RICE STRAW AND HUSK: RENEWABLE SOURCES OF BIOFUEL PRODUCTION

Abstract

To achieve the requirement of world population in petroleum sector, bio-ethanol production and utilization has been going to play a major role in near future. By utilizing the lignocellulosic by-product of rice, wheat, maize like cereals, production of huge quantity of bio-ethanol could be possible through different methods like, pre-treatment, saccharification and fermentation. Well utilization of these waste by-products through different mechanisms to produce the end product as bio-ethanol is now being the hot topic worldwide. Reduction of greenhouse gas emission, protection of human from hazardous gases and basically reduction of global warming can be achieved through this production. These energy resources are very much efficient and enhancing the achievements of researches day-by-day. Modern technologies like, genetic engineering, biotechnology, etc. are now being extensively utilized for standardizing the larger production of biofuels. Utilization of microorganisms like yeast and synthetically produced hydrolysing enzymes are the potent technology for biological fermentation process which would be less harmful to environment. Potentiality of the by-products especially from rice straw showing a larger capacity than other cereals crops, for the production of the bioethanol which has been studied well now a days by researchers and the industrial applications are also followed for the replacement of conventional petroleum products.

Keywords: lignocellulose, bio-ethanol, saccharification, fermentation, detoxification, pyrolysis, syngas

Authors

Saumya Ranjan Barik
ICAR- National Rice Research Institute
Cuttack, Odisha, India

Binod Kumar Jena
Department of Genetic and Plant Breeding
Institute of Agriculture
Visva- Bharati, West Bengal, India.

Ambika Sahoo
Centre for Biotechnology
Siksha ‘O’ Anusandhan
(Deemed to be University)
Bhubaneswar, India

Paresh Chandra Kole
Department of Genetic and Plant Breeding
Institute of Agriculture
Visva- Bharati, West Bengal, India.

Arpita Moharana
ICAR- National Rice Research Institute
Cuttack, Odisha, India

Sharat Kumar Pradhan
ICAR- National Rice Research Institute
Cuttack, Odisha, India

I. INTRODUCTION

Rice is consumed by more than fifty percent of the global population. The production of raw rice is 755.45 million tonnes which is third to the global production after maize (1,148.4 million tonnes) and wheat (765.7 million tonnes) (Statistical Year Book, World Food and Agriculture 2021). Providing major cereal grain to the world population, rice produces abundant biomass as by-product which is chiefly composed of lignocelluloses. As lignocellulosic waste material, rice straw is abundant all over the world. In each kilogram of rice grain harvesting produces 1-1.5 kg by-product as rice straw [1]. It is estimated that about 0.7554-1.1331 billion tonnes of rice straw is produced annually in world and I India alone the production of rice straw is 0.26646 billion tonnes. The maximum part of rice straw produced in developing world is being consumed as cattle feed and the remaining is left as waste. With an estimate of potential, rice straw would produce 315 billion litres of bioethanol annually [2]. Due to presence of high contents of ash and silica in rice straw, it is inferior for production of biofuel to be used as raw material [3]. Apart from rice straw and husk, other lignocelluloses biomass are wood and agricultural crop residues, *e.g.* pulp of sugar beet and wheat straw are also potential raw material for production of bioethanol or bio diesel.

In lignocellulose there are more than 80% polysaccharides [4]. The management of this bulk of rice straw is not easy because of its low density**,** slow bio-degradation, carrying of pest and diseases, and high mineral content. The natural decomposition of biomass on earth is an uncontrolled bio-degradation process and methane (CH_4) gas is released which is one of the most important factor of green house effect or global warming. If this methane (CH_4) could be harvested it would be used as clean energy by the other hand reducing the green house gas (GHG) emissions [5]. Now a days due to use of combined harvesters, rice straw is burnt in the Sit u is the practice for cleaning of Field, which increases the air pollution in the local biosphere and consequently affects public health [6]. Burning of rice by-products increases the quantum of green house gases (GHG) among which CH_4, CO, CO_2, SO_2, N_2O, NO_2, etc. are the factors of most concern of global climatic change of growing global warming and is a direct threat to development [7]. The anticipated threat of the unused agro-industrial residues, growing energy crisis in developing world and development of science and technology, it is pertinent to find the alternative uses of this biomass for energy applications. If these agricultural waste materials could be used technically and efficiently it will replace fossil fuels which are not friendly to world environment. The biofuel is termed as green product as its use does not create environmental hazards. It is convinced that rice biomass has the potential to meet future energy needs With a great deal. In contrast to traditional fuels, fermentation ethanol is friendly and its combustion is not a cause of greenhouse effect. The potential energy of paddy straw is dependent on lowering heating value (LHV) and LHV further depends on the composition of ash in paddy straw [8].

The concept of bioethanol to be the fuel of future generation for combustion engines is due to its neutrality of carbon content and as the biofuel could be produced from the renewable agricultural biomass resources such as lignocellulose, which is ultimately a product of agriculture. Rice straw generally composes hemicellulose 19-27%, lignin 5-24%, cellulose 32-47% and ash 18.8%. In hemicelluloses pentose sugars are pre-dominant and hemicellulose is composed up of hexoses, pentoses (xylose) and arabinose. Glucose content

of rice straw is 41-43.4%. The other compositions of rice straw in carbohydrates are 14.8-20.2% xylose, 2.7-4.5% arabinose, 1.8% mannose and 0.4% galactose [1, 9]. There are many hindrances in production of biofuel from rice straw *i.e.*, pre-treatment, hydrolysis and fermentation process. A brief discussion on various processes of pre-treatment, hydrolysis, saccharification and fermentation for bioethanol production is Presented here that will resolve technological problems to frame low cost, efficient, economic and environment friendly processes of ethanol extraction.

The oil wealth of allies made defeated the Axis countries *i.e.*, Germany, Italy and France in the World War II. In the fourth war between Arabs and Israel in October 1973, the Arab countries imposed sanction to restrict supply of petroleum to western countries which increased the international oil price more than double the existing price and it was the first international oil crisis which traced back the GDP more than 3% of almost all countries. The warring countries had felt their energy crisis during war and following World War II there was rapid development of the world economy which increased greatly the demand on mineral oil or energy and the concept of energy security became equally important as the national security. In November 1974, under the leadership of United States with 16 industrialized western countries, International Energy Agency (IEA) was constituted whose prime objective was to address the disruption of energy supply of the member countries. Now it is in reality that the development of a nation is highly dependent on both of its strong hold on food security and energy security.

Ethanol is one type of alcohol it is made from plant sugars obtained from agricultural biomass through the process of fermentation. The agricultural biomass may be a crop plant, plant product or residue of crop plant after harvest of crop that contains the sugars. World mineral oil reserves are depleting rapidly and ethanol has become as one hope of alternate liquid fuel to accelerate the wheel of development which is dependent of consequent energy source or most remarkably on mineral oils. The directions of economy of production and the impact of its production on environment are being critically studied by researchers. The history of production of alcohols and alcoholic beverages is very old as civilization of human society. It is viewed that pure ethanol with better distillation technique was produced during twelveth to fourteenth century. At that time alcohol was produced for use in medical drugs and preparing of painting materials. During 12th century in Ireland ethanol was first produced from starchy materials perhaps for beer industry. Ethanol was well known as lamp illuminant in 1850s and about 410 million litres of ethanol was produced in U.S. in 1890s. The cheap rate of kerosene as illuminant suppressed the production of ethanol during that period. When the economic distillation process was used in ethanol production in 19^{th} century, the production of ethanol turned to be an industry with its huge production need. In the beginning of the twentieth century alcohol was known to be used as fuel for automobiles or combustion engines. The assumed oil crisis during 1980s, the emphasis on production of bioethanol was renewed ease the difficulties of dependence on mineral oil. Twenty-five federal agencies carried out different programs on ethanol production research technologies and the National Alcohol Fuels Commission (NAFC) was constituted. The need of biofuel use worldwide has increased knowing that the mineral oil reserves will exhaust in near future and the concern of climate change which is ultimate cause of using of mineral oils.

II. POTENTIAL FOR RICE STRAW IN BIO-ETHANOL PRODUCTION

Production of ethanol from biomass is gaining popularity as an alternative to mineral oil or gasoline. But it may be termed as calling another crisis by solving one crisis if ethanol would be started being produced from foods or grains that is meant as food/feed for humans or animals and ethanol production will unnecessarily compete with the food supply. So the desired processes or protocol designs of bio-ethanol production should be from inedible plant parts that are having abundant cellulose and hemicellulose as carbohydrates which on fermentation could yield ethanol with the use of microorganisms fermenting sugars to ethanol.

There are so much bulks of biomass in world, but the biochemical nature of rice straw makes it a very good source of raw material for production of bioethanol. Upon hydrolysis the contents of hemicellulose and cellulose yields fermentable sugars. The chemical composition of rice straw consists of cellulose, hemicelluloses and lignin [10, 11, 12, 13]. Cellulose, hemicellulose and lignin are main components of rice straw and in this the xylose (14.8-20.2%) the pentose sugar is the most dominant carbohydrate [11, 9]. The contents of different carbohydrates and the potential of ethanol production of rice biomass (straw) and theoretical ethanol yield of rice straw has been depicted in Table 1. The bio-chemistry of the biomass composition greatly affects the efficiency in terms of quantity and quality of biofuel production. The chemical property of rice husk and straw and wheat straw has been shown in Table 2. High content of ash of biomass or feedstock is a bottleneck for biofuel generation and it degrades the quality of biomass to be a good feedstock for biofuel or energy generation. The composition of ash is 10-17% in rice straw whereas it is about 3% in wheat straw and there is presence of about 75% SiO_2 (silica) in rice straw and its silica content of wheat straw is around 55%. [14]. The fact of rice straw having the advantage of being feedstock is it has low alkali (Na_2O and K_2O) content of 10-17% as compared to the more than 25% of the alkali content of wheat straw [15].

Table 1: Composition of Different Carbohydrates and Potential Ethanol of Rice Straw

Components	Composition
Cellulose	38.6%
Hemicellulose	19.7%
Theoretical Ethanol yield(L/kg dry)	0.42
Theoretical Ethanol yield (gal/MT dry)	110

Source: It has been adapted from Biomass feedstock Composition and Property database, "*Ref*-[16]". It is assumed that the xylose is main fractions of hemicellulose.

Table 2: Approximate Fractions of Different Elements in Rice Straw and Husk and Wheat Straw

	Rice straw	Rice husk	Wheat straw
Proximate analysis (% dry fuel)			
Fixed carbon	15.86	16.22	17.71
Volatile matter	65.47	63.52	75.27
Ash	18.67	20.26	7.02
Elemental composition of Ash (%)			
SiO_2	74.67	91.42	55.32
CaO	3.01	3.21	6.14
MgO	1.75	<0.01	1.06
Na_2O	0.96	0.21	1.71
K_2O	12.30	3.71	25.60

Source: "*Ref*-[17]"

It may be informed that the quality of straw production varies between seasons and also in different locations of production or rice growing areas. The alkali and alkaline components of straw leaches away when exposed to rain in the field which improves the quality of feedstock.

III. AVAILABILITY OF STRAW

Since Asia produces more than 90% of world's rice production, similarly large amount of rice straw of about 786 million tonnes is produced in Asia annually and this quantum of paddy straw has the potential of yielding or producing 332 billion liters of ethanol. But a major quantity of straw is burnt in the rice field for cleaning the field for growing of next crop. The production of ethanol or biofuel from this biomass seems to be emerging important in particular, with the sky reaching price rise of mineral oils and global demand for reducing green house emissions from combustion of these oils and air pollution [18].

