Review

Advances in Biological Nitrogen Removal of Landfill Leachate

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Abstract: With the development of economy and the improvement of people’s living standard, landfill leachate has been increasing year by year with the increase in municipal solid waste output. How to treat landfill leachate with high efficiency and low consumption has become a major problem, because of its high ammonia nitrogen and organic matter content, low carbon to nitrogen ratio and difficult degradation. In order to provide reference for future engineering application of landfill leachate treatment, this paper mainly reviews the biological treatment methods of landfill leachate, which focuses on the comparison of nitrogen removal processes combined with microorganisms, the biological nitrogen removal methods combined with ecology and the technology of direct application of microorganisms. In addition, the mechanism of biological nitrogen removal of landfill leachate and the factors affecting the microbial activity during the nitrogen removal process are also described. It is concluded that the treatment processes combined with microorganisms have higher nitrogen removal efficiency compared with the direct application of microorganisms. For example, the nitrogen removal efficiency of the combined process based on anaerobic ammonium oxidation (ANAMMOX) technology can reach more than 99%. Therefore, the treatment processes combined with microorganisms in the future engineering application of nitrogen removal in landfill leachate should be paid more attention to, and the efficiency of nitrogen removal should be improved from the aspects of microorganisms by considering factors affecting its activity.

Keywords: landfill leachate; microorganism; nitrogen removal

1. Introduction

In recent decades, the output of municipal solid waste has been increasing year by year with the acceleration of urbanization, the changes of lifestyle and the growth of population [1–3]. According to the statistics of China Statistical Yearbook (2009–2018), the annual output of municipal solid waste reached 22.818 billion tons. Therefore, solid waste treatment has become an important part of urban management. Many cities will face the phenomenon of “garbage siege” if it is not properly handled [4].

There are several ways to treat municipal solid waste, such as thermochemical treatment and biological treatment [5,6]. At present, landfill and incineration are the main treatment technology for municipal solid waste in China [7]. It is reported that there are a total of 1091 municipal solid waste treatment sites in China, including 994 waste incinerators and landfills [8]. However, a secondary pollutant, landfill leachate, is inevitably produced in the process of incineration and landfill disposal [9–11]. Landfill leachate is a kind of high-concentration organic wastewater [12], which contains a variety of organic and inorganic pollutants [13,14], such as humus, heavy metals, inorganic salts, ammonia nitrogen and so on [8]. High ammonia nitrogen content is a typical feature of mature
landfill leachate, and ammonia nitrogen content generally does not decrease with the increase in landfill age [15]. It is easy for landfill leachate coupled with the high content of organic matter to cause surface water and groundwater pollution. Therefore, improper disposal of landfill leachate leads to a series of serious environmental contamination.

The treatments of landfill leachate are generally divided into physical–chemical method and biological method [16]. However, the physical–chemical method has high cost and is prone to causing secondary pollution [17]. In compost, biological treatment is widely used for nitrogen removal of leachate due to its low cost [18,19], such as the traditional nitrification/denitrification process, the new ANAMMOX nitrogen removal process, the new enhanced biological nitrogen removal process using dominant bacteria [20] and the nitrogen removal in leachate by constructing artificial wetland in ecological engineering [21]. However, it is very important for the landfill leachate treatment to select the reasonable process of nitrogen removal due to the different properties of leachate. Therefore, this paper reviews the biological treatment of leachate from municipal solid waste. The focus is on nitrogen removal process, mechanism, various other nitrogen removal methods combined with microorganisms and the factors affecting microbial activity during the process. The purpose of this review is to provide necessary information for the rational selection of nitrogen removal treatment process for leachate from municipal solid waste.

2. Methods and Techniques of Biological Nitrogen Removal from Landfill Leachate

2.1. Biological Nitrogen Removal Processes and Reactors of Landfill Leachate

Biological treatment has been considered as one of the most favorable nitrogen removal technologies for mature landfill leachate [19], which involves nitrification in aerobic reactor and denitrification in anoxic reactor. The traditional biological treatment process of landfill leachate usually includes two stages: nitrification and denitrification. However, the traditional approach requires a large amount of oxygen in the nitrification process and a large number of carbon sources in the denitrification process [22]; therefore, the operation cost is relatively high [23]. At present, a new process of nitrogen removal of landfill leachate, which is a traditional process combined with an ANAMMOX process, is commonly used in the reactor [24]. This new process has the advantages of high nitrogen removal capacity [25,26], low energy consumption and low sludge yield, and it does not need additional carbon sources during operation [27,28]. Most of the processes used in the reactor can be divided into the following categories: the combination process of nitrification and denitrification, the combination process of partial nitrification, nitrosation, denitrification and ANAMMOX, biofilm treatment process based on ANAMMOX and membrane bioreactor, etc. Table 1 shows the types of different processes and reactors, the sources and basic characteristics of leachate, the efficiency of nitrogen removal, and the microorganisms and dominant strains involved in the process of nitrogen removal.

