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Analysis of Biofuel Production from Fruit Wastes using Co-cultures

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ABSTRACT: Production of Ethanol from renewable sources for fuel or fuel additives is known as bioethanol production. Since the demand of bioethanol has been increasing, the production of bioethanol must be enhanced using cheaper and eco-friendly raw materials. On the basis of these characteristics, novel sources can be considered as cheaper and eco-friendly. In this study, we used different fruit wastes as raw material for the production of bioethanol by using co-cultures of *Saccharomyces cerevisiae* and *Aspergillus niger*, *Saccharomyces cerevisiae* and *Rhizopusoryza* through with simultaneous saccharification and fermentation process. This study established that wastes from fruits that contain fermentable sugar could be converted to useful products like bioethanol that can serve as an alternative energy source. Both aerobic and anaerobic fermentation were carried out.

KEYWORDS: Bioethanol, novel sources, saccharification, fermentable sugar.

I. INTRODUCTION

The largest single use of ethanol is as a motor fuel and fuel additive. It is also an important industrial ingredient and has widespread use as a base chemical for other organic compounds and used in medical wipes and most commonly as an antibacterial hand sanitizer gels and as an antiseptic. Additionally, ethanol from biomass-based waste materials is considered as bioethanol [2]. Alcohol is an organic compound that has one or more hydroxyl (OH) groups attached to a carbon atom. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste [10]. Ethanol fermented from renewable sources for fuel or fuel additives is known as bioethanol. Bioethanol, unlike petroleum, is a form of renewable energy source that can be produced from agricultural feed stocks [5]. The first generation of ethanol production used corn as a substrate, later corn was considered as a feedstock lead to the second generation of production of ethanol which used microorganisms and different wastes as substrates [14]. The cheapest and easily available source for the production of bio ethanol is fruit wastes. It is a potential energy source, from which ethanol can be obtained. Fruit waste which is thrown away has very good antimicrobial and antioxidant potential. In this study, comparing of the ethanol efficiency produced by fermentation process from different fruit wastes such as grapes, pomegranate peel and orange peel.

Here we used co-cultures of *Saccharomyces cerevisiae* & *Aspergillus niger* and *Saccharomyces cerevisiae* & *Rhizopusoryzae* for the fermentation process since it yields more ethanol due to the combined saccharification and fermentation process.

Grapes are the fruit of a wine (*Vitis vinifera*). The whole fruit, skin, leaves and seed of the grape plant are used as medicine. Grape wastes can be used as a substrate for the production of ethanol. The yield of ethanol amounted to greater than 80% of the fermentable sugar consumed [15]. Peels of fruits contain a greater amount of carbohydrates

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and could be used as a potential source for the production of bioethanol. Here, we selected pomegranate and orange peels as substrates since they contain higher amounts of carbohydrates [3]. Pomegranate peels have a carbohydrate content of about 80.50 ± 1.52 g/100g dry peel and about orange peels are 53.27 ± 0.10 g/100g dry peel. Ripe grapes have a sugar content of about 150 to 250 g/L [11].

II. MATERIALS AND METHODS

Materials and Equipments

The chemicals that are used in this research are YPD medium, 20.0 g of malt extract, 0.1 N HCl & 0.1 N NaOH, Agar (20.0g), Potato dextrose agar, Iodine reagent, Starch, Peptone water, 5% potassium permanganate (KMnO₄), 50g of sucrose, Urea, Chromic acid, Glucose & Distilled water.

The equipments used are Incubators, Distillation unit, Hot air oven, Autoclave & Spectrophotometers.

Isolation of microorganisms

Saccharomyces cerevisiae

Saccharomyces cerevisiae strain was obtained in the form of pellets. The strain was revived in 100ml of YPD medium with shake flask culture and it was maintained on PDA slants at 4° C. From the slant, culture was developed as inoculum with same YPD medium [13].

Aspergillus niger

Soil samples were collected from different areas in sterilized polythene bags. The soil suspension was prepared by dissolving one gram of soil in 250 ml conical flask containing 100 ml of sterilized distilled water. Different dilutions such as 10⁻⁴ -10⁻⁶ were prepared from this stock solution in sterile distilled water under aseptic conditions. One milliliter of this dilute suspension was then transferred to individual petri plates containing malt extract agar medium. The malt extract agar medium was prepared by dissolving 20.0 g of malt extract in approximately 900 ml of distilled water. The pH of the medium was maintained at 4.8 by 0.1 N HCl or 0.1 N NaOH. The agar (20.0g) was then added. After raising the volume up to 1000 ml, the medium was heated for about 10 min to obtain a homogeneous mixture. The petri plates were gently rotated clockwise and anticlockwise to facilitate a uniform spreading of soil suspension. The petri plates were then incubated at 30±1°C in an incubator for 3-4 days.

