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Anammox reactor treating low strength domestic wastewater: a review

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Abstract. Anaerobic ammonia oxidation (anammox) is a promising biological wastewater treatment process. It has been widely implemented in many industries due to lower oxygen consumptions besides being an environmental friendly method for wastewater treatment. However, there are several challenges in the process for potential application such as low anammox bacterial abundance and slow growth rate that lower the efficiency of the reaction. Therefore, several factors are being considered during operation of the anammox process. There are various anammox bacteria, which may be enriched using special techniques. Lower nitrogen content of influent brings great challenges for anammox performance due to inhibition caused by ammonium-oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in nitrification process. This review highlighted the anammox process in various industry as well their anammox bacteria morphology, mechanism and strategies to enrich the bacteria. Coupled anammox process with low strength domestic wastewater requires more research to enable potential application in wastewater treatment.

1. Introduction

The supportive wastewater treatment for primary and secondary treatment is the tertiary wastewater treatment [1]. The objective of tertiary process is to exclude the residual organic, inorganic compounds, the refractory and dissolved substances to the standard of mandatory. Biological wastewater treatment is applied to terminate the residual contaminants after the primary treatment [2]. At the pulp and mill wastewater treatment plant, the biological treatment process is crucial as bacteria has the capability to decompose the toxic and hazardous wastes [3].

Biological wastewater treatment methods were divided into two types; with the availability of oxygen is known as aerobic treatment while without oxygen as anaerobic treatment, respectively. The two preferable biological processes, biological oxidation and biosynthesis are carried out by using selected microorganisms for the decomposition of the organic matter [4].

According to Salar et al. [5], the examples of aerobic treatment may be categorised into two groups; (i) suspension of microbial species is the activated sludge process while (ii) attached growth system are as following fixed film or biofilm, trickling filters, rotating disk reactors and airlift reactors. In the anaerobic process, carbon dioxide, methane and excess biomass are the by-products [6-7]. The upflow anaerobic sludge blanket (UASB) reactor efficiently is been used to treat the wastewater containing medium to high organic content (COD > 1000 ppm) and rich with organic content, particularly beverage industry's wastewater [8].

According to Anijiofor et al. [9], for treating the low strength wastewater with COD less than 1000mg/L the aerobic biological system is beneficial, therefore, the treatment process requires oxygen.

The aerobic system able to yield high quality effluent compared to anaerobic system because it is capable to remove higher amount of soluble organic material and produce well-flocculated biomass, which may result in lower effluent concentration.

The conventional biological treatment of sewage was activated sludge process, whereby it requires two separate tanks similarly to aeration and settling tanks. To overcome the challenges faced by the activated sludge process, the new technology called aerobic granular sludge process was been introduced. The benefits of aerobic granular sludge process was it required one tank only (aeration tank) with smaller footprint, which allow retention of high biomass concentrations in the bioreactor. In the conventional nitrogen removal process, the ammonium will undergo nitrification while the nitrate or nitrite will undergo denitrification; it will be associated with simultaneous aerobic and anaerobic/anoxic conditions, respectively [10].

According to Vázquez-Padín et al. [11], currently the combination of partial nitritation and anammox (PN/A) processes was discovered as an advanced strategy for the removal of nitrogen from the wastewater. While according to Strous et al. [12], in the partial nitration process the AOB will oxidize a portion of the ammonium in the wastewater into nitrite. Next, the anammox bacteria will utilize the produced nitrite as electron acceptor to oxidize the remaining ammonium into nitrogen gas and residual quantities of nitrate. According to Morales et al. [13], the advantages of the PN/A processes compared to the conventional process are as like the reduction of cost for aeration by 40%, the emissions of the greenhouse gases like CO_2 and N_2O may be reduced by 83% and approximately 90% of the sludge production may be suppressed.

Anammox process is highly associated 10 to 12 days of longer doubling time by the anammox bacteria [14] especially in low-strength and low temperature condition. The long doubling time subsequently leads to problems in mainstream treatment such as (i) slow biomass growth rate because the operating temperature is below the optimum range and (ii) the less net biomass production because of insufficient nitrogen content in the stream [13]. To overcome the challenges, high solids retention time (SRT) is required by applying the granular sludge, which enhance the biomass concentration in the system besides ensures the development of anammox [15]. According to Liu et al. [16], to enhance the granular sludge in anaerobic conditions, many types of reactor designs were been suggested for the anammox process implementation. The UASB reactor is the most feasible reactor as compared to others due to the presence of high biomass retention capacity [17]. Nevertheless, mainly it had operated at upflow velocities (Vup) of as low as 0.5– 1.5 m h^{-1}, which subsequently helps to promote the external mass transfer limitations due to absences of homogenous mixing in the sludge bed [18]. This paper reviews previous studies on anammox applications for the treatment of low strength wastewater. This paper will benefit researchers in understanding the factors influencing the anammox growth and its usage in wastewater treatment.

2. Wastewater treatment in Malaysia

According to Muralikrishna & Manickam [19] wastewater is the liquids and waterborne solids originated from domestic, industrial, and commercial uses or the other waters that have been used in man's activities, whose quality has been degraded and are discharged into a sewage system. There are three types of wastewater including domestic wastewater originating from households, municipal wastewater sourced from communities or sewage and industrial wastewater from industrial activities.

Based on the Department of Statistics Malaysia, the estimated population of Malaysia in 2018 is approximately 32.4 million and expected to have 33.8 million in 2020. As the population density increases, it subsequently may develop the water pollution, since large amount of waste maybe generated and disposed into the water bodies. The estimated volumes of wastewater produced by municipal and industrial sectors are 2.97 billion cubic meters per year. In Malaysia, the [20] is responsible for the wastewater effluent quality through the Environmental Quality Act 1974 and its regulations such as the Environmental Quality (Sewage) Regulations 2009 and Environmental Quality (Industrial Effluent) Regulations 2009.

