Process Parameters for Fermentation in a Rotary Disc Reactor for Optimum Microbial Cellulose Production using Response Surface Methodology

Khairul Azly Zahan, [a, c] Norhayati Pa'e,[a] and Ida Idayu Muhamad [a,b,*]

In this study, microbial cellulose production by *Acetobacter xylinum 0416* using standardized liquid pineapple waste was carried out in a 4-L rotary disc reactor (RDR). The objective of this study was to optimize the process parameters for production of microbial cellulose in the RDR. The effects of the disc rotation speed (5 to 12 rpm), pH (3.5 to 7.5), fermentation period (3 to 6 days), and inoculum concentration (3 to 20% v/v) on the microbial cellulose production were investigated. The optimum microbial cellulose yield was obtained using 10% (v/v) of inoculum concentration, whereby four days' duration gave the most productive yield. In addition, the highest production of microbial cellulose was obtained at a low disc rotation speed of 7 rpm and a pH of 5.0. Analysis of data performed a high coefficient of determination value (R^2=0.875) represented by a mathematical model of optimized microbial cellulose production, $Y = -200.437 + 7.180X_1 + 69.869X_2 + 4.369X_3 + 1.867X_4 – 0.512X_1^2 – 6.766X_2^2 – 0.585X_3^2 – 0.079X_4^2$. From the results, it can be concluded that the foremost factors that affect the production of microbial cellulose in RDR were pH followed by inoculum concentration, disc rotation speed (rpm), and fermentation period.

Keywords: Microbial cellulose; Rotary disc reactor; Process parameters; Optimization; Response surface methodology; Pineapple waste; Acetobacter xylinum 0416

Contact information: a: Bioprocess Engineering Department, Faculty of Chemical Engineering, Univerisiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia; b: Cardio Engineering Centre IJN-UTM, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia; c: Section of Bioengineering Technology, Malaysian Institute of Chemical and Bioengineering Technology, Universiti Kuala Lumpur, Lot 1988, Bandar Vendor Tabuh Naning, 78000 Alor Gajah, Melaka, Malaysia;
** Corresponding author: idayu@cheme.utm.my*

INTRODUCTION

Microbial cellulose, also known as Nata, is very pure. It has a higher degree of polymerization and crystallinity compared to cellulose obtained from plants. This is because the cellulose fibrils in plants are embedded with hemicelluloses, lignin, and waxy aromatic substances (Ross *et al.* 1991). Because of its high water holding capacity and tensile strength, microbial cellulose has become an important raw material for products such as high fidelity acoustic speakers, paper, and desert foods (Iguchi *et al.* 2000; Ross *et al.* 1991; Norhayati *et al.* 2011). In addition, it also has been used in the production of pharmaceutical and beauty products (Czaja *et al.* 2006). Microbial cellulose can also be used in the production of industrial materials such as ultrafiltration membranes, binders for powders, thickeners for ink, adhesives, paint, and cement (Iguchi *et al.* 2000).

There are few different methods used for the production of microbial cellulose that have been previously reported. For the static culture method, a long fermentation

period and intensive manpower are required, thus resulting in a low production yield and high labour cost (Norhayati *et al.* 2011). The agitated culture method might convert microbial cellulose production strains into cellulose negative mutants. Because of their rapid growth, it becomes more enriched than the wild type strain, thus reducing the production of microbial cellulose (Kim *et al.* 2007). In airlift and stirred tank reactors, microbial cellulose production is decreased due to the adhesion of the culture broth to the reactor wall and the upper part of the equipment (Krystynowicz *et al.* 2002).

Critical factors that affect the production of microbial cellulose by *Acetobacter xylinum 0416* can be divided into two major groups. Firstly, the fermentation composition, including the carbon, mineral, and nitrogen sources used in the medium (Keshk and Sameshima 2005). Traditional sources of carbon for microbial fermentation are sugars such as glucose, fructose, and sucrose (Bae and Shoda 2005). More recently, unconventional feedstocks from renewable resources and waste streams have been investigated (Zeng *et al.* 2011). These include fruit juices (Kurosumi *et al.* 2009), sugar cane molasses (Keshk and Sameshima 2006), sweet potato pulp (Shigematsu *et al.* 2005), rotten apples (Gupta *et al.* 2010), and maple syrup (Zeng *et al.* 2011). The second factor is operating conditions such as dissolved oxygen (Kouda *et al.* 1997), temperature, pH of the growth medium (Noro *et al.* 2004), inoculation ratio (Hutchens *et al.* 2007), and inoculum age. Both groups of factors must be at optimized levels to ensure optimum production of microbial cellulose.

Response surface methodology (RSM) is widely used for multivariable optimization studies in several biotechnological processes such as optimization of media, process conditions, catalyzed reactions conditions, oxidation, production, fermentation, and biosorption of metals (Chang *et al.* 2006; Preetha and Viruthagiri 2007; Soo *et al.* 2006; Wang and Lu 2005).

This research aimed to study and optimize the process parameters of fermentation in a designed rotary disc reactor (RDR) so that the production yield could be increased and fermentation time could be reduced. The RDR uses the concept of a rotating biological contactor that exposes bacteria to the air for better aeration. The use of RDR can reduce the problems faced using the traditional method in static tray such as low volumetric yields, lack of large scale production capacity, long fermentation period, manpower, and large spatial demands to produce the microbial cellulose. In previous research (Norhayati *et al.* 2011), fermentation of microbial cellulose in RDR achieved 85% greater yield compared to that under stationary conditions of growth.

Table 1. Characteristics of Liquid Pineapple Waste

Composition	Liquid Waste	
	Before sterilization	After sterilization
COD (g/L)	100.8	103.7
Reducing sugar (g/L)	39.20	41.20
Total sugar (g/L)	100.0	100.9
Dextran (g/L)	1.50	1.50
Raffinose (g/L)	2.60	1.50
Sucrose (g/L)	40.1	40.1
Glucose (g/L)	23.6	23.6
Galactose (g/L)	1.70	2.10
Fructose (g/L)	14.0	15.6
pH	4.00	4.00

Source: Sasaki *et al.* 1991

Pineapple waste is a good source of nutrients for microorganisms because it consists of high carbon levels and other elements important for survival of the microorganism (Sasaki *et al.* 1991). Table 1 shows the chemical composition of liquid pineapple wastes. In addition, pineapple industries produce large quantities of solid and liquid wastes. Thus, the use of pineapple waste as fermentation medium in RDR for microbial cellulose production is a beneficial waste to wealth program.

