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Coffee cherry processing waste to produce value-added products through clean production biorefineries

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ABSTRACT

Indonesia is the 4th largest coffee-producing country in the world. Coffee cherry can produce 60% CCPW, more than the core product of coffee beans as the main product produced. CCPW contains lignocellulosic material that can be converted into value-added products. This research optimizes the production of xylose and xylanase enzymes as an integrated process with a biorefinery model to increase the added value of CCPW using the Research Surface Methodology (RSM). Enzyme production is carried out using a bioprocess using *Trichoderma viride*. Xylose production is carried out using the dregs of xylanase production as raw material and xylanase enzymes which are produced in the integrated process. The xylanase and xylose produced are in the liquid phase. The treatment design for xylanase production is substrate concentration and incubation time, while for xylose production it is substrate concentration and xylanase concentration. Based on the optimization conducted, the optimum condition of xylanase production was 75% substrate concentration with an incubation time of 72 hours, which will result in xylanase an enzyme with an activity of 29.275 U/ml with desirability of 99.55%. On the other hand, the optimum condition of xylose production was 12.54% substrate concentration with a xylanase concentration of 4.01%, which will result in 0.53 g/L xylose content with desirability of 94.06%. These findings show that xylose production efficiency can be achieved through integrated two-stage biorefinery production for efficient and sustainable utilization of CCPW.

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INTRODUCTION

Coffee processing begins with washing, soaking, and peeling the outer skin, and will produce 65% coffee beans and the rest is coffee skin. The coffee beans will then be processed into coffee powder, but the coffee skins will not undergo processing and will only become waste. The coffee processing process is generally divided into two, namely wet processing and dry processing. Both methods will create solid waste in the form of red outer skin resulting from the fruit peeling process, skin flesh resulting from the incubation and washing process, and seed shells or horn skin resulting from the process of stripping the horn skin from coffee beans. According to the Central Statistics Agency (BPS), coffee production can be estimated to produce 410 thousand tons of coffee waste per year (Direktorat Jendral Perkebunan, 2021). Of course, this will disrupt the natural balance and environmental damage due to the accumulation of waste. Coffee bean shell waste contains lignocellulose which has the potential to be used as a source of xylan in enzyme production (Jędrzejczyk et al., 2019). The use of lignocellulosic materials for food purposes is more attractive compared to starchy materials because they do not compete with their position as a source of food (Fatma et al., 2018).

Xylanase is an enzyme that can hydrolyze xylan with a protein weight of between 15,000 to 30,000 Daltons, active at a temperature of 55°C (Bhardwaj et al., 2019). Xylanases can be grouped into β-xylosidase, exo-xylanase, and endoxylanase (Elegbede & Lateef, 2019). β-xylosidase can hydrolyze short chains of

xylooligosaccharides into xylose (Kamble & Jadhav, 2012). Exoxylanse can hydrolyze the xylan polymer chain located at the reduction end, thereby producing the main product xylose, and several short-chain oligosaccharides (Istiqomah et al., 2022a). Endoxylanase can break the β-1,4 bonds in the inside of the xylan chain regularly (Ernawati et al., 2014). Determination of bond breaking is based on the length of the substrate chain, the presence or absence of substitution groups, the degree of branching, and the breaking pattern of the enzyme (Wösten, 2019).

Xylose sugar is a sugar that has a sweet taste and contains low calories compared to sucrose crystal sugar (Galvan et al., 2022). Diabetics or people who need to control their weight can consume xylose sugar as a substitute for sucrose (Norazlina et al., 2021). Xylose does not have negative side effects from the sugar produced, xylose is excreted from the kidneys and is not metabolized by humans (Norazlina et al., 2021).

Previous research focused more on the use of CCPW as a natural fertilizer material, this research adopts the conversion of lignocellulosic waste materials into xylose (J. Cerino-Córdova et al., 2020; Lee et al., 2023; Pudjiastuti et al., 2019; Rahmah et al., 2023; Ravindran et al., 2019; Scully et al., 2016). This research aims to increase the optimization of the use of CCPW to xylose and xylanase using sustainable substrates. The first production process is the production of xylanase using CCPW as a bioprocess substrate using *Trichoderma viride*, carried out at the beginning because microorganisms require optimum nutrition for their growth. The second stage of the production process is the production of xylose from CCPW dregs resulting from xylanase production. This process is expected to increase the yield because there has been a biological breakdown of lignocellulosic bonds in the substrate. The main objective of this research is to evaluate the use of CCPW as a substrate in a multistage biorefinery process in producing xylanase and xylose. The research hopefully can add more insight into the existing knowledge of the topic discussed, especially clean production biorefineries in Indonesia.

