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Original Article

Evaluation and Optimization of Bioethanol Production from Pomegranate Peel by *Zymomonas mobilis*

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Abstract

Introduction: The large amount of production and use of pomegranate in Iran has made the waste of this important product a suitable source for the production of bioethanol. This research examined the ability of *Zymomonas mobilis* for producing bioethanol from Pomegranate peel.

Materials and Methods: The hydrothermal treatment method and enzymatic hydrolysis were used to release the fermentable sugars from PP particles. Cellulase loading of 30 U/g solid was used for enzymatic hydrolysis. *Zymomonas mobilis* PTCC 1718 was used as the ethanol-producing strain. The Response Surface Methodology experimental design was performed to optimize the fermentation process conditions for maximum ethanol production and minimum fermentation time.

Results: The amount of sugar released after hydrolysis was about 18.37% of the fresh PP weight. The amount of meat peptone and yeast extract (as nitrogen sources added to the medium), bacterial dry weight (as inoculum) and fermentation time were considered as the effective factors in the RSM experimental design. The maximum amount of 0.218 g ethanol was produced at 0.37 g meat peptone, 0.28 g yeast extract, 0.021 g bacterial dry weight and 30 h fermentation time (in 50 ml of culture medium). The maximum ethanol production yield of 45.5% (which is 89.2% of the theoretical yield) was achieved in this process.

Conclusions: *Z. mobilis* has good ability for producing bioethanol from PP. However, more research should be conducted in order to industrialize the process.

Keywords: Bioethanol, Pomegranate Peel, *Zymomonas mobilis*, Response Surface Methodology
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Introduction

Increasing energy consumption in today's world has promoted the search for new sources of energy. Also, fossil fuels, as the world's current leading source of energy, have declining resources, fluctuating prices, and adverse environmental impacts.[1-3] All these have led to a global effort to develop new energy sources. One of these alternative sources of energy is bioethanol produced through the fermentation of sugars via microorganisms, thereby making this a promising renewable fuel. Furthermore, the utilization of this fuel can also help solve one of the most serious environmental problems, i.e. air pollution; and greenhouse gas emissions.[4,5]

Various feedstocks can be employed to produce bioethanol; such as edible crops containing fermentable sugars (first-generation bioethanol) and inedible lignocellulosic waste materials (second-generation bioethanol). The latter is one of the most low-priced and abundant resources on the planet. Although second-generation bioethanol production requires a higher cost of hydrolysis, it is a viable option for the economical production of renewable bioethanol fuel.[5-7]

Pomegranate (*Punica granatum*) has recently received a great deal of attention because of its beneficial properties to humans' health. The growing consumption of this fruit and its progressive popularity in food industries have led researchers to work more on Pomegranate Peel (PP) as a valuable by-product.[8] The PP constitutes about 30 to 50% of the total weight of the fresh fruit. The chemical composition of PP has been investigated by many researchers in the literature and found to be a rich source of commercially important products.[8-11] For instance, phenolic compounds constitute about 10-20% of the PP weight and have antioxidant and anti-cancer properties,[9] with pectins making up around 20-25% of PP.[8] Another important component of PP is polysaccharides such as cellulose and neutral sugars, making PP an interesting source for fuel bioethanol production. The cellulose and neutral sugars content of dietary fiber of PP have been reported to be 17.43- 22.71% and 16.88-19.66% respectively.[8] In another study, Alaa reported that PP consists of 27.9% pectin, 10.8% hemicellulose, 26.2% cellulose, and 5.7% lignin.[12]

Iran is one of the largest producers and exporters of

pomegranate in the world and produces about 800,000 metric tons of pomegranate annually, accounting for approximately 40% of the total world production.[10] According to the literature, the non-edible portion of Iranian pomegranates is about 35-62% of the total fruit weight. Thus, a considerable amount of PP waste is produced annually in this country, which can be used as a promising source for bioethanol production.[13]

