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A NOVEL FUNGAL-BASED APPROACH FOR THE SUSTAINABLE TREATMENT OF LANDFILL LEACHATE

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ABSTRACT

Leachate, a wastewater generated from a solid waste landfill, usually falls in the high-strength wastewater category, containing both organic and inorganic pollutants. In this study, an attempt was made to utilize the *Aspergillus* and *Mucor* fungi for leachate treatment, as fungi possess excellent recalcitrant compound degradability for wastewater treatment. Their faster growth, enzyme versatility, nutrient removal abilities, and biosorption properties make them promising technologies for future research and applications. Three identical lab scale reactors of 3 L were used with different fungal species i.e. *Aspergillus* (R_A), *Mucor* (R_B), and combination of *Aspergillus* and *Mucor* (R_C). Stainless steel pall rings were packed in each reactor which occupied 35% of reactor volume, which provided the support for the growth of the fungi and for abundant surface area for fungal growth. The reactors were investigated simultaneously for leachate treatment. The entire experiments have been carried out using various leachate dilutions, starting from 10% to 100% (with 20% interval), with a hydraulic retention time (HRT) of 7 h with recycling. The reduction of various pollution parameters, specifically COD and TSS, was measured in a batch scale. The reactor with *Mucor* fungi performed superior to other two at all dilution level. Hence, fungi can be provided sustainable and economical option for pre-treatment of leachate.

Keywords: Leachate, Fungi, *Aspergillus*, *Mucor,* Stainless steel pall rings, Biosorption

INTRODUCTION

The common treatment method of treating the solid waste is secured landfill (El Ghammat et al., 2019; Aroua-Berkat and Aroua, 2022; Zaidi et al., 2023). Particularly in India, there is no proper information available regarding the amount and composition of urban waste. Manufacturing industries also produces waste in solid and semi solid form which may have characteristics like self-reactivity, ignitability, explosive, toxicity, and radioactivity. Because of such characteristics they are generally disposed in landfill. A contaminated liquid that is generated through the compacted solid waste, which is dumped into the landfill, can be termed as leachate (El Kharmouz et al., 2013; Kouassi et al., 2014; Saadi et al., 2014; Bouaouine et al., 2015; Kozak and Cirik, 2024). The characteristics of the leachate will be different for every landfill as the composition and the type of solid waste dumped will be different. So, the major contaminants present in the landfill leachate are inorganic contaminants and large amount of organic contaminants, i.e., the chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammonia, hydrocarbons, suspended solids, concentrations of heavy metals and inorganic salt (Sbai and Loukli, 2015; Ziati et al., 2018; Swarnakar et al., 2023; Mohamad et al., 2024).

The waste disposed of in landfills, generally, produces leachate, which is a byproduct of the treatment method. Leachate is produced when the water through rainfall or surface water percolates through the solid waste present in the landfill. As water moves through the waste, it extracts soluble or suspended materials, including organic matter (Haouchine et al., 2024), metals, and other contaminants, creating a highly toxic liquid. Landfill leachate may be characterized as a water-based solution consisting of four groups of contaminants: (i) dissolved organic matter, such as alcohols, acids, aldehydes, and short chain sugars (Aw et al., 2016); (ii) inorganic macro components, which include common cations and anions, e.g., sulfate, chloride, iron, aluminum, zinc, and ammonia (Bacha and Achour, 2023); (iii) heavy metals, i.e., Pb, Ni, Cu, Hg, etc…(Ghomri et al., 2013; Larakeb et al., 2014; Achour et al., 2017); and (iv) xenobiotic organic compounds such as halogenated organics, e.g., PCBs, dioxins, etc...(Achour et al., 2002; Achour and Chabbi, 2014; Adamcová et al., 2017). The disposal of these contaminated effluents into receiving waters can cause environmental damages, directly influencing the aquatic ecosystem, public health, and even human being (Spina et al., 2012; Faye, 2017; Baba Hamed, 2021; Ihsan and Derosya, 2024; Chadee et al., 2024). As the amount of solid waste generation has been increased now a days, and so significantly increasing the environmental concern and requires careful management to prevent contamination of the environment (Jayasena et al., 2021; Pandey et al., 2022). Thus, landfill leachate alters the physicochemical parameters and heavy metal concentration in surface water, groundwater, soil, and plants and so it becomes necessary to treat leachate before discharging it.

Several technologies can be used to treat leachate depending on its characteristics. Commonly used treatment technologies include biological, chemical, physical, and membrane filtration. Each of these treatment technologies has advantages and limitations, and the appropriate technology or combination of technologies will depend on the specific characteristics of the leachate and the regulatory requirements for its disposal or reuse (Collado et al., 2019; Ghyadh et al., 2019).

Physical treatment cannot remove all the contaminants and chemical treatment is costly (Haddad and Ghoualem, 2014; Laghzal and Salmoun, 2014; Bouchemal and Achour, 2015). Biological treatment is cost effective as pollutants are removed using microorganisms like bacteria, fungi, algae etc. (Gaouar and Gaouar, 2016)

Due to advancement biological treatment, the use of different kind fungi has provided more benefit to the treatment technology then other physico-chemical treatment due to less use of chemicals, cost effective, low sludge generation; fungi have also been proven to survive in the toxic environment. So, the use of fungi for leachate treatment needs to study well as it is still in its early stages, and more research is needed to fully understand its potential and optimize its use (Bhadouria et al., 2020).

