



Valorization of *Zymomonas mobilis* for bioethanol production from potato peel: fermentation process optimization

Davood Mazaheri¹ · Ali Pirouzi²

Received: 23 April 2020 / Revised: 17 June 2020 / Accepted: 19 June 2020 / Published online: 1 July 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

In this study, *Zymomonas mobilis* was used to produce bioethanol from potato peel (PP) wastes. For this purpose, enzymatic hydrolysis was first performed on potato peel to release the fermentable sugars. Then, the effect of enzyme dosages and solid loading was investigated on the hydrolysis process. Following the hydrolysis step, to evaluate the ability of *Z. mobilis* in bioethanol production from PP, the effects of five important factors (initial sugar concentration, bacterial dry weight, peptone and yeast extract weights, and fermentation time) were studied, after which the factors were optimized using response surface methodology (RSM) experimental design. It was observed that a maximum of 88.2 g/l reducing sugar was produced using 50 U α -amylase and 12 U amyloglucosidase with 20%PP solid loading. The optimized conditions for fermentation step were as follows: 61.3 g/l initial sugar, 0.024 g bacterial dry cell, 0.35 g meat peptone, 0.35 g yeast extract, and 31-h fermentation time. The maximum ethanol concentration of 23.3 ± 0.015 g/l was produced under the optimum conditions. Time courses of ethanol production and sugar consumption were also studied, revealing consumption of 78.9% of initial sugars under optimum conditions.

Keywords Bioethanol · Potato peel · *Zymomonas mobilis* · Enzymatic hydrolysis · Response surface methodology

1 Introduction

Fossil fuels are recognized as the world's main source of energy. However, some problems such as the depletion of oil resources and environmental pollution are among the factors that have prompted scientific studies and industrial efforts in recent years to develop alternative sources [1]. Bioethanol is one of these alternative sources that can be used as a sustainable energy source thanks to its renewability, as well as its effectiveness in reducing air pollution and preventing global warming [1–3].

Bioethanol can be produced from different sources, among which inedible resources such as agro-industrial and lignocellulosic wastes have received more attention [1–3]. Starch-based waste that is widely available worldwide is one of the

feedstocks that can be used for bioethanol production. Such wastes contain large amounts of starch that can be converted into fermentable sugars [4]. For example, potatoes are one of the most important food crops in the world and are widely used in all kitchens, restaurants, and food industries. As a result, large quantities of potato peel (PP) are produced as a by-product that can be used as a cheap feedstock for bioethanol production [5]. In the literature, the starch content of PP has been reported to be about 20% (in native sample) [6] and 48 to 52% (on dry basis) [5, 7]. The native PP also contains about 10% hemicellulose, 4% cellulose, and 6% lignin [6]. The starch and cellulose content of potato peel makes it a promising feedstock for bioethanol production.

In the bioethanol production from starch-based waste, a hydrolysis step must be performed before the fermentation step to produce fermentable sugars [8]. Acidic or enzymatic hydrolysis methods can be employed for this purpose. Enzymatic hydrolysis of starchy compounds occurs in two steps by applying two types of enzymes: In the first step, α -amylase enzyme is used to produce maltose and maltodextrins by hydrolyzing the α -(1–4) chemical bonds of starch (liquefaction step). In the next step, the partially hydrolyzed starch from the liquefaction step is converted to glucose in a process

✉ Davood Mazaheri
dmazaheri@mahallat.ac.ir

¹ Faculty of Engineering, Mahallat Institute of Higher Education, Mahallat, Iran

² Department of Chemical Engineering, University of Science and Technology of Mazandaran, P.O. Box 48518-78195, Behshahr, Iran

called saccharification by applying glucoamylase enzyme. Then, the produced glucose can be fermented to ethanol using appropriate microorganisms [7–10].

Saccharomyces cerevisiae, the most conventional strain for production of bioethanol, has been used in some research for bioethanol production from PP [5–7, 11, 12]. Arapoglou et al. hydrolyzed a number of batches of PP with a combination of various enzymes (amylase, glucoamylase, and cellulase) and used *S. cerevisiae* for fermenting the resulting sugars to ethanol. They reported production of 7.6 g/l ethanol after fermentation, demonstrating that PP (as a zero-value waste of potato industries) exhibits a high potential for bioethanol production [5]. A comparative study between acidic and enzymatic hydrolysis of PP by Khawla et al. indicated that a combination of amylase UEB-S (produced from *Bacillus* sp. UEB-S) and amyloglucosidase under optimal conditions resulted in production of 69 g/l reducing sugars and 21 g/l ethanol by *S. cerevisiae* [7]. For reducing the time, energy consumption, and capital costs, the simultaneous saccharification and fermentation method was used for bioethanol production from PP. In this process, a maximum bioethanol concentration of 22.54 g/L was obtained under optimal conditions [6]. Although in all of these studies, the high value of PP for bioethanol production has been identified, and most of them have focused on optimizing the hydrolysis process and paid less attention to the fermentation process.

Although *S. cerevisiae* is a traditional yeast for bioethanol production, researchers have been studying alternative strains for increasing the ethanol production yield and reducing production cost. *Zymomonas mobilis* is a gram-negative bacterium with interesting properties such as high sugar consumption, high ethanol yield, and low bacterial biomass production [13, 14]. Lower biomass production by this bacterium is both effective in increasing ethanol production yield and causing fewer problems in industrial equipment. In addition, *Z. mobilis* as a bacterium has simpler and cheaper culture media than that of the *S. cerevisiae*. In this regard, various studies have shown the successful use of *Z. mobilis* for bioethanol production from various feedstocks [15–20]. In the study of bioethanol production from agricultural wastes, Braide et al. reported the ethanol yield percentage of 5.17% from PP by *Z. mobilis* and 5.51% by *S. cerevisiae*, indicating the good ability of *Z. mobilis* for bioethanol production from PP [19]. Another study comparing the performance of *Z. mobilis* and *S. cerevisiae* showed that *Z. mobilis* had better performance in producing bioethanol from PP [20]. However, in the abovementioned studies, the effect of some parameters and optimization of the fermentation process by considering the parameters interactions were not included, and more investigations were still required.

