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EFFECT OF THE LIGNOCELLULOLYTIC SUBSTRATES AND FERMENTATION PROCESS PARAMETERS ON MYCO-COAGULANT PRODUCTION FOR WATER TREATMENT

MAROUA FELLAH[1], MD ZAHANGIR ALAM[1*], ABDULLAH AL MAMUN[2], NASSERLDEEN AHMED KHABBASHI[1], NURUL SAKINAH ENGLIMAN[1] AND SONIA HADJ ARAB[1]

[1]Department of Chemical Engineering and Sustainability,
[2]Department of Civil Engineering,
Kulliyyah of Engineering, International Islamic University Malaysia,
Jalan Gombak, 53100 Kuala Lumpur, Malaysia

[*]Corresponding author: zahangir@iium.edu.my

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ABSTRACT: In the present research, a fungal strain was used to produce a myco-coagulant via solid-state bioconversion to reduce water turbidity. The production of myco-coagulant was achieved using several low-cost lignocellulolytic substrates, namely coco peat, sawdust, palm kernel cake, and rice bran as sources of carbon and nitrogen. This research involves the study of both the effect of lignocellulolytic substrates and the parameters involved in the fermentation process for myco-coagulant production. Coco peat was chosen as a suitable lignocellulolytic substrate to serve as a carbon source for producing myco-coagulant, potentially reducing the turbidity by 84.6% from the kaolin suspension. Sawdust, palm kernel cake, and rice bran showed 33.06%, 30.18, and 21.18 %, respectively. Furthermore, a statistical approach to the Plackett-Burman design was conducted to evaluate the significant parameters that affect the production of myco-coagulant. Eleven fermentation process parameters were selected: concentration of coco peat (2- 4 %), incubation time (5-9 days), temperature (25-35 °C), pH (5-9), glucose (0-2 %), malt extract (1-2 %), yeast extract (0-2%), wheat flour (0-2 %), ammonium sulfate (0-1 %), inoculum size (1-3 %) and potassium dihydrogen phosphate (0-0.5 %). The selected variables were assessed through statistical analysis (main effects) based on their significance. Based on the main effect of each variable on flocculation activity, three variables, namely glucose, malt extract, and pH influenced high levels. On the other hand, the remaining eight variables did not significantly affect the production of myco-coagulant. Furthermore, a deeper study was conducted to further optimize the three effective variables involved in the fermentation process to evaluate these factors' influence on flocculation activity.

ABSTRAK: Penyelidikan ini adalah berkenaan strain fungus yang digunakan bagi menghasilkan miko-koagulan melalui penukaran-bio berkeadaan pepejal bagi mengurangkan kekeruhan air. Miko-koagulan dihasilkan dengan menggunakan beberapa substrat lignoselulolitik berkos rendah, iaitu habuk kelapa, habuk papan, hampas kelapa sawit, dan dedak padi sebagai sumber karbon dan nitrogen. Penyelidikan ini mengkaji kesan substrat lignoselulolitik dan faktor-faktor yang terlibat dalam proses fermentasi bagi menghasilkan miko-koagulan. Habuk kelapa dipilih sebagai substrat lignoselulolitik yang sesuai berfungsi sebagai sumber karbon dalam menghasilkan miko-koagulan, berpotensi mengurangkan kekeruhan sebanyak 84.6% daripada ampaian kaolin. Sebaliknya, habuk papan, hampas kelapa sawit, dan dedak padi menunjukkan 33.06%, 30.18, dan 21.18 %, masing-masing. Tambahan pula, pendekatan statistik ke atas reka bentuk Plackett-Burman telah dijalankan bagi menilai parameter penting yang mempengaruhi penghasilan miko-koagulan. Sebelas

parameter proses penapaian telah dipilih: kepekatan habuk kelapa (2- 4 %), masa pengeraman (5-9 hari), suhu (25-35 C), pH (5-9), glukosa (0-2 %), ekstrak malt (1-2), tepung gandum (0-2 %), ammonium sulfat (0-1%), saiz inokulum (1-3 %) dan Kalium dihidrogen fosfat (0-0.5 %). Pemboleh ubah yang dipilih dinilai melalui analisis statistik berdasarkan kepentingannya. Berdasarkan kesan utama setiap pemboleh ubah terhadap aktiviti penggumpalan, tiga pemboleh ubah ini adalah glukosa, ekstrak malt, dan pH yang memberi kesan tertinggi. Sebaliknya, lapan pemboleh ubah lain tidak mempengaruhi penghasilan miko-koagulan dengan ketara. Tambahan lagi, kajian yang lebih mendalam telah dijalankan bagi membaiki tiga pemboleh ubah utama yang terlibat dalam proses fermentasi bagi menilai kesan yang mempengaruhi aktiviti penggumpalan.

