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pH-values

Relative Saccharification of Sawdust Materials at Different Incubation

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ABSTRACT

The uncontrolled production of waste is a daily phenomenon that is experienced by most global communities, and the situation worsens due to the lack of effective waste management procedures. Solid waste such as sawdust is primarily produced by the forestry industry and although it is utilized by certain countries as briquettes to make fire or as an absorbent to clean fluid spillage as well as a component of ceilings, most of the sawdust along the Lagos Lagoon in Nigeria is left unattended as waste, contributing to environmental pollution. Cellulose, composed of glucose units is a structural component of sawdust and when saccharified the resulting glucose can be fermented into renewable substances such as bio-ethanol. The cellulose degradation process can be performed with a cellulase enzyme such as available in the fungus Aspergillus niger and during the current investigation, this enzyme system was used to bio-convert the cellulose component of sawdust from ten different trees along the Lagoon into glucose. To increase the cellulase action all sawdust materials were delignified before cellulase action with the main aim of determining the optimum pH value for maximum degradation of the various sawdust materials. The pH-related saccharification profile of each type of sawdust was constructed as well as the relative percentage of saccharification and it was concluded that all the materials were optimum degraded at acidic pH-values which varied between pH 5.0 and pH 6.0 that are like optimum pH-values reported for the other types of cellulose materials.

INTRODUCTION

Environmental pollution will become more topical as the amount of waste produced by the global population increases with the negative effect thereof on water resources, in air as well as on land already observed and described (Li et al. 2021). Solid waste is one of the various sectors of rubbish with organic waste a major component of trash composed of materials produced by agriculture, forestry, and households (Janakiram & Sridevi 2010). Organic waste refers to substances of plant origin such as food waste, garden waste (plants, grass, trees), and sawdust which potential to be degraded by cellulase enzymes into glucose, a fermentable sugar at different incubation pH-value was determined during the current investigation. Sawdust is a waste product produced by forestry during the felling of trees tons of this wood material are produced annually along the Lagos Lagoon in Nigeria because of the activities of numerous sawmills (Faremi et al. 2021). Efforts to develop waste through recycling into useful products or commodities are widely applied with the aim not only to protect natural resources but also to limit the use of fossil fuels that could have a positive effect on the environment specifically to control the effects of climate change (Paritosh et al. 2017). Although sawdust has several applications such as an efficient adsorbent for dye removal (Chikri et al. 2020), insulation material (Okino et al. 2021), biosorbent (Giwa et al. 2016),

forms part of ceiling boards (Zeleke & Rotich 2021), its potential to be developed as a resource for the development of bio-chemicals or bio-pharmaceuticals is yet to be realized.

The chemical structure of sawdust makes it a suitable resource for the synthesis carbon carbon-related substances that would replace fossil fuels as feedstock (Babu et al. 2022). Cellulose a glucose bio-polymer is one of the major structural components of sawdust and if degraded into glucose this fermentable sugar could be used as a renewable feedstock for the synthesis of many bio-products such as bioethanol (Amaefule et al. 2023). Although cellulose exhibits a relatively high resistance towards different degradation procedures it can be saccharified through the action of cellulase a hydrolytic enzyme (Sartori et al. 2015). To make the cellulose more susceptible to cellulase-catalyzed degradation this biopolymer can be delignified (Kurian et al. 2014), a process that destroys its interaction with lignin, another biopolymer in plant materials. Cellulase enzymes are available from different bacterial (Sethi et al. 2013) and fungal (Ja'afaru 2013) sources with cellulases from Trichoderma reesei (De Paula et al. 2018) and Aspergillus niger (Lee et al. 2011) known as effective enzyme systems acting on cellulose causing the release of free glucose. The enzymatic or cellulase-catalyzed degradation of cellulose is subjected to several catalytic variables that could affect the effective outcome of the cellulose saccharification process in terms of glucose production. These variables include incubation temperature, incubation time, the pH value at which the incubation takes place, the concentration of the cellulase enzyme used during the degradation process, and the amount of cellulose used during each incubation process. One of the aims of cellulose degradation is to design a saccharification process that results in the maximum amount of sugar produced and this process should include the optimization of catalytic properties of the cellulase enzymes as well as the application of physical and chemical pretreatments rendering the cellulose more susceptible for cellulase catalyzed degradation (Abolore et al. 2024). It is however important to mention that the pretreatment action should be environmentally benign thus making acid and alkaline pretreatment agents less favorable.

