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Perspectives on Biological Treatment of Sanitary Landfill Leachate

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Abstract

Landfilling, one of the prevailing worldwide waste management strategies, is presented together with its benefits and environmental risks. Aside from biogas, another non-avoidable product of landfilling is landfill leachate, which usually contains a variety of potentially hazardous inorganic and organic compounds. It can be treated by different physico-chemical and biological methods and their combinations. The composition and characteristics of landfill leachate are presented from the aspect of biotreatability. The treatment with activated sludge, mainly consisting of bacterial cultures under aerobic and anaerobic conditions in various reactor systems, is explained, including an extensive literature review. The potential of fungi and their extracellular enzymes for treatment of municipal landfill leachates is also presented, with a detailed review of the landfill leachate treatment studies. The future perspectives of biological treatment are also discussed.

Keywords: Activated sludge treatment, biotreatability, fungal treatment, landfill leachate

1. Introduction

Landfilling is still widely accepted and used in any waste management strategy, but it can constitute a hazard for the environment. This method generally offers lower cost of operation and maintenance when compared to other methods, such as incineration. Besides households and urban activities, the industry is directly associated with the production of large amounts

of solid wastes. Several methodologies and strategies have been developed for the integrated management of these wastes. They start with pollution prevention, waste minimization (*zero waste*), reuse of products or their parts, as well as material and/or energy recovery. But in spite of all environmental policies, the majority of municipal and industrial wastes still end up at the landfill and the amount of deposited wastes is significant worldwide. Landfill still accounted for nearly 40% of municipal waste treated in the European Union in 2010. In the 25 countries of the European Union, 502 kg of municipal waste was generated per person in 2010, while 486 kg of municipal waste was treated per person: 38% was landfilled, 22% incinerated, 25% recycled, and 15% composted.

In the deposited wastes, organics are still present even after thorough waste separation, mainly due to the dirty packages and other remains that could not be completely separated; thus, microbial processes dominate the stabilization of the waste and lead to the generation of the landfill gas, and dictate the amount and composition of the leachate. Landfill leachate is defined as wastewater formed due to precipitation, deposited waste moisture, and water, formed within the body of the landfill. Untreated leachates can permeate groundwater or mix with surface waters and contribute to the pollution of soil, ground water, and surface waters. Careful site management can reduce the quantity and increase the purity of the formed leachate, but it cannot completely eliminate it. Its composition is therefore site- and time-specific, based on the characteristics of deposited solid wastes, physico-chemical conditions, rainfall regime that regulates moisture level, and landfill age. Even within a single landfill site, variability is frequently evident [1, 2, 3]. Significant components of leachate at the beginning of landfill operation are heavy metals and degradable organics, while persistent organic pollutants usually appear later as a result of biotic (i.e., living components that constitute an ecosystem) and abiotic (i.e., non-living chemical and physical components that affect living organisms and the performance of ecosystems) processes in the system. Among these substances are several compounds classified as potentially hazardous: bio-accumulative, toxic, genotoxic (chemical compounds that damage the genetic information within a cell causing mutation that may lead to cancer), and they could have endocrine disruptive effect [2]. Hazardous substances from the leachate should be caught and removed properly, to avoid spreading in the receiving environment. Efficient treatment methods must be matched to the actual characteristics of a particular leachate and they could vary with time. Often, biological processes are employed if biotreatability in terms of low toxicity and at least moderate biodegradability of the leachate is indicated [2, 4].

Biodegradability of the wastewaters and also leachates is usually determined using various non-standardized laboratory or pilot-scale long-term tests with activated sludge as the source of active microorganisms [5]. Toxicity tests must be accomplished prior to the biodegradability determination to assess the impact of landfill leachate components on microorganisms of the aerobic or anaerobic activated sludge. Biodegradability assessment of leachates usually starts with the determination of ready biodegradation in common environmental conditions, it is upgraded with the assessment of biodegradation potential in an inherent biodegradability assessment test under optimal conditions, and it is finally concluded with a simulation of biodegradation in the wastewater treatment plant. All of the mentioned tests are based on the

measurement of summary parameters, such as chemical oxygen demand (COD) or dissolved organic carbon (DOC) removal, O₂ consumption, etc. Inherent biodegradability assessment tests provide data on adsorption potential of the sample to the activated sludge and allow estimation of its impact on the biological wastewater treatment plant. Preliminary estimations should then be verified in a laboratory or a pilot-scale aerobic treatment plant to determine the actual impact of the wastewater on the activated sludge processes [5-7]. The connection between the biodegradation and changes in toxicity of the sample represents the stabilization study, where leachate is diluted in a batch reactor to avoid significant toxicity, and it is aerated and stirred until the biodegradation reaches the final plateau. Among other parameters, toxicity is monitored during biodegradation by using the appropriate method. Stabilization (ageing) allows us to assess the toxic fraction as permanent or biodegradable [8-10].

After the complete examination of the landfill leachate characteristics, the appropriate treatment process should be considered. In the case of significant biodegradability, various biological processes could be involved. Biological treatment is reliable, simple, highly cost-effective, and provides many advantages in terms of biodegradable and nitrogen and phosphorous compounds removal [11, 12]. It can be accomplished with microorganisms in different types of reactors, in aerobic and anaerobic conditions. Classical systems with activated sludge, sequencing batch reactor (SBR), biofilters, membrane bioreactors, as well as up-flow anaerobic sludge blanket processes and fluidized bed reactors are often considered [13]. In the last few decades, researchers also confirmed the great potential of white rot fungi for removal of hazardous as well as toxic pollutants. They produce various extracellular ligninolytic enzymes, including laccase (Lac) and manganese peroxidase (MnP), which are involved in the degradation of lignin in their natural lignocellulosic substrates [14], and according to the literature data [15], offer also an interesting potential for the landfill leachate treatment.

A considerable amount of work has been done in the field of landfill leachate biological treatment in the past decades. But the strict implementation of environmental legislative demands and the ageing of existing landfills put pressure on managers and operators of landfills to implement more efficient processes for landfill leachate treatment. Nevertheless, the results, obtained during decades of research, indicate some future research guidelines.

2. Solid waste management

The industrial and economic growth of many countries around the world has resulted in a rapid increase in industrial and municipal solid waste generation [1]. Many countries are still trying to implement separate collection and disposal of waste based on the Reduce-Recycle-Reuse principle, which should lead to zero waste management practice in the future. However, the improved waste management strategy, based on the waste management hierarchy pyramid (Figure 1) clearly pointed out the disposal of solid wastes, such as landfilling, as the least favorable option leading to severe environmental impact and degradation. It also results in a loss of natural resources [16]. Different recovery options, such as energy recovery, result in the utilization of energy potential of the waste, while recycling is even preferred due to the

recovery of materials. In both cases, some loss of material and/or energy is noticed, and thus the differences regarding the impacts on the environment are relatively small [17]. The measures of waste prevention eliminate the need for the above-mentioned measures, thus lowering the impact that the waste has on the environment. The waste prevention measures can be any form of reducing the quantities of materials used in a process, or any form of reducing the quantity of harmful materials that may be contained in a product. At the same time, the replacement of hazardous materials should also be considered. Prevention and minimization also include processes or activities that avoid, reduce, or eliminate the waste at its source, or result in its reuse or recycling [18]. In recent years, these goals were incorporated in European environmental policy to make a resource-efficient Europe, while cutting off the burden of wastes and emissions. Effective implementation of these waste policies demands an understanding of what has been achieved so far and a set-up of targets and broader goals for the future. The first EU legislation covering the generation and treatment of waste was introduced in 1975, and now, there are more than 20 legislative documents currently in force on waste management [19-22].

Municipal solid waste is defined differently in different European states, but according to Eurostat, "Municipal waste is mainly produced by households, though similar wastes from sources such as commerce, offices, and public institutions are included. The amount of municipal waste generated consists of waste collected by or on behalf of municipal authorities and disposed of through the waste management system" [23]. Other wastes, similar in nature and composition, but collected by the private sector, are also included in this definition. In the EU Landfill Directive, municipal solid waste is defined broader as the "Waste from households, as well as other waste which, because of its nature or composition, is similar to waste from households" [24]. This seems to be the definitively more appropriate definition, referring to the type of the waste and not to who produced or collected it [25].

However, due to different definitions and methodologies used across EU countries, the efficiency of improved waste prevention in the last decade (2001-2010) is not easy to estimate. The average EU-27 value still varies around 500 kg for wastes generated per capita. 21 countries generated more municipal wastes per capita in 2010 than in 2001, and 11 cut per capita municipal waste generation [25]. On the other hand, there was a clear shift up the waste hierarchy, from landfilling to energy recovery and recycling. In this period of time, landfilling decreased by almost 41 million tons, whereas incineration increased by 15 million tons, and recycling grew by 28 million tons within EU-27. In the study where, together with the EU-27 countries, Croatia, Iceland, Norway, Switzerland, and Turkey were also taken into account, the decrease of landfilling in 2001-2010 is evident in Table 1 [25]. Each country can be included in several waste management categories, so the total number of countries is sometimes more than 32. When Table 1 is considered, the shift in recycling is noticeable; however, in 2010, in 50% of the countries landfill still presented more than 50% of municipal waste. The data on the trends in the recycling of materials and biowaste could be used to evaluate changes in the composition of deposited solid wastes. This parameter is also affected by the implementation of waste hierarchy, not only by the quantity of the deposited wastes. The total recycling rate increased between 2001 and 2010, mainly due to the fact that many countries have increased

recycling of materials such as glass, paper and cardboard, metals, plastic, and textiles. Eight out of 32 investigated countries increased their material recycling rate by more than 10%, and 11 countries achieved an increase of 5%-10% [25].

