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Comparative Study of Ethanol Production from Microbial Pretreated Agricultural Residues

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ABSTRACT: Bioconversion offers a cheap and safe method of not only disposing the agricultural residues, but also it has the potential to convert lignocellulosic wastes into usable forms such as reducing sugars that could be used for ethanol production. This paper reports a preliminary study on the microbial pretreatment and fermentation of the agricultural residues like wheat straw, rice straw, rice husk and bagasse. A combination of six different fungi obtained from screening were used for pretreatment and *Saccharomyces cereviseae*(NCIM 3095) was used for carrying out fermentation. In case of Wheat straw and rice straw, pretreatment with *Aspergillus niger* and *Aspergillus awamori* and fermentation yielded highest amount of ethanol(2.5gl^{-1} & 2.2g l^{-1} respectively), for rice husk and bagasse it was with *Aspergillus awamori* and *Pleurotus sajor-caju* (8.5g l^{-1} & 9.8g l^{-1} respectively). @JASEM

Bioethanol produced from renewable biomass has received considerable attention in current years. Using ethanol as a gasoline fuel additive as well as transportation fuel helps to alleviate global warming and environmental pollution.

In the last decade, most research has tended to focus on developing an economical and ecofriendly ethanol production process. Much emphasis is being given to the production of ethanol from agricultural and forestry residues and other forms of lignocellulosic biomass.(Kadam *et al*. 2000). Changes in how agricultural field residues are managed further complicate farming economies. In the past, disposal of straw by burning was an accepted practice. This practice is now being challenged due to concern over the health effects of smoke from burning fields. Further the cellulosic plant material represents an as–of–yet untapped source of fermentable sugars for significant use, especially non-food lignocellulosic waste products like wheat straw, rice straw, baggasse, rice husk etc. In these waste products, the polysaccharides, cellulose and hemicellulose are intimately associated with lignin in the plant cell wall(Ballerini.*et al*. 1994). The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further transformation processes. Therefore, the pretreatment is a necessary process for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars.Bioconversion of cellulosic biomass into fermentable sugar, for production of ethanol using microorganisms, especially cellulose degrading fungi, makes bioethanol production economic, environmental friendly and also renewable.

Cellulose is the major constituent of organic matter of plant origin. Lignocellulosic materials are most abundant and renewable resources on earth, which makes them attractive for production of ethanol (Zsolt Szengyel 2000).Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment is required to alter the structures of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.* 2005) and to cellulase producing microorganisms.

There are several ways to increase the digestibility of cellulose before it is exposed to enzyme or microbial conversion: mechanical, physical chemical or biological pretreatment, as well as the combination of these methods (Bollok 1999).

In this work a study is made on the ethanol production from raw materials which have been treated with various combination of the fungal strains obtained after screening.

MATERIALS AND METHODS

Raw materials

1. Wheat straw obtained from local fields of Davanagere district
2. Bagasse from local sugar factory (Davangere sugar limited, Kukkawada).
3. Rice straw from local fields
4. Rice husk from local rice mill

Each raw material was powdered and sieved into a 1mm seiver. Powder of each raw material was used as carbon source.

Microorganisms: Screening of fungi capable of degrading cellulose(Abdul *et al*.1999) was done from the soil of local paddy and wheat fields and six fungi were selected and were sent for identification to NCIM, Pune and were identified as viz. *Aspergillus niger , Aspergillus awamori, Trichoderma reesei, Trichoderma viride,*

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Phenerochaete chrysosporium, Pleurotus sajor-caju. These were isolated and preserved on PDA slants.

Saccharomyces cereviseae (NCIM3095) was obtained from NCIM, Pune.

