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Research Article

Optimizing Bioethanol Production Via Consolidated Bioprocessing: The Potential of *Aspergillus niger* B2484

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ABSTRACT

Consolidated bioprocessing (CBP) integrates enzyme secretion, hydrolysis, and fermentation into a single-step process, eliminating the need for costly separate enzyme production in bioethanol manufacturing. While CBP aims to utilize naturally occurring cellulolytic microbes, no single microorganism has been identified to efficiently perform all required processes. One of the key challenges in CBP is optimizing culture conditions to maximize bioethanol yield. This study investigates the potential of *Aspergillus niger* B2484 as a single-culture bioethanol producer and optimizes the physicochemical parameters for converting pretreated paddy straw into bioethanol through CBP. Key parameters, including saccharification and fermentation duration, temperature, substrate loading, and medium composition, were evaluated using the One-Factor-At-a-Time (OFAT) method and further optimized via Response Surface Methodology (RSM). The optimal conditions were determined to be 66.7 hr of saccharification at 29.8°C, followed by 32.3 hr of fermentation at 30.2°C, with a substrate loading of 2.6% (w/v) and a medium level of 14.8% (v/v). The actual ethanol yield (0.63 g/L) closely matched the RSM-predicted yield (0.61 g/L), confirming the reliability of the optimization model. This study demonstrates the feasibility of *A. niger* B2484 as a single-culture bioethanol producer in CBP, highlighting its potential for commercial application either as a standalone microbial agent or as part of a customized enzymatic system to enhance bioethanol yield.

Key words: Bioethanol, consolidated bioprocessing, OFAT method, response surface methodology, single culture

INTRODUCTION

Over the years, the governments and industries have expanded their research scope to explore, innovate, and promote new technologies for an alternative fuel such as bioethanol, which is derived from plant biomass or agrowaste (Ali *et al*., 2016). Bioethanol production involves the conversion of lignocellulosic raw material into fermentable sugar and, consequently, converting it into ethanol by microbial fermentation (Alabdalall *et al*., 2023). However, the conversion process can be extremely challenging as the protective structural and biochemical nature of the lignocellulose will hinder the saccharification of the polysaccharides to fermentable sugars (Wu *et al*., 2018). Bioethanol production from cellulosic materials has been successfully commercialized, with a few production plants existing across the globe based on various technologies and a few approaches for fermentation, such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) (Duarah *et al*., 2022; Singhania *et al*., 2022). Normally, the lignocellulosic materials to sugars and later into bioethanol requires a multistep process, but the steps can be reduced by developing a strategy that can convert lignocellulosic materials directly into bioethanol in a single step, known as consolidated bioprocessing (CBP) (Devi *et al*., 2022).

The highest point of the integration process for bioethanol production is achieved by CBP, in which all biological processes such as enzyme production, degradation of cellulose into glucose, fermentation of hexoses (galactose, glucose & mannose), and fermentation of pentoses (arabinose & xylose) are performed in a single-step process (Bušić *et al*., 2018; Dempfle *et al*., 2021). This strategy improves the efficiency of cellulose conversion and reduces the overall cost of bioethanol production (Periyasamy *et al*., 2023). However, few studies show that CBP produces low ethanol yields and requires a longer fermentation period of more than 3 to 4 days (Saini *et al*., 2015). In addition, the development of CBP demands in-depth research to find a powerful microorganism that can perform SSF without using any additional enzymes (Cunha *et al*., 2020).

Although fungi are not widely recommended as CBP microorganisms, several filamentous fungi species have been reported to have the ability to directly convert cellulose to bioethanol, such as *Trichoderma*, *Aspergillus*, *Neurospora*, *Monilia*, *Rhizopus*, and *Fusarium* (Kamei *et al*., 2012; Olguin-Maciel *et al*., 2019). Existing research has evaluated the potential of fungi to produce bioethanol via CBP, considering the natural propensity of certain fungi species to degrade lignocellulosic material in the environment, and several species are usually exploited for commercial production of cellulases, such as *Aspergillus* and *Trichoderma* spp. (Ghazal *et al*., 2016; Wilkinson *et al*., 2017).

