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Enhancing Single-cell Protein Produced by *Aspergillus terreus* UniMAP AA-1 from Palm-pressed Fiber via Response Surface Methodology

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ABSTRACT

The increased demand for aquaculture has led to significant implications for fish feed supply since its cost contributes to most of the operational cost. The primary protein source in feed ingredients comes from fish meal (FM). However, increasing FM prices and overfishing concerns substantially challenge its sustainable production. Alternatively, single-cell protein (SCP) derived from microbial biomass has demonstrated efficacy as a protein substitute. This study enhanced microbial protein production of *Aspergillus terreus* UniMAP AA-1 cultivated on palm-pressed fiber (PPF) through the response surface methodology (RSM) approach. Screening of media compositions comprising potassium dihydrogen phosphate (KH_2PO_4), ammonium nitrate (NH_4NO_3), sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$), copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$), and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) was conducted using the Plackett-Burman design (PBD). The Central Composite Rotatable Design (CCRD) approach was further used to optimize the significant parameters influencing SCP production. Screening by the PBD signifies that NH_4NO_3 and $MgSO_4.7H_2O$ are significant factors enhancing SCP production, with the highest SCP produced of 551 mg/L. Subsequently, optimization by CCRD revealed that the optimum concentration for NH_4NO_3 and $MgSO_4.7H_2O$ were 0.85% and 0.05% (w/v). This optimization strategy has increased the SCP

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production to 673 mg/L, 22% higher than those obtained earlier. The data gathered in this study may be used for scale-up production of SCP from PPF by *A. terreus* UniMAP AA-1.

Keywords: Central Composite Rotatable Design, fish feed application, media composition optimization, palm-pressed fiber, Plackett-Burman Design, single-cell protein, solid-state fermentation

INTRODUCTION

The aquaculture industry is one of the fastest-growing food sectors globally, driven by the increasing demand for fish and shellfish on a global scale. The aquaculture sector has grown at an average yearly rate of 8.9% since 1970 (Huang & Nitin, 2019). The surge in demand has led to significant implications for fish feed supply. Feed costs typically account for 30%–70% of total operational costs, significantly impacting investment profitability (Bratosin et al., 2021). The primary protein source in feed ingredients is fish meal (FM). However, the increasing costs for FM and overfishing concerns substantially challenge its sustainable production. FM production is expected to struggle to meet global demand by 2050 (Bratosin et al., 2021). Therefore, exploring alternative protein sources is crucial, as they have the potential to be substitutes for fish meals. One promising alternative is a single-cell protein (SCP) derived from microbial biomass, which has demonstrated efficacy as a protein substitute for fish and soybeans. SCP is obtained from cultured cells and can be isolated as dried cells or purified protein. Various microorganisms can produce it, including bacteria, fungi, and algae. Microbial protein is cost-effective and offers better nutritional value than other protein sources. Its rapid growth rate also enables large-scale production (Bajić et al., 2023).

Oil palm holds significant importance in Malaysia's agricultural landscape alongside rubber, cocoa, rice, and coconut (Jafri et al., 2021). The oil palm plantations covered 5.67 million hectares as of 2022, producing 1.04 million tons (MT) of palm kernel oil and about 15.72 MT of palm oil (Malaysian Oil Palm Council [MPOC], 2024). The oil palm tree's biomass makes up 90% of its mass, with palm oil making up only 10% of this biomass (Jafri et al., 2021). Oil palm biomass refers to residues from the industry's replanting, pruning, and milling activities. Among these residues, palm-pressed fiber (PPF) emerges as a promising substrate for SCP production. PPF is made of a mixture of mesocarp fiber, crushed kernel, kernel shell, and debris extracted from the mesocarp of oil palm fruits after the oil is extracted (Neoh et al., 2011). Rich in cellulose and hemicellulose, PPF offers an ideal carbon source for fungi to produce SCP, thereby serving as a valuable ingredient in feed ingredients (Amara & El-Baky, 2023).

