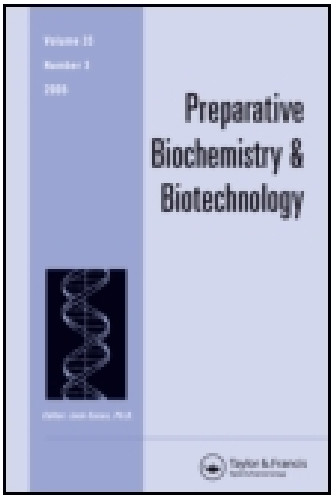

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EVALUATION OF BIOETHANOL PRODUCTION FROM CAROB PODS BY *Zymomonas mobilis* AND *Saccharomyces cerevisiae* IN SOLID SUBMERGED FERMENTATION

Saeed Saharkhiz, Davood Mazaheri, and Seyed Abbas Shojaosadati
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□ *Bioethanol production from carob pods has attracted many researchers due to its high sugar content. Both* Zymomonas mobilis *and* Saccharomyces cerevisiae *have been used previously for this purpose in submerged and solid-state fermentation. Since extraction of sugars from the carob pod particles is a costly process, solid-state and solid submerged fermentations, which do not require the sugar extraction step, may be economical processes for bioethanol production. The aim of this study is to evaluate the bioethanol production in solid submerged fermentation from carob pods. The maximum ethanol production of* $0.42\,g\,g^{-1}$ *initial sugar was obtained for* Z. mobilis *at* 30° *C, initial pH 5.3, and inoculum size of 5% v/v, 9 g carob powder per 50 mL of culture media, agitation rate 0 rpm, and fermentation time of 40 hr. The maximum ethanol production for* S. cerevisiae *was* $0.40\,g\,g^{-1}$ *initial sugar under the same condition. The results obtained in this research are comparable to those of* Z. mobilis *and* S. cerevisiae *performance in other culture mediums from various agricultural sources. Accordingly, solid submerged fermentation has a potential to be an economical process for bioethanol production from carob pods.*

Keywords bioethanol, carob pods, response surface methodology, saccharomyces cerevisiae, solid submerged fermentation, *Zymomonas mobilis*

INTRODUCTION

The continuous depletion of conventional fossil fuels, increasing energy consumption, shortages of energy resources, and greenhouse gas emissions have motivated many world countries to work on renewable energy sources. Bioethanol is considered to be the most interesting alternative liquid fuel, since it is a sustainable and environmentally friendly fuel and can be produced by fermentation of agricultural-based renewable materials.[1]

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Raw materials containing fermentable sugars (e.g., sugar cane, sugar beet, sweet sorgum and carob), hydrolyzable polysaccharides (wheat, maize, and other starch-containing grains) and lignocellulosic materials are the main types of feedstocks for bioethanol production. Among these different feedstocks, carob (*Ceratonia siliqua*), which has high carbohydrate content, may be an intersting source for bioethanol production. Carob is an evergreen shrub or tree, native to the Mediterranean area and southwest Asia, capable of being grown in arid lands. It contains large amounts of fermentable sugars (glucose, fructose, and sucrose) and as a result, can be used for ethanol production without any hydrolysis process. For instance, analysis of some Turkish carob pods yielded 102–115 g kg^{-1} of fructose, 33.0–36.8 of glucose and 299–384 of sucrose.[2] Energy and greenhouse gas emissions balance for ethanol refinery from carob is reported to be 10.94 MJ/kg ethanol and 0.92 Kg-eq CO_2/kg ethanol, respectively.[3]

Saccharomyces cerevisiae is currently used as the major ethanol-producing microorganism. The first study of bioethanol production from carob pod using *Saccharomyces cerevisiae* was carried out by Roukas.[4–6] In a recent study, Sanchez et al. studied the global process of bioethanol production from carob pods by *S. cerevisiae*, including physical pretreatment, sugar extraction, hydrolysis of carob pod, fermentation of aqueous extracts, distillation and dehydration, and simulation of water–bioethanol mixtures.[7] The extraction conditions and fermentation factors, such as pH, inoculum size, and nitrogen source, in the process of bioethanol production from carob extract with *S. cerevisiae* were also evaluated by Turhan et al.[8] The process design and economic performance of a bioethanol production plant using carob pod as feedstock and *S. cerevisiae* as the microorganism were investigated by Sanchez-Segado et al.[9] The ethanol production cost and discounted cash flow rate of return were estimated to be 0.55 € L^{-1} and 7%, respectively.[9]

