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Unexpected Insights: Raw Sub-Bituminous Coal Outperforms Gamma-Irradiated Coal in Bio-solubilization with *Phanerochaete chrysosporium*

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ABSTRACT

Purpose of the study: The purpose of this study is to investigate the effect of gamma irradiation treatment on sub-bituminous coal in the bio-solubilization process using *Phanerochaete chrysosporium* and to characterize the bio-solubilization products of raw and irradiated coal under laboratory conditions.

Methodology: This study used sub-bituminous coal from South Sumatra, gamma irradiated with an Irpasena irradiator, and biosolubilized with *Phanerochaete chrysosporium*. Instruments included a Precision® shaker incubator, Novel® microscope, Hanna® pH meter, Shimadzu® GC-MS, and Shimadzu® UV-Vis spectrophotometer. Data were analyzed statistically using T-test with SPSS version 20 software.

Main Findings: The results revealed that raw coal exhibited higher solubilization efficiency compared to irradiated coal, indicated by a greater increase in soluble phenolic (0.95 mg/mL) and aromatic compounds (1.42 mg/mL), as well as higher protein production. GC-MS analysis confirmed that raw coal generated more diverse and abundant metabolites, including phenolic and aromatic derivatives, while irradiated coal produced fewer compounds. Statistical analysis (T-test, $p < 0.05$) supported the significant differences between treatments. These findings suggest that gamma irradiation does not enhance, and may even inhibit, coal bio-solubilization ..

Novelty/Originality of this study: This study provides new evidence that gamma irradiation does not enhance, but rather reduces, the bio-solubilization efficiency of sub-bituminous coal by *Phanerochaete chrysosporium*. The findings challenge the common assumption about irradiation benefits and contribute to advancing knowledge by highlighting the superior performance of raw coal in producing energy-equivalent products.

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1. INTRODUCTION

The global demand for energy continues to increase in line with population growth, economic development, and rising per capita consumption. Indonesia has long relied heavily on fossil fuels, particularly petroleum, to meet its energy needs [1]-[3]. However, crude oil production data in Indonesia show a significant downward trend in recent years [4], [5]. This decline has created an urgent need to explore more sustainable and reliable alternative energy sources as long-term reserves [6], [7].

On the other hand, alternative energy sources such as coal still hold considerable potential in Indonesia. Although coal is a solid energy source that produces high emissions and pollutants when burned directly, it can be converted into more eco-friendly forms through various bioconversion technologies [8]-[10]. These technologies offer promising approaches to transform coal into products with higher environmental and economic value [11]-[13]. One promising technology is bio-solubilization , a process in which microorganisms—both fungi and bacteria—transform coal into a liquid or more easily degradable form that can be utilized as an alternative fuel or as a source of high-value chemicals [14], [15].

Bio-solubilization is driven by extracellular enzymes such as lignin peroxidase, manganese peroxidase, and laccase [16], [17]. These enzymes break down complex aromatic compounds and polymer structures present in coal [18], [19]. Recent studies have shown that white-rot fungi, such as *Phanerochaete chrysosporium*, possess a high degradation capacity toward lignin and other aromatic compounds and are also employed in pretreatment to enhance the efficiency of biodegradation or biogasification. For example, the study "Promotion Effect and Mechanism Analysis of Different Strain Pre-Treatment on Methane Conversion from Lignite" (2025) reported that pretreatment using *P. chrysosporium* improved lignite degradation and methane yield in the biogasification process [20].

In the context of physical or chemical pretreatment, gamma irradiation has been investigated as a method to enhance coal solubility by breaking complex bonds and reducing the degree of polymerization. Although earlier studies (from the 1990s to the early 2000s) demonstrated that certain doses of gamma radiation can increase the extractability of coal or lignite in specific solvents, there is still limited recent research exploring the combination of gamma irradiation and specific microorganisms such as *Phanerochaete chrysosporium* on sub-bituminous coal [21].

The study "Recent progress on the biological degradation and solubilization of coal" by Sekhohola-Dlamini et al. [16] reviews developments from 2014 to 2024, including the identification of new microorganisms capable of degrading coal, the use of microbial consortia, and both aerobic and anaerobic mechanisms in coal biodegradation. This review highlights that, despite significant mechanistic and applied advances, further research is still needed to improve the efficiency and productivity of bio-solubilization , as well as to characterize bio-solubilization products to enable large-scale applications [16].

In Indonesia, there are examples of studies on lignite bio-solubilization by indigenous fungi isolated from coal mining soils in South Sumatra, where product characterization using GC-MS revealed hydrocarbon compounds with properties similar to gasoline and diesel from certain isolates [22]. owever, the application of *Phanerochaete chrysosporium* as an agent isolated from local mining sites, combined with gamma irradiation pretreatment of sub-bituminous coal, remains underexplored, particularly in the context of local conditions and varied irradiation doses.

Therefore, this study aims to fill this gap by comparing the bio-solubilization of raw and gamma-irradiated sub-bituminous coal using *Phanerochaete chrysosporium* in submerged culture. Several doses of gamma irradiation will be applied, and analyses will include pH measurements, fungal colonization, phenolic and conjugated aromatic compound content, UV-Vis spectroscopy, extracellular protein assays, enzymatic activity (e.g., FDA hydrolysis), and product analysis using GC-MS. Gamma irradiation pretreatment is expected to enhance the degree of bio-solubilization and produce higher-quality products suitable as substitutes for fossil fuels.

Thus, this research is expected to contribute to (1) a deeper understanding of the effects of physical pretreatment (gamma irradiation) on the structure of sub-bituminous coal and the bio-solubilization capability of *P. chrysosporium*, and (2) the characterization of bio-solubilization products to approach biofuel or liquid fuel standards, thereby opening opportunities for larger-scale applications in Indonesia.

2. RESEARCH METHOD

2.1. Materials and Instruments

The primary instruments used in this study included a Precision® shaker incubator for controlled agitation and temperature, a Novel® compound microscope for microscopic observations, and an Irpasena gamma irradiator for coal sterilization. Analytical instruments consisted of a Hanna® digital pH meter for pH monitoring, a Shimadzu® UV-Vis spectrophotometer for absorbance analysis, and a Shimadzu® GC-MS for identification of bio-solubilization products. Sub-bituminous coal was sourced from a mining site in South Sumatra, Indonesia. Culture media included Sabouraud Dextrose Agar (SDA) for fungal cultivation, Plate Count Agar (PCA) for bacterial enumeration, Mineral Salt Solution (MSS) as the basal medium, MSS+ medium (MSS supplemented with 2% sucrose, 0.03% urea, and 5% coal powder), and bacto agar. Additional reagents comprised immersion oil, Lowry reagent solutions 1 and 2 for protein assays, and fluorescein diacetate (FDA) for enzyme activity determination.

