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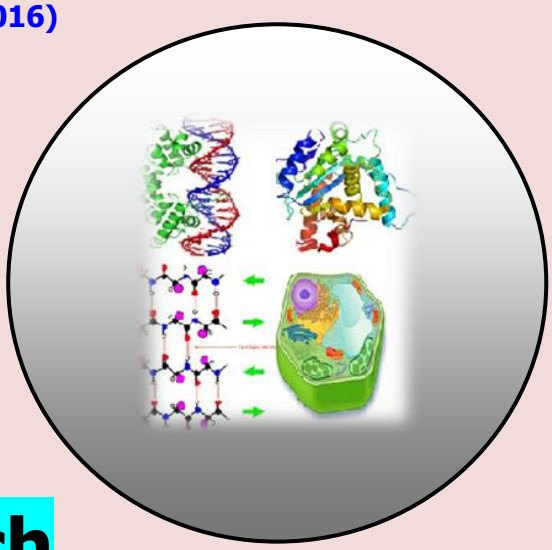

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RESEARCH PAPER

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Production of Ethanol from Fruit wastes using Saccharomyces *cerevisae*

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ABSTRACT

Yeast is a unicellular eukaryotic fungus, very common in the environment and is mostly saprophytic. Orange, pineapple and pomegranate fruit rinds (wastes) were locally available and thus serve as readily available raw materials for fermentation studies. In this study, maximum ethanol production by the yeast was recorded from pineapple waste (8.6g/ml) at 48 hrs of incubation period in batch culture, where as minimum ethanol yield in pomegranate waste was obtained and from orange peels at 48 - 60 hrs of incubation period in batch culture. There was an steady increase in reducing sugars content in different fruit wastes were observed as inoculated with Saccharomyces cerevisae. The result of this work has shown that different fruit wastes can serve as raw material for the production of bio ethanol. This process is cost effective and does not yield any toxic residues. Hence it can be run as a small scale industry.
Key words: Saccharomyces cerevisae, Fruit wastes and Fermentation studies.

INTRODUCTION

Due to rapid exhaustion of fossil fuels, there is an urgent need to resort to alternative fuels like ethanol. The first large scale use of ethanol as a fuel happened during the early 1900s when petroleum supplies in Europe were short. Though ethanol is conventionally produced from petroleum by-products, bio ethanol can alternatively be produced by fermentation technology using renewable raw materials (Singh and Jain, 1995).

Saccharomyces cerevisiae is the most popular organism used for ethanol production due to its high ethanol yield tolerance. Now a days, crops are the main source used for ethanol production. To achieve significant economic and environmental benefit large amount of food wastes can be utilized to produce ethanol. Utilization of fruit waste for bio ethanol production is one of the best options. One example of raw material is pineapple waste that is converted to bio ethanol (Hossain *et al*.,2008). The wastes contain valuable components such as sucrose, glucose, fructose and other nutrients (Sasaki *et al*., 2002). Lignocellulose is the major structural component of woody plants and non woody plants. The least expensive and easily available raw material for the production of bio ethanol is fruit waste. It is a potential source, from which ethanol can be produced. Fruit waste which is discarded has great antimicrobial and cell reinforcement potential (Reddy, 2007). 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 bio ethanol. *Saccharomyces cerevisiae* is used in the fermentation process since it converts sugar with oxygen to give carbon dioxide (Asli, 2010).

Yeast is a unicellular eukaryotic fungus, very common in the environment and is mostly saprophytic. The commercial importance of stains of yeast species *Saccharomyces cerevisiae* has made it a modal organism of study in both research and industrial applications (Legras, *et al.,* 2007). Fermenting wild yeast species are being isolated from the natural sources are being used in various fermentation processes. Yeast has been isolated from variety of natural sources like leaves, flowers and fruits (Spencer and Spencer, 1997; Tournas, 2005; Li *et al.,* 2008). Being a sugar-loving microorganism, it is usually isolation from sugar rich materials. Fruits contain high sugar concentration and hence yeast species are naturally present on these and can be easily isolated from fruits. Because of yeast fermentative characteristic, there is always a need for yeast stains with better feature of fermentation especially high ethanol tolerance for production of ethanol as bio fuel on commercial scale (Colin, *et al.,* 2006). Keeping these views in mind, an attempt was made to study on production of alcohol from fruit wastes using *Saccharomyces cerevisiae.* The present study was intended to determine the potential of the waste of ripe orange, pineapple and pomegranate fruits for alcohol production and to study the effect of various parameters on ethanol production like pH, turbidity, sugar production fermented with yeast.