As a potential alternative to presently used yeast for ethanol production, a gram-negative bacterium, *Zymomonas mobilis*, can also be used. This bacterium has higher specific rate of sugar uptake, higher ethanol yield, lower biomass production, and higher volumetric sugar uptake and ethanol productivity.[10] *Zymomonas mobilis* is one of the few facultative bacteria metabolizing glucose and fructose via the Entner–Doudoroff pathway. However, the only utilizable sugars for *Z. mobilis* are glucose, fructose, and sucrose,[11] and since these kinds of sugars exist in the sugar profile of carob pods, *Z. mobilis* can be used for ethanol production from carob pods. In the former study on the production of bioethanol from carob extract by *Z. mobilis*, Vaheed et al. reported that 0.34 g ethanol g^{-1} of initial sugar was produced,[12] comparable to the maximum yields of 0.37,[8] 0.47,[7] 0.21,[13] and 0.48[14] g g^{-1} achieved by *S. cerevisiae*. We also examined the potential of *Z mobilis* for bioethanol production from the mixture of carob pods

and wheat bran in solid-state fermentation.[15] The maximum of 0.30 g ethanol g^{-1} initial sugar was produced under the optimized condition in SSF. The successful results of *Z. mobilis* in submerged fermentation (SMF)[12] and solid-state fermentation (SSF)[15] encouraged us to study the ethanol production by this microorganism in solid submerged fermentation.

Therefore, in this research, the capabilities of both *Z. mobilis* and *S. cerevisiae* in ethanol production from carob pods in solid submerged fermentation were investigated for the first time. In addition, the effects of important independent factors on ethanol production were studied. Response surface methodology (RSM) was used to optimize the process conditions.

EXPERIMENTAL

Microorganism

Zymomonas mobilis PTCC 1718 was obtained from the Persian Type Culture Collection. It was activated and grown for 17 hr at 30°C in a conical flask shaken at 120 rpm in medium containing 10 g L^{-1} peptone from meat, 10 g L^{-1} yeast extract, and 20 g L^{-1} glucose. *Saccharomyces cerevisiae* was obtained as baker's yeast from Fariman Co., Iran, and cultivated in a flask containing 20 g L^{-1} glucose, 60 g L^{-1} sucrose, 30 g L^{-1} peptone from meat, 30 g L^{-1} yeast extract, and 30 L^{-1} KH_2PO_4 at 30°C for 24 hr. All of the chemicals used for medium preparation were purchased from Merck, Germany.

Preparation of Carob Pods

Carob pods were obtained from a Cypriot local market. After removing the seeds, dried kibbles were ground into fine particles and passed through a sieve of 0.2 mm opening. This carob powder was used as the carbon source in all experiments.

Solid Submerged Fermentation

The fermentations were carried out in 100-mL Erlenmeyer flasks containing a certain amount of carob powder in 50 mL distilled water. The medium was inoculated with optimized cell concentrations of *Z. mobilis* or *S. cerevisiae*. The pH of the medium (5.2 ± 0.1) is in the optimum range for cultivation of both microorganisms. In each experiment the inoculum size, amount of carob powder, agitation rate, and fermentation time were chosen according to the experimental design (Table 1 for *Z. mobilis* and

TABLE 1 RSM-Designed Experiments and Response Obtained as Ethanol Produced by *Z. mobilis*

Run	Amount of Inoculum (% v/v)	Amount of Carob Powder (g)	Agitation Rate (rpm)	Fermentation Time (hr)	Ethanol Produced (g)
1	3.8	8	150	26	1.24
2	2.6	2	100	38	0.32
3	3.8	19	50	49	2.94
4	1.4	8	50	26	0.93
5	2.6	14	200	38	2.16
6	0.2	14	100	38	1.72
7	3.8	8	50	49	1.25
8	3.8	19	150	49	2.47
9	1.4	8	50	49	1.02
10	1.4	19	50	26	1.04
11	2.6	14	100	38	2.21
12	2.6	14	100	38	2.21
13	2.6	14	100	38	2.17
14	2.6	14	100	15	0.18
15	1.4	19	150	26	0.79
16	2.6	14	100	38	2.10
17	2.6	14	100	38	2.22
18	2.6	14	0	38	2.23
19	3.8	19	150	26	1.25
20	3.8	8	150	49	0.05
21	1.4	19	150	49	2.86
22	1.4	19	50	49	2.80
23	2.6	14	100	60	2.59
24	2.6	14	100	38	2.34
25	2.6	25	100	38	2.63
26	3.8	8	50	26	1.10
27	1.4	8	150	49	1.19
28	5.0	14	100	38	2.10
29	3.8	19	50	26	1.34
30	1.4	8	150	26	0.95

Table 2 for *S. cerevisiae*). The flasks were incubated anaerobically at 30°C in the specified agitation rate and the fermentation time based on the experimental design (Table 1 for *Z. mobilis* and Table 2 for *S. cerevisiae*).