2.2. Sample Preparation and Sterilization

Coal samples were air-dried, ground using a mechanical grinder, and sieved to obtain a uniform powder of 100–150 mesh particle size. Portions of 5 g coal powder were weighed and sealed in sterile polyethylene bags for irradiation. All glassware and culture vessels were sterilized using an autoclave at 121 °C and 1 atm for 15 min, while heat-sensitive tools were sterilized with 70% ethanol. Gamma irradiation of coal powder was performed using an Irpasena gamma irradiator at room temperature with ^60Co as the radiation source. Samples were exposed to doses of 2.5, 5, 10, 20, and 40 kGy at a dose rate of approximately 5 kGy/h. Post-irradiation, sterility was verified by spreading 0.1 g of irradiated coal on PCA (for bacteria) and SDA (for fungi) followed by incubation at 30 °C for 48 h.

2.3. Preparation of Culture Media and Inoculum

The MSS medium was prepared by dissolving 0.1 g $MgSO_4 \cdot 7H_2O$, 0.2 g KH_2PO_4, and 0.2 g K_2HPO_4 in 1 L of distilled water, adjusted to pH 6.0. The MSS+ medium contained MSS supplemented with 20 g sucrose, 0.3 g urea, and 50 g coal powder per liter. All media were autoclaved at 121 °C for 15 min. *Phanerochaete chrysosporium* was maintained on SDA slants and sub-cultured every 7 days. For inoculation, spores were harvested by adding 10 mL of sterile distilled water containing 0.01% Tween-80 to a 7-day-old culture and filtered through sterile gauze to obtain a uniform spore suspension (~1×10^6 spores/mL).

2.4. Bio-solubilization Experiments

Sterile 250 mL Erlenmeyer flasks were filled with 50 mL MSS+ medium and 5% (w/v) coal powder (either raw or gamma-irradiated). Each flask was inoculated with 1 mL of *P. chrysosporium* spore suspension and incubated in a Precision® shaker incubator at 30 °C and 150 rpm for 21 days. Triplicate flasks were prepared for each irradiation dose. Uninoculated flasks served as abiotic controls.

2.5. Monitoring and Analytical Procedures

Medium pH was measured every three days using a calibrated Hanna® pH meter. Fungal colonization was visually monitored by photographing coal particles under a Novel® microscope at 400× magnification. Extracellular protein concentration was determined using the Lowry method with bovine serum albumin as a standard. Enzymatic activity was assessed by hydrolysis of fluorescein diacetate (FDA), measured spectrophotometrically at 490 nm. The degree of coal solubilization was quantified by measuring the absorbance of culture supernatants at 250 nm and 450 nm with a Shimadzu® UV-Vis spectrophotometer.

2.6. GC-MS Analysis of Bio-solubilization Products

After 21 days, culture broths were filtered to separate fungal biomass and insoluble coal residue. The filtrates were extracted with dichloromethane (1:1 v/v) and concentrated using a rotary evaporator. Concentrated extracts were analyzed using a Shimadzu® GC-MS equipped with an Rtx-5MS column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at 1.0 mL/min. The oven temperature program was set from 40 °C (held 5 min) to 300 °C at a rate of 10 °C/min and held for 10 min. Detected peaks were identified by comparison with the NIST 2011 mass spectral library.

2.7. Statistical Analysis

All experiments were performed in triplicate. Data on pH, absorbance, protein concentration, and enzyme activity were expressed as mean ± standard deviation. Statistical significance between treatments was evaluated using independent T-tests or one-way ANOVA followed by Tukey's post hoc test, where appropriate, using SPSS version 20, with a significance level set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Sterilization Test of Gamma-Irradiated Coal

The sterilization test results showed that higher doses of gamma irradiation led to lower bacterial and fungal growth on coal (Table 1). A dose of 5 kGy was sufficient to eliminate bacteria, while a dose of 20 kGy was required to suppress fungal growth. Based on these findings, a dose of 20 kGy was selected as the sterilization treatment for coal and was used in subsequent experiments.

Table 1. Bacterial and Fungal Counts under Various Gamma Irradiation Doses

Dose (kGy)	Bacteria (CFU/g)	Fungi (Propagule/g)
0	6.47×10^{14}	2.17×10^{13}
2.5	3.84×10^{12}	3.84×10^{12}
5	0	1.24×10^{15}

10	0	2.17×10^{13}
20	0	0
40	0	0

The difference in irradiation doses required for bacteria and fungi is attributed to their distinct cellular morphology. Bacteria, as prokaryotic cells, lack a nucleus, and their chromosomes are freely located within the cytoplasm. In contrast, fungi are eukaryotic organisms with a nucleus enclosed by a nuclear membrane and possess chitin in their cell walls. Their cell sizes also differ, with bacteria (prokaryotes) being smaller, ranging from 0.1–10 µm, whereas fungi (eukaryotes) range from 10–100 µm. Both bacteria and fungi present in coal exist in a dormant state as spores. Spores and vegetative cells can be inactivated by gamma irradiation due to damage to cell structures and DNA [23], [24].

These findings differ from those reported by Sugoro [25] in a study using lignite coal, in which the radiation dose required for fungal sterilization was 10 kGy and for bacteria 20 kGy. This discrepancy may be explained by differences in coal type. Sub-bituminous coal generally contains fewer microorganisms compared to lignite, leading to lower irradiation doses required for sterilization [25]. This is likely due to the more complex structure of sub-bituminous coal, which makes it less favorable for microbial survival, in addition to the influence of physical and chemical conditions during the coalification process.

3.2. Evaluation of Coal Bio-solubilization with *Phanerochaete chrysosporium*

3.2.1 pH Changes in the Culture Medium

The pH of the medium resulting from coal bio-solubilization with *P. chrysosporium* showed a similar trend but with different values between the two treatments (Figure 1). The medium pH of the sterilized coal treatment (B) was higher than that of the raw coal treatment (A). The pH range for the sterilized coal treatment was 4.13–4.42, while that for the raw coal treatment was 4.08–4.29. Statistical analysis using the T-test indicated a significant difference in medium pH between the two treatments ($P \leq 0.05$).