Optimizing the composition of the culture medium is essential for enhancing biomass accumulation and producing desired products. However, each element requires the appropriate concentration, ratios, and chemical forms to provide optimal growth conditions

(Bentahar & Deschênes, 2022). Fermentation medium optimization frequently involves either the one-factor-at-a-time (OFAT) or more advanced statistical optimization techniques. Although OFAT is simple, it can be expensive and time-consuming due to the multiple tests required to reach conclusions. Furthermore, OFAT overlooks the interactions among different factors under investigation.

On the contrary, statistical optimization methods offer advantages like nutrient balancing, enriching essential components, and eliminating unnecessary ones while considering factor interactions. These approaches also reduce the number of experiments needed, thus reducing costs and enhancing the production process's efficiency (Guo et al., 2019). Response surface methodology (RSM), Plackett-Burman design (PBD), and factorial design are common methods for achieving statistical optimization, which produces ideal results like increased cell proliferation or volumetric production. Thus, RSM, a robust mathematical tool, allows for the efficient testing of multiple process variables, reducing the number of trials required to identify significant components impacting microbial processes (Choi et al., 2021). Therefore, this study aims to investigate the performance of PPF as a substrate for SCP generation by *Aspergillus terreus* UniMAP AA-1. The PBD approach was selected to screen the most significant media components. Through the central composite rotatable design (CCRD), RSM was employed to further optimize the parameters that affect SCP production.

MATERIALS AND METHODS

Collection of Raw Materials and Preparation of Substrate

The PPF used in this study was provided by Norstar Palm Oil Mill Sdn. Bhd. in Kuala Ketil, Kedah (5°34′09.3″N 100°42′01.8″E). Dust, sand, and other contaminants were eliminated from the PPF by rinsing it with tap water, followed by drying at 60°C for 24 hours in the oven. Then, it was ground and sieved into 500 µm particle size before being kept in a dry container prior to further use.

Pretreatment of Substrate

About 1 L of 5% (w/v) NaOH (Merck, Germany) was used to treat 50 g of dried PPF. The substrate mixture was then incubated according to Rahman et al. (2021). Following the draining of the NaOH solution, the substrate was cleaned with dH_2O until the pH reached a neutral level and oven-dried at 60°C.

Inoculum Preparation

A. terreus UniMAP AA-1 (Gunny & Arbain, 2012) was obtained from the culture collection of the Faculty of Chemical Engineering and Technology, Universiti Malaysia Perlis. The

culture was cultivated for 7 days at 30°C on potato dextrose agar (PDA) (HiMedia, India). About 30 ml of sterilized dH_2O was added to the 7-day-old spores. After rubbing, the spores were filtered through Whatman Grade No. 1 filter paper, and the spore was adjusted to the concentration of 10^7 CFU/ml using a hemocytometer (De la Cruz-Quiroz et al., 2019). The filtrate was kept at 4°C before usage.

Growth Media Preparation and Solid-state Fermentation

A 250 ml conical flask was filled with approximately 6 g of substrate. About 50% moisture content was achieved by adding 6 ml of solution to the substrate, consisting of 20% inoculum and 80% growth media solution. The composition of the media solution was varied by altering the concentration of seven media components which include potassium dihydrogen phosphate (KH_2PO_4; Bendosen, Malaysia), ammonium nitrate (NH_4NO_3; HmbG, Germany), sodium chloride (NaCl; HmbG, Germany), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$; HmbG, Germany), ferrous sulfate heptahydrate ($FeSO_4.7H_2O$; HmbG, Germany), copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$; Bendosen, Malaysia), and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$; HmbG, Germany) based on the experimental design. The mixture was thoroughly mixed prior to incubation at 30°C for 5 days (Rahman et al., 2021).