METHOD

CCPW is all waste produced from processing Cherry coffee, including exocarp, mesocarp, and endocarp. The CCPW used is a mixture consisting of 50% Arabica coffee and 50% Robusta coffee and was obtained from PDP Kahyangan Jember, East Java, Indonesia. Before use, CCPW raw materials are washed thoroughly using running water until the washing water is clear. CCPW waste is then dried using sunlight for 24 hours, crushed then sieved with fine flour measuring 60 mesh. Based on the Chesson analysis method, the CCPW used contains 19.74% lignin, 29.98% cellulose, and 14.05% hemicellulose. The research employed chemicals that were acquired from Merck.

Extracellular Xylanase Production (Yuliana et al., 2019)

Xylanase production is carried out using cultivation media consisting of 1.5 g (NH4)2SO4, 2 g KH2PO4, 0.35 g urea, 0.03 g CaCl2.2H2O, and 0.2 g MgSO4.7H2O in 1000 mL of distilled water. The CCPW substrate that has been dissolved in the cultivation medium is wet sterilized using an autoclave at 121C for 15 minutes. The cultivation medium is used as a solvent in xylan incubation by *T. viride* with the composition ratio referring to the cultivation medium. In contrast, the CCPW substrate concentration used is according to the RSM design, and the concentration of *T. viride* used is 10% of the volume of the cultivation medium used. The working volume used in the research was 100 ml so for each treatment, 10 ml of *T. viride* inoculum solution was used which was made by dissolving the *T. viride* rejuvenation results on PDA agar slant media into 10 ml of 0.85% NaCl and checking the turbidity using UV-Vis spectroscopy. at λ = 660 nm with standard Mc Farland solution 3. The absorbance of the inoculum is a minimum of 0.616 which is equivalent to a cell number of 3x108 cfu/ml. Incubation was carried out under anaerobic conditions in an incubator with a duration according to the treatment in the RSM design. The next process is enzyme extraction which is carried out by adding 0.85% NaCl 3 times the working volume of the cultivation medium and the solution is homogenized using an orbital shaker at a speed of 100 rpm for 1 hour at a temperature of 25C anaerobically. Enzyme separation was carried out using the centrifugation method at a speed of 1000 rpm at a temperature of 25C for 15 minutes. The resulting solid is a substrate that will be used in xylose production, while the solution obtained is a crude xylanase extract that will be used in xylose production at the next stage.

Xylose Production Using Crude Xylanase (S. O. N. Yudiastuti et al., 2018)

The solid by-product resulting from xylanase production is the substrate used in xylose production. Previously, the substrate was dried using an oven at 80C for 18 hours then ground and filtered to a size of 60 - 80 mesh. The substrate was then dissolved in 0.05 M acetate buffer solution at pH 5 and then autoclaved at 121C for 15 minutes. Xylose production is the second stage in the implementation, carried out using crude xylanase produced in stage one. The crude xylanase used is the result of optimization in the first stage of the integrated process. The xylose concentration used and the enzyme concentration used are based on the results of the RSM design. The xylose production method used is the fed-batch hydrolysis method, hydrolysis is carried out for 96 hours at a temperature of 38C.

Analysis Method

The lignocellulose composition was determined using the Chesson method (SON. Yudiastuti et al., 2018). Xylanase enzyme activity and xylose concentration were analyzed using the DNS method (S. Yudiastuti, 2021). The protein enzyme concentration was analyzed using the Bradford method. The growth of *T. viride* cells can be measured based on their spore density using a hemocytometer (S. Yudiastuti et al., 2020).

Research Design

The CCD RSM method was used in this integrated biorefinery process research, both in the first and second stages with the design as shown in Table 1.

Table 1. Treatment design optimization

Run	Stage 1			Stage 2		
	Treatment Variable		Response	Treatment Variable		Response
	Substrate Concentration (%)	***Incubation Time (hour)***	***Xylanase enzyme activity (U/ml)***	***Substrate Concentration (%)***	***Xylanase Concentration (%)***	***Xylose Content (g/L)***
1	50	81,9411	Y_1	10	1	Y_1
2	25	24	Y_1	20	1	Y_1
3	50	48	Y_1	10	5	Y_1
4	75	72	Y_1	20	5	Y_1
5	75	24	Y_1	15	3	Y_1
6	50	48	Y_1	15	3	Y_1
7	50	48	Y_1	15	3	Y_1
8	85,3553	48	Y_1	15	3	Y_1
9	25	72	Y_1	15	3	Y_1
10	50	14,0589	Y_1	7.929	3	Y_1
11	50	48	Y_1	15	0.172	Y_1
12	14,6447	48	Y_1	15	5.828	Y_1
13	50	48	Y_1	22.071	3	Y_1

RESULTS AND DISCUSSION

The Growth Phase of Mold Cells Isolates *Trichoderma viride*

The growth curve of *T. viride* is shown in Figure 1. *T. viride* fungal cells have a cell adaptation growth time of 0-36 hours, a logarithmic phase of 36 - 72 hours, a stationary phase of 72-120 hours, and a death phase of 120 - 192 hours. cell growth.

