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Efficient entrapment of *Kluyveromyces marxianus* DBKKUY-103 in polyvinyl alcohol hydrogel for ethanol production from sweet sorghum juice

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Abstract: The aim of this investigation was to optimize immobilization conditions to entrap *Kluyveromyces marxianus* DBKKUY-103 during ethanol fermentation from sweet sorghum juice using a response surface methodology. The effects and interactions of variables involved in cell entrapment using polyvinyl alcohol and sodium alginate were studied through a fractional factorial design. The results suggested that the primary variables involved in cell entrapment that significantly affected ethanol fermentation were sodium sulfate concentration, polyvinyl alcohol, and sodium alginate contents ($P < 0.01$). Subsequently, these variables were optimized for the preparation of cell entrapment based on response surface methodology and using central composite design. The results indicate that cell entrapment can achieve an ethanol concentration of 81.56 g L^{-1} under the following optimal conditions: bead gel diameter of 4.0 mm, calcium chloride concentration of 0.3 M, polyvinyl alcohol content of 10.09% (w v^{-1}), sodium alginate content of 3.39% (w v^{-1}), and sodium sulfate concentration of 0.44 M. Furthermore, statistical analysis of the model reveals that it adequately fit the experimental data ($P < 0.01$, $R^2 = 0.9879$). Therefore, this model can be used to efficiently prepare entrapped cells for ethanol production.

Key words: Cell entrapment, *Kluyveromyces marxianus*, ethanol fermentation, sweet sorghum juice, polyvinyl alcohol, sodium alginate, interpenetrating polymer networks, response surface methodology, central composite design

1. Introduction

Depletion of energy resources and abundance of fuel consumption require the development of renewable and environmentally friendly resources. Bioethanol produced from renewable biomass is recognized as one of the most promising biofuels, and could play an important role in the reduction of carbon emissions (Mussatto et al., 2010). Typically, several sugar and starch crops are used as carbon sources for ethanol fermentation, including sugar beet, sugarcane, cassava, maize, and wheat (Bai et al., 2008). Sweet sorghum is an alternative source of ethanol production because it has numerous advantages over other types of biomass, such as high ethanol productivity, drought resistance, water logging resistance, saline-alkaline tolerance, and low consumption of fertilizer and water (Ratnavathi et al., 2010). Throughout the world, the yeast *Saccharomyces cerevisiae* is commonly used as the major ethanol-fermenting microorganism because of its high capability for ethanol production. Recently, thermotolerant yeast *Kluyveromyces marxianus* has received considerable attention. This yeast strain is not only capable of growing and fermenting a high yield of ethanol at high temperatures, but also possesses several benefits for ethanol fermentation, such as a decreased risk of contamination, as well as energy conservation through a reduction in cooling costs (Limtong et al., 2007; Fonseca et al., 2008; Abdel-Banat et al., 2010). Furthermore, immobilization of the yeast cells during ethanol fermentation offers several advantages, such as simple cell separation from the medium, better operational stability and cell viability during several cycles of operation, reduced substrate and product inhibition, and enhanced yield and ethanol tolerance (Behera et al., 2010b; Razmovski and Vučurović, 2011).

There are several methods of cell immobilization, including adsorption, covalent binding, cross-linking, encapsulation, and entrapment (Kourkoutas et al., 2004). Although each method has distinct advantages and disadvantages, the most suitable approach for cell immobilization may be cell entrapment because of its simple preparation, cost-effectiveness, and high cell viability and activity (Behera et al., 2010a; Nikolić et al., 2010). The use of natural and synthetic carriers for entrapped cells has been widely studied (Phisalaphong et al., 2007; Yu et al., 2007; Zhang et al., 2007; Behera

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et al., 2010b). Currently, interpenetrating polymer networks (IPNs) are widely applied in biotechnological and biomedical applications, mainly due to their unique biophysical properties, such as simple fabrication into various geometrical forms, a soft and rubbery texture, minimal mechanical irritation to the surrounding tissues, and remarkable stability against biological fluids (Makas et al., 2010; Pulat et al. 2013, 2014). IPNs are defined as a blend of 2 or more polymers in a network form, at least 1 of which is synthesized and/or cross-linked in the immediate presence of the other(s) (Sperling and Hu, 2003). Among several types of IPNs, synthetic polyvinyl alcohol (PVA) is recognized as a promising carrier for immobilization because it is cheap, is nontoxic for microbes, and has high mechanical strength and durability (Wirawan et al., 2012; Wang et al., 2013). The simplest procedure to immobilize microbial cells using PVA is the PVA-boric acid method. However, it reduces cell viability, and PVA beads usually agglomerate due to the highly acidic conditions and slow cross-linking process (Hashimoto and Furukawa, 1987; Wu and Wisecarver, 1992). To overcome these problems, the addition of sodium alginate to the PVA beads and the treatment of the beads with sodium sulfate were examined (Takei et al., 2011; Zain et al., 2011). Therefore, optimal conditions for the preparation of the beads are necessary to achieve high stability of hydrogel beads.

