Vol.2 Issue.2 2026

ISSN : 3007-4738

Bioconversion of wheat straw through alkaline pretreatment and enzymatic hydrolysis

Sama A. F. ALRubai

College of Science, Wasit university, Al kut, Iraq

Article Information
Received: 7-5-2026
Accepted: 1-6-2026
Published: 1-7-2026

Abstract
The enzymatic hydrolysis of wheat straw after pretreated of alkaline for saccharification was studied. Sub-strate wheat straw was pretreated with different concentrations of NaOH (1.0, 2.0 and 3.0 wt%) and treated with different autoclave times (20, 40, 60 min) at 121°C. Results are showed the percentage of cellulose was increased with increasing alkali concentration up to 2.0%, and the maximum cellulose content and lignin removal (58.74% and 43.25%) respectively, was observed after 40 minutes of sterilization. Enzymatic hy-drolysis of treated wheat straw was carried out using different enzyme loads (0.5, 1.0, 1.5, 2.0, 2.5) of cellu-lase. As a result of the analysis of reducing sugars, it was found that the highest saccharification (56%) was obtained. Also, the optimal enzyme concentration and incubation period were recorded at 1% (w/v) and (1 h), resulting in a saccharification of 56%.

Keywords: lignocellulose, wheat straw, alkali pretreatment, enzymatic hydrolysis and saccharification

Introduction
Lignocellulose biomass is source of renewable available, it is the most abundant in nature in the world and can be biologically converted into biofuels or produce values added where target chemical compounds is a monosaccharide which are environmentally friendly compounds [1]. Among the lignocellulose biomass, wheat straw produced as a by-product after wheat cultivation was investigated. It is used as livestock livestock, but is burned in large quantities in the fields causing environmental pollution. Generally, wheat straw consist nearly 40% of cellulose, 25% of hemicellulose, and 12% of lignin. Critical steps of lignocellulose bioconversion are decreasing cellulose crystallinity and increasing enzymatic digestibility [2].
Since cellulose and hemicellulose in plant cells are covered with lignin, therefore the important step for lignin analysis is pretreatment to release cellulose and increased access of enzyme to

the saccharificationm. [3]. Among the pretreatment methods, alkaline pretreatment, especially with NaOH. It was important because it degrades lignin and breaks down the acetyl bonds to expose the cellulose content, and allows the enzymes hydrolysis to be properly work.

[4] stated that pretreatment with dilute alkali leads to the swell of cellulose, which leads to a lower degree crystallinity. The delignification and degrade structure were favorable for increased access of enzyme to hydrolysis of cellulose, which resulted in increased reducing sugar released. After pretreatment, enzymatic hydrolysis a main step was performed for depolymerizing lignocellulose to release sugars. The expensive of enzymes used currently has hampered the commercial application of bioconversion, therefore pretreatment of lignocellulose biomass was one way to degrease the enzyme loading. [5], [6]

The hydrolysis of enzymatic can be performed by cellulolytic enzymes which break down cellulose to reduce sugars. The enzymatic hydrolysis process can be carried out under mild conditions, resulting in a high yield of sugar, as well as formed of less inhibitors during hydrolysis [7]. Simultaneous saccharification has been applied by several researchers to overcome this obstacle. Compared to chemical hydrolysis, enzymatic hydrolysis offers several advantages over hydrolysis using chemicals. As known, the hydrolysis using chemicals requires to operate in high pH conditions and high temperatures whereby toxic pollution and generate more inhibitors, whereas the hydrolysis using enzymes may be done under mild conditions of temperature and pH, which reduces manufacturing costs and contributes to environmental conservation. In addition, enzymatic hydrolysis allows produce of polysaccharides in higher yields, compared to chemical hydrolysis.

As a type of hydrolysis reaction, enzymatic hydrolysis of lignocellulosic biomass is catalyzed by several enzymes such as hemicellulases and cellulases acting on sugars in the biomass.

The aimed of this study was to production of reducing sugars from chemically treated wheat straw. Alkaline pretreatment was evaluated by enzymatic hydrolysis.

Material and Methods

2.1.Materials

Wheat straw as locally sourced was used obtained from farm in Wasit Governorate, Iraq. Samples were sun dried, ground, sieved to determine particle size less than 2 mm, and stored at room temperature (25) °C until use.

