Australian Journal of Basic and Applied Sciences, 10(17) Special 2016, Pages: 90-100

AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Optimization Of Sorbitol Production Using Immobilized Of *Lactobacillus Plantarum* Strain (BAA-793) Via Solid State Fermentation (SSF) Process: Response Surface Methodology (RSM)

[1]Zuriana Sidi Ahmad, [2]Mimi Sakinah Abdul Munaim, [3]Farhan Mohd Said

[1]*University of Malaysia Pahang (UMP), Faculty of Chemical Engineering and Natural Resources, 2500 Kuantan Pahang, Malaysia*.
[2]*University of Malaysia Pahang (UMP), Faculty of Technology Engineering, 2500 Kuantan Pahang, Malaysia*.
[3]*University of Malaysia Pahang (UMP), Faculty of Chemical Engineering and Natural Resources, 2500 Kuantan Pahang, Malaysia*.

Address For Correspondence:
Faculty of Technology Engineering, University of Malaysia Pahang (UMP) Kuantan, Pahang, Malaysia.
E-mail: mimi@ump.edu.my/ mimisakinah@gmail.com, tel: 09-5492825/+6019-9221475

ARTICLE INFO

Article history:
Received 19 August 2016
Accepted 10 December 2016
Published 31 December 2016

Keywords:
solid state fermentation, immobilized of Lactobacillus plantarum, respond surface methodology, sorbitol.

ABSTRACT

Background: The process introduced in this work is the production of sorbitol via solid state fermentation (SSF) using immobilized cells of bacteria. The raw material that was used in this study is *Meranti* wood sawdust (MWS) that was obtained from Kilang Kayu Aman, Gambang Kuantan, Pahang. The bacterium used was *Lactobacillus plantarum* (BAA-793) and was immobilized using the entrapment technique (entrapped in sodium alginate). The pretreatment processes used for the treatment of the MWS to recover the cellulose from MWS are the physical pretreatment and chemical pretreatments. The last phase of this works was fermentation using solid state (SSF) process to convert the cellulose to sorbitol. **Objective**: This study aim to optimize sorbitol production via solid state fermentation (SSF) process using response surface methodology (RSM) and the central composite design (CCD) was used in order to reduce the total number of experiments; besides, to determine the best combination of parameters for optimization of the process. **Results**: The result shows that the interactions between parameters like moisture content and substrate amount have a very significant effect and the p-value was < 0.0001. The highest yield of sorbitol production (13.607 g/L) was obtained at the condition comprised of 50 % of moisture content, 4 h of fermentation time and 1.0 gram of substrate amount. **Conclusion:** The production of sorbitol will increase when all the process parameters in the solid state fermentation (SSF) process have been optimized. The RSM was also suitable for fitting a quadratic surface and it also helped to optimize the effective parameters with a minimum number of experiments as well as to analyze the interaction between the parameters.

INTRODUCTION

Malaysia generates an abundance of agricultural wastes such as Meranti wood sawdust (MWS), sugar cane baggage, rice husk, rice straw, rubber wood dust, palm kernel cake and many other waste materials. These wastes result in a significant environmental problem if not disposed of in a proper manner. The Agro-industrial wastes like sawdust have a great potential as a substrate for sorbitol production and others biochemical products. Such utilization would further increase the profitability of the sorbitol and other biochemical industries besides solving an environmental problem. As a renewable energy source, woody biomass like MWS is expected to play an important role in the future metallurgical application. Nowadays biomass such as wood biomass can be used in power generation and production of various chemicals such as biofuels. However, sawdust and others wood wastes will be making an important contribution as biomass in this study since woody biomass like MWS

To Cite This Article: Zuriana Sidi Ahmad, Mimi Sakinah Abdul Munaim, Farhan Mohd Said., Optimization Of Sorbitol Production Using Immobilized Of *Lactobacillus Plantarum* Strain (BAA-793) Via Solid State Fermentation (SSF) Process: Response Surface Methodology (RSM). ***Aust. J. Basic & Appl. Sci.,*** *10(17): 90-100, 2016*

consists mainly of cellulose, hemicellulose, and lignin. The woody biomass can also be potentially used as feedstock for other industrial process where it can provide the energy source for the sustainable metallurgical operations (Strezov *et al*., 2004).

Solid state fermentation (SSF) is the cultivation of microorganism in the absence of free water under controlled conditions. SSF has been utilized to convert moist agricultural polymeric substrate like soy, rice, sawdust, wheat and other substrates into fermented food products like industrial enzymes, fuel and nutrient enriched animal feeds (Pandey *et al*., 2001a, 2004). The processes of solid state fermentation (SSF) can involve pure culture of microorganisms or mixture culture of pure strains. In this research, solid state fermentation was preferred to the liquid state fermentation because of its simple technique, low waste water output (liquid waste is not produced), fewer chances of contamination, low capital investment (cheaper), lower levels of catabolized repression, better product recovery, less time-consuming, and high quality of product (Manpreet *et al*., 2005).

Currently, production of biochemical products such as lactic acid, sorbitol, bioethanol and others biochemical product provides about 50 % of the world's supply through free cell fermentation, but productivity is very low in the fermentation process. However, employing cell immobilization technique that provides high cell density can increase the productivity of biochemical products. The immobilization process is also one of the most attractive methods for maintaining high cell concentration in the bioreactor during biochemical processes (Chang, 1996). Basically, immobilization process can be seen as the localization of intact cell to a certain defined region of space or physical confinement with the preservation of some desired activity (Karel *et al*., 1995). Immobilization also consists of immobilizing cells of microorganism inside or on the surface of a carrier in a way that preserves their catalytic activity (Jack and Zajic, 2006). The process of cell immobilization and their application in bioprocessing has been interesting for about 30 years now (Behera *et al*., 2010). The cell immobilization system offers a great advantage in several industrial processes such as been making it easy to handle biocatalyst, easy separation of biological material from the reaction medium, employs high cell loading capacity and improved the production rate of products (Massalha *et al*., 2007). The process of immobilization such as entrapment in Na-alginate is the most widely used procedure for lactic acid bacteria immobilization (Yan *et al*., 2001).