Currently, there is little information on the optimum process parameters of fermentation in RDR. Therefore, the objective of this study was to evaluate the major factors that can influence the microbial cellulose production yield in order to determine the optimum process parameters of fermentation in RDR. Data from this work will provide valuable information and better understanding on the production of microbial cellulose in RDR, thus increasing production capacity and helping to fulfil the world demand of microbial cellulose.

EXPERIMENTAL

Experimental Setup using RDR

Preparation and set up for fermentation using the rotary disc reactor (RDR) was in accordance with the work of Norhayati *et al.* (2011). The RDR consists of a series of discs, which are mounted on a shaft. The shaft is connected to a driven motor so that it could rotate the shaft together with the discs. The discs were put in a horizontally designed trough that contained a biological medium in which at least a portion of the contained discs were submerged. A driven motor was used in order to give rotational force to the shaft and discs. The discs would alternately soak the organisms in nutrient medium and expose them to air during each rotation. Figure 1 shows a schematic diagram of RDR, while Table 2 shows the specifications of RDR used in this study.

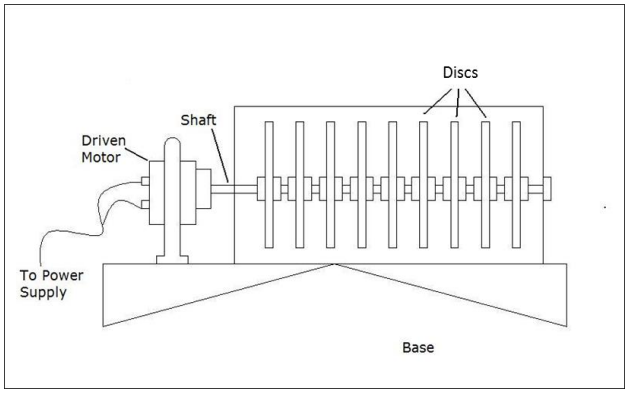

Fig. 1. Schematic diagram of RDR (Pa'e 2009)

Table 2. Specifications of RDR Used in this Study

Parameter	Unit	Specifications
Disc diameter	cm	13
Disc submergence	%	30
Total surface area for discs	cm^2	2124
Total volume	cm^3	10000
Working volume	cm^3	4000
Number of discs	-	8

Source: Pa'e 2009

Preparation of Inoculum *Acetobacter xylinum 0416* (Pa'e *et al.* 2007)

Four hundred millilitres of pineapple waste were sieved into a beaker. Then, 6.00 g/L of yeast extract, 1.49 g/L of bactopepton, 1.08 g/L of KH_2PO_4, and 0.06 g/L of $MgSO_4$ were added to the medium. The medium was stirred until all ingredients were completely dissolved. The pH of the medium was adjusted with 2 M NaOH to pH 5.0 using a pH meter. Then, the medium was poured into a Schott bottle and then autoclaved at 121 °C for 15 min. After being cooled to 28±1 °C, about 10 mL of *Acetobacter xylinum 0416* (obtained from Biotechnology Research Centre, MARDI, Serdang) was added to the medium using aseptic technique. The medium was mixed apparently by manually shaking the bottle gently and slowly using hand. Then, the Schott bottle was kept at 28±1 °C for 3 days before further use.

Preparation of Fermentation Medium (Pa'e *et al.* 2007)

Four litres of pineapple waste were sieved into a 4-L tray. Then, 6.00 g/L of yeast extract, 1.49 g/L of bactopepton, 1.08 g/L of KH_2PO_4, and 0.06 g/L of $MgSO_4$ were added to the medium. The medium was stirred until all ingredients were completely dissolved. The pH of the medium was adjusted with 2 M NaOH to pH 5.0 using a pH meter. Then, the medium was transferred into a Schott bottle and autoclaved at 121 °C for 15 min. After being cooled to 28±1 °C, the medium was ready for the fermentation process. The medium with inoculum was poured into the RDR. The RDR was covered and left for fermentation at 28±1 °C with various ranges of disc rotation speed (5, 7, 9, and 12 rpm), pH (3.5, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.5), fermentation period (3, 4, 5, and 6 days), and inoculum concentration (3, 5, 10, and 20% v/v).

Optimization using Response Surface Methodology (RSM)

Response surface methodology (RSM) is a collection of mathematical and statistical approaches that are useful for the modelling and analysis of data in which a response of interest will be affected by more than one factor and the main target is to determine the optimum conditions of factors to predict desired responses (Myers and Montgomery 2002). In this study, culture conditions were initially determined using experiments based on one-factor-at-a-time (OFAT) variations. In order to optimize the fermentation process in RDR, central composite design (CCD) was selected. With four variables, 17 experiments were carried out. The variables (independent factors) used in this study were: disc rotation speed (rpm), pH, fermentation period (days), and inoculum concentration (% v/v), where X_1 is the disc rotation speed (rpm), X_2 is pH, X_3 is the fermentation period (days), and X_4 is inoculum concentration (% v/v), as shown in Tables 3 and 4. The results from OFAT were then entered into the design experiment spreadsheet in Statistica 8.0 to evaluate the production performance and obtain the equation for microbial cellulose production in RDR based on the variables purposed. In order to evaluate the experimental results, the response factors were fitted with a second-order model in the form of quadratic polynomial equation given below,

$$Y = \beta_0 \pm \beta_i X_i \pm \beta_{ii} X^2_i \pm \beta_{ij} X_i X_j \quad (1)$$

where Y is the predicted response (microbial cellulose production in RDR) used as dependent factor; X_i (i = 1, 2, 3, and 4) are the independent factors, β_0 is the intercept coefficient, β_i, β_{ii} and β_{ij} (i = 1, 2, 3, and 4; j = 1, 2, 3, and 4) are the model coefficients, respectively.