The young *A. niger* colonies were picked up and transferred to potato dextrose agar (PDA) slants. The potato dextrose agar medium was prepared by dissolving 39.0 g of PDA in approximately 800 ml of distilled water and raising the final volume up to 1000 ml.

This was heated for 10-15 min while constant stirring until a clear solution formed. The pH was maintained at 5.6 by 0.1 N HCl or 0.1 N NaOH. About 4.5 to 5.0 ml of this medium was poured into individual test tubes. The tubes were cotton plugged and sterilized in an autoclave at 15.0 lbs/in² pressure (121°C) for 15 min. After sterilization, the test tubes were kept in slanting position (at an angle of about 30°) to increase the surface area. The PDA slants were then inoculated by transferring a small amount of *A. niger* conidia from the petri plates and incubated at 30°C (4-6 days) for maximum sporulation. The cultures were stored at 4°C for further studies.

Rhizopusoryzae

The pure culture of *Rhizopusoryzae* MTCC8784 (Microbial Type Culture Collection, Chandigarh) was obtained and it was cultured on potato dextrose agar medium [12].

Yeast Viability

Yeast cell viability of the starter culture and during fermentation was determined by standard plate counts. From the initial sample, 1 mL was aliquot into 9 mL of buffered peptone water (20 g/L) and tenfold serial dilution was performed. One hundred microliters of the diluted suspension was spread plate onto malt extract agar (50 g/L). The agar plates were incubated at 30°C for 24–48 hrs and examined for the growth of colonies. Plates showing between 30–300 colonies were selected and counted. Viability was expressed by calculating the colony forming units per mL. [1],[6].

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Inoculum preparation (*Aspergillusniger*)

The conidia from 4-6 days old cultures were wetted by adding 10 ml of 0.005% Monoxal O.T (Diocetyl ester of sodium sulpho succinic acid) to each slant. The conidia were scratched with a sterilized inoculating needle and the tube was shaken gently. The supernatant containing conidia was decanted off aseptically and the suspension was used as an inoculum.



Fig.1 Streak culture of *R. Oryzae*



Fig.2 Slant culture of *S. cerevisiae*



Fig.3 Streak culture of *A. niger*

Preparation of the substrate for the fermentation process

About 60g of grape fruit waste was weighed and was taken in a beaker, which was washed with 5% potassium permanganate solution and then rinsed with distilled water.

Grape waste was crushed in a mixer and collected in beaker. 0.3g of urea, 15g of sucrose and 9g of *Saccharomyces cerevisiae* was mixed well in warm water in a separate beaker. This mixture and inoculum of *Aspergillusniger* was taken as an inoculum for fermentation process. The fruit mash and inoculum were transferred into 0.5 liter conical flask and made up to the final volume 300ml with distilled water. The sample is subjected to the fermentation process. The same procedure was followed for the preparation of *R.oryzae* and *Saccharomyces cerevisiae* inoculum preparation [7], [16].



Fig.4 Grape waste



Fig.5 Pomegranate peel



Fig.6 Orange peel

Simultaneous Saccharification and Fermentation (SSF)

Ethanol fermentation was carried out in 500 ml flasks containing 5g powdered orange & pomegranate peels in 96 ml distilled water. The flasks were sterilized by autoclaving at 121°C for 30 min and a 4% (v/v) inoculum of *Aspergillusniger* and 3% (w/v) inoculum of *Saccharomyces cerevisiae* was added. Fermentation was done for 7 days and

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the ethanol content was measured every 24 hours. The same procedure was followed for the preparation of *R.oryzae* and *Saccharomyces cerevisiae* inoculum preparation[8].



Fig.7 Co cultures with orange peel as substrate



Fig.8 Co cultures with pomegranate peel as substrate

Estimation of Ethanol [9]

The ethanol content in the fermentation medium was estimated after appropriate incubation time using chromic acid method adopted by Caputi et al. (1968). 10 ml of fermented medium was taken and underwent distillation process. Approximately 1ml of distillate was collected and combined with 5ml of chromic acid and content was made up to 10ml with sterile distilled water and kept in water bath at 80°C for 15 minutes, after cooling read at 600nm using UV VISIBLE SPECTROPHOTOMETER-118 SYSTRONICS. Thus, the obtained values were plotted with standard values.



