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Temperature-related Saccharification of Delignified Sawdust Materials from the Lagos Lagoon in Nigeria

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ABSTRACT

Sawdust, a product of the forest industry is mostly left untreated as solid waste. This phenomenon is well observed along the Lagos Lagoon in Nigeria where hundreds of trees are cut daily by sawmills to deliver wood for mainly the furniture industry. Different types of trees are utilized in this manner and the massive amounts of sawdust produced as a result of these activities are polluting the environment causing health risks for humans and animals. Cellulose, a glucose bio-polymer is a major structural component of sawdust and could be developed as a renewable energy resource should the cellulose be degraded into glucose, a fermentable sugar. This saccharification was done with *Aspergillus niger* cellulase and to make the cellulose more susceptible for cellulase action the sawdust was delignified with hydrogen peroxide. Both delignified and non-delignified sawdust were treated with the cellulase enzyme at incubation temperatures of 30°C, 40°C, 50°C, and 60°C. Delignification proved to be effective as an increased amount of sugar was released from all delignified sawdust materials relative to the non-delignified materials when saccharified with *A. niger* cellulase. Most of the materials were degraded at an incubation temperature of 40°C and 50°C and the highest percentage saccharification of 58% was obtained during the degradation of delignifed cellulose from the tree, *Ricindendron heudelotti*

INTRODUCTION

Sawdust is a major waste product of the wood industry mainly produced by sawmills when cutting trees in forests such as the rain forest on the banks of the Lago Lagoon in Nigeria. Currently, limited economic procedures are in place to deal effectively with this massive amount of wood waste produced annually resulting in this plant material being classified as organic solid waste. Sawdust is mostly used as a feedstock for the production of low value-added applications such as the absorbent for nitro-glycerine or wastewater containing heavy metals, as plastic fillers, wood compost, and cardboard (Dong et al. 2022, Weber et al.1993).

The accumulated amount of sawdust along the Lagos Lagoon is a great environmental concern as it not only causes soil and water pollution but also has the potential to catch fire which results in air pollution, a major threat to human and animal lives. Airborne sawdust is another health concern especially when inhaled and it is of environmental concern when greenhouse gases are released from decomposed wood waste when left unattended for long periods. With increasing concerns regarding the effect of fossil fuel consumption on the environment, it is important to identify and develop renewable resources. It is also important to consider the utilization of energy resources with a lower carbon footprint and renewable biomass such as sawdust has emerged as an alternative source of biofuels and bio-based products (Chen et al. 2020).

As a result of the effect of fossil fuel combustion on the environment, the development of a bio-economy is thus an important strategy to counteract this negative phenomenon, and the utilization of nano-based biodegradable polymers is a suitable example of the application of renewable substances (Kargarzadeh et al. 2018). The cellulose component of lignocellulosic material, a glucose-based polysaccharide is not only described as inexhaustible, but it offers a great potential as feedstock when saccharified into glucose a fermentable sugar. Lignocellulosic materials are made up of cellulose (38-50%), hemicelluloses (23-32%), and lignin (15-25%) in a complex structure. Enzymatic hydrolysis is one of the best methods used to convert cellulosic materials into soluble sugars and

requires low energy demand regardless of the difficulty of the low cellulose accessibility due to the strong linkage of cellulose with lignin (Gupta et al. 2011). To increase cellulolytic enzyme activity in terms of speed and efficiency the lignocellulosic materials can be physically and chemically pretreated to remove or modify the lignin and or hemicellulose thus increasing the pore space and allow more enzyme accessibility to the cellulose micro-fibrils (Raymond & Maazuza 2015).

The cellulose structure is mostly crystalline while hemicellulose exhibits an amorphous composition because of its branched structure. Hemicellulose is therefore relatively easy to hydrolyze to its monomer sugars compared to the degradation of cellulose (Guerra-Rodrı´guez et al. 2012). In industry, cellulases are used for the preparation of medicines, perfumes, resins, starch, baking, treatment of waste, and mostly for bioethanol production from lignocellulosic biomass (Sudhanshu & Ramesh 2016). Cellulase enzymes have been isolated from various fungal resources such as species from *Aspergillus*, *Trichoderma*, *Penicillium,* and *Neurospora* as well as bacterial resources from species like *Clostridium*, *Cellulomonas*, *Pseudomonas,* and *Ruminococcus* (Sajith et al. 2016). The saccharification action of cellulase on various waste cellulose materials such as paper (Van Wyk & Sibiya 2016) and kitchen waste (Ga et al. 2015) has been reported.

The current investigation reveals information on *A. niger* cellulase-catalyzed saccharification of sawdust from five different types of trees along the Lagos Lagoon in Nigeria. This research aimed to investigate the possibility of producing fermentable sugars from the cellulose content of sawdust from various trees. To increase the susceptibility of the cellulose to cellulase action the various sawdust materials have been delignified with the Kraft process (Gustafson et al. 1983) as well as hydrogen peroxide treatment (Ndukwe et al. 2009). The amount of sugar released from the delignified sawdust was compared with the extent of sugar formation from the non-delignified materials and the importance of delignifying cellulose from waste cellulose before cellulase-catalyzed saccharification was confirmed.

MATERIALS AND METHODS

Sawdust Substrate and Cellulase Enzyme

Non-delignified and delignified sawdust samples (10 mg) from five different trees along the Lagos Lagoon in Nigeria were transferred in triplicate into test tubes. Names of these sawdust samples are *Erythropleum suaveolens, Symphonia globulifera, Ricindendron heudelotii, Pterygota macrocarpa,* and *Milicia excelsa*. Commercially obtained *A. 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}. This enzyme solution was used to perform the saccharification of the various delignified and non-delignified sawdust samples.

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 NaS_2 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.