Table 3. Variables Study

Variable	Symbol	Coded Levels		
		-1.0	0	+1.0
Disc Rotation speed (rpm)	X_1	5	7	12
pH	X_2	5.0	6.0	6.5
Fermentation period (day)	X_3	3	5	6
Inoculum concentration (% v/v)	X_4	3	10	20

Table 4. Coded Factor Levels for Central Composite Design

Run	Disc Rotation Speed (rpm)	pH	Fermentation Period (day)	Inoculum Concentration (% v/v)
1	5.00	5.50	4.00	10.00
2	7.00	5.50	4.00	10.00
3	9.00	5.50	4.00	10.00
4	12.00	5.50	4.00	10.00
5	7.00	3.50	4.00	10.00
6	7.00	5.00	4.00	10.00
7	7.00	5.50	4.00	10.00
8	7.00	6.00	4.00	10.00
9	7.00	6.50	4.00	10.00
10	7.00	5.00	3.00	10.00
11	7.00	5.00	4.00	10.00
12	7.00	5.00	5.00	10.00
13	7.00	5.00	6.00	10.00
14	7.00	5.00	4.00	10.00
15	7.00	5.00	4.00	3.00
16	7.00	5.00	4.00	5.00
17	7.00	5.00	4.00	20.00

The model was evaluated using the Fisher's statistical test for analysis of variance (ANOVA). The F-value is the ratio of the mean square due to regression to the mean square due to error. The value of F is compared to the table value F (p-1, n-p, α). If the value of F is smaller than F (p-1, n-p, α), then the null hypothesis is accepted at the α level of significance. Therefore, if the null hypothesis is true, it means that the model is a good predictor of the experimental data. Finally, two-dimensional contour plots and three-dimensional curves of the response surfaces were generated using the same statistical approach.

RESULTS AND DISCUSSION

Optimization of Process Parameters based on OFAT Approach

Four experimental factors, *i.e.*, disc rotation speed, pH, fermentation period, and inoculum concentration, were considered to have the most significant effect on the microbial cellulose production by *Acetobacter xylinum 0416* in RDR. The effect of these four factors was obtained in optimization by the OFAT approach. In this approach, when the effect of one factor was investigated, the other three factors were fixed at the presumptive optimum values as follows: disc rotation speed of 7 rpm, pH of 5.5, fermentation period of 4 days, and 10% (v/v) of inoculum concentration. These factors were examined in the range of parameters, *i.e.*, disc rotation speed (5, 7, 9, and 12 rpm),

pH (3.5, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.5), fermentation period (3, 4, 5, and 6 days), and inoculum concentration (3, 5, 10, and 20% v/v). Microbial cellulose production was considerably affected by changes in these factors. These approximately optimized values (disc rotation speed of 7 rpm, pH of 5.0, fermentation period of 4 days, and inoculum concentration of 10% v/v) were set at the middle level in CCD for further optimization.

Statistical Analysis of Results Obtained using Response Surface Methodology (RSM) through Central Composite Design

The effect of process parameters (disc rotation speed, pH, fermentation period, and inoculum concentration) was investigated using response surface methodology (RSM) through central composite design (CCD). The results were analysed in RSM to visualize the effect of independent factors on responses. Table 5 shows the results of each experiment performed. An empirical relationship between the response and the independent variables has been expressed by the following quadratic model,

$$Y = -200.437 + 7.180X_1 + 69.869X_2 + 4.369X_3 + 1.867X_4 - 0.512X_1^2 - 6.766X_2^2 - 0.585X_3^2 - 0.079X_4^2 \quad (2)$$

where Y is the dried mass of microbial cellulose produced in RDR, X_1 is the disc rotation speed (rpm), X_2 is the pH of fermentation medium, X_3 is the fermentation period, and X_4 is the inoculum concentration.

Table 5. RSM Result of Process Parameters for RDR

	Disc Rotation Speed (rpm)	pH	Fermentation Period (day)	Inoculum Concentration (% v/v)	Dried Mass of Cellulose (g)
1	5.00	5.50	4.00	10.00	20.75
2	7.00	5.50	4.00	10.00	20.75
3	9.00	5.50	4.00	10.00	20.25
4	12.00	5.50	4.00	10.00	10.75
5	7.00	3.50	4.00	10.00	4.70
6	7.00	5.00	4.00	10.00	28.30
7	7.00	5.50	4.00	10.00	22.80
8	7.00	6.00	4.00	10.00	18.10
9	7.00	6.50	4.00	10.00	13.50
10	7.00	5.00	3.00	10.00	23.34
11	7.00	5.00	4.00	10.00	28.30
12	7.00	5.00	5.00	10.00	22.29
13	7.00	5.00	6.00	10.00	21.09
14	7.00	5.00	4.00	10.00	20.34
15	7.00	5.00	4.00	3.00	18.67
16	7.00	5.00	4.00	5.00	19.31
17	7.00	5.00	4.00	20.00	18.70

The actual (observed) values for the combined effects of all factors (disc rotation speed, pH, fermentation period, and inoculum concentration) were used to assess the model responses in terms of specific numerical values in addition to the statistical evaluation of the model. The comparisons of actual and predicted values of microbial cellulose production by *Acetobacter xylinum 0416* in RDR are shown in Table 6 and Fig. 2. The plot of predicted values versus experimental dry weight of microbial cellulose is

shown in Fig. 2 with R^2=0.875, thus indicating that the proposed model is highly adequate.