According to Conesa *et al*. (2017), the cellulase secreted by *Trichoderma reesei* has been the most powerful enzyme to

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break down the crystalline structure of cellulose, but the activity of converting cellobiose into glucose has proven to be slow due to the species' inability to produce a relatively strong β-glucosidase enzyme. On the contrary, *A. niger* is presented with its abundant amount of β-glucosidase, which is commonly applied to complete the cocktail of cellulolytic enzymes for the saccharification of lignocellulosic materials (Zhao *et al*., 2020). This fungal species is valuable in the industry for its ability to raise levels of proteins secreted, has a high capacity for fermentation, and produces a variety of enzymes for diverse applications, including the integration of enzymes for the hydrolysis of polysaccharides in the plant cell wall (Junior *et al*., 2014). The hydrolysis of paddy straw by *A. niger* yielded the highest number of monosaccharides compared to *T. harzianum* and relatively produced the highest yield of ethanol when integrated with *Saccharomyces cerevisiae* for fermentation at optimal conditions (Ahmad *et al*., 2018). According to Mbaneme-Smith and Chinn (2015), *A. niger* has great potential to secrete cellulolytic enzymes required for the saccharification of raw materials to monosaccharides, consequently converting the sugars into ethanol by fermentation under aerobic conditions as this strain is reported to have capabilities to produce feruloyl esterases, which can separate different links in the structure of cellulose and relatively improve the entire hydrolysis performance.

However, until today, there are no natural microorganisms with great performance for CBP in bioethanol industries that have been discovered yet. Hence, creating and finding a single culture or microbial consortium with high efficiency for CBP is necessary (Kricka *et al*., 2014; Jouzani & Taherzadeh, 2015). *Aspergillus niger* B2484 was selected in reference to an earlier study by Syazwanee *et al*. (2019), where this strain was able to produce the highest endoglucanase (5.60 ± 0.43 U/mL)**.** There are several optimization strategies that have been implemented involving CBP, and it is necessary to perform an optimization study to optimize the parameters involved in the conversion of lignocellulosic materials into bioethanol (Madhuvanthi *et al*., 2022). Hence, this study aims to evaluate the potential of *Aspergillus niger* B2484 as a promising single-culture candidate for bioethanol production and to optimize its physicochemical parameters via the OFAT method and Response Surface Methodology (RSM) using Design Expert Software.

MATERIALS AND METHODS

Collection of fungal isolates

Aspergillus niger strain B2484 was obtained from the Laboratory of Mycology at the Department of Biology, Faculty of Science, Universiti Putra Malaysia, and cultured on Potato Dextrose Agar (PDA) for 7 days at 28 ± 2°C.

Culture medium conditions

The spore suspensions of *A. niger* B2484 were collected from completely sporulated colonies and left suspended in sterilized distilled water. The concentrations of the spore suspensions were adjusted to 1 × 10^6 spores/mL using a haemocytometer (Mauch *et al*., 1988).

In a conical flask, 25 mL basal medium was prepared with 10% (v/v) (KH_2PO_4 2.0 g/L; $MgSO_4.7H_2O$ 0.3 g/L; $(NH4)_2SO_4$ 1.4 g/L; $CaCl_2$ 0.3 g/L; $CoCl_2$ 2.0 g/L) mixed with 1% (w/v) pretreated paddy straw (size 5 mm pretreated with 2% (w/v) NaOH) (Syazwanee *et al*., 2018) and 1 mL of trace element ($FeSO_4.7H_2O$ 5.0 g/L; $MnSO_4.H_2O$ 1.56 g/L; $ZnSO_4.7H_2O$ 1.4 g/L), sterilised for 15 min at 121 ± 0.5°C. An amount of 1 × 10^6 spores/mL *A. niger* B2484 was inoculated once the culture was cooled to room temperature (approximately 28 ± 0.5°C).

One-factor-at-a-time (OFAT) method

Several factors that affect the production of bioethanol were analyzed by performing OFAT experiments. The culture was prepared by mixing 1 × 10^6 spores/mL of *A. niger* B2484 into a sterilized 10% (v/v) basal medium culture and supplemented with 1% (w/v) pretreated paddy straw. The culture was left incubated for 3 days of saccharification, followed by another 3 days of fermentation based on the preliminary study at 150 rpm, 30 ± 0.5°C. Six parameters for bioethanol production were subjected to OFAT method are number of days (1 to 5 days) and temperature of saccharification (25°C - 45 ± 0.5°C), number of days (1 to 5 days) and temperature of fermentation (25°C - 45 ± 0.5°C), level of media (10% - 90%, v/v), and substrate concentration (1% - 7%, w/v). The Megazyme® Ethanol Assay Kit (Megazyme, Ireland) was used to assess the amount of bioethanol produced following the procedure by the manufacturer (Cutzu & Bardi, 2017). The absorbance difference (ΔA) at 340 nm was measured before and after the addition of alcohol dehydrogenase (EC 1.1.1.1). Ethanol concentration was calculated relative to the standard according to Equation (1):

$$Ethanol\left(\frac{g}{L}\right) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard} \times F$$

