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Optimization and Scale-Up of Solid-State Bioconversion for Microbial Coagulant Production toward Sustainable Water Treatment

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ABSTRACT: Microbial coagulants are emerging as an eco-friendly alternative to chemical coagulants owing to their non-toxicity, biodegradability, and sustainability. However, their use is limited by high production costs linked to expensive nutrient media and scalability issues. To address these challenges, this study developed a cost-effective microbial coagulant based on a fungus using cocopeat as a substrate. Growth conditions (malt extract, glucose, and pH) were optimized using the Face-Centered Central Composite Design (FCCCD) under Response Surface Methodology (RSM) to maximize coagulant yield. The performance in water treatment was evaluated using jar tests, and large-scale production techniques were investigated using a tray bioreactor and an agitated drum bioreactor (ADB). The fungal strain used in this study was identified as *Phanerochaete concrescens*, which demonstrated high turbidity-reducing efficiency, achieving a maximum flocculating activity of 93.06% in a standard jar test. These results were obtained under statistically optimized growth conditions, specifically 5 days of incubation at 25°C, 70% moisture content, 3% malt extract, 2.5% glucose, and pH 7, confirming model predictions. *Phanerochaete concrescens* was successfully scaled using a tray fermenter, achieving a flocculation rate of 92% after 5 days of incubation at 70% moisture, pH 7, and a 3 cm substrate thickness. However, in a 30 L agitated bioreactor drum, flocculating activity was recorded at 29% due to insufficient fungal growth at the tested agitation rate. This study confirms the feasibility of a cost-effective microbial coagulant that can be scaled appropriately, offering a sustainable and biodegradable alternative to chemical coagulants.

ABSTRAK: Koagulan mikrob muncul sebagai alternatif mesra alam pada koagulan kimia kerana sifatnya yang tidak toksik, terbiodegradasi, dan mampan. Walau bagaimanapun, penggunaannya terhad kerana kos pengeluaran tinggi disebabkan oleh media nutrien yang mahal dan isu kebolehskalaan. Bagi menangani cabaran ini, kajian ini membangunkan koagulan mikrob kos efektif berdasarkan kulapuk kokopit sebagai substrat. Keadaan pertumbuhan (ekstrak malt, glukosa, dan pH) dioptimumkan menggunakan Reka Bentuk Komposit Berpusat Muka (FCCCD) di bawah Kaedah Gerak Balas Permukaan (RSM) bagi memaksimum hasil koagulan. Prestasi dalam rawatan air dinilai menggunakan ujian balang, dan teknik pengeluaran skala besar dikaji menggunakan bioreaktor dulang dan bioreaktor dram bergoncang (ADB). Strain kulat yang digunakan dalam kajian ini dikenal pasti sebagai Phanerochaete concrescens, menunjukkan kecekapan tinggi dalam mengurangkan kekeruhan,

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mencapai aktiviti pengflokulan maksimum 93.06% melalui ujian piawai balang. Keputusan ini diperoleh di bawah keadaan pertumbuhan yang optimum secara statistik, iaitu 5 hari pengeraman pada suhu 25°C, kandungan lembapan 70%, ekstrak malt 3%, glukosa 2.5%, dan pH 7, mengesahkan model ramalan. Phanerochaete concrescens berjaya diskala menggunakan penapai dulang, dengan kadar pengflokulan 92% dicapai selepas 5 hari pengeraman di bawah lembapan 70%, pH 7, dan ketebalan substrat 3 cm. Walau bagaimanapun, dalam dram bioreaktor bergoncang 30 L, aktiviti pengflokulan direkodkan pada 29% disebabkan oleh pertumbuhan kulat yang tidak mencukupi pada kadar goncangan yang diuji. Kajian ini mengesahkan kebolehlaksanaan koagulan mikrob kos efektif pada skala wajar, menawarkan alternatif mampan dan terbiodegradasi pada koagulan kimia.

KEYWORDS: *microbial coagulant, response surface methodology, solid-state bioconversion, scale-up, water treatment.*

1. INTRODUCTION

Water is vital for human survival and ecological balance, underscoring the global necessity of universal access to clean, safe water. However, population growth, industrialization, and water scarcity due to climate change pose substantial challenges to water resources, underscoring the need for innovative and sustainable water treatment technologies [1, 2]. Several technologies have been employed in water treatment, ranging from conventional methods such as sedimentation and filtration to advanced techniques such as reverse osmosis, ozonation, and membrane filtration [3, 4, 5, 6]. Among these, the coagulation-flocculation process is valued for its simplicity, cost-effectiveness, and efficiency in water treatment, removing organic matter, suspended solids, and heavy metals [7]. The coagulation-flocculation process involves adding coagulants to neutralize the surface charges of suspended particles, facilitating their aggregation into larger flocs that can be removed by sedimentation, thereby enhancing water clarity and quality [8,9,10]. Coagulants can be categorized into chemical coagulants (inorganic, such as aluminum sulfate and ferric chloride), synthetic organic coagulants (such as polyacrylamide derivatives), and natural coagulants (plant, animal, and microbial origin).

Water treatment has traditionally used chemical coagulants, both inorganic (such as polyaluminum chloride and ferric chloride) and organic (such as polyacrylamide). Despite their widespread use, they pose significant environmental and health risks due to low biodegradability and potential toxicity, as well as concerns regarding cost and local availability [11,12]. Consequently, there has been an increasing trend towards seeking safer, sustainable alternatives that can effectively demonstrate coagulation-flocculation performance while minimizing environmental impact and health risks [13]. Natural coagulants are organic, non-toxic substances sourced from plants (e.g., starch, guar gum, Moringa oleifera), animals (e.g., shellfish extracts, chitosan), and microorganisms (e.g., bacteria, yeast, fungi, algae). They are regarded as sustainable and can be locally available. Within this broad category, microbial coagulants are emerging as a promising sustainable alternative to chemical coagulants, largely due to their rapid reproducibility, biodegradability, environmental friendliness, and effectiveness across applications such as heavy metal removal, dye wastewater treatment, drinking water purification, and microalgae harvesting [14, 15]. Microbial coagulants produce a wide range of biomolecules, including proteins, glycoproteins, polysaccharides, and extracellular polymeric substances (EPS), which effectively remove suspended particles, organic matter, and contaminants [16, 17].

Most research on microbial coagulants is largely limited to laboratory studies, highlighting a considerable challenge for large-scale industrial production and application. The main obstacle hindering this advancement is the high production cost. To reduce production and application costs, various strategies have been implemented, including exploring low-cost alternative media. Solid-state bioconversion is identified as a cost-effective alternative that reduces overall resource demands by employing low-cost agro-industrial residues, enhancing growth conditions, and using affordable lignocellulosic substrates, relying on specific microorganisms to convert these solid substrates into high-value products [18,19]. Among microorganisms, filamentous fungi are well-suited for solid-state bioconversion due to their strong enzymatic activity and distinctive hyphal growth, which facilitate effective penetration and nutrient assimilation in solid matrices, thereby enhancing substrate conversion efficiency and yield [20, 21, 22].

