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Original Research

Nitrogen Converters in Various Landfill Leachates

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Abstract

Nitrogen has significant adverse effects on the environment and leads to operational failure in biological treatment units. Therefore, it is necessary to investigate and implement new nitrogen conversion pathways in order to expand alternatives for *in-situ/ex-situ* leachate treatment systems. In this study, microbial species responsible for nitrogen conversion were quantitatively investigated based on both phylogenetic and functional gene markers using real-time PCR in nine different leachate samples in Turkey. Real-time PCR studies revealed that landfill leachate harbored diverse nitrogen-converting microbial communities that include ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, anammox bacteria, and ammonia-oxidizing archaea (AOA). *Nitrosomonas* and *Nitrospira* species were found to be the dominant ammonia- and nitrite-oxidizing bacteria, respectively. In contrast to the estimates, on average *Nitrospira* species were detected as 5 times more abundant than ammonia-oxidizing bacteria species. The presence of anammox and AOA revealed that partial nitrogen removal may occur inside landfills.

Keywords: ammonia-oxidizing archaea, anammox, landfill, leachate, nitrogen converter

Introduction

Landfilling is an effective solid waste disposal method in developing countries. Although it is a relatively cheap and simple way to eliminate waste, the treatment of leachate with high pollutant concentrations is a serious issue. Nitrogen elimination is one of the main problems of leachate treatment, especially in Turkey. High nitrogen concentrations of up to 5,000 mg L^{-1} cause operational failure in anaerobic and aerobic biological treatment units [1]. Treating and managing leachate has been an ongoing challenge for landfill designers all over the world. Different factors, such as landfill age, meteorological factors, and seasonal variations may influence the organic matter and nitrogen concentrations in landfill leachate. Organic matter and nitrogen concentrations significantly vary, showing highest concentrations in summer because of evaporation, and lower concentrations in winter because of dilution with precipitation. Apart from this variation, organic matter decomposes while nitrogen concentration increases over time in the landfill. In order to design and operate a leachate treatment plant efficiently, scientists have to analyze seasonal and long-term variations in landfill leachate.

The removal of nitrogen compounds in leachate is of high priority to eliminate its inhibitory effects on treatment processes. In order to remove nitrogen from leachate, several *ex-situ* technologies have been

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developed such as biological processes (nitrification/denitrification or Sharon/Anammox) [2-3], air stripping [4], struvite precipitation [5], adsorption by activated carbon [6], zeolite [7], and composite materials [8]. In recent years, *in-situ* removal of nitrogen has become an attractive alternative technology in bioreactor landfill studies [9-11]. Researchers have studied *in-situ* (forced bottom aeration-recirculation) or partially *in-situ* denitrification (*ex-situ* nitrification-recirculation) at the laboratory and full-scale studies [11-13]. Since the ex-situ removal of ammonia from high-strength leachate can be difficult and costly [11], *in-situ* removal technologies are being investigated. However, the mechanisms of *in-situ* nitrogen removal in an aerated municipal solid waste (MSW) bioreactor are not yet understood. Hao [14] observed that the amount of ammonium consumed is much higher than that of the nitrite and nitrate produced in an MSW bioreactor. High temperature and pH within the landfill sites may favor the formation of free ammonia, hence air stripping may be the dominant pathway of ammonia removal.

New developments related to microbial tools have explored new pathways and players in the global nitrogen cycle [15-18]. The recent discovery of new players, anoxic ammonium oxidizer (anammox) has upended our prejudice that nitrogen can only be eliminated in the presence of oxygen. Anammox bacteria were first discovered in a denitrifying pilot plant reactor in Delft. The process involves the oxidation of ammonium nitrogen to nitrogen gas, with nitrite nitrogen as the electron acceptor under an anoxic environment [17]. After the discovery of anammox, AOA has also been identified as playing a key role in the global nitrogen cycle. Moreover, some scientists have indicated that the abundance of ammonia-oxidizing archaea is more numerically dominant than ammonia-oxidizing bacteria in some environments [15-16]. In recent years, researchers have shown that members of the nitrite-oxidizer genus *Nitrospira* are capable of complete nitrification and grow by the oxidation of ammonia to nitrate in a single organism [18]. Therefore, these new mechanisms in landfill sites and the microbial communities responsible for these processes have to be exposed in order to highlight alternative in/*ex-situ* leachate treatment systems.