According to the literature, most studies on PP have so far focused on its constituents and extraction of materials such as phenolic compounds and pectin,[8-11] and little attention has been paid to ethanol production from this valuable source. Recently, Demiray et al. investigated the production of bioethanol from PP by two conventional microorganisms, called *Saccharomyces cerevisiae* (for the fermentation of six-carbon sugars) and *Pichia stipitis* (for the fermentation of five-carbon sugars) after a dilute sulfuric acid pre-treatment, and optimized the influencing factors in the process.[14] According to the results, the maximum amount of ethanol produced under the optimum conditions was 5.58 g/L for *S. cerevisiae* and 2.95 g/L for *P. stipitis* using various nitrogen sources and metal salts in the fermentation medium. Applying a hydrothermal processing, Talekar et al. simultaneously recovered pectin and phenolics from PP and then produced ethanol by *S. cerevisiae* after an enzymatic hydrolysis step.[15] It was reported that the hydrothermal process used for the recovery of pectin and phenolics enhanced the release of glucose in enzymatic hydrolysis process, thereby increasing the ethanol production with a yield of about 88% of the theoretical yield.[15]

S. cerevisiae is the most well-known microorganism for ethanol production. One of the alternatives is *Zymomonas mobilis*, a gram-negative bacterium that can produce bioethanol by fermenting glucose, fructose, and sucrose.[16,17] *Z.mobilis* was successfully used for bioethanol production from many feedstocks in both submerged and solid-state fermentations.[18-21] Since PP contains phenolic compounds, the tolerance of microorganisms to these compounds is important. In a study of various phenolic compounds derived from lignocellulosic materials, Gu et al. reported that *Z. mobilis* tolerance to phenolic aldehydes was similar to that of *S. cerevisiae*, but *Z. mobilis* showed stronger tolerance to phenolic acids.[22] These properties can make *Z. mobilis* an appropriate option for bioethanol production from PP.

However, to the best of our knowledge, the ability of *Z. mobilis* for producing bioethanol from PP has not yet been investigated. In this study, we applied a hydrothermal process for feedstock pre-treatment and removing pectin plus phenolic compounds followed by an enzymatic hydrolysis step to produce fermentable sugars from lignocellulosic materials. Finally, *Zymomonas mobilis* was employed for the first time to produce ethanol, and the effect of different factors in the process was investigated where the process factors were optimized for the maximum ethanol production and minimum fermentation time using RSM experimental design.

Materials and Methods

Microorganism and Substrate

Zymomonas mobilis (PTCC 1718) was purchased from the Persian Type Culture Collection. To prepare the inoculum, *Z.mobilis* was grown at 30 °C and 120 rpm for 17 h in a medium containing 10 g meat peptone (Merck), 10 g yeast extract (Merck) and 20 g glucose (Merck) per liter of distilled water. The PP was obtained from a local market in Mahallat, Iran. Once transferred to the laboratory, PP was ground into smaller pieces and sieved to obtain the particle size between 4 and 6 mm.

Pre-treatment of PP Particles

For pre-treatment of PP particles, the hydrothermal method recently developed by Talekar et al.,[15] was used under the reported optimum conditions. In this method, 50 g of fresh ground PP was mixed with water at a liquid-solid ratio of 10 in a batch vessel, and then; autoclaved at 115 °C for 40 min. In the next step, the whole vessel content was centrifuged (B. Braun Biotech International B 16) at 4000 rpm for 10 min. The sediment was used as a substrate for the hydrolysis process.

Enzymatic Hydrolysis and Fermentation

After the hydrothermal pre-treatment, enzymatic hydrolysis was used to release the fermentable sugars from the lignocellulosic material. This process was performed through cellulase loading of 30 U/g with a solid loading of 12% at 50 °C, pH 5 and 150 rpm for 15 h in a batch vessel.[15]

The fermentation step was carried out in 100 ml conical flasks containing 30 ml PP hydrolysate (which has 0.54 g glucose sugar) with suitable amounts of meat peptone and yeast extract added to the medium according to the experimental design (Table 1). The entire flask was then sterilized at 121 °C for 15 min in an autoclave. Subsequently, the medium was inoculated with an appropriate volume of *Z. mobilis* cells' suspension (providing the specific amount of bacterial dry weight based on the experimental design). The inoculated mixture volume was then adjusted to 50 ml by adding distilled water and then incubated anaerobically at 30 °C and 80 rpm for a specific time. In each experiment, the amounts of bacterial dry weight, fermentation time, peptone, and yeast extract weight were chosen according to the experimental design (Table 1).