In the present study, a filamentous fungus was used to produce an effective microbial coagulant via solid-state bioconversion of cocopeat, a natural, low-cost agricultural by-product, with the aim of optimizing growth conditions to maximize microbial coagulant yield and turbidity removal efficiency at the laboratory scale [23,24]. The optimization employed Face-Centered Central Composite Design under response surface methodology to assess the key variables influencing microbial coagulant production, including malt extract, glucose, and pH. Additionally, these optimized parameters were applied in the first reported scale-up of this process, utilizing several bioreactor configurations, including a tray bioreactor and an agitated bioreactor drum (ABD). This study demonstrates that using agricultural residues and low-nutrient cultivation methods can substantially lower production costs without compromising effectiveness. Furthermore, this sustainable and eco-friendly approach shows strong potential for industrial-scale up, thereby enhancing the commercial feasibility of microbial coagulant production for water treatment.

2. Materials and Methods

2.1. Microorganism collection

The present study employed a fungus as a microorganism that was obtained from the Biotechnology Laboratory of the International Islamic University of Malaysia (IIUM). The strain was preserved through successive subcultures on potato dextrose agar (PDA) and maintained on agar at 4°C.

2.2 Molecular identification of microbial coagulant-producing fungal strain

DNA samples were extracted from a one-week-old PDA pure culture of a fungal strain for molecular identification. The extraction, PCR amplification of ITS, purification, and sequencing were carried out by Apical Scientific MBS Laboratories in Malaysia. Genomic DNA was sequenced using the 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, and 28S rDNA gene. These sequences were then compared to related sequences using BLAST in GenBank, leading to the construction of a phylogenetic tree via pairwise alignments and the neighbor-joining method.

2.3. Preparation of inoculum (mycelial suspension)

In this study, the inoculum was prepared by harvesting fungal cultures after 7 days of incubation, washing them with 20 ml of sterile distilled water to create a uniform mycelial suspension for solid-state bioconversion, ensuring consistency in coagulant production.

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2.4. Substrate collection and preparation

In this study, cocopeat, sourced from the International Islamic University Malaysia, was processed into a fine powder, sieved, and stored for fermentation. This method utilizes a cost-effective agricultural byproduct, enhancing sustainability and reducing material costs.

2.5. Experimental design

In this study, the production of the desired microbial coagulant was systematically optimized. Growth conditions were designed and analyzed using a Face-Centered Central Composite Design (FCCCD) within Response Surface Methodology (RSM) via Design Expert® 7.0.0. The total number of samples examined under RSM was 20 experimental runs, each analyzed in triplicate, including six center points, as shown in Table 1.

Table 1. Response surface design (FCCCD) of three independent parameters

Run	Malt extract [% w/w]	pH	Glucose [% w/w]
1	2.00	4.00	1.00
2	4.00	4.00	1.00
3	2.00	8.00	1.00
4	4.00	8.00	1.00
5	2.00	4.00	3.00
6	4.00	4.00	3.00
7	2.00	8.00	3.00
8	4.00	8.00	3.00
9	2.00	6.00	2.00
10	4.00	6.00	2.00
11	3.00	4.00	2.00
12	3.00	8.00	2.00
13	3.00	6.00	1.00
14	3.00	6.00	3.00
15	3.00	6.00	2.00
16	3.00	6.00	2.00
17	3.00	6.00	2.00
18	3.00	6.00	2.00
19	3.00	6.00	2.00
20	3.00	6.00	2.00

The flocculating activity rate (%) was selected as the design experiment's response (Y). For the variable assessments, the independent factors were adjusted at three levels: low (−1), medium (0), and high (+1). The parameters employed were malt extract concentration (2-4 % w/w), pH (4-8), and glucose concentration (1-3 % w/w). The relationship between the flocculation activity rate and independent factors was modeled using a second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 \quad (1)$$

where Y indicates the flocculation activity rate, while X_1, X_2, and X_3 stand for malt extract, pH, and glucose, respectively. The coefficients include β_0 (intercept), β_1, β_2, and β_3 (linear coefficients), β_{12}, β_{13}, and β_{23} (interaction coefficients), and β_{11}, β_{22}, and β_{33} (quadratic coefficients). The optimal conditions were determined using analysis of variance (ANOVA) and contour plots, with model adequacy evaluated by the coefficient of determination (R^2) to assess goodness of fit [25]. For each experimental run, a microbial coagulant was produced via

solid-state bioconversion by moistening 3 g of cocopeat with 8 mL of a variable-composition production medium. This medium's malt extract, glucose concentrations, and pH were adjusted for each run as per the experimental design matrix. The medium was sterilized at 120°C for 15 minutes, inoculated, and then incubated at 25°C for five days.

2.6. Extraction process of microbial coagulant

In this study, a modified method from [26] was used to extract the produced microbial coagulant after the incubation period. The procedure for obtaining the crude extract involved adding 2 g of the fermented substrate and 20 ml of pH 7.0 buffer, shaking at 250 rpm for 1 hour at 30°C, and then filtering.

2.7. Jar procedure for microbial coagulant performance assessment

The jar test method using an SW6 UK Flocculator was used to assess the effectiveness of the produced microbial coagulant in reducing turbidity. This procedure involved adding 10 mL of the extracted microbial coagulant to a 300 mL sample. The sample was rapidly mixed for 7 minutes at 250 rpm. This was followed by a slow mixing process for 22 minutes at 90 rpm. The mixture was then left to settle for 1 hour. The turbidity of the treated water and raw water samples was determined using a turbidity meter, Model 2100Q. The rate of flocculating activity was determined from kaolin suspension tests using the method proposed by [27], where A is the initial turbidity (NTU) and B is the final turbidity (NTU). All tests were performed in triplicate.

$$Flocculating\ activity\ rate\ (\%) = \frac{A-B}{A} \times 100 \quad (2)$$

2.8. Experimental validation

Design-Expert software was used to optimize experiments based on the parameter values. Manual adjustments were made based on the equations derived from the RSM simulation to maximize the flocculating activity rate (%), as shown in Table 2. A comparison of predicted values and experimental results demonstrated the model's accuracy in predicting flocculation activity rates (%) using percentage error analysis.

Table 1 . Validation for the FCCCD model

Run	Malt extract [% w/w]	pH	Glucose [% w/w]
1	4	8	3
2	3	7	2.5
3	3	7.5	2

2.9. Scale-up procedure of microbial coagulant

For the production of microbial coagulant, two types of bioreactors, namely a tray bioreactor (Figure 1(a) and a 30 L agitated drum bioreactor (Figure 1(b), have been used to carry out the scale-up process for large-scale production through solid-state bioconversion.

