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Review

A Critical Review on the Microbial Ecology of Landfill Leachate Treatment Systems

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Abstract: Sanitary landfilling is still considered worldwide as one of the most common methods applied for the management of the municipal solid waste. As a consequence, vast amounts of landfill leachate are generated annually, which are characterized by variability in physicochemical composition, owing to the stabilization process that occurs over the years. However, sustainable management of landfill leachate is a challenging issue, due to diverse chemical composition and high concentration in heavy metals and xenobiotics. Despite the fact that several studies have been reported on the biotreatment of landfill leachate, only in recent years has the microbial composition in such systems have been examined. In the present review, the key role of the microbial ecology involved in depurification and detoxification of landfill leachate in activated sludge and anaerobic systems is interpreted and ecological considerations influencing landfill leachate treatment are stated. Apart from the assessment of landfill toxicity on certain model organisms, this work provides an extensive overview on microbial communities performing key biological processes during landfill leachate treatment, including nitrification-denitrification, anammox and anaerobic digestion. Moreover, microbial aspects affecting nutrient removal efficiency in such biosystems are discussed.

Keywords: landfill leachate generation; leachate pollution impact; toxicity of leachate; activated sludge population; microbial ecology of landfill leachate

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1. Introduction

1.1. Municipal Solid Waste Composition (MSW)

An overview of recent surveys on the composition of waste products in various countries across the globe gives an idea of how they are represented, mostly by fractions of putrescible organics (residues of the culinary processing), as well as paper and plastic (mainly packaging). Organic waste (putrescible, yard waste, paper and plastic) in most of the countries account for 80% of the total waste produced (Table 1).

Table 1. MSW composition in various countries, as % on wet basis.

Country	Putrescible Organic (%)	Paper (%)	Glass (%)	Metal (%)	Plastic (%)	Miscellaneous Combustible (%)	Textile (%)	Special/ Hazardous Waste (%)	Fines (%)	References
Argentina	53.8	7.5	6.6	1.2	9.8	-	-	-	-	[1]
Canada	51.7	6.6	2.8	6.8	15.0	4.0	-	13	-	[2]
China	42.8–50.8	4.0–11.6	1.9–3.5	0.5–1.7	6.7–13.7	1.2–5.9	1.9–4.2	0.3–3.7	19.2	[3]
Denmark	42.2	15.8	2.1	2.3	12.6	17.6	3.3	0.7	-	[4]
Finland	24.2	15.5	2.5	3.8	21.6	20.1	10.5	1.7	-	[5]
India	43.0	6.0	2.0	5.0	6.0	11.5	5.0	3.0	-	[6]
Italy	22	20	8	3.7	13.4	-	3.3	7.0 *	-	[7]
Malaysia	27.9–42.9	24.0–30.8	0.4–1.4	0.7–1.4	15.8–25.2	1.1–1.9	3.0–4.1	0.4–1.1	-	[8]
Norway	25.8	31.5	5.3	5.5	13.2	11.8	3.8	-	-	[9]
Russia	24.2	20.0	12.5	0.6	18.7	1.9	2.0	0.6	11.2	[10]
South Africa	21.3	16.2	7.8	6.7	22.6	6.4	0.2	19.1	-	[11]
Spain	79.5–82.4	2.2–3.1	0.4–2.1	0.2–0.3	4.5–6.0	-	0.02–0.3	-	7.5–8.4	[12]
USA	21.6	23.1	4.2	8.8	12.2	6.2	5.8	12.1 *	-	[13]

* yard trimmings.

1.2. Municipal Solid Waste Management Approaches

The chemical composition of urban waste includes various heavy metals and other toxic substances [14]. During uncontrolled disposal and dumping, such pollutants can disperse in aquatic and terrestrial environments, affecting surface and ground waters [15], soil [16] and atmosphere [17], while they intrude in some cases into the human food chain [18]. Many vital functions are compromised or altered by heavy metals, which permanently bind to enzymes, inhibiting their activity and causing health issues [19].

That is why waste needs to be managed properly with a consistent and coherent strategy. People should not be left to manage waste on their own, because they are affected by public opinion syndromes: NIMBY (not in my backyard), NIMO (not in my office time) and BANANA (build absolutely nothing anywhere near anybody) [20].

The European Directive 2008/98/EC [21] focuses on minimizing the negative effects of waste production on the environment and human health. Some operations and techniques should be preferred to others, because of their environmental impact; for example, waste disposal in landfills is a major cause of greenhouse gas (GHG) emissions into the atmosphere (more than 20% of GHG emissions globally, about 30% in the US) [22–24], while recycling of waste can decrease natural sources exploitation. In this frame, processes should be evaluated for sustainability reasons, meaning economic advantage, small environmental impacts and social endurance. Since the application of the directive in the EU Member states, there has been a general decline in the disposal of landfilled waste (from 63% to 25%), and of putrescible waste [25]. In past decades in the EU, the amount of produced waste followed an upward trend, while the amount of landfilled waste was limited. Interestingly, 121 million tonnes (286 kg per capita) were landfilled in 1995, whereas the respective amount was only 53 million tonnes in 2019, corresponding to an average annual decrease of 3.3%. In the EU, the percentage of MSW directed to material recycling, incineration, landfilling and composting is 30%, 27%, 24% and 17%, respectively [25]. In the United States of America (U.S.A.), the proportion of municipal waste landfilled is double compared to that in the EU. The percentage of MSW in the U.S.A. directed to material recycling, incineration, landfilling and composting is 24%, 12%, 50% and 8%, respectively [13]. According to Ding et al. [26], in China, a country producing more than 10% of the urban solid wastes globally, 52% of the 228 million tonnes of MSWs are landfilled, 45% are incinerated and 3% are composted.

