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Effect of Potassium Hydroxide (KOH) Pretreatment on Solids Recovery, Delignification and Total Sugars of Cotton Stalk

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ABSTRACT

Bioethanol is one of the promising alternative fuels to gasoline in the transport sector. Ethanol may be produced either from petroleum products or from biomass. In the world around 80% of the ethanol produced is still obtained from fermentation and the reminder comes largely by synthesis from the petroleum product ethylene (Tanaka, 2006). Cotton stalks have been regarded as potential sources for cellulosic ethanol production owing to their high cellulose content being almost 37% (Binod *et al.,* 2012). Hence, there is a need of utilizing available biomass resources for production of ethanol to reach energy demand. Compounding the challenges is the fact that the country lacks mature technologies for ethanol production from lignocellulosic biomass which is by far the most abundant renewable resource that may be exploited (Lynd *et al.,* 2002; Zhang, 2008). The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. This study is aimed at investigating the effect of potassium hydroxide (KOH) on cotton stalks for optimization of pre-treatment conditions on solids recovery, delignification and total sugars. Potassium hydroxide (KOH) pre-treatment was performed at 50 °C, 70 °C with residence times of 6, 12, and 24 h and 120 °C with residence times of 0.25, 0.5 and 1 h each. All the temperature–time pretreatment combinations were performed with potassium hydroxide (KOH) concentrations of 1, 2 and 3%. Solid recoveries, AIL, ASL, total sugars, cellulose and hemicelluloses content after each pretreatment ranged between 49.60-85.04%, 10.19-23.96%, 1.51-1.95%, 268.01-419.51(mg/g dry biomass), 33.00-53.54% and 1.17-14.04% respectively. Maximum lignin reductions at different temperatures were all obtained at the combinations of highest KOH concentrations and longest treatment times, which indicated a close relationship between pretreatment severity and lignin reduction.

Introduction

Currently the world is mostly dependent on fossil fuels for meeting its energy demand and more than 80% of the total global energy is obtained by burning fossil fuels, of which 58% alone is consumed by the transport sector. Rapid increase in the consumption of all kinds of fossil fuels due to the growing industrialization and motorization of the world has resulted in fast depletion of these non-renewable fuels. The limited reserves of the fossil fuels have been anticipated to be exhausted by the next 40-50 years and fossil fuels has the contribution to greenhouse gas emissions and global warming that cause climate change, rise in sea level and loss of biodiversity and urban pollution (Anon, 2013). Therefore, it is necessary to find out an alternative energy source for our industrial economies and consumer societies by using renewable, sustainable, efficient and cost effective feedstocks with lesser emission of greenhouse gases, where bioethanol would be an attractive alternative option due to its ease of production.

Ligno cellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose, and lignin. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin. They have been considered as alternative energy sources because they can capture CO_2 during their growth so that their combustion does not generate net CO_2. Cellulose and hemicellulose are polysaccharides that can be used for ethanol production, while lignin is a complex aromatic polymer that stiffens and surrounds the fibres of polysaccharides.

Cotton is a major commercial crop grown in almost all the Agro-climatic zones of Karnataka. All the four cultivated species of cotton *viz., Gossypium arboreum, G. herbaceum, G. barbadense,* and *G. hirsutum* are grown in the state where in *Gossypium hirsutum* has the major share of the hybrid cotton grown. Cotton stalk is one of the important by products of cotton crop and about 23 million tonnes of cotton plant stalks are generated in India annually. On an average about 2 to 3 tonnes of stalk are generated in one hectare of land. Most of the stalk is treated as waste, though a small part of it (15%) is used as fuel. The lignocellulosic nature of cotton stalk favours to use as renewable material for variety of commercial applications. In conventional method, bulk of the stalk is burnt in the fields after the harvest of the cotton crop although it is not desirable since it causes air pollution. Hence, it is required to utilize cotton stalk for ethanol production instead of burning in the field.

