Biofiltration of Volatile organic compounds Using Chir Pine Cone Nuts Inoculated with *Pseudomonas putida*

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Abstract. Volatile organic compounds (VOCs) and hazardous air pollutants (HAPs) are the major pollutants in industrial and agricultural emissions. This study targets the efficiency and applicability of biofiltration to remove methanol and n-hexane, two common air pollutants, using Chir pine cone nuts as filter media and Pseudomonas putida as the inoculant. The designed biofilter was operated between 25-35°C, with an airflow rate of 0.35 m^3 h^{-1} and nutrient supply of 1-2 L Day^{-1}. From a 60-day operating period, methanol's removal efficiency was higher than n-hexane, with a maximum removal efficiency of 93.91% achieved at an inlet loading rate of 101.39 g $m^{-3}h^{-1}$ for methanol and 87.96% at 398.46 g $m^{-3}h^{-1}$ for n-hexane. The effects of varying loading rates on the elimination capacity for both pollutants were also studied. In addition, the temperature profile of the biofilter, microbial analysis, and the BOD concentration of leachate was also studied during the operation period. The findings offer insights into the potential use of Chir pine nuts as filter media for the biodegradation of methanol and n-hexane and provide a foundation for future research to optimize the biofilter system's design and operation to increase its removal efficiency of other contaminants.

Keywords: Air pollutants, Biofiltration, Pseudomonas putida, Chir Pine Cone Nuts, VOC.

1. Introduction

Today's world faces a significant challenge with air pollution, primarily caused by increased human requirements, accelerating industrialization. The resulting air pollution has drawn much attention, focusing on removing volatile organic compounds (VOCs), significantly contributing to air quality degradation. Around 25% of VOCs in our global atmosphere are estimated to come from anthropogenic activities, with industrial operations related to petrochemical, wood, pulp, and paint production playing a major role in VOC emissions. Numerous physical, chemical, and biological treatments for removing VOCs and other gases have been suggested in the literature (Bouzaza et al., 2004; Wang et al., 2007; Ralebitso-Senior et al., 2012; Padhi & Gokhale, 2014; Kureel et al., 2018; Pui et al., 2019). Of these treatments, biological treatments for VOCs have

proven to be more advantageous due to lower economic and operational investments and a better removal rate of pollutants, leading to less secondary pollution (Álvarez-Hornos et al., 2011; Zamir et al., 2015; Yang et al., 2019).

Methanol and n-hexane are two common VOCs frequently present in significant amounts. Methanol is usually formed from various industries and oil and gas extraction plants and has been responsible for major suicidal, accidental, and epidemic poisonings (Barbusinski et al., 2017; Prikyai et al., 2020). Similarly, n-Hexane is a naturally occurring chemical released during crude oil and natural gas extractions (Pan et al., 2017). Significant emitters of n-hexane are chemical plants, paints and adhesives production units, and industries that handle hexane for various operations (Tu et al., 2015; Karimnezhad et al., 2014; Volckaert et al., 2016). Inhalation of n-Hexane or even short-term direct exposure to high levels of this organic compound can cause central nervous system (CNS) effects (Chang et al., 1993; Sendur et al., 2009). While these incidents are extreme examples, they demonstrate the potential dangers of exposure to n-hexane and methanol and the importance of proper handling and safety precautions when working with these chemicals.

In a biofilter, the performance of VOC removal depends on the capacity of microorganisms to consume pollutant gases as a source of energy. These microbes convert the gaseous components into biomass and other inorganic products. The selection and employability of these microorganisms depend on various conditions to ensure adequate removal capacity. A schematic view of a biofilter and processes involved in pollutant removal is shown in Figure 1. Studies have demonstrated that different media filters can eliminate n-hexane and methanol in a monolith bioreactor (Jin et al., 2008; Rybarczyk et al., 2019). These studies confirm that gas flow rate and inlet concentration fluctuations are challenging to manage in a bioreactor, making this technology somewhat limited (Sercu et al., 2006).

Pinus roxburghii, commonly known as Chir pine, is a pine tree species found in the Himalayan regions. Chir pine is the most common of India's six species of pine trees and exists in the altitude range of 500 to 2000 meters (Kumar et al., 2019; Dhyani et al., 2022). NaOH-modified pine cones from *Pinus roxburghii* have been used as a biosorbent in South Africa (Ofomaja et al., 2009), and Pinus bark has been suggested as a medium in a low-cost and effective decentralized drinking water treatment facility (Shah et al., 2015). Despite this abundance of *Pinus roxburghii*, no studies have shown its ability to remove VOCs.

No prior investigation into the simultaneous biofiltration of n-hexane and methanol, particularly in exploring their mutual interactions and the potential for n-hexane removal under

such conditions. It is essential to consider that the performance evaluation of the bioreactor is critical since it is impossible to control environmental conditions outside the laboratories. Several media, including perlite, sterilized expanded clay, Sal wood chips, compost, and peat, have been explored to eliminate VOC via biofiltration.

The primary objective of this study was to assess the performance of a biofilter when treating n-hexane and methanol, considering different composition ratios. Still, after having such an abundance of *Pinus roxburghii*, the authors could not find any studies regarding its VOC removal abilities. The study evaluates the performance of Indian chir pine as a biofilter media for treating methanol and n-Hexane using *Pseudomonas putida*. The filtration capacity and the chir pine cone nuts media's efficiency were checked for the n-Hexane and methanol, respectively. Factors like temperature variation and microbial analysis of the leachate and filter media were studied along with the BOD and conductivity variations of leachate.