The popularity of biological treatments is increasing because of their affordability and sustainability. Bacteria are the most commonly used microorganisms in such treatments, but fungi have also emerged as a suitable option due to their ability to tolerate and resist toxicity, especially in the treatment of leachate (Collado et al., 2019).

Fungi are a diverse group of eukaryotic organisms that include yeasts, molds, and mushrooms. They are distinct from plants, animals, and bacteria, and have their own unique characteristics. Fungi are well known as great decomposers in many ecosystems, breaking down dead organic matter and recycling nutrients. They also form symbiotic relationships with other organisms, such as *mycorrhizal* fungi that form mutualistic associations with plant roots (Akhtar and Mannan, 2020; Bhadouria et al., 2020; Ferreira et al., 2020).

The cells of fungi are oblong and have large trichomes that contain organelles and intracellular structures. While some fungi grow on dead organic matter as saprophytes, others are parasites. Fungi are absorptive heterotrophs that secrete digestive enzymes onto their substrates to break down organic matter, which they then absorb. Fungal hyphae have small volumes but large surface areas, which allow them to have a high absorptive capacity. For example, white rot fungi can produce extracellular lignin peroxidase (LiP) and manganese–dependent peroxidase (MnP) that is essential for lignin degradation. It also secretes one or more of the three principal ligninolytic enzymes, i.e. lignin peroxidase, Mn-dependent peroxidases (Abdullah et al., 2013). Actually, fungal extracellular oxidative enzymes with non-selective catalytical activity are the responsible agents of the degradation of recalcitrant compounds with high redox potential, which are instead hard to handle by other organisms, such as bacteria (Hultberg et al., 2020; Tigini et al., 2013). Fig. 1 shows how fungi can degrade the pollutants.

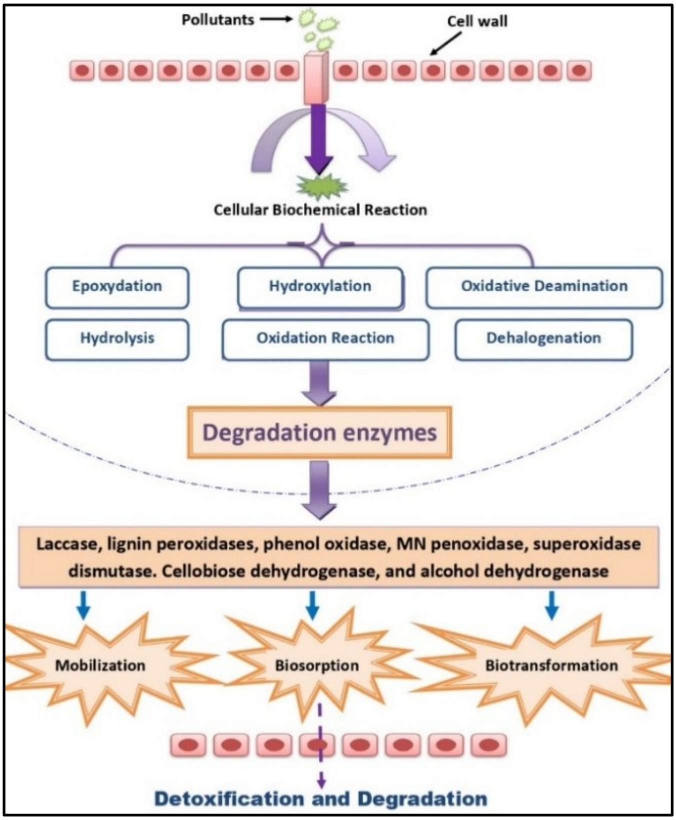


Figure 1: Mechanism of fungi degrading pollutants

Among other microorganisms, white-rot fungi have attracted attention for their unique and versatile metabolism, which is capable of degrading a wide range of xenobiotics through the activity of their intracellular and extracellular enzyme systems (Zhuo and Fan, 2021). In addition, white-rot fungi have shown excellent resistance to highly toxic waters without any prior acclimatization process, unlike other biological treatments such as conventional activated sludge. While white rot fungi have been the focus of much research due to their powerful lignin-degrading capabilities and effectiveness in breaking down complex organic pollutants, *Aspergillus* and *Mucor* species bring a different set of advantages to wastewater treatment. Their faster growth, enzyme versatility, nutrient removal abilities, and biosorption properties make them different. *Aspergillus* is a genus of fungi that belongs to the Ascomycota phylum, which is characterized by the production of sexual spores (ascospores) within a sac-like structure called an ascus. *Aspergillus* fungi can play a role in the treatment of leachate (Ghyadh et al., 2019). Leachate can contain a range of pollutants, including organic matter, nitrogen, and heavy metals, which can have harmful effects on the environment and public health. *Mucor* is a group of fungi that belongs to the Zygomycota phylum. While there is limited research on the role of *Mucor* in leachate treatment, some studies have suggested that certain species of *Mucor* may have potential for use in bioremediation of wastewater and industrial effluents (Chitale et al., 2022).