1.3. Sanitary Landfilling

Sanitary landfilling is considered the most common method for the management of the waste in the world, with the majority of collected waste being disposed of in landfills [27]. The minimization of the impact on public health and the environment takes place in sanitary landfilling using engineered barriers that prevent the dispersion of pollutants contained in waste and favor the decomposition of organic matter through the appropriate amount of water [28]. Barriers need to be of containment purposes, such as bottom liners and capping for draining purposes and the use of coarse medium and the pipes. However, the presence of water cannot be avoided in the bulk, due to initial moisture and rain percolating through the capping [28].

As landfill matures, a succession of phases is observed [29]. Shortly after MSW is disposed of, organic substances undergo multistep biochemical degradation. The oxygen that is initially entrapped within solids allows a limited aerobic degradation of the organic matter. This process is considered quite brief as the penetration of oxygen is restricted and rapid consumption occurs as a consequence of the high organic load inside the cell. The exothermic reactions that take place result in an increase in the temperature within the cell, which also leads to an increase in the solubility of salts, thus increasing the electrical conductivity of the landfill leachate.

During the stabilization process, leachate is produced, and methane is released, which poses a negative effect on the environment. After the hydrolysis of the polysaccharides, mainly hemicelluloses and celluloses, which are the main constituents of the organic fraction of solid waste (45–60% of organic matter), the decomposition process is carried out by fermentative bacteria, which further converts mono- and di-saccharides into volatile fatty acids (VFAs) and low molecular weight (M.W.) alcohols. In a secondary fermentation process named acetogenesis, volatile fatty acids (VFAs) and low M.W. alcohols are converted to acetate in synergy with methanogenic archaea, otherwise such conversion is non-thermodynamically favored [30,31]. Thus, a syntrophic relationship with the hydrogenotrophic methanogenic archaea is required to achieve acetogenesis. Finally, methane production takes place either by the action of acetoclastic methanogens *Methanosaeta* and *Methanosarcina*, or through the reduction in carbon dioxide in the presence of hydrogen by hydrogenotrophic methanogens.

After a few decades, methanogenesis is limited due to the restricted degradation of organic matter and landfill emissions are reduced.

Even though several studies have focused on the biotreatment of landfill leachate, the microbial composition in these landfill leachate treatment systems have been just recently uncovered. Thus, this systematic review aims to provide a comprehensive understanding of microbial communities ruling biological processes during landfill leachate treatment and to identify how microbial community structure is linked with the nutrient removal efficiency of these biosystems.

2. Methodology

A keyword survey was conducted by formulating and developing a search statement. Following identification of the main concepts of the research topic, multiple keyword searches were performed through exploration of the words "ecology", "landfill leachate", "diversity" and "landfill leachate", as well as "microbial communities" and "landfill leachate". From an initial search of 806 scientific publications in Scopus under the above-reported keyword searches, 122 references were selected to be included in the systematic literature review. Then, the article was structured into four sections, where Section 1 was an introductory to landfilling and landfill leachate generation and treatment; Section 2 reported the conducted methodology; Section 3 referred to landfill leachate toxicity; Section 4 provided a comprehensive evaluation of the microbial communities in the activated sludge of landfill leachate treatment systems and Section 5 stated the conclusions of this systematic study.

3. Toxicity of Landfill Leachate

3.1. Comparative Evaluation of Landfill Leachate Toxicity

One critical aspect of landfills is leachate production, not only during operation, but also during the post-closure period which may last decades or even centuries [32]. Toxicity of the leachate constitutes another challenging issue of this wastewater (Table 2).

Table 2. Toxicity of landfill leachate at LC50 level.

Test Organism	pH	NH_4^+ -N (mg/L)	Initial COD (% *v*/*v* Landfill Leachate)	Test Time (h)	References
Artemia salina	8.04	381	12,161 mg/L COD	-	[33]
Artemia salina	8.00	-	3324 mg/L COD (20% *v*/*v*)	-	[34]
Daphnia magna	-	1955	17,988 mg/L COD (12.5% *v*/*v*)	48	[35]
Macrobrachium lanchesteri	8.30	1693	3583 mg/L COD (1–7.5 % *v*/*v*)	96	[36]
Mytilus sp. (mussels)	8.15	1526	4925 mg/L (0.53% *v*/*v*)	96	[37]
Danio rerio (zebrafish)	7.95	2700	5123 mg/L COD (1.4% *v*/*v*)	96	[38]
Danio rerio embryos, *D. rerio* larvae *	6.55 ± 0.34	394 ± 24	1400 mg/L COD	96	[39]
Oreochromis mossambicus	7.35	43	51,200 mg/L COD (3.2% *v*/*v*)	96	[40]
Oreochromis niloticus	8.80		57.69 mg/L	96	[41]
Pangasius sutchi S., 1878 *Clarias batrachus* L., 1758	8.20	880	10,234 mg/L COD (3.2 & 5.9% *v*/*v*)	96	[42]
Poecilia reticulata	6.12–8.31	952–1078	8880–66,420 mg/L COD (1.22 & 12.35% *v*/*v*)	96	[43]
Poecilia reticulata	7.90	283	1500 mg/L COD (47% *v*/*v*)	96	[44]
Rasbora sumatrana	8.30	1693	3583 mg/L COD (0.82–1.39% *v*/*v*)	24	[36]
Eisenia andrei	7.80	2398	2.32 & 1.34 µL/ cm^2 paper 77.83 & 53.94 mL/kg soil	48/72 168/336	[45]
Senna macranthera seeds	9.00	595	6.25% *v*/*v* (5592 mg/L COD)	168	[46]

* 84.75 & 82.64% *v*/*v* of population died.

