Optimization of process parameters for improved production of biomass protein from *Aspergillus niger* using banana peel as a substrate

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Abstract This study was aimed to optimize the process variables for improved production of biomass protein using *Aspergillus niger* from banana fruit peel by the use of response surface methodology. A five-level-four factors central composite rotatable design was applied to elucidate the influence of process variables viz. temperature (20–40 °C), pH (4–8), substrate concentration (5–25%), and fermentation period (1–5 days) on biomass and protein content. The second-order polynomial models were established, which effectively explicated the variation in experimental data and significantly epitomized the

appreciable correlation between independent variables and responses. After numerical optimization, the predicted optimum conditions (temperature of 31.02 °C, pH of 6.19, substrate concentration of 19.92%, and the fermentation period of 4 days) were obtained with biomass of 24.69 g/L and protein of 61.23%, which were verified through confirmatory experiments.

Keywords Biomass · Protein · *Aspergillus niger* · Banana peel · Optimization · Response surface methodology

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Introduction

Protein deficiency is one of the major key challenges especially in the developing countries (Umesh et al., 2017). The main reasons behind this issue are a fast increase in population growth rate, scarcity of agricultural land, as well as changes in lifestyle. Current status revealed that around 12.5% of people all over the world have been affected by chronic hunger, malnutrition and lack of nutritious food (FAO, 2013). Protein-calorie malnutrition (PCM) generally causes impaired mental growth and low immunity in children (Umesh et al., 2017). The demand for protein-rich food against its supply is quite high due to increasing world population, which creates insufficiency in the supply chain (Rajoka et al., 2012). About 25% of people have been suffering from protein deficiency, which is a glaring example of protein gap worldwide (Azam et al., 2014). Therefore, it is essential to search for alternative or novel protein sources in order to bridge this gap (Rajoka et al., 2012). Several attempts were practiced to satisfy this upsurge demand, i.e. techniques including elimination, purification, and recycling (Saadia et al., 2008).

Production of bio-protein through microbial fermentation of agricultural wastes is one of the most promising approaches for increasing the availability of proteins (Sahed et al., 2016). Almost 50% of the agricultural products are not used as a food or feed (Foyle et al., 2007), which are often improperly disposed causing enormous environmental disorders (Essien et al., 2005). Bioconversion of agro-industrial wastes into valuable components by fermentation is a standard approach to convalesce resources (Jin et al., 2001; Kandari and Gupta, 2012; Rajoka et al., 2012), which in turn can play a significant role to achieve food supply stability (Jamal et al., 2005; Yunus et al., 2015). Moreover, the transformation of these by-products using microbial fermentation can be a promising energy source for the production of value-added products, e.g. single cell protein (SCP) at a reasonable cost. It can also reduce the encumbrance of environmental pollution (Ghori et al., 2011).

Single cell protein (SCP) or biomass protein refers to edible unicellular microorganisms, which are dead, dry microbial cells or total protein. They are recovered from pure microbiological culture, e.g. yeast, fungi, bacteria and algae (Anupama and Ravindra, 2000), grown on different carbon sources. SCP possesses a high amount of protein (around 60–82%) and other valuable nutrients, such as vitamins, carbohydrates, minerals, nucleic acids, and fats (Jamal et al., 2008). SCP could be a promising, economic, and better alternative protein source instead of expensive sources, e.g. soybean and fish. Furthermore, SCP can be implemented to the food products as a protein additive (Gad et al., 2010; Ghasem, 2015). However, the direct use of SCP in food products is prohibited due to the presence of higher amount (6–10%) of nucleic acids in SCP (Yadav et al., 2016), which in turn can elevate the serum uric acid levels in the human body (Nasseri et al., 2011). Therefore, SCP might be associated with health complications due to high ribonucleic acid (RNA) content, toxins produced by microbes, and harmful substances derived from the feedstock (Ritala et al., 2017; Yadav et al., 2016). Furthermore, the cell wall of the microorganisms might be non-digestible and could bear unacceptable color and flavors (Adedayo et al., 2011). Despite many complications, a number of studies reported that *Aspergillus niger* is safe for the production of enzymes and widely applied in agricultural, industrial, and medical sectors (Schuster et al., 2002). It is also used for waste management and biotransformation (Mbah and Edeani, 2014; Schuster et al., 2002).