MATERIALS AND METHODS

The fruit rinds (wastes) *viz.,* orange, pomegranate, pineapple was collected from in and around fruit stalls of Tenkasi (TK.), Tirunelveli, Tamil Nadu. These wastes were sliced into small pieces and shade dried. The dried wastes were ground into fine powder using a mixer. For alcoholic fermentation, yeast granules *i.e. Saccharomyces cerevisae* was procured from Local Bakery shop, Tenkasi (TK).

Medium preparation

Malt extract yeast liquid medium was prepared in 250 ml Erlenmeyer flasks containing malt extract (2.0%), yeast extract (2.0%), peptone (1.0%) dissolved in 100 ml of distilled water. pH 6.0 was adjusted to using a pH meter. Liquid medium was prepared individually in 250ml Erlenmeyer flasks containing fruit wastes (2%), glucose (1%), K_2HPO_4 (0.2%), KH_2PO_4(0.2%), $NaNO_3$(0.2%), Pinch of $FeSO_4$ dissolved inwater pH was adjused to6.0 using pH meter . These flasks were plugged with non-adsorbent cotton and autoclaved at 121˚C for 15 to 20 minutes at 15 1bs pressure and cooled. The sterilized flasks were inoculated with 0.1 ml of yeast suspension (prepared by dissolving one gram of yeast cells in 1ml of sterile dissolved water). The uninoculated flasks served as controls. All the inoculated flasks were kept in rotary shaker (Remi , India) for slow agitation. For every 12 hrs interval, the broth was analysed for change in pH, cell count by spread plate method (Aneja, 2005), turbidity in terms of growth, sugar content (Nelson Somiyil Method) and ethanol production (Kingsley otulugbu, 2012). All the experiments were carried out in replicates.

RESULTS AND DISCUSSION

Yeast is a unicellular eukaryotic fungus, very common in the environment and is mostly saprophytic. Orange, pineapple and pomegranate fruit rinds (wastes) were locally available and thus serve as readily available raw materials for fermentation studies. Yeast cells were grown in a pure culture on MGYP medium. After 24 to 48 hrs, white to cream coloured colonies appeared on agar plates. The colonies were smooth, shiny and flat with entire margin. Observation of the colonies under microscope showed that the obtained budding /oblong shaped colonies were of yeast and belonged to the same morphological group. Similar results were observed by Nasreen *et al* . (2014) worked on production of alcohol by yeast isolated from apple, orange and banana.

As the incubation period increases, there was an increase in cell count. Cell growth and highest number of yeast cells were found between 36 to 48 hrs of incubation period (1.8 to 2.7×10^6) (Table 1and Table 5). Mishra (2012) recorded crude protein and crude fat in banana peels. The result of alcohol/ethanol production from fermented orange, pineapple and pomegranate fruit wastes should be increased that the increased incubation period, that enhanced the ethanol yield (Table 4). In this study, maximum ethanol production by the yeast was recorded from pineapple waste (Table 4) at 48 hrs of incubation period in batch culture, where as minimum ethanol yield in pomegranate was obtained and from orange peels at 48 - 60 hrs of incubation period in batch culture. Viability of *Saccharomyces spp*. also studied by Moaris *et al*. (1996) and Aguilar *et al*. (2010). Manikandan *et al*. (2008) isolated various strains of indigenous yeast capable of producing ethanol from local fermented pineapple juice. Lavarack *et al.* (2012) and Hossain *et al.* (2008) did comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

Table: 1. Growth of *Saccharomyces cerevisiae* in batch culture.

S.No	Time interval (hr)	Total no. of yeast cells
1.	0	0.00
2.	12	1.5×10^4
3.	24	2.2×10^5
4.	36	1.8×10^6
5.	48	2.7×10^6
6.	60	1.1×10^4

Table: 2. pH level at different time intervals inoculated with *Saccharomyces cerevisae* in fruit wastes.