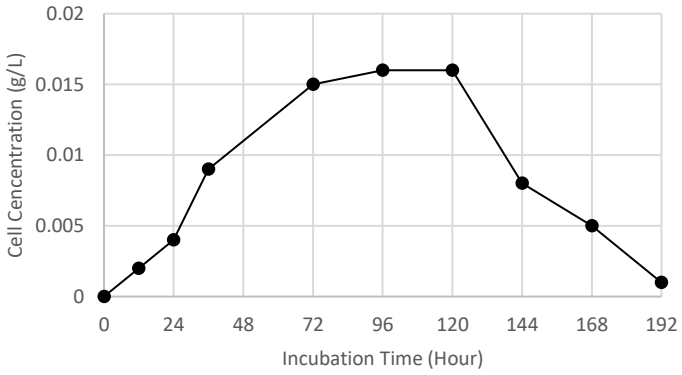

Figure 1. The Growth Curves of *T.viride*

Based on Figure 1, it can be seen that the right time to harvest *T. viride* cells so they can be used in the incubation process is when the cells are alive for 96 hours. When they enter growth, they remain in the stationary phase. Very accelerated growth conditions occur in the 72-96 hour interval between the exponential and stationary phases. At that time, optimum production of primary products and metabolites will likely occur. This growth curve was used as a basis for determining the upper limit of incubation time used in the research. The growth curve of *T.viride* cells will be different in different incubation media or substrates, this is because

it is determined by the number of initial cells inoculated, physiological and morphological conditions, as well as the cultivation media in the substrate used (Windari et al., 2014).

Optimization of Stage 1: Xylanase Production

The objection of stage one of the integration process is to optimize xylanase enzyme production from the CCPW substrate. The experiment was conducted for 4 days using CCPW concentration according to the RSM design. The amount of xylose produced from pure xylan as a substrate was used to calculate the xylanase enzyme activity obtained from CCPW. The optimization results are presented in Table 2.

Table 2. Response Data for Incubation Time and Substrate Concentration in Xylanase Enzyme Activity

Run	Stage 1		
	Treatment Variable		Response Variable
	Substrate Concentration (%)	***Incubation Time (hour)***	***Xylanase Enzyme Activity (U/ml)***
1	50	81,9411	22.367
2	25	24	23.354
3	50	48	24.998
4	75	72	29.603
5	75	24	20.064
6	50	48	26.643
7	50	48	24.340
8	85,3553	48	27.630
9	25	72	20.722
10	50	14,0589	21.051
11	50	48	25.656
12	14,6447	48	22.696
13	50	48	24.669

Table 2 shows that the xylanase enzyme activity gained varied from 20.722 U/ml at 25% substrate concentration, and 72 hours of incubation time to 29.603 g/L in run 4 at 75% substrate concentration, and 72 hours of incubation time. In crystal sugar, the proportion of glucose is greater than that of xylose. This is so because biomass substrates contain both cellulose and hemicellulose, which can be converted to glucose. Another co-product of the hydrolysis of lignocellulosic biomass is glucose, which is utilized as a co-substrate in the incubation process to produce xylitol from xylose. As incubation time increased, there was an increase in xylanase enzyme activity due to longer incubation time giving *T. viride* the opportunity to reproduce. Based on the cell growth curve, the stationary time for *T. viride* cells is 72-120 hours of life. The right substrate concentration must also support the longer incubation time to produce the xylanase enzyme with the desired enzyme activity so that the treatment with the longest incubation time produces enzyme activity that is not the greatest because the substrate concentration used is not sufficient.

ANOVA, or analysis of variance, was carried out using the chosen model as a basis (Table 4). Table 3 shows the model selection that RSM recommended. The quadratic R^2 value is 0.6900, indicating that the quadratic models are significant based on Table 3. The RSM program did not recommend the 2FI or linear models, or they were aliased, meaning the model was not accurate by the research design and did not display any mathematical equations. As a result, the models were not chosen. Because the model is significant, the cubic model was selected. The P value illustrates the model's significance; a P value of less than 0.05 ($P<0.05$) denotes.