Sugar Measurement

The total sugar (glucose, fructose, and sucrose) content of the samples was quantified by hydrolysis with 1 *M* HCl at pH 1 and 80–85°C for 30 min, and neutralization with 1 *M* NaOH. The 3,5-dinitrosalicylic acid (DNS) method[16] was used to determine the sugar content as glucose. Reducing sugar was determined by the same method but without hydrolysis. The standard curve was in the range of 0.1–1 ($g L^{-1}$) glucose solution ($R^2 = 0.98$).

TABLE 2 RSM-Designed Experiments and Response Obtained as Ethanol Produced by *S. cerevisiae*

Run	Amount of Inoculum (% v/v)	Amount of Carob Powder (g)	Agitation Rate (rpm)	Fermentation Time (hr)	Ethanol Produced (g)
1	3.8	8	150	26	1.24
2	2.6	2	100	38	0.32
3	3.8	19	50	49	2.94
4	1.4	8	50	26	0.93
5	2.6	14	200	38	2.16
6	0.2	14	100	38	1.72
7	3.8	8	50	49	1.25
8	3.8	19	150	49	2.47
9	1.4	8	50	49	1.02
10	1.4	19	50	26	1.04
11	2.6	14	100	38	2.21
12	2.6	14	100	38	2.21
13	2.6	14	100	38	2.17
14	2.6	14	100	15	0.18
15	1.4	19	150	26	0.79
16	2.6	14	100	38	2.10
17	2.6	14	100	38	2.22
18	2.6	14	0	38	2.23
19	3.8	19	150	26	1.25
20	3.8	8	150	49	0.05
21	1.4	19	150	49	2.86
22	1.4	19	50	49	2.80
23	2.6	14	100	60	2.59
24	2.6	14	100	38	2.34
25	2.6	25	100	38	2.63
26	3.8	8	50	26	1.10
27	1.4	8	150	49	1.19
28	5.0	14	100	38	2.10
29	3.8	19	50	26	1.34
30	1.4	8	150	26	0.95

Ethanol Measurement

The fermented medium was centrifuged (B. Braun Biotech International B 16) at 4000 rpm for 10 min, and 10 mL of the supernatant was distilled at atmospheric pressure. Produced ethanol was measured as w/v% by the Arthur Caputi Jr. method.[17] Absolute ethanol (Merck) was used to prepare a standard curve in the range of 0.1–0.7 g ethanol per 100 mL ($R^2 = 0.99$).

Statistical Experimental Design

There was no former published study regarding feasibility of *Z. mobilis* fermentation on carob pod powder in solid sumberged fermentation. As a result, after preliminary experiments, four independent variables were

indentified affecting the production of ethanol. The variables are *A* (inoculum size, % v/v), *B* (amount of carob powder, g), *C* (agitation rate, rpm), and *D* (fermentation time, h). RSM was used to design experiments in order to optimize the conditions for maximum ethanol production. The levels of factors in the experiments based on CCD for *Z. mobilis* are shown in Table 1. The levels for *S. cerevisiae* were the same, unless the high level of carob (25 g) and the low level of time (16 hr) were different. Thirty designed experiments and the responses obtained for *Z.mobilis* and *S.cerevisiae* are presented in Tables 1 and 2, respectively.

Biokinetic Experiments

Biokinetic model variables can be evaluated by observing product formation with time in batch fermentations and then fitting the data with an appropriate model. In this study, the Monod model was used to determine the optimal kinetic coefficients of *Z. mobilis*. In order to determine the kinetic of cell growth, substrate utilization, and ethanol production, samples were removed at specified time intervals. For this experiment, the fermentation was conducted under the optimum conditions for maximum ethanol production. For biomass estimation, the carob solid particles were separated by filtration from the medium and the filtrate was centrifuged at 5000 rpm for 10 min. The sediment was dried at 70°C until a constant weight was attained. The growth of the organisms was determined as dry weight.

RESULTS AND DISCUSSION

Carob-Powder Analysis

The raw-carob-pod-particle analysis showed moisture content of $8.9 \pm 0.1\%$ (w/w), $53 \pm 1.5\%$ (w/w) total sugars, and 18.5% (w/w) reducing sugars.

