176.
[61] G. Sin, K. Villez, P.A. Vanrolleghem, Application of a model-based optimization methodology for nutrient removing SBRs leads to falsification of the model, Water Science & Technology 53 (4–5) (2006) 95–103.
[62] L. Yang, J.E. Alleman, Investigation of batchwise nitrite build-up by an enriched nitrification culture, Water Science & Technology 26 (5–6) (1992) 997–1005.
[63] S.S. Hu, Acute substrate-intermediate-product related inhibition of nitrifiers, MSc Thesis, School of Civil Engineering, Purdue University, West Lafayette, Indiana, USA, 1990.
[64] D. Castignetti, H.B. Gunner, Differential tolerance of hydroxylamine by an *Alcaligenes* sp., a heterotrophic nitrifier, and by *Nitrobacter agilis*, Canadian Journal of Microbiology 28 (1982) 148–150.
[65] R. Stüven, M. Vollmer, E. Bock, The impact of organic matter on nitric oxide formation by *Nitrosomonas europaea*, Archives of Microbiology 158 (1992) 439–443.
[66] E. Bock, H.P. Koops, H. Harms, Cell biology of nitrifying bacteria, in: J.I. Prosser (Ed.), Nitrification, IRL, Oxford, 1986, pp. 17–38.
[67] R. Van Kempen, J.W. Mulder, M.C.M. van Loosdrecht, Overview: full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering, Water Science & Technology 44 (2001) 145–152.
[68] A. Pollice, V. Tandoi, C. Lestingi, Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate, Water Research 36 (10) (2002) 2541–2546.
[69] A. Pollice, T. Valter, L. Carmela, Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate, Water Research 36 (10) (2002) 2541–2546.
[70] Y.-H. Ahn, I.-S. Hwang, K.-S. Min, ANAMMOX and partial denitritation in anaerobic nitrogen removal from piggery waste, Water Science & Technology 49 (5–6) (2004) 145–153.
[71] B. Molinuevo, M.C. Garcia, D. Karakashev, I. Angelidaki, Anammox for ammonia removal from pig manure effluents: effect of organic matter content on process performance, Bioresource Technology 100 (2009) 2171–2175.
[72] Q.X. Yang, M. Yang, S.J. Zhang, W.Z. Lv, Treatment of wastewater from a monosodium glutamate manufacturing plant using successive yeast and activated sludge systems, Process Biochemistry 40 (2005) 2483–2488.
[73] A. Mosquera-Corral, F. González, J.L. Campos, R. Méndez, Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds, Process Biochemistry 40 (2005) 3109–3118.
[74] A. Zepeda, A.-C. Texiera, E. Razo-Flores, J. Gomeza, Kinetic and metabolic study of benzene, toluene and m-xylene in nitrifying batch cultures, Water Research 40 (2006) 1643–1649.
[75] G. Camilla, G. Lena, D. Gunnel, Comparison of inhibition assays using nitrogen removing bacteria: application to industrial wastewater, Water Research 32 (10) (1998) 2995–3000.
[76] A.M. Eilersen, M. Henze, L. Kloft, Effect of volatile fatty acids and trimethylamine on nitrification in activated sludge, Water Research 28 (1994) 1329–1336.
[77] T.G. Tomlinson, A.G. Boon, C.N.A. Trotman, Inhibition of nitrification in the activated sludge process of sewage disposal, Journal of Applied Bacteriology 29 (1966) 266–291.
[78] J. Surmacz-Gorska, K. Gernaey, C. Demuynck, P.A. Vanrolleghem, W. Verstraete, Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements, Water Research 30 (1996) 1228–1236.
[79] Y. Peng, X. Song, C. Peng, J. Li, Y. Chen, Biological nitrogen removal in SBRs bypassing nitrate generation accomplished by chlorination and aeration time control, Water Science & Technology 49 (5–6) (2004) 295–300.
[80] A.A. van de Graaf, A. Mulder, P. De Bruijn, M.S.M. Jetten, L.A. Robertson, J.G. Kuenen, Anaerobic oxidation of ammonia is a biologically mediated process, Applied & Environmental Microbiology 61 (1995) 1246–1251.
[81] A.A. van de Graaf, P. De Bruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen, Autotrophic growth of anaerobic ammonium oxidation on the basis of ^{15}N studies in a fluidized bed reactor, Microbiology 143 (1997) 2415–2421.
[82] M. Strous, J.J. Heijnen, J.G. Kuenen, M.S.M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, Applied Microbiology & Biotechnology 50 (1998) 589–596.