Table 3. Fit Summary Test Result

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Selection Model
Linear	0.1587	0.0143	0.1695	-0.4230	*Not significant*
2FI	0.0080	0.0515	0.5952	0.3039	*Not significant*
Quadratic	**0.0062**	**0.3726**	**0.8785**	**0.6900**	**Suggested**
Cubic	0.1710	0.9997	0.9161	0.9454	**Aliased**

Table 4. The outcome of ANOVA Result

Source	Sum of square	df	Mean square	f-value	p-value prob > F	
Model	88,57	5	17,71	18,35	0,0007	*Significant*
A- **Substrate Concentration**	19,75	1	19,75	20,46	0,0027	
B- **Incubation Time**	9,61	1	9,61	9,96	0,0160	
AB	37,03	1	37,03	38,37	0,0004	
A^2	0,0168	1	0,0168	0,0174	0,8987	
B^2	21,95	1	21,95	22,74	0,0020	
Residual	6,76	7	0,9653			
Lack of Fit	3,42	3	1,14	1,37	0,3726	*Not significant*
Pure Error	3,33	4	0,8335			
Cor Total	95,33	12				
R^2	0,9291					
Adjusted R^2	0,8785					
Predicted R^2	0,6900					
Adeq Precision	13,8285					

The quadratic models in Table 3 have P values <0.05, or 0.0062 respectively. This indicates that a value is significant if it is less than 0.05 ($\alpha = 5\%$). The significant model is quadratic, as demonstrated by the influence of Xylanase enzyme activity obtained from the CCPW substrate and incubation time. With a P value less than 0.05, the independent variables that affect the response to the xylanase enzyme activity generated are the CCPW substrate concentration variable (P = 0.0027) and the incubation time variable (P = 0.0160). Both combinations can influence the xylanase enzyme activity since the combination treatment value has a P value of 0.0004. Insufficient fit value. Given that the optimization's P value is greater than 0.05, the lack of fit value is not significant. A non-significant lack of fit value suggests that there is no variation in the data.

The R^2 value obtained is close to 1, indicating a model with good data. The difference between the adjusted R^2 and the predicted R^2 is less than 0.2, or 0.0506. A difference value of less than 0.2 suggests that there are no issues with the model or the data that were used. The model in this study can be used to explore the RSM design space because it demonstrates a good adequate precision ratio value of 13.8285 or greater than 4 (adeq prediction > 4). Equation 1 shows the mathematical model equation that was derived from RSM to optimize the set of independent variables on the response of xylanase enzyme activity.

$$Y = 25{,}26 + 1{,}57A + 1{,}10B + 3{,}04AB - 0{,}0492A^2 - 1{,}78B^2 \quad (1)$$

where:
Y = xylanase enzyme activity (U/ml)
A = CCPW Substrate concentration (%)
B = Incubation time (hour)

When using variable values for the concentrations of CCPW substrate, and incubation time that differ from the conducted research data, the equation can be used to calculate the xylanase enzyme activity value produced in the production of xylanase enzyme from CCPW. The equation produced by the Design Expert 13 software describes the relationship between substrate concentration and incubation time on the enzyme activity response in quadratic form. This shows that the enzyme activity response is influenced by substrate concentration, incubation time, and the relationship between the two. Substrate concentration has a greater influence on the enzyme activity response compared to incubation time (El-Gendi et al., 2021). This is known from the value of coefficient A which is greater than the value of coefficient B. The relationship between the two has a greater influence on the enzyme activity response if the combination used is balanced. However, if excessive or insufficient substrate concentration and incubation time are used, it will decrease the enzyme activity response.

The higher substrate concentration causes the high xylan content and also causes *T.viride* to produce xylanase enzymes. The higher the xylan, the more substrate will be converted into xylose, so the xylanase enzyme produced by mold will also be higher (Mardawati et al., 2020). The selection of a balanced combination of substrate concentration and incubation time greatly influences enzyme activity. The higher the substrate concentration and incubation time, the greater the enzyme activity produced.

The relationship between substrate concentration and incubation time with the enzyme activity response is displayed in the form of a contour graph shown in Figure 2 and a 3D surface graph in Figure 3. The red dots

on the contour graph and 3D surface graph represent the 13 treatment combinations that have been carried out. The contour graph which can be seen in Figure 2 shows that the enzyme activity value increases along with the higher substrate concentration and incubation time used. The lowest enzyme activity values are shown in the green to blue areas, while the highest enzyme activity values are shown in the red areas.

