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Solid-state Fermentation for Production of Bio-coagulant on Rice Bran using *Candida utilis, Trichoderma* sp., *Phanerochaete chrysosporium* for Turbidity Removal

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ABSTRACT: Solid-state fermentation is a microbial culture technique used to produce compounds and products of industrial interest. In this study, rice bran, a by-product of the rice processing industry, was used as a coagulant substrate for *Candida utilis*, *Trichoderma* sp., and *Phanerochaete chrysosporium* in solid-state fermentation (SSF). To the best of our knowledge, no study has reported the production of coagulants by *Trichoderma* sp. *and Candida utilis*. Moreover, no attempts have been made to utilize rice bran as a substrate for the production of coagulants in solid-state by these three microorganisms. Two different substrate proportions were used to produce natural coagulants. Bio-coagulants produced in this research were used to remove turbidity from the kaolin solution. The experiments were conducted with artificially turbid water and a conventional jar test apparatus. The findings demonstrate that Substrate 2, distinguished by its lower water content, exhibited the highest turbidity removal efficiency compared to Substrate 1, which possessed higher water content. Moreover, *Trichoderma* sp. showed a maximum turbidity removal of 71%. While the maximum turbidity removal by the coagulants produced from *C. utilis* and *P. chrysosporium* was 59 %. The microscopic observation of the microorganism's growth on rice bran substrate has also been performed. It was revealed that the three microorganisms fully colonized the substrate surface. Further optimization of the SSF process parameters is expected to achieve efficient turbidity reduction in wastewater.

ABSTRAK: Penapaian keadaan pepejal ialah teknik kultur mikrob yang digunakan untuk menghasilkan sebatian dan produk yang mempunyai kepentingan industri. Dalam kajian ini, dedak padi, iaitu hasil sampingan daripada industri pemprosesan beras, digunakan sebagai substrat koagulan bagi *Candida utilis*, *Trichoderma* sp., dan *Phanerochaete chrysosporium* dalam penapaian keadaan pepejal (*solid-state fermentation*, SSF). Sepanjang pengetahuan kami, belum terdapat kajian yang melaporkan penghasilan koagulan oleh *Trichoderma* sp. dan *Candida utilis*. Selain itu, belum ada usaha yang dilaporkan untuk memanfaatkan dedak padi sebagai substrat bagi penghasilan koagulan secara keadaan pepejal oleh ketiga-tiga mikroorganisma ini. Dua nisbah substrat yang berbeza telah digunakan untuk menghasilkan koagulan semula jadi. Bio-koagulan yang dihasilkan dalam kajian ini digunakan untuk menyingkirkan kekeruhan daripada larutan kaolin. Eksperimen dijalankan menggunakan air keruh buatan dan radas ujian balang konvensional. Dapatan kajian menunjukkan bahawa Substrat 2, yang dicirikan oleh kandungan air yang lebih rendah, mempamerkan kecekapan penyingkiran kekeruhan yang paling tinggi berbanding Substrat 1 yang mempunyai kandungan air lebih tinggi. Selain itu, *Trichoderma* sp. menunjukkan penyingkiran kekeruhan

maksimum sebanyak 71%, manakala penyingkiran kekeruhan maksimum oleh koagulan yang dihasilkan daripada *C. utilis* dan *P. chrysosporium* ialah 59%. Pemerhatian mikroskopik terhadap pertumbuhan mikroorganisma pada substrat dedak padi juga telah dijalankan, dan menunjukkan bahawa ketiga-tiga mikroorganisma tersebut telah mengkolonikan permukaan substrat sepenuhnya. Pengoptimuman lanjut terhadap parameter proses SSF dijangka dapat mencapai pengurangan kekeruhan yang lebih berkesan dalam air sisa.

KEYWORDS: *Bio-Coagulant, Rice bran substrate, Large-scale, Solid-state fermentation, Turbidity removal.*

1. INTRODUCTION

Chemical flocculants are frequently used in downstream processing, the food, fermentation, wastewater, and drinking treatment industries, owing to their great flocculating efficiency and cost-effectiveness. However, because of their widespread use, there are significant environmental and health risks [1]. Therefore, biotechnology research has focused on developing more environmentally friendly and safe coagulants for pollutant removal in water treatment to eliminate the hazards associated with chemical coagulants. Thus, bio-coagulants have recently attracted global attention due to their enormous potential to replace chemical flocculants [2].

Bio-coagulants are mainly polysaccharides and proteins [3] that can be isolated from plant, animal, or microbial tissues [2]. Because of their biodegradability, they are more environmentally benign than both organic and inorganic coagulants [3]. Bio-coagulants provide several advantages over chemical coagulants, including minimal toxicity, low residual sludge generation, and low cost [4]. These qualities make bio-coagulants useful for treating water and as potential substitutes for chemical coagulants. However, compared to synthetic organic flocculants, bio-coagulants have limited applicability in water treatment because they require sophisticated extraction techniques and high operational costs. Accordingly, it is necessary to have a coagulant/flocculant with low operating expenses and high flocculation efficiency [5].

Solid-state fermentation is a fungal or bacterial culture technique used to produce compounds and products of industrial interest [6], including pre-treatment of agricultural wastes for producing simple sugars (e.g., glucose) [7], antibiotics (e.g., griseofulvin) [8], pigments [9], enzymes (e.g., cellulases) [10], flavor and fragrance compounds [11], organic acids [12] and organic compounds (e.g., surfactants) [13]. In SSF, the required microorganisms are cultivated on an inert or natural substrate as a solid support, with minimal or no free water [14]. Many researchers have found that using SSF has various biotechnological benefits, including higher fermentation productivity, higher product end concentrations, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates or mixed cultivation of diverse fungi, and finally, fewer sterility demands owing to the low water activity used in SSF [9]. Furthermore, SSF provides many benefits over conventional submerged fermentation (SF), including a simpler process, lower energy use, less pollution, greater product recovery, and a more closely simulated native microorganism environment [14].