Table 1: Initial composition of the raw materials

Sl. No	Raw material	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Nonreducing sugar (mgg^{-1})	Moisture (%)	Total solids (%)	Organic carbon (%)	N_2 (%)
1	Wheat straw	0.4	0.0175	0.685	5.265	94.735	36.18	0.126
2	Rice straw	0.7	0.0175	0.382	1.83	98.62	36.93	0.448
3	Rice husk	2.6	0.0325	2.5675	6.69	93.31	29.87	0.574
4	Bagasse	1.3	0.175	1.125	8.34	91.66	36.18	0.448

*Inoculum preparation***:** Fungal cultures were inoculated onto PDA medium in the Petri plate. After 4-5 days, culture is used for inoculation.

*Culture medium***:** Mandles medium was prepared by adding (gl^{-1}): urea 0.3, $(NH_4)_2SO_4$ 1.4, KH_2PO_4 2, $CaCl_2$ 0.3, $MgSO_47H_2O$ 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements were also added, using a 1% (v/v) solution of salts(mll^{-1}):$FeSO_47H_2O$ 0.5, $MnSO_4$ 0.16, $ZnSO_4$ 0.14, $CoCl_2$ 2. pH was adjusted to 5.5-6.0 before sterilization(Bollok and Reczey 2000).

*Culture conditions***:** 10g /l of each residue was taken in conical flask containing 200ml of Mandle's medium. The conical flasks were plugged with cotton and sterilized at 15lbs per sq.inch for 20 minutes. Each flask was inoculated with 4-5 discs of different fungi . These flasks were incubated at room temperature for 5days on an orbital shaker. After five days mycelium was separated by filtration through Whatman filter paper No.1. The filtrate was used for further studies (Abdul *et al.* 1999).

*Determination of total carbohydrate***:** The carbohydrate content of untreated and pretreated raw materials in the culture broth was measured by phenol sulphuric acid method (Thimmaiah 1999) with glucose as standard.

*Determination of reducing sugars***:** Reducing sugars in untreated and pretreated raw materials in the culture broth were determined by dinitrosalicylic acid (DNS) method (Miller 1959) with glucose as standard.

Determination of protein: The protein content of culture broth was determined by Lowry et al. method (Thimmaiah 1999) with bovine serum albumin as standard.

FPU assay: Cellulase enzyme production was studied by FPU assay(Ghose 1987)

Fermentation: Culture filtrate was further inoculated with Saccharomyces cereviseae strain and allowed for fermentation for seven days (Sandhu *et al*. 1998). After fermentation it was filtered and ethanol content was determined.

*Ethanol estimation***:** Determination of ethanol content was done by spectrophotomentric method.(Caputi *et al*. 1968)

RESULTS AND DISCUSSION

Total sugar, reducing sugar, nonreducing sugar, organic carbon, Nitrogen, total solids, moisture content of each raw material was determined. Initial composition of each raw material is given in the table 1.

Autoclaving for sterilization has affected and resulted in increase in sugar content. With fungal treatment still increase in the yield of sugars was observed. Than individual fungal treatment, the combination of two fungi resulted in high yield of sugars. In case of wheat straw the treatment with *Aspergillus niger* and *Aspergillus awamori* , *Aspergillus niger* and *Trichoderma reesei*, *Aspergillus awamori* and *Phenerochaete chrysosporium* were found to be effective, the highest yield being with *Aspergillus niger* and *Aspergillus awamori.* Similar effects were observed as that of reducing sugar yield for protein and cellulase activity. Highest FPU of cellulase was observed in *Aspergillus niger* and *Aspergillus awamori* treated wheat straw.(Table 2).

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Table 2. Effect of fungal treatment on Wheat straw