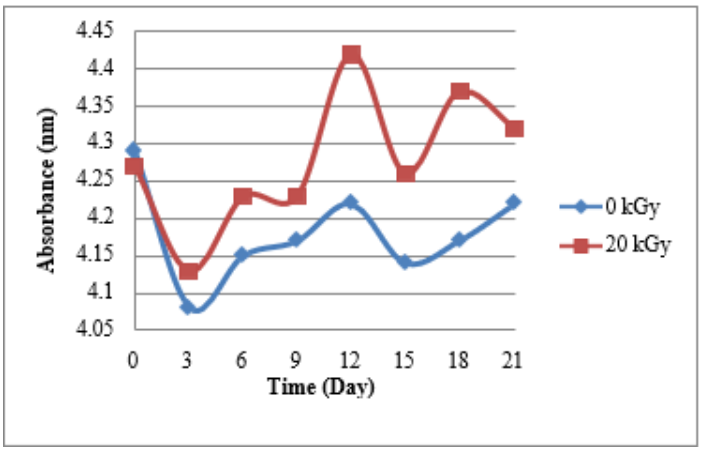


Figure 1. pH values of MSS++ medium with raw coal + *P. chrysosporium* (A) and MSS++ medium with sterilized coal + *P. chrysosporium* (B)

Most fungi are able to grow within a wide pH range, from 2 to 8.5 (Pelczar & Chan, 2005). The solubilization process carried out by almost all fungi generally tends to produce acidic pH [26]. The initial pH value of the raw coal treatment (A) was higher compared to the sterilized coal treatment (B). This is because gamma irradiation reduces the pH, making it more acidic, as irradiation breaks down complex hydrocarbon chains into simpler compounds, releasing organic acids such as humic acid, fulvic acid, and carboxylic acid [27], [28].

A decrease in pH during the process can also be attributed to coal desulfurization. Desulfurization refers to the removal of sulfur during solubilization [29], [30]. The dissolution of sulfur in the form of sulfate ions (SO_4^{2-}) into the liquid medium leads to the formation of sulfuric acid [31], [32]. Medium acidity is also associated with the production of phenols, aldehydes, and ketone groups during the bio-solubilization process. The presence of these organic acids is closely linked to fungal degradation activity involving enzymes such as lignin peroxidase, phenol oxidase, and manganese peroxidase [33].

The pH of the raw coal treatment (A) decreased until day 3, reaching 4.08. It then increased until day 12 (pH 4.22) before decreasing again by day 15 and finally rising again until the end of incubation on day 21. A similar pattern was observed in the sterilized coal treatment (B). The initial decrease in pH reflects the active growth of *P. chrysosporium* in biomass formation. The decrease in culture medium pH was also caused by the inoculation of *P. chrysosporium* spores, which created a more acidic environment.

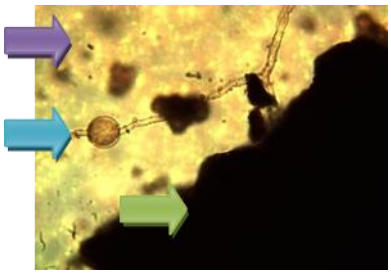
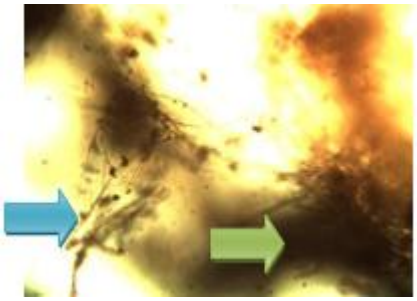
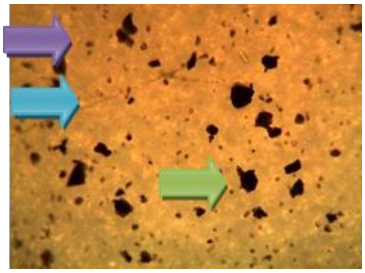
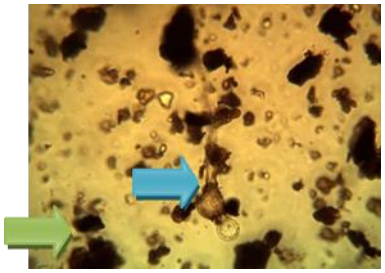
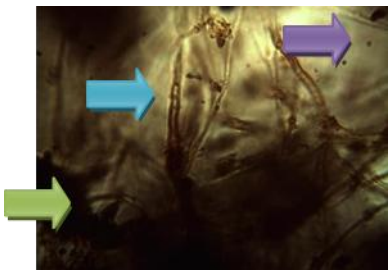
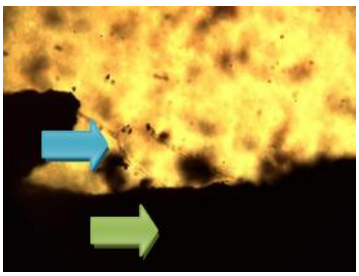
The raw coal treatment (A) exhibited a more acidic pH than the sterilized coal treatment (B) (Figure 1). This difference can be attributed to the combined metabolism of *P. chrysosporium* and the indigenous microbes naturally present in raw coal. In contrast, the sterilized coal treatment (B) exhibited higher pH values because the solubilization process was solely carried out by *P. chrysosporium*, as indigenous microbes were eliminated during coal sterilization.

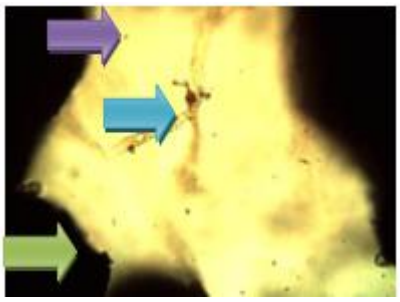
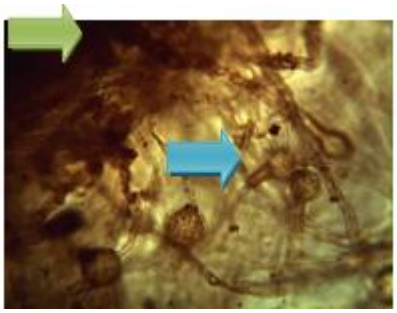
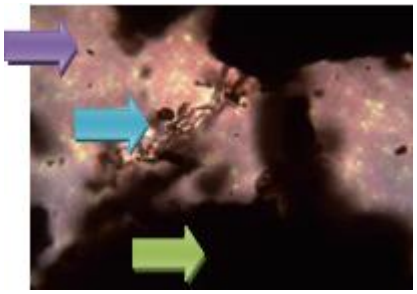
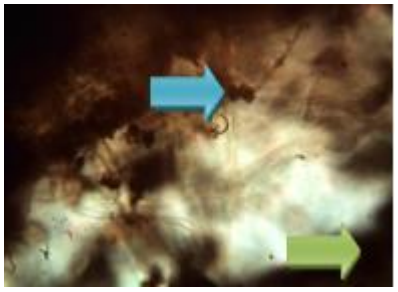
The increase in pH observed during later incubation stages was likely due to the depletion of nutrients in the medium, as well as the accumulation of toxic metabolites causing the death of indigenous bacteria and *P. chrysosporium*. Microbial competition for dominance within the substrate also contributed to this phenomenon [34], [35]. Dead cells within the medium were then reutilized as a nitrogen source for surviving microorganisms, leading to a buffering effect [36], [37].