Cellulase Incubation and Sugar Analyses

The weighed sawdust material in the tubes was incubated with the *A. niger* cellulase enzyme solution (200 uL) and Tris buffer solution pH 5,0 (800 uL) for 2h at different temperatures of 30°C, 40°C, 50°C, and 60°C. The concentration of sugars released from the sawdust materials during cellulase-catalyzed 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 as a result 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 bioconverted 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

Cellulose a glucose-based biopolymer is an important structural component of plant materials and this substance is described as the most underdeveloped and thus also the greatest under-utilized global renewable energy resource (Fatma et al. 2018). A reason why this bio-compound is not commercially developed as an energy resource could be because fossil fuel technology is well established and the global economy is completely dependent and developed on this energy source (Olson & Lenzmann 2016). Another issue in preventing the use of cellulose as a resource for the development of renewable substances could be the fact that it is classified as waste and the bio-recycling technology is not well developed. With the negative effects of fossil fuel consumption on the environment becoming more evident it will convince more scientific agencies to consider alternative and renewable energy resources as feedstock for the synthesis of many commodities. Currently, waste cellulose is a major structural component of plant-derived materials and is classified as organic waste which is discarded without being considered as a potential clean energy resource. Cellulose, based on its richness in glucose, a fermentable sugar, is a suitable contender to be developed as an energy resource should glucose be freed effectively from the cellulose structure. Glucose obtained from waste cellulose in plant materials could not only be fermented into an energy-rich compound such as bio-ethanol which is environmentally friendly, but the process would also have a positive effect on the environment as the amount of organic solid waste occupying value land could be limited (Kaur et al. 2013).

The saccharification of waste cellulose is a process that can be achieved by strong alkaline (Glaus & Van Loon, 2008) as well as acidic (Zhou et al. 2021) treatments but these processes are not environmentally benign as both used acid and alkaline agents would have also negative effects on the environment. These acidic and alkaline hydrolytic substances need therefore to be neutralized before being released in the environment, mostly in wastewater resources and these steps will further increase the cost of effectively recovering glucose from cellulose. The enzymatic catalyzed degradation of cellulose employing a hydrolytic enzyme system known as cellulase into glucose is an attractive means of developing waste cellulose as an energy resource in an effective and environmentally clean way (Agostinho et al. 2015). Effective enzymatic catalyzed reactions depend on several catalytic properties such as enzyme concentration (Kaschuk et al. 2019), substrate concentration (Carver 2019), pH (Ye et al. 2017) as well as the effect of reaction temperature (Li et al. 2019). This investigation obtained information on the relative saccharification of different delignified and non-delignified sawdust materials when bioconverted by the *A. niger* cellulase into sugars.

Delignified (10 mg) and non-delignified sawdust (10 mg) obtained from various trees along the Lagos Lagoon have been exposed to different incubation temperatures of 30°C, 40°C, 50°C and 60°C in the absence as well as in the presence of *A. niger* cellulase enzyme. This investigation was to conclude the incubation temperature for the optimum bio-conversion of these waste cellulose materials into glucose. The incubation of sawdust in the absence of the cellulase enzyme served as a control to determine the amount of free sugar released from these materials when not degraded by the enzyme.

Fig. 1 represents the concentration of sugar released from delignified and non-delignified *Erythropleum suaveolens* sawdust in the absence as well as in the presence of *A. niger* cellulase. The resultant amount of sugar released from the delignified as well as non-delignified sawdust bio-converted with the cellulase enzyme is summarized in Fig. 2.

From the results represented in Fig. 1, it can be concluded that the amount of sugar released from non-delignified sawdust in the absence of cellulase when incubated at all the temperatures resulted in a concentration that varies between 1.2-1.7 $mg.mL^{-1}$. In the case of the delignified materials, the amount of sugar released when no cellulase was acting on the sawdust at the various incubation temperatures was slightly higher than the amount of free sugar released from the non-delignified sawdust at values varied between 1.8 and 3.3 $mg.mL^{-1}$. The general trend for sugars released from the non-delignified sawdust when incubated with the cellulase enzyme is higher than the amount of sugars released from the corresponding sawdust materials not treated with the cellulase enzyme and the values varied between 2.6 and 3.9 $mg.mL^{-1}$ when incubated at the various temperatures. A similar observation was made when the delignified cellulose was treated with the cellulase enzyme and during this incubation at the various incubation temperatures, the sugars produced varied between 3.8 and 6.7 $mg.mL^{-1}$ when incubated at the various temperatures.

The resultant amount of sugar released from the non-

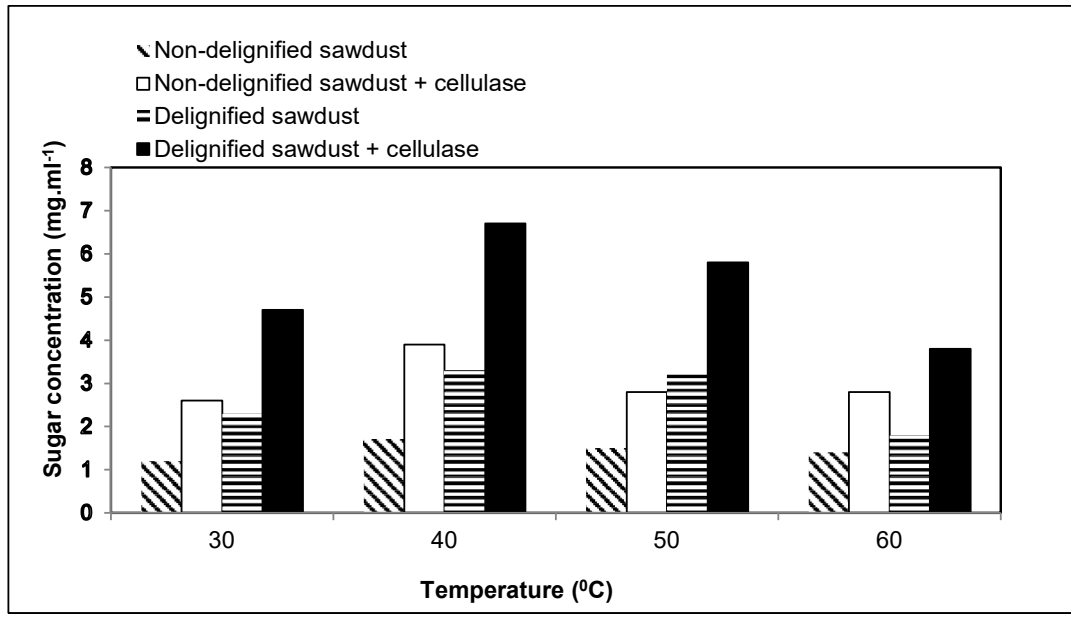

Fig. 1: Amount of sugar released from non-delignified and delignified *Erythropleum suaveolens* sawdust in the absence and presence of *A. niger* cellulase.