Table 6. Comparison between Observed and Predicted Values of Results

Run	Observed (Actual)	Predicted	Residues
1	20.75000	21.09677	-0.34677
2	20.75000	23.07144	-2.32144
3	20.25000	21.05913	-0.80913
4	10.75000	10.56506	0.18494
5	4.70000	5.22204	-0.52204
6	28.30000	23.58869	4.71131
7	22.80000	23.07144	-0.27144
8	18.10000	19.23447	-1.13447
9	13.50000	12.07777	1.42223
10	23.34000	23.48964	-0.14964
11	28.30000	23.58869	4.71131
12	22.29000	22.73892	-0.44892
13	21.09000	20.94036	0.14964
14	18.67000	23.58869	-4.91869
15	19.31000	18.93221	0.37779
16	20.34000	20.93943	-0.59943
17	18.70000	18.73526	-0.03526

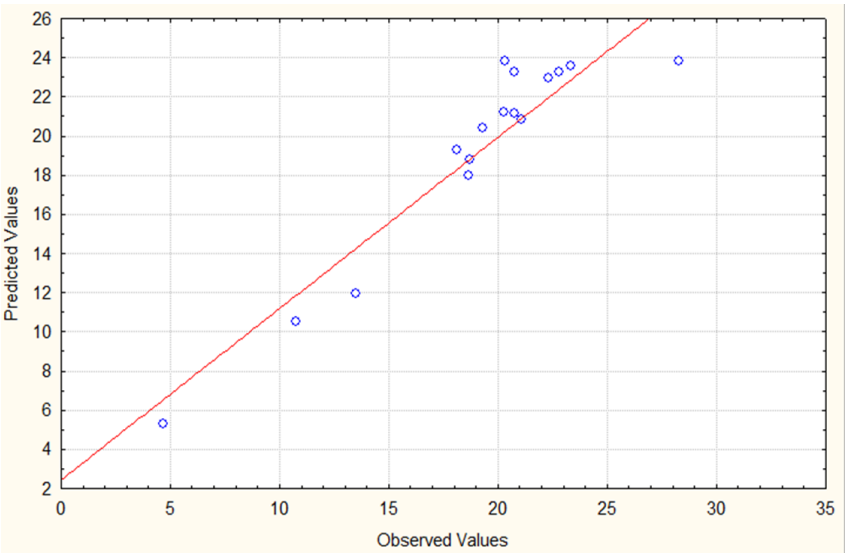

Fig. 2. Observed (actual) *versus* predicted values

Analysing the Accessibility of the Model using ANOVA

Analysis of variance was calculated to determine the accessibility of the model. The analysis of variance of the responses has been presented in Tables 7 and 8. To evaluate the accuracy of the model, the coefficient of variation (the ratio of the standard error of estimate to the mean value expressed as a percentage) was determined and the F-value test was performed. The F-value in the ANOVA table is the ratio of the model mean square (MS) to the appropriate error mean square. The larger the ratio, the larger will be the F-value and the more likely that the variance contributed by the model is significantly larger than random error. As a general rule, the coefficient of variation should not be greater than 10%. ANOVA is required to test the significance and

adequacy of the model. The Fishers variance ratio F-value $=MS_{regression}/MS_{residual}=(SSR/DF_{regression})/(SSE/DF_{residual})$ is the ratio of the mean square owing to regression to the mean square owing to an error. It is the measure of variation in the data about the mean.

Table 7. Analysis of Variance

Sources	Sum of Squares (SS)	Degree of Freedom (DF)	Mean Squares (MS)	F-value	$F_{0.05}$
Regression (SSR)	463.9138	8	57.9892	7.00	3.44
Residual	66.2232	8	8.2779		
Total (SST)	530.1370	16			

Table 8. ANOVA Results

Factor	SS	df	MS	F	p
(1) RPM (L)	66.5458	1	66.5458	8.03897	0.021970
RPM (Q)	42.2767	1	42.2767	5.10718	0.053730
(2) pH (L)	64.1602	1	64.1602	7.75078	0.023777
pH (Q)	338.7998	1	338.7998	40.92822	0.000210
(3) Fermentation Period (L)	4.2676	1	4.2676	0.51554	0.493182
Fermentation Period (Q)	2.0081	1	2.0081	0.24259	0.635573
(4) Inoculum conc. (L)	0.3861	1	0.3861	0.04664	0.834423
Inoculum conc. (Q)	48.6098	1	48.6098	5.87224	0.041637
Error	66.2232	8	8.2779		
Total SS	530.1370	16			

From the results, the ANOVA of the regression model demonstrates that the model is significant as evident from the calculated F-value (7.00) and a very low probability value ($P \leq 0.002693$). The *P* values are used as a tool to check the significance of each of the coefficients, which in turn may indicate the patterns of the interaction among the variables. Values greater than 0.10 indicate the model terms are not significant. This implies that the quadratic effects of disc rotation speed ($p = 0.053730$), pH ($p = 0.000210$), and inoculum concentration ($p = 0.041637$) are more significant. Table 9 shows the analysis of variance (ANOVA) of regression parameters for the predicted response surface quadratic model for the production of microbial cellulose in RDR using the results of all experiments performed.

Table 9. Regression Coefficients

Factor	Regr. Coeff.	Std Error	t(8)	p	-95% Cnf.Limt	+95% Cnf.Limt
Mean/Interc.	-200.437	46.84635	-4.27860	0.002693	-308.465	-92.4090
(1) RPM (L)	7.180	4.00866	1.79123	0.111030	-2.064	16.4244
RPM (Q)	-0.512	0.22648	-2.25991	0.053730	-1.034	0.0104
(2) pH (L)	69.869	10.60530	6.58816	0.000171	45.414	94.3253
pH (Q)	-6.766	1.05753	-6.39752	0.000210	-9.204	-4.3269
(3)Ferm. Period (L)	4.369	10.97718	0.39805	0.701003	-20.944	29.6829
Ferm. Period (Q)	-0.585	1.18738	-0.49253	0.635573	-3.323	2.1533
(4)Inoculum conc. (L)	1.867	0.79105	2.35984	0.045970	0.043	3.6909
Inoculum conc. (Q)	-0.079	0.03265	-2.42327	0.041637	-0.154	-0.0038

From Table 9, the value of coefficient of determination (R^2 = 0.875) indicates that only 12.49% of the total variation could not be explained by the empirical model and expresses good enough quadratic fits to navigate the design space. Joglekar and May (1987) suggested that the R^2 value should be at least 0.80 for a good fit of a model. The R^2 value (0.875) obtained indicated that the regression models explained the reaction well. Thus the response surface model developed in this study for predicting the production of microbial cellulose was considered to be satisfactory.

Effect of Factors for Responses

The response surface curves were plotted to understand the interaction of the variables and to determine the optimum level of each variable for maximum response. The elliptical nature of the contour signifies that the interactive effects between the variables were significant and that optimum values of the test variables could be obtained. The response surface curves for the production of microbial cellulose are shown in Figs. 3 through 8. Each 3-D plot represents the number of combinations of two-test variables. The figures also show the optimal values for the process conditions.