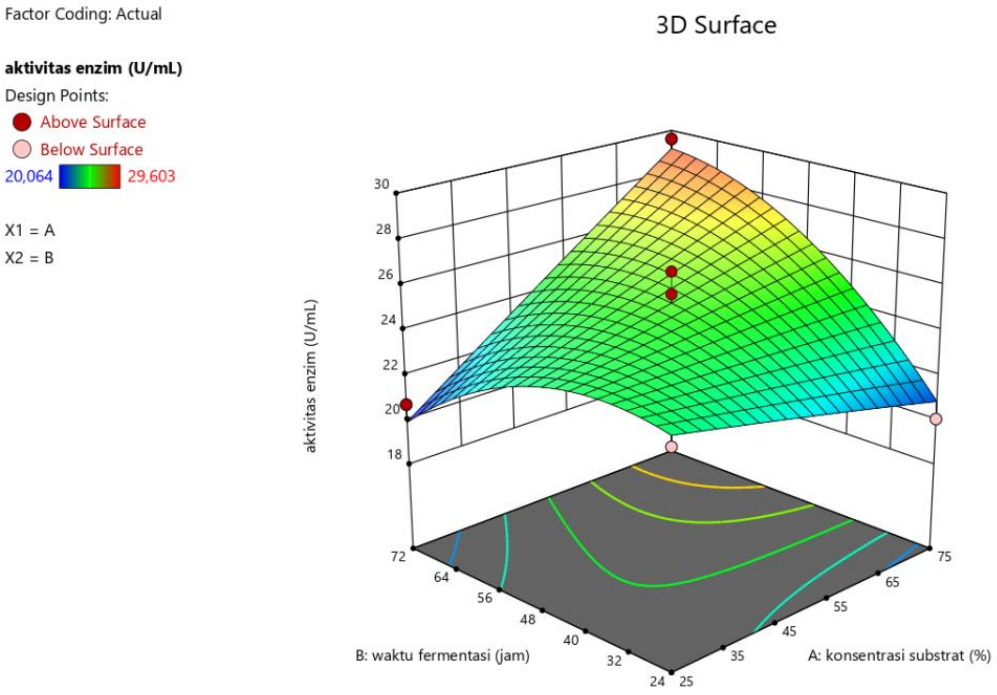

Figure 2. Substrate Concentration and Xylanase Concentration to Concentration of Xylose

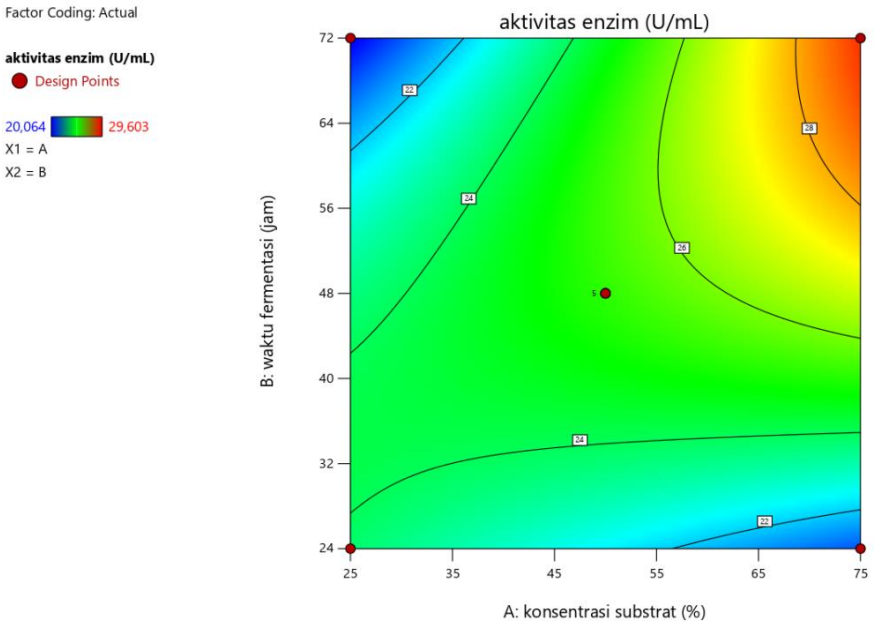

Figure 3. Ideal State of the Graphic Contour

The graph also shows that an imbalance in the combination of substrate concentration and incubation time can have an impact on low enzyme activity values. For example, in the combination of a substrate concentration of 75% and an incubation time of 24 hours, the lowest yield was obtained, namely 20.064 U/ml. Meanwhile, the balance of substrate concentration and incubation time can have an impact on the magnitude of the enzyme activity value produced, such as the combination of a substrate concentration of 75% and an incubation time of 72 hours, the highest result was obtained, namely 29,603 U/ml. The enzyme activity

increases as the number of molds increases, which is also balanced with the high concentration of substrate used (Mardawati et al., 2020).

