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Effect of Nutrient Ratios of Palm Oil Mill Effluent (POME) on the Removal of Impurities by *Aspergillus brasiliensis*

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Abstract

This study is focussing the removal of impurities from Palm Oil Mill Effluent (POME) by a filamentous fungus (*Aspergillus brasiliensis*) which contains high levels of nutrients (organic pollutants) such as Biochemical Oxygen Demand (BOD), Total Suspended Solid (TSS) and Ammoniacal Nitrogen (NH_3-N). These pollutants can highly affect water quality and aquatic life and lack of oxygen in water bodies if not properly treated. The objective in this study is to evaluate the fungal' efficiency in removing impurities in POME at different dilution of POME (10%, 30% and 50%). For that, the trends of biomass growth of fungal (*Aspergillus brasiliensis*), removal percentage of BOD, TSS and NH_3-N were measured while pH and Dissolve Oxygen (DO) were observed periodically. Finally, the correlation between fungal with water quality parameter was determined. The results of this study showed that the *A. brasiliensis* growth well in 50% dilution of POME and *A. brasiliensis* effectively removes BOD_5, and TSS from POME over 5 days, with optimal removal outcomes depending on dilution level. A 30% POME dilution yielded the highest BOD_5 removal (90%)**,** NH_3-N shows no removal**,** and TSS removal was most efficient at 30% dilution with 13.33%. Low regression correlations between biomass and pollutant removal (R^2 = 0.001–0.7) suggest that the treatment efficiency of *Aspergillus brasiliensis* is governed more by enzymatic activity and physicochemical interactions than by biomass growth alone. The study highlights that *Aspergillus brasiliensis* efficiently removes BOD_5, NH_3-N, and TSS from POME, with optimal removal depending on dilution, and underscores the significance of enzymatic activity and physicochemical interactions over biomass growth, contributing valuable insights to fungal-based bioremediation of industrial effluents.

Keywords: Ammoniacal Nitrogen, *Aspergillus brasiliensis*, Biochemical Oxygen Demand, POME, Total Suspended Solids

1. Introduction

Palm Oil Mill Effluent (POME) is an important waste product produced during the palm oil processing which contain high pollutant material such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), phenolic substances, and suspended solids. These impurities present a serious environmental risk, as untreated discharge can cause oxygen depletion in water bodies and threating aquatic life. According to previous study by Albuquerque et al., (2024), the researcher was estimated that about 2.5 to 3.0 tons of POME has been created for every ton of crude palm oil produced by the palm oil industry. An effective treatment method is required because of the environmental impact of POME. The traditional methods of POME treatment, such as ponding systems and anaerobic digestion, though widely used, have their limitations. Ponding systems involve huge amounts of land and time; these systems mostly do not remove the impurities completely. Even though chemical treatments can effectively reduce some pollutants, they can be expensive and produce hazardous byproducts that make waste disposal even more difficult (Ofon et al., 2024; Razak et al., 2022).

Ideally, POME should be treated efficiently to meet the environmental standards so that there is no pollution of water bodies or soil. However, existing treatment technologies, such as anaerobic digestion, coagulation, and flocculation, usually cannot remove impurities perfectly, especially colour and phenolic compounds, which are harmful to aquatic life. The methods also lead to high energy consumption, the generation of secondary waste, and are not economically feasible for small-scale palm oil mills. Therefore, the inefficiency in the treatment process results in partially treated POME being discharged; this causes water pollution, eutrophication, and hence eventually damages the ecosystems. Therefore, there is an increasing interest in biological treatment, especially those the use of fungi because biological treatment methods are sustainable and environmentally friendly.