Analytical Methods

The bacterial dry cell mass in the inoculum culture was determined as g by measuring the Optical Density (OD) at 600 nm by using a standard curve.

For ethanol and sugar determination, samples were taken from the fermentation medium and centrifuged at 4000 rpm for 10

min. The supernatant was used for further investigations. Reducing sugars were determined using the 3,5 dinitrosalicylic acid (DNS) method.[23] To determine the produced ethanol, the supernatant was first distilled at atmospheric pressure, after which the Arthur Caputi Jr. method was used to determine the ethanol content.[24]

Experimental Design

After studying the literature and performing some preliminary experiments, four independent variables that were effective in the fermentation process were identified: A = weight of peptone from meat (g), B = weight of yeast extract (g), C = inoculum bacterial dry weight (g) and D = fermentation time (h). The amount of the produced ethanol (g) was considered as the response variable. A Central Composite Design (CCD) and Response Surface Methodology (RSM) were employed to optimize the effective variables for the maximum ethanol production within the minimum fermentation time. Thirty designed experiments as well as the low and high levels of each variable are presented in Table 1.

Table 1. The Effective Factors Selected for Optimization with Their Low and High Values Plus RSM-designed Experiments and the Results of Produced Ethanol as the Response

Run	A	B	C	D	Ethanol (g)
1	0.13	0.13	0.015	30	0.138
2	0.38	0.13	0.015	30	0.203
3	0.13	0.38	0.015	30	0.176
4	0.38	0.38	0.015	30	0.203
5	0.13	0.13	0.025	30	0.173
6	0.38	0.13	0.025	30	0.218
7	0.13	0.38	0.025	30	0.212
8	0.38	0.38	0.025	30	0.219
9	0.13	0.13	0.015	42	0.166
10	0.38	0.13	0.015	42	0.203
11	0.13	0.38	0.015	42	0.169
12	0.38	0.38	0.015	42	0.222
13	0.13	0.13	0.025	42	0.197
14	0.38	0.13	0.025	42	0.213
15	0.13	0.38	0.025	42	0.215
16	0.38	0.38	0.025	42	0.204
17	0.00	0.25	0.02	36	0.169
18	0.50	0.25	0.02	36	0.210
19	0.25	0.00	0.02	36	0.201
20	0.25	0.50	0.02	36	0.222
21	0.25	0.25	0.01	36	0.201
22	0.25	0.25	0.03	36	0.212
23	0.25	0.25	0.02	24	0.211
24	0.25	0.25	0.02	48	0.207
25	0.25	0.25	0.02	36	0.198
26	0.25	0.25	0.02	36	0.220
27	0.25	0.25	0.02	36	0.210
28	0.25	0.25	0.02	36	0.220
29	0.25	0.25	0.02	36	0.199
30	0.25	0.25	0.02	36	0.210

Indication	Factor	Unit	Low level	High level
A	Meat Peptone	g	0	0.5
B	Yeast extract	g	0	0.5
C	Bacterial dry weight	g	0.01	0.03
D	Fermentation time	h	24	48

Table 2. Factors' Coefficient Estimates and Significance Levels of the (a) CCD Model for Ethanol Production and (b) Simplified CCD Model for Ethanol Production