3.2.2 Mycelial Colonization of *P. chrysosporium* on Coal

The colonization of *P. chrysosporium* on coal showed differences at each observation period (Table 2). Mycelial colonization on sterilized coal (B) increased at the end of incubation compared to raw coal (A). However, observations of treatments A and B on day 0 after inoculation with *P. chrysosporium* spores appeared similar, in which only spores were observed and no hyphae had developed. The observed spores were transparent or colorless.

Table 2. Observation of Colonization on Raw Coal + *P. chrysosporium* (A) and MSS++ Medium with Sterilized Coal + *P. chrysosporium* (B) at 1000× Magnification.

Time (Days)	(A) 0 kGy	(B) 20 kGy
9	 A decrease in mycelial aggregation (+) was observed, and indigenous bacteria were present	 An increase in mycelial aggregation (++) was observed, and no indigenous bacteria were present
12	 Hyphae attached to the coal (+) were observed, and indigenous bacteria were present.	 Hyphae attached to the coal (+) were observed, and no indigenous bacteria were present
15	 Numerous mycelial aggregates (+++) were observed, and indigenous bacteria were present	 Increased hyphae (+) were observed, and no indigenous bacteria were present.

18	 Hyphae attached to the coal (+) were observed, and indigenous bacteria were present	 Mycelial aggregates (+++) were observed, and no indigenous bacteria were present
21	 Terlihat hifa yang melekat pada batubara (++) dan ada bakteri indigenus	 Mycelial aggregates (+++) were observed, and no indigenous bacteria were present.

Description:

Batubara: +++ : > 75%

Spore/mycelium: ++ : 50-75%

Bacteria: + : <50%

Hyphae were observed in the medium after the 3rd day of incubation, and by the 6th day mycelial growth of *P. chrysosporium* had begun. The amount of mycelial aggregates decreased on the 12th day compared to the previous observation, suggesting that the fungus had entered the exponential phase or that many microorganisms had started to die. This corresponds with the increase in pH values. On the 15th day, fungal growth resumed, as indicated by the increasing number of mycelial aggregates compared to the 12th day.

The fungus was able to grow well until the 21st day, and it is assumed that the fungus could degrade coal, as evidenced by the presence of mycelial aggregates attached to the coal surface. Gamma irradiation also played a role in coal degradation, since higher irradiation doses facilitated fungal decomposition of coal into smaller particles. Gamma irradiation was found to influence the amount of coal trapped in the fungal matrix [38], [39]. The greater the number and length of hyphae, the more coal particles could be entrapped by the fungus.

Microscopic observations also revealed the presence of bacteria (purple arrow) along with fungi (blue arrow) in treatment A (Table 2). This indicated a decrease in pH due to the metabolic activity of both *P. chrysosporium* and indigenous microbes in coal. The occurrence of colonization demonstrates that the fungus utilized coal as a substrate for its metabolism with the aid of enzymes, leading to the bio-solubilization process.

3.2.3 Analysis of Phenolic Compounds and Conjugated Aromatics

The phenolic and aromatic compounds resulting from coal bio-solubilization with *P. chrysosporium* exhibited similar fluctuating patterns in both treatments (Figures 9 and 10). Analysis of phenolic compounds in the raw coal treatment (A) showed a higher rate compared to the sterilized coal treatment (B). The phenolic compound rate in raw coal (A) was 0.113/day, whereas in sterilized coal (B) it was approximately 0.095/day, observed on day 3. Figure 2 also shows the highest absorbance value of 0.7 on day 12 in the raw coal treatment (A). The higher solubilization yield in raw coal (A) compared to sterilized coal (B) is consistent with the findings of Shi et al. [40], who reported that an increase in UV–Vis absorbance reflects a greater amount of bio-solubilization products formed.

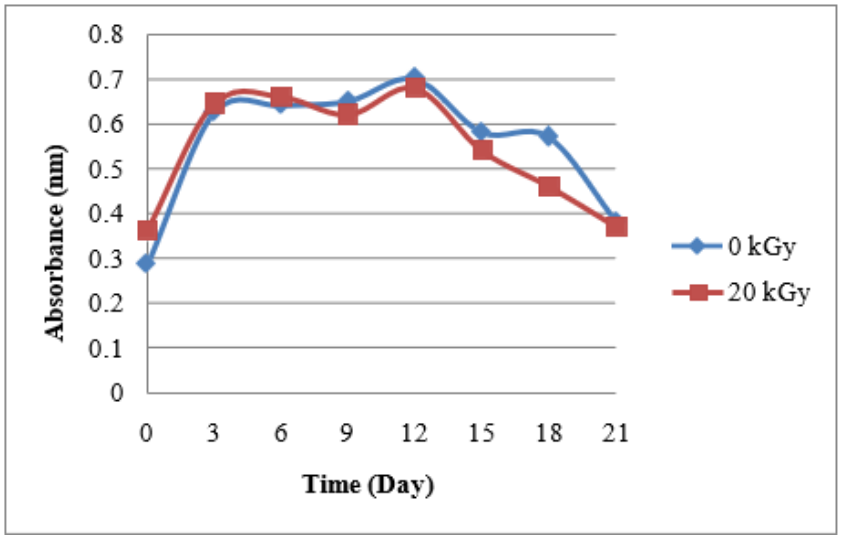


Figure 2. Absorbance values of phenolic compounds measured using a UV-Vis spectrophotometer at a wavelength of 250 nm from the bio-solubilization of coal by *P. chrysosporium*..

The test results corresponded to the supernatant collected during sampling, which showed color variations from day 0 to day 21. The initial sample appeared clearer, whereas by day 21 it became slightly turbid, with the collected sample appearing denser and more viscous compared to the early incubation stage. This indicates that the bio-solubilization process of coal had occurred.

