Chapter 2

Innovative Nitrogen and Carbon Removal

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Additional information is available at the end of the chapter

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Abstract

The aerobic systems have been the most widely biological treatment used for municipal and industrial wastewater but show serious problem with sludge sedimentation, high energy demand and microbial inhibition. On the other hand, the anaerobic digestion (AD) of wastewater is considered the best alternative to remove the organic compounds and to recover energy via methane production. Nevertheless, AD has a problem: the treatment of industrial wastewater with high organic nitrogen content reaches high free ammonia (NH_3) concentrations due to the protein degradation. NH_3 inhibits the methanogenic process and is toxic to the environment, and then, it must be removed before its final disposition. Several physicochemical processes have been evaluated for the recovery or/and treatment of ammonium from wastewater. The most frequent treatments are gas stripping and magnesium ammonium phosphate precipitation. These methods are effective, but they are very expensive compared to biological treatments. Moreover, these techniques usually require more power consumption than the biological process. The technologies based on partial nitrification and Anammox (PN-A) are the ones with better performance. Thus, this chapter mainly focuses on biological processes based on AD, denitrification and PN-A for the removal of carbon and nitrogen from industrial wastewater with recovery of energy and water.

Keywords: anammox, anaerobic digestion, nitrogen removal, carbon removal, partial nitrification, REMON

1. Introduction

Anaerobic digestion (AD) of high load wastewater is considered one of the best alternatives to remove the organic compounds and to recover energy via the production of methane, which has significant advantages over other forms of bioenergy production. The bottleneck of many industry wastewater AD is the high content of generated total ammonia nitrogen (TAN, ammonium + ammonia) reaching inhibitory concentrations for methanogenic bacteria, which result in suboptimal production of methane. Anaerobic reactors fed with high ammonia concentrations also produce an effluent with high TAN concentrations, which require to be treated [1].

In addition, physicochemical processes have been evaluated for the recovery and/or treatment of ammonia from wastewaters. Recovery is usually done with struvite precipitation; on the other hand, the most common treatment is gas stripping [2]. Nevertheless, these processes require the addition of chemicals and a previous carbonate treatment to avoid the precipitation on the equipment. Therefore, the physicochemical treatment is more expensive than the biological treatment [3].

Among several biological processes for the abatement of nitrogen species, we will discuss the different biological technologies based on AD, denitrification, partial nitrification and anaerobic ammonium oxidation (Anammox). Most methods can be applied to treat municipal wastewater, agricultural residues and high nitrogen wastewaters from chemical processes.

The classic biological treatment for nitrogen removal from wastewaters has been the coupled nitrification/denitrification processes, but, in the last 20 years, the partial nitrification-Anammox (PN-A) technology has proved to be efficient in nitrogen removal [3]. The PN-A process is a completely autotrophic technology that compared with the conventional nitrification/denitrification process shows many advantages: (1) consumes 60% less oxygen since a partial nitritation is needed; (2) produces 85% less of sludge mainly due to the slow biomass growth of autotrophic bacteria; (3) no organic matter is needed, which makes it an excellent process to use with anaerobic digestion and (4) releases less greenhouse gases (CO_2, N_2O, etc.) to the atmosphere [4–8]. Even more, in this chapter, we show a new biological technology using the concept of AD and PN-A with water reuse that reduces cost from the annual fresh water consumption and heating.

2. Ammonium rich wastewaters

Industrial activities, summarized in **Table 1**, generate wastewaters rich in organic matter expressed as chemical oxygen demand (COD) and/or a nitrogen-rich wastewater (expressed as TAN, total Kjeldhal nitrogen (TKN) or total nitrogen (TN)) [9–29]. High levels of TAN discharged to the environment can cause serious damage. Emissions of TAN on aquatic systems cause hypoxia: ammonia is oxidized to nitrite and nitrate, promoting biomass growth (mainly algal biomass), and then, eutrophication of water bodies occurs, affecting aquatic life and decreasing the biodiversity because of low availability of dissolved oxygen [30]. Moreover, nitrogen contamination can affect even human health. Consumption of polluted water with nitrate can lead reproductive diseases, methaemoglobinaemia and cancer [31]. Thus, environmental regulations set maximum values allowed to release into the environment.

AD process is an excellent alternative to treat wastewaters with high COD concentration (>3000 mg COD/L) because it does not require oxygen and has low sludge production, and

