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Bioconversion of paddy straw for bio-ethanol production

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Abstract
To investigate the effect of enzymatic saccharification and ethanol fermentation of paddy straw supplemented with fruits waste, NaOH was used to prepare paddy straw hydrolysate. Results revealed that bioethanol production from alkali (2% NaOH) pretreated paddy straw supplemented with mixed fruit peels under simultaneous saccharification and co-fermentation using yeast strains *viz.*, *Saccharomyces cerevisiae* HAU-1 and *Candida sp.* was done at different temperature and incubation period. Maximum ethanol (4.4%) production was recorded after 72 h incubation at 35 ^{0}C.

Keywords: Fermentation, fruits peel, paddy straw, bio-ethanol

Introduction
The increasing demand for ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives, has necessitated increased production of ethanol. Ethanol is a most important chemical product with emerging potential as a biofuel that replace fossil fuels. Biofuel, a clean and renewable energy source, which can be produced through fermentation of sugars, has drawn much attention. Agricultural residues generated as wastes during or after processing of agricultural crops are one of such renewable and lignocellulose-rich biomass resources available in huge amounts for bioethanol production [1]. Use of lignocellulosic material from agricultural wastes provides low cost fermentative substrate. Lignocelluloses biomass like wood and agricultural crop residue such as straw and sugar beet pulp are potential raw materials for producing several high value products like fuel ethanol and biodiesel. Lignocellulose contains up to 80% of the polysaccharides [2]. These renewable raw materials look promising for replacing environmentally unfriendly fossil hydrocarbon raw materials and, hence creating “green” products. In contrast to traditional fuels, fermentation ethanol does not contribute to the greenhouse effect, being a carbon dioxide natural resource. Paddy straw is a by-product of rice production and great bioresource. It is one of the most abundant lignocelluosic waste and huge amount of straw as solid biomass wastes produced annually [3]. It contains 32-47% cellulose, 19-27% hemicelluloses 5-24% lignin and 18.8% ash [4]. India is the second largest producer of paddy in the world after China. The structural complexity of paddy straw due to the presence of lignin is a major constraint for enzymatic and microbial attacks [5]. The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further hydrolysis processes. Therefore, the pretreatment is a necessary process for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars. Production of ethanol from lignocellulosic biomass contains three major steps: pretreatment, hydrolysis and fermentation. Pretreatment breaks the lignin structure and disrupts the crystalline structure of cellulose thus enhancing enzyme accessibility to the cellulose during hydrolysis [6].
Fruit peels are the by-products which are obtained during the processing of various fruits and these are good source of various bioactive compounds that posses various beneficial effects. But when these fruit peels are discarded as waste by processing industries cause environmental degradation. These fruit peel wastes can be used as feedstock for bio ethanol production which is renewable, biodegradable and nontoxic [7]. In the present investigation, efforts have been made to enhance bioethanol production from paddy straw supplemented with fruit peels by simultaneous saccharification and co-fermentation process.

Material and Methods
Pretreatment and Analysis of Paddy straw: Paddy straw (Basmati-1121) was procured from farmer’s field, Hisar, Haryana, India.

Pretreatment of paddy straw was done using 2% NaOH solution and delignified by autoclave at 15 psi for 1hr and residue was collected and washed with tap water 6-7 times to make it alkali free and neutralized with 0.1 M acetic acid and dried at 50 °C for further use. Commercial cellulase enzymes (palkonol and palkosoft) were used for hydrolysis of paddy straw. The hexoses and pentose utilizing yeast strains *Saccharomyces cerevisiae* (HAU-1) and *Candida* sp. were obtained from culture collection, Department of Microbiology, CCSA HAU, Hisar. All the strains were maintained on yeast extract peptone dextrose (YEPDA) slants. For inoculum preparation, yeast extract peptone sucrose (YEPS) media was used.

The cellulose, hemicelluloses and lignin content of paddy straw was estimated by determining acid detergent fiber (ADF) and neutral detergent fiber (NDF) in the sample as described by the standard procedure [8]. Ethanol concentration was estimated by spectrophotometric method described [9].

