

## Development of Alkaline/Organosolv Pretreatment of Rice Straw to Enhance High Solid Loading Saccharification

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**Abstract.** Lignocellulose represents a promising starting material for conversion to fuels and chemicals in biorefinery; however, its efficient conversion to sugar requires a prerequisite pretreatment step. In the present research, the pretreatment of rice straw by alkaline and organosolv processes was studied aiming to separate high quality lignin and enhance enzymatic digestibility of the cellulose-enriched solid. Effects of alkaline (Tri-ethyl amine, TEA) and organosolv pretreatment using ethyl acetate (EA) and ethanol (EtOH) at varying temperature (80°C, 90°C, 100°C) were studied. Ethyl acetate was shown to be better result more than ethanol in terms of cellulose selectivity and enzymatic digestibility. The highest sugar yield of 178.32 mg sugar/g native rice straw was obtained by using ethyl acetate as a solvent with an operating temperature of 90°C for 30 min and subsequent enzymatic hydrolysis. Furthermore, addition of TEA over EA can further promote the sugar yield. The work therefore shows potential of alkaline pretreatment in organic solvent system for increasing digestibility of lignocelluloses in biorefinery.

**Keywords:** rice straw; alkaline; organosolv; pretreatment

### 1. Introduction

Nowadays, the demand for fossil fuel has been continually increasing opposite to the depleting supply of petroleum resources which leads to rising oil price during the last decade. The use of alternative energy from renewable resources is a promising solution not only in economic aspects but also for long term environmental sustainability. Bioethanol is a widely used biofuels in automobiles. Its current production is from sugars and starch-based feedstock. However, with the "Food v.s. Fuel" controversy issue, production of ethanol from underused lignocellulosic plant biomass is thus of great challenge and has received considerable interest from industries in recent years. New biotechnological solutions for the decomposition of lignocellulosic biomass are required to improve the production efficiencies and reduce the costs of cellulosic biofuel production [1].

Thailand is an agricultural-based country with high potential for development of biofuel industry from its plenty lignocellulosic resources. Lignocellulosic biomass has a complex structure comprising (i) cellulose: a linear homopolymer of glucose which formed into highly organized microfibrils; (ii) hemicelluloses: a branched heteropolymer of pentoses and hexoses and (iii) lignin: a complex polymer of phenolic subunits, shielding plants from external stresses and microbial attacks. This complex structure results in recalcitrance of biomass to enzymatic hydrolysis. Pretreatment is required to disrupt the structure of lignocellulosic materials in cellulosic ethanol production [2]. A pretreatment step using chemical or thermochemical methods is needed to increase digestibility of lignocelluloses which can be achieved by removal of lignin, solubilization of hemicelluloses, or decrystallization of cellulose. This leads to increasing sugar yield from the subsequent enzymatic saccharification step for further conversion to bioethanol by fermentation.

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Organosolv is a versatile pretreatment method, with the major effect on lignin solubilization. Lignin extracted in the organosolv process was found to be highly phenolic, and it had low molecular weight, which made it attractive for utilization in the production of chemicals [3]. Several organic solvents for examples alcohols and ketones have been used in organosolv processes. The use of acid catalysts in organosolv has been reported which led to enhancement of sugar yields and selectivity of the reaction. In this study, the use of alkaline on organosolv process in different organic solvent systems was studied. Effects of operational temperature on enzymatic digestibility and reaction selectivity were investigated. The work provides a promising alternative method for efficient lignocelluloses pretreatment in biorefinery.

## 2. Experiment

### 2.1. Materials

Rice straw (RS) was collected from a local area in Pathumthani province, Thailand. It was milled by pass through 2mm screen in cutting mill (Retsch SM2000) and dried it until weight constant before use it. The composition in rice straw contained 35.8 wt% cellulose, 21.5 wt% hemicelluloses, 24.4 wt% lignin, and 15.0 wt% ash (standard NREL method). All of organic solvents and chemicals were purchased from chemical suppliers i.e. Sigma-Aldrich, Merck, and Fluka.

### 2.2. Pretreatment

The organosolv pretreatment was performed in a 600-ml Parr reactor equipped with a thermocouple inside the reactor and a cooling system.

The alkaline/organosolv pretreatment reaction contained 2% w/w of dried rice straw in 190 ml of the ethyl acetate with 10 ml of tri-ethyl amine as the catalyst. The organosolv reaction in the absence of alkaline contained 2% w/w of the biomass in 200 ml of the solvent (ethanol and ethyl acetate). The reaction was treated at 80-100°C for 30 min under a pressurized conditions at 20 bars using N<sub>2</sub> with mixing at 300 rpm. The solid and liquid fractions after pretreatment were separated by vacuum filtration. After that, the solid fraction was washed by deionized water until pH equal 7 and dried it in oven at 60°C overnight.

