A comperative study of Bioethanol Production ability of *Bacillus subtilis* and *Sacchromyces cerevisiae* using Banana and Orange Peels

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Abstract— In present in present investigation a comparetive analysis of bioethanol production ability of *S. cerevisiae* and *B. subtilis* is done on varies parameters such as temperature, pH and incubation time to determine the best optimal conditions of bioethanol production using Banana and orange peels with these micro- organisms. Banana and Orange peels choosen as substrate in solid state fermentation for bioethanol production due to its cheap, easy and whole year availibilty.Collected plants material were dried and converted into fine power, then a pretreatment was given to plant material to delignifie the plant material.The enzymatic hydrolysis of both pretreated substrates were done with cellulase that depolymerized cellulose to monomeric glucose, that converted in to ethanol finally. S. cerevisiae and B. subtilis both exibited maximum yield of bioethanol at 400C, 4.07% (v/v) and 4.10% (v/v) respectively for orange peels. For banana peels *S. cerevisiae*, *B. subtilis* both has shown maximum ethanol production at 300C which is 2.86% (v/v) and 3.89% (v/v) repectively. For both orange and banana peels *B. subtilis* showed maximum ethanol production at pH 7 but *S. cerevisiae* shown maximum ethanol production at pH 5 from orange peel and at pH 4 from banana peel. Produced bioethanol purification were done through fractional distillation. Specific-gravity and Iodine values were determined for best optimal conditions to cheak the purity of bioethanol produced. Specific-gravity and iodine values of bioethanol produced through fermentation with *S. cerevisiae* was found to more close to pure ethanol.

Index Terms— *S. cerevisiae*, *B. subtilis*, Banana peel, Orange peel, Solid state fermentation.

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1 INTRODUCTION

Ethanol is one of the most advanced liqid fuel because it is environment friendly [1]. Its carbon content has a vegetable origin and as a consequence, when it is released during the combustion process, it does not contribute to the increase of carbon dioxide in the atmosphere, reducing global warming [2] [3] [4]. Biomass is the earth's most attrative alternative among fuel sources and sustainable energy resource. As per the FAO statistics, India is the largest producer of banana in the world and accounts for nearly 30% of the total world production of banana. Though banana peel is a fruit residue, it accounts for 30–40% of the total fruit weight [5] and contains carbohydrates, proteins, and fiber in significant amounts. Since banana peels contain lignin in low quantities [6], it could serve as a good substrate for production of value-added products like ethanol. Citrus fruits is the one of the major fruit crop cultivated world wide.In the citrus processing industry citrus peel is the major solid by-product and compaises around 50% of the fresh fruit weight and can be utilized as substrate for bioethanol production. There are major limitations to efficient ethanol production from agricultural residues. These limitation includes the close physical and chemical association between lignin and plant cell wall polysaccharides together with cellulose crystallinity. Lignin froms a protective shield around cellulose and hemicellulose, protecting the polysaccharides from enzymatic degradation. To convert the biomass in to ethanol, the cellulose must be readily available for cellulose enzyme.

Thus by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermation. Therefore, a pretreatment was applied to degrate the lignin in the peel residue, decrease cellulose crystallinity and increase the surface area for enzymatic activity. In present study change in ethanol concentration was investigated on temperature, pH, and incubation period to estimate the production ability of *S. cerevisiae* and *B. subtilis* with change in temperature, pH and incubation period.

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2 MATERIAL AND METHOD

2.1 Collection of Raw Material

The raw material used in the process of fermantation for the the production must be economical and its availatity must be easy to a large degree they determine the economics of the process, the production method and the quality of the product. If the raw material is naturally ocurring subtances it must be readily available throught most of the year. So kepping all these things in mind banana and orange peels had been choosen as substrate for bioethanol production. Banana and orange peels was collected from local market of Allahabad and washed twiced with Distled water and wiped with 70% ethanol, chopped in to small pieces and dried at room temperature and stored at 40C for further use.

2.2 Isolation of Bacterial species

Pure culture of *B. subtilis* and *Sacchromycees cerevisiae* were procured and maintained on potato Dextrose agar medium at 40°C. The slants were grown at 280C for 3 days. The spores were harvested using sterilized water with 0.1% Tween80. For innoculation 3ml of spore suspension was used. Stock cultures

of *B. subtilis* were grown on nutrient agar slants at pH 6.8. They were periodically sub-cultured by transfer onto fresh agar medium. Liquid culture of B. subtilis was prepared in nutrient broth from pure culture of bacterium. For innoculation 3ml of bacterial culture was used.