In general, the efficiency of nitrogen removal is not high in a simple process, and a variety of processes is needed to coordinate to improve the efficiency of nitrogen removal. At the same time, the appropriate process should be selected to improve its efficiency according to the different properties of landfill leachate. When the COD concentration in the leachate reaches above 10,000 mg/L, the leachate is young leachate (landfill time is less than 5 years); when the COD concentration reaches between 4000 and 10,000 mg/L, the leachate is intermediate leachate (landfill time is between 5–10 years); when the COD concentration reaches less than 4000 mg/L, the leachate is old leachate (landfill time is larger than 10 years) [47]. The problem of carbon source should be solved in the process of denitrification due to the low C/N ratio of the aged leachate. According to Table 1, partial nitrification and integrated fermentation–denitrification (PNIFD) system, modified sequencing batch reactor (SBR), the combination process of traditional process and ANAMMOX (PN/A, PD/A, SNAD and PNA) and dynamic membrane bioreactor (DMBR) are the main bioreactors for nitrogen removal of aging leachate at present. These processes can solve the problem of carbon source shortage in the traditional denitrification process.
well. In addition, the organic matter can be first converted to CO$_2$ or CH$_4$ by an improved SBR reactor or a membrane bioreactor for biological nitrogen removal of leachate since the COD concentration of the intermediate and young leachate can reach about 10,000 mg/L.

Among them, the combined process based on ANAMMOX has a higher efficiency of nitrogen removal in the biological treatment for landfill leachate. It is a biological process in which Anammox microorganisms use nitrite nitrogen as an electron acceptor to react with ammonia nitrogen to generate nitrogen and then remove it under anaerobic or anoxic conditions [27,48]. Many microorganisms will participate in it, mainly taking anaerobic ammonox and autotrophic bacteria as the core [24]. Therefore, the combined process based on ANAMMOX has great development prospects in the biological treatment for landfill leachate in the future. In addition, more attention should be paid to the dynamic changes of microbial strains to improve the efficiency of nitrogen removal from the perspective of microorganisms.

Table 1. The efficiency of nitrogen removal and dominant microbial strains in different processes and reactors.

| Types of Reactors | Sources and Basic Characteristics of Leachate | Efficiency of Nitrogen Removal | Dominant Microbial Strains | References |
|------------------|---------------------------------------------|--------------------------------|---------------------------|------------|
| Partial nitrification and integrated fermentation–denitrification process (PNIFD) | Liulitun municipal solid waste sanitation landfill site (Beijing, China) TN = 2023 ± 75 mg/L, COD = 2109 ± 200 mg/L, pH = 8.0 ± 0.2 | Total nitrogen (TN) removal efficiency of 95.0%, average nitrogen removal rate (NRR) of 0.63 kg/m$^3$d | Anaerolineaceae, Acidimicrobiaceae, Thauera | [29] |
| A combined process consisting of ex situ nitrification and in situ denitrification | Tianziling Landfill, Hangzhou, China, TN = 889–2100 mg/L, COD = 2980–11,800 mg/L, pH = 7.04–8.23 | Maximum total oxidizing nitrogen removal rate of 67.2 g N t$^{-1}$ TS$_{wasted}$ | Azoarcus tolulyticus | [30] |
| A modified sequencing batch reactor (SBR) operated at the anaerobic–aerobic–anoxic mode | Liulitun Municipal Solid Waste (MSW) sanitation landfill site (Beijing, China), NH$_4^+$–N = 1200–2000 mg N/L, COD = 1000–6000 mg/L, pH = 7.8–8.2 | Total nitrogen (TN) removal above 98% | Ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), GAOs | [31] |
| A novel biological system coupling a UASB and an SBR using anaerobic–aerobic process | Liulitun municipal landfill site in Beijing, China, TN = 838–2564 mg/L, COD = 1237–13,500 mg/L, pH = 7.1–8.5 | Total nitrogen (TN) removal efficiency of 99.5%, Ammonia (NH$_4^+$-N) removal efficiency of 99.1% | Ammonia-oxidizing bacteria (AOB) dominant, nitrite-oxidizing bacteria (NOB) | [32] |
| A system combined ASBR with pulsed SBR (PSBR) using anaerobic–aerobic process | Liulitun Landfill Leachate Treatment Plant (Beijing, China), TN = 1278–1578 mg/L, COD = 7341–10,488 mg/L, pH = 7.7–8.2 | Total nitrogen (TN) removal rate of 97.03–98.87% | phosphate accumulating organisms (PAOs), glycogen accumulating organisms (GAOs) | [33] |
| A continuous-flow combined process based on partial nitrification-Anammox and partial denitrification-Anammox (PN/A + PD/A) | A sanitation landfill site which had been operated for 20 years, TN = 1021–1049 mg/L, COD = 2231–2448 mg/L, pH = 8.0–8.6 | Total nitrogen (TN) removal rate of 98.8% | Candidatus Brocadia, Candidatus Kuenenia, Candidatus Jettenia | [34] |
Table 1. Cont.