Generally, respond surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for modeling and analysis of experiments especially where a response of interest is influenced by several variables. The main objective is to optimize/maximize the response of a process with minimal input (Shankar *et al*., 2013). Currently, RSM is widely used in quality improvement, product design and uncertainty analysis. RSM can also determine the optimum process conditions by combining the experimental designs with interpolation by first- or second- polynomial equation in the sequential testing procedure (Ferreira *et al*., 2009). Therefore, this study mainly focused on the optimization of sorbitol production from MWS using the central composite design (CCD) of the RSM as a tool for maximum yield.

Methods:

Substrate and Pretreatment:

The raw material used in this study was MWS obtained from Kilang Kayu Aman, Gambang Kuantan, Pahang. The MWS is a hardwood that is highly composed of cellulose when compared with others wood types. The MWS was taken from the plant and dried under the sunlight for about 5 h in order to make it easier to grinding using a grinder. The pretreatment processes in this study were divided into two which are physical pretreatment and chemical pretreatment. The physical pretreatment processes were actually used to enhance the removal of lignin and hemicelluloses from the MWS. The MWS was ground using the grinder in order to get a uniform size of the around ≤ 0.5mm. The materials were then sorted into sizes by using the sieve shaker machine to get the uniform particle size and the particle size of the sawdust that was used in this study was ≤ 0.5 mm. The reduction in particle size leads to an increased availability of specific surface area and a reduction of the degree of polymerization (DP) of the fibers. The reduction of the DP, shearing and increase in the specific surface area contributed to the increase in the total hydrolysis yield of the lignocellulose material and the reduction in the technical digestion time. This size reduction also will lead to an effective pretreatment (Taherzadeh & Karimi, 2008). After sorting into sizes, the MWS was autoclaved at 121 ^oC for 15 min and later dried in the oven at 60 ^oC for 48 h in order to achieve a constant weight with a moisture content of less than or equal to 10 % (Kholic *et al*., 2011). The moisture content of the MWS was determined by using the weight loss equation shown in Eq. 1 (Kholic *et al*., 2011).

$$MC =(\text{(Mass initial of sawdust-Mass dried of sawdust))/(Mass initial of sawdust)x100\%} \quad (1)$$

Then, during the chemical pretreatments, treatment was performed in phases-the pre-delignification treatment, first stage treatment and second stage of treatment. For the process of pre-delignification, 18 % (w/v) of NaOH solution was prepared and the MWS soaked into it at a ratio of 6:1 (L:S) w/w in an oil bath shaker for 90 min at 110 ^oC. This was performed to bleach and also to pre-break off the link of lignin, hemicelluloses, and cellulose in the biomass. After that, the samples were washed using hot deionized water until the wash water attained or nearly become pH 7. The samples were then dried in the oven for 24 h at 60 ºC and then followed by first stage treatment. All the experiment were done using 1000 mL Erlenmeyer conical flask.

The purpose of the first stage pretreatment process was to remove the lignin content from MWS using peracetic acid (PAA). The previous study showed that the peracetic acid (PAA) can highly remove lignin from biomass material such as MWS (Zhoa *et al*., 2008). The samples were soaked in PAA solution at 80 ºC at a ratio of 6:1(L:S) w/w for 90 min (Zhao *et al*., 2008). After that, all the sample were washed using hot de-ionized water until the washed water neared pH 7 before drying in the oven at 60 ºC for 24 h. All the experiment was carried out using 1000 ml Erlenmeyer conical flask placed in a water bath shaker. For the second stage of treatment, it was performed using sulfuric acid (H_2SO_4) at a concentration of 1.1 %. The samples were soaked in 1.1 % sulfuric acid solution at 123 ºC at a ratio of 6:1(L:S) w/w for 80 min. After that, the samples were washed using hot de-ionized water until the washed water pH was close to 7 and the samples were then dried in the oven at 60 ºC for 24 h and kept until further processing (Zhao *et al*., 2008). The percentage of lignin removed after every step of the treatment was measured using the Kappa Number and Technical Association of Pulp and Paper Industry standard method (TAPPI method).

Microorganism:

The type of bacteria used was *Lactobacillus plantarum* strain (BAA-793) and was purchased from America Type Culture Collection (ATCC). This strain is anaerobic condition type. To handle this bacterium, the anaerobic condition must be applied in order to get the good growth of those bacteria.

Immobilization Cells:

The bacteria (*Lactobacillus plantarum*) cells that were previously grown in MRS broth were mixed with an equal volume of 2 % Na-alginate solution (1:1, v/v). Then, the alginate-cell solution was dropped slowly into 0.2 % solution of calcium chloride ($CaCl_2$) using a syringe needle. Upon addition to the $CaCl_2$ solution, the alginate solidified and formed gel beads, thus entrapping the bacteria cells. The beads were allowed to harden in the $CaCl_2$ solution for 30 min before been washed with 0.85 % of NaCl solution and distilled water to remove the excess calcium ion and cells. The beads were stored at 4 °C for further experiment. To improve the immobilization results, the ratio of $CaCl_2$ and NaCl that was used in solution preparation was 1:1 v/v (Modification from El-Borai *et al*., 2013; Bangrak *et al*., 2011; Yan *et al*., 2001).