Identification of Significant Media Compositions Using Plackett-Burman Design (PBD)

The screening was conducted using the PBD (Design Expert v12). The design can decrease the number of experiments required to achieve goals efficiently. The first-order model in Equation 1 served as the basis for the Plackett-Burman experimental design:

$$Y = ß_0 + \sum ß_i X_i \quad [1]$$

where, Y is the SCP production, mg/L, $ß_0$ is the model intercept, and $ß_i$ is the variable estimates. A significant factor contributing to SCP production is denoted by a confidence level greater than 95%. A total of 12 runs were generated, which included seven media components. The experimental runs were replicated three times, and the response was the average of triplicates. The range used for each component is depicted in Table 1.

Table 1
Plackett-Burman Design of different media compositions and levels

Factor	Term	Unit	Level	
			-1	+1
NH_4NO_3	A	% (w/v)	0.30	0.70
KH_2PO_4	B	% (w/v)	0.15	0.35
NaCl	C	% (w/v)	0.00	0.20
$MgSO_4.7H_2O$	D	% (w/v)	0.10	0.20
$FeSO_4.7H_2O$	E	% (w/v)	0.00	0.20
$ZnSO_4.7H_2O$	F	% (w/v)	0.00	0.20
$CuSO_4.5H_2O$	G	% (w/v)	0.00	0.20

Optimization of Media Components by Central Composite Rotatable Design (CCRD)

The optimization was conducted using the CCRD (Design Expert v12). The significant media components identified by PBD were selected as variables in the CCRD. They were NH_4NO_3 and $MgSO_4.7H_2O$, and the levels were set, as shown in Table 2.

As indicated by the PBD, the $FeSO_4.7H_2O$, KH_2PO_4, NaCl, $CuSO_4.5H_2O$, and $ZnSO_4.7H_2O$ concentrations were kept at their optimal levels. About 13 runs were performed, including 5 duplicates at the center points. The analysis was done in triplicates. According to Equation 2, a second-order polynomial response surface approach was fitted using the experimental data in the statistical package Design Expert v12 (StatEase Inc. Minneapolis, USA):

$$Y = ß_0 + ß_1X_1 + ß_2X_2 + ß_{11}X^2_1 + ß_{22}X^2_2 + ß_{12}X_1X_2 \quad [2]$$

where, Y is the SCP (mg/L), $ß_0$ is the intercept, $ß_1$ and $ß_2$ are the linear coefficients, $ß_{11}$ and $ß_{22}$ are the squared coefficients, and $ß_{12}$ is the interaction of coefficients.

Protein Recovery and Determination

The fermentation sample was dried for 24 hr at 60°C. The dried samples were ground using a mortar and pestle to obtain a smaller and smoother substrate. In a centrifuge tube, 0.5 g of the dried sample and 25 ml of 1 N NaOH were mixed and soaked for 24 hours at room temperature. Subsequently, the mixture was centrifuged for 10 min at 10,062 *g* and the supernatant was kept at 4°C (Rahman et al., 2021). Protein concentration was determined using the Lowry method.

Total Reducing Sugar Content

In a test tube, 1 ml of 3,5-dinitrosalicylic acid (DNS) reagent and 1 ml of sample supernatant were mixed. The mixture was incubated for 10 min in an 80°C water bath and then cooled under running water. Absorbance was measured by a UV spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 540 nm (Rahman et al., 2021).

Table 2
The factors and levels applied for Central Composite Rotatable Design (CCRD)

Factor	Unit	Level				
		$-\alpha$	−1	0	+1	α
NH_4NO_3	% (w/v)	0.64	0.70	0.85	1.00	1.06
$MgSO_4.7H_2O$	% (w/v)	0.00	0.00	0.05	0.10	0.12

RESULTS AND DISCUSSION

Screening of Media Compositions by Plackett-Burman Design (PBD)

PBD is a tool used to screen the effects of process variables on yield. This tool can reduce the number of experiments needed in the subsequent optimization study using RSM. The effects of different media compositions on SCP production were determined by performing 12 runs given by the PBD (Table 3). The highest protein concentration was obtained in run 2, with 551 mg/L, while the lowest was obtained in run 3, with 90 mg/L.