Raw effluents				
Effluent	**COD (mg/L)**	**TAN (mg/L)**	**TKN (TN) (mg/L)**	**Reference**
Fish industry effluent	5000–32,000	39–1940	n.d.	[9]
Winery wastewater	50	4000–6000	n.d.	[10]
Olive mill wastewater	40,300 ± 1000	n.d.	(240 ± 50)	[11]
Optoelectronic industrial wastewater	13.5 ± 0.7	3712 ± 120	3799 ± 9	[12]
Swine wastewater	3000–15,000	400–1400	(600–2100)	[13]
Cheese whey	73,000–86,000	58–150	(897–1200)	[14]
Tannery wastewater	2400–2600	200–230	n.d.	[15]
Abattoir wastewater	5800–6100	130–280	530–810	[16]
Domestic sewage WWTP	160–320	47–76	(50–89)	[17]
Coke wastewater	630–6500	50–400	250–550	[18]
Poultry manure	43,000 ± 4800	2443 ± 260	n.d.	Our group, unpublished
Piggery wastewater	19,990 ± 2458	740 ± 56	n.d.	[19]
Pharmaceutical wastewater	415–843	123–257	n.d.	[20]
Recycled fish meal effluents	5000–6300	n.d.	480–800	[21]
Brewery wastewater	1300–2300	15–28	(30–37)	[22]
Glass	n.d.	300–650	n.d.	[29]
Coal gasification	n.d.	<1000	n.d.	[29]
Explosives	n.d.	<1503	n.d.	[29]
Landfill leachate	554 ± 97	634 ± 143	n.d.	[23]
Monosodium glutamate wastewater	25,000 ± 5000	19,000 ± 1000	n.d.	[24]
Anaerobic digestion effluents (ADE)				
Effluent	**DQO (mg/L)**	**TAN (mg/L)**	**TKN (TN) (mg/L)**	**Reference**
ADE of sludge	1500–2000	800–900	n.d.	[25]
ADE of fish canning wastewater	914 ± 291	324 ± 36	n.d.	[26]
ADE of abattoir wastewater	800 ± 200	1388 ± 70	n.d.	[16]
ADE of poultry manure	11,860 ± 1270	2533 ± 326	n.d.	Our group, unpublished data
ADE of piggery farm	1980	1200	(1240)	[27]
ADE of slaughterhouse	544–3240	485–783	n.d.	[28]

TAN, total ammonia nitrogen; TKN, total kjeldhal nitrogen; TN, total nitrogen; n.d., not determined.

Table 1. Organic matter and nitrogen rich industry wastewaters.

it is a sustainable process because of the biogas production. Nevertheless, the TAN concentration increases during the AD and is produced from proteins, urea and nucleic acids degradation. Although TAN is an important nutrient for microbial growth, it is inhibitory at concentrations between 1500 and 3000 mgTAN/L and pH 7.4–7.6, and it is toxic for biomass at concentrations over 3000 mg TAN/L [32]. Free ammonia (NH_3) inhibits the methanogenic process by increasing the maintenance energy requirement, affecting the intracellular pH, depleting the intracellular potassium and inhibiting specific enzyme reactions, principally of archaea populations [33].

Ammonium inhibition at large-scale AD leads to serious economic and operational problems. In fact, many full-scale anaerobic digesters operate in an ammonia-induced "inhibited steady-state," with up to 30% losses of potential methane production yield [1]. Finally, an effluent with a high concentration of ammonia requires treatment before its final disposition, which can be possible with biological treatment or chemical treatment.

Table 1 was divided into two sections: the first one includes raw effluent from industry, which have high content of organic matter and/or nitrogen, whereas the second section groups include anaerobic digestion effluents, which have less organic matter content and more nitrogen content than the respective raw effluent. Optoelectronic industrial wastewater seems to be an ideal substrate for autotrophic processes such as PN-A due to its low COD content; nevertheless, it lacks of essential trace elements [12]. Then, the addition of trace elements is needed to be able to perform a biological treatment. Cokes wastewater has been considered the most toxic one since it contains toxic compounds such as phenols, polynuclear aromatic hydrocarbons, heterocyclic compounds containing nitrogen, oxygen and sulfur, cyanides, thiocyanate and ammonia, nevertheless to employ biological treatment is feasible [18]. Finally, it has been demonstrated that most of effluents presents in **Table 1**, such as poultry manure, slaughterhouse, fish canning, fish industry, cheese whey, etc., can be treated with biological process.

3. Partial nitrification and Anammox process

Autotrophic nitrogen removal technology is used without organic matter, and it is considered the best sustainable treatment for rich nitrogen wastewater. Anaerobic ammonia-oxidizing bacteria (anAOB) are responsible for the anaerobic ammonium oxidation (Anammox) process [34]. Anammox reaction consists of the ammonium oxidation using nitrite as an electron acceptor (Eq. (1)) [35]. Nitrite can be obtained from nitritation (oxidation of ammonium to nitrite) by aerobic ammonia-oxidizing bacteria (aerAOB) (Eq. (2)) [4, 8]. The PN-A process has proved to be an efficient nitrogen removal technology (Eq. (3)) [36].

$$\begin{aligned} NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ &\rightarrow 0.986\,N_2 \\ + 2.02\,H_2O + 0.071CH_{1.74}O_{0.031}N_{0.15} &+ 0.161NO_3^- \end{aligned} \tag{1}$$

$$\begin{aligned} \mathrm{NH_4^+} + 0.0743\,\mathrm{HCO_3^-} + 1.404\,\mathrm{O_2} &\rightarrow 0.985\,\mathrm{NO_2^-} \\ + 0.0149\,\mathrm{C_5H_7O_2N} + 1.911\,\mathrm{H^+} &+ 1.03\,\mathrm{H_2O} \end{aligned} \tag{2}$$

$$NH_4^+ + 0.804\ O_2 + 0.071 HCO_3^- \rightarrow 0.436 N_2 + 0.111 NO_3^- + 0.009 C_5H_7O_2N + 0.028 CH_2O_{0.5}N_{0.15} + 1.038 H^+ + 1.46\ H_2O \quad (3)$$

