

ETHANOL PRODUCTION FROM JERUSALEM ARTICHOKE BY *ZYMOMONAS MOBILIS* IN BATCH FERMENTATION

Tatcha Onsoy¹, Pornthap Thanonkeo^{2*}, Sudarat Thanonkeo³ and Mamoru Yamada⁴

¹Department of Biotechnology, Graduate School,
Khon Kaen University, Khon Kaen 40002, Thailand

²Department of Biotechnology and Fermentation Research Center for Value
Added Agricultural Products (FerVAAP), Faculty of Technology,
Khon Kaen University, Khon Kaen 40002, Thailand

³Walai Rukhavej Research Institute, Mahasarakham University,
Mahasarakham 44001, Thailand

⁴Department of Biological Chemistry, Faculty of Agriculture,
Yamaguchi University, Yamaguchi 753-8515, Japan

ABSTRACT

Jerusalem artichoke (*Helianthus tuberosus* L.) is one of the most suitable materials for ethanol production as it contains nearly 20% of carbohydrates, 70-90% of which is inulin. In the present study, the batch ethanol fermentation of Jerusalem artichoke juices by the bacterium *Zymomonas mobilis* TISTR548 was investigated. Acid and enzymatic hydrolysis of inulin in Jerusalem artichoke juices were compared and the results show that acid hydrolysis at 80°C for 40 min using concentrated sulfuric acid gave the maximum reducing sugars content as well as ethanol yield (0.42 g g⁻¹ utilized sugars) and ethanol productivity (0.65 g l⁻¹ h⁻¹) with 83.19% of the theoretical ethanol yield. Effect of initial pH of ethanol production medium and the inoculum size of *Z. mobilis* on ethanol production of acid hydrolyzed Jerusalem artichoke juices was determined. The results reveal that initial pH of 5.0 and 10% inoculum size exhibited the highest ethanol yield (0.47 g g⁻¹ utilized sugars) and ethanol productivity (1.33 g l⁻¹ h⁻¹) with 92.75% of the theoretical ethanol yield, as compared to other conditions tested.

KEYWORDS: Jerusalem artichoke, ethanol fermentation, inulin, *Zymomonas mobilis*, renewable energy

*Corresponding author: Tel: 66-4336-2121 Fax: 66- 4336-2121
E-mail: porttha@kku.ac.th

1. INTRODUCTION

The increased oil price for nonrenewable oil resources has stimulated worldwide interest in the utilization of fermentation ethanol as a potential liquid fuel [1]. Great attention is focused on renewable sources in fuel ethanol production and Jerusalem artichoke is one of the most interesting materials among unconventional raw materials [2]. Jerusalem artichoke is a plant native in temperate regions of North America. This plant is a rich source of carbohydrates (11-20% by weight), where 70-90% of them is inulin. Inulin consists of linear chain of *D*-fructose units in the β (2 $\rightarrow$ 1) position. The chain is terminated by a *D*-glucose residues linked to fructose by an α (1 $\rightarrow$ 2) bond [3]. The potential advantages of Jerusalem artichoke over the traditional agricultural crops include the following: a) minimal fertilizer requirements, b) good growth in poor soil, c) high tolerance to frost and various plant diseases, and d) very high carbohydrate yields per acre [4 -6].

Zymomonas mobilis is a gram-negative, obligately fermentative bacterium found in association with plants containing high concentrations of sugars in their saps and fruit juices [7]. This microorganism is unique in employing the Entner-Doudoroff (ED) (2-keto-3-deoxy-6-phosphogluconate, KDPG) pathway for sugar catabolism and produces ethanol and carbon dioxide as dominant fermentation products [8]. The utilizable sugars for ethanol production of *Z. mobilis* are restricted to glucose, fructose and sucrose. It does not readily ferment high molecular weight β -fructosides such as inulin. An inulin hydrolysis is thus needed prior to fermentation [9]. In this present research, the effects of inulin hydrolysis processes as well as initial pH of the ethanol production medium and inoculum size of *Z. mobilis* on the ethanol fermentation yield from Jerusalem artichoke juices were investigated.

2. MATERIALS AND METHODS

2.1 Biological materials

Jerusalem artichoke tubers (cultivar KKKU AC001) were obtained from the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Thailand. The whole tubers were washed with water and ground into a mash using a food grinder. The juices obtained after pressing the mashed tubers through cloth bags were used in classical batch fermentation processes.

2.2 Microorganism

Zymomonas mobilis TISTR 548 obtained from the Thailand Institute of Scientific and Technological Research, Bangkok was used in this study.

2.3 Inoculum preparation

Zymomonas mobilis was cultured in YPG medium containing: glucose, 30g l⁻¹; yeast extract 3 g l⁻¹; and peptone 5 g l⁻¹ at 30°C with shaking at 100 rpm until the cell density reached 0.8-0.1 (1x10⁸ cells ml⁻¹), and then used as inoculum.