Landfill leachates may contain important amounts of heavy metals, such as manganese (Mn), zinc (Zn), nickel (Ni), copper (Cu), chromium (Cr), arsenic (As), lead (Pb), cadmium (Cd) or mercury (Hg), as a result of the disposition of metal-containing wastes into sanitary landfills. During maturation, the solubility of metals, like Zn, Mn and Mg, decreases significantly (Table 3), due to both pH and alkalinity increase. Moreover, the presence of significant organic content in all stages of landfill operation leads to the formation of metal-organic complexes in leachate, further hindering its sustainable management [47–49].

Table 3. Age of leachate and changes in the concentration of heavy metals in the landfill leachate.

Parameter (mg/L)	BOD/COD > 0.3	References	0.15 < BOD/COD < 0.3	References	BOD/COD < 0.15	References
Zn	0.16–8.00	[50–53]	0.05–7.51	[54]	0.09–2.78	[55–59]
Cd	ND–0.57	[51–53,60]	0.06–0.54	[54,61–63]	ND–0.45	[52,64]
As	0.02–0.33	[60]	0.12–0.94	[61,63]	0.02–0.38	[56,64]
Cr	ND–2.24	[50–53,60]	0.01–2.21	[61,63]	0.04–1.90	[56,58,59,64]
Co	0.07–0.30	[52]	0.1	[52]	0.01–0.04	[52,56]
Cu	0.01–2.69	[50–53,60]	0.03–0.19	[61–63]	0.03–2.94	[55–57,59]
Fe	1.17–20.6	[50–53,65]	1.20–23.2	[54,61–63]	2.82–22.9	[55–59]
Pb	0.08–0.83	[50–53,60]	0.01–0.66	[54,61–63]	0.003–0.57	[55,56,64]
Mn	0.01–18.5	[52,60]	0.01–9.70	[54,61,63]	0.06–1.09	[56,58,64]
Hg	0.01–2.4	[52,60]	0.03–1.21	[52]	0.001–0.16	[52]
Ni	0.02–4.80	[50–53]	0.33–1.20	[54,61,63]	0.03–1.45	[55–59]
Mg	32–1800	[50,51,53,66,67]	24.1–300	[65,67]	31.1–98	[55,56,58]

Environmental pollution and health risks need to be prevented by applying sustainable leachate treatment techniques in order to eliminate the content of organic and inorganic pollutants prior to their discharge into the water system. Unfortunately, their content in landfill leachate is inconstant, making difficult the selection of the appropriate design and operating procedure [68]. Öman and Junestedt [69] analysed leachates from 26 different landfills and detected around 400 substances of both organic and inorganic origins. Some of them were detected in traces, but others, like benzenes, reached a concentration of 15 mg/L. Escape of chemical substances present in leachate can occur in the food web and bioaccumulate in various aquatic species [70].

Risk assessment regarding landfill leachate may be accomplished through toxicological methods, which indicate the possible impact on aquatic organisms. Knowledge of the composition and toxicity of substances is necessary to determine any long-term adverse effects on the aquatic ecosystems [71].

Landfill leachate toxicity changes over season and climate conditions [72]. Even diluted landfill leachate is enough to induce toxic effects on a variety of aquatic model organisms, including crustaceans, mussels and fish (Table 2). The adverse effects of landfill leachate on aquatic organisms should be attributed to the high ammonium content and the presence of various toxic compounds, including a wide range of xenobiotics and heavy metals [73–75].

The toxicity of landfill leachate has been assessed using aquatic crustaceans, mussels, fishes and seeds. Interestingly, leachate toxicity (expressed as LC50) initiates even at 58 mg/L COD in the case of *Oreochromis niloticus* and expands above 3.3 g/L COD, when *Artemia salina* serves as the test organism. The resistance of this brine shrimp species could be attributed to its saline nature, a fact that permits it to overcome the relatively high salinity of landfill leachate and to tolerate the synergistic effect of high organic content and salinity (Table 2). The possible interface of ammonia concentration with the acute toxicity of landfill leachate should be also taken into consideration [76].

3.2. Landfill Leachate Toxicity on Activated Sludge Microbiota

de Albuquerque et al. [77] have reported that pretreatment with alkali and air stripping highly improved the performance of activated sludge, a fact that may be indicative of high ammonia concentration, permitting the achievement of high BOD and COD removal efficiencies. A higher sensitivity to eukaryotes compared to prokaryotes was identified by increasing the proportion of landfill leachate in the treated effluent. By increasing landfill leachate proportion in the composition of a synthetic influent, activated sludge dehydrogenase and esterase activities were found to be reduced by approximately 80%, indicating the strong inhibition of activated sludge microbiota by the presence of landfill leachate [78], which should be considered as a major drawback in the case that the biomass is not acclimatized. A shift in the microbial composition was observed, determining greater proportion of 3-hydroxy and cyclopropane fatty acids [78]. Phosphate concentration can also be a limiting factor affecting activated sludge microbiota [79]. Moreover, activated sludge microbiota appeared to produce more exopolymeric substances (EPS) as a protective mechanism against landfill leachate toxicity [80].

4. Microbial Communities for Sustainable Landfill Leachate Treatment

A few studies have been carried out in the last decade to reveal microbial composition in raw leachate and in activated sludge and anaerobic systems treating landfill leachate of various ages. Thus, this systematic overview gives an in-depth evaluation of microbial ecology of landfill leachate treatment systems, providing for the first time the key microbiota influencing biological processes during biotreatment of landfill leachate and the major factors shaping microbial community structure in these biosystems.

4.1. Microbial Communities in Raw Landfill Leachate

In particular, members of *Bacteroidales* and *Pusillimonas*-like bacteria capable of degrading recalcitrant compounds, together with anaerobic fermentative bacteria of the class *Clostridia* have been detected in the raw landfill leachate (Table 4). A significant proportion of microbial population in the untreated leachate consists of sulphate reducers, such as *Desulfobacter* spp.