2.2 Preparation of Substrate

Banana and orange peels were dried seperately and powered in a grider to get the small size of substrate. The substrates were then treated separately with 2% alkali NaOH. The treted substrate was subjected to heat treatment by autoclaving for one houres at 121^{0}C. After the heat treatment, substrates were washed using distilled water and then neutralized by acetic acid and sodium hydraoxide. The substrate was dried at 600C in oven for 12 hours. Enzmatic hydrolysis was carried out in reaction mixture cotaining 5gm of pretreated substrates (Banana and orange peel) in 100ml 0.1M citrate buffer with 5µl of concentrated crude cellulase enzyme, pH was adjusted to 4.5 in all the four flasks. The reaction mixtures were incubated on rotary shaker at 300C, 75rpm for 24 hours. After the 24 hours of incubation, reaction mixtures were boiled for 2 minutes to denature the enzyme and the centrifuge at 5000rpm for 15 minutes. The supernetents collected and used for fermantation.

2.3 Effect of pH on Bioethanol Production

Fermantation was carried out by solid state fermantation. The volume of all the four flasks was adjusted to 100ml. The pH of suspention were adjusted. The flasks containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, autoclave for 15 minutes at 121^{0}C and allowed to cool at room temperature. A pinch of Ammonium sulphate is added in each flask as a nitrogen source. The sterilized flasks were innculated with 3ml of 24 hours old cultures and incubated under anaerobic condition.

2.4 Effect of Temperature on Bioethanol Production

Optimization of fermantation process had been done at different parameters to estimate the ethanol production ability of *S. cerevisiae* and *B. subtilis*. Various optimization parameters were pH (4.0, 5.0, 6.0 & 7.0), fermantation temperature (10^{0}C, 20^{0}C, 30^{0}C & 40^{0}C), and time of fermentation (48, 72, 96 & 120 hours) to obtained maximum bioethenol production.

2.5 Effect of Incubation Period on Bioethanol Production

After different time periods, the fermented substrates were filtered and dispensed into round bottle flask fixed to a distillation column enclosed in running tap water. A conical flask was fixed to a distillation column to collect the the distillate. A heating mantle with temperature adjusted to 650C for 6 hours was used to heat the round bottomed flask contaning fermanted broth.

3 Result and Discussion

3.1 Effect of Temperature on Bioethanol Production

Temperature is important factors that effect the fermantation process and product formation. It is commanly belived that 20-35^{0}C is the ideal range for fermantation (Wu et al; 1998, Ballestron et al. 2004, Aldiguer et al; 2004, Phisalaphong et al; 2006, Gao et al; 1988) but when the temperature was increased to 45^{0}C, *S. cerevisiae* will show a high cell growth and ethanol production rate [7]. In the present investigation fermantation process is carried out at 10, 20, 30, and 40^{0}C to determine the optimal temperature of Bioethanol production with banana and orange peel using *S. cerevisiae and B. sutilis*.

As from the results obtained in present study maximum ethanol production was obtained at 40^{0}C from orange peels by fermantation using *S.cerevisiae* and Banana peels shows maximum ethanol production at 300C with *S.cerevisiae*. *S. cereviciae* are known to convert sugar in to bioethanol at temperature range of 25^{0}C to 30^{0}C [8]. The Best optimum temperature for *S. cerevisiae* is 30^{0}C in banana peel [9]. The Best optimum temperature found to be 33^{0}C using *S. cerevisiae* mutant strain 4 for bioethanol production using banana peel. [10]. Gomma (2012) founded more ethanol yield than *S. cerevisiae* using B. subtilis by orange peel fermantation that shows aggriment with results obtained in present study. According to Gomma optimum temperatur for bioethanol production is 35^{0}C by orange peels using B. subtilis than 400C in present investigation.

