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Full Length Research Paper

A comparative study of ethanol production from various agro residues by using *Saccharomyces cerevisiae and Candida albicans*

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In this study, ethanol produced from fruits of pineapple, orange and sweet lime was investigated. Effect of different constant times [24 and 72 h, and 8 days (in submerged fermentation)]; pH (3.5 to 8.5), temperature (78°C) and autoclave pre-treatment (121°C, 20min) were also studied to improve the yield of ethanol in fruits. Yeast (*Saccharomyces cerevisiae* and *Candida alb* cans) are used for fermentation. Fermentation process used solid state fermentation and submerged fermentation methods. The results showed that there is a substantial increase in the quantity of ethanol produced in submerged fermentation as compared to that produced by the solid state fermentation. Optimal ph and temperatures for the better yield of ethanol were 3.5 to 8.5 and 78°C respectively. Autoclave pre treatment protected the samples from contamination and increased the volume of ethanol.

Key word: Ethanol, fruits, sugar concentration, *Saccharomyces crevasse* and *Candida albicans.*

INTRODUCTION

Ethanol is a fossil fuel which is made from sugars found in plants. In the India, it is usually made from corn or grain sorghum. Ethanol can also made from many other plants or plants of parts, such as wheat, sugarcane, sawdust and yard clippings. Bioethanol used for production of gasoline can reduce vehicle carbon dioxide emission by 90% (War and Singhs, 2002). Inorder to reduce the cost and quantity of petrol consumption, Government of India uses a mixture of 10% ethanol to the petrol. The method of ethanol production from various agro residues is of prime importance as the raw materials are easy available and cheap in cost. So the pineapple peeling is the favourite choice generally used for production of ethanol along with *Saccharomyces cerevisiae.* Earlier research suggests that the solid state fermentation used for the setup has shown to give a high production of ethanol (Avril, 2008). Production of ethanol has taken a high toll due to the higher demand of the fossil fuels and this has contributed to increase in the use of the. *Wasingtonia robista* fruits are used in U.S. in the above lines with commercial yeast (Mehmet, 2010)

Carbohydrate is the main source for ethanol production which is easily found in various plant-parts. Dake et al. (2010) reported that natural resources along with *S. cerevisiae* are the highest bidders for the commercial production of ethanol. Several published work also suggest the role of pesticides found in the agro residues adding to the yield in fermentation by products. The separation then requires detailed GC-MS analysis to understand and later lessen the percentage of pesticide contamination as reported in wine production from grapes by Cus et al., 1999, in their food control paper. In our case however, the extraction of bioethanol found has less chances of contamination as it has already been treated in the city limits and pre-treatment is done by the fruit

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vendors prior to obtaining the agro residues. Other researchers like Hernández-Sánchez et al., 2011, have also reported the use of zeolite effects the production of ethanol as well as the cell biomass. In our study we have not incorporated zeolite for production of ethanol. Hence the yield is variable from the earlier reported data. Scientific literature also suggests that fungicides play an important role in effecting the aroma, colour and other physic-chemical properties of bioethanol extracted. Such studies are more important for determining food toxicity pertaining to ethanol production. Our work shows a maximum yield without the use of fungicides.

MATERIALS AND METHODS

Firstly the pure culture plates of two strains *S. cerevisiae* and *Candida albicans* were collected from the CDRI laboratories, Lucknow. These two strains were inoculated into solid media and the liquid submerged media under sterile conditions and kept for growth. The two methods used for the production of ethanol are: 1. Solid state fermentation. 2. Submerged fermentation. The agro residues of the samples of pineapple, orange and sweet lime were collected from the local market and carefully taken in sterilized sheets. For the present work, only the peels of the three fruits were considered.

Solid state fermentation

Collection, preparation, and total sugar determination of pineapple, orange and sweet lime peels

Waste peels were collected from various agro residues. These were then ground into pulp using a blender, placed in a sterile container, and stored for the subsequent sugar concentration analysis. The container was then labelled for identification in preparation for the total sugar determination. Twenty grams of peel powder was subjected to auto chemical analysis of its total sugar content. Benedict's test was employed for the analysis of sugar concentration present in the peels. Test showed that the glucose content of the substrate peels was suitable for saccharification and fermentation. Having determined the glucose content of the peels to be used for solid state fermentation, 100 g of peels were cut into smaller particles and ground into pulp for fifteen minutes using a blender to make the samples more susceptible to enzyme attack. The sugar concentration of the peels was determined using Benedict's method.

Preparation of the culture media

Two 100 ml flasks of glucose-yeast-peptone (GYP) medium were prepared by diluting 20 g of glucose and 10 g of peptone in 1 L of distilled water and sterilized for 20 min at 121°C. All glasswares and laboratory apparatus were autoclaved for 1 h at 121°C.