2.9.1. Microbial coagulant scale-up in Tray Bioreactor (TB)

The first scaled-up process for the target microbial coagulant involved using a tray bioreactor, where a sterilized cocopeat substrate mixture was added and incubated for 5 days at 25°C. Some of the critical factors that are part of the process of scaling up this coagulant, such as the moisture content (60-80%) of the substrate bed, the substrate bed thickness (1-3

cm), and the pH level of the medium (7-9), were also tested to assess their effect on the coagulant produced.

2.9.2. Microbial coagulant scale-up in Rotary Drum Bioreactor (RDB)

For the large-scale production of the desired microbial coagulant through solid-state bioconversion, the process was carried out using a 30 L rotary drum bioreactor with 10 kg of sterilized cocopeat substrate at 70% moisture level, and the stirring process was performed for 2 hours a day over 5 days using the optimized growth condition at laboratory scale. Daily measurement of coagulant efficiency was performed using a jar test.

Figure 1. Scale-up procedure of microbial coagulant, a) tray foil, b) rotatory drum bioreactor.

3. RESULTS AND DISCUSSIONS

3.1. Molecular identification of fungal strain

The potential fungal strain used in this study was identified as belonging to the genus *Phanerochaete concrescens.* Molecular identification based on the rRNA operon gene (18S rRNA, ITS1 spacer, 5.8S rRNA, ITS2 spacer, and 28S rRNA) sequences showed the highest similarity to *Phanerochaete concrescens*. Sequence similarity analysis of the fungal rRNA operons was conducted using 10 related strains of *Phanerochaete concrescens* from GenBank. The isolated filamentous strain exhibited 100% sequence identity with the type strain of *Phanerochaete concrescens*. Morphological and phylogenetic analyses confirmed the strain's identification as *Phanerochaete concrescens*. A phylogenetic tree was constructed to compare this strain with similar sequences available in GenBank (Fig 2). The tree was generated from rRNA operon gene sequences using BLAST pairwise alignments and the neighbor-joining method, resulting in an unrooted tree. However, to the best of our knowledge, this fungus has not been reported as a microbial coagulant-producer. These findings identify a new fungal strain capable of producing microbial coagulants, highlighting its potential for eco-friendly coagulation and flocculation applications.

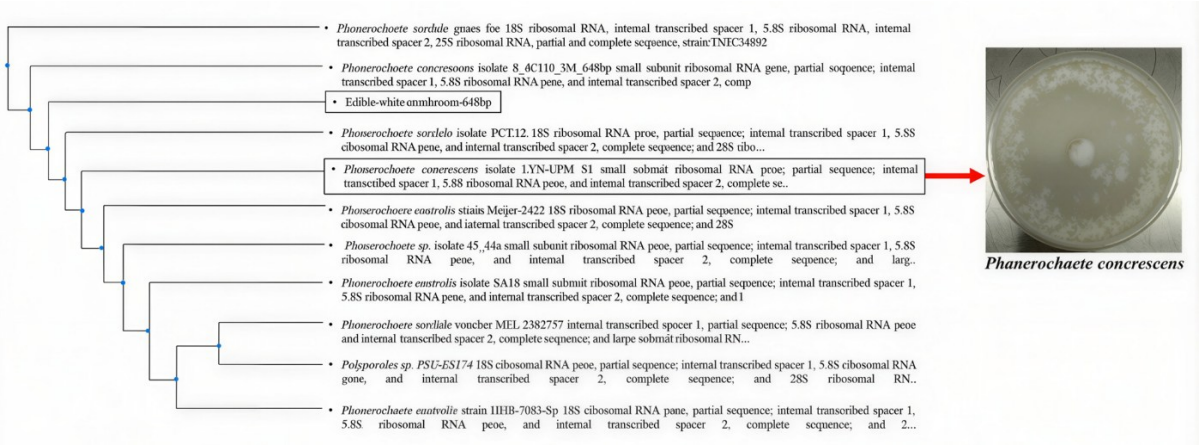

Figure 2. A phylogenetic tree based on 16 rRNA gene sequences of strain *Phanerochaete concrescens*.

3.2. Optimization of growth conditions for efficient microbial coagulant production

Solid-state fermentation using cocopeat as a lignocellulosic substrate is an effective method for producing a microbial coagulant and offers a sustainable alternative to traditional submerged fermentation [23, 24]. In the current research, response surface methodology (RSM) was employed to optimize microbial coagulant production and assess its efficacy in turbidity removal, thereby revealing its potential as a sustainable water treatment technology. The Face-Centered Central Composite Design (FCCCD) was employed in the RSM approach to effectively explore nonlinear variable relationships among the variables, thereby enabling the optimization of factors that enhanced microbial coagulant yield and flocculating activity. Three critical independent variables, including malt extract concentration, pH, and glucose concentration, were selected based on their significant influence on the growth and metabolite production of the fungal strain responsible for coagulant production [23, 24].

As shown in Table 3, the flocculating activity rate varied significantly across experimental runs, ranging from 79.00% (run 2) to 93.03% (run 8), indicating the sensitivity of the fungal strain's metabolism to both nutrient composition and environmental pH. The range also indicates that, although the fungal strain exhibited the lowest flocculating activity at 79% under unfavorable conditions, the experiment underscores the importance of precision in controlling environmental conditions. Conversely, the highest percentage of flocculating activity, at 93.03%, underscores the effectiveness of the synergism between the optimized nutrient composition and the pH achieved during the experiment. Additionally, the repetition of activity levels at the central points of the experiment validated its reliability.

The optimization of experimental conditions was performed to enhance the flocculation activity rate (%), improve statistical interactions between factors, and reduce experimental costs. A second-order polynomial model was used to describe the empirical relationship between the dependent variable (flocculation activity rate (%)) and the independent variables: malt extract (A), pH (B), and glucose (C). The resulting regression equation was

$$Y(Flocculation\ activity\ rate, \%) = +90.45 - 1.04A + 1.19B + 1.77C - 1.06A^2 - 0.93B^2 - 0.59C^2 + 1.92AB - 2.29AC - 0.97BC \quad (3)$$

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Table 2. FCCCD experimental design and the response

Run	Malt extract [% w/w]	pH	Glucose [% w/w]	Flocculation activity rate (%)	
				Experimental	Predicted
1	2.00	4.00	1.00	89.36	89.19
2	4.00	4.00	1.00	79.00	78.68
3	2.00	8.00	1.00	89.19	89.68
4	4.00	8.00	1.00	86.83	86.85
5	2.00	4.00	3.00	90.07	90.10
6	4.00	4.00	3.00	89.19	88.75
7	2.00	8.00	3.00	86.33	86.70
8	4.00	8.00	3.00	92.80	93.03
9	2.00	6.00	2.00	91.14	90.43
10	4.00	6.00	2.00	87.83	88.34
11	3.00	4.00	2.00	87.43	88.33
12	3.00	8.00	2.00	91.81	90.71
13	3.00	6.00	1.00	88.10	88.09
14	3.00	6.00	3.00	91.82	91.63
15	3.00	6.00	2.00	91.00	90.45
16	3.00	6.00	2.00	89.50	90.45
17	3.00	6.00	2.00	88.24	90.45
18	3.00	6.00	2.00	90.76	90.45
19	3.00	6.00	2.00	91.23	90.45
20	3.00	6.00	2.00	91.54	90.45