Given the rising environmental threat posed by untreated leachate and the limitations of conventional wastewater treatment methods, there is a growing need to investigate innovative and sustainable biological treatment techniques. Fungal species *Mucor* and *Aspergillus* offer a promising approach owing due to their unique enzymatic systems, resilience in harsh environments, and low-cost operation. However, while initial research is promising, further studies are required for optimization, understanding their interaction, and scaling up for real-world applications.

FUNGI USED FOR LEACHATE TREATMENT

Several studies have been conducted on different types of *white-rot* fungi and *Pleaurotusostereatus* and that to in suspended growth process. Where these fungi have been found to give best results for chemical oxygen demand (COD) and the colour reduction of the leachate. *White-rot* fungi are commonly used for treating landfill leachate as well as industrial wastewater due to their ability to degrade a wide range of persistent and toxic pollutants. They have nonspecific degradation mechanisms that allow them to break down chemicals such as lignin and hazardous pollutants in response to low levels of key nutrients. *White-rot* fungi produce lignin per oxidation and manganese-dependent peroxides, which are enzymes that degrade insoluble chemicals. They can tolerate high concentrations of toxic pollutants like cyanide and can degrade complex mixtures of pollutants without requiring preconditioning. *White-rot* fungi can be grown in soil using inexpensive substrates such as corn cobs and wood dust, as well as in liquid culture. Several studies have also been done on the combined treatment of physical and biological treatment process where also it has achieved the great results for the chemical oxygen demand (COD) (Díaz et al., 2022; Wan Razarinah et al., 2015, Wang et al., 2018).

In suspended growth process the treatment was done using various concentrations of the fungi and various parameters like Biochemical Oxygen Demand (BOD) and leachate toxicity was also measured. Less study has been conducted using attached growth process and that to with other species rather than the *White-rot.*

Major fungi used for the leachate treatment are *Porostereus Spadiceum, Tramets Pubescences, Bjerkandera Adusta, T Menziesii* (Razarinah, Wan., Noor Zalina, M. and Noorlidah, 2011; Wan Razarinah et al., 2015)*., Ganoderma Austral Mycelia* (Abdullah et al., 2013; Legorreta-Castañeda et al., 2020; Shah et al., 2021)*, Saccaromuces Cerevisiac*, and other types of filamentous fungi (Ferreira et al., 2020; Sankaran et al., 2010).

In addition to *White-rot* fungi, *yeast* is also utilized in landfill leachate treatment due to its ability to break down and absorb complex and persistent pollutants in the leachate, including humic substances. *Yeast* is capable of degrading complex organic compounds, and there are several genera of yeast, such as *Candida, Rhodotorula, Yarrowia,* *Hansenula, and Saccharomyces cerevisiae*, that have been reported to have this capability (Abbasi, 2018; Brito et al., 2012; Sankaran et al., 2010).

GROWTH CONDITION REQUIRED FOR THE FUNGI

The basic condition required for the adequate growth of fungi include all physical, chemical, and biological factors like temperature, pH, oxygen, nutrient source etc. According to the research studies, it is found that most of the fungi are mesophiles and maximum temperature that can grow is approximately 20°C - 30°C. As the temperature has a significant impact on fungal growth rate, metabolism, nutritional needs, enzymatic reactions, and cell permeability. The composition and structure of fungal cell membranes can also be altered by temperature (Mustafa et al., 2023; Tkaczuk and Majchrowska-Safaryan, 2023).

As pH of the culture can affect the morphology of fungi, with changes observed in the shape of fungal pellets it is found that the fungi generally show their majority of growth in the pH range of 5.0 to 7.0. Fungi are typically strict aerobes, meaning they require oxygen at some or all stages of their life cycle. When oxygen is limited, the nutritional demand increases, which can reduce fungal growth.

Studies has been carried out by the various researchers on the fungal treatment for industrial wastewater and for leachate pollution reduction.

ROLE OF FUNGI IN WASTEWATER POLLUTION REDUCTION

Different kinds of fungi have been previously used in beaker or column studies to treat different types of wastewaters for pollution reduction parameters like BOD, COD, TSS, colour, heavy metal, toxicity, etc. Fungi have been employed in different forms, including precultured fresh spores, powdered form, grown on various attached media, and pellets.

Numerous studies have demonstrated that fungi play an important role for reduction in pollutants in a various type of wastewater. Leachate colour was drastically decreased by 51% and 60% by *White Rot* fungi, *Autochthonous* fungi, and *Allochthonous* fungi, respectively, while *P. Ostreatus* assisted in reducing toxicity (Tigini et al., 2013; Spina et al., 2018; Adamcová et al., 2017).

Color reduction in textile and pharmaceutical wastewater has been successfully achieved by *B. Adusta* MUT2295 (Spina et al., 2012). *T. menziesii* enhanced leachate treatment by diminishing BOD by 89.2% and COD by 2.1%; however, another study found even improved pollution reductions in conductivity (89.9%), turbidity (86.7%), BOD (92.8%), and COD (92.8%) (Wan Razarinah et al., 2015; Raghab et al., 2013). Additionally, there were substantial reductions in COD, BOD, ammoniacal nitrogen, and colour observed using *B. Panacihumi*, *Ganoderma Australe Mycelia*, and *Saccharomyces* fungal species (Er et al., 2018; Abdullah et al., 2013; Brito et al., 2012).