The PBD generates a regression equation that predicts the factors influencing the response, such as SCP production. This equation, expressed through the coefficient of R^2, showed a value of 0.9376 for SCP, confirming the design's significance in predicting variable effects on *A. terreus* UniMAP AA-1's SCP production. An analysis of variance (ANOVA) using Fisher's *F*-test and *F*-value further validated the model's significance (Table 4). The significance of the model is indicated by its *F*-value of 8.58, which has a 2.76% probability of happening owing to random variation. The *p*-value is useful for determining each coefficient's significance and identifying interaction patterns between variables. A smaller *p*-value (p-value < 0.05) signifies greater coefficient significance. In this study, only three parameters—NH_4NO_3, $MgSO_4.7H_2O$, and $FeSO_4.7H_2O$—showed significant model terms, as detailed in Table 4.

The main effects (*t*-value) of the analyzed factors on SCP production are graphically illustrated in Figure 1. The *t*-value with a large magnitude indicates the high significance

Table 3
The Placket-Burman experimental runs and resulting single-cell protein (SCP) of A. terreus *UniMAP AA-1 cultivated on palm-pressed fiber*

Run	A	B	C	D	E	F	G	SCP (mg/L)
1	0.7	0.35	0.2	0.1	0.0	0.0	0.2	452
2	0.7	0.35	0.0	0.1	0.0	0.2	0.0	551
3	0.3	0.35	0.0	0.2	0.2	0.0	0.2	90
4	0.7	0.15	0.0	0.1	0.2	0.0	0.2	393
5	0.3	0.35	0.2	0.2	0.0	0.0	0.0	250
6	0.3	0.15	0.2	0.1	0.2	0.2	0.0	291
7	0.7	0.15	0.2	0.2	0.0	0.2	0.2	364
8	0.3	0.35	0.2	0.1	0.2	0.2	0.2	273
9	0.7	0.15	0.2	0.2	0.2	0.0	0.0	312
10	0.3	0.15	0.0	0.2	0.0	0.2	0.2	345
11	0.3	0.15	0.0	0.1	0.0	0.0	0.0	338
12	0.7	0.35	0.0	0.2	0.2	0.2	0.0	381

Note. Variables listed in % (w/v): A = NH_4NO_3, B = KH_2PO_4, C = NaCl, D = $MgSO_4.7H_2O$, E = $FeSO_4.7H_2O$, F = $ZnSO_4.7H_2O$, G = $CuSO_4.5H_2O$

Table 4
Statistical analysis of Plackett-Burman design for each variable

Variable	*df*	*F*-value	*p*-value	Confidence level (%)
Model	7	8.58	0.0276	97.24
NH_4NO_3	1	28.53	0.0059	99.41
KH_2PO_4	1	0.081	0.7907	20.93
NaCl	1	0.926	0.3904	60.96
$MgSO_4.7H_2O$	1	11.76	0.0265	97.36
$FeSO_4.7H_2O$	1	11.93	0.0260	97.40
$ZnSO_4.7H_2O$	1	5.21	0.0846	91.54
$CuSO_4.5H_2O$	1	1.61	0.2727	72.73

Note. Highlighted in grey is the significant model term (*p*-value <0.05)