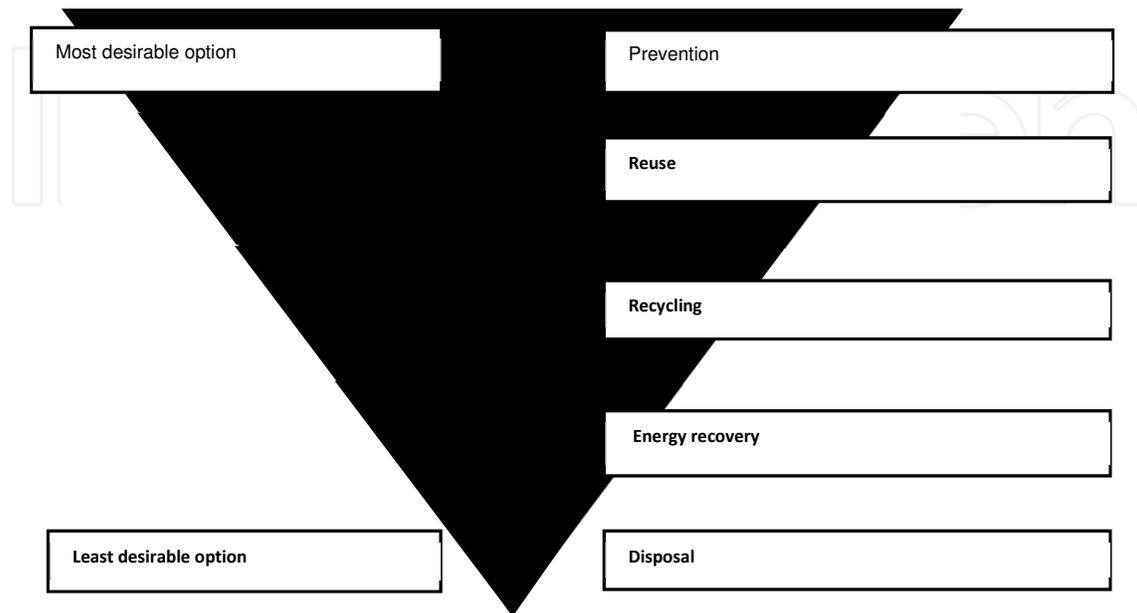


Figure 1. Waste management hierarchy [18].

Waste management	Number of countries	
	Year 2001	Year 2010
> 25 % Recycling	11	16
> 25 % Incineration	8	10
> 50 % Landfilling	22	19
> 75 % Landfilling	17	11

Table 1. Number of countries at different levels of municipal waste management in 2001 and 2010 [25].

In contrast to material recycling, biowaste recycling is not comparably efficient over the same period of time (EEA, 2013); one country increased its municipal waste-derived biowaste recycling by more than 10%, and only six of them improved it by 5%-10%. Eighteen countries even sustained a very low level of biowaste recycling (0%-10% of municipal waste generated). This could be a consequence of several factors affected by the methodology of data collection and the legislation. The fact is that material and biowaste recycling potential depends on the share of each waste type in the total collected municipal waste. In most countries, the biowaste recycling potential is lower than the material recycling potential because biowaste represents a smaller proportion of the total municipal waste [25]. This leads to the conclusion that different

policy instruments should be implemented and combined in different countries to achieve maximal impact in terms of municipal waste management.

3. Landfilling of municipal waste

Currently, the deposit in a landfill is still the most widely used method for municipal solid waste disposal within almost all European states (Table 1). The landfill can be considered as a complex environment or even a biochemical reactor, where many interacting physical, chemical, and biological processes take place. The degradation process of municipal solid wastes in landfills is a long-term event. A major problem regarding disposal of wastes is the lack of available landfilling sites, as well as the production of landfill leachates and biogas, consisted mainly of carbon dioxide and methane, which has 28 times higher global warming potential than CO_2 in a 100-year cycle [26]. In Slovenia, for example, net emissions of greenhouse gases due to municipal waste management have been decreasing constantly since 1999 [27]. This could also be the consequence of a better municipal waste management (e.g., recycling of biowaste), resulting in lower biodegradable fraction landfilled. It can be assumed that the direct emissions of GHCs from landfilling will continue to decrease in the coming years, but for several years ahead, considerable amounts of greenhouse gases will continue to be emitted from landfills because biowaste landfilled in the previous years will continue to generate methane for several decades [27]. However, with appropriate entrapment and utilization, biogas is usually efficiently exploited for energy purposes at the site, while leachates could pose a serious risk for nearby soil, surface, and underground waters [28]. At the sites, where there is no need for energy or where the methane content is very low, methane is flared to avoid its migration in the atmosphere. Landfill top covers or so-called biocovers are often used at landfills to reduce methane emissions. They optimize environmental conditions for development of methanotrophic bacteria to help oxidize any fugitive methane. Biocovers are usually spread over the entire surface of the landfill and they are made of compost, dewatered sewage sludge, or other waste material. Landfills with gas collection and recovery systems had a methane recovery efficiency of 41%-81%. Methane emissions could range from 2.6 kg h^{-1} to 60.8 kg h^{-1} , with the lowest emissions from the small and old landfills and the highest emissions from the larger landfills [29].

3.1. Landfill leachate

Appropriate management can reduce the quantity and quality of the leachate, but it cannot be completely eliminated. Landfill leachate is generated as a mixture of rainwater percolation through the wastes, water produced from the (bio) degradation of wastes, and the water present in the wastes at the time of deposition [2]. Its composition is based on the type and the amount of waste deposited, as well as its maturity, and the construction of the landfill site [4]. Main sequential and distinct consecutive stages involved in landfill stabilization are [30, 31]:

1. Aerobic phase, characterized by processes enabled by oxygen present. In this acclimation phase, sufficient moisture develops to support active microbial communities. Initial

changes in compounds occur in order to establish the appropriate conditions for further biochemical degradation.

2. Hydrolysis and fermentation stage, where complex molecules are broken into smaller fragments and an aerobic environment is transferred to an anaerobic one, the amount of entrapped oxygen is drastically reduced and the reducing conditions occur. The main electron acceptors are nitrates and sulfates.
3. Anaerobic acetogenic stage, characterized by continuous hydrolysis of solid wastes, preceded by the production of volatile fatty acids at high concentrations. Low hydrogen levels promote the activity of methanogenic bacteria, which produce methane and carbon dioxide from organic acids. This stage could be recognized by a high concentration of metals in the leachate, due to their increased mobility because of the lower pH, even below 4.
4. Anaerobic methanogenic stage, where significant methane production is evident. pH is again increased to 7-8, due to the degradation of intermediate acids and the buffering capacity of bicarbonates. The concentration of heavy metals is reduced again, due to their complexation and precipitation. During this stage, the mesophilic bacteria, which are active in temperatures of 30°C-35°C, and thermophilic bacteria, active in the range of 45°C-65°C, dominate the microbial population.
5. Maturation stage, as the final stage of landfill stabilization, could be recognized by low microbial activity due to degradation of biodegradable fraction and limiting impact of nutrients. As a result, the methane production decreases, as does the amount of pollutants in the leachate, which usually stays at a constant level. Slow degradation activity of the resistant organic pollutants can be observed by the production of humic and fulvic substances.

The duration of each phase is dependent upon many factors, and the development and activity of microorganisms are dependent upon sustaining appropriate conditions. Landfill leachates are characterized by high concentrations of numerous toxic and carcinogenic chemicals, heavy metals, and organic as well as inorganic matter. Among the organic compounds detected in the landfill leachate, the main compounds are different hydrocarbons, esters, alcohols, and ketones, as well as aromatic and heterocyclic compounds [32]. Additionally, the leachates can also be contaminated with bacteria, including aerobic, psychrophilic and mesophilic bacteria, coliform and fecal coliforms, spore-forming-bacteria, and with numerous fungi [32, 33]. Typical concentrations of landfill leachate compounds as a function of landfill age and stabilization are presented in Table 2 [30, 31, 34].

Concentrations of organic compounds, expressed as COD and biological oxygen demand 5-day test (BOD₅), in the young leachate are high (aerobic and acid formation phase), while leachates from stabilized landfills (methane formation and maturation phase) contain lower levels of organic matter [28]. Several authors have reported that in young landfills, COD could vary from 5,000 to even more than 60,000 mg L⁻¹, with BOD₅ starting from 3,000 L⁻¹ to even 40,000 mg L⁻¹ [35]. Those values are significantly lower in leachates from mature landfills, as a result of biotic stabilization processes in the body of the landfill [36]. The total quantity of

the produced landfill leachate can be estimated by using empirical data based on flow measurements, or by using water mass balance between precipitation, evapotranspiration, surface runoff, and capacity of moisture storage. Waterproof covers and different covering liners contribute a lot to the reduction of landfill leachate quality, but they cannot completely reduce it. Both parameters (leachate quality and quantity) affect the attempts of uniform design of leachate treatment systems. The optimal treatment solution may change over time because of the changeable quality and quantity of the leachate, and the development of new technologies and the legislation [37].

Parameter	Aerobic phase	Acid formation phase	Methane formation phase	Maturation phase
pH (/)	6.7	4.7–7.7	6.3–8.8	7.1–8.8
COD (mg L ⁻¹)	480–18,000	1500–71,000	500–10,000	<1000
BOD ₅ /COD (/)	>0.5	0.5–1.0	0.5–1.0	<0.1
Organic compounds (/)	Various	80% of volatile fatty acids	5%–30% of volatile fatty acids + fulvic and humic acids	fulvic and humic acids
Ammonium nitrogen (mg L ⁻¹)	>100	<1000	<500	<500
Heavy metals concentration (/)	Low	Low - Medium	Low	Low
Conductivity (μS cm ⁻¹)	2,500–3,300	1,600–17,100	2,900–7,700	1,400–4,500
Biodegradability	Important	Important	Medium	Low

Table 2. Typical concentrations of landfill leachate concentrations as a function of landfill stabilization [30, 31, 34].

3.2. Landfill leachate treatment

The landfill treatment and disposal represents one of the major landfill operational costs. Its management is not accomplished by the closure of the landfill, because its characteristics must be monitored even further, as defined by particular legislative requirements.