Crude cellulase was obtained from Sigma-Aldrich. All chemicals were purchased from local markets.

2. 2. Alkaline pretreatment

Wheat straw treatment was performed as described by [8] with some modifications. Briefly, wheat straw was soaked in different concentrations of NaOH (1.0, 2.0 and 3.0 by weight) in a ratio of 1:10 (solid/liquid) and autoclaved at 121 °C for different times (20, 40, 60 min). After autoclaved, the samples were immediately cooled to room temperature and separated into solid and liquid fractions by vacuum filtration. The solid residue was washed with distilled water until it was neutral and it was then dried in an oven dried at 65 °C for further analysis.

2. 3. Enzymatic Hydrolysis:

Different enzyme concentrations (0.5, 1, 1.5, 2 and 2.5) % (w,) were used to achieve the best results of reducing sugars. Samples of each concentration were withdrawn at (1-5) hours. [9].

The reaction conditions were 50 mM citrate buffer, pH 4.8 for 20 min at 50 °C. Scarification yield was calculated as milligrams of glucose per gram of dry raw wheat straw.

2. 4. Analytical methods

Determination of wheat straw component

The compositions of cellulose, hemicellulose, and lignin were determined according to [10] method.

Ash content was determined by burning the samples at 500 °C for 3 hours.

2. 5. Determining of reducing sugar

Reducing sugars were determined using 3,5 dinitrosalysilic acid method [11]. A 5 ml sample was placed in a test tube, then (1 ml) of 3,5 dinitrosalysilic acid was added, the solution was mixed and boiled until solution was turned reddish-brown. Samples were cooled in a cool water for 10 minutes. Determining of reducing sugar content using a UV-visible spectrophotometer at 540 nm.

Saccharification rate (%) = reducing sugar (mg/ml) / substrate used (mg/ml) x 100.

Lignin removal content was determined according to Irfan et al. (2014) method using the following formula:

Lignin removal % = (crude lignin - processed lignin) / crude lignin x 100

2. 6. Statistical analysis

Statistical analysis was carried out in triplicates using the SPSS program. The value differences were presented as probability values (P 0.05).

Results

3. 1. Chemicals composition of wheat straw

The chemical composition of wheat straw and previous publications is shown in Table 1. The main fractions in the composition of the wheat straw sample were cellulose, hemicellulose and lignin with contents (35.44%), (23.30%) and (23.30%). (19.51%) respectively.

Table 1. Composition (wt%) of wheat straw in this study and in the previous literature

Prametars	Wheat Straw	[14]
Dry mater	93.35	96.41
Cellulose%	35.46	45
Hemicellulose%	31.97	21.30
Lignin%	20.53	19
Ash%	4.43	3.88

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%.

3. 2. Effect of alkali pretreatment on the chemical composition

Table 2 shows the effect of alkaline treatment on the chemical composition. The results showed the effect of different concentrations of NaOH at different soaking times. Table 2 shows the chemical fractionation of cellulose, hemicellulose and lignin after pretreatment. The fraction of cellulose increased from 37.34 to 58.74% after alkaline treatment, while lignin and hemicellulose decreased in pre-treated alkaline wheat straw.

Table 2. Percentage of main contents of wheat straw after alkaline treatments

Means within a column, row and their interactions followed with the same letters are not significantly different from each other according to Duncan multiple ranges test at significant level of 5%

Run	NaOH %	Time min	cellulose	hemicellulose	lignin	Delignification
1	1	20	37.34	18.2	14.14	18.54
2	1	40	39.28	19.3	13.65	19.88
3	1	60	39.58	18.14	14.25	22.2
4	2	20	42.68	14.25	13.37	25.34
5	2	40	54.47	14.61	13.55	13.16
6	2	60	58.74	13.81	12.14	43.25
7	3	20	50.59	13.97	9.36	36.58
8	3	40	48.64	12.33	9.29	35.86
9	3	60	47.28	12.28	9.21	36.54

The results obtained showed the effect of concentration of NaOH on cellulose content and lignin removal at different soaking times, when using of 1% NaOH, the maximum cellulose content and lignin removal was observed after 60 minutes of soaking and values were (39.58% and 22.2%) respectively. On the other hand, the maximum produced of cellulose content and lignin removal were (58.74% and 43.25%) respectively was observed after 40 minutes for 2% NaOH at same soaking times, while the effect for 3% NaOH was observed at same times, cellulose content and lignin removal were (50.59% and 36.58%) respectively was observed after 20 minutes. Increasing the pretreatment time to 90 minutes reduced the cellulose content to (52.74%).