The kinetic parameters of the bacterial groups responsible of the process are present in **Table 2**. Different configurations have been designed to allow a properly balanced process considering the kinetic parameters such as: (1) the low duplication time of aerAOB and anAOB is an advantage because of the low sludge production and in turn is a disadvantage because a high biomass retention is needed [8]. (2) The Anammox activity is temporarily inhibited with dissolved oxygen (DO) at values higher than 0.032 mg/L, but oxygen can be consumed by aerAOB when working with a one-stage system. Then, a correct control of DO is needed. (3) Nitrite oxidizing bacteria (NOB) are an undesirable microorganism, typically present in the process. NOB compete for oxygen with aerAOB and for nitrite

Parameter	Symbol	Value	Unity	Reference
aerAOB				
Maximum growth rate	*Umax aerAOB*	1.36	1/d	[29]
Oxygen saturation coefficient	$k_{O_2}^{aerAOB}$	0.3	g O_2/m^3	[29]
ammonia saturation coefficient	$k_{NH_3}^{aerAOB}$	1.1	g N/m^3	[29]
Decay rate	b_{aerAOB}	0.068	1/d	[38]
NOB				
Maximum growth rate	*Umax NOB*	0.79	1/d	[29]
Oxygen saturation coefficient	$k_{O_2}^{NOB}$	1.1	g O_2/m^3	[29]
ammonia saturation coefficient	$k_{NH_3}^{NOB}$	0.51	g N/m^3	[29]
Decay rate	b_{NOB}	0.04	1/d	[38]
anAOB				
Maximum growth rate	*Umax anNOB*	0.052	1/d	[39]
ammonia saturation coefficient	$k_{NH_3}^{anAOB}$	0.03	g N/m^3	[38]
Nitrite saturation coefficient	$k_{HNO_2}^{anAOB}$	0.005	g N/m^3	[38]
Oxygen inhibition coefficient	$k_{O_2}^{anAOB}$	0.01	g O_2/m^3	[40]
Decay rate	b_{anAOB}	0.0026	1/d	[38]

aerAOB, aerobic ammonia-oxidizing bacteria; NOB, nitrite-oxidizing bacteria; anAOB, anaerobic ammonia-oxidizing bacteria.

Table 2. Kinetic parameters of the partial nitrification and Anammox process.

with anAOB. Thus, the NOB suppression of the system is a priority step to reach high efficiencies in the process. (4) Organic matter can inhibit the entire process because of the fast development of heterotrophic bacteria (HB), which competes for oxygen with aerAOB and for living space with anAOB. (5) Finally, different environmental conditions such as temperature, concentrations of free nitrous acid and free ammonia control the process efficiency [8, 37].

As a conclusion, the success of the process is dominated by two great premises: the type of operation strategies (two or one stage and type of reactor) and the environmental conditions related to the inhibition or process optimization.

3.1. Operation strategies: number of stages and type of reactor

The application of the coupled processes of partial nitrification and Anammox can be performed in two different units or in a single one. The first experience with a full-scale Anammox—two stages process was in the Rotterdam wastewater treatment plant (WWTP) in 2002. The Anammox reactor was coupled to a previous Single reactor system for High Ammonium Removal Over Nitrite (SHARON®) to remove the nitrogen from a side stream [41]. Thus, the first large-scale proposal for the autotrophic removal of nitrogen was composed of two stages: partial nitrification (PN) and anammox (A). SHARON® was designed to produce a partial nitrification by controlling the effluent composition (equal concentration of ammonia and nitrite), the temperature (near to 30°C), the solid retention time (SRT) equal to the HRT, short HRT (1 day) and the pH value through DO concentration. With those strategies, the growth of aerAOB is favored over that of NOB [3, 41]. The NOB suppression has been one of the main challenges of the PN-A systems. Some of strategies are as follows: (1) increasing free ammonia concentration working at high pH values and thus limiting the growth of NOB due to their higher sensitivity to free ammonia than aerAOB [42], (2) decreasing the dissolved oxygen concentration due to the low oxygen affinity of NOB compared to aerAOB [29], (3) operating at temperatures above 25°C since the maximum specific growth rate of aerAOB will be higher than that of NOB at these conditions.

The advantages of a two stages PN-A process are as follows: (1) the organic material can be depleted in the first stage avoiding the anAOB inhibition, (2) all inhibition strategies of NOB can be applied in the first stage, (3) there is no risk of oxygen inhibition of anAOB, and (4) in summary, the aerobic and anaerobic metabolisms can be optimized separately [43].

Operation parameters for two stages processes have been extensively reported. Values for SRT, HRT and mixed liquor suspended solids (MLSSs) ranges in the first partial nitrification unit are 1–13 d, 1–1.25 d and 0.27–20 g MLSS/L, respectively. On the other hand, the second units (anammox reactors) show operation parameter such as HRT and MLSS of 0.5–1.7 d and 0.2–35 g MLSS/L, respectively; SRT is a parameter little measured, because the systems are oriented in retaining the greater quantity of biomass [36, 44–46]. Most nitrogen load rate (NLR) and nitrogen removal efficiency ranges of the combined systems are 0.35–1.2 kg N/m^3 d and 72–89%, respectively [36, 44–46]. Nevertheless, the highest nitrogen load and removal efficiency have been reported for the Rotterdam anammox reactor with more than 6.5 years of operation period with a high granular biomass concentration of 35 g MLSS/L

[46]. This two stages process has a common NLR and efficiency of 7 kg N/m^3 d and 95%, respectively [46].

