

BIOETHANOL PRODUCTION AS BIOFUEL FROM POTATO PEEL USING *Saccharomyces cerevisiae* PTCC 5052 AND *Zymomonas mobilis* PTCC 1718

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ABSTRACT

Bioethanol is a renewable and environmentally friendly fuel with minimum contamination that can be obtained from the fermentation or distillation of several raw materials. In the present research, potato peel waste was used as a pure carbon source due to its high fermentable carbohydrate content. In order to promote the conversion of starch to glucose, acid hydrolysis, as a low cost method was used. Two types of microorganisms including *Saccharomyces cerevisiae* and *Zymomonas mobilis* were used to synthesize the bioethanol. The effect of different biochemical conditions (fermentation time and yeast extract concentration) was assessed on the ethanol yield in both types of microorganisms. The results showed that the best fermentation time for *Z. mobilis* occurred after five days, and the best yeast extract concentration added to fermentation was 3 g·L⁻¹, which had the highest yield for bioethanol production (0.521 %). The best fermentation time for *S. cerevisiae* was obtained after seven days without adding yeast extract, which had the highest yield for bioethanol production (0.180 %). In conclusion, the results obtained here showed that *Z. mobilis* had the best performance in the synthesis of bioethanol from potato peels, and the duration of 120 hours for fermentation in both microorganisms led to better results for ethanol production due to approaching the stationary phase.

Additional keywords: Acid hydrolysis, fermentation, organic wastes, yeasts

RESUMEN

Producción de bioetanol partir de piel de papa usando *Saccharomyces cerevisiae* PTCC 5052 y *Zymomonas mobilis* PTCC 1718

El bioetanol es un combustible renovable y ecológico que produce un mínimo de contaminación y que se puede obtener de la fermentación o destilación de diferentes materias primas. En la presente investigación, se utilizaron residuos de piel (peridermis) de papa como fuente de carbono debido a su alto contenido de carbohidratos fermentables. Para la conversión del almidón en glucosa se utilizó hidrólisis ácida como un método de bajo costo, así como el empleo de dos tipos de microorganismos (*Saccharomyces cerevisiae* y *Zymomonas mobilis*) para sintetizar el bioetanol. El efecto de diferentes condiciones bioquímicas (tiempo de fermentación y concentración de extracto de levaduras) se evaluó en el rendimiento de etanol en ambos tipos de microorganismos. Los resultados mostraron que el mejor tiempo de fermentación para *Z. mobilis* ocurrió después de 5 días y la mejor concentración de extracto de levaduras agregado a la fermentación fue de 3 g·L⁻¹, el cual produjo el mayor rendimiento de bioetanol (0,521 %). Para *S. cerevisiae* el mejor tiempo de fermentación se obtuvo después de 7 días, sin agregar extracto de levaduras, con un rendimiento en bioetanol de 0,180 %. En conclusión, los resultados mostraron que *Z. mobilis* tuvo el mejor desempeño en la síntesis de bioetanol a partir de la piel de la papa, y la duración de 120 horas de fermentación en ambos microorganismos condujo a los mejores resultados en la producción de etanol dado que se aproximaron a su fase estacionaria.

Palabras clave adicionales: Fermentación, hidrólisis ácida, levaduras, residuos orgánicos

INTRODUCTION

With an ever-increasing world's population and modernization, the global need for energy which is mainly provided from fossil fuel sources (oil, gasoline, gas, coal) is constantly increasing

(Sun and Cheng, 2002).

The main sources of greenhouse gas emissions, the major responsible for the destruction of the ozone layer and global warming are fossil fuels. Furthermore, fossil fuels are non-renewable and their supplies will be depleted if the consumption

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trend continues at current rate (Crookes, 2006). According to the International Energy Agency (IEA), the use of fossil fuels will double over the next twenty years, so that due to this global energy crisis and the increasing prices of fossil fuels, the need for new and renewable source of energy seems necessary (Liu and Lin, 2009).