KEYWORDS: *Myco-coagulant, solid-state bioconversion, lignocellulolytic substrates, water treatment, turbidity removal, flocculation activity, PBD.*

1 INTRODUCTION

High turbidity and suspended solids (SS) are significant problems that affect rivers due to wastewater discharge, terrain conditions, land cover, rainfall, soil type, agriculture, stirred bottom sediments, algal blooms, and other development activities. These problems indicate the essential need to protect the aquatic environment and life by diminishing the turbidity and residual levels in the rivers using both conventional and advanced technologies in water: from treatment such as sedimentation and filtration to more complex methods, including ultrafiltration, ozonation, and reverse osmosis, to process raw water sources before supplying it to consumers and to ensure that the treated water can meet the effluent discharge requirement before it is discharged to water bodies [1,2].

The coagulation-flocculation process is considered one of the most straightforward approaches to accelerate the removal of suspended impurities in water efficiently. Even with advanced technologies, coagulation-flocculation remains one of the essential treatment processes for removing impurities (mainly suspended particles) in water treatment plants. This process requires adding components called coagulants/flocculants [3,4]. Coagulants/flocculants are commonly applied in many industrial processes, such as potable water purification and wastewater treatment. Generally, coagulants/flocculants are classified into three types: synthetic organic polymer flocculants like polyacrylamides, inorganic flocculants such as aluminium sulfate, iron sulfate, and iron chloride, and those that are naturally occurring, such as sodium alginate, chitosan, and microbial coagulants/flocculants [3,4]. Due to flocculating efficiency, chemical coagulants-flocculants are widely used in conventional water treatment processes [5,6,7]. However, using chemical coagulants/flocculants in water treatment has been limited due to several disadvantages [8,9]. As reported in many studies, its usage can cause several environmental problems and human health concerns [10,11,12].

Since the coagulants/flocculants play a significant role in the coagulation/flocculation process, developing highly efficient alternative coagulants/flocculants has always remained one of the most challenging research areas [13]. Biocoagulants/flocculants have piqued the interest of many researchers due to their numerous advantages: biodegradability, nontoxic properties, and potential as an alternative for conventional coagulants/flocculants [11,14]. Microbial coagulants/flocculants are extracellular biopolymers secreted by microorganisms such as bacteria, yeasts, fungi, and algae [2]. They contain mainly glycoproteins, polysaccharides, and proteins produced by microorganisms during active secretion [15]. However, the application of microbial coagulants/flocculants has been hindered by the

challenge of producing them on a large scale. Currently, researchers produce microbial coagulants/flocculants by applying synthetic media which contain simple sugar (glucose, sucrose, lactose, fructose, maltose), alcohols, and organic acid as carbon sources through the liquid-state fermentation method. Despite the potential applications of microbial coagulants/flocculants, the high production costs still limit their use.

Interestingly, new strategies to produce microbial coagulants/flocculants have been identified. The preference for SSF is more economically attractive due to the usage of low-cost materials such as agricultural and industrial byproducts via solid-state fermentation to produce bioactive compounds. SSF is of interest as an alternative to other conventional fermentation methods that are more costly and mainly chemically driven [16]. These bioprocessing technologies are devoted to developing cost-effective measures by utilizing inexpensive fermentation substrates to meet the current market demand for bioproducts [16], which may reduce production costs on industrial scales. Thus, an appropriate, inexpensive, and abundant substitute for these substrates, such as byproducts and agricultural residues, should be utilized to replace the conventional substrates to solve this problem. Solid-state fermentation may be better for producing a microbial coagulant/flocculant using filamentous fungi [17,18]. Thus, this research will investigate a coagulant-produced fungus called myco-coagulant via solid-state fermentation using agricultural waste as a low-cost substrate.

The present study aims to select the best lignocellulolytic substrates via a solid-state fermentation (SSF) process to produce an efficient myco-coagulant to reduce the turbidity of the water. It was also designed to study the impact of various fermentation process parameters, namely concentration of coco peat, incubation time, temperature, pH, glucose, malt extract, wheat flour, ammonium sulfate, inoculum size, and potassium dihydrogen phosphate (KH_2PO_4) on the production of coagulants from a fungal strain using a Placket-Burman experimental design.

2 MATERIALS AND METHODS

2.1 Microorganisms

The microorganism used in the present study was a fungal strain collected from a supermarket in Kuala Lumpur, Malaysia. The fungal strain was maintained on potato dextrose agar (PDA), stored in the chiller at 4º C, and kept at room temperature (30±2º C) for further use.

2.2 Substrate collection and preparation

In this study, different lignocellulolytic (LC) substrates, namely coco peat, sawdust, palm kernel cake, and rice bran, were collected from the International Islamic University Malaysia, Kuala Lumpur. The substrates were used as the supplementary media throughout this study, which were ground and milled into a fine powder using a domestic blender. The fine powders passed through a 50 mm-mesh sieve and were stored at room temperature for solid-state fermentation (SSF). These substrates may be seen as an inexpensive and abundant agricultural waste model, making the entire SSF process feasible and low-cost.