Preparation and inoculation of the S. cerevisiae and C. albicans

5 ml suspensions of *S. cerevisiae and C. albicans* strains obtained were inoculated into the prepared broths separately. Both cultures were incubated at room temperature on a rotary shaker at 200 rpm for 24 h prior to inoculation into the fermentation medium.

Preparation and experimentation of the peels with S. cerevisiae and C. albicans through simultaneous saccharification and fermentation

Twenty four flasks were used for the experiment and labelled depending on the type of solid state fermentation manipulation they would undergo. Three substrate contained in 24 flasks was poured into 8 flasks giving 3 sets. Each set was divided into two groups A and B. Each subgroup is composed of four flasks each, containing 15 g of pulp per flask. 50 ml of prepared 4.0% sodium hydroxide (NaOH) buffer were distributed to each flask of group A, and 100 ml of the same buffer were distributed to each flask of group B. Groups A and B were inoculated each with 5 ml suspension of Candida albicans and Saccharomyces cerevisiae yeast. The first two flasks of the first group, labelled A_1, were allowed to ferment for 24 h, while the second pair of flasks from the same group, labelled A_2, was allowed to ferment for 72 h. Likewise, the first two flasks of the second group, labelled B_1, were allowed to ferment for 24 h, while the second pair of flasks of the same group, labelled B_2, was allowed to ferment for 72 h respectively. Bioethanol evaporation was prevented and aerobic conditions were maintained by placing cotton plugs on all flasks. After their respective fermentation times, the pulp broth was filtered through Whatmann filter paper 1, and the filtrate of each flask was immediately subjected to distillation.

The same procedure was also followed for submerged fermentation. However a slightly different way was followed for the substrate preparation in the process of submerged fermentation: In this method all the extracts of all the various agro residues was prepared. One hundred grams waste of each sub substrate was taken and 500 ml distilled water was added. After preparation of extract, the extract was inoculated with the suspension of *S. cerevisiae* and pure culture of *C. albicans*. After inoculation the extract was left for fermentation and noted at different time intervals (24, 48 and 72 h). The extract was filtered after fermentation and left for production of ethanol in the distillation unit.

Distillation

After fermentation the yeast cells were separated by filtration. The liquid part was distilled by Claisen condenser apparatus .The fractions were collected up to 78.5°C. Collected fractions were analysed for ethanol percentage (v/v) by optical density method using a colorimeter.

RESULTS AND DISCUSSION

The present study revealed certain interesting facts about ethanol production. Figure 1 shows the glucose-yeast-peptone (GYP) medium. Figure 2 shows condensed bioethanol produced in a condenser. Total amount of sugar in pineapple, sweet lime and orange was 0.5,1 and 0.8% with a colour variation of green, yellow and light yellow respectively. The presence of reducing sugar was thus confirmed and the data is represented in Table 1. Table 2 shows the estimation of ethanol from *S. cerevisae* and *C. albicans* by solid state fermentation method. It follows that the optical density decreases as the percentage of ethanol produced decreases. In the solid state fermentation, pineapple agro residue gives a maximum yield around 2.16% with yeast. With a change of strain to *C. albicans*, pineapple still gives a high yield of 1.08% for group A in 50 ml capacity as shown by Table

Figure 1. Glucose yeast peptone medium.

Figure 2. Production of bioethanol in a condenser apparatus.

2. Similarly for the group B and 100 ml capacity flasks, pineapple gives a maximum of 1.36% in 72 h. Table 2 show *C. albicans* gives a greater yield of 1.32% in sweet lime in 72 h. Table 3 depicts the ethanol production by submerged fermentation. Pineapple gives a maximum yield of 1.87% with *S. cerevisae* as shown in Table 3.

Table 1. Reducing sugar determination.

Serial number	Substrate	Colour	Sugar concentration (%)
1	Pineapple	Green	0.5
2	Sweet lime	Yellow	1.0
3	Orange	Light yellow	0.8

Table 2. Estimation of ethanol by solid state fermentation.

Serial number	Substrates	Time (h)	O.D	Ethanol%(v/v)	g/l
		Group A (50ml): For *S. cerevisiae*			
1	Pineapple	24	0.99	2.16	17.2
		72	0.54	1.562	12.3
2	Sweet Lime	24	0.50	1.09	8.6
		72	0.564	1.25	9.9
3	Orange	24	0.418	0.91	7.2
		72	0.359	0.79	6.2
		Group A (50ml): For *C. albicans*			
1	Pineapple	24	0.41	0.92	7.1
		72	0.47	1.08	8.4
2	Sweet Lime	24	0.29	0.51	5.2
		72	0.32	0.71	6.4
3	Orange	24	0.28	0.50	5.2
		72	0.38	0.77	6.9
		Group B (100ml): For *S. cerevisiae*			
1	Pineapple	24	0.568	1.35	10.7
		72	0.622	1.36	10.7
2	Sweet lime	24	0.586	1.27	10.1
		72	0.473	0.81	6.4
3	Orange	24	0.492	1.07	8.5
		72	0.512	1.25	9.3
		Group B (100ml): For *C. albicans*			
1	Pineapple	24	0.20	0.42	4.4
		72	0.24	0.50	5.1
2	Sweet Lime	24	0.41	1.02	7.4
		72	0.41	1.32	9.1
3	0range	24	0.29	0.67	7.2
		72	0.38	0.77	6.9