In the recent years, several agro-industrial wastes such as papaya waste, rice husk, potato residue, wheat bran, poultry fodder, pineapple waste, orange peels etc. have been used to produce SCP (Nwufu et al., 2014; Rajoka et al., 2004; Rosma et al., 2005; Shipra and Diksit, 2017; Umesh et al., 2017; Yunus et al., 2015). Banana peel

constituted a considerable amount of lignocelluloses and other nutrients (Anhwange et al., 2009), which can be used to support microbial growth and SCP production (Yabaya and Ado, 2008). *Aspergillus niger* is the most common fungus, which is capable of fast propagation on agricultural or farming trashes and thereby enhancing the production of protein content (Anupama and Ravindra, 2000). The improved production of bio-protein might possible by selecting suitable strains and substrates as well as optimal process conditions (Jamal et al., 2008).

The successful design of a fermentation process includes optimizing the media composition, fermentation conditions, and fermenter design as well as developing superior strains by mutation (Singh et al., 2017). The classical optimization method for the medium and culture condition involves varying one parameter at a time while keeping the others at a constant level. This method is inappropriate for optimization having various disadvantages since the effects of interaction among variables are neglected and do not guarantee the determination of optimal conditions (Luo et al., 2009). As the process is time-consuming, the determination of optimum levels would be expensive and a number of experiments are required. Limitations of these problems can be eliminated by employing response surface methodology (RSM), which has successfully been used in the optimization of bioprocesses (Alara et al., 2018; Hao et al., 2010; Luo et al., 2009). RSM is useful to develop, improve, and optimize the processes, and it can evaluate the effect of the variables and their interactions (Dinarvand et al., 2017; Shishir et al., 2016).

Therefore, the present study was intended—(1) to investigate the effects of culture conditions, i.e. temperature, pH, substrate concentration, and fermentation period for the production of biomass and single cell protein using *A. niger* from banana waste as the substrate, and (2) to optimize the process parameters for the improved production of biomass and single cell protein from *A. niger*. The results of this study might open a new horizon for a wide range of industrial applications of microbial protein and could contribute to satisfying the present demand for protein.

Materials and methods

Collection of culture strain

Culture strains of *A. niger* were collected from the Laboratory of Plant Pathology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.

Inoculum preparation

Inoculum preparation (spore suspension) was carried out according to the procedure of Jamal et al. (2005) with some alteration. Potato dextrose agar (PDA, 3.9%) was taken to culture fungal strain and kept for 7 days at 32 °C. It was then shifted to Erlenmeyer flask using 100 mL of sterile distilled water followed by shaking at 150 rpm for 24 h. Then the sample was filtered and the filtrate was counted as inoculum after measuring its concentration (spores/mL). The spore suspension obtained was counted by a haemocytometer (Labtronics, Korea, Model No. 37) and spore concentration was adjusted to 2×10^7 spores/mL. The suspension inoculums were subcultured at 2 weeks interval and kept in the chiller at 4 °C until further use. All flasks, funnels, filter papers, and distilled water were sterilized in a sterilizer (LS-2D, Rexall Industries Co. Ltd., Taiwan) prior to use.

Collection and preparation of the substrate

Fresh banana (*Musa sapientum*) peels were collected from fruits vendors near to Hajee Mohammad Danesh Science and Technology University, Dinajpur. Peels were washed properly with water and then dehydrated in a cabinet dryer (Model-136–120, Seoul, Korea) at 60 °C to a constant weight to cease the destructive activity of microorganisms and biological reactions. The dried peels were grounded to prepare powder by a laboratory grinder (Jaipan CM/L-7360065, Japan) and screened to 2 mm size through a sieve (Sieve no.MIC-300). The screened powder was packed in high-density polyethylene (HDPE) pouches and kept at 4 °C until further use.

Preparation of fermentation media

Banana peel powders were degraded to convert cellulose content into more available sugars by chemical treatments. For this purpose, 10% (w/v) HCl was added to the banana peel powder in conical flask maintaining liquid to solid ratio of 10:1. The mixture was heated in a water bath (VS-1205SW1, Vision Scientific Company Ltd.) at 100 °C for 1.5 h followed by allowed to cool, and it was filtered using Whatman No. 1 filter paper. The residue was washed with 10% NaOH until neutralization. The filtrates were diluted with sterile distilled water at different concentrations and autoclaved at 121 °C for 15 min, which was used as a carbon and nitrogen source for biomass production.