Figure 3 shows the combined effect of disc rotation speed and pH in the RDR where maximum production of microbial cellulose was recorded at a pH of 5.0 and a disc rotation speed of 7 rpm. Clearly, it can also be seen that the high speed of disc rotation and low value of pH had a significant effect on the production of microbial cellulose. From the interrelation between these two factors, pH had a more significant effect compared to the disc rotation speed in the RDR.

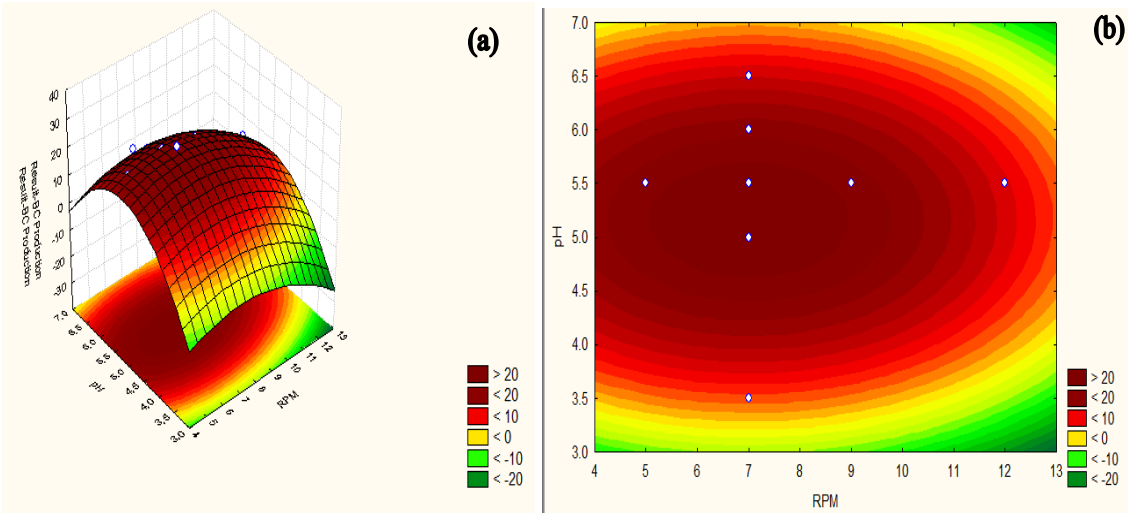

Fig. 3. (a) Composite graph for disc rotation speed (rpm) and pH *versus* microbial cellulose production. (b) Surface response for disc rotation speed (rpm) and pH *versus* microbial cellulose production

The mutual effects of disc rotation speed (rpm) and fermentation period are illustrated in Fig. 4. The highest production of microbial cellulose was obtained at 7 rpm and four days of fermentation period. The disc rotation speed evidently had a more significant effect to the production of microbial cellulose in RDR compared to the fermentation period.

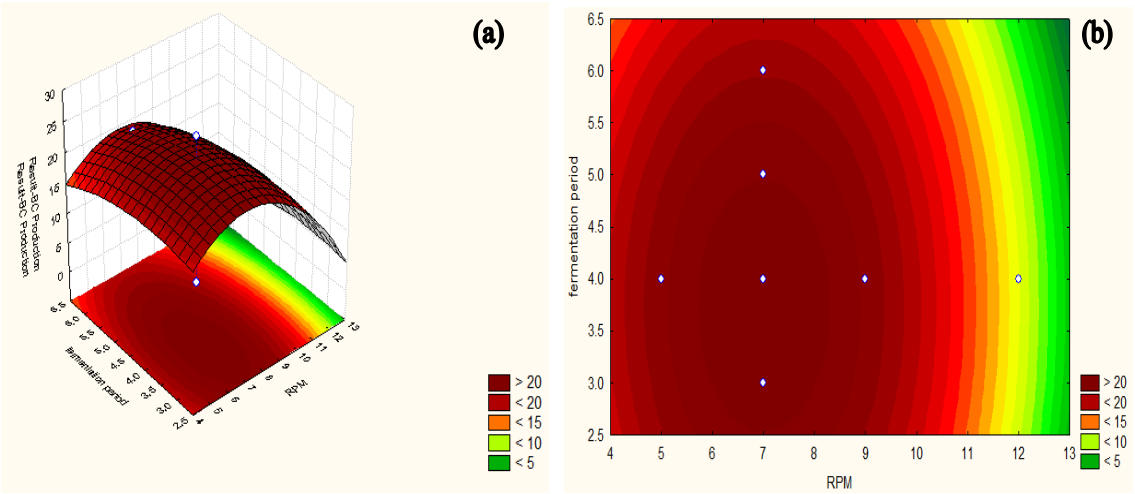

Fig. 4. (a) Composite graph for disc rotation speed (rpm) and fermentation period *versus* microbial cellulose production. (b) Surface response for disc rotation speed (rpm) and fermentation period *versus* microbial cellulose production

The effect of disc rotation speed (rpm) and inoculum concentration on the production of microbial cellulose can be observed in Fig. 5. The optimum conditions were recorded at 7 rpm and 10% (v/v) of inoculum concentration. At high disc rotation speeds (above 10 rpm), the production of microbial cellulose was decreased dramatically. Meanwhile, only a slight effect was observed in production if the inoculum concentration was too low (below 3% v/v) or too high (above 20% v/v). Hence, disc rotation speed had more significant effects when compared to the inoculum concentration.