Fermentation and Optimization

According to the literature review and some pretests, we considered the most effective factors and the high and low levels for each factor. In a study of molasses fermentation, the range of 24–48 hr for fermentation time and $0.2\,g\,L^{-1}$ of *Z. mobilis* were used.[18] The low and high values of agitation rate were zero and 180 rpm, respectively. Temperature of 30°C and pH range of 5.0–7.0 were reported as optimum conditions. Sreekumar et al. reported the optimum pH of 5.5 and glucose concentration of $120.4\,g\,L^{-1}$ in *Z. mobilis*

ethanol fermentation.[19] The highest ethanol yield from artichoke juice was observed at pH 5 and static condition.[20] It was reported that *Z. mobilis* grows best within the temperature range of 30–35°C and is able to grow at pH values of 4.0–7.0. The optimal condition for *Z. mobilis* ethanol production from kitchen garbage was 30°C and pH 5.[21] Therefore, interesting factors including inoculum size, amount of carob powder, agitation rate, and fermentation time are selected for further study at 30°C and pH 5–5.2. Thirty experiments were designed as presented in Table 1 for *Z. mobilis* and Table 2 for *S. cerevisiae*. Responses (produced ethanol) were analyzed by the Design Expert (dx7) software trial version. The coefficient estimates of coded and significance level of factors presented in Table 3a. for *Z. mobilis* and Table 4a for *S. cerevisiae*.

The R^2 value was 0.97 for *Z. mobilis* and 0.95 for *S. cerevisiae*. For *Z. mobilis* analysis of the results indicated that *A* (inoculum size), *B* (amount of carob powder), *C* (agitation rate), *D* (fermentation time), *BD*, B^2, and D^2 were significant factors in the proposed model ($p = 0.05$). Lack of fit was insignificant ($p = 0.05$). The insignificant terms were dropped from the model. In this simplified model, the coefficients were just changed slightly

TABLE 3 Estimated Factor Coefficients and Associated Significance Levels of the CCD Model for Response (Produced Ethanol) by *Z. mobilis*

Factor	Coefficient Estimate	*p*-Value
Part a		
Model		<0.0001
Intercept	1.75	
A, Inoculum size (% v/v)	0.092	0.0064
B, Amount of carob powder (g)	0.47	<0.0001
C, Agitation rate (rpm)	−0.062	0.0510
D, Fermentation time (hr)	0.30	<0.0001
AB	0.055	0.1422
AC	−0.052	0.1645
AD	−0.068	0.0768
BC	0.032	0.3870
BD	0.16	0.0004
CD	0.051	0.1742
A^2	−0.025	0.3644
B^2	−0.18	<0.0001
C^2	-3.557×10^{-3}	0.8977
D^2	−0.16	<0.0001
Part b		
Model		<0.0001
Intercept	1.46	
A, Inoculum size (% v/v)	0.092	0.009
B, Amount of carob powder (g)	0.47	<0.0001
C, Agitation rate (rpm)	−0.062	0.0680
D, Fermentation time (hr)	0.30	<0.0001
BD	0.16	0.0005

TABLE 4 Estimated Factor Coefficients and Associated Significance Levels of the CCD Model for Response (Produced Ethanol) by *S. cerevisiae*

Factor	Coefficient Estimate	*p*-Value
Part a		
Model		<0.0001
Intercept	2.21	
A, Inoculum size (% v/v)	0.076	0.1108
B, Amount of carob powder (g)	0.47	<0.0001
C, Agitation rate (rpm)	−0.031	0.4917
D, Fermentation time (hr)	0.49	<0.0001
AB	-2.8×10^{-3}	0.9596
AC	−0.038	0.4927
AD	−0.085	0.1394
BC	−0.055	0.3276
BD	0.40	<0.0001
CD	−0.017	0.7661
A^2	−0.11	0.0166
B^2	−0.22	<0.0001
C^2	−0.042	0.3312
D^2	−0.24	<0.0001
Part b		
Model		<0.0001
Intercept	2.16	<0.0001
B	0.47	<0.0001
D	0.49	<0.0001
BD	0.40	<0.0001
A^2	−0.11	0.0177
B^2	−0.22	<0.0001
D^2	−0.24	<0.0001

(Table 3b). The R^2 value was reduced to 0.95, and lack of fit was insignificant ($p = 0.05$). The final equation for the model as a function of the coded factors is given as Eq. (1) for *Z. mobilis*.

$$\begin{aligned}\text{Response (ethanol produced, g)} = {} & -2.64804 + 0.076649\,A + 0.14894\,B \\ & - 1.23292 \times 10^{-3}\,C + 0.11518\,D \\ & + 3.55208 \times 10^{-3}\,BD - 8.49055 \times 10^{-3}\,B^2 \\ & - 1.55559 \times 10^{-3}\,D^2 \qquad (1)\end{aligned}$$

The specified factor ranges for Eq. (1) were as follows: inoculum size, 0.2–5% v/v; amount of carob powder, 2–20 g; agitation rate, 0–200 rpm; fermentation time, 20–60 hr; ethanol produced, 0.26–2.42 g.