[83] A.A. van de Graaf, P. De Bruijn, L.A. Robertson, M.S.M. Jetten, J.G. Kuenen, Autotrophic growth of anaerobic ammonium-oxidizing microorganisms in a fluidized bed reactor, Microbiology 142 (1996) 2187–2196.
[84] M.S.M. Jetten, M. Wagner, J. Fuerst, M. van Loosdrecht, G. Kuenen, M. Strous, Microbiology and application of the anaerobic ammonium oxidation ('ANAMMOX') process, Current Opinion in Biotechnology 12 (2001) 283–288.
[85] M. Schmid, B. Maas, A. Dapena, K. van de Pas-Schoonen, J. van de Vossenberg, B. Kartal, L. Van Niftrik, I. Schmidt, I. Cirpus, J.G. Kuenen, M. Wagner, J.S. Sinninghe Damsté, M.M.M. Kuypers, N.P. Revsbech, R. Mendez, M.S.M. Jetten, M. Strous, Biomarkers for in situ detection of anaerobic ammonium oxidizing (Anammox) bacteria, Applied & Environmental Microbiology 71 (2005) 1677–1684.
[86] H. Siegrist, S. Reithaar, G. Koch, P. Lais, Nitrogen loss in a nitrifying rotating contactor treating ammonium-rich wastewater without organic carbon, Water Science & Technology 38 (8–9) (1998) 241–248.

[87] R. Chouari, D. Le Paslier, P. Daegelen, P. Ginestet, J. Weissenbach, A. Sghir, Molecular evidence for novel planctomycete diversity in a municipal wastewater treatment plant, Applied & Environmental Microbiology 69 (2003) 7354–7363.
[88] M. Kuypers, A.O. Sliekers, G. Lavik, M. Schmid, B.B. Jorgensen, J.G. Kuenen, J.S. Sinninghe Damsté, M. Strous, M.S.M. Jetten, Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, Nature 422 (2003) 608–611.
[89] M. Trimmer, J.C. Nicholls, B. Deflandre, Anaerobic ammonium oxidation measured in sediments along the Thames Estuary, United Kingdom, Applied & Environmental Microbiology 69 (2003) 6447–6454.
[90] T. Dalsgaard, B. Thamdrup, Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments, Applied & Environmental Microbiology 68 (2002) 3802–3808.
[91] M. Strous, J.A. Fuerst, E.H.M. Kramer, S. Logemann, G. Muyze, K.T. Van De Pas-Schoonen, R. Webb, J.G. Kuenen, M.S.M. Jetten, Missing litotroph identified as new planctomycete, Nature 400 (1999) 446–449.
[92] M. Schmid, K. Walsh, R. Webb, W.I.C. Rijpstra, K. van de Pas-Schoonen, M.J. Verbruggen, T. Hill, B. Moffett, J. Fuerst, S. Schouten, J.S. Sinninghe Damsté, J. Harris, P. Shaw, M. Jetten, M. Strous, Candidatus "*Scalindua brodae*", sp. nov., Candidatus "*Scalindua wagneri*", sp. nov., two new species of anaerobic ammonium oxidizing bacteria, Systematic & Applied Microbiology 26 (2003) 529–538.
[93] K. Furukawa, P.K. Lieu, H. Tokitoh, T. Fujii, Development of single-stage nitrogen removal using anammox and partial nitritation (SNAP) and its treatment performances, Water Science & Technology 53 (6) (2006) 83–90.
[94] J. Schalk, H. Oustad, J.G. Kuenen, M.S.M. Jetten, The anaerobic oxidation of hydrazine: a novel reaction in microbial nitrogen metabolism, FEMS Microbiology Letters 158 (1998) 61–67.
[95] J.G. Kuenen, M.S.M. Jetten, Extraordinary anaeribic ammonia-oxidizing bacteria, ASM News 67 (2001) 456–463.
[96] S. Schouten, M. Strous, M.M.M. Kuypers, W.I.C. Rijpstra, M. Baas, C.J. Schubert, M.S.M. Jetten, J.S. Sinninghe Damsté, Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria, Applied & Environmental Microbiology 70 (2004) 3785–3788.
[97] M.R. Lindsay, R. Webb, M. Strous, M.S.M. Jetten, M.K. Butler, R.J. Forde, J.A. Fuerst, Cell compartimentalisation in planctomycetes: novel types of structural organisation for the bacterial cell, Archives of Microbiology 175 (2001) 413–429.
[98] L.A. Van Niftrik, J.A. Fuerst, J.S. Sinninghe Damsté, J.G. Kuenen, M.S.M. Jetten, M. Strous, The anammoxosome: an intracytoplasmic compartment in anammox bacteria, FEMS Microbiology Letters 233 (2004) 7–13.