Biochemical oxygen demand (BOD) and suspended solids (SS) are the crucial parameters, which are been measured to provide a cleaner and safer environment as well to improve the living conditions of Malaysians. Therefore, high BOD is significant because it indicates that sewage will quickly consume all the DO available in the streams, rivers and lakes, subsequently causing the death of all aquatic life and rendering the water septic and foul-smelling. High SS indicates that the sludge deposits in the waterways and leading significant environmental deterioration.

2.1 Wastewater treatment

In Malaysia, common practices for wastewater treatment includes several processes as described by the local WWTP. The preliminary stage is to remove the rags, grit, oil and grease, followed by the primary phase to remove the settable and floatable materials. The secondary phase or biological treatment is to exclude organic and suspended solids. In tertiary phase, the removal of nutrients, toxic substances (heavy metals), suspended solids and organic will take place. According to the local WWTP currently, there is no plan to build tertiary treatment systems in Malaysia. Hence, the local WWTP has been focusing on the basic standard of preliminary, primary, and secondary treatments, respectively. Wastewater treatment technologies or advanced wastewater treatment methods may broadly classified into three sub divisions, which includes physical, chemical and biological treatment method.

2.2 Anammox applications for the low strength wastewater treatment

PN/A process has some limitations due to the inhibition caused by the substrates and exogenous compounds including biodegradable COD [21], optimum temperature in the mesophile range and slow start-up [14,22]. Subsequently, these limitations had caused the full-scale application relatively restricted to some wastewaters, especially the reject water line of WWTPs. According to Cao et al. [23], the primary challenges of implementing anammox in mainstream treatment are the low activity of anammox bacteria at low temperatures besides interruption from NOB when treating low strength wastewater.

In the nitrification process, the AOB and ammonium-oxidizing archaea (AOA) are responsible for the production of nitrite through oxidising the ammonia. Next, an oxidation process by NOB into nitrate [24]. However, the preferable substrate by the anammox bacteria is nitrite, rather than nitrate [25]. According to Wang et al. [26], the municipal WWTPs with low strength ammonium loading have proven that AOB has larger population than AOA, which referring that AOB supplied more nitrite for anammox than AOA. Table 1. summarizes the application of anammox process in low strength wastewater treatment.

Table 1. Application of anammox process in low strength wastewater treatment

Reactors	Wastewater	Effluent concentration	References
Air-lift reactor	50 mg/L ammonia in wastewater	Nitrogen removal efficiencies: 71.8 ± 9.9%	[27]
Upflow anammox sludge blanket (UAnSB)	Synthetic influent (35 mg N-$NH_4^+L^{-1}$ and 35 mg N-$NO_2^-L^{-1}$)	Nitrogen removal efficiencies: 80 ± 3%	[28]
Sequencing batch reactor (SBR)	51.2 to 67.5 mg-N/L ammonium in wastewater	High NRR: 73 mg-N/(L.d)	[29]
Moving bed biofilm reactor (MBBR)	Influent ammonium concentration of >500 mg-N/L	Effluent ammonium concentrations: 50 mg-N/L	[30]
Upflow anaerobic sludge blanket (UASB)	NH_4^+-N: 16.87 ± 2.09 mg/L, NO_2^--N: 20.57 ± 2.31 mg/L, NO_3^--N: 13.97 ± 3.99 mg/L and soluble COD: 25.54 ± 6.94 mg/L	Nitrite removal efficiencies: 94.35% and ammonium removal efficiencies: 92.81%,	[31]

3. Anaerobic ammonia oxidation (Anammox)

3.1 Definition of anammox

Anammox is a novel process, which occurs in the anaerobic condition, by utilizing nitrite as the electron acceptor and ammonium as the electron donor, to convert the ammonium into nitrogen gas [1]. In a study to elucidate anammox underlying molecular mechanisms, it was been discovered that the process does not produce nitrous oxide (NO_2)[32]. In the development of autotrophic nitrogen removal technologies, anammox is been categorised as one of them because its more cost-effective and environmental friendly compared to conventional activated sludge systems which consumes lower oxygen for nitrogen removal [33-34].

3.2 Factors affect anammox activity

The challenges faced by the anammox for efficient process are the low bacterial abundance and slow growth rate in the sewage treatment systems. A number of key factors were discovered as the primary drivers for affecting the development of the anammox bacteria.

3.2.1 Substrate concentrations

In the treatment process, the bacterial like AOB, anammox and NOB have a certain range of adaptation to the substrate concentrations. The most difficult aspect in the anammox stability performance is the presence of nitrite, which is the electron acceptor in the reaction [35]. The nitrite is a main substrate for NOB growth. The activity of AOB and anammox bacteria will been inhibited due the competition among the NOB versus AOB and anammox bacteria, against substrate called oxygen and nitrite with the presence of COD [36]. Besides that, a portion of nitrate production also will been contributed by the NOB.

According to Bao et al. [37] and Miao et al. [38], NOB have a long lag period during the conversion phase from anaerobic to aerobic phase. Hence, AOB was preferred as it is more convenient to change into aerobic environment than NOB and intermittent aeration maybe substituted to cause inhibition of NOB growth. DO is important to be maintained to inhibit the growth of the NOB. Based on Bao et al. [37], maintaining DO concentrations in the aerobic zone at 0.3 mg/L besides decreasing the quantity of influent air to the reactor operation may complete the nitrification process and inhibit the growth of NOB.

3.2.2 pH

According to the analysis of reaction rates and kinetics of anammox [39], the pH may directly affect the bacterial [40]. According to Strous et al. [12], the preferable pH range to enhance the growth and activity of anammox bacteria in wastewater treatment is 6.7 to 8.3. While Tang et al. [17], determined the anammox activity at the pH range of 6.5 to 9.3.

According to Zhu et al. [41], the active anammox bacteria had discovered in the low and high pH freshwater, at pH 3.88 and 8.91, respectively. The research has proven that the factors that able to secure the anammox bacteria from acidic and alkaline conditions are the low permeability of the bacteria membrane and restricted penetration of protons. The low pH may cause a decrease in the free ammonia concentrations and an increase in the free nitrous acid concentrations. While in high pH, an increase in the free ammonia concentrations and drop in the free nitrous acid concentrations [42] might cause. When the pH is lower, the reactor could perform effectively with higher nitrite level [43].