Typically, homogeneous agricultural products or lignocellulosic wastes are excellent substrates for SSF because they contain the carbon, nitrogen, and various mineral elements (K, Mg) necessary for microbial growth and metabolite synthesis [14]. However, only a few studies have used solid-state fermentation (SSF) to produce bio-coagulants. Luthfi et al. (2020) used SSF to produce a bioflocculant from Aspergillus niger DWB, employing oil palm empty fruit

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bunch fiber as the substrate [15]. Mamun et al. (2023) and Nedjai et al. (2024) employed SSF with coco peat as the substrate to produce a myco coagulant [16,17]. Nedjai et al. (2025) also used coco peat as the substrate to produce a powdered myco coagulant from *Phanerochaete concrescens* [18]. A variety of substrates have been used for the growth of fungi, including seeds, roots, rice, wheat bran, and wood pieces. Several factors affect substrate selection, but the two most crucial ones are availability and cost [19].

Rice bran is produced as a byproduct of milling during the processing of paddy. Rice is the second-most widely grown crop worldwide, particularly in developing countries. Milling of paddy yields 8% rice bran [20], with one tonne of milled rice yielding 60-80 kg of rice bran [21]. Despite the enormous volume of rice bran produced, it is still not used enough for human consumption [20]. Thus, the use of rice bran as a substrate is an efficient solution to the disposal of large amounts of waste and serves as a waste-to-wealth resource, as leftover rice bran is used to produce a beneficial coagulant that could help many countries address their wastewater treatment crises.

Consequently, the main objective of this research is to use rice bran to produce coagulants via SSF with Candida utilis, Trichoderma sp., and Phanerochaete chrysosporium, using the tray method for semi-industrial production. To the best of our knowledge, no research has reported on the production of coagulants by *Trichoderma sp.* and *Candida utilis*. Furthermore, no attempts have been made to use rice bran as a substrate for producing coagulants via solid-state fermentation by these three microorganisms. Natural coagulants were produced using two distinct substrate proportions. Natural coagulants were evaluated for turbidity removal from a kaolin solution. So, the experiments were carried out using artificially turbid water and a standard jar test apparatus. Scanning electron microscopy (SEM) was also used to observe the growth of the three microorganisms on the rice bran substrate.

2. MATERIALS AND METHODS

2.1. Materials

The fungal strains (*Trichoderma* sp. and *Phanerochaete chrysosporium*) and the bacterial strain (*Candida utilis*) were obtained from the Department of Chemical Engineering and Sustainability, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM). The microbial strains were transferred into a refrigerator and preserved on Potato Dextrose Agar (PDA) at 4°C for future use. Oxoid Ltd. provided the Malt Extract Broth (MEB). We bought potato dextrose agar (PDA) from Difco™. R&M Chemicals provided NaOH, kaolin, D(+)-glucose, and a buffer solution at pH 7. The rice bran was crushed and sieved to obtain fine powders that passed through a 50 mm mesh.

2.2. Methods

2.2.1. Preparation of Media

In this investigation, MEB and glucose were employed. 3% MEB and 2% D (+)-glucose were combined with distilled water to create 100 mL of medium. After adjusting the mixture's pH to 6 with NaOH using a pH meter (METTLER TOLEDO, PB-10 Sartorius, Germany), it was combined with rice bran and autoclaved for 15 minutes at 121°C in an autoclave (Hirayama, HV-110, HVE-50).

2.2.2. Microbes Isolation and Cultures

20 mL of PDA medium, prepared according to the manufacturer's instructions, was used to culture microbes in Petri dishes. To ensure that everything had completely dissolved, the

PDA was suspended in 1 L of distilled water, heated for about 60 s, and then vigorously stirred using a hot plate (Jenway Ltd., UK, Model 1000). Before being placed in sterilized Petri dishes, the mixed components were autoclaved at 121°C for 15 minutes and allowed to polymerize for at least 30 minutes. Microbe strains were inoculated. The yeast *Candida utilis* and *the basidiomycete Phanerochaete chrysosporium plates were incubated at 30 °C for 2-3 days [7] to achieve* sufficient growth. The Trichoderma sp. plates were incubated at 30 °C for 5 days [21] to complete mycelial growth. The isolated microbes were purified and further inoculated onto PDA plates in a biological safety cabinet (ESCO Technologies Inc., USA).

2.2.3. Preparation of Kaolin Solution

In this experiment, kaolin was used to replace water. A specific quantity of kaolin powder was dissolved in 10 liters of distilled water to prepare the stock kaolin solution with an initial turbidity of 750 ± 10 NTU. A Jar test device (Model SW6, Bibby Stuart, UK) was used to gently mix the liquid at 200 rpm for 1 h to achieve a uniform dispersion of kaolin particles. Afterward, the suspension was allowed to stand for a day to ensure that the kaolin was completely hydrated [16].

2.2.4. Cell and Spore Suspensions Preparation

The suspensions of *Candida utilis* cells, *Trichoderma* sp., and *P. chrysosporium* spores were prepared from their cultures. The yeast cells of *Candida utilis* were washed by suspending them in sterile distilled water and then collected using a micropipette. In contrast, the fungi were collected by scraping the media on the plates with a sterile loop and then transferring them to a beaker filled with sterile distilled water. The breakers were shaken using shakers (SK71 model, Jeio Tech Co., Ltd., Korea) to separate spore aggregates. The volume of distilled water was subtracted from the 18% inoculum volume used [18].

