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Cost effective method for production of ethanol from sugar beet and its estimation by modified dichromate method

Nakade Dhanraj B.

Dept. of Microbiology, Govt. Rajaram college, Vidyanagar, Kolhapur (MS) India.

ABSTRACT

Sugar beet, cultivated Beta vulgaris, is a plant whose root contains a high concentration of sucrose. It is grown commercially for sugar production. The root of the beet contains 75% water, about 20% sugar and 5% pulp. By considering this in the present study, Sugar beet roots were used to produce ethanol using Saccharomyces cerevisiae. After fermentation at 30^{O} c for 48 hours ethanol was purified and estimated by modified dichromate method. For estimation 10ml of Acid dichromate solution was taken in BOD bottle. The ethanol sample from fermented broth was kept in hanging position in 2ml eppendroff vial for 24 hours. The ethanol from sample was converted in to ethanolic acid by dichromate solution. Remaining unused dichromate solution was then estimated with thiosulphate solution to determine ethanol percentage. Results indicated that this method is 99 % effective in estimation of ethanol and can be used as one of the best method for ethanol production.

Keywords: Ethanol, Beta vulgaris, *Saccharomyces cerevisiae* ,Sugarbeet roots, sugar beet.

INTRODUCTION

Fossil fuels provide 80% of the energy needs worldwide and the combustion of fossil fuels account for 73% of worldwide carbon dioxide emissions [1,2]. The gradual depletion of fossil fuel reserves, increasing energy demand and concern over the greenhouse gas emissions is a need for the research and development of alternative and renewable energy sources.

International crude price has crossed $100 per barrel and is increasing day by day, therefore it is the high time to think about the fuel need of today's world. Today's energy demand has been fulfilled by fossil fuels like oil, coal and natural gas, but the resources are limited hence it is necessary to switch over for alternative energy sources[3]. Ethanol is one of the major solution to fulfill energy demand and Government of India has taken the decision to mix 5 % of ethanol in petrol and diesel and is likely to increase 20% up to 2017.

World ethanol production has reached 102 billion liters, In India there are 595 distilleries producing 2600million liters of ethanol as against annual requirement of 3120 million liters[4,5]. This provides an immense scope for agricultural sector. Secondly, production of ethanol from sugarcane molasses is expensive and needs to search for more chiefer substrate that can be easily available and ecofriendly. As there is a great problem in disposal of distillery spent wash generated from cane molasses.

Several countries are using different food crops such as wheat, maize, barley, sweet sorghum, lignocellulosic material, biomass straw, However sugar beet is easily cultivated in adverse climatic conditions and available in sufficient quantity in India.

As it is a short term crop requires 5 months period for harvesting, fit well for crop rotation which is of special value for crop formers, it can be cultivated by crop rotation which helps to increase soil fertility and it is possible to develop superior variety. ***Beta vulgaris*** grows in adverse climatic conditions, requires less water and fertilizers as compared to sugar cane that's why it is cultivated on large scale.

By considering this in the present study, Sugar beet roots were used to produce ethanol using ***Saccharomyces cerevisiae*** and quantity was estimated using newly developed dichromate method.

MATERIALS AND METHODS

1]Collection of sugar beet: Healthy sugar beet roots were procured from farmers of village Mangaon Ta: Hatkanangale Dist: Kolhapur, Maharashtra, India.

Soil was removed from roots by brush and packed in polythene bags and brought to laboratory.

2] Extraction of juice for fermentation: A total of 6 kg of pulp was prepared by crushing chopped beet root pieces in mixer grinder by adding 10 % distilled water and filtered through musline cloth to obtain raw juice concentrate. Sugar content was determined as per AOAC [6,7,8]. A total of three one liter flasks containing 500 ml of juice concentrate were used for fermentation.

3] Preparation of starter culture: Yeast *Saccharomyces cerevisiae* procured from distillery was used for experiment. One loopful inoculum of *Saccharomyces cerevisiae* was transferred to a test tube containing 5 ml of sterilized Yeast extract peptone broth. It was incubated at room temperature for 12 hours. Five ml of inoculum containing 10^8 CFU/ml of Yeast cells was inoculated in to 500ml of beet juice.

4] Fermentation: Flasks were incubated at 30^OC for 48 hours. It was centrifuged to remove Yeast cell and filtrate was used for estimation by modified dichromate method.

5] Estimation by Williams and Reese modified dichromate method[9].

- *Principle*

This method uses a redox titration to find the concentration of ethanol in a distillate solution. The ethanol is oxidized to ethanoic acid by reacting with an excess of potassium dichromate in acid.

The amount of unused dichromate was then determined by adding potassium iodide solution which is also oxidized by the Potassium dichromate releasing iodine.

The iodine was then titrated with a standard solution of sodium thiosulfate till it becomes colorless (End point, Fig.1-4) and the titration results were used to calculate the ethanol content of the original solution.

