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Production of Acetic Acid by Fermentation Using Sugar Beet Molasses

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Abstract

Background: Acetic acid is a molecule central to biochemistry and is produced in some amount by nearly all forms of life.

Aim: Production of acetic acid from sugar beet molasses by the process of fermentation using the suitable yeasts

Method: Ethanol was produced from sugar beet molasses having (10% and 12% sugar concentration.) using the suitable yeasts, Saccharomyces cerevisiae or Saccharomyces pastorianus with or without 2% fruit ingredients at a high pitching rate, controlled pH 5.5, and constant temperature 40^{o}C. This ethanol was converted to acetic acid by the oxidative method.

Results: In both cases, 10% or 12% sugar concentration; Saccharomyces cerevisiae with 2% fruit ingredients produced a large amount of alcohol, 4.6 and 4.9 gm/100ml respectively, and it produced 4.8 ml of acetic acid. On the other hand, Saccharomyces pastorianus with 2% fruit ingredients produced 2.8 and 2.7 gm/100ml alcohols respectively, which correspond to 2.7 ml of acetic acid.

Conclusion: This work has confirmed that the production of acetic acid from sugar beet molasses is technically possible by fermentation with the consumption of specific yeast by the use of fruit ingredients. Furthermore, Saccharomyces cerevisiae produced large quantity of ethanol. Changing the sugar concentration from 10% to 12% did not had any impact on the quantity of ethanol produced.

Keywords: Acetic acid, Sugar Beet Molasses, Vinegar,Saccharomyces cerviceae,Saccharomyces pastorianus, Fermentation

Introduction

The genus of Acetobacter is named for its ability to produce acetic acid [1]. As such, acetic acid is produced naturally in fruits and some other food spoils, and it is one of the oldest chemicals known to mankind. The global demand of acetic acid is around 6.5 million Tons Per Annum (MTPA), of which approximately 1.5 MTPA is met by recycling; the remaining 5 MTPA is manufactured from petrochemical feed stocks or from biological sources. In 2018, the global production capacity of acetic acid reached almost 18 MTPA, and it is forecast to increase to some 21.66 MTPA by 2023 [2].

Acetic acid is produced both synthetically and by bacterial fermentation. Most acetic acid made for industrial use is made by one of the three chemical processes: methanol carbonylation, butane oxidation, or acetaldehyde oxidation [3]. Acetic acid can also be produced by fermentation: for this purpose, two types of fermentation are used, oxidative fermentation and anaerobic fermentation. For most of the human history, bacteria of the genus Acetobacter, given sufficient oxygen have made acetic acid, in the form of vinegar [4,5,6]. These bacteria can produce vinegar from a variety of alcoholic food stuffs. Commonly used, feeds include apple cider, wine, fermented grain, malt, rice, potato mashes [7], and mango juice [8]. A variety of sources can be used to supply carbohydrates and other nutrients for fermentation. In the manufacture of sugar from sugar beet, the principal by-product is “molasses”. Sugar beet (Beta vulgaris) roots contain a high concentration of sucrose [9]. Sugar beet is grown commercially for sugar. It produces a large (1-2kg) storage root whose dry mass is 15-20% sucrose by weight 3.5 % molasses 4.5 % dried pulp and a varying amount of filter cake [10].

Agular et. al. worked on the influence of low-cost carbohydrates as carbon sources on Brettanomyces bruxellensis growth [11], acetic acid and ethanol production in order to ascertain the viability of this yeast to eventually become an industrial acetic acid producer. Use of maize; Zea mays Ganga-5, hybrid variety, as a substrate for ethanol production by batch fermentation using Zymomonas mobilis was earlier demonstrated [12]. In this work we intend to demonstrate the production of acetic acid from sugar beet molasses by the process of fermentation using suitable yeasts extracts of Saccharomyces Cerevisiae (SC) and Saccharomyces Pastorianus (SP) and comparing the production of ethanol by using different fruit ingredients. Figure 1, highlight the biochemical reactions involved in the production of acetic acid.