Time interval (hr)	Yeast culture	Orange	Pineapple	Pomegranate
0	6.5	6.5	6.5	6.5
12	6.0	6.2	6.0	6.3
24	5.7	5.8	5.5	6.0
36	5.5	5.6	5.2	5.8
48	5.2	5.4	4.9	5.6
60	4..9	5.0	4.7	5.3

Table:3 Production of Residual sugar (mg/ml) in different fruit wastes inoculated with *Saccharomyces cerevisae.*

Time interval (hr)	Yeast culture	Pineapple	Orange	Pomegranate
0	0	0	0	0
12	0.03	0.01	0.06	0.0
24	0.05	0.04	0.01	0.02
36	0.06	0.06	0.03	0.02
48	0.08	0.05	0.03	0.06
60	0.05	0.04	0.05	0.07

Table: 4 Ethanol production (ml/g) in different fruit wastes inoculated with *Saccharomyces cerevisae*.

Time interval (hrs)	Yeast culture	Pineapple	Orange	Pomegranate
0	0	0	0	0
12	1.2	2.3	1.2	0.5
24	2.0	4.5	1.8	1.2
36	2.7	7.3	2.7	1.8
48	4.6	8.6	3.5	2.5
60	4.0	5.2	4.4	3.2

Table 5 Cell growth of *Saccharomyces cerevisae* at different time intervals.

Time interval (hrs)	Yeast culture	Pineapple	Orange	Pomegranate
0	-	-	-	-
12	++	++	++	+
24	+++	+++	++	++
36	++++	++++	+++	++
48	++++	++++	+++	+++
60	++	+++	++	++

(++++) – Excellent; (+++) – Good; (++) – Fair; (+) – Moderate

pH value has signification influence on alcoholic fermentation. pH of bioethanol produced from different fruit wastes were determined . As the incubation period increases, there was a steep decrease in pH of fast inoculated yeast liquid medium as well as in different fruit wastes broth culture. In pineapple waste there was a sharp decline in pH observed at 60 hrs of incubation period when inoculated with *Saccharomyces cerevisae* (Table 2).

pH value has significant influence on alcoholic fermentation. Results were positively correlated with Reddy (2007) were the pH value of ethanol produced by the process of fermentation ranges from 4 to 6 yeast *i.e. Saccharomyces cerevisae* survives in a slightly acidic environment with pH ranges from 4 to 6. Among this range, ethanol produced from fruit wastes had on higher alcoholic content at pH 5.0 especially in pineapple waste after steadily declined 48 hrs of fermentation and later. Prolonged fermentation in orange and pomegranate wastes had on less alcoholic content at pH 4.7 and 5.3. Ban-koffi and Han (1990), isolated yeast strains from ripe banana peels for ethanol production and found, that isolates fermented 40% glucose at 30 degree Celsius to yield 3.6 and 5.8% ethanol respectively. As the fermentation progressed, these was an change in broth culture *i.e.*, From dark brown to light yellow in colour, due to the growth of yeast cells (Table 5). There by an steady increased in cell growth/ accumulation of biomass, also exhibited high flocculation ability. The highest yeast biomass was recorded pineapple waste and lowest one was on orange and pomegranate waste as compared to the yeast culture during 48 hrs of fermentation (Table5). Mishra (2012) recorded crude protein and crude fat in banana peels. Protein is an essential nutrient for yeast growth while fat is vital for the structure and biological functions of the cell and can be utilized as alternative source of energy by the cells.

There was an steady increase in reducing sugars content in different fruit wastes were observed as inoculated with *Saccharomyces cerevisae.* The maximum reducing sugars content was seen in pineapple waste and minimum amount was observed in orange and pomegranate waste as compared to the yeast culture during the 36 to 48 hrs of incubation period and a sudden decline further prolonged the incubation period (Table 3). Temperature plays a major role in the production of ethanol; since the rate of alcoholic fermentation increases with the increase in temperature. The optimum temperature of ethanol ranges between 25 degree celsius to 40 degree celsius which depends on room temperature. Bio Ethanol produced from pineapple, orange and pomegranate wastes have same temperature of about 30 degree and ethanol from yeast culture has 27 degree Celsius temperature. When temperature goes below the optimal range, their ability to catalyze the intended reaction slows down. On the other hand, when the temperature increases enzymes begin to denature / unfold and thus become inactive. Each enzyme will have a different temperature range, where it becomes inactive. Even if one essential enzyme stops working, the organism fails to grow. Here, the first essential enzyme that gets deactivated defines the maximal temperature at which that organism can grow at the lower end, it gets more complicated. Usually the enzymes are not inactivated, but rather just slow down (Sanchez, 2007). The result of this work has shown that different fruit wastes can serve as raw material for the production of bio ethanol. From this comparative study, it is clear that the maximum yield of ethanol was obtained from pineapple wastes at pH 5.0, temperature 30ºC. Finally, comparative studies of bio ethanol production from higher efficiency than other fruit wastes such as orange and pomegranate wastes such as orange and pomegranate wastes as inoculated with yeast cells *i.e., Saccharomyces cerevisiae.* This process is cost effective and does not yield any toxic residues. Hence it can be run as a small scale industry.