The conventional method for the optimization of experimental parameters involves altering one variable while holding all other variables constant. The disadvantages of this approach are that the effects and interactions of all parameters cannot be extensively evaluated, and a considerable number of experiments are required. However, statistical methods are alternative and economical procedures (Montgomery, 2005). Response surface methodology (RSM) has been widely used to evaluate effects and interactions of the parameters to determine optimal conditions for several biotechnological processes (Demir, et al., 2012; Öngen et al., 2012; Awad et al., 2013; Bisht et al., 2013). RSM is usually used in first-order or second-order polynomial equations to evaluate the relationship between response and independent variables. Central composite design (CCD) is a common design for fitting second-order response surfaces because it is relatively efficient with respect to the number of experiments. In general, a CCD in k factors requires a 2^k factorial design with $2k$ axial treatments and at least 1 center point, though 3 to 5 center points are typically used (Montgomery, 2005). In the present study, immobilization parameters that significantly affected ethanol production were examined using a fractional factorial design. CCD was then utilized to determine the optimal conditions to entrap cells during ethanol production from sweet sorghum juice.

2. Materials and methods

2.1. Materials and microorganisms

PVA (degree of polymerization: 2000) and sodium alginate were obtained from Sigma-Aldrich (St Louis, MO, USA). Boric acid, calcium chloride, and sodium sulfate were purchased from Ajax Finechem (Auckland, New Zealand). Sweet sorghum juice, *Sorghum bicolor* (L.) Moench KKU-40, was obtained from the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University (Khon Kaen, Thailand). To avoid storage issues, the juice was concentrated to 750 g L^{-1} and kept at –20 °C until ethanol fermentation. *K. marxianus* DBKKUY-103 was kindly acquired from Associate Professor Pornthap Thanonkeo, Department of Biotechnology, Faculty of Technology, Khon Kaen University (Khon Kaen, Thailand). Yeast cells were cultured in yeast and malt extract medium (10.0 g of glucose, 5.0 g of peptone, 3.0 g of yeast extract, and 3.0 g of malt extract in 1.0 L of distilled water). The cells were harvested by centrifugation in the late exponential phase of growth. The collected cells were washed in sterilized 0.9% (w v^{-1}) NaCl solution and resuspended in distilled water. The cell suspension was used in the subsequent experiments. The density of yeast cells, including dead and viable cells in the suspension, was determined using a hemocytometer.

2.2. Entrapment of *K. marxianus* DBKKUY-103 in PVA hydrogel

To prepare PVA hydrogel beads, the seed culture was centrifuged at 8000 × g for 20 min, washed, and then suspended in 50 mL of deionized water. The supernatant was removed and the yeast pellet was mixed with 50 mL of 2% (w v^{-1}) sodium alginate and 8% (w v^{-1}) PVA. The mixture was developed into beads by dropping it into chilled, sterile 0.1 M $CaCl_2$ containing saturated boric acid. The beads were incubated for 30 min and then immersed in 0.5 M Na_2SO_4 for 1.5 h. The beads were hardened by incubation in 0.1 M $CaCl_2$ for 24 h at 4 °C. Prior to use as biocatalysts for ethanol fermentation, the beads were washed with sterile distilled water to remove excess amounts of calcium ions and unentrapped cells (Quintana and Dalton, 1998; Takei et al., 2011).

2.3. Ethanol production in batch fermentation

The concentrated juice was diluted with distilled water to give a total sugar content of 220 g L^{-1} and $(NH_4)_2SO_4$ was added for a final concentration of 0.5 g L^{-1}. The pH of the mixture was adjusted to 5.0 with 0.1 M HCl and it was sterilized using an autoclave. The mixture was then stored at 4 °C until it was used for ethanol fermentation. The yeast inoculum was added to flasks containing the sterile juice at an initial yeast cell concentration of 1.0×10^7 cells mL^{-1}. The flasks were incubated in a rotating shaker at 40 °C for 84 h at 100 rpm.