3.2.3 Temperature

In recent years, excellent anammox activity results were obtained at the optimum temperatures ranging between 30 to 40 °C [44]. According to Fernández et al. [45], at the extreme low temperature the anammox activity may be affected as the anammox process turns unsteady due to nitrite accumulation. At the mainstream line conditions, the factors that may affect the microbial activity and dynamics are the low-strength nitrogen concentrations and changes in the temperature due to weather condition [46]. As compared to AOB and NOB, anammox bacteria have lower growth rates. Commonly anammox-doubling time is 15 to 30 days [47] but at low temperature operation, the doubling time may result beyond 79 days [48]. The anammox activity may reduce because of temperature drop; subsequently will cause the ammonium and nitrite to allocate in one-stage PN/A reactors. When temperature was lowered, nitrite concentration raised due to higher nitritation than anammox rate [30, 49].

3.3 Applications of anammox process

Compared to the traditional biological nitrogen removal processes, the implementation of the anammox process in wastewater treatment have contributed to significant energy reduction of 60% and greenhouse gas emission reduced up to 90% [50-52]. While at the lab-scale, anammox process has been developed successfully in variety types of wastewater treatment such as the landfill leachate treatment, domestic wastewater treatment, municipal wastewater treatment, monosodium glutamate wastewater and pharmaceutical wastewater [23, 53-56]. According to Strous et al. [57], in the practical implementation, anammox reaction is restricted due to low bacterial abundance and slow growing time, which are about 10 to 14 days. Currently, many researches had being conducted regarding anammox start-up process for the laboratory and big full-scale. Nevertheless, the rapid start-up of anammox process is challenging for actual practical application [58]. Table 2. summarizes the applications of anammox in recent years.

Table 2. Application of anammox in 2018 to 2019

Reactor	Influent fed	Effluent concentrations	Anammox activity	References
Four fertilization treatments as 1) Control, 2) Chemical fertilizer, 3) Pig composted manure plus chemical fertilizer and 4) Straw returned to soil plus chemical fertilizer	Different water contents, 70% of field capacity, alternate wetting and drying, flooding I (D.O 5.8 mg L^{-1}), and flooding II (D.O 2.6 mg L^{-1}).	1) Water treatments: Lowest: 70% of field capacity (0.61 nmol $N_2 \cdot g^{-1} \cdot h^{-1}$) and Highest: Flooding II (1.14 nmol $N_2 \cdot g^{-1} \cdot h^{-1}$) 2) Soil treatments: Lowest: PMCF (0.76 nmol $N_2 \cdot g^{-1} \cdot h^{-1}$) and Highest: SRCF (1.01 nmol $N_2 \cdot g^{-1} \cdot h^{-1}$)	Anammox rate: straw returned to soil plus chemical fertilizer with flooding II (1.47 nmol $N_2 \cdot g^{-1} \cdot h^{-1}$)	[59]
Denitrifying ammonium oxidation reactor	Fluoride 552 mg/L. containing semiconductor wastewater with NH_4^+-N (100mg/L) and NO_2^--N (130mg/L).	Nitrogen removal efficiency: 98% and total NRR: 4.11 kg/($m^3 \cdot d$)	NRR of anammox: 3.11kg/($m^3 \cdot d$)	[60]
Moving bed biofilm reactor (MBBR)	Dewatering facility of a municipal WWTP	NRR: 2.5 g-N m^{-2} d^{-1}	Specific anammox activity: 19 ± 2 mg-N g-VSS^{-1} h^{-1}	[61]
Subsurface flow constructed wetlands	Synthetic wastewater of KNO_3 and NH_4Cl (TN:15 mg L^{-1})	TN removal efficiencies: 97.96% and TN concentrations: 0.28 mg/L,		[62]
Expanded granular sludge bed (EGSB) reactor	Leachate from municipal solid waste	Total nitrogen removal efficiency: 71.9% removal of NH_4^+-N: 100% and NO_2^--N: 98.2%	Concentrations of EPS proteins: 128.2 mg/g VSS and ratio of proteins to	[63]

			polysaccharides: 1.18	
Sequencing batch reactor (SBR) fed with zero valent iron nanoparticles	Synthetic wastewater (NH_4^+-N: 20–50 mg/L and NO_2^--N: 30–70 mg/L)	2–6 fold of NH_4^+-N removal rates increased and 4–6 fold of NO_2^--N removal rates increased.	Specific anammox activity: 0.11 gN_2/log copy anammox.day	[64]

3.4 Mechanism of anammox process

Anammox process requires nitrite, which is unusual in actual treatment of wastewaters. However, nitrite might been obtained through partial nitrification process [65]. Hence, as a solution for the shortcomings of anammox process, within a single reactor the collaboration of anammox reaction and partial nitrification was been introduced, which includes both anammox bacteria and AOB, respectively [66]. The AOB will initiate the process of converting ammonium into nitrite via partial oxidation by controlling DO at low concentrations. Subsequently, the anammox bacteria will utilise the generated nitrite to oxidize the residual ammonium into nitrogen gas [67].

According to Dapena-Mora et al. [68], the anammox process has proven oxygen requirement and external carbon source usage were decreased 60% and 100%, respectively, no sludge production and N_2O emission compared to the conventional nitrification and denitrification processes, respectively. However, PN/A process has a better prospective due to the avoidance of external carbon addition, reduction in sludge production and aeration energy essential, respectively [69].