Otherwise, the one-stage operation parameters such as SRT, HRT and MLSS are 15–40 d, 0.075–4 d and 2–3.5 g MLSS/L, respectively [12, 36, 47, 48]. In addition, the NLR and nitrogen removal efficiency of this one step process are 0.46–1.4 kg N/m^3 d and 50–89%, respectively [12, 36, 47, 48]. Clearly, greater NLR and efficiencies values are expected in two-stage systems. Despite these advantages of the two-step configuration, 88% of all plants are operated as single-stage systems [46]. The one-stage systems have advantages such as: (1) continuous consumption of nitrite avoiding inhibitions in both aerAOB and anAOB, (2) smaller operational units are needed, (3) simplification of the operation control and (4) lower N_2O emissions compared to two stages systems [49]. In a one-stage reactor, the process has been registered with different names; CANON: Completely Autotrophic Nitrogen removal Over Nitrite process [50]; ELAN: Spanish acronym for ELiminación Autotrófica de Nitrógeno-(autotrophic nitrogen removal) [51]; DEMON: DE-amMONnification [52, 53]; ClearGreen: Cyclic Low Energy Ammonium Removal [46]; NAS: New Activated Sludge [54], OLAND: Oxygen-Limited Autotrophic Nitrification–Denitrification [55]; SNAD: Simultaneous partial Nitrification, Anammox and Denitrification [56].

Some one-stage characteristics are as follows: (1) CANON process is based on the control of parameters such as pH, DO, and redox potential; aeration and shear stress applied to biomass allows the development of a granular biomass aerAOB (external zone) and anAOB (internal zone). (2) ELAN system is operated in cycles of 3 or 6 h where the feeding to the reactor and the aeration s continuous during the most of the time cycle (90–95%). Short periods of settling time are used to allow the washout of NOB flocculent biomass. (3) DEMON is a system with a hydrocyclone that keeps the granular biomass in the reactor and eliminates the small flocculent biomass. (4) ClearGreen is operated with a three-period cycle: At period 1 feeding, mixing, aerobic period and anoxic periods carried out. At period 2 is a settling period and at period 3 withdrawal occurs. Due to the nitrate removal during the anoxic periods nitrogen removal reaches 90%. (5) NAS is in an active sludge with a portion of anAOB. This process shows the combination of batch-fed partial nitritation, anammox, denitrification and nitrification reactors in a four-stage configuration plant with internal recycling lines. (6) OLAND is carried out on biodiscs under microaerobic conditions with coexistence of aerAOB and anAOB.

FISH analyses [55, 57] revealed that anaerobic ammonium oxidation in all aforementioned processes is performed by anAOB. In addition, the coupled reactions of PN-A leave 11% of residual nitrogen in the form of nitrate due to the reaction stoichiometry (see Eq. (4)); thus, in the presence of organic carbon, the remaining nitrate can be used by denitrifying bacteria as an electron acceptor, improving the N removal efficiency. This new process is known as Simultaneous Nitrification, Anammox and Denitrification (SNAD) process [56].

Beyond the regime used (sequencing batch or continuous reactors), all reactor designs for PN-A pursue to retain the biomass in the system due to their long duplication time. Initially, the Anammox process was operated in continuous biofilm reactors [58, 59]. In order to improve the biomass retention and the stability of the process, the sequencing batch reactor

(SBR) has been extensively used [49, 60] where mixing was achieved either by mechanical stirring or by gas flow stirring. More than 50% of all PN-A industrial installations are SBR [46]. In SBR or airlift reactors with suspended biomass, the biomass settling properties determine the retention and are related to the microbial aggregate morphology as floc or granule and size. Granules are defined as compact and dense aggregates with an approximately spherical external appearance that do not coagulate under decreased hydrodynamic shear conditions and settle significantly faster than flocs [61]. In terms of physical properties, large granules are preferable for suspended-growth applications.

Granular biomass allows the development of aerAOB at the external layers of the granule, while anAOB can grow in the anoxic core of granule, but still close to the bulk liquid and to the layer of the aerAOB [51]. In an one-stage PN-A processes, the aggregates sizes not only influence settling properties but also affect the proportion of microbial nitrite production and consumption; low aerAOB activity and high anAOB activity have been observed in large aggregates [62]. Better performances in terms of cost efficiency have been obtained when granular systems were used in a PN-A process [49].

In summary, the most used configuration for PN-A process is a one-stage reactor, mainly because of the lower investment cost compared to a two-stage reactor and for its easy operation. The type of biomass structure depends mainly on the reactor design or regime, where best results have been observed with granular biomass.

3.2. Environmental conditions

The PN-A process is very sensitive to oxygen, temperature, and concentrations of organic matter, free nitrous acid and free ammonia. The anoxic recovery of an autotrophic process is a typical answer to the temporal inhibition of Anammox biomass when DO is near to 0.032 mg O_2/L [40]. Otherwise, when a one-stage PN-A system is operated, the aerobic community such as aerAOB, heterotrophic biomass or even NOB can remove the oxygen before reaching the anAOB cell [63]. As a counterpart, during this symbiosis, anAOB can consume the NO_2, which is toxic for all bacterial populations in the consortium [8]. The PN-A process saves aeration costs because only half of the ammonium needs to be oxidized to nitrite (partial nitritation). Thus, the avoidance of high DO concentrations prevents the growth of NOB and avoids the inhibition of anAOB. NOB has lower affinity for oxygen than aerAOB, and it competes for nitrite with anAOB [8, 64].