2.3 Inoculum preparation (Mycelium suspension)

The mycelial suspension was prepared by fully grown plates of fungi cultures from the incubator after seven days of incubation. A wriggled L-shaped rod was gently scraped off each plate and washed carefully with 20 ml of sterilized distilled water. The suspended mycelial suspension was used as the main inoculum.

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2.4 Solid-state bioconversion (SSB)

The solid-state bioconversion was conducted by moistening 3 g of each substrate, namely coco peat, sawdust, palm kernel cake, and rice bran, with 1 ml of fungal mycelial suspension and 7 ml of production media in a petri dish, which was sealed with a parafilm to avoid contamination with unwanted microbes. The medium consisted of 20 g of malt extract dissolved into 1000 ml of distilled water (2% w/v), and its initial pH was adjusted to 7 using 1 M NaOH or 2 M HCl. The production medium was autoclaved at 120º C for 15 minutes. The inoculated plates were incubated at 30º C for seven days. The major activities involved in the research are shown in Fig. 1.

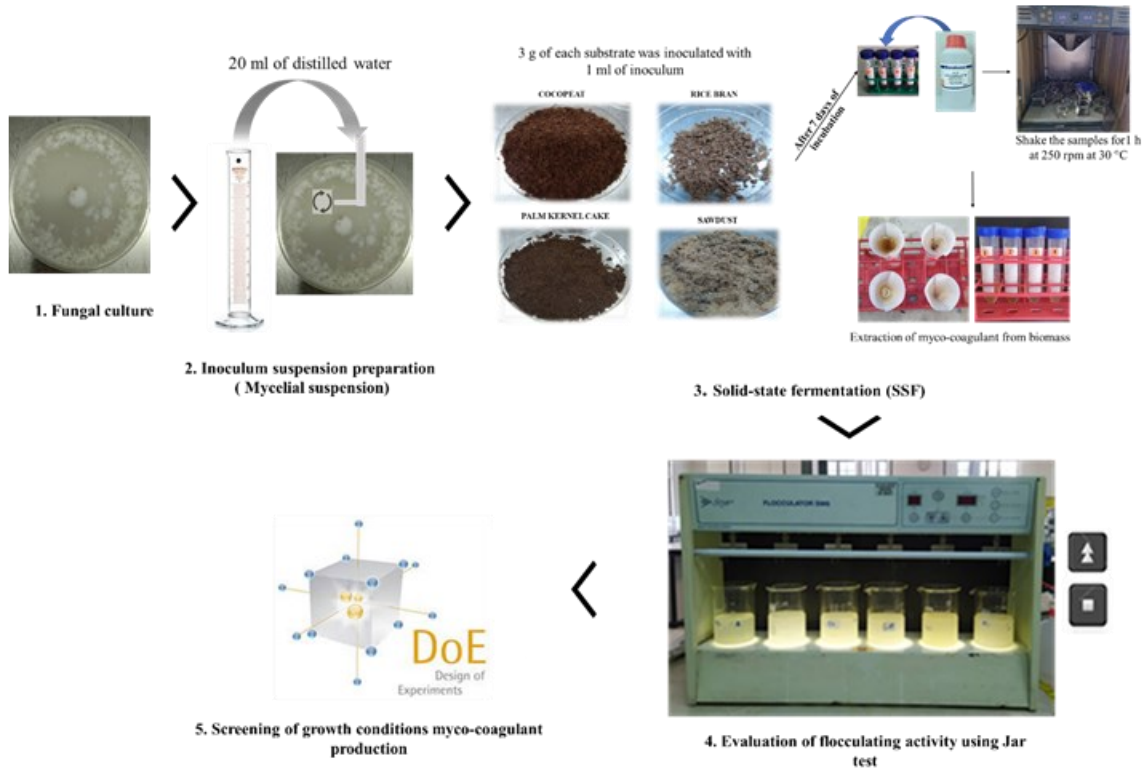

Fig. 1. Overall scheme of the major activity to produce myco-coagulant using solid-state fermentation.

2.5 Surface Morphology Analysis

Scanning electron microscopy (SEM) (SEM-Sipma-VP03-67, Zeiss, and P-Sigma, Munich, Germany) was used to observe the fungus's morphological surface and the mycelium formation in various lignocellulosic media.

2.6 Extraction of myco-coagulant

In this study, the extraction of the myco-coagulant from the biomass was conducted using an aqueous buffer solution at pH 7. After seven days of incubation, the fermented medium of SSF from each plate (2 g of the substrate) was mixed with 20 ml of aqueous buffered solution at pH 7. The mixture was kept in a shaker incubator for 1 hour at 250 rpm at 30º C. It was then filtered using muslin, followed by a Whatman paper. The clear supernatant was collected as

the main myco-coagulant and used to determine their flocculating activity using the Jar apparatus method.

2.7 Determination of turbidity

The turbidity meter (Model 2100Q: HACH, Loveland, USA) was switched on before the data recording, and a blank sample of 10 mL distilled water was poured into the turbidity vial. 10 mL of the supernatant from each untreated water sample was poured into the turbidity vial. The outer vial was cleaned and placed into the turbidity meter before the 'read' button was pressed to get the turbidity reading in NTU. Finally, the average readings were calculated for record purposes.