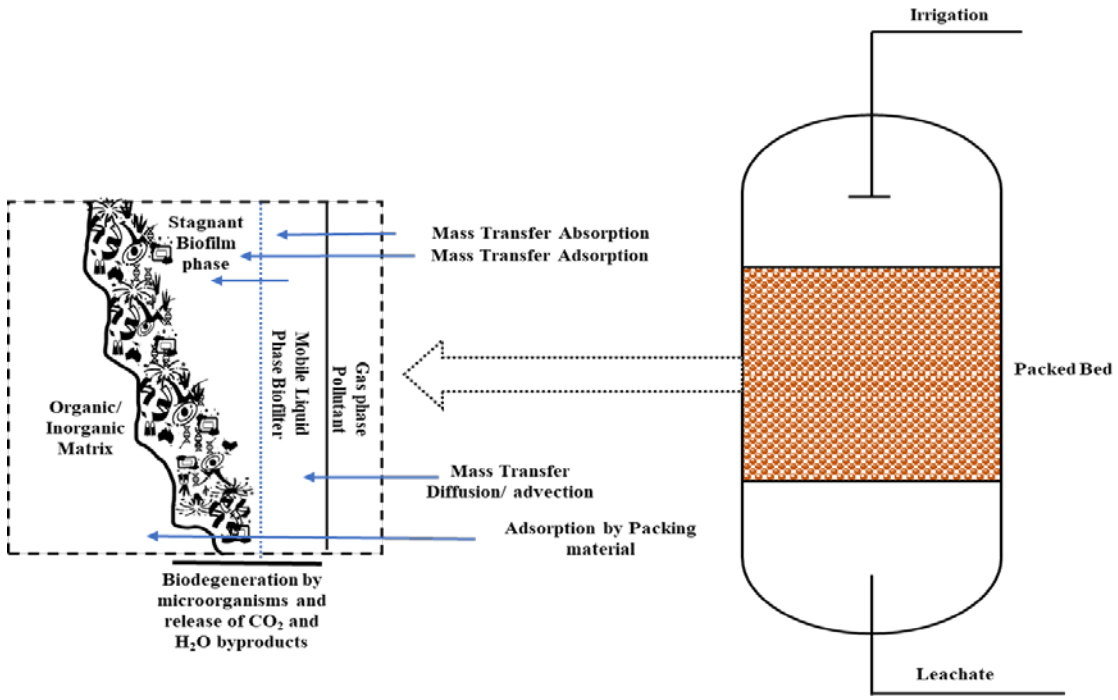

Figure 1. Illustration depicting the processes engaged in pollutant removal within biofilters

2. Materials and Methodology

2.1 Microorganism and Media

The bacterial strain *Pseudomonas putida* (MTCC-102) was obtained from the Microbial Type Culture Collection (MTCC) in Chandigarh, India, and cultivated in a nutrient broth solution at 28°C. A nutrient solution was prepared according to the composition shown in Table 1 to support

the growth of these heterotrophic microorganisms in the biofilter. The filter bed was packed with chir pine (*Pinus roxburghii* Sarg) cone nuts media sourced from Tehri Garhwal, Uttarakhand. The pine cones were washed, dried in sunlight, and oven-dried at 45°C. The size of the nuts ranged from 1.5 cm to 4 cm. The *Pseudomonas putida* strain was inoculated in the media to initiate the VOC removal process. The various stages of media (Chir pine cones) during the VOC removal process are illustrated in Figure 2. The concentration of n-Hexane and methanol was analyzed with the help of a Gas Chromatograph.

Table 1. Composition of Nutrient Solution.

Nutrient Salt	**Quantity (g L^{-1})**
$NaNO_3$	6
Glucose	2
$MnSO_4.H_2O$	0.00136
$ZnSO_4.7H_2O$	0.00058
$KHPO_4$	1.3
KH_2PO_4	0.5
$MgSO_4.7H_2O$	0.5
$CaCl_2$	0.055
$FeSO_4.7H_2O$	0.00136
$CoCl_2.6H_2O$	0.00024
$CuSO_4.5H_2O$	0.0002