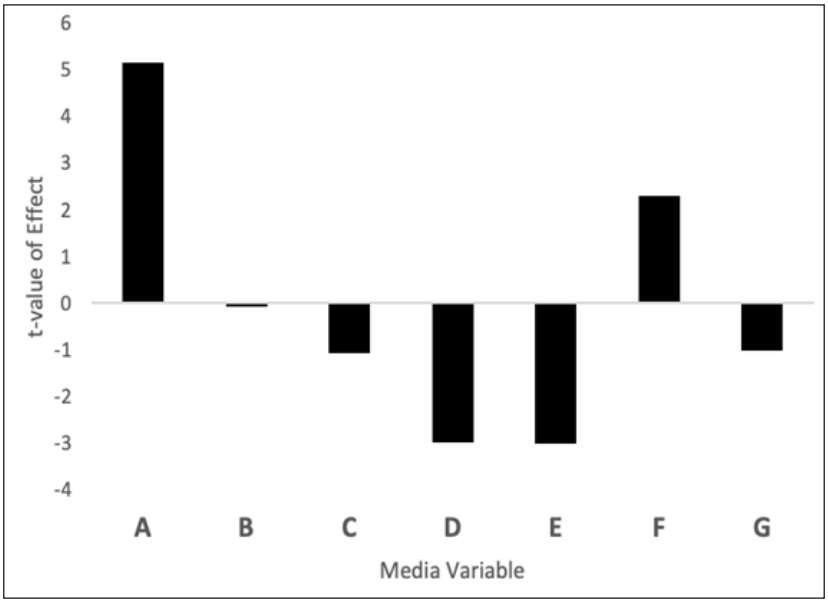

Figure 1. Main effects of media compositions for single-cell protein production

Note. A = NH_4NO_3, B = KH_2PO_4, C = NaCl, D = $MgSO_4.7H_2O$, E = $FeSO_4.7H_2O$, F = $ZnSO_4.7H_2O$, G = $CuSO_4.5H_2O$

of the equivalent coefficient. Based on Figure 1, the most significant factor is NH_4NO_3, and the positive value indicates that by increasing the parameter concentration, the production of SCP will be enhanced. Besides that, $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$ are the other two significant factors that affect SCP production. The *t*-values of these factors are negative values, indicating that they are needed at lower concentrations for an enhanced production of SCP.

Nitrogen sources are an essential nutrient component for microbial growth and building blocks of protein. Organic or inorganic sources can supply nitrogen. Ammonium is the preferred nitrogen source because it promotes faster growth compared to other nitrogen sources available (Chen et al., 2022; Chutrakul et al., 2022; Jiang et al., 2019; Martin-Jézéquel et al., 2015). In this study, nitrogen in the form of NH_4NO_3 was added to the media, and the PBD identified this compound as the most significant compound for protein production at a concentration of 0.7% (w/v). An optimum amount of nitrogen source is vital for mycelium growth due to the formation of metabolic by-products (Nguyen et al., 2023). Akintomide and Antai (2012) also observed the effect of inorganic nitrogen supplementation on SCP production. They investigated how various inorganic nitrogen sources affected the development of microorganisms and discovered that adding $(NH_4)_2SO_4$ to *Saccharomyces cerevisiae* (21.3%) and *Aspergillus niger* (16.78%) resulted in the highest crude protein yields (Akintomide & Antai, 2012).

Moreover, supplementing a nitrogen source in the form of NH_4 can stimulate mycelial growth in fungi, as Dulay et al. (2020) reported, consequently enhancing both biomass and protein yields simultaneously. On the contrary, although $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$ played significant roles in SCP production, their amount is needed in small amounts as they are the trace elements for fungi growth. The elevated concentrations of Mg^{2+} and Fe^{2+} are known to cause cell growth inhibition, leading to lagging of doubling time (Chen et al., 2022; Nepal & Kumar, 2020). Zięba et al. (2021) reported that the most significant decrease in *Plurotus djamor* productivity was observed when the media was supplemented with the highest Mg concentration of 4200 mg. The finding in this study was further corroborated by Prakash et al. (2015), who cultivated *Fusarium venenatum* in combination with jaggery water, date extract, and mineral salts (KH_2PO_4, K_2HPO_4, and $MgSO_4$). They observed that the highest protein concentration (4.9 g/L) was obtained at a low level (50 mg/L) of $MgSO_4$ (Prakash et al., 2015). The observed results suggested that although Mg^{2+} and nitrogen are essential elements in organisms, excess quantities may hamper protein production (Nepal & Kumar, 2020; Nguyen et al., 2023).

