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Utilization of Pineapple Wastes for Bio-Ethanol Production

***Barate DL and Ingle SS**

Department of Microbiology, Shri. Shivaji College of Arts, Commerce and Science, Akola, India.

ABSTRACT:

The generation of various types of fruit waste is increasing day-by-day. The fruit waste containing essential components can be utilized for the production of valuable products. In the present study bio-ethanol production from pineapple waste using *Saccharomyces cerevisiae* and *Aspergillus niger* with different combinations of pineapple waste (as substrate) and microorganism (as an inoculum) was performed by fermentation. The results obtained showed that, the highest amount of bio-ethanol yield produced which was 17.75% (v/v) consisting of pulp as a substrate and *Aspergillus niger* as an inoculum. The bio-ethanol yield obtained from *Saccharomyces cerevisiae* and peels set was 8.84%. The bio-ethanol yield from *Aspergillus niger* was highest as compared to *Saccharomyces cerevisiae* this might be due to *Aspergillus niger* has the ability to utilize sugar present in fermentation medium at a faster rate producing higher concentration of bio-ethanol.

KEYWORDS: bio-ethanol, pineapple waste, fermentation, *Saccharomyces cerevisiae*, *Aspergillus niger.*

INTRODUCTION:

Bio-ethanol has gained its importance, not just as the chemical feedstock, an industrial solvent or a beverage, but in recent years, it is emerging as a fuel option for automobile particularly in countries like United States of America, Brazil, and Canada (Qian *et al*., 2014). Due to the rapid depletion of the world's energy supply, there is an increasing global interest in alternative energy sources (Lin & Tanaka, 2006). The burning of petroleum-based fossil fuels in machines and vehicles accompanied by evolution of dangerous gases has been a major cause of increase in environmental problems. This, thus call for an alternative source of fuel which can be obtained by focusing attention to greener technology in the production of bio-fuel using various renewable materials. The production of bio-fuel for domestic and external uses following advancement of biotechnology has been one of the most prominent features of the 20th century which has greatly moved to the 21st Century (Mushimiyimana *et al*., 2015).

According to Indian Agriculture Research Data Book (2004), the losses in fruits and vegetables are to the tones of 30 percent. Taking estimated production of fruits and vegetables in India at 150 millions tones, the total waste generated comes to 50 million tons per annum. Efficient management of these wastes can help in preserving vital nutrients of our foods and feeds and bringing down the cost of production of processed foods, besides minimizing pollution hazards (Gautham and Gularia, 2007).

Bio-ethanol is non-toxic, biodegradable and does not cause environmental pollution as compared to the fossil fuel (Hossain *et al*., 2010). Bio-ethanol is produced by the conversion of carbon based feedstock coming after fermentation technology. Agricultural waste feed stocks are considered as renewable components because of getting energy from the sun using photosynthesis (Hossain *et al*., 2011). One of the major drivers of Bio ethanol promotion worldwide is the concern about climate change and the potential of bio fuels to reduce the Green House Gas (GHG) emissions (Micic and Jotanovic, 2015).

Investigations have carried out in production of ethanol from food and agricultural waste now a day by bio-processing in research. Fruits Wastes such as banana, orange, pineapple peels were saccharification and fermentation by co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* (Girisha Malhotra, 2013).The best source for the production of bio-ethanol from fruit waste is pineapple. Pineapple is the third most important tropical fruit in the world after banana and citrus. India ranks 5th on the list of world producers of pineapple as well as is the leading producer of pineapple in World. Production and consumption of pineapple releases great amount of organic waste also known as pineapple waste which includes fruit peel and crown comprised of lignin, hemicellulose, and cellulose, these are considered to be lignocellulosic materials that can be used in the production of bio ethanol. It has high fiber content which made it as a potential bio-ethanol feedstock (Casabar *et al*., 2019).

The production of bio-ethanol in a larger volume can be done by using *Saccharomyces cerevisiae and Aspergillus niger.* These two microorganisms are the best source for fermentation studies in bio ethanol extraction from the pineapple wastes. The recent advances in microbiological field resulted in betterment in the fermentation process using these micro-organisms.