[99] J.S. Sinninghe Damsté, M. Strous, W.I.C. Rijpstra, E.C. Hopmans, J.A.J. Geenevasen, A.C.T. Van Duin, L.A. Van Niftrik, M.S.M. Jetten, Linearly concatenated cyclobutane lipids form a dense bacterial membrane, Nature 419 (2002) 708–712.
[100] W.R.L. van der Star, A.I. Miclea, U.G.J.M. van Dongen, G. Muyzer, C. Picioreanu, M.C.M. van Loosdrecht, The membrane bioreactor: a novel tool to grow anammox bacteria as free cells, Biotechnology and Bioengineering 101 (2008) 286–294.
[101] K. Isaka, Y. Date, T. Sumino, S. Yoshie, S. Tsuneda, Growth characteristic of anaerobic ammonium-oxidizing bacteria in an anaerobic filtrated reactor, Applied Microbiology & Biotechnology 70 (2006) 47–52.
[102] A. Dapena-Mora, I. Fernandez, J.L. Campos, A. Mosquera-Corral, R. Mendez, M.S.M. Jetten, Evaluation of activity and inhibition effects on Annamox process by batch tests based on the nitrogen gas production, Enzyme and Microbial Technology 40 (2007) 859–865.
[103] M. Strous, J.G. Kuenen, M.S.M. Jetten, Key physiology of anaerobic ammonium oxidation, Applied & Environmental Microbiology 65 (1999) 3248–3250.
[104] C. Fux, Biological nitrogen elimination of ammonium-rich sludge digester liquids, PhD Thesis, ETH-Zürich, Switzerland, 2003.
[105] K. Egli, U. Fanger, P.J.J. Alvarez, H. Siegrist, J.R. Van Der Meer, A.J.B. Zehnder, Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate, Archive of Microbiology 175 (2001) 198–207.
[106] J. Dosta, I. Fernández, J.R. Vázquez-Padín, A. Mosquera-Corral, J.L. Campos, J. Mata-Álvarez, R. Méndez, Short- and long-term effects of temperature on the Anammox process, Journal of Hazardous Materials 154 (2008) 688–693.
[107] L. Dexiang, L. Xiaoming, Y. Qi, Z. Guangming, G. Liang, Y. Xiu, Effect of inorganic carbon on anaerobic ammonium oxidation enriched in sequencing batch reactor, Journal of Environmental Sciences 20 (2007) 940–944.
[108] K. Pynaert, B.F. Smets, S. Wyffels, D. Beheydt, S.D. Siciliano, W. Verstraete, Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor, Applied & Environmental Microbiology 69 (2003) 3626–3635.
[109] Z. Yang, S. Zhou, Y. Sun, Start-up of simultaneous removal of ammonium and sulfate in an anaerobic ammonium oxidation (anammox) process in an anaerobic upflow bio-reactor, Journal of Hazardous Materials 169 (2009) 113–118.
[110] M. Strous, E. Van Gerven, J.G. Kuenen, M.S.M. Jetten, Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge, Applied & Environmental Microbiology 63 (1997) 2446–2448.
[111] M. Ruscalleda, H. López, R. Ganigué, S. Ouig, M. Balaguer, J. Colprim, Heterotrophic denitrification on granular anammox SBR treating urban landfill leachate, Water Science & Technology 58 (9) (2008) 1749–1755.
[112] P.C. Sabumon, Anaerobic ammonia removal in presence of organic matter: a novel route, Journal of Hazardous Materials 149 (2007) 49–59.
[113] C. Tang, P. Zheng, C. Wang, Q. Mahmood, Suppresion of anaerobic ammonium oxidizers under high organic content in high-rate Anammox UASB reactor, Bioresource Technology 101 (2010) 1762–1768.
[114] N. Chamchoi, S. Nitisoravut, J.E. Schmidt, Inactivation of ANAMMOX communities under concurrent operation of anaerobic ammonium oxidation (ANAMMOX) and denitrification, Bioresource Technology 99 (2008) 3331–3336.
[115] D. Güven, A. Dapena, B. Kartal, M.C. Schmid, B. Maas, K. van de Pas-Schoonen, S. Sözen, R. Mendez, H.J.M. Op den Camp, M.S.M. Jetten, M. Strous, I. Schmidt, Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria, Applied & Environmental Microbiology 71 (2005) 1066–1071.
[116] W. Jianlong, K. Jing, The characteristics of anaerobic ammonium oxidation (ANAMMOX) by granular sludge from an EGSB reactor, Process Biochemistry 40 (2005) 1973–1978.
[117] B.E. Rittmann, P.I. McCarty, Environmental Biotechnology: Principles and Applications, McGraw-Hill Companies, 2001.