The environmental impact of fossil fuel consumption as an energy source or feedstock during the synthesis of chemical-related substances is well-researched and published (Lak et al. 2024). As a result of the negative environmental effects caused by fossil fuel use alternative and renewable energy resources must be identified and developed. Linked to this environmental observation and of concern is the increasing volumes of non-manageable solid waste especially organic waste produced annually. These two phenomena could be resolved simultaneously by developing the cellulose component of organic waste such as sawdust as a resource for bioproduct synthesis thus limiting the amount of solid waste and the dependence on fossil fuels as an energy resource and feedstock for synthetic purposes.

The development of waste cellulose such as sawdust and wastepaper (Mokatse & Van Wyk 2021) as a resource of bio-energy should be initiated by making it more susceptible to degradation and the use of cellulase enzymes should be advisable as the process is environmentally benign (Verma & Kumar 2022). This investigation dealt with the saccharification of sawdust from different trees along the Lagos Lagoon with the cellulase enzyme from *Aspergillus niger* with a focus on the effect of changing incubation pH values when degrading the delignified sawdust materials.

MATERIALS AND METHODS

Sawdust Substrate and Cellulase Enzyme

Delignified sawdust samples from ten different trees along the Lagos Lagoon in Nigeria were transferred in triplicate into test tubes. Names of these sawdust samples are *Erythropleum suaveolens*, *Symphona globulifera*, *Ricindendron heudelotii*, *Pterygota macrocarpa*, *Milicia excels*, *Ipomoeu asarifolia*, *Hallelea ciliate*, *Sacoglottis gabonensis*, *Pycnanthus angolensis*, and *Terminalia superb*. Commercially obtained *Aspergillus niger* cellulase enzyme (0.1g) was dissolved in 0.005 mol.dm⁻³ pH 5.0 tris buffer resulting in an enzyme solution concentration of 2.0 mg.mL⁻¹.

Delignification of Sawdust - Kraft Pulping and Hydrogen Peroxide Treatment of the Wood Sawdust

To ensure a maximum cellulose exposure to the cellulase enzyme the various sawdust materials were delignified by subjecting 2kg of each of the different sawdust materials (2.8-5.0 mm particle size) to 350g of NaOH and 140g NaS₂ during the Kraft pulping process. The Kraft pulping chemicals were dissolved in 8 L water and the delignification of the lignocellulosic materials (sawdust) was carried out in a rotary steel digester at 170°C and a pressure of 200 kPa for 1 h 45 min at cooking liquor to the wood ratio of 4:1. After the Kraft pretreatment, the extracted cellulose fibers were washed in turns with deionized water until they were free of the Kraft reagents (Ndukwe et al. 2009).

To remove residual lignin from these Kraft-treated cellulose all these sawdust materials (10 g) were treated with 30% hydrogen peroxide (60 mL) at 40°C for 25-30 min. The relatively small waste volumes of the Kraft and delignification process were kept in containers for further purification treatments.

Cellulase Incubation and Sugar Analyses

The delignified sawdust materials (10 mg) were transferred in triplicate in test tubes and incubated with the *A. niger* cellulase enzyme solution (200 ul) and Tris buffer solutions at pH-values varied between pH 4.0 and pH 7,0 (800 ul) for 2h at an incubation temperature of 50°C. The concentration of sugars released from the sawdust materials during cellulasecatalyzed degradation was determined from a standard glucose calibration curve constructed with glucose standard solutions at concentrations of 0.50 mg.mL⁻¹, 2.00 mg.mL⁻¹, 4.00 mg.mL⁻¹, 6.00 mg.mL⁻¹ and 8.00 mg.mL⁻¹. The DNS method as described by Miller was used to calculate the concentration of the sugar produced during *A. niger* action on the waste sawdust (Miller 1959).

Calculation of Resultant Amount of Sugar Produced and Percentage Saccharification

The resultant amount of sugar produced from the delignified and non-delignified sawdust was calculated by subtracting the amount of sugar released from each type of sawdust in the absence of cellulase action from the amount of sugar released when the sawdust was treated with the cellulase enzyme. This amount of sugar known as the resultant amount of sugar was released because of the cellulase action on each type of sawdust material.

The percentage saccharification of each sawdust material was calculated by dividing the resultant mass of sugars produced through cellulase action by the total mass of the sawdust incubated multiplied by a hundred. These values indicate to what extent the sawdust was bio-converted into sugars and can also be used to conclude the relative saccharification of the various sawdust materials.

Statistical Analysis

All the experimental analyses were performed in triplicate, and the mean values with standard deviations were determined with Microsoft Excel.

