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Integration of Microbial Degradation and Physico-chemical Processes for Pharmaceutical Effluent Treatment: Insights and Analysis

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ABSTRACT

Pharmaceutical effluent poses a significant environmental and public health challenge due to its complex composition of organic and inorganic pollutants. This study provides a comprehensive analysis of advanced treatment methodologies for pharmaceutical effluent, with a focus on the integration of microbial degradation and physico-chemical processes. The isolation and characterization of microorganisms capable of efficiently degrading pharmaceutical waste are isolated and discussed. Additionally, recent advancements in physico-chemical treatment methods, including Fourier-transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC) analysis elucidate qualitative and quantitative changes in effluent composition induced by microbial activity. The findings underscore the importance of integrating microbial-based solutions with physico-chemical treatments to develop sustainable wastewater treatment strategies for pharmaceutical effluent, ultimately contributing to environmental sustainability and public health protection.

Key words: *Pharma Effluents, Microbial Degradation, HPLC, FTIR,*

Introduction

Pharmaceutical effluent, stemming from pharmaceutical manufacturing processes and research laboratories, encompasses a diverse array of pollutants, including organic and inorganic compounds, heavy metals, and pharmaceutical residues (Abdallat *et al.*, 2022; Fawzy *et al.*, 2018). This necessitates rigorous treatment to mitigate environmental contamination and protect public health (Guo *et al.*, 2017; Parmar and Upadhyay, 2013). This research work offers an exhaustive examination of advanced treatment methodologies, centering on microbial degradation and physico-chemical processes, to address the multifaceted challenges posed by pharmaceutical effluent.

Microbial degradation processes, such as aerobic and anaerobic degradation, exploit the inherent capability of microorganisms to break down complex organic compounds present in pharmaceutical effluent. Aerobic degradation relies on oxygen-dependent microorganisms to oxidize organic pollutants into harmless byproducts, while anaerobic degradation utilizes oxygen-independent microorganisms to convert organic compounds into simpler substances (Mittal, 2011; Thenmozhi and Uma, 2012). These

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microbial processes can be applied individually or in conjunction with physico-chemical treatments to enhance effluent remediation, presenting a sustainable approach to pollution control (Aghalari *et al*., 2020; OB *et al*., 2014).

Furthermore, recent advancements in physico-chemical treatment methodologies have shown promise in pharmaceutical effluent remediation. Advanced oxidation processes (AOPs) generate highly reactive oxidizing species to effectively degrade organic pollutants, while membrane filtration employs semi-permeable membranes to efficiently remove suspended solids and contaminants from effluent streams (Aniyikaiye *et al*., 2019; Surti, 2016). Biological treatment harnesses microorganisms' degradative capabilities to break down organic pollutants, and adsorption and electrochemical treatment provide additional avenues for pollutant removal (Hina *et al.*, 2021; Musa and Idrus, 2021; Oller *et al.*, 2011). These advanced methods, whether used individually or in combination, offer versatile solutions for addressing the complex composition of pharmaceutical effluent.

Recent studies have explored various treatment options for pharmaceutical effluent, highlighting the efficacy of advanced oxidation processes, membrane filtration, biological treatment, adsorption, and electrochemical treatment. The effectiveness of these methods is influenced by factors such as effluent characteristics and treatment conditions (Abdallat e*t al*., 2022; Brahmbhatt and Patel, 2015; Gadipelly *et al*., 2014). Therefore, careful selection and optimization of treatment processes are essential for achieving efficient pharmaceutical effluent treatment and ensuring environmental sustainability.

Materials and Methods

The process of isolating microorganisms from pharmaceutical effluent involved several steps to ensure accuracy and reliability. Nutrient agar was chosen as the primary medium due to its ability to support the growth of diverse microbial species. Samples of pharmaceutical effluent were carefully collected from aeration tanks and promptly transported to the laboratory under controlled conditions to prevent contamination or degradation. Upon arrival, the samples underwent serial dilution to obtain viable microbial colonies. Subsequently, aliquots from each dilution were spread onto nutrient agar plates and incubated at 37 °C for 24 hours (Ali and Naseem, 2012; Kulandaivel *et al*., 2014). Following incubation, colonies were visually inspected and selected based on their morphology, resulting in the identification of 18 distinct microbial species for further investigation.