MATERIAL AND METHODS:

Collection of Raw Material: - Fresh pineapple fruit was bought from the market of Akola city. The fruit waste containing pulp and peels. It was washed and chopped into smaller pieces and then subjected to size reduction using a blender. Peels were stored in a beaker and refrigerated. Then the waste sample was dried on sterile stainless steel trays inside a hot air oven at 60°C for 5 days, until a constant weight was obtained. The dried waste was blended into fine powder with a Philips house-hold blender and stored in refrigerator for further experimental studies.

Source of Inoculum: - Active dry yeast *Saccharomyces cerevisiae* was purchased from store. The process of rehydration was conducted using distilled water with yeast at 30°C for 30 min. After rehydration process, the rehydrated yeast was used for fermentation process. *Aspergillus niger* procured from Dept. of Microbiology Shri Shivaji College Akola.

Production of bio-ethanol by fermentation :- Fermentation was carried out in 1000ml Erlenmeyer flasks containing pineapple pulp juice measuring 250ml pulp and 250ml distilled water and 50g powdered pineapple peels in 450ml of distilled water respectively. The mono culture fermentation was inoculated with 2ml of freshly harvested inoculums of *Saccharomyces cerevisiae* and 2ml inoculums of *Aspergillus niger*. The fermentation was carried out in an incubator and it was set up at 30°C for 3 days. The pH of the pineapples was measured. The original pH for pineapple was from range 5-5.5. After 3 days, the samples were taken out from the incubator for distillation process. The fermentation was carried out using the different combination of pulp, peels and microorganisms as following table :-

Table 1:- Different combinations of pineapple waste and microorganism.

Set	Substrate	Inoculum
Set 1	Pulp	*Saccharomyces cerevisiae*
Set 2	Peels	*Saccharomyces cerevisiae*
Set 3	Pulp	*Aspergillus niger*
Set 4	Peels	*Aspergillus niger*
Set 5 (Control)	Glucose	*Saccharomyces cerevisiae*
Set 6 (Control)	Glucose	*Aspergillus niger*

Extraction and Estimation of bio-ethanol:-

The extraction of bio-ethanol was done by distillation process and further determination of final yield was calculated by bio-ethanol estimation methods. The first method used for the determination of bio-ethanol was specific gravity analysis method (Tupe *et al.,* 2018) and second was potassium dichromate method which involves reactions of different concentrations from yeast culture using $K_2Cr_2O_7$ reagent (Seo *et al*., 2008).

RESUTLS:

In the present study, the sample distillate sets comprises of different substrates and inoculums shows varied amount of bio-ethanol yield (Table 1). The specific gravity analysis method was used for the determination of bio-ethanol yield from respective sample distillate sets. The sample distillate set 1 which had pulp as a substrate and *Saccharomyces cerevisiae* as an inoculum showed 3.92% (v/v) percentage of bio-ethanol yield. The sample distillate set 2 which had peels as a substrate and *Saccharomyces cerevisiae* as an inoculum showed 8.84% (v/v) percentage of bio-ethanol yield. The sample distillate set 3 which had pulp as a substrate and *Aspergillus niger* as an inoculum showed 17.75% (v/v) percentage of bio-ethanol yield. The sample distillate set 4 which had peels as a substrate and *Aspergillus niger* as an inoculum showed 10.09% (v/v) percentage of bio-ethanol yield. The sample distillate set 5 which had glucose (control) as a substrate and *Saccharomyces cerevisiae* as an inoculum showed 19.93% (v/v) percentage of bio-ethanol yield. The

sample distillate set 6 which had glucose (control) as a substrate and *Aspergillus niger* as an inoculum showed 23.79% (v/v) percentage of bio-ethanol yield (Fig 1).

