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RESEARCH ARTICLE

WASTE POTATOES AS AN ALTERNATIVE SOURCE FOR THE PRODUCTION OF BIOETHANOL BY CO-CULTURE OF SACCHAROMYCES CEREVISIAE AND ASPERGILLUS NIGER

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ABSTRACT

Bioethanol is one of the energy sources that can be produced by renewable sources. Waste potatoes relatively inexpensive as compared with other feedstock considered as food sources. However, a pretreatment process is needed. In Chitrakoot a much amount of potatoes are produced every year. This research was carried out to find out the optimum conditions for bioethanol production. The protocol was used by co-cultures of *Saccharomyces cerevisiae* and *Aspergillus niger* under simultaneous saccharification and fermentation (SSF). Bioethanol production was done at different temperature (25 to 35°C), pH (4 to 6) and fermentation protocol was done at 96, 120, 144 hours. The optimal condition for the production of bioethanol was 6 pH and 30°C temperature. The fermentation of potatoes was the highest at 144 hours with yeast concentration 4 to 6 %.The maximum ethanol yield from waste potatoes was 11.14% and amount of ethanol content increased with the increase in fermentation time.

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INTRODUCTION

Bioethanol is an alcohol, made by fermentation, mostly from carbohydrates produced from a large variety of natural renewable materials such as agricultural crops, forest products, as well as from industrial and domestic waste such as paper, textile and beverages. Ethanol can be used as a fuel in vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Another benefit over fossil fuels is the green house gas emissions. In the present scenario, there is a growing interest for ecologically and economically sustainable biofuels production. In the past years, feasibility of lignocelluloses containing materials for ethyl alcohol production has been explored depending upon the availability in the region (Shindo and Tachibana, 2006). In recent years, in the most developed countries, more than 90% of total energy comes from non-renewable fuel sources and they impart fuel sources for the energy (Lee *et al.,* 2012).This relationship causes global energy crisis because of their non-sustainability. An ethanologenic microorganism capable of fermenting all sugars released from lignocellulosic biomass through a saccharification process which is essential for secondary bioethanol production (Yanase *et al*., 2012).

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Ethanol produced from biomass-based waste materials is considered as bio-ethanol. Bioethanol as an alternative source of energy and it has received special attention worldwide due to depletion of fossil fuels. Bioethanol production from potatoes is based on the utilization of waste potatoes. Waste potatoes are produced from 5-20 % of crops as byproducts in potato cultivation. (Ghosal *et al.,* 2013) The cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and expensive. Under such circumstances a novel approach is to use renewable substrates such as potato waste. Renewable energy attracts attention for the protection of the environment and supplies our energy needs by reducing dependency on petroleum and non-renewable energy sources (Mishra *et al.,* 2014). Ethanol has been widespread used as a solvent of substances intended for human contact or consumption, including perfumes, flavours, colourings and medicines.

The economics of ethanol production by fermentation is significantly influenced by the cost of raw materials, which accounts for more than half of production cost. In recent years efforts have been directed towards the utilization of cheap renewable agricultural resources such as banana peel, waste paper, sugarcane waste as alternative substrate for ethanol production (Pranavya *et al.,* 2015). Fermentation processes are being extensively used in the biotechnology, pharmaceutical, food and beverage industries. Typically, fermentations utilize microorganisms (bacteria and yeast) to produce a desired product from a substrate. Ethanol produced from glucose in the presence of oxygen by aerobic fermentation. With a huge agricultural resources, abundant livestock, and cost competitiveness, India is fast emerging as a sourcing hub for processed beverage. The Indian beverage processing industry accounts for 32 per cent of the country's total beverage market. (Chandra and Mishra, 2015) In Chitrakoot huge amount of potatoes and sweet potatoes are produced, which are expected to be used as the promising source of bioethanol. For the production of potato, farmers deprived of retail price every year. Alternate use of potato like bioethanol production would ensure potato price at farmer's level. Therefore, this research work was done considering the availability of raw materials and optimum condition in Chitrakoot.

