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Research Article

DILUTE ACID HYDROLYSIS OF PADDY STRAW AS SUBSTRATE FOR A.B.E FERMENTATION

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Abstract- Lignin presence in the fermentation substrate make it hard to breakdown by microbial activity since it is made of complex polymers, like phenolic propane units which are linked to one another by ether/carbon-carbon bonds. Dilute acid hydrolysis of paddy straw biomass was carried out for the depolymerization of hemicelluloses into xylose and other forms of sugars; this pretreatment was aimed in the intention of removal of lignin, improving the formation of fermentable sugars, degradation of carbohydrates. The acid employed in the dilute acid hydrolysis was orthophosphoric acid (H_3PO_4). The acid hydrolysis of paddy straw was employed in breaking down the hemicelluloses into pentose and hexose sugars for its utilization by microbial culture for more easier and simple bioconversion of sugars to solvents. The maximum sugars released were observed for 5% orthophosphoric acid concentration, the concentration of sugars in the acidified hydrolysate was 35mg/g.

Keywords- Paddy straw, Hydrolysis, Monosaccharide, Cellulose, Hemicelluloses.

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Introduction

The global bio fuels market is predicted to reach $247 million by 2020. In India still the major contribution of energy is from coal which is 55 % and the Indian crude oil import for the year 2013 is 196 billon dollars. The switching over to biofuels for a sustainable development in 2020 can only reduce the dependence on fossil fuels. Biobutanol is acquired by fermentation of sugars released from biomass by A.B.E. process. The process produces these solvents in a ratio of 3:6:1 (acetone: butanol: ethanol). The feedstock for biobutanol fermentation are the same as for ethanol which include energy crops such as sugar beets, sugar cane, corn grain, wheat and cassava, non-edible energy crops as well as agricultural residues, post harvest biomass such as straw and bagasse. Lignocellulosic materials are the most abundant and low cost biomass available to the world [3]. The polysaccharides present in the lignocellulosic materials can be depolymerized to monosaccharide and then converted to ethanol/ butanol via appropriate processes. Thus, other than grains and sugarcane, paddy straw biomass is considered to be the alternative resource for alcoholic fuel productionLignin content in rice straw is lower than as corn stover and wheat straw [4]. Paddy straw is an abundant agricultural waste biomass available around the world, estimated to be about 700‑800 million tons per annum [5]. The main components of lignocellulosic biomasses are cellulose (35–50 % of dry weight), hemicelluloses (25–35 %) and lignin (10–25 %) [1], additionally, the use of non-edible biomasses instead of foodstuff is desirable. By using by-products and waste materials, also sustainability, efficiency, and waste reduction can be enhanced. Molasses, starch from potato and corn have been the traditional staples for production of biobutanol in industrial scale [2]. Dilute acid hydrolysisis technique employed for the depolymerisation of hemicelluloses into xylose and other sugars. The acid hydrolysis was employed for the separation lignin and monosaccharides.

Pre-treatments are applied when a high yield of conversion into glucose is intended. The hydrolysate obtained by dilute acid pre-treatment is rich in sugars (mainly pentose) that can also be used to produce ethanol. Lignin can be used to produce energy.

MaterialsandMethods

Methods 1

Acid hydrolysis

In order to carry out A.B.E (Acetone Butanol Ethanol) fermentation using paddy straw biomass, the pretreatment of biomass was carried out using dilute orthophosphoric acid for hydrolysis. Acid hydrolysis was carried out with different diluted concentrations of 2.5, 5.0, 7.5 and 10.0% of orthophosphoric acid for the total solids percentage of biomass, 7.5, 10.0 and 12.5%. The purity of orthophosphoric acid was 85%, its molecular weight was 92g and its specific gravity was 1.82. The liquid hydrolysate (substrate) obtained from the acid hydrolysis of paddy straw of 100g sample with 2.5% concentration of Orthophosphoric acid was 950 ml. Similarly, hydrolyses for different concentrations of orthophosphoric acid, total solids and time of 60, 120 and 180 minutes at 100 °C were extracted and stored in sterile containers, to be used as substrates for fermentation.