Factor	Coefficient estimate	*F*-value	*p*-value
a			
Model		13.71	< 0.0001
Intercept	0.21		
A-Peptone	0.012	52.46	< 0.0001
B-Yeast extract	4.54×10^{-3}	7.99	0.0143
C-Bacterial dry mass	0.014	50.97	< 0.0001
D-Time	-1.32×10^{-4}	6.76×10^{-3}	0.9357
AB	-8.07×10^{-3}	16.09	0.0015
AC	-5.18×10^{-3}	6.62	0.0232
AD	-5.70×10^{-3}	8.01	0.0142
BC	1.95×10^{-3}	0.94	0.3508
BD	-5.57×10^{-3}	7.67	0.0159
CD	5.73×10^{-4}	0.081	0.7804
A^2	-5.77×10^{-3}	15.95	0.0015
B^2	-2.72×10^{-4}	0.035	0.8539
C^2	-7.97×10^{-3}	17	0.0012
D^2	-8.97×10^{-4}	0.38	0.5458
b			
Model		22.62	< 0.0001
Intercept	0.21		
A- Peptone	0.012	66.21	< 0.0001
B- Yeast extract	4.752×10^{-3}	10.67	0.0045
C-Bacterial dry mass	0.014	61.49	< 0.0001
D- Time	8.530×10^{-5}	3.439×10^{-3}	0.9539
AB	-7.747×10^{-3}	18.22	0.0005
AC	-5.503×10^{-3}	9.19	0.0075
AD	-5.372×10^{-3}	8.76	0.0088
BD	-5.247×10^{-3}	8.36	0.0102
A^2	-5.589×10^{-3}	18.64	0.0005
C^2	-7.748×10^{-3}	19.41	00004

Results and Discussion

Pre-treatment and Hydrolysis of PP Particles

Before the main hydrolysis process, a pre-treatment step is usually used to enhance the yield of hydrolysis.[1] In this study, we used the hydrothermal pre-treatment method in which pectin and phenolic compounds can be simultaneously extracted from PP particles. Removing pectin can improve the access of enzymes to the cellulosic parts of the PP cell walls. In this way, the recalcitrant cellulosic structure of PP can be degraded more easily in the hydrolysis step. Meanwhile, phenolic compounds were identified as a microbial growth inhibitor. Thus, eliminating phenolics from PP can help improve the production of ethanol. The solvent in the hydrothermal treatment is water acting as an acid catalyst; hence, acid is no longer used for pectin extraction from PP.[25] In a recent study, Talekar et al.[15] evaluated the hydrothermal process for pre-treatment of PP and optimized the conditions. By performing the hydrothermal treatment at 115 °C and 40 min with a liquid-solid ratio of 10 (the optimized condition), the authors reported the maximum pectin and phenolics recovery yields of 20.8% and 11.5%, respectively. It was also reported that the percentage of sugar loss during the hydrothermal step was around 5-4% of the initial sugar, which is not significant.[15] In this study, we employed the same procedure for the pre-treatment of PP particles to help improve the release of sugars in the hydrolysis step and the growth of bacteria in the fermentation step.

For hydrolysis of lignocellulosic substrates, several methods such as acid, alkali, and enzymatic hydrolysis have been used.[5] Dilute sulfuric acid hydrolysis has recently been employed for hydrolysis of PP particles in the process of bioethanol production, where the maximum ethanol concentration was achieved when H_2SO_4 1% at biomass loading of 10 g/L was used.[14] One of the most widely used methods for hydrolysis of cellulosic materials for releasing glucose is the use of cellulase enzyme. Cellulase can break down the bonds in cellulose and turn it into glucose, which can be utilized by microorganisms.[5] Since PP contains pectin and cellulose, Pocan et al. used cellulase and pectinase simultaneously to hydrolyze PP. They observed that the use of pectinase alongside cellulase can help boost the glucose production yield.[26] The authors also mentioned that due to the low lignin content of PP, the pre-treatment processes were not preferred for lignin removal before the enzymatic hydrolysis step.

Enzymatic hydrolysis was also used by Talekar et Al. for saccharification of PP for ethanol production. The cellulase loading of 30 U/g and solid loading of 12% for 12 h were reported as the best hydrolysis conditions.[15] In this study, we did not optimize the hydrolysis process and used the optimized condition reported in the above-mentioned study.[15] However, based on our preliminary experiments, prolongation of the hydrolysis time to 15 h resulted in more glucose production. The results of the sugar measurement in our experiments showed that the amount of glucose in PP particles after the enzymatic hydrolysis process was about 18.37 % (w/w) of the PP weight, which is comparable to the amount of 17.7% glucose reported by Talekar et al.[15]

Preliminary Experiments and Evaluating the Experimental Design Variables

Based on the literature review and our preliminary experiments, we identified four independent factors as influencing factors and selected their high and low values (Table 1).