The aim of this study was to investigate the potential of *Z. mobilis* for producing bioethanol from potato peel and optimize the fermentation parameters. To this end, the appropriate conditions for enzymatic hydrolysis of PP were investigated by two enzymes. Then, the fermentation process was examined based on *Z. mobilis*, and various factors affecting the process were studied with the optimum conditions for maximum ethanol production obtained via response surface methodology (RSM).

2 Materials and methods

2.1 Microorganism

The strain used in this study for fermenting the sugars to ethanol was *Zymomonas mobilis* PTCC 1718 purchased from the Persian Type Culture Collection. The inoculum was prepared in a conical flask at 120 rpm and 30 °C for 17 h in the culture medium containing 10 g peptone from meat, 10 g yeast extract, and 20 g glucose per liter of deionized water under aerobic condition. All materials were purchased from Merck, Germany.

2.2 Materials

Potato peel was obtained from the university canteen in Mahallat Institute of Higher Education, Mahallat, Iran, in November. Then, PP was dried in an oven at 50 °C for 48 h, ground into smaller pieces by a laboratory grinder, and sieved to obtain a particle size of around 1–2 mm. The ground PP particles were stored at 4 °C for further investigations.

Termamyl (α -amylase) with enzyme activity of 3000 U/ml and amyloglucosidase with enzyme activity of 260 U/ml were purchased from Sigma-Aldrich.

2.3 Enzymatic hydrolysis of PP particles

Specifically, 20 g of dried PP was mixed with 100 ml deionized water in 250-ml flasks. For liquefaction, the mixture was treated with various units of α -amylase enzyme (10–70 units) at 90 °C, pH 7, and 120 rpm for 2 h. Then, the saccharification step was performed by applying various units of amyloglucosidase (1–15 units) to the slurry at 60 °C, pH 5, and 120 rpm for 24 h. To evaluate the amount of released glucose, samples were withdrawn from the flasks at different time intervals and heated at 100 °C for 5 min. After cooling, the samples were centrifuged at 4000 rpm for 10 min. The supernatants were used for determining the content of reducing sugars via 3,5-dinitrosalicylic acid (DNS) method [21]. All the experiments were carried out in triplicate.

Table 1 List of fifty RSM-designed experiments of ethanol production optimization

| Run | Factor A: initial sugar (g/l) | Factor B: bacterial dry weight (g) | Factor C: peptone weight (g) | Factor D: yeast extract weight (g) | Factor E: time (h) | Response: ethanol (g/l) |
|-----|----------------------------------|---------------------------------------|---------------------------------|---------------------------------------|-----------------------|----------------------------|
| 1 | 61.3 | 0.016 | 0.355 | 0.355 | 31 | 21.0 |
| 2 | 61.3 | 0.024 | 0.355 | 0.145 | 31 | 22.6 |
| 3 | 48.7 | 0.016 | 0.145 | 0.355 | 31 | 18.3 |
| 4 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 20.9 |
| 5 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 20.5 |
| 6 | 48.7 | 0.024 | 0.355 | 0.355 | 31 | 17.8 |
| 7 | 48.7 | 0.016 | 0.355 | 0.145 | 41 | 17.5 |
| 8 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 18.8 |
| 9 | 55.0 | 0.020 | 0.250 | 0.250 | 48 | 19.1 |
| 10 | 61.3 | 0.016 | 0.145 | 0.355 | 41 | 17.3 |
| 11 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 20.1 |
| 12 | 48.7 | 0.024 | 0.355 | 0.355 | 41 | 16.7 |
| 13 | 48.7 | 0.016 | 0.355 | 0.355 | 31 | 17.8 |
| 14 | 61.3 | 0.024 | 0.145 | 0.355 | 31 | 22.0 |
| 15 | 48.7 | 0.016 | 0.145 | 0.145 | 41 | 17.4 |
| 16 | 55.0 | 0.020 | 0.250 | 0.250 | 24 | 19.6 |
| 17 | 61.3 | 0.016 | 0.355 | 0.355 | 41 | 22.3 |
| 18 | 61.3 | 0.016 | 0.145 | 0.355 | 31 | 18.3 |
| 19 | 55.0 | 0.030 | 0.250 | 0.250 | 36 | 20.2 |
| 20 | 48.7 | 0.024 | 0.355 | 0.145 | 31 | 17.9 |
| 21 | 48.7 | 0.024 | 0.145 | 0.355 | 31 | 17.7 |
| 22 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 19.8 |
| 23 | 55.0 | 0.020 | 0.000 | 0.250 | 36 | 15.7 |
| 24 | 61.3 | 0.024 | 0.145 | 0.145 | 31 | 17.9 |
| 25 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 19.1 |
| 26 | 48.7 | 0.024 | 0.355 | 0.145 | 41 | 17.6 |
| 27 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 20.3 |
| 28 | 55.0 | 0.020 | 0.500 | 0.250 | 36 | 16.4 |
| 29 | 48.7 | 0.016 | 0.355 | 0.355 | 41 | 17.5 |
| 30 | 55.0 | 0.020 | 0.250 | 0.500 | 36 | 20.6 |
| 31 | 55.0 | 0.020 | 0.250 | 0.250 | 36 | 20.3 |
| 32 | 48.7 | 0.016 | 0.355 | 0.145 | 31 | 17.1 |
| 33 | 48.7 | 0.024 | 0.145 | 0.145 | 41 | 16.8 |
| 34 | 61.3 | 0.024 | 0.355 | 0.355 | 41 | 21.1 |
| 35 | 61.3 | 0.016 | 0.355 | 0.145 | 41 | 21.0 |
| 36 | 61.3 | 0.016 | 0.355 | 0.145 | 31 | 21.0 |
| 37 | 48.7 | 0.016 | 0.145 | 0.355 | 41 | 16.1 |
| 38 | 55.0 | 0.010 | 0.250 | 0.250 | 36 | 19.6 |
| 39 | 61.3 | 0.024 | 0.355 | 0.355 | 31 | 23.3 |
| 40 | 61.3 | 0.016 | 0.145 | 0.145 | 41 | 17.2 |
| 41 | 48.7 | 0.016 | 0.145 | 0.145 | 31 | 16.8 |
| 42 | 61.3 | 0.024 | 0.355 | 0.145 | 41 | 22.1 |
| 43 | 48.7 | 0.024 | 0.145 | 0.355 | 41 | 18.5 |
| 44 | 55.0 | 0.020 | 0.250 | 0.000 | 36 | 18.7 |
| 45 | 61.3 | 0.024 | 0.145 | 0.355 | 41 | 22.3 |
| 46 | 40.0 | 0.020 | 0.250 | 0.250 | 36 | 13.1 |
| 47 | 61.3 | 0.016 | 0.145 | 0.145 | 31 | 15.1 |
| 48 | 61.3 | 0.024 | 0.145 | 0.145 | 41 | 20.3 |