3.5 Morphology of anammox bacteria

According to Liu et al. [70], the granular sludge from the anammox reactor was observed using scanning electron microscopy (SEM). The anammox cell morphology was highly compact, in the shape of round or oval. According to Duan et al. [71], anammox cell viewed under a transmission electron microscope (TEM) has the diameter between ranges of 0.6–1.0 μm. Meanwhile, in Liu et al. [70] observation, under the ocular micrometer, the coccoid or anammox bacteria was being within the diameter of 0.8 to 1.1 μm. It indicates that ocular micrometer is more precise than TEM. A high degree of compactness was seen inside the anammox granular sludge because each cell was been closely integrated with other. Subsequently they became microunits with little space between them.

In Kuenen et al. [72] observation, the cryosubstituted "*Candidatus* Brocadia anammoxidans'' using TEM has shown clear structural representation of the anammox bacteria. A single bilayer membrane, in where catabolism process occurs and known as the anammoxosome, bound the anammox bacteria compartment. The anammox cytoplasm consists of (i) paryphoplasm (outer region), (ii) riboplasm (circulated by an intracytoplasmic membrane) and (iii) anammoxosome (inner ribosome-free layer) [72].

3.6 Anammox bacteria

The bacteria, archaea, and eukaryotes are the three domains of life, which construct the intracellular structure of the anammox bacteria [73]. The *Ca.* Scalindua, *Ca.* Brocadia, and *Ca*. Aestuarianus are the three common genera of anammox bacteria, which had indicated by the phylogenetic analysis [74]. The quantitative PCR (qPCR) and fluorescence in situ hybridization (FISH) methods are been applied to observe the growth and distribution pattern of anammox bacteria and AOB in PN/A reactors [75]. According to Li et al. [76], an efficient tool to investigate anammox bacteria is the hydrazine oxidoreductase gene (*hzo*), which encodes a key anammox enzyme.

Meanwhile, Yang et al. [77] observed *Ca*. Brocadia and *Ca*. Kuenenia as the dominants of anammox genera while *Nitrosomonas* as dominant genus of AOB. According to Rodriguez-Sanchez et al. [78], the *Ca.* Brocadia or the sole anammox genus will be the dominant at the low temperature of 10 to 20 °C in many cases of PN/A. The species for heterotrophic denitrification are *Ignavibacterium,* *Comamonadaceae* and *Rhodocyclaceae* because they may help for the nitrogen removal in PN/A reactor through the process of nitrite or nitrate reduction [79].

3.7 Anammox enrichment techniques
According to Ibrahim et al. [80], to determine the primary parameters and the presence of anammox activity, the batch assay might been conducted upon the seeding sludge. While Tsushima et al. [81] studies have proven this analysis able to verify the compatible of the anammox culture initiator for the continuous enrichment process in the bioreactor. The inoculation should been conducted in an anaerobic chamber because prior to terminate oxygen from batch experiments, therefore, at the beginning of culture process, the anammox cultures will been sparged with inert gases as argon or nitrogen [82]. Based on Kartal et al. [83], one of the challenge in anammox enrichment using the bioreactor is the prolong time required for the start-up of anammox activity because of their longer timing for doubling and slow metabolism. Hence, anammox bacteria required a good configuration bioreactor having large specific surface area for the anammox activity to perform and at low substrate concentrations able to have effective biomass retaining ability, which is suitable for long-term operations [84].

4. Perspectives and further research
In the research area, PN/A is the effective approach to remove the nitrogen from wastewater. Anammox process is been applied by utilizing the nitrite as electron acceptor and ammonium as electron donor for the transformation of ammonium into nitrogen gas under anaerobic conditions. The key factors that are capable to affect the performance of granule based anammox bioreactor were substrate concentrations, pH and temperature besides the size of granules integrated with anammox.

The discharge of wastewater without proper treatment may lead to serious problems for the environment and human well-being. In the area of biological WWTP, aerobic granular sludge is of the promising biotechnologies. Hence, the application of the anammox process incorporate with granular sludge in wastewater treatment can contribute to significant energy reduction and greenhouse gas emission.

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References

[1] Karthikeyan, O. P., & Joseph, K. (2014). Anaerobic Ammonium Oxidation (Anammox) Process for Nitrogen Removal – a Review. *Biological Methods of Waste Treatment and Management in South India*, (November 2016), 102–111.https://www.researchgate.net/publication/239930760_anaerobic_ammonium_oxidation_anammox_process_for_nitrogen_removal_-_a_review

[2] Samer, M. (2015). Biological and Chemical Wastewater Treatment Processes. *Wastewater Treatment Engineering*, 1–50. https://doi.org/10.5772/61250

[3] Vashi, H., Iorhemen, O. T., & Tay, J. H. (2017). Aerobic granulation: a recent development on the biological treatment of pulp and paper wastewater. *Environmental Technology & Innovation.* https://doi.org/10.1016/j.eti.2017.12.006

[4] Gray N. F. (2005). Water Technology: An Introduction for Environmental Scientists and Engineers (2nd Edition), Elsevier Science & Technology Books, ISBN 0750666331, Amsterdam, The Netherlands.

[5] Salar R.K., Gahlawat S.K., Siwach P., & Duhan J.S. (2013). Biotechnology: Prospects and Applications, Springer Science & Business Media, ISBN 978-81-322-1683-4, New Delhi, India.

[6] Cakir, F. Y., & Stenstrom, M. K. (2005). Greenhouse gas production: A comparison between aerobic and anaerobic wastewater treatment technology. *Water Research*, **39**(17), 4197–4203. https://doi.org/10.1016/j.watres.2005.07.042

[7] Chan, Y. J., Chong, M. F., Law, C. L., & Hassell, D. G. (2009). A review on anaerobic-aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal*, **155**(1–2), 1–18. https://doi.org/10.1016/j.cej.2009.06.041

[8] Oliveira, S. C., & Von Sperling, M. (2009). Performance evaluation of UASB reactor systems with and without post-treatment. *Water Science and Technology*, **59**(7), 1299–1306. https://doi.org/10.2166/wst.2009.138

[9] Anijiofor, S. C., Jamil, N. A. M., Jabbar, S., Sakyat, S., & Gomes, C. (2017). Aerobic and anaerobic sewage biodegradable processes: The Gap Analysis *International Journal of Research in Environmental Science*, **3**(3), 9–19. https://doi.org/http://dx.doi.org/10.20431/2454-9444.0303002

