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EFFECTIVENESS OF FUNGAL PRETREATMENT OF ARECA NUT (*ARECA CATECHU* L.) HUSK WASTE ON BIOETHANOL PRODUCTION BY YEASTS AND *ZYMOMONAS MOBILIS*

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ABSTRACT

Arecanut husk waste is most abundant renewable energy source that may be considered as potential feedstock for ethanol production by microbial fermentation. The arecanut husk waste was initially subjected to microbiological pretreatment for obtaining reducing sugars. Such treated substrates with and without obtaining the filtrate were further subjected to fermentation. Among these, filtrate fermentation showed maximum production of ethanol and *Zymomonas mobilis* was efficient in conversion to ethanol from the substrate. The maximum ethanol yield was observed in *Aspergillus niger* and *Phanerochaete chrysosporium* pretreated raw material. After pretreatment, the filterate was collected and treated with different yeast strains and *Zymomonas mobilis*. The minimum ethanol yield was observed in *Candida shehatae* (1.3 ± 0.10 mg/g) and the maximum ethanol yield was observed in *Saccharomyces uvarum* (4.8 ± 0.05 mg/g) treatment. The medium ethanol yield was observed in *Phanerochaete chrysosporium* pretreated raw material. The minimum ethanol yield was observed in *Saccharomyces cerevisiae* (2.0 ± 0.10 mg/g) and the maximum ethanol yield was observed in *Pichia stipitis* (5.3 ± 0.05 mg/g) treatment. The minimum ethanol yield was observed in *Aspergillus niger* pretreated raw material. The minimum ethanol yield was observed in *Schizosaccharomyces pombe* and *Zymomonas mobilis* (1.4 ± 0.10 mg/g) and the maximum ethanol yield was observed in *Saccharomyces uvarum* (2.9 ± 0.05 mg/g) treatment. When both fermentation methods were compared, maximum ethanol was produced in filtrate fermentation method (without solids). It is essential to identify better combination of pretreatment methods and efficient yeast strains for exploiting the arecanut husk waste for ethanol production.

INTRODUCTION

Renewable energy is one of the important energy alternatives to reduce world dependence on petroleum. Ethanol has been recognized as a potential renewable energy to petroleum-derived transportation fuels (Sassner *et al.*, 2008). Developing biofuel such as ethanol from renewable biomass or lignocellulosic materials such as arecanut husk waste would provide strategic, environmental, and societal benefits (Prasad *et al.,* 2007; Samsuri *et al.,* 2008; Naveenkumar *et al.,* 2012). Lignocellulosic materials are considered the most abundant renewable resource available for the production of ethanol. The utilization of lignocellulosic biomass for fuel ethanol is still under development (Afifi *et al.,* 2011). Production of fuel ethanol from lignocellulosic material has the potential to reduce the net contribution of greenhouse gases to the atmosphere because ethanol could reduce net emissions of carbon dioxide (Samsuri *et al.,* 2008).

Arecanut husk waste (*Areca catechu* L.) is a potential renewable biomass resource for ethanol production (Prasad *et al.,* 2007). Arecanut (*Areca catechu* L.) popularly known as betelnut or supari, is one of the most importtant plantation crops of india (Mohankumar, 2008; Narayanamurthy *et al.,* 2008). It is cultivated in india covering an area of about 2.6 lakh ha with an annual production of 3.13 lakh tonnes. Arecanut is a most important commercial plantation crop in Shivamogga district of Karnataka state, cultivated in 26,725 ha with an annual production of 37,458 tonnes (Rajan *et al.,* 2005; Narayanamurthy *et al.,* 2008). Arecanut husk, which constitutes about 60-80%, is a solid residue generated as

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waste of which a large quantity is disposed off without proper treatment, thereby causing environmental pollution (Prasad *et al.,* 2007; Narayanamurthy *et al.,* 2008).

