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Chapter 1

# Fungi in Landfill Leachate Treatment Process

Yanan Ren and Qiuyan Yuan

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**Abstract**

The landfill leachate has high concentration of COD, ammonia and other recalcitrant composition compounds. The amount of eachwhich is mainly largely dependent on the age of the landfill. The conventional leachate treatments can be classified as chemical-physical treatments and biological treatments. Using fungi to treat leachate is an emerging research topic. Fungi, with their excellent recalcitrant compound degradability, have been used to treat industrial wastewater that contains toxic or recalcitrant compound. Due to the complex composition and toxicity of landfill leachate, fungi have showed shown better removal efficiency in terms of COD, toxicity and color removal than the conventional leachate treatment. White rot fungi species and yeast are so far the two species that have been studied in treating landfill leachate. Future research should be extended to the other fungi species as well asand also on the impact of ammonia in landfill leachate on the fungi treatment process.

**Keywords:** Fungi, landfill leachate, recalcitrant compound, COD removal

## 1. Introduction

Landfill leachate is produced by the seeping of liquids through landfilled waste. Rain water or melted snow percolating into the waste, as well as the original water content or humidity of the waste itself and the degradation and compaction of the organic fraction, all contribute to the generation of leachate[1,2]. Landfill leachate contains dissolved organic matter, inorganic macro components, heavy metals, and xenobiotic organic compounds such as halogenated organics. These contaminants play an important role in groundwater and soil pollution. Due to the complexity of the pollutants in the leachate, the treatment of landfill leachate is complicated, usually requiring various processes to reduce COD, nitrogen, and phosphorus all of which make the treatment of landfill leachate expensive.

The conventional landfill leachate treatment includes physico-chemical treatments, and biological treatments. Physico-chemical treatments are usually used to reduce suspended solids, colloidal particles, color, and certain toxic compounds. However the cost associated with this type of treatment usually is high. On the other hand, biological treatment has been shown to be very effective in removing organic and nitrogenous matter from the leachate, especially when the BOD/COD ratio is high (>0.5)[3]. Biological treatments is gaining more popularity due to its relatively low cost and high sustainability. Among the various biological treatment, bacteria are the most common microorganisms that are used. Recently, fungi, with their high tolerance and resistance to toxicity, have been recognized as an excellent candidate for treating leachate.

Fungi were first studied to treatas the treatment for industrial wastewater to remove recalcitrant compounds. Fungi showed excellent degradability of recalcitrant compounds. In addition, good removal of COD and color was achieved. Lately fungi, especially white-rot fungi, have been applied in leachate treatment. Research has shown that white-rot fungi have developed nonspecific mechanisms to degrade an extremely diverse range of very persistent or toxic environmental pollutants [4]. The biodegradation capacity of organic pollutants by white-rot fungi is correlated with their ability to secrete extracellular enzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP), and laccases [5]. Besides white-rot fungi, yeast is the other fungi specie that has been studied. Yeast has a high capacity of to breaking and assimilating assimilate difficult degradation pollutants in leachate. Several genera of yeast have been documented as been able to degrade complex organic compounds [6].

This literature review aimed [1] to understand the unique characteristics of leachate and the current treatment methods, [2] to review the fungi treatment process in wastewater and leachate, and [3] to examine the operating constraints and the important affecting factors of the fungal process. In addition, the future of fungal treatments on leachate will also be discussed.



## 2. Landfill leachate characteristics

Landfill leachate are any liquid that passes through wastes and different artificial layers that is collected in the bottom of landfill. The leachate flow rate is influenced by precipitation, surface run-off, and infiltration or intrusion of groundwater percolating through the landfill. Leachate production depends on the water content and the degree of compaction of the waste. The production of leachate is generally greater whenever the waste is less compacted, since compaction reduces the filtration rate [7].

Landfill leachate may be characterized as a water-based solution consisting of four groups of contaminants: (1) dissolved organic matter, such as alcohols, acids, aldehydes, and short chain sugars; (2) inorganic macro components, which include common cations and anions (e.g.,

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sulfate, chloride, iron, aluminum, zinc, and ammonia); (3) heavy metals (i.e., Pb, Ni, Cu, Hg, etc.); and (4) xenobiotic organic compounds such as halogenated organics (e.g., PCBs, dioxins, etc.)[1].