Where:

ΔA_{sample} = absorbance change of the sample at 340 nm

$\Delta A_{standard}$ = absorbance change of the ethanol standard

$C_{standard}$ = concentration of ethanol standard (g/L), typically 0.05 g/L

F = dilution factor applied to the sample before the assay

Response Surface Methodology

The optimization of the RSM set-up was fulfilled via Design-Expert 6.0.8 software (Stat-Ease Inc., Minneapolis, MN, USA) along with the quadratic model that has been fully expressed. The optimum points for each response were estimated based on

the input variable and the second order of polynomials using the quadratic model.

All factors were studied in five levels (-α, -1, 0, +1, +α) via Central Composite Design (CCD), where the coded variables were represented as the axial point (±1), the central point (0), and the predicted point (±α) (Table 1). All experimental runs in the RSM design were conducted in triplicate to ensure reproducibility, and the mean values were used for analysis. The quantified amount of ethanol for each setup was subjected to analysis of variance (ANOVA).

Table 1. The optimizations of each variable with five coded levels in the Central Composite Design

Variables	Symbol	Coded				
		-α	-1	0	+1	+α
Independent variables						
Saccharification hour (hr)	A	-7.88	36.00	60.00	84.00	127.88
Saccharification temperature (°C)	B	22.43	27.00	29.50	32.00	36.57
Fermentation hour (hr)	C	-31.88	12.00	36.00	60.00	103.88
Fermentation temperature (°C)	D	22.43	27.00	29.50	32.00	36.57
Level of media (%, v/v)	E	-13.28	5.00	15.00	25.00	43.28
Level of substrate (%, w/v)	F	-0.33	1.5	2.5	3.5	5.33

RESULTS

One-Factor-At-A-Time (OFAT) method

The OFAT method was carried out in accordance with the sequence of the first parameter evaluated, which was the fermentation temperature, while keeping the rest of the parameters constant (Table 2). The temperature ranges were set between 25 °C to 45°C. Based on the first OFAT method, the highest ethanol produced was at 30°C (0.04 ± 0.02 g/L) after 3 days (72 hr) of saccharification at 30°C and 3 days (72 hr) of fermentation using 1% (w/v) paddy straw substrate in 10% (v/v) media level. At any other temperature, the fermentation failed to produce any amount of ethanol. The number of days for both saccharification and fermentation processes was set from 1 to 5 days. However, the results show that the most optimal days of the saccharification process were 3 days (0.04 ± 0.02 g/L), and the amount of ethanol produced started to decline from Day 4 onwards. The number of days for the fermentation process shows that the highest production of ethanol was on Day 1 of fermentation, but the amount of ethanol was decreasing as the incubation time exceeded more than 1 day. Despite the estimation of 6 days for both saccharification and fermentation processes, the OFAT method revealed that the optimal total number of days for both processes was only 4 days (96 hr). Until these parameters were met, there were no significant changes or improvements in terms of the volume of bioethanol produced. The amount of ethanol started to rise after the fourth parameter was tested: the substrate level (1–7%, w/v). The substrate level is the amount of pretreated paddy straw mixed with the base media. The 2% (w/v) substrate level produced 0.83 ± 0.05 g/L of ethanol, which is a lot more than the previous parameters. The amount of ethanol keeps going up as the OFAT method moves on to the fifth parameter, the level of media (%, v/v), which is also known as the amount of basal media used in the CBP process. The optimization of media (%, v/v) for *A. niger* B2484 shows an improvement in the amount of ethanol produced, from 0.83 ± 0.05 g/L to 0.90 ± 0.03 g/L using 20% (v/v) media. The last parameter tested for the OFAT method was the saccharification temperature. In this study, the optimum temperature of saccharification for *A. niger* B2484 was 30 ± 0.5°C. The volume of ethanol produced by *A. niger* B2484 increases as it reaches 30°C, but drops as the temperature rises. With the last parameter's result, the OFAT method found that 30 ± 0.5°C was the best temperature for both the fermentation and saccharification processes in CBP, as shown in Figure 1.