2.2.5. Production of Coagulants

To produce the coagulant, rice bran was mixed with an additional amount of media. There were two different proportions used. In Substrate 1, 41.25 g of rice bran and 124.87 mL of media were combined, while in Substrate 2, 83.25 g of rice bran and 83.25 mL of media were combined. The mixture was poured into aluminum trays and covered with aluminum foil. The mixes were then autoclaved for 15 minutes at 121ºC. Afterward, the mixtures were poured into the sterilized trays. The trays were inoculated with suspensions of *Candida utilis* cells, *Trichoderma* sp., and *P. chrysosporium* spores to promote additional inoculum expansion. After covering the trays with aluminum foil, they were incubated at 30 °C for 10 days using a laboratory incubator (Memmert Model).

2.2.6. Coagulants Extraction

4 g of each culture was placed into conical flasks and combined with 20 mL of buffer solution after various days of incubation (1-10 days) (pH 7). At room temperature and 250 rpm, the flasks were agitated for 1 hour. The coagulant was separated from the biomass using a centrifuge (Hettich® Universal 320R, Germany) at 9000 rpm for 5 minutes at 25 ºC. The supernatants were collected, and their flocculating activity was determined by the Jar apparatus method [17].

2.2.7. Evaluation of Produced Coagulants

The kaolin clay suspension method was used to measure the flocculation activity. The initial and final turbidity were measured using a turbidity meter (EXTECH Instruments, TB400, China). In separate beakers containing 300 mL of kaolin solution (750 ± 10 NTU), 10 mL of coagulant was added. After that, the beakers were then shaken for several durations at

different rates, including 7 minutes of rapid mixing at 250 rpm and 22 minutes of slow mixing at 90 rpm. The suspensions were allowed to settle for 60 minutes after agitation was stopped. Using a pipette, samples were taken from the center of the supernatant to determine the final concentration.

The flocculation activity was determined using the following equation:

$$Turbidity\ efficiency\ (\%) = \frac{(initial\ turbidity - final\ turbidity)}{final\ turbidity} \times 100 \qquad (1)$$

2.2.8. Statistical Analysis

All analyses were performed in triplicate. Graphs and statistical analyses were carried out using Microsoft Excel (version 16.69.1). One-way ANOVA was used to analyze the turbidity removal results. Statistically significant differences among microbes were determined by Tukey's test.

2.2.9. Scanning Electron Microscopy (SEM)

A JEOL-IT 100 SEM device was used to examine the morphology and microstructure of microorganisms on rice bran. The morphology of the isolates' colonies was examined after 3 days of incubation. All samples were placed on carbon tape, transferred to a metallic stub, and then allowed to dry in air under a hood. Afterward, using a Quorum Q300TD with a sputter current of 20 mA, the samples were sputter-coated with a thin layer of gold (10–20 nm) for 50 seconds. Cell size measurements were performed using the measurement tool on the JEOL IT100 SEM (InTouchScope^{TM}).

3. RESULTS AND DISCUSSION

3.1. Morphological Structure of Microorganisms

After the incubation period, the plates were examined, and microscopic observation was performed (Figure 1).

Figure 1 (a) shows *C. utilis* cultivated on PDA media for 3 days at 30ºC. On PDA, *C. utilis* appears as smooth, white-to-cream colonies. They had an entire margin and a convex elevation. A microscopic structure of *C. utilis is* presented in Figures 1(b) and 1(c). *C. utilis* yeasts were single, unicellular, oval cells. Some budding (not separated) cells were also observed.

Trichoderma sp. was grown on PDA media for 5 days at 30 °C (Figure 1(d). After 24 hours of incubation at 30°C, conidial germination was first observed, and the colony reached a radius of 45 mm after 5 days. Cottony white at first; eventually, the colony changed to light green, then dark green, as sporulation spread over the agar plate during the incubation period. Microscopic observation of *Trichoderma* sp. showed the presence of dark green conidial masses (Figure 1 (e)). The conidia of *Trichoderma* sp. were subglobose to ovoidal (Figure 1 (f)). The chlamydospores were not observed.

Figure 1 (g) shows the growth of *P. chrysosporium* on PDA media for three days at 30ºC when the mycelial mats filled the Petri dish. The *P. chrysosporium* colony structures were thin, white, cottony, and sticky. A microscopic observation of *P. chrysosporium* showed unbranched, smooth-walled hyphae (Figure 1(h), 1(i)). Chlamydospores were also observed (Figure 1(i)).

The rapid growth and fluffy appearance of the microorganisms' colonies on PDA provide clues about potential bio-coagulant production, which is efficient in removing turbidity from water.

