

Characterization and Application of Biochar Immobilized Microbes for Palm Oil Mill Effluent Treatment

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Abstract Palm Oil Mill Effluent (POME) is a high-strength wastewater generated from palm oil processing industries, containing significant levels of organic matter that pose environmental threats if left untreated. Conventional methods, such as open lagoon systems, while widely used, are often inefficient and environmentally detrimental due to their large land requirements and greenhouse gas emissions. This study proposes an integrated treatment approach that utilizes thermophilic microbes immobilized in coconut shell-derived biochar, combining the advantages of biological degradation and physical adsorption. Thermophilic fungal strains from *Aspergillus* spp. were isolated and immobilized on biochar via direct adsorption and entrapment in sodium alginate beads. The immobilized systems were applied to raw POME and monitored over a short-term batch experiment. The characterization of POME before and after treatment involved measuring changes in COD, BOD, TSS, and pH. The results demonstrated significant pollutant removal, with COD reduced from 6750 mg/L to 640 mg/L and BOD from 624 mg/L to 173.2 mg/L. The pH value shifted from acidic (4.64) toward neutral (6.72-6.75), indicating effective microbial activity and environmental stabilisation. However, while substantial reductions were achieved, the final values of BOD and COD still exceeded the discharge

limits set by Indonesian Regulation (Regulation of the Ministry of Environment and Forestry No. 16/2019), highlighting the need for further optimization. This biochar-immobilized microbes method offers a promising, sustainable pre-treatment alternative for the palm oil industry, especially in resource-limited regions, and contributes to circular bioeconomy principles by repurposing agricultural waste into functional bioremediation materials.

Keywords Biochar, Thermophilic Fungi, POME, Wastewater Treatment

1. Introduction

Palm Oil Mill Effluent (POME) is a liquid waste produced from the process of processing *crude palm oil* (CPO). This waste generally contains a high concentration of organic matter, including carbon, nitrogen, and phosphorus compounds, as indicated by chemical oxygen demand (COD) and biological oxygen demand (BOD) values that exceed industrial wastewater quality standards [1], [2], [3]. The abundant organic content of POME makes it a potential environmental pollutant if disposed of without

adequate treatment, as it can lower dissolved oxygen levels in water, trigger eutrophication, and produce greenhouse gases, such as methane, through natural anaerobic degradation processes [4], [5]. Therefore, effective POME treatment is urgently needed to prevent environmental pollution and to meet strict liquid waste standards. The use of biochar in this system provides a synergistic advantage. Its porous structure and large surface area allow microorganisms to attach securely, form stable biofilms, and remain active throughout the treatment process [5], [6]. At the same time, biochar adsorbs organic contaminants, bringing them into close contact with the immobilized microbes. This dual function not only improves mass transfer and accelerates biodegradation but also enhances overall treatment efficiency, resulting in faster pollutant removal and more stable system performance [7], [8].

In recent years, biological treatments have gained significant attention as a sustainable alternative to POME remediation due to their efficiency in degrading complex organic compounds while minimizing secondary pollution [9], [10]. Among emerging approaches, the use of immobilized microorganisms has proven successful, as it improves microbial stability, tolerance to fluctuating environmental conditions, and reuse in sustainable processing systems [11]. Biochar, a porous carbon material derived from agricultural biomass, has been identified as an excellent support matrix for microbial immobilization due to its large surface area, favorable microstructure, and the presence of functional groups that facilitate microbial adhesion and activity [8], [12], [13]. The integration of immobile microorganisms with biochar thus offers a double advantage: efficient biodegradation of POME through microbial metabolism and simultaneous adsorption of rogue pollutants by the biochar matrix. This hybrid strategy not only improves treatment performance but also aligns with the principles of the circular bioeconomy by valorizing agricultural residues into functional materials for wastewater management [5], [14], [15].

2. Materials and Methods

2.1. Materials

The materials used in this study included biochar, palm oil mill effluent (POME), and thermophilic fungi. Biochar was produced from coconut shells obtained from smallholder plantations in Riau Province, Indonesia. POME samples were collected from the second treatment pond of a palm oil processing mill in the same province. Thermophilic fungi were isolated from a natural hot spring located in West Sumatra Province, Indonesia, and incubated at temperatures between 27 and 45 °C. Two thermophilic fungal strains identified as *Aspergillus* sp. were used in this study and labeled as strain 304 and strain

308.

2.2. Biochar Preparation

The coconut shells were first thoroughly washed with clean water to remove adhering dust, soil particles, and any residual organic impurities. After washing, the shells were air-dried and then oven-dried to remove residual moisture, ensuring optimal thermal conversion during pyrolysis. The dried shells were then subjected to a slow-pyrolysis process at 450 °C for 2 hours under limited oxygen conditions, allowing thermal decomposition of lignocellulosic components into a carbon-rich solid material. After pyrolysis, the biochar was allowed to cool naturally in a sealed container to prevent oxidation. The resulting biochar was then mechanically ground and sieved to obtain a uniform particle size corresponding to particles passing through a 50-mesh sieve, ensuring consistent surface area exposure and enhanced contact efficiency during the subsequent immobilization and adsorption experiments.