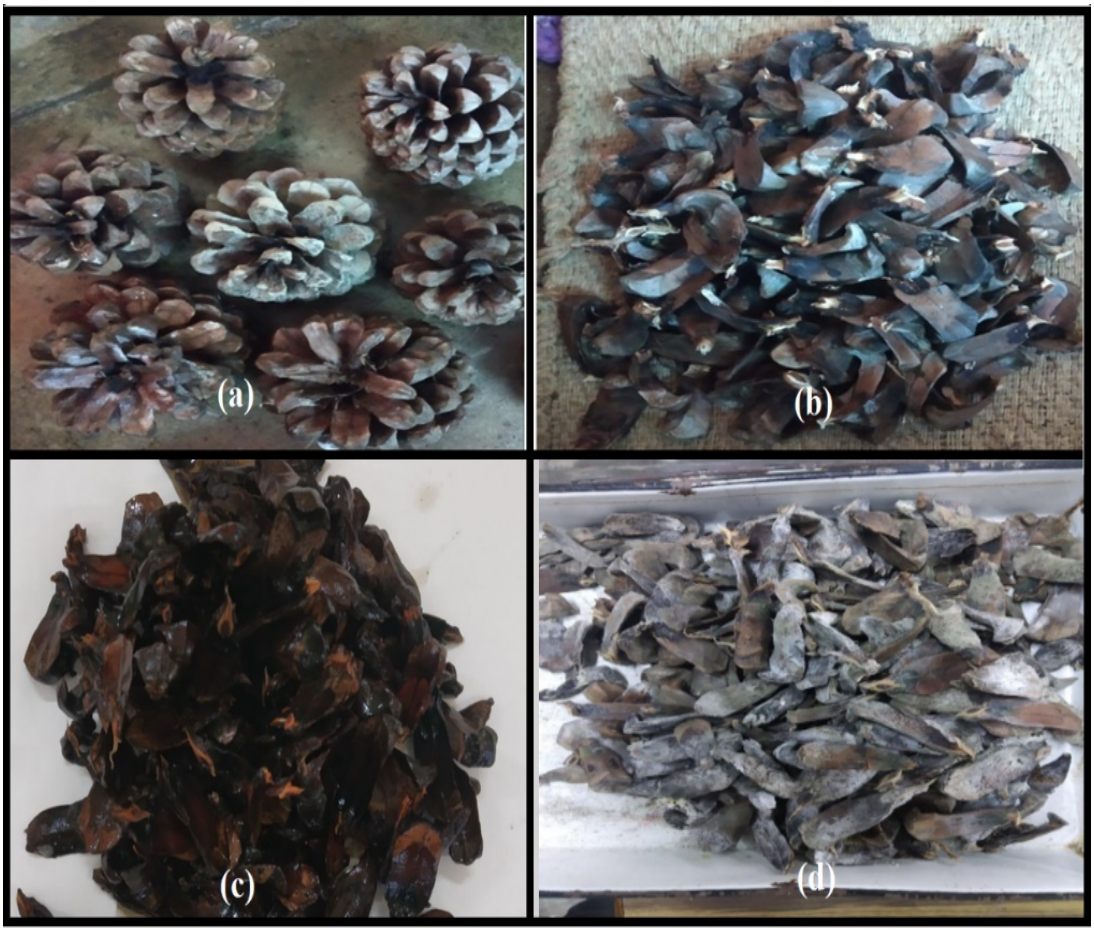

Figure 2. (a) Chir Pine Cones used as media, (b) Media immediately after inoculation, (c) Media after seven days of inoculation, and (d) Media after Completion of Run

2.2 Biofilter

The filter is designed into four sections joined with the help of screws. To make the biofilter airtight High vacuum silicon grease was applied. The total packed depth of the bed was limited to 70.5 cm, whereas the calculated bed volume was 4.65 liters. Two rotameters were installed to measure the airflow rate going to n-Hexane and methanol vessels. It ensured the controlled concentration of pollutants in the inlet stream. Using the inlet, outlet, and sampling ports, samples were collected at regular intervals using an airtight syringe. The schematic diagram and the various components of the experimental setup are mentioned in Figure 3. The experimental operating conditions of the biofilter are given in Table 2.