Table 4. Major microbial communities identified in raw landfill leachate and stabilization ponds.

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
Raw Landfill Leachate and Stabilization Ponds						
Raw landfill leachate	Fresh, intermediate & Mature/ pyrosequencing	NA	1.21–2.32 g/L/ 1.68–2.34 g/L	NA	*Pseudomonas* (11.4%, in fresh), *Syntrophomonas* (3.4–11.4%), *Sedimentibacter* (3.4–4%), *Proteiniphilum* (3.1–7.1%), *Ureibacillus* (1.4–3.1%), *Anaerobranca* (1–2.9%), *Desulfobacter* (1.1–1.8%), *Petrimonas* (1.1–1.9%), *Clostridium* (0.8–1.8%), *Desulfotomaculum* (0.8%, in fresh), *Syntrophothermus* (0.9%, in mature)	[81]
Raw landfill leachate	Mature/ Illumina	NA	9.8 g/L/ 1,33 mg/L	NA	*Candidimonas*, *Pusillimonas*, *Leucobacter*, *Paralcaligens*, *Castellaniella*, *Eoetvosia*, *Parapusillimonas* and *Pseudomonas* accounted for more than 42% of relative abundance, with *Pusillimonas*-like bacteria representing 34.7% of the total reads	[82]
Stabilization pond (pilot scale)	Intermediate/ FISH	25–42/ (16 months)	3.2 g/L/ 0.9 g/L	56%/ 82%	***Bacteria:*** 75% of DAPI, of which 10% of DAPI are nitrifying bacteria and 25% are Sulphate-Reducing Bacteria. ***Methanogenic Archaea:*** 25% of DAPI	[83]

FISH: Fluorescence in situ hybridization; DAPI: 4′,6-diamidino-2-phenylindole; NA: Not applicable.

4.2. Microbial Communities in Biological Nitrogen Removal Sytems Treating Landfill Leachate

In anoxic-oxic bioreactors, *Thauera*, *Truepera*, *Pseudomonas*, *Paracoccus* and *Luteimonas* are commonly detected in the activated sludge of such systems, followed by *Anaerolinae* and *Hydrogenophaga* members (Table 5). These key taxa are typical denitrifiers capable of converting the nitrates formed by the nitrifiers to dinitrogen gas, contributing to the removal of the high nitrogen content of middle age and mature landfill leachate in anoxic-oxic treatment systems. Regarding nitrification, *Nitrosomonas* and *Nitrosospira* are the main ammonia-oxidizing taxa identified under aerobic conditions during the treatment of landfill leachate, whereas nitrite oxidizers of the genus *Nitrobacter* seem to proliferate in such systems (Table 5).

Due to high ammonium concentration and the low COD content of recalcitrant nature, anammox process is advantageous to remove nitrogen content without the need for external carbon source as electron donor and high energy consumption for complete nitrification. *Candidatus* Brocadia and *Candidatus* Kuenenia are the key anammox bacteria in partial nitrification/anammox bioreactor systems treating landfill leachate. Considering the conversion of ammonia to nitrite, *Nitrosomonas* spp. are the main ammonia oxidizers during partial nitrification, while common inhabitants of landfill leachate treatment systems, such as *Truepera*, *Thauera*, *Limnobacter* and *Pusillimonas*, are the major heterotrophic representatives under low-dissolved oxygen conditions (Table 6).

Typical core denitrifiers of wastewater treatment plants, such as *Thauera* and denitrifiers, specialize in the degradation of non-easily degradable compounds, including xenobiotics, present in high concentrations in landfill leachate are favored in activated sludge systems that are used to treat this effluent. *Thauera* processes the metabolic versatility to cope with a broad spectrum of organic substrates, including aromatic compounds, whereas recent findings have revealed that representatives of this genus are highly competitive in treating low carbon effluents [84], which is also the case of landfill leachate. Similarly, *Truepera* and *Pusillimonas* appear to be favored under low C/N conditions [85]. *Luteimonas* is a gammaproteobacterium capable of degrading complex polysaccharides, such as chitin and cellulosic substrate, a fact that favors its growth. Interestingly, Remmas et al. [86] reported high b-glucosidase induction as a mechanism to catabolize the least biodegradable organic fraction, due to the low organic carbon availability. *Limnobacter* spp. as thiosulphate-oxidizing bacteria are involved in the sulfur process in anoxic-oxic activated sludge systems treating landfill leachate. *Pusillimonas*-like bacteria and *Pseudomonas* species are well-known specialized microbiota with the ability to degrade a broad range of recalcitrant compounds under moderate salinity [82,87–89], a fact that favors their adaptation in landfill leachate.

In several studies, it is striking that the genus *Truepera* within the class *Deinococci*, consisting of a single species, was the dominant taxon in untreated and treated landfill leachate. This 'exotic' bacterium has been reported to be favored in environmental conditions, similar to those established in landfill leachate treatment systems, due to its ability to grow under poly-extreme conditions, including alkaline pH, moderate salinity, high radiation and temperature [90]. Indeed, the alkaline pH and moderate salinity of landfill leachate, as well as the natural weathering of landfill sites, which expose leachate to solar radiation and its metal resistance, facilitate the adaptation of this *Deinococcus*/*Thermus* member [90,91].

Table 5. Major microbial communities identified in aerobic and anoxic/oxic landfill leachate treatment systems.