| Types of Reactors                                                                 | Sources and Basic Characteristics of Leachate                                                                 | Efficiency of Nitrogen Removal                                                                 | Dominant Microbial Strains                                                                 | References |
|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------|
| Simultaneous partial nitrification, Anammox and denitrification (SNAD) with intermittent aeration | Liulitun municipal solid waste sanitation landfill site (Beijing, China), TN = 2300 ± 75 mg/L, COD = 1900 ± 200 mg/L, pH = 8.0 ± 0.2 | Ammonia conversion efficiency of 99.3 ± 0.3%, total nitrogen (TN) removal efficiency of 99 ± 0.1% | Aerobic ammonia-oxidizing bacteria (AOB), anaerobic ammonia-oxidizing bacteria (AnAOB)      | [35]       |
| A combined continuous-flow process of nitritation and Anammox                     | Liulitun Municipal Solid Waste (MSW) sanitation landfill site (Beijing, China), TN = 1387–1684 mg N/L, COD = 2193–2540 mg/L, pH = 8.1–8.5 | Total nitrogen (TN) removal efficiency of 94%                                                   | Aerobic ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB)                  | [13]       |
| Long-term performance of a two-stage partial nitritation (PN)–anaerobic ammonium oxidation (ANAMMOX) process | A landfill (jiangmen, China), NH$_4^+$ – N = 1040 ± 322 mg/L, COD = 1818 ± 188 mg/L, pH = 8.84 ± 0.38       | High-rate nitrogen removal over 4 kg N/m$^3$/d was observed in the ANAMMOX reactor in the first three months. However, during long-term operation, the ANAMMOX reactor can only stably operate under nitrogen load of 1 kg N/m$^3$/d, with 85 ± 1% of nitrogen removal. | In the PN-SBR ammonium-oxidizing bacteria (AOB) affiliated to Nitrosomonas sp. IWT514, Nitrosomonas eutropha, Nitrosomonas eutropha; In the ANAMMOX reactor anaerobic ammonium-oxidizing bacteria (AnAOB) affiliated to Kuenenia stuttgartiensis | [36]       |
| The combined processes of pre-denitrification, highly efficient partial nitritation in a ZBAF and Anammox | A municipal solid waste landfill plant located in Zengcheng, Guangdong Province, China, NH$_4^+$ – N = 1000–1250 mg/L, COD = 1200–2000 mg/L, pH = 8.20–8.80 | Nitrogen removal efficiencies (NRE) of 90.0%, nitrogen removal rates (NRR) of 0.490 kg (m$^3$ day)$^{-1}$ | At the phylum level Bacteroidetes (36.4%), Proteobacteria (31.5%), Chloroflexi (11.2%); In the Anammox reactor Proteobacteria (37.9%), Chloroflexi (20.1%), Planctomycetes (16.6%), Brocadiae (11.2%, Candidatus Kuenenia, Candidatus Nitrosooglobus, Candidatus jettania and Candidatus brocadia); At the class level Gammaproteobacteria (USB 25.6% and ZBAF 51.4%); At the genus level Paracoccus, Comamonas | [37]       |
| Simultaneous partial nitrification, Anammox and denitrification (SNAD) process   | Changshengqiao MSW landfill (Chongqing, China), TN = 2045 ± 23 mg/L, COD = 1027 ± 14 mg/L, pH = 8.4 ± 0.3  | Ammonia (NH$_4^+$–N) removal efficiency of 98.9–99.9%, total nitrogen (TN) removal efficiency of 90.7–94.9% | ammonium-oxidizing bacteria, Anammox bacteria, phyta Chloroflexi, Chlorobi, genera Nitrosoconus, Ignavibacterium, Aminiphilus | [38]       |
| Types of Reactors                                                                 | Sources and Basic Characteristics of Leachate                                                                 | Efficiency of Nitrogen Removal | Dominant Microbial Strains                                                                 | References |
|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------|------------------------------------------------------------------------------------------|------------|
| A pilot-scale low dissolved oxygen (DO) composite biological system (LDOCBS)      | Wuhan Landfill Treatment Engineering Project (located in Wuhan city, China), TN = 414.67 mg/L, COD = 2078.22 mg/L, pH = 8.2 | Ammonia (NH$_4^+$-N) removal efficiency of 99.92%, total nitrogen (TN) removal efficiency of 84.06% | At the phylum level, Proteobacteria 58.83–68.11%, Bacteroidetes 14.24–34.06%, Deinococcus-Thermus and Verrucomicrobia achieved NH$_4^+$-N removal by nitrification Acidobacteria, Chloroflexi and Actinobacteria achieved TN removal by denitrification. At the class level, Betaproteobacteria (Thauera, Azorarcus, Nitrosonomonas), Gammaproteobacteria, Sphingobacteria; At the genus level Thauera (16.40–24.02%), Arminonas (10.53–15.96%), Azorarcus (4.98–11.97%), Hydrogenophaga (3.41–6.29%). Denitrifying bacteria, pseudomonas (1.68–2.55%), Ottowia (0.76–1.66%), Thiapsudomonas (0.66–1.54%) | [39]       |
| A lab-scale two-stage Anammox system using a sequencing biofilm batch reactor    | Liulitun Municipal Solid Waste (MSW) sanitation landfill site (Beijing, China), NH$_4^+$-N = 3000 ± 100 mg/L, COD = 3000 ± 1000 mg/L, pH = 8.0 ± 0.2 | Ammonia (NH$_4^+$-N) removal efficiency of 95% | Anammox bacteria heterotrophic bacteria                                                 | [40]       |
| A novel partial nitrification-Anammox biofilm reactor (PNABR) operated under high dissolved oxygen (DO) with pre-anoxic-aerobic-anoxic operational mode | Liulitun Municipal Solid Waste (MSW) sanitation landfill site (Beijing, China), NH$_4^+$-N = 1600 ± 20 mg/L, COD = 2000 ± 100 mg/L, pH = 8.0 ± 0.2 | Nitrogen removal rate (NRR) of 396.6 g N/m$^3$/d, nitrogen removal efficiency (NRE) of 96.1% | Ammonia oxidation bacteria (AOB) (including Nitrosonomonas), nitrite-oxidizing bacteria (NOB) (including Nitrospira), Anammox bacteria (including Candidatus Kuenenia belonging to family Brocadiaceae) | [41]       |
| A membrane bioreactor (MBR)                                                      | Anthemounta landfill site (Northern Greece), TN = 1000–1200 mg/L, COD = 2970 mg/L, pH = 8.21 ± 0.22          | MBR unit resulted in NH$_4^+$-N removal efficiencies of 86.7%, 97.0% and 97.6% during bioreactor operation with 50%, 75% and 100% v/v landfill leachate, respectively. | TM7 (candidate phylum Saccharibacteria)                                               | [42]       |
| Dynamic membrane bioreactor (DMBR)                                               | A non-hazardous, municipal solid waste (MSW) landfill situated in northern Italy, TN = 1760–1780 mg/L, COD = 1461–1916 mg/L, pH = 7.56–8.66 | Ammonia (NH$_4^+$-N) removal efficiency of more than 98%, total nitrogen (TN) removal efficiency of 90% | Candidatus, Burkholderia genus, Betaproteobacteria (Thauera, Nitrosononas europaea, Alicycliphilus) | [43]       |
Table 1. Cont.

