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Bioethanol production from white onion by yeast in repeated batch

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Abstract

Considered to be the cleanest liquid fuel, bioethanol can be a reliable alternative to fossil fuels. It is produced by fermentation of sugar components of plant materials. The common onions are considered to be a favorable source of fermentation products as they have high sugar contents as well as contain various nutrients. This study focused on the effective production of ethanol from an endemic Iranian white onion (*Allium cepa* L., Dorche cultivar) by the yeast "*Saccharomyces cerevisiae" in* repeated batch. The results showed that the total sugar concentration of onion juice was77.3 g/l. The maximum rate of productivity, ethanol yield and final bioethanol percentage was 8 g/l/h (g ethanol per liter of onion juice per hour), 40 g/l (g ethanol per liter of onion juice and 93 %, respectively.

Keywords*: Allium cepa*; biofuels; crop waste; ethanol; *Saccharomyces cerevisiae*

1. Introduction

Due to the potential exhausting of traditional fossil fuels and the increasing price of petroleum together with environmental concerns, the search for alternative renewable fuels has attracted great attention in recent years [1].

Attention has been devoted to the conversion of biomass into fuel ethanol, which is the cleanest liquid fuel alternative to fossil fuels [2]. Biologically produced alcohols, most commonly ethanol, were used as a fuel source for cars well until the 1930s. After World War II, however, little interest remained in using agricultural crops for liquid fuel production because of the abundant and cheap supply of fuel from petroleum and natural gas [3]. With the current limited oil supplies, there is a renewed focus on the need for alternative energy sources; Fuel ethanol remains an attractive option. It benefits farmers by creating a substantial new market for crop supplies and by creating new jobs in economically depressed rural areas and small communities. Ethanol is produced by the action of microorganisms and enzymes through the fermentation of sugars, starches or cellulose [2]. Yeast is one of the most important microorganisms in the production of ethanol but now many microorganisms can replace yeast for ethanol production. In addition to ethanol, butanol has been introduced as a fuel [3].

Onion (*Allium cepa* L.) is a major vegetable product in Iran with many native cultivars including red and white [4]. In addition, the onion is the most widely cultivated horticultural crop in Europe, with a reported annual production of more than 5 million tons [5]. Onions have significant nutritional value as they contain sugars, vitamins, e.g. vitamin C, and minerals, in particular calcium, phosphorus and potassium. However, large amounts of onion annually produced worldwide are disposed of as agricultural waste or sold in the market as sub-standard quality [6]. Thus, a new product from onion waste is very affordable economically. The common onion is considered a favorable source of fermentation products because of its high sugar and nutrient content. Bioethanol produced from onions could be used as an advantageous substrate for acetic fermentation to obtain onion vinegar. This vinegar contains a higher amount of minerals, and organic acids [7]. While fuel ethanol production is currently an energy-efficient process, additional research is underway to improve its long-term economic viability [8]. A repeated batch process using yeast is widely recognized as a promising method for effective alcohol production, easy cell recycle and stable operation [9]. Because the worthless onions are seasonal agricultural products and the available quantity is limited, the

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repeated batch process is suitable for the onion alcohol production rather than a continuous process in terms of its operability [9]. Since onion is an important vegetable product in Iran, this kind of study on this plant has various applications. In this study, bioethanol from an Iranian endemic white onion was produced by yeast for the first time in repeated batch.

2. Materials and methods

2.1. Strain and medium

Saccharomyces cerevisiae was taken from the Persian Type Culture Collection (PTCC) of the Iranian Research Organization for Science and Technology. The strain was maintained on agar slants containing YPD medium and the inoculums cultures were inoculated from the yeast on the slant by means of sterile inoculation loops. YPD medium containing 1g glucose/dextrose, 1 g yeast extract, and 2 g peptone per 100ml distilled water, was used for the inoculums medium except as stated.

An Iranian endemic white onion (Dorche cultivar) from Isfahan city was used as the raw material for alcohol fermentation. Onions were cut and pressed in a mechanical juicer. The extract was collected in a glass vial (l000ml) and autoclaved at 120°C for 20 min. The autoclaved juice was cooled and then filtered using Whatman (No. 1) filter paper.

2.2. Experiments

Onion juice for bioethanol production was obtained as follows. Onion was pressed in a mechanical juicer and the extract was promptly autoclaved for 30min at 120°C. The extract was then filtered twice, using 6.0 pm pore-size membrane to remove coarse particles and a 0.4pm pore-size membrane to remove microbes. By this procedure, 60 wt% of the onions processed was recovered as onion juice. The filtered juice was stored in a refrigerator at 4.0 °C and used as needed. Then S. cerevisiae was cultured in the YPD medium broth in the shaker incubator (VS-8480SF, Korea) at a temperature of 30°C at 120 rpm for 24 h. The growth of yeast was measured by spectrophotometer (600 nm) [7].