Table 2. The pre-selected ranges used in the OFAT method and the suggested optimal condition for the highest bioethanol production by *Aspergillus niger* B2484

Parameters tested	Control setting	Ranges	Optimum point
Fermentation temperature	30 ± 0.5°C	25°C - 45 ± 0.5°C	30°C
Saccharification days	3 days	1 day – 5 days	3 days
Fermentation days	3 days	1 day – 5 days	1 day
Level of substrate	1%	1% - 7%	2%
Level of media	10%	10% - 90%	20%
Saccharification temperature	30 ± 0.5°C	25°C - 45 ± 0.5°C	30°C

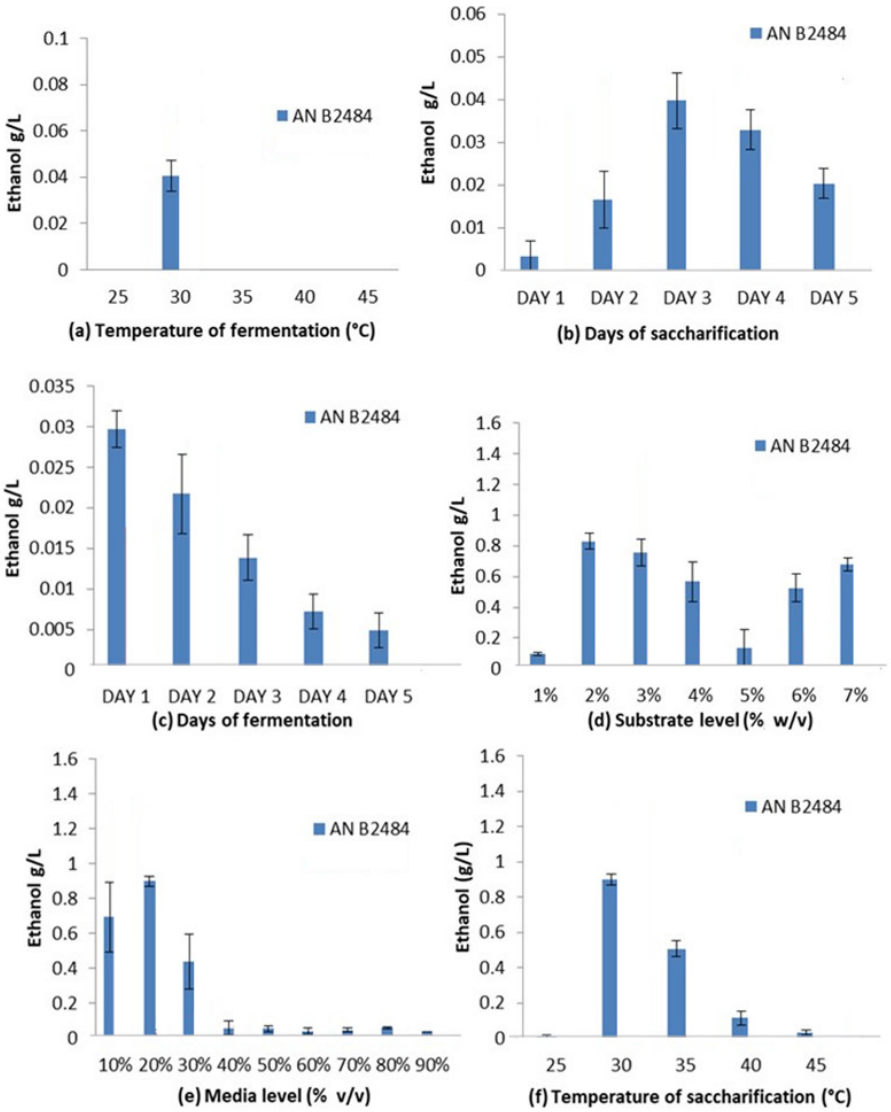

Fig. 1. Optimization of all significant parameters using the OFAT method, which were carried out in a sequential pattern from parameter (a) temperature of fermentation to parameter (f) temperature of saccharification using *A. niger* B2484.