Rice straw and rice husk are two prime residues, where rice straw is left when raw rice is harvested and rice husk is produced when raw rice milled. Straw production is 1.0-1.5 times of raw rice production whereas the quantity of husk is about 18-22% of raw rice milled. There are already some established technologies for use of rice husk. But there is no commercially used technology for using rice straw for renewable energy generation. The preferred commercial use of rice husk is that these materials are readily available at rice mills for a fairly long period during rice milling and its collection and transportation is easy from these commercial units; but the rice straw is available only during harvesting time and its collection and transportation is laborious and time consuming. Its availability is limited to only harvest time. Availability of rice straw in major producing countries is presented in Table 3.

Table 3: Availability of Rice Straw Potential of Ethanol Yield

Country	Rice straw availability (million MT)	Theoretical ethanol yield (billion litres)
Africa	20.93	8.83
Asia	786.00	332
Europe	3.92	1.65
North America	10.95	4.62
CentralAmerica	2.77	1.17
South America	23.52	9.92

Source: Adapted from "*Ref*-[18]"

IV. PRODUCTION OF ETHANOL FROM RICE STRAW

Rice straw is a biomass composed of assimilated carbon in different forms of carbon compounds and also the ethanol is one bio-carbon compound or organic compound in form of oil. The main constituents present in rice straw are hemicellulose, cellulose and lignin. Basically ethanol is an alcohol and production of ethanol from biomass is not a new thing and it dates long back to the existence of human civilization. Production technology of ethanol from rice straw biomass follows two platforms. One platform of ethanol production is known as the sugar platform and in this platform hemicellulose and cellulose is first converted to sugar which then undergoes fermentation and yield bioethanol. The other platform of ethanol production is synthesis gas (or syngas) platform (Fig. 1). Glucose, galactose, xylose, mannose and arabinose under fermentation produce ethanol in the sugar platform. Hemicelluloses and cellulose on hydrolysis produce above sugars by using acids or enzymes [19]

Gasification is the process followed for production of ethanol in syngas platform. In this process, treatment of biomass is carried out by heating with total absence of oxygen or one third of oxygen that is needed for complete combustion of biomass. Biomass is converted first to a gaseous phase of products mostly having hydrogen and carbon monoxide and this gas mixture is known as synthesis gas or syngas that means this gaseous mass will be utilized for synthesis of bioethanol. The syngas is fermented catalytically by some gas fermenting microorganisms to produce the end product ethanol. This process is more efficient and more environment friendly for ethanol production than other methods. In this process there is no byproducts is left at end and where as in other processes there is some more or less quantity of residue is left over which is again a problem for its safe disposal as it may affect the health and hygiene of organisms living on earth. The carbohydrates in the sugar platform are used for ethanol production and in synthesis gas method of ethanol production all components lignin, celluloses and hemicelluloses are used [19].

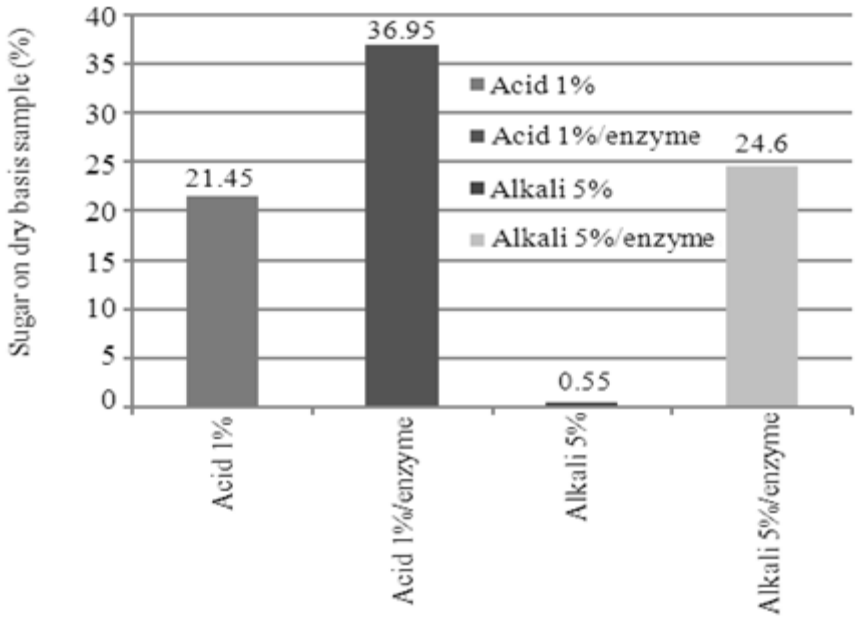
V. DIFFERENT PRE-TREATMENTS OF BIOMASS

The first step of the biofuel or ethanol production of biomass conversion is called pre-treatment. In this pre-treatment process the lignin present in biomass disrupted or lignin structure broken down and also pre-treatment disrupts the crystalline structure of cellulose by

which way enzymes or chemicals (acids or alkalis) can get access of the biomass and hydrolyze the cellulose. There are different kinds of pre-treatment methods, *i.e.*, pyrolysis; milling and grinding; high pressure steaming of acid or alkaline hydrolysis; high energy radiation; gas treatment with ozone, sulfur dioxide and chlorine dioxide; treatment with H_2O_2; treatment with organic solvents; steam explosion; wet oxidation; hydrothermal treatment and biological treatment processes [20, 21, 22].

At present there are many processes of pre-treatments of lignocellulosic waste materials for conversion of these biomass into fermentable sugars or gaseous mass (syngas method), but all methods are not environment friendly. There is one environment friendly green technique which is known as pre-treatment with sub critical water (SCW) [23, 24]. In SCW instead of using of not environment friendly organic solvents, extraction of organic compounds could be done. Thus with having merits SCW is used for hydrolysis of organic compounds. There is no information of using ultrasonic sound pre-treatment in combination with enzymatic conversion of biomass to ethanol. In this chapter it has been taken care to have an over view of different pre-treatment methods with hydrolysis and fermentation for conversion of lignocellulosic biomass such as paddy straw and husk into biologically, commercially and economically important products of biofuel or bioenergy for solving human need.

Now the process of conversion of biomass to fermentable sugars using combined methods of physical and chemical treatments and fermentation of sugars by using the yeast *Saccharomyces cerevisae* for production of biofuel is frequently used.



Source: "*Ref*-[25]

Figure 1: Graph of Pretreatment of Acids and Alkalis With or Without Enzymes to Sugars.

With a concentration of 1-9%, sulfuric acid can be used as acid pre-treatment of lignocellulogic biomass for conversion into bioethanol. Sodium hydroxide with concentration of 1-5% can be used for alkaline pre-treatment of biomass conversion into fermentable sugars. At temperature of 160^{o}C and pressure of 5 bars and at temperature of 200^{o}C and

pressure of 15 bars SCW pre-treatment is carried out for a time period of 10 minutes. The pre-treated samples need to be fermented by *S. cerevisae* and the efficiency of the pre-treatment method is accessed by quantification of the ethanol the end product.

After treatment with different concentrations of acids and alkalis for pre-treatment of biomass to convert to bioethanol it was observed that an acid treatment at a temperature of 121^{o}C for 15 minutes was found to be effective as pre-treatment method to convert lignocelluloses to sugars. The sugar yield was measured to be 21.45% w/w of biomass. When enzymatic treatment is carried consequently with acid or alkali pre-treatment drastically the content of sugar yield increased which was the aim of experiment. Application of enzymatic treatment in acid treatment gives sugar yield of 37% w/w and it is 28% in enzymatic treatment of pre-treatment process with use of alkalis. A 17% (w/w) sugar yield could be achieved in SCW treatment at temperature of 200^{o}C for 10 minutes and followed by enzyme treatment. In the combined treatment of acid and ultrasonic sound the sugar yield was found to be 44% w/w of biomass. After 3 days of fermentation of rice straw about 55-65% sugar will be converted to bioethanol. It was also found that even after 6 days of fermentation the left out sugars do not convert to bioethanol [25].

1. **Alkali pre-treatment:** Fifty gram of 2cm length cut rice straw was treated with different concentrations of ammonia with 1%, 2%, 3%, 4% and 5% in a ratio of 1:10 (w/w) rice straw and NaOH. Incubation of sample in water was carried out for 1 hour and after 1hour water of treatment the hydrolysate was pressed by cheese cloth. The quantity of sugar in the juice was measured with use of Luff schoorl method [26].

2. **Acid pre-treatment:** Fifty gram of 2cm length cut rice straw was treated with different concentrations of sulfuric acid with 1%, 3%, 5%, 7% and 9% in a ratio of 1:10 (w/w) rice straw and H_2SO_4. Autoclaving of sample at 121^{o}C was carried out for 15 minutes and after 15 minutes treatment the hydrolysate was pressed by cheese cloth. The quantity of sugar in the juice was measured with use of Luff schoorl method.

3. **Alkali-enzyme pre-treatment:** It is well known that chemical reactions can be enhanced considerably by use of enzymes in which enzymes enhance the rate and efficiency of the process but after the end of process enzymes could be recovered and may be reused. Similarly enzymes could be used in the alkali pretreatment of lignocellulosic biomass. In this process the sample after treatment with NaOH was pressed with cheese cloth and distilled water was mixed with the ratio of 10:1 (w/w) of weight of rice straw into the left over pulp. An enzyme mixture of 0.8% v/w of rice straw equivalent was mixed with sample containing pulp and distilled water. Then the sample was incubated in water bath at a temperature of 55^{o}C, pH of 4 for 12 hours (First enzyme). After that the sample was again pressed to separate the solution and pulp by same method and the process was repeated for a mixture of 2nd enzyme. The juice of both enzyme treatment and first non enzyme treatments were conducted for measurement of sugar content.

4. **Acid-enzyme pre-treatment:** The most conducive acid pre-treatment condition was taken for acid/enzyme pre-treatment. The samples were treated for enzymatic hydrolysis as it is has been followed in alkali-enzyme pre-treatment process. SCW (Subcritical water pre-treatment): In this process a high pressure tube (diameter = 15 mm. volume = 80 ml) with pressure gauge and thermocouple was used. At a constant temperature the

heating of subcritical water treatment tube was conducted in an oil bath [25].

Chopped dry rice straw and distilled water was mixed with a ratio of 10:1 (w/v) of distilled water and rice straw. After that mixture of straw and water was put in SCW tube and the experiment was carried for 10 minutes was mixed with distilled water in the ratio of 1:10 (w/v) rice straw and water. The mixture was poured in subcritical water tube and the SCW tube was heated for 10 minutes in an oil bath at 160 and 200^{o}C. After the SCW, the tube was cooled in water and the content was kept in a beaker. After that, enzyme mixture of 0.8% v/w on straw basis and pH of 4 was treated with the samples at a temperature of 55^{o}C for 12 hours. Treated samples were pressed to get out the dissolved solution of sugar and the sugar content of the pressed solution was quantified.

5. **Ultrasound or ultrasonic pre-treatment:** The biomass was treated with 1% acid and kept in a beaker. The acid treated sample was subjected to treatment of ultrasonic sound at 40 watt for 10 minutes with temperature < 50^{o}C. Again enzymatic treatment with 4% v/w of rice straw equivalent at pH of 4 was done for ultrasonic treated sample. The sugar dissolved solution was pressed out and quantity of sugar was measured.

6. **Detoxification:** Detoxification is a novel process in bioethanol conversion in which acid or alkali or ultrasonic pre-treated samples were detoxified of toxic substances and heavy metals to make the end product more efficient for use and environment friendly. The above pretreated samples of 20 parts (w) are mixed with 1 part (w) activated charcoal (charcoal from wood, sample and charcoal ratio 20:1) and it was agitated at room temperature for 48 hours on magnetic stirrer. After completion of the charcoal treatment the samples were filtered using filter No. 5 (Whatman, Germany) to remove out charcoal. Then the filtrate was carried for measurement of sugar.