Experimental design

In this study, four factors and five levels central composite rotatable design (CCRD) was employed to investigate and

optimize the effect of process variable on the maximum yield of biomass and protein. Fermentation temperature (20–40 °C), pH (4–8), substrate concentration (5–25%), and fermentation period (1–5 days) were selected as independent variables (Table 1), whereas biomass and protein were selected as the response. A total number of thirty experimental runs with six center points (used to estimate experimental error) were ascertained (Table 1). All the measurements were performed in triplicate and calculated the mean value. A second-order polynomial model was established to fit the experimental data and define the correlation between independent variables and responses. The general formula of the second-order polynomial model is presented (Shishir et al., 2016) as follows:

$$Y = \beta_0 + \sum_{j=1}^4 \beta_j X_j + \sum_{j=1}^4 \beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^4 \beta_{ij} X_i X_j$$

where, Y-response; X_i and X_j -independent variables (i and j fluctuated between 1 and 4); β_0 -model intercept coefficient; β_j , β_{jj} , and β_{ij} -interaction coefficients. Design of expert (version 11.0.5.0, State-Ease Inc., Minneapolis, USA) was utilized to construct and evaluate the experimental design and observed data. Numerical optimization of the independent variables was performed by Derringer's desired function methodology. Duplicate additional experiments were performed to confirm and validate the optimal condition.

Fermentation process

Submerged fermentation was carried out in 250 mL Erlenmeyer flask having 100 mL media as per experimental design (Table 1). In all experiment, the media was initially adjusted to different pH level according to the design using 1 N H_2SO_4 and/or 1 N NaOH to obtain the maximum biomass. Appropriate amounts of each medium were transferred into 250 mL Erlenmeyer flasks and sterilized at 121 °C for 15 min. Inoculum (2×10^7 cfu/mL) from the suspension of culture strain (microbes) was aseptically transferred into each medium. However, fermentation was conducted in a shaking incubator (Vision Scientific Co. LTD., Model No. VS-8480SN) at different conditions following the experimental design (Table 1).

Harvesting of biomass

After fermentation, the biomass was collected by centrifugation in a laboratory centrifuge (MF-300, Human Lab Instrument Co., Korea) at $3500 \times g$. The biomass was filtered by vacuum filtration using Whatman No. 1 filter paper and washed triplicates using distilled water (approximately 20 mL). It was shifted to an aluminum disk

Table 1 Central composite rotatable design (CCRD) matrix of factors and real values along with biomass and protein as a response

Independent variables	Symbols	Level				
		− 2	− 1	0	+ 1	+ 2
Temperature (°C)	A	20	25	30	35	40
pH	B	4	5	6	7	8
Substrate concentration (%)	C	5	10	15	20	25
Fermentation period (Day)	D	1	2	3	4	5
Run order	Uncoded process variables				Responses	
	Temperature (°C)	pH	Substrate conc. (%)	Fermentation period (Day)	Biomass (g/L)	Protein (%)
1 ^a	30	6	15	3	19.90	47.46
2	25	5	10	2	9.13	20.89
3 ^a	30	6	15	3	20.77	52.87
4	30	6	15	1	9.12	10.26
5	20	6	15	3	7.84	15.49
6	30	6	15	5	24.68	60.68
7 ^a	30	6	15	3	19.23	51.00
8	25	7	10	2	9.39	18.50
9	25	7	20	4	18.44	38.91
10	25	5	10	4	11.63	31.16
11	30	6	5	3	14.19	40.95
12	35	7	10	2	11.33	33.39
13 ^a	30	6	15	3	19.47	52.86
14	35	5	10	2	11.87	23.64
15	30	6	25	3	22.28	59.87
16	25	5	20	2	9.72	22.50
17	30	8	15	3	15.09	45.28
18	35	7	20	4	22.73	61.02
19 ^a	30	6	15	3	20.77	53.24
20	35	5	20	4	18.83	54.38
21	30	4	15	3	12.84	37.21
22	25	5	20	4	16.79	37.05
23 ^a	30	6	15	3	18.49	46.84
24	25	7	20	2	10.13	25.54
25	35	7	10	4	16.47	55.34
26	35	7	20	2	12.87	35.24
27	35	5	10	4	15.19	49.43
28	25	7	10	4	10.76	32.06
29	35	5	20	2	9.91	26.49
30	40	6	15	3	10.32	47.14

^aCenter point

and then dried in a hot air oven (101C-3B, Shanghai Experimental Apparatus Co. Ltd.) at 103–105 °C for 1 h followed by cooling in desiccators. The collected biomass was then weighed and analyzed for biomass protein.