Conversion Cellulose to Sorbitol using SSF Process:

In the solid state fermentation (SSF) process, several parameters play important roles in order to produce a high yield of sorbitol. In this study, the effect of several parameters such as the fermentation time, moisture content, and substrate amount were studied. Two grams of samples were added into a 100 mL Erlenmeyer conical flask and moistened with 50 % of distilled water (Sabu *et al*., 2008). All the apparatus and materials were sterilized at 121 ºC for 15 min and all the studies were performed under the laminar flow to avoid contamination and loss of viability. After the sterilization of the apparatus to be used in the study, 10 % beads of immobilized cells were put into the samples (Modification from Hsieh and Yang, 2004; Yan *et al*., 2001; Bangrak *et al*., 2011; El-Borai *et al*., 2013). The samples were later purged with nitrogen gas (N_2) in order to replace oxygen gas to maintain the anaerobic condition and then it was incubated at 35 °C.

Analysis Methods:

The sorbitol production was determined using High-Performance Liquid Chromatography, (HPLC Agilent, 1200 series). The column for the quantification of sorbitol was Rezex Chromatographic Method, RCM Monosaccharides 300 X 7.8 mm with water as a mobile phase. The sugar was eluted with deionized water at a flow rate 0.6 mL/min and the column, maintained at 75 ºC with the retention time is 30 min. This method used Refractive Index, RI as a detector (Saha and Nakamura, 2003).

Experimental Design:

In this part, the experimental design for sorbitol production from MWS using immobilized cells via solid state fermentation (SSF) process was done by choosing the parameters which had a considerable effect on the sorbitol production during the screening phase of the study. These parameters include the fermentation time, the moisture content of substrate and the substrate amount. The parameters were chosen from the OFAT studies and the optimum points set at 4 h for fermentation time, 50 % for moisture content and 1.0 gram for the substrate amount. These parameters will most likely influence the production of sorbitol. In addition, the experiment was designed using the CCD module of the Design Expert V 6.0.8. The statistical optimization study was carried out in order to study the effect of varying the studied parameters on the yield of sorbitol. The central composite design (CCD) was used because the number of varied parameters was not more than three. The RSM was also suitable for a quadratic response surface and it is also used to optimize the effective parameters with a minimum number of experiments. It also helps to analyze the interaction between parameters (Myers and Montgomery, 2002). The RSM has already been successfully applied for the optimization of the fermentation process and other bioprocesses (Amini *et al*., 2008; Hao *et al*., 2010).

RESULT AND DISCUSSION

Central Composite Design (CCD) and Response Surface Methodology (RSM):

The response of sorbitol production (g/L), as a function of fermentation time, moisture content, and the substrate amount was evaluated using the CCD. All the experiments were carried out under these conditions, fermentation time of 2-6 h, the moisture content of 40- 60 %, and the substrate amount of 0.5-1.5 gram. The range of these parameters was chosen based on the experimental results achieved when using one factor at a time (OFAT) to screen for the parameters. The three factors were initially screened using the full factorial design with 2 replicates of factorial point and 2 replicates of the axial point, 4 center points ($\alpha = 2$) which resulted in a total of 32 experimental runs. The CCD design of the experiments and the response of sorbitol yield are shown in Table 1. A full quadratic model was proposed by the Design Expert V6.0.8.

Table 1: The experimental layout and results of central composite design (CCD)

Std	Run	Factor 1: Fermentation time (Hours)	Factor 2: Moisture content (%)	Factor 3: Substrate amount (g)	Sorbitol production (g/L). Actual value	Sorbitol production (g/L). Predicted value
1	1	3.00	45.00	0.75	10.55	10.62
2	2	3.00	45.00	0.75	10.57	10.62
3	3	5.00	45.00	0.75	11.89	12.14
4	4	5.00	45.00	0.75	12.81	12.14
5	5	3.00	55.00	0.75	8.96	8.90
6	6	3.00	55.00	0.75	8.73	8.90
7	7	5.00	55.00	0.75	10.55	10.66
8	8	5.00	55.00	0.75	10.56	10.66
9	9	3.00	45.00	1.25	11.24	11.12
10	10	3.00	45.00	1.25	11.23	11.12
11	11	5.00	45.00	1.25	11.60	11.52
12	12	5.00	45.00	1.25	11.55	11.52
13	13	3.00	55.00	1.25	11.27	11.49
14	14	3.00	55.00	1.25	11.28	11.49
15	15	5.00	55.00	1.25	11.72	12.14
16	16	5.00	55.00	1.25	12.69	12.14
17	17	2.00	50.00	1.00	10.09	9.97
18	18	2.00	50.00	1.00	10.07	9.97
19	19	6.00	50.00	1.00	12.03	12.14
20	20	6.00	50.00	1.00	12.02	12.14
21	21	4.00	40.00	1.00	10.69	10.83
22	22	4.00	40.00	1.00	10.65	10.83
23	23	4.00	60.00	1.00	9.93	9.73
24	24	4.00	60.00	1.00	9.84	9.73
25	25	4.00	50.00	0.50	8.41	8.41
26	26	4.00	50.00	0.50	8.41	8.41
27	27	4.00	50.00	1.50	10.61	10.38
28	28	4.00	50.00	1.50	10.13	10.38
29	29	4.00	50.00	1.00	13.61	13.57
30	30	4.00	50.00	1.00	13.52	13.57
31	31	4.00	50.00	1.00	13.59	13.57
32	32	4.00	50.00	1.00	13.56	13.57

Using the Design Expert V6.0.8 (Stat Ease Inc., Minneapolis, USA), an analysis of variance was conducted for the evaluation of the effects of the variables and their probably existed interactions. Table 2 shows the lack of fit tests for the three models (Linear, 2FI, and Quadratic). The selected model had an insignificant lack of fit. From the Table 2, the quadratic model has an insignificant lack of fit with high multiple correlations of coefficients of R^2 (0.9777) respectively and a full quadratic model was proposed by expert V6.0.8. Table 3 shows the ANOVA for the response surface quadratic model (partial of some square) and response for the sorbitol production. From the Table 3, the computed F and prob>F values were 107.28 and < 0.0001, respectively, which implied that the model was highly significant with low probability. Besides that, the results adequately suggested that the present mathematical model was good for the prediction of the experimental result. The percentage error between the actual and predicted value of the response was less than 5 %, which means that the empirical model developed was considerably accurate for the response terms of sorbitol production. The multiple correlation coefficients of R^2 was calculated to be 0.9777, showing that there was a good agreement between the experimental and predicted value; as well as depicting that 97.77 % of the variability in the response could be well explained by the model.

