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Evaluation of different pretreatment methods on bioconversion of wheat straw and corn strover

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ABSTRACT

Lignocellulosic biomers have been found to possess great potential as substrates for various bioconversion processes; this is due to their vast availability coupled with their renewable nature. The direct conversion of lignocellulosic substrates is however hindered by their rigid and complex structural composition, which must be broken down via a process called pretreatment. This research aimed at comparing the effects of different methods used in pretreating lignocellulose. Samples of the selected biomers were subjected to various methods of pretreatments (mechanical, chemical, physicochemical and a combination method). Compositional analysis of each biomer was carried out before and after each pretreatment method, the pretreated biomers were then subjected to microbial hydrolysis using *Trichoderma viride* and *Aspergillus niger* for 5 days, after which estimation of reducing sugar present was carried out. Analytical contents of the samples showed high cellulose, hemicellulose and lignin contents (36.39, 26.52, 13.62 and 43.30, 32.30, 6.42% respectively). The combination of 2 or more methods proved more efficient than other individual methods, significantly increasing cellulose contents from 36.39% to 58.61% and 43.30% to 61.09% in wheat straw and corn stover respectively, while eliminating the lignin barrier from 13.62% to 3.82% and 6.42% to 2.38% respectively. The reducing sugar results proved that *Trichoderma viride* had the most sugar content of 0.55 g/g in corn stover after pretreatment with the combination method, making it more effective in cellulose hydrolysis.

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1. Introduction

Increase in human population, coupled with diversification and depletion of available resources, as well as the drastic changes in global climate (1) have convinced academics, governments and other stakeholders of the need to develop sustainable energy in a bid to cushion the effect of greenhouse gas emissions (2).

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Also, reduction in energy reserves globally is happening at a relatively rapid rate, due to a global increase in the utilization of fossil fuels given rise to by technological advancements and motorization of the world (3).

Currently, fossil fuels are still regarded as the main source of global energy and compose of about 88% of global energy usage (4), combustion of these fuels however results in the production of greenhouse gases, particularly CO_2 (5). Hence, substituting the use of these fuels with those of renewable nature provides a viable solution in solving these problems and thus reducing the rapid change in global climate caused by these fuels (6).

Renewable energy can be described as any and every type of energy gotten from the renewable fraction of municipal waste, biodiesel, solid biofuels, other liquid biofuels, and so on. In the same vain, biofuels can be referred to as any fuel gotten in one way or another from sources relating to biomass (7). The term Biomass are regarded to as one of the biggest sources of energy available, they also make up the largest and most valuable option for renewable energy in our day and are also being used in the day-to-day production of different other energy sources (8).

A very major advantage of biomass is their state of being carbon neutral, this is with regards to the fact that very insignificant amounts of CO_2 is produced during utilization, and that the little quantity generated is equivalent to that used up by green plants during the process of photosynthesis (9).

The biggest and most promising options in this group are the lignocellulosic biomass, which are regarded as viable and highly efficient in energy generation.

Lignocellulosic biomasses are natural resources obtained from plant sources which include brittle and fibrous tissues, generally referred to as dry matter. This biomass has long been presented as very vital components of what is termed a sustainable society, and this has been so for several years. They are generally regarded as extremely viable replacement to fossils. The 2016 global biofuel production was found to be around 137 billion L (3.3 EJ) (10). Other potential application lignocellulosic biomass includes polymers and fertilizers generation; however transportation fuels are regarded as the main users of all energy sources. Other production processes used in the production of certain substances including chemicals are regarded as being ripe for production through biological paths, a major example of this include organic acid production, which are now being successfully carried out biologically (11).

The complex composition of lignocelluloses has however been a major drawback to their effective use/conversion. This challenge is currently being overcome by the compulsory inclusion of what is generally referred to as a fractionation stage. Fractionation, which is also referred to as pretreatment, is a highly important procedure involved in the conversion of these vast substances. First, fractionation is highly important in the used to change the structural lattice of the biomass in other to easily access the cellulose and hemicellulose contents of the biomass for bioconversion (12). However, the most important reason for the fractionation step is the removal of all barriers including cell wall as well as the effective reduction of the crystallinity of the cellulose fraction present (13,14). This step of fractionation/pretreatment must carry out as economically as possible in other to reduce the overall operating and capital costs and hence the total processing cost b implication (15). Highly regarded as one of the foremost steps in the conversion process of lignocellulosic biomass, it is generally done to increase access to the raw materials by microbes, enzymes etc. determination of the best fractionation/pretreatment method for all situations and raw materials is a relatively difficult process, however, it is important that some essential goals be maintained such as maintaining a good recovery of all separate polymers as well as compounds in the biomass, also, the process must maintain at the barest minimum, the generation of all forms of toxic or inhibitory components in other to avoid the possibility of negative effects during the conversion process, thereby reducing the general efficacy of the entire process (11).