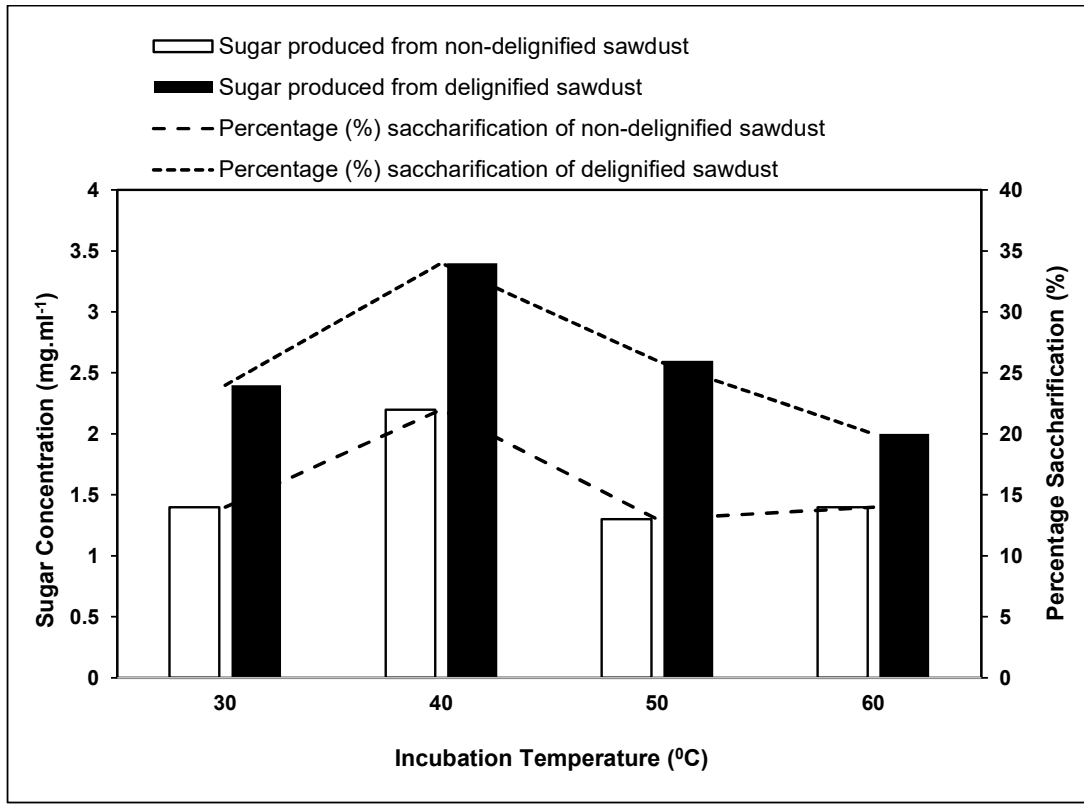

Fig. 2: Resultant amount of sugar produced (mg.mL^{-1}) from non-delignified and delignified *Erythropleum suaveolens* sawdust when bioconverted with *A. niger* cellulase.

delignified and delignified sawdust (Fig. 2) indicates that more sugar was produced from the delignified sawdust at all the incubation temperatures than the concentration of sugars released from the corresponding sawdust not delignified before cellulase catalyzed saccharification. The sugar concentrations obtained from the non-delignified sawdust varied between 1.3 and 2.2 mg.mL^{-1} whilst the amount released from the delignified sawdust varied between 2.0 and 3.4 mg.mL^{-1}. When the non-delignified material was bio-converted with the *A. niger* cellulase the highest sugar concentration was obtained at a temperature of 40°C which resulted in a sugar concentration of 2.2 mg.mL^{-1} and a 22% saccharification. The highest degree of saccharification was obtained from the delignified sawdust when it was incubated at a temperature of 40°C producing a sugar concentration of 3.4 mg.mL^{-1} and 34% saccharification. The amount of sugar produced from the delignified sawdust at the optimum incubation temperature of 40°C was 154% higher than the maximum amount of sugar released from the non-delignified sawdust released when incubated at the optimum incubation temperature of 40°C.

Fig. 3 represents the concentration of sugar released from delignified and non-delignified *Symphona globuifera* sawdust in the absence as well as in the presence of *A. niger* cellulase. The resultant amount of sugar released from the delignified as well as non-delignified sawdust bio-converted with the cellulase enzyme is summarized in Fig. 4.

From the results represented in Fig. 3, it can be concluded that the amount of free sugar released from non-delignified sawdust (in the absence of cellulase) when incubated at all the temperatures resulted in a concentration that varies between 1.5-2.2 mg.mL^{-1}. In the case of the delignified materials, the amount of free sugar released in the absence of cellulase at the various incubation temperatures was slightly higher than the amount of free sugar released from the non-delignified sawdust at values varied between 1.8 and 2.7 mg.mL^{-1}. The general trend for sugars released from the non-delignified sawdust when incubated with the cellulase enzyme is higher than the amount of sugars released from the corresponding sawdust materials not treated with the cellulase enzyme and the values varied between 3.1 and 4.9 mg.mL^{-1} when incubated at the various temperatures. A similar observation was made when the delignified cellulose was treated with the cellulase enzyme and during this incubation at the various incubation temperatures, the sugars produced varied between 3.8 and 6.2 mg.mL^{-1} when incubated at the various temperatures.

The resultant amount of sugar released from the non-delignified and delignified sawdust (Fig. 4) indicates that more sugar was produced from the delignified sawdust at all the incubation temperatures than the concentration of sugars released from the corresponding sawdust not delignified before cellulase catalyzed saccharification. The sugar concentrations obtained from the non-delignified sawdust varied between 1.3 and 3.4 mg.mL^{-1} whilst the amount released from the delignified sawdust varied between 2.0