Optimization of set-up using Central Composite Design (CCD) via Response Surface Methodology (RSM) analysis

Analysis using RSM software was an attempt to understand the interactions between the parameters tested. In this analysis, the time taken for both saccharification and fermentation processes was converted into hr instead of days for higher accuracy and precision. The software generated 86 settings, including 10 center points, and each of the setups was tested in triplicate. The fit summary compiles the key statistics used to determine an appropriate starting point for the final model. Model adequacy was validated by examining the statistical properties reported in the fit summary table, including ANOVA, R^2, adjusted R^2, predicted R^2, and adequate precision. The ANOVA test evaluated the relationship between the coded variables (A, B, C, D, E & F) in ethanol production by *A. niger* B2484 (Table 3). The obtained F-value of 3.24 for *A. niger* B2484 indicates that the model is significant, with only a 0.01% probability that this result is due to noise. The null hypothesis was rejected as the Prob > F value was <0.05, suggesting less than a 5% chance that the regression parameters were zero. The range of R-square (R^2) model was between 0 to 1, and the model can be expressed as a larger section of variance as the value closer to 1. The value of R^2 for model *A. niger* B2484 was 0.60, indicating the calculated model could explain 60% of the results (Table 4). Although this value is lower than the ideal (>0.9), it is still acceptable in biological systems where inherent variability often reduces the predictive power of statistical models. A negative value for predicted R^2 indicates the total mean is a better predictor of the variables compared to the existing model. This was suggested for the *A. niger* B2484 model, with an adjusted R^2 of 0.42 with predicted R^2 was -0.01. The ratio of signal-to-noise in Adequate Precision should be at least 4 and above. In this study, *A. niger* B2484 had an adequate precision ratio of 8.23, which shows that the signal was strong enough and the model was fit for estimation. Although the model's predicted R^2 value was insufficient, the ratios of adequate precision demonstrated the model's ability to navigate the design space, thereby enabling effective navigation of three-dimensional (3D) structures (Figure 2).

Table 3. Statistical summary of ethanol production by *A. niger* B2484

Source	Sum of squares	DF	Mean square	F-value	Prob > F
Model	3.08	27	0.11	3.24	< 0.0001
Hours of saccharification (A)	0.99	1	0.99	28.24	< 0.0001
Temperature of saccharification (B)	0.03	1	0.03	0.72	0.40
Hours of fermentation (C)	0.04	1	0.04	1.06	0.31
Temperature of fermentation (D)	0.10	1	0.10	2.69	0.11
Medial level (E)	0.03	1	0.03	0.98	0.33
Substrate level (F)	0.06	1	0.06	1.80	0.19
Residual	2.04	58	0.04		
Lack of fit	2.04	49	0.04		
Pure error	0.00	9	0.00		
Correlation total	5.12	85			

Table 4. The summation of statistical analysis on the quadratic model of *Aspergillus niger* B2484

Source	Value
Std dev.	0.19
Mean	0.22
R-Squared (R^2)	0.60
Adjusted R^2	0.42
Predicted R^2	-0.01
Adequate Precision	8.23

$$Y = + 0.58 + 0.11A + 0.02B + 0.02C + 0.03D + 0.02E + 0.03F -0.07A^2 - 0.07B^2 -0.06C^2 - 0.05D^2 - 0.07E^2 - 0.06F^2 - 0.01AB + 0.02AC + 9.25E\text{-}003AD + 7.25E\text{-}003AE + 0.01AF - 5.50E\text{-}003BC + 0.01BD - 4.97E\text{-}003BE - 0.02BF + 0.02CD - 0.01CE + 0.02CF + 8.75E\text{-}004DE + 9.41E\text{-}003DF - 7.03E\text{-}003EF \quad \text{- Equation 2}$$

Y represents *A. niger* B2484's ethanol production in the CBP process (Equation 2). The plus and minus symbols in these equations represented interaction and incompatible consequences between the parameters, respectively. Thus, the model shows a positive interaction resulted in the production of ethanol by *A. niger* B2484 between A, B, C, D, E, F, AC, AD, AE, AF, BD, CD, CF, DE, DF, while other terms showed antagonistic effects. The three-dimensional (3D) plots (Figure 2) embody the visual illustration of the regression equation to establish the optimum values of the independent variables within the considered ranges. The form of the corresponding contour reveals the significant interactions between the independent variables.