The production of biofuels (bioethanol, biodiesel and biohydrogen) has increased in recent years. Production and consumption of bioethanol have globally reached a limit of one billion liters per year, and this trend will continue to increase in the future. Currently, the United States and Brazil are the world's largest bioethanol producer, with 63.0 and 24.4 % of the total world production (Demirbas, 2009).

Bioethanol production process consists of three main stages, including acid or alkaline hydrolysis (the process of starch-to-sugar conversion), microbial fermentation of carbohydrates (especially sugars with 5 and 6 carbons under specific microbial conditions) and separating ethanol from the solution by distillation method (Alonso et al., 2010; Alonso et al., 2013; Sadeghinezhad et al., 2014). The hydrolysis step is carried out either by acid or enzymatic, or a combination of both of them.

Various factors such as viscosity, physical form of raw material and the addition of foods such as fat affect the degree of hydrolysis. For instance, with increasing the viscosity, the degree of hydrolysis increases, while the presence of fat decreases the degree of hydrolysis (Kilpimaa et al., 2009).

To date, various microorganisms such as *Starmarella bacillaris*, *Pichia stipitis*, *S. cerevisiae*, *Hanseniaspora uvarum*, *Choiromyces marianus*, *Aspergillus niger*, *Mucor mucedo* and *Zymomonas mobilis* have been employed for production of bioethanol. Among them, *Z. mobilis*, a gram-negative bacterium member of GRAS classification, has shown higher yield and more ability to the conversion of starch to glucose (Galbe and Zacchi, 2007). Meanwhile, *S. cerevisiae* is the most common microorganism for production of bioethanol (Tasić and Veljković, 2011).

The materials that can be converted to bioethanol are divided into three main groups including sugar, starch, and cellulose. Potato peel

contains cellulose, hemicellulose, fermentable sugars and starch that represents about 58 % of its dry weight (Israilides et al., 2008), a concentration higher than that found in the whole tuber, so it may be a very important source for bioethanol production. Even though, Mishra et al. (2016) found that waste potatoes are suitable to produce bioethanol, so the authors encourage farmers to increase potato growing area so that the waste can be an important source for bioethanol production.

During harvesting and processing of the potato, 5-20 % of it remains as the waste. Additionally, it has been reported that during production of potato chips, approximately 18 % of potatoes are disposed as waste. The production of biofuels from these by-products is an effective step in the recovery of these wastes for the output of value-added products (Minal and Deshpande, 2010).

Das and Khan (2016) conducted a research to establish a process design for the production of bioethanol using potato peels as feed material in Pakistan. The research focused on proposing an entire bioethanol manufacturing process for a small scale production.

To the best of our knowledge, there are no study devoted to optimizing the production of bioethanol from potato peel waste (PPW) using both the yeast *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis*. Therefore, in order to investigate the effectiveness of these microorganisms on ethanol production and the effect of adding yeast extracts, this work aims to find out the best biochemical conditions (fermentation time and yeast extract concentration) to produce bioethanol with highest yield.

MATERIALS AND METHODS

The PPW was obtained from a food chips factory (with humidity of 7.5 % and pH of 5.94). *S. cerevisiae* and *Z. mobilis* were obtained from Persian Type Culture Collection (PTCC).

Acid Hydrolysis of PPW. At the first step, potato peels were dried at 80 °C for overnight in an oven and then powdered using a grinder. For acid hydrolysis of dried peels, 10 mL of concentrated hydrochloric acid (0.5 % v/v) were added to 240 g of the sample. Afterward, the volume was made

up to 2 L using distilled water. The resulting mixture was placed in water bath at a temperature of 75 °C for half an hour. Then, the hydrolyzed material was autoclaved at 121 °C. Finally, the solution was filtered (45 µm, 4 Whatman GFD) and the pH adjusted to 6.3 by sodium hydroxide (Sheikh et al., 2016).