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
Aerobic granular SBR (GSBR)	Fresh/ FISH	0.5 d/ NR	0.9 g/L/ 0.3 g/L	32–50%/ 66–70%	Betaproteobacterial AOB (16.6 ± 11.9%), Most *Accumulibacter* (3.3 ± 1.2%), *Anammox* (2.5 ± 0.9 %), cultured *Tetrasphaera* PAO (4.3 ± 2.1%), *Comamonadaceae* (5.6 ± 3.5%), *Acidovorax* (5.4 ± 3.6%), *Curvibacter* (4.8 ± 1.6%), *Paracoccus* (4.2 ± 2.9%), *Zooglea* not *Z. resiniphila* (4.1 ± 2.8%), *Azoarcus* (2.8 ± 0.6%), *Thauera* (2.4 ± 0.4%)	[92]
Anoxic/multi-oxic stages full-scale treatment plant	Mixture of fresh and mature leachate/Ion torrent	0.3 d/NR	5.16–25.78 g/L/ 41–2330 mg/L	NR/ >80%, as TN	*Azoarcus*, *Cellulosibacter*, *Clostridium*, *Comamonas*, *Nitrosococcus*, *Nitrosomonas*, *Nitrosospira*, *Paracoccus*, *Petrimonas*, *Pseudomonas*, *Proteiniborus*, *Syntrophomonas*, *Thauera*, *Tissierella*	[93]
A/O MBR	Intermediate to mature/ pyrosequencing	5 d/ infinite	1.5–3.0 g/L/ 708–927 mg/L	32–58%/ 41–86%	*Nitrosomonas* (*amoA*), *Nitrospira* (*amoA*), *Arenimonas* (*nirS*), *Bradyrhizobium* (*nirS*), *Sulfuritalea* (*nirS*), *Acidovorax* (*nirS*), *Thauera* (*nirS*), *Dechloromonas* (*nirS*), *Parococcus* (*nirS*)	[94]
A/O MBR	Fresh to Intermediate /Illumina	5 d/ infinite	0.90–1.60 g/L/ 300–800 mg/L	39.9–56.8%/ 81.1–83.0% (as TN)	**Bacteria:** Candidate Saccharibacteria (50% at 50 & 100% *v*/*v*), while SR1, *Sinobacteraceae*, *Truepera*, *Thauera*, *Limnobacter*, *Paracoccus*, *Phyllobacteriaceae*, *Nitrosomonas and Dokdonella* accounted for 21% at 100% *v*/*v*) **Fungi (Rozella clade):** Cryptomycota (Rozellomycota)-based community (80%) at 100% *v*/*v*	[86]
A/O MBR	Mature/ Pyrosequencing	4.2 d/ 20 d	5.0–6.9 g/L/ 2200–3035 mg/L	43–51%/ 36–38%	**Mixed liquor:** *Pseudomonas* (7.6%), *Aequorivita* (7%), *Ulvibacter* (3.4%), *Taibaiella* (4.5%), *Thermus* (3.7%), *Pusillimonas* (1.7%), *Halomonas* (0.7%) **Biofilm:** *Aequorivita* (7.3%), *Thermus* (7.3%), *Taibaiella* (7.0%), *Pseudofulvimonas* (3.2%), *Candidatus* Microthrix (3.0), *Phyllobacterium* (2.1%), *Paracoccus* (1.1%)	[95]
A/O MBR	Fresh/ PCR-DGGE	4 d/No excess sludge wastage	4.6 g/L/ 489 mg/L	99%/ 99%	**Bacteria:** *Pseudomonas* (intense band), *Bacillus* (intense band), *Sphingomonas* (intense band) *Nitrosomonas* (intense band), *Others: (Dechloromonas*, *Rastolia*, *Rhodopseudomonas*, *Nitrosococcus*, *Nitrosospira)* **Archaea:** *Methanosphaerula* (intense band), *Methanosaeta* (intense band), *Methanoregula* (intense band) *Methanosarcina* (intense band), Others (*Methanobacterium*, *Methanobolus*, *Methanococcoids*, *Methanogenium*)	[96]

Table 5. *Cont.*

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
Two-stage anoxic/oxic combined MBR	Intermediate/ Illumina	168 h/ *	4–20 g/L/ 1.5–2.1 g/L	81%/ 99%	*Nitrosomonas* (3.6–10.0%), *Nitrobacter* (6.5–9.5%), *Truepera* (2.1–6.7%), *Planctomyces* (3.1–5.6%), *Acidovorax* (0.69–5.8%), *Pseudomonas* (1.6–5.5%), *Nitrolancea* (1.6–3.3%), *Rhodobacteraceae* (1.4–2.3%), *Azospira* (0.6–1.2%), Others (*Pedosphaera*, *Thalassospira*, *Flovobacterium*)	[97]
Two-stage A/O-MBR	Intermediate to mature/Illumina	NR/14 d	5.3–17.9 g/L/ 1589–2031 mg/L	77.6–85.6%/ 99.1–99.3%	*Thiopseudomonas* (2–25%), *Amaricoccus* (6–14%), *Nitrosomonas* (3–10%), *Luteimonas* (4–7%), *Tissierella* (0.5–7%), *Ottowia* (2.5–6%), *Moheibacter* (2.5–5%), *Tepidisphaera* (2.5–5%), *Truepera* (1.5–3%), *Nitrobacter* (1–3%), *Nitrospira* (0.5–2%), Others (*Thauera*, *Paracoccus*, *Petrimonas*, *Pusillimonas*, *Halobacteriovorax*, *Brevundimonas*, *Flavobacterium*, *Geminicoccus*, *Phycisphaera*)	[98]
Anoxic tank-aerobic MBR	Mature/ Illumina	10 d/ 16–19 d	1.5–1.9 g/L/ 1.8 g/L	13.1%, as DOC/ 99.3%	*Thauera* (18.8–23.5%), *Candidatus* Nitrospira defluvii (10.0%), *Burkholderia* (4.0%), *Nitrosomonas europaea* (7.5%), *Alicycliphilus* (2.9%), *Rhodospirillum* (2.7%), *Pseudomonas* (2.6%), *Spiribacter* (2.3%), *Bordetella* (2.2%), Others (*Thiomonas*, *Nitrobacter*, *Chloracidobacteria*, *Gallionelaceae*)	[99]
A/O full-scale system combined with ultrafiltration	Mix of fresh, intermediate & mature leachate/ Illumina	NR	4.8–27.5/ 0.9–4.8 g/L	87–95%/ 95–99.9%	*Phycisphaerae*, *Flavobacteriales*, *Anaerolineaceae*, *Ignavibacteriales*, *Thauera* (0.4–5.5%), *Truepera* (0.6–2.8%), *Nitrosomonas* (2.1–3.3%), *Nitrosospira* (0.1–0.6%), dominant uncultured bacteria (~ 10%)	[100]
Landfill simulation reactor with aerobic and anoxic zones	Mature/ Illumina	13.8 L/m^3/d (HLR)/NR	3.02 g/L/2947 mg/L	77.67–80.61%/ 72–98%	*Methylocaldum* (11.61–26.94%), *Methylobacter* (0.73–4.22%), *Nitrosomonas* (0.62–2.75%), *Nitrobacter* (0.81–0.86%), *Anaerolimnae* (0.98–10.20%), Others (*Nitrospira*, *Paeniglutamicibacter*, *Planococcus*, *Pseudomonas*, *Truepera*)	[101]
Multistage biological contact oxidation reactor	Mix of synthetic and landfill leachate/Illumina	1–1.5 d/ NR	0.2–5 g/L/ 100–832 mg/L	84.26–90.51%/ 31.7–72%	**Major taxa:** *Saprospiraceae*, Family_XI, *Comamonadaceae*, *Trichococcus*, Others (*Acinetobacter*, *Anaerolinaceae*, *Arcobacter*, *Bargeyella*, *Christensenellaceae*, *Desulfobulbaceae*, *Desulfobulbus*, *Desulfomicrobiu*, *Enterococcus*, *Flavobacterium*, *Hydrogenophaga*, *Leucobacter*, *Saccharibacteria*, *Stenotrophomonas*, *Thermomonas*, NS9 marine group, vadinBC27)	[102]