Comparison between the predicted amount of ethanol production (0.61 g/L) with the actual amount of ethanol production (0.63 g/L) was calculated to validate and subsequently verify the RSM set-up (Table 5). The results show there was no significant difference, hence confirming the stability and reliability of the setup. The response of *A. niger* B2484 using the setup by RSM was lower (0.63 g/L) compared to the value of ethanol produced using the OFAT method (0.90 g/L).

DISCUSSION

Optimizing important parameters such as hour (hr) and temperature (°C) of saccharification, hour (hr) and temperature (°C) of fermentation, media level (%, v/v) as well as substrate concentration (%, w/v) are extremely important as these parameters play a crucial part in the ethanol production from pretreated paddy straw and have the potential to improve the performance of *A. niger* B2484 in CBP. However, choosing an optimum temperature set-up during operation has become a challenge in CBP as cellulolytic enzymes and ethanologenic microorganisms such as *Saccharomyces cerevisiae* work at different temperatures (Cunha *et al*., 2020). Since the nature of ethanol is volatile, temperature has a huge impact on the amount produced. Typically, researchers set the temperature range for culturing microorganisms between 28°C and 30°C and observed that a temperature exceeding 30°C inhibits the microorganism's growth, thereby ceasing ethanol fermentation (Gawande & Patil, 2018). Ethanol production under mesophilic conditions is considered more favourable, not only because it enhances fermentation efficiency but also because it improves economic viability by reducing energy requirements, which becomes an advantage particularly relevant in countries with temperate climates (Hashem *et al*., 2021). Dinarvand *et al*. (2017) reported that the optimum growth temperature for *Aspergillus niger* lies within the range of 30–35°C, which is consistent with the present findings.

The incubation period during CBP also affects the efficiency and productivity of bioethanol production; hence, the incubation period was set within 4 days for both saccharification and fermentation processes to take place. According to Aggarwal *et al*. (2017), the endoglucanase production by *A. niger* BK01 yielded a maximum level of 9.06 ± 0.06 U/gds after 96 hr (4 days) of incubation period, and the enzyme production started to decline afterwards. Extending the incubation period beyond 4 days resulted in a decrease in ethanol production, as the prolonged incubation period weakened and suppressed the microorganisms' metabolism due to unfavourable conditions (Wu, 2019). However, it depends on the species of lignocellulosic fungi, as Dahir & Al-Dossary (2023) reported that *Aspergillus flavus* produced the highest amount of bioethanol at 5.570 g/L and 6.363 g/L after 4 days of incubation using barley and corn waste, respectively.

The substrate loading range was set low (<10% w/v), as a high substrate concentration can severely reduce the amount of ethanol yield for commercialization and raise the production cost, particularly in the distillation process (Zhao *et al*., 2015). Commonly, the greater the substrate loading, the greater the amount of glucose is expected. However, adjusting the substrate loading between 2% and 5% swiftly increased the ethanol yield (Zeghlouli *et al*., 2021). As the substrate loading goes up, more sugars become accessible for the saccharification process. However, because the number of microbial cells added is not

proportional to the substrate loading, the cells will become unable to digest the large amounts of sugar, which will lead to a low ethanol yield (Olawale *et al*., 2021). Amiri *et al*. (2014) reported that a 5% (w/v) solid loading of pretreated paddy straw produced 1.43 g/L ethanol, 5.10 g/L butanol, 1.23 g/L butyric acid, 1.18 g/L acetic acid, and 1.34 g/L acetone. Another key factor affecting the fermentation of ethanol is the medium volume in the culture conditions. The ideal range for media level is between 10% to 15% (v/v). Further increment in medium volume will cause a high level of aerobic metabolism, where the fungi will only use sugar substrate with no ethanol production (Arifa & Sarwar, 2012; Syazwanee *et al*., 2022). Choosing a suitable culture medium for bioethanol production is imperative, as a high concentration of sugar in culture media can lead to substrate inhibition and result in low ethanol production due to osmotic stress (Chang *et al*., 2018).