Other fungal species have also shown substantial pollution reduction results in the treatment of wastewater (Lacina et al., 2003). Heavy metals, BOD, and COD have all been eliminated from raw wastewater by *Phanerochaete - chrysosporium*, whereas a combination of *Aspergillus japonicus, Funaliatrogii*, and *Phanerochaete chrysosporium* reduced heavy metals by 10% and dye content by 90% (Legorreta-Castañeda et al., 2020). In leachate, *P. chrysosporium* has further decreased colour, BOD, and COD (Díaz et al., 2022). These experimental results clearly demonstrate the immense potential of fungal species as an efficient and sustainable method of treating wastewater pollution.

Conventional wastewater treatment techniques frequently encounter difficulties with sustainability, cost, and efficiency, demonstrating the need for alternate approaches. Fungal bioremediation has received concerns because of its biosorption capabilities, enzymatic versatility, and ability to degrade pollutant compounds effectively. This study assesses the potential of *Aspergillus* and *Mucor* fungi for sanitary landfill leachate treatment, which can offer a sustainable and cost-effective solution for pollutant reduction. This study aims to enhance the viability of large-scale applications and optimize bioreactor design by assessing the performance of several fungal species under various leachate dilutions. Hence, the study's goal is to fill the gap between fungal bioremediation potential and its practical implementation, which will enhance the landfill leachate management.

This study investigates and suggests a low-cost and sustainable biological treatment approach for sanitary landfill leachate, a prevalent environmental issue at municipal solid waste disposal sites. This approach can be enhanced and incorporated as a pre-treatment in existing leachate treatment processes by using fungal species like *Mucor* and *Aspergillus*, which are easily cultivated and naturally occurring. The use of fungal species for biofilm formation in the packed bed reactors offers a practical design for field application, requiring minimal energy and infrastructure compared to conventional physicochemical processes. This approach effectively reduces pollutant loads (i.e. COD and TSS) in landfill leachate prior to advanced treatment. As a result, it decreases the overall operational costs, enhance the treatment efficiency, and contributing to sustainable and environmentally friendly waste management practices.

MATERIAL AND METHODS

Fabrication of the reactors

In this study, three identical lab scale reactors were used for almost six months in batch scale at 7 h hydraulic retention time (HRT) with different leachate dilutions i.e. starting from 10% to 100% (at an interval of 20%). The reactors then labelled as R_A for *aspergillus*, R_B for mix culture and R_C for *mucor.* To ensure the effective contact time, stainless steel pall rings (94 numbers in each reactor) and sprayed sprinkler recycling facility was implemented. An additional pipeline connection was constructed alongside the outlet to facilitate the recycling. The treated leachate would drain out at the bottom of the column and was recycled using a pump for up to 7 h.

The inlet was designed to accommodate both the effluent inlet and the recycled inlet through a single inlet. To increase the contact time and provide sufficient surface area, a sprinkler was installed at the inlet. This allowed the effluent to be sprayed over the media, thereby enhancing the contact time and surface area.

The leachate from the inlet tank was pumped through the inlet using the sprinkler. The leachate was then sprayed over the media containing fungi, where it was treated and retained at the bottom of the reactor. The bottom of the reactor had an outlet and a recycle pipe. The retained leachate was recycled for a retention time of seven hours. Table 1 shows the dimensions of the reactor. Fig. 2 indicates the schematic diagram of the reactor and the reactors' set up during the experiments.

Table 1: Physical dimension of the reactor

Sr no	Parameters	Dimensions
1.	Thickness of reactor material	6 mm
2.	Total height	60 cm
3.	Inlet diameter	9 cm
4.	Total volume	3.6 L
5.	Packing media used in each reactor	SS Pall rings (94 nos.)
6	Media height	20 cm
7	Specific surface area of the media	350 $m^2.m^{-3}$
8	Dimensions (Diameter X Height)	17 mm X 18 mm
9.	Volume of sample (leachate)utilized in each cycle	1 L