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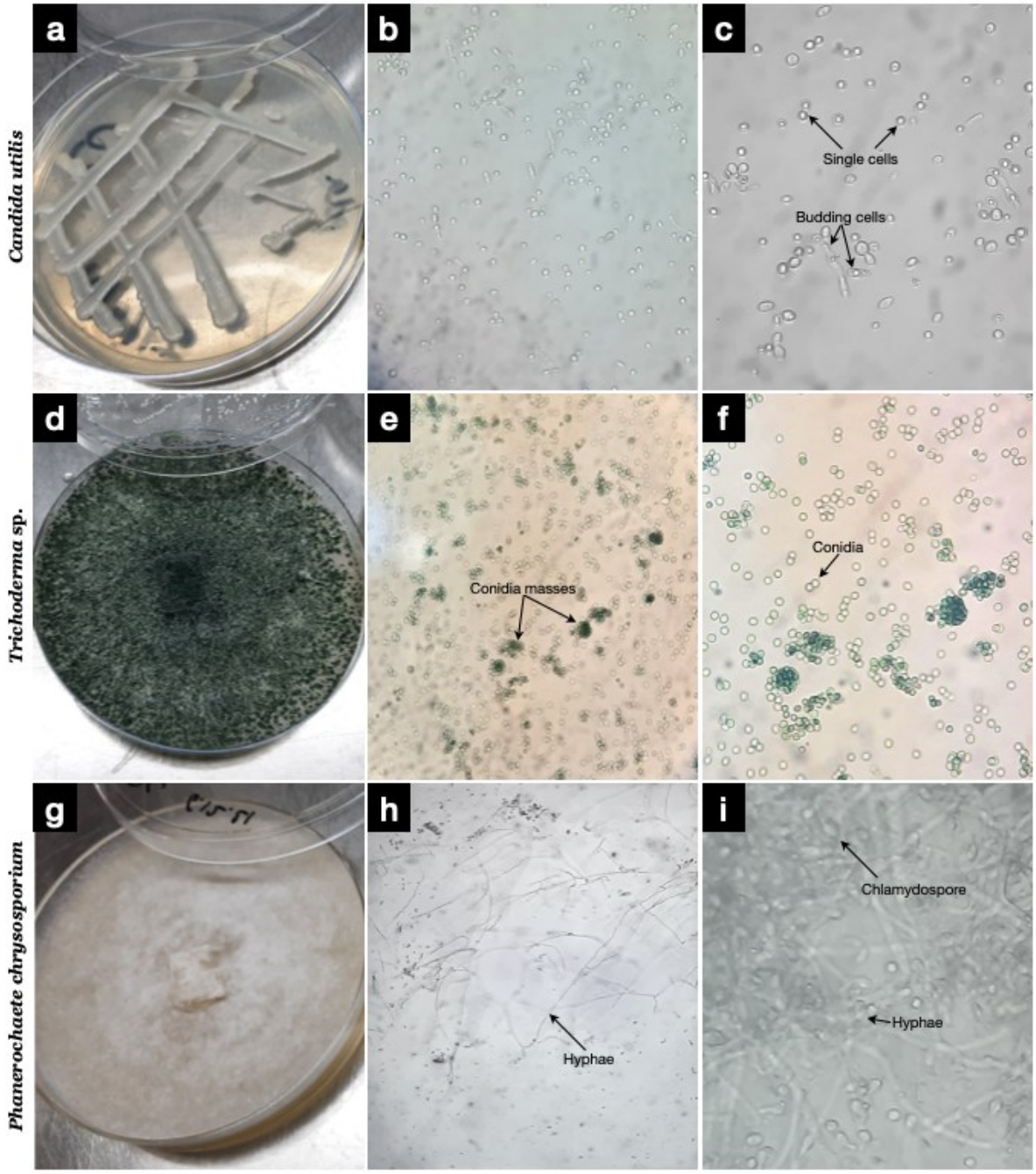

Figure 1. Macroscopic and microscopic features of yeast cells of *Candida utilis*: (a) Colony appearance on PDA plate, (b) magnification ×10, (c) magnification ×40; *Trichoderma* sp.: (d) Colony appearance on PDA plate, (e) magnification ×10, (f) Conidia magnification ×40; and *Phanerochaete chrysosporium*: (g) Colony appearance on PDA plate, (h) magnification ×10, (i) magnification ×40.

3.2. Microorganisms Growth on the Substrates

Three distinct microorganisms and two substrate ratios were used to produce coagulants, with the aim of determining the optimal growth conditions using the tray method. Microbial cultures on the rice bran after different days (3, 4, and 5 days) are shown in Figure 2. Within 5 days, the microorganisms grew and colonized the entire surface of the substrates, as observed. The color of the surface substrates changed over these three days due to an increase in intensity

caused by microorganism growth. Moreover, the microorganism's growth on the substrate prepared by Substrate 2 (Figure 3) was much higher than that on Substrate 1 (Figure 2).