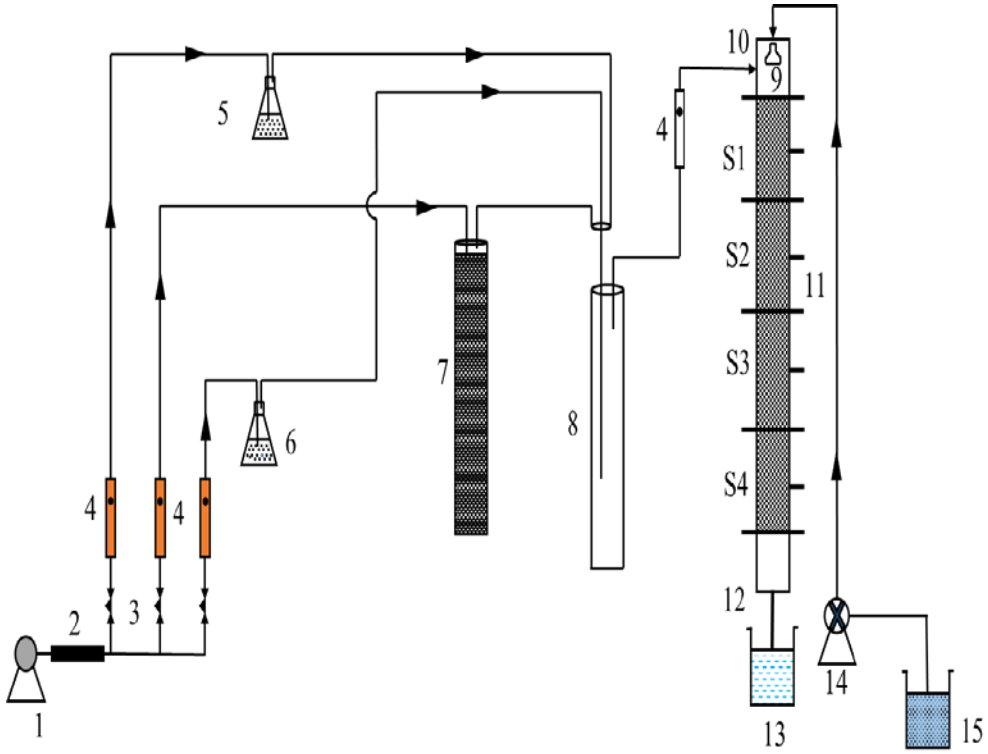

Figure 3. Schematic Diagram of Experimental Setup. 1. Air pump 2. Moisture/Carbon trap 3. Mass flow controller 4. Rotameter 5. Methanol container 6. n-Hexane container 7. Humidifier 8. Mixing compartment 9. Spray nozzle 10. Inlet 11. Biofilter 12. Clean air outlet/ Leachate collection port 13. Leachate collection vessel 14. Peristaltic pump 15. Nutrient solution flask

2.3 Performance Evaluation Parameters

The evaluation of this study is done in terms of following terms:

$$Inlet\ Loading(IL)\ in\ \ g\ m^{-3}h^{-1} = (Q \times C_1)/V \quad (1)$$

$$Removal\ Efficiency\ (RE)\ in\ (\%)\ =\ \left[1-(C_o/C_1)\right]\ *100 \quad (2)$$

$$Elimination\ Capacity\ (EC)\ in\ g\ m^{-3}h^{-1} =\ \left[Q\times(C_1-C_0)\right]/V \quad (3)$$

$$Empty\ Bed\ Residence\ Time\ (EBRT)\ in\ hours\ =\ V/Q \quad (4)$$

The parameters are defined as follows: C_1 = concentration of contaminant at inlet (gm^{-3}), C_o = concentration of contaminant at outlet (gm^{-3}), Q = volumetric gas flow rate (m^3h^{-1}), and V = volume of filter bed (m^3).

Table 2. Operating Conditions of Biofilter.

Parameter	Value
Pollutants	n-Hexane and methanol
Support media	Chir pine cone nuts
Microorganism	Pseudomonas Putida
Dimensions of packing media	5-8 cm broad and 8-12 cm long
Porosity of packing media	64%
Density of packing media ($g/cm^{3)}$	0.7127
Height of Biofilter column (cm)	70.5
Diameter of Biofilter column (cm)	9.4
n-Hexane concentration at the inlet ($g/m^{3)}$	2.34-6.35
Methanol concentration at the inlet ($g/m^{3)}$	1.25-3.16
Airflow rate (m^3/h)	0.36
Temperature of the Biofilter (°C)	28-40
Volume of nutrient solution added (L Day^{-1})	1-2
EBRT (seconds)	50

3. Results and Discussion

3.1. Start-up of Bacterial Biofilter

In the initial stage, bacterial culture was inoculated onto the packing medium, and during this stage, the contaminated stream was not allowed into the biofilter. This was done to ensure bacterial growth on the material bed. After a week, bacterial growth was observed, and the color of the chir pine cone nuts turned dark. The biofiltration was started with an initial loading of 151.13 – 192.44 g m^{-3} h^{-1} and 80.74 – 123.34 g m^{-3} h^{-1} for n-Hexane and methanol, respectively. All sections of the biofilter showed bacterial growth with a regular supply of nutrient solution at a rate of 1 L/day. Section 1 of the biofilter exhibited the maximum bacterial growth, whereas the top section (i.e., the 4th section) had the most negligible bacterial growth.

3.2.Temperature profile of biofilter

Temperature profiles for all four biofilter sections were recorded and shown in Figure 4. The temperature profile fluctuated due to moisture content variations, incoming gas temperature, and microbial activity on the bed's surface. The contaminated gas flow rate was maintained at 0.35 m^3h^{-1} with an EBRT of 50 seconds. The bottom section (Section 1) had a higher recorded

temperature than the topmost section due to the cooling effect of the gas stream from the point of entry to exit. The maximum recorded temperature was observed at the bottom section, which indicated higher biodegradation activity and maximum growth of microbes, resulting in higher elimination of targeted contaminants. Removal efficiency for n-Hexane and methanol and temperature variations are depicted in Figure 5. The study confirms that temperature affects the removal efficiency of both contaminants, with the maximum removal efficiency observed for n-Hexane and methane at temperatures of 36°C and 33°C, respectively.