The conversion of lingo cellulosic biomass to ethanol is more challenging. The process of ethanol production from lingocellulosic biomass constitutes three stages: (a) pretreatment of biomass to reduce lignin content and cellulose crystallinity, (b) hydrolysis of pretreated biomass for sugar generation, and (c) fermentation of sugars into ethanol.

Pretreatment of biomass has been found to change its macromolecular structure and increase surface area and pore size, making it conducive for hydrolytic enzymes to attach themselves to the carbohydrate matrix for generating sugars, which are subsequently converted to ethanol through bacterial or yeast fermentation. Pretreatment can be divided into three main categories: (a) physical, (b) chemical, and (c) biological. Physical pretreatment processes have proven to be energetically unviable and biological pretreatment methods can be expensive and time consuming. Chemical pretreatment techniques on the other hands have been the most widely studied and alkaline pretreatment in particular has seen considerable success (Vijayakumar *et al.*, 2016).

Silverstein *et al.,* (2007) investigated chemical pretreatment of cotton stalks and reported that, among four pretreatment methods (NaOH, H_2SO_4, H_2O_2, and ozone pretreatments), NaOH pretreatment resulted in the highest level of delignification (65.63% at 2% NaOH, 90 min, 121 °C) and cellulose conversion (60.8%).

Shi *et al.,* (2009) studied the fungus, *Phanerochaete chrysosporium,* to pretreat cotton stalks with two methods, shallow stationary and agitated cultivation, at three supplemental salt concentrations. Pretreatment efficiencies were compared by evaluating lignin degradation, solid recovery and carbohydrate availability over a 14-day period. Shallow stationary cultivation with no salts gave 20.7% lignin degradation along with 76.3% solid recovery and 29.0% carbohydrate availability. The highest lignin degradation of 33.9% at a corresponding solid recovery and carbohydrate availability of 67.8% and 18.4%, respectively, was obtained through agitated cultivation with Modified NREL salts.

Keshav *et al.,* (2016) reported that cotton stalk pretreated to steam explosion in the range 170-200 °C for 5 min. Steam explosion at 200 °C and 5 min led to significant hemicellulose solubilization (71.90±0.10%). Alkaline extraction of steam exploded cotton stalk (SECOH) using 3% NaOH at room temperature for 6 h led to 85.07±1.43% lignin removal with complete hemicellulose solubilization. Besides, this combined pretreatment allowed a high recovery of the cellulosic fraction from the biomass. Enzymatic saccharification was studied between steam exploded cotton stalk (SECS) and SECOH using different cellulase loadings. SECOH gave a maximum of 785.30±8.28 mg/g reducing sugars with saccharification efficiency of 82.13±0.72%. Subsequently, fermentation of SECOH hydrolysate containing sugars (68.20±1.16 g/l) with *Saccharomyces cerevisiae* produced 23.17±0.84 g/l ethanol with 0.44 g/g yield.

Potassium hydroxide is a relatively less explored pretreatment agent but could potentially be used for lignocellulose pretreatment due to its reported reactivity with carbon nanofibers and carbon nanostructures and its ability to deacetylate biomass.

Keeping this in view, the effect of alkaline pretreatment conditions on delignification and total sugar content of cotton stalks was investigated.

Materials and Methods

Cotton stalk is selected as raw material in this study for testing its feasibility for production of cellulosic ethanol. The cotton stalks available in the University of Agricultural Sciences, Raichur campus was selected. The cotton stalks were cut into small chips and oven dried at 70 °C in a forced air oven for 72 h. Then oven dried samples were ground to pass through a 2 mm sieve in a hammer mill and stored at room temperature in zip-locked plastic bags, and used for further experimentation.