2.4. Analytical methods

During batch fermentation, the pH value of the sample was measured with a pH meter using a glass electrode. The total sugar content in the juice was estimated utilizing the phenol-sulfuric acid method (Dubois et al., 1956). Ethanol concentration was analyzed by gas chromatography (Shimadzu GC-14B, Kyoto, Japan; solid phase: polyethylene glycol (PEG-20M); carrier gas: nitrogen, 150 °C isothermal packed column; injection temperature: 180 °C; flame ionization detector temperature: 250 °C; GC Solution Analysis Version 2.30) and 2-propanol was used as an internal standard (Ratnavathi et al., 2010).

2.5. Optimization procedure

In this study, the evaluation of the optimal conditions for cell entrapment was conducted in 2 steps. Fractional factorial design was employed to investigate the influence of parameters on the cell entrapment via PVA hydrogel during ethanol fermentation. These parameters were bead gel diameter, sodium alginate, and PVA contents, as well as calcium chloride and sodium sulfate concentrations (Idris and Suzana, 2006; Sheng et al., 2007; Takei et al., 2011). The variables are coded according to Eq. (1):

$$\chi_i = \frac{X_i - X_0}{\Delta X_i}, \quad (1)$$

where; χ_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the center point, and ΔX_i is the step change value of the variables.

The values and levels of the variables are presented in Table 1. The significance of the variables was evaluated according analysis of variance (ANOVA).

RSM was used to determine the optimal conditions for cell entrapment during ethanol fermentation. CCD was utilized to fit a second-order response surface model (Myers et al., 2009). To predict the optimal point, a quadratic model is expressed according to Eq. (2):

$$y = b_0 + \Sigma b_i \chi_i + \Sigma b_{ii} \chi_i^2 + \Sigma b_{ij} \chi_i \chi_j, \quad (2)$$

where; y is the response variable, b_0 is intercept, b_i are linear coefficients, b_{ii} are squared coefficients, b_{ij} are interaction coefficients, and x is the coded levels of the independent variable.

2.6. Statistical analysis

Design Expert software (version 7.0.0; STATEASE Inc., Minneapolis, MN, USA) was used for regression and graphical analysis of the data. The statistical significance of the model was examined using Fisher's F-test. The accuracy and general ability of the model were evaluated by the coefficient of determination, R^2. Each experimental treatment was performed in duplicate and the mean values were reported.

3. Results

3.1. Screening of influential parameters for cell entrapment during ethanol production

Parameters playing the most important roles in cell entrapment via PVA hydrogel are depicted in Table 1. Effects of the variables on ethanol fermentation were examined using a fractional factorial design (Montgomery, 2005). The experimental design and the results are outlined in Table 2. The results indicate that ethanol concentration fluctuates from 62.23 to 79.46 g L^{-1} according to the experimental treatments. Lower ethanol yields were obtained (<70 g L^{-1}) when the contents of sodium alginate and PVA were applied at minimal levels. However, higher ethanol yields were observed (>70 g L^{-1}) when the contents of sodium alginate and PVA were employed at maximal levels. In addition, lower ethanol concentrations were obtained (<70 g L^{-1}) when PVA content and sodium sulfate concentration were used at their minimal and maximal levels, respectively. In contrast, higher ethanol concentrations were acquired (>70 g L^{-1}) when PVA content and sodium sulfate concentration were utilized at their respective maximal and minimal levels. These results suggest that these variables have the greatest potential to affect ethanol yields. Statistical analysis indicates that the 3 parameters of sodium sulfate concentration, PVA, and sodium alginate contents have significant effects on

Table 1. Levels of the independent variables for 2^{5-1} fractional factorial design.

Independent variables	Symbols	Levels		
		−1	0	+1
Sodium alginate content [% (w v^{-1})]	X_1	1.0	2.0	3.0
Calcium chloride concentration (M)	X_2	0.1	0.3	0.5
PVA content [% (w v^{-1})]	X_3	6.0	8.0	10.0
Bead gel diameter (mm)	X_4	2.0	4.0	6.0
Sodium sulfate concentration (M)	X_5	0.5	1.0	1.5

Table 2. Fractional factorial design for screening of the independent variables affecting ethanol production using immobilized *K. marxianus* DBKKUY-103.