Table 1 (continued)

| Run | Factor A: initial sugar (g/l) | Factor B: bacterial dry weight (g) | Factor C: peptone weight (g) | Factor D: yeast extract weight (g) | Factor E: time (h) | Response: ethanol (g/l) |
|-----|----------------------------------|---------------------------------------|---------------------------------|---------------------------------------|-----------------------|----------------------------|
| 49 | 48.7 | 0.024 | 0.145 | 0.145 | 31 | 18.1 |
| 50 | 70.0 | 0.020 | 0.250 | 0.250 | 36 | 21.2 |

2.4 Ethanol fermentation of PP hydrolysates

The fermentation step was carried out in 250-ml Erlenmeyer flasks containing different volumes of PP hydrolysates. The initial sugar concentration in the fermentation medium could be adjusted according to the experimental design (Table 1) by changing the volume of PP hydrolysates. Then, the PP hydrolysates were supplemented with different amounts of peptone from meat and yeast extract (Table 1), with the pH of the medium adjusted to 5.5. The entire flask was then sterilized at 121 °C for 15 min in an autoclave. For inoculation, subsequently, an appropriate volume of *Z. mobilis* cells' suspension (providing a specific amount of bacterial dry weight based on the experimental design) was added to the medium. The working volume of the flask was then adjusted to 100 ml by adding sterilized distilled water. Finally, the mixture was incubated anaerobically at 30 °C and 80 rpm for a specific time (according to the experimental design) (Table 1).

2.5 Analytical methods

The bacterial dry cell mass in the inoculum culture as well as the fermentation medium was calculated as gram by measuring the optical density (OD) of the mixture at 600 nm using a standard curve. For evaluating the amount of produced ethanol, samples were taken from fermentation flasks and centrifuged at 4000 rpm for 10 min. The supernatant was then used for measuring the reducing sugar content via DNS method. For ethanol determination, the supernatant was first distilled at atmospheric pressure, and subsequently, the Caputi Jr method [22] was performed.

2.6 Optimization by response surface methodology

The response surface methodology (central composite design) was used to optimize the fermentation process conditions to achieve the maximum ethanol production within the shortest