Various types and kinds of pretreatment methods used for lignocellulosic biomass have been discovered/analyzed, they generally include the following

1. Mechanical pretreatment which involves the reduction in the size of the various biomer particle, hence increasing their surface area to volume ratio.
2. Chemical Fractionation involves the use of chemicals to break down certain structural barriers, is carried out using alkalis, diluted acids, or organic solvents.
3. Physicochemical pretreatment uses heat under pressure to liberate biomass composition, it includes; steam explosion and hot water.
4. Biological Pretreatment involves the utilization of microbes or their enzymes (16).

The goal of this research is to access the effectiveness of different pretreatment/fractionation methods individually and when combined on corn stover and wheat straw samples.

2.Materials and Methods

2.1.Sample Collection

The selected samples (wheat straw and corn stover) were collected from sites within south Western Nigeria. The samples were washed thoroughly; sun dried and subjected to various pretreatment methods.

2.2.Compositional analysis of straws

The determination of the compositional analysis was carried out on the samples according to AOAC (17). The compositional analysis of the selected substrates was done on the raw untreated samples as well as on the processed/pretreated samples. The Cellulose content evaluation was carried by a process known as colorimetric method. The selected samples in powdered form underwent boiling to 100^{o}C using a 1:8, v/v composition of nitric acid for a duration of one h, repeated centrifugation and dilution with sulfuric acid (67% H_2SO_4) was then carried out in order to eliminate the hemicellulose lignin, and xylosans contents. Cold anthrone reagent was then used to evaluate the Cellulose content at 620 nm.

Evaluation of lignin and hemicellulose compositions of the selected samples was also done. Residue generated in the previous step which is rich in the aforementioned fractions was subjected to heating with 5 ml of 72% (w/w) sulfuric acid solution for duration of four and half h in a bid to breakdown hemicellulose.

Filtration of the generated suspension was then carried out; the solid fraction obtained was then dried out at 105°C for twenty-four h and weighed, this was designated W1. Generated residue was again transferred to a dry crucible whose weight had been previously determined, it was then heated at 600°C for about five h and, then it was left to cool down, and the weight was again determined as w^2. The difference between the first and second weights was then used to determine the lignin content.

Generated filtrate from the sulfuric acid treatment which had released sugar contents from was vigorously homogenized, after which the reducing sugar and glucose contents were then evaluated and used in the estimation of hemicellulose.

$$\text{Hemicellulose (\%)} = \left(\frac{w}{s}\right) x\ (C2 - C1) x\ \left(\frac{v}{m}\right)\ x\ 100$$

S = scarification yield, W = molecular weight ratio of the polymer and monomer pentose, C2 = determined reducing sugar C1 = glucose concentration, M = dry weight of the sample (g), and V = total volume of sugar solution (L).

2.3. Pretreatment Lignocellulosic Biomers

2.3.1. Physical Pretreatment method

Mechanical pretreatment is majorly carried out in order to disrupt the crystallinity of the lignocellulosic biomass and also to reduce the particle size to achieve a certain surface area to enhance the action of enzymes and decrease the degree of polymerization (18).

Physical and chemical alteration of the selected lignocellulosic samples was carried out by heating, mixing and shredding using an extruder. Shortening of the lignocellulosic biomers were achieved by the control/adjustment of Screw speed and temperature of the barrel. After extrusion the final particle size was found to be 0.5 mm.

2.3.2. Chemical Pretreatment Method

The selected lignocellulosic samples were subjected to alkaline fractionation using a one molar sodium hydroxide (NaOH) solution, in a ratio of 10% w/v. The lignocellulosic samples were steeped in the solution for about an hour at ambient temperature. They were then washed using distilled water and oven dried at 100°C (19).