Trial number	Coded levels of the independent variables					Ethanol concentration (g L^{-1})
	X_1	X_2	X_3	X_4	X_5	
1	1	−1	−1	1	1	66.31
2	−1	1	1	1	−1	78.65
3	−1	−1	−1	1	−1	67.75
4	−1	−1	1	−1	−1	78.50
5	−1	1	−1	1	1	62.23
6	−1	1	1	−1	1	76.18
7	1	−1	1	1	−1	79.46
8	1	1	−1	1	−1	70.58
9	−1	−1	−1	−1	1	64.62
10	−1	−1	1	1	1	75.54
11	1	1	−1	−1	1	65.66
12	1	−1	−1	−1	−1	69.69
13	−1	1	−1	−1	−1	68.44
14	1	1	1	−1	−1	79.46
15	1	−1	1	−1	1	76.77
16	1	1	1	1	1	77.25
17	0	0	0	0	0	71.42
18	0	0	0	0	0	71.41
19	0	0	0	0	0	71.12
20	0	0	0	0	0	70.79

ethanol production with a confidence level of greater than 99% ($P < 0.01$). Interactions between these variables were also detected ($P < 0.05$). This model adequately fits the experimental data ($P < 0.01$) with $R^2 = 0.99$, as illustrated in Table 3.

3.2. Optimization of cell entrapment during ethanol fermentation

According to the results of the fractional factorial design, sodium sulfate concentration, sodium alginate, and PVA contents were identified as having significant effects on ethanol yields and were thus further optimized. A 2^3 full factorial CCD with 6 star points and 6 replicates at the center points, comprising 20 experiments, was used to verify the interactive effects of the independent variables on ethanol yields. The experimental design and ethanol concentration are displayed in Table 4. The results indicate that the optimal conditions for preparation of entrapped cells are close to the center points of the independent variables. Higher ethanol concentrations were achieved when the variables were set to their center points, but lower ethanol concentrations were obtained when the variables were adjusted to other points. To correlate the

Table 3. Analysis of variance for fractional factorial design.

Source	Mean square	F-value	P-value
Model	35.80	724.77	<0.0001
X_1	11.01	222.79	0.0007
X_2	0.002	0.046	0.8445
X_3	467.97	9472.98	<0.0001
X_4	0.15	3.04	0.1796
X_5	48.90	989.78	<0.0001
X_1X_2	0.17	3.36	0.1641
X_1X_3	1.64	33.30	0.0103
X_1X_4	1.95	39.53	0.0081
X_1X_5	0.15	3.12	0.1756
X_2X_3	0.47	9.43	0.0545
X_2X_4	0.016	0.33	0.6064
X_2X_5	0.83	16.86	0.0262
X_3X_4	0.15	2.96	0.1837
X_3X_5	3.34	67.61	0.0038
X_4X_5	0.32	6.40	0.0854

Table 4. Central composite design with experimental values of ethanol production using immobilized *K. marxianus* DBKKUY-103.

Trial number	Levels of the independent variables actual (coded) values			Ethanol concentration (g L^{-1})	
	X_1	X_3	X_5	Experimental values	Predicted values
1	4.0 (+1)	9.0 (-1)	0.7 (+1)	65.08	65.48
2	4.0 (+1)	11.0 (+1)	0.3 (–1)	74.27	74.52
3	2.0 (–1)	9.0 (–1)	0.3 (–1)	66.38	66.70
4	1.32 (–1.68)	10.0 (0)	0.5 (0)	74.42	74.10
5	4.0 (+1)	11.0 (+1)	0.7 (+1)	66.27	66.56
6	3.0 (0)	10.0 (0)	0.84 (+1.68)	63.42	63.00
7	3.0 (0)	11.68 (+1.68)	0.5 (0)	69.11	68.87
8	4.68 (+1.68)	10.0 (0)	0.5 (0)	77.75	77.22
9	2.0 (–1)	9.0 (–1)	0.7 (+1)	66.01	66.36
10	2.0 (–1)	11.0 (+1)	0.7 (+1)	69.42	69.57
11	3.0 (0)	10.0 (0)	0.16 (–1.68)	70.42	69.98
12	3.0 (0)	8.32 (–1.68)	0.5 (0)	65.85	65.24
13	4.0 (+1)	9.0 (–1)	0.3 (–1)	72.97	73.42
14	2.0 (–1)	11.0 (+1)	0.3 (–1)	69.72	69.93
15	3.0 (0)	10.0 (0)	0.5 (0)	81.09	80.72
16	3.0 (0)	10.0 (0)	0.5 (0)	80.42	80.72
17	3.0 (0)	10.0 (0)	0.5 (0)	81.81	80.72
18	3.0 (0)	10.0 (0)	0.5 (0)	79.17	80.72
19	3.0 (0)	10.0 (0)	0.5 (0)	79.50	80.72
20	3.0 (0)	10.0 (0)	0.5 (0)	82.16	80.72

