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Review

Nitrogen Removal from Mature Landfill Leachate via Anammox Based Processes: A Review

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Abstract: Mature landfill leachate is a complex and highly polluted effluent with a large amount of ammonia nitrogen, toxic components and low biodegradability. Its COD/N and BOD_5/COD ratios are low, which is not suitable for traditional nitrification and denitrification processes. Anaerobic ammonia oxidation (anammox) is an innovative biological denitrification process, relying on anammox bacteria to form stable biofilms or granules. It has been extensively used in nitrogen removal of mature landfill leachate due to its high efficiency, low cost and sludge yield. This paper reviewed recent advances of anammox based processes for mature landfill leachate treatment. The state of the art anammox process for mature landfill leachate is systematically described, mainly including partial nitrification–anammox, partial nitrification–anammox coupled denitrification. At the same time, the microbiological analysis of the process operation was given. Anaerobic ammonium oxidation (anammox) has the merit of saving the carbon source and aeration energy, while its practical application is mainly limited by an unstable influent condition, operational control and seasonal temperature variation. To improve process efficiency, it is suggested to develop some novel denitrification processes coupled with anammox to reduce the inhibition of anammox bacteria by mature landfill leachate, and to find cheap new carbon sources (methane, waste fruits) to improve the biological denitrification efficiency of the anammox system.

Keywords: anaerobic ammonium-oxidizing bacteria; nitrification; denitrification; biological denitrification; mature landfill leachate

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1. Introduction

With urbanization, population growth and industrialization, the amount of municipal solid waste has increased sharply, and landfill is the most common way to dispose solid waste, but this has also led to the production of a large amount of landfill leachate [1]. The special characteristics of landfill leachate, including high age dependent concentrations of ammonia, refractory organic matter and heavy metal ions, can potentially have hazardous and toxic effects on the environment and endanger human health [2].

Landfill leachate, especially mature landfill leachate, is difficult to treat using conventional biological processes. As the landfill time increases (>10 years), organic nitrogen in the leachate is gradually converted to ammonia nitrogen, while the organic matter, especially the bioavailable part, decreases, forming mature landfill leachate characterized by high ammonia nitrogen, low C/N, and (Biochemical Oxygen Demand) BOD_5/(Chemical Oxygen Demand) COD [3]. So far, there are many ways to treat landfill leachate, mainly physiological–chemical processes and biological methods. At present, ion exchange, reverse osmosis, advanced oxidation, ammonium stripping and chemical precipitation have been commonly used to treat mature landfill leachate [4–6]. Although the physic–chemical processes described above can remove most of the toxic and harmful substances in mature landfill leachate, they are expensive and produce secondary pollution, and the physic–chemical process usually needs to be used in combination with biological methods [7]. In contrast, biological treatment is widely used in the denitrification of mature landfill leachate due to its high economic benefits [2]. In practical application, the traditional nitrification and denitrification process is one of the most commonly used biological denitrification technologies, such as the conventional activated sludge process, moving bed biofilm reactor (MBBR) biological filter and sequencing batch reactors (SBR) [8]. However, the process has some disadvantages in long term operation, such as high aeration energy consumption, large greenhouse gas production, the high cost of an external carbon source and large floor area, which restricts its further popularization and application. Especially in mature landfill leachate, COD/N and BOD_5/COD ratios are low, which is not suitable for the nitrification and denitrification process [9].

As a new nitrogen removal process, the anammox process can directly reduce ammonia nitrogen to nitrogen with nitrite nitrogen as an electron donor. Currently, the process has been widely used to treat high ammonia nitrogen wastewater, such as sludge digestion wastewater, semiconductor wastewater, and mature landfill leachate [10,11]. Compared to traditional nitrification–denitrification processes, the anammox process has the merit of saving the carbon source and aeration energy.

Anammox as a single process for nitrogen removal has been developed and attracted much attention. Granular sludge systems or biofilm systems, or a one stage system or multistage system, all have applied in treating mature landfill leachate. The sidestream anammox process has been applied in engineering and more than 100 high ammonia wastewater treatment plants have been established [12]. Although the anammox process is considered to be the suitable treatment method for mature landfill leachate with the highest economic value, there are still some difficulties in its practical application. Firstly, anammox bacteria (AnAOB) are autotrophic bacteria with a long doubling time and need a sufficient nitrogen load, which prolongs the anammox process start up [13]. Secondly, theoretically, 11% of the nitrate produced in the anammox process leads to the failure of the effluent total nitrogen, which means that the effluent needs to be further treated. Lastly, the components of leachate are complex, and its endogenous substances have positive or negative effects on the anammox process [14,15]. In order to solve these problems, many studies have optimized the process and obtained a series of excellent experimental results, including good nitrogen removal performance.