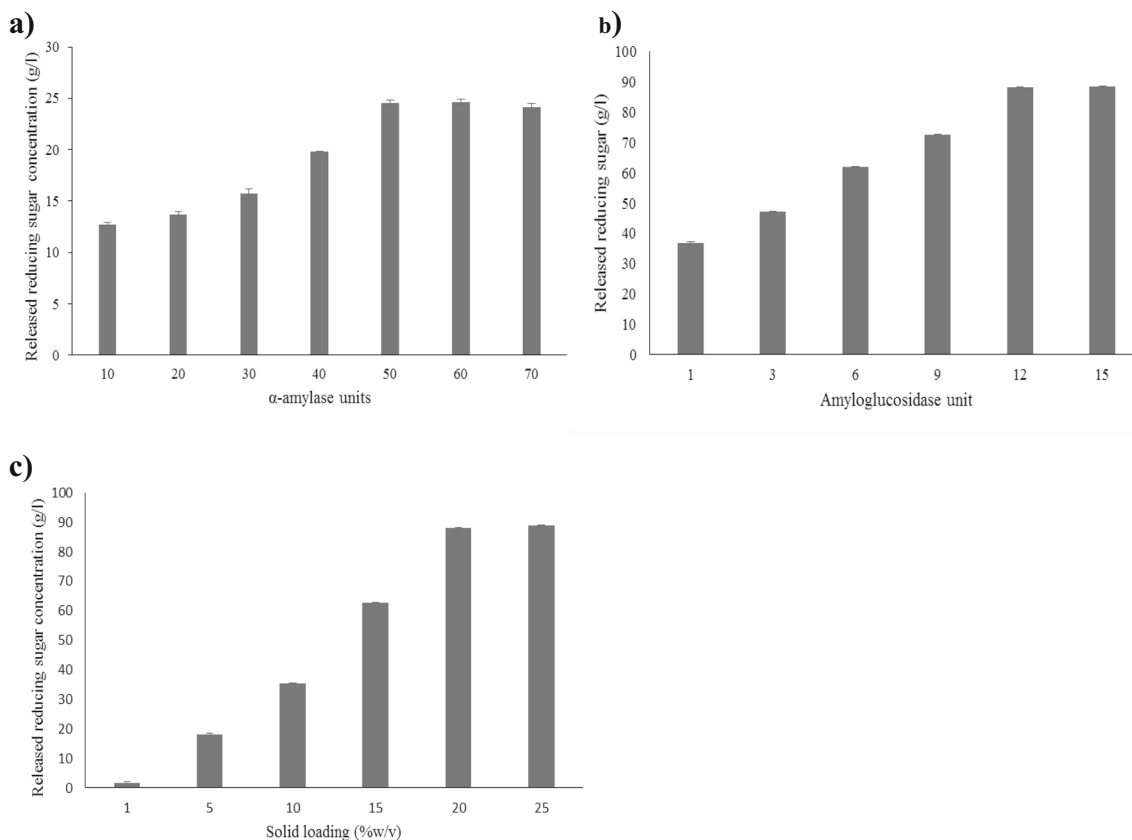


Fig. 1 The concentration of reducing sugars released in enzymatic hydrolysis process. **a** Effect of amylase dose (using 20% solid loading), **b** effect of amyloglucosidase dose (with 50 U amylase and 20% solid loading), and **c** effect of PP solid loading (with 50 U amylase and 12 U amyloglucosidase)

time. Factors affecting the process and their higher and lower levels in the experimental design were determined according to the other researchers' results [15, 16, 18, 19, 23] and some preliminary experiments. Accordingly, five important factors (initial sugar concentration, bacterial dry cell weight, peptone and yeast extract weight, and fermentation time) were selected, with the amount of ethanol produced considered as the response. The results of the 50 designed experiments are presented in Table 1.

3 Results and discussion

3.1 Enzymatic hydrolysis of potato peel

The enzymatic hydrolysis of PP has been investigated previously in several studies [5–7, 9, 12]. Nevertheless, due to the different potato cultivars used in this study, we first tried to examine the effect of the most important factors on enzymatic hydrolysis in order to obtain the highest sugar release. For this purpose, the effects of the dosage of the two enzymes were applied, and the solid PP loading were investigated. Firstly, the impact of α -amylase (Termamyl) enzyme on the PP solids

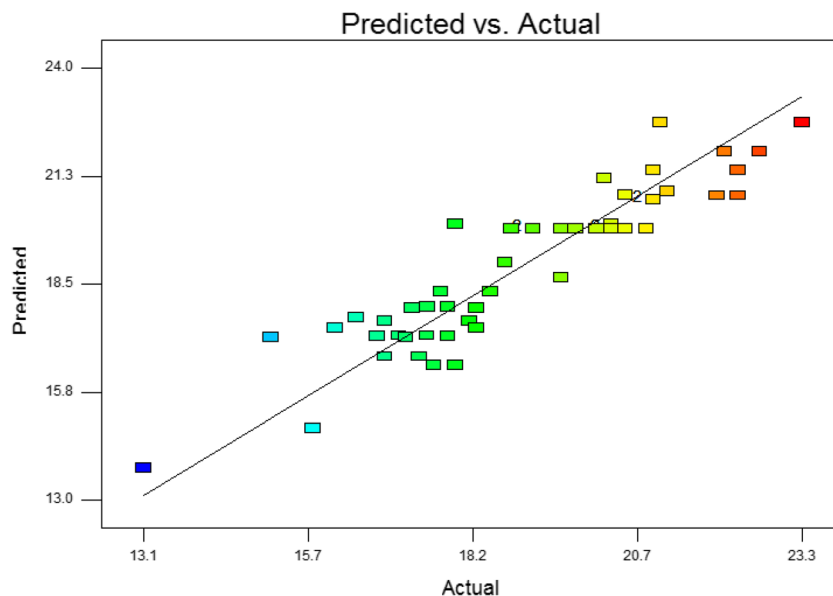
was evaluated to determine the best amount of required enzyme. The appropriate temperature, pH, and time required for this stage were reported 90–95 °C, 6.5–7, and 2–3 h, respectively, in previous studies [5–7, 12]. Figure 1a displays the effect of different α -amylase units on the reducing sugars released in 2 h, when the solid loading was 20%. The figure exhibits that the amount of released sugar increased as α -amylase dosage was elevated. However, beyond the enzyme dose of 50 units, no significant increase in glucose production was observed. In addition, as Liu [9] reported earlier, the use of amylase alone has little effect on glucose release.

Figure 1b illustrates the effect of different doses of amyloglucosidase enzyme on the amount of released sugar with a fixed amylase dosage of 50 U in 24 h. In this step, the optimum temperature, pH, and time of about 60 °C, 4.5, and 24 h, respectively, were reported by other researchers [5–7, 12]. As can be seen in this figure, the higher the enzyme dosage, the higher the amount of released sugar was. A maximum amount of 88.4 g/l reducing sugar was obtained in the enzyme combination of 50 U amylases and 15 U amyloglucosidases. However, upon elevating the enzyme dosage from 12 to 15 units, no significant increase was observed in the amount of reducing sugar. The trend of the

