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Ethanol production from various plant sources using *Saccharomyces cerevisiae*

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Abstract
The comparative analysis on ethanol production by *Saccharomyces cerevisiae* from different carbohydrate rich sources like Sweet potato, Cassava, Sugar beet, Mahua flower, Sal seed and Grapes was studied. The optimum temperature to get maximum ethanol yield varied from 29 ºC to 35 ºC. The values for Cassava, Grape, Sweet potato, Sugar beet, Mahua flower and Sal seed were 29, 35, 31, 30, 31 and 34 ºC respectively. The optimum pH for ethanol yield in Mahua and Grape was 5.0 where as in Sugar beet and Sweet potato was at 5.5. The value for Sal seed and Cassava were 4.5 and 6.0 respectively. In batch fermentation after 8th day highest ethanol production of 27.8% was noticed in case Mahua flower and lowest 5.9% in grapes. Ethanol yield from Sweet potato was 14.2% followed by Sal seed 13.6%, Sugar beet 12.6% and Cassava 7.9%. The present findings showed positive relationship of carbohydrate content to that of ethanol yield from the substrates used and Mahua flower may be best use for commercial ethanol production.

Keywords: Substrates, ethanol, *Saccharomyces cerevisiae*, batch fermentation, carbohydrate

Introduction
Ethanol is a colorless natural byproduct of plant fermentation resulted through microbial conversion of biomass from different carbohydrate rich sources or chemically produced in the industry. It is a common ingredient of manufacturing various products of various industries. In many countries ethanol is used as an alternative fuel on blended with petrol or gasoline. It is widely accepted as to show clean burning (Reddy and Reddy, 2007) [13]. Many researchers have studied on ethanol production using various raw materials (Pramanik and Rao, 2005) [11]. By using *Saccharomyces cerevisiae*, ethanol can be produced from the sources like Mahua flower, Sweet potato, Sugar beet, Grapes, Cassava and Sal seed (*Shorea robusta*). These examples of substrates used in this work due to the high content of fermentable carbohydrates (sugar and starch) which can be directly used as fermentable substrates without any modification. The selected sources are preferable commercial production, as the sources like Mahua flower and Sal seed used, are cheap and easily available. Mahua flower and Sal seed are locally collected from forest areas of Odisha without expenditure towards special cultivation. Traditionally, the village people of Odisha use to produce country-wine from Mahua flower. Cassava, Sweet potato and Sugar beet, the common edible tubers are the rich source of starch. The grape fruits, though production is expensive but it is widely accepted as the source of tasty wine. *Saccharomyces cerevisiae* have been still used as prime species for ethanol production than other microorganisms (Bai *et al.* 2008) [5].
Bio-ethanol is more preferable as it is prepared from a renewable source where as the petroleum ethanol is produced from non-renewable source like fossil products. Production of bio ethanol is also cost effective as the investment is very low for the production units. Beside this the price of the substrates used for this purpose are also cheaper than the fossil products.

Materias and methods
Preparation of slurry of different substrates
Sweet Potato
Freshly harvested 500 gm Sweet potato, variety Gouri was collected from Regional Centre of CTCRI, Bhubaneswar. It was cleaned, washed, thoroughly and the skin was removed. Then peeled and sundried for four to five days and grounded to fine powder. The sweet potato slurries was prepared adding water to the fine powder and autoclaved for 30 minutes at 120 ºC under 15 lb pressure.

Cassava and Sugar Beet
Likewise 500gm of Cassava fine powder was prepared collecting freshly harvested cassava variety Sree Rekha from Regional Center of CTCRI, Bhubaneswar. Cassava slurry was also prepared by the same method as above. Sugar beet slurry was prepared from variety Subhra collected from local market following the same method (Vucurovic and Razmovski, 2012) [17].

Mahua
Fresh Mahua Flowers was collected from the forests of Keonjhar district of Odisha state. Washed two to three times with clean water. These flowers were then sun dried for four to five days to reduce the water content. The dried Mahua flowers were grinded with a blender adding distilled water five times by weight. The slurry was autoclaved in 20 lb pressure for 20 minutes and kept for fermentation.