The composition of landfill leachate mainly depends on the age of the landfill. When water percolates through the waste, it promotes and assists the process of decomposition by microorganisms. During the decomposition process, the by-products are released either in the leachate or as the gas. The decomposition process also rapidly uses up available oxygen creating an anoxic environment followed by anaerobic environment. Young landfill, usually it contains large amounts of biodegradable organic matter, which leads to a rapid anaerobic fermentation. This results in the production of volatile fatty acids (VFA) as the main fermentation products [8]. The early phase of a landfill's lifetime is called the acidogenic phase and leads to the release of large quantities of free VFA, which can be as much as 95% of the organic content [9]. In the mature landfill, the methanogenic phase occurs when the methanogenic microorganisms develop in the waste. In this phase, methanogenic microorganisms convert VFA to biogas ($CH_4$, $CO_2$). The organic fraction of leachate decreases as the landfill age increases. Eventually, the main compounds in a matured landfill leachate are nonbiodegradable. Table 1 illustrates characteristics in different landfill age phases [3,10].

| | **Young** | **Intermediate** | **Old** |
|---|---|---|---|
| Age | < 5 | 5-10 | >10 |
| pH | 6.5 | 6.5-7.5 | >7.5 |
| COD | >10,000 | 4,000-10,000 | <4,000 |
| BOD/COD | >0.3 | 0.1-0.3 | <0.1 |
| Organic compounds | 80% VFA | 5-30% VFA + Humic and fulvic acids | Humic and fulvic acids |
| Heavy metals | Low-medium | Low | Low |
| Biodegradability | High | Medium | Low |

**Table 1.** Landfill leachate characteristics [1,2]

Leachate, when it emerges from a typical landfill site, is black, yellow or orange in color, and can be slightly cloudy. The smell of leachate is acidic, offensive and pervasive due to the presents of hydrogen, nitrogen and sulfur rich organic species such as mercaptans [11]

## 3. Conventional leachate treatment

Conventional leachate treatments can be classified as chemical, physical, and biological treatments. However, in order to meet stringent quality standards for direct discharge of leachate into the surface water, an integrated method of treatment is commonly used [12].

### 3.1. Physical and chemical treatment

Physicochemical treatments are usually used for preliminary leachate treatment and final polishing treatment, including reduction of suspended solids, colloidal particles, color, and toxic compounds.

#### *3.1.1. Coagulation and flocculation*

Coagulation and flocculation are widely used as a pretreatment, prior to biological or reverse osmosis step, or as a final polishing treatment step in order to remove nonbiodegradable organic matter. Aluminum sulfate, ferrous sulfate, ferric chloride, and ferric chlorosulfate are commonly used as coagulants [10].

Several studies have been conducted on the examination of coagulation and flocculation for the treatment of landfill leachates. Those studies aimed at performance optimization, i.e., selection of the coagulant, determination of operational conditions, evaluation of the effect of pH, and investigation of the addition of flocculants [13]. Depending on the landfill age and type of coagulant, the COD removal rate is in the range of 20% to 90%.

#### *3.1.2. Chemical oxidation*

Chemical oxidation is used to treat leachate that contains soluble organic substance (which cannot be removed by physical separation), nonbiodegradable, and/or toxic substance not suitable for biological oxidation [14].

Recently, there has been growing interest in advanced oxidation processes (AOP). Most of them, except simple ozonation ($O_3$), use a combination of strong oxidants, e.g., $O_3$ and $H_2O_2$, irradiation, e.g., ultraviolet (UV), ultrasound (US), or electron beam (EB), and catalysts, e.g., transition metal ions or photocatalyst [3]. For instance, the efficiency of COD removal by using a Fenton reagent varied from 60% to 75% for mature and biologically pretreated leachate, respectively [15].

#### *3.1.3. Air stripping*

Air striping is the most commonly used method for eliminating a high concentration of NH4+-N in the wastewater. High levels of ammonium nitrogen are usually found in landfill leachate. In many applications, air stripping was used successfully in the removal of ammonium nitrogen present in the leachate [16].