Table 2 Analysis of variance (ANOVA) of the developed RSM model

| Source | Sum of squares | df | Mean squares | F value | P value Prob > F | Remarks |
|----------------------|----------------|----|--------------|---------|---------------------|-----------------|
| Model | 203 | 20 | 10.20 | 11.40 | < 0.0001 | Significant |
| A-Initial sugar | 95.3 | 1 | 95.30 | 107.00 | < 0.0001 | |
| B-Bacterial dry cell | 12.2 | 1 | 12.20 | 13.70 | 0.00088 | |
| C-Peptone | 15.4 | 1 | 15.40 | 17.30 | 0.00025 | |
| D-Yeast extract | 5.75 | 1 | 5.75 | 6.47 | 0.01650 | |
| E-Time | 0.104 | 1 | 0.10 | 0.12 | 0.73400 | |
| AB | 7.76 | 1 | 7.76 | 8.74 | 0.00614 | |
| AC | 17.8 | 1 | 17.80 | 20.00 | 0.00011 | |
| AD | 2.61 | 1 | 2.61 | 2.94 | 0.09720 | |
| AE | 1.09 | 1 | 1.09 | 1.22 | 0.27800 | |
| BC | 5.54 | 1 | 5.54 | 6.24 | 0.01840 | |
| BD | 0.00551 | 1 | 0.005 | 0.006 | 0.93800 | |
| BE | 0.249 | 1 | 0.25 | 0.28 | 0.60100 | |
| CD | 3.01 | 1 | 3.01 | 3.39 | 0.07580 | |
| CE | 0.6 | 1 | 0.60 | 0.67 | 0.41800 | |
| DE | 1.82 | 1 | 1.82 | 2.05 | 0.16300 | |
| A ² | 12.1 | 1 | 12.10 | 13.60 | 0.00091 | |
| B ² | 0.0153 | 1 | 0.01 | 0.017 | 0.89600 | |
| C ² | 24.2 | 1 | 24.20 | 27.30 | < 0.0001 | |
| D ² | 0.0539 | 1 | 0.05 | 0.060 | 0.80700 | |
| E ² | 0.361 | 1 | 0.36 | 0.407 | 0.52900 | |
| Residual | 25.8 | 29 | 0.89 | | | |
| Lack of fit | 22.2 | 22 | 1.01 | 1.980 | 0.17900 | Not significant |
| Pure error | 3.56 | 7 | 0.51 | | | |

Fig. 2 Predicted vs. actual values plot



results is in agreement with the previous researches' findings [7, 9]. For evaluating the best solid loading, different PP solid loadings were used in the enzyme combination of 50 U amylases and 12 U amyloglucosidases in 24 h. Figure 1c reveals that at low PP solid loadings, the amount of released reducing sugars is not significant; however, by increasing the solid loading, a higher amount of reducing sugars was released. According to this figure, the best solid loading was 20% which gave 88.2 g/l of reducing sugars. At solid loadings greater than 20%, there was little increase in the amount of reducing sugars. In addition, high solid loading can enhance the viscosity of the medium which can inversely affect the process [9]. For comparison, Khawla et al. [7] reported the released reducing sugar concentration of 63 g/l using 45 units of amylase combined with 9 units of

amyloglucosidase and 15% of PP solid loading in enzymatic hydrolysis of PP wastes.

3.2 Ethanol fermentation

3.2.1 Factors considered in RSM experimental design

Following the PP hydrolysis process, the hydrolysate was used for ethanol fermentation by *Z. mobilis*. Since there were few previously published study on fermentation of PP by *Z. mobilis*, we evaluated and optimized the effective parameters in ethanol fermentation process in this section using RSM central composite design. Based on the previous studies [15, 16, 18–20, 23] on *Z. mobilis* as well as some preliminary experiments, we identified five important factors and checked

Table 3 Analysis of variance (ANOVA) of the simplified RSM model

| Source | Sum of squares | df | Mean squares | F value | P value | Remarks |
|----------------------|----------------|----|--------------|---------|----------|-----------------|
| | | | | | Prob > F | |
| Model | 193 | 9 | 21.5 | 24.1 | < 0.0001 | Significant |
| A-Initial sugar | 95.3 | 1 | 95.3 | 107 | < 0.0001 | |
| B-Bacterial dry cell | 12.2 | 1 | 12.2 | 13.7 | 0.000652 | |
| C-Peptone | 15.4 | 1 | 15.4 | 17.3 | 0.000166 | |
| D-Yeast extract | 5.75 | 1 | 5.75 | 6.45 | 0.015100 | |
| AB | 7.76 | 1 | 7.76 | 8.7 | 0.005300 | |
| AC | 17.8 | 1 | 17.8 | 19.9 | < 0.0001 | |
| BC | 5.54 | 1 | 5.54 | 6.21 | 0.016900 | |
| A ² | 12.1 | 1 | 12.1 | 13.6 | 0.000682 | |
| C ² | 24.5 | 1 | 24.5 | 27.5 | < 0.0001 | |
| Residual | 35.7 | 40 | 0.892 | | | |
| Lack of fit | 32.1 | 33 | 0.974 | 1.91 | 0.189000 | Not significant |
| Pure error | 3.56 | 7 | 0.509 | | | |

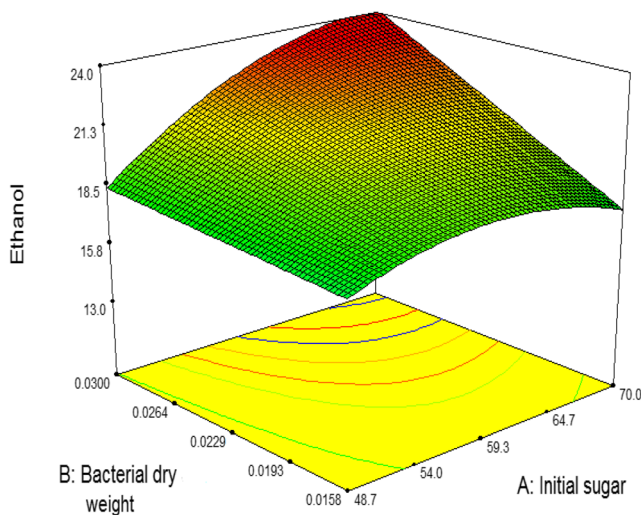


Fig. 3 Response surface curve for the interaction effects of initial sugar concentration and bacterial dry weight on ethanol (peptone and yeast extract weight, 0.2 g; fermentation time, 30 h)

their high and low levels for optimization process by RSM. The factors were initial sugar concentration, bacterial dry cell weight, peptone and yeast extract weight, and fermentation time. In this regard, Vaheed et al. [15] also identified the same factors as dominant factors by screening the effective factors using Plackett-Burman design in ethanol fermentation of carob pods by *Z. mobilis*. The initial glucose concentration and dried bacterial weight ranges of 4–7 g glucose, and 0.01–0.02 g per 100 ml of fermentation medium were reported in their study, respectively. The amount of peptone and yeast extract, as nitrogen sources, was considered within 0–0.43 g (0–9.67 g/l) in the fermentation time of 24–48 h. They also revealed that the effect of pH within 5–7 had no significant