| Types of Reactors | Sources and Basic Characteristics of Leachate | Efficiency of Nitrogen Removal | Dominant Microbial Strains References |
|-------------------|----------------------------------------------|---------------------------------|---------------------------------------|
| An aerobic moving bed biofilm reactor (MBBR) | A waste incineration plant, located in Henan Province, China, 
NH$_4^+$ – N = 2100 mg/L, 
COD = 4000–7000 mg/L, 
pH = 7.8–8.2 | Ammonia (NH$_4^+$-N) average removal efficiency of 97% | Proteobacteria, Bacteroides, Firmicutes [44] |
| An aged refuse bioreactor | A landfill cell of Shanghai Laogang sanitary landfill treatment plant, 
TN = 1900 ± 151 mg/L, 
COD = 2326 ± 370 mg/L, 
pH = 7.9 ± 0.3 | Total nitrogen (TN) removal efficiency of more than 90% under the nitrogen loading rate (NLR) of 0.74 g/kg (vs) d | Proteobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Gemmatimonadetes [45] |
| An aged refuse bioreactor | Shanghai Laogang waste disposal plant, 
TN = 540–1100 mg/L, 
COD = 2326 ± 370 mg/L, 
pH = 7.9 ± 0.3 | Ammonia (NH$_4^+$-N) average removal efficiency of 89% | Planctomycetes Anammox bacteria [46] |

2.2. Biological Nitrogen Removal Methods Combined with Ecology

2.2.1. Algae-Bacteria Combined System

It is also a promising treatment method to use microalgae to treat landfill leachate [49,50], which has good nutrient recovery capabilities and additional benefits of bio-lipid production [51]. Microalgae are single-celled photosynthetic microorganisms living in marine or freshwater ecosystems. They can convert solar energy, nutrients and carbon dioxide into biomass and biofuels [52]. Various studies have also shown that the cultivation of microalgae may have high potential as a commercial and environment-friendly energy source [53–55]. At the same time, microalgae can absorb a large amount of inorganic nitrogen, such as ammonium [54], which can reduce the nitrogen content in pollutants; therefore, it can be used to remove nitrogen from landfill leachate [50,56,57]. Wu et al. introduced anaerobic digestion as the ammonization pretreatment of the leachate from food waste before the subsequent microalgae treatment. The concentrations of COD and TN in this leachate from food waste are about 12,000 and 552 mg/L, respectively, and the pH value is 5.53, meaning it is a kind of young leachate. It significantly improved the performance of the subsequent microalgae treatment compared with the single microalgae treatment. D. tertiolecta could remove 98.99% of total nitrogen and 65% of total phosphorus, while C. aponinum could remove 80% of total nitrogen and more than 65% of total phosphorus [58]. Nguyen et al. also found that leachate can be treated at an appropriate dilution with simultaneous electricity generation in algae cathode MFC [59]. Previous studies have shown that removal of ammonia nitrogen by microalgal assimilation can remove 65–85% of nitrogen from leachate during growth and deposition of organic nitrogen in a stabilizer tank [60]. In addition, compared with the single treatment of microalgae, the coexistence of microalgae and bacteria in the photo-bioreactor can obviously improve the efficiency of nitrogen removal of landfill leachate [55,56,61]. Tighiri and Erkurt et al. evaluated the efficiency of biological treatment of landfill leachate, combined microalgae with bacteria and observed that the symbiotic relationship between microalgae and bacteria in the leachate did not restrict the growth of the culture. In both batches, the concentrations of COD and TN in leachate are about 10,000 and 3800 mg/L, respectively; ammonia nitrogen was completely removed from the leachate; and the second batch removed nitrate over 90% [61]. Zhao et al. also found that the combination of microalgae and bacteria was effective in the treatment of landfill leachate. The removal efficiency of ammonia nitrogen and total nitrogen in landfill leachate was up to 95% and 90% under the culture condi-
tion with the total nitrogen concentration of 221.6 mg/L and the peak ratio of leachate of 10% [56]. Chang et al. used a membrane photo-bioreactor (M-PBR) to increase the concentration of microalgae biomass from 0.66 in the traditional photo-bioreactor (T-PBR) to 0.95 g/L. M-PBR was significantly higher than T-PBR in terms of the recovery efficiency of nitrogen and phosphorus [51]. Sniffen et al. studied a remediation system for the removal of nitrogen from a young landfill leachate in which COD concentration is more than 10,000 mg/L by a mixed algae–bacteria culture. The results showed that the maximum efficiency of nitrogen removal was 9.18 mg N/(L day), and the maximum biomass density was 480 mg biomass/L. The efficiency of ammonia nitrogen removal increased with the increase in initial ammonia concentration. The maximum nitrogen removal occurred when the ammonia concentration was 80 mg N-NH$_3$/L [55]. Xu et al. used cultures of *Chlorella vulgaris*, *Scenedesmus obliquus*, *Spirulina platensis*, and aerobic activated sludge in raw municipal wastewater, whose results showed that the highest total nitrogen removal efficiency was 2.34 d$^{-1}$ during summer and autumn with aeration [62]. Sniffen et al. found the accumulation of nitrite and nitrate within the algal–bacterial leachate treatment system was attributed to high relative abundances of ammonia- and nitrite-oxidizing bacteria [63]. What is more, studies have shown that the high diversity of microalgae has higher nitrogen removal efficiency from landfill leachate compared with single culture [64]. Martins et al. showed that the mixed microalgae strains dominated by chlamydia had a nitrogen removal efficiency of 75–99% in the treatment of landfill leachate [60]. Martins et al. screened five kinds of microalgae mixed with natural algae to treat landfill leachate, and the removal efficiency of ammonia nitrogen was 99.9% [65].