Table 3. ANOVA of the developed model

Source	Sum of Squares	Mean Square	*F*-Value	*p*-Value
Model	160.00	17.78	15.40	< 0.0001
A-malt extract	10.90	10.90	9.44	0.0118
B-pH	14.18	14.18	12.29	0.0057
C-glucose	31.44	31.44	27.23	0.0004
AB	29.45	29.45	25.51	0.0005
AC	41.91	41.91	36.30	0.0001
BC	7.59	7.59	6.57	0.0282
A^2	3.10	3.10	2.68	0.1324
B^2	2.36	2.36	2.04	0.1833
C^2	0.95	0.95	0.82	0.3867
Lack of Fit	3.59	0.72	0.45	0.7981

R^2 = 0.9327, **Adjusted R^2** = 0.8721, **CV** = 1.21, **Adequate precision** = 18.879

The analysis of variance (ANOVA) of the developed model, summarized in Table 4, was employed to assess the adequacy and statistical validity of the proposed regression model. The F-value and corresponding p-value are key indicators for evaluating both the overall model significance and the interactions among the independent variables. The model exhibited an F-value of 15.40 with a p-value < 0.0001, indicating that the probability of such a large F-value arising from random noise is less than 0.01%, thereby confirming the model's statistical significance. According to standard statistical criteria, model terms with p-values < 0.05 are considered significant, whereas values > 0.1 indicate insignificance. As shown in Table 4, the linear terms malt extract (A), pH (B), and glucose (C) exerted significant effects on flocculating activity ($p < 0.05$). In addition, the interaction terms AB, AC, and BC were statistically significant, indicating strong synergistic interactions among the studied factors. In contrast, the quadratic terms A^2, B^2, and C^2 were not significant ($p > 0.1$), suggesting that pronounced curvature effects were not present within the investigated experimental domain. The lack-of-

fit test was non-significant (p = 0.7981), indicating good agreement between the experimental and predicted responses and confirming the model's suitability. The model's efficiency and predictive accuracy were further supported by the high coefficient of determination (R^2 = 0.9327) and adjusted R^2 (0.8721), indicating that 93.27% and 87.21% of the total variation in flocculating activity were explained by the model, respectively. The adequate precision ratio, which measures the signal-to-noise ratio, was 18.879, well above the desirable threshold of 4, indicating an adequate and reliable signal. Moreover, the low coefficient of variation (CV = 1.21%) reflects high experimental precision and reproducibility. Commonly, a model is deemed reasonably reproducible 79if its coefficient of variation (CV) is less than 10% [28].

3.3. Analysis of the response surfaces model

To further elucidate the interactions among medium components and their concentrations in optimizing microbial coagulant production, response surface methodology was employed. The relationships between variables were visualized using three-dimensional (3D) response surface plots and two-dimensional (2D) contour plots. The elliptical contour patterns observed in these plots confirmed the presence of significant interactions among the tested parameters, consistent with the ANOVA results.

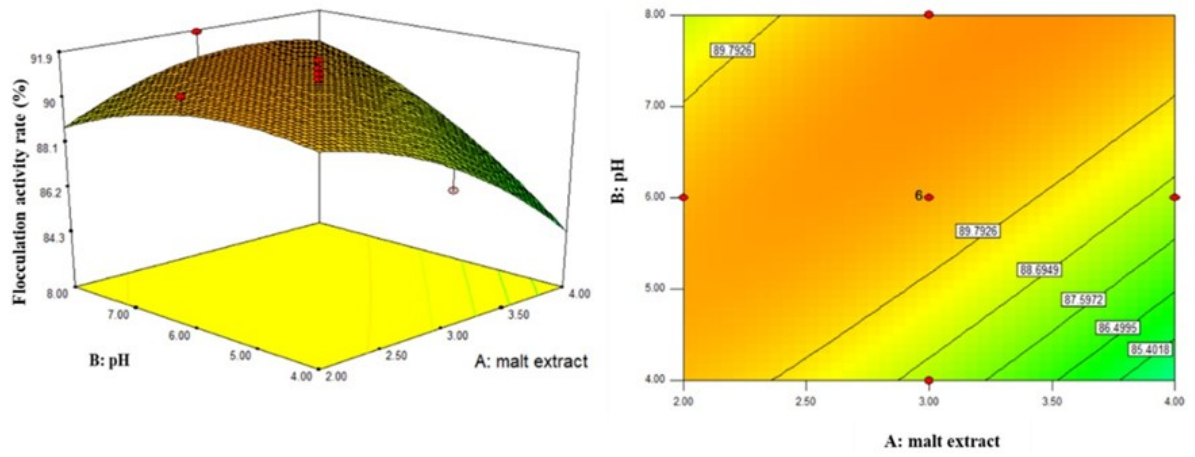

Figure 3. 3D and contour plots representing the effect of malt extract and pH on flocculation activity.

Figure 3 illustrates the interaction effect of pH and malt extract concentration on the flocculation activity rate (%), providing insight into the optimal conditions for achieving maximum flocculation efficiency. As shown in Figure 1, it is clear that the flocculation activity rate is significantly affected by both pH and malt extract concentration. The maximum flocculation activity of about 91.9% was achieved at a moderate pH range (6.0–7.5) and an optimal malt extract concentration of 2.5–3.5. The increase in pH was found to improve the flocculation activity, which can be attributed to enhanced charge neutralization and polymer bridging mechanisms. These results are in agreement with the study by [29], whereby *Aspergillus flavus* MCB 271 and *Aspergillus niger* MCBF 08 produced a bioflocculant optimally at pH 7. However, excessive malt extract concentrations reduced flocculation efficiency, which may be attributed to overdosing that hinders effective particle aggregation. The interaction between pH and malt extract concentration is crucial for determining the flocculation response, underscoring the importance of optimizing both factors simultaneously. Statistical analysis confirmed that the interaction between pH and malt extract significantly affected the flocculation rate, as indicated by the corresponding p-values presented in Table 4. These results emphasize the synergistic influence of pH and malt extract on flocculation

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performance and validate the response surface model as an effective tool for optimizing process conditions.

