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RESEARCH ARTICLE

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OPTIMIZING THE EFFECT OF CHEMICAL PRETREATMENT ON LIGNOCELLULOSIC PROPERTIES OF WHEAT STRAW

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ABSTRACT

The current research was postured for optimizing the pretreatment of wheat straw with three different chemicals i.e., 1% NaOH at 121°C for 1 hour, 4% lime at 80°C for 40 minutes, similarly 4% acetic acid at 121°C for 1 hour and studying their effect on lingo cellulosic structure of wheat straw. It was observed that NaOH treatment was most suitable maximum degradation of lignin and least disruption of cellulosic contents. NaOH pretreatment was optimized for three main process parameters i.e., temperature, pretreatment time and chemical concentration. It was analyzed that at same concentration by varying treatment time and temperature, hemicelluloses and lignin contents decreased with the increase of both parameters. Concentration of chemical was optimized at boiling temperature of 105°C for 10 minutes, revealed that with the increase in NaOH concentration lignin and hemicelluloses contents degrade more. Time parameter was optimized applying treatment of 2, 5 and 8 days. Lignin degradation was highest at 8% NaOH concentration for 2 days. Lignin contents increased abruptly at 8% NaOH and 8 days of treatment. Optimum conditions obtained for cellulose and hemicellulose are 2% NaOH, 105°C for 10 minutes.

KEYWORDS

Wheat Straw, Chemical Pretreatment, Lignin degradation, optimization.

1. Introduction

Lignocellulosic biomass is one of the most suitable recourse for biogas production because it is easily and abundantly available and also inexpensive. Lignocellulosic material has wide range of uses i.e., cellulose received from lignocelluloses is used in textile industry for fiber making and also widely used in fulfilling world's increasing energy demands. Hemicellulose another main constituent is used in pharmacy and edible items. Third important constituent is lignin, has its use in low cost energy production. Lignocellulosic material obtains from wheat straw is one of the most acceptable options for low cost energy generation. It is estimated that out of total cultured land in the world, one sixth portion of the land is cultivated for wheat. It grows easily and expeditiously in coarse land (Haque et al., 2012). Remain of wheat crop after removal of chaff and grain is a dry stalk named as wheat straw.

Moreover, it is the second largest agricultural remain, making it one of the most available raw material for different products, and it is an amazing feedstock for biogas production because it has reduced our need to use fossil fuels and reduced the emission of greenhouse gasses (Caesar, 2011). With estimation 21% of the world's food demand is based on wheat crop, meanwhile with the growing human population demand for the growth of wheat crop is increasing side by side.

Lignocellulosic biomass has involved and complex structure hunged by cellulose, hemicelluloses and lignin. These three essential components form approximately 90% of total formation of lignocellulosic material (Wu et al., 2014). Wheat straw contains high amount of cellulose,

hemicelluloses and lignin i.e., 34-40%, 14-18% cellulose, 26-35% hemicelluloses and 4.93% extractives. Protective sheet of lignin against plant cell wall provides major hindrance during enzymatic hydrolysis involved in the process of biogas formation. There is severe need of breakdown of hemicelluloses and cellulose into their smallest units and such form in which they are easily degradable and accessible by microorganisms. Extraction of digestible material from lignocellulosic materials can be achieved by chemical pre-treatment by employing various acids and base for efficient digestion of lignin and hemi-cellulose thus enhances rate of energy production.

These treatments have varied residence time, some pre-treatment takes short time but some takes several hours or days. Optimization of temperature is crucial in all chemical pre-treatments. All chemical treatments require high temperature except ammonia and lime; they can perform at low temperature and longer residence time. NaOH is stronger base as compared to lime, so can perform more efficiently at low temperature. Proper concentration of chemical is another important parameter to gain higher energy yield at boiling temperature and for fixed residence time. By differing the concentration of chemicals morphological characteristics of wheat biomass can be altered. To utilize agricultural waste in the shape of wheat straw and evolve a method for successive detachment of lignin, cellulose and hemicelluloses, for the rehabilitation of complex mass hemicelluloses. Keeping view the above discussion following research is aimed on optimizing the best chemical pretreatment for lignin degradation keeping in view the effect on cellulose and hemi cellulose found in wheat straw.