Fig.9 Simple Distillation Unit



Fig.10 Chromic acid

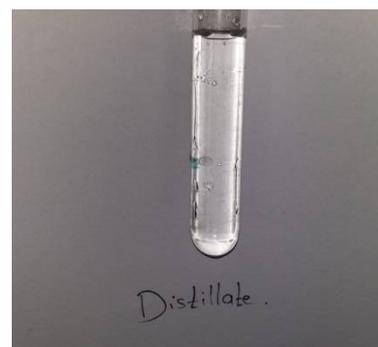


Fig.11 Distillate

III. RESULTS AND DISCUSION

In this study, it has been shown that ethanol was produced from different fruit wastes. Thus the comparative study has been carried out to check the efficiency of ethanol produced from these fruit wastes using different co-cultures. The observation is presented as follows:

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Table 1 OD values obtained during fermentation of grape wastes with co cultures

Fermentation time (Hrs)	OD at 600 nm		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	0.869	1.150	1.392
48	1.079	1.301	1.702
72	1.102	1.374	1.725
96	1.221	1.564	1.821
120	1.317	1.680	1.923
144	1.377	1.721	1.926
168	1.384	1.725	1.949

Table 2 OD values obtained during fermentation of pomegranate peels with co cultures

Fermentation time (Hrs)	OD at 600 nm		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	0.687	1.584	1.384
48	0.803	1.725	1.586
72	0.924	1.843	1.816
96	1.041	1.965	1.882
120	1.156	2.051	2.020
144	1.280	2.142	2.050
168	1.346	2.165	2.059

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Table 3 OD values obtained during fermentation of orange peels with co cultures

Fermentation time (Hrs)	OD at 600 nm		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	0.121	0.346	0.246
48	0.190	0.446	0.320
72	0.207	0.497	0.346
96	0.289	0.513	0.365
120	0.316	0.575	0.460
144	0.347	0.655	0.515
168	0.361	0.675	0.523

Table 4 Bio ethanol yield from grape wastes

Fermentation time (Hrs)	Production of Ethanol (in %)		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	7.6	10.03	12.2
48	9.45	11.4	14.93
72	9.65	12.04	15.13
96	10.7	13.71	15.98
120	11.54	14.73	16.87
144	12.01	15.1	16.9
168	12.13	15.13	17.1

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Table 5 Bio ethanol yield from pomegranate peels

Fermentation time (Hrs)	Production of Ethanol (in %)		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	5.99	13.89	12.13
48	7.01	15.13	13.91
72	8.08	16.17	15.93
96	9.11	17.24	16.51
120	10.12	18.0	17.73
144	11.21	18.8	17.99
168	11.79	19.0	18.07

Table 6 Bio ethanol yield from orange peels

Fermentation time (Hrs)	Production of Ethanol (in %)		
	<i>S. cerevisiae</i>	<i>S. cerevisiae&A.niger</i>	<i>S. cerevisiae&R.oryzae</i>
24	1.01	2.99	2.11
48	1.62	3.87	2.76
72	1.77	4.32	2.99
96	2.31	4.46	3.16
120	2.73	5.01	3.99
144	3.0	5.71	4.48
168	3.12	5.89	4.55

Estimation of ethanol content

Sample calculation

Using the equation $y = 0.1136x + 0.0063$ (Caputi et al. 1968), ethanol content was found out.

Ethanol content in pomegranate peel substrate with co cultures of *S. cerevisiae&A.niger* after 168 Hrs = $(2.165 - 0.0063)/0.1136 = 19.00\text{g}/100\text{ml}$

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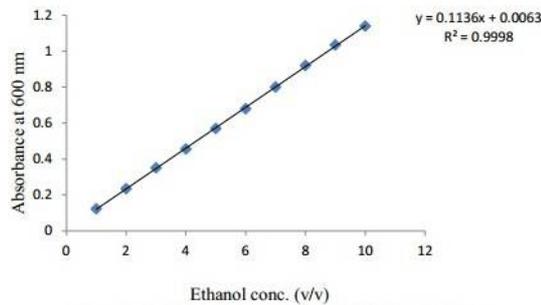