Traditionally, biological treatment is the most widely-used treatment strategy for wastewaters, mostly because of its low operational costs and complete as well as rapid destruction of pollution [2]. However, biological treatment is not always effective enough for the toxic and recalcitrant leachates (e.g., methane formation and maturation phase), in which case physico-chemical treatment can take place. Usually, the leachate treatment involves a combination of various biological and chemical methods. Conventional landfill leachate treatment can be classified into three major groups:

1. Recycling and combined treatment with domestic sewage. In the past, it was common to treat landfill leachate mixed with municipal wastewater [2, 38]. This option is not so favorable nowadays, due to the identified presence of hazardous persistent compounds in the leachate, which are not removed in the conventional municipal treatment plant. On

the other hand, the municipal wastewater represents an important source of nitrogen and phosphorous, which would otherwise be limiting factors in the biological treatment of landfill leachate alone [39]. The new trends include recycling of landfill leachates, to manage the landfill as a bioreactor with moisture and air control to enhance the establishment of conditions for efficient biodegradation of present organic fractions. However, this can decrease the concentration of organic constituents in the landfill leachate, but it can also increase the concentration of ammonium nitrogen, which should then be removed by additional treatment processes. Recycling of the leachate is a viable option especially in developing countries, to reduce its environmental risks and to avoid as many multi-process treatment methods as possible [40]. Another important aspect is also the reduction of time needed to stabilize the deposited waste, from several decades to a few years [41].

2. Biological treatment employing mainly aerobic biodegradation processes. Biological treatment is reliable, simple, highly cost-effective, and provides many advantages in terms of biodegradable matter and nitrogen compounds removal. However, its efficiency is strongly limited in the presence of refractory or inhibitory compounds in wastewaters, which are also typical for mature landfill leachates. All of the aspects of the biological treatment of applications will be presented in the next chapters.
3. Chemical and physical methods, such as chemical oxidation, adsorption, coagulation/flocculation, membrane techniques and air stripping [2]. Membrane-based treatment processes gained a lot of attention recently, including reverse osmosis, nanofiltration, ultrafiltration, and microfiltration [2, 31]. Chemical oxidation processes are viable options for effective landfill leachate treatment, but they are relatively expensive for the complete mineralization of landfill leachate pollutants, because the oxidation intermediates, formed during treatment, tend to be more and more resistant to the complete chemical degradation. Fenton oxidation or ozonation in a pre-treatment process can convert persistent and non-biodegradable organic compounds into more biodegradable intermediates, which would be subsequently treated by a biological treatment process. On the other hand, the polishing of remained organics in the effluent after biological treatment using one of the oxidation processes could also reduce the environmental impact of landfill leachates. Several authors have reported that Fenton's process can achieve 60%-90% of COD removal of organics from landfill leachate [42]. Moreover, if leachates were pretreated by biological processes, the Fenton's oxidation added additional 63% of COD removal [43]. Some authors also suggested electrocoagulation as a suitable method for leachate treatment, accompanied by a flotation of formed sludge [44]. In this study, 40-73% of COD and color at 245 nm were removed, depending upon different cathode material (graphite or aluminum). However, the treatment lasted for 210 minutes and the energy requirement per kg of removed COD was 135 kWh, accompanied by 40% and 0% of nitrate and ammonium nitrogen removal, respectively.

3.3. Selection of the treatment process

It is well recognized that the selection of appropriate treatment method is strictly dependent upon the leachate characteristics and composition. For effective biological treatment, its

biotreatability must be evaluated. In the case of a low-treatment efficiency of the biological plant, other treatment methods should be investigated.

Any determination of biological treatability of the wastewater must include data on toxicity and biodegradability. Toxicity could be assessed using one of the tests with mixed culture of microorganisms (activated sludge), which plays an essential role in a biological wastewater treatment plant. The test with measurement of inhibition of oxygen consumption and the test where growth inhibition of activated sludge is measured are widely applied [45]. If the impact of the leachate to anaerobic microorganisms is assessed, then the reduction of biogas production is often measured [46].

Method	Group of pollutants removed	Data obtained
Filtration at different pHs	Suspended solids	<ul style="list-style-type: none"> · Toxicity is related to soluble or insoluble material · Metals form insoluble complexes at higher pHs and are removed during filtration
Ion exchange	Inorganic compounds ions	<ul style="list-style-type: none"> · Toxicity is related to inorganic compounds or ions
Biodegradability testing	Biodegradable fraction	<ul style="list-style-type: none"> · Decrease in toxicity due to the biological treatment · Toxicity is related to recalcitrant or biodegradable compounds · The extent of biodegradation of wastewater at investigated condition · Possible sorption of pollutants to microorganisms. · Biodegradability or mineralization potential
Oxidant reduction	Oxidants	<ul style="list-style-type: none"> · Toxicity is related to oxidants.
Metal chelation (EDTA)	Cationic metals (no Hg)	<ul style="list-style-type: none"> · Toxicity is related to metals.
Air stripping at different pHs	Ammonia Volatile organics	<ul style="list-style-type: none"> · Toxicity is related to volatile organics · At low pH (pH = 3) small molecular weight organic acids will be effectively removed, while at higher pH (pH = 11) they may be dissociated or form salts and are not purged out · Toxicity is related to ammonia · Ammonia could be stripped out at higher pH.
Adsorption	Adsorbable organics	<ul style="list-style-type: none"> · Toxicity is related to adsorbable organics · Presence of compounds causing color · Results very dependent upon adsorbent used and experimental conditions
Oxidation	Oxidizable organics	<ul style="list-style-type: none"> · Toxicity is related to oxidizable organics · Results very dependent upon oxidant used and experimental conditions

Table 3. Commonly used methods in Toxicity Identification Evaluation (TIE) procedures [12, 49, 57].

Biodegradability of the wastewaters is usually determined by using various non-standardized laboratory or pilot-scale long-term tests with activated sludge as a source of active microorganisms [5, 6]. At the same time, some of the standardized test methods, developed for biodegradability assessment of pure chemicals, could be applied [7]. Biodegradability assessment usually starts with the determination of ready biodegradation in common environmental conditions and it is upgraded with the assessment of biodegradation potential in the inherent biodegradability assessment test under optimal conditions [5]. All of the above mentioned tests are based on the measurement of summary parameters, such as COD or DOC removal, O₂ consumption, etc. [47, 48]. A combination of different measurement techniques to follow biodegradation is recommended to distinguish between the biodegradation and the complete mineralization of the sample [5]. Inherent biodegradability assessment tests provide the data on adsorption potential of the sample to the activated sludge and allow us to estimate its impact on biological wastewater treatment plants [49]. Preliminary estimation should then be verified in an actual laboratory or a pilot-scale aerobic treatment plant, to determine the impact of the wastewater on activated sludge processes. Another possibility to assess biotreatability is also the stabilization study, which represents a link between toxicity and biodegradability, to correlate the changes of toxicity versus the extent and rate of the biodegradation. The initial and final biodegradability testing of the test mixtures allows us to confirm the measured degradation or the persistency of final residue [10]. If the wastewater consists of mainly degradable components, resulting in the toxicity elimination, the biological treatment is a good alternative, while in the case of poorly biodegradable wastewater with negligible decrease in toxicity, other treatment methods should be considered [8-10].

However, the discussion on the selection of the treatment method is based on the knowledge on wastewater quantity and quality, as well as the required effluent quality. The costs and the availability of the land are also very important; a detailed cost analysis should therefore always be made prior to the final process selection and design. The main characteristics, which should be considered are [12]: i) soluble organics responsible for oxygen consumption; ii) suspended solids; iii) priority substances that have hazardous environmental impact due to their persistency, toxicity, bioaccumulation potential and they could pose endocrine disruptive effect; iv) heavy metals; v) substances and particles causing color and turbidity; vi) nitrogen and phosphorous content; vii) refractory substances; viii) floating oils and grease; ix) volatile compounds (organics and H₂S), etc. For wastewaters containing nontoxic and biodegradable organics, the process design criteria can be obtained from the data from laboratory or pilot studies, while more defined screening procedures are often needed for more complex and changeable wastewaters, such as landfill leachates. To set up appropriate treatment technology, the toxicity identification (TIE) approach is sometimes feasible, especially when a biological treatment is considered [53]. The TIE is a wastewater-specific study to isolate, identify and confirm the causative agents of toxicity. It is based on procedures, developed by the United States Environmental Protection Agency (USEPA). The Toxicity Reduction Evaluation (TRE) procedure is used as a tool to identify toxic components that may be removed or reduced in an effluent to reduce toxicity problems.

Combined processes	Experimental scale	Type of the leachate	Measured parameters	Removal efficiency	Reference
<ul style="list-style-type: none"> · Sequencing batch reactor (SBR) · Coagulation with polyferric sulphate + Fenton system · Upflow biological aerated filters 	Full	Mature/ Stabilized	COD NH ₃ -N TP SS	97.3% > 99% < 1 mg/L-1 < 10 mg/L-1	[62]
<ul style="list-style-type: none"> · Sequencing Batch Biofilter Granular Reactor · With/no ozone · Solar photo-Fenton 	Laboratory	Medium aged	Toxicity: -respirometry - <i>Vibrio fischeri</i> - <i>Lepidium sativum</i> COD DOC	High High High 95.3% 95%	[63]
<ul style="list-style-type: none"> · FeCl₃ coagulation · Magnetic ion exchange · Reverse osmosis · Nanofiltration 	Laboratory	Mature/ Stabilized	DOC UV245 adsorbing OM Salts DOM	45–71%* 84–94%* > 93%* > 99%*	[64]
<ul style="list-style-type: none"> · Aerated lagoon · Solar photo-Fenton · Conventional biological WWTP (with nitrification/denitrification) 	Pilot	Young/ After lagooning	DOC TN Biodegradability	90% 56%–90%* Increased	[61]
<ul style="list-style-type: none"> · Agitation/stripping · FeSO₄ coagulation · SBR, mixed with sewage: anoxic-aerobic-anoxic conditions · Sand and carbon filtration 	Laboratory	Mature/ Stabilized	COD TOC BOD ₅ SS NH ₃ -N	97.4% 92.3% 94.4% 97.5% 99.2%	[66]
<ul style="list-style-type: none"> · TiO₂/UV photolysis · Bioreactors with various inoculums (raw leachate/soil extract/activated sludge) 	Laboratory	Mature/ Stabilized	COD BOD ₅ NH ₃ -N	87% 90% 43%–79%*	[65]
<ul style="list-style-type: none"> · Coagulation/flocculation · Fenton · Solar photo-Fenton 	Laboratory Pilot	Mature/ Stabilized	Toxicity: -respirometry COD Biodegradability	Remains low 89% Increased	[67]

*...Depends upon the combination of treatment processes and landfill leachate sample characteristics.