Fungi, especially *Aspergillus* species, have shown potential in the breakdown by enzymes of complex chemical molecules. Research clearly focusing on *Aspergillus brasiliensis's* application to POME treatment is still limited, but the fungal species has shown promise for bioremediation methods (Thegarathah et al., 2020). Filamentous fungi, particularly species such as *Aspergillus* and *Trametes*, exhibit significant capabilities in the treatment of industrial wastewater. These fungi demonstrate resilience to harsh environmental conditions and effectively remove persistent pollutants, including heavy metals and pharmaceuticals, through mechanisms such as biosorption and biodegradation. Specifically, *Trametes versicolor*, a white-rot fungus, produces ligninolytic enzymes like laccases and peroxidases that efficiently break down complex pollutants, including dyes and lignin-like compounds (Ghosh et al., 2023). Additionally, species of *Aspergillus* are recognized for their dual roles in contaminant binding and microbial degradation, which enhances their applicability in sustainable wastewater treatment processes (Hultberg & Golovko, 2024). Although fungi have demonstrated efficiency in wastewater treatment, their use in POME treatment is currently still relatively rare. Since using fungi as treatments are eco-friendly, economical, and effective at break down complex contaminants, this would be the most appropriate method of approach. However, the other study from Tang, (2023) focus on chemical methods and bacteria, which creates a gap in knowledge regarding the precise mechanisms and methods of optimization for treatments using fungi. Due to the lack of in deep research, effective POME treatment with fungi solutions is not sufficiently produced, which leads to a continuous need for less sustainable methods. To fill this gap and create an affordable treatment system using fungi that can more effectively address POME contamination, the current research aims to study the effectiveness of *Aspergillus brasiliensis* in eliminating POME contaminants. The optimum scenario is for fungi treatment methods to be accurate and reliable despite changes in POME's nutrition levels. However, the amount of carbon and nitrogen sources, as well as other nutrients, greatly affects how well fungal treatments perform (Dominic & Baidurah, 2022). According to study from Low et al. (2021), the fungal growth and the rates at which impurities are removed can be greatly impacted by variations in POME composition, such as dilution or concentration, which might result in treatment effects that are unpredictable.

Due to this variation, growth of fungal-based systems is limited and efforts to comply with regulatory standards for effluent discharge are complicated. Thus, the purpose of study is (1) to determine the trends of biomass growth of fungal *(Aspergillus brasiliensis)* in 10%, 30% and 50% dilution of POME, (2) to determine the efficiency of *Aspergillus brasiliensis* to remove BOD, TSS, NH_3-N at different dilution of POME and finally (3) to determine the correlation between *Aspergillus brasiliensis* biomass with pollutant's removal. This study is significant to address the environmental issues raised by POME. Besides, this study aims to present a cost-effective treatment for POME through the application of *Aspergillus brasiliensis*, a naturally occurring on fungal. These findings highlight a potentially cost-effective alternative to chemical treatment for POME, which can reduce operating costs for palm oil producers while ensuring compliance with environmental regulations. These findings can help the government in developing and enforcing regulations on wastewater treatment standards, especially in the palm oil industry, helping to reduce environmental degradation.

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2. Research Method

2.1 Preparation of POME media

The POME was obtained from environmental laboratory 1 from School of Civil Engineering Uitm Shah Alam, Selangor and was diluted for further used as shown in Figure 1.

Figure 1. Dilution of POME for 10%, 30% and 50%

Distilled water was chosen because it is accessible and contain nutrients for fungi. Briefly, 3 different dilution of POME which are 10%, 30% and 50% with distilled water were prepared in this study. This methodology enables the identification of optimal concentration levels for effective biodegradation while evaluating the fungal tolerance to organic strengths. Furthermore, it simulates realistic wastewater conditions, which are crucial for potential applications in diverse treatment scenarios wherein filamentous fungi serve as key agents for the bioremediation of organic pollutants typically found in wastewater systems (Qader & Shekha, 2022). Briefly, about 100 ml of POME sample is added with 900 ml of distilled water for 10% whereas for 30% was prepared about 300 ml of POME sample is added with 700 ml of distilled water and followed by 50% dilution has been prepared about 500 ml of POME sample is added with 500 ml of distilled water. The initial concentration of BOD, TSS, NH_3-N, DO and pH was measured for each dilution and shown in **Table 1**.