2.8 Jar test experiment

The purpose of a jar test experiment was to observe the coagulation and flocculation processes of water treatment on the laboratory scale. The equipment needed in conducting jar tests was a Flocculator unit (SW6, UK) that consisted of six paddle mixers to which respective beakers were placed and a control panel to adjust the stirring speed and time. Four operating conditions of the jar test were set as the independent variables: the initial pH value, coagulant dosage of myco-coagulant, rapid mixing speed, and settlement time. After that, the required amount of myco-coagulant was added to synthetic turbid water and succeeded by rapid mixing to simulate the coagulation process. It was then followed by the slow mixing that demonstrated the flocculation process. Then, the beakers containing treated wastewater were left aside for one hour to allow flocs sedimentation. After the flocs had settled, the supernatant of the treated wastewater was taken and analyzed for its final turbidity.

2.9 Evaluation of flocculation activity via Jar test

The Kaolin suspension was used to determine the flocculation activity of the myco-coagulant in its capacity to reduce the turbidity level. First, 0.7 g/l kaolin clay was suspended in 1500 ml of distilled water and pH 7 using 1M NaOH or 2M HCl. Initial turbidity was recorded at 480 NTU. 10 ml of the supernatant (myco-coagulant) was added to each jar containing 300 ml kaolin suspension. Then, the jar was set up and operated at three stages: fast mixing at 250 rpm for 7 minutes, then 90 rpm for 22 minutes, and finally settling for one hour. Next, the top layer of the water in each jar was collected with a micropipette, and the flocculation activity was calculated based on the percentage removal of turbidity. All the experiments were conducted in triplicate. The flocculating activity was calculated according to Eq. (1).

$$\text{Turbidity removal efficiency (\%)} = \frac{A-B}{A} \times 100 \quad (1)$$

where A is the initial turbidity of kaolin suspension directly after preparation (NTU) and B is the final turbidity of kaolin suspension after the settling period (NTU). A sartorius PB-10 pH meter was used to measure the pH value. The turbidity was measured by using a nephelometric turbidity unit (NTU) (standard method 2130 B) with a portable turbidimeter (2100Q HACH, USA).

2.10 Screening of growth conditions using Plackett-Burman design

Statistical experimental design plays an essential role in developing fermentation bioprocesses by screening for the main factors of interest and then further optimizing these to improve industrial process performance. Different nutritional and environmental variables were evaluated to determine the variables affecting flocculation activity by the production of the myco-coagulant. The Plackett–Burman statistical experimental design "Design Expert®

7.0.0" was used to identify the critical variables required to produce a myco-coagulant to reduce turbidity. The Plackett-Burman design (PBD) is an easy and fast method appropriate to screen multiple variables in a single experiment and is often used to evaluate the most significant variables affecting the culture requirements for fermentation and enzyme production.

Based on previous studies, several potential factors may affect the yield of the myco-coagulant. In this study, eleven factors were chosen. The eleven different independent variables are shown in Table 1, including the physical/chemical parameters (temperature, incubation time, inoculum size, cocopeat concentration, pH), and nutrients (malt extract, glucose, wheat flour, yeast, ammonium sulfate $(NH_4)_2SO_4$ and potassium dihydrogen phosphate (KH_2PO_4)). Each variable is represented at both high and low, denoted by (+) and (−). Flocculation activity was used as the response variable.

Table 1: Variables and their levels employed in Plackett–Burman design to screen culture conditions affecting myco-coagulant production by the fungus

Variables	Code	Low level (-)	High level (+)
Temperature (ºC)	A	25	35
Incubation time (Days)	B	5	9
pH	C	5	9
Cocopeat concentration (g)	D	2	4
Malt extract	E	1	2
Ammonium sulfate $(NH_4)_2$ SO_4 (%)	F	0	1
Glucose (%)	G	0	2
Wheat flour (%)	H	0	2
Yeast extract (%)	J	0	2
Potassium dihydrogen phosphate KH_2PO_4 (%)	K	0	0.5
Inoculum size (ml)	L	1	3

3 RESULTS AND DISCUSSION

3.1 Estimation of biomass in solid-state fermentation

The fungal mycelium penetrates deep into solid-state fermentation and remains attached to the solid substrate particles. As a result, it is difficult to separate the microorganisms from the solid particles. The present study used standard methods to estimate the biomass in solid-state fermentation [19,20,21].

3.1.1 Prescreening test for estimation of fungal growth

Estimating biomass is a critical step in a variety of microbial fermentation processes. In the present study, a prescreening test was performed to monitor and compare the growth rate of fungal strains on four distinct lignocellulosic substrates. As shown in Fig. 2 on cocopeat substrates, the fungal strain grew swiftly and created intact homogenous and filamentous mycelium composite structures. In addition, sawdust showed slow growth during this time of incubation. On the other hand, the fungal strain grew on palm kernel cake and rice bran; you can barely see the growth.