Simultaneous Saccharification and Co-fermentation (SSCF)

Alkali pretreated dry paddy straw was suspended in distilled water at 1:10 (solid:liquid). The enzymes (palkosoft) were added at a concentration of 6.5 FPU/g and 1.0% (v/v) inoculums of yeast with 0.3% urea and 5.0% fruit waste (peel). The flasks were incubated at different temperature (25, 35 and 45 °C). Samples were analyzed for ethanol content at 24 h intervals.

Results and Discussion

Pretreatment of Paddy straw

In alkali pretreatment, lignin component is dissolved in alkali and removed in liquid fraction while hemicelluloses and cellulose fractions are recovered together in solid fraction [10]. The Rice straw pretreatment was optimized by soaking in 2% H_2SO_4 followed by autoclaving at 15 psi for 90 min. It was observed that 25% solubility of cellulose along with decrease in hemicelluloses and lignin contents [11].

Analysis of Paddy straw

Paddy straw used in present investigation contained 38.9% cellulose, 25.5% hemicellulose and 5.5% lignin whereas alkali treated paddy straw contained cellulose (72.0%), hemicelluloses (16.2%) and lignin (2.2%). Similar results were reported [12] that paddy straw contained 35% cellulose, 22% hemicelluloses and 12% lignin in paddy straw. It was found that lignin content decreased with increase in cellulose content by alkali treatment. The maximum lignin removal was achieved with 0.5 mm mesh size (2.2%) as compared to 2.0 mm mesh size (6.1%).

Simultaneous Saccharification and Co-fermentation (SSCF)

For enhancing ethanol production simultaneous Saccharification and co-fermentation of delignified paddy straw supplemented with urea (0.3%) and 5% mixed fruit peel wastes was carried out using palkosoft enzyme and co-culture of *Saccharomyces cerevisiae* and *Candida sp.* The ethanol production was studied with respect to temperature, incubation period and different fruit peel wastes. Fermentation study revealed increased ethanol concentration with increased in incubation period for all three temperatures with co-cultures of yeast strains. Maximum production of ethanol 4.4% was observed after 72 h incubation with T-4 (sapota, grapes and kinnow) at 35 ^{0}C (Figure 1) followed by T-5 (mango, papaya and banana) i.e. 3.8% and T-6 (watermelon, muskmelon and peach) i.e. 3.2% (Table 1). Simultaneous Saccharification and co-fermentation of alkali pretreated paddy straw with co-culture of *Saccharomyces cerevisiae P. tannophilus* and *Candida sp*. showed maximum production of ethanol 24.94g/L after 96 hr incubation at 35 ^{0}C [13]. The fermentation of the dilute acid pretreatment hydrolysate of mixed fruit pulps (banana and mango) showed maximum ethanol production of 35.86% corresponding to a fermentation efficiency of 70.31% at 48 hr of incubation [14].