[10] Bengtsson, S., de Blois, M., Wilén, B. M., & Gustavsson, D. (2018). Treatment of municipal wastewater with aerobic granular sludge. *Critical Reviews in Environmental Science and Technology*, **48**(2), 119–166. https://doi.org/10.1080/10643389.2018.1439653

[11] Vázquez-Padín, J. R., Morales, N., Aiartza, I., Pedrouso, A., Campos, J. L., Val del Rio, A., & Mosquera-Corral, A. (2018). Pilot-scale ELAN ® process applied to treat primary settled urban wastewater at low temperature via partial nitritation-anammox processes. *Separation and Purification Technology*, **200**, 94–101. https://doi.org/10.1016/j.seppur.2018.02.017

[12] Strous, M., Gijs Kuenen, J., & Jetten, M. S. M. (1999). Key Physiology of Anaerobic Ammonium Oxidation. *Applied and Environmental Microbiology*, **65**(7), 3248–3250. https://doi.org/papers2://publication/uuid/E9A1573A6D62420E94D0CA7C84 D0FEB9

[13] Morales, N., Val del Río, Á., Vázquez-Padín, J. R., Méndez, R., Mosquera-Corral, A., & Campos, J. L. (2015). Integration of the Anammox process to the rejection water and mainstream lines of WWTPs. *Chemosphere*, **140**, 99–105. https://doi.org/10.1016/j.chemosphere.2015.03.058

[14] Dosta, J., Fernández, I., Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L., Mata-Álvarez, J., & Méndez, R. (2008). Short- and long-term effects of temperature on the Anammox process. Journal of Hazardous Materials, **154**(1–3), 688–693. https://doi.org/10.1016/j.jhazmat.2007.10.082

[15] Fernández, I., Vázquez-Padín, J. R., Mosquera-Corral, A., Campos, J. L., & Méndez, R. (2008). Biofilm and granular systems to improve Anammox biomass retention. *Biochemical Engineering Journal*, **42**(3), 308–313. https://doi.org/10.1016/j.bej.2008.07.011

[16] Liu, Y., Xu, H., Yang, S., & Tay, J. (2003). Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor, *Water Research*, **37**, 661–673. https://doi.org/10.1016/S0043-1354(02)00351-2.

[17] Tang, C. J., Zheng, P., Wang, C. H., Mahmood, Q., Zhang, J. Q., Chen, X. G., & Chen, J. W. (2011). Performance of high-loaded ANAMMOX UASB reactors containing granular sludge. *Water Research*, **45**(1), 135–144. https://doi.org/10.1016/j.watres.2010.08.018

[18] Latif, M. A., Ghufran, R., Wahid, Z. A., & Ahmad, A. (2011). Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. *Water Research*, **45**(16), 4683–4699. https://doi.org/10.1016/j.watres.2011.05.049

[19] Muralikrishna, I. V., & Manickam, V. (2017). Wastewater Treatment Technologies. *Environmental Management*. https://doi.org/10.1016/b978-0-12-811989-1.00012-9

[20] Department of Environment (DOE)(2019). Acts. Retrieved from www.doe.gov.my.

[21] Jin, R. C., Yang, G. F., Yu, J. J., & Zheng, P. (2012). The inhibition of the Anammox process: A review. *Chemical Engineering Journal*, **197**, 67–79. https://doi.org/10.1016/j.cej.2012.05.014

[22] Van der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J. W., Tokutomi, T., Strous, M., & van Loosdrecht, M. C. M. (2007). Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research*, **41**(18), 4149–4163. https://doi.org/10.1016/j.watres.2007.03.044

[23] Cao, Y., van Loosdrecht, M. C. M., & Daigger, G. T. (2017). Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. *Applied Microbiology and Biotechnology*, **101**(4), 1365–1383. https://doi.org/10.1007/s00253-016-8058-7