Arecanut husk disposed off in this way still contains considerable amount of fermentable sugars, which can be used as a cellulose source for the production of bioethanol employing solid state fermantation. As on date no literature is available on the production of bioethanol using Arecanut husk waste by fermentation. The husk is about 15-30% of the weight of the raw nut. The arecanut husk fibers are predominantly composed of cellulose and varying proportions of hemicellulose, lignin, pectin and protopection. The total hemicellulose content varies with the development and maturity, the mature husk containing less hemicellulose than the immature ones. The lignin content proportionately increases with the development until maturity. The arecanut husk constitutes about 60-80% of the total weight and volume of the fresh fruit. The husk fiber is composed of cellulose with varying proportions of hemicellulose (35-64.8%), lignin (13.0-26.0%), pection and propection.

Cellulose, hemicellulose and lignin are major components of the lignocellulosic biomass (Nigam, 2001; Kuila *et al.,* 2011). Biological pretreatment methods have not been developed as intensively as physical and chemical methods, due to its slow rate of reaction (Zhu *et al.,* 2009; Thirmal *et al.,* 2011). Biological pretreatment offers major advantages over conventional processes (chemical and physical processes) (Ueng *et al.,* 1981), which includes greater yields, minimal by product formation, absence of substrate loss due to chemical modifications, low energy requirement, mild operating conditions and low chemical disposal cost (Rao *et al.,* 1985; Shrestha *et al.,* 2008; Sherief *et al.,* 2010; Kuila *et al.,* 2011).

The present research was aim to obtain a novel process for the production of ethanol from Arecanut husk waste. Fermentation processes were carried out by simultaneuous saccharification and fermentation. Simultaneuous saccharification and fermentation (SSF) is the most promising process for the production of ethanol from lignocellulosic materials.

MATERIAL AND METHODS

Collection of raw material

Arecanut husk waste were collected from the local farmer in Shankaraghatta region, Bhadravathi taluk, Shivamogga district, Karnataka, India and cleaned. Moisture content was recorded and stored in a laboratory condition.

Milling of raw material

The arecanut husk waste were oven dried at 45°C and mechanically powdered to one mm size and used for the further experiments (Narayanamurthy *et al.,* 2008; Seema, 2008; Ballesteros *et al.,* 2010; Shwetha, 2010; Naveenkumar *et al.,* 2012).

Selection of fungal and yeast cultures

The two fungal cultures used for microbiological pretreatment were, *Phanerochaete chrysosporium* (NCIM 1197) *and Aspergillus niger* were maintained on Potato dextrose agar (PDA) medium plate containing 200g of potato filtrate, 20g of dextrose, 0.1yeast extract, 20g of agar, 1000 distilled water. The standard yeast strains used for the fermentation process were *Saccharomyces cerevisiae* (NCIM 3095), *Candida shehatae* (NCIM 3500), *Saccharomyces uvarum* (NCIM 3455), *Pichia stipitis* (NCIM 3498), *Schizosaccharomyces pombe* (NCIM 3457) maintained on MGYP medium (yeast extract 3g, malt extract 3g, peptone 5g, glucose 10g, agar 16g, distilled water 1000 ml) and bacterium *Zymomonas mobilis* (NCIM 2915) was maintained on *Zymomonas mobilis* medium (1% beef extract, 0.5% sodium chloride, 1%peptone, 2% glucose and 1.5% agar) (Amin and Doelle, 1990; Gurav and Geeta, 2007; Naveenkumar *et al.,* 2012).

Fermentation without substrate

Ten grams of finely powdered substrates were taken in 250 ml flasks and sterilized for 90 minutes at 15 lb pressure. The flasks were inoculated with the respective combinations of fungal strains at the rate of 5 disks in each flask. After seven days of incubation, 100 ml of sterile distilled water was added to each flask under aseptic condition and was filtered. The filter extractant of substrates were inoculated with bacteria and the yeast strains at the rate of 2% inoculum. The flasks were incubated under aerobic condition for 24 hours and thereafter anaerobic condition was created by plugging the flasks with cork making a provision for trapping carbon dioxide and incubated at room temperature for 7 days. After incubation, to estimate the ethanol content, reducing sugar and non-reducing sugar content in the sample (Gurav and Geeta, 2007; Havannavar and Geeta, 2010).