Methods 2

Sugar analysis

For the determination of reducing sugars 0.5ml of extractive solution was taken and added with 2.5ml of water, then 3ml of DNS reagent was added to it. The solution was kept in boiling water bath for 5 minutes, then 1 ml of 40% Rochelle salt solution was added to it. The solution was cooled and the sample was added to it. The solution was cooled and the sample was analysed in a spectrometer at 510nm bandwidth. The DNS reagent was prepared with 2.5g of dinitro salicylic acid, 0.5 g of phenol, 0.125 g of sodium sulphate, and 2.5g of NaOH and was made up to 250 ml with distilled water. Rochelle salt of 40% was prepared with 40g of sodium potassium tartrate in 100 ml of distilled water.

Methods 3

Lignin analysis

ASTM D 1106-96 standard procedure [6] was adopted in the determination of lignin in biomass samples. Biomass sample of 0.3g was added to 3 ml of 72 % of H2SO4, the solution was mixed well and incubated for 60 minutes at 30°C. Then it was diluted with 84 ml of distilled water to 4% concentration. It was then autoclaved at 121°C for 1 h. The solution was then filtered and the liquid filtrate obtained was the acid soluble lignin and carbohydrates. The residue contains the acid insoluble lignin, and then it was dried until constant weight was noted after which it was placed in a muffle furnace in a silica crucible at 575±25°C. The ash obtained was weighed and the lignin was calculated using the following expressions, the oven dry weight (ODW) extractives free sample was calculated as,

$$\text{ODW} = [(\text{Weight}_{airdry}) \times (\%\ \text{Total solids})] / 100$$

The weight percent acid insoluble residue (AIR) was calculated using the following expression,

$$\%\text{AIR} = [(\text{Weight}_{(Crucible+AIR)}) - \text{Weight}_{(crucible)}] \times 100 / (\text{ODW sample})$$

Weight of the acid soluble lignin (AIL) on an extractives free basis was derived from the formula,

$$\%\text{AIL} = [(\text{Weight}_{(Crucible+AIR)}) - \text{Weight}_{(crucible)}] - [(\text{Weight}_{(Crucible+ash)}) - \text{Weight}_{(crucible)}] - [\text{Weight}_{Protein}] \times 100 / (\text{ODW sample})$$

Where, weight of protein was the amount of protein present in the acid insoluble residue. The amount of acid soluble lignin (ASL) on an extractive free basis,

$$\%\text{ASL} = \{[\text{UV}_{abs} \times \text{volume of filtrate} \times \text{dilution}] / [(\varepsilon) \times \text{ODW}_{(sample)} \times \text{Path length}]\} \times 100$$

Where, UV_{abs} reading was the absorption reading displayed in the spectrometer, the average ultraviolet visible absortance for the sample at appropriate wave length of 560 nm. ε was the absorptivity of the biomass at specific wavelength, was 25 nm and the path length was the path length of ultra violet visual cell in cm, which was 1cm. The dilution was derived as,

$$\text{Dilution} = [(\text{Volume of sample}) - (\text{Volume of diluting sample})] / (\text{Volume of sample})$$

Total amount of lignin on an extractives free basis was calculated as,

$$\%\text{Lignin}_{(Extractives\ free)} = \%\text{AIL} + \%\ \text{ASL}$$

The total lignin obtained or received from the analysis was estimated using the formula,

$$\%\text{Lignin}_{(as\ received)} = \%\text{Lignin}_{(Extractives\ free)} \times [(100 - \%\text{extractives}) / 100].$$