2.3.3. Psychochemical pretreatment Method

This was carried out using a modified steam explosion method. 1 kg of each straw was chopped into bits using a knife and soaked in dilute sulfuric acid solution overnight. The impregnated straws were then filtered, after which the straws were then steam treated using a 10 L steam reactor at 180°C and 15 bar for 10 min. The relatively high pressure within the reactor was then suddenly released by the opening of the valve knob; this was done to generate a sudden and rapid change in pressure referred to as shock. This pressure shock which resulted in a sudden difference between the pressure within and that of the surrounding environment caused an explosion of the fiber.

The generated material was then rinsed with water for 30 min. It was then stored at 4^{o}C for later use.

2.3.4.Combination of methods

This involved the use of several methods including Mechanical, physical and chemical methods. The selected lignocellulosic samples were initially extruded in others to reduce particle size, after which the somewhat powdered samples were then treated using Dilute Acid Pre-hydrolysis (DAPH-100-121) and alkaline delignification using NaOH as described by Olugbenga and Ibileke (21). The dilute sulfuric acid was first used by autoclaving the selected previously ground samples in 1.25% H_2SO_4 at 121°C for 16 min. The pulp like solid fraction was then obtained and dried, after which they were again subjected to another round of autoclaving at 120°C for 80 min using a 2% solution of NaOH in a 1:20 g/g ratio. The solid materials were again collected and filtered, after which they were rinsed thoroughly with distilled water to remove all leftover chemicals. They were then dried and subjected to analysis.

2.4.Microbial hydrolysis of pretreated Straw

After the completion of the fractionation stage using various methods, the selected biomers were then weighed into separate flasks and distilled water was then added to make up one thousand milliliters. The flasks were then covered and autoclaved at 121°C for 15 min, after which the flasks were then left to cool down.

The cooled flasks were then inoculated with previously purified cultures of *Trichoderma viride* and *Aspergillus niger*, after which standardization of pH was then carried out to 5.5 and the flask left to sit at ambient temperature for 72 h. A specific quantity of the solution within the flask was obtained at the end of the 72 h and was used to determine the presence and quantity of reducing sugar produced by each organism (22).

2.5.Estimation of Reducing Sugar

The reducing sugar contents of each of the flasks from above were determined using the DNSA method as described by Rahnama et al. (23).

A specific quantity of samples from each of the flasks from above was introduced into a test tube, after which activated charcoal was then added to the test tube. The test tubes were then homogenized by vigorous agitation, after which it was then filtered to get a colorless liquid, which was then transferred into another test tube. DNSA was then added to the colorless liquid in the second test tube and allowed to boil for about five minutes. After 72 h, the absorbance of the sample was then determined using a spectrophotometer at 540 nm. The reducing sugar content was then graphically determined.

2.6.Data Analysis

Collected data were then subjected to various statistical analyses. The standard deviation from the mean was evaluated using SPSS 20. Data obtained from each parameter were analyzed for significant difference using the one way ANOVA. The means were compared at 0.05% confidence interval.

3.Results

Proximate composition of selected lignocellulosic material is shown in table 1. Corn Stover had higher fiber (39.40%), fat (3.83%), protein (4.40%) and moisture content (4.25%), while wheat straw had higher ash content (8.73%). The mineral contents of the selected biomers (wheat straw and corn stover) is shown in table 2, wheat straw was observed to have higher carbon (49 mg/g), nitrogen (0.77 mg/g) and potassium (1.24 mg/g), while corn stover showed higher sodium (1.48 mg/g), calcium (6.48 mg/g), magnesium (1.12 mg/g) and phosphorous (8.03 mg/g). The structural content of the utilized biomers (wheat straw and corn stover) is shown in table 3, corn stover had a higher cellulose and hemicellulose content (43.30%) and (32.30%) respectively while wheat straw had a higher lignin content (13.2%).

Table 1. Proximate composition of the utilized lignocellulosic biomers (%)

Parameters	Wheat straw	Corn stover
Moisture content	2.82 ± 0.10^{b}	4.25 ± 0.12^{b}
Ash Content	8.73 ± 0.15^{c}	6.45 ± 0.20^{c}
Crude Fiber	37.82 ± 0.08^{e}	39.40 ±0.37^{e}
Crude fat	1.50 ± 0.20^{a}	3.83 ± 0.28^{b}
Crude Protein	4.35 ± 0.32^{b}	4.40 ± 0.00^{bc}

Table 2. Mineral content of the utilized lignocellulosic biomers (mg/g)