Determining new players in the nitrogen cycle is also important in terms of bioaugmentation practices. It has been reported that bioaugmentation can be used in *in-situ* leachate treatment, which may enhance ammonia removal – especially at high concentrations [19]. By understanding the nitrogen removal mechanisms in landfills, the effectiveness of bioaugmentation can be increased.

Microbial population monitoring with the use of molecular tools can account for the nitrogen removal mechanisms in landfills. Until now, however, only limited data have been published on *in-situ* removal of ammonia in bioreactor landfills by using molecular microbiological studies [10, 20]. The presence of sensitive nitrogen converters AOB, NOB, AOA, and anammox bacteria were investigated in landfill leachate samples taken directly from 8 different cities in Turkey. To the best of our knowledge, this would be the first study to evaluate microorganisms taking part in the nitrogen cycle in samples taken from a landfill.

Material and Methods

Landfill Sites

Leachate samples from several solid waste landfills in Turkey (Istanbul Komurcuoda and Odayeri, Ankara, Antalya, Bursa, Gaziantep, Samsun and Trabzon) were collected to investigate the abundance of microorganisms taking part in the nitrogen cycle. In addition to molecular microbiological analysis, physical and chemical characterizations of these samples were conducted in leachate samples. These cities are located in different regions of Turkey and exhibit different climatic conditions, populations, and regional properties. Additionally, the composition of solid wastes and landfill structures are different.

Odayeri and Kömürcüoda sanitary landfills are located in the European and Asian sides of Istanbul, respectively. Although leachate samples were taken from both active and closed sites in Odayeri Landfill, Kömürcüoda leachate sample was taken from the active section of the landfill. Bursa Hamitler Sanitary Landfill is located in the southern Marmara Region, which consists of five different storage areas. Although the landfill site is 15 years old, the leachate sample was taken from the equalization tank that combines active and old landfill leachate. Ankara Sanitary Landfill is composed of 2 storage areas where municipal solid wastes and salt industry wastes are stored separately. The leachate sample was taken from the municipal waste storage area. Ankara is a new landfill and leachate is not treated; leachate is recirculated back to the landfill body. The sanitary landfill site of Antalya has been accepting solid waste since 2003 and it is composed of 7 storage areas. The leachate sample was taken from the active storage area. Gaziantep is the largest city in Turkey's southeastern Anatolia Region. Gaziantep sanitary landfill site has been accepting solid waste since 1996. Since it is composed of only one storage cell, the landfill site is still active. Leachate is recirculated to the landfill body and an excess amount is transported to the municipal wastewater treatment plant. Trabzon and Samsun are cities on the Black Sea coast of northeastern and northern Turkey and have been accepting solid waste since 2007 and 2008, respectively.

Physical and Chemical Properties of Leachate Samples

Leachate samples were kept at 4ºC until analyzed in order to prevent changes in the physical and chemical

Table 1. Measurement methods for the analyses conducted to characterize leachate samples.

Analyses	Measurement Method
pH	4500-H^+ B. Electrometric Method
Total Alkalinity	2320 B. Titration Method
Chemical Oxygen Demand	5220 D. Closed Reflux Colorimetric Method
Biochemical Oxygen Demand	5210 D. Respirometric Method
Ammonium Nitrogen	4500 C. Titrimetric Method
Total Kjeldahl Nitrogen	4500-N_{org} Nitrogen- Semi Micro-Kjeldahl Method
Total Solids	2540 B. Total Solids Dried at 103-105ºC
Total Suspended Solids	2540 D. Total Suspended Solids Dried at 103-105ºC
Volatile Suspended Solids	2540 E. Fixed and Volatile Solids Ignited at 550ºC
Total Organic Carbon	5310 B. High Temperature Combustion Method

parameters. The analytical methods were conducted according to [2]. Experimental analyses and methods of measurement are listed in Table 1.