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Fig. 2. Culture of fungus on different substrates after seven days of incubation (a) culture of fungus on cocopeat; (b) culture of fungus on sawdust; (c) culture of fungus on PKC; (d) culture of fungus on rice bran.

3.1.2 Evaluation of fungal growth by scanning electron microscope (SEM)

A scanning electron microscope (SEM) was used to observe the fungus's morphological surface and the mycelium formation in various lignocellulosic media. Fig. 3 shows a significant variation in the density of the filamentous fibre of the fungus in various lignocellulosic media. On the other hand, the SEM indicates no change in the morphological surface due to the use of the same strains. Fig. 3 illustrates mycelium morphology observed in SEM with randomly arranged and oriented filaments on cocopeat, sawdust, palm cake, and rice bran substrates, respectively. The mean hyphae filament diameter was 0.6 ± 0.66 µm. In contrast, the other substrates show a hyphal density of less than coco peat with a diameter of filaments with a mean of 0.7 µm, 0.9 µm, and 1 µm for sawdust, palm kernel cake, and rice bran, respectively.

In general, there is no difference in the morphological structure, as shown in Fig.3, which refers to using the same fungal strain. However, the density and size of filaments differed from one substrate to another. The variation of the branching density can be explained by the environmental conditions here, where various lignocellulosic media were used. Fungal mycelial growth is aided by the substrate, which contains modest concentrations of carbohydrates, lipids, proteins, inorganic substances, and water [22]. Therefore, the physical characteristics of the substrate may reveal differences in mycelium growth [23,24].

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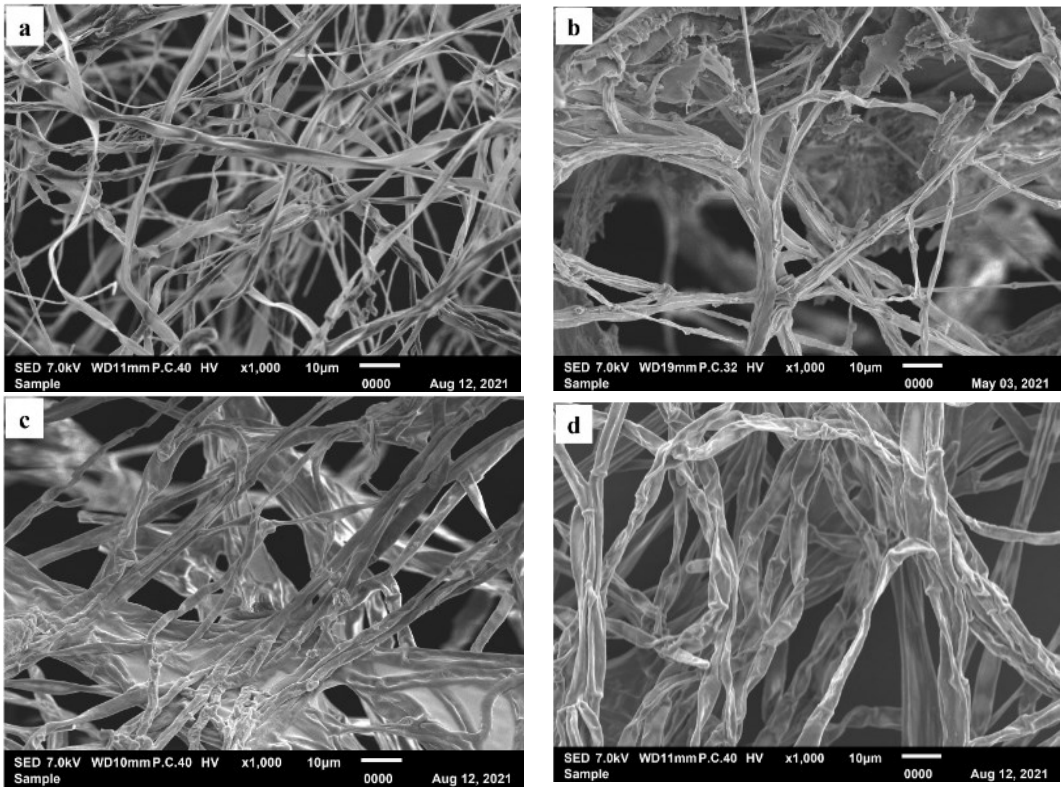

Fig. 3. Scanning Electron Microscopy (SEM) observations of fungal colonies on different substrates after seven days of incubation (a) culture of fungus on cocopeat; (b) culture of fungus on sawdust; (c) culture of fungus on PKC; (d) culture of fungus on rice bran magnification at X1000.

3.2 Analysis of myco-coagulant activity

Microbial secondary metabolites are regarded as valuable products due to their large number of biological activities. The secondary metabolites are synthesized in a fermentation medium during the growth of the microbes [19].