Estimation of biomass protein

The protein content of the biomass was measured spectrophotometrically following the method proposed by Bradford (1976) with slight modification. 0.5 g of the sample was taken in a beaker and then 10 mL of distilled water was added into it. Then the sample was stirred with a

magnetic stirrer and filtered using a filter paper. 500 μL filtered samples were then taken into a falcon tube and diluted to 4500 μL with distilled water. Afterward, 5 mL of Bradford reagent was added and mixed by vortex (KMC-1300 V, Korea) for a few minutes. The concentration of protein in the solution was determined from the absorbance at 595 nm against the blank (containing the same reagents without sample). Protein content was determined using the following formula by a comparison of the values obtained with the standard curve of bovine serum albumin (BSA) and the results were expressed as a percentage.

$$\% \text{ Protein} = \frac{X (\text{mg/mL}) \times \text{Volume made (mL)}}{\text{Weight of sample (g)}} \times 100$$

Here, X = amount of protein (mg/mL) from standard curve equation.

Results and discussion

Model fitting and statistical analysis

The experimental data in terms of the biomass and protein yield were recorded in Table 1. These experimental values were employed as raw data in the RSM program to produce the best-predicted model and its statistical analysis. Multiple regression analysis was accompanied by the

experimental data to develop second-order polynomial equations, including linear, quadratic, and interactive terms, which can define the correlation between independent variables and responses. The developed final models with coded factors obtained after excluding the insignificant model terms are as follows:

$$\begin{aligned} \text{Biomass Yield (g/L)} = & 19.77 + 1.17A + 0.57B + 1.66C \\ & + 3.23D + 1.36CD - 2.86A^2 \\ & - 1.63B^2 - 0.57C^2 - 0.90D^2 \end{aligned}$$

$$\begin{aligned} \text{Protein Yield (\%)} = & 49.81 + 7.32A + 2.11B + 3.11C + 10.58D \\ & + 3.10AD - 5.45A^2 - 2.97B^2 - 4.41D^2 \end{aligned}$$

where, A, B, C, and D are the temperature, pH, substrate concentration and fermentation time, respectively.

Analysis of variance (ANOVA) was employed to evaluate the statistical significance of the developed models. The regression coefficients (β) and p value for the second-order polynomial equations are shown in Table 2 and it indicates that the equations adequately represent the correlation between the response and their significant variables. ANOVA of the regression analysis (Table 2) reveals the models' significance was corroborated by high F -value (40.49 for biomass and 49.12 for protein) with a very low p value ($p < 0.0001$). These indicate that the developed models could explain most of the variation in the response. The high values of coefficient of determination (R^2)

Table 2 Analysis of variance (ANOVA) for response surface quadratic model for the production of biomass and protein

Source	df	Biomass				Source	df	Protein			
		β	Sum of square	F value	p value			β	Sum of square	F value	p value
Model	9	19.77	656.71	40.49	< 0.0001	Model	8	49.81	5791.80	49.12	< 0.0001
Temperature (A)	1	1.17	33.06	18.35	0.0004	Temperature (A)	1	7.32	1285.02	87.18	< 0.0001
pH (B)	1	0.57	7.67	4.26	0.0523	pH (B)	1	2.11	106.76	7.24	0.0137
Substrate Conc. (C)	1	1.66	66.15	36.71	< 0.0001	Substrate conc. (C)	1	3.11	231.73	15.72	0.0007
Fermentation period (D)	1	3.23	250.99	139.29	< 0.0001	Fermentation period (D)	1	10.58	2687.52	182.32	< 0.0001
CD	1	1.36	29.76	16.51	0.0006	AD	1	3.10	154.17	10.46	0.0040
A ²	1	- 2.86	223.62	124.10	< 0.0001	A ²	1	- 5.45	830.62	56.35	< 0.0001
B ²	1	- 1.63	73.27	40.66	< 0.0001	B ²	1	- 2.57	246.17	16.70	0.0005
C ²	1	- 0.57	8.82	4.89	0.0387	D ²	1	- 4.41	544.19	36.92	< 0.0001
D ²	1	- 0.90	22.31	12.38	0.0022	Lack of fit	16		268.25	2.03	0.2229
Lack of fit	15		31.99	2.64	0.1449						
R^2			0.9480			R^2			0.9493		
Adjusted- R^2			0.9246			Adjusted- R^2			0.9299		
Predicted- R^2			0.8419			Predicted- R^2			0.8504		
Adequate precision			21.456			Adequate Precision			24.822		
C.V. %			8.95			C.V. %			9.71