Molecular Microbiological Studies

For molecular analysis, a single sample was analyzed for each landfill area. Microorganisms in landfill leachate samples were harvested by centrifuging at 7,000 rpm for 30 min. Concentrated samples were stored in a freezer at -20ºC prior to molecular analyses. Nucleic acid extraction was performed according to the protocol provided with the FastDNA SPIN kit (Q-BIOgene) with minor modifications [21]. Final DNA concentrations were quantified according to the protocol provided with the Quant-iT PicoGreen dsDNA reagent kit (molecular probes) with minor modifications. The PicoGreen reagent concentrated solution in DMSO was prepared by 80-fold dilution (instead of 200-fold) in 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Extracted DNA samples diluted in 10 µl of TE buffer were mixed with 10 µl PicoGreen working solution to reach a final volume. Measurements were calculated as a result of triplicate analysis.

Real-time PCR was performed using the LightCycler Instrument (Roche, Germany) with the FastStart DNA Master SYBR Green I kit (Roche) following the manufacturer's protocol. The quantitative PCR program included an initial denaturation for 10 min at 95ºC, followed by 40 cycles of denaturation at 95ºC for 10 s, annealing for 10 s at 57ºC, and extension for 45 s at 72ºC. The temperature transition rate was 20ºC s^{-1} at all steps. SYBR Green real-time PCR reaction mixture consisted of 2.0 µL, 10x Mastermix; 2.0 µL, 25 mM $MgCl_2$; 1.25 µM of each primer; 2 µL, template DNA (with a dilution factor of 10 to prevent PCR inhibition), and up to 20 µL of double distilled pure water. Six sets of primers – 341f-907r for partial 16S rRNA bacterial gene, amoA1F-amoA2R for *amoA* ammonia oxidizing bacterial gene, FGPS872-FGPS1269 for 16S rRNA *Nitrobacter* sp., NSR1113F-NSR1264R for 16S rRNA *Nitrospira sp.*, Arch-amoAF-Arch-amoAR for *amoA* ammonia oxidizing archaeal gene, and PLA46f-Amx667r for anammox bacteria – were used to amplify a target sequence from 9 different leachate samples [22]. Potassium chloride with a final concentration of 18 mM was added to real-time PCR mixtures to provide the correct ionic strength targeting the 16S rRNA gene of *Nitrobacter* [23]. In all experiments, appropriate negative controls containing no template DNA were subjected to the same procedure to exclude or detect any possible contamination. Real-time PCR results were evaluated using the LightCycler data analysis software (version 4.0). Melting curve analysis was performed with a programmed temperature transition rate of 0.1ºC s^{-1} from 65ºC to 95ºC to confirm specificity of amplification and minimize nonspecific amplification and rule out primer-dimer generation.

Real-time PCR quantification is accomplished by constructing an external DNA standard curve using a series of 10-fold dilutions of known copy numbers of target genes. PCR fragments excised from agarose gel (0.8%) were purified using a MinElute Gel Extraction Kit (Qiagen). Quant-iT PicoGreen dsDNA reagents and kit were used to determine the concentration of DNA [24]. R^2 values were always greater than 0.99 for the entire standard curves. Quantification is then done in the unknown sample by calculating from the standard curve.

Results and Discussion

Leachate Characterization

Physico-chemical analyses have shown that great variations in terms of both composition and characteristics existed among the leachate samples. Physico-chemical characterization of leachate samples is depicted in Table 2.

Table 2. Physical and chemical characterisation of samples.