[24] Peng, Y., & Zhu, G. (2006). Biological nitrogen removal with nitrification and denitrification via nitrite pathway. *Applied Microbiology and Biotechnology*, **73**(1), 15–26. https://doi.org/10.1007/s00253-006-0534-z
[25] Zhu, G., Peng, Y., Li, B., Guo, J., Yang, Q., & Wang, S. (2008). Biological removal of nitrogen from wastewater. *Reviews of Environmental Contamination and Toxicology*, **192**, 159–195. https://doi.org/10.1007/978-0-387-71724-1_5
[26] Wang, S., Peng, Y., Ma, B., Wang, S., & Zhu, G. (2015). Anaerobic ammonium oxidation in traditional municipal wastewater treatment plants with low-strength ammonium loading: Widespread but overlooked. *Water Research*, **84**, 66–75. https://doi.org/10.1016/j.watres.2015.07.005
[27] Chen R., Ji J., Chen Y., Takemura Y., Liu Y., Kubota K., Ma H., & Li Y. (2019). Successful operation performance and syntrophic micro-granule in partial nitritation and anammox reactor treating low-strength ammonia wastewater. *Water Research*, **155**, 288–299. https://doi.org/10.1016/j.watres.2019.02.041
[28] Reino, C., & Carrera, J. (2017). Low-strength wastewater treatment in an anammox UASB reactor: Effect of the liquid upflow velocity. *Chemical Engineering Journal*, **313,** 217–225. https://doi.org/10.1016/j.cej.2016.12.051
[29] Wang, S., Miao, Y., Li, B., Zhang, Q., Yang, Y., Zhang, L., & Peng, Y. (2016). Start-up of single-stage partial nitrification-anammox process treating low-strength swage and its restoration from nitrate accumulation. *Bioresource Technology*, **218**, 771–779. https://doi.org/10.1016/j.biortech.2016.06.125
[30] Gilbert, E. M., Agrawal, S., Karst, S. M., Horn, H., Nielsen, P. H., & Lackner, S. (2014). Low temperature partial nitritation/anammox in a moving bed biofilm reactor treating low strength wastewater. *Environmental Science and Technology*, **48**(15), 8784–8792. https://doi.org/10.1021/es501649m
[31] Ma, B., Peng, Y., Zhang, S., Wang, J., Gan, Y., Chang, J., & Zhu, G. (2013). Performance of anammox UASB reactor treating low strength wastewater under moderate and low temperatures. *Bioresource Technology*, **129**, 606–611. https://doi.org/10.1016/j.biortech.2012.11.025
[32] Strous, M., Jetten, M. S. M., Janssen-Megens, E. M., Geerts, W., Op den Camp, H. J. M., Cirpus, I., & Harhangi, H. R. (2011). Molecular mechanism of anaerobic ammonium oxidation. *Nature*, **479**(7371), 127–130. https://doi.org/10.1038/nature10453
[33] Gonzalez-Martinez, A., Osorio, F., Morillo, J. A., Rodriguez-Sanchez, A., Gonzalez-Lopez, J., Abbas, B. A., & van Loosdrecht, M. C. M. (2015). Comparison of bacterial diversity in full-scale anammox bioreactors operated under different conditions. *Biotechnology Progress*, **31**(6), 1464–1472. https://doi.org/10.1002/btpr.2151
[34] Zhang, H., Sung, S., Li, X., Badgley, B. D., He, Z., & Sun, S. (2016). Nitrogen removal by granular nitritation–anammox in an upflow membrane-aerated biofilm reactor. *Water Research*, **94**, 23–31. https://doi.org/10.1016/j.watres.2016.02.031
[35] Jin, R. C., Yang, G. F., Zhang, Q. Q., Ma, C., Yu, J. J., & Xing, B. S. (2013). The effect of sulfide inhibition on the ANAMMOX process. *Water Research*, **47**(3), 1459–1469. https://doi.org/10.1016/j.watres.2012.12.018
[36] Zhang, X., Zhang, H., Ye, C., Wei, M., & Du, J. (2015). Effect of COD/N ratio on nitrogen removal and microbial communities of CANON process in membrane bioreactors. *Bioresource Technology*, **189**, 302–308. https://doi.org/10.1016/j.biortech.2015.04.006
[37] Bao, P., Wang, S., Ma, B., Zhang, Q., & Peng, Y. (2017). Achieving partial nitrification by inhibiting the activity of Nitrospira-like bacteria under high-DO conditions in an intermittent aeration reactor. *Journal of Environmental Sciences*, **56**, 71–78. https://doi.org/10.1016/j.jes.2016.09.004
[38] Miao, Y., Peng, Y., Zhang, L., Li, B., Li, X., Wu, L., & Wang, S. (2018). Partial nitrification-anammox (PNA) treating sewage with intermittent aeration mode: Effect of influent C/N ratios. *Chemical Engineering Journal*, **334**, 664–672. https://doi.org/10.1016/j.cej.2017.10.072

[39] Puyol, D., Carvajal-Arroyo, J. M., Garcia, B., Sierra-Alvarez, R., & Field, J. A. (2013). Kinetic characterization of Brocadia spp.-dominated anammox cultures. *Bioresource Technology*, **139**, 94–100. https://doi.org/10.1016/j.biortech.2013.04.001
[40] Wang, Y. F., & Gu, J. D. (2014). Effects of allylthiourea, salinity, and pH on ammonia/ammonium-oxidizing prokaryotes in mangrove sediment incubated in laboratory microcosms. *Applied Microbiology and Biotechnology*, **98**(7), 3257–3274. https://doi.org/10.1007/s00253-013-5399-3
[41] Zhu, G., Xia, C., Shanyun, W., Zhou, L., Liu, L., & Zhao, S. (2015). Occurrence, activity and contribution of anammox in some freshwater extreme environments. *Environmental Microbiology Reports*, **7**(6), 961–969. https://doi.org/10.1111/1758-2229.12341
[42] Mosquera-Corral, A., González, F., Campos, J. L., & Méndez, R. (2005). Partial nitrification in a SHARON reactor in the presence of salts and organic carbon compounds. *Process Biochemistry*, **40**(9), 3109–3118. https://doi.org/10.1016/j.procbio.2005.03.042
[43] Jaroszynski, L. W., Cicek, N., Sparling, R., & Oleszkiewicz, J. A. (2011). Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors. *Bioresource Technology*, **102**(14), 7051–7056. https://doi.org/10.1016/j.biortech.2011.04.069
[44] Daverey, A., Chei, P. C., Dutta, K., & Lin, J. G. (2015). Statistical analysis to evaluate the effects of temperature and pH on anammox activity. *International Biodeterioration and Biodegradation*, **102**, 89–93. https://doi.org/10.1016/j.ibiod.2015.03.006
[45] Fernández, I., Dosta, J., Fajardo, C., Campos, J. L., Mosquera-Corral, A., & Méndez, R. (2012). Short- and long-term effects of ammonium and nitrite on the Anammox process. *Journal of Environmental Management*, **95**(SUPPL.), S170–S174. https://doi.org/10.1016/j.jenvman.2010.10.044
[46] Akaboci, T. R. V., Gich, F., Ruscalleda, M., Balaguer, M. D., & Colprim, J. (2018). Assessment of operational conditions towards mainstream partial nitritation-anammox stability at moderate to low temperature: Reactor performance and bacterial community. *Chemical Engineering Journal*, **350**, 192–200. https://doi.org/10.1016/j.cej.2018.05.115
[47] Lotti, T., Kleerebezem, R., Abelleira-Pereira, J. M., Abbas, B., & van Loosdrecht, M. C. M. (2015). Faster through training: The anammox case. *Water Research*, **81**, 261–268. https://doi.org/10.1016/j.watres.2015.06.001
[48] Laureni, M., Weissbrodt, D. G., Szivák, I., Robin, O., Nielsen, J. L., Morgenroth, E., & Joss, A. (2015). Activity and growth of anammox biomass on aerobically pre-treated municipal wastewater. *Water Research*, **80**, 325–336. https://doi.org/10.1016/j.watres.2015.04.026
[49] Lotti, T., Kleerebezem, R., Hu, Z., Kartal, B., Jetten, M. S. M., & van Loosdrecht, M. C. M. (2014). Simultaneous partial nitritation and anammox at low temperature with granular sludge. *Water Research*, **66**, 111–121. https://doi.org/10.1016/j.watres.2014.07.047
[50] Jetten, M. S. M., Horn, S. J., & Van Loosdrecht, M. C. M. (1997). Towards a more sustainable municipal wastewater treatment system. *Water Science and Technology*, **35**(9), 171–180. https://doi.org/10.1016/S0273-1223(97)00195-9
[51] Siegrist, H., Salzgeber, D., Eugster, J., & Joss, A. (2008). Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Science and Technology*, **57**(3), 383–388. https://doi.org/10.2166/wst.2008.048
[52] Kartal, B., Kuenen, J. G., & Van Loosdrecht, M. C. M. (2010). Sewage treatment with anammox. *Science*, **328**(5979), 702–703. https://doi.org/10.1126/science.1185941
[53] Wang, Q., Wang, Y., Lin, J., Tang, R., Wang, W., Zhan, X., & Hu, Z. H. (2018). Selection of seeding strategy for fast start-up of Anammox process with low concentration of Anammox sludge inoculum. *Bioresource Technology*, **268**, 638–647. https://doi.org/10.1016/j.biortech.2018.08.056
[54] Wijaya, I. M. W., Soedjono, E. S., & Fitriani, N. (2017). Development of anaerobic ammonium oxidation (anammox) for biological nitrogen removal in domestic wastewater treatment (Case study: Surabaya City, Indonesia). *AIP Conference Proceedings*, **1903**(2017). https://doi.org/10.1063/1.5011532
[55] Shen, L. D., Hu, A. H., Jin, R. C., Cheng, D. Q., Zheng, P., Xu, X. Y., & Hu, B. L. (2012). Enrichment of anammox bacteria from three sludge sources for the startup of monosodium