The maximum ethanol production using the minimum amount of carob powder occurred at inoculum amount of 5% v/v, carob powder amount of 9.4 g, agitation rate of 0 rpm, and fermentation time of 48 hr. Through the present optimum condition, maximum ethanol of 0.42 g g^{-1} initial sugar was produced.

For *S. cerevisiae* analysis of the results indicated that *B* (amount of carob powder), *D* (fermentation time), *BD*, A^2, B^2, and D^2 were significant factors in the proposed model ($p = 0.05$). Lack of fit was also significant ($p = 0.05$). The insignificant terms were dropped from the model. In this simplified model, the coefficients were changed slightly (Table 4b. The R^2 value was reduced to 0.94. The final equation for the model as a function of the coded factors is given as Eq. (2) for *S. cerevisiae*.

$$\begin{aligned}\text{Response (ethanol produced, g)} = {} & -1.95759 + 0.44873\ A + 0.026991\ B \\ & + 0.10122\ D + 6.16908 \times 10^{-3}\ BD \\ & - 0.074002\ A^2 - 6.51229 \times 10^{-3}\ B^2 \\ & - 1.87901 \times 10^{-3}\ D^2 \end{aligned} \quad (2)$$

The specified factor ranges for Eq. (2) were as follows: inoculum size, 0.2–5% v/v ; amount of carob powder, 2–25 g; agitation rate, 0–200 rpm; fermentation time, 15–60 hr; ethanol produced, 0.32–2.86 g.

The maximum ethanol production using the minimum amount of carob powder occurred at inoculum amount of 3% v/v, carob powder amount of 8 g, agitation rate of 140 rpm, and fermentation time of 40 hr. Through the present optimum condition, maximum ethanol of 0.40 g g^{-1} initial sugar was produced.

The effects of significantly affecting factors on the response are shown in Figures 1 and 2 for *Z. mobilis* and in Figures 3 and 4 for *S. cerevisiae*.

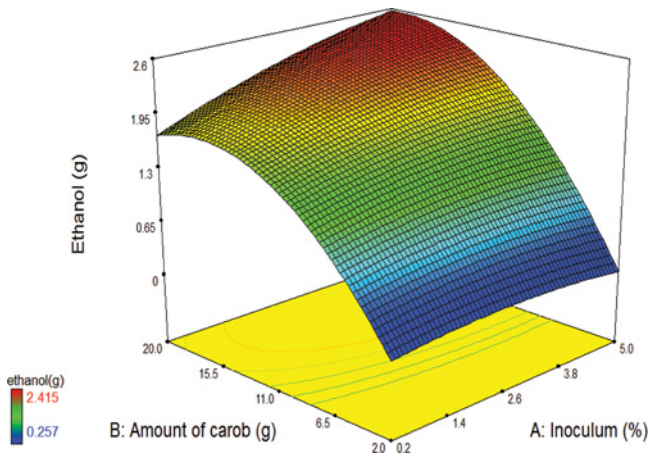

FIGURE 1 Interactions of amount of carob powder and inoculum size on response (produced ethanol, g). Actual factors: agitation rate, 50 rpm; fermentation time, 45 hr for *Z. mobilis* (color figure available online).

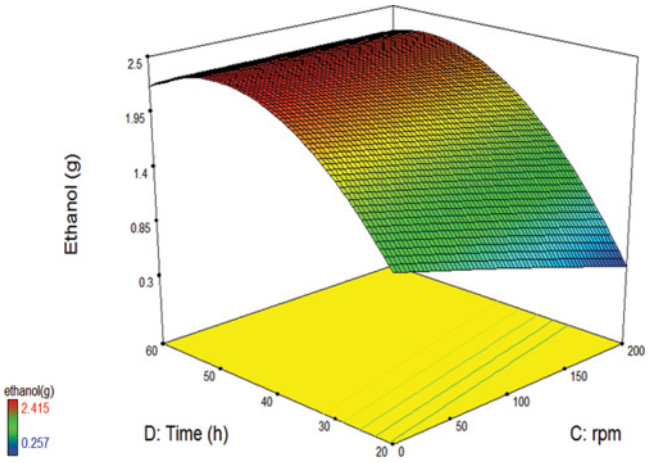

FIGURE 2 Interactions of fermentation time and agitation rate on response (produced ethanol, g). Actual factors: inoculum size, 4.0% (v/v); amount of carob powder, 15 g for *Z. mobilis* (color figure available online).