The pH values were inversely related to the solubilization values. When solubilization was high, the pH was low. Conversely, solubilization values were directly proportional to the mycelial colonies observed in both raw coal (A) and sterilized coal (B) treatments. When solubilization was higher, the observed mycelial colonies were also more abundant (Table 2).

Analysis of aromatic compounds also revealed fluctuating results. Aromatic compounds in the raw coal treatment (A) showed higher rates compared to sterilized coal (B). The highest rate of aromatic compounds in raw coal (A) occurred on day 6, reaching 0.02/day, while sterilized coal (B) reached approximately 0.013/day. The highest product value was also observed on day 6, with 0.21 in the raw coal (A) treatment. Statistical analysis using the t-test showed no significant differences in the phenolic and aromatic compound contents between the two treatments ($P \leq 0.05$).

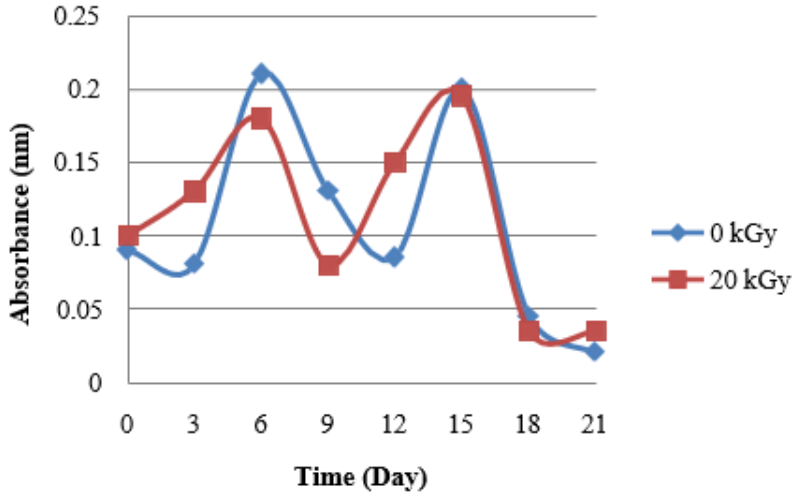


Figure 3. Absorbance values of aromatic compounds measured using a UV-Vis spectrophotometer at a wavelength of 450 nm from the bio-solubilization of coal by *P. chrysosporium*.

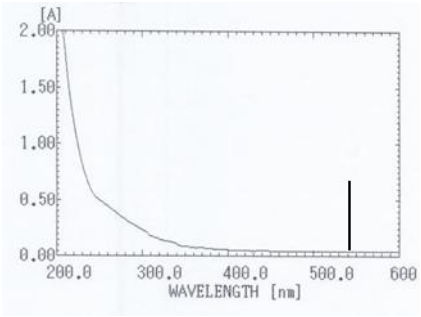
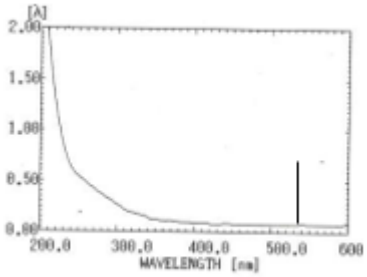
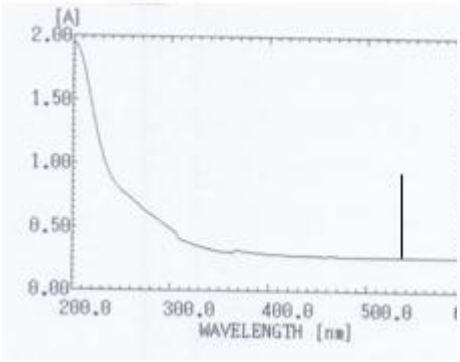
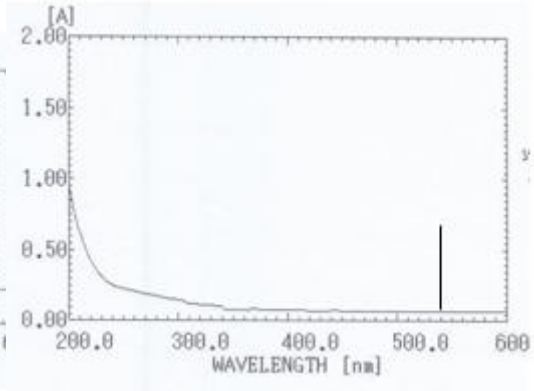
The decrease in absorbance values of phenolic compounds was attributed to the decomposition of coal bio-solubilization products into simpler compounds [41], [42]. This was caused by the activity of both the fungus and indigenous microbes present in the coal. Phenolic and conjugated aromatic compounds are recognized as products of the bio-solubilization process (Selvi et al., 2009). The increase in absorbance values of conjugated aromatic compounds was due to the release of substances such as humic acids from the coal surface through the activity of *Trichoderma* sp.

3.2.4 UV-Vis Spectrophotometric Scanning Results (200–600 nm) of Phenolic and Aromatic Compounds

Scanning measurements in this experiment were carried out at wavelengths of 200–600 nm, but the results appeared less consistent. The incubation days 3 and 6 showed the highest absorbance rates for phenolic and aromatic compounds (Figures 2 and 3), therefore scanning was only conducted for these two samples. The scanning results of samples A and B on days 3 and 6 showed peaks around 538 nm, which corresponds to aromatic compounds (Table 3). The highest absorbance was observed in sample A on day 6 (0.275), while the lowest was in sample A on day 3 (0.048). In contrast, sample B showed the highest absorbance on day 3 (0.085) and the lowest on day 18 (0.023).

Peak determination appeared similar across all wavelengths. The absorbance results confirmed the presence of compounds, as indicated by the broad absorption bands within the wavelength range of 200–600 nm [43]. The highest absorbance values indicated the presence of aromatic compounds in the coal. These elevated values suggested that *P. chrysosporium* and indigenous microbes in the medium were able to solubilize coal by breaking down complex compounds into simpler ones.

Table 3. Scanning results at 200–600 nm using a UV-VIS spectrophotometer on MSS^+ medium with raw coal + *P. chrysosporium* (A) and MSS^+ medium with sterilized coal + *P. chrysosporium* (B).

