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Research paper



Fermentation Optimization on Bacterial Cellulose Production by *Acetobacter Xylinum 0416*

Faezah Esa[1], Norliza Abd. Rahman[1*], Mohd Sahaid Kalil[1], Siti Masrinda Tasirin[1]

[1]Chemical Engineering Programme, Research Centre for Sustainable Process Technology (CESPRO), Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
**Corresponding author E-mail: norlizajkkp@ukm.edu.my*

Abstract

Bacterial cellulose (BC) has received extensive attention in various field due to of its high purity, mechanical strength, crystallinity and biodegradability. Determination of optimum fermentation condition for the improvement of cellulose production is essential especially in industrial scale. The production of bacterial cellulose by *A.xylinum 0416* was fermented in matured coconut water medium and the suitable culture conditions were predicted statistically via Central Composite Design of Response Surface Methodology. Effect of varying temperature, fermentation period, initial pH, inoculum size and parameters interaction were studied in five-levels Design of Experimental approach. Temperature, inoculum size and fermentation period had significant effects on the cellulose production. By aiming to minimize the temperature and fermentation period for maximum cellulose production, the optimized parameters predicted for cellulose production of 1.12829 g dry weight were temperature: 27˚C, fermentation period: 5 days, initial pH: pH 8 and inoculum size: 15% with high desirability of 78.2%.

Keywords: *Acetobacter xylinum 0416; bacterial cellulose; central composite design; fermentation; optimum parameter.*

1. Introduction

Bacterial cellulose is a biopolymer that synthesized extracellular by some bacteria especially acetic acid bacteria such as *Acetobacter xylinum* [1]. It has similar chemical formula with plant cellulose, exhibit pure cellulose matrix and has superior physiochemical properties than plant. About 30% of production cost has been spent on fermentation medium [2] and low productivity limits their industrial and commercial application. Thus, improving fermentation system and utilizing low cost medium are crucial in maximizing cellulose productivity. Mature coconut water is a common agriculture waste from coconut-based market such as coconut oil, copra, desiccated coconut and coir. Its nutritious medium content and availability in large amount provide matured coconut water as a potential fermentation medium for bacterial cellulose production.
Central Composite Design (CCD) is an experimental design under Response Surface Methodology (RSM). RSM consist of three main procedures in analyzing relationship between variable and response parameters: performing statistically designed experiments, estimating the coefficients in an empirical model and predicting the response and checking the adequacy of the model [3]. CCD allows estimation of parameters into second order model (quadratic) and its prediction process manages to reduce the cost and time for experimental trial. This statistical analysis focuses on determining the optimal condition of bacterial cellulose production by examining the impact of variables on it production yield.

2. Materials and methods

2.1. Bacterial strain

Acetobacter xylinum 0416 was received from The Malaysian Agricultural Research and Development Institute. The bacterial culture was maintained in growth medium containing following constituents per litre: D-glucose 80 g, ammonium sulphate 5g, acetic acid glacial and coconut water. The medium was autoclaved at 120°C for 15 minutes to achieve sterility, inoculated with 10% bacterial culture, and incubated in agitated condition at 150 rpm, 30˚C for 3 days.

2.2. Fermentation medium

The matured coconut water was collected from a copra dealer and the medium pH was adjusted with glacial acetic acid or sodium hydroxide to desired value. Then, the medium was sterilized in autoclave machine at 120˚C for 15 min.

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2.3. Experimental design and data analysis

An appropriate amount of *A. xylinum* was added into coconut water medium to make a total of 100 ml and left undisturbed in an incubator that was maintained to certain condition for cellulose production. The fermentation conditions were determined by a Central Composite Design with five level of selected variables consisting of temperature, initial pH, fermentation period and inoculum size (Table 1). Thirty sets of experimental run were repeated for three times. The response surface contour was used to analysed the interaction between independent variables and their impact on the response variable. The second order polynomial quadratic equation (equation 1) was applied to describe this model:

$$Y_i = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n-1} \sum_{i=i+1}^{n} \beta_{ij} x_i x_j + \sum_{i=1}^{n} \beta_{ii} x_i^2 \quad (1)$$

where Yi = response variable (Y = cellulose production); xi and xj represent the coded independent variables (x1 = temperature, x2 = initial pH, x3 = fermentation period, x4 = inoculum size). βo was the value of fitted response at the centre point of design and βi, βii and βij were the linear, quadratic and cross-product regression coefficients respectively.