TP = Total phosphorous
 TN = Total nitrogen
 SS = Suspended solids
 COD = Chemical Oxygen Demand
 DOC = Dissolved Organic Carbon
 OM = Organic Matter
 DOM = Dissolved Organic Matter
 WWTP = wastewater treatment plant

Table 4. Some of the recently investigated combinations for treatment of heavily polluted landfill leachates [61-67].

The TIE methodology uses the responses of the test organisms to detect the presence of toxic substances in the sample before and after the samples are subjected to a series of physical and chemical treatments. This combination of physical/chemical manipulations of toxic samples, followed by the toxicity testing, allows one to isolate and identify the problematic group of compounds [50, 54, 55]. The most often used procedures are listed in Table 3. Results could be efficiently utilized to set up the appropriate treatment procedure for the particular wastewater, because it can be clearly estimated which treatment method is efficient in the removal of the particular group of pollutants and where the toxicity of the wastewater comes from [56]. Usually these simple, cost-effective methods could reduce the need for a complex and detailed characterization of the wastewater before setting up treatment procedure. However, they have to be designed and performed with caution (blank sample) to avoid any impact of the applied method (pH manipulation, addition of chemicals, etc.) to the final characteristics of the sample.

According to the results of the methods described in Table 3, suitable treatment methodology could be set up. If, for example, the wastewater contains a significant fraction of a non-biodegradable organic fraction (determined by biodegradability testing) and it contains a lot of oxidizable organics (proved by oxidation experiment), one of the advanced oxidation processes would seem to be to be the most viable treatment option. On the other hand, if it contains organics that are able to mineralize almost completely in the biodegradability test, and it contains a lot of ammonia, one of the biological treatments involving nitrification/denitrification would seem to be to be the best choice. It can be clearly concluded that, in the case of municipal landfill leachates, a technically and economically viable methodology for the effective treatment has yet to be designed. The available options are similar to those used in the treatment of industrial wastewaters, involving a combination of physical, chemical, and biological processes. Primarily due to their low costs, the biological processes, in their various forms according to redox regime (aerobic, anaerobic, anoxic), a type of biomass (a mixed or a pure bacterial culture, fungi, etc.) and a biomass fixation (dispersed, attached), remain the most widely implemented type of treatment processes [2, 58, 59].

However, a combination of biological and physico-chemical processes is usually employed for heavily polluted leachates. Many examples of efficient treatment combinations could be found in literature. As presented in [60], an aerobic biological treatment, a chemical coagulation, an advanced oxidation process (AOP), and some combined treatment strategies were compared. Laboratory experiments were done with 200 mL samples in a glass vessel. The efficiency of these treatment procedures was evaluated by analyzing the COD and color removals. In the extended aeration process, the maximum COD and color removals were 36% and 20%, respectively. They could be achieved during the optimum retention time of 7 days. Chemical coagulation with an optimum aluminum sulphate dose of 15,000 mg/L at pH = 7.0, gave the maximum COD and color removals of 34% and 66%, respectively. Using Fenton oxidation process at optimum pH = 5.0 and optimum dosages of reagents, with H₂O₂/Fe²⁺ molar ratio of 1:3, the highest removals of COD and color were 68% and 87%, respectively. The combined treatment, the extended aeration followed by Fenton oxidation, was found to be the most suitable.

Some additional, recently investigated and proposed treatment designs are presented in Table 4.

A large-scale multistage treatment system was also designed for the treatment of a mature raw landfill leachate [61]. The system consisted of an activated sludge biological oxidation (ASBO) reactor for aerobic and anoxic conditions (volume 3.3 m³) and a solar compound parabolic collector (CPC) for photo-Fenton process (total collector surface 39.52 m² and illuminated volume 482 L). The raw leachate was characterized by a high concentration of humic substances, representing 39% of the DOC content and high nitrogen content, mostly in the form of ammonium nitrogen. In the first biological oxidation step, a 95% removal of total nitrogen and a 39% mineralization in terms of DOC were achieved. The following photo-Fenton reaction led to the depletion of humic substances > 80% of low-molecular-weight carboxylate anions > 70% and other organic micropollutants, thus resulting in a total biodegradability increase of > 70%. The neutralized photo-bio-treated leachate was finally treated with the second stage biological oxidation, where the rest of biodegradable organic carbon and nitrogen content were eliminated. This way, a high efficiency of the overall treatment process was achieved.

4. Biological treatment of the landfill leachate

4.1. Treatment with activated sludge

Biological treatment has become one of the most often used treatment processes; it is the most common method for the removal of organic, nitrogen, or phosphorus components from wastewaters. One of the main reasons for the selection of this process is its capability to achieve high elimination efficiency of these pollutants, and at the same time, it is relatively less expensive than physico-chemical or chemical processes. The pollution is completely destroyed to the level of non-hazardous, simple products, and not only transformed into another form. Nowadays, it is used not only for the treatment of sewage, but also for the removal of different xenobiotics such as pharmaceuticals, personal care products, and cleaning agents from the sewage and the heavily polluted industrial wastewaters and landfill leachates [12]. Biological degradation of pollutants is caused by the metabolic activity of microorganisms, in particular by the bacteria and fungi that live in natural environments. However, its efficiency is strongly reduced in the presence of refractory or inhibitory compounds in wastewaters, typical also for mature landfill leachates [33, 66, 68]. To achieve good removal efficiency, high BOD/COD ratio is recommended (>0.5) [13].

When biological treatment is discussed, mainly microorganisms that grow in a controlled environment through a complex sequence of biochemical reactions, forming the vital steps of their metabolic activities are considered [13]. The prevailing species are the saprotrophic bacteria, there is also an important protozoan flora present, composed mainly of amoebae, *Spirotrichs*, *Peritrichs* including Vorticellids, and a range of other filter-feeding species. Fungi could also contribute to the diversity of present populations. Other important constituents include motile and sedentary rotifers. The most important seems to be the bacteria, found in all types of treatment processes. The nature of the population changes continually, in response

to variations in the composition of the wastewaters and to environmental conditions [69]. Generally, biological treatment of wastewaters involves bacterial community in aerobic and anaerobic conditions, which could be dispersed or attached, in small flocks, granulated or forming biofilms, while treatment with fungi and their enzymes also lately received more attention [70]. However, fungal treatments have not yet found a wider recognition, due to the difficulty in selecting organisms that are able to grow and remain active in the actual wastewater [71].

4.1.1. Treatment with activated sludge under aerobic conditions

The most often applied processes for biological treatment are aerobic. In an aerobic environment (concentration of dissolved oxygen $>2 \text{ mg L}^{-1}$), organic matter is used as a food source for microorganisms. The suspended organics are removed by entrapment in the biological activated sludge flocks. The colloidal organics and a small amount of soluble organics are also partially adsorbed and entrapped by the sludge flocks. Therefore, approximately up to 85% removal of the total COD can be achieved after 10 min to 15 min of retention time. The remaining degradable soluble organic fraction undergoes biological reactions [11, 13]. A portion of organic compounds (about 50% of organic carbon) is oxidized to CO_2 and H_2O , and the rest of it is incorporated into a new biomass. Approximately about 60% of the energy content in wastewater organics is consumed for synthesis of the new biomass and the rest represents reaction heat loss [13]. At the same time, an efficient removal of ammonium nitrogen should also be achieved to protect the sensitive water bodies from eutrophication [12, 54].

A combination of aerobic and anoxic environment is necessary for the accomplishment of organic and nitrogen pollutants' removal from wastewater. On the other hand, the combination of anaerobic and aerobic environment is required to biologically remove phosphates from wastewater, so the systems could not always be characterized as completely aerobic or anaerobic. The upgrading of biological processes, from removal of carbonaceous organics to the nitrogen and phosphorus removal, significantly impacted the system configuration. Not only do the system configuration and its operation increased in complexity, but also the new legislative demands on effluent quality have to be met. Thus, the system must be well-designed, optimized, and operated at its optimum in order to fulfill these criteria [72]. Some of the bioreactors, also applicable for aerobic treatment using microorganisms, are summarized in Table 5. Aerobic biological systems, based on suspended-growth biomass, have been widely studied and also applied [2]. Also recently, attached-biomass systems have been developed, such as moving bed bioreactors (MBBR) and with different options of biofilters. A promising alternative also are membrane bioreactors (MBR), which represent an advanced biological treatment process, replacing a secondary clarifier in activated sludge process for removal of biomass with membrane module. It can be incorporated as an internal or an external unit of aeration basin to achieve better effluent quality, process stability, increased biomass retention time, and low sludge production [73]. Some of the systems where biological treatment represents the most effective stage will be briefly overviewed and discussed in this chapter. It should be emphasized that sometimes it is difficult to distinguish between aerobic and anaerobic treatment plants, due to the fact that most of the systems apply a combination of different regimes (aerobic, anaerobic, anoxic) to achieve the optimal treatment efficiency.