glutamate industrial wastewater treatment system. *Journal of Hazardous Materials*, **199–200**, 193–199. https://doi.org/10.1016/j.jhazmat.2011.10.081

[56] Tang, C. J., Zheng, P., Chen, T. T., Zhang, J. Q., Mahmood, Q., Ding, S., & Wu, D. T. (2011). Enhanced nitrogen removal from pharmaceutical wastewater using SBA-ANAMMOX process. *Water Research*, **45**(1), 201–210. https://doi.org/10.1016/j.watres.2010.08.036

[57] Strous, M., Heijnen, J., Kuenen, J., & Jetten, M. (1998). The sequencing batch reactor as a powerful tool for the study.pdf. *Applied Microbiology and Biotechnology*, **50**, 589–596. https://doi.org/10.1007/s002530051340

[58] Azari, M., Walter, U., Rekers, V., Gu, J. D., & Denecke, M. (2017). More than a decade of experience of landfill leachate treatment with a full-scale anammox plant combining activated sludge and activated carbon biofilm. *Chemosphere*, **174**, 117–126. https://doi.org/10.1016/j.chemosphere.2017.01.123

[59] Abbas, T., Zhang, Q., Jin, H., Li, Y., Liang, Y., Di, H., & Zhao, Y. (2019). Anammox microbial community and activity changes in response to water and dissolved oxygen managements in a paddy-wheat soil of Southern China. *Science of the Total Environment*, **672**, 305–313. https://doi.org/10.1016/j.scitotenv.2019.03.392

[60] Li, X., Yuan, Y., Huang, Y., & Bi, Z. (2019). Simultaneous removal of ammonia and nitrate by coupled S 0 -driven autotrophic denitrification and Anammox process in fluorine-containing semiconductor wastewater. *Science of the Total Environment*, **661**, 235–242. https://doi.org/10.1016/j.scitotenv.2019.01.164

[61] Kowalski, M. S., Devlin, T., di Biase, A., Basu, S., & Oleszkiewicz, J. A. (2019). Accelerated start-up of a partial nitritation-anammox moving bed biofilm reactor. *Biochemical Engineering Journal*, **145**, 83–89. https://doi.org/10.1016/j.bej.2019.02.015

[62] Chen, D., Gu, X., Zhu, W., He, S., Wu, F., Huang, J., & Zhou, W. (2019). Denitrification- and anammox-dominant simultaneous nitrification, anammox and denitrification (SNAD) process in subsurface flow constructed wetlands. *Bioresource Technology*, **271**, 298–305. https://doi.org/10.1016/j.biortech.2018.09.123

[63] Liu, Z., Sun, D., Tian, H., Yan, L., Dang, Y., & Smith, J. A. (2019). Enhancing biotreatment of incineration leachate by applying an electric potential in a partial nitritation-Anammox system. *Bioresource Technology*, **285**, 121311. https://doi.org/10.1016/j.biortech.2019.121311

[64] Erdim, E., Yücesoy Özkan, Z., Kurt, H., & Alpaslan Kocamemi, B. (2019). Overcoming challenges in mainstream Anammox applications: Utilization of nanoscale zero valent iron (nZVI). *Science of the Total Environment*, **651**, 3023–3033. https://doi.org/10.1016/j.scitotenv.2018.09.140

[65] Iliaszewicz, P., & Miodoński, S. (2017). Start-up analysis of partial nitrification process in SBR reactors for different initial conditions. *E3S Web of Conferences*, **17**, 00031. https://doi.org/10.1051/e3sconf/20171700031

[66] Daverey, A., Su, S. H., Huang, Y. T., Chen, S. S., Sung, S., & Lin, J. G. (2013). Partial nitrification and anammox process: A method for high strength optoelectronic industrial wastewater treatment. *Water Research*, **47**(9), 2929–2937. https://doi.org/10.1016/j.watres.2013.01.028