Fig. 1 Diagnostic plots for model competency of biomass (A–C) and protein (D–F)

(0.9480 for biomass and 0.9493 for protein) clearly verified that the established models are precise, which exhibits a good correlation between the response and independent variables. The value of adjusted- R^2 for biomass (0.9246) and protein (0.9299) was also high, referring to a good relationship between the experimental and predicted values. In this study, the predicted- R^2 values for biomass (0.8419) and protein (0.8504) were in line with the adjusted- R^2 (0.9246 for biomass and 0.9299 for protein). Moreover, the low CV (coefficient of variance) values (Table 2) for biomass (8.95%) and protein (9.71%) referred to the low deviations between experimental and predicted values, a high degree of precision and reliability in experimental design (Shishir et al., 2016). Adequate precision was observed to be greater than 4, which indicates the best fit of developed models. The “fitness” of the models were assessed through the lack of fit test, which was not significant ($p > 0.05$) relative to the pure error indicating the acceptability of models (Shishir et al., 2016).

Analysis of model competence

It is essential to check the model suitability because it can produce ambiguous results unless the model shows a reasonable fit. Therefore, the model adequacy was assessed by several influential plots (predicted vs actual, normal % probability vs internally studentized residual, and predicted values vs internally studentized residual) and the results were shown in Fig. 1A–C for biomass and Fig. 1D–F for protein. It was found that the data stretched out on the diagonal lines indicated a good relationship between experimental and predicted data. The residual values were low and seemed to be distributed randomly within the range. Therefore, it could be mentioned that the established model is precise and could predict the experimental data as shown in Fig. 1A–F.

Influence of process variables on the responses

The main and interactive effects of independent variable e.g. temperature, pH, substrate concentration and fermentation period were observed on the response variables (biomass and protein). Following sections describe the details about the influences of these variables on the responses.

Effect on biomass production

The yield of biomass from *A. niger* was ranged from 7.84 to 24.68 g/L (Table 1). All the process parameters, i.e. temperature, pH, substrate concentration, and fermentation period showed a positive and significant ($p < 0.05$) effect on the biomass yield (Table 2). For biomass response, the

Fig. 2 Response surface plot showing the effect of process parameters on biomass production: 2D contour plot (A) and 3D contour plot (B)

magnitude of β value (Table 2) indicates that fermentation period ($\beta = 3.23$) had the maximum positive effect on biomass yield followed by substrate concentration ($\beta = 1.66$), temperature ($\beta = 1.17$) and pH ($\beta = 0.57$), respectively. The positive signs of quadratic linear terms (CD) revealed that biomass production increased with the increase of linear independent variables. However, all the quadratic square terms (A^2 , B^2 , C^2 , and D^2) exhibited a negative impact on biomass yield (β value: -2.86 , -1.63 , -0.57 , and -0.90 , respectively), which were significant at 5% level of significance as presented in Table 2.

The 2D and 3D contour plots as shown in Fig. 2(A, B, respectively) were generated for the fitted model to visualize the combined effect of different variables on biomass yield. Only the interaction term of substrate concentration and fermentation period (CD) had a significant ($p < 0.05$)

influence on the biomass production (Table 2). Increase in fermentation period (2–4 days) with a combination of substrate concentration (10–20%) resulted in an intensification of biomass yield, while other parameters were constant at their middle value (Fig. 2). These results were corroborated by other studies for enzyme production using microbial strains (Managamuri et al., 2016; Mohan et al., 2014). In addition, carbon and energy sources are the most important nutrient required for the growth of microorganism and biomass formation (Nasseri et al., 2011; Suman et al., 2015). Microorganisms can transform the banana peel into reducing sugar during fermentation process due to the presence of high amounts of lignocellulosic compounds. Therefore, sugar components of the substrate are metabolized by fungal strains resulting in enhanced production of biomass (Saheed et al., 2016; Yabaya and Ado, 2008). Similar outcomes were also found by previous researchers (Essien et al., 2005).