Results and Discussion

The proximate analysis was carried out for the paddy straw sample and the initial lignin percent in raw untreated biomass was 14%. The cellulose and hemicelluloses content in the raw biomass were 42.9% and 19.0%, respectively. The moisture content in the biomass was 6.5% and its ash content was 18.2%, reducing sugars was 34.2%. The paddy straw biomass was subjected to the different pretreatments and the results are discussed in this section. The pretreatment of paddy straw was carried out with total solids of 7.5, 10.0 and 12.5%, orthophosphoric acid concentration of 2.5, 5.0, 7.5 and 10.0%, at temperatures of 100°C for three different reaction time of 60, 120 and 180 minutes. The sugars released during the different hydrolysis the fermentable sugars released were recorded and the results are represented in this section. [Table-1] depicts the sugars released from the paddy straw biomass hydrolysis by dilute orthophosphoric acid and the corresponding lignin percentage in the hydrolysate for the four different acid concentrations, three different time periods for the total solids of 7.5% at a temperature of 100°C.

The the sugars released for the different acid concentrations at different times of treatment at 100°C. It is observed that, at 5% of the H3PO4 concentration, the maximum sugars released was 27.30 mg/g [Fig-1] at 60 min treatment at 100°C and the lowest sugar yield was observed to be 15.8 mg/g for 2.5% of acid concentration for 60 min trial at 100°C. From the figure it can be noticed that the sugar release for the acid concentration of 5% for the different time intervals of is high whereas post the increase in the acid concentration to 7.5% and above the sugars released follow a skinny pattern in which the maximum difference between the treatments were not more than 3% as it is evident in the treatment period of 60 and 120 min for the acid concentration of 5% the sugars released for were 27.30 and 26.98 mg/g. In all the trials conducted, there was an instant surge in the sugars released at 5% of acid concentration irrespective of the time of the trials, then there was drop in the sugars released at 7.5% of acid concentrations and again sugar release at the 10% improved gradually. However the maximum amount of sugar released was for the 5% acid concentration at 60 min.

Table-1 *Sugars released and lignin in acidified paddy straw hydrolysate of 7.5% total solids at 100°C*

No.	H_3PO_4 , %	Sugars, mg/g		
		60 min	120 min	180 min
1.	2.5	15.8	23.10	19.66
2.	5.0	27.3	26.98	22.66
3.	7.5	23.4	22.03	22.74
4.	10.0	25.2	22.26	23.36
No.	**H_3PO_4 , %**	**Lignin, %**		
		60min	**120min**	**180min**
1.	2.5	9.10	9.10	9.10
2.	5.0	8.48	8.48	8.48
3.	7.5	8.68	8.68	8.68
4.	10.0	8.47	8.47	8.47

[Fig-2] describes the lignin percentage in the acidified paddy straw hydrolysate (10% total solids) after the pretreatment with dil. H_3PO_4 at different time periods of treatment. It is observed that, the lignin percent in the acid concentration of 2.5% at 60 min trial was the 10.1%, which decreased to 8.32% and 6.84% when the treatment times were increased to 120 and 180 minutes. Similarly, for the 5% of the acid concentration the lignin percentage decreased from 8.26 to 6.54, then to 6.11% for the trials of 60,120 and 180 min. This similar trend was observed to be predominant for the acid concentrations of 7.5 and 10%. From the overall treatments with 10% total solids in paddy straw the lowest value of lignin recorded was 6.11% for 180 min trial with 5% H_3PO_4 concentration and the highest value of lignin 10.1% for the 60 min trial with 2.5% H_3PO_4.

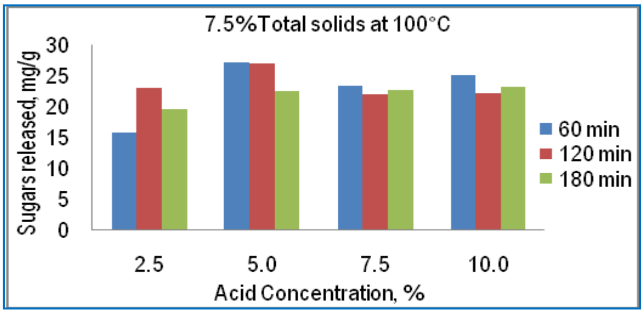

Fig-1 Fermentable sugars released from hydrolysis of paddy straw biomass with 7.5% total solids for different acid concentrations and time at 100°C