Table 1. The initial concentration of each parameter of POME

Dilution percentage (%)	0 (original)	10	30	50	Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977 DOE (2009)
pH	4.43	7.975	8.985	9.355	5.5-9.0
BOD_5 (mg/L)	263.6	133.82	119.11	104.70	100
DO (mg/L)	0.9	3.24	3.40	3.94	>5
NH_3-N (mg/L)	20.4	16.38	7.81	3.13	10
TSS (mg/L)	7060	3285	2125	690	400

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2.2 The fungi culture preparation

A 20 uL of fungal spores at age 7 days was inoculated in the middle of sterilised potato dextrose agar (PDA, HiMedia, India) and incubated for 3 days at 30 °C. After the formation of mycelium, the fungi plug (1 cm diameter) was cut and transfer into the samples (Hamzah et al., 2024). Each POME dilution was prepared for 2 samples in obtain an accurate result. The pellet plug was inoculated into 50 ml of prepared POME and incubated in an incubator shaker at 150 rpm, 30 °C for 5 days. The sample was harvested daily for analysis. Filtration method was chosen for removal of fungal pellet from the POME using an 8 µm filter paper. This filtration method only removes fungal pellet and larger particles of wastewater but not contribute with changes of colour the wastewater. The biomass was filtered and dried in 60 °C oven until consistent weight was observed. The biomass was determined using Equation1.

$$\text{Biomass Concentration, mg/L} = (W_i - W_f)/V \qquad \text{Equation 1}$$

Where W_i and W_f indicate the initial and final weight of biomass without filter paper respectively, while V is volume of water sample used in the experiment.

2.3 Determination of Removal percentage of POME water quality

The following parameters such as BOD_5, DO, pH, TSS and NH_3-N were measured on sample before and after treatment with fungi in 5 days according to standard method provided. All the analysis methods were adapted from Standard Methods for the Examination of Water and Wastewater APHA (2017). The performance of *Aspergillus brasiliensis* in treating POME was determine by measuring the removal percentage of BOD, TSS, NH_3-N based on Equation 2 while pH and DO were monitored throughout the experiment duration.

$$\text{Percentage removal (\%)} = (C_i - C_f)/C_i \times 100 \qquad \text{Equation 2}$$

Where C_i is initial concentration of pollutant, and C_f is final concentration of the pollutant.

2.4 Statistical Analysis

All the data was collected and evaluated into Microsoft Excell 2016 to obtain the result including the regression analysis and to obtain the significant P (>0.05) from Two-Way ANOVA for POME wastewater characteristics (Abdulsalam et al., 2020). This analytical approach was selected because it enables the concurrent evaluation of both the individual and combined influences of two independent variables on a continuous dependent variable. Consequently, it provides a robust statistical framework for analyzing treatment performance across various experimental conditions (Trivedi & Chhaya, 2022).

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3. Results and Discussion

3.1 Biomass trend for *A. brasilliensis*

Figure 2 shows the results of biomass production by growth of fungi in various dilution of POME for five days. Based on the result, the growth of fungi is strongly determined by nutrient concentration in POME, which reflects the observed trend through the 10%, 30%, and 50% dilution.

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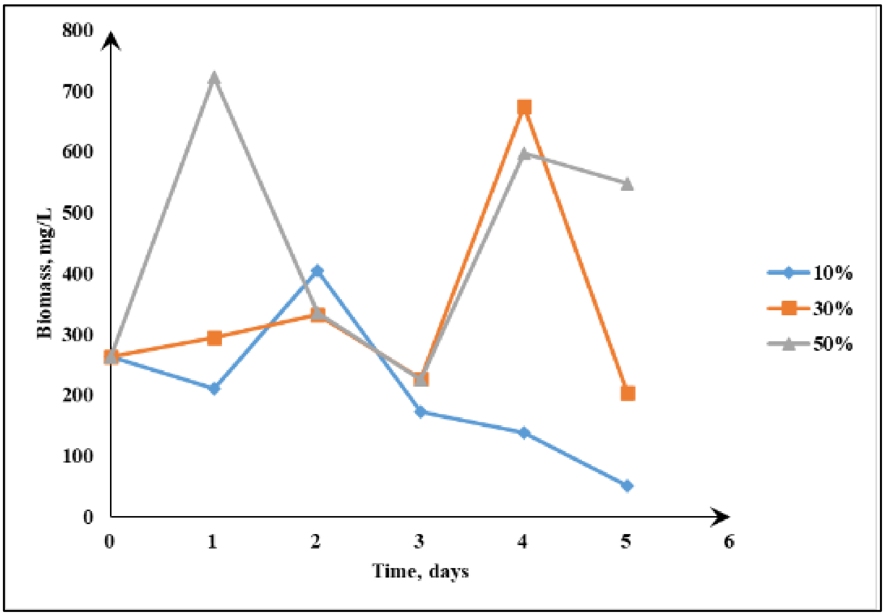

