Production of Bioethanol from *Muntingia calabura* Fruits using *Saccharomyces cervisiae* and *Schizosaccharomyces pombe*

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Abstract

Bioethanol is the ethanol that has been produced from the biological source and the production of bioethanol otherwise can be called as wine preparation, or technically known as "Venification". The Jamaica cherry (Muntingia calabura L.) is tropical to near-tropical and production of bioethanol from the fruits of Jamaica Cherry was done successfully; process was optimized using two inoculums,Saccharomyces cervisiae and Schizosaccharomyces pombe at optimum conditions. The optimum conditions for the production of bioethanol using Saccharomyces cervisiae were determined as follows. The ethanol concentration was found to be optimum at pH 6.0 with a yield of 7.709% (w/v) by titrimetry and 7.172%(w/v) by GC-MS. The optimum temperature was found to be 34°C with yield of 7.250% (w/v) of ethanol titrimetrically and 7.810% (w/v) as per GC-MS method. The inoculum size of 8% was found to be optimum with a yield of 7.900% (w/v) of ethanol by titrimetry and 7.241% (w/v) by GC-MS. Furthermore, the optimum conditions for the production of bioethanol using Schizosaccharomyces pombe were determined as pH 6, temperature 31°C and inoculums size of 8%, yielded ethanol concentration of 5.228% and 5.290% (w/v), 5.770% and 5.670% (w/v), 5.228% and 7.241% (w/v), respectively. Saccharomyces cervisiae can produce ethanol more efficiently in comparison with Schizosaccharomyces pombe.

Keywords: Bioethanol, Fermentation, Jamaican fruits, Saccharomyces cervisiae, schizosaccharomyces pombe

Introduction

Bioethanol is ethanol that has been produced from a biological source. Bioethanol as a fuel additive decreases environmental pollution and reduce the stress of the decline in fossil fuel availability which is becoming popular in an increasing manner. Biomass for the production of ethanol includes cereal grains, lignocelluloses, or algae. The plant is commonly called in various names as "Singapore Cherry", "Jamaica Cherry", "Panama Cherry", "Strawberry Tree", "Jam Tree" and "Cotton Candy Berry". Usually, children are fond of fruits because of its candy like taste. The tree Muntingia calabura L. belongs to Muntingiaceae, the tree has several advantages such as: it is often cooked in tarts and made into jam. The leaf infusion is drunk as a tea-like beverage. Several works which has been done on leaves of Muntingia calabura L. will clearly claim the antibacterial capacity of the plant. Zakaria et al. (2006) shown that antibacterial potential of the plant is comparable to that of standard antibiotics used. Maragatham and Panneerselvam (2011) investigated on the preparation of wine from papaya. Sibounnavong et al. (2010) conducted experiment to produce wine from star gooseberry (Phyllanthus acidus (L.) Skeels and Carambola (Averrhoa carambola L.) by fermentation with Saccharomyces cerevisiae. The aim of the present study is to optimize fermentable conditions and Saccharomyces strains for the production of bioethanol using Muntingia calabura fruits.

Materials and Methods

Muntingia calabura fruits were collected from the botanic garden of Karnatak University, Dharwad (India) during March 2014. The fruits were first surface sterilized and macerated using pestle and

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mortar. The 1500ml of fruit juice was taken and added with 0.8ml of sodium metabisulphate to inhibit the growth of other microorganisms. The 0.75g of ammonium sulphate was added as a source of nitrogen. The juice was then pasteurised, cooled and inoculated separately with Saccharomyces cervisiae NCIM 3045 and Schizosaccharomyces pombe NCIM 3457.

After filtration and supplementation, the fruit juice was subjected to check for its pH. The 1500ml fruit juice was divided into 1000ml and 500ml. The 1000ml is set at a pH of 6.5. The rest 500ml of juice was divided into five parts in five conical flasks to set different pH of 3, 4, 5, 6, and 7. To set pH, 0.05N hydrochloric acid and 1N sodium hydroxide are used. After setting the pH, 5% inoculum was added to each flask. The pH set was now ready for fermentation and fermentation was carried out at room temperature. Similarly, the rest 1000ml of pH 6.5 is divided into 10 parts of 100ml each in 10 different conical flasks. Five of them are inoculated with different inoculum size such as 2%, 5%, 8%, 11% and 14% and kept for fermentation at room temperature. The rest flasks after inoculating with 5% starter culture are kept for fermentation at different temperatures such as 25, 28, 31, 34 and 37 °C using incubators. The 100ml of fruit juice was kept as a standard for each set. All the flasks were kept for fermentation for 15 days. After 15 days, the fermented product was subjected to titrimetric and GC-MS analysis for the estimation of ethanol. All the determinations were made in triplicate and standard deviation was calculated to evaluate the significance of data.