[118] M. Kumar, J. Lin, Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal—strategies and issues, Journal of Hazardous Materials (2010), doi:10.1016/j.jhazmat.2010.01.077.
[119] D. Paredes, P. Kuschk, T.S.A. Mbwette, F. Stange, R.A. Müller, H. Köser, New aspects of microbial nitrogen transformations in the context of wastewater treatment—a review, Engineering Life Sciences 7 (1) (2007) 13–25.
[120] B. Kartal, J. Rattray, L.A. van Niftrik, J. van de Vossenberg, M.C. Schmid, R.I. Webb, S. Schouten, J.A. Fuerst, J.S. Damsté, M.S.M. Jetten, M. Strous, *Candidatus* "*Anammoxoglobus propionicus*" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria, Systematic and Applied Microbiology 30 (1) (2007) 39–49.
[121] Z. Quan, S. Rhee, J. Zuo, Y. Yang, J. Bae, R. Park, S. Lee, Y. Park, Diversity of ammonium-oxidizing bacteria in a granular sludge anaerobic ammonium-oxidizing (anammox) reactor, Environmental Microbiology 10 (11) (2008) 3130–3139.
[122] T. Awata, T. Kindaichi, N. Ozaki, A. Ohashi, The activity of the Anammox in the presence of organic matter, in: International Conference on Civil and Environmental Engineering, ICCEE-2009/Oct. 2009/Pukyong National University, 2009.
[123] S.K. Toh, N.J. Ashbolt, Adaptation of anaerobic ammonium-oxidising consortium to synthetic coke-ovens wastewater, Applied Microbiology and Biotechnology 59 (2–3) (2002) 344–352.
[124] S.K. Toh, R.I. Webb, N.J. Ashbot, Enrichment of autotrophic anaerobic ammonium-oxidizing consortia from various wastewaters, Microbial Ecology 43 (2002) 154–167.
[125] B. Kartal, M. Koleva, R. Arsov, W. van der Star, M.S.M. Jetten, M. Strous, Adaptation of a freshwater anammox population to high salinity wastewater, Journal of Biotechnology 126 (2006) 546–553.
[126] K. Windey, I. De Bo, W. Verstraete, Oxygen-limited autotrophic nitrification–denitrification (OLAND) in a rotating biological contactor treating high-salinity wastewater, Water Research 39 (2005) 4512–4520.
[127] G. Cema, J. Wiszniowski, S. Zabczynski, E. Zablocka-Godlewska, A. Raszka, J. Surmacz-Górska, Biological nitrogen removal from landfill leachate by deammonification assisted by heterotrophic denitrification in a rotating biological contactor (RBC), Water Science & Technology 55 (8–9) (2007) 35–41.
[128] K. Isaka, T. Sumino, S. Tsuneda, High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions, Journal of Bioscience and Bioengineering 103 (5) (2006) 486–490.
[129] B. Szatkowska, E. Plaza, Temperature as a factor influencing the Anammox process performance, Water and Environmental Management Series. Young Researchers 2006 (2006) 51–58.
[130] S. Rysgaard, R.N. Glud, N. Risgaard-Petersen, D. Dalsgaard, Denitrification and anammox activity in arctic marine sediments, Limnology & Oceanography 49 (2004) 1493–1502.
[131] K. Pynaert, B.F. Smets, D. Beheydt, W. Verstraete, Start-up of autotrophic nitrogen removal reactors via sequential biocatalyst addition, Environmental Science & Technology 38 (2004) 1228–1235.
[132] M. Strous, Microbiology and application of anaerobic ammonium oxidation, PhD Thesis, TU Delft, 2000, 144 p.
[133] T. Yamamoto, K. Takaki, T. Koyama, K. Furukawa, Long-term stability of partial nitritation of swine wastewater digester liquor and its subsequent treatment by Anammox, Bioresource Technology 99 (2008) 6419–6425.
[134] C. Trigo, J.M. Campos, J.M. Garrido, R. Mendez, Start-up of the Anammox process in a membrane bioreactor, Journal of Biotechnology 126 (2006) 475–487.
[135] B. Arrojo, A. Mosquera-Corral, J.L. Campos, R. Méndez, Effects of mechanical stress on Anammox granules in a sequencing batch reactor (SBR), Journal of Biotechnology 123 (2006) 453–463.
[136] S. Wyffels, P. Boeckx, K. Pynaert, D. Zhang, O. Van Cleemput, G. Chen, W. Verstraete, Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification denitrification process, Water Science & Technology 49 (5–6) (2004) 57–64.