Figure 2. The growth trends of *Aspergillus brasiliensis* biomass in POME at 3 dilution levels (10%, 30%,50%)

Higher percentages of POME, especially at 50%, contain more nutrients and hence support better growth of fungal biomass; this has similarly been observed from previous findings regarding nutrient-rich wastewater that are conducive for microbial growth (Gad et al., 2023). Besides, the growth patterns across different dilution were also analyse in this research. Biomass growth in the 10% POME dilution is continuously low over the research, reaching a high of 405 mg/L on day 2. This delayed peak indicates that fungal growth and adaptability are negatively impacted by the nutrient limit. In contrast, biomass grows more rapidly at the 30% dilution, reaching early on day 4 at 675 mg/L before decreasing. The 50% dilution, on the other hand, shows the highest biomass growth, rising sharply to 722 mg/L on day 1 and then decreasing to day 3 and increase again into day 4, when it peaks once more at 597 mg/L. According to study on wastewater treatment systems, these results prove the hypothesis that increased biomass production is supported by higher nutrient availability in more concentrated POME (Nur et al., 2019). Lastly, the consistent decrease in biomass on day 5 across all dilutions indicates is due to greater competition or lack of nutrients in the fungal culture system. These findings indicate the significance of medium optimization or periodic nutrient replacement to maintain biomass production, which is a crucial factor for large-scale bioremediation process. The significance of choosing the right nutrient concentrations for effective fungal production, especially in wastewater treatment applications, is further highlighted by the significant rise in the 50% dilution from study by Palanisamy et al., (2023).

3.2 Efficiency of *A. brasiliensis* to remove BOD, TSS and NH3-N at different dilution

The data showed in **Figure 3** indicate the trend of wastewater pollutant removal of the POME at different dilution levels. The data showed a notable decrease in BOD_5 (**Figure 3(A)**) with percentages at range from 67 % to 90 %. This finding indicates active organic matter degradation by the fungal treatment. Despite fluctuations, the overall reduction suggests effective microbial activity. Variability may result from changes in substrate availability, fungal growth dynamics, or environmental factors influencing biodegradation efficiency throughout the treatment period (Ardiati et al., 2022). Meanwhile, **Figure 3 (B)** shows that *A. brasiliensis* inefficiently remove NH_3-N which no significant removal was observed during the treatment for all dilution.

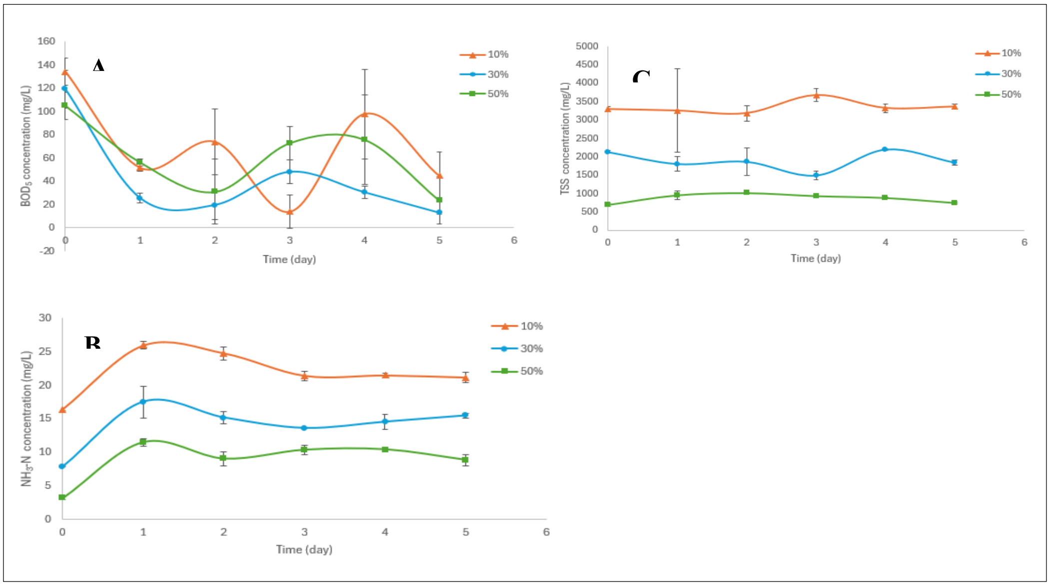

Figure 3. Removal of (A) BOD, (B) TSS and (C) NH_3-N during the incubation period for different dilution of POME