Indeed, all these assumptions led to the first start-up strategies of the PN-A process, which were focused on acclimation to low oxygen concentrations. ELAN® and Cleargreen® started their process with DO concentrations below 0.5 and 0.8 mgO_2/L, respectively [46]. In addition, OLAND® process and DEMON® processes started with DO below 0.65 and 0.3 mgO_2/L, respectively [46]. Nevertheless, several authors have proposed to start-up with high oxygen concentrations, such as 1 [62], 4.6 [6] and even 6.6 mgO_2/L [5]. The development of a strong nitrifying layer, to increase the protection of anAOB as well as to increase the granular biomass concentration is the main arguments for a high DO concentration at the start-up [51]. However, a higher DO concentration means a more expensive operation. Also, with more oxygen the granular diameter increases, by one side, this leads to a larger sedimentation capacity, but on the other side, with a diameter above 2.20 mm granules floatation may occur, which hinders the operation [8].

Otherwise, nitrate build-up has been reported in 50% of the large-scale plants, this means that the DO concentration control not always provides a good correlation with the nitrogen removal [46]. Higher oxygen concentration and a small nitrifying layer (due to a low oxygen start-up strategy) lead to an oxygen penetration and NOB activity in the core of the granular biomass. Finally, the type of oxygen strategies used to start up a PN-A process is very important because it affects the granules properties.

Temperature is currently the most investigated parameter. The main aim is to introduce the PN-A process to the mainstream of WWTP, and this innovation will open new possibilities in the design of energy production processes [65]. The Anammox reaction has been assayed at incubation temperature between 6 and 43°C [8]. The slope of activity drops quickly after temperature below 20°C [65]. To understand the influence of temperature on the Anammox activity, it is necessary to understand its influence on the activation energy. The activation energy of anAOB is similar to aerAOB (63–72 kJ/mol) [66]. A correct determination of the effect of the temperature on PN-A process considers different temperature coefficients depending on the experimental range and on the biomass history [65]. Unlike other biological processes, the Arrhenius equation considers different slopes for different temperature ranges. On the other hand, acclimated biomass to lower temperatures presents higher specific rates with a major effect on anAOB biomass compared to the aerAOB biomass [65]. Consequently, the temperature effect increases at lower temperatures, but the importance of this effect is closely related to the biomass specie.

When both PN and Anammox processes are carried out in one stage in the presence of organic matter, the development of heterotrophic bacteria (HB) can destabilize the nitrogen removal process. HB have higher growth rates than autotrophic bacteria and thus, competing for living space and substrates. Moreover, HB outcompete aerAOB and anAOB for oxygen and nitrite, respectively [67, 68]. Nevertheless, if suitable operational conditions and inlet $COD_{biodegradable}/N$ ratios are provided, balanced activities among aerAOB, anAOB and HB can be achieved maintaining a high nitrogen removal efficiency [37]. Stoichiometrically, coupled reactions of partial nitrification and Anammox are capable of removing maximum of 89% of ammonium, leaving the remaining 11% of nitrogen in the form of nitrate. In the presence of organic matter, the remaining nitrate can be used by HB as an electron acceptor for the oxidation of organic carbon approaching the theoretical removal of 100% of nitrogen by the combined action of these three bacterial groups. This trabajofinalizado new process has been called SNAD process. Since its appearance in 2009 [56], the number of published articles of SNAD has grown compared with other N removal processes [67, 68]. The first difficulty of the system is the organic load, since an excess of COD destabilizes the bacterial consortium. Generally, the inlet COD/N ratio reported in the literature takes into account the total COD; however, only the biodegradable fraction of organic matter should be counted because it is the available substrate for heterotrophic growth. Most reported SNAD process working at $COD_{biodegradable}/N$ ratios lower than 0.7 have shown good performances [37].

The inhibition by free nitrous acid and free ammonia concentrations is influenced by pH. Free nitrous acid effect is the most dramatic; indeed, aerAOB catabolic processes present 50% inhibition at 0.40–0.63 mg H-NO_2-N/L under aerobic condition and anabolic process presented complete inhibition at 0.40 mg H-NO_2-N/L under aerobic condition [69]. These concentrations decrease for NOB population. Under aerobic condition, NOB anabolic process has presented completely inhibition at 0.02 mg H-NO_2-N/L and did not present any inhibition for catabolic process up to 0.024 mg H-NO_2-N/L [69]. On other hand, NOB inhibition at free ammonia occurs with concentrations below 1 mg N/L, whereas aerAOB showed inhibition above 16 mg/L [42, 69].

Finally, the operational parameters such as oxygen concentration, temperature, organic matter in the influent, free nitrous acid and free ammonia concentrations are essential for the correct performance of the process.

3.3. Removal of organic matter and nitrogen species (REMON)

The **REM**oval of **O**rganic matter and **N**itrogen species (called REMON) process is based on the sequential and parallel reactions of PN-A (Eq. (3)) and denitrification (Eq. (4)).

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.47N_2 + 0.056C_5H_7O_2N + 1.68H_2O + HCO_3^- \quad (4)$$

The REMON process has been validated in a bubble column reactor (BCR) in a continuous regimen. BCR was selected because the mixing is performed sparging recirculated gas, and this reactor configuration requires less energy than mechanical stirring [64]. Also, the process has been assayed in SBR with mechanical agitation, but the performance is very sensitive to the type of agitation and to oxygen modifications [70]. In the BCR, the upper section had a three-phase separator for granular and the flocculent biomass separation [64].