In summary, it would be ideal to use algae as the main biological component of the young landfill leachate treatment system, because the natural growth of algae requires nitrogen and phosphorus. Besides, ammonia is the preferred nitrogen source for many types of algae, and the concentration of COD is high in some young landfill leachate. The use of existing waste sources, such as landfill leachate, as the growth medium for algae, not only reduces the current costs of disposing of these wastes in landfills but also facilitates the development of the algal biomass production industries.

2.2.2. Constructed Wetlands

The cost of using physical, chemical and biological methods to treat landfill leachate is high, while land or natural systems are of low price in developing countries. They have certain economic benefits and are another important way to treat landfill leachate, such as waste stabilization ponds and constructed wetlands [66]. Among them, constructed wetlands (CWs) are engineering systems that use wetland vegetation, soil and related microorganisms to treat nitrogen-rich wastewater. The mechanism of nitrogen removal in constructed wetlands includes volatilization, nitrification, denitrification, anaerobic ammonia oxidation, plant absorption, nitrogen fixation and substrate adsorption [67]. Sawittayothin and Polprasert et al. showed that a constructed wetland unit with a hydraulic retention time of 8 d had the highest efficiency in treating high nitrogen and high bacterial content of sanitary landfill leachate (COD = 1820–4100 mg/L, TKN = 140–1260 mg/L) under tropical conditions (temperature is about 30 °C), and the TN removal efficiency could reach 96%. In addition, 88% of the total nitrogen input was absorbed by plant biomass based on the mass balance analysis of the total nitrogen content, plant biomass, dissolved oxygen and redox potential value [21]. Tanveer Saeed et al. co-treated landfill leachate and municipal by a three parallel two-stage *Phragmites*– or *Vetiver*-based constructed wetland mesocosms and found that second-stage wetland mesocosms achieved higher nitrogen removal (85–92%) [68]. Their research identified the factors associated with organics and nutrients removal performance based on four hybrid wetland systems dosed with landfill leachate, whose nitrogen removal percentage ranged between 50% and 93% [69]. The research of Bialowiec showed that the final total nitrogen, nitrate and chemical oxygen demand of reed ponds and willow ponds were significantly lower than those of unplanted ponds on the nitrogen removal of landfill leachate from reed and willow
constructed wetlands. These might be achieved through plant absorption and the effects of increased oxygen and organic carbon levels in the rhizosphere soil on nitrification and denitrification [70]. However, Ye et al. reviewed that the nitrogen removal efficiency of the constructed wetland treatment system based on ANAMMOX was lower and more unstable than that of the conventional nitrogen removal process because of its inhibitory effect of organic matter and high-strength nitrogen on Anammox bacteria. Therefore, the constructed wetland treatment system based on ANAMMOX could be used to treat the low-intensity wastewater from the traditional leachate treatment process [71].

2.2.3. Aquatic Plant

Aquatic plants (helophytes), such as bryophytes, can absorb inorganic compounds to meet their own nutritional needs [72,73]; therefore, they can be used in all kinds of wastewater treatment systems. Among them, high-quality DOM is released mainly through the leachate of plant leaf litter [74,75], which enhances the removal of nitrogen and promotes the heterotrophic activity of the microbial combinations [76]. Ribot et al. tested the effects of iris and reed leaf litter leachate on the structure and activity of freshwater biofilm grown in flumes fed by effluent from a wastewater treatment plant (WWTP) and found that all DOM sources significantly enhanced the aerobic respiration and denitrification of biofilm, and increased the total abundance of microorganisms, thus improving the absorption of N and C by microorganisms [77]. Therefore, many Helophytes can be included in the bioengineering treatment of landfill leachate system to increase the cooperative nitrogen removal pathway.