Systems with suspended-growth biomass	Systems with attached/immobilized biomass
Lagoons: <ul style="list-style-type: none"> · Aerated · Non-aerated 	Biofilters: <ul style="list-style-type: none"> · Tricking filters · Submerged biological filters
Constructed wetlands: <ul style="list-style-type: none"> · Horizontal system · Vertical system 	Moving bed bioreactors (MBBR): <ul style="list-style-type: none"> · Rotating biological contactors · Suspended carrier biofilm reactors
Activated sludge (AS) systems: <ul style="list-style-type: none"> · Continuous flow reactors · SBR 	
Membrane bioreactors (MBR): <ul style="list-style-type: none"> · External membrane module · Submerged/Immersed membrane module 	

Table 5. Typical treatment system with aerobic microorganisms applied in landfill leachate treatment [2, 13, 73].

When aerated and non-aerated lagoons are discussed, together with artificial or natural wetlands, there is usually a combination of aerobic-anaerobic systems. The upper part is usually aerated, while the bottom part is anaerobic [74]. Such combination is well-illustrated in [75]. Four connected on-site lagoons were used for the treatment of mature landfill leachate in the aging methanogenic state. The landfill leachate contained a relatively low COD value (mean value 1,740 mg L⁻¹) and a relatively high ammonium nitrogen concentration (mean value 1,241 mg L⁻¹). The pH of the raw leachate was in the range of 7.0-8.0 and the temperature was 16.7°C, which is higher than the mean ambient temperature (13.5°C). Volumes of the lagoons varied from 60-80 m³. The leachate was mixed and aerated by compressed air (4-6 h) through diffuser pipes at the base of lagoons. The facultative aerobic system was obtained where sequential aerobic and anaerobic stages were maintained. The total COD removal was 75% in 56 days. Ammonium nitrogen removed 99%, while an average of 9 mg L⁻¹ remained. However, the authors calculated that the conditions at the site could still be toxic to fish and the treated leachate should be diluted before its release into the environment. Due to aerobic conditions, nitrification occurred and the concentration of the nitrate was considerably higher in the effluent of the lagoons than in the influent; however, 80% of nitrogen was removed. The bacterial community profile also differed from one lagoon to another.

The study by [76] compared efficiency of horizontal- and vertical-constructed wetlands for landfill leachate, containing 2,930-14,650 mg L⁻¹ of COD, 170-4,012 mg L⁻¹ of ammonium nitrogen, and 44-153 mg L⁻¹ of orthophosphate-P. The experiments were run in a continuous mode, in three subsurface wetland systems; two of them operated in a vertical flow mode and one in a horizontal flow mode. The system was planted by *Typha latifolia*. Basins were 100 cm in length, 50 cm wide and 40 cm deep. The systems were filled with different heights of gravel and sand when zeolite was added in the third lagoon to increase its adsorption and ion exchange capacity. The leachate was introduced intermittently (10 min h⁻¹) to assure hydraulic retention time of 8-12.5 days. In the vertical systems, COD removal was 15%-42%, while it

reached up to 61% in the horizontal one. Orthophosphate-P removal in the vertical systems was 30%-83%, while in the horizontal one, it varied between 26.3 and 61.0%, depending on the climate conditions (a month of determination). The removal of $\text{NH}_4\text{-N}$ was better in vertical systems (36.8-67.4) in comparison to the horizontal one (17.8-49.0). The authors also presented the removal of heavy metals from the leachate. The concentration of Cr and Zn increased in the effluent, suggesting that they were washed out of the system. The iron removal decreased with time and was below 50%. The removal of lead varied between 30%-90%. It was concluded that the vertical system with zeolite layer was beneficial, especially in terms of ammonia removal.

In the study of [77], the activated sludge system with sequencing batch laboratory reactor mode was employed for the treatment of landfill leachate, containing 4,298-5,547 mg L^{-1} of COD, 913-1,017 mg L^{-1} of BOD_5 , 13,971-17,421 mg L^{-1} of TDS, and 72-374 mg L^{-1} of $\text{NH}_4\text{-N}$. Biomass was in the form of granules (0.36-0.60 mm, in cylindrical shape), the working volume of the reactor was 3 L in the operational mode of 12-h cycle (60 min of feeding, 640 min of aeration, 5 min of settling, and 5 min of effluent discharge). The reactor operated for three months with 182 cycles. The average COD removal was 82.8%-84.4%, while the ammonium removal efficiency reached $62\pm 8\%$. The authors confirmed the high impact of initial ammonium N concentration on the performance of nitrification, which was confirmed by the simultaneous experiment with pretreated landfill leachate, where $\text{NH}_4^+\text{-N}$ was reduced. To obtain a high removal of organics, a pretreatment in terms of ammonium removal was proposed.

Removal of nutrients, especially ammonium nitrogen, from landfill leachates using a biological system was also studied by [78]. A batch reactor ($V=150$ L) was used for the treatment of homogenous mixture of old and freshly produced leachate from the body of the Tunisian landfill. The first treatment step involved an anoxic process preceded by aerobic processes. In the anoxic phase, COD reduction reached 46%, TOC was reduced to 65%, while ammonium N removal was 45%. Afterwards, the treated leachate was led to three aerobic submerged biological reactors, where a 7-day retention time was employed. As a result, high overall treatment efficiencies were obtained in BOD_5 , COD, and the $\text{NH}_4^+\text{-N}$ removal was 95%, 94%, and 92%, respectively.

MBR also gained a lot of attention lately for the treatment of landfill leachate [73]. They are often referred to as an efficient and robust alternative to other systems, in spite of their higher operational costs. They are essentially composed of two parts; i) a bioreactor dealing with the removal of organics and ii) a membrane module for the separation of the treated leachate and biomass. In comparison to the conventional activated sludge systems, they allow for complete retention of the biomass in the system; this way, the settling characteristics of the sludge are less important. As a result, the system can be operated at much higher concentrations of biosolids, up to 20 mg L^{-1} , with very clean effluent. Additional important advantages are also higher loading rates, smaller volumes, lower production of excess sludge, and easier development of microorganisms with lower growth rates. MBR systems are usually designed as ultrafiltration or nanofiltration in a hollow fiber, plate, or frame; they could be in a flat or tubular configuration with continuous stirred tank reactors, plug-flow systems or sequencing batch reactors.

A submerged MBR for the treatment of heavily polluted landfill leachate, containing 18,685 mg L⁻¹ of COD and 310 mg N per liter was used in reference [3]. Biologically treated leachate was additionally filtrated, using nanofiltration and reverse osmosis. The biological stage was very effective, removing 89% of COD and 85% of the total Kjeldahl nitrogen (TKN). In MBR, polyeter sulfone ultrafiltration membrane was installed as a submerged module. A system with the volume of 4 L operated as SBR. The landfill leachate was fed daily (300 mL) and the lack of phosphorus was overcome by the addition of KH₂PO₄. The system reached steady-state operational mode after 4 months with 9 g L⁻¹ of biomass. COD removal stabilized at 89%. Some portion of inert COD was entrapped in the reactor. Due to the unstable pH conditions, effective nitrification was not achieved; it was probably also reduced due to the air stripping of NH₄-N at pH=8.6 and the consequent lack of the ammonium.

However, sometimes it is impossible to distinguish clearly between systems with suspended and attached biomass, since the combination of advantages of both treatment systems is sometimes the most optimal to assure the environmentally and legislatively acceptable performance. A combination of a cross-flow MBR and MBBR for the treatment of stabilized leachate was, for example, used in reference [79]. The treatment was focused on the removal of ammonium N, present in stabilized landfill leachate up to 3,000 mg L⁻¹. A combination of pure oxygen MBR and the subsequent MBBR was used for the nitrification and the denitrification, respectively. The volume of the membrane bioreactor was 500 L and it contained ultrafiltration ceramic membrane, while the volume of the MBBR was 540 L. Ammonium was oxidized only to a nitrite to reduce oxygen consumption for 25%, in comparison with the much higher oxygen consumption in the case of its complete conversion to nitrate. The authors obtained a 90% conversion of the ammonia N to nitrite with the sludge retention time of over 45 days. The system also enables up to 40% savings in the COD demand for nitrification. It was possible to oxidize more than 95% of total N inflow, and effluent ammonia concentrations were always below 50 mg L⁻¹.

Fixed bed filters offer a higher resistance to the toxic compounds and lower temperatures [80]. Aerated filters have been used for efficient treatment of landfill leachate [81]. In the study of [82], two systems using attached biomass were compared. Two MBBR systems using small free-floating polyurethane elements and granular activated carbon (GAC) as biomass carriers were set up. The laboratory SBR system had a volume of 6 L, and the inflow leachate contained NH₄-N, COD, and BOD₅ in the average values of 1,800 mg L⁻¹, 5,000 mg L⁻¹, and 1,000 mg L⁻¹, respectively. According to the low BOD₅/COD ratio=0.2 and pH=7.5, the landfill leachate could be characterized as a stabilized one. The study was conducted in two separate treatment cycles, the first one with cube-shaped polyurethane (30 g/reactor), while in the second one, 90 g of GAC (1,100 m² g⁻¹) was added. Nitrification and denitrification processes also occurred, but the need for additional external dosage of carbon source was emphasized. However, these processes efficiently and almost completely removed nitrogen, accompanied by the sufficient removal of COD (up to 81%), BOD₅ (up to 90%), and turbidity.

A bioreactor cascade with a submerged biofilm was also successfully used for a young landfill leachate treatment. Three reactors, each with the working volume of 18 L were used. The biofilm support was made from PVC synthetic fiber (57 m² m⁻³). The reactors were inoculated

with sludge from wastewater treatment plant and the biofilm consisted of various microorganisms, while bacterial groups such as *Bacillus*, *Actinomyces*, *Pseudomonas*, and *Burkholderia genera* were assumed to be responsible for the simultaneous removal of organic carbon and nitrogen. Bioreactor operated at a hydraulic retention time of 12 h, under organic loading charges 0.6 to 16.3 kg TOC m³ day⁻¹. TOC removal rate varied between 65% and 97% and the total reduction of COD reached 92% without initial pH adjustment. The removal of total Kjeldahl nitrogen for loading charges of 0.5 kg N m³ day⁻¹ reached 75%. However, pH increased during the experiments and caused biofilm separation and a decrease of attached solids concentration, which consequently reduced the carbon and nitrogen removal. When pH was adjusted to 7.5, nitrogen removal improved to 85% at a loading charge of 1 kg N m³ day⁻¹ [83].