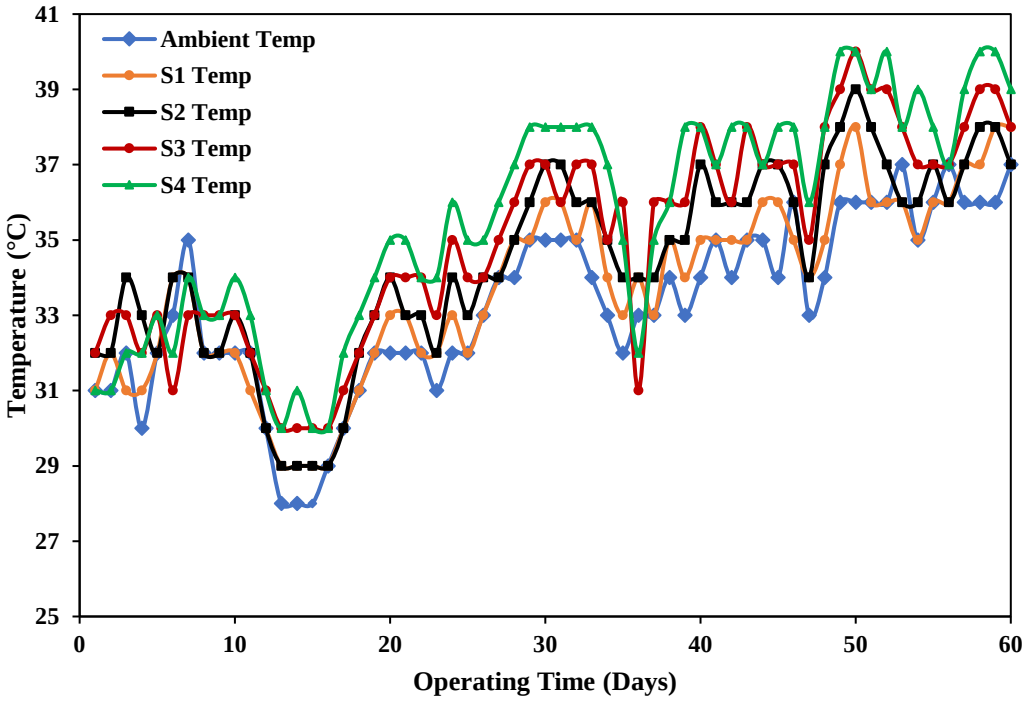

Figure 4. Temperature Variations at Various Sections of Biofilter

3.3. Removal Efficiency

3.3.1. For Phase I

The efficiency of the Chir pine-based biofilter was evaluated in two phases, namely Phase I and Phase II. During Phase-I, a start-up period of 30 days was carried out with a low loading rate range of 151.12 to 192.45 g m^{-3} h^{-1} for n-Hexane and 80.73 to 123.35 g m^{-3} h^{-1} for methanol while maintaining a constant air flow rate and EBRT. As shown in Figure 4, the removal efficiency for n-Hexane increased from 30.45% on the first day to 48.69% on the 30th day, while for methanol, the biofilter showed an efficiency of 83.89% in the beginning, increasing to 91.35% on the 30th day of operation. After two weeks, the degradation rate was almost constant, with temperatures ranging from 28°C to 40°C.

3.3.2. For Phase II

For the second phase, the performance evaluation of the biofilter was checked at higher loading rates. A varied loading rate ranging from 364.25-410.47 g m^{-3} h^{-1} was selected for n-Hexane, whereas Methanol loading varied from 192.48 to 204.08 g m^{-3} h^{-1}. On the 40th day, the maximum elimination capacity of n-hexane was 235.09 g m^{-3} h^{-1}, which was 79.61%. The maximum removal efficiency of 87.96% for n-Hexane was observed on the 38th day of operation. In the case of methanol, the maximum removal efficiency of 92.97% was achieved on the 34th day of operation (Figs 6 and 7).

On the 43rd day of biofilter operation, the maximum elimination capacity for n-Hexane was 235.07 g m^{-3} h^{-1}, achieved on the 40th day at an inlet loading of 408.15 g m^{-3} h^{-1} and corresponding to a removal efficiency of 57.61%. The maximum elimination capacity for methanol was 182.76 g m^{-3} h^{-1}, achieved at an inlet loading of 198.91 g m^{-3} h^{-1} and corresponding removal efficiency of 91.93%. The higher concentrations of n-Hexane and methanol in the inlet air stream resulted in a decline of microbial count in the chir pine cone nuts media from 7.20×10^9 CFU to 5.80×10^9 CFU, which could be attributed to the toxicity caused by the higher concentrations of methanol and n-Hexane to the microorganisms.

Table 3. Summary of Biofilter Operating Conditions.

Phase of Operation	**Flow Rate (m^3/h)**	**EBRT (s)**	**Methanol Inlet conc. (g/m^3)**	**n-Hexane Inlet conc. (g/m^3)**	**Methanol Loading Rate (g m^{-3} h^{-1})**	**n-Hexane Loading Rate (g m^{-3} h^{-1})**	**Operation Days**
Phase I	0.35	50	1.25-1.91	2.34-2.98	80.73-123.35	151.12-192.45	1-30
Phase II	0.35	50	2.98-3.16	5.64-6.35	192.05-204.07	364.23-410.08	31-60