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2. MATERIALS AND METHODS

2.1 Wheat Straw collection

Wheat straw was taken from biogas plant of University of Agriculture Faisalabad. Collected wheat was cleaned by removing foreign particles like, stones, dirt and other straws. It was then cut into smaller pieces of size 5-10mm by a local chopper, air dried and stored in plastic bags.

2.2 Chemical Pretreatments

Three different chemical pre-treatments were applied separately to analyze the best suitable treatment.

2.3 Sodium Hydroxide treatment

1% NaOH solution was formed by adding 10g NaOH in 1000ml of water, mixed property with a strrir untill solution formed.10 g of wheat straw is weighted using electrical balance. 100ml NaOH solution was taken in 500 ml beaked and weighted weight straw was added in it and then mixed properly with a glass rod.Beaker was covered with plastic sheet to avoid enterance of air in the beaker and placed in oven for 1.5 hours at a temperature of 121°C. After 1.5 hour sample was taken out and washed with warm distilled water for 5-6 times untill washed water became clear. The sustrate was then allowed to stand for a while to dry.It was then placed in washed and cleaned china dish and kept again in oven to dry at temperature of 105°C until it gained constant weight. Sample was then taken out and put in dessicator for almost 15 minutes to absorb moisture if any.This sample was then stored in sealed plastic bags.Two more samples having same weight were treated by the same procedure reported above.

2.4 Lime water treatment

Lime water was treated with 10g of wheat straw at 80°C with residence time of 40 minutes. 100ml lime water was taken in 500ml beaker and then stirred properly using a glass, 10 g weighted wheat straw was added in it. It was then stirred properly using a glass rod. This beaker was then covered with plastic sheet to avoid entrance of air in beaker. Beaker is labeled and placed in electric oven at a temperature of 80°C at residence time of 40 minutes. After 40 minutes sample was taken out and washed with hot distilled water until washed water became colorless. The substrate was then strained using strainer and put aside for few minutes to dry. Substrate was then placed in washed and clean china dish and put in oven at temperature of 105°C until constant weight was gained. This was then placed in desiccators to absorb extra moisture if any. Two more sample of the same weight were treated by the same procedure and then stored in sealed plastic bags.

2.5 Acetic acid treatment

10 g of wheat straw was treated with 4% acetic acid at 121°C for residence time of 1hour. Substrate was soaked with 4% acetic acid having wheat to liquid ratio of 1:20. 200ml of 4% acetic acid is taken in 500ml beaker and 10g wheat straw is added in it. The mixer was stirred well using a glass rod. The beaker was then covered with plastic cover to avoid entrance of air in beaker. Beaker was labeled and placed in electric oven at a temperature of 121°C at residence time of 60 minutes. After 60 minutes sample was taken out and washed with hot distilled water until washed water became colorless. The substrate is then strained using strainer and put aside for few minutes to dry. Substrate was then placed in washed and clean china dish and put in oven at temperature of 105°C until constant weight is gained. This was then placed in desiccators to absorb extra moisture if any. Two more sample of the same weight were treated by the same procedure and then stored in sealed plastic bags.

2.6 Determination of hemicelluloses concentration

Hemicellulose concentration was determined using NDF and ADF analyzing methods.

2.6.1 Preparation of NDF solution

NDF solution of made using 1000ml of distilled water,18.6 g Disodium EDTA, 5.5 g disodium hydrogen phosphate,30g sodium laurul sulphate,7 g sodium tetra borate. The solution was mixed well by glass rod on hot plate. 1g wheat straw was taken in a conical flask; 0.5 g sodium sulphite was weighted and added in flak. In the same flask 100ml NDF solution was added stirred by using glass rod. Flask was covered by using lid and placed on a hot plate. Bubbles formed in the flask were continuously removed by opening the lid after some time, the sample was heated slowly for about 1

hour. Sample was cooled and filtered. After filtering it was washed with warm distilled water for 4-5 times until clear water was obtained and then washed with acetone to fasten drying process. This was allowed to stand for a while. A crucible was washed and dried after that weighted using an electrical balance. Sample was transferred into weighted crucible and then placed in electric oven at temperature of 105°C for almost 4 hours this was then placed in desiccators to absorb extra moisture if any to attain constant weight. Crucible having NDF residue was again weighted on balance.