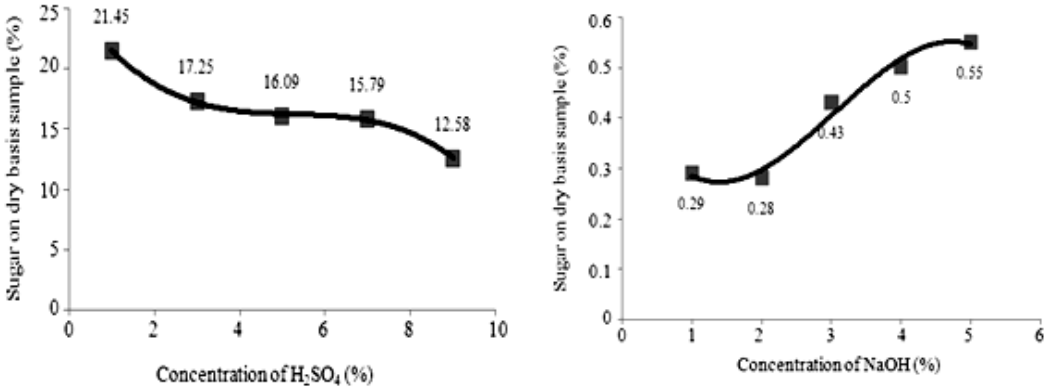
7. **Fermentation:** Fermentation of sugar into ethanol is a biological reduction process in which the microbes reduce the sugars *i.e.* glucose, fructose, sucrose, etc. into ethanol. Reduction of 1 mole of glucose by microbes in fermentation produces 2 moles of ethanol, 2 moles of CO_2 and 2 moles of ATPs. ATPs are utilized by microbes (*Saccharomyces cerevisae)*, ethanol and CO_2 are byproduct of microbe activity. So this kind of fermentation is called biological ethanol fermentation. The different pretreated samples are first detoxified or may not be detoxified and then the samples are subjected to biological fermentation by microorganism yeast *Saccharomyces cerevisae.* After 3 days and 6 days of fermentation the ethanol yield (EY) was quantified. The EY could be estimated by the following formula,

$$\text{Ethanol Yield} = \frac{\text{Measured Ethanol in Sample (g)}}{\text{Theoretical Ethanol (g)}}$$

$$\text{Theoretical ethanol(g)} = \text{Amount of initial sugar content(g) in fermentation solution X 0.5}$$

Effect of acid or alkali pre-treatment methods on sugar yield has been shown in figure 2 and 3 respectively. There is reverse relationship between concentration of acid treatment and sugar yield. This is due to the digestion of sugar monomers. Sugar yield is

found to be highest (21-45% of straw equivalent) at the acid treatment of 1% sulfuric acid. In regards of alkali treatment higher concentration leads to slight increase of sugar yield.



Source: "*Ref*-[25]

Figure 2: Concentration of Sugar in Acid Pre-Treatment of Straw (Rice)

Table 4: Content of Sugar in SCW Pre-Treatment and Enzyme Treatment of Straw (Rice)

Situation	**Temp (°C)**	**% of sugar***
SCW-Enzyme	160	7.4
	200	16.9
Only Enzyme	30	3.4

Equivalent of rice straw **(***Source: "*Ref*-[25]")

Table 5: Conversion of Lignocellulose to Sugars of Different Treated Straw (Rice)

Pre-treatment	**Sugars (%)***
Acid- 1%	21.45
Acid- 1% + enzyme (0.8%v/w)	36.96
Acid- 1% + enzyme (4%v/w)	39.10
Alkali-5%	0.55
Alkali- 5%/ + enzyme (0.8%v/w)	24.60
SCW- enzyme (0.8%v/w)	16.90
Enzyme- (0.8%v/w)	3.40
Acid-1% + ultrasonic + enzyme (4%v/w)	43.93
Acid1% + ultrasonic + enzyme (4%v/w/and detoxified)	32.29

*Equivalent of rice straw **(***Source: "*Ref*-[25]")

In absence of pre-treatment, enzyme treatment has no effect on sugar yield (only 3.4% equivalent of rice straw).

Effect of SCW: In Table 4 effect of SCW and enzymatic treatment for converting lignocelluloses to sugars has been shown and it has been observed that when there is increase of temperature from 160ºC to 200ºC, the sugar concentration increases more than two times (7.4%-17%).

Effect of enzyme concentration: When there will be increase in concentration of enzymes in acid pre-treated samples of lignocellulose from conc. 0.8 v/w (equivalent of rice straw) to 4.0%; there will be similar increase in conc. of sugars in the enzyme treated sample from 36.96% to 39.1% (Table 5).

Table 6: Concentration of sugar(g/100g) in fermentation

Fermentation period (days)	**1**	**2**	**3**	**4**
0	4.35	5.13	4.02	4.49
3	2.00	2.13	1.67	1.98
6	1.60	1.77	1.51	1.75

*1 = No ultrasonic and no detoxification; 2 = with ultrasonic and no detoxification; 3 = No ultrasonic with detoxification; 4 = with ultrasonic and detoxification **(*****Source: ‘‘Ref-[25]’’)

Combined Ultrasonic and acid pre- treatment effects: When there will be combined treatment of acid pre-treated lignocellulosic samples with ultrasonic and enzymatic treatment (concentration of enzyme 4% v/w equivalent of rice straw), the sugar yield of this sample was high as 43.93% as compared to 39.1% sugar yield in treatment of sample without ultrasonic treatment (Table 5). It confirms that ultrasonic sound treatment has positive effect for sugar yield in biomass conversion.

Effect of detoxification on sugar yield: During detoxification the total content of sugar yield decreases as some part of sugar remains attached to the surface of activated charcoal (Table 5).

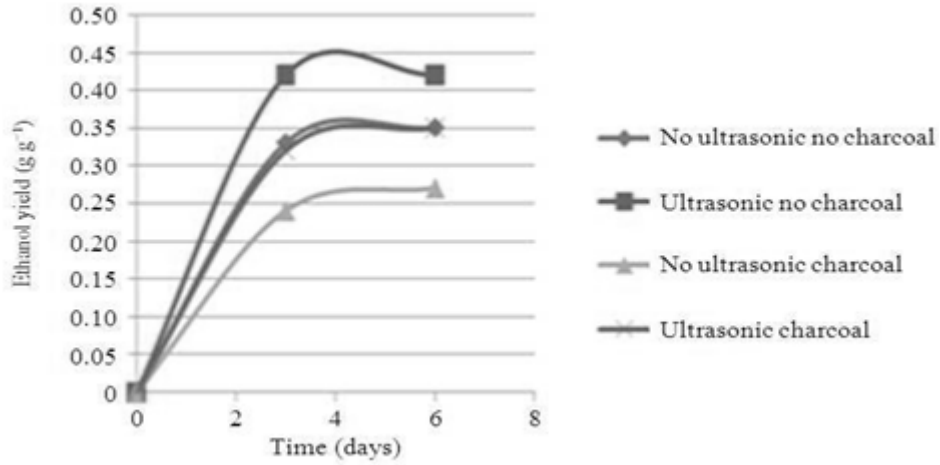


Figure 4: Yield of Ethanol in Fermentation (*Source: “*Ref*- [25]”)

Fermentation: The effect of duration of fermentation on production of bioethanol from reducing sugars in acid pre-treated and with or without treatment of ultrasonic accompanied with enzyme treatment of biomass of rice straw has been shown in figure 4. Fermentation for duration of 72 hours by yeast *Saccharomyces cerevisae* will convert all fermentable sugar to bioethanol (Table 6). After 6 days of fermentation there will be 55-65% of conversion of sugar to ethanol.

The enzyme application in the alkali pre-treatment process and conversion of sugars to bioethanol has positive effect. (Fig. 5). It may be concluded that that if the acid pre-treated hemicellulose materials are treated with enzymes there will be distinct increase in sugar yield (21.45%) and this trend is not observed in the alkali pre-treatment conversion of hemicellulose to glucose and by treatment of enzymes to the alkali pre-treated sample there is drastic increase in conversion of hemicellulose to sugars (24.6%).

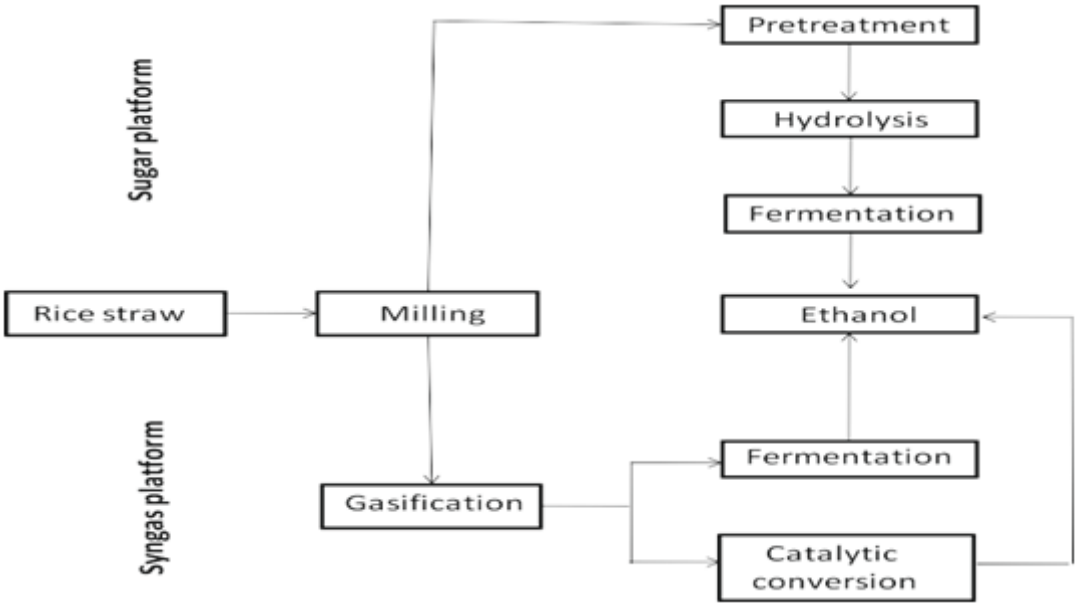
VI. IMPORTANCE OF PRE-TREATMENT

Rice straw consists of hydrocarbon polymers. The straw is composed of hemicellulose and cellulose very densely and both of these two components are covered by layers of lignin that protect the carbohydrates against hydrolysis. As long as lignin layer is on the surface of straw, the cellulose and hemicellulose could not be brought under enzymatic hydrolysis process and hence restricts to decompose. Therefore it is necessary to break lignin layers of rice straw by a pre-treatment process. By the process of pre-treatment lignin layers are broken down, the crystallinity of glucose decreases with increase in its biomass surface area and hemicellulose are removed. By the process of pre-treatment cellulose becomes accessible for enzymatic activities, which increases enzymatic hydrolysis, glucose converts to fermentable sugars with higher rate and with more sugar yields [27].

Types of Pre-treatments

1. **Physical pre-treatment:** In physical pre-treatment there will be increase in the surface area and size of pores in cellulose that makes the biomass more accessible to enzymes and decrease in crystallinity in cellulose. The different physical pre-treatments are grinding and milling, steaming, temperature, irradiation and pressure.

2. **Grinding and milling:** It is the first and foremost step of pre-treatment of biomass that mainly aims to reduce the size of particle and crystallinity of biomass. When steam exploded biomass is treated with super fine grinding proves better than grinding by hydrolysis [28] but there is more requirement of energy. The energy requirement of any commercial work is vital and takes a decision of employing any process to carry out or not. Wet disk milling of rice straw is proved better than ball milling and in this grinding there is more recovery of glucose content and more saving of energy [29]. The different grinding and milling methods in pre-treatment of biomass of rice straw are roll milling, ball milling, wet disk milling, etc. Wet disk milling and roll milling are important and several types of grinding and milling methods have been devised depending on the biomass.



