Bio-ethanol production from Strawberry by *Saccharomyces cerevisiae* in repeated batch

Abstract

Bio-ethanol, is considered the cleanest fluid fuel, and can be a dependable substitution to non-renewable energy sources. The maturation of sugar parts of plant materials produces it. Due to their high sugar content and abundance of nutrients, berries are thought to be an excellent source of fermentation products. This examination saw how well the yeast "*Saccharomyces cerevisiae*" created ethanol from strawberries (Fragaria ananassa) in rehashed bunches. According to the findings, strawberry juice had a total sugar concentration of 41.87 g/l. The final bioethanol percentage, ethanol yield, and maximum productivity were, respectively, 6 g/l/h (g ethanol per liter of strawberry juice per hour), 35 g/l (g ethanol per liter of raspberry juice), and 7 g/l (g ethanol per liter of strawberry juice).

Key words: *Fragaria ananassa*. bio-fuels. ethanol. *Saccharomyces cerevisiae*

1. Introduction

“Because of the scarcity of petroleum products, an ever-increasing number of endeavors have been made to deliver substitute energy from sustainable sources through microbial maturation” [17]. “The transformation of biomass into fuel ethanol, the cleanest fluid fuel option in contrast to petroleum derivatives, has gotten a ton of consideration” [13]. “Biologically produced alcohols, most notably ethanol, were utilized as vehicle fuel up until the 1930s” [20]. “As a result of the plentiful and modest accessibility of fuel from oil and gaseous petrol after WWII, in any case, there was little interest in utilizing farming harvests for fluid fuel creation” [13]. Microorganisms and enzymes ferment sugars, starches, or cellulose to produce ethanol [11]. The US is the world's biggest maker of strawberries. California, Florida, and Oregon are the top three strawberry-producing states. More than 50,000 acres of land in California are devoted to strawberry cultivation, and the state produced approximately 2.7 billion pounds of strawberries in 2013 [11]. Additionally, the strawberry is the most widely grown horticultural crop in the United States, with over 60,000 acres currently under cultivation. 9]. However, every year, almost 10% of mature strawberry crops are sold as subpar quality or discarded as agricultural waste. 14]. Consequently, a brand-new product made from strawberry waste is particularly eco-friendly [21]. “In light of its high sugar and wholesome substance, the normal strawberry is a decent wellspring of maturation items. In order to produce raspberry vinegar, raspberry bioethanol could serve as an advantageous substrate for acetic fermentation. This vinegar has a more noteworthy mineral and natural corrosive substance” [14]. “Albeit the assembling of fuel ethanol is now an energy-productive interaction, further exploration is being led to building its drawn-out monetary feasibility” [9]. “A repeating batch method based on yeast is widely regarded as a promising technology for stable operation, efficient alcohol synthesis, and easy cell recycling” [11]. “In terms of operability, it is preferable to use a repeated batch method rather than a continuous process for the production of raspberry alcohol because useless raspberries are seasonal agricultural crops with limited supply” [17,21].

2. Materials and Methods

2.1 Strain and Medium

The Iranian Investigate Organization for Science and Innovation's Persian Type Culture Collection (PTCC) contained *Saccharomyces cerevisiae*. The strain was kept up on agar slants containing YPD medium and the inoculum's social orders were inoculated from the yeast on the inclination by suggests of sterile vaccination circles. For the inoculums' medium, the following ingredients were used: 100 ml refined water, 1 g glucose/dextrose, 1 g yeast extricate, and 2 g peptone. Raspberry from Rasht City was used as the starting material for the fermentation of alcohol. A mechanical juicer was used to cut and squeeze the raspberries. The concentrate was gathered in a glass vial (1,000 ml) and autoclaved at 120 °C for 20 min. After being cooled, the juice from the autoclave was sifted using a Whatman (No. 1) paper channel.