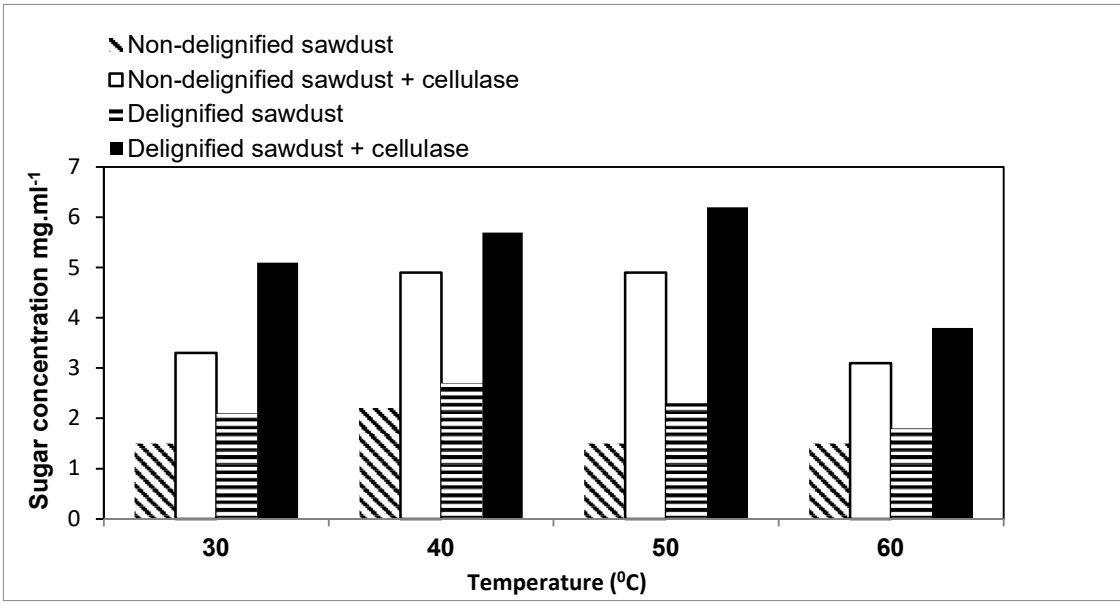

Fig. 3: Amount of sugar released from non-delignified and delignified *Symphona globuifera* sawdust in the absence and presence of *A. niger* cellulase.

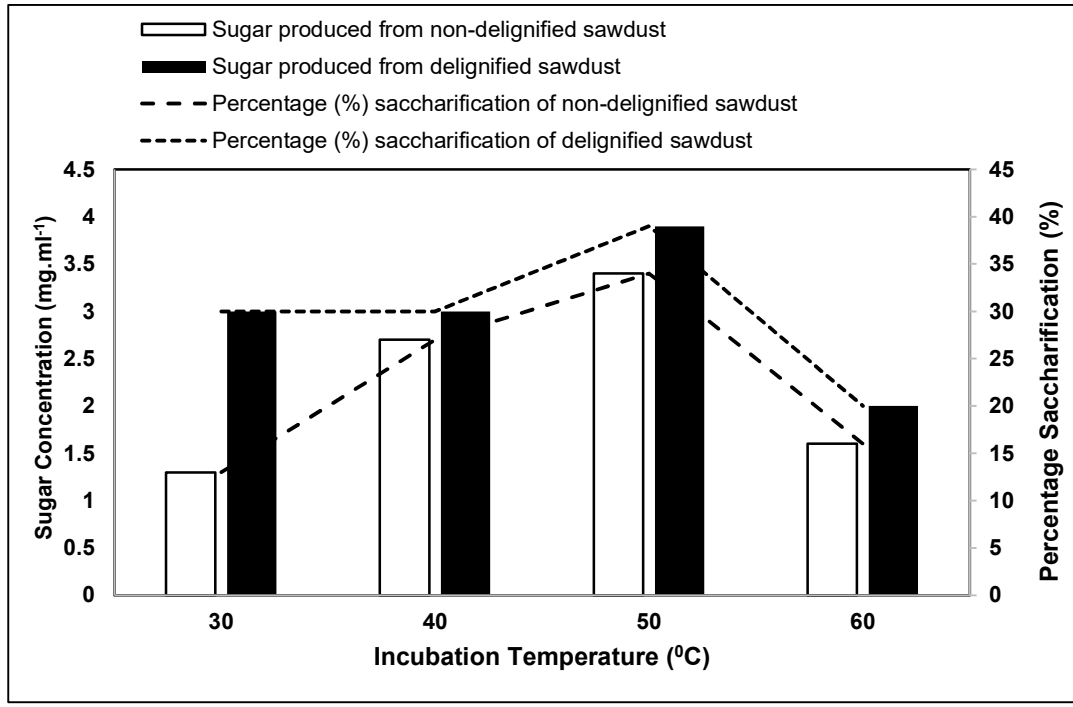

Fig: 4: Resultant amount of sugar produced (mg.mL^{-1}) from non-delignified as well as delignified *Symphona globuifera* sawdust when bioconverted with *A. niger* cellulase.

and 3.9 mg.mL^{-1}. When the non-delignified material was bio-converted with the *A. niger* cellulase the highest sugar concentration was obtained at a temperature of 50°C which resulted in a sugar concentration of 3.4 mg.mL^{-1} and a 34% saccharification. The highest degree of saccharification was obtained from the delignified sawdust when it was incubated at a temperature of 50°C producing a sugar concentration of 3.9 mg.mL^{-1} and 39% saccharification. The amount of sugar produced from the delignified sawdust at an optimum incubation temperature of 50°C was 114% higher than the maximum amount of sugar released from the non-delignified sawdust released when incubated at an optimum incubation temperature of 50°C.

Fig. 5 represents the concentration of sugar released from delignified and non-delignified *Ricindendron heudelotti* sawdust in the absence as well as in the presence of *A. niger* cellulase. The resultant amount of sugar released from the delignified as well as non-delignified sawdust bio-converted with the cellulase enzyme is summarized in Fig. 6.

From the results represented in Fig. 5, it can be concluded that the amount of free sugar released from non-delignified sawdust when incubated at all the temperatures resulted in a concentration that varies between 2.6-3.4 mg.mL^{-1}. In the case of the delignified materials, the amount of free sugar released at the various incubation temperatures was slightly higher than the amount of free sugar released from the non-delignified sawdust at values varied between 6.5 and 9.9 mg.mL^{-1}. The general trend for sugars released from the non-delignified sawdust when incubated with the cellulase enzyme is higher than the amount of sugars released from the corresponding sawdust materials not treated with the cellulase enzyme and the values varied between 5.5 and 6.8 mg.mL^{-1} when incubated at the various temperatures. A similar observation was made when the delignified cellulose was treated with the cellulase enzyme and during this incubation at the various incubation temperatures, the sugars produced varied between 10.2 and 13.7 mg.mL^{-1} when incubated at the various temperatures.