The optimal conditions for response were obtained by further numerical analysis of the response surface using the software. The solution to the maximal response for *Z. mobilis* was inoculum size, 5%; amount of carob powder, 9 g; agitation rate, 0 rpm; fermentation time, 40 hr. A confirmation upon the experiment, under the optimal condition mentioned earlier, was conducted. The result was proven to be 1.89 g

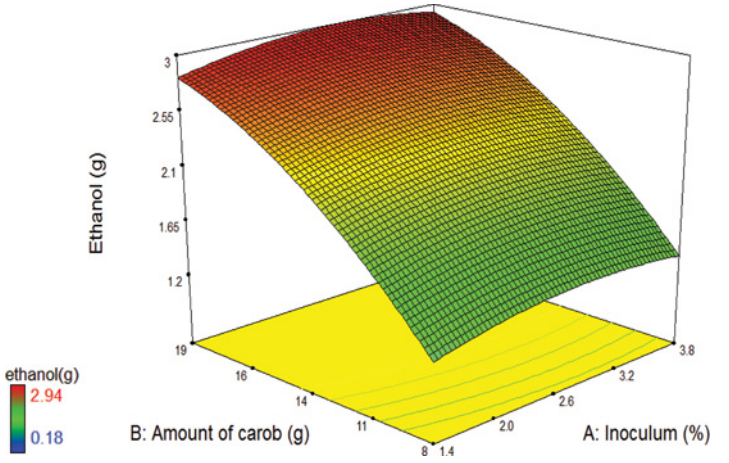

FIGURE 3 Interactions of amount of carob powder and inoculum size on response (produced ethanol, g). Actual factors: agitation rate, 50 rpm; fermentation time, 45 hr for *S. cerevisiae* (color figure available online).

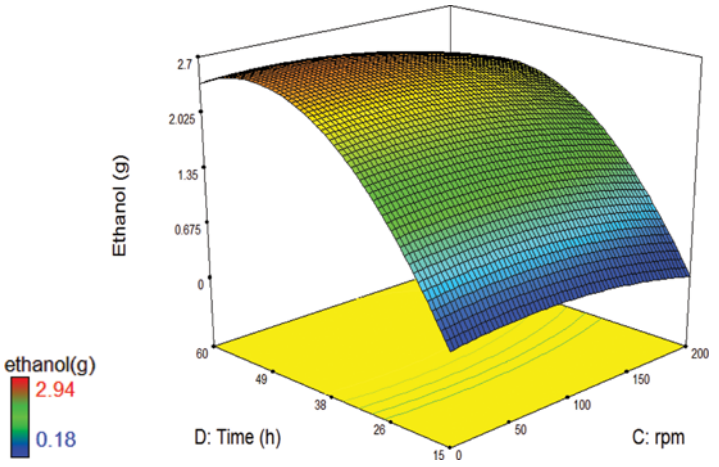

FIGURE 4 Interactions fermentation time and agitation rate on response (produced ethanol, g). Actual factors: inoculum size, 4.0% (v/v); amount of carob powder, 15 g for *S. cerevisiae* (color figure available online).

ethanol, which was within the 95% confidence interval of the prediction (1.43–2.34 ethanol, g).

The maximal response for *S. cerevisiae* was at inoculum size, 2.9%; amount of carob powder, 9 g; agitation rate, 95 rpm; and fermentation time, 42 hr. A confirmation upon the experiment within the 95% confidence interval, under the optimal condition mentioned earlier, was conducted. The result was proven to be 1.80 g ethanol.

Ethanol Productivity

Ethanol productivity by *Z. mobilis* was calculated as g ethanol produced per liter per hour. It was also analyzed by the software, and then Eq. (3) was obtained for productivity prediction as a function of coded factors ($p = 0.05$). The maximum productivity was 1.23 g L^{-1} hr^{-1}.

$$\begin{aligned}\text{Response (ethanol productivity, g L}^{-1}\text{h}^{-1}) = {} & 0.87 + 0.054\,A + 0.22\,B \\ & - 0.036\,C - 0.012\,D + 0.032\,AB \\ & - 0.027\,AC - 0.042\,AD + 0.020\,BC \\ & + 0.019\,BD + 0.034\,CD - 8.281 \\ & \times 10^{-3}\,A^2 - 0.096\,B^2 - 2.344 \\ & \times 10^{-3}\,C^2 - 0.081\,D^2 \qquad (3)\end{aligned}$$

$$\begin{aligned}\text{Response (ethanol productivity, g L}^{-1}\text{h}^{-1}) = {} & 1.16 + 0.053\ A + 0.22\ B \\ & - 0.016\ C + 0.037\ D \\ & + 6.125 \times 10^{-3}\ AB - 0.011\ AC \\ & - 0.063\ AD - 0.035\ BC \\ & + 0.016\ BD - 3 \times 10^{-3}\ CD \\ & - 0.049\ A^2 - 0.11\ B^2 \\ & - 0.010\ C^2 - 0.16\ D^2 \end{aligned} \quad (4)$$

Equation (4) was also obtained for productivity prediction as a function of coded factors for *S. cerevisiae*.