REMON shows same reaction of SNAD process, but the operational strategies are different. The NOB suppression, granular biomass selection during the continuous process and the good response to high organic matter concentration are main goals of the REMON process.

The NOB inhibition in a PN-A process has been widely studied. Different technologies based on the PN-A process consider strategies such as control of SRT, pH, DO limitation, aeration intensity, redox potential and concentrations of free ammonia [50, 52, 53, 55]. The REMON process has evaluated single-parameter strategies only with oxygen limitation [64]. The best nitrogen removal was 75.36% using a DO of 0.2 mgO_2/L. In addition, at this DO, molecular analyses demonstrated that the NOB group was the most abundant bacteria (**Figure 1**). Understanding the inhibition as the loss of metabolic activity and the suppression as bacterial lysis, the oxygen limitation promoted NOB inhibition without NOB suppression. Thus, the design of a NOB suppression strategy prior to the adaptation of the PN-A biomass to organic matter is crucial [64].

Thus, for NOB suppression, a multiparametric strategy was sized [36]. In order to achieve a robust REMON process capable of tolerate the addition of organic matter, a three stages stabilization strategy was implemented: NOB suppression by free ammonia overload with oxygen limitation, recovery of ammonium oxidizing activity and promotion of aerAOB growth, and

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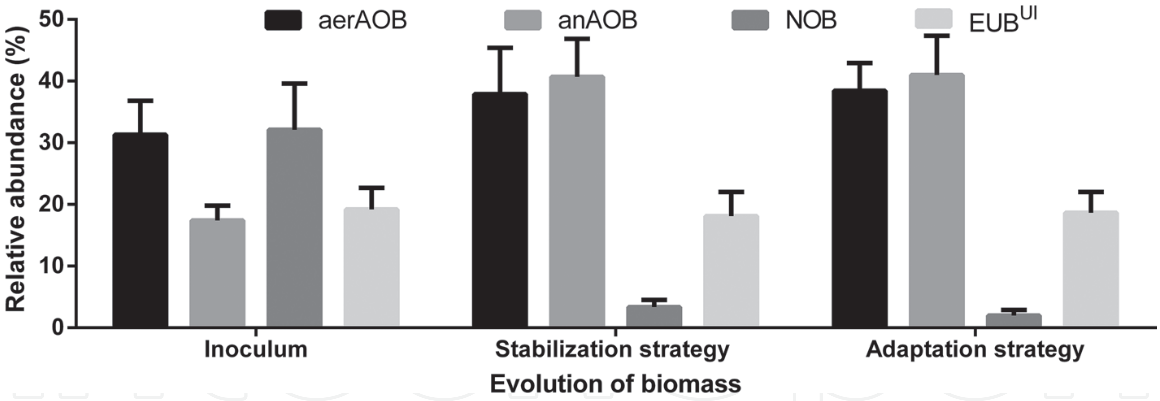

Figure 1. Microbiological characterization of the granular PN-A Inoculum biomass and PN-A biomass during different stages of adaptation. Aerobic ammonia-oxidizing bacteria (aerAOB), anaerobic ammonia-oxidizing bacteria (anAOB), nitrite-oxidizing bacteria (NOB) and unidentified eubacteria (EUB^{UI}) [71].

finally, DO decrease to induce anAOB activity recovery. On the one hand, the FISH analysis confirms a strong decrease of the NOB group in the granular biomass and at the end of the stabilization period, the relative abundance of aerAOB, anAOB, NOB and unidentified eubacteria (EUB^{UI}) was 37.88, 40.67, 3.34 and 18.11%, respectively. On the other hand, the relative abundances of the inoculum were 31.3, 17.4, 32.1 and 19.2%, respectively (**Figure 1**). These results revealed a decrease of nearly 90% of NOB abundance, which support the effectiveness of the start-up implemented strategy. These results agree with the strategies described for the start-up of the PN-A process [50, 52, 53, 55, 64], since so far the literature only reports the inhibition of NOB without considering a bioprospecting of the bacterial consortium in the biomass that will ensure the suppression of undesirables species [71]. Then, an adaptation strategy was performed in four steps, corresponding to different increasing feeding ratios of 25, 50, 75 and 100% (v/v) of anaerobic digester/total substrate ratio (mixed anaerobic digester and synthetic substrate). The aim of a gradual adaptation of the REMON biomass to the organic matter was to avoid an excessive growth of heterotrophic flocculent biomass through the control of the SRT with a slight modification of the separation system. The proposed control was gradual in order to maintain a denitrifying activity on the reactor, prevented also in other systems such as CANON [50]. In addition, the growth of denitrifying bacteria over the granular biomass should be avoided in order to increase the process efficiency. This was successfully accomplished by using a HRT larger than the inverse of the specific growth rate of the heterotrophic bacteria, about 4 h to prevent forming granules and to promote its free floc state [37].

During the experimental work, the biomass concentration in the reactor was 6.5 g VSS/L, and the SRT was 10 d. After a slight modification of the separation system, the biomass concentration on the reactor decreased to 3.5 g VSS/L, and the SRT was 5.4 d. Moreover, the washed-out biomass mainly corresponded to flocs, achieving good granular biomass retention. The control of HRT and SRT allowed the suppression of NOB biomass (**Figure 1**) and the development of denitrifying biomass in the reactor. Summarizing, a greater efficiency was achieved when the adaptation was completed (100%, v/v real substrate), and a maximum of 91.68% total nitrogen removal was reached with a COD/N ratio of 2.63 (organic load of 864 mg COD/L d^{-})

(**Figure 2**). However, for high COD/N ratios, an effective biomass separation system in the SNAD reactor is essential for both the outflow of suspended biomass and the retention of granules. A bad separation system design can lead to a reactor clogging or a fully biomass retention, and the process will collapse [71].