Back to table 2, also the results showed the effect of 3% concentration of NaOH in periods different soak. A decrease in the content of cellulose, hemicellulose and lignin is observed in the pretreated wheat straw. The percentages of cellulose, hemicellulose and lignin contents were (47.28), (12.28) and (9.21), respectively.

3. 3. Enzymatic analysis

Wheat straw pretreated for enzymatic hydrolysis was evaluated by commercially available cellulase. The effect of enzyme dosage and incubation time was studied.

Figure 1 shows the effect of incubation time on the reducing sugar released process. The results reviled that the maximum sacchrification (43%) was obtained during the 4-hour incubation period. Decreasing or increasing incubation time beyond this limit lead in the decline the reducing sugar yield.

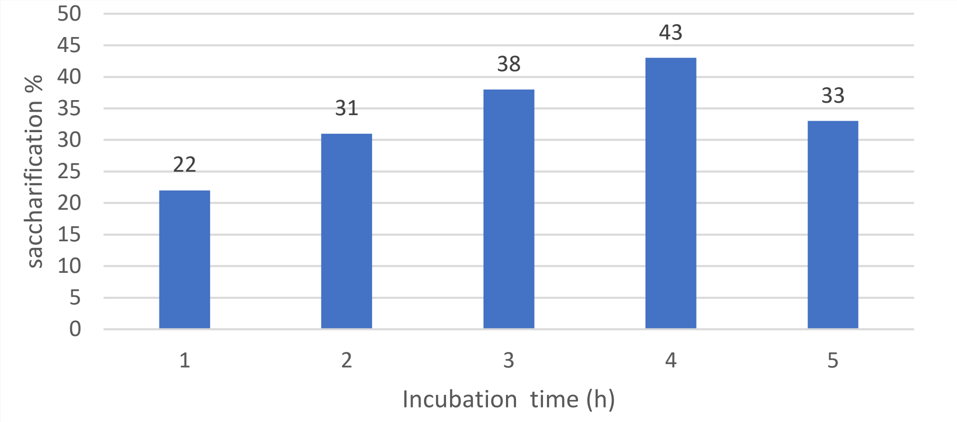

Figure 1. Effect of different incubation periods at pH 5.0 and 50 °C. on sacchrification.

The enzymatic hydrolysis conditions, the concentration of the enzyme (2900 IU/mL CMCase activity and 1500 FPU/mL filter paper activity) was improved by varying the enzyme concentration (0.5% to 3%), the results from (Fig. 2) showed that the maximum sacchrification (56%) was achieved at 1% enzyme concentration. Higher concentrations (2.5%) of the enzyme do not increase the reducing sugar yield.