Effect on protein content

The F and p values in Table 2 reveal that all the process variables exhibited a positive and significant effect on the protein yield from biomass. The β -value in Table 2 clearly illustrates that fermentation period ($\beta = 10.58$) is the most dominant factor on the protein yield followed by temperature ($\beta = 7.32$), substrate concentration ($\beta = 3.11$) and pH ($\beta = 2.11$). This outcome revealed that protein production increased with the increase of the fermentation period. This is possibly due to the high content of lignocellulosic compounds in the banana peel, which are converted to sugars during fermentation that helped to the proper distribution of nutritional contents required for the growth of microorganisms at different fermentation period (Isaac and Chiedu, 2016). It is clearly identified in Table 2 that all the quadratic square terms of temperature, pH, and fermentation period (A^2 , B^2 , and D^2) had negative and significant ($p < 0.05$) effect on protein content with the exception of the substrate concentration (C^2).

To obtain a better understanding of the parameters effect, response surface 2D and 3D contour plots were illustrated in Fig. 3(A, B, respectively) from the developed mathematical model for protein content in the biomass. The interaction between temperature and fermentation period (AD) was found to be a positive and statistically significant on the protein content (Table 2). The results exhibited that the protein content increased with the increasing temperature from 25 to 35 °C and fermentation period from 2 to 4 days (Fig. 3). Generally, the temperature of the cultivation medium is one of the most important variables for the growth of microbes. The range of temperature affects growth rate, nutritional requirement, as well as chemical and enzymatic composition of the cells

Fig. 3 Response surface plot showing the effect of process parameters on protein: 2D contour plot (A) and 3D contour plot (B)

(Umesh et al., 2017). The optimum temperature for the growth of *Aspergillus* species was reported to the range between 30 and 35 °C, which supports the present findings (Dinarvand et al., 2017; Shehu and Bello, 2011).

Optimization of the process parameters

Numerical optimization was performed using the developed models for the biomass and protein yield. The independent variables, such as temperature, pH, substrate concentration, and fermentation period were set in the range of 20–40 °C, 4–8, 5–25%, and 1–5 days, respectively. While the responses, i.e. biomass and protein were set at maximum (since we desired to be the highest yield of biomass and protein). The design expert program was run for the optimum conditions and the solutions. The best solution was found with maximum desirability (99.9%) selected as the optimum conditions for enhanced

Table 3 Comparison of experimental values with predicted values at the optimized condition

Response	Predicted value	Experimental value (Mean $\pm$ SEM ^a)
Biomass (g/L)	24.69	23.74 $\pm$ 0.27
Protein (%)	61.23	60.15 $\pm$ 0.21

^aStandard error of mean (n = 3)

production of biomass and protein content. Therefore, the predicted optimum conditions were obtained as the temperature of 31.02 °C, pH of 6.19, substrate concentration of 19.92%, and the fermentation period of 4 days.

Verification of the models

To verify the established model, duplicate experiments were carried out under the recommended optimum condition with a slight modification in temperature by 31 °C, pH by 6 and substrate concentration by 20% in exchange of 31.02 °C, 6.19, and 19.92%, respectively. The experimental values and predicted values of various responses are presented in Table 3. The obtained experimental values were adequate with the predicted values of the response surface model because the experimental values were very close to the predicted values (Table 3), which satisfy the predicted model.

In conclusion, from the experimental results, second order polynomial mathematical models were developed for the responses with a high coefficient of determination (R^2) values. The optimal conditions to obtain the maximum yield of biomass and protein were determined as follows: temperature of 31.02 °C, pH of 6.19, substrate concentration of 19.92%, and the fermentation period of 4 days. Under these optimal conditions, experimental yield (biomass of 23.74 $\pm$ 0.27 g/L and protein of 60.15 $\pm$ 0.21%) was very close to the predicted values (biomass of 24.69 g/L and protein of 61.23%), indicating the suitability of developed models within the acceptable range of the responses. Overall, the results of the present study suggest that the model obtained through response surface methodology is adequate for the improved production of biomass and protein from *A. niger* using banana peel as a low-cost substrate. This work highly appreciates further studies on the large-scale production as well as exploring the safety levels of the produced biomass before using as protein.

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Compliance with ethical standards

Conflict of interest We the authors have no conflict of interest to disclose in this work.

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