2.3. Microbial Immobilisation Procedure

Microbial immobilization was performed using an adsorption-based method. In this process, thermophilic fungal cells adhere to the biochar surface via interactions with functional groups, such as amino, carbonyl, carboxyl, and hydroxyl groups, which are naturally present on the biochar. Initially, fungal cultures were grown in liquid medium until the logarithmic growth phase (day 8). The liquid fungal cultures were then mixed with the prepared biochar and gently agitated for 48 h to facilitate attachment. After incubation, the mixture was filtered, and the biochar was spread as a thin layer on Petri dishes for rapid drying. It was then stored in a sealed container at room temperature.

2.4. Characterization and Analytical Methods

The surface area and morphology of the biochar immobilized fungi were characterized using Brunauer–Emmett–Teller (BET) surface area analysis and Scanning Electron Microscopy (SEM), respectively. These analyses were used to evaluate the structural characteristics and potential mechanisms of the immobilized fungi in degrading organic compounds.

The POME quality parameters, including pH, chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS), were measured in accordance with the national standards outlined by the Ministry of Environment and Forestry of the Republic of Indonesia (Regulation No. 21/2018). The treatment system was designed as a pre-treatment unit, aiming to lower COD and BOD concentrations before further conventional or advanced wastewater treatment processes.

3. Results and Discussion

3.1. Analysis of Functional Group of Biochar Immobilized Fungi

Biochar is a solid carbon material produced through the pyrolysis of biomass under limited oxygen conditions. Biochar derived from coconut shells, empty fruit bunches (EFB) of oil palm, or other agricultural residues has been widely utilized as an adsorbent due to its superior physical and chemical properties, including high porosity, large surface area, thermal stability, and the ability to bind both organic compounds and heavy metals [16], [17].

When applied as an immobilization matrix, biochar serves not only as a habitat for microorganisms but also aids in the adsorption of various pollutants present in palm

oil mill effluent (POME). Its hydrophobic nature and surface functional groups can attract organic compounds such as fatty acids and lignin, which are commonly found in POME. The alterations in the functional groups of biochar before and after microbial immobilization are illustrated in Figure 1 and Figure 2.

Figure 1 presents a comparison of FTIR spectra between pristine coconut shell biochar and biochar after the microbial immobilization process, revealing a distinct difference. In the pristine biochar, transmittance values range between 90% and 95%, indicating that the material possesses a relatively simple composition dominated by characteristic functional groups of carbonized structures. These include the O–H stretching vibration around 3300 cm^{-1} , C–H stretching near 2900 cm^{-1} , and aromatic C=C stretching around 1600 cm^{-1} .