independent variables with ethanol concentration, a second-order polynomial equation was employed, and the polynomial coefficient for each term of the equation was examined through multiple regression analysis using Design Expert software. This model consists of 3 linear effects, 3 two-factor interaction effects, and 3 squared effects. The following model describes ethanol concentration according to Eq. (3):

$$Y = 80.72 + 0.93X_1 + 1.08X_3 - 2.07X_5\text{-}1.90X_1X_5 - 1.79X_1^2 - 4.83X_3^2 - 5.03X_5^2, \quad (3)$$

where; Y is the predicted response (ethanol concentration) and X_1, X_3, X_5 are the coded values of the independent variables (sodium alginate and PVA contents as well as sodium sulfate concentration).

Table 5 illustrates the statistical significance of the model using Fisher's F-test and ANOVA. The calculated F-value of 90.99 and P-value of 0.0001 suggest that the model is significant at a high confidence level ($P < 0.01$). Moreover, the significance of the parameters indicates that these variables influence ethanol yields ($P < 0.01$), and positive coefficients of the independent variables imply a linear effect for elevated ethanol concentrations (Montgomery, 2005). The model with R^2 value of 0.9879 indicates a high correlation between the experimental and predicted values. Adequate precision of the model is determined by the signal-to-noise ratio. The ratio of 25.88 reveals that the model provides adequate precision for the fabrication of entrapped cells.

Response surface plot was performed to observe the effects and interactions of the independent variables on the response. Effects of sodium alginate and PVA contents on ethanol concentration are displayed in Figure 1. When sodium alginate content was increased at a low content of PVA, ethanol yields were slightly enhanced from 71.56 to

Table 5. Analysis of variance of the response of ethanol production using immobilized *K. marxianus* DBKKUY-103.

Source	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Model	767.00	9	85.22	90.99	<0.0001
X_1	11.74	1	11.74	12.53	0.0054
X_3	15.87	1	15.87	16.95	0.0021
X_5	58.78	1	58.78	62.75	<0.0001
$X_1 X_3$	2.27	1	2.27	2.42	0.1507
$X_1 X_5$	28.96	1	28.96	30.91	0.0002
$X_3 X_5$	0.0002	1	0.0002	0.0002	0.9886
X_1^2	46.11	1	46.11	49.22	<0.0001
X_3^2	336.33	1	336.33	359.08	<0.0001
X_5^2	364.47	1	364.47	389.12	<0.0001
Residual	9.37	10	0.94		
Lack of fit	1.99	5	0.40	0.27	0.9114
Total	776.37	19			

Coefficient of determination (R^2) = 0.9879, adequate precision = 25.883.