Fermentation with substrate

Ten grams of finely powdered substrates were taken in 250 ml flasks and sterilized for 90 minutes at 15 lb pressure. The flasks were inoculated with the respective combinations of fungal strains at the rate of 5 disks in each flask and incubated at room temperature for 7 days. After microbiological pretreatment, fungal cultures were heat killed and 100 ml of sterile distilled water was added to each flask under aseptic condition. Yeast and bacterial cultures were added to the substrates directly and fermentation was carried out along with the substrate. The flasks were incubated under aerobic condition for 24 hours and thereafter anaerobic condition was created by plugging the flasks with cork making a provision for trapping carbon dioxide and incubated at room temperature for 7 days. After incubation, to estimate the ethanol content, reducing sugar and non-reducing sugar content in the sample (Gurav and Geeta, 2007).

Analytical Methods

Determination of reducing sugar

The reducing sugars were estimated by Dinitrosalicylic acid method. The aliquots of extract were pipette out from 0.5 to 3 ml in test tubes the volume was equalized to 3 ml with water in all the tubes. Then 3 ml of DNS reagent was added, mixed and heated for 5 min. on a boiling water bath. After the colour has developed, 1 ml of 40% Rochelle salt solution was added and mixed. The tubes were cooled under running tap water and the absorption was read at 510 nm. The amount of reducing sugar in the sample was calculated using standard graph prepared from working standard Glucose (Sadasivam and Manickam, 1996; Agblevor *et al.,* 2006).

Determination of Non-reducing Sugar

Non-reducing sugars present in the extracts were hydrolyzed with sulphuric acid to reducing sugars. Then the total reducing sugars were estimated by DNS method. About 100 mg of the sample was taken and the sugars were extracted with 80 % alcohol (hot) twice (5ml each time). The supernatant was collected and evaporated on water bath. Ten ml of distilled water was added to dissolve the sugars. One ml of extract was pipette in to a test tube and 1ml of 1N H_2SO_4 was added. The mixture was hydrolyzed by heating at 49^0 C for 30 min. and then 1 or 2 drops of methyl red indicator was added. The contents were neutralized by adding 1N NaoH drop wise from a pipette. Appropriate reagent blanks were maintained. Then total non-reducing sugar was estimated by DNS method (Sadasivam and Manickam, 1996; Agblevor *et al*., 2006; Seema, 2008).

Determination of ethanol concentration

The amount of ethanol content was estimated by colorimetric method as described by Caputi *et al.,* (1968).

RESULTS

The arecanut husk waste was initially subjected to microbiological pretreatment for obtaining reducing sugars. Such treated substrates with and without obtaining the filtrate were further subjected to fermentation. Among these, filtrate fermentation showed maximum production of ethanol and *Zymomonas mobilis* was efficient in conversion to ethanol from the substrate.

Initial chemical composition of the raw material

The total reducing sugar (3.18 ± 0.02 mg/g), non-reducing sugar (0.37 ± 0.01 mg/g), Protein (5.80 ± 0.10 mg/g), Total carbohydrate (2.82 ± 0.01 mg/g), Moisture (2.90 ± 0.10 %), Total solids (75.29 ± 0.61 %), Organic carbon (29.38 ± 0.12 %), Nitrogen (0.05 ± 0.01 %), Cellulose (33.5 ± 0.74 %), Hemicellulose (32.4 ± 0.19 %) and Lignin content (11.5 ± 0.35 %) (Table1).

Fungal pretreatment of Arecanut husk waste (without substrate)

The maximum ethanol yield was observed in *Phanerochaete chrysosporium* pretreated raw material and than compared to control. This raw material was treated with different yeast strains and *Zymomonas mobilis*. The minimum ethanol yield was observed in *Saccharomyces cerevisiae* (270 ± 0.58 mg/L) and the maximum ethanol yield was observed in *Pichia stipitis* (450 ± 0.58 mg/L) treatment. The medium ethanol yield was observed in *Aspergillus niger* and *Phanerochaete chrysosporium* pretreated raw material. The minimum ethanol yield was observed in *Candida shehatae* (320 ±1.0 mg/L) and the maximum ethanol yield was observed in *Saccharomyces uvarum* (410 ±1.53 mg/L) treatment. The minium ethanol yield was observed in *Aspergillus niger* pretreated raw material. The minimum ethanol yield was observed in *Zymomonas mobilis* (270 ± 0.58 mg/L). The maximum ethanol yield was observed in *Saccharomyces cerevisiae* (390 ±0.58 mg/L) treatment. When all the treatments are compared *Zymomonas mobilis* showed maximum production of ethanol (Table 2, 3 and 4).