No	Treatment	Scanning 200-600 nm
1	A	 Absorbance 538 nm  Absorbance 538 nm
2	B	 Absorbance 538 nm  Absorbance 538 nm

3.2.5 Extracellular Protein Test Analysis Results

The extracellular protein content of *P. chrysosporium* with raw coal and sterilized coal exhibited a fluctuating pattern and differed between the two treatments (Figure 4). In treatment A, the highest result was observed on day 18 with a value of 0.42, while in treatment B the highest value was 0.40 on day 15. The increase in absorbance values indicates an increase in extracellular protein levels, as the fungus secretes extracellular enzymes and facilitates the degradation of coal (Sugoro et al., 2009). Statistical analysis using the T-test showed that there was no significant difference in extracellular protein content between the two treatments ($P \leq 0.05$).

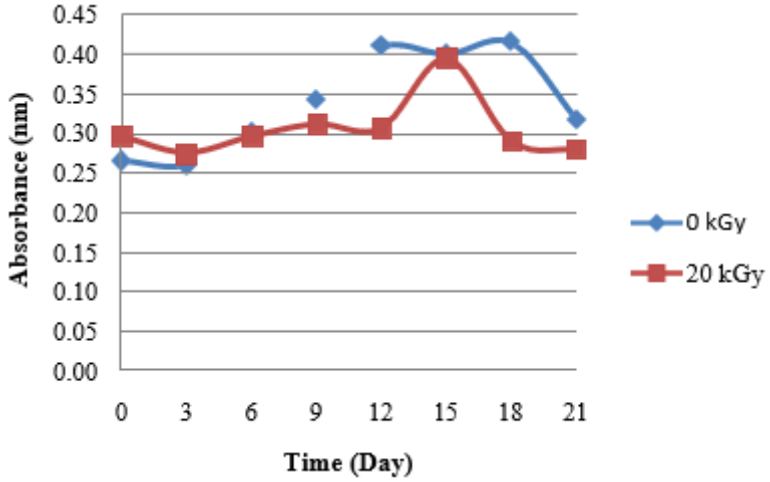


Figure 4. Extracellular protein content measured using UV-VIS spectrophotometer with the Lowry assay of *P. chrysosporium*

The high extracellular protein values were consistent with the large amount of mycelial aggregates observed in Table 2, indicating that greater fungal growth corresponded to higher levels of extracellular protein produced. The fungus was able to utilize urea present in the medium to synthesize protein. This is in line with the findings of Grzyb et al. [44], who emphasized that microorganisms utilize carbon as an energy source and nitrogen as a key nutrient for protein synthesis and cell growth. Although the extracellular protein levels were high, the pH of the medium increased. This finding is consistent with the study *"Nitrogen source orchestrates pH modulation and secondary metabolism in Trichoderma harzianum*," which reported that cultures of *Trichoderma* exhibited elevated pH values when extracellular protein levels were high, a condition possibly linked to cell lysis [45].

3.2.6 Hydrolyzed Fluorescein Diacetate (FDA) Measurement Results

The measurement of hydrolyzed fluorescein diacetate (FDA) in this study was conducted to determine esterase enzyme activity during the coal bio-solubilization process [42]. The concentration of hydrolyzed FDA in both treatments exhibited different patterns (Figure 5). Treatment B showed higher hydrolyzed FDA levels compared to treatment A.

The highest product of FDA hydrolysis by the fungus was observed on day 15 with a value of 0.055 for sample A and on day 12 with a value of 0.105 for sample B. The highest hydrolysis rate was 0.40/day, occurring on day 12 with 2.33/day for raw coal (A) and on day 15 with 2/day for sterilized coal (B). The fungal isolate in sample A showed a decrease on day 3, followed by an increase from day 6 to day 12, and then a decline until day 21. In contrast, the fungal isolate in sample B increased from day 3 to day 15, and subsequently decreased on day 21. Statistical analysis using the T-test revealed that there was no significant difference in enzyme content between the two treatments ($P \leq 0.05$).

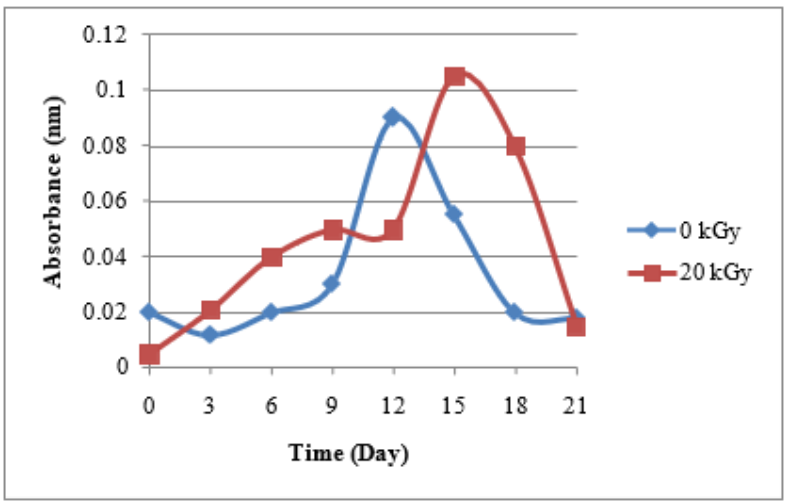


Figure 5. Results of FDA hydrolysis at a wavelength of 490 nm by *P. chrysosporium*

It is known that the higher the measured absorbance value, the higher the fluorescein concentration, which indirectly indicates the level of microbial enzymatic activity [46], [47]. The concentration of enzymes measured using the FDA method represents enzymes involved in the coal bio-solubilization process [11], [48]. The fluctuation observed in FDA hydrolysis is due to the production of extracellular enzymes by fungi and bacteria that hydrolyze complex molecules from the substrate. Hydrolyzed FDA began to increase on day 6, which can be attributed to the release of coal degradation products into the medium. This is consistent with the pH results, where high microbial activity in the medium was indicated by the low pH observed on day 15. The subsequent decline in FDA hydrolysis results is due to the breakdown of complex substrates into simpler compounds, thereby reducing the activity of extracellular enzymes.

3.2.7 Bio-solubilization Product Analysis by GC-MS

The GC-MS analysis was conducted to identify the compounds present in the bio-solubilization products. The samples selected were those showing the highest absorbance values at wavelengths of 250–450 nm, namely on day 6 for both treatments. The use of samples with the highest absorbance was expected to yield products comparable to gasoline and diesel.

The GC-MS results revealed 20 compounds in sample A and 30 compounds in sample B (Table 4). Sample B contained more compounds than sample A, which may be attributed to the effect of gamma irradiation. Gamma irradiation is known to break complex molecular bonds into simpler ones, thereby facilitating the degradation of subbituminous coal by the fungus.