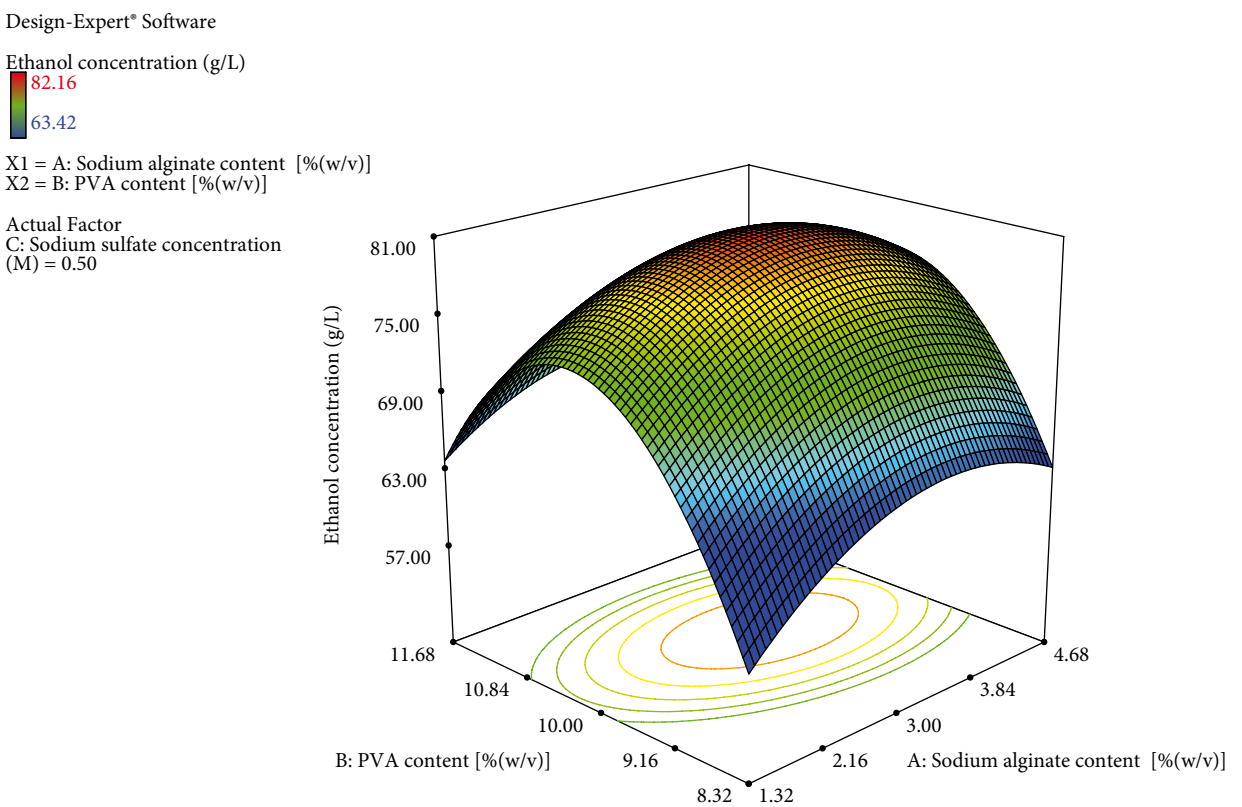

Figure 1. Response surface plot of the combined effects of PVA and sodium alginate contents on ethanol concentration using immobilized *K. marxianus* DBKKUY-103 with constant sodium sulfate concentration of 0.5 M.

74.47 g L^{-1}. However, when sodium alginate content was increased at a high content of PVA, ethanol yields were almost constant, ranging between 74.78 and 75.57 g L^{-1}. The results indicate that increasing sodium alginate content within the experimental ranges is beneficial to ethanol yields. In addition, effects of sodium alginate content and sodium sulfate concentration on ethanol concentration are demonstrated in Figure 2. Ethanol yields increased from 73.14 to 78.80 g L^{-1} when sodium alginate content was increased at a low concentration of sodium sulfate. On the other hand, the yields were reduced from 72.80 to 70.85 g L^{-1}, when sodium alginate content was increased at a high concentration of sodium sulfate. These results suggest that raising the sodium sulfate concentration deteriorates the ethanol yields. Effects of PVA content and sodium sulfate concentration on ethanol concentration are illustrated in Figure 3. Higher ethanol yields were obtained at a low sodium sulfate concentration, while the yields steadily increased when PVA content was increased. These results suggest that an increase in PVA content is advantageous for ethanol yield.

3.3. Model validation

Model validation was performed under the optimal conditions for entrapped cells. The ethanol concentration was in agreement with the predicted response. The difference between the experimental and predicted responses was only 0.4%. To achieve an ethanol concentration of 81.56 g L^{-1}, the optimal conditions for the entrapped cells were calcium chloride concentration of 0.30 M, sodium sulfate concentration of 0.44 M, sodium alginate content of 3.39% (w v^{-1}), PVA content of 10.09% (w v^{-1}), and bead gel diameter of 4.0 mm. The maximal level of ethanol concentration was 22.24% higher than the minimal level of response obtained from the experimental design.

4. Discussion

In this study, the 3 parameters had significant effects on ethanol production ($P < 0.01$). Sodium alginate and PVA contents were jointly used to retain the stability, mechanical strength, and surface area of the entrapped cells during fermentation, which were important to maintain high conversion of substrate to product (Sheng et al., 2007; Kumar et al., 2012). The role of sodium sulfate concentration was to induce cross-linkage of PVA for fabricating hydrogel beads and to preserve the viability of the entrapped cells (Idris and Suzana, 2006; Takei et al.,