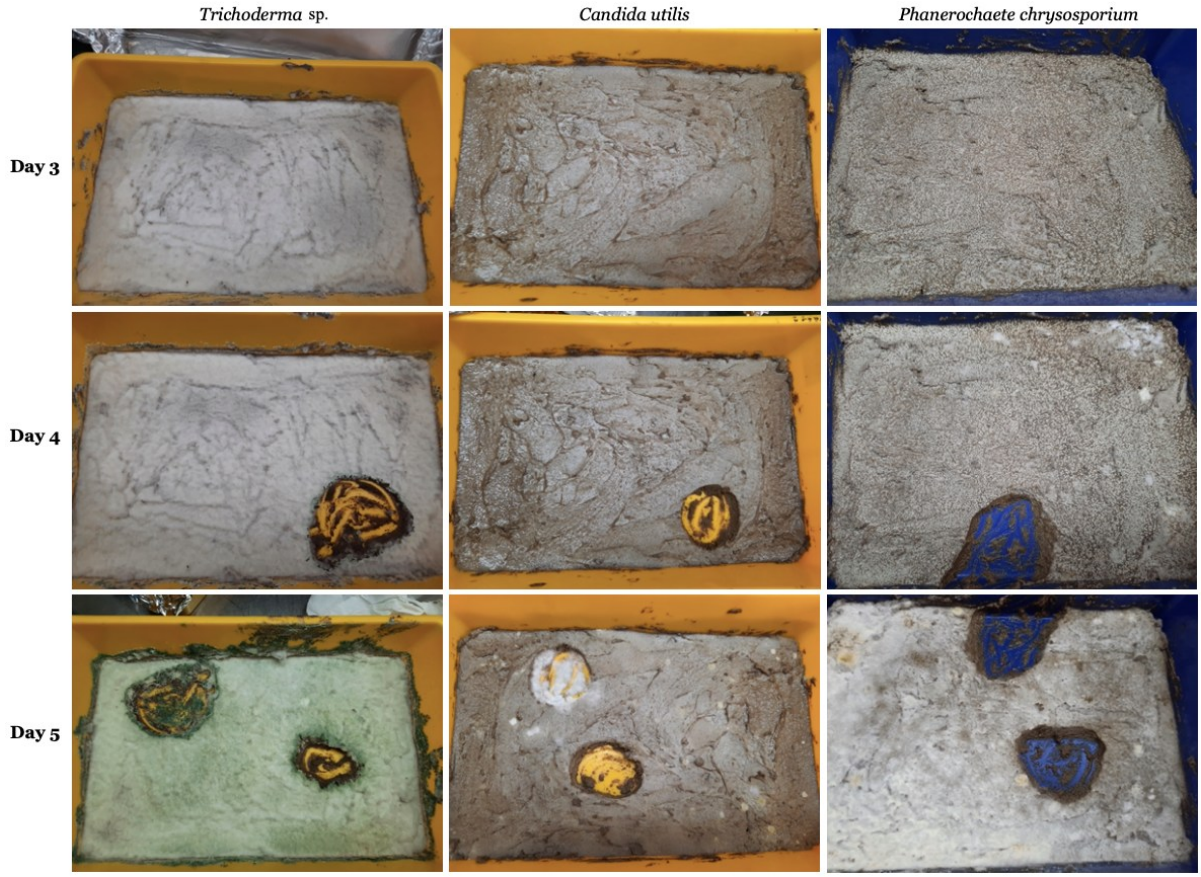

Figure 2. Microbe cultures on substrate 1 after different days (3, 4, and 5 days).

The composition of the substrate influences the concentration of extracted bio-coagulants. Substrates prepared with 83.25 mL of media and 83.25 g of rice bran yielded higher concentrations of bio-coagulants than those prepared with 124.87 mL of media and 41.62 g of rice bran. This difference is attributed to the higher water content present in substrates containing 124.87 mL of media and 41.62 g of rice bran. Excess water content beyond the saturation level that the solid substrate can absorb would reduce interparticle air space and impede oxygen transfer into the SSF [22]. Moisture is generally required for fungi to thrive. However, an oversaturated environment can hinder the life cycle of certain fungi. Rintaro Hattori et al. (2014) reported that excess water inhibits fungal spore germination and hyphal growth [23]. It can be seen that growth on the substrate surface is usually higher due to increased oxygen availability [24].

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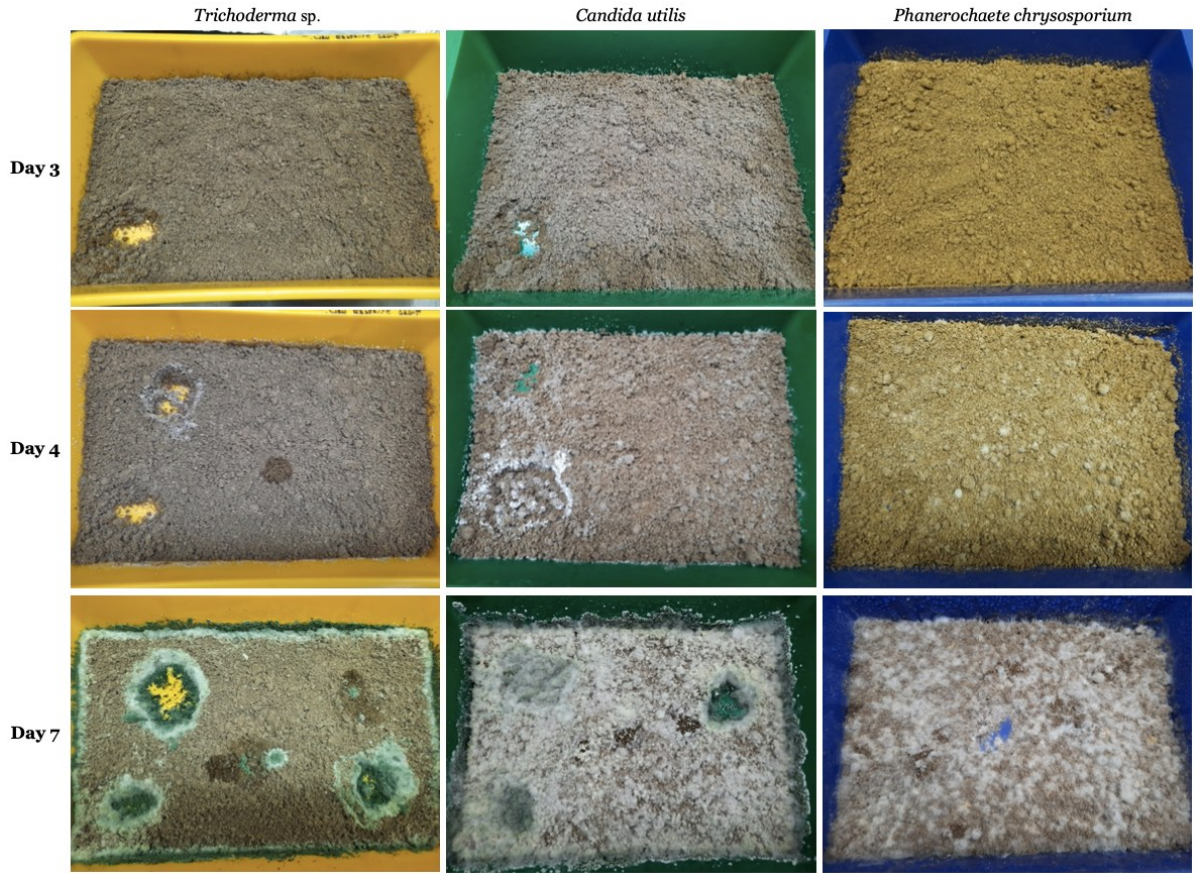

Figure 3. Microbe cultures on Substrate 2 after different days (3, 4, and 5 days).

