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INDUCED MUTATIONAL STUDIES ON *SACCHAROMYCES CEREVISIAE* FOR BIO-ETHANOL PRODUCTION FROM AGRO WASTE

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ABSTRACT
The fresh (Musa Cavendish) pseudostem and paddy straw was collected from nearby agricultural field. The efficient rice straw degrading microorganism was identified as *S.cerevisae. S.cerevisae* cellulase was produced by the solid state culture on Banana pseudostem and rice straw medium. Rice straw exhibited different susceptibilities towards cellulase totheir conversion to reducing sugars. The present study showed also that, the general trend of banana pseudostem and ricestraw bioconversion with cellulase was more than the general trend by *S.cerevisae.* The acid pretreatment and alkali treatment resulted the highest conversion of lignocellulose in rice straw and banana pseudostem to sugar, highest ethanol concentration after 7 days fermentation with mutant strain of yeast *S.cerevisae*. The ethanol yield (EY/ml) n this study was about 8.9ml and non mutant strain producing ethanol of 7.9ml.

KEYWORDS: S. Cerevisae, rice straw residues, Banana Pseudostem, Acid and alkali hydrolysis, Bioethanol.

INTRODUCTION
Bio ethanol is made biologically by fermentation of sugars derived from a variety of sources. The use of ethanol as a motor fuel begun with its use in the internal combustion engine invented by Nikolas Otto in 1897 (Ahindra, 2008). Alcohols have been used as fuels since the inception of the automobile. ‘’First generation bio ethanol’’ is made from sugar feedstock such as cane juice (in Brazil) and molasses (in India) or from starch rich materials such as corn (in US) second generation ethanol from non food lignocellulosic materials such as agricultural residues, wood, paper and municipal solid waste and dedicated energy crops (viz. miscanthus, switchgrass, sweet sorghum, etc..,) which constitute the most abundant renewable organic component in the biosphere (Claassen *et al*., 1999).

Banana pseudostem (BPS) was used as raw material for the production of bioethanol. High concentration of holocellulose (72%) with low lignin content (10%) and environment. High concentration of holocellulose (72%) with low lignin content (10%) and its easy availability makes BPS as potential lignocellulosic biomass which could be used for the production of bioethanol. Rice straw is a by - product of rice production and great bioresource. Rice straw predominantly contains cellulose 32 -47%, hemicellulose, 19 – 27 %, lignin 5-24% and ashes 18 %. The Pentose is a dominant in hemicelluloses which contains xylose. Xylose is the most important sugar followed by arabinose and hexoses.

The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For the conversion of biomass to fuel, the cellulose and hemicelluloses must be broken down into their corresponding monomers (sugars), so that microorganisms can utilize them. Three major hydrolysis processes are typically used to produce a variety of sugars suitable for ethanol production: dilute acid, concentrated acid and enzymatic hydrolysis.

MATERIALS AND METHODS
Method of Sample Collection
The fresh *(Musa Cavendish)* pseudostem and paddy straw was collected from nearby agricultural field. The fresh pseudostem and paddy straw was washed in running water to remove the dirt. It was cut into small pieces and shade dried. Dried peudostem and paddy straw was grinded in a mixer and grinder thereby reducing the particle size and stored at room temperature for further use.

Preparation of yeast strains: Yeast was isolated from commercial yeast. *Sacchromyces cerevisiae* were grown on petriplate containing yeast – peptone dextrose (YPD) agar. Single cell isolated colonies were inoculated in conical flask containing 50ml of yeast – peptone dextrose (YPD) media. *Sacchromyces cerevisiae* culture was incubated for 18 to 24 hours prior to inoculation.

Induction of Mutation By UV

Grow an overnight culture of the desired yeast strain in 25 ml YPD medium at 30°c.To transfer the culture by inoculation loop and four plate was taken and culture was streaked in petriplate in the time intervals 5 sec, 20 sec, 30 sec, and 60 seconds interval.