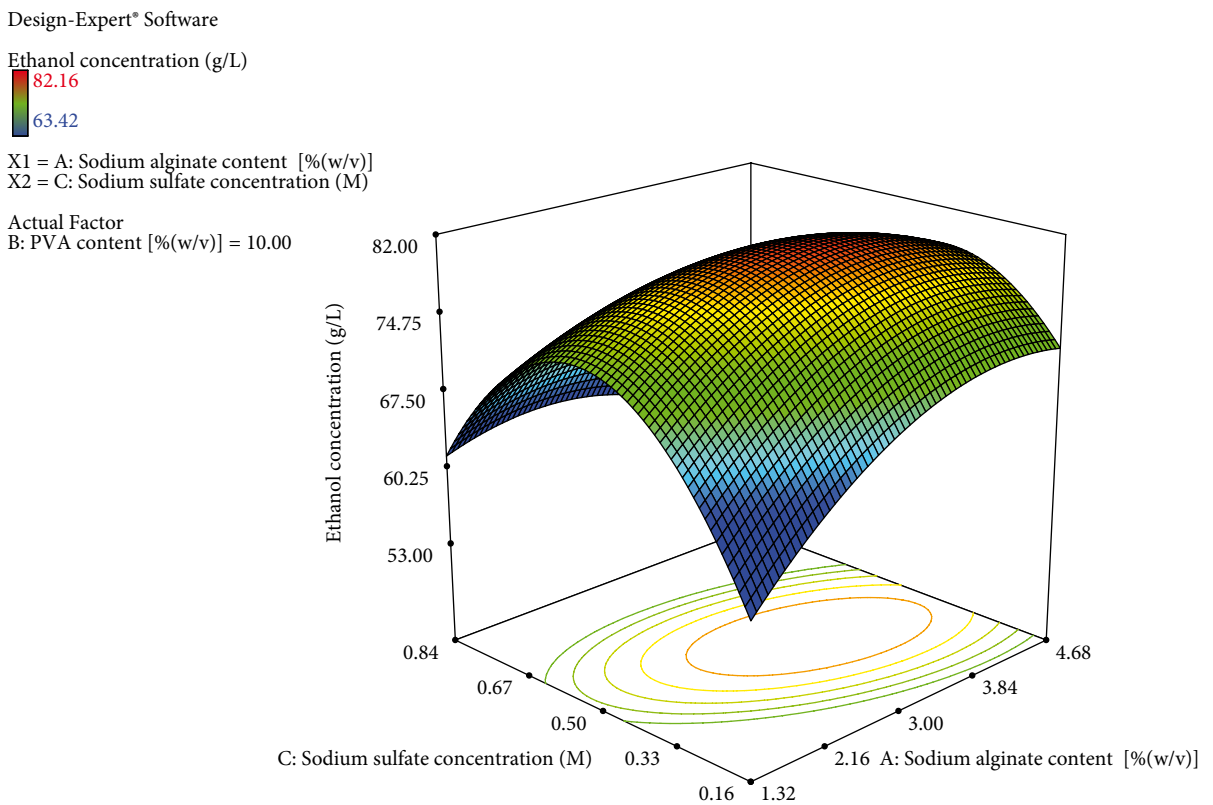

Figure 2. Response surface plot of the combined effects of sodium alginate content and sodium sulfate concentration on ethanol concentration using immobilized *K. marxianus* DBKKUY-103 with constant PVA content of 10.0% (w v^{-1}).