RESULTS AND DISCUSSION

A major environmental problem facing cities and towns in Nigeria, especially along the Lagos Lagoon is the improper disposal of waste generated daily by activities of sawmills. Waste production relates to living and cannot be avoided, and such is the continuous production of sawdust by sawmills in the Lagos Lagoon area with an estimated number of 2000 sawmills (Sibiya et al. 2020). The abandonment of sawdust at the sawmills causes aesthetic problems as well as air pollution resulting in respiratory problems for many humans similar is the open-air combustion of sawdust which causes the release of carbon dioxide, smoke as well as NOx and the loss of potential useful energy into the environment. The indiscriminate incineration of sawdust is also responsible for producing greenhouse gases (Oluoti et al. 2014). With most sawdust management procedures resulting in negative environmental effects, it is important to consider environmentally benign management procedures



Fig. 1: Effect of changing pH-value on the degradation of delignified sawdust from Erythropleum suaveolens by A. niger cellulase.

and such an action could be the enzymatically catalyzed bioconversion of the cellulose component of sawdust into glucose a fermentable sugar that could be further developed as a feedstock for the synthesis of bioproducts such as bioethanol. The pH value at which the cellulase enzyme acts on sawdust is one of the catalytic variables which must be optimized during the saccharification with *A. niger* cellulase enzyme and the degradation of the delignified as well sawdust was performed at pH-values ranging between pH 4.0 to pH 7.0.

Fig. 1 represents the effect of changing pH values on the degradation of delignified sawdust from *Erythropleum*

suaveolens. The optimum pH for cellulase action was observed at pH 5.0 which showed the highest sugar production of 3.9 mg.mL⁻¹ and a 39% saccharification. The lowest sugar production was obtained at pH 6.0 resulting in a concentration of 1.5 mg.mL⁻¹ and a 15% saccharification while the highest amount of sugar produced at pH 5.0 was 2.6 times higher than the lowest amount of sugar produced. The saccharification of delignified cellulose from *Symphona globulifera* is illustrated in Fig. 2 showing optimum sugar production at a pH-value of 6.0 which is less acidic than the optimum pH-value of 5.0 that was observed during the maximum degradation of cellulose from *Erythropleum suaveolens*.



Fig. 2: Effect of changing pH-value on the degradation of delignified sawdust from Symphonia globulifera by A. niger cellulase.



Fig. 3: Effect of changing pH-value on the degradation of delignified sawdust from Ricindendron heudelotii by A. niger cellulase.



Fig. 4: Effect of changing pH-value on the degradation of delignified sawdust from Pterygota macrocarpa by A. niger cellulase.

The maximum amount of sugar produced from this *Symphonia globulifera* cellulose was calculated at a concentration of 2.8 mg.mL⁻¹ and a 28% saccharification. This sugar concentration was 2.2 times higher than the lowest amount of sugar produced at a concentration of 1.3 mg.mL⁻¹ during the saccharification at pH 4.5. The percentage saccharification at this lowest degree of saccharification was 13% while the second highest amount of sugar production was observed at a pH value of 5.0 producing a sugar concentration of 2.3 mg.mL⁻¹ and a 23% saccharification. Incubation of this cellulose at a pH value of 7,0 produced also a relatively high sugar amount at a concentration of 2.2 mg.mL⁻¹ and a 22% saccharification.

The saccharification of delignified cellulose from *Ricindendron heudelotti* (Fig. 3) showed optimum degradation at pH-values between 5.0 and 6.0 with the maximum amount of sugar produced at a concentration of 3.5 mg.mL⁻¹ produced at a pH value of 5.0 which decline to a value of 3.0 mg.mL⁻¹ when this bio-polymer was degraded at pH 6.0. During this degradation process the corresponding percentage of degradation declined from 35% to 30% saccharification with the lowest degree of saccharification observed at the pH-values of 4.0, 4.5, and 7.0 producing sugars at concentrations between 2.2 and 2.4 mg.mL⁻¹. The maximum amount of sugar produced at pH 5.0 was 1.6 times higher than the lowest sugar concentration produced when degraded at a pH value of 4.0.