However, there are a few drawbacks to this technology. One drawback is the exhausted air which is mixed with NH3 needs to be treated with either $H_2SO_4$ or HCl before it is released into the atmosphere. Other drawbacks are the calcium carbonate scaling of the stripping tower when lime is used for pH adjustment, and foaming when a large stripping tower is used [17].

#### *3.1.4. Membrane filtration*

Membrane filtration is the process that separates solid immiscible particles from the liquid stream. It is based primarily on size difference. It includes microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (RO). Membrane filtration cannot be used alone in leachate treatment, and usually is used as pretreatment for other membrane processes. Membrane filtration can achieve an over 90% COD removal rate (Table 2) [18–20]; however, cost is a concern. Membrane filtration requires high energy input. In addition, residue needs to be further treated and properly disposed which increases the cost of the treatment.

| Process | COD Removal Rate | Reference |
|---|---|---|
| Microfiltration | 25-35% | [3] |
| Ultrafiltration | 50% | [4] |
| RO | >90% | [4] |
| Nanofiltration | 60-80% | [5] |

**Table 2.** The performance of different membrane process on leachate treatment

### 3.2. Biological treatment

Biological processes are very effective in removing organic matters and nitrogen, especially from young landfill leachate when the BOD/COD ratio has a high value (>0.5). When landfill operation time is longer than 10 years, the major presence of refractory compounds (mainly humic and fulvic acids) in leachate tends to limit effectiveness of biological treatment.

#### *3.2.1. Aerobic treatment*

Aerobic treatment of leachate can be performed in suspended growth microorganisms in activated sludge as well as attached growth microorganisms. Both systems, which are commonly applied to municipal wastewaters treatment, can be adapted to treat leachate. Aerobic treatment can achieve a partial decrease in biodegradable organic compounds and can result nitrification to transfer ammonia to nitrite and nitrate.

Aerobic biological processes have been widely studied and adopted. There are two types. One is based on suspended-growth biomass, such as aerated lagoons, conventional activated sludge processes and sequencing batch reactors (SBR). The other is based on attached- growth biomass, such as membrane bioreactor and different biofilters. Table 3 summarizes the typical performances of the most commonly used aerobic treatment processes in leachate [21–24].

| Process | COD Removal Rate | $NH_4^+$-N Removal Rate | Reference |
|---|---|---|---|
| Activated sludge | 46%-97% | 87.5% | [6,7] |
| SBR | 48-91% | >99% | [8] |
| Trickling Filter | 87% BOD | 90% | [9] |

**Table 3.** The performance of different aerobic process on leachate treatment

### *3.2.2. Anaerobic treatment*

The anaerobic digestion process involves biological decomposition of organic and inorganic matter in the absence of molecular oxygen. As a result of conversion, a variety of end products, including methanol ($CH_4$) and carbon dioxide ($CO_2$), is produced. It is particularly suitable for treating leachate with high strength organic content, such as leachate streams from young landfill [25]. Anaerobic treatment includes suspended-growth biomass processes such as anaerobic digester, up-flow anaerobic sludge blanket reactor (UASBR), and attached-growth biomass processes (anaerobic filter). Table 4 summarizes the typical performances of common anaerobic treatment processes in leachate [8,21,26]

| Process | COD Removal Rate | $NH_4^+$-N Removal Rate | Reference |
|---|---|---|---|
| Digester | 20%-96% | --- | [10] |
| UASBR | 45-91% | --- | [11] |
| Anaerobic filter | 60-95% | 87% | [6] |

**Table 4.** The performance of different anaerobic process on leachate treatment

## 4. Microbiology of fungi

### 4.1. Characteristic of fungi

A fungus is any member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. Like other eukaryotes, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA with noncoding regions called introns and coding regions called exons. They are comprised of soluble carbohydrates and storage compounds, including sugar alcohols, disaccharides and polysaccharides [27].