2.2 Experiments

Strawberry juice for bio-ethanol age was gotten as follows. Strawberry was crushed in a mechanical juicer and the concentrate was immediately autoclaved for 30 min at 120 °C. The concentrate was by then filtered two times, using a 6.0 pm pore-size layer to remove coarse particles and a 0.4 pm pore-size film to oust creatures. 60 percent of the prepared raspberries were turned into raspberry juice using this method. The sifted juice was taken care of in a refrigerator at 4.0 °C and used depending on the situation. The development of *S. cerevisiae* was measured using a spectrophotometer (600 nm) after it was refined in the YPD medium broth in the shaker hatchery (VS-8480SF, Korea) for 24 hours at 30 °C. Then, 50 milliliters of raspberry extricate and 5 milliliters of inoculums were added to the four Erlenmeyer jars—two for the standard strain of *S. cerevisiae* and two for the Japanese hereditarily adjusted strain. The Gathering test was by then completed in carafes fitted with air flow gear. In order to maintain an anaerobic environment, alcohol aging was initially carried out at 30 °C with CO2 bubbling. 15]. Four initial yeast development tests were performed at 30 °C for 48 hours, and alcohol maturation tests were performed at room temperature for 72 hours. The ethanol product produced by ordinary yeast and a hereditarily modified strain was compared.

2.3 Analytical

All out natural acids in raspberry juice were estimated by Superior execution liquid chromatography (HPLC, 8020 model, Tosoh CO., Tokyo, Japan) and free amino acids were estimated by the NBD-F (4-Fluoro-7-nitrobenzofurazan) procedure in blend with HPLC. Inductively coupled plasma nuclear emanation spectroscopy (ICP, Hitachi, Japan) was used to select a few minerals. The phenol–sulphuric acid method was used to determine the total number of sugars [10]. Thusly, aging tests were centrifuged (7,000 rpm) at 4 °C for 6 min and the supernatant was used for the confirmation of ethanol by HPLC.

3. Results and Discussion

Ethanol's emotional generation capacity is increasing as a result of its renewable and environmentally friendly properties. *S. cerevisiae* is still the most commonly used microorganism for the production of ethanol [3]. In this consideration, the entire sugar centralization of raspberry juice was 42.6 g/l; It was discovered to range from 35.7 to 47.4 g/l in other analyses [5]. “Because ethanol is produced by the activity of microorganisms and enzymes during the aging of sugars, it appeared that the examined raspberry could be an excellent source for the production of bioethanol. In a nutshell, how raspberry juice came to be: Raspberry juice contained 882.22 mg/l of total organic acids, 954.57 mg/l of free amino acids, K 784.9 mg/l, PO4 130 mg/l, Mg 114.5 mg/l, Ca 130.2 mg/l, Na 1 mg/l, Mn 0.82 mg/l, Zn 0.64 mg/l, and Fe 0.69 mg/l, as well as 10.42, 29.76, and 26.01 g/l of sucrose, fructose, pH was 3.2. Separately, the highest efficiency, ethanol absorption, and final ethanol percentage were 6 g/l/h, 35 g/l, and 87 %. Compared to other substances, raspberry juice produces bioethanol at higher rates than any other (6.5 g/l/h, 30.6 g/l, and 91.9 %, respectively)” [19]. “Complete natural acids and free amino acids were 882.22 and 954.57 mg/l, individually. Free amino acids and minerals are important because yeast uses them as supplements and ethanol production is tightly linked to yeast cell development” [18]. The release of CO2 further reduces the pyruvate to ethanol in anaerobic conditions. The ordinary yeast tube has more CO2 than the ethanol with an ethanol yield of 43 g/l yet the ethanol gives up of the adjusted yeast was 46 g/l, asserting that the ethanol age of the changed strain has more critical flawlessness. It was essential to use discarded raspberries as a medium source of sugar and supplement substance because they had been rejected primarily due to their shape or estimate [16]. “A hereditarily altered strain of *S. cerevisiae* and ordinary *S. cerevisiae* were compared in their ability to produce ethanol. Above the ethanol, the altered strain had more CO2. The utilization of changed yeast to further develop ethanol age from glucose might be a key in many biofuels age programs” [2].

4. Conclusion

Strawberry crops are widely produced and have a high potential for ethanol production. The scientific community and policymakers are currently focusing on environmentally friendly and prudent management of its huge amount of bagasse for bioenergy production. Several pretreatment strategies are available to achieve high ethanol yields through economically viable pretreatment, enzymatic hydrolysis, and bagasse fermentation. There are numerous hurdles to overcome at the key step in bioethanol synthesis, such as physicochemical and biological pretreatment followed by enzymatic saccharification. However, substantial study is required to produce cost-effective, new bioconversion pretreatment technology options, as well as the right selection of efficient procedures. The effective delignification, inhibitory compound removal with low sugar loss, and the utilization of simultaneous saccharification and fermentation (SSF) can make it more successful and valuable for economically industrial ethanol production.