### 2.3. Enzymatic hydrolysis

Enzymatic hydrolysis reaction with the total volume of 1 ml contained 5% (w/v) of pretreated rice straw in 50 mM sodium citrate buffer (pH 4.8) with 20 FPU/g of Accellerase® 1500 (Danisco, Rochester, NY) and 5 % w/v sodium azide. The reaction was incubated at 50°C with vertical mixing at 30 rpm for 72 hours.

## 3. Results and Discussion

In this study, the effects of tri-ethyl amine on organosolv pretreatment of rice straw were studied in organic solvent, ethyl acetate, at varying operating temperatures under pressurized conditions.

### 3.1. Organosolv pretreatment in the absence of alkaline

Pretreatment of rice straw using organosolv in the absence of alkaline catalyst led to a slight increase in sugar yield compared the native rice straw that is highly recalcitrant to hydrolysis. Relatively low sugar yields from enzymatic hydrolysis were obtained using different solvents (Fig. 1). Glucose was the major released sugar from enzymatic hydrolysis followed by xylose and arabinose, respectively. Pretreatment using ethyl acetate was then studied at varying temperatures. The highest total sugar yield is 171.40 mg/g pretreated biomass at 80°C. Glucose was the major products with substantial cross contamination of pentoses. This was equivalent to the glucose recovery yield of 26.95% from the native biomass.

Table 1: Sugar recovery from organosolv pretreatment of rice straw using different organic solvents in the absence of alkaline.

Organic solvent	Total sugar yield (mg sugar/g pretreated RS)	Total sugar yield (mg sugar/g native RS)	Glucose recovery yield	%wt loss
ethyl acetate	165.60	125.67	26.48	24.11
ethanol	171.40	132.39	26.95	22.76

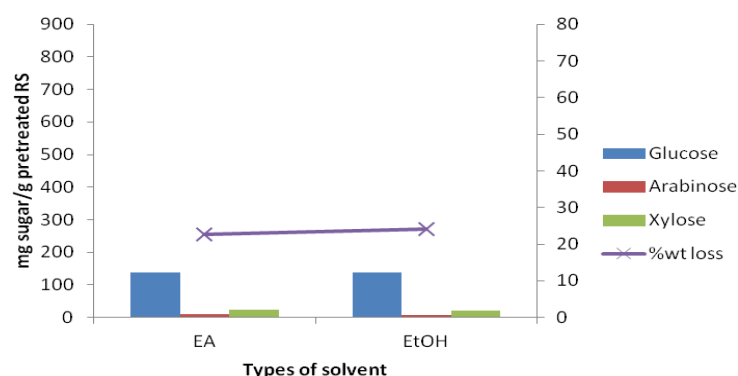


Fig. 1: Sugar recovery from enzymatic hydrolysis of rice straw pretreated by organosolv in the absence of alkaline. The pretreatment reaction contained 2% (w/v) rice straw in different solvents and treated at 80°C for 30 min at 20 bars with mixing at 300 rpm.

### 3.2. Alkaline pretreatment of TEA in Deionized water

Pretreatment of rice straw in deionized water with alkaline catalyst led to increasing sugar yield from enzymatic hydrolysis of rice straw compared to the pretreatment reaction in the absence of alkaline. Temperature showed a marginal effect on sugar yield under the experimental conditions. The highest glucose yield of 360.53 mg/g pretreated biomass was obtained at 100°C with very low cross contamination of xylose and arabinose (Fig. 2). The results showed high selectivity of hemicellulose and lignin removal leading to enrichment of cellulose in the solid fraction. The highest total sugar recovery was 293.91 mg/g native rice straw which was equivalent to 60.73% glucose recovery from the native biomass (Table 2).