Figure 4 presents the interactive effect of malt extract and glucose on the flocculation activity rate. The highest response values, reaching approximately 90.6%, were observed within the orange region of the response surface, indicating the optimal concentration range for both factors. In contrast, further increases in either glucose or malt extract beyond this optimal range resulted in a noticeable decline in flocculation activity, as illustrated by the green region. These findings demonstrate that both malt extract and glucose play a crucial role in modulating the flocculation response, likely through their influence on microbial growth, metabolic activity, and extracellular polymer production. The existence of an optimal concentration range suggests that a balanced nutrient supply is essential for achieving maximum flocculation efficiency. At ideal concentrations, malt extract and glucose provide sufficient carbon and energy sources to support microbial metabolism without inducing substrate inhibition or metabolic imbalance. Conversely, excessive nutrient levels may disrupt microbial physiology or interfere with floc formation, thereby reducing flocculation performance. The findings were consistent with other studies, which reported that glucose is the most preferred carbon source [30]. However, other studies have shown that peptone is the preferred source of nitrogen [29].

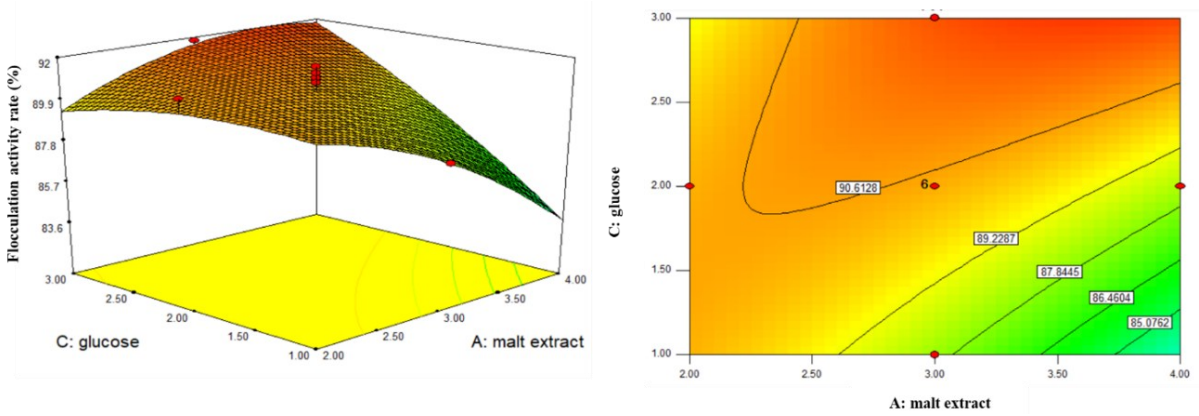

Figure 4. 3D and contour plots representing the effect of malt extract and glucose on flocculation activity.

Figure 5 shows the relationship between pH and glucose (m/m) concentration on the flocculation activity rate. The three-dimensional response surface displays a clear parabolic shape, confirming a quadratic relationship between these variables and the response. Both pH and glucose concentration positively influence flocculation activity, with the response increasing as these factors rise, up to an optimal point beyond which no further gains occur. The parabolic pattern between pH and glucose indicates that carbon flux through biosynthetic pathways depends heavily on enzyme stability within a narrow pH range, with slightly alkaline conditions promoting extracellular enzyme secretion and microbial coagulant formation [20]. This explains the decline in flocculation efficiency at extreme pH values, where enzymatic activity and metabolic balance may be disrupted. Additionally, the curvature of the response surface emphasizes the importance of maintaining optimal pH–glucose conditions to avoid substrate inhibition and metabolic stress. The close match between experimental data points and the predicted surface further confirms the good fit, accuracy, and predictive power of the developed model, demonstrating the effectiveness of response surface methodology for optimizing flocculation performance.

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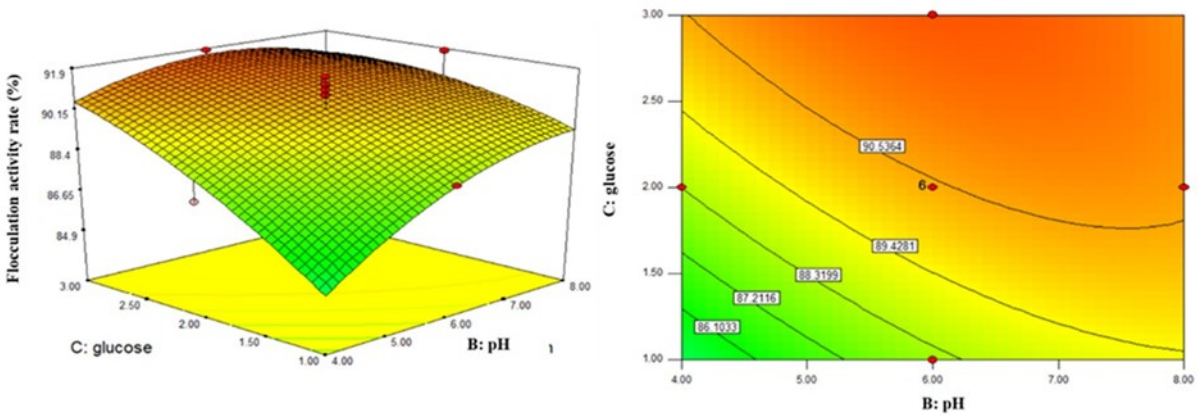

Figure 5. 3D and contour plots representing the effect of glucose and pH on flocculation activity.

3.4. Validation of the optimized parameter

Model validation evaluated the reliability of optimization outcomes by comparing predicted and experimental flocculating activity values. The Design Expert software's point prediction function assessed model accuracy, while high and low levels of key factors were manually adjusted to refine predictions of optimal conditions.

Additional extraction of the desired microbial coagulant was performed to validate the generated model and confirm the best results, as presented in Table 5. The optimized condition, obtained at 3% malt extract, 2.5% glucose, and pH 7, yielded a maximum flocculating activity of 93.06%, significantly reducing water turbidity from 672 to 46.63. However, in Run 3, a lower flocculating activity resulted in only a 60.55 NTU reduction. The experimental values were compared with the predicted values based on a standard error below 10%, confirming high predictive accuracy. The fact that these findings were so closely related proved that the response model accurately represented the predicted optimization.

Table 4. Validation of the experimental model

Run	Malt extract	pH	Glucose	Flocculation activity (%)		Error (%)
				Predicted	Experimental	
1	4	8	3	92.02	91.14	0.88
2	3	7	2.5	91.30	93.06	1.76
3	3	7.5	2	90.81	90.99	0.18

3.5. Large production of microbial coagulant

Significant progress has been made in researching microbial coagulants; however, their scaling up is still under development. In this context, the current study is conducted to enhance the production of microbial coagulant through solid-state bioconversion using different bioreactor types, including tray fermenters and agitated drum bioreactors, thereby providing an environmentally friendly solution for large-scale water treatment.