This finding suggest that *Aspergillus* alone is ineffective at removing ammoniacal nitrogen, as it lacks the metabolic pathways necessary for both nitrification and denitrification. Its treatment efficacy largely revolves around the degradation of organic pollutants rather than nitrogenous compounds. Research indicates that efficient removal of ammoniacal nitrogen usually involves specific bacterial species and aerobic conditions (Dałecka et al., 2020). Similarly, the results for TSS removal as shown in **Figure 3 (C)** was minimal, with slight reduction only at 10% dilution, likely due to lower particulate load. *Aspergillus* primarily degrades dissolved organics, not solids, and may contribute to TSS through biomass shedding (Hamad & Saied, 2021). Higher concentrations exceeded its capacity, indicating the need for additional physical or microbial processes for effective TSS removal. All parameters show statistically significant ($P<0.05$) indicates percentage dilution play significant role in removing pollutants in POME.

3.3 Monitoring of pH and DO during treatment process

As shown in **Figure 4 (A),** the results of pH indicated slight variations in pH values across dilutions, with the 10% dilution showing the lowest pH (7.95), followed by 30% (8.91) and 50% (9.38). The lower pH at 10% dilution was attributed to reduced nutrient concentrations, which likely influenced microbial metabolic activities and pH control (Jaya and Gunawan 2024). Higher nutrient concentrations in the 50% and 30% dilutions seemed to enhance microbial metabolism, thus increasing pH levels. This finding was statistically significant, with a p-value of less than 0.05 indicating a strong effect of dilution on pH levels. Regarding DO (**Figure 4 (B)**), the 10% dilution showed the lowest DO initially, followed by the 30% and 50% dilutions, suggesting that microbial oxygen consumption was initially higher at higher POME concentrations. However, DO levels increased over time at all dilutions, reflecting a balance between re-aeration and microbial oxygen consumption. The 50% dilution showed a peak in DO around day 3, likely due to a balance between oxygen uptake and microbial activity. The 30% dilution exhibited more consistent DO levels, suggesting it maintained an optimal environment for microbial activity. While the 10% dilution showed a gradual increase in DO, indicating more sustained growth, it appeared to limit fungal oxygen demand due to lower nutrient concentrations. Overall, the study found no significant impact of dilution on DO levels, with a p-value > 0.05 indicating that the treatment process did not significantly affect DO levels, which remained within a range supportive of microbial life (Chan et al., 2020). These findings suggest that while pH is significantly impacted by dilution levels, DO is relatively stable across dilutions, supporting the microbial health and activity essential for effective POME treatment.