The resultant amount of sugar released from the non-delignified and delignified sawdust (Fig. 6) indicates that more sugar was produced from the delignified sawdust at all the incubation temperatures than the concentration of sugars released from the corresponding sawdust not delignified before cellulase catalyzed saccharification. The sugar concentrations obtained from the non-delignified sawdust varied between 2.9 and 3.7 mg.mL^{-1} whilst the amount released from the delignified sawdust varied between 3.5 and 5.8 mg.mL^{-1}. When the non-delignified material was bio-converted with the *A. niger* cellulase the highest sugar concentration was obtained at a temperature of 50°C which resulted in a sugar concentration of 3.7 mg.mL^{-1} and a 37%

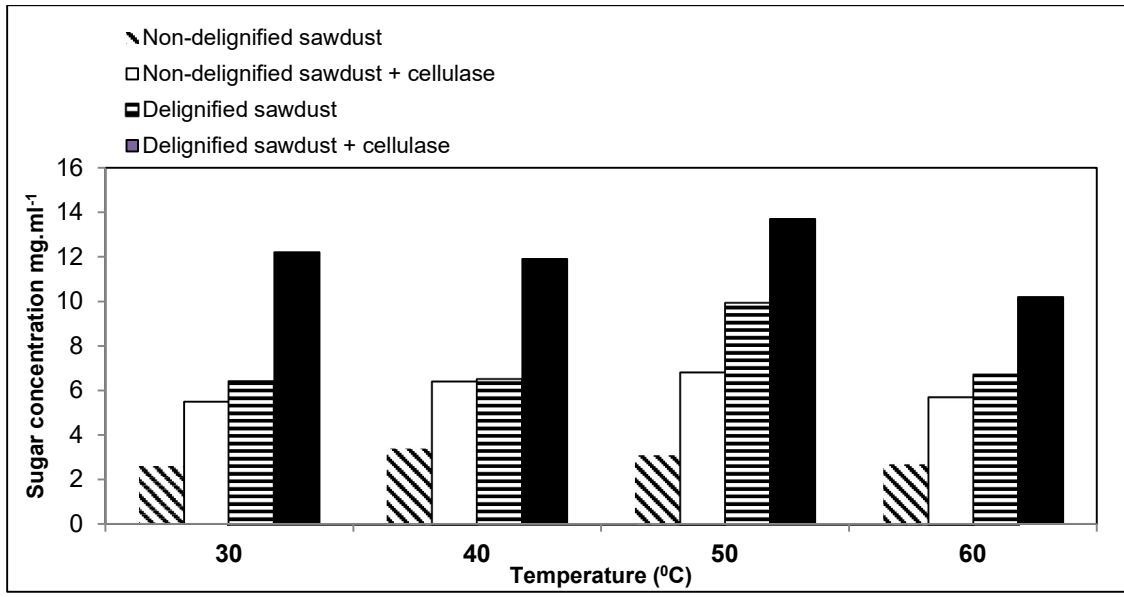

Fig. 5: Amount of sugar released from non-delignified and delignified *Ricindendron heudelotti* sawdust in the absence and presence of *A. niger* cellulase.

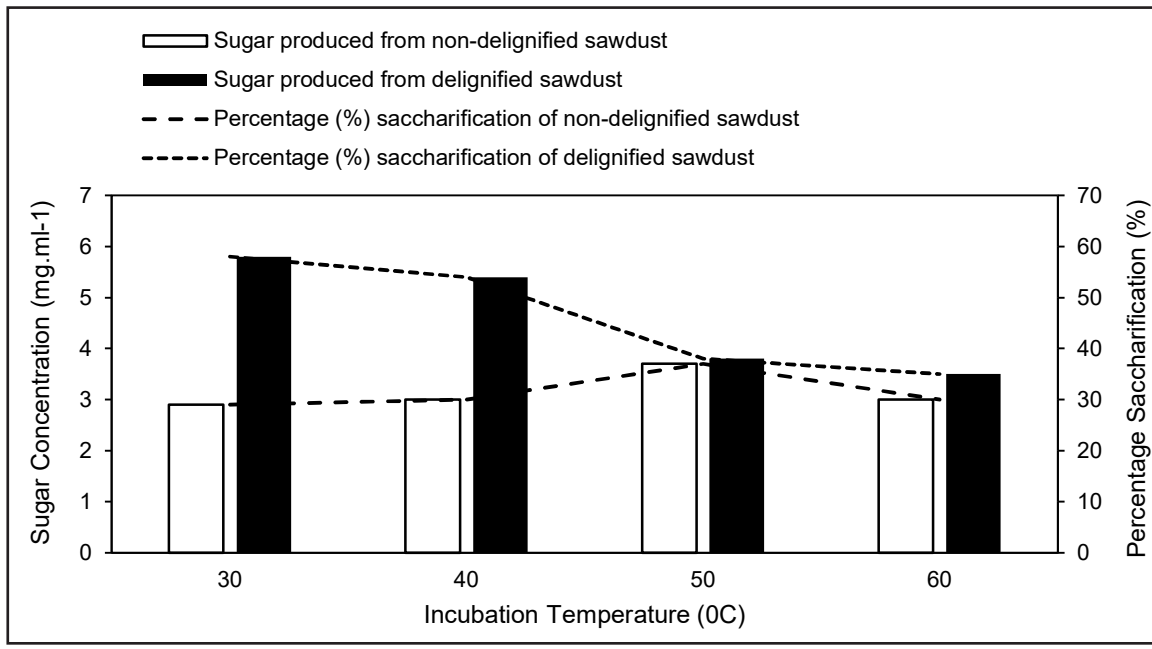

Fig. 6: Resultant amount of sugar produced (mg.mL^{-1}) from non-delignified as well as delignified *Ricindendron heudelotti* sawdust when bioconverted with *A. niger* cellulase.

saccharification. The highest degree of saccharification was obtained from the delignified sawdust when it was incubated at a temperature of 30°C producing a sugar concentration of 5.8 mg.mL^{-1} and 58% saccharification. The amount of sugar produced from the delignified sawdust at the optimum incubation temperature of 30°C was 156% higher than the maximum amount of sugar released from the non-delignified sawdust released when incubated at the optimum incubation temperature of 40°C.

Fig. 7 represents the concentration of sugar released from delignified and non-delignified *Pterygota macrocarpa* sawdust in the absence as well as in the presence of *A. niger*

cellulase. The resultant amount of sugar released from the delignified as well as non-delignified sawdust bio-converted with the cellulase enzyme is summarized in Fig. 8.