In this research, the desirable secondary metabolite was a myco-coagulant secreted during fungus growth on different lignocellulosic media. Fig. 4 shows the flocculation activity rate of the myco-coagulant using four lignocellulosic media. From the results, it can be clearly seen that coco peat recorded the highest flocculation rate at 84.59 % in removing suspended solids from kaolin suspension. On the other hand, the remaining LC substrates, namely sawdust, palm kernel cake, and rice bran, were recorded at 33.06 %, 23.91 %, and 21.18 %, respectively. Based on the results, coco peat was chosen as the best media to serve as an LC substrate for the fungal strain and produced an efficient myco-coagulant.

Selecting an appropriate substrate suitable for fungal growth and the target of myco-coagulant synthesis is crucial in developing an efficient technology for myco-coagulant production. Four substrates, namely coco peat, sawdust, palm kernel cake, and rice bran, were used in this study, which was collected from the biotechnology engineering laboratory of the International Islamic University Malaysia (IIUM). These solid substrates were considered to evaluate their ability to produce an efficient myco-coagulant to reduce the turbidity from water.

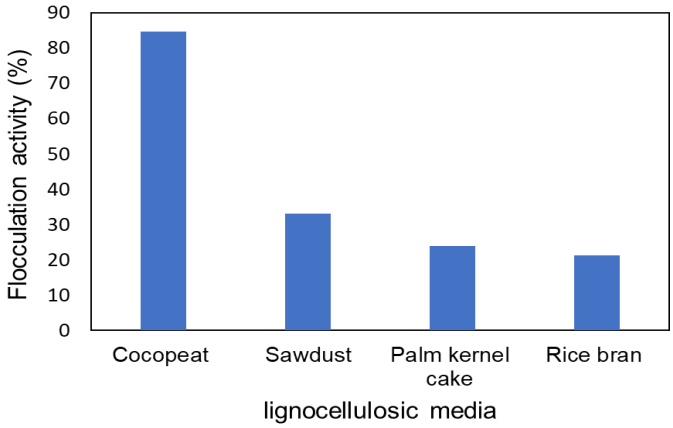

Fig. 4. Flocculation activity of the myco-coagulant in four different lignocellulosic media.

The supernatants of the used fungus extracted from biomass were tested to determine the flocculation ability in terms of turbidity reduction from synthetic turbid water (Kaolin suspension). The initial turbidity was recorded at 600 ± 30 NTU for kaolin suspension.

The flocculation activity rate of the treated synthetic turbid water by the fungal supernatants extracted from the biomass showed a wide variation in flocculation activity all over the fourth biomass ranging from 18.7 % to 89.19 %. Among the four fungal supernatants, the fungal supernatant extracted from coco peat from 623 to 96 NTU 84.59% showed the highest flocculation activity. On the other hand, sawdust, palm kernel cake, and rice bran showed 33.06%, 30.18, and 21.18 %, respectively.

The myco-coagulant was evaluated to test its ability to remove turbidity by flocculating the particles' kaolin suspension using the Jar apparatus. Based on the results, the fungal strain has proven its ability to produce an efficient myco-coagulant via solid-state using cocopeat as a substrate in reducing turbidity.

Coco peat was the best supplement that offered the best carbon source in yielding a myco-coagulant, with the highest FA (84.59 %) obtained with kaolin clay suspension. Therefore, the myco-coagulant extracted from coco peat that exhibited the best flocculating activity was chosen for further analysis, and the remaining substrates were eliminated from the remaining part of the study. As reported by Luthfi et al. [25], *Aspergillus niger* DWB showed a good flocculation activity rate of 60.5 % from oil palm empty fruit bunch (OPEFB) fiber. Qi et al. [26] reported that *Alcaligenes faecalis subsp. phenolicus* ZY-16 showed a good removal efficiency (90.05%) of citrus peel wastes as substrates.

3.3. Screening of process conditions for the SSF using PBD

The effects of different nutritional components on myco-coagulant production using coco peat were studied to develop a medium that requires minimal nutritional supplementation for enhanced enzyme production. A Plackett-Burman design (PBD) was applied in this study to determine which variables significantly affected the flocculation activity by producing a myco-coagulant using solid-state fermentation. The PB design is a simple and fast method suitable for screening multiple variables in a single experiment and is often used to evaluate the most significant variables affecting fermentation and enzyme production culture requirements.

The impacts of nutritional factors, including carbon and nitrogen sources and mineral salts, were studied using a PB design with 12 trials for 11 variables. As a first step, the corresponding responses in Table 2 were carried out to identify the significant variables on myco-coagulant

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production to reduce turbidity. PB experiments showed a wide variation in flocculation activity over all the different experiments, ranging from (18.7 to 89.19 %) presented in Table 2, highlighting the importance of further media optimization to attain a high yield of the interesting product myco-coagulant with high flocculation activity.