The R^2 value of this equation was 0.949. The productivity obtained at optimized conditions was calculated as $1.15 \pm 0.28\,\text{g L}^{-1}\,\text{hr}^{-1}$. This value fell within the 95% confidence interval of the prediction (1.43–$0.87\,\text{g L}^{-1}\,\text{hr}^{-1}$).

The maximum ethanol productivity was $1.30\,\text{g L}^{-1}\,\text{hr}^{-1}$.

Figure 1 shows the interaction effects of carob powder amount and inoculum size. When the agitation rate is 50 rpm and the fermentation time is 45 hr, predicted conditions for maximum response are the factor combination of about 4.5% inoculation and 16 g carob powder. The interaction effect of fermentation time and agitation rate on ethanol production by *Z. mobilis* is shown in Figure 2. This figure predicts that when the inoculum size is 4% (v/v) and the amount of carob powder in the medium is 15 g, produced ethanol would be at its maximum amount for approximately 23 rpm and 49 hr fermentation time.

According to Figure 1, the high sugar content in the fermentation medium causes an increase in the osmotic pressure, which has a damaging effect on the cells. The same trend can be seen in Figure 3 for *S. cerevisiae*. The difference between the inoculum size of *S. cerevisiae* and *Z. mobilis* and the amount of carob powder used for each microorganism show that *S. cerevisiae* has greater resistance to the sugar than *Z. mobilis*. It can be observed that by increasing the amount of carob powder to more than 18 g in the medium of *Z.mobilis*, the amount of produced ethanol was decreased, while a similar decrease was observed when the amount of carob powder is more than 22 g for *S. cerevisiae*. According to Figure 2, by increasing the rate of agitation, the solubility of oxygen in the medium was increased and the amount of ethanol production decreased. Consequently, the metabolism pathway of bacteria was changed from anaerobic to aerobic.

Based on the optimum conditions reported in the preceding, the major difference between optimal conditions of ethanol production for the two microorganisms was agitation rate. The higher optimal agitation

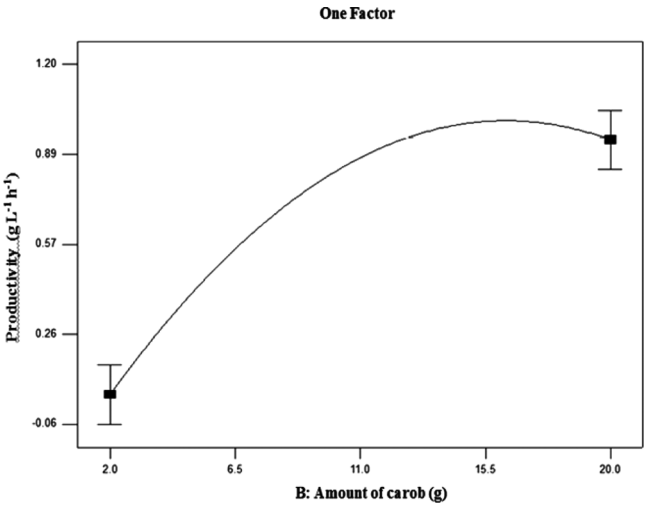

FIGURE 5 Effect of amount of carob powder on ethanol productivity for *Z. mobilis*. Actual factors: agitation rate, 50 rpm; fermentation time, 45 hr; inoculum size, 4.0% (v/v).

rate of the yeast showed that *S. cerevisiae* is more resistant to shear stress and aeration in the same condition than *Z. mobilis*. By comparing these two microorganisms, the amount of produced ethanol (g ethanol per g initial sugar) by *Z. mobilis* was 0.42 and by *S. cerevisiae* was 0.4. It can be concluded that *Z. mobilis* had slightly higher yields than *S. cerevisiae*.

The maximum ethanol productivity was 1.30 g L^{-1} hr^{-1}. It is predicted that productivity can be improved by increasing the amount of carob from 2 to 16 g. Thereafter, the productivity will decrease (Figure 5). This trend is similar to the results for ethanol production in Figure 1. In addition, productivity showed an upward trend with decreasing the agitation rate (Figure 6). The increasing trend of productivity with increasing fermentation time lasting for 40 hr, and after that, less ethanol will be produced. The same method was performed for *S. cerevisiae* and the maximum ethanol productivity was 1.38 g L^{-1} hr^{-1}.