[137] X. Hao, J.J. Heijnen, M.C.M. van Loosdrecht, Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process, Biotechnology and Bioengineering 77 (3) (2001) 266–277.
[138] M. Nielsen, A. Bollmann, O. Sliekers, M. Jetten, M. Schmid, M. Strous, I. Schmidt, L.H. Larsen, L.P. Nielsen, N.P. Revsbech, Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor, FEMS Microbiology Ecology 51 (2) (2005) 247–256.

[139] P. Veys, H. Vandeweyer, W. Audenaert, A. Monballiu, P. Dejans, E. Jooken, A. Dumoulin, B.D. Meesschaert, S.W.H. Van Hulle, Performance analysis and optimization of autotrophic nitrogen removal in different reactor configurations: a modeling study, Environmental Technology, in press.
[140] J.R. Vazquez-Padin, I. Figueroa, A. Mosquera-Corral, J.L. Campos, R. Méndez, Post-treatment of effluents from anaerobic digesters by the Anammox process, Water Science & Technology 60 (2009) 1135–1143.
[141] S. Lackner, A. Terada, B.F. Smets, Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: results of a modelling study, Water Research 42 (2008) 1102–1112.
[142] Z. Gong, F. Yang, S. Liu, H. Bao, S. Hu, K. Furukawa, Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox, Chemosphere 69 (2007) 776–784.
[143] O.A. Sliekers, K. Third, W. Abma, J.G. Kuenen, M.S.M. Jetten, CANON and Anammox in a gas-lift reactor, FEMS Microbiology Letters 218 (2003) 339–344.
[144] B. Szatkowska, G. Cema, E. Plaza, J. Trela, B. Hultman, One-stage system with partial nitritation and Anammox process in moving-bed biofilm reactor, Water Science and Technology (8–9) (2007) 19–26.
[145] L. Kuai, W. Verstraete, Ammonium removal by the oxygen-limited autotrophic nitrification–denitrification system, Applied & Environmental Microbiology 64 (1998) 4500–4506.
[146] K.A. Third, O. Sliekers, J.G. Kuenen, M.S.M. Jetten, The CANON system (Completely Autotrophic Nitrogen-removal Over Nitrite) under ammonium limitation: interaction and competition between three groups of bacteria, System & Applied Microbiology 24 (2001) 588–596.
[147] A. Hippen, K.-H. Rosenwinkel, G. Baumgarten, C.F. Seyfried, Aerobic deammonification: a new experience in the treatment of wastewaters, Water Science & Technology 35 (10) (1997) 111–120.
[148] B. Wett, Development and implementation of a robust deammonification process, Water Science & Technology 56 (7) (2007) 81–88.
[149] C. Helmer, S. Kunst, S. Juretschko, M.C. Schmid, K.-H. Schleifer, M. Wagner, Nitrogen loss in a nitrifying biofilm system, Water Science & Technology 39 (7) (1999) 13–21.
[150] C. Helmer-Madhok, M. Schmid, E. Filipov, T. Gaul, A. Hippen, K.-H. Rosenwinkel, C.F. Seyfried, M. Wagner, S. Kunst, Deammonification in biofilm systems: population structure and function, Water Science & Technology 46 (1–2) (2002) 223–231.
[151] O.A. Sliekers, N. Derwort, J.L. Campos-Gomez, M. Strous, J.G. Kuenen, M.S.M. Jetten, Completely autotrophic nitrogen removal over nitrite in a single reactor, Water Research 36 (2002) 2475–2482.
[152] H. De Clippeleir, S.E. Vlaeminck, M. Carballa, W. Verstraete, A low volumetric exchange ratio allows high autotrophic nitrogen removal in a sequencing batch reactor, Bioresource Technology 100 (2009) 5010–5015.
[153] G. Sun, D. Austin, Completely autotrophic nitrogen-removal over nitrite in lab-scale constructed wetlands: evidence from a mass balance study, Chemosphere 68 (2007) 1120–1128.
[154] Z. Dong, T. Sun, A potential new process for improving nitrogen removal in constructed wetlands—promoting coexistence of partial-nitrification and ANAMMOX, Ecological Engineering 31 (2007) 69–78.
[155] A. Hippen, C. Helmer, S. Kunst, K.-H. Rosenwinkel, C.F. Seyfried, Six years' practical experience with aerobic/anoxic deammonification in biofilm systems, Water Science & Technology 44 (2–3) (2001) 39–48.
[156] T. Gaul, E. Filipov, N. Schlösser, S. Kunst, C. Helmer-Madhok, Balancing of nitrogen conversion in deammonifying biofilms through batch tests and GC/MS, Water Science & Technology 46 (4–5) (2002) 157–162.