Table 1 Initial chemical composition of the Arecanut husk waste

Sl.No.	Content	Composition
1	Reducing sugar **(**mg/g)	3.18 ± 0.02
2	Non- Reducing sugar (mg/g)	0.37 ± 0.01
3	Protein Content (mg/g)	5.80 ± 0.10
4	Total Carbohydrate Content (mg/g)	2.82 ± 0.01
5	Moisture content (%)	2.90 ± 0.10
6	Total solids (%)	75.29 ± 0.61
7	Organic carbon (%)	29.38 ± 0.12
8	Nitrogen (%)	0.05 ± 0.01
9	Cellulose (%)	33.5 ± 0.74
10	Hemicellulose (%)	32.4 ± 0.19
11	Lignin (%)	11.5 ± 0.35

Note: The value of each combination consisted of mean ± S.D. of three replicates.

In *Phanerochaete chrysosporium* pretreatment, the minimum residual reducing sugar was observed in *Saccharomyces cerevisiae* (2.0 ± 0.29 mg/g) and the minimum residual non reducing sugar was observed in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (0.2 ± 0.01 mg/g) treatment. The maximum residual reducing sugar was observed in *Pichia stipitis* (5.3 ± 0.10 mg/g) and the maximum residual non reducing sugar was observed in *Saccharomyces uvarum* and *Pichia stipitis* (0.7 ± 0.03 mg/g) treatment (Table 3).

In *Aspergillus niger* and *Phanerochaete chrysosporium* pretreatment, the minimum residual reducing sugar was observed in *Candida shehatae* (1.3 ± 0.12 mg/g) and the minimum residual non reducing sugar was observed in *Pichia stipitis* (0.5 ± 0.02 mg/g) treatment. The maximum residual sugar was observed in *Saccharomyces uvarum* (5.3 ± 0.15 mg/g) and the maximum residual non reducing sugar was observed in *Candida shehatae* (1.8 ± 0.05 mg/g) treatment (Table 4).

In *Aspergillus niger* pretreatment, the minimum residual reducing sugar was observed in *Schizosaccharomyces pombe* and *Zymomonas mobilis* (1.4 ± 0.02 mg/g) and the minimum residual non reducing sugar was observed in *Saccharomyces uvarum* and *Schizosaccharomyces pombe* (0.2 ± 0.01 mg/g) treatment. The maximum residual sugar was observed in *Saccharomyces uvarum* (2.9 ± 0.05 mg/g) and the

Table 2 Ethanol production in *Aspergillus niger* pretreated Arecanut husk waste (with and without substrate)

Sl.No.	Treatments	Residual Total sugar (mg/g)		Residual Reducing sugar (mg/g)		Residual Non-reducing sugar (mg/g)		Ethanol (mg/L)	
		With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate
1	Control	6.9 ± 0.06	7.2 ± 0.05	4.4 ± 0.03	5.7 ± 0.05	2.5 ± 0.05	1.5 ± 0.03	60 ±1.53	110 ±1.53
2	*Saccharomyces cerevisiae*	1.2 ± 0.10	3.0 ± 0.06	0.8 ± 0.05	2.3 ± 0.03	0.4 ± 0.03	0.7 ± 0.05	100 ±0.58	390 ±0.58
3	*Saccharomyces uvarum*	1.0 ± 0.06	3.1 ± 0.08	0.6 ± 0.03	2.9 ± 0.05	0.4 ± 0.03	0.2 ± 0.04	20 ±0.58	360 ±1.0
4	*Schizo saccharomyces pombe*	2.8 ± 0.06	1.6 ± 0.03	2.6 ± 0.03	1.4 ± 0.02	0.2 ± 0.01	0.2 ± 0.02	20 ±1.0	290 ±1.0
5	*Pichia stipitis*	2.8 ± 0.06	3.0 ± 0.10	2.0 ± 0.06	1.9 ± 0.03	0.8 ± 0.01	1.1 ± 0.06	130 ±1.0	290 ±0.58
6	*Candida shehatae*	2.4 ± 0.03	2.9 ± 0.02	2.2 ± 0.03	2.1 ± 0.06	0.2 ± 0.03	0.8 ± 0.02	100 ±0.58	320 ±1.0
7	*Zymomonas mobilis*	2.1 ± 0.10	1.8 ± 0.02	0.8 ± 0.03	1.4 ± 0.03	1.3 ± 0.03	0.4 ± 0.05	150 ±1.0	270±0.58