Apart from the contour graph, the increase in enzyme activity values due to the influence of substrate concentration and incubation time used is also shown on the 3D surface graph. In the 3D surface graph, it can be seen that the graph shape curves upwards and the color becomes redder due to the increase in substrate concentration and incubation time used. From these results, it can be said that as the incubation time and substrate concentration increase, there will be an increase in enzyme activity which is caused by the longer incubation time and the higher substrate concentration, which will allow Trichoderma to reproduce (Chatterjee et al., 2016). *Trichoderma* will get abundant nutrition from substrates derived from xylan under the right conditions (Imanisa et al., 2023).

The enzyme activity resulting from research on the production of xylanase enzymes from coffee fruit skin waste obtained a greater enzyme activity value compared to research on the production of xylanase enzymes from soybean residues carried out (Cunha et al., 2018; Imanisa et al., 2023; Istiqomah et al., 2022b). In this study, the highest enzyme activity was obtained with an enzyme activity value of 29,603 U/ml, its higher than xylanase production obtained from soybean residue with the highest enzyme activity value of 11.84 U/ml (Cunha et al., 2018). Other xylanase production produced by *Trichoderma sp* using corn cobs as a substrate obtained a higher enzyme activity of 181.22 U/g (Istiqomah et al., 2022b). The differences in enzyme activity obtained from each study were due to the different types of substrate and microorganisms used in producing the xylanase enzyme (Wösten, 2019). The success of xylanase enzyme production is influenced by the type of substrate and substrate concentration used during the xylanase enzyme production process (Sugiharto et al., 2016).

Table 5. Model Validation

Run	Substrate Concentration (%)	Incubation Time (hour)	Xylanase enzyme activity (U/ml)	Validation (%)
Prediction	75	72	29.146	99,55%
Actual	75	72	29.275	

By performing optimization, the optimal combination of treatments can be found, allowing for the expected identification of the response. Thirteen treatment combination solutions are offered by the Design Expert 11 program, which is based on the research's optimization process. High optimization target value (desirability), that is, a desirability value near 1 indicates the optimal treatment combination solution. Solution 4, which combines treatment with a substrate concentration of 75% and an incubation period of 72 hours, has the highest desirability value in this study at 0.933. The xylose concentration of 29.275 U/ml, which is in line with the optimization target of 29.246 U/ml, will be produced by this combination of treatments.

Optimization of Stage 2: Xylose Production

Optimizing the production of xylose from the CCPW substrate is the second stage of the integration process aim. The RSM design was done during the 96 hours of the experiment, using xylanase produced from stage one and substrate from the dregs waste of stage one too. Table 2 displays the results of the optimization.

Table 6. Response Results of Xylose Levels

Run	Stage 2		
	Treatment Variable		Response Variable
	Substrate Concentration (%)	***Xylanase Concentration (%)***	***Xylose Concentration (g/L)***
1	10	1	0.457
2	20	1	0.439
3	10	5	0.514
4	20	5	0.616
5	15	3	0.516
6	15	3	0.52
7	15	3	0.632
8	15	3	0.617
9	15	3	0.558
10	7.929	3	0.419
11	15	0.172	0.358
12	15	5.828	0.566

13	22.071	3	0.588

Table 6 shows that the xylose content in the hydrolyzate ranges from 0.3 mg/mL to 0.6 mg/ml. This relatively low xylose yield can be caused by the xylanase enzyme used that has not been purified. Another method that can be used to increase hydrolysis results is the preliminary pre-treatment of the substrate with NaOH (Jönsson & Martín, 2016). This aims to reduce lignin in the substrate so that the hemicellulose and cellulose levels become higher(Huang et al., 2022). The NaOH solvent can damage the lignin walls because at high temperatures the NaOH ions will more quickly break down the intermolecular bonds of lignin which form a barrier to the cellulose and hemicellulose complex. High lignin content can also be reduced by releasing pectin on the substrate using a NaOH solution (Quintero-Ramirez, 2014).

Another method is by adding surfactants to increase the performance of xylanase in the hydrolysis process, thereby increasing the xylose sugar content. The addition of surfactants in the hydrolysis process can increase enzyme work by reducing surface tension, expanding the contact surface between the enzyme and cellulose and hemicellulose by removing lignin, and reducing the ease with which the enzyme binds to the substrate (Anita et al., 2021). One surfactant that can be used is Tween 80, which is a non-ionic surfactant that can increase the production of reducing sugars.