3.5.1. Large production of microbial coagulant in a tray fermenter

Tray fermenters are used economically for solid-state bioconversion, in which tray covers hold the substrates and inoculum for growth. The factors examined in this study included pH, moisture content, and substrate thickness, and a 5-day incubation at 25°C was used for coagulant-scale production by Phanerochaete concrescens.

3.5.1.1. Effect of pH value on flocculating activity rate (%) in a tray fermenter

One of the most influential factors is the medium's pH, which significantly affects the production of the microbial coagulant during solid-state bioconversion [31]. Thus, the effect of medium pH was evaluated over the range 6 to 9, as shown in Figure 6. Based on the obtained results, the highest flocculating activity was observed at 91.5% at pH 7 after 5 days of incubation. The flocculating activity decreased at acidic pH, while the moisture content (70 %) and agitation (3 cm) were considered. The optimal pH for bioflocculant production by A. niger was 7, as reported by [27, 32].

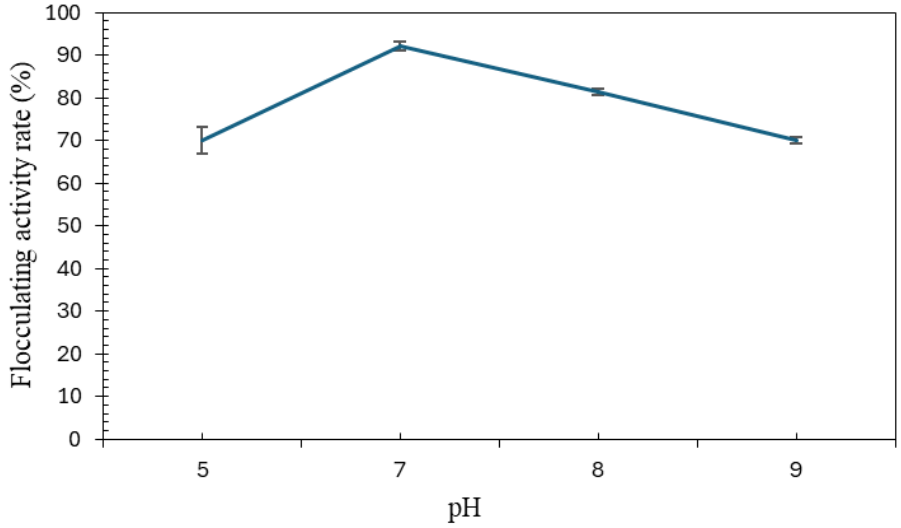

Figure 6. Effect of pH on flocculating activity rate (%) in a tray fermenter.

3.5.1.2. Effect of moisture content on flocculating activity rate (%) in a tray fermenter

For microbial coagulant production, moisture content is a crucial factor [33]. Thus, different moisture contents in the fermentation media were evaluated to assess the optimal level that supports better growth of the used fungal strain and, hence, better substrate utilization, resulting in maximum microbial coagulant production.

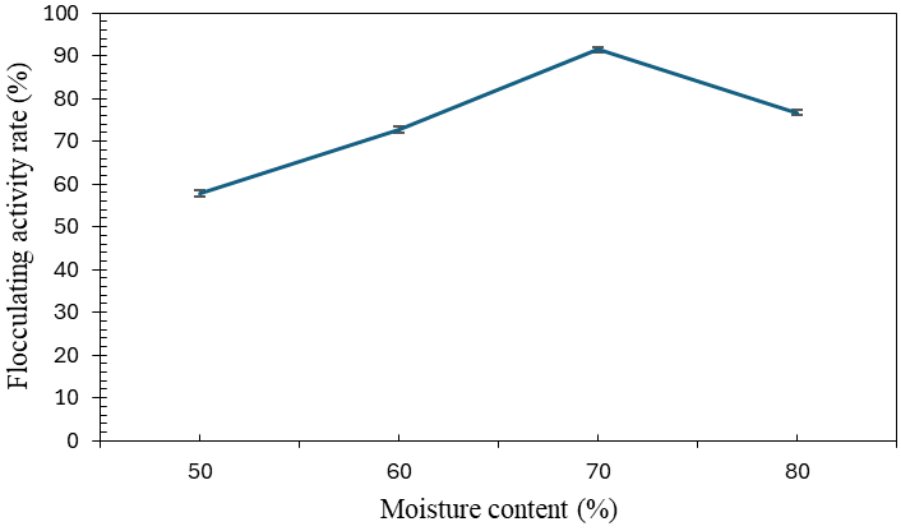

Figure 7. Effect of moisture content on flocculating activity rate (%) in the tray fermenter.

As indicated by the result presented in Figure 7, the highest flocculating activity of 91.5% was found at a moisture content of 70%, whereas the lowest flocculating activity rate of 54.5%

was noted at a moisture content of 50%. It was justified that *Phanerochaete concrescens*. required a considerable amount of medium moisture. This could be explained by the fact that the coco-peat substrate holds water. The moisture content facilitates proper substrate swelling, thereby providing microorganisms with an opportunity to use it effectively.

3.5.1.3. Effect of thickness of substrate on flocculating activity rate (%) in a tray fermenter

In this study, the thickness of the substrate layer was examined to assess its impact on the production of the target microbial coagulant during the solid-state bioconversion process. A layer thickness of 3 cm was found optimal, yielding a maximum microbial production of 92%. A layer thickness of 3 cm was found to be optimal for maximum microbial coagulant output. Substrate thicknesses of 1 and 2 cm resulted in lower flocculating activity rates of 60% and 71.2%, respectively (Figure 8). This could be due to lower thickness not supporting the full growth of fungal mycelia, resulting in reduced production yield. Conversely, increasing the layer thickness can hinder microbial respiration and aeration, leading to poor growth. Additionally, a thicker substrate can reduce product diffusion, potentially lowering enzyme activity due to feedback inhibition [34].

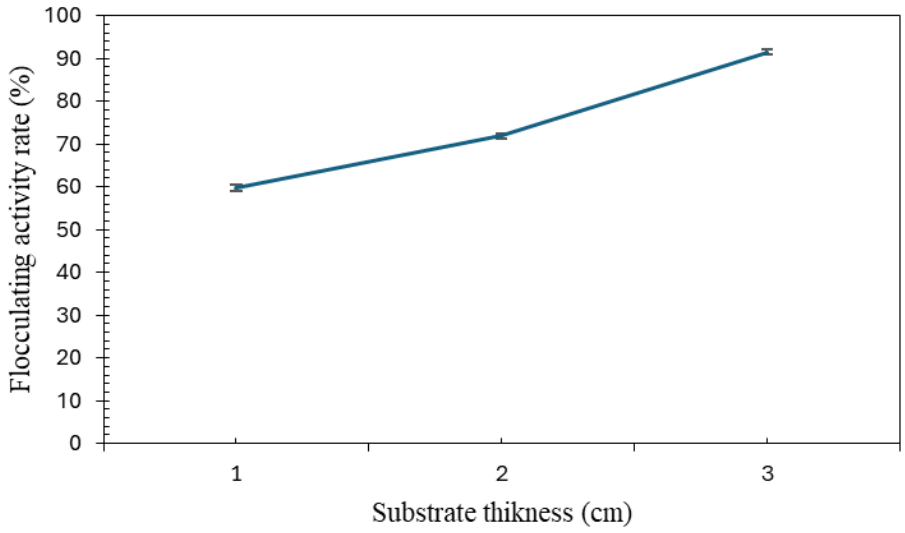

Figure 8. Effect of substrate thickness on flocculating activity rate (%) in the tray fermenter.