	Antalya	Ankara	Bursa	Gaziantep	İstanbul Odayeri-1	İstanbul Odayeri-2	İstanbul Kömürcüoda	Samsun	Trabzon
pH	6.5	8.6	7.9	8.2	7.4	8.2	7.7	7.4	7.5
Conductivity, µS/cm	33800	23500	30300	38500	34200	34900	27700	17800	12880
Total Alkalinity, mg/ L as $CaCO_3$	14850	3000	12400	15500	13950	14850	10100	10500	4800
Total Suspended Solids, mg/L	4800	633	138	1810	2840	529	3440	2120	1220
Volatile Suspended Solids, mg/ L	3360	340	135	1190	1160	329	2100	1150	760
Total Solids, mg/ L	47056	16840	15200	28500	34496	18128	24200	24410	8590
Chemical Oxygen Demand, mg/ L	69018	1480	4048	17846	40600	11289	28716	28554	6665
5 Day-Biochemical Oxygen Demand, mg/ L	37394	140	1380	9913	27000	4500	17230	15611	3415
Total Organic Carbon, mg/ L	22094	623	1120	8678	13612	4575	10774	8466	3031
Total Kjeldahl Nitrogen, mg N/L	3642	163	2920	3900	3445	4230	3200	2836	1038
Ammonia nitrogen, mg N/L	2220	91	2280	3550	2740	3460	2560	2400	920
BOD_5/COD	0.54	0.09	0.34	0.56	0.67	0.40	0.60	0.55	0.51

In general, the stabilization phase of landfill is mostly determined by considering BOD_5, COD, pH, and BOD_5/COD ratio. In literature, COD and BOD_5 in acid phase leachates were mostly reported to be between 6,000 to 60,000 mg L^{-1} and 4,000 to 40,000 mg L^{-1}, respectively [25]. These parameters dramatically change through the progression of waste stabilization [26]. In previous studies, BOD_5/COD ratio in acid phase was shown to vary between 0.4 [27] and 0.6 [1, 28], with such high ratios implying high biodegradability. However, the BOD_5/COD ratio can decrease even down to 0.1 [27] in response to the aging of the landfill. Another indicator of the stabilization phase, pH, could vary between 4.5 and 7.5 in the case of acid phase, and 7.5 and 9.0 in the case of methanogenic phase [25]. In light of the above information, it is concluded that Antalya, Gaziantep, İstanbul Odayeri-1, İstanbul Kömürcüoda, and Samsun landfills are in acid phase. Bursa Landfill seems to be in methanogenic phase with low organic content since the sample was taken from the equalization tank, which combines the old and young landfill lots. Because Trabzon Landfill is located in the rainiest region in Turkey, the pollutant loads were mostly found to be low. However, BOD_5/COD ratio and pH of leachate samples indicated that Trabzon Landfill is in the acid-intermediate phase. İstanbul Odayeri-2 Landfill is in the intermediate-methanogenic phase with high pH and relatively low organic content. During the time of sampling, Ankara Landfill was a newly constructed landfill and a small amount of produced leachate was transferred back to the landfill storage lot. Therefore, the chemical characteristics suit neither the acid nor methanogenic phases. At least 2,000 mg L^{-1} of TKN concentrations were observed in all landfill sites except Ankara and Trabzon. It is interesting to note that in Gaziantep and Istanbul, TKN concentrations exceed 4,000 mg L^{-1}, and such high concentrations are seldom found in literature [29-30]. High ammonia concentrations are expected to occur in MSW landfills as a result of nitrogen-rich food waste or climatic conditions. High nitrogen concentrations and their difficulty of treatment at high efficiencies take nitrogen to focus interest in leachate management. Therefore, it is necessary to find economical solutions for the *in-situ* treatment of nitrogen in landfill leachate. In order to find such solutions, it is essential that all possible microbial-driven processes/mechanisms taking part in the nitrogen cycle be unravelled, and at the same time, abundance and diversity of the players in the nitrogen cycle be determined in leachate.