Weight of crucible = AWeight of crucible + NDF residue = B NDF = A-B = C

2.6.2 Preparation of ADF solution

NDF%

ADF solution was made in 1000ml distilled water by adding. 0.1N sulphuric acid was made. 2.27 ml of 0.1N sulphuric acid and 2g cetyl tri methyl ammonium bromide in 600 ml. Magnetic stirrer bar was used to mix properly and removing formed crystals after that remaining 400ml was also added. 1.0 g wheat straw was taken in a conical flask; 0.5 g sodium sulphite was weighted and added in flak. In the same flask 100 ml ADF solution was added stirred by using glass rod. Flask was covered by using lid and placed on a hot plate. Bubbles formed in the flask were continuously removed by opening the lid after some time, the sample was heated slowly for about 1 hour. Sample was cooled and filtered. After filtering it was washed with warm distilled water for 4-5 times until clear water was obtained and then washed with acetone to fasten drying process. This was allowed to stand for a while. A crucible was washed and dried after that weighted using an electrical balance. Sample was transferred into weighted crucible and then placed in electric oven at temperature of 105°C for almost 4 hours this was then placed in desiccators to absorb extra moisture if any and attain constant weight. Crucible having ADF residue was again weighted on balance.

= $(A-B/\text{ weight of sample}) \times 100$

Weight of crucible = AWeight of crucible + ADF residue = B ADF = A - B = D ADF % $= (A - B / weight of sample) \times 100$

2.6.3 Calculation of hemicellulose

Hemicellulose contents were calculated by taking difference of both ADF and NDF residues (Chen et al., 2018).

2.6.4 Calculation of cellulose

Weight of wheat straw sample = x gWeight of wheat straw sample = ADF residue after drying = y gWeight of crucible = ADF residue treated with sulphuric acid = z gConcentration of cellulose contents = y-z g

2.6.5 Lignin Calculation

Weight of cellulose residue + weight of crucible = lignin contents % Lignin = (lignin contents/ weight of sample) \times 100

3. RESULTS AND DISCUSSION

After pre-treatment of wheat straw with 1% NaOH at 121°C for 60 minutes, cellulose contents in wheat straw were increased from 40.3% to 57.4% and hemicelluloses contents decreased from 21.1% to 14.4% like hemicelluloses, reduction was also seen in the lignin contents i.e., from 20.33% to 11.7%. Aim of the pre-treatment was to increase sample porosity and cellulose contents, breaking the lignin at the same time partial degradation of hemicelluloses which is beneficial for the enzymatic hydrolysis of wheat straw. Previous study of 1% NaOH at 121°C for

1.5hours showed increase in cellulose contents to 44%, decrease of hemicelluloses and lignin contents to 44% and 42% respectively (Xiong et al., 2017).

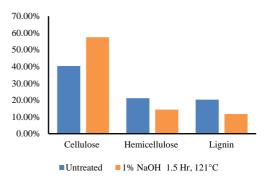


Figure 1: Lignocellulosic properties of wheat straw after treatment of 1% NaOH at 121° C for 1.5 hour

Wheat straw was pretreated with 4% lime at 80°C for 40 minutes reduced hemicelluloses contents from 21.1% to 20% and lignin from 20.33% to 18% at the same time cellulose contents slightly increased up to 41%. In the previous research it was stated that most researches declared lime pretreatment as effective as other treatments but the difference in effectiveness comes with the variance of time and temperature. No significant lignin degradation was found after lime treatment at 20° C, also no increase in cellulose contents was observed. It was observed that lime treatment is less effective as compared to NaOH treatment for lignin removal. Furthermore, lime pre-treatment requires longer residence time and temperature (Soares, 2015). The reason for least degradation of lignin with lime treatment was the bonding of lignin with calcium, lignin remained present in biomass instead after pre-treatment and difficult to degrade (Sträuber et al., 2015).