The initial composition of cotton stalk was analyzed using Laboratory Analytical Procedures (LAP) adopted by National Renewable Energy Laboratory (NREL) for the measurement of total solids, acid insoluble lignin (AIL), acid soluble lignin (ASL) and ash (Sluiter *et al.*, 2005a, 2005b; Sluiter *et al.*, 2008). While the structural carbohydrates (cellulose and hemicelluloses) represented by total reducing sugars of biomass was estimated by the 3, 5-dinitrosalycylic acid (DNS) method (Ghose, 1987; Miller, 1959). Whereas, the crude protein, crude fibre and crude fat present in cotton stalk were estimated by the AOAC method (AOAC, 2005). Cellulose was estimated by standard cellulose solution methods and hemicellulose was estimated by determining (ADF) acid detergent fibre and (NDF) neutral detergent fibre.

In this experiment, potassium hydroxide (KOH) pretreatment of cotton stalk at different elevated temperatures ranging from 50 to 120 °C with various combinations of residence times and KOH concentrations was explored. Pretreatment of cotton stalk samples were performed at 50, 70 with residence times of 6, 12, and 24 h and 120 °C with residence times of 0.25, 0.5, and 1 h each. All the temperature–time pretreatment combinations were performed with potassium hydroxide (KOH) concentrations of 1, 2 and 3 per cent (w/v).

Five grams of cotton stalk sample was mixed with 50 ml of KOH solution in 125 ml bottles using glass rods, and the bottles were sealed before pretreatment and kept in a water bath (Plate 1) and autoclave (Plate 2).

The pretreated samples were filtered through pre-weighed filter paper in vacuum flask using a vacuum pump. The bottles were rinsed with 50 ml DI water to recover the residual solids. All solids accumulated on the filter papers in the filtration set up were quantified by oven drying and considered in solid recovery calculations.

5 g of wet biomass was drawn from each pretreated sample and dried at 105 °C in conventional hot air oven for estimation of solid recovery. A similar amount was placed for vacuum drying at 40 °C in vacuum oven to obtain samples for estimation of acid insoluble lignin (AIL), acid soluble lignin (ASL), total sugar, cellulose and hemicellulose content to study the effect of pretreatment conditions on cotton stalk. All treatments in this study were conducted in triplicate.

Results and Discussion

Composition of cotton stalk

The initial composition of cotton stalk used in this study is presented in Table 1. The carbohydrate (total reducing sugars) of cotton stalk was estimated to be 58.66%. Cellulose and hemicelluloses were 42.63% and 16.03% respectively. Total lignin (including AIL and ASL), which is the major non-carbohydrate component, was determined to be 32.72 %, ash and total solids were estimated to be 5.56% and 92.08% respectively. The crude fibre, fat and protein of sample were observed to be 49.71%, 0.9% and 1.7% respectively.

Effect of pretreatment conditions

Pretreatment conditions had varying effects on solid recovery, acid insoluble lignin and acid soluble lignin, total sugar, cellulose and hemicellulose in the biomass. Intensity of treatment increased with increasing KOH concentration and treatment temperature.

Solid recovery

On an average, solid recoveries after pretreatment at different temperature-time combinations using various concentrations of KOH ranged between 64.85 and 85.04% at 50°C, 59.13-81.29% at 70°C, and 68.59-49.6% at 120°C (Table 2).

It was observed that the maximum solids of 85.04% in the sample pretreated at 50 °C, 6 h combination with 1% KOH, whereas, it was minimum 49.60% in the sample pretreated with 3% KOH at 120 °C, 1 h. It was observed that lesser solids were recovered as intensity of the pretreatment increased.

Acid insoluble lignin

Acid insoluble lignin after pretreatment at different temperature-time combinations using various concentrations of KOH ranged between 16.1-23.96% at 50 °C (Fig. 1), 14.1-20.25% at 70 °C (Fig. 2) and 10.19-16.8% at 120 °C (Fig. 3). The maximum acid insoluble lignin of 23.96% was recorded in the sample pretreated at 50 °C, 6 h combination with 1% KOH, whereas, it was minimum (10.19 %) in the sample pretreated with 3% KOH at 120 °C, 1 h.

It was observed that increasing pretreatment, a decrease in acid insoluble lignin in all the samples with three concentrations of KOH loaded at all the temperature-time combinations were recorded.