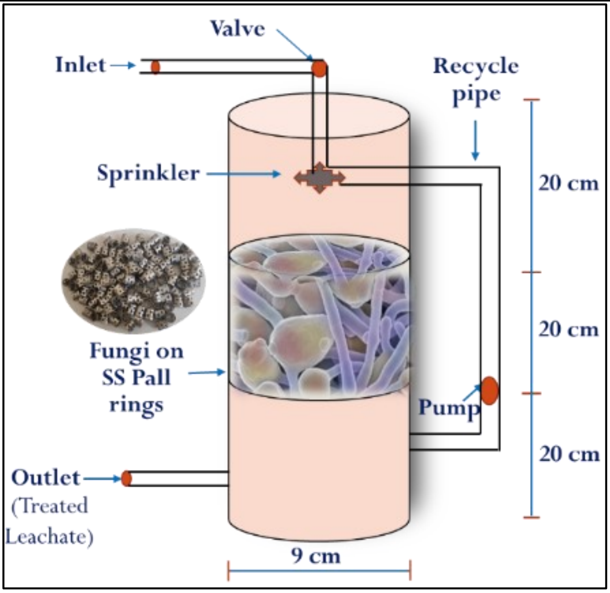


Figure 2: Schematic diagram of the reactor

Characteristics of the leachate

Municipal solid waste landfill sites are important sources of groundwater contamination due to the leakage of leachate. Landfill leachate is undoubtedly one of the most challenging wastewaters in terms of treatment (Adamcová et al., 2017; Spina et al., 2012). The landfill leachate for the study was collected daily from a landfill in the urban area of Ahmedabad, Gujarat. The leachate was characterized based on several pollutant parameters such as BOD, COD, pH, TDS, TSS, heavy metals. The procedure followed during the testing of parameters were in accordance with standard method for the examination of water and wastewater (APHA, 1998). Table 2 shows the initial characteristics of collected landfill leachate.

Table 2: Characteristics of the collected leachate

Serial Number	Parameters	Value
1	COD	11,440 ± 432 mg/l
2	BOD	3526 ± 211 mg/l
3	TSS	3100 ± 314 mg/l
4	TDS	18.49 ± 11 ppt
5	pH	8.7 ± 0.5

Some of the assumptions have been made in the design and execution of this study to simplify and control the experimental conditions. It was considered that the selected fungal species of *mucor* and *aspergillus* remain active throughout the study period in the given lab conditions. The study also presumed that the pall rings as an attached media for fungal growth provided sufficient and uniform surface area in all three reactors. Additionally, the diluted leachate was considered the representative of actual landfill leachate in terms of pollutant load and characteristics. Finally, the selected 7 h HRT with leachate recycling was thought to be adequate for effective fungal interaction and pollutant removal in the experiment.

Growing and seeding of the fungi

To initiate the practical work, it was necessary to develop the fungi in the pall rings. So, the following procedure was followed to grow the fungi.

Fungi and subculture of fungi

The subculture of *aspergillus* and *mucor* fungi was collected from Microbiology laboratory of Gujarat University, Ahmedabad. The fungi were maintained on malt extract (MEA) (Oxoid) agar slants. The spores and the microscopic view of the fungi has been shown in the Photo 1 (a to d).

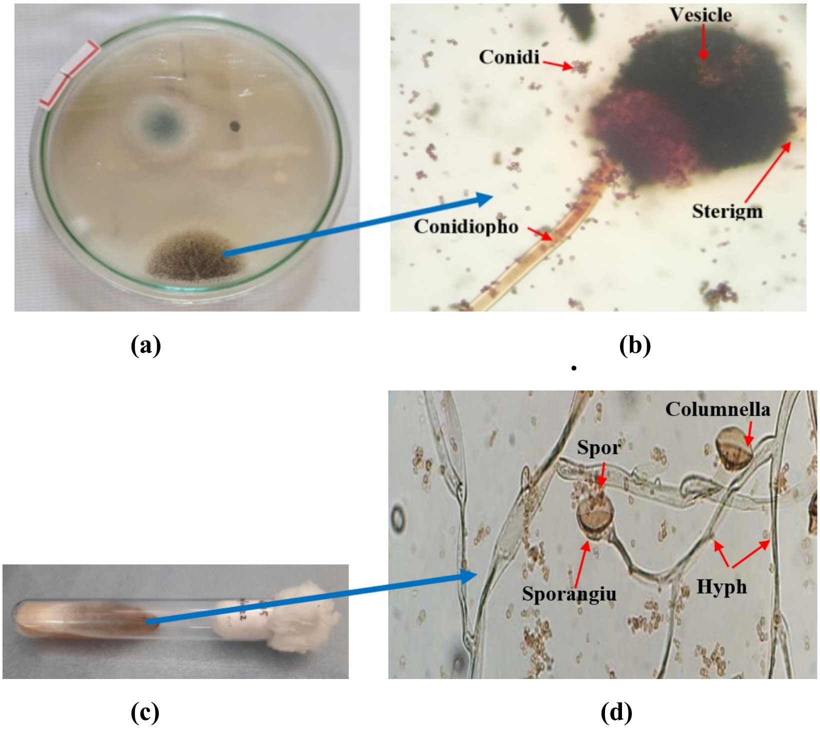


Photo 1: Subculture of selected *aspergillus* and *mucor (a)* Subculture of *aspergillus* fungi (b) Microscopic view of *aspergillus* fungi (400X) (c) Subculture of *mucor* fungi (d) Microscopic view of *mucor* fungi (400X)

Preparation of mycelial suspension

To grow the fungi, it is necessary to provide the media and proper nutrients. For that, the mycelia suspension was prepared from glucose (20 g) and peptone (10 g) in a liter of distilled water under sterile conditions. The glucose and peptone were added in the ratio 2:1. The pH of the medium was adjusted to 6.5. The mixture was poured into a sterile flask, which was then plugged with cotton to maintain aseptic conditions, and it was autoclaved at 121°C for 20 minutes. After sterilization, the medium was allowed to cool and remain steady for 24 h. The mycelial suspension was then prepared in the flask for further use in the experimental work.