Note: The value of each combination consisted of mean ± S.D. of three replicates.

Table 3 Ethanol production in *Phanerochaete chrysosporium* pretreated Arecanut husk waste (with and without substrate)

Sl.No.	Treatments	Residual Total sugar (mg/g)		Residual Reducing sugar (mg/g)		Residual Non-reducing sugar (mg/g)		Ethanol (mg/L)	
		With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate
1	Control	6.9 ± 0.06	7.2 ± 0.05	4.4 ± 0.03	5.7 ± 0.05	2.5 ± 0.05	1.5 ± 0.03	60 ±1.53	110 ±1.53
2	*Saccharomyces cerevisiae*	1.8 ± 0.05	2.2 ± 0.21	0.8 ± 0.03	2.0 ± 0.29	1.0 ± 0.10	0.2 ± 0.01	130 ±1.53	270 ±0.58
3	*Saccharomyces uvarum*	2.3 ± 0.05	3.2 ± 0.03	1.5 ± 0.08	2.5 ± 0.03	0.8 ± 0.05	0.7 ± 0.02	100 ±0.58	320 ±1.0
4	*Schizo saccharomyces pombe*	2.3 ± 0.12	2.4 ± 0.03	1.5 ± 0.03	2.2 ± 0.10	0.8 ± 0.01	0.2 ± 0.01	130 ±0.58	340 ±0.58
5	*Pichia stipitis*	1.5 ± 0.03	6.0 ± 0.29	1.3 ± 0.05	5.3 ± 0.10	0.2 ± 0.02	0.7 ± 0.03	180 ±1.0	450 ±0.58
6	*Candida shehatae*	2.1 ± 0.15	3.8 ± 0.08	1.3 ± 0.08	3.0 ± 0.06	0.8 ± 0.01	0.8 ± 0.03	180 ±0.58	430 ±0.58
7	*Zymomonas mobilis*	4.7 ± 0.03	2.9 ± 0.03	4.5 ± 0.05	2.5 ± 0.03	0.2 ± 0.02	0.4 ± 0.03	340 ±1.0	360 ±1.0

Note: The value of each combination consisted of mean ± S.D. of three replicates.

Table 4 Ethanol production in *Aspergillus niger* and *Phanerochaete chrysosporium* pretreated Arecanut husk waste (with and without substrate)

Sl.No.	Treatments	Residual Total sugar (mg/g)		Residual Reducing sugar (mg/g)		Residual Non-reducing sugar (mg/g)		Ethanol (mg/L)	
		With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate	With substrate	Without substrate
1	Control	6.9 ± 0.06	7.2 ± 0.05	4.4 ± 0.03	5.7 ± 0.05	2.5 ± 0.05	1.5 ± 0.03	60 ±1.53	110 ±1.53
2	*Saccharomyces cerevisiae*	3.3 ± 0.08	4.5 ± 0.06	2.7 ± 0.03	3.2 ± 0.05	0.6 ± 0.05	1.3 ± 0.03	130 ±1.73	390 ±1.15
3	*Saccharomyces uvarum*	5.4 ± 0.13	6.5 ± 0.06	4.8 ± 0.05	5.3 ± 0.15	0.6 ± 0.05	1.3 ± 0.07	180 ±1.0	410 ±1.53
4	*Schizo saccharomyces pombe*	3.6 ± 0.08	3.7 ± 0.01	3.4 ± 0.01	2.2 ± 0.06	0.2 ± 0.05	1.5 ± 0.11	100 ±0.58	340 ±1.0
5	*Pichia stipitis*	1.5 ± 0.08	2.3 ± 0.08	1.3 ± 0.08	1.8 ± 0.09	0.2 ± 0.03	0.5 ± 0.02	130 ±1.0	360 ±0.58
6	*Candida shehatae*	1.5 ± 0.01	3.1 ± 0.10	1.3 ± 0.05	1.3 ± 0.12	0.2 ± 0.02	1.8 ± 0.05	80 ±1.0	320 ±1.0
7	*Zymomonas mobilis*	2.2 ± 0.12	4.3 ± 0.20	1.8 ± 0.08	2.7 ± 0.03	0.4 ± 0.05	1.5 ± 0.02	100 ±1.0	360 ±1.53