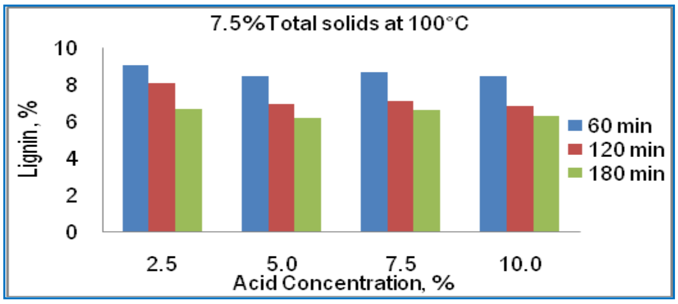

Fig-2 Lignin percent in acidified paddy straw hydrolysate with 7.5 % total solids treated with different acid concentrations and time at 100°C

The [Table-2] shows the sugars released from the paddy straw biomass hydrolysis by dilute H_3PO_4 and the corresponding lignin percentage in the hydrolysate for the four different acid concentrations, three different treatment times for the total solids of 10% at 100°C.

Table-2 *Sugars released and lignin in acidified paddy straw hydrolysate of 10% total solids at 100°C*

No.	H_3PO_4 , %	Sugars, mg/g		
		60 min	120 min	180 min
1.	2.5	19.98	25.02	18.98
2.	5.0	35.01	34.09	22.44
3.	7.5	22.99	22.03	21.07
4.	10.0	30.26	22.26	23.36
No.	**H_3PO_4 , %**	**Lignin, %**		
		60 min	**120 min**	**180 min**
1.	2.5	10.10	8.32	6.84
2.	5.0	8.26	6.54	6.11
3.	7.5	9.18	7.14	6.63
4.	10.0	8.67	7.02	6.25

The [Fig-3] represents the sugars released for the different acid concentrations at different times of treatment at 100°C. It is observed that, at 5% of the H3PO4 concentration, the maximum sugars released was 35mg/g for 60 minutes treatment at 100°C and the lowest sugar yield was observed to be 18.98 mg/g for 2.5% of acid concentration for 180 min trial at 100°C [Fig-3]. From the figure it can be noticed that the sugar release for the acid concentration of 5% for the different time intervals was greater whereas post the increase in the acid concentration to 7.5% and above the sugars released follow a weedy pattern in which the maximum difference between the treatments in the range of 2 to 3% for 60, 120 and 180 minutes trial. This similar path was observed to be prevalent for the acid concentration of 10% for 120 and 180 min trials whereas the sugars released during the 60 min treatment did not have the same traits, there was a surge in sugar level in this treatment. The sugar released in this treatment was 30.26 mg/g for the acid concentration of 10% for 60 min treatment time. It can be inferred from the graph that for the total solids of 10% the maximum sugars released was for the acid concentration of 5% for 60 min trial at 100°C and lowest value of sugars released was for 2.5% H_3PO_4 for 180 minutes trial at 100°C. [Fig-4] describes the lignin percentage in the acidified paddy straw hydrolysate (10% total solids) after the pretreatment with dilute H_3PO_4 at different time periods of treatment. From the graphical representation it can be noted that the lignin percent in the acid concentration of 2.5% at 60 min trial was the 10.1% which decreased to 8.32% and 6.84% when the treatment times were increased to 120 and 180 min. Likewise for 5% of the acid concentration the lignin percentage decreased from 8.26 to 6.54, then to 6.11% for the trials of 60,120 and 180 min. This similar trend was observed to be predominant for the acid concentrations of 7.5 and 10%. From the overall treatments with 10% total solids in paddy straw the lowest value of lignin recorded was 6.11% for 180 min trial with 5% H_3PO_4 concentration and the highest value of lignin 10.1% for the 60 min trial with 2.5% H_3PO_4.