[157] N. Jardin, A. Hippen, C.F. Seyfried, K.-H. Rosenwinkel, F. Greulich, Deammonifikation des Schlammwassers auf der Kläranlage Hattingen mit Hilfe des Schwebebettverfahrens, GWF 142 (2001) 479–484, in German.
[158] A. Cornelius, K.-H. Rosenwinkel, Aerob/anoxische Deammonifikation stickstoffhaltiger Abwässer im KALDNES®-Biofilmverfahren, KA-Wasserwirtschaft, Abwasser, Abfall 49 (2002) 1398–1403, in German.
[159] G. Innerebner, H. Insama, I.H. Franke-Whittle, B. Wett, Identification of anammox bacteria in a full-scale deammonification plant making use of anaerobic ammonia oxidation, Systematic and Applied Microbiology 30 (2007) 408–412.
[160] A. Joss, D. Salzgeber, J. Eugster, R. Knig, K. Rottermann, S. Burger, P. Fabijan, S. Leumann, J. Mohn, H. Siegrist, Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR, Environmental Science and Technology 43 (2009) 53.
[161] S. Liu, Z. Gong, F. Yang, H. Zhang, L. Shi, K. Furukawa, Combined process of urea nitrogen removal in anaerobic Anammox co-culture reactor, Bioresource Technology 99 (2008) 1722–1728.
[162] A. Gali, J. Dosta, M.C.M. van Loosdrecht, J. Mata-Alvarez, Two ways to achieve an anammox influent from real reject water treatment at lab-scale: Partial SBR nitrification and SHARON process, Process Biochemistry 42 (2007) 715–720.
[163] Y. Xue, F. Yang, S. Liu, Z. Fu, The influence of controlling factors on the start-up and operation for partial nitrification in membrane bioreactor, Bioresource Technology 100 (2009) 1055–1060.
[164] C. Fux, V. Marchesi, I. Brunner, H. Siegrist, Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors, Water Science & Technology 49 (11–12) (2004) 77–82.
[165] K. Udert, C. Fux, M. Munster, T. Larsen, H. Siegrist, W. Gujer, Nitrification and autotrophic denitrification of source-separated urine, Water Science & Technology 48 (1) (2003) 119–130.
[166] S. Wyffels, S.W.H. Van Hulle, P. Boeckx, E.I.P. Volcke, O. Van Cleemput, P.A. Vanrolleghem, W. Verstraete, Modelling and simulation of oxygen-limited partial nitritation in a membrane-assisted bioreactor (MBR), Biotechnology & Bioengineering 86 (2004) 531–542.
[167] Y.-J. Feng, S.-K. Tseng, T.-H. Hsia, C.-M. Ho, W.-P. Chou, Partial nitrification of ammonium-rich wastewater as pretreatment for anaerobic ammonium oxidation (Anammox) using membrane aeration bioreactor, Journal of Bioscience and Bioengineering 104 (2007) 182–187.
[168] R. Ganigue, H. Lopez, M.D. Balaguer, J. Colprim, Partial ammonium oxidation to nitrite of high ammonium content urban land fill leachates, Water Research 41 (2007) 3317–3326.
[169] Z. Liang, J. Liu, Landfill leachate treatment with a novel process: Anaerobic ammonium oxidation (Anammox) combined with soil infiltration system, Journal of Hazardous Materials 151 (2008) 202–212.
[170] S. Qiao, T. Yamamoto, M. Misaka, K. Isaka, T. Sumino, Z. Bhatti, K. Furukawa, High-rate nitrogen removal from livestock manure digester liquor by combined partial nitritation–anammox process, Biodegradation 21 (2010) 11–20.
[171] M. Strous, J.G. Kuenen, J.A. Fuerst, M. Wagner, M.S.M. Jetten, The Anammox case—a new experimental manifesto for microbiological eco-physiology, Antonie van Leeuwenhoek 81 (2002) 693–702.
[172] M. Strous, E. Van Gerven, Z. Ping, J.G. Kuenen, M.S.M. Jetten, Ammonium removal from concentrated waste streams with the Anaerobic Ammonium Oxidation (Anammox) process in different reactor configurations, Water Research 31 (1997) 1955–1962.
[173] A. Dapena-Mora, J.L. Campos, A. Mosquera-Corral, M.S.M. Jetten, R. Mendez, Stability of the ANAMMOX process in a gas-lift reactor and a SBR, Journal of Biotechnology 110 (2004) 159–170.
[174] I. Tsushima, Y. Ogasawara, T. Kindaichi, H. Satoh, S. Okabe, Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors, Water Research 41 (8) (2007) 1623–1634.