3.5.2. Large-scale production of microbial coagulant in an agitated drum bioreactor

Further research is underway to improve the design of the solid-state bioconversion bioreactor for producing the microbial coagulant, which is effective at removing water turbidity. However, scaling this experiment from the laboratory to industrial scale has been a significant challenge, with no prior attempt to increase production of this particular microbial coagulant. Therefore, this study is considered the first attempt to scale up the production of a microbial laccase using a 30 L agitated drum bioreactor. Scale-up production in a 30 L agitated drum bioreactor did not yield the desired microbial coagulant. Based on the results in Figure 9, flocculating activity ranged from 22 to 29 % over 7 days of incubation. It can be observed that within the first three days of incubation, the fungus grows in the substrate with daily mixing for 1 hour. However, no growth was observed on the fourth day of incubation. Therefore, it is clear that the stirring process negatively affected the growth of the fungus as the hyphae were damaged.

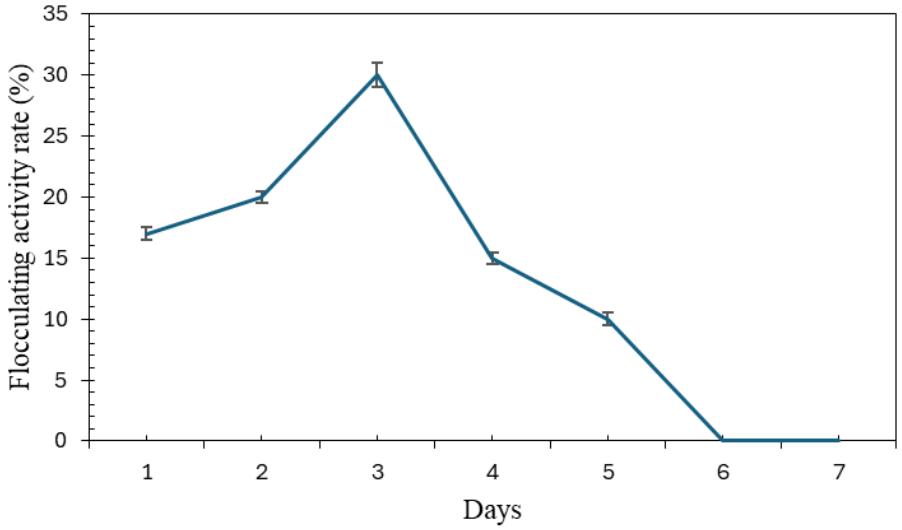

Figure 9. Flocculating activity rate (%) in an agitated drum bioreactor during 7 days.

4. CONCLUSIONS

In conclusion, the study presents a cost-effective method for producing microbial coagulants from cocopeat through solid-state bioconversion. Optimal conditions for large-scale production were established as 3% malt extract, 2.5% glucose, pH 7, 70% moisture, and 25°C for 5 days, resulting in 93.06% flocculating activity. A comparison of production methods revealed that the tray fermenter maintained 92% activity under these conditions, while the agitated drum bioreactor showed only 29% effectiveness. This approach uses agro-industrial waste and optimized fermentation to produce microbial coagulants for water treatment, providing a sustainable and cost-effective approach.

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REFERENCES

[1] Gizewski J., van der Sande L., Holtmann D. (2023) Contribution of electrobiotechnology to sustainable development goals. Trends Biotechnol. 41(9):1106–1108. https: //doi.org/10.1016/j.tibtech.2023.02.009

[2] Qu J., Liu H., Liu G. (2022) Science and technology for combating global water challenges. Engineering 9:1–2. https://doi.org/10.1016/j.eng.2022.01.007

[3] Chakraborty R., Asthana A., Singh A.K., Jain B., Susan A.B.H. (2022) Adsorption of heavy metal ions by various low-cost adsorbents: a review. Int. J. Environ. Anal. Chem. 102(2):342–379. https://doi.org/10.1080/03067319.2020.1722811

[4] Tan X., Wei H., Zhou Y., Zhang C., Ho S.-H. (2022) Adsorption of sulfamethoxazole via biochar: the key role of characteristic components derived from different growth stage of microalgae. Environ. Res. 210:11296. https://doi.org/10.1016/j.envres.2022.112965

[5] Elmoutez S.S., Abushaban A., Necibi M.C., Sillanp M., Liu J., Dhiba D., Chehbouni A., Taky M. (2023) Design and operational aspects of anaerobic membrane bioreactor for efficient

wastewater treatment and biogas production. Environ. Challenges 10:100671. https://doi.org/10.1016/j.envc.2022.100671

[6] Lanzetta A., Papirio S., Oliva A., Cesaro A., Pucci L., Capasso E.M., Esposito G., Pirozzi F. (2023) Ozonation processes for color removal from urban and leather tanning wastewater. Water 15(13):2362. https://doi.org/10.3390/w15132362

[7] Ang W.L., Mohammad A.W. (2020) State of the art and sustainability of natural coagulants in water and wastewater treatment. J. Clean. Prod. 262:121267. https://doi.org/10.1016/j.jclepro.2020.121267

[8] Renault F., Sancey B., Badot P.M., Crini G. (2009) Chitosan for coagulation/flocculation processes an eco-friendly approach. Eur. Polym. J. 45(5):1337–1348. https://doi.org/10.1016/j.eurpolymj.2008.12.027

[9] El-Gaayda J., Titchou F.E., Oukhrib R., Yap P.-S., Liu T., Hamdani M., Ait Akbour R. (2021) Natural flocculants for the treatment of wastewaters containing dyes or heavy metals: A state-of-the-art review. J. Environ. Chem. Eng. 9(5):106060. https://doi.org/10.1016/j.jece.2021.106060

[10] Metin S., ifi D.. (2023) Chemical industry wastewater treatment by coagulation combined with Fenton and photo-Fenton processes. J. Chem. Technol. Biotechnol. 98(5):1158–1165. https://doi.org/10.1002/jctb.7321

[11] Ahmad A, Abdullah S.R.S., Hasan H.A., Othman A.R., Ismail N.I. (2021) Plant-based versus metal-based coagulants in aquaculture wastewater treatment: Effect of mass ratio and settling time. J. Water Process Eng. 43:102269. https://doi.org/10.1016/j.jwpe.2021.102269