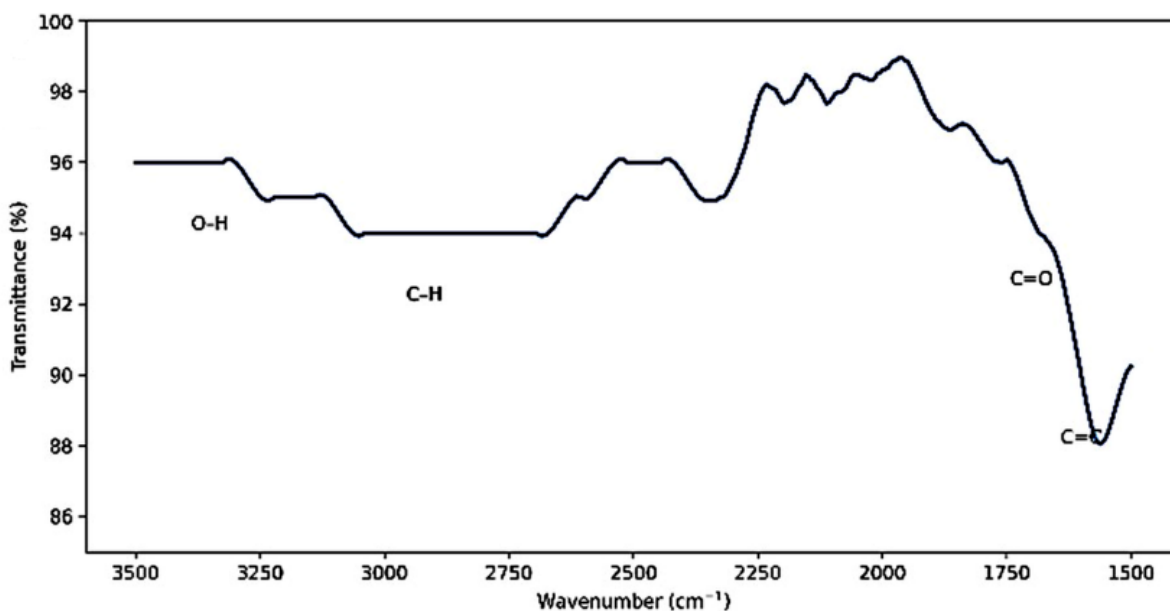
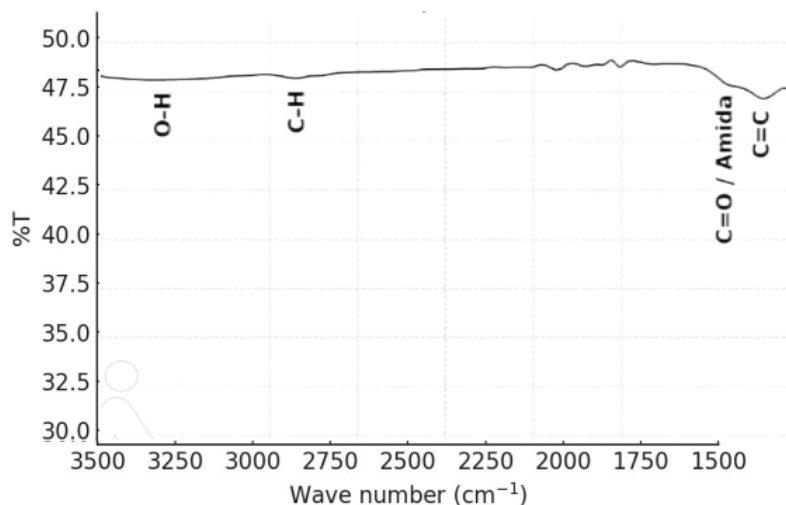
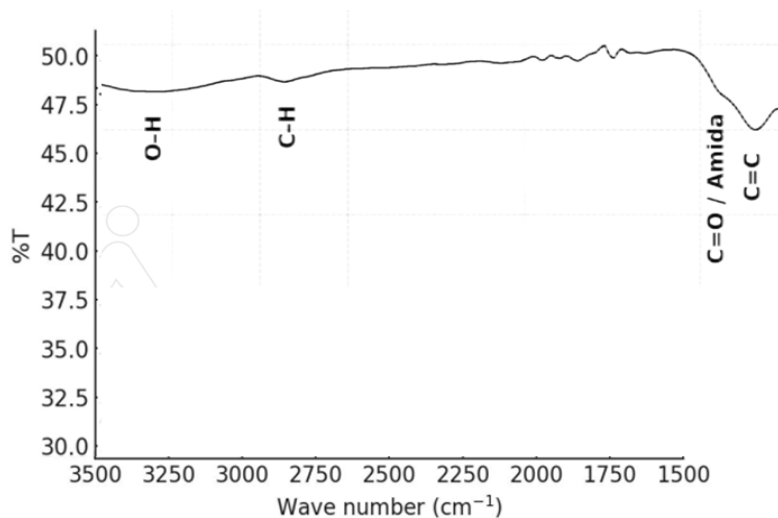


Figure 1. Functional groups of coconut shell's biochar



(a)



(b)

Figure 2. Functional groups of biochar immobilized fungi strain 304 (a) and 308 (b)

Following microbial immobilization, the transmittance intensity decreases dramatically to approximately 45–50%, suggesting an enhanced ability of the material to absorb infrared radiation. This phenomenon implies that the biochar surface has been enriched with microbial biomolecules, such as proteins, lipids, and polysaccharides, each contributing active functional groups that broaden the absorption spectrum. This transformation is particularly evident from the appearance of new bands at approximately 1650 cm^{-1} and 1540 cm^{-1} , corresponding to Amide I and Amide II, respectively, which are indicative of proteins or peptides not prominently observed in the pristine biochar [14], [18].

Although the aromatic $\text{C}=\text{C}$ band remains visible, signifying the persistence of the biochar's carbon framework, the overall FTIR profile reflects a shift toward a more complex biochar-biomolecule matrix. This structural modification not only confirms the successful immobilization of microorganisms but also highlights the dual role of biochar as both a stable and reactive substrate, providing a supportive environment for microbial attachment and activity.

The FTIR spectrum of the microbial-immobilized biochar sample reveals the presence of new absorption bands that are not prominent in the pristine coconut shell biochar, specifically at approximately 1650 cm^{-1} and 1540 cm^{-1} . These two bands are identified as Amida/Amide I and Amide II, which are characteristic signatures of peptide bonds in proteins and polypeptides. The Amide I band, appearing near 1650 cm^{-1} , primarily arises from the stretching vibrations of the carbonyl group ($\text{C}=\text{O}$) within the peptide bond structure. This band is highly sensitive to protein conformation, making it a useful marker for detecting the presence and secondary structure of proteins, such as α -helices and β -sheets.

Meanwhile, the Amide II band, located around 1540 cm^{-1} , results from a combination of $\text{C}-\text{N}$ stretching and $\text{N}-\text{H}$

bending vibrations within the same peptide bond, albeit through different vibrational modes. Thus, Amide I and Amide II do not represent different chemical groups but rather two distinct vibrational modes of the amide functional group ($-\text{CONH}-$), which is a hallmark of protein-based compounds. The absence of these bands in pristine biochar can be explained by its composition, which is largely derived from the pyrolysis of lignocellulosic biomass. During pyrolysis, most proteins and nitrogen-containing compounds are degraded, leaving behind primarily oxygenated functional groups such as $\text{O}-\text{H}$, $\text{C}-\text{H}$, aromatic $\text{C}=\text{C}$, and $\text{C}-\text{O}$. However, after being utilized as a microbial immobilization matrix, the biochar surface becomes covered with microbial biomass and extracellular polymeric substances (EPS) secreted by microorganisms. The presence of proteins, enzymes, and peptide components within the EPS accounts for the clear appearance of the Amide I and Amide II bands in the immobilized biochar sample, thereby providing strong evidence of successful microbial colonization and biofilm formation on the biochar surface.