2.3. Biological Nitrogen Removal in Conjunction with Other Solid Organic Wastes

According to the reactor processes reviewed above, the combined processes of nitrification, denitrification and ANAMMOX are commonly used to treat mature landfill leachate. However, the low COD/TN ratio (C/N < 3) strongly inhibits the denitrification process due to the severe lack of organic carbon sources as electron donors [78]. Therefore, the addition of external carbon sources is generally considered as a necessary condition for effective nitrogen removal [79]. However, traditional carbon sources, such as methanol [80], glycerol [80,81], peptone [82] and so on [83–87], are too costly, which limits their feasibility [88]. At present, organic solid wastes such as activated sludge [89,90] and food waste [91–93] can be regarded as a promising carbon source due to their high organic matter content. Yan et al. fermented the de-oiled food waste and the oily food waste and applied it as an external carbon source in the aerobic/anoxic membrane bioreactor for biological nitrogen removal of old landfill leachate. Finally, the effluent could meet the general emission standards of total nitrogen and ammonia nitrogen in China and the stricter emission standards in some special areas [94]. Kaczorek and Ledakowicz et al. used sodium acetate as external carbon source in a double-sludge sequential intermittent reactor system to remove nitrogen from landfill leachate, which the concentrations of COD and TN in leachate are about 2480–4850 mg/L and 1950–2450 mg/L, respectively and the pH value is 7.5–7.86. This reactor system could remove 99% of inorganic nitrogen compounds [95]. Zhang et al. added activated sludge to the old landfill leachate and developed an innovative process (PN-SBR and IFD-SBR) to improve the nitrogen removal capacity of the mature landfill leachate with low carbon/nitrogen (1:1). Finally, it reduced the nitrogen content in the landfill leachate. Moreover, the total nitrogen removal efficiency was 95.0%, and the average nitrogen removal rate (NRR) was 0.63 kg/m$^3$ d in the final operation stage [29]. Xie et al. also found the technical viability of a bioreactor packed with a mixture of slag and aged refuse at a v/v ratio of 2:1 for landfill leachate pollutant removal [96]. In general, it is beneficial for nitrogen removal and can also save costs to add activated sludge, food waste and other solid organic waste as external carbon sources in the old landfill leachate treatment process. However, in order to avoid introducing a large amount of nitrogen into the treatment system to increase its burden, organic solid wastes with high C/N ratio should be selected as far as possible since these organic wastes also...
contain nitrogen. For example, acid-producing liquid derived from activated sludge [97] or food waste [85] can be used as an external carbon source to accelerate the denitrification process [84,85]. In addition, it is relatively easy to produce high-quality carbon sources for denitrification and the cycle time is short by controlling only the hydrolysis and acidification of anaerobic fermentation [98]. Tang et al. used fermentation liquid of food waste (FLFW) as an external carbon source to treat domestic wastewater with low COD/TN ratio in a pilot-scale anoxic-/oxic-membrane bioreactor (A/O-MBR) system. The results showed that the total nitrogen removal increased from less than 20% to 44–67% over 150 days of operation with the addition of FLFW [99].

2.4. Microbiological Processes for Direct Nitrogen Removal of Leachate

Nitrogen removal of landfill leachate is inseparable from the role of microorganisms including aerobic ammonia-oxidizing bacteria (AOB), anaerobic ammonium oxidation bacteria (AnAOB), nitrite-oxidizing bacteria (NOB), heterotrophic denitrifying bacteria and so on. Some processes improve the efficiency of nitrogen removal by inoculation with some strong denitrifying bacteria. Some processes can be used to find and screen out some efficient nitrogen-free bacteria. Other processes use bacteria-loaded materials to improve nitrogen removal performance. Several microbiological processes for direct nitrogen removal of leachate are shown below.

2.4.1. To Find, Screen and Isolate Highly Effective Bacteria from the Process

Generally, nitrification and denitrification are used to remove nitrogen from leachate. However, the nitrogen removal efficiency is limited by their different conditions. The discovery of heterotrophic nitrification-aerobic denitrification (HN-AD) effectively solved the above problems. Acinetobacter tandoii MZ-5, which is capable of HN-AD, was isolated from the sediment of a polluted river for the first time. It used NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N as sole nitrogen sources with maximum removal rates of 2.28, 1.18 and 1.04 mg L$^{-1}$ h$^{-1}$, respectively. Simultaneous nitrification and denitrification were observed when using mixed N sources, and NH$_4^+$-N was preferentially utilized. High nitrogen removal efficiencies (>90%) were achieved under the following conditions: C/N ratio 11–18, pH 6–8, 25–30 $^\circ$C and dissolved oxygen 7.35–7.66 mg L$^{-1}$ [20]. Three novel strains capable of heterotrophic nitrification-aerobic denitrification were isolated from the landfill leachate treatment system, which were Agrobacterium sp. LAD9, Achromobacter sp. GAD3 and Comamonas sp. GAD4. The maximum aerobic nitrification-denitrification rate was achieved by the strain GAD4 followed by LAD9 and GAD3 [100]. Chen and Ni et al. also newly isolated Agrobacterium sp. LAD9 with heterotrophic nitrification-aerobic denitrification capability for nitrogen removal [101]. In addition, other bacteria can be screened to improve the efficiency of nitrogen removal. Yang et al. screened out AOB with efficient and stable ammonia-nitrogen removal capability through various methods [102]. Feng et al. isolated mixed Marine bacterial for ammonia-removal nitrogen from Marine sediments [103]. Wei found that Proteobacteria, Chloroflexi, Acidobacteria and Firmicutes were the dominant phyla in the Anammox-denitrification biomass [104]. Therefore, efficient bacteria can be screened and isolated from the processes, and their nitrogen removal performance can be enhanced through domestication, which is more conducive to the improvement of nitrogen removal efficiency.

2.4.2. To Cultivate and Inoculate Efficient Bacteria

Cultivation or direct inoculation of bacteria with high nitrogen removal capacity in the reactor is also an approach to improve the system’s nitrogen removal capacity. Isaka et al. cultured nitrifying bacteria with high nitrification rate in a polyethylene glycol PEG gel carrier at 10 $^\circ$C for more than 1 year, and Nitrosomonas sp. was the dominant ammonium-oxidizing bacteria in the gel carrier at low temperature [105]. Dadrasnia developed a novel resident strain microbe that can survive in high ammonia nitrogen concentrations. Bacillus salmalaya strain 139SI, which removed 78% of ammonia nitrogen when the concentrations
of \(\text{NH}_4^+\)-N in leachate, are about 2000 mg/L [106]. He et al. nitrified the landfill leachate under the conditions of pH 7.5–8.5 and DO 0.5–2.0 mg/L in a continuous stirred tank reactor (CSTR) by inoculating the ammonia-oxidizing bacteria (AOB) AHAA-4, and the ammonia removal was over 95% [107].