Pretreatment Optimization

Pretreatment with Acid

Pretreatment was carried out in Erlenmeyer flasks(100ml). The powdered *Musa Cavendish* peudostem and paddy straw was treated with different acid i.e., HCL and H_2SO_4 at different concentrations of 0.5 %, 1%, 1.5 % and 2 % (w/v) for 1g and 2g of dried sample. The mixture was autoclaved at 120 ^{o} C, 15 lbs pressure for 15 minutes and further cooled down to room temperature. The hydrolysate was filterate was collected and analysed for the reducing sugar content by using DNS test.

Pretreatment with Alkali

Pretreatment was carried out n Erlenmeyer flasks (100ml). The powdered *Musa Cavendish* peudostem and paddy straw was treated with alkali i.e., NaOH and KOH at different concentrations of 0.5 %, 1%, 1.5% and 2% (w/v) for 1g and 2g of dried sample. The mixture was autoclaved at 120^{o} C, 15 lbs pressure for 15 minutes and further cooled down to room temperature. The hydrolysate was filterate was collected and analysed for the reducing sugar content by using DNS test.

Fermentation

Fermentation was carried out in Erlenmeyer conical flasks. On obtaining high sugar content, the pretreated hydrolysate was taken for further fermentation process. 50ml of pretreated sample was fermented in 100 ml of Erlenmeyer flasks. The ph was adjusted to 6 – 7 and the 1ml of *Sacchromyces cerevisiae* was inoculated and incubated in room temperature. At an interval of 3 days, 6 days, 9 days, 12 days, and 15 days the reducing sugar and ethanol was estimated.

Estimation of Reducing Sugar

Total reducing sugar was estimated by dinitrosalicylic (DNS) acid reagent (Miller,1959). The hydrolysate obtained after pretreatment was estimated for reducing sugar by DNS method. The whole reaction mixture was incubated at boiling water bath for 15 minutes. Subsequently observance was measured at 540 nm. Following the similar procedure, the filtrate obtained at different acid and alkali concentrations was estimated for reducing sugar by adding DNSA reagent.

Dns Preparation

1.0.5g of dinitrosalicylic acid(DNS) and chopped in morter pestle.

2. weigh 2g of NaOH, is dissolved in 50 ml of distilled water.

3. Then to chopped DNS, add NaOH drop by drop, to avoid crystal like substances.

4. Then weigh 30g of sodium potassium tartarate and dissolved in 40 ml of distilled water.

5. Then filter the DNS solution and add sodium potassium tartarate to it, make it to 200 ml with distilled water.

Moisture content (TAPPI Standards, 1980)

A Sample weighing of 5g (A) was taken in crucible. It was dried in oven for 2 hours at 105^{0} C then cooled. Then after 1 day crucible was weighing and until a constant weight (B) was obtained. The moisture content was collect using formula.

Moisture content= ((A-B)/A) × 100.

Ash content (TAPPI Standards, 1980)

The ash content was determined by heating a sample in muffle furnace. 5g of sample in placed crucible and weighted. The crucible was transferred to a muffle furnace and the ash continued in 750^{0} C, after 1 day until a constant weight was obtained.

Lignin content (TAPPI standard, 1980)

5 grams of pseudostem powder was weighed out accurately in weighing bottle and transferred in a 100 ml beaker. 11.2 ml of cold (10-15 °C) 72% sulphuric acids was added carefully with a pippet and the mixture was stirred with a small glass rod. After the specimen is dispersed, cover the beaker with watch glass and keep it in a bath at 20 °C for 2 hours. Add about 40 ml of water to a flask and transfer to material from the beaker to the flask. Rinse and dilute with water to 3% concentration of sulphuric acid. Boil the solution for 4 hours, maintaining constant volume by addition of hot water. Allow the insoluble material (lignin) to settle keeping the flask in an inclined position. Without stirring up the precipitate, decant off the supernatant solution through a filtering crucible. Wash the lignin with hot water. Dry the crucible with lignin in an oven at 105°C to the constant weight. Cooled in a desiccator and weighed.