Table 3 depicts as well the 1.45% yield by pineapple in 72 h by submerged fermentation with *C. albicans*. Figures 3 and 4 show crude fermentation product from sweet lime and pineapple, Figures 5 and 6 shows the bioethanol

Table 3. Estimation of ethanol from submerged fermentation.

Serial number	Substrate	Time (h)	O.D	Ethanol%(v/v)	g/l
		For S. cerevisiae			
1	Pineapple	72	0.625	1.87	11.4
2	Sweet Lime	72	0.622	1.46	10.3
3	Orange	72	0.564	1.32	9.8
		For C. albicans			
1	Pineapple	72	0.467	1.45	9.5
2	Sweet Lime	72	0.452	1.41	9.3
3	Orange	72	0.452	1.41	9.3

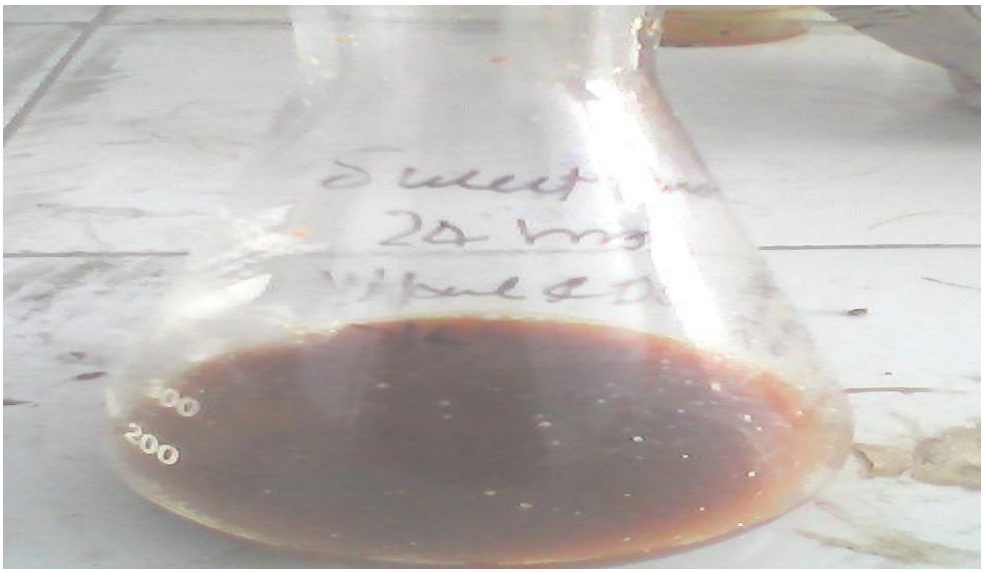

Figure 3. Sweet lime crude.

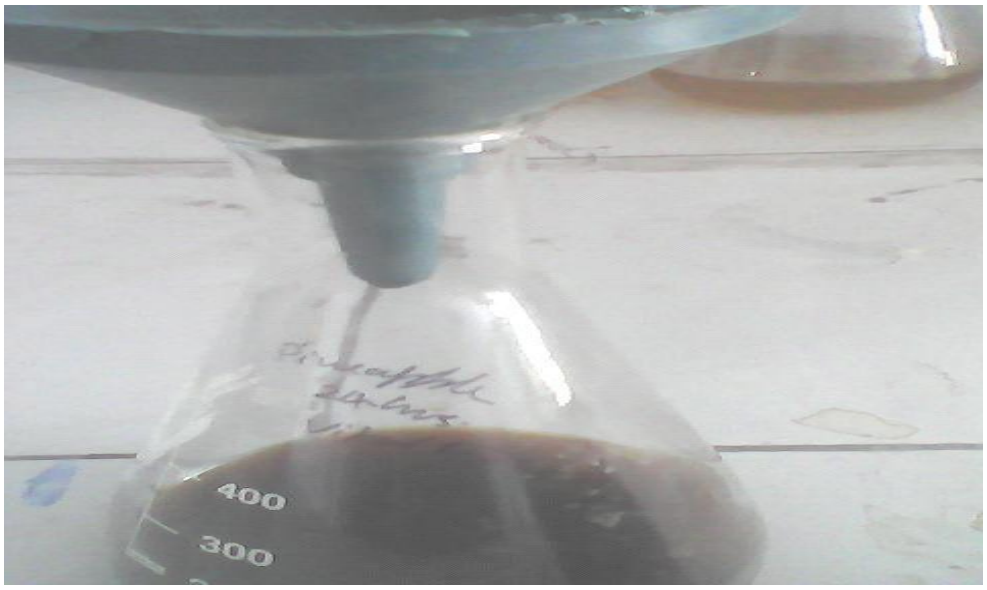

Figure 4. Pineapple crude extract.