. *Source: *Ref*-[3]

Figure 5: Process of Ethanol Production from Rice Straw

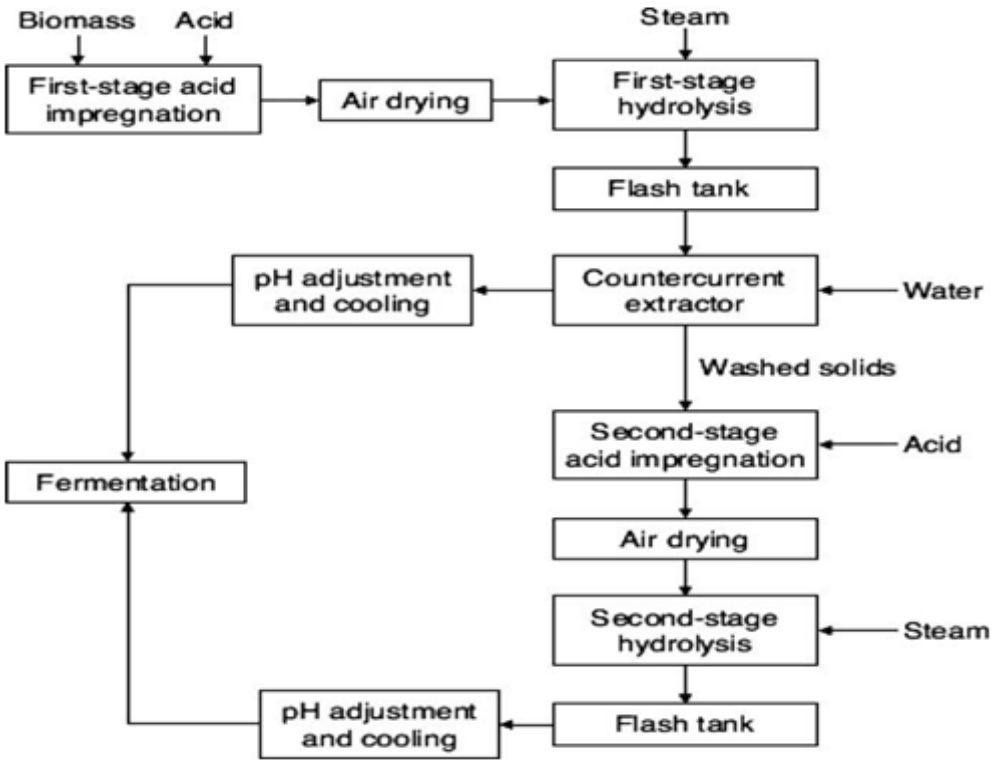
3. **Electron beam irradiation:** The lignocellulose biomass can be pretreated by irradiation of electron beam and the cellulose can be degraded to loose fibers of low molecular weight cellobiose and oligosaccharides [30]. In electron beam irradiation of lignocellulose there is glucosidal bonds of cellulose polymers which dissociates preferentially in presence of lignin. This process has many difficulties and it is expensive requiring high energy. Physical pre-treatment of milled rice straw using irradiation of accelerated electron beams [31] with subsequent enzymatic hydrolysis of 132 hours produces a glucose yield of 52.1% whereas the glucose yield of untreated electron beam irradiation sample is 22.6%. At controlled temperatures the production of inhibitory substances could be minimized in the acid or alkaline pre-treatment methods.

4. **Microwave pre-treatment:** Heating efficiency is high in microwave irradiation, due to this factor and easy operation of microwave irradiation has wide use. Ultra structure of cellulose is changed [32] by microwave irradiation and it helps in degrading hemi-celluloses and lignin and in increasing the susceptibility of biomass to enzyme activity [33]. Hydrolysis of lignocellulose to reducible sugars could be increased by microwave pre-treatment in the presence of water and enzyme [33, 34]. Enzymatic hydrolysis of dry rice straw could be enhanced in the glycerine medium also with less amount of water [35]. Alone the microwave irradiation has no advantage over other pre-treatments as it has same hydrolysis rate and sugar yield with those of raw straw [16].

5. **Chemical pre-treatments:** The important chemical pre-treatments are pre-treatments using alkali (NaOH) and ammonia (NH_3). For effective enzymatic conversion of biomass to fermentable sugars chemical pretreatment is required.

6. **Alkali pre-treatment:** Alkaline solutions such as sodium hydroxide (NaOH) or potassium hydroxide (KOH) are used in alkali; NaOH or KOH helps to remove lignin and a portion of hemicellulose and it makes the cellulose accessible to enzymatic activity. This type of pre-treatment could be conducted at low temperature with a long time period with high concentration of base and alkali pre-treatment increases the saccharification yield. When compared with acid or oxidative reagents, alkali treatment seems as the most effective method for conversion of biomass to high sugar yield. Fragmentation of hemicellulose polymers could be checked by 2% NaOH (alkaline) treatment of rice straw [36].The ester bonds between cellulose, hemicellulose and lignin could be broken efficiently by alkali treatment than acid treatment [37]. The porosity and external surface area of the rice straw increases due to separation and exposure of microfibrils and it increases the process of hydrolysis by increasing the enzymatic activity [38].

7. **Ammonia (NH_3) treatment:** NH_3 being as a pre-treatment reagent it is an excellent swelling reagent and used in the treatment of lignocellulosic biomass. It is volatile and possesses high selectivity with lignin than that of carbohydrates. Due to volatile nature of NH_3 it can be recovered from the reaction system and could be reused which is a great quality of reagents for chemical reactions. Ammonia is noncorrosive and doesn't be polluted in reaction process. Aqueous ammonia cleaves the C-O-C bonds in lignin, ether and ester bonds in lignin- carbohydrate complex [39]. In ammonia recycle percolation (ARP) process, NH_3 is pumped into a heap of hemicellulosic biomass maintained at a temperature of 170°C. In this ARP process there is delignification of 85% of biomass and about 100% of theoretical sugar yield would be achieved [19]. The xylan and glucan contents in biomass sample is protected by soaking the sample at mild temperature of 40-90^oC for a longer reaction time and it is then fermented by using the simultaneous saccharification and co-fermentation (SSCF) process [39, 40]. NH_3 treatment is highly selective in removing of lignin than other alkali treatments and it has considerable swelling effect on the biomass of lignocellulose [41]. The temperature of pre-treatment strongly determines the effectiveness of the soaking in aqueous ammonia (SAA) process. Anhydrous ammonia is used in the ammonia fiber/freeze explosion/expansion (AFEX) process. Anhydrous ammonia due to its high volatile nature can be recovered and reused in AFEX process. The exit stream in AFEX process is a mixture of NH_3 and water vapour. The biomass components remain as solid biomass and hence there is not any loss of carbohydrates of the lignocelluloses. There is no need of adjustment of pH as the ammonia evaporates very quickly. AFEX-treated biomass with enzymatic hydrolysis will produce >90% glucose and 80% of xylose and there is no formation of any inhibitory compounds [19]. AFEX process has only 3% sugar loss in entire process and hence it is an effective pre-treatment process. [42]. "*Ref*-[43]" observed pre-treatment of rice straw by the process of Ammonia Pressurization and Depressurization (PDA) using a laboratory scale ammonia reactor that consists of a 4-L reactor with adequate support equipments. There is considerable increase in the yield of sugar in this pre-treatment with enzymatic hydrolysis. "*Ref*-[44]" conducted aqueous ammonia (NH_4OH) pre-treatment with 21% optimum conc. at 69°C for 10 hours. In the use of AFEX in conjunction with b-glucosidase, 60 FPU (filter paper units) of cellulase/g-glucan and xylanase and other supplements, the yield of glucose was 60-90% after hydrolysis of 72-168 hours [45].



(*Source: *Ref*-[19]).

Figure 6: Schematic flow Diagram of the NREL's Two-Stage Dilute Sulphuric Acid Pre-Treatment Process.

8. **Acid pre-treatment:** There will be increase in anaerobic degradation of cellulosic biomass with acid pre-treatment at favourable temperatures. In general the impact of dilute acid pre-treatment is very little in degradation of lignin. Acid pre-treatment solubilizes hemicellulose and the cellulose is better accessed to the treatment by enzymes. HCL and H_2SO_4 (mineral acids) are generally used in acid pre-treatment of cellulosic biomass. Dilute acid is used in acid pre-treatment, and then enzyme cellulase is utilized for hydrolysis of remaining carbohydrates in treated biomass. Biomass is treated with dilute H_2SO_4 in dilute acid pre-treatment at a suitable concentration of acid, temperature for a specific period of time. A schematic diagram of this process is shown in Fig. 6. There is very less literature pertaining to dilute acid treatment and hydrolysis of lignocellulogic biomass and the process is unable to remove lignin and low sugar yield [46].

9. **Pre-treatment with oxidising agent:** Hydrogen peroxide or per acetic acid is added to lignocellulogic biomass in oxidative pre-treatment and the acid remains in water. Lignin and hemicellulose are removed from pre-treated biomass to make cellulose access for enzymatic hydrolysis. Several reactions take place during oxidative pre-treatment. Different reactions such as displacement of side chain, electrophilic substitution, electrophilic substitution and breaking of alkyl aryl ether links takes place [47]. In pre-treatment using hydrogen peroxide oxidative delignification occurs to detach and for solubilisation of lignin and loosening of lignocellulosic biomass and hence improves enzymatic degradation [48]. "*Ref*-[49]" studied the effect of hydrogen peroxide pre-

treatment about the changes that occurs in the structural features and the enzymatic hydrolysis of rice straw. The changes in the content of lignin, loss of weight of biomass, cadoxen solvent accessibility, changes in water holding capacity of the biomass, and changes of crystallinity of straw is measured during the pre-treatment process to get the quantitative estimate of the changes of lignocellulosic structure of rice straw. The rate and the extent of enzymatic hydrolysis, adsorption of cellulose, and accumulation of cellobiose at the start of hydrolysis were found out to study the pre-treatment effects of lignocellulosic biomass of straw. Oxidative pre-treatment of lignocellulose biomass at 60°C for 5 hours with 1% (w/w) H_2O_2 and NaOH resulted 60% delignification, weight loss of 40%, cadoxen accessibility increased five times, doubling of the water holding capacity and only a little decrease in crystallinity in comparison to the parameters of untreated rice straw. The oxidative pre-treatment method could be improved with an increase in the initial alkalinity and the temperature of the pre-treatment of the H_2O_2 solution. The improvement in the structural features of the pre-treatment biomass could happen if the weight ratio of H_2O_2 to dry rice straw will be more than 0.25g H_2O_2 /g straw with 1% w/w NaOH at temperature of 32^{o}C in alkaline hydrogen peroxide. The initial rate and count of hydrolysis, adsorption of cellulase, and accumulation of cellobiose in enzymatic hydrolysis of pre-treated biomass were increased according to the improved structural features of rice straw. The enzymatic hydrolysis will increase 4 folds for 24 hours that attributing to the alkaline H_2O_2 peroxide pre-treatment. There are some reports available of per acetic acid pre-treatment of rice straw [50, 51]. The quantitative changes occurring in the treated straw composition, extracted cellulose, susceptibility of treated straw and the crystallinity of treated straw with treatment of per acetic acid results in slight loss in contents of cellulose , and susceptibility of the treated straw with per acetic acid results into a slight loss in hemicellulose and cellulose contents in the straw. There is no or little breakdown in the crystalline structure of cellulose in treated straw by per acetic acid treatment.