Sal (*Shorea robusta*)
Raw seeds of *Shorea robusta* (Sal) were collected in the month of April-May from the forests of Angul district of Odisha state. The wings and fruit coat were removed and seeds were harvested, sun dried thoroughly for 3-4 days. The seeds were grinded to fine paste. The paste was mixed with water and sterilized under 15 lb pressure for 20 minutes and slurry was kept for fermentation.

Grapes
Fully ripened grapes (black variety) were collected from the local market of Bhubaneswar city of Odisha state. The 250gm fruits were cleaned and washed with distilled water 3-4 times and blended. This fresh slurry was used for fermentation.

Preparation of Yeast Culture (Inoculums)
The yeast strain was obtained from RMRC, Bhubaneswar and multiplied in Yeast extract peptone dextrose (YEPD) agar medium. The fermentation inoculums were prepared growing the yeast in sterile YEPD broth in a shaking incubator.

Fermentation
The sterile slurry of different substrates was inoculated with 3 day's old 2% inoculums for batch fermentation in 500 Erlenmeyer flask. The pH was maintained to 5.5 and kept at temperature of 30 ^{o}C, in a shaking incubator with 110 RPM for 8 days in shaking condition (Saritha *et al.* 2010) [14].

Estimation of Ethanol
Quantification of Ethanol was done taking 10ml of sample in every 24 hours interval up to 8 day of inoculation. The sample was centrifuged at 10000 rpm for 15 minutes. The supernatant was added with 25 ml of potassium dichromate reagent, kept in water bath at 80 ^{o}C for 15 minutes. Then 1 ml of 40% sodium potassium tartarate was added and the OD was measured by spectroscopy at 600 nm. The ethanol quantity was estimated from a standard graph of absolute alcohol (Caputi *et al.* 1968) [6].

Estimation of Carbohydrate, Protein and Ash content of substrates
The carbohydrate content of the substrates was estimated by DNS method (Miller, 1959) [9], Protein By Lowry Method (Lowry, 1951) [8] and Ash by dry oxidation method (ASTM E1755-01, 2003) [4].
To determine the optimum temperature and pH for fermentation of different sources, one separate experiment was conducted. For pH, the flasks were kept at 28 ^{o}C maintaining pH from 3.0 to 7 at 0.5 intervals. The pH was maintained by adding H_2SO_4 and NaOH. For determination of optimum temperature the flasks were kept at different temperature ranging from 26 ^{o}C to 36 ^{o}C at 1 ^{o}C intervals in shaking incubator for seven days and quantification of ethanol was done.

Results
Quantification ethanol production was done after 24 hours of incubation up to 8th days in all the substrates. After 8 days of inoculation the total ethanol production in Mahua flower was 27.8% which was highest among the substrate of test and lowest in Grapes with 5.9%. Next to Mahua flower, 14.2% ethanol production was recorded in Sweet Potato followed by Sal Seed 13.6%, Sugar beet 12.6% and Cassava of 7.9% (Table-1, Figure-1). After 24 hours of incubation highest production in ethanol was observed in Sal Seed and lowest in Grapes. The rate of ethanol production was increased up to 5th day of incubation then after the production rate decreased in almost all the substrates

Table 1: Ethanol production (%) in different plant sources

Days of Inoculation	Sources/Ethanol Production (%)					
	Sweet potato	Cassava	Sugar beet	Mahua	Sal seed	Grape
1	0.8	1.0	1.1	2.3	5.9	0.5
2	3.1	1.9	2.0	4.7	7.8	1.2
3	5.9	2.8	2.9	7.4	9.7	2.1
4	8.7	3.6	4.8	12.5	11.5	2.9
5	12.1	5.1	6.6	18.5	12.3	3.9
6	13.5	5.9	9.1	22.5	12.9	4.3
7	14.0	6.2	11.3	26.5	13.2	5.1
8	14.2	7.9	12.6	27.8	13.6	5.9