Fungi are heterotrophic organisms and require organic compounds as energy sources. They reproduce by both sexual and asexual means and spores. Some species grow as unicellular yeasts that reproduce by budding or binary fission. The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structure called hyphae, which may contain multiple nuclei and extend at their tips [28]. In common with some plant and animal species, more than 60 fungal species display the phenomenon of bioluminescence [29]. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions. Fungi are the only organisms that combine both glucans and chitin structural molecules in their cell wall [28].

### 4.2. Growth requirements of fungi

#### *4.2.1. Temperature*

Most fungi are mesophiles and relatively few can grow at or above 37°C or even above 30°C, whereas many bacteria can grow at this temperature. The upper limit for growth of any fungus (or any eukaryote) is about 62°C [27]. Temperature affects their growth rate, metabolism, nutritional requirements, regulation mechanisms of enzymatic reactions, and cell permeability. The structure and composition of cytoplasmic membranes in cells are also altered by temperatures that determine the substrate utilization rate of fungi [30]. In addition, temperature also plays a major role in determining fungal spore survival [31].

Most fungi have a maximum growth at a temperature of 25°C with reduced growth at temperatures below 20°C and above 35°C [32]. Thermophilc fungi dominate at a high temperature environment (above 35°C). They are no more efficient in substrate utilization than the mesophiles [27].

*4.2.2. pH*

Many fungi will grow over the pH range 4.0–8.5, or sometimes 3.0–9.0, and they show relatively broad pH optima of about 5.0–7.0 [27]. Acidophilic fungi, able to grow down to pH 1 or 2, are found in a few environments such as coal refuse tips and acidic mine wastes; many of these species are yeasts. Alkalophilic fungi are able to colonize alkaline environments with pH of 10, and they include specialized species of filamentous fungi. The morphology of fungi is also affected by the pH. Typically, the morphological change attributed to pH variation is in the shape of the fungal pellet. This varies from fluffy to clumpy and compact depend on pH [33]. Fungi can rapidly change the pH of the culture by selective uptake or exchange of ions; therefore, the responses of fungi to pH of the culture need to be assessed in strongly buffered media [27].

*4.2.3. Oxygen*

Most fungi are strict aerobes; they require oxygen at some, if not all the stages of their life cycle. Therefore, fungi are usually found growing on or near the surface of the substrate that open to the air. Some fungi are facultative aerobes. They can survive in oxygen-limited environments, including sewage sludge and polluted waters. Insufficient oxygen supply increases the nutritional demand and thereby decreases fungal growth [34].

*4.2.4. Nutrients*

Fungi have quite simple nutritional requirements. They need a source of organic nutrients to supply their energy and to supply carbon skeletons for cellular synthesis [27]. Fungi absorb simple, soluble nutrients through the wall and plasma membrane. In many cases, this is achieved by releasing enzymes to degrade complex polymers to simple nutrients and then absorbing them.

**a.** Carbon source

Fungi differ widely in their abilities of using different carbon sources. The utilization efficiency of a defined carbon source by fungi may be influenced by the medium composition and the culture conditions. Usually, the carbon sources can be cellulosic, $CH_4$, monosaccharide, disaccharides, and different types of wastes. The substrates that have different carbon compositions can be used for growing different type of fungi. For example, although both white- and brown-rot fungi are known for their ability to degrade lignin and cellulose, white-rot fungi perform better in the degradation of both simultaneous and selective lignin, while the brown-rot fungi degrade the cellulose and hemicellulose [35].

**b.** Nitrogen

Fungi do not fix atmospheric nitrogen, but they can use many combined forms of nitrogen such as nitrates, nitrites, ammonium, or organic nitrogen sources. All fungi can use amino acids as a nitrogen source. Often they need to be supplied with only one type of amino acid such as glutamic acid or glutamine. Then they can produce all the other essential amino acids by transamination reactions [27].

Most fungi can use ammonia or ammonium as a nitrogen source. After uptake, ammonia/ ammonium is combined with organic acids, usually to produce either glutamic acid or aspartic acid. Many fungi can also use nitrate as their sole nitrogen source. They produce nitrate reductase and nitrite reductase to convert nitrate to ammonium [27].