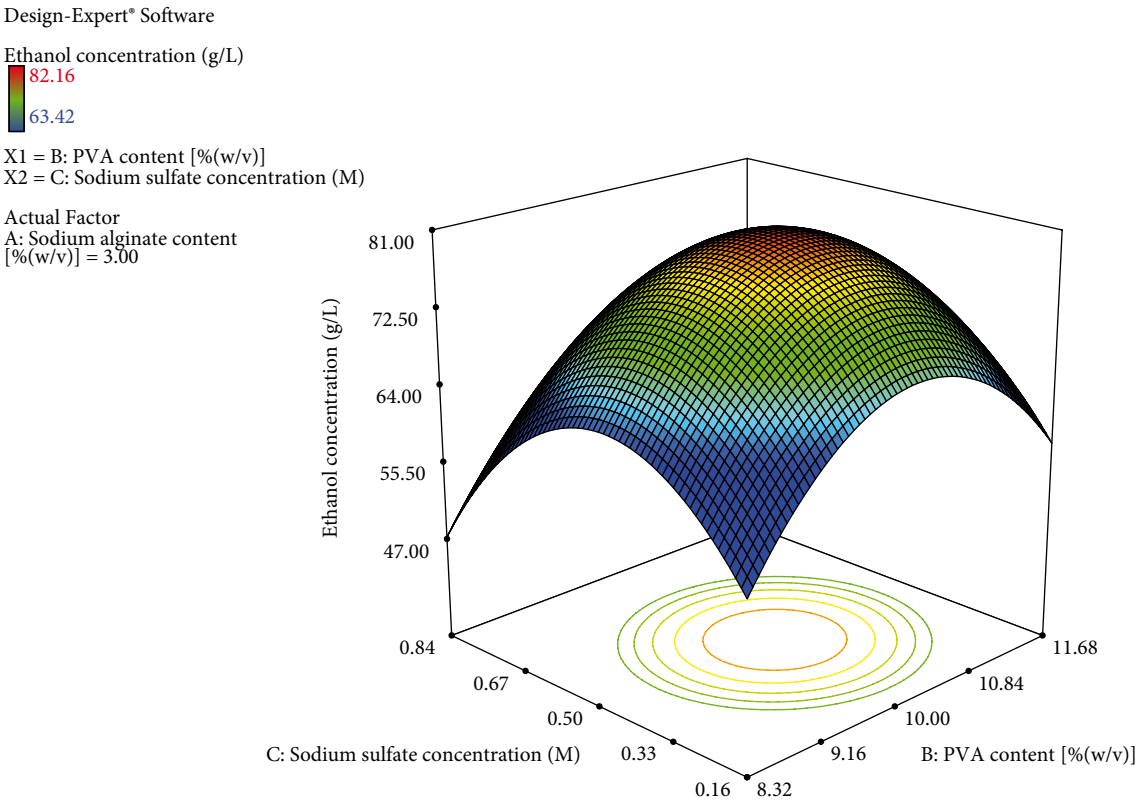

Figure 3. Response surface plot of the combined effects of PVA content and sodium sulfate concentration on ethanol concentration using immobilized *K. marxianus* DBKKUY-103 with constant sodium alginate content of 3.0% (w v^{-1}).

2011). Furthermore, increasing sodium alginate and PVA contents within the experimental ranges was beneficial to the ethanol yields. This could improve surface area and eliminate agglomeration of the hydrogel beads as well as retain the stability and mechanical strength of the entrapped cells (Kumar et al., 2012). However, rising sodium sulfate concentrations deteriorated the ethanol yields. This could be associated with the substantial mortality of the entrapped cells (Takei et al., 2011). The results of this study were consistent with previous reports. Entrapment of *Rhizopus oryzae* in PVA-alginate hydrogel during lactic acid production was investigated. The results suggest that sodium alginate content impacts both bead structure and product yields. Addition of sodium alginate enhanced the production of lactic acid by increasing the numbers of calcium ion binding sites, and an extensively cross-linked gel was observed. Increasing the PVA content also tends to improve product yields because the stability of gel beads is detected. Therefore, PVA and sodium alginate contents should be optimized to obtain appropriate mechanical strength, bioactivity, and mass transfer resistance (Wang et al., 2013). Preparation of entrapped cells from *Pseudomonas* sp. a3 for the treatment of mixed aromatics wastewater was performed. The results suggest that mechanical stability is enhanced at an elevated content of PVA and sodium alginate, whereas the degradation efficiency of nitrobenzene is diminished due to a low mass transfer (Wu et al., 2012). Moreover, immobilization of *S. cerevisiae* NBRC-0216 in PVA-alginate hydrogel was examined. The results indicate that cell viability appears to be diminished with the increase of sodium sulfate concentration owing to osmotic stress and denaturation of several proteins in the yeast cells (Takei et al., 2011).

The present study demonstrated PVA-alginate entrapment as a promising method for ethanol production from sweet sorghum juice using *K. marxianus* DBKKUY-103. The optimal conditions for immobilization were evaluated by CCD and an ethanol concentration of 81.56 g L^{-1} was achieved. Compilation of ethanol fermentation from sugar feedstock suggests that PVA-alginate entrapment is an efficient method to prepare biocatalysts for ethanol fermentation (Table 6). This could be associated with its unique biophysical properties, such as remarkable stability, high mechanical strength, and durability (Takei et al., 2011; Wirawan et al., 2012).

Table 6. Compilation of ethanol fermentation from sugar feedstock performed with the immobilized yeast cells.

Yeast strain	Type of support	Medium	Fermentation condition	Ethanol content	Reference
S. cerevisiae M30	Alginate-loofa matrix	Cane molasses	pH 5.0, 33 °C for 48 h	77.80 g L^{-1}	Phisalaphong et al., 2007
S. cerevisiae DTN	Alginate-maize stem disks	Sugar beet juice	pH 5.5, 30 °C for 48 h	69.18 g L^{-1}	Razmovski and Vučurović, 2013
K. marxianus DMKU 3-1042	Alginate-loofa matrix	Cane juice	pH 5.0, 37 °C for 72 h	70.0 g L^{-1}	Eiadpum et al., 2012
K. marxianus DBKKUY-103	PVA – alginate hydrogel	Sweet sorghum juice	pH 5.0, 40 °C for 84 h	81.56 g L^{-1}	This work

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