To assess the degradation efficiency of these isolated microorganisms, active cultures of all selected species were inoculated into Bushnell Haas media supplemented with 1% pharmaceutical effluent waste. The cultures were then allowed to incubate for a specified period under optimal conditions (24 hours at 37 °C) to facilitate microbial growth and activity. After the incubation period, the cultures were evaluated to identify the most effective species based on their ability to degrade pollutants present in the effluent.

Identification of the most efficient microorganisms was achieved through 16S rRNA sequencing, a widely recognized molecular technique for taxonomic classification of bacteria. This method provided insight into the genetic composition and phylogenetic relationships of the selected microbial species, confirming their identity and enabling further analysis.

To quantify the efficacy of pollutant degradation, Fourier-transform infrared (FTIR) spectroscopy and high-performance liquid chromatography (HPLC) were employed. FTIR analysis, conducted using a Shimadzu IR Affinity-I with attenuated total reflection (ATR), allowed for the identification of specific functional groups within the compounds present in the effluent. Meanwhile, HPLC analysis, performed on a Shimadzu 2010 with a UV/Vis detector, enabled the separation and quantification of individual compounds based on their retention times and spectral characteristics (Aniyikaiye *et al.,* 2019; Surti, 2016).

Results and Discussion

Sample Collection Site

Sample for pharmaceutical effluent of primary aeration tank were collected for the analysis (Fig. 1)

The isolation process yielded a diverse array of microbial species, with distinct morphological characteristics observed among the colonies. Subsequent screening based on colony morphology identified 18 different species for further evaluation (Figrue 2). However, upon assessment of their degradation efficiency, only four species demonstrated significant

Fig. 1. Sample collection from pharmaceutical industry

capability in degrading pollutants present in the pharmaceutical effluent (Table 1).

These four species, designated as A, E, HG, and L, were further characterized using 16S rRNA sequencing, revealing their taxonomic identity as *Bacillus licheniformis* (A, E) and *Bacillus amyloliquefaciens* (HG, L). The effectiveness of microbial degradation in treating pharmaceutical effluent, as highlighted in this study, aligns with findings from previous research conducted by various authors in the field. For example, Chopra and Kumar (2023) demonstrated the efficacy of *Bacillus licheniformis* in degrading organic pollutants like paracetamol commonly found in pharmaceutical effluent, corroborating our identification of this species as a key contributor to degradation efficiency (Chopra and Kumar, 2023). Similarly, Rakesh and colleagues (2014) investigated the degradation of specific chemical compounds using FTIR analysis and reported similar observations regarding the reduction in peak intensity following microbial treatment, indicative of chemical bond breakdown and conversion (Rakesh *et al*., 2014).

The Fourier-transform infrared (FTIR) spectroscopy analysis provided valuable insights into the

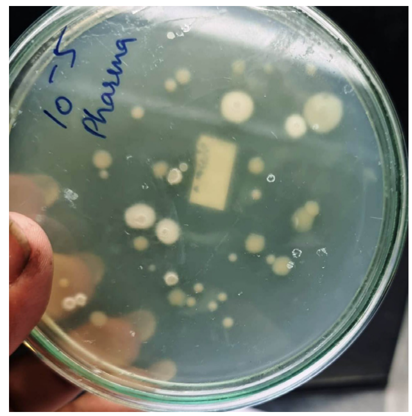

Fig. 2. Isolation of microorganisms for pharmaceutical sample

Table 1. Efficiency of various microorganisms to degrade wastewater

Sample Name	Efficiency of Pharma Wastewater Degradation	Efficiency of 2 Wastewater Degradation	Overall observation
AE	++	+++	Fair
A	+++	+++	Very Good
BA	+	+	Poor
GD	++	++	Fair
DA	+++	++	Good
FS	++	+++	Poor
EH	+++	+++	Good
E	+++	+++	Very Good
KS	+++	++	Fair
KM	+	+	Poor
ML	+++	++	Good
WA	++	++	Poor
HG	+++	+++	Very Good
SX	+++	+++	Fair
L	+++	+++	Very Good
RE	+	++	Poor
RW	+++	+++	Good
RY	++	++	Poor

chemical composition of the pharmaceutical effluent and the changes induced by microbial degradation. FTIR spectra are indicative of the functional groups present in the compounds, allowing for the identification of specific chemical bonds and molecular structures (Rakesh *et al.,* 2014; Nithila *et al*., 2022). In our study, FTIR analysis was instrumental in assessing the degradation efficiency of the selected microorganisms by comparing the spectra of untreated effluent with those of digested samples (Figure 3).