Kinetics of Ethanol Production

The kinetic parameters were determined from experimental data. From Figure 7, which shows both the time course of ethanol production and the time course of total sugar utilization by *Z. mobilis*, it may be concluded that the lag phase was about 5 hr. The maximum ethanol production can be observed at around 40 hr. However, the increase in produced ethanol concentration is not significant after 30 hr.

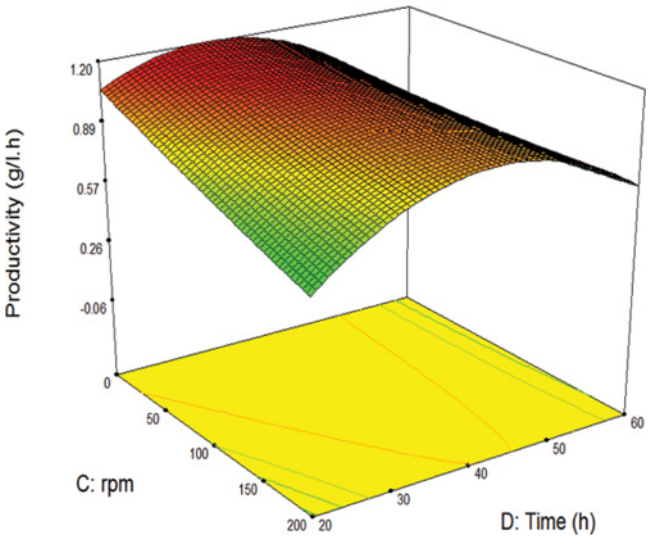

FIGURE 6 Interactions of fermentation time (hr) and agitation rate (rpm) on response (productivity) for *Z. mobilis*. Actual factors: inoculum size, 4.0% (v/v %); amount of carob powder, 15 g (color figure available online).

The total sugars remaining after fermentation were 0.65 g, corresponding to a fermentation of about 85% of total sugars. The maximum yield of the process is $Y_{P/S} = 0.48$. From the experimental data, it can be calculated that the maximum specific growth rate of *Z. mobilis* in this condition is $\mu_{max} = 0.13\,hr^{-1}$, and specific productivity of ethanol is $q_P = 5.24\,hr^{-1}$.

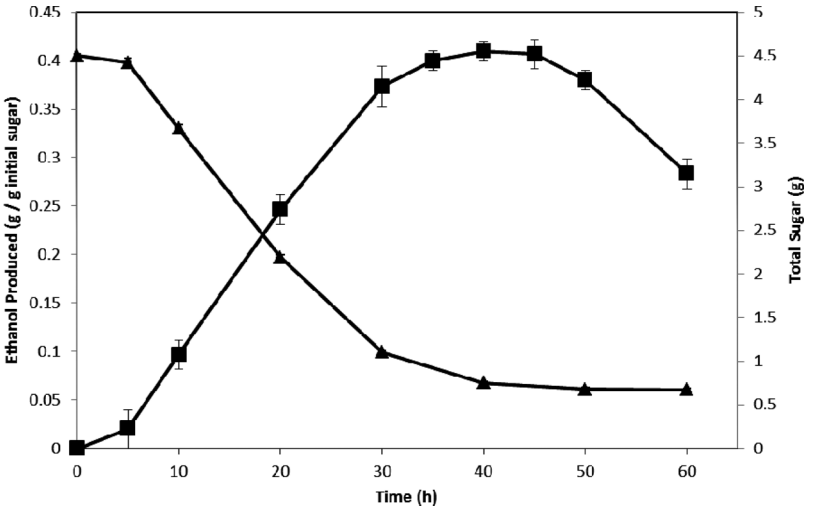

FIGURE 7 Time courses for ethanol produced concentration (■) and total sugars (▲) in the solid submerged medium.

CONCLUSIONS

The present study showed that *Z. mobilis* can utilize the carob sugars and produce ethanol in solid submerged fermentation. The data obtained in this research are comparable to the results of *Z. mobilis* and *S. cerevisiae* performance in other culture mediums from various agricultural sources. The ethanol yield by *Z. mobilis* could be comparable with traditional type of fermentation by *S. cerevisiae*. Response surface methodology was a useful method to optimize the conditions for maximum ethanol production from carob powder by solid submerged fermentation. As demonstrated, for ethanol production by *Z. mobilis*, there was no need to aerate submerged carob powders medium. The main advantage of the solid submerged fermentation is that there is no need to extract carob pod sugars and the extraction step can be omitted.

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