Because alcoholic Distillate may contain other oxidisable substances that could interfere with the titration, the dichromate solution was placed in a BOD bottle and the alcoholic Distillate sample was suspended in a small Eppendroff tube above it.

Equipment Used
300 BOD bottles with toppers, burette, 2 ml Eppendroff vials, Distillate sample 10ml and 1 ml pipettes, 10 Incubator .
Solutions Used: Acid dichromate solution: (0.01 $molL^{-1}$) in 5.0 $molL^{-1}$ sulfuric acid)
Starch Indicator-1% solution., Sodium thiosulfate solution: (1.0 $molL^{-1}$)
Potassium Iodide solution : (1.0$molL^{-1}$)

METHOD
10 ml of Potassium dichromate was inoculated in two 300 ml BOD bottle . One bottle was labeled as Blank and other as sample(Fig.1).

In the sample bottle Eppendroff tube containing 1 ml of distillate sample was inserted above the dichromate solution.

Both the bottle were incubated at 30^OC for 24 hours.

After incubation Eppendroff tube was removed from the bottle and to the dichromate sample 1 ml Potassium Iodide was added. Here unreacted dichromate reacts with Potassium Iodide and releases Iodine(Fig.2).

It was added with 1 ml of 1 % Starch indicator (Fig.3) and titrated with Sodium thiosulphate solution till solution becomes colorless (Fig.4).

Then by subtracting blank reading from sample reading amount of thiosulphate required was determined and alcohol concentration was determined as per the following equation.

1 mol of Sodium thiosulphate required is equivalent to 0.25 mol of Ethanol.

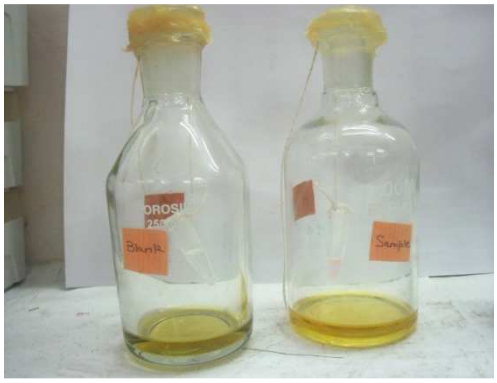

Fig.1.Initial Blank and Sample

Fig.2.After addition of KI with release of Iodine (Brown)

Fig.3. After addition of Starch

Fig.4. End point (Colourless)

RESULTS AND DISCUSSION

In this research, ethanol production from raw sugar beet juice was studied in comparison with ethanol from cane molasses. The main goal of this investigation was to develop a accurate method for estimation of alcohol.

The initial sugar content of juice was 16 % further sugar percentage was increased by adding 4 % starch. Total concentration was made 20%. Total yield of ethanol after 36 hours was 63 g/L with cane molasses and 58 g/L with sugar beet. Ethanol percentage was found to be 93.4 %.

A relatively high yield 63 g/L with cane molasses and 58 g/L with sugar beet corresponding to a 93.4 % conversion efficiency was obtained using the natural pH of the juice, which was close to a pH of 4.5 Ogbonna also found similar results[10].
Balcerek [11] studied the efficiency of alcohol fermentation using thick juice of sugar beet, they obtained yield of 87.54% however, our study indicated the yield 93.4 %, which was higher than reported.

Dodic [12] investigated the effect of initial sugar concentration on ethanol production using commercial baker's yeast for 72 h at 30°C. They observed, that if the initial sugar concentration was increased from 20 to 25% , the ethanol concentration decreased. However, our results indicated that increase in sugar concentration from 16 % to 20% increased ethanol concentration. The study of Dodic showed that intermediate products of sugar beet

processing, such as thick juice can be used for ethanol production and is just as efficient as molasses. We also found even the diluted juice of sugar beet as efficient as that of molasses. Similar results with [13,14,15].

The chemical composition of juice used for this study was typical sugar beet. The high content of saccharose is useful to high yield of ethanol.

CONCLUSION

On the basis of results obtained it was clear that sugar beet can be used successfully for ethanol production as the sugar beet contains sufficient amount of nitrogen and phosphorous, which supports the growth of yeast there is no need to add nutrients and inorganic salts for growth. The ethanol produced from sugar beet has a considerable impact on the final price of ethanol as a fuel. The cost of ethanol production by cane molasses is 35 to 40 rupees per liter where as the cost of ethanol from sugar beet was just 10 rupees per liter. Thus sugar beets are very much feasible for ethanol production and can be used on large scale for ethanol production. Further modified dichromate method used for estimation gives 99 % accurate results we recommend this method for estimation of ethanol. Further it can be concluded from this work that tropical sugar beet is a viable crop for ethanol production that requires no additional nutrients, enzymes and no additional water or pH adjustments to obtain a high yield of ethanol.

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