Inoculation of *Saccharomyces cerevisiae*. The *S. cerevisiae* colonies were inoculated in yeast mold agar (YMA) culture medium in inclined tubes, then incubation was performed. In the next step, the tubes were stored in the refrigerator at 4 °C. In order to preparation of inoculum culture liquid, the active growing cells from the freshly prepared sloped culture medium were inoculated into 100 mL of Erlenmeyer flask containing yeast mold broth (YMB). The medium was put in a shaker incubator at 24 °C with 150 rpm for 72 hours.

Inoculum culture of *Z. mobilis*. These colonies were cultivated in Zymomonas agar (ZMA) using slant culture technique. Then, incubation was performed and the tubes were kept in the refrigerator. The active growing cells of the freshly prepared slant culture medium were inoculated in Erlenmeyer flask containing 100 mL Zymomonas broth (ZMB) to obtain inoculum culture. The incubation was then performed at 30 °C for 48 hours.

Ethanol fermentation medium. To prepare this medium, 1.5 g of K₂HPO₄, 1.8 g of KH₂PO₄, 0.75 g of MgSO₄·7H₂O and 0.75 g of NaCl were added to 1500 mL of hydrolyzed and filtered PPW. After adding the materials, the pH was adjusted to 7 using NaOH (1 M). The culture medium was distributed in 30 mL sterile vials and autoclaved for 15 min. Later, in order to promote ethanol production, inoculations with 1.5 g/L of the yeast or the bacterium in the fermentation medium were performed in separate assays.

Effects of adding yeast extract. Different extract concentrations of *Saccharomyces cerevisiae* (0, 1.5, 3, 4.5, 6 g·L⁻¹) were added to vials containing 30 mL of fermentation medium. The samples were incubated.

Effect of fermentation time. The fermentation medium with culture of *S. cerevisiae* as inoculum and addition of yeast extract was incubated at 24 °C using a shaker incubator at 150 rpm over time of 5 and 7 days. The same procedure was followed

in the medium with inoculum culture of *Z. mobilis* with addition of yeast extract, added at 30 °C, during 5 and 7 days as well, in an incubator. Then, they were evaluated to determine their capacity to synthesize ethanol.

Growth rate of cells. Cell growth was measured daily by spectrophotometry method using a spectrophotometer (Perkin Elmer Lambda 25 UV-visible). First, a mixture of fermentation medium was diluted with 1 mL distilled water, and then the absorbance of the mixture was read at 600 nm.

Measuring ethanol by gas chromatography (GC). The measurement was carried out using solid phase micro-extraction method. Briefly, 20 mL of the upper portion of each test sample was centrifuged at 1000 g and the sample was transferred to a container with 1.5 g sodium chloride. The top of the container was sealed with silicone. Afterward, the micro-extraction syringe solid phase was placed in the upper phase of the sample (sample temperature of 50°C, a spin rate of 600 rpm, and a 5-minute micro-extraction time). After micro-extraction from the upper phase, the content was injected using this syringe to an American Agilent 6890N gas chromatography apparatus equipped with a flame ion detector and a splitless injection valve (DB-Wax, 30 m X 0.25 mm id, film thickness 0.25 µ. Restek).

The initial temperature of the oven was maintained at 40 °C for 3 min and then increased at the rate of 15 °C per minute to 140 °C and kept for 2 min at the same temperature. Nitrogen gas as carrier was used at a flow rate of one and 45 mL per minute. The injection valve temperature was set at 200 °C, the desorption time was 10 seconds, and the detector temperature was set at 250 °C (Maleki et al., 2009).

Statistics. A randomized design with factorial arrangement of the treatments was used to evaluate the impact of yeast extract concentration (0, 1.5, 3, 4.5 and 6 g·L⁻¹) and time of fermentation (5 and 7 days) to ethanol production by *S. cerevisiae* and *Z. mobilis*. The measurements were carried out in duplicate.

Statistical analysis of the data was conducted by Anova, and mean separation by least significant difference (LSD) test using Statistix v.10 software.

RESULTS AND DISCUSSION

The effect of the studied factors including fermentation time, type of microorganisms and concentrations of yeast extract on the cell growth and yield of ethanol were significant ($P \leq 0.05$ or 0.01). Interaction were significant as well, but with higher probability of the experimental error, so the main effects will be considered in the analysis (Table 1).