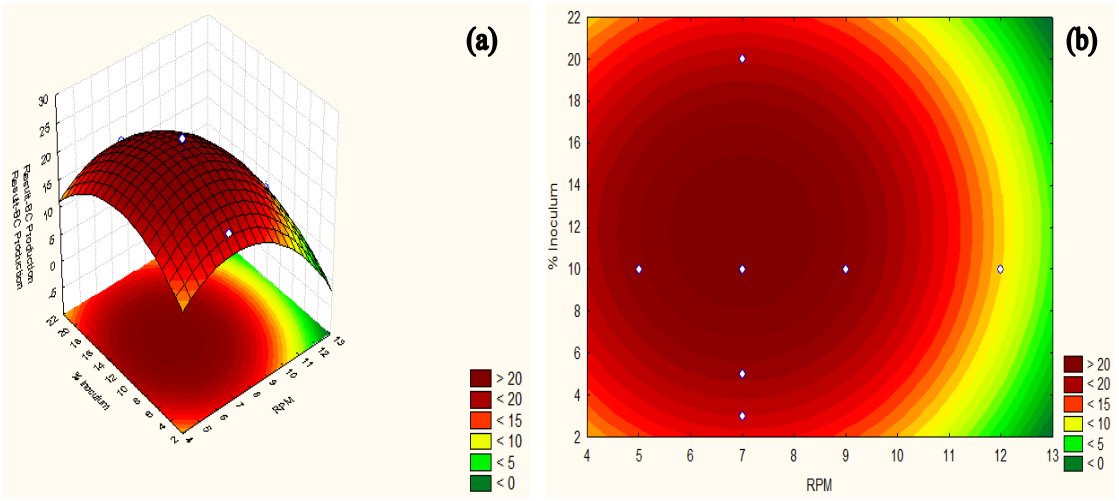

Fig. 5. (a) Composite graph for disc rotation speed (rpm) and inoculum concentration *versus* microbial cellulose production. (b) Surface response for disc rotation speed (rpm) and inoculum concentration *versus* microbial cellulose production

The effects of pH and fermentation period are presented in Fig. 6. The optimum conditions for production of microbial cellulose were noted at a pH of 5.0 and a fermentation period of four days. However, the fermentation period did not have a huge effect on the production of microbial cellulose compared to pH, since a pH value that was too low (below 4.0) or too high (above 6.5) can cause dramatic decrease in microbial

cellulose production because *Acetobacter xylinum 0416* is highly sensitive to pH changes. As a consequence, pH had a more significant effect over the fermentation period.

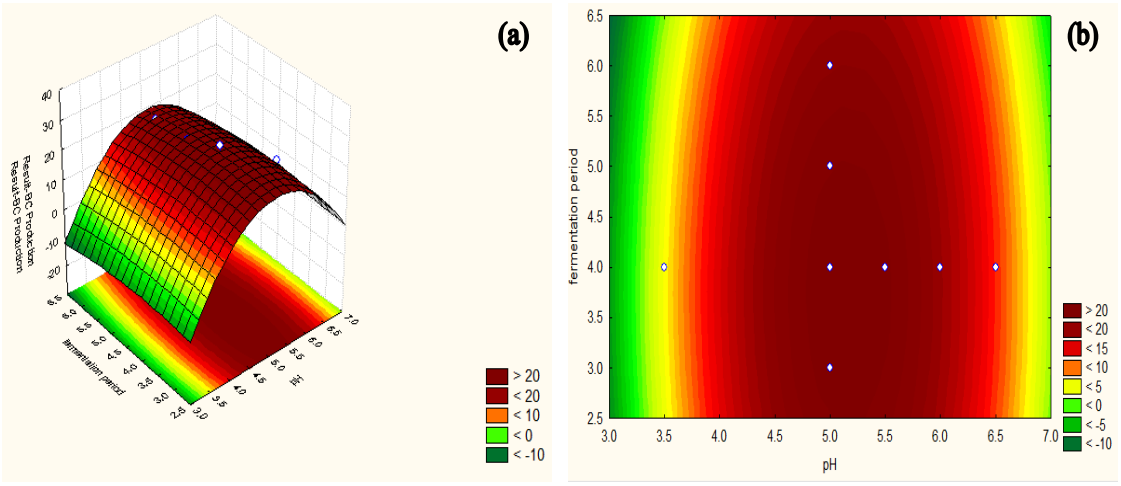

Fig. 6. (a) Composite graph for pH and fermentation period *versus* microbial cellulose production; (b) Surface response for pH and fermentation period *versus* microbial cellulose production

As shown in Fig. 7, the maximum production of microbial cellulose was recorded at a pH of 5.0 and 10% (v/v) of inoculum concentration. As can be seen in the graph, changes in pH values also had a significant effect on production where the desired production yield could only be achieved between a pH of 4.5 and 6.0 (more than 15 grams per litre), whereas the inoculum concentration did not show much effect on the production process. Thus pH gives a more significant effect compared to the inoculum concentration in the RDR.

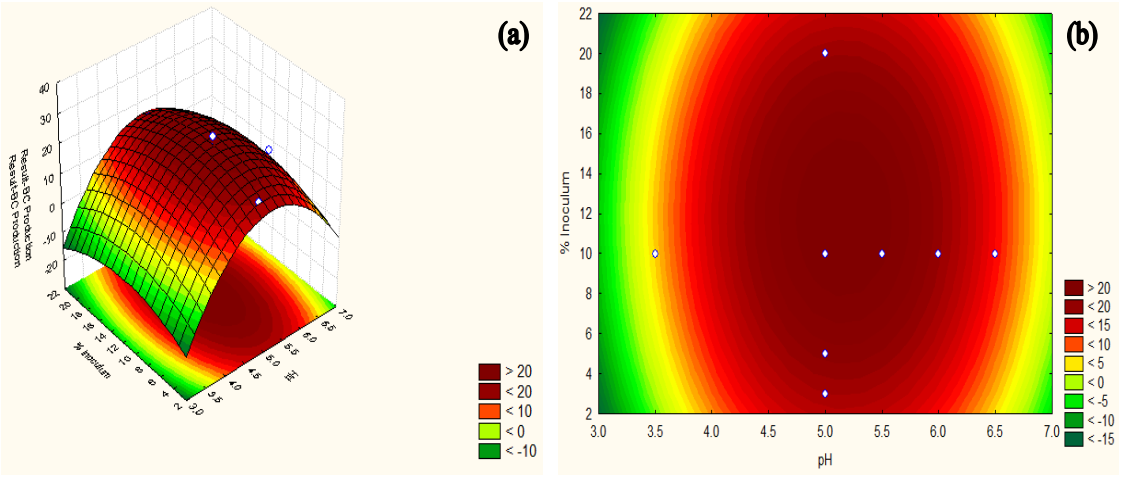

Fig. 7. (a) Composite graph for pH and inoculum concentration *versus* microbial cellulose production; (b) Surface response for pH and inoculum concentration *versus* microbial cellulose production.