From the results represented in Fig. 7, it can be concluded that the amount of free sugar released from non-delignified sawdust when incubated at all the temperatures in the absence of cellulase resulted in a concentration that varies between 2.7-2.9 mg.mL^{-1}. In the case of the delignified materials, the amount of free sugar released at the various incubation temperatures was slightly higher than the amount of free sugar released from the non-delignified sawdust at values varied between 3.5 and 8.7 mg.mL^{-1}. The general

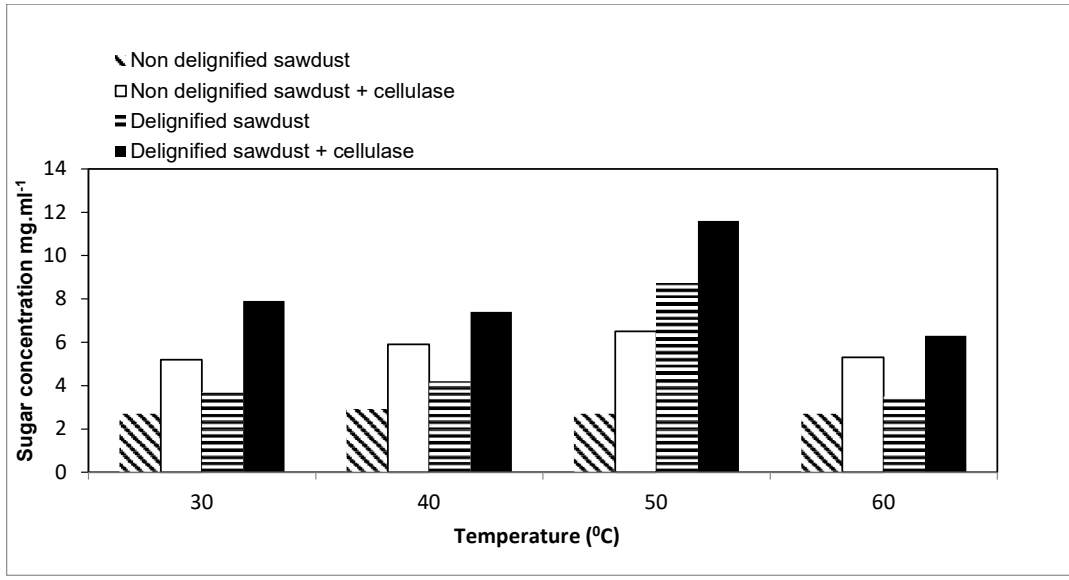

Fig. 7: Amount of sugar released from non-delignified and delignified *Pterygota macrocarpa* sawdust in the absence and presence of *A. niger* cellulase.

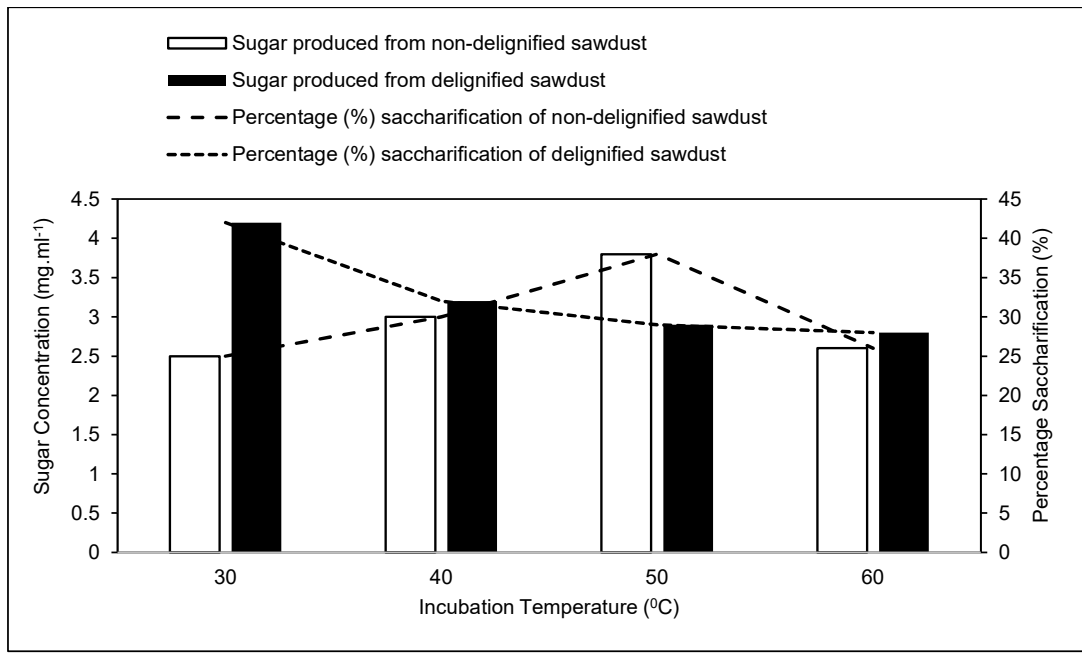

Fig. 8: Resultant amount of sugar produced (mg.mL^{-1}) from non-delignified as well as delignified *Pterygota macrocarpa* sawdust when bioconverted with *A. niger* cellulase.

trend for sugars released from the non-delignified sawdust when incubated with the cellulase enzyme is higher than the amount of sugars released from the corresponding sawdust materials not treated with the cellulase enzyme and the values varied between 5.2 and 6.5 mg.mL^{-1} when incubated at the various temperatures. A similar observation was made when the delignified cellulose was treated with the cellulase enzyme and during this incubation at the various incubation temperatures, the sugars produced varied between 6.3 and 11.6 mg.mL^{-1} when incubated at the various temperatures.

The resultant amount of sugar released from the non-delignified and delignified sawdust (Fig. 8) indicates that more sugar was produced from the delignified sawdust at all the incubation temperatures than the concentration of sugars released from the corresponding sawdust not delignified prior to cellulase catalyzed saccharification. The sugar concentrations obtained from the non-delignified sawdust varied between 2.5 and 3.8 mg.mL^{-1} whilst the amount released from the delignified sawdust varied between 2.8 and 4.2 mg.mL^{-1}. When the non-delignified material was bio-converted with the *A. niger* cellulase the highest resultant sugar concentration was obtained at a temperature of 50°C which resulted in a sugar concentration of 3.8 mg.mL^{-1} and a 38% saccharification. The highest degree of saccharification was obtained from the delignified sawdust when it was incubated at a temperature of 30°C producing a sugar concentration of 4.2 mg.mL^{-1} and 42% saccharification. The amount of sugar produced from the delignified sawdust at the optimum incubation temperature of 30°C was 110% higher than the maximum amount of sugar released from the non-delignified sawdust released when incubated at the optimum incubation temperature of 40°C.