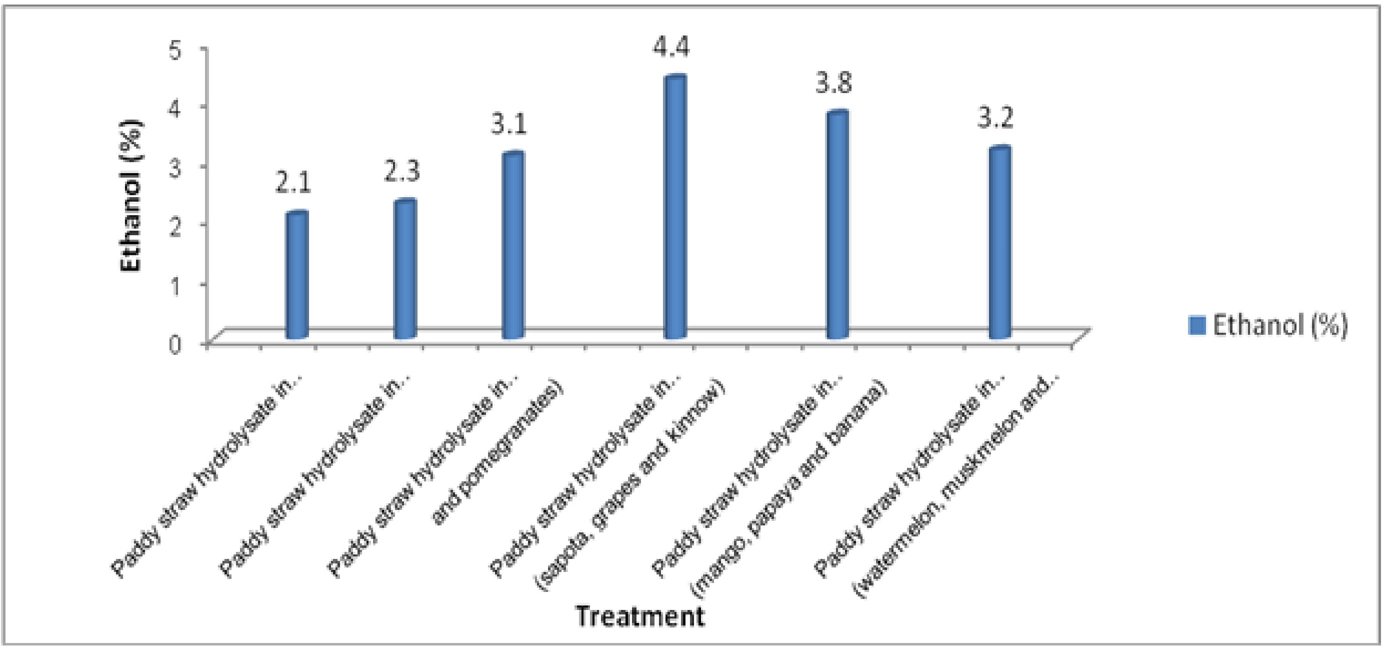

Fig 1: Ethanol production from paddy straw supplemented with mixed fruit peel wastes at 35^{0}C under stationary condition

Table 1: Ethanol Production from Paddy straw Supplemented with Mixed Fruit Peel Wastes under SSCF at different Temperatures

Treatments	Ethanol %								
	25^0C			35^0C			45^0C		
	Incubation period (hrs)								
	24	48	72	24	48	72	24	48	72
T-1	0.95	1.30	1.65	1.65	1.80	2.20	0.65	0.90	1.15
T-2	1.20	1.55	1.85	1.80	2.05	2.30	0.85	1.05	1.30
T-3	1.45	1.65	1.25	2.55	2.75	3.05	1.05	1.15	1.40
T-4	2.85	2.05	1.75	2.85	3.60	4.45	1.75	1.80	2.10
T-5	2.35	2.65	2.15	2.55	3.10	3.85	1.25	1.65	1.85
T-6	2.20	2.45	2.75	2.45	2.65	3.25	0.95	1.20	1.45
SE (D)	0.077	0.029	0.073	0.094	0.083	0.073	0.089	0.083	0.039
CD	0.204	0.076	0.192	0.247	0.217	0.192	0.235	0.217	0.102

SE (D)-Standard error (deviation), CD- Critical difference, MFPW- Mixed fruit peel waste
T-1 Paddy straw hydrolysate in water
T-2 Paddy straw hydrolysate in water+ 0.3% urea
T-3 Paddy straw hydrolysate in water+0.3% urea+5.0% MFPW -1 (apple and pomegranates)
T-4 Paddy straw hydrolysate in water + 0.3% urea + 5% MFPW-2 (sapota, grapes and orange)
T-5 Paddy straw hydrolysate in water + 0.3% urea + 5% MFPW-3 (mango, papaya and banana)
T-6 Paddy straw hydrolysate in water + 0.3% urea + 5% MFPW-4 (watermelon, muskmelon and peach)

Conclusions

Maximum ethanol (4.4%) was obtained from paddy straw by addition of mixed fruits peel (sapota, grapes and kinnow) under Simultaneous Saccharification and co-fermentation at 35 °C after 72 hrs. Supplementation of 0.3% urea and 5.0% mixed fruit peel to paddy straw suspended in water resulted in production of 2.1 to 4.4% ethanol by *Saccharomyces cerevisiae* and *Candida* sp. The amount of ethanol content increased with the increase in fermentation time. Therefore, the finding of that work was suggested that the addition of fruit peels during fermentation enhanced the quantity of ethanol production from paddy straw.

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