10. **Organosolv pre-treatment process:** This is an efficient pre-treatment method which could mostly be relied on as in this process organic solvents are used in pre-treatment for delignification of treated biomass and removal of hemicellulose and providing a cellulose rich pre-treated biomass for highly efficient enzymatic hydrolysis for production of reducible sugars. This process increases the enzymatic degradation and the sugar yield in this process is almost equal to the theoretical glucose yield. Lignin and hemicellulose could be recovered for further production of high value co-products. The change occurring in the crystallinty of cellulose is not clear. The swelling of cellulose strongly depends on the species and quantity of organic solvent used and the temperature of pre-treatment in organosolv pre-treatment [52, 53]. Hot organic solvents are used in organosolv process. *i.e.*, ethanol with acidic pH is used to fractionate biomass components into distinct groups of lignin, hemicellulose and cellulose. Earlier this process was used for paper making and now it has been devised for pre-treatment of lignocellulosic biomass for production of bioethanol. Though the cost of expenditure is high in organosolv pre-treatment in comparison with other leading pre-treatments, the advantage of organosolv process is that the solvents used in this process could be separated and reused which reduces the total operational cost of using organosolv pre-treatment process. It needs fully controlled conditions since the organic solvents are highly volatile in nature. The solvents need to be removed from the pre-treated mass before enzymatic hydrolysis and fermentation otherwise it may inhibit the efficiency or

activity of enzymes used in the process of conversion of pre-treated biomass to sugars and then to bioethanol [54]. Ethanol and methanol with low boiling points are commonly used organic solvents, alcohols with high boiling points like glycerol, ethylene glycol and tetrahydrofurfuryl alcohol and other organic compounds such as ethers, phenols, dimethylsulfoxide and ketones are also used in organosolv pre-treatment process [55]. When organosolv process is carried out at high temperature 185-210^{o}C there is no need for addition of acid but the process at lower temperature needs addition of catalyst [56]. "*Ref-[*57]" reported that rice straw pulping can be carried out using diethylene glycol, mixture of diethylene glycol and ethylene glycol at atmospheric pressure. Pre-treatment of lignocellulosic biomass by organosolv process at higher boiling point organic solvents increase the delignification. The organosolv pre-treatment of biomass could be achieved at normal atmospheric pressure when treating the biomass with high boiling point alcohol which is a remarkable advantage of this process. "*Ref*-[58]" reported acetic acid pre-treatment of rice straw by 80% acetic acid with 0.6% H_2SO_4 as catalyst at 80^{o}C for 120 minutes there is maximum dissolution of pentosans.

11. **Biological pre-treatment:** By concept biological pre-treatment offers advantages that it is a controlled slow chemical reaction and low energy using process but remarkably a very rapid biochemical system is yet to be seen and all of its mechanism is also has not been disclosed to scientific community due to lack of getting a complete similar system out of the body of living organism. Chemical pre-treatment processes have the limitations that it requires specialized equipments that should be highly corrosion resistant, frequent cleaning or washing is required and the greatest headache is the disposal of chemical wastes produced out of chemical pre-treatments which may be very hazardous to environment. In these regards biological pretreatments are safe and environmentally sound process for removing lignin from lignocellulosic biomass by using biological organisms. The microorganisms are the most promising biological organisms for carrying out biological pre-treatment of biomass. Microorganisms such as white rot fungi, belonging to the fungal class Basidiomycetes [59] are used for biological pre-treatment. The four white rot fungi *Trametes versicolor*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* were studied on basis of structural and quantitative changes occurring in the components of pretreated biomass of rice straw. These microorganisms are widely used in this biochemical process of cleavage of lignin bonds, release of sugar molecules by enzymatic hydrolysis and fermentation of sugars to ethanol, CO_2, and free energy. Of these four fungi of Basidiomycetes, *P. ostreatus* was selective in degrading lignin fraction of rice straw rather than holocellulose fraction. On pretreating rice straw with *P. ostreanus* for 60 days duration there was 25% and 41% total weight loss of biomass and the degree of degradation of klosan lignin respectively. The residual quantities of hemicelluloses and cellulose left in the residues were 52% and 83% respectively. The findings after enzymatic hydrolysis with a commercial cellulase after 48 hours 44% cellulose and 52% holocellulose solubilized in the pretreated straw. There was a net sugar yield of 33% of total soluble sugar from holocellulose and 32% for glucose from cellulose [59]. In general the biological pre-treatment brings structural loosening of cells of treated biomass by increasing its pore space. The scanning electron microscopic (SEM) study showed that the pre-treatment of lignocellulosic biomass with *Pleurotus ostreatus* resulted into an

increase in the susceptibility of rice straw to enzymatic hydrolysis due to semi degradation of lignin which restricts entry of cellulase in the rice straw. "*Ref*-[60]" studied the pretreatment and fermentation on waste produce of agricultural sector by microbes and the agricultural residue used was rice straw in particular. *Aspergillus awamori*, *Phenerochaete chrysosporium*, *Aspergillus niger*, *Trichoderma reesei* and *Pleurotus sajorcaju* were used for pretreatment and the well known fermenting yeast *Saccharomyces cerevisae* was used for fermentation in the experiment. The result was that pre-treatment of rice straw with *Aspergillus niger* and *Aspergillus awamori* and later fermentation by *S. cerevisae* produced highest amount of ethanol (2.2g/L).

12. **Combined pre-treatment:** Different pretreatment processes of treating agricultural biomass particularly rice straw for conversion of bioethanol has been described. Combination of pre-treatments of biomass [61] which is the photocatalysis assisted alkali treatment of rice straw where there is efficient changes in the microstructure and physical properties of rice straw and it resulted in the decrease of contents of lignin and the decrease in lignin content makes the substrate susceptible for enzymatic hydrolysis and there is increase in the enzymatic hydrolysis. In the absence of hydrogen peroxide, alkali pretreatment of biomass of rice straw favours to solubilize small molecular size hemicellulose that is rich in glucose and originates from a-glucan and the treatment with alkaline hydrogen peroxide enhances the dissolving of larger molecular size hemicelluloses that are rich in xylose [62]. Combined application of microwave pre-treatment with other pretreatment methods proves to be better and efficient [63]. Several combined application of microwave pretreatments have been reported that pre-treatments of rice straw along with acid and alkali in which acid removes the hemicellulose and alkali removes the lignin, and the more lignin is removed by microwave compared to pre-treatment with only alkali. The results indicates that The pretreatment application of higher microwave power with shorter pre-treatment time and the lower microwave power with longer pre-treatment time have the same reaction effect on the weight loss and composition of pretreated biomass reaction mixture with consumption of same energy. The pre-treatment of microwave process increases some reactions in the pre-treatment of biomass. Radiation pre-treatment of rice straw biomass was reported [64] in the presence of ammonium hydroxide solutions by using the electron beam accelerator and in result it was observed that lignocellulosic structure of treated biomass was altered by electron beam which makes easy entry of NaOH solution to the structure of lignocelluloses complex and increases the reaction rate that favours the elimination of lignin in the treatment mixture. Irradiation scissors out the hemicelluloses or cellulose that helps in increasing the enzymatic accessibility. "*Ref*-[28]" studied the pre-treatment combination of steam explosion with superfine grinding of biomass of rice straw and its enzymatic hydrolysis. When superfine grinding pre-treatment of rice straw was combined with another pre-treatment of low severity steam explosion for treating the rice straw there is shortening of the grinding time, avoiding of the inhibitors, saving of energy cost, and high enzyme mediated hydrolysis. There is quite difference in enzyme mediated hydrolysis in chemical composition, polymer characteristics and composed cells contents of the superfine ground steam exploded rice straw product and the ground steam exploded rice straw residue. The hydrolysis of superfine ground

product by enzyme treatment gained maximum hydrolysis rate and produced more sugars, than the sugars produced without enzyme treatment of rice straw. The steam explosion and superfine grinding process is low energy requiring and decreases the particle size and improves reaction surface area to the largest content.

13. **Enzymatic hydrolysis:** The second step of conversion of lignocellulogic biomass to bioethanol is enzymatic hydrolysis of pre-treated biomass. In this process there is cleavage of different polymers of cellulose and hemicelluloses to reducible sugars by the use of enzymes. Cellulose is generally composed of glucans and hemicellulose is composed of different sugar polymers such as xylan, glucan, mannan, arabinan, mannan and galactan. Cellulose on hydrolysis yields glucose and on hydrolysis hemicelluloses produces many pentose and hexose sugars [65]. Lignin contents of hemicelluloses hinder the accessibility of enzymes for enzymatic hydrolysis. Therefore when there is more lignin in the pre-treated biomass the efficiency of enzymatic hydrolysis decreases considerably. Suitable pre-treatment methods need to be used to break the lignin content of the lignocellulosic biomass. Glucose and cellobiose also act as inhibitors of cellulase enzymes [66].

The enzymatic hydrolysis of pre-treated biomass is a complex process. There are many factors that influence the conversion of lignoceellulose to different monomers of sugars and by-products. These factors are ratio of liquid and solid in the solution, particle size, length of macromolecule, polymerization degree of cellulose, kind and concentration acid used, reaction time, temperature, cellulose chain configuration, association of cellulose with other protective structure of other polymers such as pectin, lignin, proteins, hemicelluloses, and minerals present in plant cell wall. Enzymatic hydrolysis is operated under mild conditions of longer treatment time and low pressure**.** "*Ref*-[67]" carried out a study on hydrolysis of dried rice straw using 5%–10% sulphuric acid at 80–100ºC. The report of best sugar yield was at temperature of 100ºC with 10% sulphuric acid for a time period of 240 minutes. "*Ref*-[68]" studied enzymatic hydrolysis of hemicellulose portion of straw with treatment of 2% H_2SO_4 at temperatures of 110–120ºC, where it was possible to hydrolyze more than 70% of pentoses in comparison to the total pentoses in dry straw basis. "*Ref*-[69]" conducted the enzymatic hydrolysis of rice straw with different acids of varying conc. (0.5–1% H_2SO_4, 0.5-1% H_3PO_4 and 2–3% HCl) and it was observed after 3 hours time duration that rice straw pentoses converted to a solution of monosaccharides suitable for fermentation. "*Ref*-[9]" studied effects of different conc. of H_2SO_4 and retention time in production of sugars and by-products from rice straw at relatively low temperature of 121˚C and long treatment time (10–30 minutes) in a 350L batch reactor. The 1% concentration of acid and retention time of 27 minutes was found to be optimum condition at which the hydrolysis attains high and yield of 77% xylose. "*Ref*-[70]" reported the pre-treatment of biomass of rice straw with dilute sulphuric acid that resulted in 0.72 g/g sugar yield in 48 hours of enzymatic hydrolysis, which was higher than steam pre-treated (0.60g/g) and untreated rice straw (0.46g/g). When there is increase in concentration of substrate from 20 to 50 and 100g/L sugar yield lowered to13% and16%, respectively.

"*Ref*-[71]" studied biochemical kinetics of glucose production from pre-treated rice straw by an Ascomycota group fungus *Aspergillus niger*. It was found that when the

particle size of the materials of pre-treated biomass decreases from 425lm to 75lm at pH range of 4.5 to 5 and optimum temperature of 45-50^{o}C there is increase in glucose yield from 43% to 87% equivalent of rice straw biomass. Photo catalysis of sugar production is supported by enzymatic hydrolysis and there is 2.56 folds increase in hydrolysis as compared with the hydrolysis carried alkali pre-treatment process. It was found that the concentration and the rate of glucose production depends on pre-treatment of straw, conc. of substrate and cell loading initially to start the process [61]. Pre-treatment of lignocellulosic biomass of rice straw increased the production of sugar monomers from 11% to 61% [72]. Use of combination of enzymes such as xylanases, cellulose, and pectinase increase the hydrolysis efficiency [42] but the cost of the process increases.