Cellulose content (Halliwell, 1965)

5g of sample was added 10 ml petroleum ether the extraction was taken in a oven. The sample were boiled in 0.5% ammonium oxalate for 1 hour. They were using 1% sodium chloride in 0.05 N acetic acid boiling water bath for removing lignins. Then sample was treated with 5% potassium hydroxide 2-3 hrs. The cellulose thus obtained was stirred with a 20 fold concentrated phosphoric acid for 2 hrs at 1^{0} C, washed with 1% sodium carbonate and then with water. The residue was dried and weighted to constant weight and reported as cellulose.

Estimation of Ethanol (Caputi *et al.*, 1968)

Stock standard solution of ethanol: Different concentration (20%-100%) ethanol was diluted with water to make up the volume to 1ml.

Preparation of potassium dichromate solution
32.5ml of conc. H_2SO_4 was diluted with 40ml of distilled water in a volumetric flask is followed by 33g of potassium dichromate was added and then make up to100ml distilled water.

Preparation of standard
1.0ml of each concentration of standard solution (20%-100% (v/v). 2.5ml of potassium dichromate solution was added in test tubes. The samples were heated for 60^0 C for 20 minutes in a boiling water bath and cooled. Then the sample diluted with 5.0ml with distilled water. The colour was developed read colorimetrically at 600nm.

Determination of ethanol
0.5ml of sample with 2.5ml of potassium dichromate solution was added in each test tubes and the mixture was heated at 60^o C in a boiling water bath and cooled. The colour developed was read colorimetrically at 600nm.

RESULT AND DISCUSSION
The standard graph was plotted by using estimation of sugar by DNS method. The standard volume is 0.2, 0.4, 0.6, 0.8, and 1.0 and add distilled water to make 1ml for each tube. And add DNS reagent in 1ml for each tube. And reading od value at 540 nm.

Chemical composition of banana pseudostem was analysed and different contents was calculated in percentage basis. Lignin, moisture, ash, cellulose these are main contents present in banana pseudostem paddy straw. The moisture content was determined in banana pseudostem was 3.1%.and Paddy strawwas 2.9%. Then the ash content was estimated in banana pseudostem was obtained at 1.8% and paddy straw 1.6%.The lignin content was 7.5% and paddy strawwas 5.5%. Then the cellulose content of banana pseudostem was 20% and paddy straw 12%.

The moisture content determined in *Musa cavendish* was 95.3% ± 0.2% which was similar to the values determined by Oliveira and Medeiros, 2007 for the same type of residue. The lignin content (8.07% ± 2.11%) on a dry basis was lower than those found in most other plants studied for second-generation ethanol production (Sassner *et al*., 2006); however, lower lignin content in biomass does not mean that the delignification process can be conducted with a lower reagent or catalyst load as established by (Silva *et al*., 2009). The cellulose levels in banana tree pseudostem (44.0% ± 2.0%). (Guimaraes *et al*., 2009).

Moisturecontent Ash Content
The alkali pretreatment for the *Musa Cavendish* using NaOH and the concentration level was 0.5%, 1%, 1.5% and 2%, for 1g and 2g samples. And the best sugar produced in high level in 0.5% concentration in 1g and low level produced in 1% concentration. In 2 g the high level sugar produced in 1.5% and low level sugar in 1%. KOH pretreated with *Musa cavendish* the concentration level is same for all the treatment they are 0.5 %, 1%, 1.5%, and 2% high level sugar presented in 1g 1.5% and low in 1%. In the 2g the sugar concentration level in high in 0.5% and low in 2%.

Table -1.

SI No	Different Days	Alkali/ substrate concentration			
		KOH (1.5%) 1g UV		KOH (1%) 1g Non UV mutant	
		SC µg/ml	EY (ml)	SC µg/ml	EY (ml)
1	**3 day**	960	4.5	880	4.0
2	**6 day**	840	5.7	730	5.2
3	**9 day**	660	6.8	620	6.0
4	**12 day**	530	7.2	550	7.4
5	**15 day**	470	8.9	440	7.9

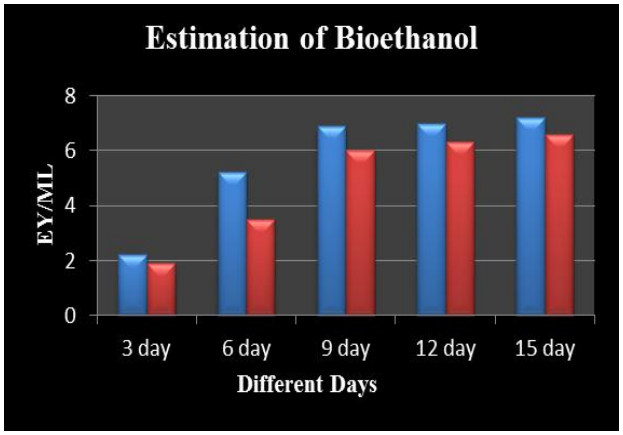


Figure. 1.