Table 1: Independent variables and their levels used for Central Composite Design (CCD)

Variable	Coded Xi	Experimental design (central composite design)				
		Coded level				
		-1.682	-1	0	1	1.682
Temperature (˚C)	X_1	23	27	31	35	39
Fermentation period (Day)	X_2	3	5	7	9	11
Initial pH	X_3	3	5	7	9	11
Inoculum size (%)	X_4	0	5	10	15	20

2.4. Isolation and purification of cellulose

The cellulose pellicle generated was harvested, rinsed with tab water and treated with 0.1 M sodium hydroxide solution for 30 minutes at 90˚C [4] to remove bacteria from the cellulose matrix. To remove the remaining base, the pellicle was washed with distilled water until its totally neutral. The dry weight was taken after drying the film at 45˚C until it reached a constant weight and was kept for further analysis.

3. Results and discussion

Selection of bacteria and types of medium are crucial factors in BC production. *A.xylinum 0416* is one of the microorganisms acknowledged as high yield cellulose producer while mature coconut water contains various types of nutrients and carbonssources that important for bacterial growth. The potential of matured coconut water as culture medium was considered by determined cultivation parameters that might significantly affect the production such as temperature, initial pH, fermentation period and inoculum size. The fermentations were run as suggested by central composite design in the form of rotatable cube (Table 2).

Table 2. Experimental design in coded forms of process variables and values of experimental data for optimization of cellulose production.

Coded process variables				Responses
x1	x2	x3	x4	Cellulose Dry weight (g/100 ml)
27	5	5	5	0.3619
35	5	5	5	0.0000
27	9	5	5	0.6466
35	9	5	5	0.0000
27	5	9	5	0.5934
35	5	9	5	0.0000
27	9	9	5	0.9878
35	9	9	5	0.0000
27	5	5	15	0.8108
35	5	5	15	0.0000
27	9	5	15	1.2992
35	9	5	15	0.0000
27	5	9	15	0.8915
35	5	9	15	0.0000
27	9	9	15	1.4983
35	9	9	15	0.0000
23	7	7	10	0.4861
39	7	7	10	0.0000
31	3	7	10	0.6912
31	11	7	10	1.5455
31	7	3	10	0.0000
31	7	11	10	1.2952
31	7	7	0	0.0000
31	7	7	20	1.7072
31	7	7	10	0.8478
31	7	7	10	1.2372
31	7	7	10	1.2816
31	7	7	10	1.2344
31	7	7	10	1.2218
31	7	7	10	0.7300

The thirty experimental data sets analyzed by Analysis of Variance (ANOVA) demonstrate that the quadratic regression was significantly fit the second-order polynomial model (Table 3) with P-value less than 0.05. The final equation of cellulose production obtained for fermentation in 100 ml medium is as below:

$$\text{Cellulose production (g/100 ml)} = -22.12563 + 1.11793*X_1 + 0.58740*X_2 + 0.81950*X_3 + 0.30809*X_4 - 0.016106*X_1^2 - 9.71858E\text{-}003*X_2^2 - 0.039140*X_3^2 - 4.20247E\text{-}003*X_4^2 - 0.013862*X_1*X_2 - 6.65937E\text{-}003*X_1*X_3 - 5.96937E\text{-}003*X_1*X_4 + 3.56354E\text{-}003*X_2*X_3 + 2.60000E\text{-}003*X_2*X_4 - 1.83125E\text{-}003*X_3*X_4 \quad (2)$$

It can be seen that within the tested range, temperature (p-value: 0.0006) and inoculum size (p-value: 0.0125) had significant effect on cellulose production. Meanwhile, the fermentation period (p-value: 0.0833) and initial pH (p-value: 0.0866) has insignificant effect on response and hence can be excluded from mathematical model. In this study, the initial pH of medium was assigned prior to sterilization and addition of inoculum which is pH 4.5 did contribute to the reduction pH value. Besides that, during fermentation, the pH of medium drop gradually due to production of gluconic acid which can reach as low as 3.5. At this rate, the bacteria may proliferate but hardly produce cellulose [5].