Parameters	Wheat straw	Corn stover
Na	0.68 ± 0.03^{a}	1.48 ± 0.16^{a}
C	49.00 ± 0.20^{b}	43.92 ± 0.24^{b}
K	1.24 ± 0.17^{a}	0.12 ± 0.02^{a}
Ca	0.53 ± 0.10^{a}	6.48 ± 0.02^{b}
N	0.77 ± 0.14^{a}	0.47 ± 0.04^{a}
Mg	0.54 ± 0.08^{a}	1.12 ± 0.02^{a}
P	0.21 ± 0.03^{a}	8.03 ± 0.17^{c}

Table 3. Structural composition of the raw untreated biomers

Substrate	Corn stover	Wheat straw
Cellulose	43.30 ± 0.06^{e}	36.39 ± 0.38^{de}
Hemicellulose	32.30 ± 0.05^{d}	26.52 ± 0.30^{d}
Lignin	6.42 ± 0.17^{a}	13.62 ± 0.10^{b}

Table 4. Efficacy of the Physical pretreatment method (Mechanical Comminution) on the utilized biomers

Substrate	Before pretreatment (%)			After pretreatment (%)		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Wheat Straw	36.39 ± 0.38[d]	26.52 ± 0.30[c]	13.62 ± 0.10[b]	36.98 ± 0.03[d]	25.55 ± 0.06[c]	10.51 ± 0.20[b]
Corn Stover	43.30 ± 0.06[e]	32.30 ± 0.05[d]	6.42 ± 0.17[a]	42.95 ± 0.11[e]	33.44 ± 0.17[d]	4.26 ± 0.12[a]

Table 5. Efficacy of the physicochemical pretreatment method (Acid catalyzed heat treatment) on the utilized biomers

Substrate	Before pretreatment (%)			After pretreatment (%)		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Wheat Straw	36.39 ± 0.38[d]	26.52 ± 0.30[cd]	13.62 ± 0.10[b]	41.75 ± 0.10[e]	15.54 ± 0.07[b]	3.89 ± 0.04[a]
Corn Stover	43.30 ± 0.06[e]	32.30 ± 0.05[d]	6.42 ± 0.17[a]	47.63 ± 0.15[e]	21.87 ± 0.42[c]	4.02 ± 0.18[a]

Table 6. Efficacy of the Chemical pretreatment method on the utilized biomers

Substrate	Before pretreatment (%)			After pretreatment (%)		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Wheat Straw	36.39 ± 0.38[de]	26.52 ± 0.30[cd]	13.62 ± 0.10[ab]	52.34 ± 0.31[f]	11.05 ± 0.32[b]	5.56 ± 0.24[a]
Corn Stover	43.30 ± 0.06[e]	32.30 ± 0.05[d]	6.42 ± 0.17[ab]	58.63 ± 0.02[f]	15.87 ± 0.27[b]	3.06 ± 0.22[a]

Table 7. Efficacy of the combination pretreatment method (Mechanical Comminution and treatment) on the utilized biomers

Substrate	Before pretreatment (%)			After pretreatment (%)		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Wheat Straw	36.39 ± 0.38[d]	26.52 ± 0.30[d]	13.62 ± 0.10[c]	58.61 ± 0.26[f]	7.84 ± 0.09[b]	3.82 ± 0.36[a]
Corn Stover	43.30 ± 0.06[e]	32.30 ± 0.05[d]	6.42 ± 0.17[b]	61.09 ± 0.34[f]	9.82 ± 0.45[b]	2.38 ± 0.16[a]

Sugar produced by the hydrolysis of the pretreated biomers with *A. niger,* and *T. viride* is shown in Figure 1 and 2. *T. viride* generated a sugar yield of 0.55 g/g in corn stover and 0.41 g/g in wheat straw pretreated with the combination method. *A. niger* had a lower yield of 4.5 and 3.1 g/g in corn stover and wheat straw respectively.