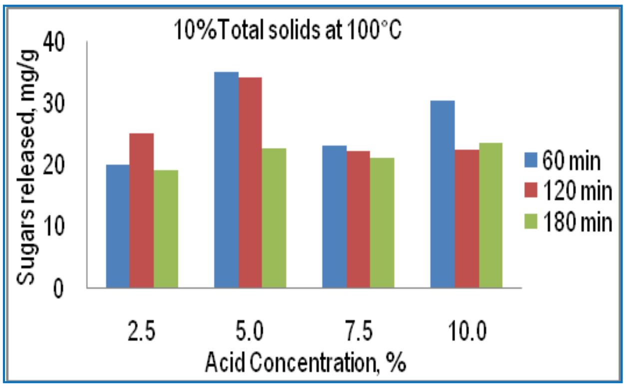

Fig-3 Fermentable sugars released from hydrolysis of paddy straw biomass with 10% total solids for different acid concentrations and time at 100°C

The [Table-3] furnishes the data of sugars and lignin in dilute H_3PO_4 hydrolysed paddy straw hydrolysate at different times for the total solids of 12.5% at 100°C.

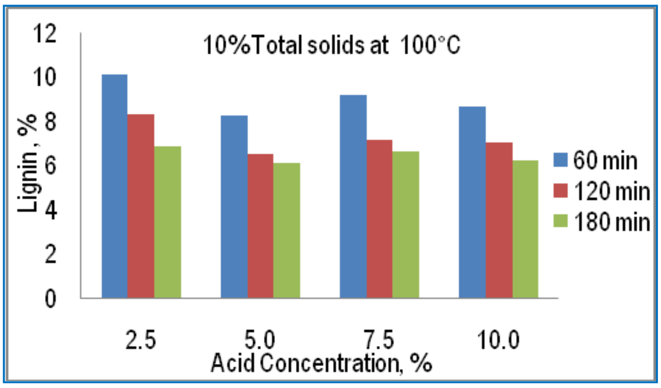

Fig-4 Lignin percent in acidified paddy straw hydrolysate with 10 % total solids treated with different acid concentrations and time at 100°C

Table-3 *Sugars released and lignin in acidified paddy straw hydrolysate of 12.5% total solids at 100°C*

No.	H_3PO_4 , %	Sugars, mg/g		
		60 min	120 min	180 min
1.	2.5	22.1	25.16	19.66
2.	5.0	35.0	34.10	22.66
3.	7.5	23.4	21.96	22.74
4.	10.0	31.5	21.78	23.02
No.	**H_3PO_4 , %**	**Lignin, %**		
		60 min	**120 min**	**180 min**
1.	2.5	9.13	8.05	5.98
2.	5.0	8.32	6.92	6.17
3.	7.5	8.55	7.08	5.91
4.	10.0	8.47	6.80	5.61

[Fig-5] exhibits the sugars released for the trials conducted with four different acid concentrations, three different times at 100°C for the total solids of 12.5%. In the graph it can be seen that the sugars released were 22.1, 35.0, 23.4 and 31.5mg/g for the orthophosphoric acid concentrations of 2.5, 5, 7.5 and 10% for the treatment time of 60 minutes at 100°C. The maximum sugars released were observed for H3PO4 concentration of 5%. The sugars released for the 120 and 180 minutes trial were 25.16, 34.10, 21.96, 21.78, 19.66, 22.66, 22.74, and 23.02 for the acid concentrations of 2.5, 5, 7.5 and 10%. Alike the previous trial of 60 minutes the maximum sugars, 34.10 mg/g was observed for the 5% acid concentration for the 120 minutes trial however the model differed for the 180 minutes trial in which 7.5 % acid concentration recorded the maximum sugar of 22.74 mg/g closely followed by 5% H3PO4 treatment with sugars of 22.66 mg/g.