The effects of fermentation period and inoculum concentration are illustrated in Fig. 8. The production of microbial cellulose had optimal conditions of a fermentation period of four days and 10% (v/v) of inoculum concentration. If the concentration of inoculum was too low (below 3% v/v) or too high (above 20% v/v) the production

process decreased significantly while changes in fermentation period did not show much effect on the production yield.

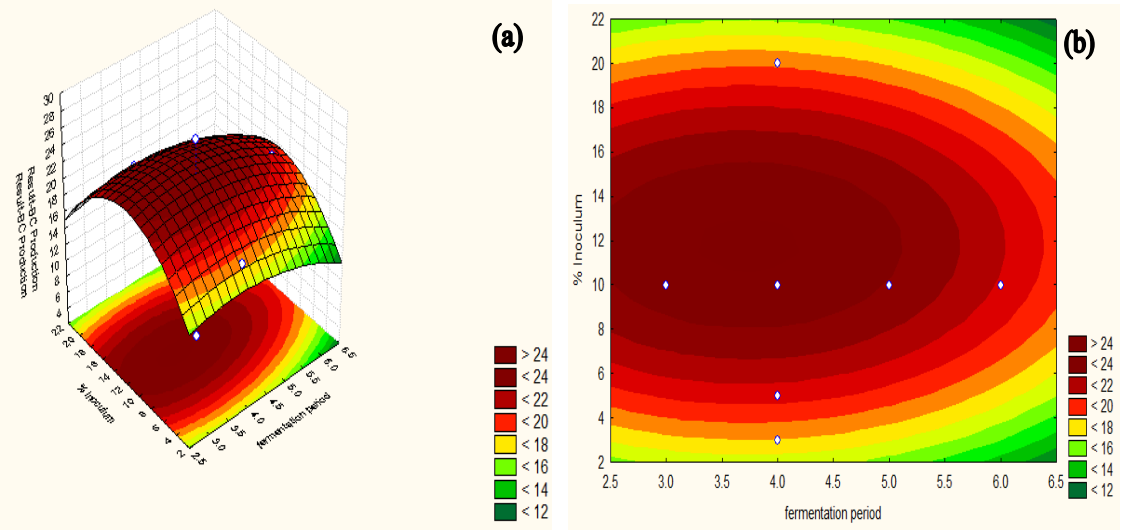

Fig. 8. (a) Composite graph for fermentation period and inoculum concentration *versus* microbial cellulose production. (b) Surface response for fermentation period and inoculum concentration *versus* microbial cellulose production

Pareto Chart of Optimized Parameters for Microbial Cellulose Fermentation in RDR

A Pareto chart highlights the category with the highest frequency of all the variables which have been discussed previously in the production of microbial cellulose in the RDR. From the Pareto chart in Fig. 9, pH showed the highest frequency among other factors that had an impact on the production of microbial cellulose in the RDR. This means the foremost factors that affect the production of microbial cellulose in RDR were pH, followed by inoculum concentration, disc rotation speed (rpm), and fermentation period. Finally, it is also worth to note that since the values for pH, disc rotation speed, and inoculum concentration were above p=0.05, only these three factors would give significant effect to the production process in RDR.

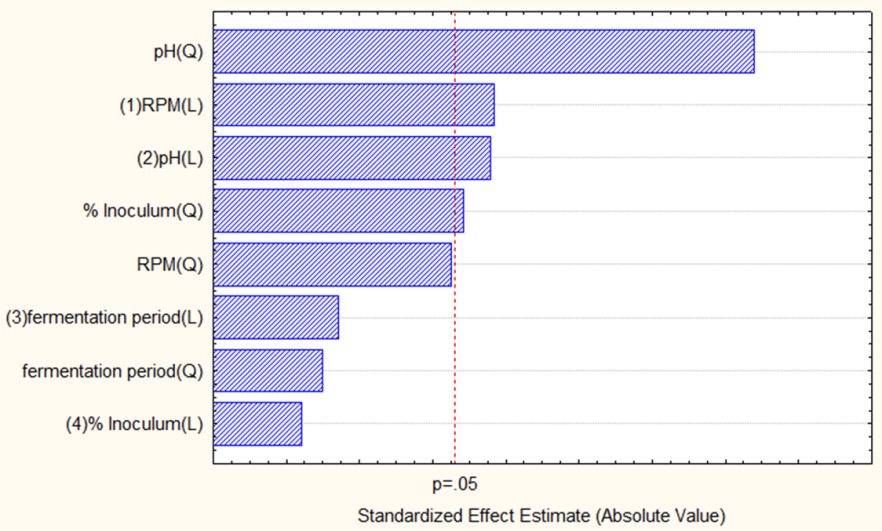

Fig. 9. Pareto chart

CONCLUSIONS

1. An empirical relationship between the response and independent variables can be expressed by the following quadratic model: $Y = -200.437 + 7.180X_1 + 69.869X_2 + 4.369X_3 + 1.867X_4 - 0.512X_1^2 - 6.766X_2^2 - 0.585X_3^2 - 0.079X_4^2$, where Y is the dried mass of microbial cellulose produced in the RDR, X_1 is the disc rotation speed (rpm), X_2 is the pH of the fermentation medium, X_3 is the fermentation period, and X_4 is the inoculum concentration (% v/v).
2. ANOVA of the regression model demonstrated that the model was significant, as evidenced by the calculated F-value (7.00) and a very low probability value ($P \leq 0.002693$). In addition, the value of the coefficient of determination ($R^2 = 0.875$) signified that only 12.49% of the total variation could not be explained by the empirical model and expressed a sufficient quadratic fit to navigate the design space.
3. Finally, pH showed the highest frequency among other factors that had an effect on the production of microbial cellulose in RDR. This means the main factors that affected the production of microbial cellulose in RDR were pH, followed by inoculum concentration, disc rotation speed (rpm), and fermentation period.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Science and Innovation (MOSTI) Malaysia, the Ministry of Higher Education (MOHE), and Research Grant (QJ130000.2544.03H98) from Research Management Centre UTM for their support of this study.