The lack of fit value was found insignificant (prob>F = 0.3080) which denoted that the model was desirably fit. The p-value of less than 0.05 indicates that the model terms were significant since values greater than 0.1000 indicates an insignificant model (Li *et al*., 2008; 2013). Furthermore, the main effect of the fermentation time (A), moisture content (B) and substrate amount (C) were found to be primarily significant on the sorbitol production process. The second order effect of fermentation time (A2), moisture content (B2), the substrate amount (C2); and the two level interactions between the moisture content and the substrate amount (BC) were also found to significantly influence the yield of sorbitol as could be substantiated with a p-value of < 0.0001. The two level interactions between the fermentation time and the substrate amount (AC); and the two level interactions between the fermentation time and the moisture content (AB) were found to show secondary effect on sorbitol production as could be substantiated with the p-values of 0.0003 and 0.3451, respectively.

Table 2: Lack of Fit Tests

Sources	Sum of square	Degree of freedom,DF	Mean square	F-value	P-value Prob >F	R-squared
Linear	44.81	11	4.07	65.73	< 0.0001	0.2993
2FI	39.13	8	4.89	78.92	< 0.0001	0.3861
Quadratic	0.40	5	0.081	1.31	0.3080	0.9777

Table 3: ANOVA for response surface quadratic model (partial some of the square), response; sorbitol production (g/L)

Source	Sum of Square	DF	Mean Squares	F-Value	P-Value Prob>F
Model	63.99	9	7.11	107.28	< 0.0001[a]
A	9.38	1	9.38	141.58	< 0.0001
B	2.42	1	2.42	36.48	< 0.0001
C	7.79	1	7.79	117.48	< 0.0001
AB	0.062	1	0.062	0.93	0.3451
AC	1.24	1	1.24	18.65	0.0003
BC	4.38	1	4.38	66.13	< 0.0001
A^2	12.63	1	12.63	190.51	< 0.0001
B^2	21.63	1	21.33	326.44	< 0.0001
C^2	34.87	1	34.87	526.22	< 0.0001
Residual	1.46	22	0.066		
Lack of Fit	0.40	5	0.081	1.31	0.3080[b]
Pure Error	1.05	17	0.062		
Cor Total	65.44	31			
Std.Dev.	0.26			R^2	0.9777
Mean	11.07			Adjusted R^2	0.9686

The value of "prob>F" less than 0.0500 indicate model terms are significant.
[a] significant
[b] not significant

The multiple regression equations for sorbitol production where A is the fermentation time, B is the moisture content, and C is the substrate amount in actual and coded terms are given in Eqs. 2 and 3. The final equation in term of coded factors is given as:

Final equation in term of Coded factors:

$$\text{Sorbitol production} = +13.57 + 0.54A - 0.27B + 0.49C + 0.062AB - 0.28AC + 0.52BC - 0.63A^2 - 0.82B^2 - 1.04C^2 \quad (2)$$

Final equation in term of Actual factors:

$$\text{Sorbitol production} = -77.82521 + 6.05709A + 2.76553B + 18.89037C + 0.012420AB - 1.11167AC + 0.41869BC - 0.62812A^2 - 0.032889B^2 - 16.70270C^2 \quad (3)$$

The Eqs 2 and 3 in coded and actual factors respectively are empirical model equations. These empirical model equations are mathematical correlation models that can be employed to predict and optimize sorbitol production within the range of varied factors in this experiment.

Interaction of Variables:

The relationship between the response and the variables was visualized by response surfaces constructed according to the full models as shown in the Figures (A, B, and C) as it shows the 3-D response surface plots of the effects of the studied parameters on the sorbitol production.

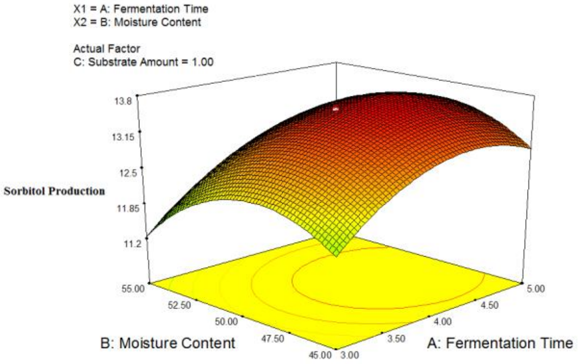

Fig. A: The three-dimensional graph (3-D) of the effect of fermentation time and moisture content on sorbitol production