In this review, the anammox processes for treating mature landfill leachate were reviewed, including the characteristics and operating efficiency of each process. Then, the relevant functional microorganisms and several challenges in real application were systematically introduced. Finally, the effective ways to achieve a high nitrogen removal performance and economic benefit in the future are pointed out. This review is aimed to serve as a guide for future research and the application of anammox processes in the treatment of mature landfill leachate.

2. Mature Landfill Leachate Treatment via Anammox-Based Processes

At present, the anammox based processes for the treatment of mature landfill leachate mainly includes the following three types: partial nitrification–anammox, the coupling of partial nitrification–anammox and denitrification, and the coupling of partial nitrification–anammox and partial denitrification. Every type of process has their own characteristic and nitrogen removal pathway, as shown in Figure 1.

2.1. Partial Nitrification–Anammox Process

The anammox process is a promising technique to treat mature landfill leachate. When treating wastewater, anammox cannot work alone, because it needs the nitrite substrate supplied by nitritation [16]. Stable partial nitrification (PN) is hard to achieve in municipal wastewater treatment due to the dominancy of nitrite-oxidizing bacteria

(NOB) over ammonia-oxidizing bacteria (AOB). Various studies have investigated high free ammonia (FA), free nitrous acid (FNA), low dissolved oxygen (DO), and high pH to inhibit NOB growth. The high ammonia nitrogen concentration in landfill leachate can naturally provide enough high FA concentration [3]. The single or two stage partial nitritation–anammox (PN/A) processes are two types of deammonification reactors which were widely used in application. The former is more economical because of its lower civil engineering cost. However, the two stage PN/A process can be preferred for landfill leachate [17]. In this configuration, the COD in the pretreated effluent will preferentially enter the PN reactor to avoid the direct impact of organic matter on AnAOB, subsequently providing a more suitable influent for the following anammox reactor. Compared to one stage PN/A, two stage is advantageous due to the high nitrogen removal rate [18]. A combined continuous flow process of nitritation and anammox produced a total nitrogen volumetric load of anammox in an UASB reactor that was increased from 0.5 kg N/(m^3·d) to 1.2 kg N/(m^3·d) [19]. In addition, a two-stage anammox SBBR system has been used to treat leachate: after 107 days of operation, the effluent TN remained below 20 mg/L with 95% of TN removed [20].

The single stage PN/A needs more precise control parameters in engineering applications under the conditions of varying influent composition [21]. Numerous studies have investigated PN/A to ensure system stability via DO and COD optimization. One study has investigated the effect of influent organics on an anammox reactor in treating mature landfill leachate, they find influent COD should be maintained below 800 mg/L. Simultaneous denitrification and anammox processes can happen in the system under an appropriate COD concentration, which will enhance the nitrogen removal efficiency and operational stability. The effect of DO on nitrogen removal is also very crucial. Especially in one stage PN/A, the optimum microaerobic conditions can greatly promote the activities of AOB, anammox, and out select NOB. When DO increases from 0.2 mg/L to 0.6 mg/L, the nitrogen removal rate (NRR) decreased by about 20%, owing to the fact that anammox activity was inhibited under the conditions of DO 0.6 mg/L [22]. However, the activity of AOB might also be restricted by low DO. A novel partial nitrification–anammox biofilm reactor (PNABR) operated under high DO concentrations (>1 mg/L) with a preanoxic—aerobic—anoxic operational way has been proposed, where aerobic biofilm was specially cultivated on the outside surface of the anammox bacteria biofilm carrier. Such a configuration enhanced the microbial resistance to high DO conditions. Eventually, NRR and nitrogen removal efficiency (NRE) finally reached 0.396 kg N/(m^3·d) and 96.1%, respectively [23]. It is possible that the high ammonia nitrogen content (>1000 mg/L) in mature landfill leachate is another key factor in the inhibition of NOB at high DO.