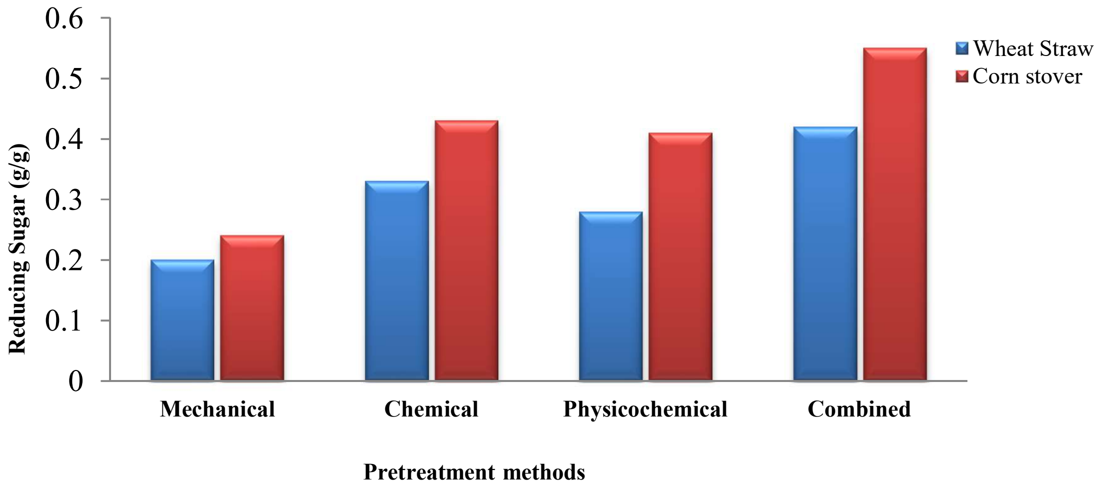

Figure 1. Reducing sugar generated by hydrolysis of pretreated straws with *T. viride*

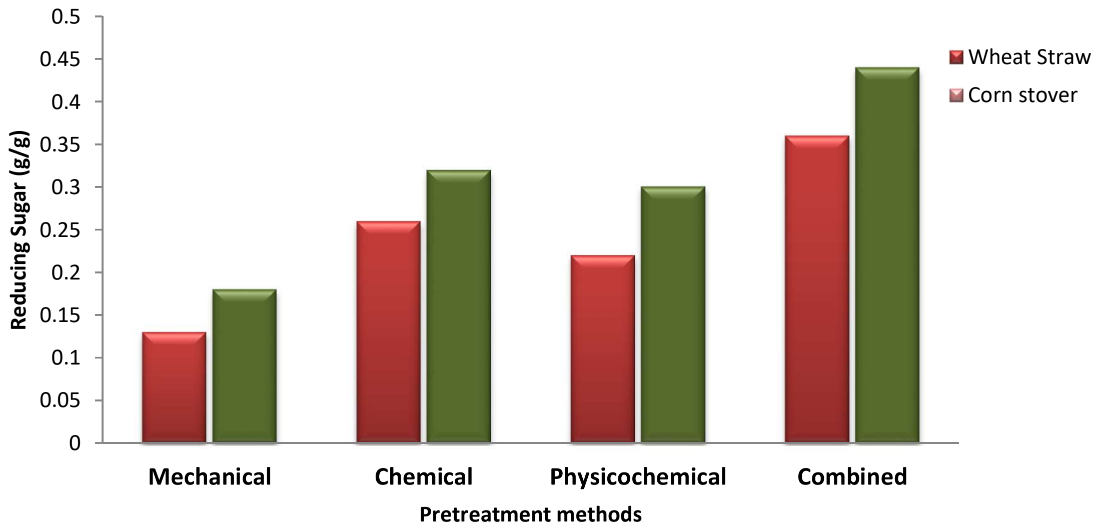

Figure 2. Reducing sugar generated by hydrolysis of pretreated straws with *A. niger*

The different pretreatment methods used had various effects on the utilized biomers and this can be seen in tables 4 to 7. Reductions in the hemicellulose compositions can be progressively observed in the different fractionation methods used, while a similar Progressive increase in the cellulose contents can also be noticed. The increase in cellulose content was lowest in the mechanical method, in which wheat straw increased from 36.39 to 36.98%, while the cellulose content of corn stover reduced from 43.30 to 42.95%. The highest increment in cellulose content was observed in the combined methods, with wheat straw increasing from 36.39 to 58.61% while corn stover increased from 43.30 to 61.09%.

4.Discussion

The proximate compositions of wheat straw and corn strover (table 1), the samples were observed to contain high fiber contents of 37% and 39.40 % respectively. The high fiber content is directly proportional to the high lignin contents of the utilized biomers. The utilized lignocellulosic biomers were observed to be low in moisture content; corn stover had slightly higher moisture of 4.25%. Okoye et al. (24) noted that certain lignocellulosic biomers had significantly higher moisture of 8.5 – 9.1% this was also the case with Pothiraj et al. (25). The ash contents of the biomers were somewhat high but notably higher in wheat straw and this can be the result of the high carbon content of the wheat straw biomer. The observed mineral contents of the biomers were somewhat different as seen in Table 2, the sodium, calcium and potassium contents of the corn stover biomer; 1.48, .48 and 8.03 mg/g respectively were significantly high. Both biomers utilized processed low nitrogen levels, as was also the case as reported by Wannapeera et al. (26).