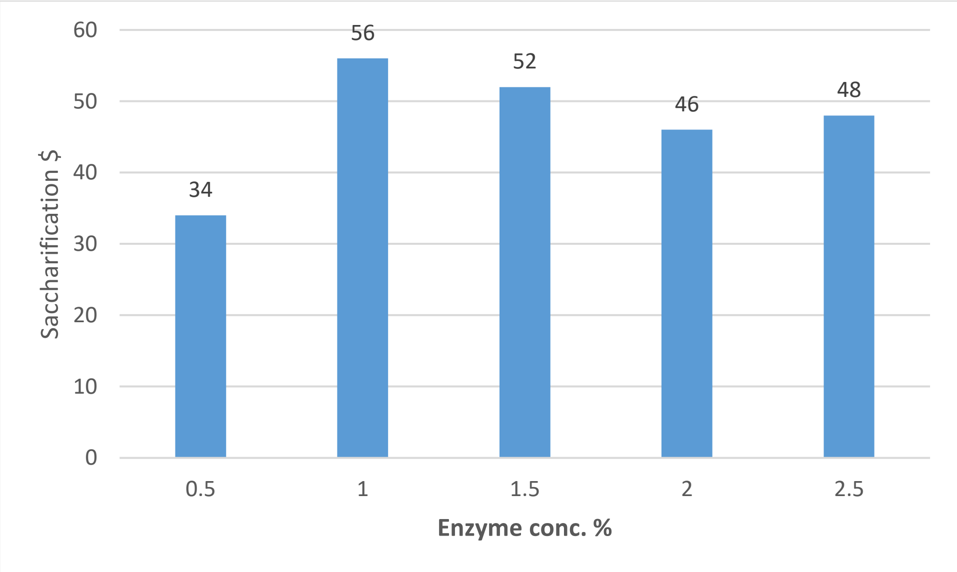

Figure 2. the saccharification process at pH 5.0 and 50 °C with enzyme concentration.

4. Discussion

4.1 Chemicals composition of wheat straw

According to data As can be seen from Table 1., the composition of the wheat straw samples in this study was similar to the previous literature, but the cellulose content (0.1%) was lower than the reported value (45%) 12]. The composition of wheat straw samples may vary depending on their location, variety and agricultural practices [13].

From preliminary reading. percentage of contents (cellulose and hemicellulose) emphasize for the selected agricultural waste to produce bioethanol, fermentable sugars and bio-products by hydrolysis or fractionation.

4.2 Effect of alkali pretreatment on the chemical composition

As can be seen from Table 2 In particular, alkaline treatment has been reported to have a strong lignin removal ability and can break the cross-links between lignin and hemicellulose [15].

Therefore, the degradation of lignin during the pretreatment resulted in a relatively high content of cellulose in the feedstock.
In reported that increased of NaOH concentration facilitated to release of lignin and increased reducing sugar yield after hydrolysis. Also, reducing sugars yield reached a plateau corresponding to a NaOH dose of 1.2% given 400 mg of oligosaccharides for gram of dry biomass. [16]
The effect of alkaline pretreatment was mainly in removing of lignin, no need to remove up to 60-80% of hemicellulose to promote sugar production, and further increase reducing sugar content was achieved after pretreatment. [17]
The results indicate that the pretreatment with 3% of NaOH for (90) minutes has a stronger effect on degraded of lignocellulosic biomass than 2% NaOH, which can also degrade the content of lignin, hemicellulose, and dissolved cellulose. [18]
We analyze the effect of alkaline treatment on the structure of the resulting wheat straw, composition and subsequent hydrolysis. The results of the current study were in agreement with those of the previous literature, although using a different lignocellulosic, pretreatment by soaking in 1% NaOH resulted in a reducing sugar of 8.9 mg per 100 mg of substrate. This is approximately the highest value was achieved. [19].
3. 3. Enzymatic analysis
According to figure 1 stated that the maximal enzymatic hydrolysis with an incubation time of 30 h at 50 °C. [20] Also, [21] they obtained maximum glucose 7.6 mg/mLfrom alkali pretreated corn cobs hydrolyzed using cellulase after a 48-hour hydrolysis time. The pretreatment intensity enhanced enzymatic hydrolysis as a result of more produce of glucose from the lignocellulose biomass [22].
While in figure 2 These values are consistent with previous studies on sawdust [23], they found that the enzyme concentration was higher than the enzymatic hydrolysis of lignocellulosic biomass such as sawdust. [24] also reported that the highest enzyme load and time were effected enzymatic hydrolysis of lignocellulosic biomass. Treated common reed was biodegraded (chemically and enzymatically), results showed that the alkali pretreatment had the highest conversion rate of cellulose (79.7%) as glucan[25].

Conclusion

The study revealed the lignin removal and cellulose content from wheat straw using alkaline treatment and enzymatic hydrolysis. The alkaline pretreatment was more successful and effective technique for release of cellulose, as maximum cellulose and lignin removal (58.74% and 43.25%) were achieved at a concentration of 2.0% NaOH for 60 minutes and the maximum sacchrification (56%) was achieved at 1% enzyme concentration. All these results indicate the importance of alkaline pretreatment and enzymatic hydrolysis before the saccharification process.

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