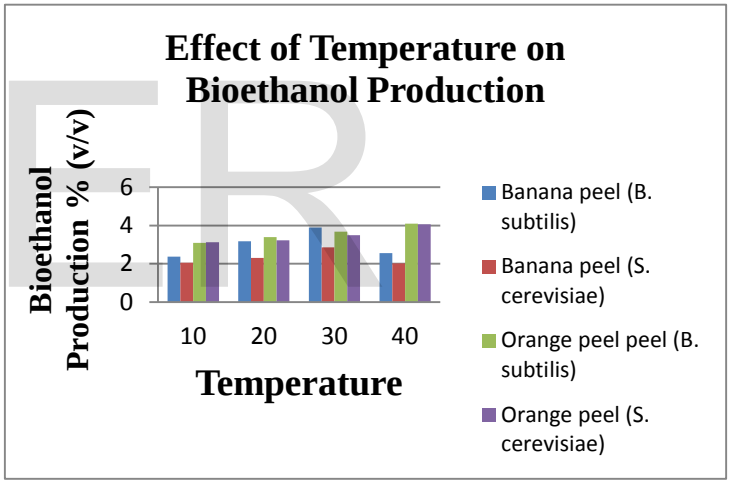


Fig 1: Effect of Temperature on Bioethanol Production

3.2 Effect of pH on Bioethanol Production

In addition to temperature, pH is also an important factor that affects the ethanol fermentation [11]. In this study change in ethanol concentration was investigated to estimate the production ability of S. cerevisiae and B. subtilis with changing pH. Investigation is done on pH 4, 5, 6, 7. Results obtained in present investigation showed that Orange peels fermented with S.cerevisiae yield maximum ethanol at pH 4 and Banana peels with S.cerevisiae shown maximum yield at pH 5. *B. subtilis* show maximum ethanol production at pH 7 with both the substrate. A pH range of 4.0-5.0 regarded as the optimal limit for the anaerobic ethanol production process using S. cerevisiae and The highest specific ethanol production rate for all the batch experiments was achieved at pH 5.0 [7].

The Best optimum pH found to be 4.5 using S. cerevisiae

mutant strain 4 for bioethanol production using banana peel [10]. S.cerevisiae shown maximum productivity of bioethanol at pH 5 for grape waste [12] [13] founded 3.81 as optimul pH for ethanol production from orange juice using S. cerevisiae According to Gomma (2012), highest ethanol production was obtained at pH 7 with orange peels using B. subtilis.

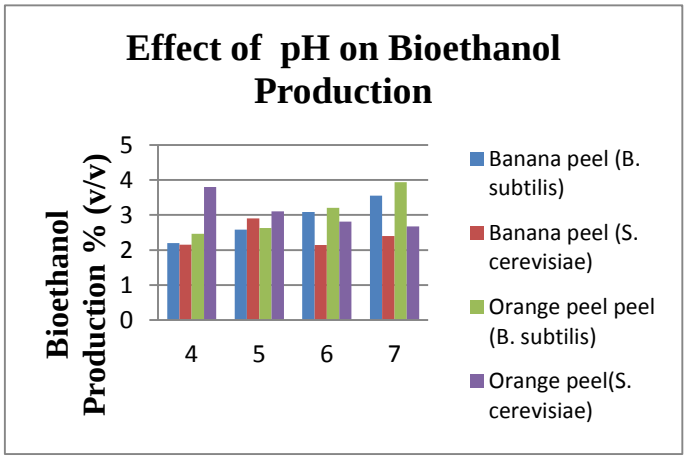
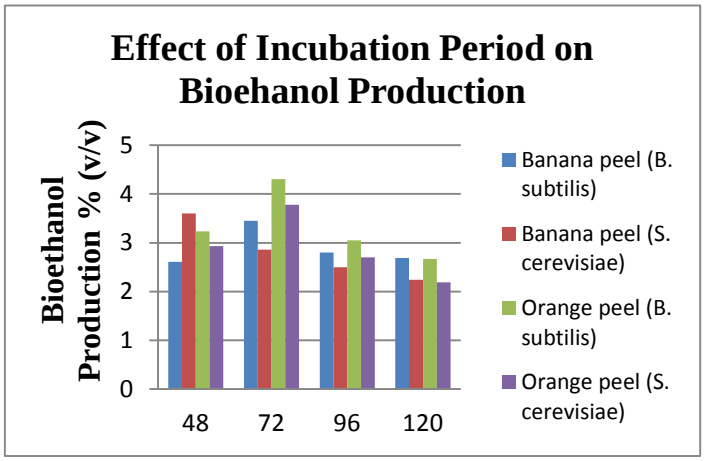