[12] Kurniawan S.B., Abdullah S.R.S., Imron M.F., Said N.S.M., Ismail N., Hasan H.A., Othman A.R., Purwanti I.F. (2020) Challenges and opportunities of biocoagulant/bioflocculant application for drinking water and wastewater treatment and its potential for sludge recovery. Int. J. Environ. Res. Public Health 17(24):9312. https://doi.org/10.3390/ijerph17249312

[13] Gautam S., Saini G. (2020) Use of natural coagulants for industrial wastewater treatment.Glob. J. Environ. Sci. Manag. 6(4):553–578. https://doi.org/10.22034/gjesm.2020.04.10

[14] Ayangbenro A.S., Babalola O.O., Aremu O.S. (2019) Bioflocculant production and heavy metal sorption by metal resistant bacterial isolates from gold mining soil. Chemosphere 231:113–120. https://doi.org/10.1016/j.chemosphere.2019.05.092

[15] Pu L., Zeng Y.J., Xu P., Li F.Z., Zong M.H., Yang J.G., Lou W.Y. (2020) Using a novel polysaccharide BM2 produced by Bacillus megaterium strain PL8 as an efficient bioflocculant for wastewater treatment. Int. J. Biol. Macromol. 162:374–384. https://doi.org/10.1016/j.ijbiomac.2020.06.167

[16] Giri S.S., Harshiny M., Sen S.S. (2015) Production and characterization of a thermostable bioflocculant from Bacillus subtilis F9, isolated from wastewater sludge. Ecotoxicol. Environ. Saf. 121:45–50. https://doi.org/10.1016/j.ecoenv.2015.06.010

[17] Sivasankar P., Poongodi S., Lobo A.O., Pugazhendhi A. (2020) Characterization of a novel polymeric bioflocculant from marine actinobacterium Streptomyces sp. And its application in recovery of microalgae. Int. Biodeterior. Biodegrad. 148:104883. https://doi.org/10.1016/j.ibiod.2020.104883

[18] Yafetto L. (2022) Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. Heliyon 8(3). https://doi.org/10.1016/j.heliyon.2022.e09173

[19] Selo G., Planini M., Tima M., Tomas S., Koceva Komleni D., Buci-Koji A. (2021) A comprehensive review on valorization of agro-food industrial residues by solid-state fermentation. Foods 10(5):927. https://doi.org/10.3390/foods10050927

[20] Farinas C.S. (2015) Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. Renewable Sustainable Energy Rev. 52:179–188. https://doi.org/10.1016/j.rser.2015.07.092

[21] Prado-Barragn L.A., Figueroa J.J.B., Rodrguez Durn L.V., Aguilar Gonzlez C.N., Hennigs C. (2016) Fermentative production methods. In: Biotransformation of Agricultural Waste and By-Products, pp. 189–217. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-803622-8.00007-0

[22] Guo J., Yang C., Peng L. (2014) Optimization of microbial flocculant production by response surface methodology. Bioresour. Technol. 152:490–498. http://10.1007/s12088-016-0631-3

[23] Fellah M., Alam M.Z., Al-Mamun A., Khabbashi N.A., Engliman N.S., Arab S.H. (2023) Effect of the lignocellulolytic substrates and fermentation process parameters on microbial coagulant production for water treatment. IIUM Eng. J. 24(1):13–26. https://doi.org/10.31436/iiumej.v24i1.2400

[24] Fellah M., Alam M., Al-Mamun A., Kabbashi N., Engliman N. (2021) Evaluation of solid-state production of microbial coagulant using various lignocellulosic media to reduce water turbidity. IOP Conf. Ser.: Mater. Sci. Eng. 1192(1):012022. https://doi.org/10.1088/1757-899X/1192/1/012022

[25] Maran J.P., Manikandan S., Nivetha C.V., Dinesh R. (2017) Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design. Arabian J. Chem. 10:1145–1157. https://doi.org/10.1016/j.arabjc.2013.02.007.\

[26] Zulkeflee Z., Snchez A. (2014) Solid-state fermentation of soybean residues for bioflocculant production in a pilot-scale bioreactor system. Water Sci. Technol.70(6):1032–1039. https://doi.org/10.2166/wst.2014.329

[27] Aljuboori, A. H. R., Uemura, Y., Osman, N. B., & Yusup, S. (2014). Production of a bioflocculant from Aspergillus niger using palm oil mill effluent as carbon source. Bioresource technology, 171,66-70. http://dx.doi.org/10.1016/j.biortech.2014.08.038.

[28] Ma, C. H., Liu, T. T., Yang, L., Zu, Y. G., Chen, X., Zhang, L., ... & Zhao, C. (2011). Ionic liquid-based microwave-assisted extraction of essential oil and biphenyl cyclooctene lignans from Schisandra chinensis Baill fruits. Journal of Chromatography a, 1218(48), 8573-8580. https://doi.org/10.3390/ijms131114294

[29] David, O.; Oluwole, O.; Ayodele, O.; Lasisi, T. Characterisation of fungal bioflocculants and its application in water treatment. Curr. J. Appl. Sci. Technol. 2019, 34, 1 9. http://10.9734/cjast/2019/v34i630159

[30] Tsilo, P. H., Basson, A. K., Ntombela, Z. G., Maliehe, T. S., & Pullabhotla, R. V. (2021). Isolation and optimization of culture conditions of a bioflocculant-producing fungi from Kombucha tea SCOBY. Microbiology Research, 12(4), 950-966. https://doi.org/10.3390/microbiolres12040070

[31] HASSAN, H. B., ALAM, M. Z., Al Mamun, A. B. D. U. L. L. A. H., & MUJELI, M. (2021). Screening of Nutrients for The Production of Microbial coagulant from Lentinus Squarrosulus for Water Treatment. Evaluation, 1, 12.

[32] Pu, S., Ma, H., Deng, D., Xue, S., Zhu, R., Zhou, Y., & Xiong, X. (2018). Isolation, identification, and characterization of an Aspergillus niger bioflocculant-producing strain using potato starch wastewater as nutrilite and its application. PloS one, 13(1), e0190236. https://doi.org/10.1371/journal.pone.0190236

[33] Ramachandran, S., Patel, A. K., Nampoothiri, K. M., Francis, F., Nagy, V., Szakacs, G., & Pandey, A. (2004). Coconut oil cake—a potential raw material for the production of α-amylase. Bioresource technology, 93(2), 169-174. https://10.1016/j.biortech.2003.10.021

[34] Krishna, P. R., Srivastava, A. K., Ramaswamy, N. K., Suprasanna, P., & D'souza, S. F. (2012). Banana peel as substrate for alpha-amylase production using Aspergillus niger NCIM 616 and process optimization. Indian journal of biotechnology, 11(3), 314-319.