The effect of shear stress on the granular biomass of a REMON continuous reactor system fed with digested poultry manure has been studied [72]. The start-up was carried out in a continuously fed granular BCR. The BCR was stabilized with synthetic substrate and then adapted to digested poultry manure until reaching a NLR of 0.4 g N/L d. After adaptation, the applied power in the BCR was increased from 8.43 to 15.72 W/m^3. The biomass was characterized physicochemical and molecularly. During the increase of the shear stress, nitrogen removal decreased from 63 to 17%. Relative abundance of aerAOB and anAOB did not show significant differences. However, the specific Anammox and nitrification activities fell 88.54 and 53.10%, respectively (see **Table 3**). In summary, there is an upper limit of the applied agitation power on a granular biomass in a REMON reactor. If this limit is exceeded, a negative effect on the activities of the biomass and in the reactor performance is shown [72].

The different operation parameters of the process have shown some limitation such as: 0.25–1 g SST/L^-, 0.13–0.5 g SSV/L, a maximum COD/N ratio of 2.63. With an optimum of 0.7 of $COD_{biodegradable}$/N [37], TAN influent concentration of 0.2–0.8 g N/L with HRT of 4–0.4 d, the NLR assay has been 0.05–1 g TAN/L d^-. The removal efficiency of the system is 20–50% of COD, with a nitrogen removal of 80–95% [71].

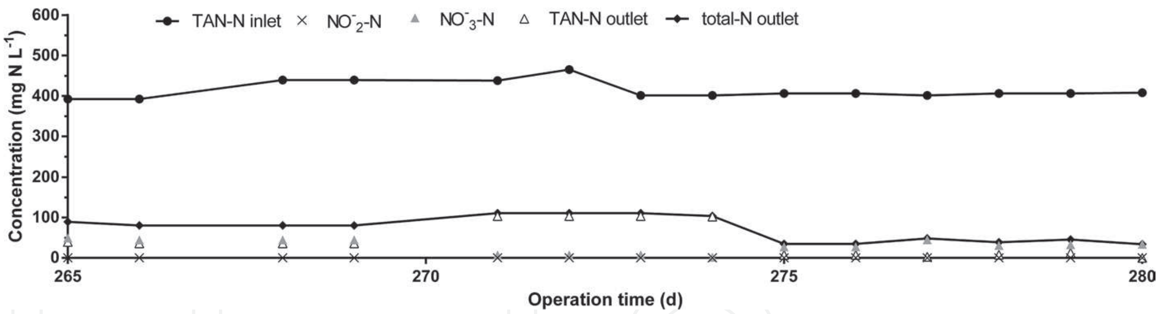

Figure 2. Profile during the adaptation of the PN-A reactor [71].

Shear stress (W/m^3)	EUB [Bacteria/g of biomass]	anAOB [Bacteria/g of biomass]	aerAOB [Bacteria/g of biomass]	NOB [Bacteria/g of biomass]	SNA [g N-NH_4^+/g SSV d]	SAA [g N_2/g SSV d]
8.43	2.86×10^8	1.08×10^8	1.02×10^8	5.18×10^6	0.314	0.113
12.07	2.76×10^8	9.98×10^7	9.13×10^7	7.97×10^6	0.218	0.042
15.72	2.26×10^8	7.02×10^7	8.08×10^7	2.79×10^6	0.036	0.053

EUB, eubacteria; anAOB, anaerobic ammonia-oxidizing bacteria; aerAOB, aerobic ammonia-oxidizing bacteria; NOB, nitrite-oxidizing bacteria; SNA, specific nitrification activity; SAA, specific Anammox activity.

Table 3. Evaluation of the effects of shear stress in REMON system [72].

As a conclusion, REMON is a novel system that optimizes the removal of organic matter and nitrogen species considering strategies that allow NOB suppression and a correct balance between denitrifying bacteria and anAOB.

4. Anaerobic digestion optimization with nitrogen removal: coupled processes

A coupled process prototype at bench scale for the treatment of nitrogen rich wastewaters was developed; the stepwise process has been validated using poultry manure [73]. The first stage comprises the AD of the substrate, where the poultry manure is diluted in order to decrease the ammonium concentration of the substrate to avoid a large inhibition of the methane production. Best results were obtained with three configurations of AD: (1) up flow Anaerobic Sludge Blanket (UASB), (2) thermal pre-treatment with UASB and (3) two stages anaerobic process with a mixed flow reactor (hydrolytic stage) and a UASB (methanogenic stage). In the first step, the diluted manure is anaerobically digested in one of the aforementioned configurations. Most of the organic matter (60–95%) is depleted, and the organic nitrogen of proteins is released in the form of ammonia, reaching high concentrations. Biogas is also generated with a high methane percentage (50–75%). A small fraction of the stabilized solid and an effluent with a remnant organic matter measured as COD is obtained at the outlet stream of the AD. In the second step, the ammonia is removed using a REMON reactor. This reactor generates a warm ammonia free effluent. From the outlet stream, a portion is recirculated to the entrance of the AD, and as a consequence, the slurry inlet stream of the anaerobic digester is diluted (see **Figure 3**).