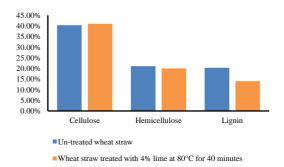


Figure 2: Lignocellulosic properties of wheat straw after treatment of 4%Lime at 80° C for 40 minutes

3.1 Acetic Acid Pre-treatment

After pre-treatment cellulose contents in wheat straw decreased from 40.3% to 37%, hemicelluloses from 21.1% to 20% and lignin from 20.3% to 14%. Acid treatment also degrades cellulose while little reduction in hemicelluloses was observed. Similar results were obtained from previous research where only 19% cellulose contents remained after 4% acetic acid treatment at 121° C. On contrary to acid treatment 24% cellulose contents were remained after 4% NaOH treatment at 121° C (Zheng et al., 2009).

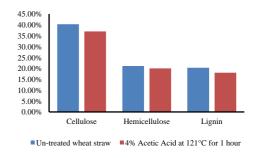


Figure 3: Properties of wheat straw after treatment of 4% Acetic acid at 121° C for 1. hour.

It was observed from the three pre-treatments that NaOH pre-treatment is most suitable. In NaOH pre-treatment cellulose contents were increased the most which are beneficial because it increases the sample porosity, which serves as instrument for efficient enzymatic hydrolysis. Lime pre-treatment also enhanced cellulose contents a little bit but. NaOH is stronger base as compared to lime so, can perform better at lower temperature. According to literature in alkali treatment some of the alkali is consumed by the biomass so, it is appropriate to use high alkali reagent in place of weak alkali reagent (Das et al., 2015). In acetic acid treatment it was observed that it decreased lignin and hemicelluloses at the same time also degraded cellulose.

Moreover, less degradation of hemi cellulose and lignin in acetic acid treatment was observed as compared to NaOH treatment. In case of NaOH mild treatment conditions are required in contrary to acid treatment (Volynets and Dahman, 2011). Owing to all above described prospective NaOH pre-treatment was optimized Optimization of sodium hydroxide pre-treatment of wheat straw Analyzation of change in lignocellulosic contents by time and temperature and keeping concentration constant. It was observed from above results that with 1% NaOH treatment by increasing time and temperature cellulose contents were also increased. Previous study found that temperature has significant effect in alkali treatment than any other treatment (Soares, 2015). As the temperature of treatment increases chances for cellulose degradation also increases because high energy tends to collapse lignocelllulosic structure (Volynets and Dahman, 2011).

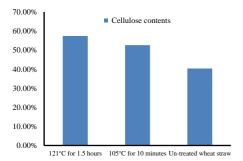


Figure 4: Changes in cellulosic contents at two different time and temperature

There was contrast found in the previous research and present, it was stated that temperature does alter hemicellulosic contents (Soares, 2015). In the present research by increasing time and temperature hemicellulosic contents also increased.

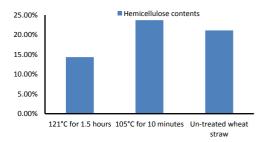


Figure 5: Changes in hemi cellulosic contents at two different time and temperature

In contrast to cellulose hemi cellulosic contents decreased with the increase in time and temperature while keeping constant concentration of chemical i.e., 1%. This reduction in cellulosic contents will aid in enzymatic hydrolysis process.