3.2. Analysis of Surface Area, Pore Volume, and Pore Structure of Biochar Immobilized Fungi Using Brunauer–Emmett–Teller (BET) Method

The characterization of the surface texture of biochar through BET analysis and the pore size distribution from the BJH method provides important information on specific surface area, total pore volume, and average pore size, which are key parameters for the effectiveness of microbial immobilization media. In this study, biochar samples were immobilized by fungi 304 and 308. The results are compared to a biochar (without fungi).

The results of BET's analysis showed that coconut shell biochar had the highest surface area of $14.60\text{ m}^2/\text{g}$. In contrast, the surface area of immobilized biochar decreased significantly, as shown in the following Table 1.

Table 1. Biochar's Surface Area

Sample	Specific Surface Area	Pore Volume
Control (biochar)	14,60 m ² /g	0.0205 cc/g
Sample 304	3,96 m ² /g	0,0085
Sample 308	4,03 m ² /g	0,0096

This decrease in specific surface area indicates the presence of pore blockage by the mass of microbes or biofilms that adhere to the biochar pore walls during the immobilization process. This phenomenon is common when pore media is used as a biological immobilization matrix [19].

The decrease in total pore volume observed in the microbial-immobilized biochar supports the hypothesis that thermophilic microorganisms, along with the exopolysaccharides (EPS) they produce, partially occupy the micro- and mesopores that were previously accessible in the raw biochar. The attachment of microbial cells and deposition of EPS within these pores effectively reduces the available internal pore space, indicating successful colonization and biofilm formation on the biochar surface. This structural change further confirms that immobilization is not merely superficial but involves physical entrapment of cells within the porous network, which enhances the stability of the microbial consortium during the treatment process.

3.3. Surface Analysis of Biochar Immobilized Fungi Using Scanning Electron Microscope (SEM)

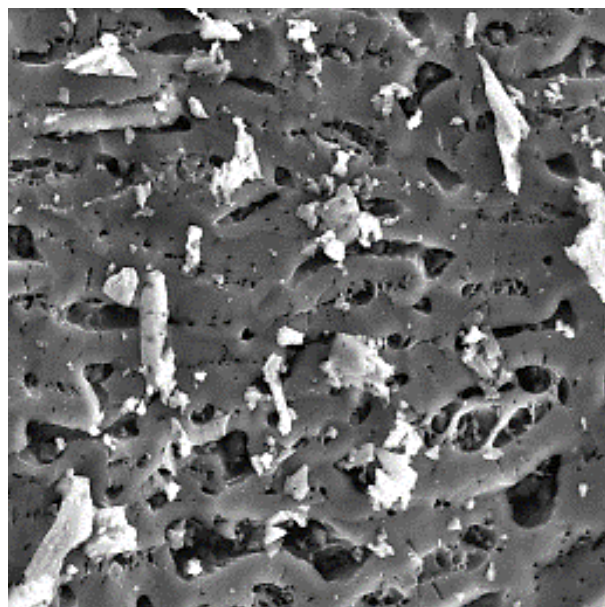
The Scanning Electron Microscope (SEM) technique is used to visualize the surface of biochar at the micrometer and even nanometer scales. Through SEM, researchers can observe details of texture, pore size, and distribution, as well as the presence of organic layers, such as biofilms or mineral deposits, that are not visible under ordinary observation. Thus, SEM enables researchers to understand how the structure of biochar affects the adsorption process and microbial growth. This analysis is then supplemented by Energy-Dispersive X-ray Spectroscopy (EDX), which provides information about the chemical composition of the material's surface. With EDX, it is possible to determine the dominant elements, such as carbon and oxygen, which are the main components of biochar, or phosphorus and sulfur, which serve as indicators of microbial biological activity.

The combination of these two techniques is beneficial for connecting the physical and chemical aspects, allowing researchers to evaluate not only the success of the biochar production process but also the effectiveness of microbial immobilization on the medium. The chemical composition of the EDX results indicates that pure biochar is primarily composed of carbon (C) and oxygen (O), which are the main characteristics of biomass-based biochar. Biochar also contains a small amount of minerals such as potassium (K) and calcium (Ca) as ash residues. The O content

indicates the presence of functional groups such as hydroxyl or carboxyl, which persist after pyrolysis and provide possible interactions with pollutants or microbes. On the other hand, organic gases (such as moisture and volatile substances) are lost through pyrolysis, allowing the pore structure to remain open and active.