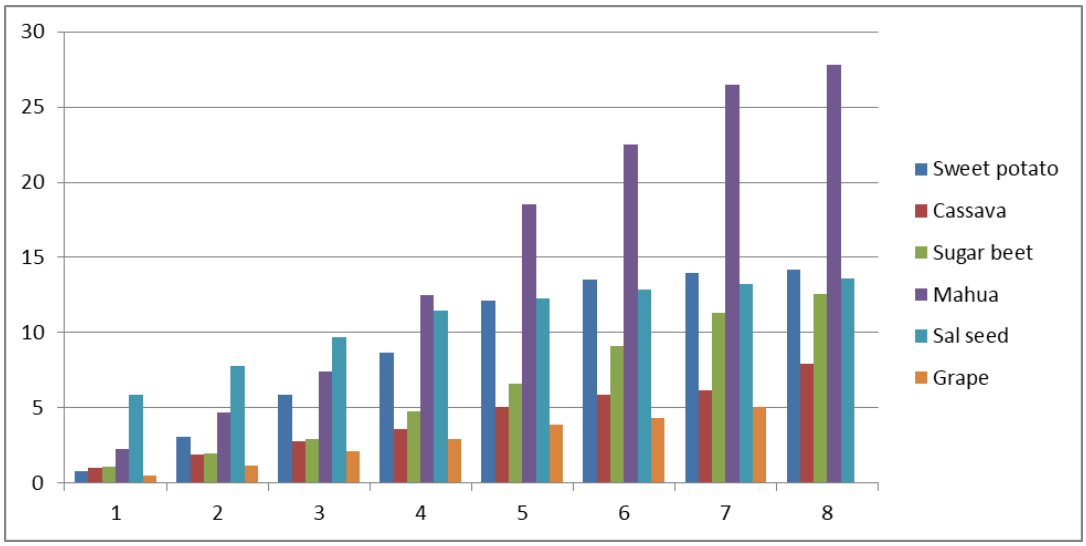

Fig 1: Ethanol production (%) in different plant sources.

Days of incubation

Optimum temperature and pH for all the substrates were determined individually (Table-2). The optimum pH for ethanol production in Cassava was 6.0, where as for Sweet Potato and Sugar beet was 5.5 pH. Mahua flower and Grape gave maximum ethanol at pH 5.0, where as ethanol production was best in Sal seed at more acidic medium (4.5 pH) (Table-2).

Table 2: Optimum pH and temperature for ethanol production of different sources

Sources/Substrates	Optimum pH	Optimum temperature(ºC)
Sweet potato	5.5	31
Cassava	6.0	29
Sugar beet	5.5	30
Mahua	5.0	31
Sal seed	4.5	34
Grape	5.0	35

The optimum temperature for ethanol production by *Saccharomyces cerevisiae* ranges from 29-35 ºC in the study. Cassava need lower temperature 29 ºC where as Grape need higher temperature 35 ºC for best ethanol production. Highest ethanol production was noticed at 31 ºC in Mahua flower and sweet potato, where as temperature of 30 ºC and 34 ºC favor ethanol production in Sugar beet and Sal seed respectively.

The carbohydrate, protein and ash content of the substrates used for ethanol production were estimated (Table-3). Highest Carbohydrate was found in Mahua flower 71.6% and lowest 28.2% in grape. Sweet potato, Sal Seed, Sugar beet and Cassava contain 69.2%, 62.7%, 50.5% and 37.9% of Carbohydrate respectively. Besides the carbohydrate, higher protein was found in Grapes 8.6% and Sal Seed 8.0% and lower in Cassava 2.1% and sweet potato 4.2%. Mahua flower contain 6.1% and Sugar beet 6.0% of protein. Highest ash content was found in Sweet Potato (4.0%) and lowest 1.5% in Mahua flower (Table-3)

Table 3: Composition of Substrates

Sources/Substrates	Carbohydrates (%)	Protein (%)	Ash
Sweet potato	69.20	4.20	4.0
Cassava	37.90	2.10	2.0
Sugar beet	50.50	6.00	7.2
Mahua	71.60	6.10	1.5
Sal seed	62.70	8.00	2.3
Grape	28.20	8.60	4.2