Fig 2: Effect of pH on Bioethanol Production

3.3 Effect of Incubation Period on Bioethanol Production

Patil and Dayanand (2006) reported that the period of fermentation depends upon the nature of medium, fermenting organisms, concentration of nutrients and process physiological conditions.For general ethanol ethanol production by yeast, the maximum fermentation time in batch process was 72 hours. [14]. Fermention of banana peel and orange peel were done at different incubation time as 48, 72, 96, 120 hours at 300C to estimate the effect of fermantation time on the ethanol production activity of S. cerevisiae and B. subtilis. Both *S. cerevisiae* and *B. subtilis* shown maximum ethanol production after 72 hours of incubation on pretreated orange peels extract. *S. cerevisiae* shown maximum ethanol production after 48 hours of incubation on pretreated banana peels and B. subtilis shown maximum ethanol production on pretreated banana peels. Diluted H_2SO_4 prereated banana fruit peels yielded a maximum of 13% ethanol with a fermentation efficiency of 27.13% at 42 hours of incubation with S. cerevisiae [10].The production of ethanol found to be maximum after 48 hours of fermantation for grape wastes using S.cerevisiae[12].The concentration of bioethanol was found to be increase with respect to time for all temperature [15] [16] [17] which supports resuls obtained in present investigation. Mishra et al (2012) founded increase in quantity of ethanol produced in submerged state fermantation as compared to the produced by solid state fermentation and founded optimal incubation period 72 hours for bioethanol production by orange peel using S. cerevisiae. Wikkins et al (2007) reported that S. cerevisiae fermented hydrolysed sugar extracted from orange peel waste and produced ethanol at 72 hours of incubation period. Gomma (2012) also recorded highest level of production after 72 hours of incubation period with orange peels using B. subtilis.



3.4 Specific gravity of produced Bioethanol at optimal condition

The specific gravity of absolute ethanol is 0.79. Specific gravity of bioethanol produced at different optimization conditions was tabulated. Result shows that specific gravity of bioethanol produced by fermantation is close to specific gravity of absolute ethanol. Specific gravity of ethanol obtained from banana peels fermanted with S.cerevisiae after incubation of 48 hours was fonded more near to absolute ethanol in compare to other substrate combinations.

Table:4 Specific gravity of produced Bioethanol at optimal condition:

Parameters	Banana peel (B. sutilis)	Banana peel (S.cerevisiae)	Orange peel (B. subtilis)	Orange peel (S.cerevisiae)
pH	7.0 (0.949)	5.0 (0.934)	7.0 (0.936)	4.0 (0.920)
Temperature (^{0}C)	30 (0.961)	30 (0.883)	40 (0.953)	40 (0.893)
Incubation period (in hours)	72 (0.946)	48 (0.824)	72 (0.991)	72 (0.931)

3.5 Iodine value of produced Bioethanol at optimal condition:

The iodine value of absolute ethanol is between 90-100. Iodine value of bioethanol produced by different substrate combinations at different optimization conditions is calcutated and tubulated. Results showed that iodine value

of ethanol obtained from banana peels fermanted with S.cerevisiae at 400C is closer to absolute ethanol in compare to other substrate combinations.

Parameters	Banana peel (B. sub-tilis)	Banana peel (S.cerevisiae)	Orange peel (B. sub-tilis)	Orange peel (S.cerevisiae)
pH	7.0 (79.23)	5.0 (76.07)	7.0 (72.73)	4.0 (73.85)
Temperature (^{0}C)	30 (69.80)	40 (88.23)	40 (80.47)	40 (82.57)
Incubation period (hours)	72 (73.85)	48 (81.34)	72 (74.94)	72 (71.07)

4 Conclusion

The present study examined the influences of temperature, pH and incubation period on ethanol production ability of *S. cerevisiae* and *B. sutilis* using the banana and orange peels as substrate. The results of this study indicates optimal temperature, pH and incubation time for fermantation using S. cerevisiae and B. subtilis which may enhance ethanol yield and minimize the cost of production could be obtained from banana and orange peels. Bioethanol production By *B. Subtilis* found more than *S. cerevisiae* with both the substrates that indicates *B. subtlis* may be used as sucessful alternative of *S. cerevisiae* in bioethanol production.

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