The effect of the fermentation time, moisture content and the substrate amount as varied parameters were further analyzed using the simulated 3-D response surface according to the quadratic model. The parameters such as fermentation time, moisture content and substrate amount were quite important and had effects on the sorbitol response. Figure A shows the response plot (3-D) of the effect of the fermentation time and moisture content on sorbitol production. From the 3-D graph in Figure A, it clearly shows that the production of sorbitol slowly increased with increases in the fermentation time and moisture content until a maximum value of sorbitol yield of 11.6496 g/L and 13.2924 g/L were achieved at 3 h and 4 h, respectively at a substrate concentration of 1.0 g. This shows that the SSF process using immobilized *Lactobacillus plantarum* (BAA-793) as a microorganism required a short time to produce a high yield of sorbitol. Also, the production of sorbitol decreased when the moisture content was increased from 50 % (13.6073 g/L) to 55% (11.450 g/L). From this plot, the maximum sorbitol production was approximately 13.6073 g/L at a fermentation time of 4 h, moisture content of 50 %, and the substrate amount of 1.0 g. These findings led to the conclusion that the moisture content (50%) was suitable for the immobilized cells of *Lactobacillus plantarum* sp. (strain BAA-793) to within a short time of fermentation convert cellulose directly to sorbitol. In addition, the optimum moisture level in SSF process has a great impact on the physical properties of the solid substrate as well as the sorbitol production. According to previous studies, the moisture level in solid state fermentation critically affects the process because of its interference in physical properties of the solid particle (Vastrad and Neelagund, 2011). Also, the result of optimization studies using RSM for the production of amylase enzyme activity was reported to have increased with an increase in the moisture level as the moisture level was the main factor in solid state fermentation (Husaini *et al*., 2016). This is supported by the reports of Mitchel *et al* (2000) and Neifar *et al* (2011) where during solid state fermentation (SSF) process, the microorganism obtained water from the moisture held within the substrate particles which is essential for their growth.

Figure B shows the 3-D plot of the effect of the fermentation time, substrate amount and moisture content on sorbitol production.

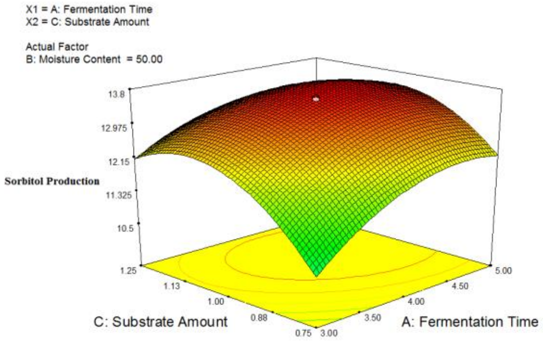

Fig. B: The three-dimensional graph (3-D) of the effect of fermentation time and substrate amount on sorbitol production.

Based on Figure B, the sorbitol production increased when the substrate amount was increased from 0.75 g (10.557 g/L) to 1.0 g (13.1916 g/L) and later decreased when the substrate amount was increased to 1.25 g (12.1509 g/L). From an industrial point of view, an important criterion in industrial or large scale production is to reduced cost of production. To achieve the optimum sorbitol production at the lowest possible level of time and cost, the immobilized cells of *Lactobacillus plantarum* sp. could be a good choice as all the parameters been used at low concentrations and a short period of time will reduce the cost of large-scale production

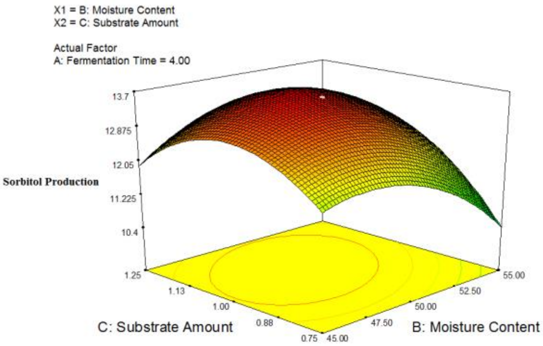

Fig. C: The three-dimensional graph (3D) of the effect of moisture content and substrate amount on sorbitol production

Figure C shows the 3-D plot for the effect of moisture content and substrate amount on sorbitol production. Based on Figure C, there was an appreciable interaction between the moisture content and substrate amount during the production of sorbitol. The production of sorbitol increased from (10.5688 g/L) at 45% to (13.6073 g/L) at 50% at a fermentation time of 4 h. Based on Figure C also, the maximum production of sorbitol was achieved and obtained at 50 % of moisture. Subsequently, the production of sorbitol began to reduce when a higher level of moisture content was introduced. Previous studies suggested that lower moisture than optimum decrease the solubility of solid substrate and lowers the degree of swelling, and produced a higher water tension; but a higher moisture content level than optimum value will cause decreased porosity and alteration in solid state particle structure (Khoramnia *et al.*,2013, Gervais and Molin 2003).

In addition, the interactions between the moisture content and the substrate amount have shown the significant effect as shown with the p-value of < 0.0001. The moisture content is related to the water activity, (aw). Actually, the water activity, (aw) usually increases with increase in temperature and pressure. For example using salt and some sugar, the water activity (aw), may reduce with increase in temperature. For small temperature increase (T1 to T2) at low aw, the relationship of water activity, (aw) and temperature can be represented as in Eq. 4.

$$\mathrm{Ln}\,((\mathrm{aw\ at\ T2})/(\mathrm{aw\ \ at\ T1})) = \Delta H/R\ \ (1/T1 - 1/T2) \qquad (4)$$

Where the is ΔH an enthalpy change (for example, absorption or mixing), R is the gas constant, T is temperature and a*w* is water activity. Then, in the solid state, the rate of the degradation reaction have been shown in order to vary with temperature and relative humidity according to humidity-corrected Arrhenius equation.

Process Optimization:

Table 4 shows a summary of sorbitol production using immobilized cells via SSF. From this table, the fermentation time, moisture content and the substrate amount were optimized at 4 h, 50 % and 1.0 g, respectively, producing a sorbitol yield of 13.607 g/L. It also shows that the difference between the actual and predicted sorbitol production was 0.3 %, which was within the agreed value of 5 %. It signifies that the model was statistically reliable for the prediction of the sorbitol yield at up to 95 % of confidence interval.