The detected compounds were predominantly hydrocarbons. Hydrocarbons are organic compounds consisting solely of carbon and hydrogen atoms in their molecular structure. The dominant compound in sample A was naphthalene ($C_{10}H_8$), with a relative abundance of 38.29%. Naphthalene is a polycyclic aromatic hydrocarbon commonly found in coal and petroleum; it functions as a gasoline additive and is known for its favorable combustion properties. In contrast, the dominant compound in sample B was tetrapentacontane ($C_{54}H_{110}$), with a relative abundance of 16.33%.

Table 4. Compounds Identified in the Bio-solubilization Products of SubbituminousCoal by GC-MS

No	Compound	% Area		
		Treatment		
		0 kGy (A)	20 kGy (B)	Product
1	Toluene (C_7H_8)	0.49	0.23	B
2	2,4-Dimethyl-1-heptene (C_9H_{18})	3.33	1.80	B
3	Styrene (C_8H_8)	-	0.06	B
4	Ethylbenzene (C_8H_{10})	-	0.04	B
5	4-Methylheptane (C_8H_{18})	0.49	0.37	B
6	o-Xylene (C_8H_{10})	-	0.05	B
7	4-Methyloctane (C_9H_{20})	13.07	1.31	B
8	1,3,5-Trimethylbenzene (C_9H_{12})	-	5.62	B
9	2,3,5-Trimethyloctane ($C_{11}H_{24}$)	-	0.59	B
10	2,3,3-Trimethylpentane (C_8H_{18})	-	0.29	B
11	Naphthalene ($C_{10}H_8$)	38.29	-	B dan D/S
12	Azulene ($C_{10}H_8$)	-	9.25	B dan D/S
13	2,4,6-Trimethylheptane ($C_{10}H_{22}$)	-	0.07	B dan D/S
14	1,2,3-Trimethylbenzene (C_9H_{12})	0.68	-	B dan D/S
15	3,7-Dimethylheptane (C_9H_{20})	1.38	-	B dan D/S
16	1,3-Dimethyl-4-ethylbenzene ($C_{10}H_{14}$)	-	0.54	B dan D/S
17	2,6,10-Trimethyldodecane ($C_{15}H_{32}$)	0.86	-	B dan D/S
18	2,6-Dimethylnaphthalene ($C_{12}H_{12}$)	0.52	-	B. D/S dan K
19	3-Methylnaphthalene ($C_{11}H_{10}$)	10.06	8.86	B. D/S dan K
20	2,3-Dimethylnaphthalene ($C_{12}H_{12}$)	1.36	-	B. D/S dan K
21	2,7-Dimethylnaphthalene ($C_{12}H_{12}$)	0.74	-	B. D/S dan K
22	1-Methylnaphthalene ($C_{11}H_{10}$)	1.3	0.62	B. D/S dan K
23	4-Buthyl-1-Methoxy-Cyclohexane ($C_{11}H_{22}O$)	4	-	B. D/Sdan K
24	Pentadecane ($C_{15}H_{32}$)	2.25	0.36	B. D/S dan K
25	Undecylcyclohexane ($C_{17}H_{34}$)	3.02	1.67	B. D/Sdan K
26	1-Ethyl-3,5-dimethylbenzene ($C_{10}H_{14}$)	-	2.29	B dan D/S
27	Octadecane ($C_{18}H_{38}$)	-	5.84	B dan D/S
28	Heptadecane ($C_{17}H_{36}$)	-	1.83	B dan D/S

29	Nonadecane ($C_{19}H_{40}$)	-	1.83	B dan D/S
30	Eicosane ($C_{20}H_{42}$)	7.65	5.82	B dan D/S
31	Heneicosane ($C_{21}H_{44}$)	4.13	6.27	B dan D/S
32	Docosane ($C_{22}H_{46}$)	3.69	-	B dan D/S
33	Tricosane ($C_{23}H_{48}$)	-	4.28	B dan D/S
34	2,6,10,15-Tetramethylheptadecane ($C_{21}H_{44}$)	-	2.74	B dan D/S
35	Tetracosane ($C_{24}H_{50}$)	2.69	1.35	B dan D/S
36	Pentacosane ($C_{25}H_{52}$)	-	5.27	B dan D/S
37	n-Hexacosane ($C_{26}H_{54}$)	-	7.56	
38	Tetracontane ($C_{40}H_{82}$)	-	6.71	
39	Tetrapentacontane ($C_{54}H_{110}$)	-	16.33	
	Total % area	100	100	
	Number of Compounds	20	30	

Description:
B = gasoline
D/S = Diesel
K = Kerosene

The compounds detected only in the raw coal treatment (A) were: *naphthalene* ($C_{10}H_8$), *3,3,5-trimethylheptane* ($C_{10}H_{22}$), *3,4,5-trimethylheptane* ($C_{10}H_{22}$), *4-methyldecane* ($C_{11}H_{24}$), *5,6-dimethyldecane* ($C_{12}H_{26}$), *5-methylundecane* ($C_{12}H_{26}$), *n-dodecane* ($C_{12}H_{26}$), *4-butyl-2-methyloctane* ($C_{13}H_{28}$), and *n-nonadecane* ($C_{19}H_{40}$). The compounds detected only in the sterile coal treatment (B) were: *styrene* (C_8H_8), *ethylbenzene* (C_8H_{10}), *n-octane* (C_8H_{18}), *3-ethyl-2-methylhexane* (C_9H_{20}), *2,3,3-trimethylhexane* (C_9H_{20}), *2,3,5-trimethylhexane* (C_9H_{20}), *azulene* ($C_{10}H_8$), *2,4,6-trimethylheptane* ($C_{10}H_{22}$), *3,3,5-trimethylheptane* ($C_{10}H_{22}$), *3,3,6-trimethylheptane* ($C_{10}H_{22}$), *undecylcyclopentane*[1] ($C_{16}H_{32}$), *n-hexadecane* ($C_{16}H_{34}$), *2-methylhexadecane* ($C_{17}H_{36}$), *n-heptadecane* ($C_{17}H_{36}$), *2,6,11,15-tetramethylhexadecane* ($C_{20}H_{42}$), *n-eicosane* ($C_{20}H_{42}$), *n-tetracosane* ($C_{24}H_{50}$), *n-hexatriacontane* ($C_{36}H_{74}$), *n-tetracontane* ($C_{40}H_{82}$), and *n-tetrapentacontane* ($C_{54}H_{110}$).