2.2. Coupling of Partial Nitrification–Anammox and Denitrification

In PN/A processes, 11% of nitrate nitrogen byproducts were generated, which limits the improvement of total nitrogen removal efficiency [24]. Indeed, the organic matter of leachate can be degraded by employing specific heterotrophic bacteria. Therefore, the coupling system of denitrification and PN/A can solve the above problems [25]. There are two coupling types: denitrification (DN) direct coupling in partial nitrification (PN)-anammox (DN + (PN-anammox)) and pre-DN followed by PN-anammox (DN-PN-anammox). DN+ (PN-anammox) have the advantage of saving reactor space, but the disadvantage is that heterotrophic bacteria have more affinity for oxygen, which first oxidized COD with oxygen, and then ammonia oxidation occurred, increasing the energy consumption of the PN section. In contrast, pre-denitrification can utilize influent carbon sources, thus avoiding the inhibition effect of organic matter on AnAOB [26]. To sum up, the combined system can achieve above 87.9% nitrogen removal efficiency, as seen in Table 1. COD removal efficiency ranged from 30% to 60%, although the effluent cannot meet COD discharge standards [27,28]. The percentage of the denitrification contribution to NO_3^--N removal decreased with higher influent organics loads, this shows the refractory organic matter in mature landfill leachate might serve as the potential electron donor for denitrification, also, they could inhibit denitrification activities. Humic acid (HA) is the dominant soluble DOM with low biodegradability in mature landfill leachate, usually accounting for 4~44% of the total organic carbon (TOC) [29]. HA could suppress the anammox bacteria activity at a certain concentration. Nevertheless, the nitrogen removal efficiency of the system rapidly returned after a short adaptation period. Controlling the abundance of Nitrospirae was the key to further improving the nitrogen removal performance of the system under HA stress. Another major drawback of the process is limited denitrification. The biochemical oxygen demand (BOD) value in mature landfill leachate is low, which limits the synergistic removal performance of carbon and nitrogen during the heterotrophic denitrification process. Concludingly, a step feed partial nitrification and simultaneous anammox and denitrification (SPNAD) process has been proposed, providing sufficient electron donors for the denitrification stage, with a final nitrogen removal efficiency of 98.7% at a total nitrogen removal load of 0.23 kg N/(m^3·d) [30].

2.3. Coupling of Partial Nitrification–Anammox and Partial Denitrification Process

NO_3^--N can be incompletely reduced by denitrifying bacteria, this process is called partial denitrification (PD), which fixes the final reduction product at NO_2^--N, saving about 40% of the carbon source compared with complete denitrification, and partial denitrification of nitrate is easier to control than partial nitrification [24,31].For mature landfill leachate, a very low BOD content wastewater and PD coupling to the PN/A process can be considered, which can further remove the byproduct nitrate nitrogen and also enhance the long-term operational stability of the system [32,33].

Under the concentration of influent ammonia and nitrate of 47.5 mg/L and 93.7 mg/L in a SBR after PN/A process, a TN removal efficiency of 84.8% was obtained with the effluent TN at less than 20 mg/L [34]. In a continuous flow system, which consisted of an upflow sludge blanket (USB-1), a multi-stage aeration and a second USB (USB-2), PD/A can also be achieved successfully. This process greatly improved NH_4^+-N and TN removal from leachate, reaching a 95% efficiency [28]. The carbon source for initiating PD has been studied with diluted landfill leachate and domestic sewage, where supplementary carbon sources were used for the denitrification of mature landfill leachate [35–37]. The related treatment cost for waste activated sludge (WAS), as a wastewater treatment byproduct, accounts for over 60% of the waste water treatment plant (WWTP) operation cost. Now, more studies focus on sludge fermentation liquid to drive partial denitrification (PD). The residual sludge provides biodegradable carbon, such as proteins, polysaccharides and/or volatile fatty acids, through the anaerobic fermentation process. Thus, researchers have carried out relevant studies to introduce the sludge fermentation IFD process to treating mature landfill leachate. The system remains stable after long term operation and achieved more than a 95% nitrogen removal rate [38].

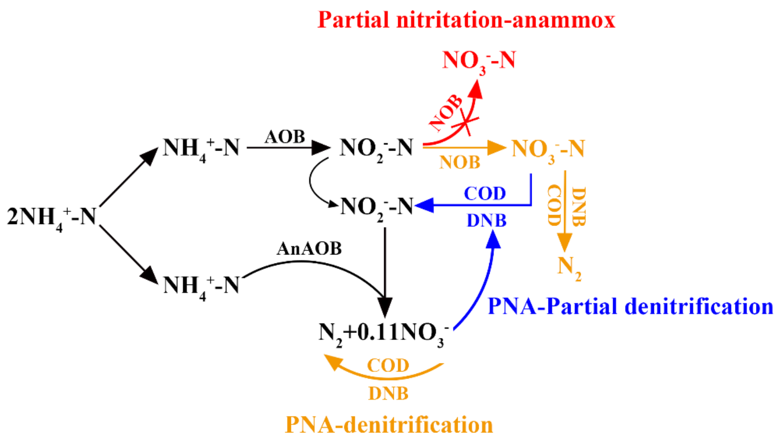

Figure 1. Nitrogen removal pathway of anammox based processes.

Table 1. Anammox based processes of treating mature landfill leachate.