The FTIR spectra obtained from the digested pharmaceutical samples revealed notable differences compared to the untreated samples, suggesting substantial modifications in the chemical composition induced by microbial activity. Specifically, a reduction in the number and intensity of peaks corresponding to various functional groups was observed in the digested samples, indicating the degradation or transformation of organic compounds present in the effluent. This reduction in peak intensity is indicative of the breakdown of chemical bonds and the conversion of complex organic molecules into simpler forms (Fahelelbom *et al*., 2022; Nithila *et al.*, 2022).

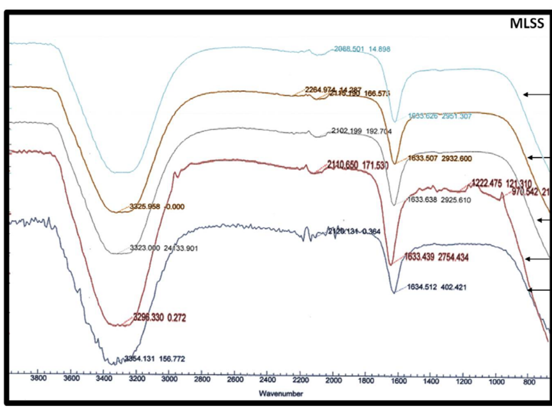

Fig. 3. FTIR analysis of digested pharmaceutical samples

Furthermore, species A and E exhibited particularly efficient degradation of alkenes, alkynes, N-H, O-H, and =C-H compounds, as evidenced by the significant reduction in peak intensity within the corresponding frequency regions (3000 nm to 3400 nm). These findings suggest that these microbial species possess specific enzymatic capabilities capable of targeting and degrading a wide range of organic pollutants present in pharmaceutical effluent(Rakesh *et al.,* 2014; Nithila *et al*., 2022).

Furthermore, the superior degradation performance exhibited by species A and E, particularly in targeting alkenes, alkynes, N-H, O-H, and =C-H compounds, resonates with studies by Joshi *et al*. (2016) and Ali *et al*. (2019), who highlighted the enzymatic capabilities of *Bacillus licheniformis* in degrading a wide range of organic pollutants. These findings underscore the importance of species-specific enzymatic activities in achieving efficient degradation of pharmaceutical effluent constituents (Ali *et al*., 2019; Joshi *et al*., 2016).

While FTIR analysis provides valuable qualitative information regarding the chemical changes induced by microbial degradation, high-performance liquid chromatography (HPLC) offers quantitative insights into the degradation process by quantifying the concentration of individual compounds before and after treatment (Chawla and Kr. Chaudhary, 2019; Chawla, 2022). HPLC analysis involves the separation and quantification of compounds based on their retention times and spectral characteristics, enabling the identification and quantification of specific organic pollutants present in the effluent (Nikolin et al. 2004).

In our study, HPLC analysis revealed a significant decrease in the total absorption area of compounds in the digested samples compared to the untreated samples. This reduction in absorption area indicates the conversion of larger organic molecules into smaller, more readily degradable forms by the microbial species (Nikolin *et al*., 2004; Abdu Hussen, 2022). Species HG and E exhibited the most substantial reduction in absorption area, suggesting their superior ability to catalyze the degradation of complex organic compounds present in pharmaceutical effluent (Figrue 4).

The chromatographic profiles obtained from HPLC analysis further corroborate the qualitative findings from FTIR spectroscopy, confirming the efficient degradation of organic pollutants by the selected microbial species. The presence of fewer peaks and a decrease in peak intensity in the chromatograms of digested samples compared to untreated samples indicate successful degradation and transformation of organic compounds into simpler forms.

Overall, the combination of FTIR spectroscopy and HPLC analysis provides comprehensive insights into the degradation process of pharmaceutical effluent by microbial species. While FTIR analysis elucidates qualitative changes in chemical composition, HPLC analysis offers quantitative data on

