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Response surface optimization for the continuous glucose isomerization process

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Production of fructose via a continuous glucose isomerization process was optimized using response surface methodology. Glucose isomerization was performed using immobilized glucose isomerase in a flow-through tubular reactor. Process factors eg pH (7.0–7.8), temperature (50–60°C), flow rate (5–17 ml min^{-1}), and glucose content (30–50% w/w) of the feedstock solution were simultaneously tested according to a central composite experimental design. Measured responses such as % isomerization, and fructose yield (g h^{-1}) had an excellent correlation with tested factors. The highest desirability, *D*, (geometric mean of % isomerization and fructose yield) was obtained when the feedstock (56–60°C) had 34–36% glucose, a pH of 7.4–7.8 and was pumped at 15 ml min^{-1}.

Keywords: immobilized glucose isomerase; response surface methodology; optimization

Introduction

Fructose is the sweetest natural sugar. Its sweetening index is about 2.34 and 1.73 times that of glucose and sucrose, respectively [17]. Therefore, enzymatic isomerization of glucose to fructose has received in recent years considerable attention as a commercial process. Immobilized glucose isomerase is currently used for the industrial production of high fructose syrup (HFS) which recently replaced sucrose in many food products [13]. Various techniques of immobilizing either partially purified glucose isomerase (GI) or cells were reported [3,4,7,9,11–13,19,20,25]. Currently, several brands of immobilized GI products are commercially available for the production of HFS [26].

Steadily increasing labor and transportation costs, competition from different producers, as well as competition from sucrose itself may drive the HFS industry into a difficult situation. Therefore, reduction in production costs is required and can be achieved by optimizing process conditions. Glucose isomerization process optimization was previously reported [21,25]. Current literature did not reveal utilization of the response surface methodology (RSM) technique in optimizing the process of glucose isomerization.

Statistical optimization has become a common practice in biotechnology. Among the numerous optimization techniques, RSM is widely applied in developing many food, fermentation and drug processes [1,8,10,15,16,22,24]. Process optimization using RSM is usually achieved by simultaneous testing of numerous factors in a limited number of experiments. Therefore, RSM consumes less time and effort compared to the traditional, one-factor-at-a-time approach. Furthermore, RSM provides quantitative measurements of possible interactions between factors, difficult information to obtain using traditional optimization techniques. Detection and quantification of the interactions between various factors are of critical importance especially for optimizing the multivariate processes of food biotechnology. The objective of this work was to study simultaneously the effect of selected process factors eg glucose concentration, temperature, pH and flow rate of the feedstock on the continuous isomerization of glucose.

Materials and methods

Chemicals

Immobilized glucose isomerase (Sweetzyme T), having an activity of 350 IGIU g^{-1}, was provided by Novo Industri A/S (Bagsvaerd, Denmark). Anhydrous glucose was obtained from Fisher Scientific (Springfield, NJ, USA). Carbazol was purchased from Sigma Chemical Company (St Louis, MO, USA). Cysteine hydrochloride was obtained from Eastman Organic Chemicals (Rochester, NY, USA). All other reagents were of analytical grade.

Continuous isomerization of glucose

Continuous isomerization of glucose was performed using a flow-through tubular reactor. Eighty grams of immobilized glucose isomerase were packed into a 3.3 cm × 27 cm reactor column (280-ml total volume and 130-ml void volume). Feedstock (FS) containing 0.24% $MgSO_4$ and desired glucose concentrations were pumped upward using a peristaltic pump at predetermined flow rates. The pH of the FS was adjusted with sodium carbonate whereas the temperature was controlled using a thermostated water-bath. The concentration of the fructose produced was determined using the modified cysteine-carbazol method [6,14].