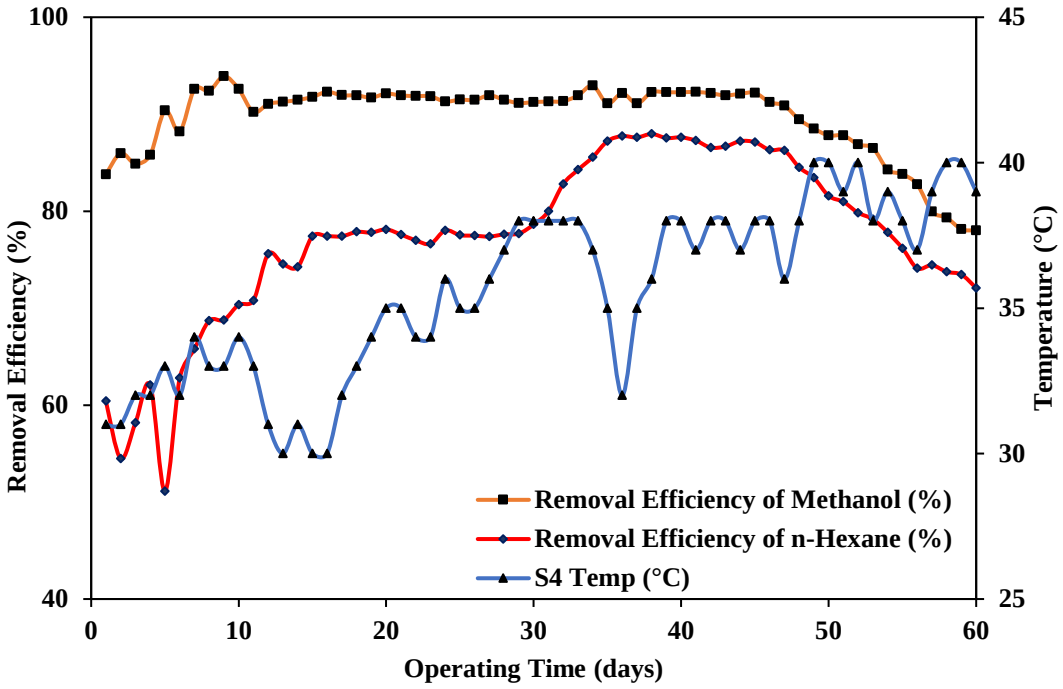

Figure 5. Variation of temperature in Section 4 and removal efficiency of methanol and n-Hexane