According to Figure 1, *S. cerevisiae* population showed a low growth rate at the first day of fermentation (lag phase) and had significant increase at the second day of fermentation (log

phase). At the third day of fermentation, *S. cerevisiae* population reached the highest growth rate approaching the stationary state (19.2×10^8 cells per mL). In the 4th and 5th days, the fermentation reached to a constant state (stationary phase), until the last day of fermentation when its counting reduced reaching death phase.

Z. mobilis bacteria showed the highest growth rate after 24 hours (20.05×10^8 cells per mL), and later, its counting showed decrease-increase while at the last fermentation day (168 hours) reduced significantly.

Table 1. Mean squares of the main effects in the trial involving two types of microorganisms (M.O.) and five concentrations of *Saccharomyces cerevisiae* at two fermentation times

Sources of change	d.f.	Cell growth	Ethanol yield
Time	1	1.28802**	0.063**
M.O. type	1	0.31212*	0.157**
Concentration	4	0.72119**	0.037**
Experimental error		0.04585	0.000002
C.V. (%)		12.64	1.01

*: $P \leq 0.05$, **: $P \leq 0.01$

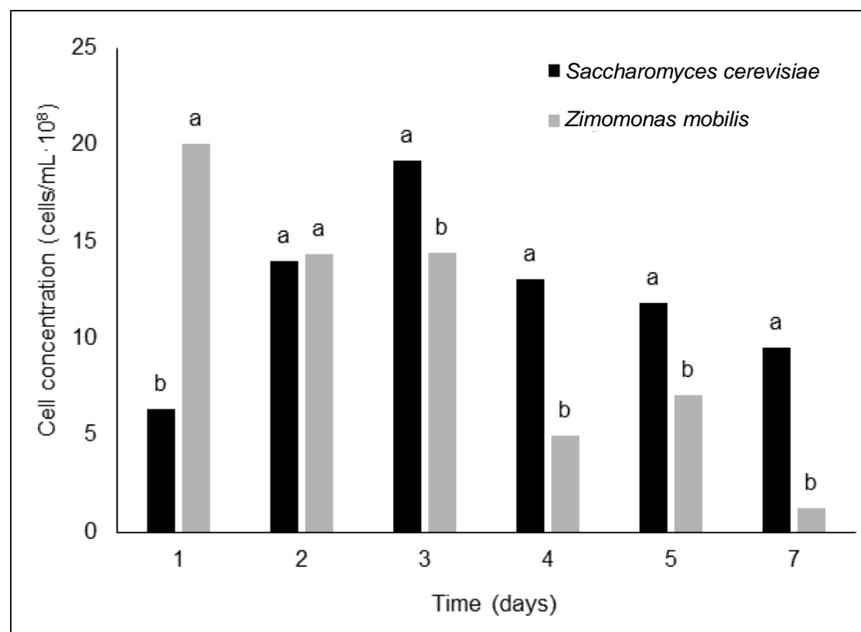


Figure 1. Microbial count of *S. cerevisiae* and *Z. mobilis* during different fermentation periods (at each day, columns with the same letter are not significantly different from each other, according to LSD test ($P \leq 0.05$))