3.3. Assessment of Turbidity Removal

Jar testing, the primary technique for assessing coagulant efficiency, was employed to evaluate the coagulants produced by three microorganisms in terms of turbidity reduction through flocculation of the kaolin suspension.

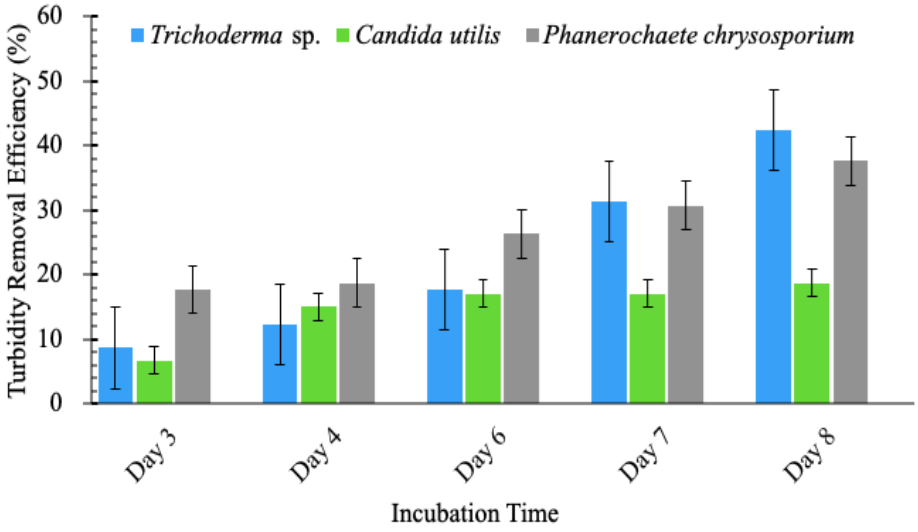

Figure 4. Turbidity Removal (%) from Kaolin Suspension (Substrate 1).

Figures 4 and 5 illustrate the turbidity removal efficiency for different coagulants produced by various microorganisms. Figure 4 presents the turbidity removal of natural coagulants

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produced on Substrate 1. Generally, it can be seen that turbidity removal increased progressively each day (from day 3 to day 8), for all produced coagulants. All coagulants achieved their highest turbidity removal on day 8. The turbidity removal by all coagulants produced from *Trichoderma* sp., *C. utilis*, and *P. chrysosporium* achieved their highest turbidity removal of 42%, 19%, and 32%, respectively, on day 8. The most effective coagulant produced in Substrate 1 is produced by *Trichoderma* sp.

From Figure 4, one-way ANOVA gives p =0.283 (p>0.05). The results show no statistically significant differences in turbidity removal efficiency among the three microbes. Tukey HSD shows no pairwise differences: *Trichoderma* sp. and *C. utilis* (p=0.550), *Trichoderma* sp. and *P. chrysosporium* (p=0.832), *C. utilis* and *P. chrysosporium* (p=0.263). All strains perform comparably for bio-coagulant screening.

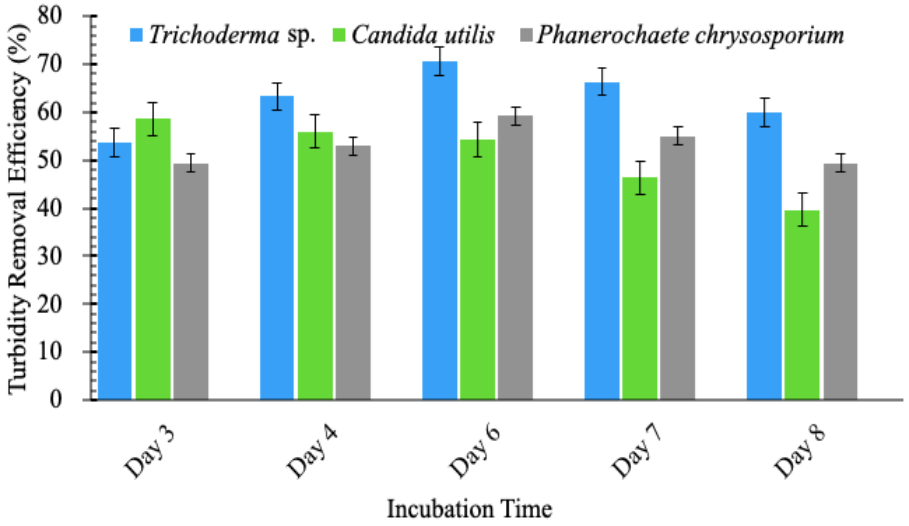

Figure 5. Turbidity Removal (%) from Kaolin Suspension (Substrate 2).

The turbidity removal of natural coagulants produced on Substrate 2 is shown in Figure 5. The findings demonstrated that the turbidity removal of *C. utilis* coagulant increased over the first three days, and this coagulant reached the maximum removal percentage of 59% on day 3. In contrast, turbidity removal by coagulants produced from Trichoderma sp. and *P. chrysosporium* continued to increase until day 6, reaching 71% and 59%, respectively, before declining to 26% and 16% by day 8, respectively. These findings are consistent with Nedjai *et al.* [17], who observed peak myco-coagulant activity on day 6. Dharsana & Prakash (2023) reported that, for a modified banana peel bio-coagulant, approximately 71% turbidity removal was obtained at the highest tested dose (around 10 wt.%) [25]. *C. utilis* and *P. chrysosporium* coagulants achieve moderate turbidity removal (~60%), comparable with several plant-derived and fungal bio-coagulants, such as orange-peel powder (61.63%) [26] and *Aspergillus niger* (60.22%) [27].