MATERIALS AND METHODS

Sample Collection

Waste potatoes were collected from the vegetable market of Sitapur, Karwi and Rajapur Chitrakoot. Before processing it was cleaned to be free from sand, stones, soil and potato foliage. Thoroughly washed unpeeled potatoes were cooked in a pressure cooker in distilled water containing 0.5% potassium metabisulphite for 30 minutes. Boiled potatoes were mashed, dried at 70°C for about 7 hours in a hot air oven and ground to fine powder.

Isolation of microorganisms and its maintenance

Soil samples were collected randomly from three different sites such as Rajaula Krishi farm, Arogyadham and Ganiwa farm from the top 2 cm of the soil profile. Approximately 50 g of soils were collected from each site and put into plastic bags and brought to the laboratory. All the soil samples were air-dried at room temperature (27±1°C) for 24 to 48 h. The dried soil samples were processed to remove stones and plant residues. 100 mg of each soil samples were transferred to labeled test tubes containing five milliliters of sterile saline (0.9% NaCl). In order to suppress bacterial growth, 30 mg/l of streptomycin was added. Each of the test tubes were vortex mixed until all soil was well dispersed throughout the tube. 100 µl of each of the suspension was evenly spread on PDA plates with a spreader and incubated at 27±1°C. Mixed colonies on the plates were observed after 5–7 days. Pure culture of *Aspergillus niger* was obtained by streak plate method. It was then maintained on PDA slants at 4°C. Yeast strain *Saccharomyces cerevisiae* (MTCC 173) was obtained from the MTCC Chandigarh. It was maintained on PDA slants at 4°C.

Starch hydrolysis test of isolated strains of *Aspergillus niger*

A loopful of pure culture was streaked on a sterile plate of starch agar medium. The inoculated plate was incubated at 27°C for 5 to 7 days. After incubation, Iodine reagent was added to flood the growth. Presence of clear zone surrounding colonies confirmed the positive result and accounts for their ability to digest the starch and thus indicates presence of alpha-amylase.

Simultaneous Saccharification and Fermentation (SSF) of Powdered Potato wastes

Ethanol fermentation was carried out in 250 ml flasks containing 5g powdered potato in 96 ml distilled water. The flasks was sterilized by autoclaving at 121°C for 30 min and inoculated with 6% (v/v) inoculums of *Saccharomyces cerevisiae* and 4% (w/v) inoculums of *Aspergillus niger*. The flasks were kept for incubation of fermentation process and the ethanol content was measured at different incubation time 96, 120 and144 hours.

Effect of temperature and pH on ethanol production

Fermentation of potatoes was carried out at different temperatures (25 to 35°C) pH (5 to 7) and incubation period 96, 120 and 144 hours. The optimum temperature and pH were obtained in the course of investigation. It was further used for fermentation at yeast concentration 4% to 6%.

Estimation of ethanol content by gas chromatography

A gas chromatograph equipped with a flame ionization detector (FID) and data acquisition system with computer software (IRIS 32) was used to analyze the ethanol concentration. The installed column was a Capillary column (30 m). Temperature programming was implemented for the liquid sample analysis by (Rath *et al.,* 2014). During the analysis, the oven temperature was maintained at 80°C.

The formula used for the calculation of percentage of ethanol is given below:-

$$\% \text{ of Ethanol} = 100 - \left\{ \frac{\text{Volume of standard ethanol x conc. of ethanol x 100}}{\text{Area of standard ethanol}} \right\}$$

RESULTS AND DISCUSSION

The result of the present investigation showed that the fermented potato produced a significant amount of ethanol. The production of ethanol varied according to the variations in temperature pH and at different incubation time.

Effect of pH on ethanol production

pH value has significant ascendancy on alcoholic fermentation. pH of bioethanol produced from different sample were determined. Yeast survives in a slightly acidic environment with pH between 4 to 6. The ethanol production was examined for inoculated sample for 96, 120 and 144 hours and the changes were noted down. The effect of pH 4, 5 and 6 on bioethanol production from waste potatoes at different incubation period 96, 120 and 144 hours in simultaneous saccharification and fermentation (SSF) at 30 °C is indicated in figure 1. The highest bioethanol production was P^H 6 (10.21%) at 144 hours and followed by pH 5 (6.24%) at 144 hours and P^H 4 (5.31%) at 144 hours. pH 6 was the most efficient yielding a higher value of ethanol as compared to other P^H 4 and 5. Therefore p^H 6 is the most efficient for the ethanol percentage increased with the increase in fermentation time. Afifi *et al,* (2011) reported the maximum ethanol yield at pH 6 from Potato Wastes in Solid State Fermentation by