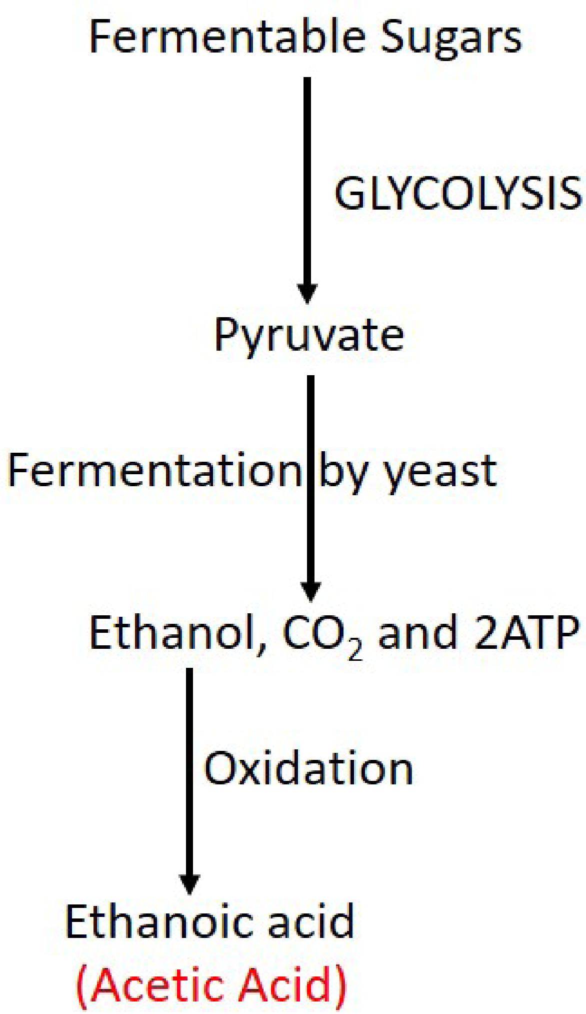

Figure 1: Illustrate the biochemical steps and process involved in the formation of acetic acid from fermentable sugars.

Material & Methods

Samples Collection

Sugar beet molasses was collected from local village which is rich in the growth of sugar cane and dry yeast; SC and SP was purchased from local bakers of these former is already known the production of fermented beverages [13].

Growth of Dry Yeast

The fungal spores were activated in nutrient broth media (Yeast extract 0.3%, and Peptone 0.5%) at pH 4.5 with 10% Volume/Volume ortho-phosphoric acid. Yeast was grown on nutrient agar medium, which was consisting of 0.5% of Peptone, Yeast extract 0.3% and agar 1.5% [14]. The fermentation ability of the yeast was evaluated by gas production in tubes containing 2.0% sugar solution (glucose or galactose) in the presence of both strain of the yeast.

Analysis of Molasses

Estimation of reducing, non-reducing, total sugars and nitrogen contents were carried out by titration methods. Molasses was clarified, at pH 4.0 molasses were boiled for half an hour. Cooled it, and at pH 6.0 CaO was added. After 24 hrs molasses were filtered. In tyndillazation, molasses were heated at 90˚C-100˚C for 30 minutes on each of three consecutive days and incubated at 37˚C in between. The pure sterilized molasses can used for further analysis.

Estimation of Sugars

Reducing sugars was estimated by titration method. 2g/100ml of glucose solution was prepared as a standard. It was titrated against Fehling solution, 3-5 drops of methylene blue were added as indicator. Reading was noted when Brick red color ppt formation occurred. Similarly, the volume of molasses required to decolorize the same amount of Fehling's reagent was noted for the determination of reducing sugars in it [15]. Sucrose in sugar beet molasses was hydrolyzed by the addition of succinic acid at concentration of 5% at the temperatures of 100˚C for 1 hour as described earlier [16,17,18]. After hydrolysis the same procedure was employed for total sugar as was employed for reducing sugar. Non-reducing sugars were determined by subtracting the reducing sugars from total sugar.