The results of SEM observations on coconut's shell biochar show a distinctive surface structure of the carbon material produced through the pyrolysis process. The surface of biochar appears porous with a complex network of ducts. The pores appear quite large and clearly indicate that the pyrolysis process is occurring optimally, resulting in an internal cavity that can serve as an active area for adsorption. This structure is particularly important because the exposed pores allow pollutant molecules such as blue methylene, as well as organic compounds in POME (Palm Oil Mill Effluent), to diffuse and be trapped in the biochar.

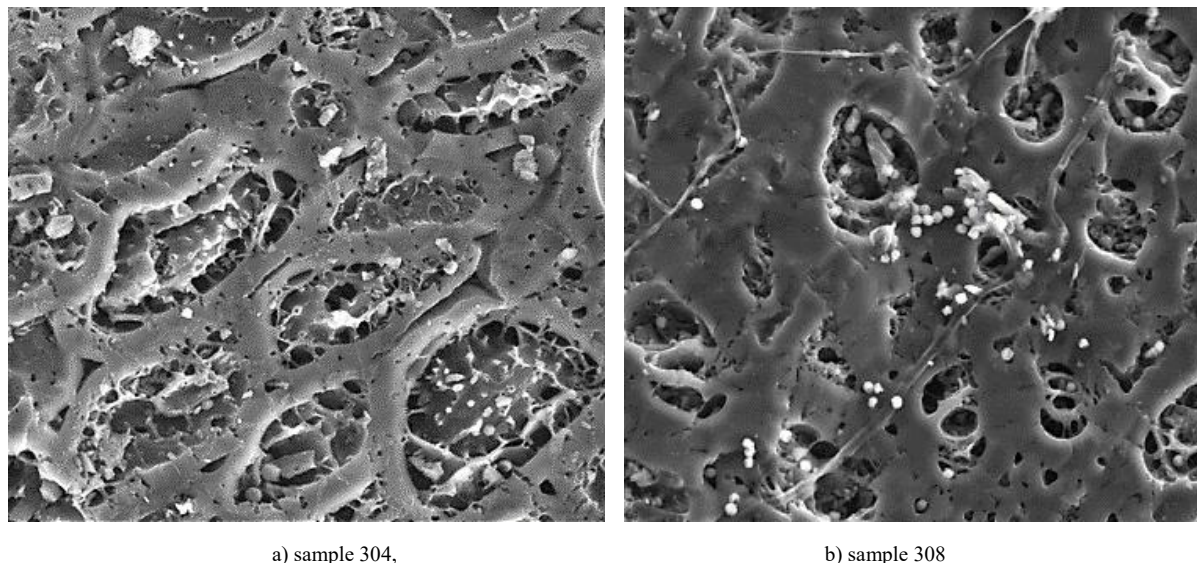
The pore walls look sharp and are not covered with organic layers, reflecting that this material has not interacted with microbial biomass. There are no visible biofilm, EPS (Extracellular Polymeric Substances), or inter-pore bridges that typically form when microbes have been mobilized (as shown in the following Figure 3 for immobilized biochar samples). The surface of pure biochar is fully exposed, making it an ideal medium for use in adsorption systems as well as a substrate ready for microbial colonization.

**Figure 3.** Biochar's surface area (2500x)

Based on the results of surface photography of biochar before and after immobilization using the Scanning Electron Microscope (SEM), it is possible to compare the shape and structure of the surface, the shape and structure of the pores, the surface conditions, and the analysis related to its adsorption, both on blue methylene dyes and organic waste such as POME, as shown in the following Table 2.

Table 2. Biochar surface analysis Using SEM and BET

Sample	Biochar Surface Analysis (SEM)	Open Pore	Biofilm	Specific Surface Area
Sample 304	Biofilm clear, bridge between pores clear	Medium	Medium	3,96 m ² /g
Sample 308	Thin biofilm, a lot of mesopores	many	rare	4,03 m ² /g



a) sample 304,

b) sample 308

Figure 4. Biochar Immobilized Microbes Surface Area. (2500x)

Figure 4 shows the surface shape of the immobilized microbes biochar, consisting of sample 304 and sample 308. All samples were taken using a Scanning Electron Microscope (SEM). In sample 304, the field of view features a porous biochar matrix that is mostly covered in a film-like layer with fine bridges spanning the inter-pores. This morphology is very consistent with the formation of microbial biofilms/EPS above the pore wall. The surface becomes smoother and rounder than pure biochar, which is usually sharp in texture. In some areas, a thin filamentous structure connects the edges of the pores, which can be interpreted as hyphae, cellular fibers or EPS fibers that dry out during preparation. Additionally, bright gray grains (both angular and spherical) are inserted within or above the film. This is likely to be inorganic particles of ash/mineral residue, or precipitated salts formed during the immobilization process. The morphology of biofilms with bridges/partitions between pores provides close contact between the biochar and the cell, allowing for a dual mechanism of adsorption and biodegradation. Mesopores with diameters of 1–5 μm that remain open are sufficient for the mass transport of small dyes/organic substances. However, if the target molecules/particles are larger, the degradation rate is likely to be slow, and the pores can become easily clogged.