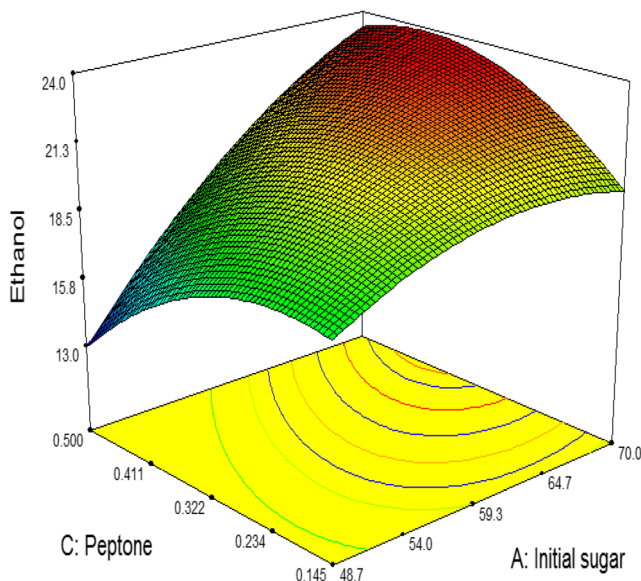


Fig. 4 Response surface curve for the interaction effects of initial sugar concentration and peptone weight on ethanol (bacterial dry weight, 0.024 g; yeast extract weight, 0.2 g; fermentation time, 30 h)

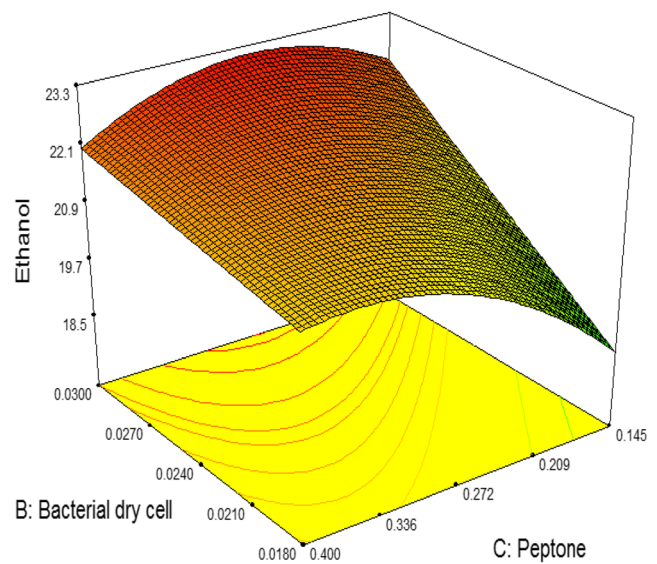


Fig. 5 Response surface curve for the interaction effects of bacterial dry weight and peptone weight on ethanol (initial sugar concentration, 60 g/l; yeast extract weight, 0.2 g; fermentation time, 30 h)

effect on the response, with shaking rates at low values (around 80 rpm) showing no negative effect on ethanol production [15]. In molasses fermentation with 0.02 g/l *Z. mobilis* in the time range of 24–48 h, the temperature of 30 °C and pH within the range of 5.0–7.0 were reported as optimum conditions [18]. Similarly, in solid-state fermentation of carob pod by *Z. mobilis*, the optimum temperature of 31 °C, pH 5.1, and 0.7 g of meat peptone (7 g/l) were also reported as the optimum conditions [16].

After identifying the effective factors and their ranges, fifty experiments were designed with the amount of produced ethanol as response measured for each experiment (Table 1). Then, the results were analyzed by Design Expert (dx-7) software.

3.2.2 Optimization of ethanol fermentation process

The analysis of the results obtained from the experimental design by the software showed that *F* value of the model was 11.04, so the resulting model is significant. The *F* values and significance levels (*P* values) of the model and its factors, resulting from the analysis of variance (ANOVA) of the

Table 4 Summary of the results obtained in this research for bioethanol production from PP by *Z. mobilis*

| | | |
|--|-----------------------------------|---------------|
| Maximum produced ethanol concentration | g/l | 23.3 ± 0.015 |
| Initial sugar concentration | g/l | 61.3 ± 0.0098 |
| Sugar utilized | % | 78.9 |
| Ethanol yield | % | 48.3 |
| Theoretical ethanol yield | % | 94.7 |
| Productivity | g l ⁻¹ h ⁻¹ | 0.776 |

model, are presented in Table 2. P values less than 0.05 indicated that the model terms are significant. In this case, the model and the model factors A (initial sugar concentration), B (bacterial dry weight), C (peptone weight), D (yeast extract weight), and their interactions AB, AC, BC, A^2 , and C^2 were significant. By excluding the insignificant terms from the model, the following equation (Eq. 1) for the model can be derived in terms of the coded factors:

$$\begin{aligned} \text{Ethanol} = & 19.9 + 1.48 A + 0.531 B + 0.596 C \\ & + 0.364 D + 0.493 AB \\ & + 0.745 AC - 0.416 BC - 0.457 A^2 - 0.651 C^2 \quad (1) \end{aligned}$$