Process	Reactor	T (°C)	C/N	Influent (mg/L)	Effluent (mg/L)	NRE (%)	NRR kgN/(m^3 d)	Ref
PN/A	SBR	30	1.37	NH_4^+ = 950 ± 20 TN = 1200	TN = 37.6	96.1	0.397	[23]
PN/A	USB	-	1.2	NH_4^+ = 900 ± 100 COD = 1300 ± 50	TN < 60	96.3	-	[22]
PN/A	SBR UASB	25–33	1.75	NH_4^+ = 1040 ± 300 TN = 1050 ± 350 COD = 1800 ± 200	TN < 50	85.1	0.75 ± 0.12	[18]
PN/A	SBBR	25–30	0.7	NH_4^+ = 3000 ± 100 COD = 3000 ± 100	TN < 20	95	0.51	[20]
SNAD	SBBR		0.5	NH_4^+ = 2004 ± 14.7 TN = 2024 ± 23.6 COD = 1026.8 ± 14.2	TN < 120	94.9		[27]
SPNAD	SBR		0.85	NH_4^+ = 1000 ± 250 TN = 1300 ± 75 COD = 1100 ± 200	TN = 37	98.7	0.23	[30]
DN-PN/A	UASB		1	NH_4^+ = 2500 ± 250 COD = 2500 ± 250	TN < 90	96	0.5	[26]
PN/A-PD/A	A/O UASB		-	NH_4^+ = 1500 ± 150 TN = 1400 ± 400 COD = 2300 ± 150	TN = 15.7	98.8		[34]
PN/A-PD/A	SBR			NH_4^+ = 804.9 COD = 1116	TN = 19.2	87.9		[32]
PN/A-PD/A	UASB A/O	30–35	2–3	NH_4^+ = 415 ± 15 TN = 430 ± 10	TN = 20	92		[35]

3. Microbiological Analysis of Stable Nitrogen Removal Performance

In the mature landfill leachate treatment using anammox based processes, the enrichment of functional microorganisms is a key factor to determine nitrogen remove efficiency. Candidatus Brocadi, Candidatus Jettenia and Candidatus Kuenenia are the most important genera of anammox bacteria. Different process types have certain selection effects on functional microorganisms, and each has its functional bacteria, which are determined by the different control strategies and the function of the reactor itself. In the two stage PN/A processes, each reactor needs different regulation methods. For example, in the PN section, AOB should be enriched and the growth of NOB should be avoided, and the effluent quality can meet the requirements of the substrate proportion for the next part of the anammox reactor [39]. Microorganisms, in one stage processes, are more complex and diverse because all kinds of biochemical reactions are taking place in a single reactor. To achieve the ideal nitrogen removal rate, more precise strategies are required, such as the time regulation of the intermittent aeration mode [40].

Intermittent aeration and endpoint pH control techniques are widely used in the anammox based processes to treat mature landfill leachate and have been proven to be effective control methods [18,31,41]. In the PN/A process, the aeration mode was changed from continuous to intermittent, and the corresponding functional microbial gene hzsB increased from 1.5×10^7 to 1.1×10^8 copies/g, and anammox activity was enhanced significantly [17]. Through the real time control strategy of pH, the time interval of aeration/anoxia in the Simultaneous nitrification, anammox and denitrification (SNAD) process has been precisely controlled. AnAOB and AOB increased by 40.6% and two times, as compared to the initial start up stage [42].

Mature landfill leachate contains refractory organic matter and toxic substances. Due to long term exposure to mature landfill leachate, the breeding of some special bacteria play an important role in maintaining the stability of the process operation. For example, *Nitrosomonas eutropha* and *Nitrosomonas communis* are AOB bacteria, but they grow to be heterotrophic in landfill leachate, having a certain degradation characteristic of the aromatic compounds, indicating that the functional bacteria will gradually adapt to such unfavorable conditions [29]. In the SNAD or PD process, specific denitrifying bacteria, such as Azoarcus tolulyticus and Ferruginibacter alkalilentus, have been reported for the biodegradation of toxic substances. *Acidobacteria,* a special denitrifying bacterial, not only can degrade HA but can also produce a copious amount of EPS; this contributed to the stability of the PN/A system [43].

4. Challenges in Anammox Based Processes for Treating Mature Landfill Leachate

Although the sidestream anammox process has been applied in engineering, more than 100 high ammonia wastewater treatment plants have been established [12]. There are also several challenges that need to be solved. The unstable influent conditions would cause the difficulty of operation. In addition, the maintenance of the activity of major functional bacteria is also facing challenges under seasonal temperature variation.