The results of observations using the Scanning Electron Microscope (SEM) on sample 308 showed the morphological characteristics of the biochar surface, which are still dominated by pure carbon structures. The surface of biochar appears to have large, clear pores, with sizes ranging from 10 to 30 μm , evenly distributed throughout

the plane. The pore walls appear smooth with firm contours, indicating that organic layers or biofilms have not primarily covered the biochar structure.

In some surface areas, micron-sized spherical particles were observed scattered on the pore walls and inside the pore canals. These particles are likely individual microbial cells that have successfully attached to the surface of the biochar. In addition, there is a thin filamentous structure that connects surface areas, which can be interpreted as the precursor to the formation of extracellular polymer or microbial hyphae. In sample 308, no continuous and thick biofilm layer was found, as seen in sample 304, indicating that the process of microbial immobilization in sample 308 has already taken place, but is still in the early stages of biofilm formation, so the microbial cells have not yet developed into a strong and organized colony.

The presence of bright grains that appear to stick to the surface of the biochar also provides essential information. These granules have an irregular morphology and are scattered in various locations, most likely derived from mineral residues such as potassium (K) and calcium (Ca). This residue is commonly found in biochar produced from biomass such as coconut shells or empty oil palm bunches.

Table 3 shows the elemental elements contained in sample 304 based on the results of the SEM-EDX test. The table data interpret the surface chemical composition of the biochar used for microbial immobilization. The data show that the dominant elements are carbon (C) and oxygen (O), with the presence of minor elements such as phosphor (P), sulfur (S), potassium (K), and calcium (Ca).

Table 3. Comparison of biochar elemental elements before and after microbial immobilization

Elements	Biochar immobilized microbes (%wt)			Information
	Biochar (%wt)	Sample 304	Sample 308	
C (Carbon)	80.94	70,8	76,7	
O (Oxygen)	15.92	26,0	21,5	
P (Phosphor)	0.00	0,68	0,31	Appears after immobilization
S (Sulfur)	0.00	0,42	0,25	Appears after immobilization
K (Kalium/ Potassium)	1.72	1,68	0,95	
Ca (Calcium)	1.42	0,40	0,25	

In biochar used for microbial immobilization, carbon is the main component derived from the biochar structure itself. The high percentage of carbon (more than 66 % wt) indicates that the surface is still dominated by a porous carbon skeleton that serves as a microbial buffer medium. This suggests that the biochar produced through the pyrolysis process has a stable carbon structure and remains dominant after the immobilization process. The presence of significant amounts of oxygen (approximately 23–30% wt) can originate from oxygen functional groups on the biochar surface, such as carboxylates, hydroxyls, or carbonyls. These clusters are essential because they play a role in the formation of hydrogen bonds and electrostatic interactions that facilitate microbial adhesion. In addition, high oxygen levels can also indicate the presence of organic compounds or EPS (Extracellular Polymeric Substances) matrices produced by microbes during the immobilization process.

Elements Phosphorus (P) and sulfur (S) indicate the presence of biological compounds derived from the cell walls of the microbes or metabolites produced. P is usually associated with membrane phospholipids, while S can be derived from amino acids. Potassium (K) is detected in moderate amounts, and it is commonly found in microbial cells because K^+ is an essential ion for maintaining osmotic balance and enzyme activity. Meanwhile, a small amount of calcium (Ca) may be derived from the residual minerals in biochar raw materials. The combination of high carbon and oxygen with these biological minor elements indicates the success of the immobilization process, since not only does the cell attach, but also a strong chemical interaction occurs between biochar and microbial biomass.

In addition to the appearance of biofilm layers as seen from the SEM microscope, changes in the composition of biochar elements after immobilization are also indicative of the success of the immobilization process. From the table, it can be seen that after immobilization, the number of element C decreases. This decrease indicates that the surface of the biochar is partially covered by a layer of microbial biomass and biofilm/EPS (Extracellular Polymeric Substances), which has a higher oxygen content. The EPS biofilm that forms replaces the carbon area that was initially exposed. The number of O elements has increased due to the presence of the O group contained in

microbial media, EPS, as well as those derived from microbial metabolism, such as carbonyl groups and hydroxyl groups.

The presence of elements P and S is related to the presence of microbes and serves as a clear indicator of living microbes bound to the surface of biochar. The greater the concentration of P and S, the thicker or more active the biofilm formed by the microbes [20].

3.4. Biochar Immobilized Microbes for Liquid Waste Palm Oil Mill Effluent (POME)

Palm oil mill effluent (POME) is a liquid waste produced from the process of processing palm fruit into palm oil or crude palm oil (CPO). This waste generally contains high concentrations of organic matter, including carbon, nitrogen, and phosphorus compounds, which is indicated by the values of chemical oxygen demand (COD) and biological oxygen demand (BOD) that far exceed industrial wastewater quality standards. This abundant organic content makes POME potentially pollute the environment if disposed of without adequate treatment, because it can reduce dissolved oxygen levels in waters, trigger eutrophication, and produce greenhouse gas emissions such as methane from natural anaerobic degradation processes [4], [15].