Table 5. *Cont.*

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
Rotating biological contactor	Mix of fresh & mature/ pyrosequencing	7 d/ 30 d	2.1 g/L/ 0.4 g/L	89%/ 99.9%	*Thauera* (16.4–24.0%), *Arenimonas* (10.5–16.0%), *Azoarcus* (5.0–12.0%), *Hydrogenophaga* (3.4–6.3%), *Truepera* (2.4–8.2%), *Pseudomonas* (1.7–2.6%), *Thiopseudomonas* (0.7–1.7%), Others (*Anaerolineae*, *Clostridium*, *Limnobacter*, *Luteimonas*, *Ottowia*, *Persicitalea)*	[103]

* 100% sludge reflux ratio & mixed liquid reflux ratio 150%. GSBR: Granular Sequencing Batch Reactor; SBR: Sequencing Batch Reactor; FISH: Fluorescence in situ hybridization; TN: Total Nitrogen; A/O MBR: Anoxic/Oxic Membrane Bioreactor; PCR-DGGE: Polymerase Chain Reaction—Denaturing Gradient Gel Electrophoresis; HLR: Hydraulic Loading Rate; DOC: Dissolved Organic Carbon; NR: Not reported.

Table 6. Major microbial communities identified in Simultaneous Nitrification-Denitrification (SND) and ANAMMOX systems treating landfill leachate.

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
PN, simultaneous Anammox and denitrification	Mature/ Illumina	NR/35 d	1.35 g/L/ 1200 mg/L	70%/ 97%	*Thauera* (25.3%), *Truepera* (10.6%), *Limnobacter* (4.1%), *Nitrosomonas* (3.0%), *Candidatus* Brocadia (2.1%), *Candidatus* Jettenia, *Candidatus* Kuenenia	[104]
DN coupled with PN-Anammox	Mature/ Illumina	1.15–1.91 d/NR	2.5 g/L/ 2550 mg/L	<10%/ >90%	**Partial nitrification:** *Thiopseudomonas* (4–40%), *Saprospiraceae* (4–8%), *Pusillimonas* (2.5–8%), *Arenimonas* (2–4%), NS9 marine group (2–4%), Others (*Denitratisoma*, *Limnobacter*, *Nitromonadaceae*, *Nitrospira*, *Novosphingobium*, *Ottowia*, *Pusillimonas)* **Anammox:** *Candidatus* Kuenenia (5–26.0%), *Candidatus* Brocadia (0.9–3.5%), SBR1031 (5–18%), *Limnobacter* (2.5–6%), JG30-KF-CM66 (2–4%), *Nitrosomonas* (0.5–2%), *Truepera* (0.5–2%). *Others*: (*Actinomarinales*, *Arenimonas*, *Denitratisoma*, *Gemmatimonadaceae*)	[105]
Single stage attached growth anammox SBR	Mature/ Illumina	2 d/NR	0.49–0.82 g/L/ 88–159 mg/L	21–32%/ 92–97%	*Candidatus* Brocadia (7–25%), *Nitrosomonas* (8–16%), *Thauera* (0.03–9%), *Candidatus* Kuenia (0.1–8.5%), *Hyphomicrobium* (0.08–4%), *Thermomonas* (0–3%), *Arenimonas* (0–3%), *Comamonas* (0.08–2%), *Steroidobacter* (0–1%), Others (*Azospira*, *Bdellovibrio*, *Candidatus Accumulibacter*, *Gemmata*, *Hydrogenophaga*, *Hyphomonas*, *Isosphaera*, *Parvibaculum*, *Pirellula*, *Roseomonas*)	[106]