In the fermentation of carob pods by *Z. mobilis*,[18] the authors used the Plackett-Burman (P-B) design for screening the factors. Then, five effective factors were considered as dominant factors, including peptone weight, yeast extract weight, initial sugar concentration, bacterial dry weight, and fermentation time. On the other hand, pH and shaking rate were found to be insignificant. Upon pH elevation from 5 to 7, a slight positive change was observed in ethanol production. Slow shaking rates (around 80 rpm) showed no negative effect on the response.[18] As mentioned in many studies, the temperature of around 30 °C and pH 5 were recognized as the suitable condition for *Z. mobilis*.[18,21,27] Similarly, in solid-state fermentation of *Z. mobilis*, the optimum temperature of 31 °C and pH 5.1 were also reported for bioethanol production from carob pods.[19,28] Thus, we performed our experiments at 30 °C temperature, pH 5, and a shaking rate of 80 rpm.

Some previous studies have used peptone and yeast extract as nitrogen sources for the production of ethanol with *Z. mobilis*. Vaheed et al. reported the use of optimum amounts of 0.43 g peptone from meat and 0.43 g yeast extract (per 50 ml of culture medium) for the maximum ethanol production in the study of evaluating the ability of *Z. mobilis* for producing ethanol from carob pods.[18] In a recent study, peptone and yeast extract were also used as organic nitrogen sources (in addition to $(NH_4)_2SO_4$) to produce ethanol from the PP with *S. cerevisiae* and *P .stipitis*.[14] Demiray et al. discovered that the addition of organic nitrogen sources (peptone and yeast extract) to the *S. cerevisiae* medium boosed the ethanol production from 3.85 g/L to 4.99 g/L.[14] In solid state fermentation of carob pods by *Z.mobilis*, it was also observed that the use of peptone from meat (0.7% w/w) positively affected the ethanol yield.[19,27]

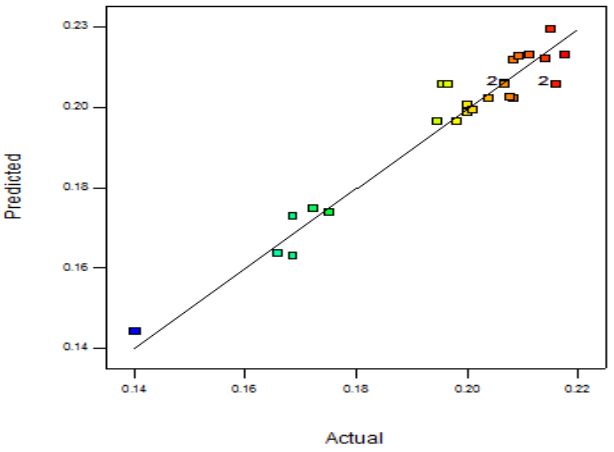

Figure 1. Predicted Versus Actual Values Plot.

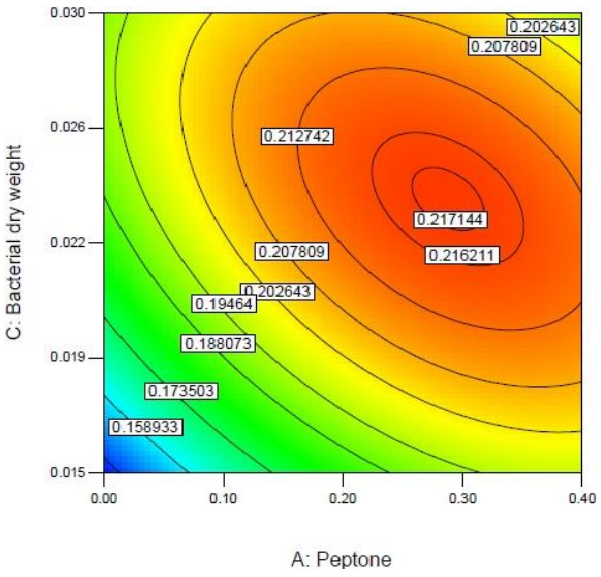

Figure 2. Interaction Effect of Bacterial Dry Weight (g) and Peptone (g) on the Response (ethanol produced, g); Yeast Extract 0.3 g; Fermentation Time 36 h.