**c.** Phosphorus

Fungi are highly adept at obtaining phosphorus, and they achieve this in several ways: (1) they respond to critically low levels of available phosphorus by increasing the activity of their phosphorus-uptake systems; (2) they release phosphatase enzymes that can cleave phosphate from organic sources; (3) they solubilize inorganic phosphates by releasing organic acids to lower the external pH; and (4) their hyphae, with a high surface area/volume ratio, extend continuously into fresh zones of soil to obtain phosphorus [36].

**d.** Other nutrients

Essential micronutrients for fungal growth are iron, zinc, copper, manganese, molybdenum, and either calcium or strontium [35]. Different fungal species can have their own specific nutrient needs. Certain fungi require vitamins in trace quantities, whereas others synthesize their own vitamins.

## 5. Fungi process in wastewater

During the late 1950s to early mid 1960s researchers started to recognize the potential of fungi in wastewater treatment process. Cooke, in the 1976, advocated the use of fungi in wastewater treatment because fungi appeared to show higher rates of degradation and showed a much greater ability to degrade cellulose, hemicellulose, and lignin materials than other microorganisms [37].

In view of the excellent recalcitrant compound degradability of certain groups of fungi, researchers have been focusing on exploring fungal degradation of toxic industrial wastewater. These research have included wastewaters from textile, olive milling, and the food-processing industries, etc. Several studies have been conducted on the ability of fungi to decolorize specific dyes [38]. Research have shown that the degradation of dye is possibly due to the fact that fungi produce the lignin-modifying enzymes laccase, manganese peroxidase, and lignin peroxidase that mineralize lignin or dyes [39]. Fungi are also very effective in degrading complex aromatic organic compounds present in wastewater. For instance, phenolic compounds present in olive mill wastewater are similar to those derived from lignin degradation [40]; therefore, fungi are an excellent candidate for treating olive milling wastewater. Fungi also have been used to treat wastewater with high COD from the food processing industry [41,42]. Table 5 summarizes the application and performance of fungi in wastewater treatment.

| Wastewater | Treatment process | Fungi species | Results |
|---|---|---|---|
| Textile wastewater | SBR | *Trametes versicolor* | Color removal : 91-95% [12] |
| Olive mill wastewater | Airlift reactor | *Pycnoporus coccineus* | COD removal: 20-50% Toxicity removal: 70% [13] |
| Potato-chip industry wastewater | Batch studies | *Aspergillus niger* | COD removal: 90%[14] |
| Starch processing wastewater | Airlift reactor | *Aspergillus oryzae* | COD removal: 47-96% [15] |

**Table 5.** The performance of fungi treatment in different industrial wastewater

## 6. Fungi in leachate treatment process

Although using fungi to treat industrial or municipal wastewater has been studied for decades, it is relatively new to use fungi to treat the landfill leachate. Very few studies have been done in this area. These studies have demonstrated that fungi can effectively decrease high COD, toxicity, and the dark color of leachate.

### 6.1. Fungi species used in leachate treatment

White-rot fungi are the most commonly used species in landfill leachate treatment. Compared to the other species, white-rot fungi have the ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants. The white-rot fungi have developed very nonspecific mechanisms for degradation [4].

In response to low levels of key sources of carbon, nitrogen, or sulfur nutrients [5], white-rot fungi produce enzymes. These are known as lignin peroxidation and manganese-dependent peroxidases, which can degrade very insoluble chemicals such as lignin or many of the hazardous pollutants. White-rot fungi have an extracellular system that enables them to tolerate considerably higher concentration of a toxic pollutant such as cyanide. In addition, white-rot fungi possess a very nonspecific nature of mechanisms to degrade very complex mixtures of pollutants. Usually, white-rot fungi do not require preconditioning to a particular pollutant. White-rot fungi can be cultivated from soil using very inexpensive growth substrate such as corn cobs and wood dust. They can also grow in the liquid culture.

Besides the white-rot fungi, yeast is the only other fungi species that is used in landfill leachate treatment. Yeast has a high capacity of breaking and assimilating difficult degradation pollutants in leachate (such as humic substances). Several genera of yeast (e.g., *Candida*, *Rhodotorula*, *Yarrowia*, *Hansenula*, *Saccharomyces cerevisiae*) have been reported to be able to degrade complex organic compounds [6].