There was an insignificant chance that the model are lack of fit (Prob > F: 0.0963) even though all interactions between factors are not significant (p>0.05) meanwhile only quadratic of temperature and pH are significant. This analysis probably leads to the coefficient of determination in moderate high, R2 = 77.74%.

Table 3: Regression summary and ANOVA table for cellulose production for uncoded values of process variables.

Source	β-Coefficient	F value	Prob > F
Model	-22.12563	3.74	0.0080
Temperature	+1.11793	18.44	0.0006
Fermentation period	+0.58740	3.44	0.0833
pH	+0.81950	3.36	0.0866
Inoculum size	+0.30809	8.05	0.0125
Temperature * Fermentation period	-0.013862	1.34	0.2651
Temperature * pH	-6.65937E-003	0.31	0.5863
Temperature * Inoculum size	-5.96937E-003	1.55	0.2318
Fermentation period * pH	+3.56354E-003	0.022	0.8837
Fermentation period * Inoculum size	+2.60000E-003	0.074	0.7898
pH * Inoculum size	-1.83125E-003	0.037	0.8510
Temperature2	-0.016106	12.40	0.0031
Fermentation period2	-9.71858E-003	0.28	0.6030
pH2	-0.039140	4.58	0.0492
Inoculum size2	-4.20247E-003	2.06	0.1715
Lack of Fit		3.37	0.0963

The contour plots and surface plots shown in Figure 1 depicts the relationship between temperature and fermentation period. It has been observed that better bacterial cellulose productivity was achieved when the temperature is about 30˚C for a week of fermentation. Increasing the temperature to 35˚C however slower its productivity as the bacterial hardly to produce cellulose at this temperature. This finding is consistent with previous study where the maximal cellulose production was observed between 28 and 30 °C [6,7,8]. Besides, increasing the inoculum size will increase the cellulose production (Figure 2).

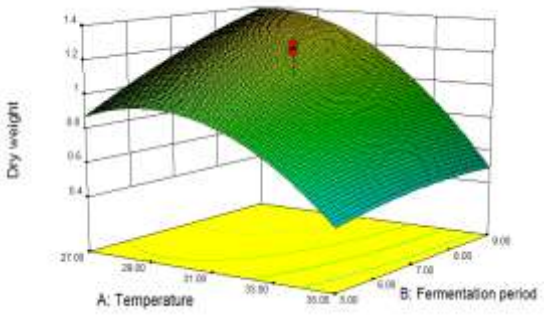


Fig.1: Effect of fermentation period and temperature on cellulose production (dry weight).

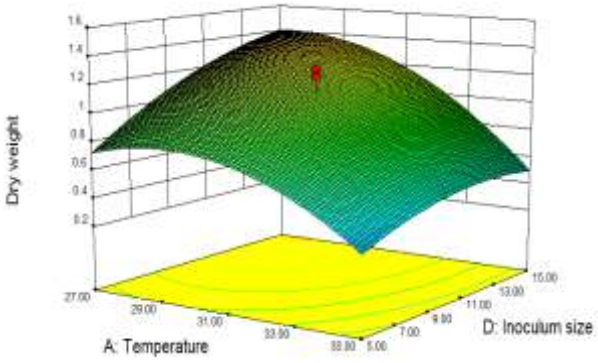


Fig. 2: Effect of inoculum size and temperature on cellulose production (dry weight).

For applying this production in a bigger scale, it is important to reduce the operation cost. Thus, the variables were adjusted to operate in minimum temperature and minimum fermentation period to have a maximum cellulose. As a result, the response surface optimization for bacterial cellulose is predicted to have conditions of temperature: 27˚C, fermentation period: 5 days, initial pH: pH 8 and inoculum size: 15% with a predicted cellulose yield of 1.12873 g dry weight per 100 ml. This prediction has a good desirability of 87.1%.

4. Conclusion

Statistical optimization on bacterial cellulose production allows reliable data received from the process without doing excessive experiments. Central Composite Design was successfully propose a mathematical model that investigate interaction effects of variables on response and predicts the optimal parameter values. Temperature alone and its interaction with fermentation period or inoculum size have significant effect on production. To achieve optimum BC yield, the fermentation can be done at 27˚C using 15% of inoculum and pH 8 coconut water medium for 5 days incubation.

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