Acid soluble lignin

After KOH pretreatment, acid soluble lignin at different temperature-time combinations using various concentrations of KOH ranged between 1.54-1.79% at 50 °C, 1.56-1.83% at 70 °C and 1.56-1.95% at 120 °C.

It was observed that acid soluble lignin increased up to 2% KOH concentration at higher temperature-time combinations.

Total sugar content

On an average, total sugar content ranged between 268.01-419.51 mg/g dry biomass (Table 3) after pretreatment at different temperature-time combinations using various concentrations of KOH.

It was observed that when the sample was pretreated with different concentrations of KOH, at 50, 70 and 120 °C, the release in sugar increased with increase in acid concentration upto 2% (v/v) KOH and it declined thereafter.

The maximum sugars of 419.51 mg/g were released, when the sample was pretreated with 2% KOH concentration at 120 °C for 1 h. Whereas, it was minimum (268.01%) in the sample pretreated at 50 °C, 6 h combination with 1% KOH.

Cellulose content

The results of cellulose content ranged between 29.02-48.54% after pretreatment at different temperature-time combinations using various concentrations of KOH (Table 4).

The maximum cellulose content of 48.54% was observed in the sample pretreated at 120 °C, 1 h combination with 2% KOH, whereas, it was minimum (29.02%) in the sample pretreated with 1% KOH at 50 °C, 6 h.

It was observed that when the sample was pretreated at 50, 70 and 120 °C with different concentrations of KOH, the release in cellulose increased with increase in akali concentration upto 2% (v/v) KOH and it declined thereafter by increasing concentration.

Hemicellulose content

Hemicellulose content ranged between 1.17-14.04% after pretreatment at different temperature-time combinations using various concentrations of KOH.

After KOH pretreatment, hemicelluloses content ranged between 8.34-14.04% at 50 °C (Fig. 4), 4.93-8.96% at 70 °C (Fig. 5), and 1.17-5.19% at 120 °C (Fig. 6).

The maximum hemicellulose content of 14.04% was observed in the sample pretreated at 50 °C, 6 h combination with 3% KOH, whereas, it was minimum (1.17%) in the sample pretreated with 2% KOH at 120 °C, 1 h.

Table.1 Composition of cotton stalk

Sl. No.	Component	Dry weight (%)
1	Total solids	92.08
2	Acid insoluble lignin	30.71
3	Acid soluble lignin	2.01
4	Total sugars	58.66
5	Cellulose	42.63
6	Hemicellulose	16.03
7	Ash	5.56
8	Crude fiber	49.71
9	Crude fat	0.9
10	Crude protein	1.7

Table.2 Effect of potassium hydroxide pretreatment on solids recovery of cotton stalk

Temperature (°C)	Time, h	Solids recovery (%)		
		KOH concentration (%)		
		1	**2**	**3**
50	6	85.04	82.89	79.36
	12	80.07	74.97	72.98
	24	79.14	68.95	64.85
70	6	81.29	74.58	69.46
	12	78.59	73.4	66.81
	24	74.89	64.85	59.13
120	0.25	68.59	63.85	61.59
	0.5	63.38	58.53	54.23
	1	59.01	51.71	49.6

Table.3 Effect of potassium hydroxide (KOH) pretreatment on total sugars of cotton stalk

Temperature (°C)	Time, h	Total sugars (mg/g dry biomass)		
		KOH concentration (%)		
		1	2	3
50	6	268.01	303.46	293.10
	12	323.16	354.71	349.64
	24	353.18	365.56	357.20
70	6	343.78	356.11	353.60
	12	359.68	367.37	363.46
	24	363.60	373.29	367.38
120	0.25	376.98	393.47	382.63
	0.5	386.99	403.84	399.69
	1	402.58	419.51	409.79

Table.4 Effect of potassium hydroxide (KOH) pretreatment on cellulose of cotton stalk