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REFERENCES

Aguilar, U, Garcia-Alvarado MGY, Gomez-Rodriguez J, Phister T, Delia ML, Strehaiano MP (2010). Growth and ethanol production of *Brettanomyces bruxellensis* at different glucose concentrations. 55 (2): 141-149.

Aneja, K.R., 1996. Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom cultivation, Wishwa Prakashan (New Age International (Pvt.Ltd), New Delhi. p.192-195.

Asli, M.S.,(2010). A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* extract from fermented siahe saedasht pomace, ***African Journal Biotechnology***, vol.9 **(20)**, pp.2906-2912.

Ban-koffi L., and Han Y.W. (1990). Alcohol production from pineapple waste. ***World J. Microbial Biotechnol.,*** 6:281-284.

Colin M, Jennifer M, Lopes De, B, Miguel JV (2006). "Generation of novel wine yeast strains by adaptive evolution". ***Am. J. Enol. Vitic,*** 57: 423–30.

Hossain ABMS, Abu Saleh A, Salleh AN, Boyce p, prothim Naquidin M (2008). Bioethanol production from agriculture waste biomass as a renewable bioenergy resource in biomaterials. ***The 4th Inter. Biomed. Engineering conference Nikko HOTEL, Kuala Lumpur, Malaysia.*** 26 Jun 2008 to 28 Jun.

Kingsley Otulugbu (2012). Production of Ethanol from Cellulose (Sawdust). Degree Thesis on Plastic Technology.

Lavarack B P, Griffin G J, Rodman D (2002). The acid hydrolysis of sugarcane bagasse hemicelluloses to produce xylose, arabinose and other productions. ***Biomass Bioenergy*** 23,367-380 .

Legras JL, Merdinoglu D, Cornuet JM, Karst F (2007). "Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history". ***Mol. Ecol***., 16: 2091– 2102.

Li H, Veenendaal E, Shukor NA, Cobbinah JR, Leifert C (2008). "Yeast populations on the tropical timber tree species *Milicia excels*". ***Lett. Appl. Microbiol***., 21: 322– 326.

Manikandan.K, Saravanan.V and Viruthagiri. T(2008). Kinetis studies on ethanol production from banana peel waste using mutant strain of *Saccharomyces cerevisiae* ***Indian Journal of Biotechnology*** Vol 7, January 2008,pp 83-88.

Mishra.J (2012). A comparative study of ethanol production from various agro residues by using *Saccharomyces cerevisiae* and *Candida albicans.* ***Journal of Yeast and Fungal Research*****:** 3(2), 12-17.

Moaris PB, Rosa CA, Linardi VR, Carazza F, Monato EA (1996). Production of fuel alcohol by *Saccharomyces* strains from tropical habitats. ***Biotechnol Lett***., 18: 1351- 56.

Nasreen Z, Jabeen S, Shafique M, Usman S, Yaseen T, Yasmeen A, Nazir S (2014). Production of alcohol by yeast isolated from apple, orange and banana. ***International Journal of Food and Nutrition Sciences***, 1(2): 016 – 019.

Reddy, V(2007). "Production of ethanol from mango fruit juice fermentation", ***Research Journal of microbiology*****,** vol.2,(10), pp 763-769.

Sasaki K, Watanabe M, Tanaka T and T.Tanaka,(2002). Biosynthesis, biotechnological production and application of 5-aminolevulinic acid. ***Appl Microbiol Biotchnol.,*** 58:23-29.

Sanchez, C., 2007. "Lignocellulosic residues: biodegradation and bioconversion by fungi", ***Biotechnology Advanced journal***, Vol.27,(2),pp.185-194.

Singh, A., and Jain, V.K, "Bath fermentation of cane molasse for ethanol production by *Zymomonas mobilis*". ***Journal of Indian chemical Engineering***. Vol.37.pp.80-94,1995.

Spencer JFT and Spencer DM (1997). "Yeasts in Natural and Artificial Habitats". Springer- Verlag, Berlin-Heidelberg, p. 381.

Tournas, V.H. (2005). "Moulds and Yeasts in fresh and minimally processed vegetable and sprouts". ***Int. J. Food Micro***., 99: 71-77.

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