A pilot-scale submerged aerobic biofilter (SAB) with a working volume of 178 L and packed with polyethylene corrugated Racshig rings was used in reference [68]. Compressed air was used for aeration. It was continuously operated, with a hydraulic retention time of 24 h, and inoculated with activated sludge biomass. The co-treatment of domestic wastewater with a large content of biodegradable organic matter, and old landfill leachate with high total ammonium nitrogen (TAN) and extremely low BOD/COD ratios, was evaluated. The leachate volumetric ratios 2 and 5 v/v.% were tested. The best results were obtained at a volumetric ratio of 2 v/v.%, where 98% of the BOD, 80% of the COD and DOC, and 90% of the total suspended solids (TSS) were removed. Here, the poorly biodegradable organic matter in leachate was removed by partial degradation. When leachate was added at a volumetric ratio of 5 v/v.%, biodegradation of the low biodegradable organic matter was less efficient and its concentration decreased primarily as a result of dilution. The total ammonium nitrogen was mostly removed (90%) by nitrification.

Generally, the treatment efficiency of the landfill leachate treatment is evaluated according to operational parameters of the investigated treatment plant, and on the basis of analyses of landfill leachate prior and after treatment. Non-specific parameters such as COD, BOD₅, and DOC are routinely determined, as well as the metallic content, concentrations of different ions and some organic pollutants. However, the identification of particular problematic contaminants (pesticides, personal care products, pharmaceuticals, endocrine disruptors, etc.) is difficult and impractical because of analytical limitations (low concentrations, complex extraction methods before analytical procedure, reactivity of the components, etc.), the uncertainty surrounding their bioavailability and the complexity of leachates [67], including possible additive, antagonistic, and synergistic effects of contaminants. As a result, there is a lack of studies dealing with complete determination of reduction of the landfill leachate hazardous environmental impact, which is most suitably determined by the battery of biotests [84]. Only a few studies addressing this problem could be found [56, 67, 70, 85].

4.1.2. Treatment with activated sludge under anaerobic conditions

The anaerobic biodegradation plays an important role in different environmental compartments, such as eutrophic lakes, soils, or sediments, while anaerobic digestion technology for waste and wastewater treatment as well as soil remediation is growing worldwide because of its economic and environmental benefits [12, 46]. The most favorable property of the anaerobic

process is the biogas production, contributing to the renewable energy generation. Aerobic systems are suitable for the treatment of low strength wastewaters ($BOD_5 < 1,000 \text{ mg L}^{-1}$), while anaerobic systems are suitable for the treatment of highly polluted wastewaters ($BOD_5 > 4,000 \text{ mg L}^{-1}$), which is usually not the case when stabilized leachates are discussed. At the same time, after the initial aerobic (acetogenic) phase, landfills actually become anaerobic digesters by themselves [2]. The leachate produced after this phase has already been subjected to anaerobic digestion, so there is little additional treatment efficiency obtained by anaerobic treatment of such leachates. At the same time, anaerobic systems produce an effluent still containing a very high concentration of ammonium nitrogen, which needs a further aerobic stage, necessary to nitrify the anaerobic effluent to be suitable for a watercourse discharge [74, 86]. On the other hand, no such second stage is needed for the aerobic process [11]. Thus, the use of anaerobic-aerobic processes can lead to a reduction in operating costs compared to aerobic treatment alone, while simultaneously resulting in higher organic matter removal efficiency, efficient removal of nitrogen, and a lower waste sludge production. Anaerobic-aerobic systems have received a great deal of attention over the past few decades due to their numerous advantages, not only with regard to the municipal wastewater, but also the sanitary landfill leachates (see Chapter 3.2). Aerobic-anaerobic systems incorporate advantages of both approaches [59]. They could be integrated bioreactors with or without physical separation of aerobic-anaerobic zones, the zones could be switched due to the sequencing mode of operation or they could employ combined culture of anaerobic and aerobic microorganism [74]. They usually achieve more than 70% of COD removal in a short hydraulic retention time (hours-days).

Anaerobic degradation of wastewaters is a very complex and dynamic system, where microbiological and physico-chemical aspects are strongly linked. This is the reason why granular anaerobic sludge is often applied in various treatment processes, allowing higher loading rates in comparison to conventional systems with dispersed sludge. One of the examples is also the upflow anaerobic sludge blanket reactor (USBAR), where wastewater is flowing through a dense bed of sludge with high microbial activity. Granules, which are formed due to the natural self-immobilization of anaerobic bacteria, have a diameter of 1-4 mm. The system could be affected by the presence of suspended and colloidal components of the influent, such as fats, proteins, or cellulose, but these components are usually not typical for landfill leachates. USBAR system is well known by its high biomass concentration, high organic loading rates and short hydraulic retention times, a lack of bed clogging, low mass transfer resistance, and large surface area. Another version of the USBAR reactor is the expanded granular sludge bed reactor (EGSB) with a high upflow liquid velocity above 4 m s^{-1} and a large height/diameter ratio (> 20) to intensify mixing [74].

Some of the typical treatment systems with prevailing anaerobic conditions, if not completely anaerobic, are presented in Table 6. The systems are in design more or less similar to aerobic ones, presented in Table 5. Anaerobic rotating biological reactors (Table 5, Table 6) are comparable to aerobic ones; they are only covered to avoid contact with air. In both systems there is a series of rotating discs, partly or completely immersed in a reactor through wastewater flows. The system is not energy demanding and it is able to deal with a wide range of flows [74].

Systems with suspended-growth biomass	Systems with attached/immobilized biomass
Activated sludge (AS): <ul style="list-style-type: none"> · Continuous flow reactors · Sequencing batch reactors (SBR) 	Filters: <ul style="list-style-type: none"> · Upflow system · Downflow system
Membrane bioreactors (MBR): <ul style="list-style-type: none"> · External membrane module · Submerged/Immersed membrane module 	Fluidized bed: <ul style="list-style-type: none"> · Sand carrier of biomass · Activated sludge carrier of biomass
	Upflow anaerobic sludge blanket (UASB): <ul style="list-style-type: none"> · Expanded granular sludge bed (EGSB)
	Moving bed bioreactors (MBBR) <ul style="list-style-type: none"> · Rotating biological contactors

Table 6. Typical treatment system with anaerobic microorganisms in landfill leachate treatment [2, 74, 86].

A possibility of biological treatment in an anaerobic submerged membrane bioreactor was studied in reference [87]. The treatment efficiency under different feeding conditions with different dilution rates of the stabilized leachate and synthetic wastewater (5-75 v/v%) was studied. It contained 2,800-5,000 mg L⁻¹, 1,950-3,650 mg L⁻¹, and 751-840 mg L⁻¹ of COD, chloride, and ammonium, respectively. The capacity of the reactor was 29 L and it contained submerged membrane bioreactor with the capillary ultrafiltration module. Reactor was fed with granular sludge, obtained from industrial wastewater treatment plants, and experiments were carried out at 35°C. The effluent from anaerobic reactor was then further treated using reverse osmosis. Treatment was the most efficient at 20 v/v.% of landfill leachate and the system was able to remove up to 90% of COD. For leachate concentration above 30 v/v.%, significant decrease of anaerobic treatment efficiency was observed, probably due to the toxicity of the landfill leachate.

The combination of anaerobic and aerobic reactors was employed in reference [88]. Here, the anaerobic sequencing batch reactor (ASBR) and the pulsed sequencing batch reactor (PSBR), both with 10 L of working volume, were combined to enhance COD and nitrogen removal from the fresh landfill leachate. Anaerobic and aerobic activated sludges from wastewater treatment plants were used to inoculate ASBR and PSBR, respectively. In ASBR, the organics from raw leachate were mainly degraded. During the 157 days long joint operation period, 89.6%-96.7% of COD and 97.0%-98.8% of total nitrogen (TN) removal were achieved. In the effluent, COD and TN were less than 910 mg L⁻¹ and 40 mg L⁻¹, respectively, without any extra carbon source addition. Most of the organics in the raw leachate were used as the carbon source during denitrification. In addition, excess organic polymers such as polyhydroxybutyrate (PHB) and glycogen, stored in biomass, acted as the internal carbon source during endogenous denitrification, confirming the possibility of nitrogen removal without the addition of an extra carbon source. These systems are recently more and more often applied, they stop nitrification at the nitrite stage (nitritation), followed directly by reduction to N₂ in anoxic conditions with carbon addition (denitrification). Nitritation/denitrification is attractive because it reduces up to

25% of the total oxygen requirements at the wastewater treatment plant and thus it could significantly reduce costs.

Another reactor system with the up-flow anaerobic sludge bed (UASB) reactor (working volume 3 L) and a 9-L sequencing batch reactor (SBR) in series was used to treat the landfill leachate, in order to enhance the organics and nitrogen removal [89]. The UASB reactor was inoculated with the anaerobic granulated sludge from the methanogenic reactor at wastewater treatment plant, while the aerobic activated sludge from the wastewater treatment plant was used to seed the SBR. Inhibition of the free ammonia on nitrite-oxidizing bacteria and process control were used to achieve the nitrite pathway in the SBR. During a 623 day long experiment, the maximum organic removal rate in the UASB and the maximum ammonium oxidation rate in the SBR were $12.7 \text{ kg}_{\text{COD}} \text{ m}^{-3} \text{ d}^{-1}$ and $0.96 \text{ kg}_{\text{N}} \text{ m}^{-3} \text{ d}^{-1}$, respectively. COD, TN, and NH_4^+ -N removal efficiencies were 93.5%, 99.5%, and 99.1%, respectively. In the SBR, the nitrite pathway was initiated at low temperatures (14.0°C - 18.2°C) and was maintained for 142 days at temperatures 9.0 - 15°C . Here, stable nitrification was predominantly done by the ammonia-oxidizing bacteria.