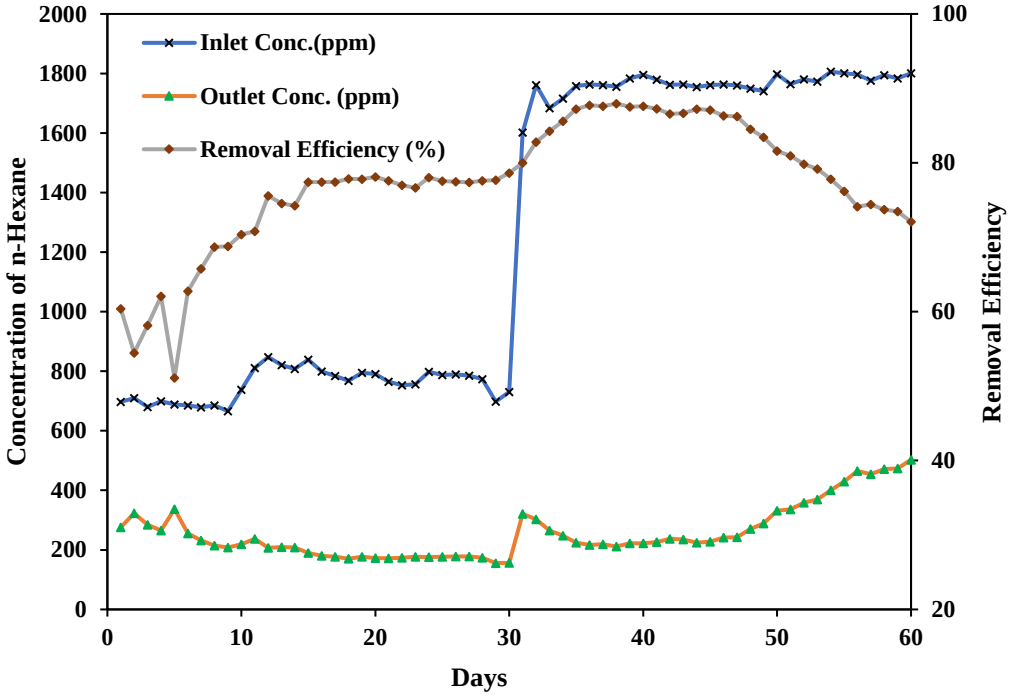

Figure 6. Biofilter performance for n-Hexane removal

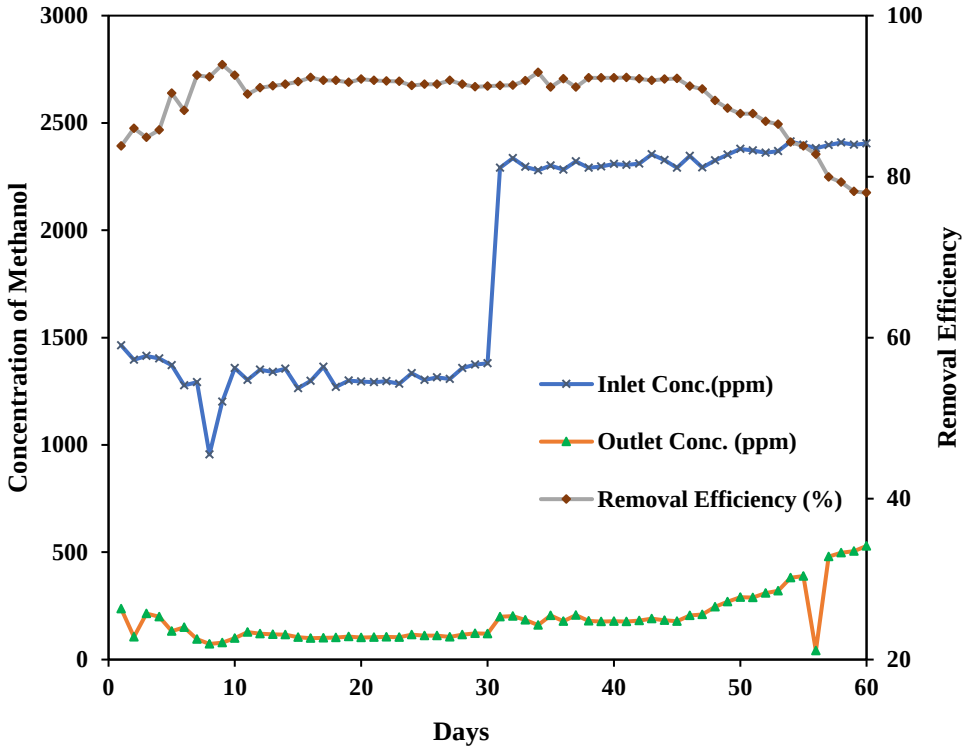

Figure 7. Biofilter performance for methanol removal

3.4. Microbial Analysis

Plate count tests were conducted on the chir pine media and leachate on days 2, 10, 18, 26, 34, 42, 49, and 54. The results confirmed a higher microbial population on the media and in the collected leachate (Fig. 8). A decline in microbial concentration was noticed on the 42nd day, which continued until the 54th day of operation. The observed data confirms that the bacterial growth declined with the increment of loading rate. The rise in temperature and higher toxicity due to the high inlet concentration of contaminants might be the reasons behind the decline. On the 34th day of biofilter operation, the test resulted in a maximum microbial concentration of 58×10^6 CFU per ml of leachate.

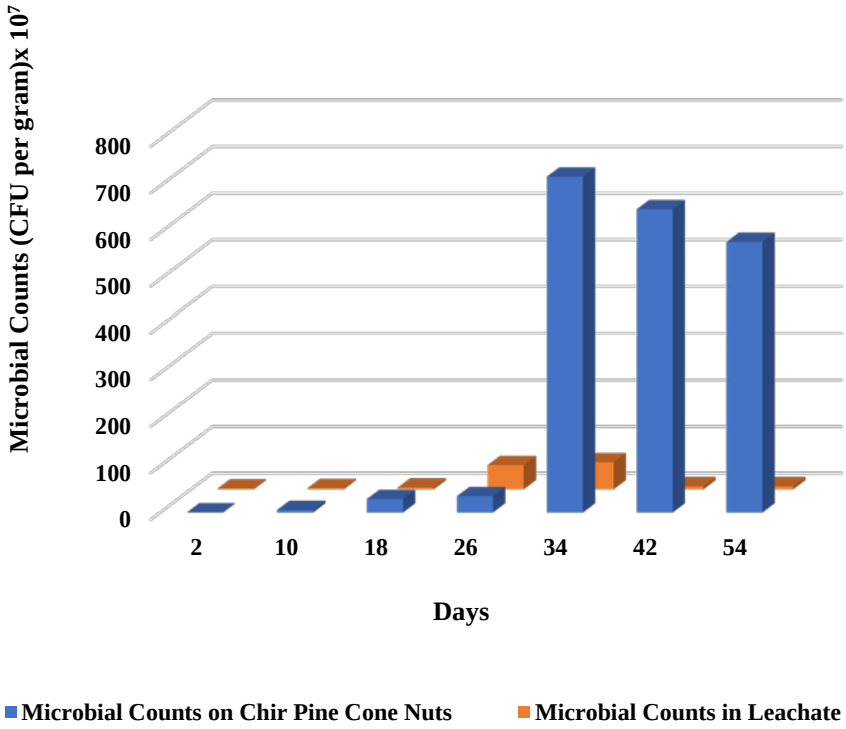

Figure 8. Bacteria population on Chir pine cone nuts media and in leachate

3.5. BOD of Leachate

The collected leachate from the biofilter was analyzed weekly for BOD values. Figure 9 shows the variation of BOD results over time for the leachate. The BOD values ranged from 250 to 350 mg/L. According to EPA 1986, the maximum value of BOD for direct wastewater discharge into the environment is around ten mg/L, and the maximum permissible value is limited to 300 mg/L for discharge into sewer systems (The Environment (Protection) Act, 1986). Therefore, treating the leachate before directly disposing of it is necessary.