Inoculating of fungal spores

From the subculture of the fungal spore and with the help of sterile loop under the sterile condition maintained, the fungal spores were inoculated into the mycelia suspension Photo 2 (a and b). After each inoculation the loop was also deep into methanol to destroy the host cells.

(a) **(b)**

Photo 2: Inoculating the fungal spores (a) Collecting of fungal Spores after disinfecting the sterile loop (b) Inoculating the fungal spores under sterile conditions

Growing of fungi

After inoculating the fungal spores the flask, were kept on the continuous shaker until the fully developed fungi was developed. It was found that within a week the fungi were developed until its mark, which is shown in Photo 3 (a to c).

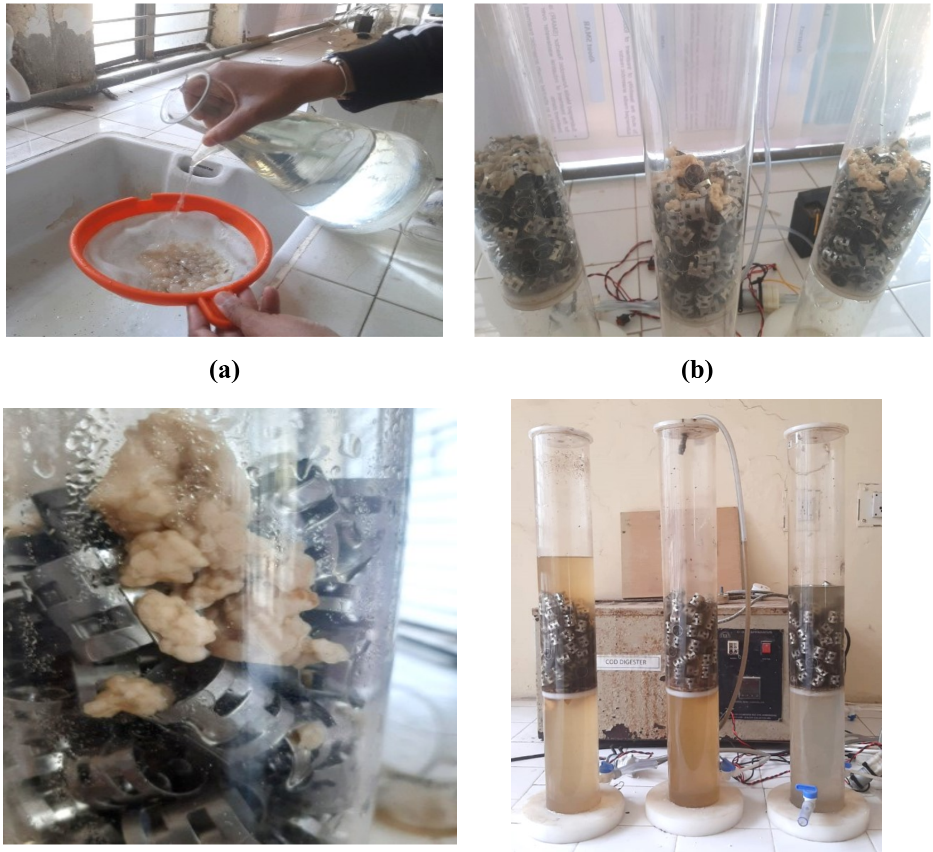
(a) **(b)** **(c)**

Photo 3: Developed form of fungi (a) *aspergillus* (b) mix culture (c) *mucor*

Seeding and acclimatizing fungi to the column

After growing the fungi, it was washed with distilled water for 2-3 times and was inserted in the column in media section. To acclimatize the fungi, it was made acclimatized under 5% concentrated leachate and glucose solution for 5 days.

After that, the column was washed with distilled water for 2 times. After that the visible growth of fungi was observed and the column was ready to sustain the higher concentration of the leachate, which is shown in Photo 4 (a to d).



(a) **(b)**

(c) **(d)**

Photo 4: Seeding and acclimatizing fungi in the reactors (a) Straining and washing of fungi (b) Fungi incubated (c) Close view of incubated fungi (d) Acclimatizing of fungi

Performance of the reactors

After the acclimatization of the fungi in the reactors, each reactor was filled with 1 L of leachate (starting from 10% dilution and increased to 100% concentration with 20% intervals) through inlet tank with the help of pump. The leachate was sprayed through the sprinkler to provide more contact time to fungi to treat leachate. The leachate would drain out at the bottom of the column and was recycled again and again until 7 h with the help of pump. For each percent dilution, runs were taken twice and average result of both the test was noted.

All the 3 identical reactors were evaluated simultaneously for 7 h HRT for COD, TSS and pH. After 40% dilution run the columns was filled with the glucose solution prepared by the ratio of 2:1 for 24 h to sustain the fungi. After which the successive run for 60% was taken.

RESULTS AND DISCUSSION

After conducting the experiment for all the diluted concentrations of leachate and providing a 7-hour contact time, the results were analyzed to determine the efficiency of the three reactors (R_A, R_B, and R_C) in reducing the COD and TSS of the leachate.

