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# Unlocking Bioethanol Potential through Novel Combination: Rice Straw Transformation using Alkali Pre-treatment and Subsequent Cellulosic Treatment

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## ABSTRACT

Cellulose is one of the most abundant carbon-based polymers which can be utilized for biofuel production. Cellulosic material present in any form can be converted into biofuels which depends on the microorganism used in fermentation stage. Cellulose is bound by lignin and hemi-cellulose and tightness of the bond is different in different species therefore different pre-treatment methods can be used for removal of lignin and hemi-celluloses. In our laboratory we successfully showed that pre-treatment of rice straw and treatment of resultant cellulose can be digested by diluted alkalis. The mixture thus gained can be used as raw material of yeast to produce bioethanol. Dried rice straw was collected and brought to the laboratory. The biomass was dried, powdered and mixed with 5% sodium hydroxide or potassium hydroxide. The mixture was then again treated with either of the diluted alkalis which converted higher polymers into fermentable sugars. Fermentable sugars were tested using DNS method. Yeast was added to the digested solution and production of ethanol was confirmed by blue green color produced by addition potassium permanganate and sulphuric acid to the fermented mixture. Ethanol was also tested at 210 nm using UV spectrophotometer.

**Keywords** Ethanol, Rice straw, Cellulose, Alkaline pre-treatment, Alkaline treatment, FTIR.

## INTRODUCTION

Alternative sources of energy are being looked into with expectations as pollution caused by production and use of fossil fuels along with their dwindling status has raised alarms (Mankar *et al*. 2021). Bio-alcohols which includes ethanol, butanol and methanol, resulting from agro-biomass, provide promising and sustainable energy alternatives (Gautam *et al.* 2020, Zhen *et al.* 2020). The concern for environment as well as health hazards of burning fossil fuels have made research and investment in developing better biofuels. Ethanol is one of the molecule which has shown promise and its production from non-edible biomass has shown promise to make bioethanol

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next generation biofuel (Taghizadeh-Alisaraei *et al*. 2019). The first generation conversion technologies used food crops for production of ethanol as biofuel which had put burden land and caused scarcity of those edible crops for human consumption (Melendez *et al.* 2022).

The process of making a system for production of food, fuel or fiber which is economically viable and ecologically safe along with it being socially excepted is called as sustainable agriculture (Singh *et al.* 2016). In case of biofuels the approach of sustainable agriculture can be extended to use of agro-biomass which are generally used traditionally. Utilizing agriculture based biomass for development of system for generating biofuels from them by utilizing them for production of enzymes as well as fuels comes under development of circular economy (Astolfi *et al*. 2019). Utilizing sustainable agriculture and developing circular economy approach for production of biofuels will reduce the burden on land and environment.

The second generation biofuels were made from any kind of biomass which contained lignocellulosic materials (Yadav *et al.* 2022). Rice is one of the most abundantly grown crops worldwide (estimated 650-975 million tons annually) (Fukagawa & Ziska 2019). In India annually 112 MMT rice husk is produce (Singh *et al*. 2016) which is generally used as fodder or making thatched roofs. It makes rice straw, a waste product from rice farming, which causes air pollution when it is burnt, easily available (Fukagawa & Ziska 2019). Rice straw is an agricultural waste which is a good source to produce alcohol as it contains cellulose which can be converted into biofuels using suitable microorganism which can be utilized in fermentation stage. In the past, burning straw was a common method for disposal causing pollution down wind. Still burning of crop stubbles is a recurrent problem causing sky lines of India to become filled with smog during winter season. However, this practice is increasingly being questioned due to growing concerns about the health risks associated with the smoke produced from burning fields. These concerns arise from the complex composition of straw, which includes polysaccharides like cellulose and hemicellulose, tightly bound together with lignin within the plant cell wall along with silica and ash content (Bhattacharyya *et al*. 2021). As lignin and cellulose are very strongly bound with each other along with some hemicellulose the removal of cellulose for enzymatic degradation becomes a difficult becomes a challenge (Ramos *et al*. 2022).

Lignocellulosic biomass conversion to bioethanol involves three steps called pre-treatment, treatment and fermentation. Pre-treatment is a process of loosen up the lignin and cellulose thereby making further processes easy to perform. It helps in reducing size of the biomass thereby increasing the surface area which helps in further degradation of the lignocellulosic material (McIntosh *et al.* 2016). This step proves particularly essential for substrates like rice straw, where it plays a key role in maximizing the yield of fermentable sugars (Ballerini *et al*. 1994). There are various chemicals and enzymes used for pre-treatment and treatment of rice straw in much other research (Malik *et al.* 2022). After pre-treatment the resulting cellulose is separated from the lignin which is dissolved in the solution and is further processed to get fermentable sugars in a process known as hydrolysis or treatment. In biofuel production technologies, hydrolysis specifically refers to the degradation of carbohydrates present in biomass (cellulose and hemicellulose) into their constituent sugars (glucose, xylose) (Kumar *et al.* 2009). Bio-alcohol, like as ethanol, can be produced via microbial fermentation of sugars which are processed from agri-biomass (Priya *et al.* 2016). It is evident that the microorganisms grow at specific temperature range as enzymes therefore for obtaining maximum yield, maintenance and monitoring of a consistent temperature within the optimal range is essential (Brethauer and Wyman 2010).