An anaerobic pilot-scale sequence batch biofilm reactor (AnSBBR) at room temperature to treat stabilized leachate from a 12-year-old landfill with two extensions, 2 and 5 years, respectively, was used in reference [90]. Leachate was collected from these two extensions. Its COD was $8,566 \pm 2,662 \text{ mg L}^{-1}$, with pH around 7.95. The volume of the reactor was 746 L. It was filled with foam cubes ($4 \times 4 \text{ cm}$ with density of 23 g L^{-1}) as inert biomass support. 110 L of the biomass obtained from the existing stabilization pond was used as inoculum. The reaction time was in a range of 5-7 days with filling time of 15 minutes, while 30 minutes were used for the emptying of the system. The treatment efficiency reached over 70% of COD. The authors also studied the kinetics of the process and confirmed that the AnSBBR reactor can be considered as a good alternative for the pretreatment of landfill leachate, if it is good or at least partially biodegradable.

It can be concluded that the selection of the biological treatment of the landfill leachate is dependent upon many factors and that the techniques, developed at particular site, could not be always efficiently applied elsewhere [91]. This is also one of the reasons for the intensive development of novel concepts, where fungal treatment seems to be one of viable options.

4.2. Fungal treatment

In the last few decades, the white rot fungi have showed great potential for the removal of hazardous and toxic pollutants. They produce various extracellular ligninolytic enzymes, including laccase (Lac) and manganese peroxidase (MnP), which are involved in the degradation of lignin and their natural lignocellulosic substrates. However, these enzymes are even capable to degrade various pollutants such as phenols, pesticides, polychlorinated biphenyls, chlorinated insecticides, organic dyes, and a range of other compounds. They have been mostly applied for treatment of textile wastewaters due to their excellent decolorization and detoxification effect [14]. A few years ago, a successful treatment of a young landfill leachate with different strains of white rot fungi was presented [14], while the fungal treatment of leachate generated in old landfills has not been investigated so far.

4.2.1. Treatment under fungal growth conditions

In general, two strategies can be applied in water pollutant degradation: (i) direct degradation by active biomass in one reactor or (ii) use of extracted enzymes from the culture medium. In the first case, the fungal growth and the enzyme synthesis, as well as the enzymatic degradation of the pollutant take place in the water solution of the pollutant that is in the wastewater. Here, the effect of the pollutant on the microbial growth and the enzyme synthesis must be taken into account. Fungal enzymes can be intracellular or extracellular products, synthesized during growth or after the growth phase. The pollutant depletion from the wastewater can happen due to the enzymatic degradation or only due to the adsorption on the biomass. Under aerobic conditions, aeration of the reactor is necessary; while under anaerobic conditions, methane is produced. Therefore, the reactor for the first strategy should be considered as a gas-liquid-solid phase system with proper mixing and aeration, since microbial biomass, especially when it is immobilized, can be treated as a solid phase [92].

4.2.2. Treatment with fungal enzymes

From the reactor design point of view, a much simpler configuration, which is only a liquid phase system with proper mixing, can be applied in the second strategy, when only enzyme is to be added to the wastewater. Here, the effect of the pollutant on the enzyme inhibition should be considered [92].

4.2.3. Factors affecting fungal activities

The fungal growth and enzyme production are influenced by numerous factors. *Media composition* has enormous effect on the fungal growth and the production of their degradation systems. In general, a special attention has to be focused on the carbon and nitrogen sources, together with mineral nutrients and other additives. The composition of landfill leachates varies with location and especially with age and is consequently a result of aerobic and later anaerobic conditions in the main body of the landfill.

A *carbon source* is necessary for the growth and enzyme production. In the research during fungal cultivation studies, organic compounds such as glucose, sucrose, starch, and similar have been used. A young landfill leachate usually contains highly biodegradable volatile fatty acids, while in an old landfill leachate, refractory humic and fulvic acids are present, and biodegradable carbon source in various forms must be added to allow fungal growth and enzyme production. A landfill leachate usually also contains toxic phenols and xenobiotics. The white rot fungi can use inorganic as well as organic *nitrogen sources*. Nitrogen demands for growth and especially enzyme production differ markedly between fungal species. In the case of *P.chrysosporium*, production of ligninolytic enzymes is more effective under the conditions of nitrogen limitation, while *B.adusta* produces more LiP and MnP in nitrogen-sufficient media. A landfill leachate usually contains higher concentrations of inhibitory ammonium nitrogen, which must be considered during research. All microorganisms have certain requirements for other medial components, such as mineral nutrients. For example, the white rot fungi need iron, copper, and manganese. However, besides the mentioned metals,

a landfill leachate usually contains excess concentrations of toxic heavy metals, which suppress microbial activities [93].

The majority of the filamentous fungi, along with the white rots, grows and produces enzymes optimally at acidic pH values. However, one must distinguish between the optimum pH for growth and enzyme production, the optimum pH for the action of isolated enzymes, and the optimal pH for pollutant degradation [93].

Temperature has to be considered from its influence on the growth and enzyme production, the enzymatic action and the temperature of the waste stream. Most white rot fungi are mesophiles with the optimal cultivation temperature of 27°C-30°C, while optimal temperatures for enzyme reactions are usually higher, but below 65°C [14, 93].

Ligninolytic fungi are obligate aerobes and therefore need oxygen for growth and ligninolytic enzyme synthesis. The oxygen demand depends on the fungus and their ligninolytic system. In addition, leachate treatment also requires oxygen. The problematic of the oxygen supply to the culture media during cultivation has been covered in numerous papers. The major problem is the low oxygen solubility in water (only 8 mg L⁻¹ at 20°C). To enhance the oxygen gas-liquid mass transfer and to satisfy the microbial oxygen requirements during cultivation, aeration and agitation are necessary. This can affect the fungal morphology and lead to the decreased rate of enzyme synthesis [94]. As a result of this, various types of bioreactors have been designed, generally divided into static and agitated configurations. The choice of the reactor depends on the particular system, although the appropriate gentle agitation gives as good or even better results as those from static conditions [14, 95].

It is important to optimize an initial leachate concentration for successful degradation in terms of BOD, COD, and ammonium nitrogen removal, as well as detoxification. Namely, leachates are usually toxic to the microorganisms, while the toxicity depends on the type and age of the leachate. This problem can be easily solved by mixing with other types of the wastewaters, mainly the municipal sewage, as already discussed in Chapter 3.2.

4.2.4. Reactors for fungal treatment

A variety of reactor configurations can be used, similar to those for fungal cultivation under submerged conditions. In general, reactors may be divided into two general groups, relying on the situation with the microbial cells. With the first type, the cells are kept in suspension, freely or attached to some support, where they grow and perform their metabolic activities. Suspension can be maintained by a mechanical device, such as a stirrer, through the sparging of air or both. Gentle mixing and aeration have usually been the necessary prerequisites for a successful biomass growth and enzyme production. These reactors are mainly used for the production of biomass or metabolites in various brands of biotechnology, and also applied as activated sludge systems in wastewater treatment technologies. On the other hand, there are many kinds of reactors in the group, where the cells are immobilized. Here, the cells are attached on some type of support, which is in a fixed position within the reactor volume. In these reactors, the attached and active biomass is used for a certain purpose, such as removal of the pollutants from the wastewater. It is shown that batch and continuous operations are

effective, both having advantages and disadvantages. Reports in several papers have demonstrated that the repeated use of mycelia over several cycles of decolorization can last from several weeks to a few months [14, 95].

Most of the studies were done under aseptic conditions, while some were effective also during non-aseptic conditions. Toxicity of the leachate strongly affects the degradation and decolorization. For a successful design of a process with the given capacity, the reactor shape and size as well as its operation mode must be selected, and operating conditions, such as concentrations, flow rates, temperature, and pH, must be defined. For the calculation of the necessary pollutant conversion, the degradation reaction rate must be estimated on the basis of literature data or laboratory experiments. This allows for the calculation of time in the batch mode or flow throughput in continuous mode. Typical bioreactors for bioremediation purposes are presented in Table 7 [96, 97].

Bioreactors with suspended-growth biomass	Bioreactors with attached/immobilized biomass
Stirred tank	Packed bed
Bubble column	Trickling filter
Fluidized bed (activated sludge)	Fixed film
	Rotating biological contactor

Table 7. Typical fungal bioreactors for removal of pollutants [96, 97].

4.2.5. Review of landfill leachate treatment studies with fungi

Various experiments were done in different types of cultivation vessels. Laboratory equipment, such as multiwell plates and Erlenmeyer flasks, as well as packed bed aerated columns with immobilized culture, was used in the experiments. The aim was mainly to reduce COD, color, and toxicity with various fungal cultures, mostly under sterile conditions. However, the experiments were done with pure and diluted landfill leachates, while the effects of ammonium-N and additional substrate were also investigated. The experiments and their results are presented in Table 8 and briefly discussed in the paragraphs that follow.

Four ecotoxicological assays were used to assess the toxicity of a raw landfill leachate and a mixture of a raw landfill leachate and effluent coming from traditional wastewater treatment plant. All tested organism indicated that both samples exceeded the legal threshold value, showing the ineffectiveness of activated sludge treatment in the reduction of toxicity. To investigate the potential of fungal enzymes for bioremediation, the autochthonous mycoflora of the two samples was evaluated by filtration, because they are already acclimatized to their toxic environment. Ascomycetes showed to be the dominant fraction in both samples, followed by basidiomycetes. However, the samples may represent a risk for human health, since some emerging pathogens were present. A decolorization screening with autochthonous fungi was done with both samples in the presence or absence of glucose. Eleven fungi basidiomycetes and ascomycetes gave promising mycoremediation results by achieving up to 38% decolorization yields [98].