Results and Discussion

Temperature is the main factor which affects the growth rate, metabolism rate, viability, enzymatic activity and sensitivity of yeast cells. Even though each specific strain of yeast is active at a specific temperature, most of the strains show optimum activity at room temperature only. Thus the fermenting batches are allowed to ferment at room temperature except those which are kept at a specific temperature to check the activity of a yeast strain. Among different temperatures like 25, 28, 31, 34 and 37°Cfor Saccharomyces cervisiae, 34°C is found to be the optimum temperature, at which 7.259g of ethanol is found to beproduced titrimetrically and 7.810g with GC-MS. For Schizosaccharomyces pombe, 31°C is found to be the optimum temperature with the production of 5.776g of ethanol per 100ml titrimetrically and 5.671 by GC-MS. It means that at this temperature the yeast species can perform at its best to produce fermentation products (Table 1).

The pH is one of the important parameter which has a direct influence on the growth of yeasts. Most yeasts are active between a pH of 2.8 to 7. Below pH 2.8 the acidic pH does not allow the growth of yeasts. And above neutral pH, the yeasts are inactive. Just like temperature, the yeasts strains are also have specific pH at which they can perform well. Among different pH, for Saccharomyces cervisiae pH 6 is found to be the optimum with the production of 7.709g of ethanol per 100 ml estimated titrimetrically and 7.172g with GC-MS. The pH 6 is found to be the optimum for

Table 1: Effect of temperature on ethanol production using Saccharomyces cervisiae.

pH of the juice	Inoculum size % (v/v)	Temperature (°C)	% of ethanol production (w/v)*	
			By titrimetry	By GC-MS
6.5	5	25	6.332±0.14	6.100±0.18
6.5	5	28	6.428±1.12	6.715±0.35
6.5	5	31	6.450±0.15	6.031±0.64
6.5	5	34	7.259±1.10	7.810±0.02
6.5	5	37	6.879±0.15	6.892±0.09

*Each value represents mean±SD of triplicate determinations.

Schizosaccharomyces pombe with the production of 5.228g of ethanol per 100ml by titrimetrically and 5.290 with GC-MS.

The standardization of inoculum size is important as sugar consumption is a balance between biomass development and ethanol production. Generally for wine production 2-3% of inoculums size is used. For the present study 5 different inoculum sizes were considered and they are 2%, 5%, 8%, 11% and 14%. The optimum inoculum size in case of Saccharomyces cervisiae is found to be 8% which gives 7.900g of ethanol per 100ml by titrimetricallyand7.241g by GC-MS. In case of Schizosaccharomyces pombe optimum inoculums size is 8% with the production of 5.228g of ethanol per 100ml titrimetrically and 5.290g with GC-MS.

Production of bio ethanol using Saccharomyces cervisiae and Schizosaccharomyces pombe were determined as follows: (1) For saccharomyces cerevisiae the ethanol concentration was found to be optimum at pH 6.0 with a yield of 7.709 % (w/v) of ethanol by titrimetry and 7.172 by GC-MS. The temperature optimum was found to be 34°C with a yield of 7.250% (w/v) of ethanol titrimetrically, 7.810 % (w/v) as per GC-MS. The inoculums size of 8% was found to be optimum with a yield of 7.900% (w/v) of ethanol by titrimetry and 7.241 % (w/v) by GC-MS. (2) For Schizosaccharomyces pombe the ethanol concentration was found to be optimum at pH 6.0 with a yield of 5.228 % (w/v) of ethanol by titrimetry and 5.290% (w/v) by GC-MS. The temperature optimum was found to be 31°C with a yield of 5.770% (w/v) of ethanol by titrimetry and 5.670% (w/v) by GC-MS. The inoculums size of 8% was found to be optimum with a yield of 5.228% (w/v) of ethanol by titrimetry and 7.241% (w/v)by GC-MS.

Conclusion

A result of the present study indicates that fruits of Jamaica cherry could be used as a fermentable substrate for the production of bioethanol using Saccharomyces cervisiae and Schizosaccharomyces pombein fifteen days. Moreover, it is evident that Saccharomyces cervisiae produces bioethanol more efficiently in comparison with Schizosaccharomyces pombe.

References

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