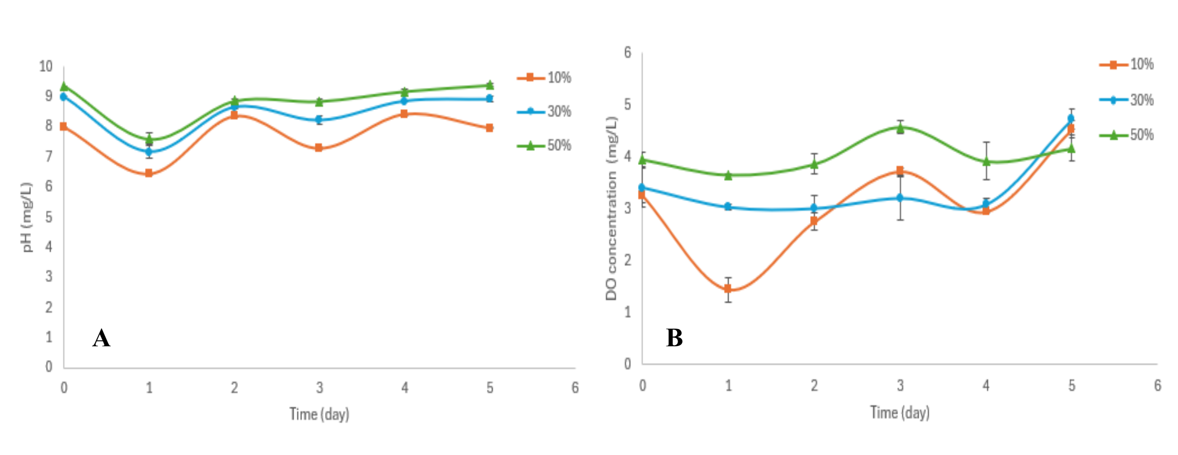

Figure 4. Observation of (A) pH and (B) DO during the incubation period for different dilution of POME

3.4 Correlation analysis between biomass and the removal of BOD, TSS and NH_3-N

Table 2 presents biomass analysis against BOD_5 in different POME dilutions. In 10% and 30% dilutions, weak negative correlations were observed between biomass and BOD, with R^2 values of 0.0224 and 0.0013, respectively, suggesting minimal impact of BOD on biomass. In contrast, the 50% dilution exhibited a strong positive correlation ($R^2 = 0.715$), indicating that higher BOD_5 levels enhance fungal biomass production. This suggests that higher POME concentrations provide more organic substrates, supporting fungal growth and pollutant removal. Optimized BOD levels are critical for enhancing fungal activities in wastewater treatment (Syazeera Zahid et al., 2020). Meanwhile, for NH_3-N in the 10% dilution, a weak negative correlation ($R^2 = 0.0583$) suggests that higher NH_3-N levels do not significantly enhance biomass production, possibly due to insufficient nitrogen. The 30% dilution showed a weak positive correlation ($R^2 = 0.0221$), indicating that smaller NH_3-N amounts may benefit biomass growth. In the 50% dilution, a stronger positive correlation ($R^2 = 0.254$) suggests that increased NH_3-N levels support fungal growth. These findings highlight that controlling nitrogen concentrations is crucial for optimizing fungal growth and biomass production in wastewater treatment (Dominic & Baidurah, 2022). For TSS, it was observed that in the 10% dilution, a weak positive correlation ($R^2 = 0.2134$) indicates a slight increase in biomass with rising TSS levels, likely due to the organic matter and nutrients in the suspended particles. The 30% dilution exhibited a stronger positive correlation ($R^2 = 0.2827$), suggesting that higher TSS concentrations offer more substrates for fungal growth. In contrast, the 50% dilution showed a weak positive correlation ($R^2 = 0.136$), implying that excessive TSS could hinder biomass production by creating unfavorable conditions for fungal development. These results emphasize the need to regulate TSS levels for optimal microbial activity in wastewater treatment (Wang et al., 2024).

Table 2. The regression analysis result that shows the equation and R^2

Dilution (%)	Biochemical Oxygen Demand (BOD)	Total suspended solid (TSS)	Ammoniacal nitrogen (NH_3-N)
10	$Y = -0.5569x+238.03$ $R^2 = 0.0224$	$Y = 0.4478x -181.96$ $R^2 = 0.2134$	$Y =-9.8421x+293.59$ $R^2 = 0.0583$
30	$Y = -0.4195x+346.24$	$Y = 0.1786x + 39.126$	$Y = 5.062x + 265.94$

	$R^2 = 0.0013$	$R^2 = 0.2827$	$R^2 = 0.0221$
50	Y =3.8203x+0.715	Y = 0.0677x + 251.57	Y = 12.216x + 208.72
	$R^2 = 0.715$	$R^2 = 0.136$	$R^2 = 0.254$.