Sl. No	Treatment	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non reducing sugar (mgg^{-1})	Protein (mgg^{-1})	FPU ($IUml^{-1}$)	Ethanol (gl^{-1})
1	Untreated after autoclaving	15	10	5	0.8	0.15	0.8
2	***Aspergillus niger(AN)***	18	15	3	1.8	0.45	1
3	***Aspergillus awamori(AA)***	20	18	2	2.2	0.35	1.5
4	***Trichoderma reesei(TR)***	17	14	3	2.0	0.32	1.2
5	***Trichoderma viride(TV)***	18	12	6	2.1	0.45	1
6	***Phenerochaete chrysosporium(PC)***	17	14	3	2.0	0.40	1.1
7	***Pleurotus sajor-caju(PS)***	20	15	2	2.1	0.22	1.4
8	AN+AA	36	22	14	2.9	0.90	2.5
9	AN+TR	35	20	15	2.3	0.40	1.5
10	AN+TV	22	18	4	2.5	0.55	1.4
11	AN+PC	23	19	3	2.2	0.21	1.5
12	AN+PS	22	12	10	2.7	0.45	1.2
13	AA+TR	25	14	6	2.5	0.60	1.5
14	AA+TV	22	15	11	2.4	0.35	1.5
15	AA+PC	20	18	2	2.8	0.45	1.2
16	AA+PS	26	13	13	3.1	0.75	2
17	TR+TV	25	13	12	3.2	0.50	1.8
18	TR+PC	20	15	5	2.4	0.32	1.5
19	TR+PS	21	14	7	2.2	0.21	1.5
20	TV+PC	19	13	6	2.0	0.19	1.6
21	TV+PS	19	15	4	2.1	0.18	1.5
22	PC+PS	19	17	2	2.4	0.30	1.8

Rice straw also yielded high amount of reducing sugar with treatment of Aspergillus *niger* and *Aspergillus awamori*. Similar effects were observed as that of reducing sugar yield for protein and cellulase activity. Highest FPU of cellulase was observed in *Aspergillus niger* and *Aspergillus awamori* treatment(Table 3).

Table 3 Effect of fungal treatment on Rice straw

Sl. No	Treatment	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non reducing sugar (mgg^{-1})	Protein (mgg^{-1})	FPU ($IUml^{-1}$)	Ethanol (gl^{-1})
1	Untreated after autoclaving	25	14	11	0.9	0.20	0.9
2	***Aspergillus niger(AN)***	28	16	12	2	0.50	1.6
3	***Aspergillus awamori(AA)***	29	16	13	2.5	0.60	1.8
4	***Trichoderma reesei(TR)***	27	15	12	2.6	0.42	1.6
5	***Trichoderma viride(TV)***	29	15	14	2.4	0.52	1.5
6	***Phenerochaete chrysosporium(PC)***	29	15	14	2.2	0.35	1.5
7	***Pleurotus sajor-caju(PS)***	30	16	14	2	0.32	1.6
8	AN+AA	42	22	19	3.1	0.92	2.2
9	AN+TR	25	16	9	3	0.28	1.8
10	AN+TV	27	15	12	2.6	0.80	1.9
11	AN+PC	25	15	10	2.5	0.2	1.7
12	AN+PS	32	16	16	2.5	0.65	1.6
13	AA+TR	33	17	16	2.7	0.68	1.7
14	AA+TV	29	15	14	2.8	0.80	1.5
15	AA+PC	29	16	13	2.8	0.90	1.9
16	AA+PS	39	17	12	3.0	0.68	2.0
17	TR+TV	27	16	11	3	0.40	1.8
18	TR+PC	27	15	12	2.5	0.30	1.7
19	TR+PS	26	15	11	2.5	0.22	1.7
20	TV+PC	26	15	11	2.2	0.18	1.6
21	TV+PS	26	16	10	2	0.30	1.5
22	PC+PS	32	17	15	2.8	0.35	1.8

But in case of rice husk good yield of reducing sugar was obtained by treatment with *Aspergillus awamori* and *Pleurotus sajor-caju* and highest FPU of cellulase was observed in *Aspergillus awamori* and *Pleurotus sajor-caju* treatment (Table 4).