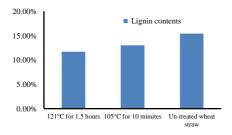


Figure 6: Changes in lignin contents at two different time and temperature

In literature 72% lignin degradation was achieved at 121° C at 2% NaOH concentration for 90-minute (Haque et al., 2012). Removal and degradation of lignin was the major purpose of treatment. Like Hemicellulose reduction in lignin contents also increased with increase in time and temperature

3.2 Effect on lignocellulosic properties of wheat straw treated at boiling temperature

Concentration of chemicals is very important parameter in pre-treatment process. Morphological characteristics of wheat biomass change with the change in concentration. Proper concentration of chemicals at fixed boiling temperature and for fixed residence time is crucial for the maintaining of efficient pre-treatment process. Wheat straw was treated with NaOH keeping temperature and time constant while changing the concentration of NaOH. It was performed to analyze optimum concentration of sodium hydroxide at boiling temperature. It was observed that by increasing NaOH concentration, cellulosic contents also increased and reached at it maximum at 2% NaOH concentration i.e., 71%. This was due to strong alkaline effect of NaOH. Same results were obtained from previous study at 105°C for 10 minutes by varying NaOH concentration from 0.5%-2% and maximum removal of lignin was found at 2% concentration i.e., 70.3% and hemicelluloses 68.2% (Haque et al., 2012).

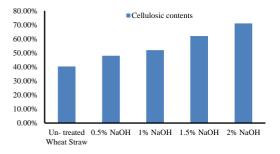


Figure 7: Change in cellulosic contents of wheat straw treated with different concentration at boiling temperature for 10 minutes

It was observed that change in lignin contents of straw after treatment. Increase in cellulosic contents of straw with the increase in concentration of NaOH was the evidence of lignin and hemicelluloses degradation which ultimately increased cellulose proportion in the straw. As the NaOH concentration increased, cellulosic contents also increased meanwhile decreasing lignin contents. Maximum removal of lignin i.e., 70.29% observed at 2% NaOH concentration. For 1.5%, 1%, and 0.5% lignin reduction was 56%, 37% and 32% respectively. Lignin is the major hindrance in enzymatic hydrolysis as it acts as a physical barrier that minimizes cellulose availability to enzymes and hence via nonproductive binding decreases the enzyme activity. Like lignin hemicelluloses contents also decreased with increase in the concentration of NaOH. It was observed that like lignin maximum reduction in the hemicelluloses i.e., 66.82% occurred at NaOH concentration of 2%. 18%, 24.17% and 47.86% reduction happened at NaOH concentration 0.5%, 1%and 1.5% respectively. Hemi cellulose forms a physical barrier for enzyme activity by interacting with cellulose. During enzymatic hydrolysis increase in the solubility of hemicelluloses, ultimately improves the digestibility of cellulose thus improves efficiency of enzymes conversion.

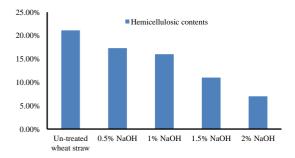


Figure 8: Change in hemicellulosic contents of wheat straw treated different concentration at boiling temperature for 10 minute

Thus, as the level of lignin and hemicelluloses degradation increase with 2% NaOH pretreatment the more favorable the straw becomes for enzymatic digestion in the process of hydrolysis.

3.3 Optimization of NaOH pre-treatment of wheat straw

Temperature is one of the most important parameter required for pretreatment of biomass. High temperature can cause over degradation and reduce the lignocellulosic material's ability for efficient production of sugar. High temperature more than 100°C may induce inhibitory byproducts. Moreover, high temperature pre-treatment is also expensive as it consumes more energy. It was observed that almost all pre-treatment require high temperature i.e., more than 100°C , therefore, optimization of temperature is required for efficient and cost effective pre-treatment (Preanger, 2014).