14. **Fermentation:** The bioethanol is produced from cellulose and hemicellulose present in the biomass by the process of fermentation. The fermentation could either be carried simultaneously with saccharification or with separate saccharification process and then the fermentation process for production of bioethanol. When both saccharification and fermentation is carried out simultaneously it is called as simultaneous saccharification and fermentation (SSF) and the other process is called as separate enzymatic hydrolysis and fermentation (SHF). SSF is widely used because of its low cost structure [73]. Since SSF minimizes the product inhibition, it gives higher ethanol yield compared to the ethanol yield of SHF. The greatest drawback of SSF is the different optimum temperatures of different enzymes for enzymatic hydrolysis and fermenting microbes. The microorganisms with good ethanol production efficiency do not tolerate the optimum temperature of 40-50^{o}C of hydrolyzing enzymes for better enzymatic hydrolysis. The microorganisms of fermentation could not tolerate this high temperature. If *Candidialus itaniae*, *Zymomonas mobilis* and *Kluyveromyces maxianus* could be used for fermentation these microorganisms could sustain the temperature of 40-50^{o}C of hydrolysing enzymes and the problem could be solved. Also the mixture of microorganisms culture like *Brethanomyces clausenii* and *Saccharomyces cerevisae* could be used in SSF to solve the problem of optimum temperature of enzymes and microorganisms [74, 75].

"*Ref*-[76]" studied the efficiency of microorganisms *Pachysolen tannophilus* and *Candida brassicae* in SSF of alkali pre-treated biomass of rice straw and found that *Pachysolen tannophilus* was associated with higher bioethanol yield than *Candida brassicae*. SSF with acid pre-treatment of biomass of rice straw with *Rhizopus oryzae*, *Saccharomyces cerevisae* and *Mucor indicus* resulted in 40-74% ethanol yield of the theoretical maximum ethanol yield [77]. Simultaneous saccharification and fermentation of alkali/ microwave and alkali pre-treated biomass of rice straw to ethanol conversion with using cellulose from *T. Reesei* and *S. serevisae* [63] under optimum conditions of pH, temperature and pressure yields ethanol of concentration 29.1g/L and the yield of ethanol was 61.3%. Ethanol production from microwave/ alkali pre-treated biomass of rice straw with shorter reaction time and lower initial enzyme loading produced ethanol with higher conc. and yield than the biomass pre-treated only with alkali. Many reports support SSF as it is superior process of fermentation and ethanol production than other processes of traditional ethanol production from the biomass of rice straw. It is because in SSF process the enzymatic hydrolysis rate is high, there is minimization of inhibitors and

there is no need of separate reactor establishment for saccharification and fermentation [78].

"*Ref*-[70]" studied the separate enzymatic hydrolysis and fermentation of lignocellulosic biomass of dry rice straw by the microorganisms such as *Rhizopus oryzae*, *Mucor indicus* and *Saccharomyces cerevisae* [70]. The study found that *Mucor indicus* is capable of producing ethanol from pentose sugars obtained from rice straw. There is one consolidated process of production of ethanol from lignocellulosic biomass in which production of cellulase, hydrolysis of biomass and ethanol fermentation are carried out together in a single container or reactor and this process is known as consolidated bioprocessing (CBP). *Clostridium phytofermentans* can directly ferment efficiently cellulose to ethanol and this microorganism can be suitable for CBP.

The lignocellulosic hydrolysates xylose and glucose are two abundantly found components which are fermented to produce ethanol. For efficient fermentation of these two kinds of sugar components two microorganisms are required. But the optimum environmental conditions for two strains of microorganisms are different and this brings difficulty in co-fermentation of xylose and glucose [79]. In the co-fermentation of xylose and glucose simultaneously fermentation of glucose takes place efficiently with traditional glucose fermenting strain and the fermentation process of xylose is relatively slow with low efficiency due to competition for oxygen requirement or due to the catabolic repression brought by glucose molecules [80, 81]. To get solution to this problem different approaches have been tried in both process engineering and strain engineering [80, 82, 83]; the immobilization of one strain [80], co-immobilization of two strains [80, 84], two stage fermentation in one bioreactor [85], and separate fermentation in two bioreactors [86, 80] have been tried.

VII. Improved Saccharification and Fermentation for Bioethanol Production

It is very nice to hear that bioethanol could be produced from lignocellulosic biomass of agricultural wastes, but at the same time it is challenging to produce ethanol from agricultural biomass efficiently, economically and environment friendly. Lignocellulose is highly complex in nature and lignin, hemicelluloses and cellulose are strongly associated in it which gives a strong and compact structure to the material or mass made up of lignocelluloses. Due to strong and compact structure of lignocelluloses, there are hindrances at every step of conversion of lignocellulosic biomass to ethanol, *i.e.*, pre-treatment, saccharification and fermentation. The crystalline nature of lignocelluloses affects negatively the saccharification yield. The crystalline nature notably affects negatively its saccharification [87]. The hemicelluloses decrease the crystallinity of cellulose and hence favours saccharification. [88]. Lignin has the most complex structure in lignocelluloses. Lignin is hydrophobic heteropolymer having with three phenyl propane units: Guaiacyl (G), syringyl (S) and p-hydroxyphenyl (H) [89]. Hemicellulose and lignin are digested during pre-treatment of biomass that makes cellulose portion of biomass more accessible for enzymes to convert cellulose into fermentable sugars. Many kinds of phenolic compounds are produced in the process of pre-treatment and these phenolic compounds inhibit the process of bioethanol production [90, 91, 92, 93]. After removal or minimization of inhibitors in pretreatment it enhances saccharification and fermentation of lignocellulosic biomass [94, 95].

For an efficient bioethanol production program, the inhibitors of the ethanol conversion process could be removed by the removal methods of activated carbon adsorption, filtration, lime treatment, advanced oxidation and solvent extraction [96, 97]. The total cost of the process increases due to application of detoxification methods in pre-treatment of biomass. Microorganisms or their enzymes could be used for minimization of effects of inhibitors or removal of inhibitors from the hydrolysate of biomass. Laccase could be used as biological method of inhibitor (phenolic compounds) removal from biomass hydrolysate [94, 98, 95]. Free laccase is generally used the in the production of ethanol from the lignocellulosic biomass of agricultural waste.

The enzymatic saccharification of pretreated lignocellulosic biomass is an expensive step in conversion of biomass to bioethanol and it is probably due to the high production cost of enzymes used in this process and the free enzymes could not be recovered after once being used. Thus the free enzymes could not be reused. Enzyme immobilization is done to overcome the unstable nature of free enzyme when it is isolated from the source [99, 100]. Immobilized enzymes are more roboust to environmental changes and mimic the natural mode of action, being attached to support surface area [101, 102]. These immobilized enzymes are not completely used in reaction mixture and hence improve stability of immobilized enzyme and allow for easy recovery and multiple uses [99, 100]. The immobilized enzymes help for the highest income in terms of reducing the costs of the enzyme while increasing hydrolysis and sugar conversion.

In the enzymatic hydrolysis of pretreated lignocellulosic biomass the enzymes involved are cellobiohydrolase (CBH), laccase, endonuclease (EG) and β-glucosidae (BGL). Cellobiohydrolase breaks end of cellulose chains and releases cellobiose, degradation of lignin and phenolic compounds are catalysed by laccase, endonuclease randomly breaks internal glycosidic of cellulose and produces glucan chains of different length and β-glucosidae plays vital role in saccharification of biomass [103] and it cleaves disaccharide cellobiose into fermentable sugars. Since a group of enzymes are required in the saccharification of biomass, it is necessary to develop enzyme cocktail that could be having all the biochemical behavior for efficient and economic conversion of biomass into saccharification products which on fermentation will produce ethanol of its maximum theoretical yield by reducing the inhibitory action of phenolic compounds [104, 105]. A cocktail having immobilized enzymes celluclast 1.5L, laccase and ß-glucosidase, degrade phenolic compounds and carry out saccharification of pretreated rice straw simultaneously [106].

1. **Cocktail of chemicals:** Celluclast 1.5L, and BGL were derived from *Aspergillus niger*, laccase derived from *Trametes versicolor* and 3-aminopropyltriethoxysilane (APTES). 2, 6- dimethoxyphenol (2, 6-DMP) and glutaldehyde (GLA), were procured for chemical cocktail preparation. Fe_3O_4 along with all other chemicals and reagents were purchased. Filter paper assay was used to measure total cellulase activity, and expressed as filter paper units (FPU) [107]. BGL activity was assayed using p-nitrophenyl-b-D-glucopyranoside (p-NPG, Sigma Aldrich) [108] and laccase activity was estimated by spectrophotometer by using 1mM of 2, 6-DMP [109].

2. **Saccharification of pretreated biomass:** Saccharification was carried out in 100-mL glass flask using the cocktails of free enzymes celluclast 1.5L, BGL, and laccase.

Reaction mixture consisting of 0.4 g biomass with a fixed dose of enzyme cocktail containing 15 FPU celluclast 1.5L and 15 international units (IU) BGL/g dry biomass in 20mL of 0.05M sodium citrate buffer- pH 5.0 was made. Tween 80 (0.2 %) was added as surfactant. 40 mg/L tetracycline and 30 mg/L cycloheximide were added to prevent microbial contamination. The reaction mixture was incubated at 50°C and 200 rpm for 48 hours. Samples were collected from the reaction mixture at specific time intervals and centrifuged at 7000 rpm for 10 minutes to separate the liquid portion. This liquid was analyzed for reducing sugars and phenolic compounds conc. The total phenol was analyzed according to Folin–Ciocalteau method [110, 111]. Amount of the reducing sugar was determined using the DNS method [112]. Saccharification yield (SY) was calculated as per the equation below,

$$\text{Saccharification Yield (\%)} = \frac{\text{Reducing sugar (mg) X 0.9}}{\text{Carbohydrate content in biomass (mg)}} \text{ X100}$$

Since any process carried out in a controlled system, huge expenditure is done, for this sake if the process would be optimized that the product of the process will keep quality and cost of product would be reasonable for use and it will enable the commercial scale application of the process. The parameters of pH, temperature, and agitation time were standardized for optimizing the process of saccharification. The optimization temperature of saccharification was kept at 35-55ºC. Similarly the optimum pH range of saccharification was 3.5-5.5 and that of agitation time was standardized within the range of 100-250rpm. The saccharification was carried out at optimum conditions as above by incubating for 48 hours. Effect of laccase on saccharification and reduction of phenolic compounds at varying enzyme doses (5–15 IU/g substrate) was investigated.

3. **Immobilization of enzymes:** Functionalization of Fe_3O_4 particles (0.5) was done with 2% GLA in phosphate buffer (100mM, pH 7.0) and incubated for 2 hours at 25ºC. Functional modification of APTES was performed in toluene using 2% (v/v) APTES and incubated for 12 hours at 25ºC while shaking at 200 rpm [107]. After three times of washing with acetone, ethanol and distilled water sequentially the particles were subjected to further activation using 2% GLA to get APTES-GLA modified particles. By loading of 100mg protein/g with the modified particles incubating at varying buffers (50mM) for 24 hours at 4ºC and shaking at 150rpm the immobilization of enzymes could be achieved. Immobilization yield (IY) and efficiency (IE) were estimated as below,

$$\text{Immobilization yield(\%)} = \frac{\text{The total amount of immobilized enzymes}}{\text{The total amount of enzyme initially added}} \text{ X100}$$

$$\text{Immobilization Efficiency (\%)} = \frac{\text{Total activity of the immobilized enzymes}}{\text{Total activity of free enzymes}} \text{ X100}$$

The total amount of immobilized enzymes effect of pH on the activity of immobilized and free enzymes was studied at pH range of 3-6 with the buffers (50mM) phosphate citrate-pH 6, sodium citrate- pH 3.0-3.5, and sodium acetate- pH 4.0-5.5. The two kinds of enzymes with optimum pH were also studied for their activities at different temperatures (35-60^{o}C). The enzymes of cocktail were immobilized on magnetic nanoparticles. Enzyme cocktail was prepared with BGL, celluclast 1.5L and laccase. The immobilized enzyme cocktail has vital role in the saccharification of the pretreated lignocellulosic biomass agricultural waste for production of bioethanol. The doses of enzymes in the saccharification process were 15IU (international units) BGL, 15 FPU celluclast 1.5L and 10 IU laccase. The encouraging property of immobilized enzymes of cocktail is that they are recovered after completion of saccharification process and could further be used repeatedly with equal effects and efficiency. The enzymes were recovered from the reaction mixture by using external electricity field and followed by washing with sodium citrate buffer-pH 5.0. Now the enzyme cocktail is ready to be used for repeated batches of saccharification process.