Compositional analysis of the substrates indicated an abundance of cellulose and hemicellulose in both substrates. However, corn stover was observed to contain higher cellulose contents (43%) than wheat straw (36.39%). Similar result was also observed in the hemicellulose contents of the substrate, however the high lignin contents observed in both substrates poses a major drawback in their use in bioconversion. However, wheat straw was seen to have considerable higher lignin content (13.62%) making its cellulose contents harder to access. The findings of this research with regard to the structural composition of the biomers were similar to that of Imran *et al.* (27) and Yingying *et al.* (28). He also noted lower cellulose and lignin contents in similar biomers analyzed and attributed this variance in structural composition of same biomers to difference in environmental, soil and growth conditions of the initial plant from which the biomers came from. Structural composition of the utilized biomers has proven that lignocellulosic biomers are indeed surrounded by a number of barriers and has hence justified the need for fractionation/pretreatment in order to achieve maximum yield from their use (29).

Efficacy of each pretreatment method was evaluated on the bases of their propensity to bring about delignification of the utilized biomers while separately

Releasing the cellulose or/and hemicellulose fraction for further use. The mechanical pretreatment alone was observed to have little or no effect on any structural component, however a slight increase in reducing sugar generation was observed, this is due to the disintegration of the various structural components during the grinding process, making the cellulose content a little more accessible ass well facilitation quicker enzyme activity (29).

The chemical and physicochemical pretreatment of the utilized biomers was seen to effectively eliminate the lignin fraction while also enhancing the cellulose recovery. However, based on the level of cellulose recovery and hydrolysis of hemicellulose fraction, the combination of two or more methods including mechanical Comminution and chemical enhanced heat treatment was noted to be more efficient than the other individual methods as seen in table 7. Cellulose content was observed to increase from 36.39 to 58.56% in wheat straw and from 43.30 to 61.09% in corn stover after treatment with the combined method. Analytical findings revealed a significant increment in the recovered cellulose fraction from rice straw biomer and an equally significant reduction in the hemicellulose and lignin fractions. The above observation was brought about by a possible acid solubilization of the hemicellulose thereby increasing the penetration of NaOH, this in turn aids in the separation of the cellulose fraction leading to the generation of a high cellulose residue (30). The observed findings and conclusions are similar to that of Liguori et al. (31), He noted that a significant increase and decrease in the structural compositions of acid pretreated brewery spent grains were evident. However, Abo-State *et al.* (32) recorded contrary findings while analyzing rice straw. These observed increase and decrease in the structural composition of the selected biomers coupled with the effective delignification are essential for microbial actions (33).

The ability of the selected organisms to hydrolyze the pretreated substrate was determined by the quantity of sugar generated after 72 h of scarification. *Trichoderma viride* was determined to be a better agent for cellulose hydrolysis than *Aspergillus niger.* Elsayed, (34), also noted better scarification of cellulose by *Trichodema sp.* when compared with *Bacillus sp*., he also noted that *Trichodema sp.* had an unrivalled ability to produce several types of cellulose enzymes such as endocellulase, exocellulase, and β-glucosidase in significantly high quantities. The combined pretreatment method yielded the highest reducing sugar in both wheat straw (0.4 g/g) and corn stover (0.54%) using *T. viride* and 0.35 and 0.44 g/g respectively using *A. niger.*

5.Conclusion

This study evaluated the efficacy of various methods used in the pretreatment of two lignocellulosic substrates. The mechanical method alone proved to be inadequate in the pretreatment of lignocellulosic materials, but proved useful when used in combination with other methods.

While other methods such as chemical and physicochemical showed some level of efficacy, the combination of two or more methods was considered the preferred method of choice for the pretreatment of lignocellulosic biomers.

The study has also confirmed the potency of *T. viride* in cellulose hydrolysis of pretreated lignocellulosic substrates, thus enhancing the bioconversion process of lignocellulosic biomers easier and more efficient.

Conflict of interest

All authors indicate that they have no conflict of interest.

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