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Table 4 Effect of fungal treatment on Rice Husk

Sl. No	Treatment	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non reducing sugar (mgg^{-1})	Protein (mgg^{-1})	FPU ($IUml^{-1}$)	Ethanol (gl^{-1})
1	Untreated after autoclaving	8	2.5	5.5	2.6	0.20	1.5
2	***Aspergillus niger(AN)***	25	13.1	11.9	12	0.30	2.8
3	***Aspergillus awamori(AA)***	17	14.3	2.7	12	0.44	3
4	***Trichoderma reesei(TR)***	25	22.5	2.5	10	0.38	2.8
5	***Trichoderma viride(TV)***	30	21.1	8.9	8	0.30	2.5
6	***Phenerochaete chrysosporium(PC)***	16	8.9	7.1	12.4	0.32	2.8
7	***Pleurotus sajor-caju(PS)***	21	15.35	5.65	12	0.80	3
8	AN+AA	17	11.35	5.65	10.4	0.70	5.5
9	AN+TR	25	16.1	8.9	10	0.60	3.2
10	AN+TV	20	14.35	5.65	12	0.50	3
11	AN+PC	24	12.75	11.25	10	0.40	3
12	AN+PS	37	28	9	8.4	0.90	4.5
13	AA+TR	37	27	10	9.4	0.85	4.8
14	AA+TV	41	30	11	14	0.95	3.5
15	AA+PC	36	32.5	3.5	8	0.80	2.5
16	AA+PS	39	34	5	12	0.96	8.5
17	TR+TV	33	17.5	15.5	11	0.60	3
18	TR+PC	37	22	15	11.5	0.50	2.5
19	TR+PS	9	6.25	2.75	10	0.35	2.8
20	TV+PC	27	13.75	13.25	8.5	0.45	3
21	TV+PS	24	19	5	9.5	0.50	3
22	PC+PS	26	25	1	13	0.60	4

Similar to rice husk in case of bagasse good yield of reducing sugar was obtained by treatment with *Aspergillus awamori* and *Pleurotus sajor-caju. (Table 5).*

Table 5 Effect of fungal treatment on Bagasse

Sl. No	Treatment	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non reducing sugar (mgg^{-1})	Protein (mgg^{-1})	FPU ($IUml^{-1}$)	Ethanol (gl^{-1})
1	Untreated after autoclaving	19	13.5	5.5	2.44	0.2	3
2	***Aspergillus niger(AN)***	30	16.85	13.15	10	0.70	5
3	***Aspergillus awamori(AA)***	57	39.58	17.5	10	0.925	8
4	***Trichoderma reesei(TR)***	74	72.75	1.25	8	0.5	6
5	***Trichoderma viride(TV)***	66	64.75	1.25	7.6	0.72	4.5
6	***Phenerochaete chrysosporium(PC)***	108	90.5	17.5	11.4	0.48	4
7	***Pleurotus sajor-caju(PS)***	60	46.5	13.5	10	0.42	6
8	AN+AA	45	32.5	12.5	10	1.10	8.5
9	AN+TR	30	18	12	10.4	0.42	8
10	AN+TV	74	49	25	12	1.27	6
11	AN+PC	57	24.5	32.5	10	0.42	6.5
12	AN+PS	25	13.5	11.5	10.4	1.0	6
13	AA+TR	47	26.2	20.8	8.4	1.04	6
14	AA+TV	64	56.5	7.5	8.4	1.48	5.5
15	AA+PC	59	37.8	13.5	10	1.17	6
16	AA+PS	66	44.8	21.2	14.4	1.6	9.8
17	TR+TV	62	41.5	20.5	11	0.4	8
18	TR+PC	53	37	16	11.4	0.25	5.5
19	TR+PS	53	30	23	9	0.25	5
20	TV+PC	52	36.5	15.5	8	0.42	4
21	TV+PS	60	36.5	13.5	4	0.729	4
22	PC+PS	65	42	23	16.4	0.840	8.2

As highest reducing sugar yield was seen in *Aspergillus niger* and *Aspergillus awamori* treated wheat and rice straws and *Aspergillus awamori* and *Pleurotus sajor-caju* treated rice husk and bagasse , the ethanol yield was also observed to be the highest in these treatments. Overall yield of ethanol was high with the bagasse and rice husk, best fungal treatment being the treatment with *Aspergillus awamori* and *Pleurotus sajor-caju.* The fungal treatment was found to be effective for conversion of cellulosic material for ethanol production.

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