4.1. Unstable Influent Condition

4.1.1. The Salinity and Heavy Metal

Mature landfill leachate is one kind of high salinity wastewater. When it is treated by biochemical methods, the impact of salinity on bacteria must be considered. Excessive salinity will lead to the disintegration of functional bacterial cells, loss of activity, and dehydration death [44]. A series of batch tests have been conducted to examine the short-term effects of different salinity on anammox bacterial activity, a significant decrease in the removal efficiency of total nitrogen was observed in the presence of over 5 g/L salinity [45–47]. In addition, salinity inhibits the activity of AOB. Nitrosomonas, the modal AOB of PN/A, was greatly suppressed at a salinity of >1.65% [48]. Interestingly, heterotrophic bacteria became dominant in the anammox based process with an increase in salt concentration, which could be a cause of anammox system failure [49]. These results showed that high salinity negatively affects the anammox based process stability when treating mature landfill leachate. AnAOB has a different tolerance to salinity, which can be domesticated in a saline environment [50,51]. A PN/A process was applied to treat NaCl amended landfill leachate. The reactor established robust nitrogen removal of 85.7 $\pm$ 2.4% with incremental salinity from 0.61% to 3.10% [52]. However, its activity would be inhibited when salinity beyond a certain range. When salinity increased from 10 to 35 g/L, the removal efficiency of one UASB-A/O decreased the biological nitrogen removal system from 99.3 to 83.9% [50]. The major difficulty is sustaining satisfactory anammox activity at high salinity. The effective utilization of marine anammox bacteria (MAB) will notably accelerate the anammox based process to treat saline wastewater [53]. However, another study thought the application of salt acclimated freshwater anammox bacteria could be a more suitable way to treat such wastewaters, MAB based PN/As can be disturbed and even deteriorated while saline wastewater was temporarily not available due to special situations because of MAB's high dependence on salinity [54]. After a period of acclimation, anammox bacteria can adapt to salinity shock and keep the nitrogen removal rate stable, but too high a salinity shock (>50 g/L) will inhibit the activity of anammox bacteria and make it difficult for the system to restore stability [55].

In addition to salinity, mature landfill leachate also contains a certain amount of heavy metals.

A high concentration of metals is commonly toxic to microorganisms because metals cannot be degraded and thus accumulate in cells [56]. Many studies have researched the inhibit effect of toxic metals in some types of wastewater, such as landfill leachate. The content of heavy metals in landfill leachate is inversely proportional to age. However, its toxic effects cannot be ignored. In the presence of both salinity and heavy metals, its inhibitory effect is stronger than that of salinity or heavy metals alone. In addition, one study points out the joint inhibitory effect of Cu (II) and Zn (II), which initially tended to increase and then reversed as the concentrations increased [57].

In a word, anammox bacterial can be adapted to a certain range of salinity and heavy metals after a period of accumulation [58]. However, it is recommended to remove them in advance to avoid the adverse impact on AnAOB activity in high salinity and heavy metals.

4.1.2. Organic Matter

Mature landfill leachate consists of organic matter, which can inhibit the activity of bacterial [59]. This part of the organic matter can be divided into two categories, one is obviously toxic to bacteria, such as phenol and antibiotics [60–62]. The other is biochemical organic matter. These toxic substances can destroy the integrity of cells due to damaged biofilm components [63]. The semi-inhibitory concentrations of phenol and oxytetracycline to anammox acute toxicity are 861.7 and 682.6 mg/L, respectively [64]. The concentration of toxic substances in mature landfill leachate tends to be lower than the lowest tolerance level [65]. However, the joint toxicity between toxic components in landfill leachate should be noted [66]. Moreover, the quantity of leachate is highly variable [67], which may result in a substrate shortage, which could markedly improve the inhibition of toxic organics on anammox activity [68].

The other is biochemical organic matter. Too high an organic carbon content will inhibit the activity of AnAOB. The main inhibition mechanism is that the growth rate of heterotrophic denitrifying bacteria is faster than that of anammox bacteria. It cannot compete with heterotrophic denitrifying bacteria for nitrite. The anammox process will gradually evolve into a heterotrophic denitrification process under high organic concentrations [69,70], as shown in Figure 2. The COD of mature landfill leachate is mainly refractory [19], and the BOD_5/COD ratio is even lower than 0.1. The growth of heterotrophic bacteria is limited by the content of organic matter, and denitrification will not dominate under BOD deficit conditions [67]. In fact, the BOD content is too low to provide sufficient electron donor for denitrification or even partial denitrification, so as to further limit the nitrogen removal efficiency [69]. Humic acid accounted for 60% of organic carbon in mature landfill leachate. It is a non-biodegradable organic matter [70]. The traditional view holds that humic acid inhibited the anammox activity and decreased the nitrogen removal efficiency. One study found that nitrogen removal performance was decreased due to humic acid inhibition on AnAOB, but it was relieved after adaptation [28]. However, when salinity and humic acid coexisted in an anammox reactor, a synergistic inhibition would occur, which was far greater than that of the single salinity or humic acid [71].