Fig. 9 represents the concentration of sugar released from delignified and non-delignified *Micilia excelsa* sawdust in the absence as well as in the presence of *A. niger* cellulase. The resultant amount of sugar released from the delignified as well as non-delignified sawdust bio-converted with the cellulase enzyme is summarized in Fig. 10.

From the results represented in Fig. 9, it can be concluded that the amount of free sugar released from non-delignified sawdust when incubated at all the temperatures resulted in a concentration that varies between 1.9-2.3 mg.mL^{-1}. In the case of the delignified materials, the amount of free sugar released in the absence of cellulase at the various incubation temperatures was slightly higher than the amount of free sugar released from the non-delignified sawdust at values varied between 5.3 and 6.2 mg.mL^{-1}. The general trend for sugars released from the non-delignified sawdust when incubated with the cellulase enzyme is higher than the amount of sugars released from the corresponding sawdust materials not treated with the cellulase enzyme and the values varied between 3.4 and 4.1 mg.mL^{-1} when incubated at the various temperatures. A similar observation was made when the delignified cellulose was treated with the cellulase enzyme and during this incubation at the various incubation temperatures, the sugars produced varied between 8.8 and 9.8 mg.mL^{-1} when incubated at the various temperatures.

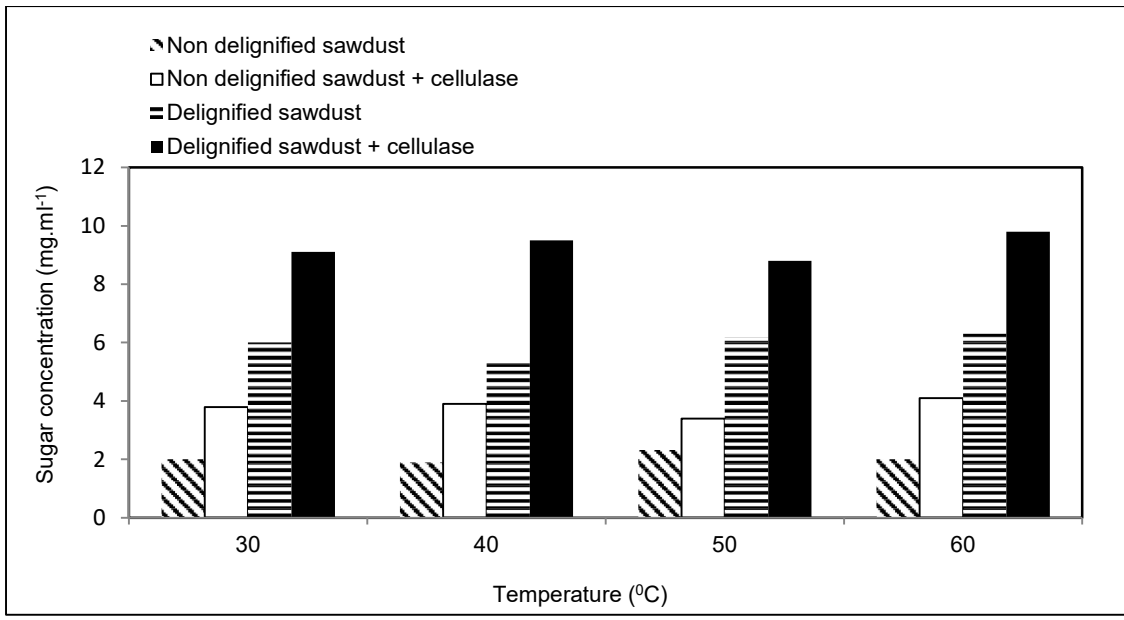

Fig. 9: Amount of sugar released from non-delignified and delignified *Micilia excelsa* sawdust in the absence and presence of *A. niger* cellulase.

The resultant amount of sugar released from the non-delignified and delignified sawdust (Fig. 10) indicates that more sugar was produced from the delignified sawdust at all the incubation temperatures than the concentration of sugars released from the corresponding sawdust not delignified before cellulase catalyzed saccharification. The sugar concentrations obtained from the non-delignified sawdust varied between 1.1 and 2.1 mg.mL^{-1} whilst the amount released from the delignified sawdust varied between 2.6 and 4.2 mg.mL^{-1}. When the non-delignified material was bio-converted with the *A. niger* cellulase the highest sugar concentration was obtained at a temperature of 60°C which resulted in a sugar concentration of 2.1 mg.mL^{-1} and a 21% saccharification. The highest degree of saccharification was obtained from the delignified sawdust when it was incubated at a temperature of 40°C producing a sugar concentration of 4.2 mg.mL^{-1} and 42% saccharification. The amount of sugar produced from the delignified sawdust at the optimum incubation temperature of 40°C was 200% higher than the maximum amount of sugar released from the non-delignified sawdust released when incubated at the optimum incubation temperature of 60°C.

Cellulase enzymes are biocatalysts responsible for the degradation of cellulose into sugars such as glucose. In case of the destruction of cellulose, the cellulase enzyme is responsible for the breaking of β-1,4-glucosidic bonds between glucose units releasing the glucose units which can further undergo changes such as fermentation by yeast