Experimental design and data analysis

According to the central composite design [2], 27 experiments were performed to study the effects of the independent variables eg glucose concentration (x_1), temperature (x_2), pH (x_3), and flow rate (x_4) of the FS on process

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responses eg % isomerization (y_1) and fructose yield (y_2) so that:

$$y_1 = \frac{\text{amount of fructose produced}}{\text{initial amount of glucose}} \times 100$$

$$y_2 = \text{amount of fructose produced h}^{-1}$$

The range of independent variables is recorded in Table 1. An overall desirability function, D, was also calculated as the geometric mean of the two responses, ie

$$D = \sqrt{y_1 \cdot y_2}$$

The Statistical Analysis system (SAS) [18] was used to fit the second order polynomial equation to the experimental data shown in Tables 2 and 3. Measured responses were correlated to the studied factors using response surface regression procedures [18]. Further model simplification steps were performed using multiple regression and backward elimination procedures [18]. After determining the optimum process conditions using RSM, the optimum response (D) was experimentally verified under the optimum conditions. The response D at the optimum process conditions was compared to values predicted by the model.

Results and discussion

Table 3 summarizes the data obtained from the 27 experiments performed according to the central composite design. Glucose isomerization (%) ranged from 27.6 to 45.5% whereas fructose yield ranged from 55.5 to 154.8 g h^{-1}. Since values of each response alone may not be fully descriptive for the process performance, a desirability function, D, was generated as the geometric mean of both

Table 1 Codes for independent variables and their corresponding values investigated in the optimization process of glucose isomerization

Independent variable	Coded symbol	Levels	
		Coded	Uncoded
Glucose (% w/w)	x_1	+2	50.0
		+1	45.0
		0	40.0
		−1	35.0
		−2	30.0
Temperature (°C)	x_2	+2	60.0
		+1	57.5
		0	55.0
		−1	52.5
		−2	50.0
pH	x_3	+2	7.8
		+1	7.6
		0	7.4
		−1	7.2
		−2	7.0
Flow rate (ml min^{-1})	x_4	+2	17.0
		+1	14.0
		0	11.0
		−1	8.0
		−2	5.0

Table 2 Experimental design and the combination of independent variables used for the various treatments

Treatment No.	Glucose conc (%)	Temp (°C)	pH	Flow rate (ml min^{-1})
1	+1	+1	+1	+1
2	+1	+1	+1	−1
3	+1	+1	−1	+1
4	+1	+1	−1	−1
5	+1	−1	+1	+1
6	+1	−1	+1	−1
7	+1	−1	−1	+1
8	+1	−1	−1	−1
9	−1	+1	+1	+1
10	−1	+1	+1	−1
11	−1	+1	−1	+1
12	−1	+1	−1	−1
13	−1	−1	+1	+1
14	−1	−1	+1	−1
15	−1	−1	−1	+1
16	−1	−1	−1	−1
17	+2	0	0	0
18	−2	0	0	0
19	0	+2	0	0
20	0	−2	0	0
21	0	0	+2	0
22	0	0	−2	0
23	0	0	0	+2
24	0	0	0	−2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0

Table 3 Response variable data obtained from optimization experiments

Treatment No.	Isomerization (%)	Fructose yield (g h^{-1})	Total desirability function
1	33.8	154.8	72.3
2	43.9	115.0	71.1
3	31.8	145.6	68.0
4	42.3	110.8	68.5
5	27.6	126.4	59.1
6	38.1	99.7	61.6
7	28.0	128.3	59.9
8	38.5	100.7	62.3
9	41.7	142.7	77.2
10	45.0	88.0	62.9
11	40.6	138.9	75.1
12	45.2	88.3	63.2
13	40.6	138.9	75.1
14	43.2	84.3	60.3
15	37.0	126.4	68.4
16	43.4	84.8	60.7
17	32.0	130.2	64.5
18	42.7	106.3	67.4
19	45.1	121.0	73.8
20	35.1	109.2	61.9
21	39.3	121.9	69.2
22	40.0	124.2	70.5
23	29.6	141.9	64.8
24	45.5	55.5	50.3
25	37.6	116.7	66.2
26	38.5	117.7	67.9
27	38.3	118.9	67.5

Table 4 Parameter estimates for full second-order polynomials and simplified models correlating responses to independent variables