In the ethanol production process with *Z. mobilis* from carob pods, the amount of bacterial dry cell for inoculation and fermentation time were 0.017 g and 36 h, respectively.[18] However, in solid-state fermentation, 0.01 g bacterial dry cell and 43 h fermentation time were reported.[19] According to these studies and our initial experiments (data not shown here), the ranges of effective factors in the experimental design were chosen and presented in Table 1.

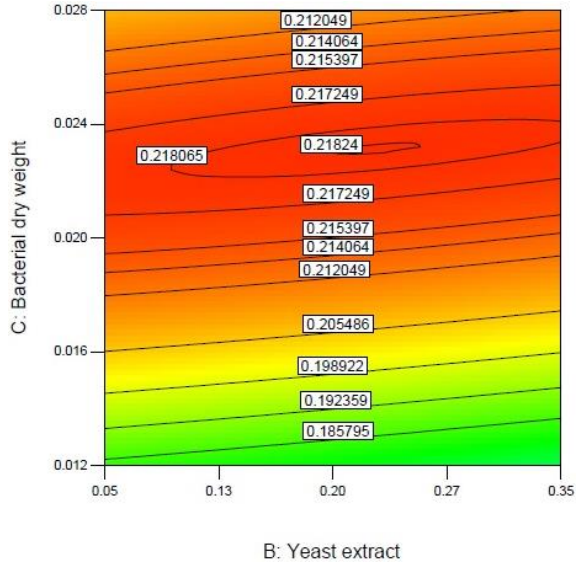

Figure 3. Interaction Effect of Bacterial Dry Weight (g) and Yeast Extract (g) on the Response (Ethanol Produced, g); Peptone 0.3 g; Fermentation Time 36 h.

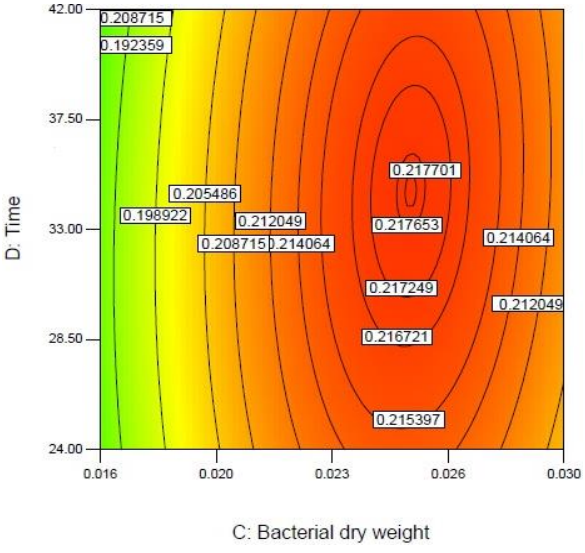

Figure 4. Interaction Effect of Bacterial Dry Weight (g) and Fermentation Time (g) on the Response (Ethanol Produced, g); Peptone 0.2 g; Yeast Extract 0.3 g.

Experimental Design of the Fermentation Step

The weight of peptone (A) and yeast extract (B) added to the culture medium as nitrogen sources, bacterial dry weight (C) used as inoculum, and fermentation time (D) were selected as the main factors for the experimental design. The weight of produced ethanol was considered as the response. To optimize the condition, RSM design according to Table 1 was performed, and the results were analyzed via Design Expert (dx-7) software using a quadratic model. According to the software calculations, the *F*-value of the resulting model was 13.71 implying that the model is significant. The *F*-value is a criterion for comparing the model variance with residual variance and is calculated based on the model variance divided by residual variance. *P* values less than 0.05 indicate that the effects of model factors are significant within the 95% confidence level. The analysis of variance (ANOVA) of the model with coefficient estimates and significance levels (*p*-values) of factors are presented in Table 2.a. The R^2 value was 0.936 and the predicted-R^2 was 0.7495, which is in reasonable agreement with the Adj-R^2 of 0.8683. Figure 1 exhibits the predicted versus actual values. ANOVA results presented in Table 2 indicate that the factors with *p*-values greater than 0.05 are insignificant. Thus, the factors A (peptone from meat), B (yeast extract), C (bacterial dry weight), AB, AC, AD, BD, A^2, and C^2 can be considered as significant terms of the model. The insignificant factors (D, BC, CD, B^2, and D^2) can be dropped from the model. The lack of fit *F*-value of the model is 0.35, suggesting that the lack of fit is not significant. The final equation of the model in terms of the coded factors after removing the insignificant factors is as follows:

$$\text{Ethanol} = +0.21 + 0.012\,A + 4.752\times10^{-3}\,B + 0.014\,C + 8.530\times10^{-5}\,D - 7.747\times10^{-3}\,AB - 5.503\times10^{-3}\,AC - 5.372\times10^{-3}\,AD - 5.247\times10^{-3}\,BD - 5.589\times10^{-3}\,A^2 - 7.748\times10^{-3}\,C^2 \quad (1)$$

The coefficient estimates and *p*-values of the simplified model (after removing the insignificant factors) are presented in Table 2.b. The effects of some significant factors on ethanol production and their interaction are shown in Figures 2-4. Figure 2 displays the interaction effect of the bacterial dry weight and peptone weight on ethanol when yeast extract weight and fermentation time were 0.31 g and 36 h, respectively. According to this figure, the maximum predicted ethanol production occurs when the amount of peptone added to the medium and the bacterial dry weight used for inoculation were 0.31 g and 0.0224 g, respectively. Figure 3 reveals the interaction effect of the bacterial dry weight used for inoculation with the amount of yeast extract added to the medium when peptone weight and fermentation time being 0.3 g and 36 h, respectively. It can be observed from this figure that the predicted condition for the maximum ethanol production

involved a bacterial dry weight of 0.023 g and yeast extract weight of about 0.24 g. In bacterial dry weights between 0.021 and 0.024 g, there was no significant change in the amount of ethanol production. Comparing Figures 2 and 3, it can be concluded that the effect of peptone weight is more significant than that of the yeast extract weight, which is consistent with the *p*-value results of ANOVA. This may be due to the better capability of peptone for supplying nitrogen sources to the bacteria.[19] Figure 4 predicts that with peptone and yeast extract weights of 0.2 g and 0.3 g, respectively, the maximum ethanol production can be observed at the bacterial dry weight and fermentation time of about 0.025 g and 34 h, respectively.

Using the polynomial model and according to the software analysis, the optimal production conditions for the maximum ethanol production and minimum fermentation time are predicted as follows: peptone weight of 0.37 g, yeast extract weight of 0.28 g, bacterial weight of 0.021 g, and fermentation time of 30 h. The estimated ethanol production under these optimum conditions was 0.222 g. To confirm the predictions, an experimental test was conducted under the optimum conditions predicted by the software. The produced ethanol under optimum condition was 0.218 ± 0.003 g, which lies within the prediction intervals (0.21-0.24 g).

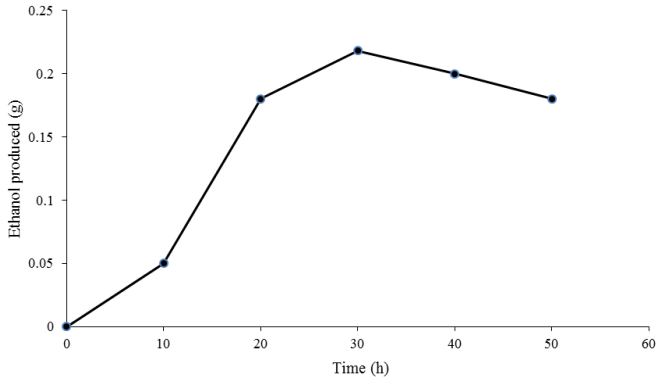

Figure 5. Produced Ethanol Content Under the Optimum Conditions.