Comparing the turbidity removal results of the produced coagulants on two substrates, Substrate 1 showed the lowest turbidity removal for the three microbes. *Trichoderma* sp. performed best on Substrate 2 (71% max), followed by *P. chrysosporium* and *C. utilis* (both 59%). Subsequently, solid-state fermentation with low water content is more advantageous than that with high water content for producing greater numbers of microorganisms, thereby yielding a high concentration of coagulants. This is due to water being a limiting factor for fungal growth in SSF [19,28]. On the other hand, oxygen is usually the major growth-limiting factor in aerobic fermentations of *C. utilis* yeast [29] because oxygen has low solubility in

water [30,31]. Therefore, increasing the water content in the solid substrate reduces yeast growth, thereby producing a low concentration of coagulants.

Coagulation activity declined after several days of microbial growth (day 3 for *C. utilis*; day 6 for fungi; Figure 5), potentially due to dosage plateaus or excess coagulant-restabilizing particles [17]. Alternatively, microbial enzymes may degrade bio-coagulants [32]. Relevant studies indicate that increasing the amount of coagulants/flocculants yields negligible improvements, or even reductions, in turbidity removal efficiency. Excess coagulant fails to interact effectively with suspended particles; instead, it adds additional colloidal solids, intensifying turbidity [17]. Another hypothesis posits that the coagulation activity post-peak might be due to bio-coagulant-degrading enzymes produced by microorganisms [32]. The three microbes exhibited distinct peak turbidity removal times (*C. utilis* on day 3*, Trichoderma* sp*., and P. chrysosporium* on day 6), reflecting species-specific differences in growth and metabolic kinetics and indicating that the production-degradation balance of their bio-coagulants is optimal at different cultivation ages.

All microbes produce extracellular polymeric substances (EPS) that act as bio-coagulants. *C. utilis* mainly produces glucomannan-type EPS [33]. *P. chrysosporium* secretes both soluble EPS (SEPS) and bound EPS (BEPS), with BEPS characterized by a high polysaccharide content [34]. *Trichoderma* sp. similarly synthesizes extracellular polysaccharides that contribute to its biocoagulant activity [35]. The substrate composition, using rice bran supplemented with glucose and malt extract, significantly influences the EPS composition produced by each microbe (monosaccharide profile, protein/polysaccharide ratio, functional groups). The differing EPS compositions of the three microbes help explain the performance differences in turbidity removal observed in Figures 4 and 5.

Trichoderm sp., a versatile lignocellulolytic fungus, likely generated diverse anionic EPS enriched with carboxyl-rich glycoproteins due to glucose induction and rice bran lignocellulosic components, thereby bridging for maximum turbidity removal [36]. *P. chrysosporium*, a lignin specialist, produced polysaccharide-heavy EPS primarily from lignocellulose degradation, supporting moderate performance through adsorption but limited bridging [37]. Strain *P. chrysosporium* excels at capturing individual particles (adsorption) but lacks the long-chain glycoprotein network required for inter-particle bridging, as *Trichoderma* sp. provides. Strain *C. utilis*, as a yeast relying on simple carbon metabolism, yielded mannan-dominant EPS [33], which favored adhesion over effective flocculation, resulting in lower removal efficiency. Thus, the lignocellulosic substrate (rice bran), combined with glucose and malt extract, likely enriched Trichoderma EPS with carboxyl-rich glycoproteins, thereby enhancing its coagulation superiority over the others.

Overall, turbidity reduction differed significantly among the three microbes on the measured days (Substrate 2), as indicated by a one-way ANOVA ($p=0.026 < 0.05$). Strain *Trichoderma* sp. exhibits the strongest turbidity removal (mean 62.8), evidencing its ideal ability to produce a bio-coagulant that can be used for turbid water. Strain *C. utilis* lags notably (mean 51.0), possibly due to slower metabolic rates, while *P. Chrysosporium* remains intermediate and stable (std 4.2). Tukey HSD reveals a significant difference in turbidity removal between *Trichoderma* sp. and *C. utilis* (mean diff -11.8, $p=0.030$), but not between *Trichoderma* sp. and *P. chrysosporium* ($p=0.073$) or *C. utilis* and *P. chrysosporium* ($p=0.872$).

An independent *t*-test was also conducted to compare turbidity removal efficiency between two microbial substrates (Substrate 1 mean 20.6 vs. Substrate 2 mean 55.6), revealing a statistically significant difference ($t = 10.60$, $p < 0.001$). These results confirm that the growth

conditions in Substrate 2 substantially enhanced the turbidity removal performance of the three microbes.

Our findings thus provide the first evidence that these *Trichoderma* sp. and *C. utilis* can achieve 71% and 59% turbidity removal, respectively, expanding the portfolio of microbial candidates for eco-friendly coagulation.

3.4. Scanning Electron Microscopy (SEM)

A morphological structure of microorganisms formed on rice bran was observed using scanning electron microscopy. The SEM micrographs of microorganisms are presented in Figure 6. After growth on the rice bran substrate for seven days at 30ºC, the microorganisms colonized the substrate surface.