The highest amount of bio-ethanol yield was produced from sample distillate set 3 which was 17.75% (v/v) consisting of pulp as a substrate and *Aspergillus niger* as an inoculum. The lowest bio-ethanol yield was produced from sample distillate set 1 which was 3.92% (v/v) consisting of pulp as a substrate and *Saccharomyces cerevisiae* as an inoculum. The bio-ethanol yield from *Aspergillus niger* was highest as compared to *Saccharomyces cerevisiae*. The *Aspergillus niger* has the ability to utilize sugar present in fermentation medium at a faster rate producing higher concentration of bio-ethanol. The second method used for the estimation of bio-ethanol was potassium dichromate method. (Fig 2, Fig 3, Fig 4).

Fig 1 :- Determination of bio-ethanol by specific gravity analysis method

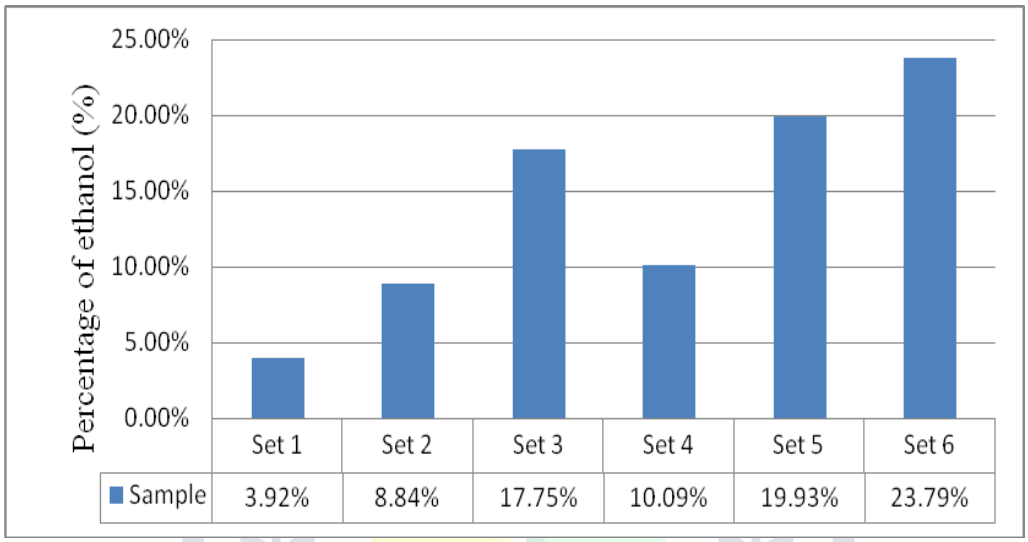


Fig.2:- Standard graph for estimation of ethanol by potassium dichromate method

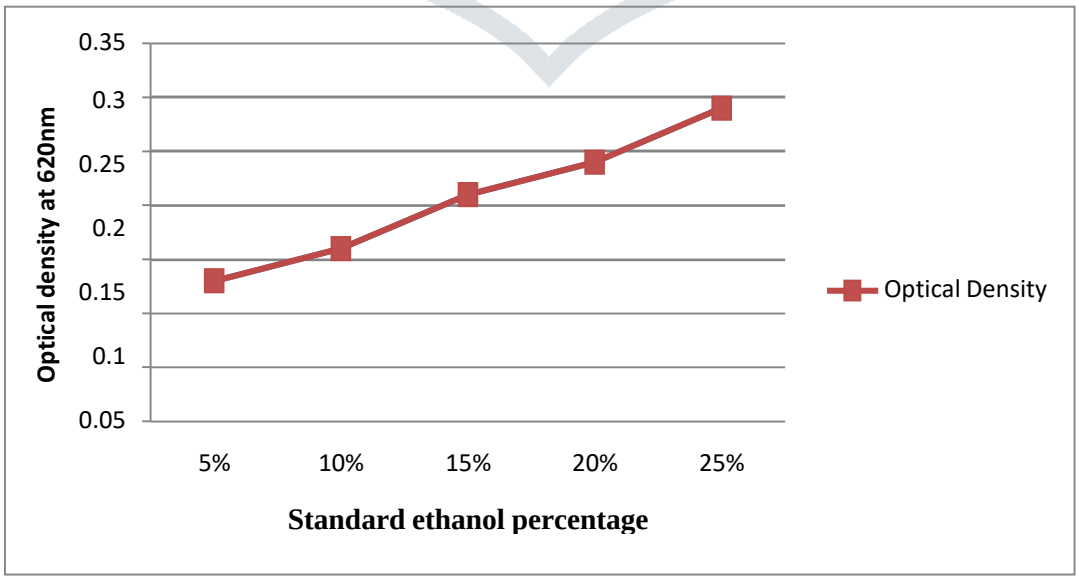


Fig. 3 :- Bio-ethanol determination by Potassium dichromate method using *Saccharomycescerevisiae*

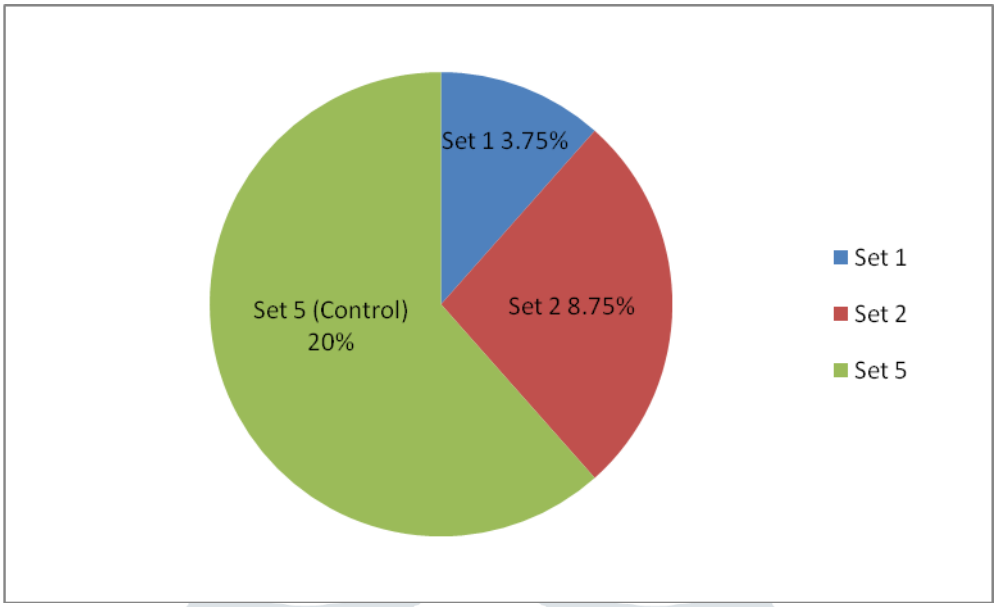
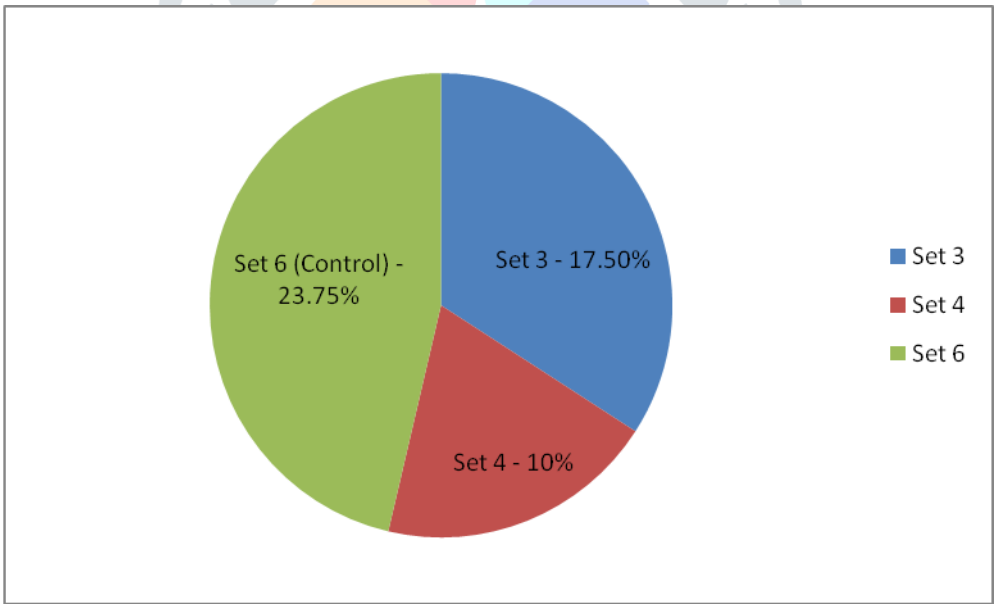
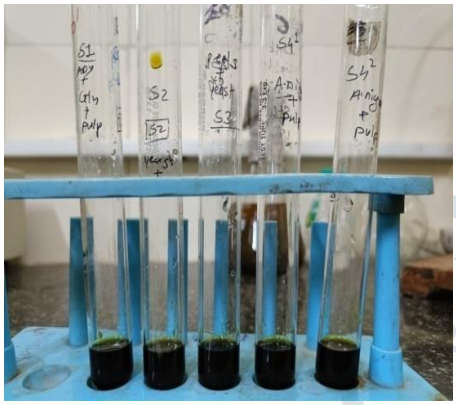