Table 4: The result of the optimum operational conditions for sorbitol production

Factors	Proposed optimal condition	Sorbitol production (g/L) (Predicted value)	Sorbitol production (g/L) (Actual value)
Fermentation time (Hours)	4		
Moisture content (%)	50	13.57	13.607
Substrate amount (g)	1.0		

Validation of Empirical Model Adequacy:

The validation process of the empirical model was required in order to confirm the prediction accuracy of the model which was generated by the regression equation for the prediction of the yield of sorbitol at a given fermentation time, moisture content and substrate amount. The result was validated by performing a set of experiment suggested by the model using the same conditions that were previously employed. Table 5 shows the result of the model validation. It involved five experimental combinations suggested by the model at optimal conditions. The actual values obtained and their associated predicted values from the selected experiments were compared for further residual and percentage error analysis.

Table 5: The validation of experimental design

No	Fermentation time (Hours)	Moisture content (% v/w)	Substrate amount (g)	Predicted	Actual	Residual	% Error
1	3.11	50	1	12.583	11.985	-0.598	4.98
2	3.20	50	1	12.732	12.202	-0.530	4.34
3	3.57	50	1	13.216	12.887	-0.329	2.55
4	4.00	50	1	13.567	13.516	-0.051	0.37
5	4.17	50	1	13.641	13.310	-0.331	2.48

From Table 5, the residual and percentage errors were calculated based on the Eqs 7 and 8. The percentage error for the validation of the experimental design for sorbitol production as shown in Table 5 ranged from 0.37 % to 4.98 %. This means that the developed empirical model was considerably accurate for the response terms of sorbitol production because the percentage errors between the actual and predicted values were well below 5 %. Furthermore, the model’s accuracy for the prediction of the sorbitol yield was statistically reliable and confident at up to 95 % prediction interval. This means that further analysis with regards to an ideal operational process for the optimal production of sorbitol from MSW would be based on this developed model.

$$\text{Residual} = (\text{Actual value} - \text{Predicted value}) \tag{5}$$
$$\%\ \text{Error} = (\text{Residual}/\text{Actual value}) \times 100\% \tag{6}$$

In addition, the validation of the experimental design for the fermentation time was set up within the range of 3 to 5 h, moisture content at 50 %, and substrate amount at 1.0 gram. This was set to be so in order to be able to achieve a good yield at low cost during industrial scale production. As seen in Table 4, the production process was economically feasible as the time of reaction to produce sorbitol was short while the yield was high. To minimize the usage of water, the moisture content should be maintained at 50 % in order to minimize the cost of producing sorbitol in the scaled-up process. The validation of the experimental design shown in Table 4 portrays that the production of sorbitol was higher when the moisture content was at 50 % and 1.0 g substrate amount.

Conclusions:

The sorbitol production was statistically optimized in the present work using the response surface methodology (RSM) in order to study the effect of the variable parameter during the solid state fermentation process using immobilized cells of *Lactobacillus plantarum* strain (BAA-793). The RSM was suitable enough for fitting a quadratic model for the prediction of the yield of the sorbitol as well as help in the optimization and analysis of the effects of the studied parameters which were considerable enough on the response. These feats were achieved with minimum experimental involvement due to the perfect utilization of the CCD model of the RSM.

REFERENCES

Amini, M., K. Mueller, K.C. Abbaspour, T. Rosenberg, M. Afyuni, K.N. Møller, M. Sarr and C.A. Johnson, 2008. Statistical Modeling of Global Geogenic Fluoride Contamination in Groundwaters. Environmental Science and Technology, 42: 3662-3668.

Amit, S., V. Vivekanand and P.S. Rajesh, 2008. Solid-State Fermentation for Gluconic Acid Productions from Sugarcane Molasses by *Aspergillus niger* ARNU-4 Employing Tea Waste as the Novel Solid Support. Bioresource Technology, 99: 3444-3450.

Anees, A., R. Mohd, S. Othman, I. Mahamad Hakimi, Y.C Yap and M.S. Bazlul, 2009. Removal of Cu(II) and Pb(II) Ions from Aqueous Solutions by Adsorption on Sawdust of *Meranti* Wood. Desalination, 247: 636-646.

Antonio, S.V., D.R. Gloria, B.O. Nabil, A. Hikmate, L.L. Rosario, M.C. Magdalena, O. Elena and G. Antonio, 2008. Inhibition of Food Poisoning and Pathogenic Bacteria by *Lactobacillus plantarum* strain 2.9 isolated from Ben Saalga, Both in a Culture Medium and In Food. Food Control, 19: 842-848.

Ashwani, S., G. Neelam, S. Jitender, K. Kalika, C. Ramesh and K.G. Vijay, 2008. Optimization of Xylanase Production using Inexpensive Agro-residues by Alkalophilic *Bacillus subtilis* ASH in Solid Sstate Fermentation. World Journal of Microbiol Biotechnol, 24: 633-640.

Bangrak, P., S. Limtong and M. Phisalaphong, 2011. Continuous Ethanol Production using Immobilezed Yeast Cells Entrapped in Loofa-reinforce Alginate Carriers. Brazilian Journal of Microbiology, 42: 676-684.

Behera, S., R.C. Mohanty and R.C. Ray, 2011. Ethanol Production from Mahula (Madhucalatifolia L.) Flowers using Free and Immobilized (in Luffacylindrical L.spongediscs) cells of *Zymomonamobilis* MTCC92. Ann Microbiol, 61: 469-474.

Chang, H.N and I.K. Yoo, 1996. Encapsulation of Lactobacillus Casei Cells in Liquid-Core Alginate Capsules for Lactic Acid Production. Journal of Enzyme and Microbial Technology, 19: 428-433.