[175] W.R.L. van der Star, W.R. Abma, D. Blommers, J.W. Mulder, T. Tokutomi, M. Strous, C. Picioreanu, M.C.M. Van Loosdrecht, Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam, Water Research 41 (2007) 4149–4163.
[176] T. Wang, H. Zhang, F. Yang, S. Liu, Z. Fu, H. Chen, Start-up of the Anammox process from the conventional activated sludge in a membrane bioreactor, Bioresource Technology 100 (2009) 2501–2506.
[177] L. Zhang, P. Zheng, C. Tang, R. Jin, Anaerobic ammonium oxidation for treatment of ammonium-rich wastewaters, Journal of Zhejiang University Science 9 (5) (2008) 416–426.
[178] H. Park, A. Rosenthal, A. Deur, K. Beckmann, K. Ramalingam, J. Fillos, K. Chandran, Molecular Based Characterisation of the Microbial Ecology and Activity of Anammox Bioreactors, Water Environment Federation, WEFTEC, 2009.
[179] K.-I. Gil, E. Choi, Nitrogen removal by recycle water nitritation as an attractive alternative for retrofit technologies in municipal wastewater treatment plants, Water Science & Technology 49 (5–6) (2004) 39–46.
[180] P. Jenicek, P. Svehla, J. Zabranska, M. Dohanyos, Factors affecting nitrogen removal by nitritation/denitritation, Water Science & Technology 49 (5–6) (2004) 73–79.
[181] S. Wyffels, P. Boeckx, K. Pynaert, W. Verstraete, O. Van Cleemput, Sustained nitrite accumulation in a membrane-assisted bioreactor (MBR) for the treatment of ammonium rich wastewater, Journal of Chemical Technology & Biotechnology 78 (2003) 412–419.
[182] M. Chen, J.-H. Kim, N. Kishida, O. Nishimura, R. Sudo, Enhanced nitrogen removal using C/N load adjustment and real-time control strategy in sequencing batch reactors for swine wastewater treatment, Water Science & Technology 49 (5–6) (2004) 309–314.
[183] D. Obaja, S. Macé, J. Costa, C. Sans, J. Mata-Alvarez, Nitrification, denitrification and biological phosphorus removal in piggery wastewater using a sequencing batch reactor, Bioresource Technology 87 (2003) 103–111.
[184] K. Poo, B. Jun, S. Lee, H. Woo, C. Kim, Treatment of strong nitrogen swine wastewater at full-scale sequencing batch reactor, Water Science & Technology 49 (5–6) (2004) 315–323.
[185] A. Tilche, E. Bacilieri, G. Bortone, F. Malaspina, S. Piccinini, L. Stante, Biological phosphorus and nitrogen removal in a full scale sequencing batch reactor treating piggery wastewater, Water Science & Technology 40 (1) (1999) 199–206.
[186] J. Chung, W. Bae, Y. Lee, G. Ko, S. Lee, S. Park, Investigation of the effect of free ammonia concentration upon leachate treatment by shortcut biological nitrogen removal process, in: IWA Speciality Symposium on Strong Nitrogenous and Agro-Wastewater, vol. 1, Seoul, Korea, June 11–13, 2003, pp. 93–104.
[187] J. Jokela, R. Kettunen, K. Sormunen, J. Rintal, Biological nitrogen removal from municipal landfill leachate: low-cost nitrification in biofilters and laboratory scale in-situ denitrification, Water Research 36 (2002) 4079–4087.
[188] S. Kalyuzhnyi, M. Gladchenko, Sequenced anaerobic-aerobic treatment of high strength, strong nitrogenous landfill leachates, Water Science & Technology 49 (5–6) (2004) 301–312.
[189] U. Abeling, C.F. Seyfried, Anaerobic aerobic treatment of potato-starch wastewater, Water Science & Technology 28 (2) (1993) 165–176.
[190] U. Austermann-Haun, H. Meyer, C. Seyfried, K.-H. Rosenwinkel, Full scale experiences with anaerobic/aerobic treatment plants in the food and beverage industry, Water Science & Technology 40 (1) (1999) 305–312.
[191] P. Deng Petersen, K. Jensen, P. Lyngsie, N. Hendrik Johansen, Nitrogen removal in industrial wastewater by nitration and denitration—3 years of experience, Water Science & Technology 47 (11) (2003) 181–188.

[192] B. Kartal, L. van Niftrik, O. Sliekers, M.C. Schmid, I. Schmidt, K. van de Pas-Schoonen, I. Cirpus, W. van der Star, M. van Loosdrecht, W. Abma, J.G. Kuenen, J. Mulder, M.S.M. Jetten, H. op den Camp, M. Strous, J. van de Vossenberg, Application, eco-physiology and biodiversity of anaerobic ammonium oxidizing bacteria, Reviews in Environmental Science and Biotechnology 3 (2004) 255–264.