Table 7. Fit Summary Test Result

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Selection Model
Linear	0.0174	0.3529	0.4665	0.2819	*Not significant*
2FI	0.3595	0.3357	0.4629	0.2633	*Not significant*
Quadratic	**0.0690**	**0.6872**	**0.6783**	**0.4117**	**Suggested**
Cubic	0.5486	0.5664	0.6457	-0.0483	**Aliased**

Statistical model analysis of responses aims to determine the most appropriate model form to describe the research data obtained. The criteria for selecting the most appropriate model to describe the optimum response are presented in Table 7. The model criteria that can be selected is a model with a p-value of less than 5% ($p<0.05$), meaning the model has an error of less than 5%. Based on the table, it is known that the model with the status "suggested" or suggested by the software is a quadratic model. The quadratic model has a p-value of 0.0690 which shows that the chance of model error is only 6.9% and has a real effect on the xylose response of 93.1%. The Linear Model has a smaller p-value, namely 0.0174, but is not recommended by the software.

The adjusted R-square and predicted R-square values of the quadratic model are 0.6783 and 0.4117. This means that the independent variable influences the response by 67.83%. The R-square value ranges from 0 to 1, the closer the value is to 1, the stronger the influence of the independent variable on the dependent variable. Based on lack of fit, the model is considered appropriate if the lack of fit p-value is greater than 0.05, which means that the model's inaccuracy is not significantly different or not statistically significant. The largest p-value of 0.6872 is found in the quadratic model. This shows that the model inaccuracy is not significant for the response of 68.72%. Based on the model selection criteria used to determine the relationship between substrate concentration and enzyme concentration on xylose levels, the model chosen was the quadratic model.

Table 8. The Outcome of ANOVA Result

Source	Sum of square	df	Mean square	f-value	p-value prob > F	
Model	0.0701	5	0.0140	6.06	0.0176	significant
A- **Substrate Concentration**	0.0130	1	0.0130	5.64	0.0493	
B- **Xylanase Concentration**	0.0349	1	0.0349	15.08	0.0060	
AB	0.0036	1	0.0036	1.56	0.2523	
A^2	0.0049	1	0.0049	2.13	0.1878	
B^2	0.0156	1	0.0156	6.75	0.0356	
Residual	0.0162	7	0.0023			
Lack of Fit	0.0046	3	0.0015	0.5270	0.6872	not significant
Pure Error	0.0116	4	0.0029			
Cor Total	0.0863	12				
R^2	0.8123					
Adjusted R^2	0.6783					

Predicted R^2	0.4117
Adeq Precision	7.6670

Based on the ANOVA table (Table 8), it is known that the quadratic model is significant for the xylose response with a p-value of 0.0176. Substrate concentration (A) has a significant influence on xylose levels because the p-value is 0.0493 ($p<0.05$), while enzyme concentration (B) has a significant influence on the response of xylose levels because the p-value is 0.0064 ($p <0.05$). The interaction between substrate concentration and enzyme concentration (AB) showed an insignificant value with a p-value of 0.2523 ($p>0.05$), meaning that the interaction between independent variables did not show a significantly different response to xylose levels. This is because when the substrate has been completely converted into a product, increasing the enzyme cannot increase the product anymore, and vice versa (Maulina et al., 2023). The substrate concentration squared factor (A^2) shows an insignificant value with a p-value of 0.1878, while the enzyme concentration squared factor (B^2) shows a significant value with a p-value of 0.0356. It can be concluded that only the factors substrate concentration (A), enzyme concentration (B) and enzyme concentration squared (B^2) have a significant effect on the response of xylose levels. The lack of fit value obtained was 0.6872 ($p>0.05$) with the statement not significant meaning that the model fits the trend of the quadratic model.

The coefficient of determination (R2) value obtained was 0.8123, meaning that the factors studied affected xylose levels by 81.23%. The adjusted R^2 value was 0.6783 and the predicted R2 was 0.4117, the difference between the two shows a result of more than 0.2. This is a problem with the model or data used, apart from that it could also be because there are independent variables that are not significant so the ability of the free variable to explain the responses obtained is very limited. The adeq precision value is 7.6670 or more than 4, so it can be said that the model can be used to navigate space in RSM. The low standard deviation of 0.0481 indicates that the model is more accurate. The quadratic model regression equation for the xylose response suggested by the ANOVA analysis is as follows:

$$Y = 0.5686 + 0.0404A + 0,0660B + 0.0300\ AB - 0.0266\ A^2 - 0.0474\ B^2 \quad (2)$$

Note :
Y = xylose concentration (g/L)
A = Substrate Concentration (%)
B = Xylanase Concentration (%)

The equation above shows that the response of xylose levels increases and is directly proportional to the increase in the components of substrate concentration and enzyme concentration which is indicated by a positive sign in the constant value. The relationship between substrate concentration and enzyme concentration on xylose content can also be depicted with a contour plot (Figure 3) and a 3D surface graph (Figure 4).