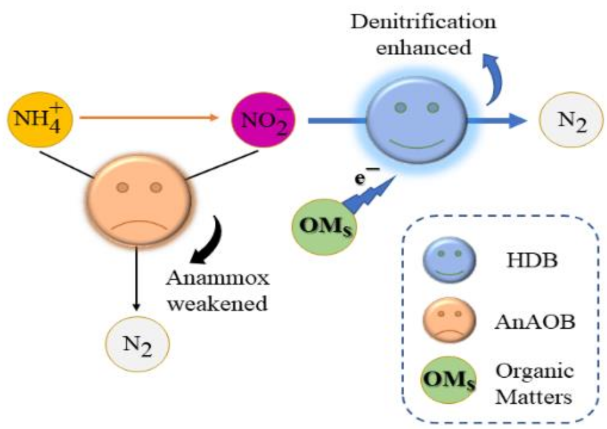

Figure 2. The high concentration organic matter inhibits the anammox process.

4.1.3. High Strength Nitrogen

The ammonia nitrogen concentration of mature landfill leachate is usually higher than 2000 mg/L. However, if the concentration is too high, it will inhibit the activity of anammox bacteria. The real inhibition is not the ammonia nitrogen itself, but the free ammonia (FA) produced by it. FA inhibits specific enzymes and destroys EPS, resulting in cell inactivation/lysis [72]. It is generally believed that FA can inhibit the activity of AnAOB by 50% and 80% at 38 mg/L and 100 mg/L, respectively. High concentrations of free nitrite acid (FNA), could also be generated in the anammox based system when treating mature landfill leachate. AnAOB was more sensitive to the inhibition of free nitrite acid due to the biological toxicity of NO_2^--N. Free nitrite acid showed inhibition to different anammox systems and AnAOB sludge. Most results suggest that when the solubility of nitrite nitrogen is higher than 320 mg/L, its possible inhibitory effect should be considered [72,73]. In addition, the inhibitory effect of FA on AOB cannot be ignored in the SNAD or PD processes. Just 25 mg/L FA could reduce the activity of AOB by 40% [74]. FA concentration in the solution is related with the NH_4^+-N concentration, pH, and temperature. The pH of mature landfill leachate is often higher than 7.5, and the physiological pH of anammox bacteria is 6.7–8.3 [9]. At leachate pH values >8.0, the FA level rises rapidly.

Fortunately, the inhibition is reversible, and the free ammonia does not change the physical properties of anammox bacteria continuously. The influence of FA and FNA on anammox reactions cannot be predicted and can only be determined through experiments. In order to ensure the efficient and stable operation of anammox systems, substrate concentration should be controlled at the predetermined threshold [75].

4.2. Difficulty in Operational Control

The cooperation and competition among functional microorganisms are crucial to the stability and performance of the anammox process, such as the active coordination between AOB and AnAOB, NOB inhibition, and HB growth control. In addition to influent conditions, the long-term operation stability is also closely related to the operational conditions. The main difficulty is how to maintain advanced nitrogen removal during long term operation [32]. The endpoint of pH, as a process control method with pH as an indicator, is a widely adopted regulation strategy in treating landfill leachate. Indeed, pH usually serves as a real time control parameter for nitritation because there is significant variation in the pH profile during the nitrification process. It has been shown that the use of the endpoint pH control technique in an SBR treating landfill leachate is feasible to achieve a stable PN performance [76]. The inflection point on the pH curve is used to determine whether the nitrification or anammox reaction is complete. The lowest point in the pH profile is known as the "ammonia valley". This "ammonia valley" indicates the termination of nitritation, preventing nitrite from being oxidized into nitrate [9].

4.2.1. The Control of DO

DO is a very important regulation parameter. The cooperation and competition among functional microorganisms are closely related to DO in the reactor. For example, too much dissolved oxygen can lead to the overgrowth of NOB. NOB growth tends to be a major challenge faced by the PN/A process. Studies have found that AOB has a higher affinity for oxygen than NOB, which means that a low DO concentration could inhibit NOB growth and activity [77]. In the one step PN/A process, when DO increases from 0.2 to 0.6 mg/L, the denitrification efficiency becomes worse. When DO = 0.6 mg/L, AnAOB activity was inhibited, leading to the accumulation of NO_2^--N in the effluent. The system has a better nitrogen removal rate at a lower DO concentration (<0.6 mg/L) [24]. Four identical sequencing biofilm batch reactors (SBBR) were used to evaluate the effects of dissolved oxygen (DO) on the performance and microbial community of a single stage PN/A system when treating mature landfill leachate. The result showed that an average total nitrogen removal efficiency (TNRE) above 90% was achieved with an optimal DO concentration of 2.7 mg/L [78]. A PNABR process treated mature landfill leachate: when DO rose to 4 mg/L in the aeration stage, the maximum NRE of the system could also reach 96.1% [34]. In the SNAD and the PN reactor, the control of DO is also different: the low DO is less than 0.5 mg/L, and the high is more than 2 mg/L [79].