### 6.2. Fungi preparation

Obtaining the desired amount of fungi to treat landfill leachate can be achieved in three steps. First, selected fungi species need to be cultivated. This should be followed by enrichment, which is achieved by mycelial suspension. The fungi will then be ready to be added to the treatment process. The following section describes the detailed technique of cultivation.

#### *6.2.1. Subculture of fungi*

The ingredients of culture medium can be different depending on the species and purpose of the experiments. For example, *Trametes trogii* have the ability to produce high activates of laccase, which can remove copper in the leachate. A basal medium can be used to optimize the production of laccase. This medium contains (per liter): glucose, 10 g; soya peptone, 9 g; diammonium tartrate, 2 g; $KH_2PO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; and trace elements solution, 1 ml. The medium is supplemented with $CuSO_4$ (0.3 mM) and ethanol (3% V/V) an inducer of laccases. The cultural medium needs to be buffered to pH 5.5 [43]. Usually, fungi can be cultivated in culture tubes or dishes within the temperature range of 25℃ and 33℃ for several days (7 days)[44].

#### *6.2.2. Mycelial suspension*

Fungi should then be further enriched by growing it in mycelial suspension. The common procedure is as follows: (1) 100 ml of sterile potato dextrose broth (PDB) is prepared in a 250-ml Erlenmeyer flask. (2) Four pieces of fungi from the culture tube are inoculated into the PDB medium by using the sterile loop. The flask is plugged with cotton and is agitated for 24 h in a rotary shaker at 150 rpm [44]. The flask is then incubated at 28 °C in an incubator. Usually, after 6–7 days, a dense mycelial mass is formed and the mycelial suspension is ready for further use [45]. Mycelia suspension can be used directly in the leachate treatment process or can be added to the fungi immobilization media.

#### *6.2.3. Immobilization of fungi*

Support materials (media) usually play an important role of fungi production and stability in the leachate. Federica Spina et al. [46] studied four inert supports (Figure 1): (A) circle industrial support; (B) net industrial support; (C) polyurethane foam PUF; and (D) stainless steel scourers. Although Supports A and B are very efficient in bacterial biofilm formation, researchers have found that they were not suitable for hyphal colonization. They also observed that fungi colonized D in a heterogeneous way with great differences in the method of colonization among supports, under an agitated condition homogeneous and persistent biomass colonization was observed on Support C. They therefore concluded that PUF is the most suitable support media in the immobilization of fungi among all the four popular media on the market.

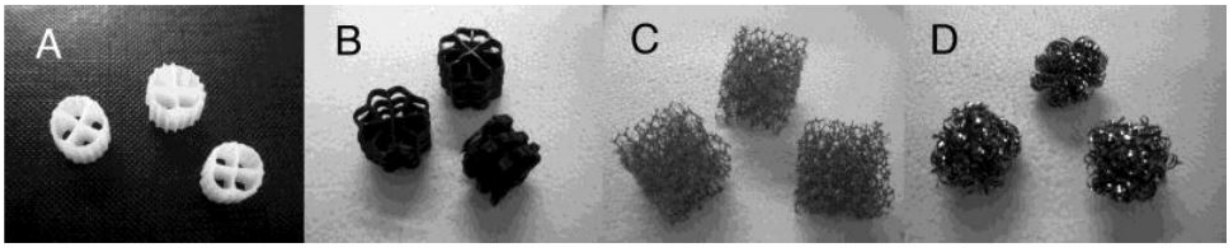


**Figure 1.** Different inert support materials used in the study conducted by Federoca Spina et al. [46].

The immobilization of fungi can be carried out by adding the media into a liquid fungi growth medium in a flask, which is seeded with a certain amount of mycelial suspension. The flask is then kept at a temperature of 30℃. Usually, it takes 4-7 days for fungi to colonize the media. After this immobilization process, fungi are ready to be used for the leachate treatment.