Identifying Nitrogen Converters

From the start-up to the stabilization of the landfill, various microorganisms play active roles in the system

Table 3. Quantitative analyses of 16S rRNA, *amoA* AOB, 16S rRNA *Nitrobacter*, 16S rRNA *Nitrospira*, *amoA* AOA, and 16S rRNA anammox bacteria by real-time PCR.

Samples	Gene copy number					
	16S rRNA	*amoA AOB*	*Nitrobacter*	*Nitrospira*	*amoA AOA*	Anammox
Antalya	2.88×10^7	9.44×10^4	1.93×10^3	2.78×10^5	2.64×10^3	2.36×10^4
Ankara	1.03×10^7	6.98×10^4	8.24×10^2	1.88×10^5	2.05×10^3	5.08×10^3
Bursa	3.18×10^7	2.31×10^5	3.52×10^3	7.97×10^5	2.83×10^3	2.13×10^4
Gaziantep	6.16×10^6	3.68×10^4	4.01×10^2	8.79×10^4	5.74×10^2	7.66×10^3
İstanbul Odayeri-1	5.00×10^7	2.70×10^5	1.18×10^4	1.15×10^6	8.06×10^3	1.88×10^4
İstanbul Odayeri-2	1.39×10^7	1.02×10^5	9.85×10^3	1.94×10^5	2.83×10^3	7.46×10^3
İstanbul Kömürcüoda	6.03×10^7	3.38×10^5	5.63×10^3	9.57×10^5	7.45×10^3	5.14×10^4
Samsun	6.28×10^6	2.15×10^4	2.82×10^2	4.60×10^4	4.03×10^2	3.68×10^3
Trabzon	2.11×10^7	1.15×10^5	1.87×10^3	2.89×10^5	2.47×10^3	6.14×10^3

while decomposing organic matter in anaerobic conditions. The composition of biogas and leachate differs due to the change of microbial activities during the landfill stabilization period. Molecular microbiological techniques have largely contributed to our understanding of the diversity and distribution of these microorganisms in the environment. Therefore, in recent years the operational criteria of landfills are investigated not only through leachate characterization and gas composition but also with the description of the diversity of microorganisms in the system [10, 31-32].

Target gene copy numbers and the fraction of nitrogen-converting organisms among whole microorganisms belonging to 9 leachate samples were estimated by copy numbers of corresponding genes using real-time PCR and are shown in Tables 3 and 4, respectively. Copy numbers and fractions for all samples analysed under the current study were calculated using the following assumption: bacterial cell has an average of 3.6 ribosomal operon copies per cell, *amoA* gene has 2 ribosomal operon copies per cell, and *Nitrospira*, AOA, and anammox cells have an average of one ribosomal operon copies per cell [33]. Total bacterial numbers were enumerated using 16S rRNA gene copy numbers as proxy. Among the 9 leachate samples that were studied, the 16S rRNA copy numbers consistently ranged from 6.16×10^6 (for Gaziantep) to 6.03×10^7 (for İstanbul Kömürcüoda) per µL of extracted DNA. Microorganisms responsible for nitrification were quantified using *amoA* gene in ammonia-oxidizing bacteria (AOB) and *amoA* gene in archaea (AOA). The results showed that the bacterial *amoA* copy numbers ranged from 2.15×10^4 (Samsun) to 3.92×10^5 (İstanbul Kömürcüoda) per µL of extracted DNA, which approximately outnumbered nearly 30 times more with respect to archaeal *amoA*. Low abundances of archaeal *amoA* can be attributed to high ammonium nitrogen concentrations in all leachate samples analyzed. There is a general agreement among researchers that archaeal *amoA* gene abundance correlated negatively to the

Table 4. Fraction of microorganisms participating in nitrogen conversion in different landfills with respect to bacterial 16S rRNA gene