Production of Alcohol

Locally available sugar beet molasses having nearly 0.3-1.6% total reducing sugar and 14% other salts was used for alcohol production by fermentation of yeast. The total

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reducing sugar was adjusted to require percentage (10% and 12%) by hydrolysis and then, pH was adjusted to 5.5 and was steamed for 30 minutes. The salts like urea 0.2% magnesium sulphate 0.05% and yeast extract 0.1% were added to the diluted molasses making the total volume to 100 ml. For fermentation two batches containing 10% and 12% reducing sugars and required salts were selected for experiment. The fermentation media supplemented with 2% of fruits ingredient (Banana, Apple, Orange and Mango) were inoculated with 10% of homogenous inoculums of SC or SP. Three control flasks were run; two without the addition of fruit supplements having one kind of yeast and the third one, without the addition of any type of yeast having only 2% of fruit ingredient. All these flasks were kept at 40°C for six days and observed on daily basis of alcohol formation and sugar readings. The samples, 5ml were removed from every flask for alcohol estimation after a time of 2 days, 4 days and 6 days as already documented in the literature [19,20,21], that this time period are enough for the estimation of alcohol. Refractive index method was used to determine the concentration of ethanol [22-25]. Alcohols separated by distillation was 90.3% pure and it was further purified by "drying" and was made pure to 95% as documented earlier. [26].

Preparation of Acetic acid

Ethanol can be oxidized using variety of oxidizing agents to acetic acid [27]. We employed the use of sulphuric acid and sodium dichromate. It is two step chemical reaction, briefed in, Figure 2. Diluted 10 ml of sulphuric acid was taken in flask, 2-3gm Sodium dichromate and a few pieces of broken porcelain were added. The contents of the flask were swirled and heated slowly to make the solution homogenized. The mixture was cooled under a running tap. Then for refluxing, the Quickfit apparatus was set and 1ml of ethanol was added drop wise down the condenser into the flask and was boiled under reflux for 20 minutes. The apparatus was rearranged for distillation and 5ml of liquid was distilled.

Estimation of Acetic acid

Acetic acid was estimated by titration method. In this method a standard solution of 4 g of acetic acid per 100 mL of distilled water was prepared. 25 ml of solution was taken in 250 ml Erlenmeyer flask and was titrated against 1.0M NaOH using phenolphthalein as an indicator. When the titration was reached to equivalence point, (the area in the solution where the drop of base falls will turn pink) the reading of used NaOH in burette was noted. The same procedure was repeated for 30 ml mixture (unknown solution).

Statistical Analysis

Data were analyzed using SPSS 21. Comparison of ethanol production by different yeasts in gm/100 ml of sugar. It was carried out using Student's t tests. A value of $P<0.05$ was considered significant.

Results & Discussion

Acetobacter, Gluconacetobacter and Gluconobacter are the principal genera for aerobic acetic acid fermentations [7, 28]. Sugar beet molasses were analyzed which was composed of 0.78% of the reducing sugar and 58.7% non-reducing sugar of the total 60% sugar. Ethanol was produced using yeasts SC or SP with or without 2% fruit ingredients and one sample was without any species of yeast and fruit ingredients. SC has earlier been used in the production of Fermented Beverages (fruit). After the addition fruits maximum incubation time was 6 days, pH was maintained at 5.5 and temperature was kept 40°C. Two batches were kept for fermentation, one with 10% sugar concentration, and the 2nd one with 12% sugar concentration. After 6th