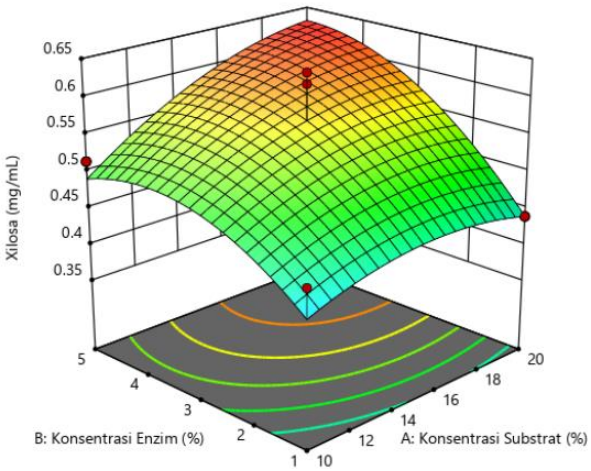

Figure 4. Substrate Concentration and Xylanase Concentration to Concentration of Xylose

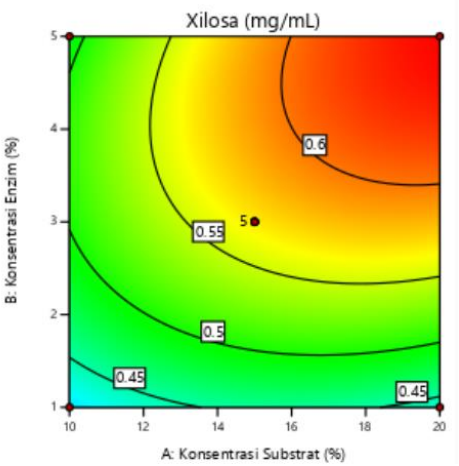

Figure 5. Ideal State of the Graphic Contour

Figure 3 shows the contour plot consisting of various color variations. The color of the contour indicates the magnitude of the xylose level response produced. Low xylose levels are green to blue, while high xylose levels are orange to red. The greater the concentration of substrate and enzyme, the closer the xylose response value is to the red area. This is because at the rate of the enzymatic reaction, the higher the substrate concentration, the more active sites of the enzyme will be bound to the substrate so that the glycosidic bonds will be broken more frequently and quickly, ultimately resulting in a higher amount of product (Maulina et al., 2023). The optimal response of xylose in the contour plot shows that it is in the range of 0.55 – 0.6 mg/mL. This value is still at the midpoint of the plot contour, so it can still be optimized to achieve a more optimal xylose value, namely in the red area. Determining the optimum conditions for maximum xylose content can also be seen from the three-dimensional curve. The 3-D graph also shows that the higher the substrate and enzyme concentration, the higher the xylose levels produced.

Table 9. Model Validation

Run	Substrate Concentration (%)	Xylanase Concentration (%)	Xylose Content (g/L)	Validation (%)
Prediction	12.54	4.01	0.55	94.06%
Actual	12.54	4.01	0.53	

The ideal set of treatments can be identified through optimization, enabling the anticipated identification of the response. Based on the research optimization process, the Design Expert 11 program provides thirteen treatment combination solutions. An optimal treatment combination solution is indicated by a desirability value that is close to 1, or a high optimization target value. In this investigation, combined treatment with a 12.54% substrate concentration and 4.01% xylanase concentration with enzyme activity of 29.275U/ml, has the highest desirability value (0.9406). This combination of treatments will result in a xylose concentration of 0.53 U/ml, which is in line with the optimization target of 0.53 U/ml.

CONCLUSION

This manuscript provides information that arabica and robusta CCPW can be processed into xylanase and xylose in one integration process using the concept of biorefinery and clean production. The processes include pre-treatment, autoclave sterilization, incubation, xylanase harvesting, centrifugation, and enzymatic hydrolysis. The substrate of xylanase was CCPW, the substrate of xylose was the dregs of xylanase production, and the xylanase enzyme produced was used in xylose production on an integration biorefinery concept. The process needs to be improved to gain more product in both xylanase and xylose. Based on the optimization conducted, the optimum condition of xylanase production was 75% substrate concentration with an incubation time of 72 hours, which will result in xylanase an enzyme with an activity of 29.275 U/ml with desirability of 99.55%. On the other hand, the optimum condition of xylose production was 12.54% substrate concentration with a xylanase concentration of 4.01%, which will result in 0.53 g/L xylose content with desirability of 94.06%.

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