In the REMON reactor, the denitrifying bacteria uses COD as an electron donor and reduces the residual nitrate to gaseous nitrogen (denitrification process) in presence of organic matter, allowing a complete nitrogen removal and the elimination of the residual biodegradable organic carbon. The integrated process of aerobic nitrification, anaerobic ammonium oxidation and facultative denitrifying bacteria with oxygen limited conditions has the potential of a nearly complete conversion of ammonia and organic carbon to nitrogen gas and carbon dioxide, respectively [71].

The economic and technical feasibility of a coupled process of AD and REMON using water reuse and energy savings applied to a full-scale poultry manure treatment plant was determined to comply with the Chilean environmental law of wastewaters disposal. The new proposed system is more economical than the nitrification-denitrification orthodox processes and offers 15% less sludge generation. The minimum volume of the AD and REMON reactors did not guarantee the minimum annual cost for the plant; on the contrary, a middle case between the minimum and maximum of an objective function of reactors volumes represents the optimal operation condition [74]. But the power consumption is 89.76 and 192.99% lower when burning and using the produced methane, respectively, which means a return of energy. The water recycle results in fresh water savings of 70% compared to the case without recycling. Moreover, the operating costs are reduced by 46%.

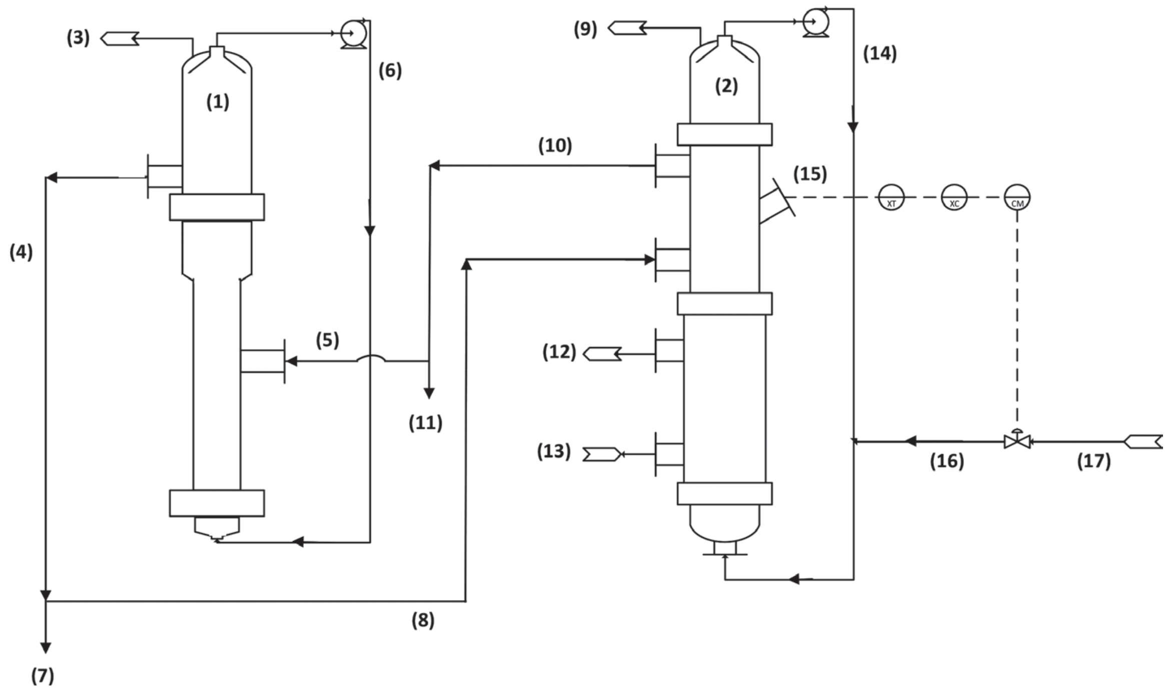

Figure 3. Scheme of coupled processes of anaerobic digester and REMON reactor. (1) anaerobic digester, (2) REMON reactor, (3) overpressure output of anaerobic digester, (4) effluent of anaerobic digester, (5) influent of anaerobic digester, (6) gas recirculation of anaerobic digester, (7) purge of biomass from anaerobic digester, (8) influent of REMON reactor, (9) overpressure output of REMON, (10) effluent of REMON, (11) purge of liquid from REMON, (12) heating water output, (13) heating water input, (14) gas recirculation of REMON, (15) dissolved oxygen [DO] measurement, (16) air make-up, (17) inlet air flow. XT: DO transmitter, XC: DO controller, CM: control module.

5. Conclusions

The correct operation strategies in a biological process (e.g., temperature, nutrient concentrations, bacterial population's interaction and reactor configuration) allow abate high organic carbon and nitrogen concentrations in wastewaters, which poses a problem and changes from a problem to an opportunity. The aforementioned process can be applied to every anaerobic digestion process with inhibitory ammonia concentrations because the need of expensive freshwater can be replaced by recycled treated water with savings of freshwater consumption and operational costs.

Finally, new solutions for ammonia removal using biological treatments reevaluate the technical and economical optimization of the anaerobic digestion projects; the latter were discarded in the past because they showed a negative total annual worth or low biogas potential. Thus, this new process contributes to all different energy matrixes.

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