2.4.3. Microbial and Photocatalytic Processes

The use of photocatalysts to improve the biodegradability and treatability of leachate has attracted much attention in recent years. Hu et al. proposed to use immobilized \textit{Phanerochaete chrysosporium} loaded with nitrogen-doped TiO\(_2\) nanoparticles to treat raw landfill leachate with a very low biodegradability ratio (BOD\(_5\)/COD) of 0.09. The most superior removal efficiencies of \(\text{NH}_4^+\)-N of landfill leachate were over 74% in 72 h at an initial COD concentration of 200 mg L\(^{-1}\) [108]. Hassan et al. used advanced oxidation processes (AOPs) by heterogeneous photocatalysis (TiO\(_2\)/UV) and persulfate (S\(_2\)O\(_8^{2-}\)) oxidation to test the effectiveness of anoxic aged refuse-based bioreactor (ARB) for biological leachate pretreatment, which degraded 81% and 92% \(\text{NH}_4^+\)-N and TN, respectively [109]. Yasmin et al. found a new landfill leachate pre-treatment by photocatalytic TiO\(_2\)/Ag nanocomposite prior to fermentation using \textit{Candida tropicalis} strain, and the degradation rate of \(\text{NH}_4^+\)-N achieved 75% when used to treat young leachate with COD and \(\text{NH}_4^+\)-N concentrations of about 24,000 and 346 mg/L and pH of 7.3, respectively [110]. However, the efficiency of photocatalytic processes combined with microorganisms is generally not high compared to biological nitrogen removal processes.

2.4.4. Microbial Fuel Cell

Microbial fuel cells are devices that use microorganisms as catalysts to convert chemical energy in compounds into electrical energy. Microbial fuel cells are also a newly developed method for nitrogen removal from leachate in recent years. The high concentration of organic carbon, nitrogen and sulfur in landfill leachate limited its treatment. Therefore, the possibility of converting leachate into a new energy source by microbial fuel cells is still inadequate [111]. Cai et al. studied the supercapacitor microbial fuel cell, whose bioanode was dominated by salt-tolerant denitrifying bacteria (38.5%), and 78.2% of ammonia nitrogen was removed [112]. Hassan et al. utilized different landfill leachate substrate to remove pollutants. In total, 66.0 ± 3.3% \(\text{NH}_4^+\)-N and 86.0 ± 0.1% NO\(_2^-\)-N were removed [113]. In general, although the nitrogen removal efficiency of microbial fuel cells is not high at present, it still has demonstrated the potential for wastewater treatment along with energy harvesting and provides a new avenue toward sustainable leachate management. In future studies, the focus can be on improving the nitrogen removal performance.

3. Mechanism of Nitrogen Removal and Factors of Microbial Activity on Nitrogen Removal Efficiency

The biological mechanism of nitrogen removal is the joint action of ammoniation, nitrification, denitrification and anammoxidation. (Figure 1) Real-time PCR studies revealed that landfill leachate harbored diverse nitrogen-convertin microbial communities that include ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, Anammox bacteria, ammonia-oxidizing archaea (AOA) and so on [114]. The activities of these microbial populations had a significant effect on the efficiency of nitrogen removal. Therefore, the efficiency of nitrogen removal can be enhanced by improving the activity of related microorganisms. Several factors influencing microbial activity are listed below, including the concentration of nitrite, free ammonia (FA), free nitrous acid (FNA) in leachate, carbon source in leachate, microbial activity, contact time between microorganism and leachate; temperature, salinity, dissolved oxygen (DO), biodegradable carbon and oxygen content, electric potential (EP) and so on.
3.1. Influences of Content of Nitrite, Free Ammonia (Fa) and Free Nitrous Acid (Fna) in Leachate

Both Anammox bacteria and heterotrophic denitrifying bacteria require the consumption of nitrites. Excessive nitrite content in the inlet water of ANAMMOX would accumulate in the reactor, and a lower nitrite supply could force a loss of specific Anammox bacteria activity due to the nitrite competition with denitrifying bacteria [115]. Spagni et al. thought that the high concentration of free ammonia (FA) in the leachate might inhibit microbial activity [116]. Tian et al. found that AOB (ammonium oxidation bacteria) had a great ability to adapt to free ammonia and nitrous acid concentration, whereas NOB (nitrite-oxidizing bacteria) were inhibited by either FA or FNA concentration to a certain extent [117].