Saccharomyces cerevisiae. Similar finding was also reported by Meenakshi and Kumaresan (2014) in which the maximum ethanol production from Corn, Potato Peel waste was found at pH 5.5 by using *Saccharomyces cerevisiae*. The maximum rate of ethanol production at pH 6 was reported by Rath *et al*, (2014) and Azad *et al,* (2014).

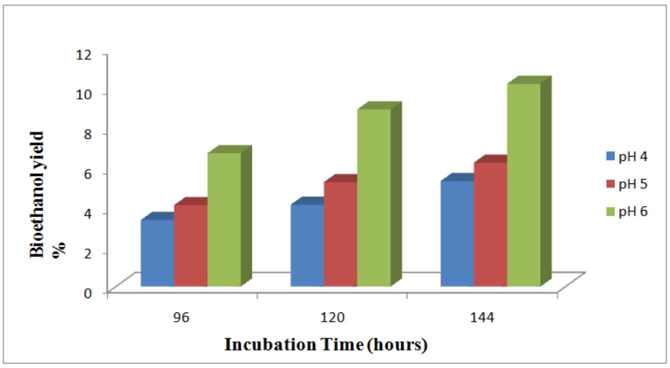

Figure 1. Ethanol yield from waste potatoes with the change in pH (4 to 6) for different incubation period at 30ºC

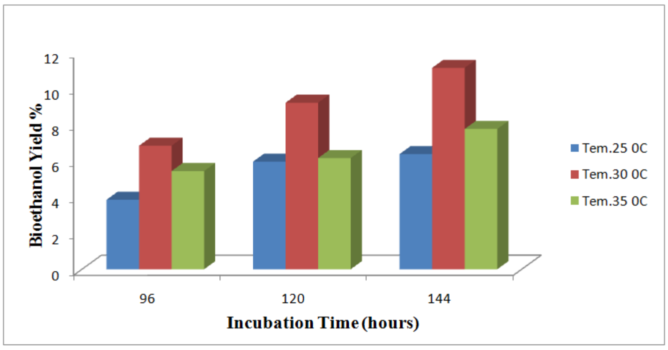

Figure 2. Variation in ethanol yield from waste potatoes with the change in temperature (25 to 35ºC) for different incubation period at pH 6

Effect of incubation temperature on ethanol production

Temperature plays a major role in the production of ethanol, since the rate of alcoholic fermentation increases with the increase in temperature between 25 to 35 ^{0}C. The ethanol production was analyzed for inoculated sample for 96, 120 and 144 hours incubation and the changes were recorded. The variation in ethanol yield from waste potatoes at incubation temperature 25, 30 and 35ºC for different incubation period 96, 120 and 144 hours at pH 6 is indicated in Figure 2, The maximum ethanol production was observed at temperature 30ºC (11.14%). The result of our findings concord with Janani *et al*. (2013) and Rath *et al.* (2014). Manikandan and Viruthagiri (2010) observed the optimum temperature to be 30ºC for the ethanol production from corn flour using *Aspergillus niger* and non starch digesting and sugar fermenting *Saccharomyces cerevisiae*. Neelakandan and Usharani (2009) found the maximum ethanol yield from cashew apple juice using immobilized yeast cells by *Saccharomyces cerevisiae* at 32 ºC.

Conclusion

Simultaneous fermentation of starch to ethanol can be conducted efficiently by using co-cultures of the sugar fermenter, *Saccharomyces cerevisiae* and fungus, *Aspergillus niger*. In Chitrakoot area, we have analyzed waste potatoes, in which starch component is present in sufficient quantities. It is suitable for production of ethanol. By encouraging farmers to increase potato production in large amount biofuels can be produced. Biofuels production would reduce dependency on traditional fuel and help in the country's economic growth and energy region will reinforce. Overall it is suggested that waste potato is very useful for the production of bioethanol.

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