[193] M. Schmid, U. Twachtmann, M. Klein, M. Strous, S. Juretschko, M. Jetten, J.W. Metzger, K.H. Schleifer, M. Wagner, Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation, Systematic and Applied Microbiology 23 (2000) 93–106.
[194] D. Woebken, P. Lam, M.M.M. Kuypers, S.W.A. Naqvi, B. Kartal, M. Strous, M.S.M. Jetten, B.M. Fuchs, R. Amann, A microdiversity study of anammox bacteria reveals a novel Candidatus Scalindua phylotype in marine oxygen minimum zones, Environmental Microbiology 10 (11) (2008) 3106–3119.
[195] B. Wett, Solved upscaling problems for implementing deammonification of rejection water, Water Science & Technology 53 (2006) 121–128.
[196] G. Nyhuis, V. Stadler, B. Wett, Inbetriebnahme der ersten DEMON-Anlage in der Schweiz zur direkten Stickstoffelimination und erste Betriebsergebnisse (Taking into operation the First DEMON installation in Switzerland for direct nitrogen removal and first process results) 6, Aachener Tagung mit Informationsforum, Stickstoffrückbelastung – Stand der Technik 2006, Aachen Germany, ATEMIS GmbH, 2006.
[197] S.E. Vlaeminck, L.F.F. Cloetens, H. De Clippeleir, M. Carballa, W. Verstraete, Granular biomass capable of partial nitritation and anammox, Water Science & Technology 59 (3) (2009) 609–617.
[198] M. Denecke, V. Rekers, U. Walter, Einsparpotenziale bei der biologischen Reinigung von Deponiesickerwasser (Cost saving potentials in the biological treatment of landfill leachate), Muell und Abfall 39 (2007) 4–7.
[199] V. Rekers, M. Denecke, U. Walter, Betriebserfahrung mit der anaeroben Deammonifikation von Deponiesickerwasser (Operational experiences with the anaerobic deammonification of landfill leachate), Depotech, 2008, Leoben, Austria, Institut für nachhaltige Abfallwirtschaft und Entsorgungstechnik, 2008.
[200] P. Johansson, A. Nyberg, M. Beier, A. Hippen, C.F. Seyfried, K.-H. Rosenwinkel, 1998, Cost efficient sludge liquor treatment, Royal Institute of Technology, Stockholm, TRITA-AMI Report 3048.
[201] M. Beier, Y. Schneider, Abschlussbericht Entwickelung von Bilanzmodellen für die Prozesse Deammonifikation und Nitritation zur Abbildung gross- and halb-technischer Anlagen (Final report development of balance models for deammonification and nitritation processes to illustrate full and half technical scale installations), Leibniz University Hannover, Hannover, 2008.
[202] A. Rosenthal, K. Ramalingam, H. Park, A. Deur, K. Beckmann, K. Chandran, J. Fillos, Anammox studies using New York city centrate to correlate performance, population dynamics and impact of toxins, in: WEFTEC 2009, 2009.
[203] R.-C. Jin, P. Zheng, A.-H. Hu, Q. Mahmood, B.-L. Hu, G. Jilani, Performance comparison of two anammox reactors: SBR and UBF, Chemical Engineering Journal 138 (2008) 224–230.
[204] A. Dapena-Mora, S.W.H. Van Hulle, J.L. Campos, R. Mendez, P.A. Vanrolleghem, M.S.M. Jetten, Enrichment of Anammox biomass from municipal activated sludge: experimental and modelling results, Journal of Chemical Technology & Biotechnology 79 (2004) 1421–1428.
[205] K.A. Third, J. Paxman, M. Schmid, M. Strous, M.S.M. Jetten, R.C. Cord-Ruwisch, Enrichment of Anammox from Activated Sludge and Its Application in the CANON Process, Microbial Ecology 49 (2005) 236–244.
[206] J. Vazquez-Padin, I. Fernádez, M. Figueroa, A. Mosquera-Corral, J. Campos, R. Méndez, Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature, Bioresource Technology 100 (2009) 2988–2994.
[207] U. Imajo, T. Tokutomi, K. Furukawa, Granulation of Anammox microorganisms in up-flow reactors, Water Science & Technology 49 (5–6) (2004) 155–164.
[208] J.R. Vazquez-Padin, M.J. Pozo, M. Jarpa, M. Figueroa, A. Franco, A. Mosquera-Corral, J.L. Campos, R. Méndez, Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR, Journal of Hazardous Materials 166 (2009) 336–341.