Note: The value of each combination consisted of mean ± S.D. of three replicates.

maximum residual non reducing sugar was observed in *Pichia stipitis* (1.1 ± 0.06 mg/g) treatment (Table 2**)**.

Fungal pretreatment of Arecanut husk waste (with substrate)

The maximum ethanol yield was observed in *Phanerochaete chrysosporium* pretreated raw material. After incubation, collected the filtrate and this filterate were treated with different yeast strains and *Zymomonas mobilis.*The minimum ethanol yield was observed in *Saccharomyces uvarum* (100 ± 0.58 mg/L). The maximum ethanol yield was observed in *Zymomonas* minimum residual non reducing sugar was observed in *Zymomonas mobilis* and *Pichia stipitis* (0.2 ± 0.02mg/g) treatment. The maximum residual reducing sugar was observed in *Zymomonas mobilis* (4.5 ± 0.05 mg/g) and the maximum residual non reducing sugar was observed in *Saccharomyces cerevisiae* (1.0 ± 0.10 mg/g) treatment (Table 3).

In *Aspergillus niger* and *Phanerochaete chrysosporium* pretreatment, the minimum residual reducing sugar was observed in *Candida shehatae* (1.3 ± 0.05 mg/g) and *Pichia stipitis* (1.3 ± 0.08 mg/g) and the minimum residual non reducing sugar was observed in

Schizosaccharomyces pombe (0.2 ± 0.05 mg/g) *Candida shehatae* (0.2 ± 0.02 mg/g) and *Pichia stipitis* (0.2 ± 0.03 mg/g) treatment. The maximum residual reducing sugar was observed in *Saccharomyces uvarum* (4.8 ± 0.05 mg/g) and the maximum residual non reducing sugar was observed in *Saccharomyces uvarum* and *Saccharomyces cerevisiae* (0.6 ± 0.05 mg/g) treatment (Table 4).

In *Aspergillus niger* pretreatment with substrate, the minimum residual reducing sugar was observed in *Saccharomyces uvarum* (0.6 ± 0.03 mg/g) and the minimum residual non reducing sugar was observed in *Schizosaccharomyces pombe* (0.2 ± 0.01 mg/g) and *Candida shehatae* (0.2 ± 0.03 mg/g) treatment. The maximum residual reducing sugar was observed in *Schizosaccharomyces pombe* (2.6 ± 0.03 mg/g) and the maximum residual non reducing sugar was observed in *Zymomonas mobilis* (1.3 ± 0.03 mg/g) treatment.When both fermentation methods (with substrate and without substrate) were compared, maximum ethanol was produced in filtrate fermentation method (without solids) (Table 2).

DISCUSSION

The arecanut husk waste treated with different fungal strains and inoculated with yeast strains and *Zymomonas mobilis* were found to produce ethanol and then compared to control after seven days of incubation period.

Production of ethanol through fermentation process from lignocellulosic biomass is dependent on its quality. Several studies in the past have described that chemical composition vary in different lignocellulosic biomasses and is also associated with environmental and genetic factors. Primarily, the major constituents of lignocellulosic material are carbohydrates (cellulose and hemicellulose) and lignin polymers. Carbohydrate contents of lignocellulosic materials are directly proportional to the commercial yield of ethanol. For producing high quality ethanol, pretreatment of biomass is essential (Mehmood *et al*., 2009; Salvachua *et al*., 2011).