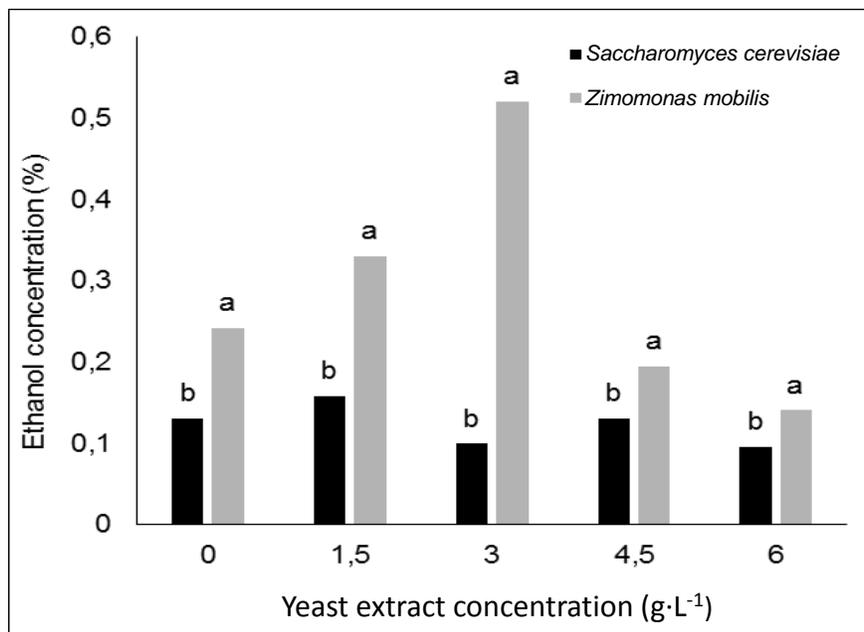


Figure 2. Effect of different concentrations of yeast extract on ethanol production by *Saccharomyces cerevisiae* and *Zymomonas mobilis* at fifth fermentation day (120 hours). At each concentration, columns with the same letter are not significantly different from each other, according to LSD test ($P \leq 0.05$)

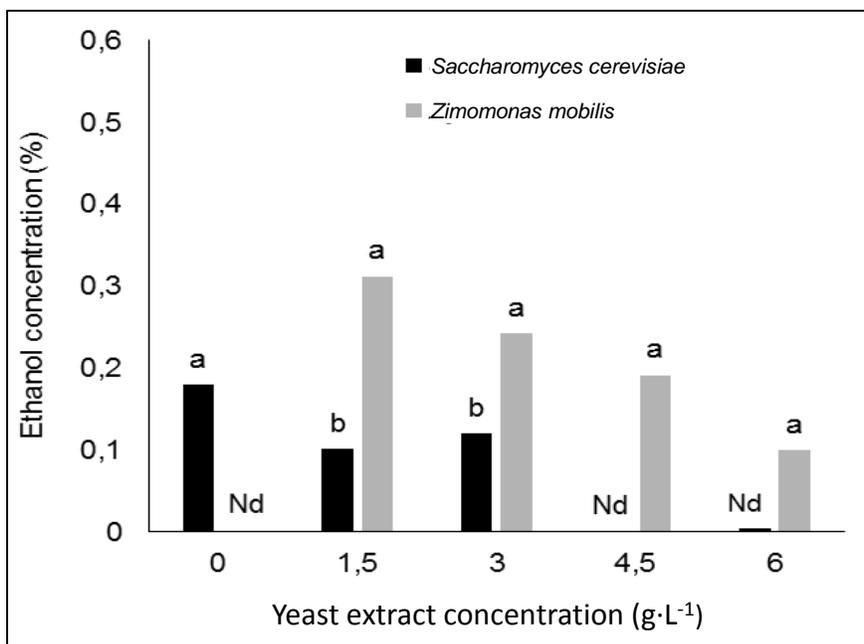


Figure 3. Effect of different concentrations of yeast extract on ethanol production by *S. cerevisiae* and *Z. mobilis* at 7th fermentation day (168 hours). At each concentration (columns with the same letter are not significantly different from each other, according to LSD test ($P \leq 0.05$)). Nd: non detectable

The performance of *Z. mobilis* and *S. cerevisiae* for ethanol production at fifth and seventh day is shown in Figures 2 and 3. *Z. mobilis* had a better performance than *S. cerevisiae* to produce ethanol at 0 g·L⁻¹ concentration of yeast (control) on the fifth day (120 hours) with 0.243 g ethanol per 100 g of potato peel. In the concentration of 1.5 g·L⁻¹ yeast extract, *Z. mobilis* showed better yields for ethanol production than *S. cerevisiae* with 0.312 and 0.331 % on the fifth and seventh day, respectively.