The effect of each nutrient component on flocculation activity is presented in Fig. 5. The main effect was estimated based on the difference between the sum of responses obtained at each component's high level (+1) and the low level (−1). Fig. 5 shows the main effect of each variable on the flocculation activity. Concerning the main effect of each variable, the results showed that three variables from the eleven different independent variables, which were malt extract, glucose, and wheat flour, affected the high flocculation activity at a high level. The level of the carbon source in the growth medium is an essential factor in the production of the myco-coagulant. In the present study, the carbon source, glucose, and wheat flour showed a significantly high effect on the production of the myco-coagulant. Previous studies reported that glucose positively affected the production of the myco-coagulant through liquid-state fermentation with *Lentinus squarrosulus* strain 7-4-2 [27].

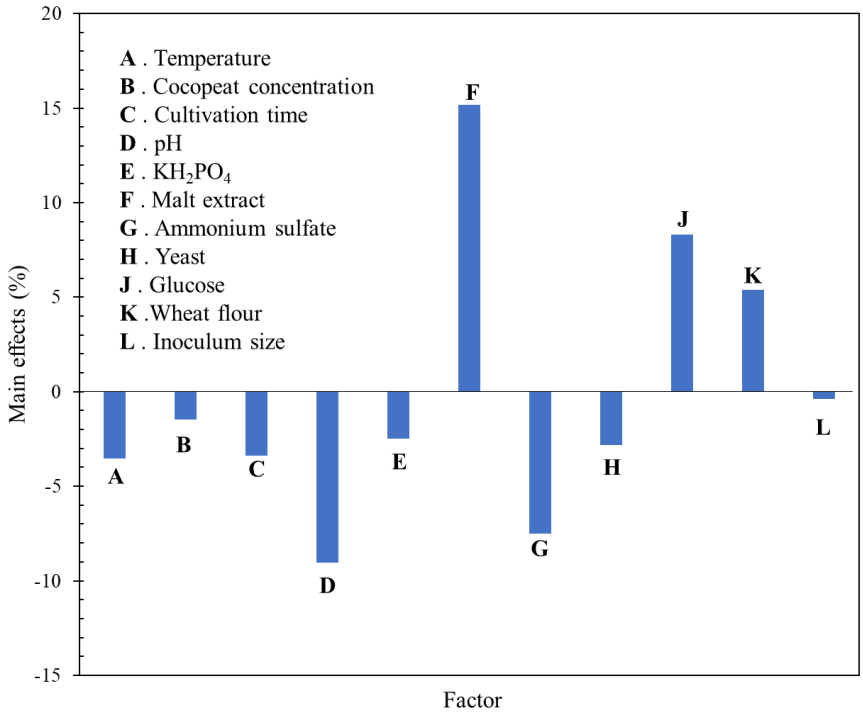

Fig. 5. Main effects of the nutritional components on Flocculation activity

Moreover, [28] reported that *B. agaradhaerens* C9 was cultured in a medium containing glucose, and yeast produced a coagulant with a flocculating activity of 91.9 %. Regarding the effect of pH a, similar results were reported by [15,29] which show the best initial pH of the medium was 6, which was in line with the best pH for bioflocculant production by *A. niger.* However, the remaining variables, temperature, coco peat concentration, cultivation time, KH_2PO_4, ammonium sulfate, pH, and inoculum size, affected the flocculation activity at a low level. The nitrogen source malt extract and ammonium sulfate showed a significant effect on the production of myco-coagulant. The most significant parameter at a low level on the main impact was pH and aluminium sulfate, whereby maintaining the fermentation at acidic pH enhanced the flocculating activity of the metabolite produced. Pu et al. [29] and Nurul et al. [30] have reported that the flocculating activity was not affected by the inoculum size, which supported our finding.

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Table 2: Plackett–Burman experimental design and obtained responses

Variables	**Temp. (°c)**	**Cocopeat concentration (%)**	**Cultivation time (day)**	**pH**	**KH_2PO_4 (%)**	**Malt extract (%)**	**Ammonium sulfate (%)**	**Yeast extract (%)**	**Glucose (%)**	**Wheat flour (%)**	**Inoculum size (ml)**	**Flocculation activity (%)**
Code	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**J**	**K**	**L**	
Run												
1	25	2.0	5	9.0	0.00	2.0	1.0	0.0	2.0	2.0	3.0	72.74
2	35	4.0	5	9.0	0.50	2.0	0.0	0.0	0.0	2.0	1.0	56.93
3	25	2.0	5	5.0	0.00	1.0	0.0	0.0	0.0	0.0	1.0	48.87
4	35	2.0	5	5.0	0.50	1.0	1.0	2.0	0.0	2.0	3.0	26.12
5	35	4.0	5	5.0	0.00	2.0	0.0	2.0	2.0	0.0	3.0	79.35
6	25	2.0	9	5.0	0.50	2.0	0.0	2.0	2.0	2.0	1.0	89.19
7	25	4.0	5	9.0	0.50	1.0	1.0	2.0	2.0	0.0	1.0	18.7
8	35	2.0	9	9.0	0.50	1.0	0.0	0.0	2.0	0.0	3.0	27.7
9	25	4.0	9	9.0	0.00	1.0	0.0	2.0	0.0	2.0	3.0	25.32
10	35	2.0	9	9.0	0.00	2.0	1.0	2.0	0.0	0.0	1.0	26.61
11	25	4.0	9	5.0	0.50	2.0	1.0	0.0	0.0	0.0	3.0	48.7
12	35	4.0	9	5.0	0.00	1.0	1.0	0.0	2.0	2.0	1.0	44.52

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The use of coco peat as a low-cost nutrient composition was earlier not reported for the higher yield of myco-coagulant from fungal fermentation. Based on this study, it was determined that the designed lignocellulosic substrate was economically significant in producing the desired myco-coagulant.