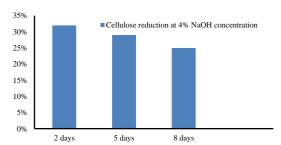


Figure 9: Change in cellulosic contents of wheat straw treated with 4% NaOH concentration at mesophilic temperature for different residence time

It was observed that cellulosic contents were higher after 2-day treatment as the residence time increases cellulose degradation also increased. Maximum degradation was observed after 8 days pre-treatment when it became up to 25%. It showed that long residence time was also not suitable for the proper treatment as it also degraded cellulose which is not beneficial for hydrolysis. For efficient pre-treatment we need maximum degradation of cellulose and lignin and minimum degradation of cellulose. After 8 days pre-treatment for 8% NaOH concentration degradation of cellulose was highest as compared to 4% and 6% NaOH treatment. Cellulose concentration was seen to decrease by increasing concentration as well as residence time. According to literature this change in cellulosic contents is not a beneficial change for hydrolysis and shows decomposition of lignocellulosic matrix (Lv et al., 2010). Both reaction time and NaOH concentration increases cellulose contents. Same results wre obtained from formal research and itis stated that it was due to reaction of NaOH with cellulose which broke cellulose structure (Yang et al., 2017).

It was observed that by increasing residence time hemicellulosic contents also decreased. Hemicellulose decreased to 17.5%, 16% and 12.5% after pre-treatment of 2, 5 and 8 days respectively. Hemicellulose degradation was 16%, 14% and 14% after 2, 5 and 8 days respectively with 6% NaOH treatment. It was higher than the degradation with 4% NaOH at same temperature. No change in hemicellulosic concentration was observed with 6% NaOH at mesophilic temperature after 5 and 8days. Hemicellulosic contents decreased to 16%, 12.5% and 11% after 2, 5 and 8 days of pre-treatment with 8% NaOH. After observing the treatments with 4, 6 and 8% NaOH it was analyzed that maximum degradation was occurred at 8% NaOH after 8 days of pre-treatment. Hemicellulose degradation was same for 4% NaOH for 8 days and 8% NaOH for 6 days. It was observed that both concentration of NaOH and time have effect on degradation of hemicelluloses but reaction time has comparatively more effect as compared to concentration.

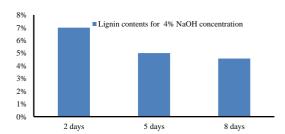


Figure 10: Change in lignin contents of wheat straw treated with 4% NaOH concentration at mesophilic temperature for different residence time

Lignin contents of wheat straw with 4% NaOH treatment after 2, 5 and days are 7%, 5% and 4.57% respectively. It was observed that by increasing residence time lignin degradation also increased till reached at

4.57% after 8 days of treatment. Lignin contents of wheat straw with 6% NaOH treatment were 8%,4% and 3.5% after 2,5 and 8 days respectively while in previous treatment with 4% NaOH they were 7%,5%,4.57% for 2, 5 and 8 days respectively. It was observed that for 2 days treatment more degradation was occurred with 4% NaOH as compared to 6% NaOH treatment. Lignin contents of wheat straw for 8% NaOH treatment were 3%,3.5% and 6% after 2, 5 and 8 days respectively. It was observed that by increasing residence time lignin contents decrease at 8% NaOH concentration.

Maximum lignin removal was occurred with 8% NaOH after 2 days of treatment. A group researcher achieved similar results at mesophilic temperature by varying NaOH concentration for residence time of 2, 5 and 8 days (Jiang et al., 2017). According to results of their research cellulose reduction of 41.2% and hemicelluloses reduction of 51.1% was achieved at 8% NaOH concentration for 8 days. NaOH alters the compositional properties of raw material, degrades lignin considerably but at a certain limit of time and concentration degradation becomes maximum and no increase in degradation was observed furthermore with the increase in time and concentration. This is the main reason of optimization of NaOH to save time and concentration of NaOH thereby reducing expenditures of treatment.

4. CONCLUSION

It was concluded that NaOH is the best treatment out of three different chemical treatments namely limes, NaOH and acetic acid treatment as it degraded maximum lignin contents. Optimization of time and temperature parameters while keeping concentration constant showed that increasing time and temperature lignin and hemi cellulose degradation also increased and cellulose contents increased. Optimization of chemical dose, keeping time constant at boiling temperature showed that lignin and hemicelluloses degradation increased with increase in dose and cellulose increased.

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