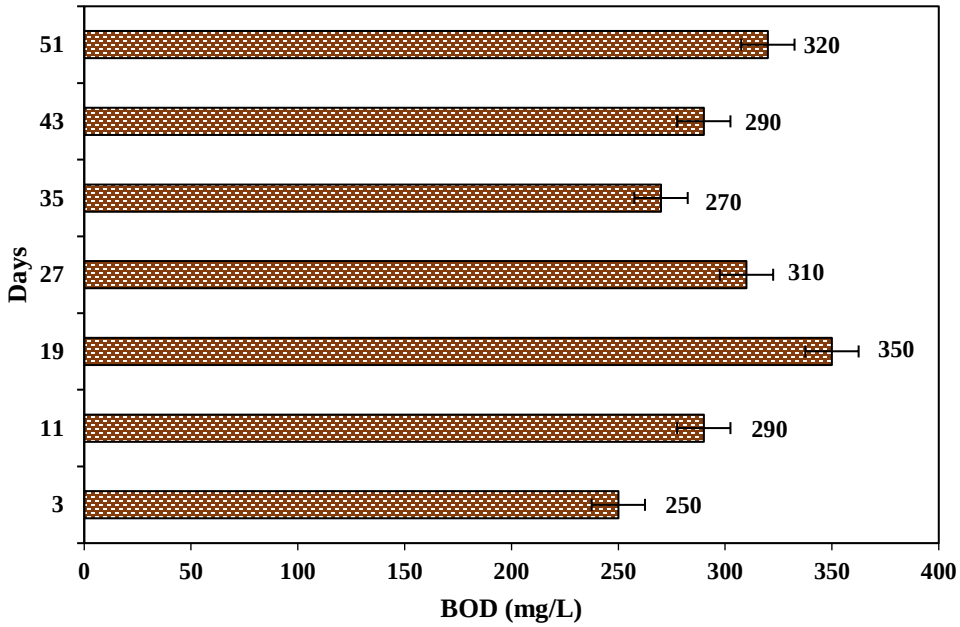

Figure 9. Variation of BOD of leachate with time

3.6. Conductivity of Leachate

To check the concentration of ions in the leachate, conductivity tests were done regularly. Before the initial supply, the solution had an average of 8.61 mS/cm conductivity. The conductivity of the original nutrient solution was always higher than the conductivity of the leachate. The analysis indicated the consumption of nutrients by the existing microorganisms on the filter media. It was noted that the conductivity of the leachate was lowest when the microorganisms were growing at a peak on the packing media. Figure 10 shows that the highest number of bacteria was reported on the 34th day with a count of 7.20×10^9, and the conductivity reported on the same day was 6.71 mS/cm. After this stage, the leachate's conductivity increased, whereas the number of bacteria decreased. Factors like bacterial growth on media, temperature, and moisture content of the media were responsible for the variation in the conductivity of the leachate.

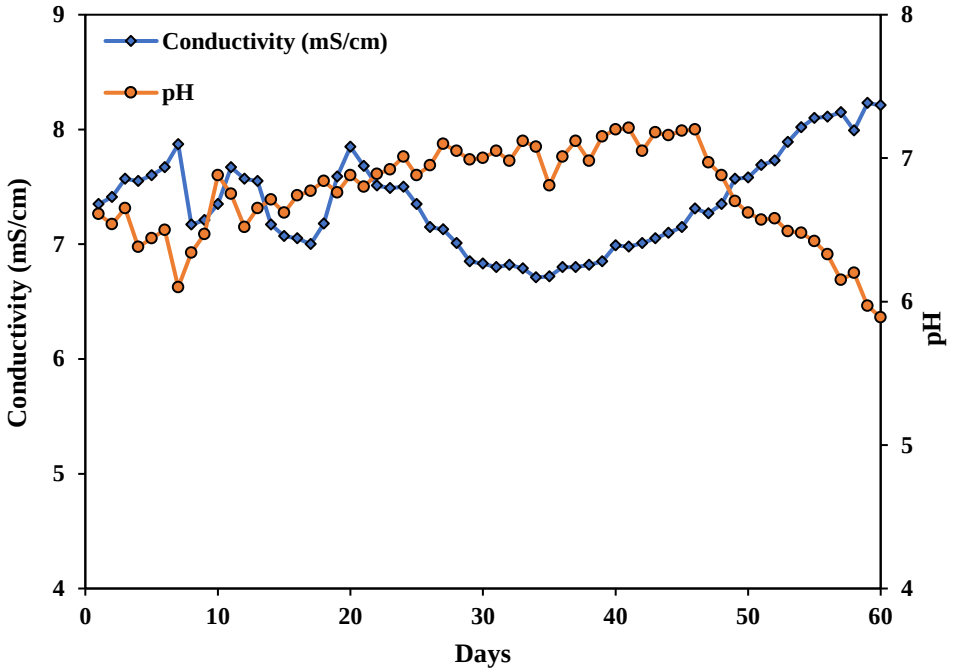

Figure 10. Variation in bacterial counts on media and conductivity of leachate with time

4. Conclusions

This study's results demonstrate that combining Chir pine cone nuts as a filter media and inoculating *Pseudomonas putida* bacteria is an effective method for removing n-Hexane and methanol from contaminated air streams. The Chir pine cone nuts media filter provides an adequate sorption capacity and high tolerance to varying loading rates, which help control fluctuations in the degradation rate. It is important to note that high loading rates of n-Hexane and methanol restrict the growth of pseudomonas putida, and continuous higher concentrations of these contaminants caused a decline in microbial growth on the filter media. The study also found that methanol adversely affected the degradation rate for n-Hexane, while the acclimation period for n-Hexane removal was higher than that of methanol.

The leachate from the biofilter showed high BOD values, indicating the need for treatment before disposal. The experimental results provide valuable insights into the potential of using Chir pine cone nuts as a filter media for the biodegradation of n-Hexane and methanol. Various factors such as microbial growth, temperature, and inlet contaminant concentrations may impact the biofilter's performance. Further studies are needed to optimize the biofilter's performance under different operational conditions and make it a more efficient and practical technology for industrial applications. Therefore, future research could focus on optimizing the biofilter system's design and operation to enhance its performance and increase the removal efficiency of other contaminants.

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