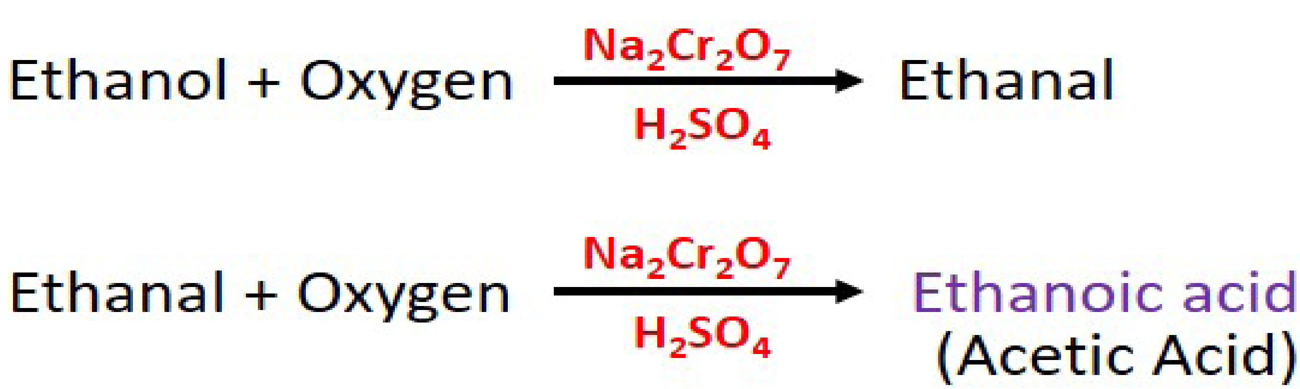

Figure 2: Illustrate the biochemical steps, process and catalyzing agents involved in the formation of acetic acid from ethanol.

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days in 10% sugar concentration media SC with 2% fruit ingredients consumed 7.9 gm sugar and produced 4.6 ml of alcohol, SP with 2% fruit ingredients consumed 5.6 gm sugar to produce 2.8 ml of ethanol. SC alone consumed 5.8 gm sugar and produces 2.9 ml of ethanol whereas SP consumed 4.7 gm sugar to produce 2.1 ml of ethanol. With 2% fruit ingredients but without yeast consumed 1.8 gm sugar and produced 0.7 ml of ethanol. These results are shown in figure 3 for 10% sugar concentration media. While in 12% sugar concentration media after 6th days SC with 2% fruit ingredients consumed 8.2 gm sugar and produced 4.9 ml of alcohol, SP with 2% fruit ingredients consumed 5.6 gm sugar to produce 2.7 ml of ethanol. Incubation of only SC consumed 6 gm sugar and produces 3.1 ml of ethanol and SP consumed 4.9 gm sugar to produce 2.2 ml of ethanol. Incubation of 2% fruit ingredients without yeast consumed 2 gm sugar and produced 1 ml of ethanol. These results are shown in table 1 for 12% sugar concentration media. For preparation of acetic acid a simple oxidation method was employed, by which pure 95% ethanol was converted to acetic acid. Then acetic acid was estimated by titration method and from 30 ml of ethanol 35.5 ml of acetic acid was obtained having percentage yield of 90.7%. In comparison of ethanol production by SC and SP (Table 1) SC produced better amount. However, change in the sugar concentration did not give any significant improvement in the production of ethanol.

Although sugar beet molasses has greater number of sugars than that of sugar cane [29] but have a little amount of reducing sugar which is not enough for fermentation. Therefore, sugar beet molasses was made suitable for fermentation by hydrolyzing it with succinic acid at concentration of 5% at the temperatures of 100˚C for 1 hour and converted approximately 60% non-reducing sugar to reducing sugar, as earlier described this is sufficient for the yeast activity [16].

Yeast are usually fully grown in two days and they convert maximum sugar to alcohol by the 6th day. SC with 2% fruit ingredients produced greater alcohol than that of the others. Because fruit ingredients provide essential nutrients for rapid growth of yeasts [30]. SP with 2% fruit ingredients produced decreased amount of alcohol as compared to SC with or without 2% fruit ingredients but produced greater amount of alcohol than that of SP. In the absence of any species of the yeast, there was no fermentation in the flask but after sixth day, due to contamination only 2% of sugar was converted to alcohol. Theoretical yield of 100% sugar is 51%. We were succeeded in obtaining 44% of actual yield. For preparation of acetic acid a simple oxidation method was employed, by which pure (95%) ethanol was converted to acetic acid. Acetic acid was estimated by titration method and from 30 ml of ethanol 35.5 ml of acetic acid was obtained having percentage yield of 118%. 100 gm molasses contain 58.21% reducing sugar, from which on fermentation 25.6 gm ethanol can be produce,

Conclusion

Fermentation is an encouraging method for the production of acetic acids from renewable biomass. In our experiment we have used bacteria-based method which can become major player in its production. We can produce 30.3 mL of acetic acid from 100 gm of sugar beet molasses. Therefore, it can be considered as the best substrate for acetic acid production under these conditions. However, limitations prevail, and requires the control of this experiment at larger scale in better sterilized conditions. Furthermore, Acetic acid thus produced needs the quality check by the quality control authority before its production at larger scale as varying manufacturing methods of Acetic acid has shown the influence on the physicochemical composition.

