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Production of Ethanol from Spoilt Banana (*Musa paradisiaca*) using Immobilized Cells

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Ethanol was produced by fermenting spoilt bananas using *Saccharomyces cerevisiae* (MTCC183). Total sugar content in banana peels; raw and ripened bananas was estimated by Fehling's method. Depending on the stage of ripening, banana peels were found to have total sugar content of 4.07%(v/v) to 7.6%(v/v), while whole ripened bananas were found to have 15.2%(v/v). *Saccharomyces cerevisiae* (MTCC183) was immobilized in sodium alginate by entrapment method. Viable count of beads was carried out to enumerate number of organisms present. 1 bead contained 9×10^7cells/ml.

Ethanol production was studied at laboratory scale and bench scale levels. Fractional distillation was carried out to recover the alcohol from the spent fermented broth, while the ethanol content in the distillate was estimated by both dichromate method and specific gravity. Fermentation medium using 100g of spoilt banana yielded 24% to 27 %(v/v) of alcohol. Ethanol yield between free cells and immobilized cell systems was drastically different. Immobilized systems yielded 23% to 27 %(v/v) alcohol while free cell system yielded only 15%(v/v) alcohol. Purity of the distillate was determined using Gas chromatography (FID) and High performance thin layer chromatography. Moisture content in the distillate was determined using Karl Fischer reagent and accounted for 10%.

Key words: Ethanol, Banana, Immobilized Yeasts.

Ethanol is generally produced from petroleum products. Massive urbanization and the ever rising industrialization signify rapid utilization of natural energy resources such as fossil fuel, petroleum and coal. These resources are suggested to last for a few years. Therefore, alternative energy sources such as ethanol, methane and hydrogen are being considered. Some biological processes have rendered possible routes for producing ethanol and methane in large volumes. A worldwide interest in the utilization of bio-ethanol as an energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production.

Ethanol made from biomass provides unique environmental, economic strategic benefits and can be considered as a safe and cleanest liquid fuel alternative to fossil fuels. There is a copious amount of lignocellulosic biomass worldwide that can be exploited for ethanol production. Significant advances have been made at bench scale towards generation of ethanol from lignocellulosics (Ravindra *et al*, 2007).

In recent years production of alcohol using fermentation technology is of considerable interest. In the United States, more than 70% ethanol is produced by synthetic methods. Brazil has been producing large quantities of ethanol since 1975 from sugarcane and Cassava. It is the second largest sugarcane producing country in the world (Inamdar 2006). Until the oil crisis of October 1973 created by OPEAC, only India appeared to appreciate the importance of fermentation alcohol as a strategic material to its

economy (Agarwal & Parihar, 2005). At present India is producing about 1.3 billion liters of ethanol mainly form sugarcane molasses in about 270 distilleries. However, since molasses being the cheapest and the richest sugary raw material in India, ethanol produced from it is apparently the cheapest. Since the sugary crops such as sugarcane and sugar beet are utilized for food purposes, their availability for the large-scale production ethanol is meager. All beverage alcohol is produced by fermentation of cereal grains, sugar molasses's and other materials with high starch and sugar contents or starch hydrolysates derived from cereal crops example: maize, barley, grain sorghum and root/tuber crops example cassava, potato, Jerusalem artichoke etc.

Fruits being highly nutritive are an important component of human diet but they possess very short post-harvest life. As they ripen they become very soft and become more prone to injuries, which make them highly perishable. In India, 30% of annual produce is wasted due to spoilage. The remaining un-consumed part also adds to the biomass waste products. Thus there is an urgent need to develop technologies to overcome post-harvest losses of fruits.

Banana is one such, commonly found fruit throughout the year. Apart from 27% of the edible portion the remaining adds to waste. Banana peels can thus be used as a substrate for production of alcohol. The presence of 27.2 % of carbohydrate in whole makes it a potential substrate for production of alcohol (Modambi & Rajagopal,1982).