Parameter	Parameter estimates					
	Isomerization (%)		Fructose yield		Desirability function	
	F[a]	S[b]	F	S	F	S
Intercept	1013.7	1065.51	3057.14	2778.11	1772.74	1377.00
x_1	−1.32	−2.56	−7.94	−8.64	−2.54	−4.18
x_2	−12.53	−11.83	−12.49	−10.25	−14.68	−4.36
x_3	−162.67	−177.41	−693.83	−631.48	−353.70	−310.74
x_4	−1.78	–	0.19	–	2.48	–
x_1^2	−0.01	–	0.02	–	0.00	–
x_2^2	0.08	0.09	−0.04	–	0.06	–
x_3^2	9.96	11.81	42.81	41.32	21.90	20.12
x_4^2	−0.01	–	−0.49	−0.49	−0.24	−0.25
x_1x_2	0.06	0.06	0.24	0.24	0.12	0.12
x_1x_3	−0.10	–	−0.32	–	−0.18	–
x_1x_4	−0.10	−0.10	−0.30	−0.30	−0.22	−0.22
x_2x_3	0.25	–	0.96	–	0.48	–
x_2x_4	0.01	–	0.25	0.25	0.08	0.09
x_3x_4	0.58	0.40	2.23	2.22	1.14	1.39

[a] Full model.
[b] Simplified model.

Table 5 Summary of ranges of independent variables at which maximum desirability was obtained

Contour plot No.	Glucose conc (%)	Temp (°C)	pH	Flow rate (ml min^{-1})
1	34–46	56–60	–	–
2	32–36	–	–	15–17
3	–	56–60	–	13–15
4	–	–	7.4–7.8	15–17
Common range (optimum conditions)	34–36	56–60	7.4–7.8	15

responses. Desirability functions serve as a single parameter used when simultaneous evaluation of different responses is required [5]. Table 4 indicates parameter estimates for the full second-order polynomial equations and corresponding simplified models. In all cases, excellent correlations ($r^2 > 0.94$) were observed among responses and studied factors. However, some of the parameters in the full models were not highly significant. Therefore, response surface graphs of the reduced model of D were applied to determine the optimum process conditions (Table 5). Desirability function increased by increasing temperature with the best obtained results between 56 and 60°C. This was due to increased enzyme activity at higher temperatures. This observation agrees with previously reported [3,21,23] temperature values (50–60°C). Similarly, at a higher pH value (7.4–7.8) optimum D was observed. Although enzyme stability was not studied in this investigation, pH and temperature ranges were chosen to minimize enzyme deactivation [23]. Straatsma *et al* [21] reported a maximum activity of glucose isomerase at a pH of 7.65 [21]. On the other hand, glucose concentration and flow rate of the FS play a rather complicated role in the isomerization process. At low concentrations of glucose, % isomerization will be high due to availability of the enzyme. However, low glucose flow rate input will result in low fructose yield. Moreover, increased flow rate will decrease % isomerization due to low enzyme–substrate contact time. Optimum FS flow rate was 15 ml min^{-1} whereas the optimum glucose concentration range was 32–46%, preferably 35%. This preferred value lies at the lower limit of substrate concentration recommended by the GI manufacturer (35–45%) [23]. Optimum conditions were presented in treatment No. 9 (Table 2). This treatment resulted in the highest D value as indicated in Table 3. Adequacy of the simplified model equation for predicting optimum response value was experimentally evaluated using a FS containing 35% glucose, pH of 7.6, temperature of 57.5°C and flow rate of 15 ml min^{-1}. This set of conditions was determined to be the optimum by the RSM optimization procedure. The experimental D value (77.2) was very close to the predicted value (85.6) indicating a good predictor model.

In conclusion, statistical central composite optimization procedure and response surface methodologies were used to obtain equations that identified significantly contributing process factors. Response surface graphic analysis allowed optimal process factors to be located. The experimental data were highly comparable to the model-predicted results (actual $D = 77.2$, predicted $D = 85.6$). The obtained optimum conditions were in close agreement with those reported in the literature.

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