Temperature (°C)	Time, h	Cellulose (%)		
		KOH concentration (%)		
		1	2	3
50	6	35.02	36.91	33.00
	12	37.26	39.77	35.12
	24	38.60	40.34	36.35
70	6	39.62	41.51	37.61
	12	42.61	45.98	41.67
	24	44.85	47.84	42.52
120	0.25	46.48	49.96	45.44
	0.5	48.78	51.93	47.51
	1	50.42	53.54	49.67

Fig.1 Acid insoluble lignin of cotton stalk pretreated with 1.0–3.0% KOH at 50 °C

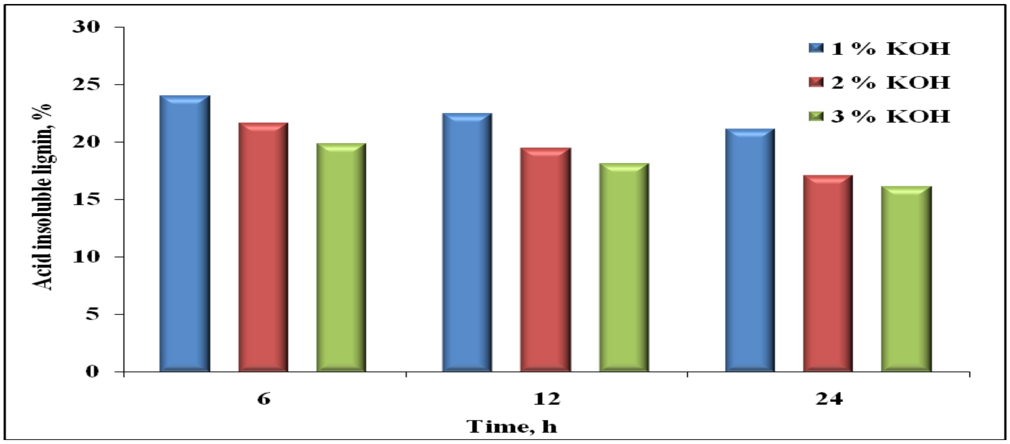

Fig.2 Acid insoluble lignin of cotton stalk pretreated with 1.0–3.0% KOH at 70 °C

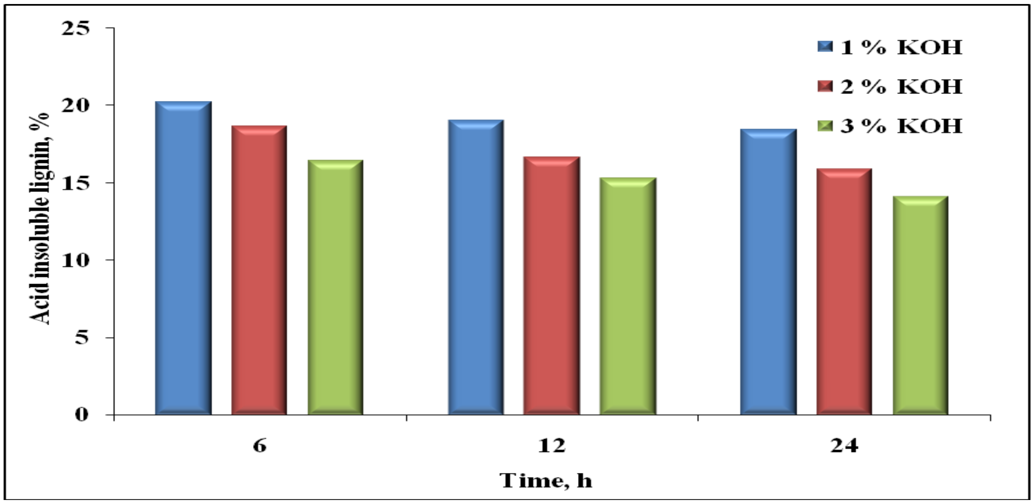

Fig.3 Acid insoluble lignin of cotton stalk pretreated with 1.0–3.0% KOH at 120 °C