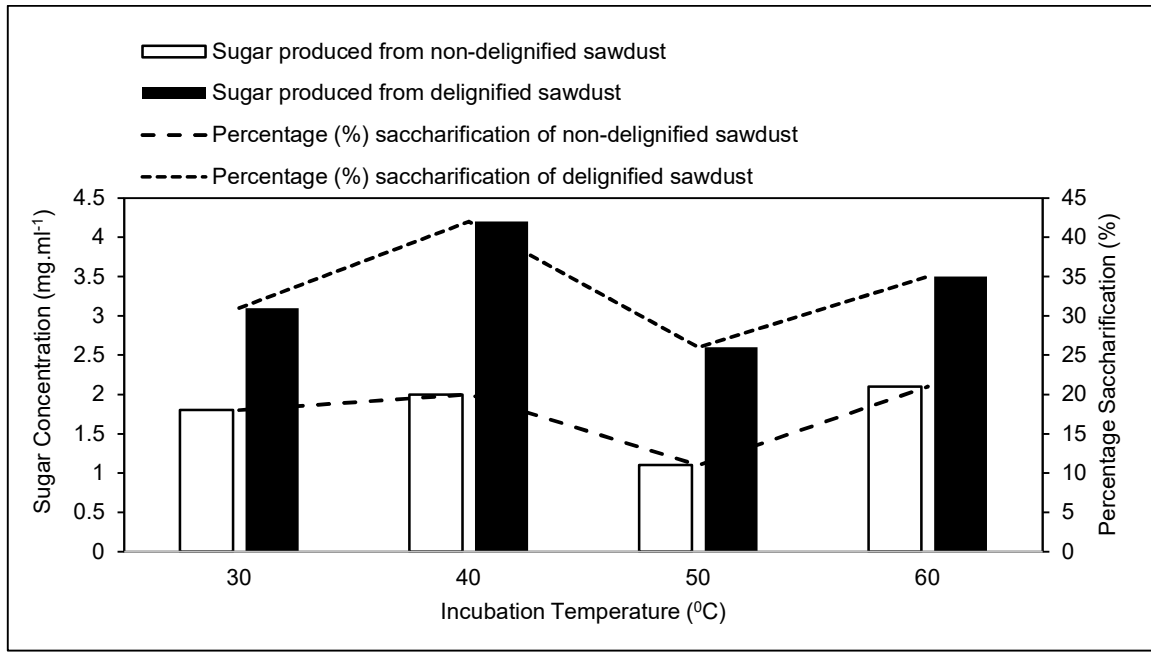

Fig. 10: Resultant amount of sugar produced (mg.mL^{-1}) from non-delignified as well as delignified *Micilia excelsa* sawdust when bioconverted with *A. niger* cellulase.

Table 1: Optimum incubation temperatures and maximum resultant sugar concentrations produced during saccharification of delignified and non-delignifoied sawdust from different trees along the Lagos Lagoon with *A. niger* cellulase.

Name of tree	Non-delignified cellulose		Delignified cellulose	
	Optimum temperature [^{o}C]	Optimum sugar concentration [mg.mL^{-1}]	Optimum temperature [^{o}C]	Optimum sugar concentration [mg.mL^{-1}]
Erythropleum suaveolens	40	2,2	40	3,4
Symphonia globulifera	50	3,4	50	3,9
Ricindendron heudelotii	50	3,8	30	5,8
Pterygota macrocarpa	50	3,8	30	4,2
Milicia excelsa	40	1,8	40	4,2

into environmentally friendly substances such as bioethanol (Zhou et al. 2023). These environmentally friendly procedures will become more in demand especially in cases such as the development of renewable energy resources as the negative effect of fossil fuel combustion will be realized by the global community (Xu et al. 2023). Various potential renewable energy resources such as solar (Khare et al. 2023), wind (Liu et al. 2023), and hydrogen (Khalil & Dincer 2023) are the focus of many development programs, with many already producing positive environmental results. Waste cellulose, which is part of organic waste, has also been identified as a potential energy resource not only for the energy released during combustion but also for its bioenergy potential when developed as a feedstock during the synthesis of bio-chemicals (Yu et al. 2023). To effectively utilize waste cellulose as a resource for bio-energy development needs an environmentally clean process to release glucose from the cellulose structure such as enzymatic catalyzed hydrolysis and incubation temperature is one of the catalytic variables which has to be optimized for maximum cellulase action in releasing glucose from the cellulose material. Another factor that would make the cellulose structure more susceptible to cellulase action and thus produce more fermentable sugar is the pretreatment of cellulose before being hydrolyzed with the cellulase enzyme. Pretreatment methods described as effective in this respect included physical methods such as the milling of cellulose (Hernandez-Varela et al. 2021) as well as chemical procedures (Wang et al. 2023). From the current investigation, it was observed that the hydrogen peroxide delignified sawdust resulted in an increased *A. niger* cellulase-catalyzed saccharification relative to non-delignified sawdust, irrespective of the incubation temperature.

The importance of optimizing the temperature at which the cellulase enzyme is acting on the cellulose structure when saccharifying this bio-polymer has been emphasized by numerous investigations. The optimum incubation temperatures for the bioconversion of both delignified and non-delignified sawdust were observed at mostly 40°C and 50°C, while a few optimal incubations were detected at 30°C. These values compare well with reported optimum cellulase catalytic incubation temperatures of 40°C when *Aspergillus niger* cellulase degrades carboxymethyl cellulose (Sulyman et al. 2020) and 55°C when cellulase from *Aspergillus fumigatus* (Saroj et al. 2022) also acting on carboxymethyl cellulose.

CONCLUSIONS

With the growing global population, the pressure on natural forests will increase as more trees will be needed for the production of wood-based commodities. During this process, more sawdust will also be produced, which currently is classified and treated as non-manageable solid waste occupying and polluting many water resources and land. The development of the cellulose component of sawdust as a bioenergy resource will not only assist in the production of a clean feedstock for the synthesis of many chemical-related substances but will also help to control environmental pollution. Cellulase-catalyzed bioconversion of cellulose is not only applicable to cellulose from sawdust but could be applied to all plant-based materials such as agricultural, kitchen, and garden waste. Although the cellulase-catalyzed saccharification of waste cellulose is a relatively clean process, its effectiveness in terms of sugar production and percentage saccharification of the cellulose materials depends on the optimization of many catalytic properties, such as incubation temperature. Since cellulose exhibits a complex structural composition, it offers a natural resistance against cellulase action, which seeks the treatment of cellulose before cellulase degradation, rendering it more susceptible to cellulase-catalyzed saccharification. Lignin is a natural component of plant structure that binds the cellulose, and the removal of lignin has a positive effect on the bio-degradation of cellulose. From this investigation, it was concluded that the delignification of cellulose before saccharification with a cellulase enzyme, as well as optimizing the incubation temperature between cellulose and cellulase, does have a positive effect on the extent of sugar production. This also indicates that more bio-products could be synthesized from waste cellulose when delignified and saccharified at optimum temperatures than from non-delignified cellulose or when incubated at a non-optimum incubation temperature.

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