Cristobal, C., R. Encarmacion, B. Ignacio, J.N. Maria and C. Eulogio, 2006. Enhanced Enzymatic Hydrolysis of Olive Tree Wood by Steam Explosion and Alkaline Peroxide Delignification. Process Biochemistry, 41: 423-429.

Fang, H., C. Zhao and X.Y. Song, 2010. Optimization of Enzymatic Hydrolysis of Steam-Exploded Corn Stover by Two approaches: Response Surface Methodology or Using Cellulase from Mixed culture of *Trichoderma resei* RUT-C30 and *Aspergillus niger* NL02. Bioresources Technology, 101: 4111-4119.

El-Borai, A.M., A.S. Youssef, A.E.A. El-Salam and S.A. El-Assar, 2013. Optimization of Keratinase Production by Aspergillus terreus Thom by Statistical and Immobilization Methods. Life Science Journal, 10(3).

Ferreira, S., A.P. Duarte, M.H.L. Ribeiro, J.A. Queiroz and F.C. Domingues, 2009. Response Surface Optimization of Enzymatic Hydroysis of *Cistus ladanifer and Cytisus striatus* for Bioethanol Production. Journal of Biochemical Engineering, 45: 192-200.

Gervais, P and P. Molin, 2003. The Role of water in Solid State Fermentation. Journal of Biochemical Engineering, 13(2-3): 85-101.

Hsieh, C and F.C. Yang, 2004. Reusing Soy for the Solid- State Fermentation of Ganoderma lucidum. Bioresources Technology, 91: 105-109.

Hao, F., Z. Chen and S. Xiang- Yang, 2010. Optimization of Enzymatic Hydrolysis of Steam-Exploded Corn Stover by Two Approaches: Response Surface Methodology or using Cellulase from Mixed Cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* NL02. Bioresources Technology, 101: 4111-4119.

How, S.P and C.O. Ibrahim, 2004. Selection and Optimization of Lipase Production from *Aspergillus Flavus* USM A10 Via Solid State Fermentation (SSF) on Rice Husks and Wood Dusts as Substrates. The 4th Annual seminar of National sciences Fellowship, pp: 132-140.

Husaini, A., S.R. Mustafa, C.N. Hipolito, H. Hussain, N. Suhaili and H.A. Roslan, 2016. Application of Response Surface Methodology for Optimizing Process Parameters in the Production of Amylase by Aspergillus flavusNSH9 under Solid State Fermentation. Brazilian Archives of Biology and Technology, 59: e16150632.

Jack, T.R and J.E. Zajic, 2006. The Immobilization of Whole Cells. Advance Biochemical Engeneering and Biotechnoloy, 5/1977, Springer Berlin, pp: 125-145.

Kamm, B., M. Kamm, M. Schmidt, T. Hirth and M. Schulze, 2006. Lignocellulose-based chemical products and product family trees. Biorefineries Industrial Processes and Products, v. 2: Status quo and future directions, Wiley-VCH.

Karel, F., J.R. Liem, T. Gabi, R. Henk and M.J. Thomas, 1995. Dorsal Differentiation of Neural Plate Cells Induced by BMP-Mediated Signals from Epidermal Ectoderm. Department of Biochemistry and Molecular Biophysics, 82: 969-979.

Kholik, A., A. Fernandes, Karmilasanti and D.D.N. Cahyono, 2011. Effect of Local Sites to Wood Quality of Shorea parvifolia Dyer. International Conference of Indonesian Forestry Researchers (INAFOR), pp: 286-290.

Khoramnia, A., A. Ebrahimpour, R. Ganbari, Z. Ajdari and O.M. Lai, 2013. Improvement of Medium Chain Fatty Acid Content an Antimicrobial Activity of Coconut Oil via Solid-State Fermentation Using a Malaysia Geotrichum candidum. Bio Med Research International, Article ID954542, 9 pages.

Ladero, V., A. Ramos, A. Wierama, P. Goffin, A. Schanok, M. Kleerebezem, J. Hugonholtz, E.J. Smie and P. Hols, 2007. High-level Production of the Low-Calorie Sugar Sorbitol by *Lactobacillus plantarum* through Metabollic Engineering. Applied Environmental Microbiology, 73: 1864-1872.

Lee, J., I. Park, S. Cho, S. Kim, Y. Choi and J. Choi, 2013. Optimization of medium composition for α-galactosidase production by Antarctic bacterial isolate, *Bacillus* sp. LX-1 using response surface methodology. African Journal of Microbiology Research, pp: 3494-3500.

Li, X.Y., Z.Q. Liu and Z.M. Chi, 2008. Production of Phytase by a Marine Yeast Kodamaea ohmeri BG3 in an Oats Medium: Optimization by Response Surface Methodology. Bioresources Technology, 99: 6386-6390.

Manpreet, S., S. Sawraj, D. Sachin, S. Pankaj and U.C. Banerjee, 2005. Influence of Process Parameters on the Production of Metabolites in Solid- State Fermentation. Malaysian Journal of Microbiology, 1(2).

Massalha, N., S. Basheer and I. Sabbah, 2007. Effect of Adsorption and Bead Size of Immobilized Biomass on the Rate of Biodegradation of Phenol at High Concentration Levels. Ind Eng Chem Res., 46: 6820-6824.

Mitchell, D.A., D.D. Do, P.F. Greenfield and H.W. Doelle, 1991. A Semimechanistic Mathematical Model for Growth of *Rhizopus oligosporus* in a Model Solid State Fermentation System. Biotechnology and Bioengineering, 38: 353-362.

Mitchell, D.A., O.F. Von Meien, L.F.L. Luz Junior and N. Krieger, 2004. Construction of Pilot-Scale Solid state Fermentation Bioreactor with Forced Aeration and Intermittent Agitation. Process System Engineering, 1-8.