VIII. FERMENTATION AFTER SACCHARIFICATION USING COCKTAIL OF CHEMICALS

Fermentation is the last step among different processes used for production of ethanol or bioethanol or biofuel from conversion of lignocellulosic biomass wastes produced in the agriculture field. The fermentation process should be so efficient that it could produce the quantity of ethanol to the tune of theoretical ethanol yield that should be produced from biomass and also the process must be economic to be used for commercial scale. In regard to purpose of use the produced ethanol should not contain heavy metals or minerals that are hazard to the environment and it should be green fuel so that it will not bring air pollution and emission of GHG. After completion of saccharification process the hydrolysate of rice straw was concentrated to increase the total sugar concentration up to 50 g/L. The concentrated hydrolysate was supplemented with nutrient medium containing 10g/L $(NH_4)SO_2$, 5g/L extract of yeast, 1.0g/L $MgSO_4.7H_2O$ and 4.5 g/L KH_2PO_4 with the pH adjusted at 5. The hydrolysate was sterilized at temperature of 110^{o}C for 10 minutes. The hydrolysate was cooled down and the cooled media was inoculated with 10% volume/ volume of active cultures of yeast *Saccharomyces cerevisae* after growing of 18 hours and maintained in yeast extract peptone dextrose (YEPD) media. The YEPD media contains 1% yeast, 2% peptone and 2% dextrose. The fermentation was conducted at 30^{o}C and 200 rpm and the samples were drawn at different time interval and conc. of ethanol, reducing sugars and yeast cell was studied at these time intervals.

Yeast cell viability and growth in the hydrolysate of rice straw was assessed by enumerating colony forming unit (CFU) on YEPD media. For this, aliquots were collected from the fermentation flask at different time intervals and then serially diluted in saline solution (0.85% NaCl) and spread onto agar plates. After that, agar plates were incubated at 30°C for 48 hours for the colonies of yeast to grow. The fermentation efficiency (%) and ethanol productivity (g/L/h) was calculated as follows,

$$\text{Fermentation efficiency (\%)} = \frac{\text{Actual ethanol yield (g/g)}}{\text{Theoretical ethanol yield (g/g)}} \text{ X100}$$

$$\text{Ethanol Productivity (g/L/h)} = \frac{\text{Maximum ethanol concentration (g/L)}}{\text{Fermentation time}}$$

Theoretical ethanol yield (g/g) = Glucan concentration x 0.51
whereas 0.51 is the coefficient for conversion of glucose to ethanol

1. **Immobilization of enzymes:** Laccase decreases the conc. of phenolics in the reaction mixture or aliquot. Laccase supplementation increases significantly the sugar yield of the cellulosic biomass of rice straw [106]. The free enzymes are unstable in soluble form and these could not be recovered after being used once in the chemical process and free enzymes could not be used in further cycles of the fermentation process. This increases the total cost of the production process. So for the economy of the process, the free enzymes need to be immobilized so that they can be separated easily after completion of one cycle of the fermentation process and can be reused for further cycles of fermentation. This could be achieved by using magnetic nanoparticles on the non magnetic enzyme supports.

   The pH of the reaction mixture significantly affects the enzyme immobilization. The immobilization of the enzyme mixture celluclast 1.5L (a mixture of CBH, BGL and EG), laccase and BGL were studied at pH range of 3.5-7.5. Immobilization yield of celluclast 1.5L, laccase and BGL were in the range of 18.2-94.0%, 38.0-79.3% and 28.2-83.3% respectively. The optimum pH values of laccase, celluclast 1.5L and BGL immobilization on Fe_3O_4 particles modified by GLA were 5.0, 7.0 and 4.0 respectively. The APTES- GLA dual modified particles showed optimum pH values for celluclast 1.5L, BGL and laccase were 7.0, 4.5 and 5.5 respectively. The maximum IY was found at 78.3, 73.2 and 62.7 for celluclast 1.5 L, BGL and laccase on GLA modified particles respectively. APTES –GLA dual modified particles showed both higher IY and IE for celluclast 1.5L (94.0 and 93.2% respectively), BGL (83.3 and 94.6% respectively) and laccase (79.3 and 88.2% respectively).

   The immobilization of enzymes on particles was ascertained by thermogravimetric analyser (TGA: It is one elemental analyzer by which the elements in any solution or suspension could be determined rapidly and accurately) analysis. There was found a considerable decrease in weight of 16.8, 15.7, and 15.1% in the presence of celluclast 1.5L, BGL, and laccase respectively as compared with only a 7.8% reduction in particles lacking the enzymes suggests the immobilization of enzymes on the APTES-GLA particles [106]. Field emission scanning electron microscope (FE-SEM) and Fourier transform infrared (FTIR) were used to confirm the immobilization of enzymes on Fe_3O_4 nanoparticles. Fluorescein isothiocyanate (FITC) dye binding to the amino functional group of the protein has been widely used to confirm successful loading of protein on nanoparticles.

2. **Characteristics of immobilized enzymes:** The characteristics of immobilized enzymes were studied in various ways with using various factors by many researchers. The temperature and pH effects on immobilized enzymes were measured on APTES-GLA modified particles. There were observed higher pH values of 5.5, 5.0 and 4.5 for immobilized celluclast 1.5L, BGL and laccase as compared to free enzymes at pH values of 5.0, 4.5 and 4.0 respectively. The higher optimum temperature values of 55^{o}C, 55^{o}C and 45^{o}C were observed for immobilized enzymes and it was 50^{o}C, 50^{o}C and 40^{o}C for free enzymes. Immobilized enzymes have higher residual activities than free enzymes. The immobilized enzymes are more stable than free enzymes.

3. **Saccharification of biomass with immobilized enzyme cocktail:** The cocktails of immobilized enzymes were used for the saccharification of pretreated biomass of rice straw [106]. Saccharification process was carried out up to 48 hours using the immobilized enzyme cocktail with and without using laccase. Saccharification was slow till first 36 hours by immobilized enzyme cocktail compared to the free enzyme cocktail. But, after 48 hours of saccharification, saccharification yield was higher of immobilized enzyme cocktail than with the free enzyme cocktail. Highest saccharification yield of 84.6% was observed after 48 hours of saccharification and when laccase was added to immobilized enzyme cocktail. In the absence of laccase there was only 68.2% saccharification yield.

It is well known that phenolic compounds act as inhibitors for saccharification of biomass. But the irony is that as the process of saccharification proceeds there is gradual increase in the concentration of phenolic compounds and hence reduces the speed of saccharification and affects the total saccharification yield. Phenolic compounds inhibit hydrolyzing enzymes in the saccharification process. Effect of laccase on phenolic compounds was studied with immobilized enzyme cocktail. When there is complete absence of laccase in the reaction mixture, the initial total phenolic conc. was 0.34g/L and it gradually increased as the reaction time proceeds and after 48 hours of incubation the conc. of phenolic compounds increased to 0.88 g/L and it resulted in 61.4% rise in conc. of total phenolics. When immobilized enzyme cocktail included laccase, the total conc. of phenolic compounds was 0.23g/L. It reported a remarkable 73.8% decrease in conc. of phenolic compounds. Inclusion of laccase increased 19.8% in saccharification yield. In nutshell laccase reduces the conc. of phenolic compounds in saccharification reaction mixture and increases saccharification yield.

The most expensive process of biomass conversion to ethanol is enzymatic saccharification of lignocelluloses of biomass. The total cost of saccharification can be managed to be low by recycling and reusing of the enzymes in different cycles of saccharification process. The enzymes could be recovered after completion of the process by application of nano magnetic particles. Nanoparticles help in covalent immobilization of enzymes of saccharification process. The immobilized enzymes show remarkable influence on the characteristics of the enzyme. Nanoparticles help in the covalent immobilization of enzymes of reaction mixture of saccharification and increases the surface area and unique structure of enzyme immobilized [100]. Covalent immobilization increases the interaction of the functional groups present on the surface of support and to overcome the limitation of leaching.

The interactions [107, 99, 100] in this study, GLA and APTES followed by GLA modification of Fe_3O_4 were used for covalent modification of enzymes celluclast 1.5L, BGL and laccase to study the effect of functional support on immobilization property. Immobilization of celluclast 1.5L and BGL is more effective than that by styrene or maleic anhydride copolymer and amine modified magnetic and silica nanoparticles. Also the laccase immobilization on APTES-GLA modified Fe_3O_4 particles was more effective than immobilized laccase on Fe_3O_4 @SiO_2@KIT-6 composite magnetic particles [113].

The SY of immobilized enzyme cocktail is 84.6% and that of cocktail of free enzyme is 77.3%. In presence of free laccase there were 17.9% high saccharification yield and 58.3% reduction in total phenolic compounds as compared to that obtained by using cocktail of free enzymes without laccase. Cocktail of immobilized enzymes resulted in 19.8% and 73.8% increase in saccharification yield and reduction in total phenolics respectively as compared to that of cocktail of free enzymes without laccase. The laccase reduces the total phenolic compounds and increases total SY. High efficiency of immobilized enzyme cocktail over cocktail of free enzymes is attributed to its higher stability and synchronized influence on saccharification process [114]. The immobilized enzyme cocktail retained 73.4% saccharification yield after the 8^{th} cycle of reuse [115,116].

Cocktail of immobilized enzymes showed more beneficial effect to have 25.2% higher growth of yeast *Saccharomyces cerevisae* than cocktail of free enzymes. The content and composition of lignin affect the saccharification of lignocellulosic biomass. Laccase reduced the concentration of phenolics in reaction mixture and it enhanced the efficiency of *Saccharomyces cerevisae*. Free laccase was previously used for detoxification of hydrolysate of lignocelluloses for bioethanol production. Use of laccase for detoxification was either before or after of saccharification [98, 94] of biomass, means the detoxification was not done during the saccharification process. This required separate detoxification method which increased the total cost of bioethanol production. The free enzymes of saccharification are highly unstable in their soluble form and they cannot be recovered and reused in further saccharification process. The cocktail of immobilized enzymes is much more stable compared to free enzyme and the cocktail enzymes maintained SY >70% after eighth cycles of reuse and results in higher saccharification yield and fermentation efficiency than the process of ethanol production with free enzymes and separate detoxification method [117, 118, 119]. Laccase in the enzyme cocktail with celluclast 1.5L and β-glucosidase reduce the total phenol compound concentration in acid pretreated rice straw by 73.8 % and increase the saccharification and ethanol fermentation yields by 33.2% and 33.3% respectively.

The cocktail of immobilized enzymes is composed of hydrolytic celluclast 1.5L, BGL and oxidizing enzyme laccase. The cocktail of immobilized enzymes degrade the phenolics and carry out saccharification of pretreated rice straw simultaneously. The cocktail of immobilized enzymes is recycled for further cycles of the process. The cocktail of immobilized enzymes have SY of >70% up to 8^{th} cycles. The repeated use of immobilized enzymes carry benefits as the total cost of the production process decreases relative to other processes using free enzymes and separate detoxification process for ethanol production. The immobilized enzymes cocktail also helps in increasing the fermentation efficiency of yeast *Saccharomyces cerevisae* and this cocktail of

immobilized enzymes enhance ethanol production from rice straw.