The study concluded that reactor R_B which contained *mucor* was the most efficient in reducing the COD of the leachate, followed by reactor R_A and then reactor R_c. This means that reactor R_B was able to treat the leachate more effectively and remove more pollutants compared to the other reactors. This suggests that *mucor* possesses superior metabolic or enzymatic capabilities under the tested conditions for breaking down or assimilating the organic load present in the leachate. This aligns with the findings of previous studies highlights the potential of fungi in wastewater treatment due to their extracellular enzyme systems and resilience to harsh conditions (Kaushik et al., 2009; Sharma et al., 2025).

Overall, the results suggest that the use of fungi in treating leachate is a promising approach, with reactor R_B showing the highest potential for efficient COD reduction i.e. 31.3% at 40% dilution. However, further studies are needed to determine the long-term sustainability and practical applications of this approach. The Fig. 3(a) and Fig. 3(b) shows the percent reduction of COD and TSS during the experimental study. pH value noted during the entire experiments between 8.4 to 8.9.

According to the discussed research findings of COD reduction, *Mucor* fungi were identified as the most effective in treating 100% concentrated leachate, achieving a reduction rate of 18.7%. On the other hand, *Aspergillus* fungi resulted in a 4.5% reduction for the same concentration, indicating its performance is significantly influenced by pollutants concentration of osmotic stress levels. While Aspergillus exhibited a high reduction rate of 40% and combined shows the 18.5% reduction in 60% dilution, suggesting possible synergistic or competitive interactions depending on dilution and substrate availability. These outcomes reinforce the adaptability of fungi in biotreatment processes in previous studies (Tiwari et al., 2024; Moradi et al., 2025).

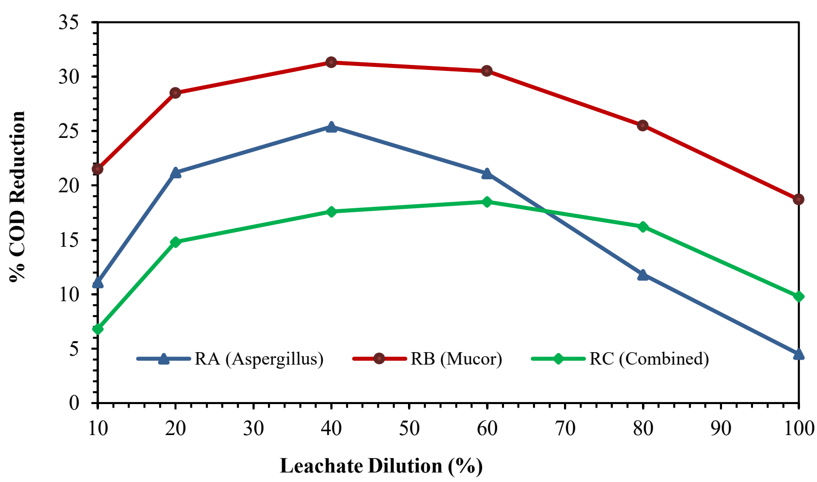
The promising pollution reduction performance of *mucor* at both low and high concentrations of leachate underscores its potential for bioremediation of leachates, consistent with previous research advocating the role of fungi in decentralized or low-energy wastewater treatment processes (Sharma et al., 2025; Yadav et al., 2024). However, further studies are required to assess the long-term operational sustainability, fungal resilience, and scalability of such biological treatment approaches under real-field conditions (Choksi et al., 2015a; Choksi et al., 2015b).

Additionally, integrating such fungal-based systems with geospatial water quality assessments and sustainable groundwater frameworks (Verma et al., 2024; Vyas et al., 2024) could enhance their applicability for holistic waste management strategies.

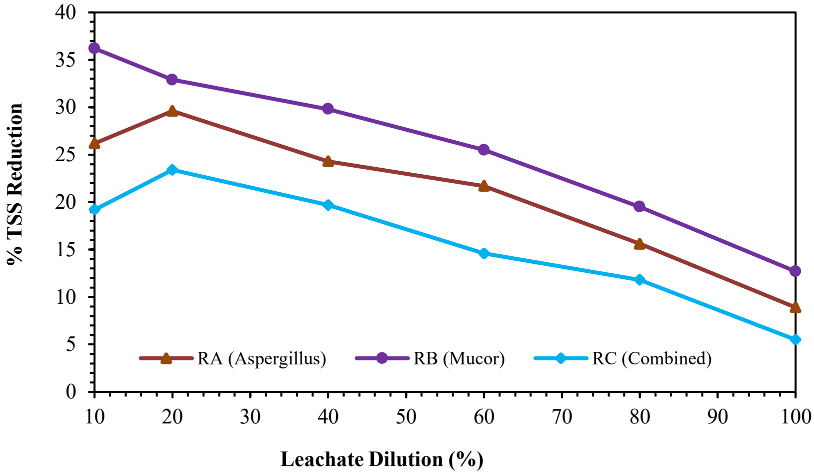
The previous studies showed that *Aspergillus niger* and *Rhizopus oligosporium* achieved significant reductions of up to 70% in COD and heavy metals in leachate over six days. *muco*r was found to be more effective than *Aspergillus niger* in removing TSS, achieving a reduction rate of 15.3% compared to 9% within a 7 h HRT (Ghyadh et al., 2019).