2.4 Hydrolysis of inulin

Jerusalem artichoke juices were amended to acid or enzymatic hydrolysis before the fermentation by *Z. mobilis*. The acid hydrolysis was conducted at pH 2.0 adjusted with sulfuric acid (H₂SO₄) and held at different temperatures; 60°C, 80°C and 100°C for 40 min. After the hydrolysis, the pH was adjusted to 5.0. The enzymatic hydrolysis was performed using the inulinase (Sigma-Aldrich,

17 U g⁻¹) from *Aspergillus niger* (0.02 g kg⁻¹ tubers) at pH 5.0, and held at 55°C for 60 mins. The enzyme was not inactivated after the hydrolysis [2].

2.5 Ethanol fermentation processes

Sterilized acid or enzymatic hydrolysate of Jerusalem artichoke juices at 110°C for 15 min was directly used as the medium for ethanol fermentation by *Z. mobilis*. All batch fermentations were carried out in 500 ml Erlenmeyer flask. Each flask contained 360 ml of acid or enzymatic hydrolysate and 1, 5 or 10% (by vol) of the inoculum. The initial pH of the hydrolysate was varied at 5.0, 6.0 and 7.0. All flasks were statically incubated at 30°C and samples were taken at regular intervals and analyzed for ethanol, total sugars and pH.

2.6 Analytical methods

The reducing sugars were estimated with 3, 5-dinitrosalicylic acid (DNS), using fructose as the standard [10]. Total reducing sugars were assayed by the same method after acid or enzymatic hydrolysis. Determination of inulin content and total sugars (carbohydrates) was determined by the phenol sulfuric acid method using inulin as the standard [11]. The inulin content was measured with the difference between total sugars and reducing sugars [12]. The pH was measured by pH meter. Ethanol concentration in the culture medium was measured by gas liquid chromatography (GLC) (Shimadzu GC-14B, Japan) with a flame ionization detector. N₂ was used as a carrier gas and isopropanol was used as an internal standard. The ethanol yield (*Y_{ps}*) was calculated as the actual ethanol produced and expressed as g ethanol per g sugar utilized (g g⁻¹). The volumetric ethanol productivity (*Q_p*) and the percentage of conversion efficiency or yield efficiency (*E_y*) were calculated by the following equations:

$$Q_p = P / t \text{ and } E_y = (Y_{ps} \times 100) / 0.51$$

Where *P* is the actual ethanol concentration produced (g l⁻¹), *t* is the fermentation time (h) giving the highest ethanol concentration and 0.51 is the maximum theoretical ethanol yield of glucose or fructose consumption.

3. RESULTS AND DISCUSSION

3.1 Effect of acid and enzymatic hydrolysis

Composition analysis of the Jerusalem artichoke juices reveals that it contained 90% of inulin (data not shown). *Zymomonas mobilis* TISTR548 used in this study lacked an inulinase activity to convert inulin into fermentable sugar, therefore hydrolysis of inulin is needed. Acid and enzymatic hydrolyses were compared in the present research and the results are summarized in Table 1. The fermentable sugar content (expressed as reducing sugar) in juices of acid hydrolysis was higher than that of enzymatic hydrolysis. The maximum reducing sugar content was achieved after acid hydrolysis at 80°C (139.38 g l⁻¹). The low amount of reducing sugars (47.85 g l⁻¹) after enzymatic hydrolysis might be due to the following: a) low amount of enzyme used and b) the present of some enzyme inhibitors in the juice. Therefore, further studies to clarify these hypotheses are needed and they are now under our investigation.

Table 1 The content of reducing sugars and inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) juices before and after acid or enzymatic hydrolysis.

Type of hydrolysis	Initial reducing sugars (g l ⁻¹)	Reducing sugars after hydrolysis (g l ⁻¹)	Inulin content (g l ⁻¹)
Sulfuric acid, at 60°C	30.92	121.54	51.23
Sulfuric acid, at 80°C	30.92	139.38	33.25
Sulfuric acid, at 100°C	30.92	137.54	33.25
Inulinase	30.92	47.85	115.03