The estimation of sugar content pretreated with HCL. The high level of sugar is presented with the concentration of 0.5% and the low level sugar is present with 1.5% in 1g. In 2 gram the sugar content was present high level in 2% and low level in 0.5%.In H_2SO_4 the sugar concentration presented *Musa cavendish* high level in 2% and low level in 0.5% for 1g. And 2 g sample high level sugar presented in 2% and low level in 0.5%.The highest yield of 7.2%(v/v) bioethanol was produced by pineapple peels followed by banana peels (5.3% v/v), bioethanol was synthesized after 7 days of fermentation by the pine apple peel(Jimoh *et al*., 2009). 8.34% (v/v) of ethanol by produced (Itelima *et al*., 2013).

Table. 2.

SI. No	Different Days	Alkali/ substrate concentration			
		KOH (1.5%) 1g UV		KOH (1%) 1g Non UV mutant	
		SC µg/ml	EY (ml)	SC µg/ml	EY (ml)
1	**3 day**	660	3.2	540	2.7
2	**6 day**	600	4.3	420	3.9
3	**9 day**	530	5.5	380	4.8
4	**12 day**	440	6.3	260	5.9
5	**15 day**	300	6.9	180	6.0

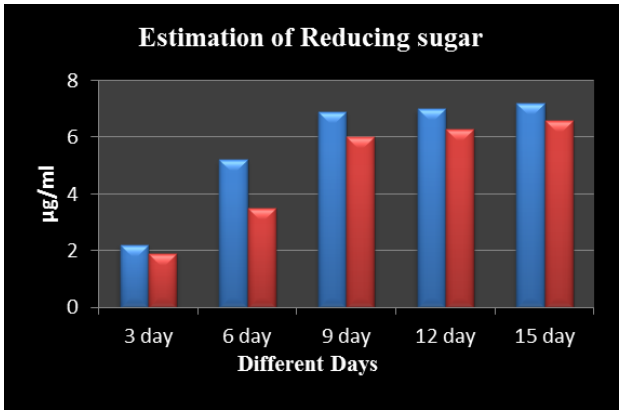
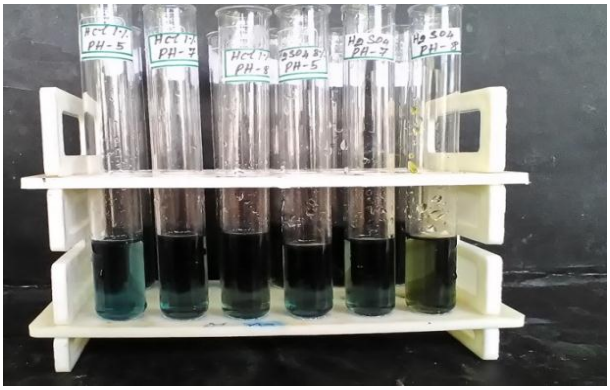


Figure. 2.

DNS Methode – Reducing Sugar



Estimation of Ethanol

The sugar presented in paddy straw for NaOH pretreated sample the high level sugar is present in 1% and low level in 2% for 1g samples. In 2g sample high level sugar in 0.5% and low level in 2%. The KOH pretreated with paddy straw for 1 g sample the sugar concentration was presented with high level in 1.5% and low level in 1%. In 2g sample the sugar concentration was presented high level in 1% and low level in 0.5%.

Table. 3.