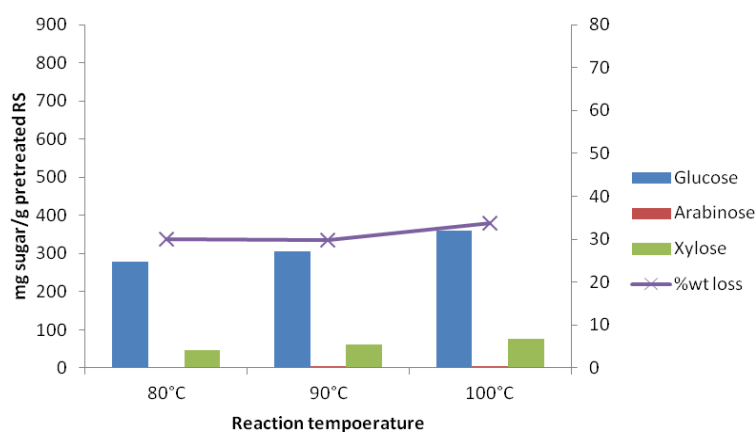


Fig. 2: Sugar recovery from enzymatic hydrolysis of rice straw from alkaline pretreatment of TEA in deionized water. The pretreatment reaction contained 2% (w/v) rice straw in 5% w/v TEA solution at varying operating temperature for 30 min.

Table 2: Sugar recovery from alkaline pretreatment of rice straw using deionized water at different temperatures

Temperature (°C)	Total sugar yield (mg sugar/g pretreated RS)	Total sugar yield (mg sugar/g native RS)	Glucose recovery yield	%wt loss
80	328.19	229.68	49.42	30.02
90	371.10	260.42	54.68	29.83
100	443.11	293.91	60.73	33.67

### 3.3. Alkaline/Organosolv pretreatment of TEA in ethyl acetate

Pretreatment of rice straw in ethyl acetate with alkaline catalyst led to increasing sugar yield compared to the reaction in ethyl acetate with no alkaline but showed relatively lower sugar yields from enzymatic

digestibility of the pretreated rice straw compared to the reaction in deionized water. Temperature also showed a marginal effect on sugar yield under the experimental conditions. The highest glucose yield of 169.92 mg/g pretreated biomass was obtained at 90°C with some cross contamination of xylose (Fig. 3). The highest total sugar recovery of 178.32 mg/g native rice straw was obtained which was equivalent to 33.68% glucose recovery from the native biomass (Table 3). It can be seen that additional of TEA can improve the pretreatment activity of EA and enhances higher sugar yield. Although the use of water can enhance higher sugar yield than EA, the benefit of EA compared to water in term of possible solvent regeneration would offer good potential of alkaline/organosolv for biomass pretreatment.

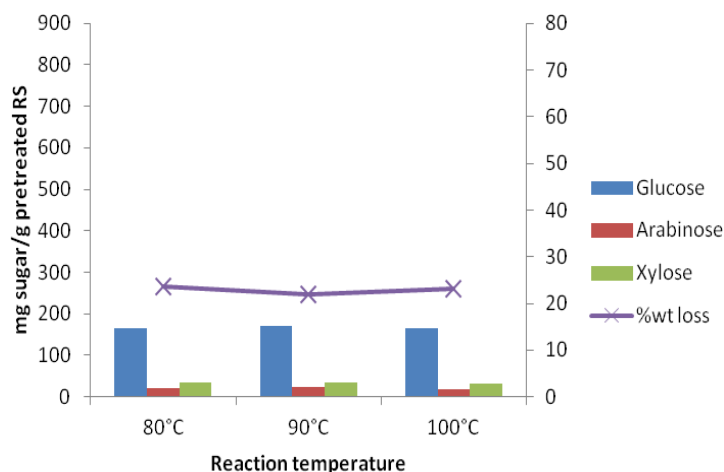


Fig. 3: Sugar recovery from enzymatic hydrolysis of rice straw from alkaline/organosolv pretreatment of TEA in EA. The pretreatment reaction contained 2% (w/v) rice straw in 190 ml EA and 10 ml TEA at varying operating temperature for 30 min.

Table. 3: Percent weight loss from alkaline/organosolv pretreatment of rice straw in ethyl acetate

Temperature (°C)	Total sugar yield (mg sugar/g pretreated RS)	Total sugar yield (mg sugar/g native RS)	Glucose recovery yield	%wt loss
80	219.04	167.22	32.13	23.66
90	228.44	178.32	33.68	21.94
100	214.20	164.44	32.02	23.23

## 4. Conclusion

From the study on the effect of types of organic solvent on alkaline pretreatment, it was found that the mixture between TEA and ethyl acetate helped increasing sugar concentration significantly compared with pretreatment only in organic solvent. Among two organic solvents, ethyl acetate was shown to be the better solvent than ethanol in term of biomass digestibility and selectivity towards cellulose in the solid fraction. Development of alkaline/organosolv pretreatment is thus suggested to be an effective method to obtain highly enrich cellulose solid with high enzymatic digestibility for further processing. Further reaction optimization and using of alternative organic alkaline are on progress.

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## 6. References

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