Optimization of Single-Cell Protein (SCP) Production by Response Surface Method (RSM) using Central Composite Rotatable Design (CCRD)

The significant parameters in the Plackett-Burman analysis were used to optimize SCP production by *A. terreus* UniMAP AA-1. The concentration of NH_4NO_3 and $MgSO_4.7H_2O$ were only considered parameters for optimization (Table 2), while $FeSO_4.7H_2O$ was not added in the media as suggested by PBD. The CCRD of the Design Expert software suggested a total of 13 runs, including five replicates at the center points, as shown in Table 5. Other parameters, such as KH_2PO_4, NaCl, $ZnSO_4.7H_2O$, and $CuSO_4.7H_2O$, were included in the growth medium at the concentration suggested optimum by Plackett-

Burman analysis. These trace elements are necessary to maintain *A. terreus* growth while increasing SCP production.

The ANOVA results (Table 6) reveal that the model is significant, with a *p*-value of 0.0004. As a result, only 0.04% of the models are insignificant due to noise. Although parameters A (NH_4NO_3), B ($MgSO_4.7H_2O$), and the interaction between AB are not significant (*p*-value > 0.05), they are still considered in the model because the higher order term, A^2, a quadratic term of NH_4NO_3, is significant with a value of less than 0.0001 because the model was built on a hierarchy. However, B^2 is insignificant and hence removed from the equation. It demonstrates that A^2 operates as a limiting factor, with a minor change in value affecting the protein concentration. In contrast to pure error, the lack of fitness is not significant, and noise has a 15.70% probability of being the source of a large lack of fit *F*-value. The model fits the data well (Elsayed & Ahmed Abdelwahed, 2020). A regression analysis was performed utilizing coded values from data estimates, as stated by Equation 3:

$$\text{SCP (mg/L)} = 639.18 + (10.78 \times NH_4NO_3) + (8.77 \times MgSO_4.\ 7H_2O) + (1.5\ NH_4NO_3 \times MgSO_4.\ 7H_2O) - (77.62 \times NH_4NO_3^2) \quad [3]$$

A quadratic model with an R^2 value of 0.9015 was proposed, indicating that the experimental variables analyzed accounted for 90.15% of the overall variation in the SCP.

Table 5
The predicted and experimental values of single-cell protein after parameters optimization by Central Composite Rotatable Design

Run	A = NH_4NO_3	B = $MgSO_4.\ 7H_2O$	Single-cell protein (mg/L)	
			Experimental	Predicted
1	0.70	0.00	553	533.43
2	0.70	0.10	576	554.45
3	0.85	0.05	673	647.02
4	1.00	0.10	591	579.01
5	0.85	0.05	663	647.02
6	0.85	0.05	635	647.02
7	1.00	0.00	562	551.99
8	0.85	0.00	597	620.83
9	0.85	0.05	640	647.02
10	1.06	0.05	497	506.02
11	0.64	0.05	453	475.54
12	0.85	0.12	616	635.65
13	0.85	0.05	636	647.02

Table 6
Analysis of variance for a quadratic model of single-cell protein production

Source	Sum of squares	df	Mean Square	F-value	p-value
Model	44459.21	4	11114.80	18.31	4.00×10^{-4}
A- NH_4NO_3	929.35	1	929.35	1.53	0.25
B- $MgSO_4.7H_2O$	536.83	1	536.83	0.88	0.37
AB	9.00	1	9.00	0.01	0.91
A^2	42580.21	1	42580.21	70.16	$<1.00\times10^{-4}$
Residual	4855.56	8	606.94		
Lack of Fit	3638.36	4	909.59	2.99	0.16
Pure Error	1217.20	4	304.30		
Cor Total	49314.77	1			

The graph model is graphically constructed from the regression model to determine the best value of SCP production. It demonstrates the interaction effect of NH_4NO_3 and $MgSO_4.7H_2O$ on SCP formation (Figure 2). The study reveals that the model has a quadratic elliptical response surface. The production of SCP increased in the orange-red circular region, as depicted in Figure 2, where the concentration of $MgSO_4.7H_2O$ ranged from 0.02% (w/v) to 0.1% (w/v), and the concentration of NH_4NO_3 ranged from 0.8% (w/v) to 0.9% (w/v). The figure also shows that $MgSO_4.7H_2O$ is close to 0.05% (w/v) and that NH_4NO_3 concentration is at 0.86% (w/v) for optimal SCP production.