3.2. Influences of Carbon Source in Leachate

The nitrogen removal process in biological denitrification requires carbon sources, but the effect of different types of carbon source on the nitrogen removal performance of landfill leachate is unclear. Glycogen accumulating organisms (GAOs) capable of storing organic compounds as poly-hydroxyalkanoate (PHA) have been used for endogenous denitrification (ED). With acetate, propionate and glucose examined as the carbon sources, their effects on yields and compositions of PHA produced by GAOs were determined and associated with nitrogen removal performance. In addition, Proteobacteria was the most abundant phylum. Among the 108 genera detected in this ED system, the genera responsible for denitrification were *Thauera*, *Paracoccus*, *Ottowia* and *Comamonadaceae_unclassification*, accounting for 46.21% of total bacteria. Especially, *Paracoccus* and *Comamonadaceae_unclassification* transformed the carbon source into PHA for denitrification, and carried out endogenous denitrification [83]. A novel system coupling an anaerobic sequencing batch reactor (ASBR) and sequencing batch reactor (SBR) was proposed without the addition of external carbon sources. The removal efficiency of total nitrogen (TN) was above 95% [118]. Du et al. studied the sequencing batch reactors (SBRs) with different carbon sources for partial-denitrification: acetate (R1) and ethanol (R2) and found *Thauera* genera were dominant in both SBRs. Different Anammox species were detected with *Candidatus Brocadia* and *Candidatus Kuenenia* in R1 and only *Candidatus Kuenenia* in R2 [119]. In general, the addition of carbon sources and different carbon sources has a significant impact on the dominant strains of microorganisms in the nitrogen removal process. Therefore, the influence of external carbon sources and their types should be considered in the subsequent process selection.

3.3. Influences of Microbial Activity, Contact Time between Microorganism and Leachate

Recent studies have shown that other processes of anaerobic ammonia-oxidizing bacteria and ammonia-oxidizing archaea (AOA) also play a role in nitrogen removal of biological systems, the dominant bacteria was *Kuenenia stuttgartiensis* genome fragment KUST_E and the uncultured Crenarchaeota clone NJYPZT_C1, respectively [120]. The experimental results of Panter showed that the removal efficiency of nitrate and nitrite was highest when the contact time was 16–24 h, and the degradation efficiency was very low when the contact time was below 8 h [121].
3.4. Influences of Salinity, Temperature, Dissolved Oxygen (DO), Biodegradable Carbon and Oxygen Content, Electric Potential (EP) and So on

Increased salinity might decrease microbial activity, resulting in the increase in N₂O production. However, the system would gradually adapt to the environment of high salinity under the impact of high salinity, and high salinity enhanced the effect of high ammonia nitrogen concentration and low DO concentration on N₂O yield. The relative proportion of *Nitrosomonas europaea* increased with the increase in salinity, and thus the efficiency of nitrogen removal was improved [122]. Temperature affected AOB activity during the treatment of landfill leachate with high nitrogen concentration by partial nitrification SBR process, which was achieved by inhibition of free ammonia (FA) and free nitrite (FNA) [123]. The ammonia nitrogen removal efficiency was positively correlated with DO concentration, and TN removal efficiency reached over 90% at the optimal DO concentration of 2.7 mg/L in the single-stage nitrogen removal using Anammox and partial nitrification (SNAP) system [124]. The diversity of microbial community in the landfill bioreactor would be significantly affected with the increase in biodegradable carbon and oxygen content, increasing the internal denitrification capacity of the whole system from 50% to 70%. Redundancy analysis (RDA) suggested that only biodegradable carbon content was the determinant of total nitrogen removal efficiency, although both oxygen and biodegradable carbon affected the microbial community structure [125]. Electric potential (EP) could also promote the removal of organic matter in the ANAMMOX process, and the removal efficiency of total nitrogen reached 71.9% at the optimal EP of 0.06 V. High-throughput DNA sequencing revealed that Anammox bacteria in the genus *Candidatus Kuenenia* were enriched for on electrodes with the applied EP. Heterotrophic denitrifying bacteria had the potential to degrade organic macromolecules, which were more abundant on the electrodes with EP compared with the control reactor [126].

In conclusion, the microbial activity in the nitrogen removal process is affected by various factors. Therefore, factors such as the composition of leachate, the nature of microorganisms and environmental conditions should be taken into account comprehensively to improve the activity of microorganisms so as to achieve efficient nitrogen removal when selecting the technology of nitrogen removal of landfill leachate.

4. Conclusions

At present, if the age of the landfill is more than 5 years, even 10 years, the high-efficiency process of nitrogen removal is mainly combined with ANAMMOX for this intermediate or old leachate with a very low biodegradability ratio (BOD₅/COD) of less than 0.3, because it can better solve the problem of carbon source shortage in the process of denitrification. For example, partial nitrification, ANAMMOX, and denitrification (SNAD) process achieved a nitrogen removal efficiency of 98.9–99.9%. Therefore, the combined process based on ANAMMOX has a great prospect in biological treatment of landfill leachate in the future. On the other hand, if the age of the landfill is less than 5 years, for this young leachate with a high biodegradability ratio (BOD₅/COD) of more than 0.3 and COD concentration more than 10,000 mg/L, microbial fuel cells or constructed wetlands can be used to remove nitrogen in leachate through microbial transformation and ecological transformation. In addition, in order to improve the nitrogen removal efficiency from the microbial perspective, it is necessary to pay attention to the dynamic changes of the microbial community and create appropriate conditions of salinity, temperature, dissolved oxygen (DO), biodegradable carbon and oxygen content, electric potential (EP) and so on. At the same time, the technology combined with ecology is also an efficient nitrogen removal method, in which the algal–bacterial combined system has an ammonia nitrogen removal efficiency of 95%. From the point of view of the direct application of microbial for nitrogen removal, the efficiency of nitrogen removal entirely dependent on microbial is not high (about 80%), because there are too many influencing factors. Considering the economic feasibility of microbial treatment, the subsequent research can focus on the screening of
efficient bacteria or bacterial modification, and selecting economical and efficient nitrogen removal technology according to the properties of leachate in practical projects.

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