Fig. 4:- Bio-ethanol determination by Potassium dichromate method using Aspergillus *niger*



Fermentation Medium

Extraction of Bioethanol by Distillation Process



Estimation by Pottasium dichromate method

Extracted Bioethanol

DISCUSSION:

The use of the lignocellulisic raw materials such as fruits, vegetable wastes, agricultural residue and much more for the production of bio-chemical is a good way to convert environmental pollutant into useful materials for maximal production of ethanol (Mohammed *et al*., 2018). In the present study, the highest bio-ethanol yield from *S. cerevisiae* was found to be 8.84% (v/v) which showed similar results with Suryaprabha *et al.*, (2017) research study. Suryaprabha *et al.*, (2017) worked on bio-ethanol production and observed that different fruit waste can serve as raw material for the ethanol, they found that from *S. cerevisiae* pineapple wastes at temperature 33℃, 6.3% (v/v) ethanol production was achieved.

Prior *et al*. (1981) found about 4% (v/v) bio-ethanol yield from pineapple cannery effluent when worked with pineapples wastes. The strain of *S. cerevisiae* used was isolated from active dry yeast. The strain showed strong abilities to ferment glucose which is an attribute that was strongly desirable for the study. Also, the strain showed ethanol tolerance at 15 % concentration. *S. cerevisiae* is commonly used for ethanol fermentation due to its high ethanol yield, high productivity and high ethanol tolerance (Tanaka, 2006).

Hemalatha *et al*.,(2015) found that the maximum ethanol percentage of treated (*Saccharomyces cerevisiae*) pineapple waste, use of mixed with *Saccharomyces cerevisiae* and Barley waste, Copra cake and Corn stalk substrates was 5% (v/v) which shows similar results as

compared with the present study. The present study shows bio-ethanol yield results using *Saccharomyces cerevisiae* were 3.92% using pulp and 8.84% using peels as a substrate of pineapple waste. Itelima *et al*., (2013) found that the pineapple peels used for the production of bio-ethanol has resulted in the highest yield of 8.34% (v/v) bio-ethanol.

CONCLUSION:

It was concluded that *Saccharomyces cerevisiae* and *Aspergillus niger* both can be efficiently produce the bio-ethanol from the pineapple waste. The *Saccharomyces cerevisiae* produced 8.84% (v/v) and *Aspergillus niger* produced 17.75% (v/v) concentration of bio-ethanol from the fermentation medium. The pineapple waste can be a good source of bio-ethanol production which also reduces the economic burden for management of waste and increasing prices of renewable sources on society.

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