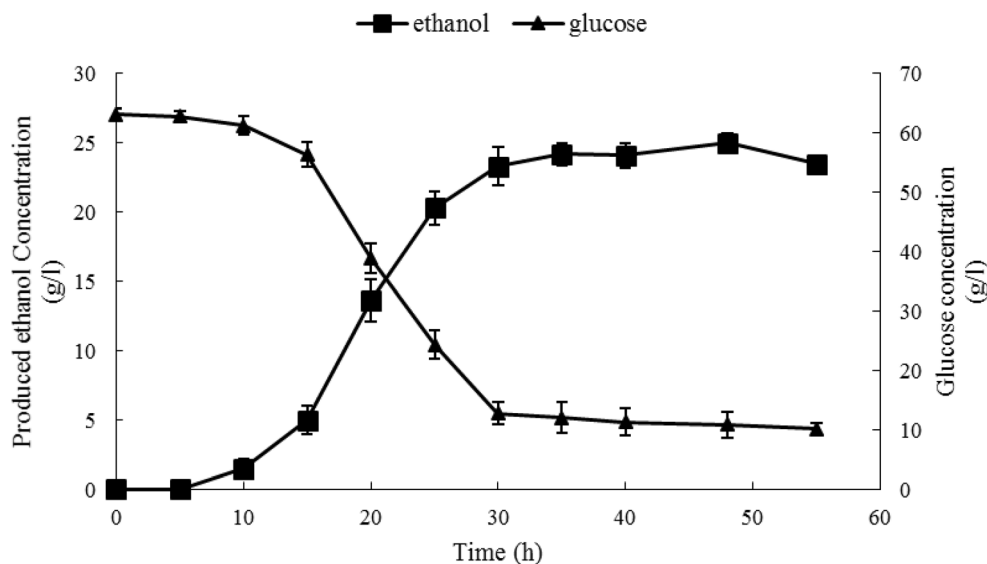
The R^2 value of 0.844 was calculated for the simplified model, where the predicted R^2 of 0.7194 is in reasonable agreement with the Adj- R^2 of 0.8092. To show this better, the model predicted values versus actual results were shown in Fig. 2. The ANOVA of the simplified model including F and P values are also reported in Table 3.

The interactive effects of the process factors on ethanol concentration are shown in response surface graphs in Figs. 3, 4, and 5. Figure 3 depicts the interaction effects of the initial sugar concentration and bacterial dry weight when peptone and yeast extract weights were both 0.2 g with 30-h fermentation time. It can be observed that the amount of produced ethanol increased with elevating of the sugar content in the medium. Ethanol production also increased with raising the inoculum concentration at constant initial sugar content. The interaction effect of initial sugar concentration and peptone weight is presented in Fig. 4. In this figure, the bacterial dry weight, yeast extract, and fermentation time were 0.024 g, 0.2 g, and 30 h, respectively. This figure also reveals the positive effect of initial sugar concentration on ethanol production.

The maximum ethanol production of about 23.8 g/l was observed with peptone weight of 0.41 g. Thus, addition of nitrogen sources such as peptone and yeast extract can help enhance ethanol production. The effect of peptone addition is also shown in Fig. 5 demonstrating the interaction effect of peptone weight and bacterial dry weight. This figure depicts that under conditions of 60 g/l initial sugar, 0.27 g yeast extract, and 30-h fermentation time, a maximum ethanol of 23.0 g/l was produced at peptone weight of 0.28 g and bacterial dry weight of 0.03 g. The same trend was observed in ethanol fermentation of carob pods by *Z. mobilis* [15].

Using the model obtained from the experimental design, the optimum condition for maximum ethanol production within the minimum fermentation time was predicted by the software as follows: initial sugar concentration of 61.3 ± 0.0098 g/l, bacterial dry weight of 0.024 g, peptone weight of 0.35 g, yeast extract weight of 0.35 g and fermentation time of 31 h. The optimum conditions predicted by the model are similar to run 39 of experimental design table (Table 1). To verify the optimum conditions, this test was carried out in triplicate, in which a maximum amount of 23.3 ± 0.015 g/l ethanol was produced. The results of reducing sugar measurements under optimum conditions revealed that the reducing sugar remaining in the medium after fermentation was 12.9 ± 0.01 g/l, indicating that 78.9% of the initial sugar was consumed. Thus, the amount of sugar consumed during the fermentation was 48.4 g/l leading to a 48.3% ethanol yield (94.7% of the theoretical yield). The summary of the results obtained in this research are reported in Table 4. In previous studies, maximum ethanol concentrations and theoretical yields of 21 g/l and 70% [7], as well as 7.6 g/l and 46% [5], respectively, were reported for bioethanol production from PP by *S. cerevisiae*. Also, in simultaneous saccharification and fermentation of PP by *S. cerevisiae*, an ethanol yield of 0.32 g/g was obtained by Chohan et al. [6].

Fig. 6 Time courses of produced ethanol and glucose concentrations in fermentation medium



In a recent study of bioethanol production from PP, 5.17% v/v of ethanol is produced after 72 h fermentation by *Z. mobilis* and 5.51% v/v by *S. cerevisiae*. In another report, the highest yield of ethanol production by *Z. mobilis* from PP was reported after 5 days, which is higher than the yield obtained by *S. cerevisiae* after 7 days. Hence, the results of this study are comparable with the findings obtained by other researchers from PP by *S. cerevisiae*, suggesting the promising ability of *Z. mobilis* for bioethanol production from PP wastes. Process optimization in this research has resulted in higher bioethanol yield compared with the similar previous researches.

3.2.3 Time course of ethanol production and sugar consumption

Under the optimum conditions, we evaluated the time variations of produced ethanol and glucose concentration in the fermentation medium. Figure 6 indicates that the lag phase was about 10 h. Then, there was a rapid increase in ethanol production and sugar consumption. After 30 h, little change occurred in the ethanol production. This result is consistent with the prediction by the model where the highest amount of ethanol within the shortest fermentation time occurred at 31-h fermentation time. Thus, a maximum ethanol of around 23–24 g/l was achieved between 30 and 40 h of fermentation time.

