Bio-ethanol Production from Green Onion by Yeast in Repeated Batch

Reza Robati

Received: 28 January 2013 / Accepted: 4 February 2013 / Published online: 16 February 2013
© Association of Microbiologists of India 2013

Abstract Considered to be the cleanest liquid fuel, bio-ethanol 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 Green onion (*Allium fistulosum* L.) by the yeast “*Saccharomyces cerevisiae*” in repeated batch. The results showed that the total sugar concentration of onion juice was 68.4 g/l. The maximum rate of productivity, ethanol yield and final bio-ethanol percentage was 7 g/l/h (g ethanol per liter of onion juice per hour), 35 g/l (g ethanol per liter of onion juice) and 90 %, respectively.

Keywords *Allium fistulosum* · Bio-fuels · Crop waste · Ethanol · *Saccharomyces cerevisiae*

Introduction

Due to scarcity of fossil fuel, more and more attempts have been focusing on alternate energy produced by microbial fermentation from renewable sources [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]. 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.

Onion (*Allium fistulosum* 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. 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. Bio-ethanol 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 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, bio-ethanol from Green onion was produced by yeast for the first time in repeated batch.

R. Robati (✉)
Department of Microbiology, Science and Research Branch,
Islamic Azad University, Fars, Iran
e-mail: rezavamp@yahoo.com

Materials and Methods

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 1 g glucose/dextrose, 1 g yeast extract, and 2 g peptone per 100 ml distilled water, was used for the inoculums medium except as stated. Green onion from Shiraz 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 (1,000 ml) and autoclaved at 120 °C for 20 min. The autoclaved juice was cooled and then filtered using Whatman (No. 1) filter paper.

Experiments

Onion juice for bio-ethanol production was obtained as follows. Onion was pressed in a mechanical juicer and the extract was promptly autoclaved for 30 min at 120 °C. The extract was then filtered twice, using 6.0 µm pore-size membrane to remove coarse particles and a 0.4 µm 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 CO₂ bubbling in order to maintain anaerobic condition. [9]. Four initial samples for yeast growth were used at 30 °C for 48 h. Following this, samples for alcohol fermentation were taken and kept at room temperature for 72 h. The ethanol product both by normal yeast and by a genetically modified strain was compared.

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 (7,000 rpm) at 4 °C for 6 min and the supernatant were used for the determination of ethanol by HPLC.

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 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 bio-ethanol production, as ethanol is produced by the action of microorganisms and enzymes through the fermentation of sugars. In brief, the composition of Green onion juice came to be; Total organic acids were 4,270 mg/l, Free amino acids were 3,930 mg/l, K 1,680 mg/l, PO₄ 405 mg/l, Mg 72.8 mg/l, Ca 68.3 mg/l, Na 8.8 mg/l, Mn 1.85 mg/l, Zn 0.88 mg/l, Fe 0.7 mg/l and the sucrose, fructose and glucose concentrations of Green onion juice were 23.2, 28.9 and 18.2 g/l. pH was 6. The maximum productivity, ethanol yield and final ethanol percentage were 7 g/l/h, 35 g/l and 87 %, respectively. Our results indicate high rates of bio-ethanol 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 4,830 and 4,438 mg/l, respectively. 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 CO₂. The normal yeast tube has more CO₂ above the ethanol with an ethanol yield of 39 g/l but the ethanol yield of the modified yeast was 44 g/l, confirming that the ethanol production of the modified strain has greater purity.

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 (propyl allysine, methyl propyl allysine) 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. The modified strain had more CO₂ above the ethanol. The application of modified yeast to improve ethanol production from glucose is a key in many biofuels production programs [12].

References

1. Jing Q, Wang J, Wu G (2012) Teth137, a conserved factor of unknown function from *Thermoanaerobacter ethanolicus* JW200 represses the transcription of the adhE gene in vitro. Indian J Microbiol. doi:10.1007/s12088-012-0339-y
2. Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69:627–642
3. Jain S, Sharma MP (2010) Stability of biodiesel and its blends: a review. Renew Sust Energ Rev 14:667–678
4. Khodadadi M, Hassanpanah D (2010) Iranian onion (*Allium fistulosum* L.) cultivars responses to inbreeding depression. World Appl Sci J 11(4):426–428
5. Khiari Z, Makris DP, Kefalas P (2008) Recovery of bioactive flavonols from onion solid wastes employing water/ethanol-based solvent systems. Food Sci Tech Int 14(6):497–502
6. Ghabel R, Rajabipour A, Ghasemi-Varnamkhasti M, Oveisi M (2010) Modeling the mass of Iranian export onion (*Allium fistulosum* L.) varieties using some physical characteristics. Res Agr Eng 56(1):33–40
7. Horiuchi J, Kanno T, Kobayashi M (1999) New vinegar production from onions. J Biosci Bioeng 88(1):107–109
8. Bothast RJ, Schleicher MA (2005) Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol 67:19–25
9. Sanda T, Hasunuma T, Matsuda F, Kondo A (2011) Repeated-batch fermentation of lignocellulosic hydrolysate to ethanol using a hybrid *Saccharomyces cerevisiae* strain metabolically engineered for tolerance to acetic and formic acids. Bioresour Technol 102(17):7917–7924
10. Masukot T, Minami A, Iwasaki N, Majima T, Nishimura SI, Lee YC (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. Analytical Biochem 339(1):69–72
11. Bai FW, Anderson WA, Moo-Young M (2008) Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnol Adv 26:89–105
12. Alper H, Moxley J, Nevoigt E, Fink GR, Stephanopoulos G (2006) Engineering yeast transcription machinery for improved ethanol tolerance and production. Science 314:1565–1568