To check the contribution of the selected factors affecting the flocculation activity of the produced myco-coagulant, Pareto analysis was performed. Analysis of the Pareto chart in Fig. 6 showed that malt extract, glucose, wheat flour, pH, and ammonium sulfate have the highest effect on flocculation activity.

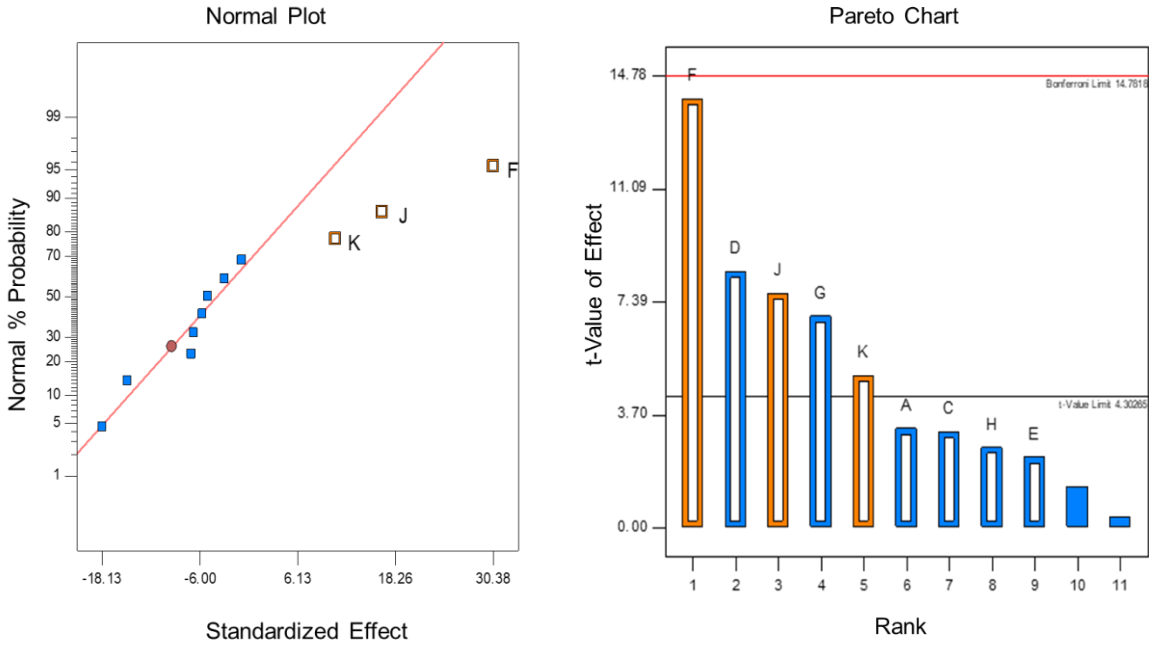

Fig. 6. Pareto chart illustrates the order and significance of the variables affecting flocculation activity using Plackett-Burman design (the blue color represents negative effects, and the orange color represent positive effects).

Plackett Burman's design does not provide a complete idea about the exact amount of each variable as well as the interaction between the variables. Thus, based on the calculations of the main effect and Pareto chart, the Plackett-Burman design selected three parameters: malt extract, pH, and glucose as the most significant factors to produce a myco-coagulant with optimum flocculation activity.

4 CONCLUSIONS

Since the cost of production of the myco-coagulant is among the challenges affecting its utilization, the results achieved in this study gave an insight into the utilization of different lignocellulosic substrates to produce an efficient myco-coagulant in reducing water turbidity as well as reduce the cost of experiments as we used abundant substrates.

In this study, among the used lignocellulosic substrate, cocopeat was identified as a suitable substrate that serves as a carbon and nitrogen source to produce the myco-coagulant using solid-state fermentation, which can potentially reduce the turbidity by 84.6% from the kaolin suspension. On the other hand, sawdust, palm kernel cake, and rice bran showed 33.06, 30.18, and 21.18 %, respectively. Coco peat, as an abundant raw material in Malaysia, may be

utilized to meet this challenge and reduce the cost of production. The produced myco-coagulant may also contribute as a safe, low-cost, and environmentally friendly coagulant alternative to current chemical coagulants. The discovery of a safe and environmental-friendly microbial coagulant that possesses the ability for sustainable water treatment is in line with the global awareness towards creating a clean and healthy environment by using green technology employing solid-state fermentation.

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