The arecanut husk waste was initially subjected to microbiological pretreatment for obtaining reducing sugars. Such treated substrates with and without obtaining the filtrate were further subjected to fermentation. This filterate was treated with different yeast strains and *Zymomonas mobilis*. In with substrate fermentation, the maximum ethanol yield was found in *Phanerochaete chrysosporium* pretreated raw material. The minimum ethanol yield was observed in *Saccharomyces uvarum* and the maximum ethanol yield was observed in *Zymomonas mobilis* in with substrate fermentation. White-rot fungi are among the most effective microorganisms for biological pretreatment of lignocelluloses (Taherzadeh and Karimi, 2008).

In without substrate (solids) fermentation, the maximum ethanol yield was found in *Phanerochaete chrysosporium* pretreated raw material. The minimum ethanol yield was observed in *Saccharomyces cerevisiae* and the maximum ethanol yield was observed in *Pichia stipitis* in without substrate fermentation. Similar work was done by Taniguchi et al., (2005) evaluated biological pretreatment of rice straw using four white-rot fungi on the basis of quantitative and structural changes in the components of the pretreated rice straw as well as susceptibility to enzymatic hydrolysis. Some bacteria can be used for biological pretreatment of lignocellulosic materials.

The medium ethanol yield was observed in *Aspergillus niger* and *Phanerochaete chrysosporium* pretreated raw material in with substrate and without substrate fermentation. The minimum ethanol yield was observed in *Candida shehatae* and the maximum ethanol yield was observed in *Saccharomyces uvarum* treatment in with and without substrate fermentation. Similar work was done by Kurkake *et al*., (2007) studied the biological pretreatment of office paper with two bacteria strains, *Sphingomonas paucimobilis* and *Bacillus circulans*, for enzymatic hydrolysis. Biological pretreatment with the combined strains improved the enzymatic hydrolysis of office paper from municipal wastes. The residual reducing sugar, total sugar and non reducing sugar decreased significantly indicating efficient utilization of sugars for the ethanol production in all the substrates.

The minimum ethanol yield was observed in *Aspergillus niger* pretreated raw material in with and without substrate fermentation. The minimum ethanol yield was observed in *Saccharomyces uvarum* and *Schizosaccharomyces pombe* treatment. The maximum ethanol yield was observed in *Zymomonas mobilis* treatment in with substrate fermentation. In without substrate fermentation, the minimum ethanol yield was observed in *Zymomonas mobilis* and *Schizosaccharomyces pombe* treatment. The maximum ethanol yield was observed in *Saccharomyces uvarum* treatment in without substrate fermentation. The uninoculated control also showed release of ethanol in arecanut husk waste indicating natural fermentation due to the competent air microflora. Similar work was done by Krishna *et al*., (1998) who carried out ethanol production by simultaneous saccharification and fermentation (SSF) of sugarcane leaves using *Trichoderma reesei* (QM 9414) and *Saccharomyces cerevisiae* (NRRL-y-132).

Autoclaving for sterilization has affected and resulted in increase in sugar content. With fungal treatment still increase in the yield of sugars was observed. The individual fungal treatment, the combination of two fungi resulted in high yield of sugars (Patel, 2007). They observed ninety-two percent conversion and increased ethanol production. When both fermentation methods were compared, maximum ethanol was produced in filtrate fermentation method (without solids) (Sree *et al*., 1999). This may be due to the available sugar for the yeast strains as carbon source. Where as in simultaneous saccharification and fermentation (SSF), there may be chance of utilization of sugar also by fungal cultures as carbon source. This results in decrease in availability of sugars to yeast strain for fermentation. Among the organisms, *Zymomonas mobilis* recorded maximum production of ethanol in all the fungal pretreated arecanut

husk waste and also in both the fermentation methods. This may be because of its higher ability of sugar up take and ethanol production, lower biomass production, higher ethanol tolerance (Gunasekaran and Chandra, 1999).

CONCLUSION

The present study, it can be concluded that the sugars obtained from microbiological pretreated arecanut husk waste yielded less ethanol. Hence, it is essential to identify better combination of pretreatment methods and efficient yeast strains for exploiting the arecanut husk waste for ethanol production.

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