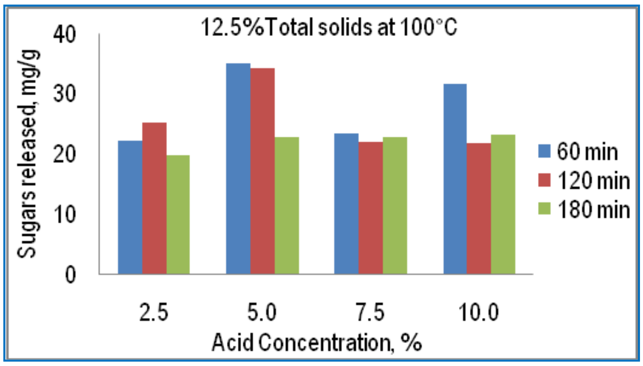

Fig-5 Fermentable sugars released from hydrolysis of paddy straw biomass with 12.5% total solids for different acid concentrations and time at 100°C

[Fig-6] demonstrates the lignin percentage in the acidified paddy straw hydrolysate treated with different concentrations of H_3PO_4 at different time periods at 121°C temperature. The curve depicting the lignin percentage after the 60 minutes trial shows that the lowest lignin percentage 8.32 was recorded for the acid concentration of 5% and the highest value of lignin was for 2.5% acid. The minimum percentage of lignin, 6.92 was recorded for the same 5% acid after 120 minutes trial and 2.5% acid treatment showed the highest lignin value of 8.05%. However, the same trend has not prevailed in the 180 minutes trial where the acid concentration of 10% showed the lowest value of lignin, 5.61% and the maximal lignin presence (6.17) was observed in 5% acid hydrolysed sample. There was a sudden drop in the lignin percentage during the trial of 180 minutes where the lignin percentage for all the trials fell to the lowest value recorded for the total solids of 12.5% at 121°C.

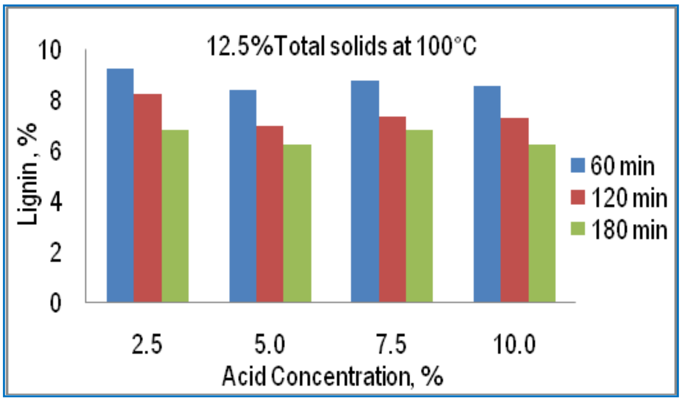

Fig-6 Lignin percent in acidified paddy straw hydrolysate with 12.5% total solids for different acid concentrations and time at 100°C

The percent lignin reduction in acidified paddy straw hydrolysate treated with 2.5, 5.0, 7.5 and 10% concentration of Orthophosphoric acid, for 60, 120 and 180 minutes at a temperature of 100 and 121°C with total solids percentage of 7.5, 10.0 and 12.5% was calculated. The percent of lignin in raw untreated paddy straw biomass was 14%. The percentage of lignin after each treatment was analysed and the percent lignin reduction was also found. The percentage lignin reductions for the different treatments carried out at 100°C are furnished in the [Tables-4, 5 & 6].

Table-4 *Percent lignin reduction at 100°C with total solids of 7.5%*

No.	H_3PO_4, %	% lignin reduction at 100°C with total solids of 7.5%		
		60 min	120 min	180 min
1.	2.5	35.00	42.00	52.00
2.	5.0	39.43	50.14	55.50
3.	7.5	38.00	49.00	52.57
4.	10.0	39.50	51.00	55.00