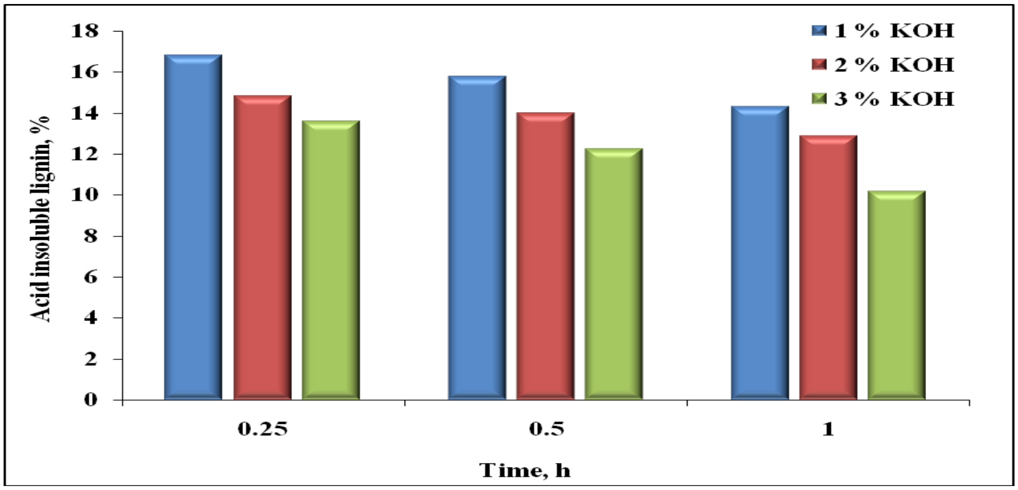

Fig.4 Hemicellulose content of cotton stalk pretreated with 1.0–3.0% KOH at 50 °C

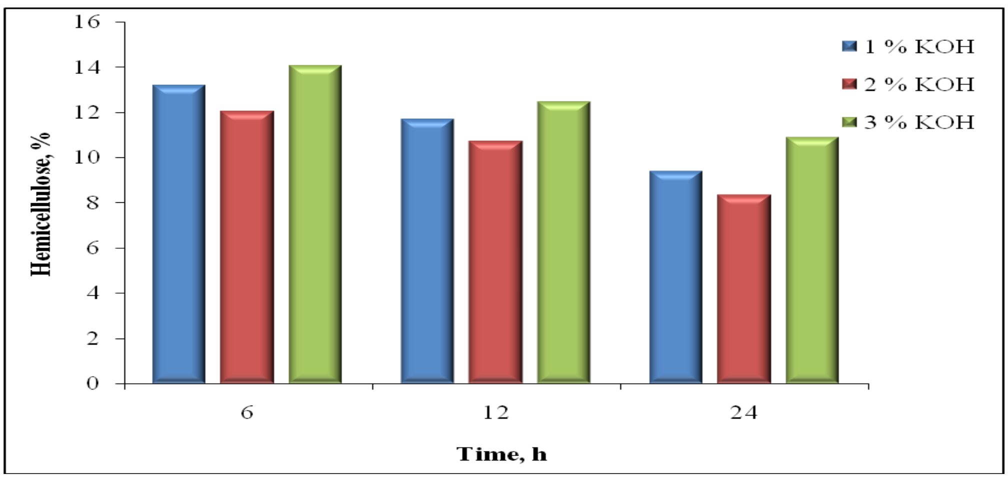

Fig.5 Hemicellulose content of cotton stalk pretreated with 1.0–3.0% KOH at 70 °C