When delignified cellulose from *Pterygota macrocarpa* (Fig. 4) was bio-converted into glucose with cellulase from *A. niger* the maximum amount of sugar was produced

at a concentration of 2.3 mg.mL⁻¹ resulting in a 3% saccharification. This amount of sugar was 1.5 times higher than the lowest sugar concentration of 1.5 mg.mL⁻¹ that was produced when the cellulose was degraded at a pH value of 4.0 causing a 15% saccharification. These results indicate that the delignified cellulose from this tree exhibits a relatively high susceptibility for degradation by the A. niger cellulase at all the pH values at which it was incubated. When Milicia excelsa cellulose (Fig. 5) was exposed to cellulase degradation the optimum degree of saccharification was obtained when the process was performed at a pH-value of 5.0 resulting in a sugar concentration of 3.0 mg.mL⁻¹ (30% saccharification). The produced sugar concentration decreased gradually when the incubation pH value was increased to 7.0 when a sugar concentration of 2.0 mg.mL⁻¹ was obtained at a saccharification of 20%. When the incubation pH value was decreased to a value of 4.0 the amount of sugar produced was also calculated at a concentration of 2.0 mg.mL⁻¹ which was 1.5 times less than the maximum amount of sugar produced.

The cellulase-catalyzed degradation of delignified sawdust from *Ipomoea asarifolia* (Fig. 6) was optimally degraded at a pH value of 5.0 resulting in a sugar concentration of 3.7 mg.mL⁻¹ and a 37% saccharification. The degree of saccharification decreased when saccharified at pH-values higher and lower than pH 5.0 with the minimum amount of sugar produced at a pH-value of 7.0 producing a sugar concentration of 1.5 mg.mL⁻¹ and a 15% saccharification. This amount of sugar produced was 2.5 times less than the highest sugar concentration whilst the second lowest



Fig. 5: Effect of changing pH-value on the degradation of delignified sawdust from Milicia excels by A. niger cellulase.



Fig. 6: Effect of changing pH-value on the degradation of delignified sawdust from Ipomoea asarifolia by A. niger cellulase.

sugar concentration was produced at 1.9 mg.mL⁻¹ (19% saccharification) when the cellulase was acting on this sawdust at a pH-value of 4.0. The degradation of cellulose from *Hallea ciliate* with *A. niger* cellulase is illustrated in Fig. 7 showing a saccharification pattern at the different pH values that are different from that obtained during the saccharification of the other sawdust materials. Optimum degradation was obtained over a relatively broad pH range which varied from pH 4.5 to pH 6.0 resulting in a sugar concentration of 2.9 mg.mL⁻¹ and a 29% saccharification. The lowest amount of sugar obtained at pH 4.0 and pH 7.0

was calculated at concentrations of 2.0 mg.mL⁻¹ and 1.9 mg.mL⁻¹, respectively.

When delignified cellulose from *Sacoglottis gabonensis* was treated with *A. niger* cellulase an optimum sugar concentration of 4.4 mg.mL⁻¹ was obtained when this bio-polymer was hydrolyzed at a pH value of 5.0 which resulted in a 44% saccharification (Fig. 8). At the higher pH values of 5.5 and 6.0, the amount of sugar produced decreased to concentrations of 3.3 mg.mL⁻¹ and 2.2 mg.mL⁻¹, respectively. During the incubation at a pH value of 7.0, the delignified cellulose showed an increased susceptibility for



Fig. 7: Effect of changing pH-value on the degradation of delignified sawdust from Hallea ciliate by A. niger cellulase.



Fig. 8: Effect of changing pH-value on the degradation of delignified sawdust from Sacoglottis gabonensis by A. niger cellulase.

the cellulase enzyme that resulted in a sugar concentration of 3.1 mg.mL⁻¹ (32% saccharification). At pH-values less than 5.0 the degradation of the cellulose component showed a lower degree of saccharification producing the lowest sugar concentration of 1.9 mg.mL⁻¹ (19% saccharification) when hydrolyzed with the cellulase enzyme at pH 4.0. The highest amount of sugar produced at pH 5.0 was 2.4 times higher than the lowest degree of saccharification obtained at a pH value of 4.0.

Fig. 9 represents the effect of changing pH values on the degradation of delignified sawdust from *Pycnanthus* angolensis. Optimum saccharification of this sawdust was obtained at two pH-values of pH 4.0 as well as at a pH value of 5.0 resulting in the formation of sugar at a concentration of 4.6 mg.mL⁻¹ (46% saccharification). The lowest degree of saccharification was observed when the incubation was performed at a pH-value of 7,0 which resulted in a sugar concentration of 1.8 mg.mL⁻¹ (18% saccharification) that was 2.5 times less than the highest amount of sugar produced. When delignified cellulose from *Terminalia superb* sawdust was degraded with the *A. niger* cellulase (Fig. 10) the amount of sugar produced showed an increased value from

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Fig. 9: Effect of changing pH-value on the degradation of delignified sawdust from Pycnanthus angolensis by A. niger cellulase.