Samples	*amoA* AOB/ 16S rRNA	*Nitrobacter*/ 16S rRNA	*Nitrospira*/ 16S rRNA	*amoA* AOA/ 16S rRNA	*Anammox*/ 16S rRNA
Antalya	0.59%	0.02%	3.47%	0.03%	0.29%
Ankara	1.22%	0.03%	6.59%	0.07%	0.18%
Bursa	1.31%	0.04%	9.02%	0.03%	0.24%
Gaziantep	1.07%	0.02%	5.14%	0.03%	0.45%
İstanbul Odayeri-1	0.97%	0.09%	8.28%	0.06%	0.14%
İstanbul Odayeri-2	1.32%	0.26%	5.04%	0.07%	0.19%
İstanbul Kömürcüoda	1.01%	0.03%	5.71%	0.04%	0.31%
Samsun	0.62%	0.02%	2.64%	0.02%	0.21%
Trabzon	0.98%	0.03%	4.94%	0.04%	0.10%

ammonium concentrations in natural and engineering systems. This is mainly because of the lower affinity of AOA toward nitrogen compared to AOB species. The AOA half-saturation constant (K_s) for ammonia is reported to be one to three orders of magnitude lower than that of AOB species by various researchers [34-37]. This means that only at low ammonium nitrogen levels can AOA be dominant, which explains the low abundances of AOA in all analyzed samples.

The copy numbers of the 16S rRNA gene of anammox bacteria in 9 leachate samples ranged from 3.68×10^3 (Samsun) to 5.14×10^4 (İstanbul Kömürcüoda) per µL of extracted DNA (Table 3). The fraction of anammox bacteria in analysed leachate samples varied between 0.10% (Trabzon) and 0.45% (Gaziantep; Table 4). The low fraction of anammox bacteria may suggest that they are not the main players in any of the studied landfills. However, the detection of the 16S rRNA gene of anammox bacteria indicates that although in low abundances, a fraction of anammox bacteria could still survive despite the presence of such high organic matter concentrations.

The percentage of *amoA* gene from ammonium-oxidizing bacteria in the total amount of microorganisms ranged from 0.59% to 1.32% in samples. The presence of the *amoA* gene in leachate samples can be taken as a measure of the genetic potential for nitrification [38]. In this study, there was a correlation between the fraction of AOB among all microorganisms and BOD_5/TKN ratio of leachate. The Pearson's product moment correlation coefficient (r_p) was used as a measure of correlation in which the strength as well as direction of such a relationship is quantified. High BOD_5/TKN ratios seemed to decrease the fraction of AOB population in the samples tested. The overall Pearson coefficient of -0.84 suggested that a moderate correlation exists between the BOD_5/TKN ratio and fraction of *amoA* gene (determined at 95% confidence interval). On the other hand, there was a complete absence of correlation between the TKN concentration and *amoA* gene fraction (r_p = -0,10), which indicates that the higher organic strength of leachate allows the heterotrophic bacteria to become dominant and does not allow for too much AOB proliferation. The effect of landfill age (which is indicated by BOD_5/COD ratios) on the *amoA* fraction was not as apparent as changes in the BOD_5/TKN ratio, as no significant correlation was found between BOD_5/COD ratios and *amoA* fractions (r_p = -0,55). The only noticeable correlation is such that, at higher BOD_5/COD ratios (which indicate young landfill leachate) the fraction of ammonia-oxidizing bacteria is much lower (in the Antalya and Samsun samples).

Among the nitrite-oxidizing bacteria, *Nitrospira* gene copy numbers were consistently higher than those of *Nitrobacter* in all cases. *Nitrobacter* only accounted for 0.02% to 0.26% of the bacterial communities. The quantification results indicated that *Nitrospira* was the dominant NOB in all leachate samples. Consistent with previous studies [22, 39-40], the percentage of *Nitrospira* biomass among all tested nitrogen converters was detected as the highest fraction. In the leachate sample taken from Bursa, this fraction was found to be 9.02%, and such a high value means that *Nitrospira* is approximately 8 times more abundant with respect to average *amoA* fractions.