4.2.2. The Control of C/N

PD can be combined with anammox to realize further nitrogen removal from landfill leachate. when challenged with an overloaded DO concentration and the failure of the real time control of pH. The PN/A-PD/A was proven to be a robust and stable alternative process [32]. However, there exists a difficulty in the accurate control of operational parameters for partial denitrification [80]. In the anammox process, coupled with partial denitrification, a collaboration between heterotrophic bacteria is crucial. Excessive proliferation of heterotrophic bacteria will compete with anammox bacteria for the substrate. The growth of denitrifying microorganisms needs to be strictly controlled. Hence, the optimal C/N ratio is a key control factor for an effective partial denitrification and anammox process. However, regarding the optimal C/N ratio, different studies have different results. This is likely due to the difference in the organic matter of influent, operating conditions, anammox bacterial activity, and the type of reactors [31].

4.3. The Seasonal Temperature Variation

The optimal temperature for an anammox based process is ranged from 30 °C to 40 °C [81]. The lower temperature range (15–25 °C) creates a bottleneck problem, meaning that the anammox process is difficult to achieve in municipal wastewater treatment. In the side stream anammox system, the treatment of landfill leachate also faces the same problem. The side stream anammox system requires a higher temperature, which usually has high activity and a nitrogen removal rate above 30 °C [82]. However, the seasonal temperature of landfill leachate varies greatly. Under the condition of low temperatures (<20 °C), the activity and growth of AnAOB bacteria and AOB will both be affected [83,84]. The temperature can also change the content of free ammonia and organic matter, which indirectly affects the activity of microorganisms [85].

Regardless of a one stage or multistage anammox process of mature landfill leachate, the nitrogen removal rate of the whole process will decrease, to a certain extent, as the temperature drops. In addition, the stability of the system is easily affected by the fluctuation in influent at low temperatures [25,86]. With the seasonal temperature decreasing from 34 °C to 11.3 °C, the total nitrogen removal rate of DN–PN–anammox system treating mature landfill leachate decreased from 1.42 kg N/ (m^3·d) to 0.49 kg N/ (m^3·d). The fluctuation in NH_4^+-N concentration in influent has a great influence on process stability, and the effluent easily deteriorates at low temperatures (<20 °C) [87]. Moreover, the influence of the toxic substances (heavy metals and organic substances) in landfill leachate on microbial community structure and functional microbial species might increase at low temperatures (See in Table 2) [88].

Table 2. Effect of organic matter, salinity and heavy metals on the performance of anammox systems.

Inhibitor	Types	Reactor	Concentration	Effect	Reference
Organic matter	Nontoxic	UASB	700 mg L^{-1} Sucrose	Nitrogen removal efficiency decreased by 98%	[89]
		MBBR	533 mg L^{-1} COD	No interruption because nitrogen load was less	[90]
		Biofilter	400 mg L^{-1} Acetate	Partial inhibition of nitrogen removal rate and increase in the heterotrophic bacteria	[91]
		Serum vials	50 mmol L^{-1} Acetate	Nitrogen removal efficiency decreased by 70%	[92]
	Toxic	UASB	50 mg L^{-1} Oxytetracycline	Activity loss of 90.4%	[61]
			150 mg L^{-1} Amoxicillin	Severely inhibited	[93]
		Serum vials	20 mg L^{-1} Chloramophenicol	Activity decreased by 36%	[94]
			200 mg L^{-1} Chloramophenicol	Activity decreased by 98%	
			100 mg L^{-1} Penicillin	Activity decreased by 36%	
Salinity	NaCl	SBR	5 g L^{-1}	Favored the formation of anammox biofilm	[95]
			15 g L^{-1}	Obversed inhibitory effect	
		RBC	6 g L^{-1}	No remarkable loss of activity	[96]
			30 g L^{-1}	Activity decreased by 95% (nonadapted) and 59% (adapted)	
		UASB	35.1 g L^{-1}	Anammox performance collapsed	[97]
			30 g L^{-1}	Activity decreased by 67.5% (nonadapted) and 45.1% (adapted)	[98]
			5–30 g L^{-1}	Performance degraded at NaCl higher than 15 g/L	[99]
	90% NaCl and 10% KCl	SBR	10 g L^{-1}	Maximum activity	[100]
			45 g L^{-1}	Decreased by 85%	
			60 g L^{-1}	The activity was lost	
	Na_2SO_4	Serum vials	11.36 g L^{-1}	Inhibition by 50%	[92]
	$CaCl_2$	SBR	5 g L^{-1}	Favored the formation of anammox biofilm	[95]