The integration of biochar with microbes presents a profitable and synergistic approach to treating palm oil mill liquid waste (POME). The utilization of biochar in this system has a dual function; Not only does it provide a large surface area for microbial colonization, but it also significantly improves the efficiency of the degradation process. This synergy facilitates increased contact between microbes and pollutants, resulting in higher degradation rates and improved processing outcomes [7], [8].

In recent years, biological treatments have garnered significant attention as a sustainable alternative to POME remediation, owing to their efficiency in degrading complex organic compounds while minimizing secondary pollution. Among the emerging approaches, the use of immobilized microorganisms has proven successful, as it enhances microbial stability, tolerance to fluctuating environmental conditions, and reuse in sustainable processing systems [11].

Meanwhile, biochar, a porous carbon material derived from agricultural biomass, has been identified as an excellent support matrix for microbial immobilization due to its large surface area, favorable microstructure, and the presence of functional groups that facilitate microbial adhesion and activity [21]. The integration of immobile microorganisms with biochar thus offers a double advantage: efficient biodegradation of POME through microbial metabolism and simultaneous adsorption of rogue pollutants by the biochar matrix.

In Indonesia, regulations related to the quality standards of liquid waste in the palm oil industry are regulated in the Regulation of the Minister of Environment and Forestry of the Ministry of Environment and Forestry No. 5 of 2014, which was updated in the Regulation of the Minister of Environment and Forestry No. 16 of 2019 concerning Wastewater Quality Standards. According to the standard, the maximum limit of the main parameters for the liquid waste of the palm oil industry is as follows in Table 4:

Table 4. POME wastewater quality standard in Indonesia

Parameter	Maximum threshold
COD (Chemical Oxygen Demand)	350 mg/L
BOD (<i>Biological Oxygen Demand</i>)	100 mg/L
TSS	150 mg/L
pH	6,0-9,0

Chemical Oxygen Demand (COD) is a key parameter in assessing the level of liquid waste pollution, including Palm Oil Mill Effluent (POME). COD measures the amount of oxygen needed to oxidize the organic and inorganic compounds present in wastewater. The higher the COD value, the greater the content of organic pollutants that are difficult to decompose naturally [22], [23]. The result is shown in Table 5. All physicochemical parameters (COD, BOD, TSS, and pH) were measured in duplicate, and results are presented as mean \pm standard deviation. The COD removal efficiency reached approximately 90.5% for Sample 308 compared to the control.

Table 5. The decrease in Chemical Oxygen Demand after treatment

Sample	COD (mg/L)
Control (POME)	6.750 \pm 16.97
S304	1250 \pm 22.63
S308	650 \pm 11.31

The COD removal efficiencies observed in this study indicate reductions in organic load after treatment. These results are consistent with previous research on biological and immobilized treatments of POME. For example, the use of *Meyerozyma guilliermondii* in POME treatment achieved approximately 72 % COD removal, supporting the feasibility of microbial-based approaches for reducing organic load in high-strength effluents [24]. Research on mixed microbial systems and indigenous bacterial treatments has similarly shown significant organic

degradation capability; for instance, *Bacillus* species and mixed cultures have been reported to remove a large fraction of COD from POME via enzymatic and metabolic processes [25].

Biochemical Oxygen Demand (BOD) is also a key parameter in assessing the quality of liquid waste, including Palm Oil Mill Effluent (POME). BOD measures the amount of oxygen required by microorganisms to decompose organic compounds in wastewater over a period of time, typically 5 days at 20 °C. A high BOD value indicates a high concentration of organic matter, which can decrease dissolved oxygen (DO) levels in the receiving water body. This condition can trigger the death of aquatic organisms, cause bad odors due to anaerobic processes, and accelerate the eutrophication process in aquatic ecosystems [8], [22], [26].

The results of the BOD measurement from this study showed a significant difference between the control sample and the treated sample with the mobilized biochar-microbial system. The measurement data are shown in Table 6. In this research, the data collected for COD, BOD, TSS, and pH have met the statistical requirements; they were collected twice (duplicates).

Table 6. BOD value before and after treatment

Sample	BOD (mg/L)
Control (POME)	624.25 \pm 1.90
S304	241.50 \pm 2.23
S308	173.25 \pm 1.66

Based on these data, POME has a high BOD of 624.25 mg/L, exceeding the quality standard. This illustrates the natural characteristics of POME, which is rich in organic compounds. So, without treatment, this waste has the potential to seriously pollute the environment. In the treated samples, BOD decreased significantly. S308 showed the best performance with a final BOD of 173.25 mg/L, followed by S304 at 241.50 mg/L. This decrease suggests that biochar systems with mobilized microbes can facilitate the biological degradation of organic compounds, in line with previous research findings that emphasize the role of microorganisms in breaking down complex compounds into simpler and less harmful molecules.

Total Suspended Solids (TSS) is the number of solid particles floating in liquid waste that can be held by filter paper with a specific pore size, typically 1.5–2.0 μ m. TSS includes both organic and inorganic materials such as fibers, mud, and other insoluble particles. In palm oil mill liquid waste (POME), the high TSS is generally due to the presence of fibers and oil residues from the extraction process. High TSS values can increase turbidity, degrade water quality, and increase the need for oxygen during biological decomposition. Therefore, TSS is a crucial parameter for evaluating the effectiveness of waste treatment and ensuring compliance with waste-disposal quality standards. TSS value in this research gave good results after treatment, as shown in Figure 5.