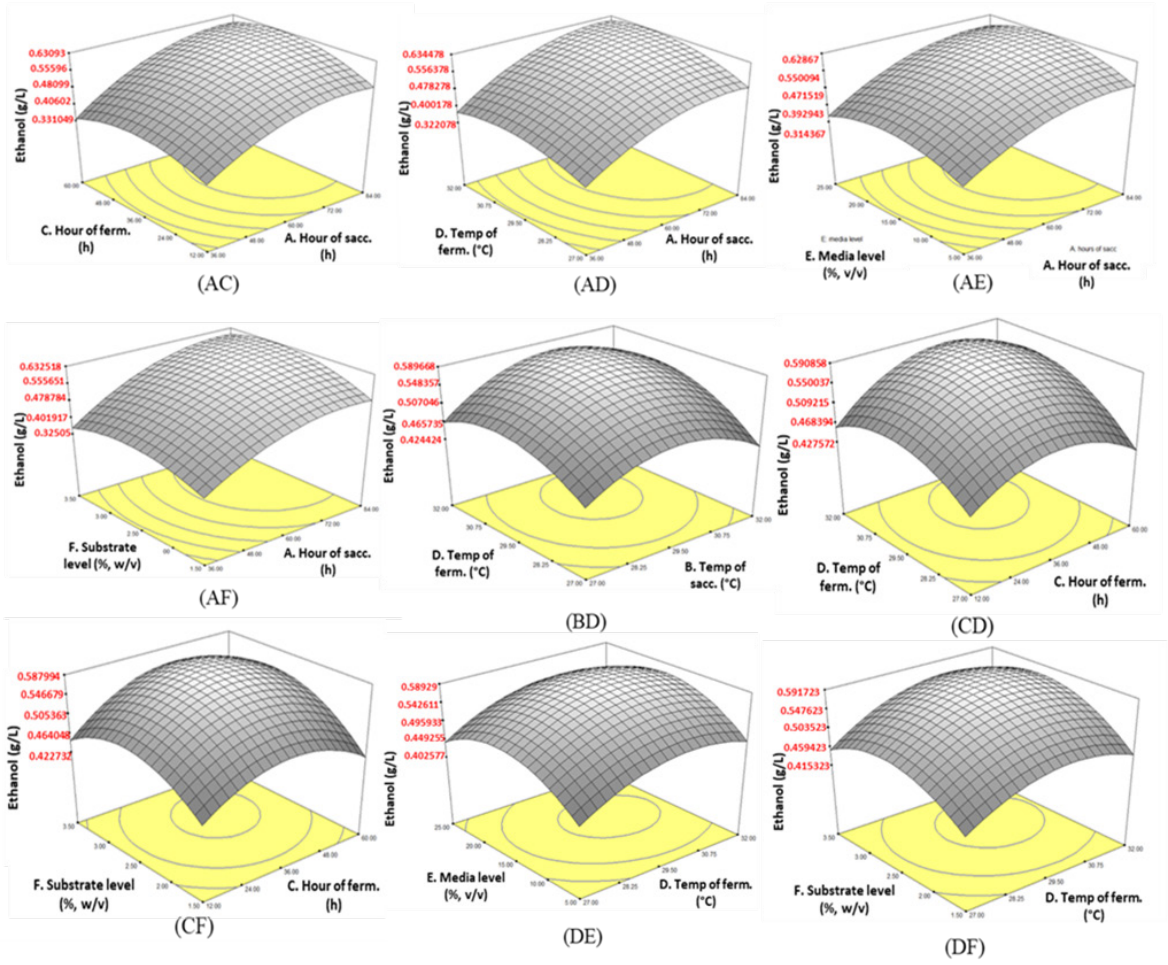

Fig. 2. The interactions between all independent variables can be observed on the 3D surface plot generated by RSM.

Table 5. Comparison between the optimized culture condition set-up generated by RSM and the set-up by the OFAT method

	Saccharification		Fermentation		Basal media		Ethanol (g/L)	
	Hours (hr)	Temp. (°C)	Hours (hr)	Temp. (°C)	Media (% v/v)	Substrate (% w/v)	Predicted (g/L)	Actual (g/L)
RSM Set-up	66.75	29.76	32.3	30.18	14.74	2.59	0.61	0.63
OFAT method	72	30	24	30	20	2		0.90