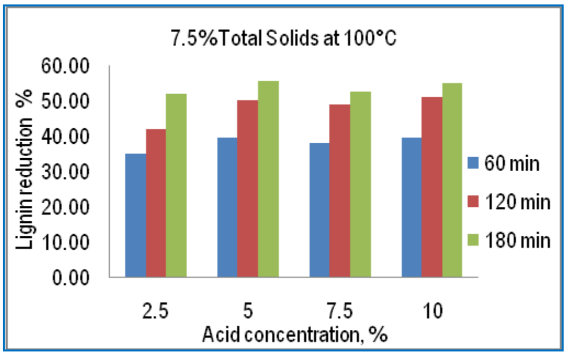

Fig-7 Percent lignin reduction in acidified paddy straw hydrolysate with 7.5% total solids for different acid concentrations and time at 100°C

The [Fig-7] shows the percent lignin reduction in the acidified paddy straw treated with 2.5%, 5, 7.5 and 10% H_3PO_4 at 100°C for 60, 120 and 180 min with 7.5 total solids. From the figure it can be noted that at the minimum percentage of lignin reduction, 35, 42 and 52% was during the treatment with 2.5% acid for all the trial periods of 60, 120 and 180 min at 100°C. The maximum reduction of percent lignin was during the 60 and 120 trial for the acid concentration of 10% while the highest percent of lignin reduction for 180 min trial was observed for 5% acid concentration, the corresponding values of the highest percent reduction of lignin were 39.50, 51 and 55.50% respectively. It can be deduced from the graph that the maximum percentage of lignin reduction achieved was 55.50% for 5% H_3PO_4 concentration during 180 min trial conducted at 100°C and the 35% lignin being the lowest value for 2.5% acid treatment at 60 minutes trial in 100°C.

Table-5 *Percent lignin reduction at 100°C with total solids of 10%*

No.	H_3PO_4, %	% lignin reduction at 100°C with total solids of 7.5%		
		60 min	120 min	180 min
1.	2.5	27.86	40.57	51.14
2.	5.0	41.00	53.29	56.36
3.	7.5	34.43	49.00	52.64
4.	10.0	38.07	49.86	55.36

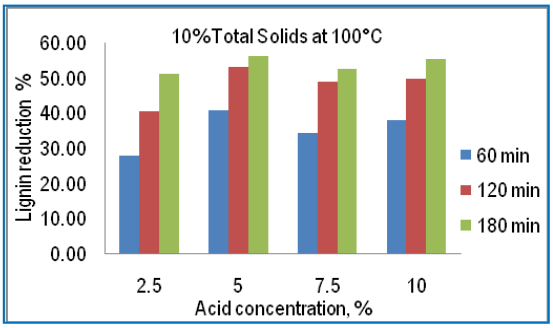

Fig-8 Percent lignin reduction in acidified paddy straw hydrolysate with 10% total solids for different acid concentrations and time at 100°C

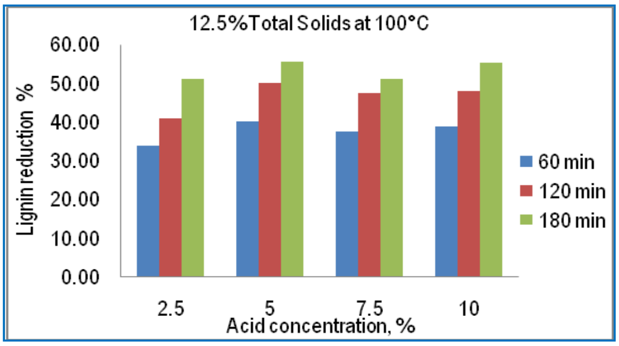

Fig-9 Percent lignin reduction in acidified paddy straw hydrolysate with 12.5% total solids for different acid concentrations and time at 100°C

From the [Fig-8], it can be depicted that the maximum percent of lignin reduction was 41.00, 53.29 and 56.36% for the acid concentrations of 5% irrespective of the time of treatment at 100°C with the substrate having total solids of 10 percent. The lowest values of percent lignin reduction were 27.86, 40.57 and 51.14% for the pretreatment with 2.5% acid concentration. It was deduced from the figure that the maximum and minimum percent of lignin reduction was noted for the 5 and 2.5% acid concentration irrespective of the time period of treatment for the paddy straw with 10% total solids at 100°C.