There are many companies involved in production of bioethanol from agriculture wastes since last decade. Many of them are facing problems and some have shut down their operations and sold the business to others. The major factors affecting the companies are losses due to high cost of production and maintaining the new renewable fuel standards along with low oil prices (Østby *et al.* 2020). This demands for production costs to be reduced and one of the methods which can help in cost reduction will be elimination of enzymes from the hydrolytic process to get fermentable sugars as enzymes increase the cost of production. Keeping this goal in mind the current study was undertaken to determine if alkali can be used for hydrolysis of pre-treated lignocellulosic biomass.

## MATERIALS AND METHODS

### Cellulosic biomass

Rice straw contains 40–50% of cellulose, 20–30% of hemicellulose, 10–18% of lignin and other substance (Yan *et al.* 2021). Rice straw is an important source of cellulose (Ramos *et al*. 2023). Rice straws were collected locally and were used as lignocellulosic substrates. The straw was washed with tap water and dried in a hot air oven at 70°C for overnight. Then, it was cut into small pieces.

### Microbes for fermentation

Baker's yeast was taken from the market and was inoculated in the treated sample at later stage.

### Alkali for pre-treatment and treatment

5% NaOH and 5% KOH solution with distilled water was used in both the processes.

#### *Alkaline pre-treatment*

10 g of washed, dried and cut rice straw sample was measured and divided into two equal halves. 100 ml 5% NaOH solution and 5% KOH solution in two different conical flasks were taken and 5g of samples were added in them for incubation period of 30 minutes. After pre-treatment, the sample was washed several times until the alkali was washed away and the pH was near 7 and all the extracellular parts of the sample except the cellulose was washed away. Using cheese cloth the pre-treated samples were completely drained of water.

Pre-treatment method was increased to 60 minutes and 120 minutes following similar protocol as stated above.

#### *Alkaline treatment*

The samples pretreated with NaOH and KOH was treated with 100 mL of 5% NaOH and 100 mL of 5% KOH respectively for 30 minutes. After treatment it was washed several times using tap water until the water after straining through cheese cloth is clean. Then the treated sample was put into 100 mL of distilled water in 250 mL conical flask with a neutral pH.

This was repeated two more times for the pre-treated 1 hour and 2 hour samples with the respective alkalis used during pretreatment for the same period of time.

### Sugar estimation

Sugar was estimated using DNS method as given by Miloski *et al.* (2008).

### Fermentation

Bioethanol fermentation was done in liquid state fermentation. The yeast *Saccharomyces cerevisiae* was used for fermentation. A pinch of Baker's Yeast was added to 10 ml of lukewarm water and then this was inoculated in sample after activation of yeast. This inoculated sample was then put into incubator at 28°C for 48 hrs.

### Ethanol estimation

After 2 days of incubation period, one mL of sample was taken in cuvette and tested in spectrophotometer at 210 nm for estimation of ethanol in the sample after incubation of all the samples as discussed by Saad *et al.* (2017). Ethanol was also tested using potassium dichromate and sulphuric acid as discussed by Sayyad *et al*. (2015).

### FTIR analysis

FTIR analysis was done on using Bruker Alpha II system as per instructions given by the manufacturer. The sample was dissolved in distilled water and pH was kept between 5–8 for the analysis.