4 Conclusions

The results of this study demonstrated the good potential of *Z. mobilis* for bioethanol production from PP in comparison with previous studies using traditional yeast, *S. cerevisiae*. For enzymatic hydrolysis process, the combination of 50 U amylases and 12 U amyloglucosidase enzymes could effectively release the fermentable sugars from PP solids. Examination of the effect of different factors on ethanol production in the fermentation process via RSM revealed that the maximum amount of ethanol (23.3 g/l) was obtained under the following optimal conditions: 61.3 g/l initial sugar, 0.24 g/l bacterial dry cell, 3.5 g/l meat peptone, 3.5 g/l yeast extract, and 31-h fermentation time. The final concentration of ethanol obtained is not high enough for industrial scales because it may cause high energy consumption in purification step. Thus, more study is required for industrializing this process.

Availability of data and material Not applicable.

Code availability Design Expert software trial version

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

- Prasad RK, Chatterjee S, Mazumder PB, Gupta SK, Sharma S, Vairale MG, Datta S, Dwivedi SK, Gupta DK (2019) Bioethanol production from waste lignocelluloses: a review on microbial degradation potential. *Chemosphere* 231:588–606. <https://doi.org/10.1016/j.chemosphere.2019.05.142>
- Toor M, Kumar SS, Malyan SK, Bishnoi NR, Mathimani T, Rajendran K, Pugazhendhi A (2020) An overview on bioethanol production from lignocellulosic feedstocks. *Chemosphere* 242: 125080. <https://doi.org/10.1016/j.chemosphere.2019.125080>
- Casabar JT, Unpaprom Y, Ramaraj R (2019) Fermentation of pineapple fruit peel wastes for bioethanol production. *Biomass Conv Bioref* 9:761–765. <https://doi.org/10.1007/s13399-019-00436-y>
- Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: an overview. *Renew Energy* 37:19–27. <https://doi.org/10.1016/j.renene.2011.06.045>
- Arapoglou D, Varzakas T, Vlyssides A, Israilides C (2010) Ethanol production from potato peel waste (PPW). *Waste Manag* 30:1898–1902. <https://doi.org/10.1016/j.wasman.2010.04.017>
- Chohan NA, Aruwajoye GS, Sewsynker-Sukai Y, Gueguim Kana EB (2020) Valorisation of potato peel wastes for bioethanol production using simultaneous saccharification and fermentation: Process optimization and kinetic assessment. *Renew Energy* 146: 1031–1040. <https://doi.org/10.1016/j.renene.2019.07.042>
- Khawla BJ, Sameh M, Imen G, Donyes F, Dhouha G, Raoudha EG, Oumèma NE (2014) Potato peel as feedstock for bioethanol production: a comparison of acidic and enzymatic hydrolysis. *Ind Crop Prod* 52:144–149. <https://doi.org/10.1016/j.indcrop.2013.10.025>
- Sanchez O, Cardona C (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol* 99:5270–5295
- Liu Q (2002) A study of enzymatic hydrolysis of starch in potato pulp. *J Food Sci* 67:2113–2117
- Batista Meneses D, Montes de Oca-Vásquez G, Vega-Baudrit JR, Rojas-Álvarez M, Corrales-Castillo J, Murillo-Araya LC (2020) Pretreatment methods of lignocellulosic wastes into value-added products: recent advances and possibilities. *Biomass Conv Bioref*. <https://doi.org/10.1007/s13399-020-00722-0>
- Hashem M, Darwish SMI (2010) Production of bioethanol and associated by-products from potato starch residue stream by *Saccharomyces cerevisiae*. *Biomass Bioenergy* 34:953–959. <https://doi.org/10.1016/j.biombioe.2010.02.003>
- Izmirliglu G, Demirci A (2012) ethanol production from waste potato mash by using *Saccharomyces Cerevisiae*. *Appl Sci* 2: 738–753 <https://www.mdpi.com/2076-3417/2/4/738>
- Panesar PS, Marwaha SS, Kennedy JF (2006) *Zymomonas mobilis*: an alternative ethanol producer. *J Chem Technol Biotechnol* 81: 623–635
- Xia J, Yang Y, Liu CG, Yang S, Bai FW (2019) Engineering *Zymomonas mobilis* for robust cellulosic ethanol production. *Trends Biotechnol* 37:1–13. <https://doi.org/10.1016/j.tibtech.2019.02.002>
- Vaheed H, Shojaosadati SA, Galip H (2011) Evaluation and optimization of ethanol production from carob pod extract by *Zymomonas mobilis* using response surface methodology. *J Ind Microbiol Biotechnol* 38:101–111

16. Mazaheri D, Shojaosadati SA, Mousavi SM, Hejazi P, Saharkhiz S (2012) Bioethanol production from carob pods by solid-state fermentation with *Zymomonas mobilis*. *Appl Energy* 99:372–378
17. Amutha R, Gunasekaran P (2001) Production of ethanol from liquefied cassava starch using co-immobilized cells of *Zymomonas mobilis* and *Saccharomyces diastaticus*. *J Biosci Bioeng* 92:560–564
18. Cazetta ML, Celligoi MAPC, Buzato JB, Scarmino IS (2007) Fermentation of molasses by *Zymomonas mobilis*: effects of temperature and sugar concentration on ethanol production. *Bioresour Technol* 98:2824–2828
19. Braide W, Oji IN, Adeleye SA, Korie MC (2018) Comparative study of bioethanol production from agricultural wastes by *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *Int J Appl Microbiol Biotechnol Res* 6:50–60
20. Maroufpour B, Rad FA, Yazdanseta S (2019) Bioethanol production as biofuel from potato peel using *Saccharomyces cerevisiae* PTCC 5052 and *Zymomonas mobilis* PTCC 1718. *BIOAGRO* 31: 177–184
21. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–428
22. Caputi A Jr, Ueda M (1968) Spectrophotometric determination of ethanol in wine. *Am J Enol Vitic* 19:160–165
23. Saharkhiz S, Mazaheri D, Shojaosadati SA (2013) Evaluation of bioethanol production from carob pods by *Zymomonas mobilis* and *Saccharomyces cerevisiae* in solid-submerged fermentation. *Prep Biochem Biotechnol* 43:415–430

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.