Table 6. *Cont.*

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
PN-SBR/Fermenter SBR/Anammox SBR	Mature/ Illumina	1.67–10 d/10 d	1.5 g/L sCOD/ 1236 mg/L	10%/ 99.6%	*Anaerolineae* (15.58% biofilm & 12.53% floc sludge), *Thauera* (0.11% & 17.29%), *Candidatus* Kuenenia (4.49% & 3.91%), *Brocadiaceae* (4.13% & 3.42%), *Acetonaerobium* (0.02% & 4.07%), *Dechloromonas* (0.004% & 1.69%), *Saprospiraceae* (1.51% & 1.51%), *Parcubacteria* (0.003% & 0.63%)	[107]
Aerobic MMBR (polyvinylidene fluoride biofillers)/SND	NR/ Pyrosequencing	NR	4–7/2.1 g/L	64.3%/ 97.4%	*Thauera* (1–10%), *Truepera* (4–10%), *Myroides* (2–8%), *Pseudomonas* (1.5–8%), *Hydrogenophaga* (1–5%), *Luteimonas* (2–4%), *Arenimonas*, *Pseudoxanthomonas*, *Serpens*, *Tissierella*, *Solitalea*, *Nitrosomonas*, *NItrobacter*	[108]
Two sequencing batch biofilm reactors (SBBRs) for simultaneous PN, ANAMMOX and denitrification	Mature/ Pyrosequencing	NR	1.0 g/L/ 1.9 g/L	59%/ 95–97%	AOB *(Nitrosomonas* & *Nitrospira)* (16.5%), Anammox (*Candidatus* Brocadia) (9.0%), *Thermomonas* (6.5%), *Bellilinea* (4.9%), *Comamonas* (3.8%), *Ferrugibacter* (3.0%), *Limnobacter* (2.8%), *Truepera* (1.7%), *Brevundimonas* (1.68%), uncultured *Chloroflexi* (1.31%)	[109]

PN: Partial Nitrification; DN: Denitrification; anammox SBR: Anaerobic Ammonium Oxidation Sequencing Batch Reactor; SND: Simultaneous Nitrification-Denitrification; SBBRs: Sequencing Batch Biofilm Reactors; NR: Not reported.

4.3. Microbial Communities during Anaerobic Digestion of Landfill Leachate

During anaerobic digestion of landfill leachate, hydrolytic and fermentative bacteria of the order *Bacteroidales* (e.g., *Petrimonas*, *Bacteroides* and *Parabacteroides*), clostridia and clostridia-like bacteria (*Clostridium*, *Eubacterium*, *Anaerovorax*, *Butyricicoccus*, *Alkaliphilus*), and *Synergistetes*-like representatives (e.g., *Synergistes*, *Thermoanaerovibrium*, *Aminobacterium*) are the common inhabitants of anaerobic bioreactor systems treating landfill leachate (Table 7). Secondary fermenters, such as *Syntrophobacter* spp., are capable of converting volatile fatty acids (VFAs) and low-molecular weight alcohols to acetate in a syntrophic relationship with methanogenic archaea. Hydrotrophic methanogens of the orders *Methanobacteriales* and *Methanomicrobiales*, such as *Methanobacterium*, *Methanobrevibacter* and *Methanoculleus*, convert hydrogen and carbon dioxide to methane, whereas *Methanosaeta* and *Methanosarcina* are key acetoclastic methanogens, producing methane from acetate. Sulphate Reducing Archaea (SRA), such as *Sulfolobus* and *Picrophillus*, are other members of the kingdom Archaea carrying out the dissimilatory sulphate reduction in anaerobic systems treating landfill leachate, converting sulphate to hydrogen sulfide (Table 7). Although the threat from potential pathogens is restricted in anoxic-oxic and anammox systems treating landfill leachate since only clostridia have been reported among the major microbial taxa (Tables 5 and 6), such concern is greater during anaerobic digestion of landfill leachate since, apart from clostridia, pathogens such as enterococci and treponema have been detected (Table 7).

Table 7. Major microbial communities identified in anaerobic digestion systems treating landfill leachate.

System	Leachate Age/Method	HRT/ SRT	COD/ NH_4^+-N	CODrem/ NH_4^+-Nrem	Microbial Community	Reference
AnMBR	Fresh/PCR-single-strand conformation polymorphism (PCR-SSCP)	7 d/ infinite	84.29 g/L/ 2800 mg/L	93.97%/ NA	**Bacteria:** *Pseudomonas*, *Alkaliphilus*, *Aminobacterium*, *Anaerovorax*, *Bacillus*, *Bacteroides*, *Brevibacterium*, *Butyricoccus*, *Clostridium*, *Eubacterium*, *Parabacteroides*, *Petrimonas*, *Synergistes*, *Thermoanaerovibrio*, *Verrucomicrobium* **Archaea:** *Ferroplasma*, *Methanocella*, *Methanoculleus*, *Methanolobus*, *Methanoplanus*, *Methanosaeta*, *Methanosarcina*, *Picrophillus*, *Thesmoplasma*, *Sulfolobus*	[110]
AnMBR	Mix of fresh & mature leachate/ pyrosequencing	2.5 d/ 125 d	13 g/L / 3.2 g/L	62%/ NA	***Bacteria:*** *Fastidiosipila* (9.25–12.2%), *vadinBC27* (6.3–16.5%), *Alkaliphilus* (0.12–2.94), *Enterococcus* (0.15–3.53%), *Petrimonas* (0.32–1.31%) ***Methanogenic Archaea:*** *Methanosarcina* & *Methanosaeta* (52.3 to 81.6%), *Methanobacterium* & *Methanobrevibactor* (45.3 to 17.6%) *Others* (*Methanoculleus*, *Methanofollis*)	[111]
Anaerobic buffled MBR/Struvite/O_3	Mature/ Illumina	6 d/NR	12.32 g/L/ 1583 mg/L	77–80%/ 22–26%	*Brevundimonas* (0.04–6.88%), *Alkalibacilum* (0.04–4.12%), *Methylophaga* (0.21–3.84%) *Percubacteria* (0.41–1.82%), *Thiopseudomonas* (0.02–1.72%), *Petrimonas* (0.002–1.22%), Others (*Acholeplasma*, *Hyphomicrobium*, *Luteimonas*, *Methylophaga*, *Pusillimonas*, *Sphaerochaeta*, *Truepera*)	[112]
Anaerobic digester	Fresh/ pyrosequencing	NR/ NR	5.63 g/L/845.2 mg/L	71.1% (mesophilic) & 77.1% (thermophilic)/NA	**Mesophilic bacteria:** *Treponema* (6.6%), *Desulfovibrio* (6.0%), E6 (3.6%), HA73 (2.3%), *Syntrophobacter* (2.0%), *Lutispora* (1.6%) **Thermophilic bacteria:** S1 (16.8%), *A55_D21* (2.5%) **Mesophilic archaea:** *Methanosaeta* (75.9%), *Methanobacterium* (14%) **Thermophilic archaea:** *Methanobacterium* (30.5%), *Methanoculleus* (28.8%), *Methanosaeta* (18.1%), *Methanosarcina* (9.5%)	[113]
					Others	
Conical flasks (fruits as C source)	Mature/ Pyrosequencing	2 d/ 2 d	0.98/1.23 g/L	87.4–93.2%/ 99.9%, at C/N 7	*Paracoccus* (59.77–69.06%), *Bacillus* (1.05–2.89%), *Tessaracoccus* (1.05–2.6%), *Sanguibacter* (0.89–2.07%), *Raineyella*, *Sacharimonadaceae*, TM7a, *Xanthomarina*, *Arenimonas*	[114]