REFERENCES CITED

Bae, S., and Shoda, M. (2005). "Statistical optimization of culture conditions for bacterial cellulose production using Box-Behnken design," *Biotechnology and Bioengineering* 90(1), 20-28.

Chang, Y. C., Lee, C. I., and Pan, T. M. (2006). "Statistical optimization of media components for the production of *Antrodia cinnamomea AC0623* submerged cultures," *Applied and Microbiology Biotechnology* 72(4), 654-661.

Czaja, W., Krystynowicz, A., Bielecki, B., and Brown Jr., R. M. (2006). "Microbial cellulose - The natural power to heal wounds," *Journal of Biomaterials* 27(2), 145-151.

Gupta, G., Basavaraj, S., and Hungund, S. (2010). "Production of bacterial cellulose from *Enterobacter amnigenus GH-1* isolated from rotten apple," *World Journal Microbiology and Biotechnology* 26(10), 1823-1828.

Hutchens, S. A., Leon, R. V., O'Neill, H. M., and Evans, B. R. (2007). "Statistical analysis of optimal culture conditions for *Gluconacetobacter hansenii* cellulose production," *Letters in Applied Microbiology* 44(2), 175-180.

Iguchi, M., Yamanaka, S., and Budhiono, A. (2000). "Bacterial cellulose: A masterpiece of nature's art," *Journal of Material Sciences* 35(2), 261-270.

Joglekar, A. M., and May, A. T. (1987). "Product excellence through design of experiments," *Cereal Food World* 32(12), 857-868.

Keshk, S., and Sameshima, K. (2006). "Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture," *Journal of Enzyme and Microbial Technology* 40(1), 4-8.

Keshk, S. M. A. S., and Sameshima, K. (2005). "Evaluation of different carbon sources for bacteria cellulose production," *Article Journal of Biotechnology* 4(6), 478.

Kim, J. Y., Kim, J. N., Wee, Y. J., Park, D. H., and Ryu, H. W. (2007). "Bacterial cellulose production by *Gluconacetobacter* sp. *RKY5* in a rotary biofilm contactor," *Applied Biochemistry and Biotechnology* 137(1), 529-537.

Kouda, T., Yano, H., and Yoshinaga, F. (1997). "Effect of agitator configuration on bacterial cellulose productivity in aerated and agitated culture," *Journal of Fermentation and Bioengineering* 83(4), 371-376.

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Goncalves-Miskiewicz, M., Turkiewicz, M., and Bielecki, S. (2002). "Factors affecting the yield and properties of bacterial cellulose," *Journal of Industrial Microbiology and Biotechnology* 29(4), 189-195.

Kurosumi, A., Sasaki, C., Yamashita, Y., and Nakamura, Y. (2009). "Utilization of various fruit juices as carbon source for production of bacterial cellulose by *Acetobacter xylinum NBRC 13693*," *Carbohydrate Polymers* 76(2), 333-335.

Myers, R. H., and Montgomery, D. C. (2002). "*Response Surface Methodology: Process and Product Optimization using Designed Experiments*," 2nd Edition, John Wiley & Sons, New York.

Norhayati, P., Khairul, A. Z., and Ida, I. M. (2011). "Production of biopolymer from *Acetobacter xylinum* using different fermentation methods," *International Journal of Engineering & Technology* 11(5), 90-98.

Noro, N., Sugano, Y., and Shoda, M. (2004). "Utilization of the buffering capacity of corn steep liquor in bacterial cellulose production by *Acetobacter xylinum*," *Applied Microbiology and Biotechnology* 64(2), 199-205.

Pa'e, N., Hui, C. C., and Muhamad, I. I. (2007). "Shaken culture fermentation for production of microbial cellulose using pineapple waste," *International Conference on Waste to Wealth.* 26-28 November, Kuala Lumpur, Malaysia Nuclear Agency (MINT).

Pa'e, N. (2009). "Rotary discs reactor for enhanced production of microbial cellulose," Master Degree Thesis, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, Skudai, Johor.

Preetha, B., and Viruthagiri, T. (2007). "Application of response surface methodology for the biosorption of copper using *Rhizopus arrhizus*," *Journal of Hazardous Materials* 143(1-2), 506-510.

Ross, P., Mayer, R., and Benzimen, M. (1991). "Cellulose biosynthesis and function in bacteria," *Microbiological Reviews* 55(1), 35-38.

Sasaki, K., Noparatnaraphorn, N., and Nagai, S. (1991). "Use of photosynthetic bacteria for the production of SCP and chemicals from agro industrial waste," in: *Bioconversion of Waste Material to Industrial Product*, A. M. Martin (ed.), Elsevier Applied Science, London, pp. 225-233.

Shigematsu, T., Takamine, K., Kitazato, M., Morita, T., Naritomi, T., and Morimura, S. (2005). "Cellulose production from glucose using a glucose dehydrogenase gene

(GDH)-deficient mutant of *Gluconacetobacter xylinus* and its use for bioconversion of sweet potato pulp," *Journal of Bioscience and Bioengineering* 99(4), 415-422.

Soo, Y. K., Jin, N. K., Young, J. W., Don, H. P., and Hwa, W. R. (2006). "Production of bacterial cellulose by *Gluconacetobacter* sp. *RKY5* isolated from persimmon vinegar," *Applied Biochemistry and Biotechnology* 131(1-3), 705-715.

Wang, Y. X., and Lu, Z. X. (2005). "Optimization of process parameters for the mycelial growth and extracellular polysaccharide production by *Boletus* spp.," *Process Biochemical* 40(3-4), 1043-1051.

Zeng, X., Darcy, P. S., and Wankei, W. (2011). "Statistical optimization of culture conditions for bacterial cellulose production by *Acetobacter xylinum BPR 2001* from maple syrup," *Carbohydrate Polymers* 85(3), 506-513.

Article submitted: November 25, 2013; Peer review completed: January 19, 2014; Revised version received and accepted: January 29, 2014; Published: February 6, 2014.