A biological process of landfill leachate treatment with the selected fungal strains on the basis of experiments under sterile conditions in Erlenmeyer flasks was developed. Samples with different concentrations (10%-100%) of young landfill leachates (LFL) with high contents of organic matter, ammonia, salts, heavy metals, phenols and hydrocarbons were treated with *Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger*. COD removal efficiencies for *P. chrysosporium*, *T. trogii* and *L. tigrinus* were of 68%, 79% and 90%, respectively, with a two-fold diluted LFL. COD reductions were accompanied by high-enzyme secretion and toxicity reduction, expressed as percent bioluminescence inhibition (< 20%). The effluent was toxic to these strains at LFL concentrations higher than 50% and caused growth inhibition. On the other hand, *A. niger* showed to tolerate raw LFL, since it grew in this media, compared to other tested strains. However, this strain is inefficient in removing phenols and hydrocarbons since toxicity reduction was very low [15].

Landfill leachates, containing high concentrations of phenols and hydrocarbons as well as ammonium nitrogen, and exhibiting high COD and high toxicity, were treated in laboratory experiments by selected strains of white rot fungi (*Trametes trogii* and *Phanerochaete chrysosporium*), with the aim of landfill leachate detoxification. Since high amounts of ammonium nitrogen are problematic for microorganisms, its effect on mycelia growth and enzyme secretion was studied first by adding NH_4Cl to the growth media. Results obtained during the 14 days of cultivation showed that ammonium chloride was not problematic below 2 g L^{-1} , while at 5 g L^{-1} , inhibition of growth and enzyme secretion occurred. Experiments with *T. trogii* and *P. chrysosporium* in 50 v/v.% of the leachate showed 79% and 68% COD removal efficiencies. High enzyme secretion for each strain and a reduction in phenols and hydrocarbons concentrations occurred and consequently, important reduction in the toxicity was achieved [99].

Biotreatability of mature municipal landfill leachate with white rot fungus and its extracellular ligninolytic enzymes was studied in Erlenmeyer flasks. Leachates were obtained from one active and one closed regional municipal landfill, which operated for many years. Both leachates were polluted by organic and inorganic compounds. The white rot fungus *Dichomitus squalens* was able to grow in the mature leachate from the closed landfill. Since it utilized present organic matter as a carbon source, the results showed 60% of DOC and COD removal and decreased toxicity to the bacterium *Aliivibrio fischeri*. In the leachate from the active landfill, the growth of the fungus was inhibited. However, when the leachate was treated with crude enzyme filtrate containing extracellular ligninolytic enzymes, 61% and 44% removal of COD and DOC was reached. In addition, a significant detoxification was proved with the bacterium *Aliivibrio fischeri* and plant *Sinapis alba*. Results showed that the fungal and enzymatic treatment is a promising biological approach for the treatment of mature landfill leachates, so this type of research should continue [70].

Color removal of a landfill leachate in a continuous experiment by immobilized *Trametes versicolor* on polyurethane foam was studied in a 3 L aerated column. Initially, batch experiments in Erlenmeyer flasks under sterile conditions were conducted to find the optimum, pH, optimum co-substrate dose, leachate dilution and contact time for microbial growth, and color removal. The same immobilized fungi under optimal conditions were used after 4 and 15 days

of initial growth in several 5-day cycles to test the reuse of fungi. Experiments with the diluted leachate were done to check the effect of organic loading on color removal. Glucose was used as a co-substrate. Results show that the same immobilized fungi can be reused for at least four cycles, each 5 days long. By using 4-day initially grown biomass, the dilution of leachate did not significantly increase the color removal efficiency without additional glucose. However, about 50% better results in color, BOD and COD removal were obtained by addition of 3 g L⁻¹ glucose to the concentrated leachate. Using 15-day initially grown biomass, slightly better color removal was achieved with concentrated leachate. About a twofold increase in color removal in five times diluted leachate was achieved with this biomass, when glucose was added. Results show that *T. versicolor* is capable of treating a highly contaminated landfill leachate and can be considered as a potentially useful microorganism [100].

Trametes versicolor and *Flavodon flavus* immobilized on PUF cubes were used for treatment of landfill leachate. Experiments were done in shaken flasks at room temperature. Effects of pH and co-substrates (glucose, corn starch, and cassava) were investigated at different contact times. Treatment efficiency was evaluated based on color, BOD, and COD removal. For both types of fungi, the optimum pH was 4, the optimum co-substrate concentration was 3 g L⁻¹ and the optimum contact time was 10 days. Depending on co-substrate and fungus, the addition of co-substrate at optimum conditions could remove 60-78% of color and reduce 52%-69% of BOD and COD. Promising results prove a potential usefulness of the white rot fungi in the treatment of landfill leachate [101].

Treatment of the landfill leachate by mycelia of *Ganoderma australe* immobilized on Ecomat (organic fibers made from oil palm empty fruit bunches), packed in a 0.63 L column, was investigated. Continuous recycling of 1 L of raw leachate and 50 v/v.% diluted leachate at a constant flow (20 mL min⁻¹) was operated for 10 cycles. The results were evaluated on the basis of pH and COD, BOD and ammonium nitrogen removal. Only slight BOD removal was achieved for the raw leachate, while no effect was observed for the diluted sample. COD removal occurred after each cycle with the diluted leachate. Higher COD removal (51%) was observed with the diluted leachate, compared to the raw leachate (23%), after the tenth cycle. Ammonium nitrogen was also reduced after cycle 8 for the diluted (46%) and the raw leachate (31%). The results suggest that the white rot fungus *G. australe* can be considered as a potential candidate in the landfill leachate treatment [102].

Combined fungal and bacterial treatment was also studied. A sequential process in two 10 L laboratory reactors was carried out using a fungal sp. (*Phanerochaete sp.*), followed by a bacterial sp. (*Pseudomonas sp.*) for the degradation and detoxification of contaminants in the landfill leachate. The optimization of cultivation and process parameters for individual fungal and bacterial isolates was done with Box-Behnken design (BBD) and response surface methodology (RSM) for three variables (C source, N source and duration), considering two responses (% of COD and color removal). Treatment in the sequential bioreactor under optimized conditions removed 76.9% of COD and 45.4% of color. In addition, no statistically significant DNA damage of the cells at the end of the treatment was observed, allowing the effluent to be discharged [103].

Type of reactor	Volume	Organism	Type of leachate	Duration	Measured parameters	Removal efficiency	Reference
Multiwell plates	2.5 mL	autochthonous mycoflora	Data not given	20 days	Color	up to 38%	[98]
Erlenmeyer flasks	100 mL	<i>Trametes trogii</i> , <i>Phanerochaete chrysosporium</i> , <i>Lentinus tigrinus</i> , <i>Aspergillus niger</i>	Young	15 days	Toxicity COD	up to 80% up to 90%	[15]
Erlenmeyer flasks	100 mL	<i>Trametes trogii</i> , <i>Phanerochaete chrysosporium</i> ,	Data not given	14 days	COD Toxicity	up to 79% up to 50%	[99]
Erlenmeyer flasks	100 mL	<i>Dichomitus squalens</i>	From young landfill From closed landfill	7 days	COD DOC, Toxicity	61% 44% 42%	[70]
Packed bed column, immobilized culture on PFU foam	3 L	<i>Trametes versicolor</i>	Young	20 days	Color	up to 78%	[100]
Immobilized culture on PFU foam	100 mL	<i>Trametes versicolor</i> , <i>Flavodon flavus</i>	Fresh	15 days	Color BOD COD	up to 78% up to 69% up to 57%	[101]
Immobilized culture	600 mL	<i>Ganoderma australe</i>	Data not given	10 cycles (50 min each)	COD NH ₃ -N	Raw leachate up to 23% Diluted leachate up to 52 % up to 46%	[102]

Table 8. Overview of some fungal landfill leachate treatment studies [15, 70, 98-102].

5. Conclusion and future perspectives

Many municipal as well as industrial wastes still end at the landfill, and consequently the amount of deposited wastes is significant worldwide. Even that waste separation is increasing, organics are still in the landfill and provide good environment for microbial processes, resulting in biogas and leachate production. The composition and the amount of leachate vary with the age and are dependent upon many factors. Significant components of the leachate are organic compounds, which are degradable at the beginning of landfill operation and become more and more persistent and potentially hazardous during the biotic and abiotic processes

in the landfill body. These toxic substances should be properly removed to prevent the environmental pollution.

Besides the physico-chemical and chemical methods of leachate treatment, the biological treatment in the form of recycling and combined treatment with domestic sewage, as well as bacterial treatment with the activated sludge under aerobic and anaerobic conditions, have gained significance in the last decade. In addition, the treatment using white rot fungi and their extracellular enzymes seems to be a promising method for the removal of biodegradable and refractory organic matter from the landfill leachate. Before application to the industrial scale (scale-up), research in laboratory and pilot-plant scale are usually required from the engineering point of view. Taking this into account, laboratory toxicity and biodegradability tests are already "a must" at the beginning of the leachate treatment process for both bacterial and fungal processes. However, the activated sludge processes are more than a step forward when compared to fungal processes. From the literature review, experiments with the active sludge have been done in pilot plant reactors with volumes from several liters and a few hundreds of liters, up to lagoons with 60-80 m³ in non-sterile conditions, which are close to an economically justified situation. It can be expected that the practical application of bacterial landfill leachate treatment will increase in the near future. On the other hand, much less data from the research with fungi is available. Even if this data is promising in regards to the biodegradability tests, it is obtained mainly in the laboratory experiments, mostly in Erlenmeyer flasks under sterile conditions. Therefore, much more time, measured in a decade or so, will be needed for the transfer to a large scale, probably only for a particular and economically tolerable process. A considerable amount of work has been done in the biological treatment of landfill leachate, but there is still a gap regarding mathematical modeling of this process, which has not gained in significance as it has in other fields of biotechnology. Therefore, this engineering tool should be further investigated and applied.

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