The acid and enzymatic hydrolysates of Jerusalem artichoke juices were directly used as the media for ethanol fermentation by *Z. mobilis* and the main fermentation parameters are summarized in Table 2. The final ethanol concentration was limited by the amount of fermentable sugars produced, which is varied depending on type of hydrolysis (Table 1). The highest ethanol yield (0.45 g g⁻¹) and conversion efficiency (83.19% of theoretical value) were consistently achieved when acid hydrolysate at 80°C was used for ethanol fermentation. The maximum ethanol yield from enzymatic hydrolysate of the juices was 0.29 g g⁻¹, with the conversion efficiency of 57.83 %. Our results are opposite from those reported by Szambelan *et al.* [9] who observed the maximum ethanol yields from the enzymatic hydrolysates of Jerusalem artichoke juices at 0.43-0.44 g g⁻¹ which are higher than those observed from acid hydrolysates (0.37-0.40 g g⁻¹). This is probably due to the differences in the cultivar of Jerusalem artichoke or strain of *Z. mobilis* used. However, our results are similar to those reported by Nakamura *et al.* [13] who observed the lower ethanol yields for Jerusalem artichoke tubers fermented by *S. cerevisiae* after enzymatic hydrolysis with inulinase. The acid hydrolysis of Jerusalem artichoke juices at 80°C gave the highest fermentable sugars as well as ethanol yield, therefore it was selected for further studies.

Table 2 Fermentation parameters of acid or enzymatic hydrolysate of Jerusalem artichoke (*Helianthus tuberosus* L.) juices by *Z. mobilis*.

Type of hydrolysate	Final pH after fermentation	P (g l ⁻¹)	Yps (g g ⁻¹)	Qp (g l ⁻¹ h ⁻¹)	Ey (%)	Fermentation time (h)
Acid hydrolysate, at 60°C	5.03	23.83	0.21	0.28	41.71	60
Acid hydrolysate, at 80°C	4.97	54.71	0.45	0.65	83.19	60
Acid hydrolysate, at 100°C	4.74	48.99	0.43	0.58	82.40	60
Enzymatic hydrolysate	5.04	32.36	0.29	0.39	57.83	60

P, ethanol concentration produced; Yps, ethanol yield; Qp, volumetric ethanol productivity; Ey, conversion efficiency or yield efficiency

3.2 Effect of initial pH and inoculum size

The effect of initial pH of ethanol production medium and inoculum size of *Z. mobilis* were tested on juices after acid hydrolysis at 80°C for 40 min. Ethanol yield was maximum when initial pH of ethanol production medium was 5.0-6.0, coincides with that reported by Swings and DeLey [7]. Inoculum size of *Z. mobilis* did not significantly affect the final ethanol concentration, but it

markedly affected the substrate consumption rate and ethanol production rate. Using 5% or 10% inoculum size, total sugars were consumed within 36 to 48 h after fermentation, whereas it took about 78 h when using 1% inoculum size (data not shown). The maximum ethanol yields obtained when using 10% inoculum size were 0.46-0.47 g g⁻¹, with 89.90-92.75% of theoretical ethanol yield (Table 3). The results obtained from Jerusalem artichoke juices fermented by *Z. mobilis* after acid hydrolysis in this research were higher than those reported by Szambelan *et al.* [2, 14].

Table 3 Fermentation parameters of Jerusalem artichoke (*Helianthus tuberosus* L.) acid hydrolysed by *Z. mobilis* under various initial pH or inoculum size.

Initial pH	Inoculum size (%)	Final pH after fermentation	<i>P</i> (g l ⁻¹)	<i>Y</i> _{ps} (g g ⁻¹)	<i>Q</i> _p (g l ⁻¹ h ⁻¹)	<i>E</i> _y (%)
5.0	1	4.3	83.18	0.45	1.1	87.59
5.0	5	4.2	98.53	0.46	1.3	89.56
5.0	10	4.3	104.20	0.47	1.3	92.75
6.0	1	4.4	85.49	0.39	1.1	76.03
6.0	5	4.3	99.56	0.42	1.3	82.92
6.0	10	4.3	102.38	0.46	1.3	89.90
7.0	1	4.8	11.11	0.25	0.1	49.81
7.0	5	4.7	26.44	0.28	0.3	55.88
7.0	10	4.7	48.28	0.30	0.6	59.39

P, ethanol concentration produced; *Y*_{ps}, ethanol yield; *Q*_p, volumetric ethanol productivity; *E*_y, conversion efficiency or yield efficiency

4. CONCLUSIONS

The hydrolysis of inulin in Jerusalem artichoke juices was necessary process for ethanol fermentation by *Z. mobilis*. Acid hydrolysis with concentrated sulfuric acid (H₂SO₄) at 80°C for 40 min gave the highest reducing sugars content as well as ethanol yield (83.19 % of the theoretical)

Initial pH at 5.0 and 6.0 showed no significant different in ethanol yield, however, pH 5.0 proved to be appropriate for ethanol production from acid hydrolysate. The highest ethanol yield of 92.75% of theoretical value was obtained at initial pH 5.0 of ethanol production medium and 10% inoculum size of *Z. mobilis*.

The fermentation of juices is easier to handle but the step of juice preparation might increase the cost of the process significantly. It is important to note that the Jerusalem artichoke juices can serve for ethanol producing microorganism growth and ethanol production without additives since they contain enough essential nutrients.

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