MATERIAL AND METHODS

Maintenance of culture

Saccharomyces cerevisiae MTCC 183 was grown in Sabourauds broth and maintained on Sabourauds agar slants overlaid with paraffin oil. During each use of the culture, Gram staining was done to check the purity of the culture.

Collection of substrate

Bananas collected from a local vendor were used for studying ethanol production and determining sugar content. Depending on the age and extent of visual deterioration bananas were selected. To carry out studies on ethanol production, spoilt ripened bananas were selected. One banana approximately weighed 70-80g hence depending on the weight of substrate required for study; numbers of bananas per study were selected. Production of alcohol at a bench scale was done using 3-5 bananas while laboratory scale used 10 bananas. Also to determine variation in sugar content each time 3-5 bananas were used. All studies were done in triplicates.

Determination of sugar content

Sugar present in raw banana, peels and whole ripened banana was determined by Fehlings method (BIS, 1984) after necessary acid hydrolysis of the samples.

Immobilization of S*accharomyces cerevisiae* (mtcc183) in alginate beads (5)

Saccharomyces cerevisiae MTCC 183 was grown in Sabourauds broth. Cells were harvested (Eltek TC 615) and washed. Wet weight of cell pellet was determined (Contech) and mixed with 4% Na- alginate solution in equal proportion. The mixture was aseptically dropped into a solution of chilled 6% $CaCl_{2;}$ the beads were allowed to cure at 20-22°C for 1h, washed with sterile distilled water and equilibrated overnight in 6% $CaCl_2$. Beads were collected and activated before use.

Bench scale studies

100ml of distilled water was added to 50g spoilt whole banana in a 250ml conical flask. The flask was autoclaved at 121° C for 20 min. After autoclaving the pH of the medium was adjusted to 6 using 1N NaOH. A culture inoculum of 25ml of activated beads was added to the medium and flask incubated at room temperature for 48 h. Similarly studies were carried out using 250g of spoilt whole banana in 500ml of distilled water.

Laboratory scale studies

1600ml of distilled water was added to 800g of spoilt whole ripened banana in a 2000ml aspirator bottle (Shcott-Duran). This aspirator bottle was autoclaved at 121 °C for 20 min. After autoclaving the pH of the medium was adjusted to 6 using sterile 5N NaOH. A culture inoculum of 400ml of activated beads was added to the medium. The aspirator bottle was incubated at room temperature for 48 h.

Comparison between free and immobilized cell systems

To compare ethanol yield between

immobilized and free cell system laboratory scale studies were carried out in aspirator bottles. Fermentation medium was prepared as described earlier. In test containing immobilized cells, a culture inoculum of 400ml of activated beads was added while free cell system a culture inoculum of 80ml containing 9×10^8cells/ml was added. After 48 h the spent medium was harvested by centrifuging (Eltek TC 4100) for 20 min at 6000 rpm and the cell free supernatant was used for distillation.

Fractional distillation and analysis

Distillation assembly was set up as per manufacturers instructions, all joints lubricated with Vaseline before starting the process. Temperature was adjusted to 78°C and distillate was collected in an ice bath. Ethanol content was studied qualitatively and quantitatively by cerric ammonium nitrate test and Dichromate method (BIS, 2002) respectively. Absolute ethanol was used as the reference standard in the dichromate method of estimation. Moisture content was analyzed by Karl Fischer (Vigomatic) while purity of the fractionally distilled sample was assessed by HPTLC (Camag linomat TLC scanner 3) and Gas chromatography (FID - Shimadzu 14 GB) injected on a column (2mm × 4mm dia) consisting of Carbowax 20M on Chromosorb W, 80-100 mesh in a Carlo Erbe (14GB) chromatograph equipped with flame ionization detectors.