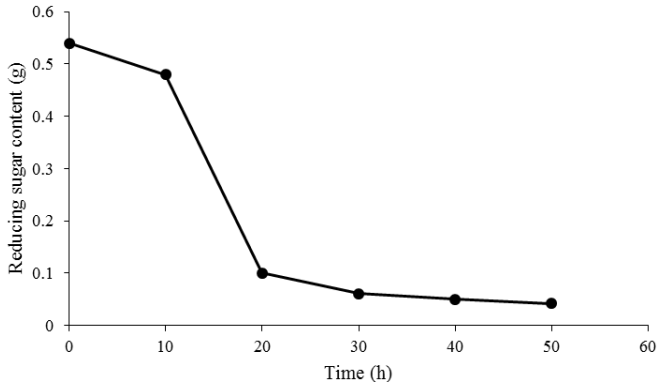

Figure 6. Reducing Sugar Content of Fermentation Medium Under the Optimum Conditions.

Table 3. Summary of the Results Obtained in Fermentation of PP by *Z. mobilis* for Bioethanol Production

Optimum conditions (in 50 ml of culture medium)		
Peptone	g	0.37
Yeast extract	g	0.28
Bacterial dry mass	g	0.021
Fermentation time	h	30.00
Final results under the optimum conditions		
Maximum ethanol production	g	0.218
Initial sugar content	g	0.54
Final remaining sugar	g	0.061
Sugar utilized	%	88.7
Ethanol yield	%	0.455
Theoretical ethanol yield	%	89.2
Productivity	g/(kg PP. h)	2.42

Using the polynomial model and according to the software analysis, the optimal production conditions for the maximum ethanol production and minimum fermentation time are predicted as follows: peptone weight of 0.37 g, yeast extract weight of 0.28 g, bacterial weight of 0.021 g, and fermentation time of 30 h. The estimated ethanol production under these optimum conditions was 0.222 g. To confirm the predictions, an experimental test was conducted under the optimum conditions predicted by the software. The produced ethanol under optimum condition was 0.218 ± 0.003 g, which lies within the prediction intervals (0.21-0.24 g).

Variation of ethanol and sugar content of the fermentation medium by time under the optimum conditions in Figure 5 and Figure 6 reveals the maximum ethanol production of about 0.218 g in 30 h. After 30 h, a slight decrease can be seen in the content of ethanol and sugar.

The initial sugar content of the medium at the beginning of the fermentation step was 0.54 g (in 50 ml of culture medium). Analysis of sugar content after fermentation showed that 0.061 ± 0.004 g of sugar remained in the medium, signifying that 88.7% of the initial amount of sugar was consumed by the bacteria during the fermentation. Thus, the maximum ethanol yield of 0.455 was achieved under the optimum condition, which was 89.2% of the theoretical yield. Table 3 summarizes the results obtained in the ethanol production process from PP using *Z. mobilis*. For comparison, in fermentation of PP using *S. cerevisiae*, the ethanol yields of 44.9% [14] and 45.1% [14,15] were reported by other researchers.

Besides, negative control tests showed that the fermentation of PP particles without enzymatic hydrolysis produced low amounts of ethanol which cannot be determined by the spectrophotometric method. Negative control tests for the interaction of hydrothermal pre-treatment and fermentation were conducted showing the bioethanol production of 0.142 g. This result exhibits the effectiveness of the hydrothermal process.

Conclusion

The results obtained in this research revealed the potential of *Z. mobilis* for the production of bioethanol from PP for

the first time. The hydrothermal pre-treatment method and enzymatic hydrolysis processes used in this research were able to produce the glucose necessary for the bacteria. Response surface methodology was successfully able to optimize the process conditions for the maximum ethanol production. The optimum conditions occurred at 0.37 g meat peptone, 0.28 g yeast extract, 0.021 g bacterial dry weight, and 30 h fermentation time (in 50 ml of culture medium) where the maximum amount of 0.218 g ethanol was produced under these conditions. According to these results, PP can be considered as a valuable, inexpensive, and abundant lignocellulosic waste material for producing a renewable source of energy.

Conflict of Interest Disclosures

The authors declare that they have no conflict interests.

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