### 6.3. Leachate treatment process

#### *6.3.1. Treatment conditions and toxicity test*

To promote the growth of fungi, the treatment conditions such as temperature, pH, mixing, etc., are very important. A few studies have been conducted to understand the optimal treatment conditions. Studies have shown that keeping the leachate temperature between 25-33°C, pH of 4-5, is necessary to achieve a desirable treatment outcome. Table 6 illustrates the effect of pH on fungal biomass concentration as well as the COD removal in the wastewater treatment process [47]. Cosubstrates such as glucose willalso result in better treatment performance. Proper mixing and aeration are the other factors that need to be considered.

| pH | Fungal biomass (mg VSS/L) | COD removal (%) |
|---|---|---|
| 3.5 | 80 | 34 |
| 4.0 | 625 | 80 |
| 4.5 | 370 | 68 |

**Table 6.** Effect of pH on fungal biomass concentration in wastewater treatment

It is also necessary to test leachate toxicity, as the raw leachate may contain some toxic compounds that are harmful to the growth of fungi. Common leachate toxicity tests are microtoxicity and phytotoxity test. The microtoxicity test is carried out by measuring the light emission of *Vibrio fischeri* and *Aliivibrio fischeri* [48]. Phytotoxicity is estimated by the determination of the germination index of *Lepidium sativum* and *Sinapis alba* seeds [49].

*6.3.2. Leachate treatment methods and results*

In the batch study conducted by Kalčíková G et al. [45], they used *Dichomitus squalens* mycelial suspension and beech wood sawdust as cosubstrate. It was found that *D. squalens* was able to grow in the mature leachate from the closed landfill. This resulted in 60% of both DOC and COD removal and decreased toxicity. They also introduced a crude enzyme containing extracellular ligninolytic enzymes filtrate to treat leachate from the active landfill, which contained inhibitory compounds. The removal levels of COD and DOC reached 61% and 44%, respectively [45].

Saetang and Bable [44] studied using immobilized *Tinea versicolor* in a continuous flow system to treat leachate for both color removal and COD removal. In this study, they used glucose as cosubstrate. With 4 days of the initial immobilization of the fungi on polyurethane foam, approximately 78% color removal and 52% of COD removal were observed.

Membrane bioreactor (MBR) was used by Brito et al. [6] to treat the leachate with yeast. In this study, a submerged microfiltration module with hollow fiber membranes of poly was used. Through the study, they gradually increased concentration of leachate while decreasing concentration of broth in the feed. Finally, with 100% raw leachate in the feed, the yeast achieved 70% COD, 82% color, and 67.7% humic substance removal rate.

As of these date, there are very few studies on using fungi to treat leachate. Based on the available literature, the performance of fungi on leachate treatment is summarized in Table 7.

| Parameters | Removal Rate Range | Reference |
|---|---|---|
| COD | 42-79% | [16–19] |
| BOD | 25-52% | [19] |
| Color | 40-82% | [16,19] |
| Toxicity | 40-50% | [16–19] |
| DOC | 40-60% | [18] |

**Table 7.** Performance of leachate treatment process with fungi

## 7. Conclusion and future challenge

Fungi can be used to treat a variety of wastewaters, ranging from municipal wastewater, industrial wastewater and landfill leachate. In terms of landfill leachate treatment, fungi showed a better removal efficiency of recalcitrant compounds than the conventional leachate treatment process. This was evident especial in the removal efficiency of recalcitrant compounds which contribute to 1) COD, 2) toxicity and 3) color of leachate. Both white-rot fungi and yeast are capable of producing special extracellular enzymes, and they are the only two species that have been studied so far. There is a need to extend the current research onto other fungi species. This requires a better understand of the characteristics of fungi. The collaboration between the microbiologist and the wastewater engineers is therefore essential.

Besides recalcitrant compounds, high ammonia concentration in the leachate is another concern. However, throughout the literature review, no information was available on the impact of ammonia on the growth of fungi as well as their ammonia removal ability. In addition, how to remove nitrogen along with other pollutants needs to be addressed in the fungi leachate treatment process. Better research and understanding of the role of fungi would help further improve the leachate treatment process. Future research should be done these challenges to develop a leachate treatment technology that is economical and easy to implement.

## Author details

Yanan Ren and Qiuyan Yuan*

*Address all correspondence to: qiuyan.yuan@umanitoba.ca

Department of Civil Engineering, University of Manitoba, Winnipeg, Manitoba, Canada

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