Table-6 *Percent lignin reduction at 100°C with total solids of 12.5%*

No.	H_3PO_4, %	% lignin reduction at 100°C with total solids of 7.5%		
		60 min	120 min	180 min
1.	2.5	33.86	40.86	51.21
2.	5.0	40.07	50.14	55.43
3.	7.5	37.43	47.57	51.14
4.	10.0	38.93	48.00	55.29

The [Fig-9] illustrates that, the highest percentage of lignin reduction in the pretreated paddy straw with 12.5% total solids was for the H_3PO_4 concentration of 5% for all the treatment times of 60, 120 and 180 minutes. The highest percent of lignin reduction were 40.07, 50.14 and 55.43%. The lowest percent reduction of lignin was found to be 33.86 and 40.86% for 2.5% H_3PO_4 for 60 and 120 minutes treatment at 100°C. While during the 180 minutes trial the lowest percent reduction of lignin, 51.14% was calculated for 10% acid concentration.

From the acid pre-treatments conducted at 100°C for the different total solids percentage, the maximum percent lignin reduction was observed for treatment with 5% acid when the total solids were 10 and 12.5% irrespective of the treatment period for 100°C. However, this trend was observed for only during the 180 min trial with 5% acid conc. when the highest percent reduction in lignin of 55.50% was worked out with total solids of 7.5%. This was closely followed by 51 and 39% of lignin reduction during 60 and 120 min treatment with acid concentration of 10%. From these set of data it can be inferred that the maximum lignin reduction percentage of 56.36% was for the total solids of 10% for the acid concentration of 5% for 180 min trial period, which could be rated as the most efficient lignin removal treatment combination among the all acid hydrolysis trials conducted at 100°C. The maximum lignin removal does not assure a maximum sugar conversion ratio. The reduction in sugars formation is attributed to an increase in the acid concentration during acid hydrolysis. Loss of fermentable sugars occurred even though the lignin degradation was higher at higher acid concentration and treatment time. The sugars released during the same treatment endorsing maximum lignin reduction percentage did not give rise to an increase in sugars, but the sugars were much lower than other treatments. This affirmed that along with an increase in lignin degradation with increase in acid concentration and time of treatment did not support a rise in fermentable sugar levels instead there was loss of sugars when compared to milder acid concentration treatments. The amount of solvents produced from the bioconversion of sugars released from the acid hydrolysis in acetone butanol ethanol fermentation are yielded 13g of acetone, 8g of butanol and 7g of ethanol for every 100g of sugars.

Conclusion

The lignin presence was analysed adhering to the ASTM D1106-96 standard [6]. The initial lignin content in the paddy straw biomass was 14%, the lowest acid insoluble lignin percentage was observed for the 5% acid hydrolysis for 180 min trial in all the cases, irrespective of the total solids percent in the substrate. The sugars released were found to be greater for the same 5% acid concentration but for the trial time of 60 min. Similar results were observed during the 60 min trials. The minimum percentage of lignin in the substrate was 6.23, 6.11 and 6.24 for 5% acid treatment at 100°C with biomass total solids of 7.5, 10.0 and 12.5 % for 180 min. Their corresponding sugar released were 22.66, 22.44 and 22.66 mg/g which was deficient in the range of 5 to 13mg of the maximal sugars released during 60 min trials, which yielded maximum sugar release. The lignin percent in the trials yielding the maximum sugars of 27.3,35 and 35 mg/g was 8.48, 8.26 and 8.33 mg/g. The difference in the lignin percentage was 2.28, 2.15 and 2.09 which was much lower when equated with the difference in sugars released, hence the maximum sugar yielding 60 minutes trial for the acid concentration of 5% for the biomass with total solids of 10 and 12.5% was adopted as carbon source for acetone butanol ethanol (A.B.E) fermentation.

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Conflict of Interest: None declared

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