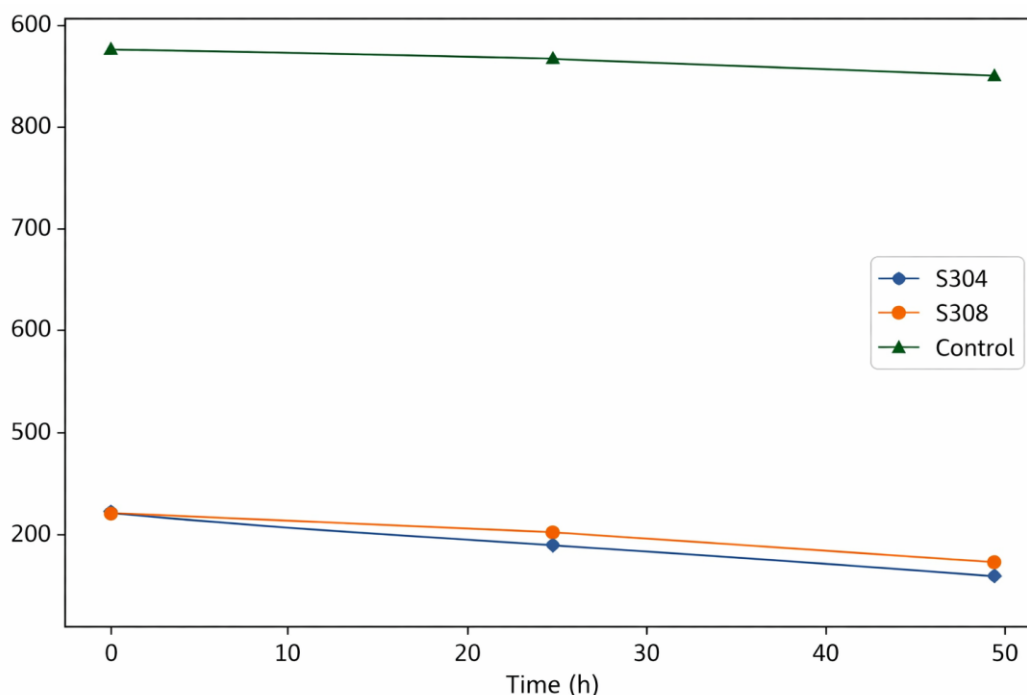


Figure 5. Decrease in TSS of POME after treatment

As shown in Table 7, the pH dynamics of POME exhibit a pattern consistent with the chemical–biological mechanisms involved in organic waste treatment. The sample (pure POME) showed only a slight increase, from 4.64 to 4.92, over 48 hours, remaining acidic. This mild increase is attributed to natural processes, such as CO₂ degassing, partial consumption of weak acids, or temperature changes. It confirms that without intervention, POME does not achieve safe conditions for further processing or disposal.

Table 7. pH measurement of POME

Sample	Initial pH	pH (24 h)	pH (48 h)
Control (POME)	4.64±0.37	4.78±0.18	4.92±0.32
Sample 304	5.75±0.19	6.14±0.39	6.75±0.30
Sample 308	5.77±0.28	6.01±0.14	6.72±0.18

4. Conclusions

The results of this study show how coconut shell biochar becomes a functional and biologically active material once thermophilic *Aspergillus* spp. are immobilized on its surface. The characterization results clearly illustrate this transformation. FTIR analysis reveals the presence of Amide I and Amide II bands, which are characteristic of proteins and peptide bonds. These bands were absent in untreated biochar, indicating that microbial biomolecules have successfully attached to the biochar surface. SEM observations visually reinforce this finding. The initially rough and open pore structure of the biochar becomes

covered by microbial cells and extracellular substances, suggesting not only attachment but also the formation of a stable microbial layer. BET analysis then completes the picture. A decrease in surface area and pore volume confirms that microbial cells and their polymers occupy the internal pores of the biochar. These three analyses collectively demonstrate that immobilization is not merely a physical contact but a true integration between biochar and microorganisms.

When applied to Palm Oil Mill Effluent, the immobilized biochar plays an active role in pollutant reduction. The combination of adsorption by biochar and biodegradation by thermophilic microbes results in a noticeable improvement in wastewater quality. COD and BOD concentrations decrease significantly, and the pH shifts from acidic to a near-neutral condition. Although the final values have not yet reached the discharge limits of Indonesian wastewater regulations, the trend of improvement shows that the system is effective as a pre-treatment method and has the potential to be optimized further.

In summary, biochar serves not only as a passive adsorbent, but as a living support system that enables microbial activity to continue efficiently. The findings confirm that coconut shell biochar is an excellent platform for microbial immobilization. Its natural porosity, functional groups, and mechanical stability make it suitable for forming a biochar–microbial matrix capable of improving wastewater treatment performance. This approach offers a sustainable option for the palm oil industry by turning agricultural waste into a valuable material for environmental remediation.

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