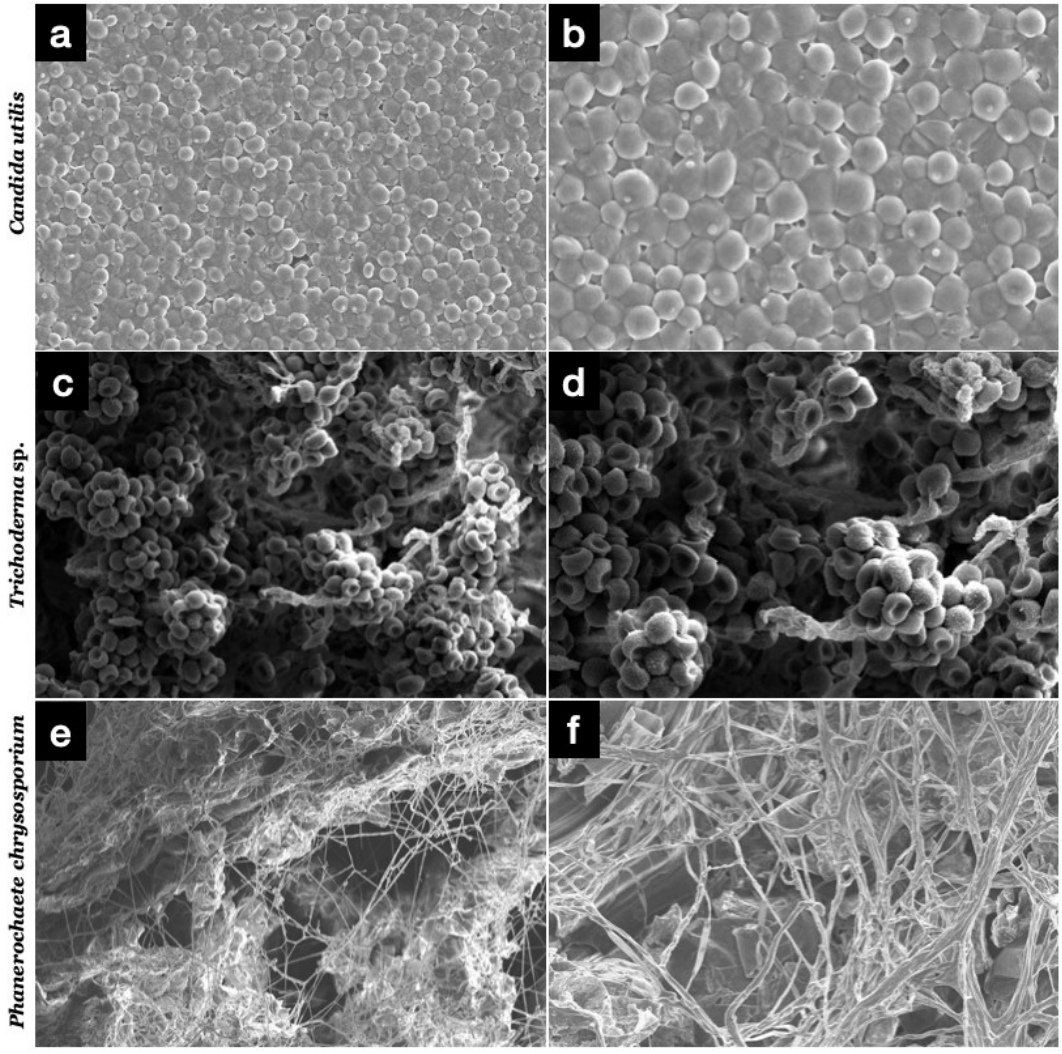

Figure 6. Scanning Electron Micrographs of Microbe Colonies on Rice Bran (a) *Candida utilis* [(a)×1000, (b)×2000], *Trichoderma* sp. [(c)×2000, (d)×3000], and *Phanerochaete chrysosporium* [(e)×180, (f)×450].

SEM examination showed that the rice bran substrate was completely colonized by *Candida utilis* yeast cells (Figure 6(a)). The shape of *Candida utilis* cells varies from circular to ellipsoidal/oval, and their length ranges from 3 to 6 µm (Figure 6(b)). Furthermore, some polar budding was observed, as shown in Figure 6(b).

Figure 6 (c) and 6 (d) show conidial balls developed from terminal phialides. The *Trichoderma* sp. conidial surface morphology appeared smooth when observed in SEM at a magnification of ×2000 (Figure 6 (c)) and in optical microscopy (Figure 1e, 1f), whereas it was more distinctly roughened and verrucose when observed in SEM at a magnification of ×3000 (Figure 6 (d)).

SEM also displayed *P. chrysosporium* growth on the surface of the rice bran substrate. The fungal mycelium of *P. chrysosporium* was filamentous and interconnected to form a spatial network. Rice bran substrate was colonized and penetrated by *P. chrysosporium*, as presented in Figure 6 (e). The hyphae of *P. chrysosporium* may be seen clearly in Figure 6 (f). Also, some spores were seen at the hyphae's terminal.

4. CONCLUSION

By introducing *Trichoderma sp*. and *C. utilis* as new fungal biocoagulants, this work opens a promising avenue for developing sustainable, microbe-based coagulation strategies in water treatment. Solid-state fermentation of *Trichoderma* sp. on the rice bran substrate proved particularly promising for producing effective natural coagulants. The results also showed that *Trichoderma* sp. was more effective at removing turbidity than the other coagulants (*Candida utilis* and *Phanerochaete chrysosporium*). Furthermore, solid-state fermentation with low water content is more advantageous than that with high water content for producing greater numbers of microorganisms, thereby yielding a high concentration of coagulants. SEM micrographs indicate that *Candida utilis*, *Trichoderma* sp., and *Phanerochaete chrysosporium* effectively colonized the solid substrate. In this study, biocoagulants, rice bran (as an abundant raw material), and tray-based solid-state fermentation (SSF) were employed, collectively reducing operational costs and dependence on expensive and complex extraction procedures.

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