RESULTS AND DISCUSSION

The present study was aimed to use spoilt banana as a substrate for ethanol production and thus to develop biofuels from fruit waste. Present day losses due to post harvesting practices are high and these may be exploited for production of ethanol. Entrapment of whole cells in alginate was carried out, as it is an effective technique whereby cell losses were minimized during fermentation. Banana a fruit commonly found throughout the year has a carbohydrate content of 27% and hence desirable as fermentation medium for ethanol production.

Bananas were selected for the present study depending on the age and extent of visual deterioration. The sugar present in them was estimated by Fehlings method. Standard deviation for standard invert sugar was found to be ± 0.11.

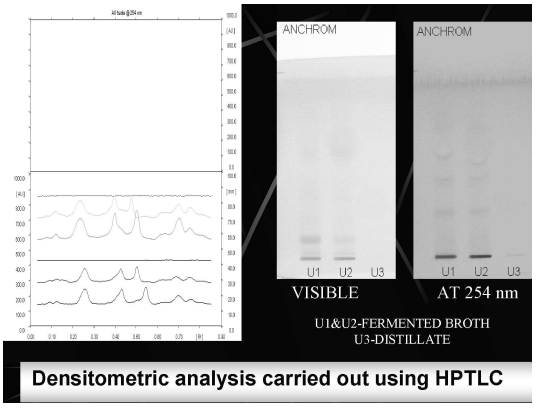
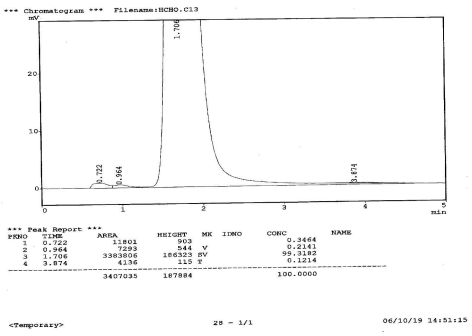


Fig. 1. Densitometric analysis of distillate



Chromatogram of distillate by GC (FID)

Fig. 2. Gas chromatogram of distillate

Fig. 3. Laboratory scale study of ethanol production

Banana peels were found to contain 4 %(v/v) of reducing sugars whereas raw banana were found to contain 7.8 %(v/v) of reducing sugars and spoilt whole bananas contained 15%(v/v) reducing sugars (Fig 1). Bansal *et al.*(2003) found that a sugar concentration of 15% v/v was optimum for ethanol fermentation.

Saccharomyces cerevisiae (MTCC183) was entrapped in sodium alginate. Beads prepared with 2% alginate concentration were found to dissolve in course of fermentation hence beads using 4% alginate were prepared and used. Viable count studies of the bead indicated presence of 3.91×10^8 cells /bead.

Qualitative detection of alcohol in distillate confirmed the presence of ethanol in the sample. Alcohol in distillates was detected by Dichromate method. Fermentation studies carried out at lab scale in 200ml(fig 3) and 1litre capacity gave an alcohol yield of 15% to 18% (v/v), scaling up the process to 2litre capacity showed no deviation in result. While relating the alcohol yield to fermentation medium using 100g of spoilt banana 24% to 27 %(v/v) of alcohol was obtained. Ethanol yield between free cells and immobilized cell systems was drastically different (Ravindra *et al,* 2007). Immobilized systems yielded 23% to 27 %(v/v) alcohol while free cell system yielded only 15%(v/v) alcohol. Moisture content analysis for fractionally distilled sample at 97°C was carried out using pyridine free Karl Fischer reagent. Sample was found to contain 10% moisture. High performance thin layer chromatography (Fig 1) showed absence of organic impurities, as also results of Gas chromatography (Fig 2).

CONCLUSION

Ethanol has been successfully produced by using a fruit waste. The technology is old but plant scale up studies would help evaluate the actual yield of ethanol. However, being an anaerobic fermentation investment cost needed for large aerobic fermentations can be minimized as also the use of immobilized cells circumvents the use of high cell concentration in a fermentor. Thus immobilized cells offer high productivity without risk of high cell loses in cell free systems.

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