In this study, the growth of *A. terreus* UniMAP AA-1 on optimized PPF media resulted in a significant increase in biomass and protein after five days of fermentation. Utilizing

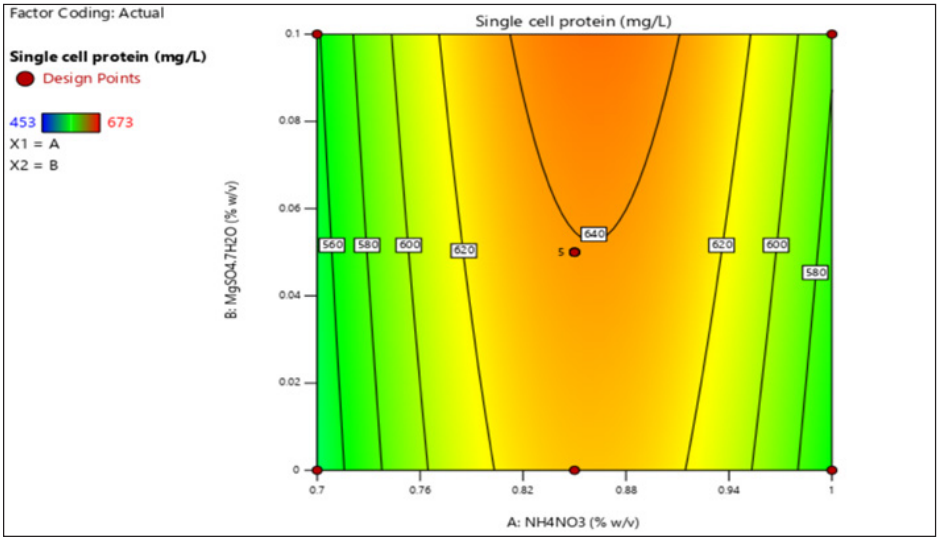

Figure 2. Contour plot for the interaction of NH_4NO_3 and $MgSO_4.7H_2O$ in response to protein concentration

the RSM approach, protein production for SCP was enhanced by 22%, rising from 551 mg/L with PBD to 673 mg/L with CCRD. In comparison, a similar enhancement in biomass production was observed by Choi et al. (2021) through PBD and CCD (RSM) studies utilizing *Lactobacillus plantarum* 200655. A total biomass concentration of 3.845 g/L was achieved in an optimal medium comprising of 31.29 g/L maltose, 30.27 g/L yeast extract, 39.43 g/L soytone, 5 g/L sodium acetate, 2 g/L K_2HPO_4, 1 g/L Tween 80, 0.1 g/L $MgSO_4 \cdot 7H_2O$, and 0.05 g/L $MnSO_4 \cdot H_2O$. It represents a 1.58-fold increase compared to the PBD medium (2.429 g/L; Choi et al., 2021). The maximum mycelial protein content of 2.11% was obtained by Tang et al. (2022) using an optimized fermentation medium designed with the Box-Behnken design to extract more mycelial soluble protein from *Ophiocordyceps sinensis*. The medium contained 20% beef broth, 0.10% peptone, 2% glucose, 0.15% yeast extract, 0.20% KH_2PO_4, and 0.02% $MgSO_4$ (Tang et al., 2022). Although media composition requirements vary among different organisms, these studies illustrate that employing the RSM approach for optimization can effectively increase protein production in microbes.