Conflict of Interest

The authors declare that they have no competing interests.

Table 1: Quantitative estimation of ethanol production by different yeasts in 10% and 12% gm/100 ml of sugar. The table compare the differences between the production of ethanol by the 6th day. SC produced good amount of alcohol in our experiments. Furthermore, no significant change is noticed in the production of ethanol after 6th day when sugar concentration is increased to 12%.

Incubated with	10% mg/100ml	12% mg/100ml
SC + Fruit Ingredients - 1	4.6	4.9
SP + Fruit Ingredients - 2	2.8	2.7
SC (Control for 1)	2.9	3.1
SP (Control for 2)	2..1	2.2
Only Fruit Ingredients	0.7	01

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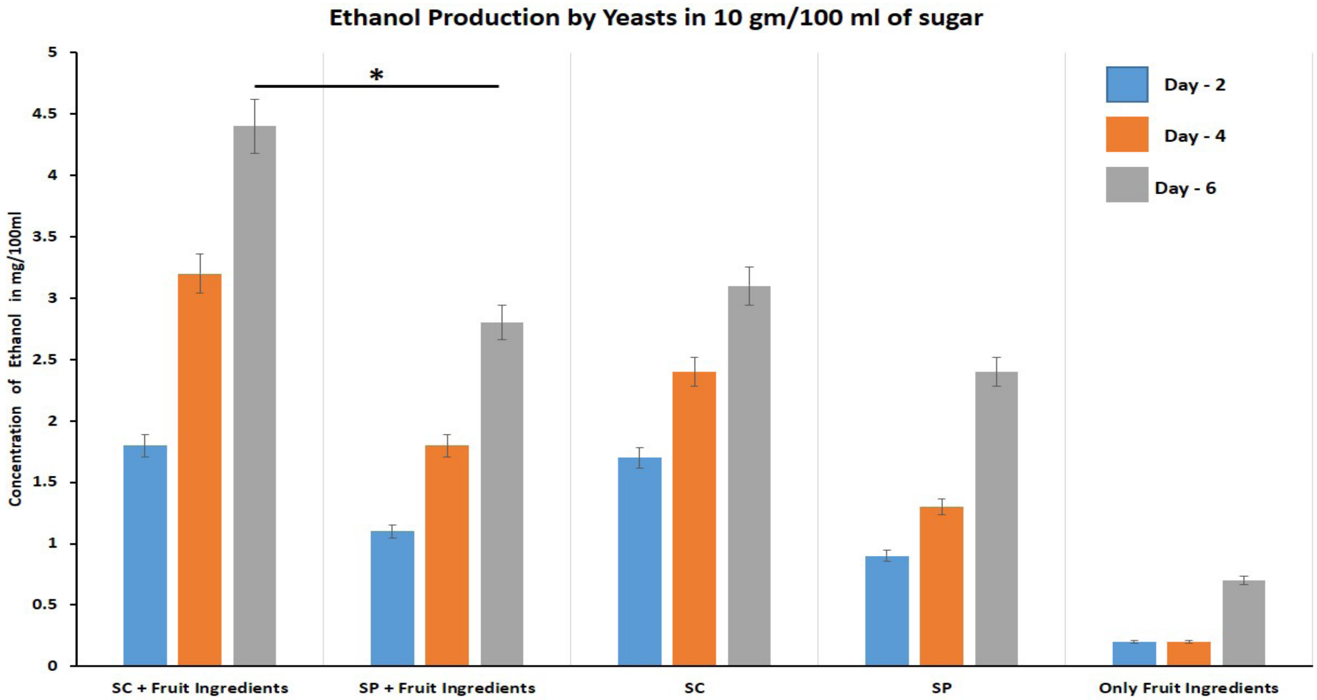

*Figure 3: Quantitative estimation of ethanol production by different yeasts in 10 gm/100 ml of sugar. The histogram represents mean ± SD from five different experiments. *P < 0.05, measured by the t-test.*

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