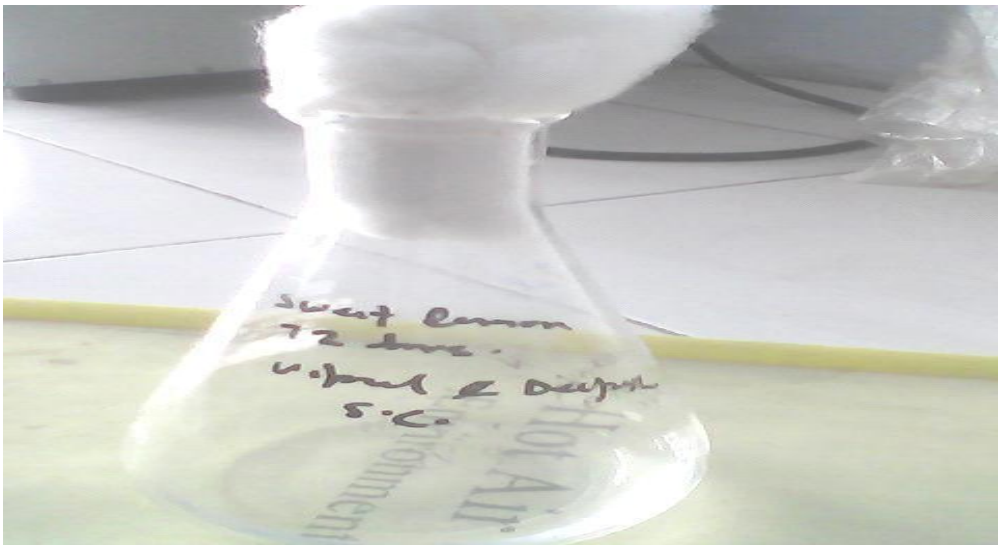

Figure 5. Ethanol Sweet lime.

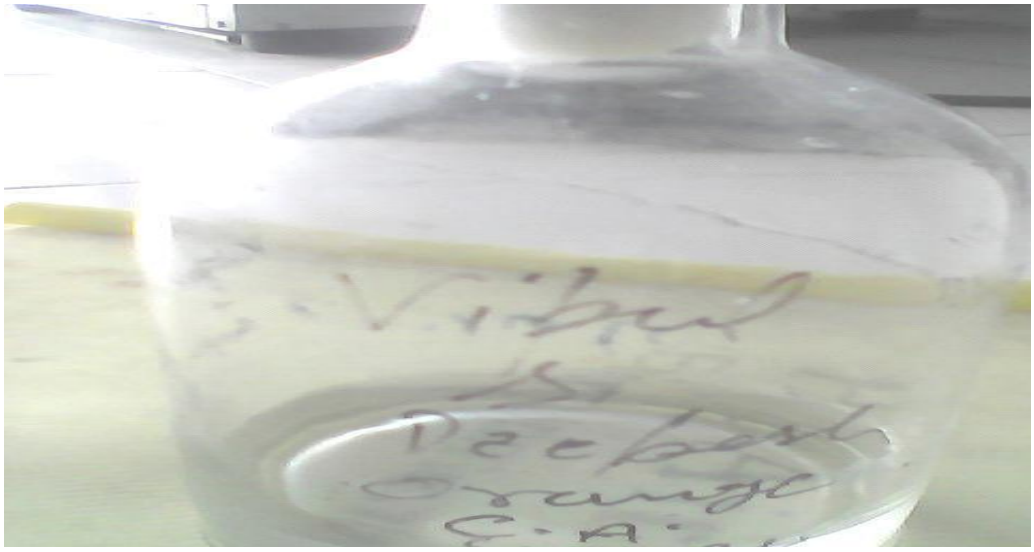

Figure 6. Ethanol orange peel.

produced from sweet lime and orange respectively.

Thus a maximum yield is obtained in submerged method for production of bioethanol suggesting that this method is better than the traditionally used solid fermentation. Also this method shows that large scale production of ethanol is strain dependent and best results are obtained with *S. cerevisae*.

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