3.5 Discussion, theoretical contributions and future applications

Although initial dilution of POME reduced most pollutant concentrations below the Department of Environment (DOE) Malaysia standards, the biological treatment with *Aspergillus brasiliensis* demonstrated differential removal efficiencies. The significant reduction in biochemical oxygen demand (BOD) indicates the fungus's enzymatic capability to degrade organic matter, consistent with its known ligninolytic and cellulolytic enzyme systems facilitating complex organic compound breakdown. This supports the role of *A. brasiliensis* in mitigating oxygen-depleting organic pollutants, which is critical in preventing eutrophication in receiving water bodies. In contrast, the negligible removal of total suspended solids (TSS) suggests that the fungal biomass and metabolic processes alone are insufficient to effectively aggregate or sediment particulate matter. This may be attributed to the physical nature of TSS, which often requires mechanical or coagulative treatment for removal. Similarly, ammoniacal nitrogen (NH_3-N) persistence indicates limited fungal involvement in nitrogen transformation pathways such as nitrification or assimilation, processes typically associated with bacterial communities. Improvements in pH and dissolved oxygen (DO) further reflect the limited enhancement of effluent quality, potentially due to reduced organic load and fungal metabolism altering the physicochemical environment. These results highlight the need for integrated treatment strategies that combine fungal biodegradation with complementary physicochemical or microbial processes to address recalcitrant pollutants like TSS and NH_3-N, thereby enhancing overall POME treatment efficacy and compliance with environmental discharge standards.

Moreover, this study enhances the theoretical understanding of fungal-based bioremediation by evaluating the performance of *Aspergillus brasiliensis* in treating diluted palm oil mill effluent (POME). Unlike many studies using synthetic wastewater, this research demonstrates how varying organic loads (10%, 30%, and 50% POME) influence fungal biomass growth and pollutant removal. The findings reveal that fungal activity is closely linked to nutrient availability, offering insights into how dilution levels affect metabolic performance and treatment efficiency. This contributes to existing knowledge by establishing the importance of substrate concentration in optimizing fungal bioremediation systems for high-strength wastewater. The limited removal of ammoniacal nitrogen highlights a theoretical gap in the fungal treatment of inorganic contaminants, suggesting that fungi alone may not be sufficient for complete remediation. This supports the need for integrated systems, such as fungal-bacterial co-cultures, to improve nitrogen removal efficiency.

For future applications, studies should focus on combining fungi with nitrifying or denitrifying bacteria, as well as scaling up the treatment using bioreactors or engineered wetlands. Enzyme analysis would further clarify the degradation pathways. These steps could lead to cost-effective, environmentally friendly wastewater treatment methods that support circular economy practices in the palm oil industry and contribute to broader sustainability goals.

4. Conclusion

This study evaluated the potential of *Aspergillus brasiliensis* for POME treatment at varying dilutions (10%, 30%, and 50%). The findings showed that fungal growth was highest at 50% dilution, suggesting a strong relationship between nutrient availability and biomass production. Although significant reduction in BOD and TSS was observed, especially at 30% dilutions, ammoniacal nitrogen removal was negligible. The variation in removal efficiency across dilutions indicates the importance of optimizing POME concentration to enhance fungal activity. Fluctuating values highlight the need for methodological refinement. Overall, *A. brasiliensis* shows potential as part of a bioremediation strategy, warranting further investigation. Additionally, the study highlights the economic and environmental advantages of using *Aspergillus brasiliensis* because it not only reduces the toxicity of POME but also helps in resource recovery, developing a circular economy within the palm oil industry. This study supports global sustainability goals by offering a creative and cost-effective wastewater management solution, which reduces the environmental impact of palm oil production. All factors considered, the effective implementation of *Aspergillus brasiliensis* in this context provides a promising path for further study and real-world application in bioremediation techniques.

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Declaration of Conflicting Interests

All authors declare that they have no conflicts of interest.

Author Contribution

Conceptualisation, **Nurhidayah Binti Hamzah**; Methodology, **Nurhidayah Binti Hamzah** & **Norizaini Mariam Binti Alias**; Validation, **Nurhidayah Binti Hamzah**; Analysis, **Nurhidayah Binti Hamzah** & **Norizaini Mariam Binti Alias**; Investigation, **Norizaini Mariam Binti Alias**; Resources, **Nurhidayah Binti Hamzah**; Data Curation, **Nurhidayah Binti Hamzah**; Writing-Draft Preparation, **Norizaini Mariam Binti Alias**; Writing-Review & Editing, **Nurhidayah Binti Hamzah**; Visualisation, **Nurhidayah Binti Hamzah**; Supervision, **Nurhidayah Binti Hamzah**; Project Administration, **Nurhidayah Binti Hamzah** & **Norizaini Mariam Binti Alias**; Funding Acquisition, **None**.

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