Mitchell, D.A., N. Krieger, D.M. Stuart and A. Pandey, 2000. New developments in solid-state fermentation. II. Rational approaches to the design operation and scale-up of bioreactors. Process Biochem, 35: 1211-1225.

Myers, R.H and D.C. Montgomery, 2002. Response Surface Methodology: Product and Process Optimization Using Design Experiment. 2ndEdition. John Wiley & Sons, New York.

Neifar, M., A. Kamoun, A. Jaouani, R. Ellouze-Ghorbel and S. Ellouze-Chaabouni, 2011. Application of asymetrical and hoke designs for optimization of laccase production by the white-rot fungus Fomesfomentarius in solid-state fermentation. Enzyme Research, 12.

Nissen, L., G. Perez-Martinez and M.J. Yebra, 2005. Sorbitol synthesis by An Engineered *Lactobacillus casei* Strains Expressing a Sorbitol-6-Phosphate Dehydrogenase Gene within the Lactose Operon. FEMS Microbial Lett, 249: 177-183.

Pandey, A., C.R. Soccol and D. Mitchell, 2000. New Developments in Solid-State Fermentation: Bioprocess and Products. *Process* Biochemistry, 35: 1153-1169.

Pandey, A., L.M.S. Palni and D. Bisht, 2001a. Dominant Fungi in the Rhizosphere of Established Tea Bushes and Their Interactions with the Dominant Bacteria Under in Situ Conditions. Microbiol. Res., 156: 377-382.

Pandey, A., P. Trivedi, B. Kumar, B. Chaurasia, S. Singh and L.M.S. Palni, 2004. Development of microbial inoculants for enhancing plant performance in mountains. In: Reddy, M.S., Khanna, S., (eds). Biotechnological Approaches for Sustainable development. Allied Publishers Pvt. Limited, New Delhi, pp: 13-20.

Pang, P.K., I. Darah, L. Poppe, G. Szakacs and C.O. Ibrahim, 2006. Xylanase Production by a Local Isolate, *Trichoderma* spp. FELT c3-2 *via* Solid state fermentation using Agriculture Waste as Substrate. Malaysian Journal of Microbiology, 2(1): 7-14.

Patrice, C., M. Kalotina, A.C. Loic, M. Armelta and M. Annick, 2001. Rapid PCR-Based Procedure to Identify Lactic Acid Bacteria: Application to Six Commom *Lactobacillus* Species. Journal of Biological Methods, 44: 139-148.

Pretzer, G., J. Snel, D. Molenaar, A. Wiersma, P.A. Born, J. Lambert, W.M. De Vos, R. Van Der Meer, M.A. Smit and M. Kleerebezem, 2005. Biodiversity-based Identification and Funtional Characterization of the Mannose Specific Adhesion of *Lactobacillus plantarum*. Journal of Bacteriol, 187: 6128-6136.

Ray, R.C., S. Mohapatra, S. Panda and S. Kar, 2008. Solid Substrate Fermentation of Cassava Fibrous Residue for Production of α-amylase, Lactic Acid and Ethanol. Journal of Environmental Biology, 29(1): 111-115.

Sabu, A., C. Swati and A. Pandey, 2006. Tannase production by *Lactobacillus* sp. ASR-SI under solid state fermentation. Proc. Biochem, 41: 575-580.

Saha, B.C and L.K. Nakamura, 2003. Production of Mannitol and Lactic acid by Fermentation with *Lactobacillus intermedius* NRRL B-3693†. Biotechnology And Bioengineering, pp: 82(7).

Sanni, A.I., J. Morhan-Guyot and J.P. Guyot, 2002. New Efficient Amylase-Producing Strainof *Lactobacillusplantarum* and *L. fermentum* Isolated from Different Nigerian Traditional Fermented Food. International Journal of Food Microbiology, 72: 53-62.

Shankar, T., R. Rama, P. Thangamathi and T. Sivakumar, 2013. Keratinase production by Bacillus megaterium RS1 using the statistical tool central composite design. African Journal of Biotecnology, 12(48): 6776-6784.

Smith, S.I.A., A.O. Aweh, K.O. Coker, D.A. Savage, Abosede and K.S. Oyedeji, 2001. Lactobacilli in Human Dental Carics and Saliva. Microbios, 105: 77-85.

Strezov, V., B. Moghtaderi and J.A.L ucas, 2004. Computational Calorimetric Investigation of the Reactions During Thermal Conversion of Wood Biomass. Biomass and Bioenergy, 27: 459-465.

Swetha, S., G. Dhanya, M.N. Kesavan, R. Carlos and A. Pandey, 2006. α-Amylases from Microbial Sources-An Overview on Recent Developments. Journal of Food Technology and Biotechnology, 44: 173-184.

Taherzadeh, M.J and K.K arimi, 2008. Pretreatment of Lignocellulosic Waste to Improve Ethanol and Biogas Production: A review. International Journal of Molecular Sciences, pp: 1621-165.

Vastrad, B.M and S.E. Neelagund, 2011. Optimization and Production of Neomycin from Different Agro Industrial Waste in Solid State Fermentation. International Journal of Pharmaceutical Sciences and Drug Research, 3(2): 104-111.

Yan, J., R. Bajpai and E. Iannotti, 2001. Lactic Acid Fermentation from Enzyme-Thinned Starch with Immobilized *Lactobacillus amylovorus*. Chem.Biochem.Eng, 15(2): 59-63.

Zhang, J., J.b. Li, S.B. Wu and Y. Lui, 2013. Advances in the Catalytic Production and Utilization of Sorbitol. Industrial & Engineering Chemistry Research, 52(34): 11799-11815.

Zhao, X., L. Zhang and D. Liu, 2008. Comparative Study on Chemical Pretreatment Method for Improving Enzymatic Digestibility of Crofton Weed Stem. Bioresources Technology, 99: 3729-3736.