Another study found that *Ganoderma australe* and *Trametesmenziesii* exhibited robust growth and sustainability in both 100% and 50% leachate concentrations, suggesting their suitability for leachate treatment (Razarinah, Wan., Noor Zalina, M. and Noorlidah, 2011). Furthermore, previous experimental work observed that fungi showed lower reductions of up to 10% for pharmaceutical wastewater but demonstrated a substantial reduction of up to 90% for dye wastewater (Legorreta-Castañeda et al., 2020).

This indicates that the performance of fungi in leachate treatment may vary depending on the specific characteristics of the wastewater. The research study was used the B. panacihumi strain ZB1 and found that it achieved an overall reduction of 39.4% in COD when combining aerobic and anaerobic treatment processes, indicating its potential for enhancing leachate treatment efficiency (Er et al., 2018).



(a)



(b)

Figure 3: Performance of reactors in various dilutions. (a) COD reduction, (b) TSS reduction

To summarize, the discussed studies indicate that different fungi exhibit varying degrees of effectiveness in leachate treatment, with *mucor* fungi showing promise in treating 100% concentrated leachate, particularly in reducing TSS. Factors such as the specific characteristics of the leachate and the targeted pollutants should be considered when selecting a suitable fungal treatment approach. After treatment the changes in the physical structure of the fungi was observed but due to the presence organic matter and the bacterial growth it was not possible to observe the physical structure of the fungi.

Table 3: Statistical analysis of the experimental work

Parameter	Most effective treatment	Statistical Significance	Correlation	ANOVA test result
COD	Reactor R_B	Yes – Strong ($p = 0.0077$)	Nearly perfect negative	$F = 2.76$, $p = 0.0077$
TSS	Reactor R_B	Average ($p = 0.0952$)	Strong negative	$F = 2.76$, $p = 0.0952$

To support the experimental noted results, a one-way ANOVA test was conducted for both COD and TSS reduction among the chosen three fungal reactors (R_A–*Aspergillus*, R_B– *Mucor*, and R_C-combined). The statistical analysis of the experimental work has shown in Table 3. The analysis showed a statistically significant difference in COD reduction efficiency among the reactors, with an F-value of 6.85 and a p-value of 0.0077 ($p<0.05$). This confirms that the selection of fungal species had a real impact on COD reduction, with *Mucor* (R_B) performing the superior. On the other hand, TSS reduction showed an F-value of 2.76 and a p-value of 0.0952, indicating that the differences among the reactors were average statistically significant. This suggests that although *Mucor* generally reduced TSS more effectively, highlighting the need for further investigation into its effect on varying operational conditions. Overall, reactor RB (*Mucor*) emerged as the most effective and reliable fungal species for leachate treatment, warranting further exploration for scale-up treatment systems.

CONCLUSION

The present study investigates the efficacy of fungal strains *aspergillus*, *mucor,* and their mixed culture for the treatment of municipal solid waste landfill leachate at varying dilutions (10% to 100%) using reactors packed with stainless steel pall rings and operated at an HRT of 7 h.

The experiments were conducted under aerobic conditions at room temperature with continuous recycling and maintaining the optimal pH range of 8-9.5. The reduction in COD exhibited a gradual increase in percent reduction up to a 40% dilution for all three cases. However, beyond that point, the percent reduction decreased as the dilution increased until reaching 100%. For *Mucor*, the highest percent reduction in COD was observed at the 40% dilution, after which it decreased. The percent removal of COD for 100% concentrated leachate was 18.7%, and the reduction in TSS was 12.7% at a pH of 8.75. *Aspergillus* followed a similar trend with lower efficiency, reducing COD by only 4.5% and TSS by 8.9% at 100% concentration (pH 8.92). The mixed culture performed best at 60% dilution, with 9.8% COD and 5.5% TSS reduction at full concentration (pH 8.79).

A one-way ANOVA test revealed a significant difference in COD reduction among the reactors (F-6.85, p-0.0077), confirming the impact of fungal species, with *Mucor* (R_B) performing best. TSS reduction showed marginal significance (F-2.76, p-0.0952), indicating moderate variation and possible influence of operational conditions.

Overall, *Mucor* emerged as the most effective and reliable fungal strain for sanitary landfill leachate treatment, followed by *Aspergillus*, while the mixed culture demonstrated intermediate results. These findings underline the promise of *Mucor* for future scale-up applications, though further research is needed to enhance conditions for TSS removal and higher operational efficiency.

FUTURE SCOPE ON THE TOPIC

Future scopes for leachate treatment using fungi include optimizing fungal strains, exploring mixed cultures and consortia, scaling up treatment systems, integrating with conventional methods, assessing environmental impacts, and evaluating economic feasibility. Long-term performance and stability in continuous or large-scale systems can be assessed. The potential for operational challenges like fungal biomass clogging on attached media, and maintenance requirements also needs further investigation. Separate investigation needed on important leachate contaminants such as heavy metals or emerging pollutants, which could influence the overall applicability of the fungal treatment in real-world scenarios. Continued research and innovation in these areas can contribute to sustainable and efficient fungal-based leachate treatment solutions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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