Then 50 ml of the onion extract and 5 ml of inoculums were added to the four Erlenmeyer flasks (two flasks for normal *S. cerevisiae* and two flasks for genetically modified strain of *S. cerevisiae*, purchased from Japan. The Batch experiment was then carried out in flasks fitted with aeration equipment. Alcohol fermentation was first performed at 30°C with CO_2 bubbling in order to maintain anaerobic condition. [9]. Four initial samples for yeast growth were used at 30°C for 48h. Following this, samples for alcohol fermentation were taken and kept at room temperature for 72h. The ethanol product both by normal yeast and by a genetically modified strain were compared.

2.3. Analytical

Total organic acids in onion juice were measured by High-performance liquid chromatography (HPLC, 8020 model, Tosoh CO., Tokyo, Japan) and free amino acids were measured by the NBD-F (4-Fluoro-7-nitrobenzofurazan) method in combination with HPLC. Several minerals were determined by inductively coupled plasma atomic emission spectroscopy (ICP, Hitachi, Japan). The amount of sugars was measured by the phenol-sulphuric acid method [10]. Subsequently, fermentation samples were centrifuged (7000 rpm) at 4°C for 6 min and the supernatant were used for the determination of ethanol by HPLC.

3. Results and discussion

Ethanol, both renewable and environmentally friendly, is leading to a dramatic increase in its production capacity. Among many microorganisms that have been exploited for ethanol production, *S. cerevisiae* remains the prime one [11].

In this study, the total sugar concentration of onion (Dorche cultivar) juice was 77.3 g/l; in other studies, it was found to vary from 46.9 to 79.6 g/l [7]. This showed that the examined onion is a good source for bioethanol production, as ethanol is produced by the action of microorganisms and enzymes through the fermentation of sugars. In brief, the sucrose, fructose and glucose concentrations of white onion juice were 26.3, 30.4 and 20.6 g/l (Table 1). The maximum productivity, ethanol yield and final ethanol percentage were 8 g/l/h, 40 g/l and 93 %, respectively.

Our results indicate high rates of bioethanol production by onion juice as compared with others (6.5 g/l/h, 30.6 g/l and 91.9%, respectively) [7].

Total organic acids and free amino acids were 4830 and 4438 mg/l, respectively. The amounts of certain minerals from white onion (Dorche cultivar) juice are shown in Table 1; these are similar, with slight variations, to those of the red onion [12]. The amounts of free amino acids and minerals are important because they are used by yeast as nutrients and ethanol production is tightly coupled with the growth of yeast cells [11].

Under anaerobic conditions, the pyruvate is further reduced to ethanol with the release of CO_2. Figure 1 shows the ethanol production by normal and modified strains of *S. cerevisiae*. The normal yeast tube has more CO_2 above the ethanol with an ethanol yield of 40 g/l but the ethanol yield of the modified yeast was 45 g/l, confirming that the ethanol production of the modified strain has greater purity.

Table 1. Composition of white onion (Dorche cultivar) juice

Parameter	Amount
Total organic acids	4830 (mg/l)
Free amino acids	4438 (mg/l)
Sucrose	26.3 (g/l)
Fructose	30.4 (g/l)
Glucose	20.6 (g/l)
K	1900 (mg/l)
PO_4	458 (mg/l)
Mg	82.3 (mg/l)
Ca	77.2 (mg/l)
Na	10.0 (mg/l)
Mn	2.1 (mg/l)
Zn	1.0 (mg/l)
Fe	0.8 (mg/l)
PH	6

Fig. 1. Ethanol production by normal *S. cerevisiae* (left) and genetically modified strain of *S. cerevisiae* (right). In the left tube more CO_2 gas is evolved above the ethanol solution

In terms of sugar and nutrient content, it was simple to use discarded onions as a source of medium as they had been rejected mainly on account of their shape or size [7]. Onions usually contain sulphur-containing amino acids and alliin, which are converted to allysine compounds (propylallysine, methyl propylallysine) by cellular alliinase during processing [9]. The allysine compounds have a keen, stimulating smell and an anti-microorganism function, which inhibits the growth of yeast. In preliminary experiments, it was found that raw onion juice was difficult to ferment by yeast. However, the allysine compounds are volatile and could be removed by autoclave treatment before fermentation [9].

Ethanol production by normal *S. cerevisiae* and a genetically modified strain of *S. cerevisiae* was compared (Fig. 1). The modified strain had more CO_2 above the ethanol. The application of modified yeast to improve ethanol production from glucose is key in many biofuels production programs [13].

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