The resulting compounds (Table 4) indicate the degradation of complex compounds into simpler ones, originating from the activity of solubilizing agents, namely mold and indigenous microbes. Table 4 shows the presence of short-chain carbon compounds, consistent with the findings of Titilawo et al. [49] and Akhtar et al. [50], who reported that low-molecular-weight carbon compounds can be utilized by fungi for bio-solubilization and that such short-chain compounds can also result from gamma irradiation, which cleaves complex bonds into simpler structures.

In addition to aromatic hydrocarbons, aliphatic hydrocarbons are also present. The presence of aliphatic hydrogen compounds indicates an enzymatic reaction carried out by the solubilizing agent, P. chrysosporium. Lignin peroxidase produced by the solubilizing agent breaks down non-phenolic lignin bonds, exposing the lignin structure. The manganese peroxidase enzyme functions to degrade phenolic bonds in coal lignin [51], [52]. Bio-solubilization results can produce compounds equivalent to gasoline (C7-C11), diesel (C10-C24), and kerosene (C12-C15). Treatment A indicated the presence of gasoline at 58.59%, while B only indicated 20.22%. Diesel (diesel) was 82.62%, B at 58.89%, and kerosene at 23.25% (A) and 11.51% (B). The percentages of all compounds showed that treatment A had a higher value than B (Figure 6).

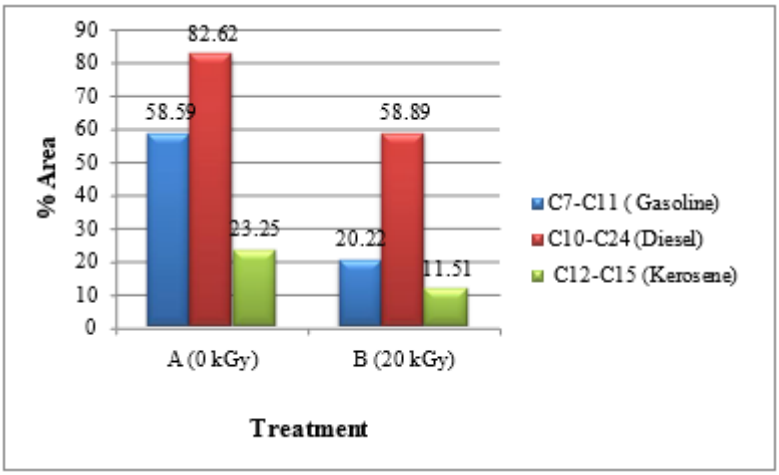


Figure 6. Percentage of the resulting area of gasoline, diesel, and kerosene compounds in treatments A (raw coal + *P. chrysosporium* mold) and B (sterile coal + *P. chrysosporium* mold) with 120 rpm agitation during 6 days of incubation

Research using Penicillium sp. mold conducted by Zahara et al. [11]produced a bio-solubilization product equivalent to gasoline of 73.24% at a dose of 10 kGy with an incubation period of 7 days, while the product equivalent to diesel fuel at a dose of 5 kGy was 48.05%. Figure 6 shows that the results of this study were superior, producing bio-solubilization products equivalent to gasoline of 58.59%, diesel of 82.62%, and kerosene of 23.25% for raw coal treatment (A) using P. chrysosoporium mold. The bio-solubilization results differed depending on the type of mold, coal, and treatment.

The sterile coal treatment (B) had a smaller area percentage than the raw coal treatment (A). This is because in the sterile coal treatment (B), only P. chrysosoporium mold acted as a solubilizer. The resulting compound yield was greater than that of the raw coal treatment (A) due to the effect of gamma irradiation, which breaks complex bonds into simpler ones, allowing the mold to degrade the coal more easily than the raw coal treatment (A). The raw coal treatment (A) had a larger surface area and fewer compounds. This is because coal contains complex compounds that are difficult to degrade. However, because treatment A contained the mold P. chrysosoporium in consortium with indigenous microbes, the solubilization process was faster and produced a higher product.

This study demonstrated that the combination of gamma irradiation and the fungus *Phanerochaete chrysosporium* effectively enhanced the biosolubilization of sub-bituminous coal into simpler hydrocarbon compounds comparable to liquid fuels. Gamma irradiation at 20 kGy successfully sterilized the coal from bacteria and fungi while breaking complex hydrocarbon chains into simpler molecules, thereby facilitating fungal degradation. GC-MS analysis revealed that the raw coal treatment produced gasoline-equivalent compounds of 58.59%, diesel of 82.62%, and kerosene of 23.25%, whereas the sterilized coal treatment yielded a greater diversity of compounds (30 types) despite lower percentages of gasoline, diesel, and kerosene. Enzymatic activity, pH fluctuations, and increased extracellular protein levels indicated the involvement of lignin peroxidase and manganese peroxidase enzymes in coal degradation, producing phenolic, aromatic, and aliphatic compounds with potential as liquid fuels.

The novelty of this research lies in the application of gamma irradiation as a pre-treatment for sub-bituminous coal combined with *P. chrysosporium* to improve biosolubilization efficiency. This approach not only accelerates coal decomposition but also generates a wider variety of short-chain hydrocarbon compounds with high energy value, enabling the production of gasoline-, diesel-, and kerosene-like products. The comparative analysis between sterilized and non-sterilized coal further provides new insights into the interactions between indigenous microbes and the fungus during coal degradation.

This research offers significant implications for biology and biochemistry education, particularly in the fields of energy biotechnology and bioremediation. The findings provide a concrete example of how microorganisms and radiation can be applied to convert natural resources into renewable energy, enriching teaching materials on ligninolytic enzymes, fungal metabolism, and microbe–substrate interactions. Moreover, the results open opportunities for further biochemical research on environmentally friendly coal processing and biofuel production, making it highly relevant for higher education and applied research in bioenergy.

4. CONCLUSION

Based on the research results, it can be concluded that gamma irradiation treatment on subbituminous coal does not have a positive effect on the bio-solubilization process using Phanerochaete chrysosporium mold. In fact, raw coal without treatment shows a better level of bio-solubilization , characterized by high rates of formation of aromatic compounds, phenolics, protein and enzyme activities, and carbon compound content equivalent to energy products such as gasoline, diesel, and kerosene. This indicates that gamma irradiation is not effective in improving the performance of coal bioconversion, and even tends to reduce the potential bio-solubilization results produced. Further research is needed on the effect of gamma irradiation using different types of mold and coal to determine the maximum results of coal solubilization.

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