At the concentration of 3 g·L⁻¹ of yeast extract, *Z. mobilis* had better performance than *S. cerevisiae* at the fifth day and consumed the highest amount of carbohydrates to convert it to ethanol, a trend that is related to approaching the stationary state phase. Comparatively, in the concentration of 4.5 and 6 g·L⁻¹ of yeast extract and at 5th day, *Z. mobilis* showed better performance than *S. cerevisiae* for ethanol production and also produced higher ethanol at 7th day than the 5th day.

In fact, the results of the Figures 2 and 3 show that both tested microorganisms produced higher ethanol at the fifth day of fermentation due to the approaching to stationary phase while ethanol production reduced at the seventh day which can be attributed to the limitation of nutrients. Moreover, *Z. mobilis* had a better ability to produce ethanol than *S. cerevisiae*, and the highest amount of ethanol was obtained at the fifth day using a small concentration of the yeast extract (3 g·L⁻¹).

Potato peels showed that can produce important yields of ethanol despite the fact that Meenakshi and Kumaresan (2014) found that the yield was lower when compared with substrates of corn.

Various microorganisms including yeasts, bacteria, and fungi are commonly used for producing ethanol. *S. cerevisiae* and *Z. mobilis* are two microorganisms that can be used to produce ethanol. Based on some scientific sources, the amount of ethanol produced by *Z. mobilis* is higher than that of *S. cerevisiae*, and thus the total cost of ethanol production can be reduced using this microorganism. For instance, Davis et al. (2006) reported that after culturing *Z. mobilis* and *S. cerevisiae*, *Z. mobilis* produced 39 g·L⁻¹ ethanol over a period of 9 hours, while *S. cerevisiae*

produced 36 g·L⁻¹ ethanol over a period of 11 hours. The amount of ethanol production by *Zymomonas* sp. is higher than *Saccharomyces* sp. In another study, Sivasakthivelan et al. (2014) compared the amount of ethanol produced by *S. cerevisiae* and *Z. mobilis*. The authors investigate the effects of pH, temperature, incubation time, inoculation and substrate concentration on yield of production. The results showed that the best conditions for production of bioethanol by *Z. mobilis* were pH of 6.5, temperature 30°C, inoculation of 10 %, concentration of substrate 5 % and a maximum of 48 hours, which yielded maximum bioethanol concentration (13.59 g·L⁻¹). Additionally, the best conditions for production of ethanol by *S. cerevisiae* were found to be pH of 5.5, temperature of 30 °C, 8 % inoculation, 3 % substrate concentration and 48 hours as maximum duration (11.46 g·L⁻¹). These results are in agreement with those observed in the present study.

In a recent research, Sheikh et al. (2016) evaluated the effect of adding yeast extract on the yield of ethanol production from potato peel by two subspecies of *S. cerevisiae*. They found that the best concentration of the extract was 2 g·L⁻¹. At this condition, 2.83 % and 2.64 % ethanol were obtained from two subspecies of this yeast. Using the minimum amount of extract concentration, they could achieve a good result to produce ethanol. Also, the best fermentation times for both species were between 72 and 96 hours, with the highest production rate of bioethanol at about 2.17 % and 2.01 % for two subspecies of *S. cerevisiae*.

CONCLUSION

Z. mobilis has a higher ability to produce ethanol than *S. cerevisiae* under various experimental conditions. The best fermentation time for *Z. mobilis* was the fifth day (120 hours), as well as the best concentration of the yeast extract added to fermentation was 3 g·L⁻¹, which provided the highest yield for bioethanol production (0.522 %). The best fermentation time for *S. cerevisiae* was the seventh day (168 hours), as well as the best concentration of the yeast extract added to fermentation was 0 g·L⁻¹ that had the highest yield for bioethanol production (0.180 %). In conclusion, *Z. mobilis*

had a better performance in the production of bioethanol from potato peel. Furthermore, the duration of 120 hours for fermentation for both microorganisms led to better results for ethanol production due to approaching the stationary phase.

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