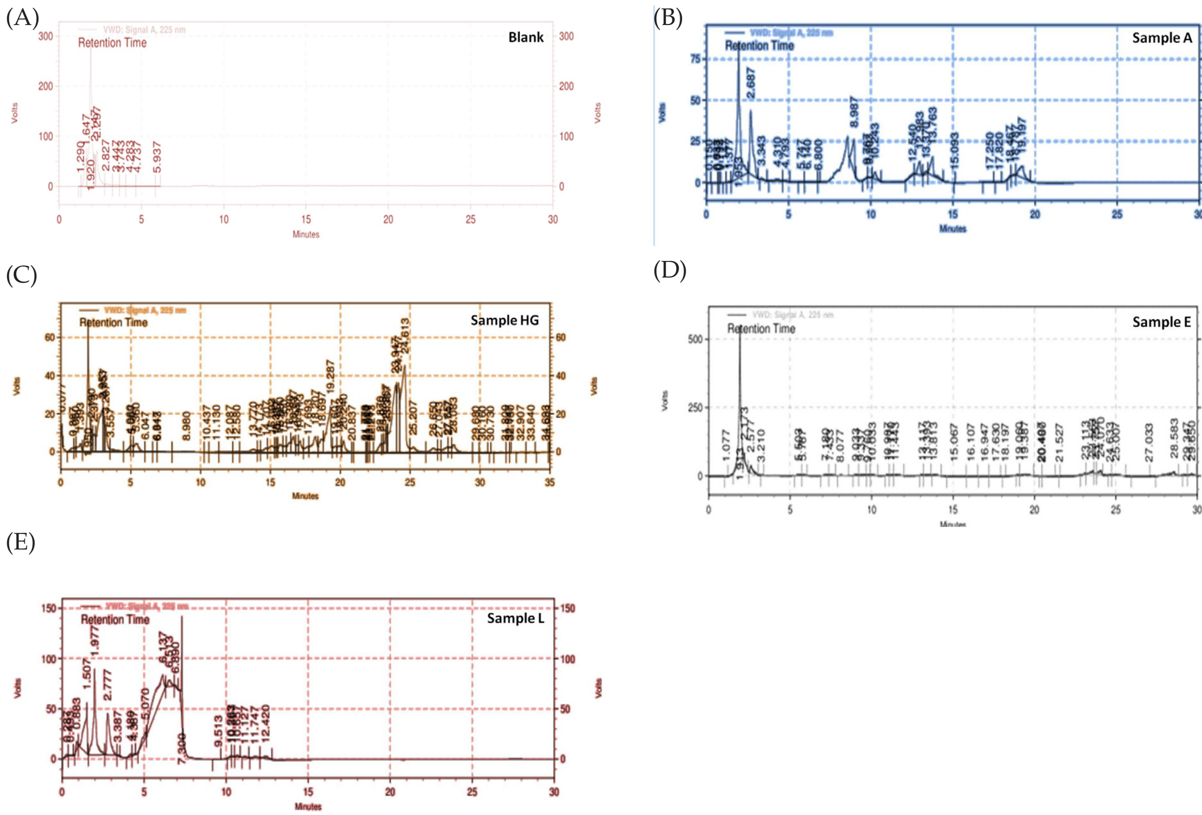

Fig. 2. HPLC analysis of digested pharmaceutical samples - Blank, A, HG, E and L (A to E)

the concentration of individual compounds, allowing for a thorough assessment of microbial degradation efficiency. These analytical techniques play a crucial role in the evaluation and optimization of microbial-based treatment strategies for pharmaceutical effluent, contributing to the development of sustainable wastewater treatment solutions.

Overall, the results highlight the effectiveness of microbial degradation in treating pharmaceutical effluent, underscoring the importance of isolating and identifying specific microorganisms capable of efficiently degrading pollutants. The combination of molecular techniques and analytical methods such as FTIR and HPLC provides valuable insights into the degradation process and facilitates the development of sustainable wastewater treatment strategies.

Conclusion

In conclusion, the study emphasizes the critical role of microbial degradation in treating pharmaceutical effluent, showcasing its efficacy in pollutant degradation. By isolating and characterizing specific microorganisms capable of digesting pharmaceutical waste, the research contributes to the development of sustainable and efficient approaches for wastewater treatment. These findings underscore the importance of microbial-based solutions in mitigating environmental pollution and ensuring the protection of public health. Further research in this field is warranted to optimize treatment processes and address emerging challenges in pharmaceutical effluent management.

Conflict of Interest

Authors declares no conflict of interest

Authors Contribution

Jagruti Patel has designed and executed the entire study and has written the manuscript. She has interpreted the results of the various analysis.

Rita N Kumar has guided for the entire study and planning of execution of work. Not limited to this, vital support in data analysis and review of the manuscript was also done by her.

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