Validation of Optimum Process Condition

In the present study, the optimal amount of NH_4NO_3 to produce the utmost protein concentration is 0.86% (w/v), while $MgSO_4.7H_2O$ is 0.1% (w/v). The validation run was performed three times. The parameters were set within a range, whereas SCP production was set to the highest level. According to Table 7, the actual reading is within the predicted range, with a mean value of 650.3 mg/L and a standard deviation of 1.26. The predicted and actual values have a minimal standard deviation and are in good agreement. Hence, the model can be considered valid.

Table 7
Comparison of the predicted and experimental values of SCP production for model validation

Replicate	NH_4NO_3 (% w/v)	$MgSO_4.7H_2O$ (% w/v)	SCP concentration (mg/L)		Percentage error (%)	Standard deviation
			Predicted value	Actual value		
1	0.86	0.10	648.4	665	2.55	1.26
2	0.86	0.10	648.4	649	0.09	
3	0.86	0.10	648.4	637	1.76	

SCP Production and Glucose Utilization by *A. terreus* UniMAP AA-1 on Optimized Media

The performance of the optimized media was evaluated over a 7-day fermentation period at 30°C. The results are depicted in Figure 3. Generally, the trends in SCP generation and

glucose utilization are similar in which it was increasing from day 1, reaching peak values, and declining towards the end of the fermentation period. Day 3 yielded the highest glucose concentration of 365.3 mg/L. In contrast, day 5 produced the highest protein concentration of 648 mg/L (Figure 3). Since *A. terreus* UniMAP AA-1 hydrolyzes cellulose to sugar, a greater concentration of glucose at the start of the fermentation process is observed. During fermentation, the fungi's consumption of these sugars promotes biomass formation and growth, which raises the amount of protein. After five days of fermentation, the protein content increased from 162 mg/L to 648 mg/L, a 4-fold increase. Hence, PPF has been demonstrated to be an invaluable substrate for the cultivation of *A. terreus* UniMAP AA-1 and is promising to produce SCP for various potential applications.

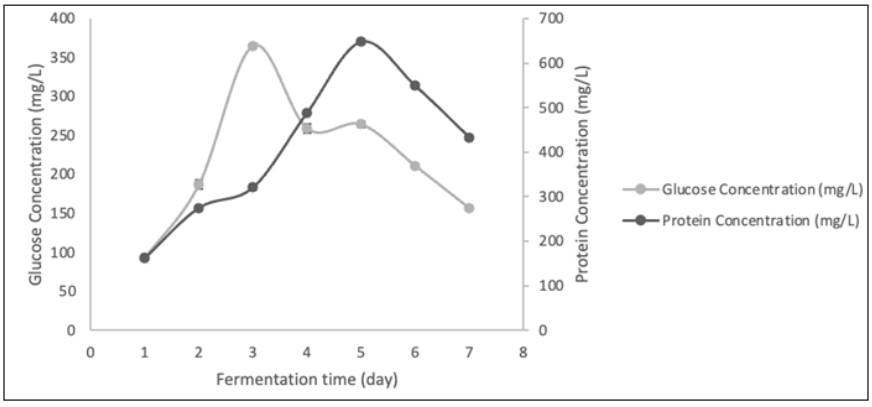

Figure 3. The single-cell protein profile and reducing sugar consumption of *Aspergillus terreus* UniMAP AA-1

CONCLUSION

In this study, SCP has been produced through the solid-state fermentation of *A. terreus* UniMAP AA-1 on PPF. $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, and NH_4NO_3 were the variables that had a major impact on SCP formation. The CCRD approach optimization revealed that the optimized values for NH_4NO_3 and $MgSO_4.7H_2O$ were 0.85 and 0.05% (w/v). However, $FeSO_4.7H_2O$ was not added to the production media according to the PBD results. The highest SCP detected was 673 mg/L, which corresponds to a 22% rise in protein compared to screening findings. The large-scale production of SCP from PPF is possible with the application of these optimization data.

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