SI. No	Different Days	Alkali/ substrate concentration			
		KOH (1.5%) 1g UV		KOH (1%) 1g Non UV mutant	
		SC (µg/ml)	EY (ml)	SC µg/ml	EY (ml)
1	**3 day**	840	2.2	600	1.9
2	**6 day**	790	5.2	510	3.5
3	**9 day**	630	6.9	460	6.0
4	**12 day**	550	7.0	340	6.3
5	**15 day**	410	7.2	220	6.6

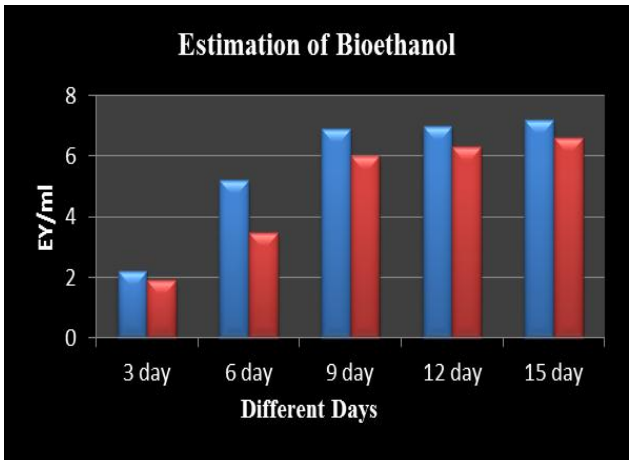


Figure. 4.

Acid Hydrolysis

Alkali Hydrolysis

The HCL pretreated with paddy straw sample the high level sugar is present 1% and low level in 0.5% for 1g sample. In 2 g sample the sugar concentration was present high level in 2% and low level in 0.5%. The pretreated paddy straw with H_2SO_4 the sugar concentration was presented in high level in 0.5% and low level sugar presented in 2% for 1g sample. In 2g sample the high level sugar concentration present with 1.5% and low level sugar present in 1%.Abedinfar *et al.* 2009 have investigated the fermentation of rice straw (pretreated with diluted acid and subsequent enzyme treatment) rice straw using *Mucorindicus* and *Rhizopus oryzae*. They have found an ethanolyield of 0.36-0.43 g g-1 using *Mucor indicus* which was comparable with the corresponding yield by *S. cerevisae*(0.37-0.45). *Rhizopus oryzae* produce 0.33-0.41 g/L ethanol. The ethanol yield in this study was about 0.42 g/L.

Table. 4.

Si. No.	Different Days	Acid/ substrate concentration			
		H_2SO_4 (2%) 1g UV		H_2SO_4(0.5%) 1g Non UV mutant	
		SC (µg/ml)	EY (ml)	SC µg/ml	EY (ml)
1	**3 day**	780	3.4	630	1.5
2	**6 day**	610	4.1	520	2.3
3	**9 day**	430	5.6	410	4.0
4	**12 day**	380	6.0	340	4.2
5	**15 day**	220	8.0	300	5.3

The standard ethanol estimation using by potassium dichromate method. To take a ethanol is 1 ml,2 ml, 3ml,…..8ml and add a distiiled water to each tube make a 10 ml and added to a 3ml of chromic acid each tube. The addition of 1ml Rochelle salt for each tube and reading OD value at 660 nm.

The high level sugar produced concentration was selected for paddy straw and *Musa cavendish*. The selected concentration used for the further test. The organism was inoculated in a selected concentration samples. The same organism was exposed on uv and inoculated in test concentration samples. At the different days were 3days, 6 days, 9 days, 12 days and 15 days. The sugar concentration presented in high level in *Musa cavendish* pretreated with HCL 0.5% 3rd and 6th day and low level sugar concentration was presented in 12th day. The paddy straw pretreated with HCL and the concentration 0.5 % uv mutant the high level sugar concentration was presented in = for1g of s.

CONCLUSION

A substrate for ethanol production by yeast strain *S.cerevisiae*. The yeast gave maximum ethanol (8.9 ml) using this hydrolysate. There is still a need to develop more efficient and economic pretreatment process a hyper cellulose producing strain for improved saccharification and an improved yeast strain capable of utilizing both pentose and hexose sugars, which in turn would increase ethanol production.

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