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IX. FAST PYROLYSIS OF RICE HUSK

Table 7: Analysis of Elements and Properties of Rice Husk

Ultimate analysis	**(wt.%)**
Carbon	39.78
Hydrogen	5.69
Nitrogen	0.71
Sulphur	0.1
Oxygen	53.72
Proximate analysis	**(wt.%)**
Moisture	14.45
Volatiles	65.76
Ash	13.27
Fixed Carbon	15.52
Heating values	**(MJ/Kg)**
HHV(High heating value)	17.32
LHV (Low heating value)	15.91

Source: "*Ref*-[120]"

About 18-22% by weight, husk is produced by the raw rice [121, 122]. It is estimated that about 151 million tonnes of rice husk is generally produced annually in world of which >90% is contributed from developing countries [123]. Most of the rice husk is underutilized for generating heat energy for cooking, brick industry or remain unutilized or burnt to ash for cleaning. Burning of rice husk creates problem as air pollution by GHG emission and disposal of a huge quantity of left over ash which may be flown by heavy wind into air and different places. Without burning the rice husk it could be decomposed and its biochemical changes could be brought to the biomass of rice husk at high temperatures as other hydrocarbons are decomposed. The decomposition of biomass by application of high temperatures is called pyrolysis and by the process of pyrolysis we can convert rice husk to bio-oils production (Table 7).

Pyrolysis is the decomposition of materials of organic or inorganic origin with high heating process and it brings the chemical changes of the treated materials *i.e.,* the material decomposes to compounds of new chemical compositions than the parent material by application of elevated heat in a neutral condition. The fast pyrolysis of rice husk is a thermal decomposition of biomass of rice husk by heating at a temperature range of 400-600^{o}C in the absence of oxygen or with one-third of oxygen that is required for the complete combustion. In this decomposition process, biomass of rice straw decomposes and produces 3 products such as bio-oils, bio-char and pyrolysis gas. The bio-oils produced in this pyrolysis are raw and are having very high viscosity. These bio-oils need to be refined or upgraded prior it is to be used. There are various processes available to upgrade bio-oils produced from rice husk by pyrolysis process. Catalytic cracking of bio-oils is one of the bio-oils upgrading methods and the catalytic cracking method is considered to be more potential in upgrading bio-oils. The catalytic cracking method is conducted at medium temperatures and at atmospheric pressure by addition of catalysts to upgrade the bio oil produced. In this process of fast

pyrolysis of rice husk catalysts such as HZSM-5 zeolite and rice husk ash are used. Rice husk ash (RHA) is used as catalyst to reduce the cost of upgrading of bio-oils. In the process of fast pyrolysis of rice husk two heating units are there for digestion of rice husk. By two heating units of pyrolysis process, upgrading of bio-oils could be done without catalyst. In catalytic cracking method of bio-oils, the catalyst promotes the de-oxygenation rate and it further improves the quality of upgraded bio-oils compared with non-catalytic cracking method of upgrading bio-oils.

There is problem in using catalytic cracking process which is the problem in activation of the catalyst caused by coking. In this case catalyst deactivation occurs from inside of the heating units to outside. To maintain the activity of catalyst [124] used tetralin as a solvent and tetralin is produced by the catalytic hydrogenation of naphthalene mixed with bio-oils at a ratio of 1:1 to lower the viscosity of the bio-oils and to improve the stability and maintain life of the catalyst. Catalyst inactivation could be avoided with naphthalene. "*Ref*-[120]" studied fixed bed cracking of bio-oils without using of additive in the experiment of fast pyrolysis of rice husk.

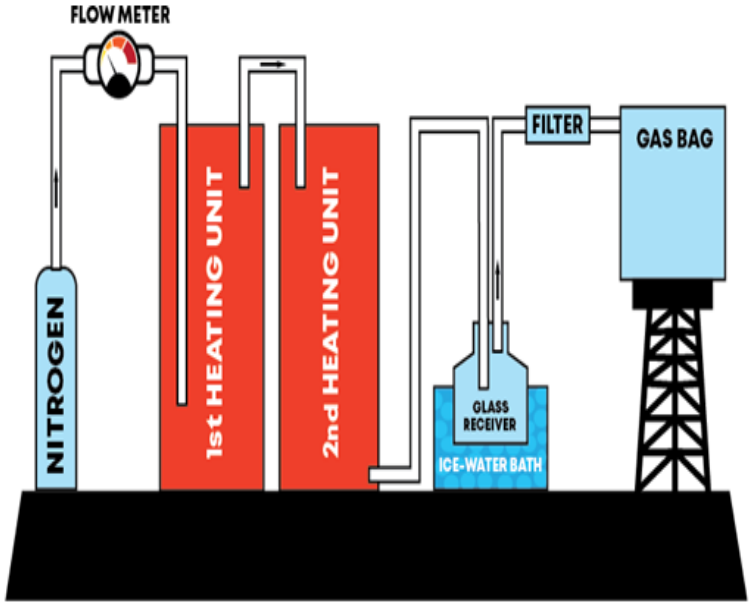

Figure 7: Fixed Bed Cracking of Bio-Oil [120]

The diagram in Fig. 7 shows the fixed bed cracking of bio-oils produced from fast pyrolysis of rice husk. The raw bio-oils are introduced into first heating unit and then after appropriate treatment the bio-oils introduced into second heating unit with or without packing catalyst. Nitrogen (N_2) is a carrier gas. As a carrier gas N_2 was fed at a flow rate of 10mL/min to first heating unit from its top for continuous withdrawal of the products and to maintain the inert atmosphere during cracking of the bio-oils. The product flowing out of the bottom of second heating unit is gaseous form and this gaseous product is condensed in a glass receiver submerged in an ice water bath. The non condensable gases are collected in a gas bag. In between ice water bath and the gas bag a filter is put there for recovering condensable vapours which might leak from the condenser. The second heating unit was filled with 15g catalyst (the catalytic cracking) while the first heating unit was filled with 30

g of raw bio-oils. The first heating unit was heated to a specific temperature after the second heating unit was heated to 500^{o}C for 60 minutes. The bio-oils produced is a complex mixture and it contains hundreds of organic compounds such as alcohols, acids, aldehydes, esters, ketones, phenols and lignin derived oligomers, etc. [125].

There are three situations we come through. In first situation the bio-oils is produced directly from rice husk by pyrolysis method and this bio-oils are named as rice husk bio-oil (RH bio-oils). In the second situation the rice husk bio-oils undergo upgrading by rice husk ash (RHA) as catalyst. By using rice husk as catalyst for upgrading of bio-oils the main theme is to reduce the cost of upgrading of bio-oils. The final bio-oils produced in the second situation of by using RHA catalyst is referred as Upgraded Bio Oil RHA catalyst. In the third situation bio-oil cracking method of bio-oils upgrading is done by HZSM-5 catalyst and the upgraded bio-oils produced by this method is referred as Upgraded Bio-oils HZSM-5 catalyst. The content of different elements and composition of different compounds vary significantly in these three ways of production of bio-oils. The water contents of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 27.14%, 19.6% and 15.4% respectively. The density of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 1.27g/ml, 1.29g/ml and 1.32g/ml respectively. The kinematic viscosity of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 12, 4.87 and 4.35 m^2/s at 45^{o}C respectively. The acid value (mg KOH/g) of rice husk oil produced from pyrolysis, upgraded bio oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 87.56, 67.4 and 54.8 mg KOH/g respectively. The high heating value (HHV) of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 16, 26.5 and 29.77MJ/kg respectively. The average pH of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 2.4, 2.3 and 2.1 respectively. The oxygen content of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 54.23, 42.2 and 37.23% by weight respectively. The carbon contents of rice husk oil produced from pyrolysis, upgraded bio-oils by RHA catalyst and upgraded bio-oils by HZSM-5 catalyst is found to be 38.76, 45.61 and 65.1% by weight respectively. The properties of bio-oil from pyrolysis of rice husk are high. There is significant decrease in the values of water content, acid value, kinematic viscosity and pH of upgraded bio-oils by catalysts than the properties of directly produced from rice husk by pyrolysis.

Table 8: Properties of Different Elements and Compounds in Different Bio-Oils

Ultimate analysis (wt %)	RH bio-oil	Upgraded Bio-oil RHA catalyst	Upgraded bio-oil HZSM-5 catalyst
Carbon	38.76	45.61	65.10
Hydrogen	7.31	7.51	7.58
Nitrogen	0.17	0.12	0.11
Sulfur	0.0	0.0	0.0
Oxygen	54.23	42.2	37.23
Element analysis			
HHV (MJ/Kg)	16	26.5	29.77
Acid Value (mg KOH/g)	87.56	67.4	54.8
Water content (wt%)	27.14	19.6	15.4
Density (g/ml)	1.27	1.29	1.32
Kinematic viscosity (m^2/s@45^{o}C)	12	4.87	4.35
pH	2.4	2.3	2.1
Ash	-	-	-

Source: "*Ref*-[120]"

Way Forward: Though the production of ethanol as alcohol or other alcohols as wine is very ancient to human civilization, the production of biofuels is not up to date till now. This process technology needs more human interest for use of this technology than need for development of production technology for biofuel production. There should be enough awareness among general public, technologist, producers, consumers, governmental staff, policy makers and international relation personnel for production and use of ethanol commercially and legality bound act that it will help in development of a clean future world with efficient recycling of energy resources. There should be systematic development and establishment of biomass recycling industries in all agricultural potential areas so that the agricultural byproducts or wastes would be systematically collected, transported for waste utilization industries/ centers. Research should also be for development rice varieties with high production and necessary straw strength that is suitable for efficient production ethanol from rice straw. This will enable all the minds that there is no agricultural waste in any form, in turn, the farmers will generate more revenue, a clean world by use of environmentally green oil (bioethanol) and no emission of GHG due to unwanted decomposition of biomass. Efficient technologies should be developed for biological conversion of biomass for production of biofuel and also processes should be developed to leave no solid waste material out of production processes of bioenergy. We all should take oath not to waste any energy source once that is generated or produced.

X. CONCLUSION

There are many viable laboratory techniques of production of biofuels by the conversion of the lignocellulosic biomass of rice straw and husk, but these technologies are yet to be used in commerce. The reason may be that the technologies needs further refinement or standardization to be used commercially or to reduce the production cost of the end product *i.e.* biofuels may not be remunerative by using expensive technologies. The other factors of non-adoption of biofuel production technologies may need awareness of

production, use and benefits of production of biofuels from agricultural wastes that it will be environment friendly, scientific and controlled use or decomposition of agricultural wastes will reduce GHG emission and the technology of collecting, heaping and transportation of agricultural wastes routinely. The policy makers should understand the dire necessity of organised organic waste collection, procedure for their beneficial use which will save world from pollution and drowning in oceans due to global warming. There should be governmental policies to develop production and production technologies of ethanol production on commercial scale as it is being followed for food grains as after food, fuel is the next essential material of sustenance of rapidly developing world. There should be all care to utilize every part of agricultural production to make the total energy production and utilization in a biosphere economic and easy. Therefore except research of only biofuel production research should be systematically and surely be diversified for utilization of agricultural by-products or wastes to produce some valuable novel products. The three processes of bioethanol production such as pre-treatment, saccharification and fermentation need to be revolutionized using the recent ideas and science as all these processes are very ancient in human use. The present modern technologies of genetic engineering, biotechnology, proteomics, met abolomics and genome editing, process engineering and extension should be used for development of technology, process, product, raw material utilization, etc. The production processes of biofuel production should sufficiently be balanced and justified for all clients such as producers, users and non users of the event. Now it is handy to produce genetically modified microorganisms (Yeast) and synthetically produced hydrolysing enzymes for development of potent technologies for biological fermentation process which would be less harmful to environment. As non-hydrolytic decomposition of biomass leaves no further solid wastes, more emphasis and research should be focussed on this as of now our world is calling for safe disposal of different wastes produced by humans from different production processes. There were very few attempts taken so far for the production of bioethanol commercially. However our government is trying to increase the production of bioethanol by developing new industry for the bioethanol from rice straw. Though this is one of the new energy source on which future world is going to depend it will be up to our researchers to focus more on these resources and to utilize these sources for the development of the society.

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