AnMBR: Anaerobic Membrane Bioreactor; PCR-SSCP: Polymerase Chain Reaction—Single-Strand Conformation Polymorphism; MBR: Membrane Bioreactor; NA: Not applicable.

4.4. Biological Systems and Factors Influencing the Biotreament of Landfill Leachate

Reduction of pollutants under certain thresholds is compulsory for treatment plants' authorizations, directing the focus of researchers on landfill leachate treatment techniques, knowing that dilution is not an acceptable option. Pollutant removal can be carried out on-site or off-site, according to the location of the treatment facility, or can be provided by performing sequential processes, combining biological and physiochemical approaches [115].

Both age of landfill and leachate composition influence the selection of the right treatment design [116]. Biological treatment is based on the growth of a specific microbial population to remove organic and inorganic components from the leachate, but its efficiency is highly dependent on landfill age and is less effective when mature leachate is treated [117]. The main parameters affecting the biodegradation rate are the sludge age, the F/M ratio, the hydraulic retention time and the dissolved oxygen concentration [118]. Biological treatment is economical and energy recovery can be achieved in some cases [119], even though unbalanced nutrients content, OLR variations, increased ammonium nitrogen concentration and insufficient phosphorus content may compromise the process [120]. Furthermore, humic and fluvic acids and xenobiotics resist degradation [120].

Satisfactory removal rates may be achieved for macropollutants, such as COD, BOD and TKN, during biological treatment of young leachates. If leachate is produced in an aged landfill, physiochemical treatment is the most suited, due to the high content of non- and low-biodegradable organic compounds. A combination of treatments can be helpful for overcoming the limitations of both methods [117].

In full- and pilot-scale Sequencing Batch Reactors (SBRs) the oxidation of ammonium nitrogen can be completed successfully, as the efficiency may reach 99%. However, in the case of increased presence of inorganic nitrogen content, the ability of the process to effectively treat landfill leachate is limited. For influent ammonia concentration of 1.3 g/L in mature landfill leachate, the efficiency of a full-scale SBR system was slightly over 70% [121], whereas the COD removal efficiency for intermediate and mature landfill leachate in full-scale SBR systems ranged between 40 and 45% [79,122].

5. Conclusions

Current quantities of the total waste generated justify the need for additional scientific research, high alert and concern regarding the management and treatment of the produced landfill leachate inside the sanitary landfills. At every stage of the stabilization process, the landfill leachate consists of high organic content, as well as substantially high ammonium nitrogen concentration. Heavy metals are constantly detected, whereas a broad range of xenobiotics and pharmaceuticals, like CHCs, PFCs, phenols, PAHs and plasticizers, enhance the recalcitrant nature of this wastewater, which in most cases may pose a toxic effect on living organisms, even at extremely low dilutions (0.53% v/v). The physiochemical methods applied for depuration of leachate may serve as pretreatment, aiding the subsequent activated sludge methods, which are mainly carried out through the operation of SBRs and MBRs. Such activated sludge systems are driven by specialized microbiota, i.e., *Thauera*, *Truepera*, *Pseudomonas*, *Paracoccus*, *Luteimonas* and *Pusillimonas*, capable of dealing with the recalcitrant nature of landfill leachate. Biological nitrogen removal is effectively achieved during landfill leachate treatment in both anoxic-oxic and anammox systems. Regarding nitrification, *Nitrosomonas* spp. are the main ammonia oxidizers during partial nitrification, while *Nitrobacter* and/or *Nitrospira* strains, as well as by members of *Candidatus* Nitrotoga, are the key nitrite-oxidizers. Due to high ammonium concentration and the low COD content of recalcitrant nature, anammox process is advantageous to remove nitrogen content without the need for external carbon source as electron donor and high energy consumption for complete nitrification. Anaerobic treatment may also be applied at early stages of landfill operation, resulting in the production of 0.30 to 0.35 CH_4/g CODrem. A concern is raised during anaerobic digestion of landfill leachate, due to the detection of pathogens such as enterococci and treponema.

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