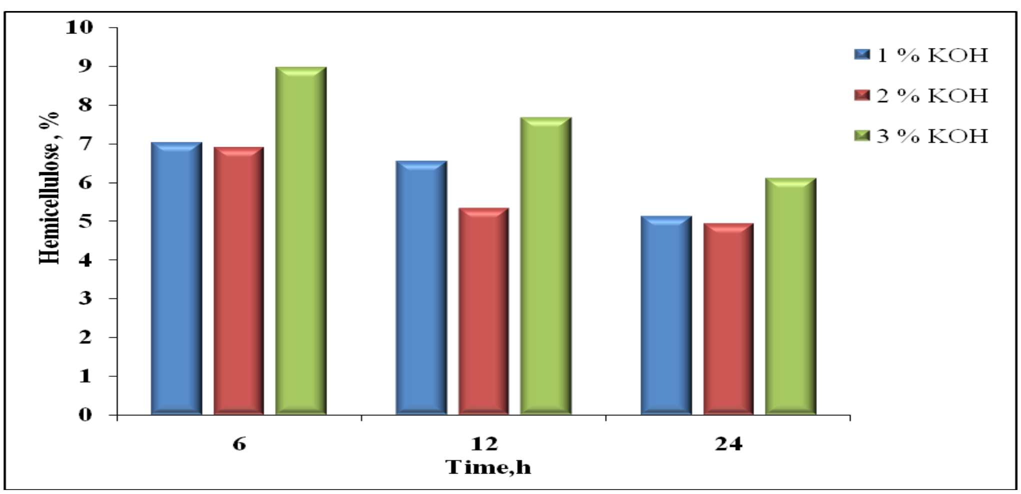

Fig.6 Hemicellulose content of cotton stalk pretreated with 1.0–3.0% KOH at 120 °C

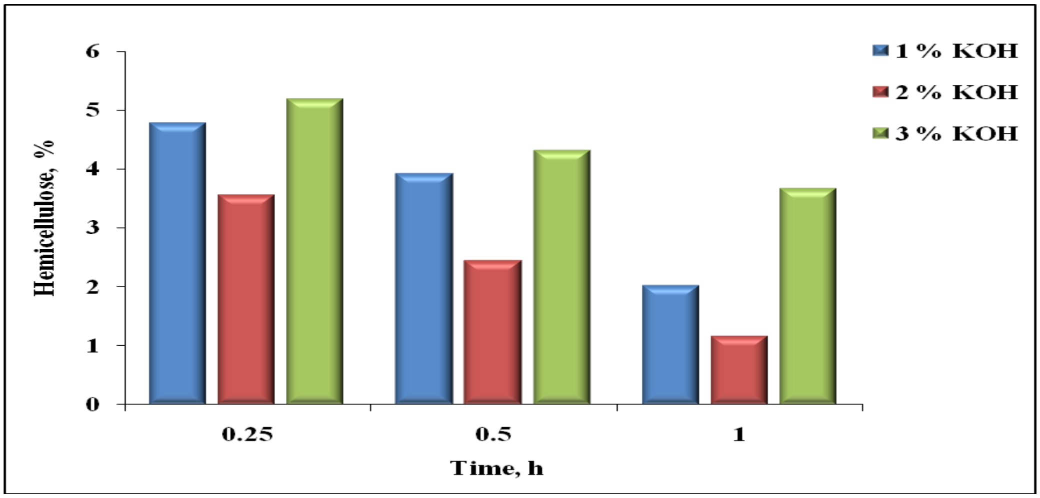

Plate.1 Samples kept in water bath for pretreatment

Plate.2 Pretreatment using fully automatic horizontal autoclave

In conclusion, pretreatment of ground cotton stalk with KOH resulted in high sugar yield with the 2% KOH, 1 h, 120 °C pretreatment indicates that even low concentrations of KOH can be effective in generating high sugars during hydrolysis. The highest carbohydrate retention of 71.51% was observed in the sample pretreated at 120 °C with 2% KOH and 1 h. Almost 53.95% of solids were dissolved at 120 °C after 1 h pretreatment with 3% KOH concentration. The corresponding maximum lignin reductions of 50.79%, 56.91% for 50, 70 °C at 24 h, 3% KOH respectively and 68.86% were obtained at 120 °C for 1 h, 3% KOH concentrations. Pretreatment with potassium hydroxide was able to degrade lignin in cotton stalks. Maximum lignin reductions at different temperatures were all obtained at the combinations of highest KOH concentrations and longest treatment times, which indicated a close relationship between pretreatment severity and lignin reduction.

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