Fig.12 Estimation of ethanol content

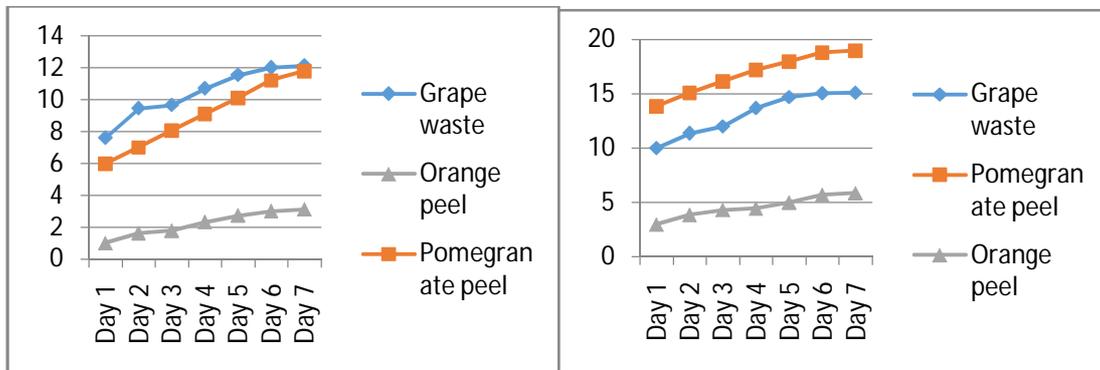


Fig.13 Comparison of bioethanol production in different fruit wastes using *S. Cerevisiae*.

Fig.14 Comparison of bioethanol production in different fruit wastes using *S. Cerevisiae* & *A. niger*.

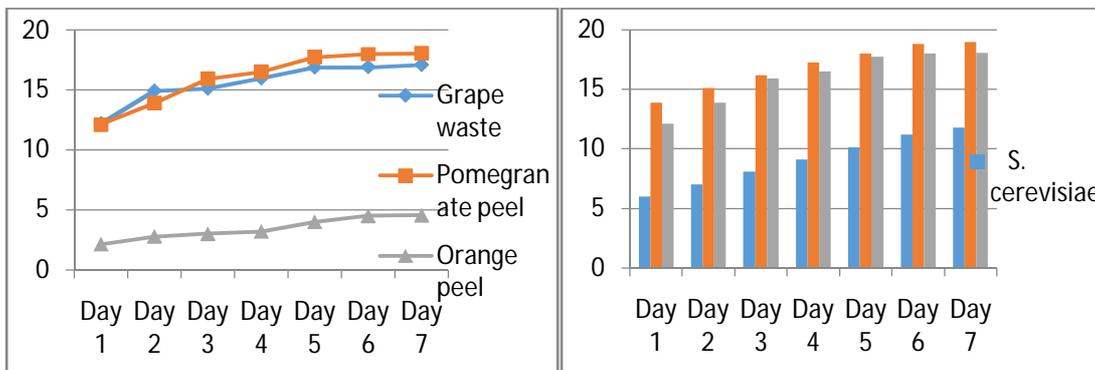


Fig.15 Comparison of bioethanol production in diff. fruit wastes using *S. Cerevisiae* & *R. oryzae*

Fig.16 Comparison of bioethanol production in pomegranate peels using different co cultures

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	Ethanol concentration (%) (<i>S.cerevisiae</i> and <i>A.niger</i>)	Ethanol concentration (%) (<i>S.cerevisiae</i> and <i>R.oryzae</i>)
Grape waste	15.13	17.1
Pomegranate peel	19.0	18.07
Orange peel	5.89	4.55

The result of this work has shown that different fruit wastes can serve as raw material for the production of bioethanol using co cultures. From this comparative study, it is clear that the maximum yield of ethanol was obtained from pomegranate peels using co cultures of *S.cerevisiae* and *A.niger*. Orange peels have comparable carbohydrate content (53.27 ± 0.10 %) with pomegranate peels (80.50 ± 1.52 %), even though it has very low bioethanol production compared to grape wastes and pomegranate peels. It may be due to the interaction of chemicals present in the orange peels with that of the microorganisms.

IV. CONCLUSION

In the present study, we have found that fruit wastes could be transferred to useful products such as bioethanol by fermentation, which could be used as an alternative fuel in motors and vehicles. It has been seen that the application of co-cultures are increasing the production of ethanol from fruit wastes. Pomegranate peel contains larger amount of carbohydrates (80.50 ± 1.52 %) and have higher conversion efficiency than orange peel wastes, and it was converted into monomers by the action of *R.oryzae* & *A.niger*. The monomers could be easily converted into ethanol by the anaerobic fermentation techniques using *Saccharomyces cerevisiae*. Also, the fungi cultures also contribute ethanol production to an extent (about 4-5%). The present study reveals greater yield of bio ethanol (19.1%) from pomegranate fruit peels while using the co cultures of *Saccharomyces cerevisiae* and *Aspergillus niger*. This process is cost effective and does not yield any toxic residues. Hence, it can be run as a small scale or even as a large scale industry.

On the basis of the current study, it may be suggested that if a method to develop and design an economic bioreactor for carrying out the process even in household scale was established, then that will serve as a fuel for vehicle purposes on a large scale. Also this method will serve as a better waste management process.

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