Table 2. *Cont.*

Inhibitor	Types	Reactor	Concentration	Effect	Reference
Heavy metal	Cu	Serum flask	12.6 mg L^{-1}	Inhibition by 50%	[101]
		UASB	5.95 mg L^{-1}	No significant inhibition	[102]
	Zn	Serum flask	20.0 mg L^{-1}	Activity decreased by 20.1%	[57]
		UASB	25 mg L^{-1}	Inhibition by 50%	[103]
	Hg	Serum vials	60.35 mg L^{-1}	Inhibition by 50%	[104]
	Ag		11.52 mg L^{-1}		
	Cd		11.16 mg L^{-1}		
	Mn	Serum vials	175.8 mg L^{-1}	Inhibition by 50%	[105]

UASB: upflow anaerobic sludge bed; MBBR: moving bed biofilm reactor; SBR: sequencing batch reactor; RBC: rotating biological contractor.

The quality of mature landfill leachate is complex and changeable. In a low temperature environment, more attention should be paid to these inhibiting factors, which puts forward higher requirements on the control methods. Both increasing effluent sludge reflux and prolonging hydraulic retention time have been proven to be effective control methods. When the temperature drops from 20–22 °C to 13–15 °C, the contribution of anammox to ammonia nitrogen removal drops from 29.3% to 11.4%. Through the regulation measures of effluent reflux and sludge reflux, the inhibitory effect of organic matter on AnAOB in a low temperature environment can be effectively reduced [86]. In addition, the acclimation and enrichment of sludge under low temperature conditions are also very important. Conducting a low-temperature acclimation process for the sludge under the condition of artificial water distribution, and then gradually introduce landfill leachate as influent after adaptation, so that the system can also achieve stability, has also been studied [25].

5. Future Perspective

Although an anammox process has the merit of saving the carbon source and aeration energy in the treatment of mature landfill leachate, its practical application still faces several challenges. With the increasingly stringent national emission standards and the emphasis on carbon neutralization and resource conservation. Recommended areas for future research are as follows:

More novel nitrogen removal microorganisms can be found and applied to treat mature landfill leachate. The coupled systems can improve the removal efficiency and reduce the treatment cost. These microorganisms can make use of ions in mature landfill leachate as an electron acceptor. Some researchers have verified the feasibility of these coupling processes [106–110]. S-anammox coupled with anammox technology has been successfully applied to treat landfill leachate in some cases, but most of them are applied in multistage [107]. The one stage coupling process needs to be further studied in terms of operation stability, control parameters, and micro microbial mechanism. Rather, most feammox only focus on the mechanism under the condition of artificial water distribution [109], and there are few reports on the treatment of actual landfill leachate.

Given the low BOD content of mature landfill leachate and the additional operating cost of external carbon sources, it is necessary to identify some cheap and easily available new carbon sources and study their applicability. For example, municipal solid waste will produce a certain amount of methane in the landfill process, and some of them will exist in the landfill leachate in the form of dissolved gas [111,112]. However, this methane is not fully utilized and is mostly discharged into the atmosphere in the form of waste. The greenhouse effect caused by methane is 28 times that of carbon dioxide. The discovery of nitrate/nitrite dependent anaerobic methane oxidation processes provides an opportunity to use methane and improve the denitrification performance of anammox based processes. However, the DAMO–anammox system has been only tested on lab scale. Therefore, further studies aimed at assessing the effects of leachate composition, such as organic matter, heavy metals, salinity, and suspended solids, should be conducted [113,114]. In addition to methane, waste fruits can also be used as an external carbon source. Four kinds of rotten fruits were utilized as new carbon sources to promote nitrogen removal from mature landfill leachate. Moreover, this carbon source is cheap and has no inhibitory effect on functional bacteria [115].

6. Conclusions

The main progress of this review is the comprehensive analysis of anammox based processes treating mature landfill leachate based on the state of the art anammox processes for mature landfill leachate. It was demonstrated that anammox based processes are suitable for the removal of nitrogen from mature landfill leachate. No matter what process type, it can achieve the desired nitrogen removal rate on the premise of proper operation. At the same time, microbiological analysis of stable nitrogen removal performance was given, and some special species in the mature landfill leachate treatment were also described.

The engineering applications also face several challenges: unstable influent condition, operational control and seasonal temperature variation. Ultimately, novel nitrogen removal coupled systems and the creation of new carbon sources as perspectives with the aim of improving the application of anammox based processes were proposed. Consequently, the value of anammox based processes for nitrogen removal from mature landfill leachate is a significant area worthy of further research.

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