As mentioned by Syazwanee *et al*. (2021), a combination of *A. niger* B2484 and *T. asperellum* B1581, with a ratio of 5:1, respectively, produced the most volume of bioethanol (1.03 g/L). The goal of co-culturing two different species in CBP is to enhance bioethanol productivity by providing several functional factors or resources. This approach is more effective as the synergistic connection in the metabolic pathways among each strain is established (Du *et al*., 2020). However, combining two different species within the same microbial environment will lead to involuntary relations that disrupt the consortium dynamics, potentially creating an unstable condition where a rapidly growing community could cause a slow-growing community to decline (Duncker *et al*., 2021). Moreover, the primary challenge associated with the utilisation of co-culturing is the intricacy of managing individual microorganisms and the entire system simultaneously. Therefore, it is advantageous to have a single microbial population that can carry out all the operations in CBP without any complications, thereby minimising production costs (Sarabana *et al*., 2015).

This study is the first to explore the potential of using *A. niger* B2484 crude enzyme as a single culture in CBP for direct ethanol fermentation, a method not previously documented in any reports. Response Surface Methodology (RSM) has generated a new

setup for the optimisation of bioethanol production using *A. niger* B2484, based on the collection of all OFAT method results. Despite RSM settings produced a lesser amount of ethanol than OFAT settings, the stability and reliability of the model has led to the selection of the RSM setting over the OFAT set-up as this method fails to develop relationships among the variables, leading to incorrect optimum setting for culture conditions, especially when the interactions between different variables are significant (Humbird & Fei, 2016). Typically, R^2 and Q^2 values above 0.5 are considered indicative of a well-fitted model, and values slightly below this threshold can still be acceptable, although showing weaker models (Zhu *et al*., 2025). The interpretation of R^2 values varies across scientific disciplines. In physical sciences and engineering, values above 0.70 are typically considered strong, whereas in ecological and biological studies, values as low as 0.20–0.50 may still be regarded as acceptable due to the inherent variability of living systems (Lin & Wiegand, 2023; Gupta *et al*., 2024). In the present study, the R^2 value of 0.60 obtained for *A. niger* B2484 is therefore within an acceptable range and considered adequate, as it reflects the complex biological variability of fermentation processes while still providing predictive value.

A low bioethanol yield was obtained from lignocellulosic materials, as the strain was unable to efficiently catabolize pentose sugars derived from hemicellulose (Pang *et al*., 2018). Although *A. niger* B2484 demonstrated the ability to produce hemicellulase (56.85 U/mL) for hemicellulose degradation (Syazwanee *et al*., 2019), the effective utilization of the substrate was still limited by the recalcitrant structure of paddy straw. Commonly, *A. niger* B2484 is considered a non-fermentative organism, but this strain has successfully displayed its potential as a single organism to carry out direct conversion of cellulosic material into bioethanol in CBP despite producing a low amount of ethanol (0.63 g/L). Although the ethanol yield obtained through RSM optimization (0.63 g/L) was lower than the maximum value observed under OFAT conditions (0.90 g/L), the RSM approach is considered more reliable, as it accounts for interactions among variables, reduces the number of experimental runs, and provides a statistically validated predictive model within a shorter timeframe (Izmirlioglu & Demirci, 2016). Despite its drawbacks, the CBP approach requires relatively low water and energy inputs, making it a promising strategy for further investigation as it offers potential pathways toward sustainable energy solutions (Wilkinson *et al*., 2017).

CONCLUSION

The OFAT method revealed that the most favourable conditions for *A. niger* B2484 were 3 days of saccharification and 1 day of fermentation at 30°C, with a substrate loading of 2% (w/v) and a medium level of 20% (v/v). Using RSM, the optimized physicochemical conditions for bioethanol production were identified as 66.7 hr of saccharification at 29.8°C, followed by 32.3 hr of fermentation at 30.2°C, with a substrate loading of 2.6% (w/v) and a medium level of 14.8% (v/v). Under these optimized conditions, ethanol production reached 0.63 g/L, demonstrating the capacity of *A. niger* B2484 to perform consolidated bioprocessing (CBP). Overall, this study successfully optimized CBP conditions for *A. niger* B2484, with optimal ethanol production achieved under short saccharification–fermentation cycles. The findings validate the strain's potential for bioethanol production, although further improvements are required to enhance yield efficiency. Future studies should investigate metabolic engineering and enzyme supplementation strategies to improve its commercial viability.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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