## RESULTS AND DISCUSSION

The rice straw has been used as raw material for pro-

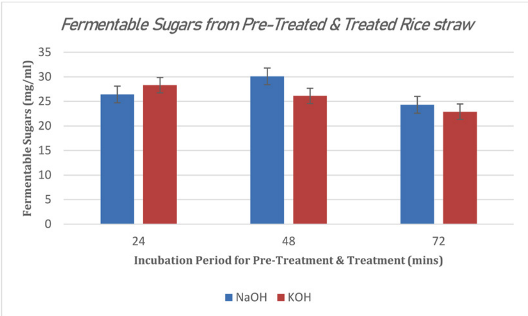


**Fig. 1.** Fermentable sugars produced from rice straw on treatment with NaOH and KOH.

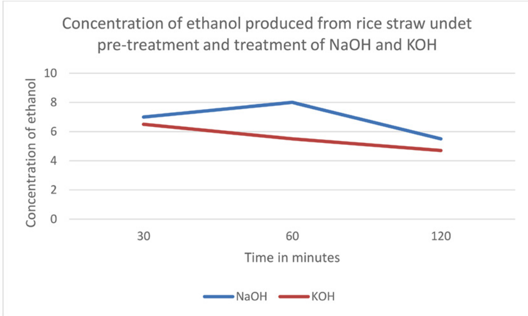


**Fig. 2.** Ethanol produced from rice straw after pre-treatment and treatment with alkalies for different time.

duction of ethanol using different pre-treatment and treatment methods. In current work we used alkaline treatment for pre-treatment as well as treatment at different time periods. The treated rice straw samples were inoculated with baker's yeast and after 48 hrs ethanol was estimated. Takano and Hoshino (2018) found fermentable sugar from rice straw around 70 g/l using cellulose enzyme. We by passed the enzymatic process as it increases the final cost of the bioethanol produced. In current study we found that using alkaline pre-treatment and treatment we were able to get around 30 g/l of fermentable sugar from rice straw (Fig. 1). In the study conducted by Takano and Hoshino (2018) it was reported that NaOH pre-treatment gave higher fermentable yield which was similar to our findings in the current study. The maximum ethanol production from both the process was 8% in NaOH treated samples. The optimum pretreatment and treatment time for ethanol production was found to be 60 minutes for NaOH while it was 30 minutes

**Table 1.** FTIR wave number and intensity with different pre-treatment and treatment methods with their suggested compounds.

| Sl. No. | Pre-treatment and treatment method | Time | Wave number | Intensity | Suggested compounds |
|---|---|---|---|---|---|
| 1 | KOH | 24 hrs | 3346.706 | 0.468 | Alcohol |
| | | | 2133.245 | 0.975 | Alkyne |
| | | | 1634.645 | 0.704 | Alkene |
| 2 | KOH | 48 hrs | 3346.055 | 0.466 | Alcohol |
| | | | 2112.596 | 0.973 | Alkyne |
| | | | 1633.295 | 0.704 | Alkene |
| | | | 2358.588 | 0.981 | Carbon dioxide |
| | | | 2341.264 | 0.981 | Carbon dioxide |
| 3 | KOH | 72 hrs | 3332.988 | 0.461 | Alcohol |
| | | | 2110.161 | 0.969 | Alkyne |
| | | | 1632.321 | 0.704 | Alkene |
| | | | 652.9215 | 0.977 | Halo compounds |
| 4 | NaOH | 24 hrs | 3345.513 | 0.466 | Alcohol |
| | | | 2113.153 | 0.975 | Alkyne |
| | | | 1635.521 | 0.702 | Alkene |
| 5 | NaOH | 48 hrs | 3334.013 | 0.463 | Alcohol |
| | | | 2109.922 | 0.972 | Alkyne |
| | | | 1632.952 | 0.704 | Alkene |
| | | | 2358.832 | 0.979 | Carbon dioxide |
| | | | 2341.665 | 0.98 | Carbon dioxide |
| 6 | NaOH | 72 hrs | 3341.395 | 0.462 | Alcohol |
| | | | 2119.606 | 0.971 | Alkyne |
| | | | 1632.743 | 0.704 | Alkene |
| | | | 636.3713 | 0.968 | Halo compounds |

for KOH (Fig. 2).

FTIR analysis was done on 3 hrs pre-treated and treated samples which were fermented for 24, 48 and 72 hrs. All the samples of 24 hrs fermentation i.e. 3 hrs pre-treated and treated samples of NaOH and KOH gave three peaks where the wavenumbers suggested presence of alcohol, alkyne and alkene (Table 1). This was evident for the samples kept fermentation for 48 and 72 hrs too. 48 hrs fermented samples of KOH and NaOH pre-treated and treated samples gave 2 more peaks, all suggesting for presence of carbon dioxide. The 72 hrs fermented samples of KOH and NaOH gave one extra peak in both suggesting presence of halo compounds.

## CONCLUSION

We conclude that using alkali for pre-treatment and subsequent treatment processes we can get enough fermentable sugars that yeast is able to ferment them into ethanol. We found that NaOH gave more fermentable sugars than KOH which also translated in more ethanol produced than KOH. In our study we found that NaOH is better pre-treatment and treatment chemical than KOH. We also found that the highest amount of ethanol produced by yeast after KOH treatments was always less than that produced after NaOH treatments. Further studies are required to estimate ethanol production after treatment on larger biomass. Along with this the treatment time if increased may give more fermentable sugars which may result in more ethanol yield. FTIR analysis also suggested presence of alcohol in all the samples while suggesting presence of alkane, alkene and alkyne in samples. Presence of halo compounds were also detected in some samples.

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