4. References

1. Akbas MY, Stark BC. (2016) Recent trends in bioethanol production from food processing byproducts. . *J Ind Microbiol Biotechnol*. 43(11):1593-1609

2. 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

3. Anderson S, Rahman P.K. (2018) Bioprocessing requirements for bioethanol: sugarcane vs. sugarcane bagasse. *Handbook of Research on Microbial Tools for Environmental Waste Management*, pp. 48-56

4. Barak. S, Rahman R.K, Neupane S, Ninnemann E, Arafin F,Laich A., A.C. Terracciano, S.S. (2020) Measuring the effectiveness of high-performance Co-Optima biofuels on suppressing soot formation at high temperature. *Proc. Natl. Acad. Sci. Unit. States Am.,* 117 (7), pp. 3451-3460

5. Bobinaiṫ e, R.; Viškelis, P.; Venskutonis, P.R. (2016) Chemical composition of raspberry (Rubus spp.) cultivars. In Nutritional Composition of Fruit Cultivars. Elsevier: *Amsterdam, The Netherlands*, pp. 713–731.

6. del Río P. G, Gullón P, Rebelo F, Romaní A, Garrote G, Gullón B. (2020) whole-slurry fermentation approach to high-solid loading for bioethanol production from corn stover. *Agronomy*, 10 (11), p. 1790

7. Ibeto, A. Ofoefule, K. Agbo. (2011) A global overview of biomass potentials for bioethanol production: a renewable alternative fuel Trends Appl. Sci. Res., 6:(5) p. 410

8. Kalemba-Drożdż M, Kwiecień I, Szewczyk A, Cierniak A, Grzywacz-Kisielewska A. (2020) Fermented Vinegars from Apple Peels, Raspberries, Rosehips, Lavender, Mint, and Rose Petals: The Composition, Antioxidant Power, and Genoprotective Abilities in Comparison to Acetic Macerates, Decoctions, and Tinctures. *Antioxidants*, *9*(11), 1121

9. Harper, J. K., S. Cornelisse, L. F. Kime, and J. Hyde. “Agricultural Alternatives: Budgeting for Agricultural Decision Making." University Park: Penn State Extension, 2013.

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. Morales C.G., Pino, M.T. and del Pozo, A. (2013). Phenological and physiological responses to drought stress and subsequent rehydration cycles in two raspberry cultivars. *Sci. Hortic*. 162: 234-241.

12. Naghshbandi M.P, Tabatabaei M, Aghbashlo M, Gupta V.K, Sulaiman A, Karimi K., Moghimi H, Maleki M. (2019) Progress toward improving ethanol production through decreased glycerol generation in *Saccharomyces cerevisiae* by metabolic and genetic engineering approaches Renew. Sustain. *Energy Rev*., 115, p. 109353

13. Nouri H, Ahi M, Azin M, Gargari S.L.M. (2020) Detoxification vs. adaptation to inhibitory substances in the production of bioethanol from sugarcane bagasse hydrolysate: a case study *Biomass Bioenergy*, 139, p. 105629

14. Pan, P.; Kang, S.; Wang, Y.; Liu, K.; Oshima, K.; Huang, Y.-W.; Zhang, J.; Yearsley, M.; Yu, J.; Wang, L.-S. (2017) Black Raspberries Enhance Natural Killer Cell Infiltration into the Colon and Suppress the Progression of Colorectal Cancer. *Front. Immunol*., 8, 997

15. Robati R. (2013) Bio-ethanol production from green onion by yeast in repeated bath. *Indian J Microbiology*. 53(3):329–331

16. Robati R*, Fathi A and gholami S. (2013) Bio-ethanol production from Ajowan plant. *Annals of biological research*, 4(3): 7-11.

17. 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

18. Siti Hajar M, Rahmath A, Siti Azmah J, Hartinie M, Jualang G, Ainol Azifa Mohd Faik, Kenneth R. (2017) Yeasts in sustainable bioethanol production: A review, *Biochemistry and Biophysics Reports*, Volume 10, P. 55

19. Vazirzadeh M and Robati R. (2013) Investigation of bio-ethanol production from waste potatoes. *Annals of biological research*, 4(1): 104-106.

20. Vohra M, Manwar J, Manmode R, Padgilwar S, Patil S. (2014) Bioethanol production: feedstock and current technologies. *Journal of Environmental Chemical Engineering*, 2 (1), pp. 573-584

21. Robati R, Mirahmadinejad E. (2022) Bio-ethanol production from Raspberry by yeast in repeated batch. Stechnolock J Case Rep, 3, 101.