Discussion

Production of bio-ethanol was studied taking six substrates (including three tuber crops) like Sweet potato, Cassava, Sugar beet, Mahua flower, Sal seed and Grape. The quantity of ethanol produced was studied after 24 hours of yeast inoculation to the slurry up to 8 days in every 24 hour intervals. The carbohydrate, protein and ash content of all the sources used were determined by DNS, Lowry and dry oxidation method (Miller, 1959; Lowry, 1951 and ASTM E1755-01, 2003) [9, 8, 4]. After 8 days among the substrate taken, Mahua flower with high carbohydrate content 71.60% give highest ethanol yield of 27.8% followed by Sweet potato having 69.2% carbohydrate yield 14.2% ethanol. As Mahua flower rich with carbohydrate and low protein, fat, calcium, magnesium and vitamins (Ward and Singh, 2002; Ward and Singh, 2005; Ward *et al* 2006) [18, 19, 20] is suitable for breaking the carbohydrate and forming alcohol by yeast fermentation. For commercial production of bio-ethanol Mahua flower and Sweet potato can be used extensively. Lesser amount (5.9%) of ethanol was produced by grape. Ethanol obtained from grape has lesser acidity 0.019gm/100 ml when the permissible limit is 0.020gm/100 ml. The aldehyde content was also lesser than 0.0068gm/100 ml. Hence ethanol from grape is suitable for wine manufacture and other various applications (Raikar, 2012) [12].

Sal seed, Sugar beet and Cassava containing 62.70%, 50.50%and 37.9% carbohydrates can yield 13.6%, 12.6% and 7.9% of bio-ethanol by yeast fermentation respectively (Table 1 and Table 2). In Cassava based on the fermentation of the hydrolysate with *Saccharomyces* after seven days resulting the bio-ethanol production which was at concentration of 8.5% (Adetunji *et al.*, 2015) [1].

To determine the optimum temperature range for bio-ethanol production separate experiment was conducted. Maximum ethanol production was noticed in Sweet potato, Cassava, Sugar beet, Mahua flower, Sal seed and Grape was at pH 5.5, 6.0, 5.5, 5.0, 4.5 and 5.0 respectively. The corresponding values of optimum temperature are 31, 29, 30, 31, 34 and 35 ^{o}C.

In case of Sal seed maximum bio-ethanol production was obtained at pH 4 (Periyasamy *et al*. 2009) [10] and at pH 4.5 (Asli 2010 and Tahir *et al.* 2010) [3, 15]. Tofighi *et al.* (2014) [16] found similar result with a novel autochthonous thermo tolerant yeast with the optimum temperature over 35 ^{o}C. Working on Sweet potato Ashok Kumar *et al.* (2014) [2] observed 35 ^{o}C was the most appropriate temperature for yeast growth and production of ethanol by *S. cerevisiae* MTCC-170 was also favored by this temperature. The present study also shows the same trends.

Conclusion

Different substrates namely Sweet potato, Cassava, Sugar beet, Mahua flower, Sal seed and Grape were used for ethanol production by yeast batch fermentation. Mahua flower gave highest percentage of bio-ethanol at the end of 8th days of study. The optimum temperature and pH for bio-ethanol production varied in between 29-35 ^{0}C and 4.5-6 pH. Mahua flower was also standardized as 31 ^{o}C and pH 5.0. If the syrup is fermented under stipulated experimental conditions with *S. cerevisie*, a substantial amount of ethanol will be produced to fulfill the industrial need of alcohol on a large scale. Though Lignocellulogic materials more difficult to hydrolyse than starchy maretials, still Sal seed showed quite encouraging result like starchy Sweet potato. From the results of this study it can be summarized that Mahua flower is an attractive substrate for bio-ethanol production. Comparatively, both Mahua flower and Sal seed seemed very cheap and can be collected from the tropical forest of India which can also facilitate the tribal people for some earnings and enhanced good forest cover.

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