[67] Ma, B., Bao, P., Wei, Y., Zhu, G., Yuan, Z., & Peng, Y. (2015). Suppressing nitrite-oxidizing bacteria growth to achieve nitrogen removal from domestic wastewater via anammox using intermittent aeration with low dissolved oxygen. *Scientific Reports*, **5**, 1–9. https://doi.org/10.1038/srep13048

[68] Dapena-Mora, A., Van Hulle, S. W. H., Campos, J. L., Méndez, R., Vanrolleghem, P. A., & Jetten, M. (2004). Enrichment of Anammox biomass from municipal activated sludge: Experimental and modelling results. *Journal of Chemical Technology and Biotechnology*, **79**(12), 1421–1428. https://doi.org/10.1002/jctb.1148

[69] Van Hulle, S. W., Volcke, E. I., Teruel, J. L., Donckels, B., van Loosdrecht, M. C., & Vanrolleghem, P. A. (2007). Influence of temperature and pH on the kinetics of the Sharon nitritation process, *J. Chem. Technol. Biotechnol*, **887**, 882–887. https://doi.org/10.1002/jctb

[70] Liu, R., R., Wu C., D., & Lu., X., J., (2016). The characteristics of immobilized granular sludge in the laboratory-scale stable partial nitrification-Anammox aquaculture water reactors. *Journal of Water Reuse and Desalination*, **6**(3), 445–453. https://doi.org/10.2166/wrd.2016.156

[71] Duan, X., Zhou, J., Qiao, S., Yin, X., Tian, T., & Xu, F. (2012). Start-up of the anammox process from the conventional activated sludge in a hybrid bioreactor. *Journal of Environmental Sciences,* **24**(6), 1083–1090. https://doi.org/10.1016/S1001-0742(11)60871-1

[72] Kuenen, J. G., Damste, J. S. S., Niftrik, L. A., Strous, M., Jetten, M. S. M., & Fuerst, J. A. (2004). The anammoxosome: an intracytoplasmic compartment in anammox bacteria. *FEMS Microbiology Letters*, **233**(1), 7–13. https://doi.org/10.1016/j.femsle.2004.01.044

[73] Kallistova, A. Y., Dorofeev, A. G., Nikolaev, Y. A., Kozlov, M. N., Kevbrina, M. V., & Pimenov, N. V. (2016). Role of anammox bacteria in removal of nitrogen compounds from wastewater. *Microbiology*, **85**(2), 140–156. https://doi.org/10.1134/S0026261716020089

[74] Fu, B., Liu, J., Yang, H., Hsu, T. C., He, B., Dai, M., & Zhang, X.-H. (2015). Shift of anammox bacterial community structure along the Pearl Estuary and the impact of environmental factors. *Journal of Geophysical Research: Oceans*, **120**(4), 2869–2883., 1–50. https://doi.org/10.1002/2014JC010554

[75] Rikmann, E., Zekker, I., Tenno, T., Saluste, A., & Tenno, T. (2018). Inoculum-free start-up of biofilm- and sludge-based deammonification systems in pilot scale. *International Journal of Environmental Science and Technology*, **15**(1), 133–148. https://doi.org/10.1007/s13762-017-1374-3

[76] Li, H., Chen, S., Mu, B. Z., & Gu, J. D. (2010). Molecular detection of anaerobic ammonium-oxidizing (Anammox) bacteria in high-temperature petroleum reservoirs. *Microbial Ecology*, **60**(4), 771–783. https://doi.org/10.1007/s00248-010-9733-3

[77] Yang, Y., Zhang, L., Cheng, J., Zhang, S., Li, X., & Peng, Y. (2018). Microbial community evolution in partial nitritation/anammox process: From sidestream to mainstream. *Bioresource Technology*, **251**(October 2017), 327–333. https://doi.org/10.1016/j.biortech.2017.12.079

[78] Rodriguez-Sanchez, A., Purswani, J., Lotti, T., Maza-Marquez, P., van Loosdrecht, M. C. M., Vahala, R., & Gonzalez-Martinez, A. (2016). Distribution and microbial community structure analysis of a single-stage partial nitritation/anammox granular sludge bioreactor operating at low temperature. *Environmental Technology (United Kingdom)*, **37**(18), 2281–2291. https://doi.org/10.1080/09593330.2016.1147613

[79] Oren, A. (2014). The Family Rhodocyclaceae. *Soil Microbiology, Ecology and Biochemistry*, 119–144. https://doi.org/10.1016/b978-0-08-047514-1.50009-3

[80] Ibrahim, M., Yusof, N., Mohd Yusoff, M. Z., & Hassan, M. A. (2016). Enrichment of anaerobic ammonium oxidation (anammox) bacteria for short start-up of the anammox process: a review. *Desalination and Water Treatment*, **57**(30), 13958–13978. https://doi.org/10.1080/19443994.2015.1063009

[81] Tsushima, I., Ogasawara, Y., Kindaichi, T., Satoh, H., & Okabe, S. (2007). Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors. *Water Research*, **41**(8), 1623–1634. https://doi.org/10.1016/j.watres.2007.01.050

[82] Chen, H., Yu, J. J., Jia, X. Y., & Jin, R. C. (2014). Enhancement of anammox performance by Cu(II), Ni(II) and Fe(III) supplementation. *Chemosphere*, **117**(1), 610–616. https://doi.org/10.1016/j.chemosphere.2014.09.047

[83] Kartal, B., van Niftrik, L., Keltjens, J. T., Op den Camp, H. J. M., & Jetten, M. S. M. (2012). Anammox-Growth Physiology, Cell Biology, and Metabolism. *Advances in Microbial Physiology*. **60**, 211-262 Elsevier Ltd. https://doi.org/10.1016/B978-0-12-398264-3.00003-6

[84] Jiang, T., Zhang, H., Qiang, H., Yang, F., Xu, X., & Du, H. (2013). Start-up of the anammox process and membrane fouling analysis in a novel rotating membrane bioreactor. *Desalination*, **311**, 46–53. https://doi.org/10.1016/j.desal.2012.10.031