The literature includes limited studies attempting to understand the microbial transformations taking part in the nitrogen cycle in engineered and natural systems. The abundance of known contributors such as AOB and NOB, and new players AOA and anammox were quantitatively analyzed at different environments using real-time PCR [22-23, 39-40]. In our previous studies, fractions of AOB, NOB, AOA, and anammox among the total community were investigated in 3 full-scale wastewater treatment plants [39], in a pilot-scale membrane bioreactor (MBR) [40], and in a full-scale leachate treatment plant [22] using molecular microbiological tools like fluorescent *in-situ* hybridization (FISH), slot-blot hybridization, quantitative real-time PCR, and sequence analysis. These findings showed that *Nitrosomonas* and *Nitrospira* species were the driving ammonia- and nitrite-oxidizing bacteria, respectively. However, *Nitrospira* species were detected as approximately 5 times more abundant than AOB species in all samples. In parallel to our case, there are some studies in the literature trying to figure out why the nitrite-oxidizing bacteria outnumbered the ammonia-oxidizing bacteria [22, 32, 39-42]. For instance, Gieseke [41] used FISH to quantify nitrifying populations in a phosphate-removing biofilm and found that the abundance of the NOB population was detected as more than one order of magnitude higher than that of AOB. Similar to our study, Yao and Peng [43] investigated nitrification activities and microbial populations of ammonium-oxidizing bacteria and nitrite-oxidizing bacteria in 10 full-scale biological nutrient removal wastewater treatment plants in Xi'an, China. They found that the average percentage of AOB was 1.27% and that of NOB was 4.02%. Using our previous experience with full-scale leachate and domestic wastewater treatment plants, we speculated that the high abundance of *Nitrospira* species may be due to alternative roles that they may have other than nitrite oxidation only [22, 33, 39-40]. The recent discovery of complete ammonia oxidizer (Comammox) organisms [18, 44-47], i.e., bacteria within the genus *Nitrospira,* which oxidize ammonia to nitrate completely in one step, can be an answer to how such a high abundance is supported in natural and engineered systems. Li [46] investigated the pathways of nitrogen (N) removal and N_2O emission in a one-stage autotrophic nitrogen removal process under anaerobic conditions. They concluded that nitrogen converters were the dominant microbes, and were probably responsible for nitrogen removal under anaerobic conditions. Martinez [47] investigated the recent discovery of *Nitrospira* strains that can conduct complete ammonium oxidation. The phylogenetic

analyses conducted in this study showed that just a few of the *Nitrospira* sequences found in the bioreactors were comammox. The higher abundance of the NOB population in previous works may point to the prominent contribution of Comammox in nitrate formation. However, further studies are needed to unveil to what extent these bacteria participate in nitrogen conversion inside landfills.

Conclusions

The molecular microbiological examinations using real-time PCR revealed that landfill leachate harboured microbial communities participating in nitrogen conversion, including ammonia- and nitrite-oxidizing bacteria, ammonia-oxidizing archaea, and anammox bacteria. *Nitrospira* species were found to be present up to 9.02% among all microorganisms, which is the highest fraction in leachate samples tested. *Nitrospira* species are believed to have more roles than only nitrite oxidation. The presence of anammox bacteria and ammonia-oxidizing archaea revealed that partial nitrogen removal may occur inside the landfills. Therefore, the bio-augmentation potential of anammox may be promising for future studies when leachate is partially nitrified *ex-situ* in the case of mature landfills. The low percentage of AOA compared to the AOB suggested that bacteria rather than archaea drive nitrogen conversion in leachate samples. In this study, the effects of denitrification and dissimilatory nitrate reduction mechanisms were not taken into consideration.

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