2.4 mg.mL⁻¹ when exposed to the enzyme at a pH-value of 4.0 to the maximum amount of sugar produced at a concentration of 3.1 mg.mL⁻¹ when degraded at pH 5.5. When exposed to the enzyme at pH-values of 6.0 and 7,0 the amount of sugar produced decreased to values of 1.9 mg.mL⁻¹ and 1.0 mg.mL⁻¹, respectively. The lowest amount of sugar produced was 3.1 times less than the highest amount of sugar released at a concentration of 3.1 mg.mL⁻¹.

The pH value at which an enzyme-catalyzed reaction is performed is an important catalytic variable that must be optimized to ensure maximum product formation while converting the reactant (cellulose) into products (sugars). Fig. 11 reflects the pH values at which optimum cellulase activity was obtained while bio-converting the sawdust from different trees along the Lagos Lagoon into sugars, mainly glucose. From these results, it can be concluded that



Fig. 10: Effect of changing pH-value on the degradation of delignified sawdust from Terminalia superb by A. niger cellulase.



Fig. 11: Optimum pH values for maximum sugar production when sawdust from different trees along the Lagos Lagoon in Nigeria are saccharified with *A. niger* cellulase.

optimum saccharification for all the sawdust materials was obtained at pH values varying between pH 5.0 and pH 6.0 with the sugar concentration varying between 2.3 mg.mL⁻¹ and 6 mg.mL⁻¹. These optimum pH values for the *A. niger* cellulase catalyzed degradation of delignified sawdust are like pH values for maximum degradation of agricultural waste at a pH 4.5 by cellulase from *Clostridium thermocellum* (Mutreja et al. 2011), filter paper with cellulase from *Phialophora sp. G5* (Zhao et al. 2012) at a pH value of 6.0,

degradation of carboxymethyl cellulose with cellulase from *Streptomyces argentomyces* at a pH-value of 5.0 (Ventorino et al. 2016) and the optimum activity of cellulase from *Trichoderma reesei* when hydrolyzing the B-D-glycosidic bond in p-nitrophenyl-B-D-cellobioside at a pH-value of 5.5 (He et al. 2019).

The difference in the amount of sugar released from the sawdust materials is an indication of the varying degree of susceptibility of these delignified materials for the *A*. *niger* cellulase enzyme. Sawdust is a major waste product of the forestry industry, and its cellulose component exhibits a strong potential for degradation into glucose a fermentable sugar that could be used as a feedstock for the synthesis of bioproducts such as bio-ethanol (Vasic et al. 2021). Delignification has been proven to be an effective

pretreatment procedure for wood materials rendering the cellulose component more susceptible to cellulase-catalyzed degradation and the positive effect of this pretreatment of sawdust is well documented (Suryadi et al. 2022). Several structural features of cellulose such as the crystallinity (Park et al. 2010) as well as the amorphous parts (Ruel et. al. 2012) influence the degree of cellulose bioconversion as the interaction between cellulose and the cellulase enzyme is dependent on the relative presence of each of these sections in the cellulose molecule. The crystalline section is less susceptible for cellulase degradation although certain components of the cellulase enzyme are aimed to degrade this part of cellulose whilst the amorphous section is more available for cellulase action in general. Delignification aims to destroy the interaction between lignin and cellulose thus rendering cellulose more available for cellulase action by exposing the various sections for attack and action by the cellulase enzyme.

Since delignification results in an increased degree of cellulase-catalyzed degradation of cellulose, the current investigation was performed on delignified sawdust from different trees determining how different incubation pH-values could affect the saccharification of the different sawdust materials when degraded with *A. niger* cellulase.

CONCLUSIONS

Environmental pollution, fossil fuel combustion, and the development of alternative and renewable energy resources are currently topical global issues. The accumulation of solid waste does not only occupy valuable land, but gases released during the fermentation of the organic part of organic solid waste have a negative effect on humans and animals. Simultaneously are gases released during the combustion of fossil fuels described as dangerous to the environment and evidence of this phenomenon is climate change already experienced by many communities. A possible solution to both these two environmental issues could be the development of waste cellulose as a renewable feedstock for the synthesis of bioproducts. In this respect sawdust could be a suitable organic waste material and the development of sawdust would not only assist in limiting the accumulation of solid waste but can also limit the dependence on fossil fuels. When subjected to cellulase-catalyzed saccharification it would be important to optimize the catalytic properties such as the pH value ensuring maximum degradation of organic waste material such as sawdust. For maximum degradation of different waste cellulose materials, it is thus important that the optimum pH value for degradation of each type of organic waste material be determined before performing a large-scale bioconversion into fermentable sugars.

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