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Development of sawdust from the Lagos Lagoon in Nigeria as a renewable feedstock for bio-product development

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Abstract

The accumulation of solid waste and consumption of fossil fuels are two phenomenons which already have a major destructive effect on the environment. The lack of alternative solid waste management procedures and shortage of the development of renewable energy resources should be addressed in order to sustain environmental quality. Sawdust is a major waste product along the Lagos lagoon with cellulose one of the predominant structural components of sawdust. The bio-conversion of waste cellulose, a glucose biopolymer into glucose a fermentable sugar has been performed with cellulase from *Aspergillus Niger*. Delignified and non-delignified sawdust from five different trees along the Lagos Lagoon have been saccharified with *A. niger* cellulase. The saccharification of these sawdust materials have been performed at different incubation temperatures of 30°C, 40°C, 50°C and 60°C. Optimum saccharification of non-delignified and delignified cellulose from the various trees along the Lagos Lagoon were optimum saccharified at different temperatures resulting in different sugar concentrations produced. A temperature of 40°C was optimum for maximum degradation of non-delignified cellulose from all the trees producing sugar at concentration between 3.0 - 4.3mg.ml^{-1}. Optimum saccharification of delignified cellulose from all the trees was obtained at a temperature of 50°C resulting in a sugar concentration of 5.9 – 8.4mg.ml^{-1}.

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Introduction

Lagos the capital city of Nigeria has a growing population and this leads to increased business activities. The city is located close to the rain forest with a lot of trees which results in many sawmills of different sizes located on the shores of the Lagos Lagoon. A lot of solid waste is generated by these sawmills which include sawdust, wood off cuts, wood backs, plain shavings and wood rejects that are not properly discarded. It is a habit that these solid waste materials are burnt in open air along banks of the Lagos Lagoon causing serious air pollution or these substances are dumped into the lagoon (Dosumnu & Ajayi, 2002). Other sources of lignocellulose that pollute the lagoon include abandoned wooden boats, seaweeds, the water hyacinth (*Eichornia crassipes*), grasses (*Paspalum* sp.*),* paper wastes, sugarcane bagasse and fruit peels which are constantly dumped into the lagoon and which decomposes after a long period of time. These lignocellulosic wastes such as sawdust and other wood related materials can be biodegraded and converted into useful products such as biofuels, bioethanol, textile dyes, food additives and chemicals of medical importance (Buraimoh *et al.,* 2015).

Sawdust is known to be carcinogenic and when not properly disposed, heaps accumulates in the environment, becomes airborne and when inhaled threatens the health and safety of residents (Odusote *et al.,* 2016). Wood waste decomposes when it is left unattended over long periods of time during which time it releases a harmful greenhouse gas such as methane and these heaps also become the breeding ground for pests and vectors of diseases (Owoyeni *et al.,* 2016). There is an estimation of more than 2,000 sawmills that exist in Nigeria and this industry produces large quantities of wood waste daily, which is dumped on land causing major environmental pollution (Oluoti *et al.,* 2014). Nigeria generates about 1.8 million tons of sawdust and 5.2 million tons of wood waste, annually (Owoyeni, *et al.,* 2016). There is a significant amount of cellulose in the plant biomass which can be utilized as a valuable carbon resource for production of value-added chemicals (Veeresh and Jin, 2014). It is also apparent that cellulose is the single most inexhaustible organic molecule on earth and the dominating waste material from agriculture. Cellulosic materials also known as lignocellulosic materials are composed of lignin, hemicellulose and cellulose**.** Wood consists mostly of cellulose and lignin, whereas cotton and paper consist of pure cellulose (Adeeyo, *et al.,* 2015) 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 applied methods used to convert cellulosic materials into soluble sugars and this process requires a low energy demand regardless of the low cellulose accessibility by cellulase enzymes due to a strong linkage of cellulose with lignin (Gupta, *et al*., 2011). Cellulose is a plant cell wall polysaccharide that is insoluble in water and is made up of repeated units of β-D-glucopyranose units which are interlinked by β-1,4-glycosidic bonds. This biopolymer is also intertwisted with lignin and hemicellulose carbohydrate polymers (George and Sabapathi, 2015). Lignin provides structural support for plants with a higher lignin content in trees than grasses. No sugars are found in lignin but it makes cellulose and hemicellulose molecules difficult to reach by covering both of them Adeeyo, *et al*., 2015).

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 increase the pore space and allowing more enzyme accessibility to the cellulose micro-fibrils (Raymond and Maazuza, 2015). The cellulose structure is mostly crystalline of nature 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-Rodriguez, *et al*., 2012). In industry cellulases are used for the preparation of medicines, perfumes, resins, starch, and for treatment of organic waste and mostly for bioethanol production from lignocellulosic biomass (Sudhandshu and 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 wastepaper and kitchen waste have been reported (Van Wyk and Sibiya, 2014; Gao *et al*., 2015).

The current investigation reveals the relative amount of sugar produced during *A. niger* cellulase catalyzed saccharification at different incubation temperatures of chemical pretreated and non-pretreated cellulose obtained from five different types of trees along the Lagos Lagoon in Nigeria. The sawdust materials have been pretreated by means of delignification with the Kraft process (Gustafson *et al*., 1983) as well as hydrogen peroxide treatment (Sun *et al*., 2000) and it also illustrate the effective role of delignification in rendering cellulose more susceptible for cellulase catalyzed bio-conversion into glucose, fermentable sugar.

Materials and methods

Sawdust substrate and cellulase enzyme

Non-delignified and delignified sawdust samples (10mg) from five different trees were transferred in triplicate into test tubes. Names of the trees from which these sawdust samples were collected are, *Ipomoea asarifolia, Hallea ciliate, Sacoglottis gabonensis, Pycnanthus angolensis* and *Terminalia superb*. 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 2.0mg.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 dehydrated sawdust materials (2.8-5.0mm particle size) to 350g of NaOH and 140g NaS_2 during the Kraft pulping process. The Kraft pulping chemicals was dissolved in 8 dm^3 water and the delignification of the lignocellulosic materials (sawdust) was carried out in a rotary steel digester at 170°C and a pressure of 200kPa for 1h 45 min at cooking liquor to wood ratio of 4:1. After the Kraft pretreatment, the extracted cellulose fibers were washed in turns with deionized water until free of the Kraft reagents (Ndukwe, *et al.,* 2009). To remove residual lignin from these Kraft-treated cellulose all these sawdust materials (10g) were treated with 30% hydrogen peroxide (60ml) at 40°C for 25-30 min.

Cellulase incubation and DNS analyses

The weighed sawdust material (10mg) in each tube was incubated with the *A. niger* cellulase enzyme solution (200ul in each test tube) and Tris buffer solution pH 5,0 (800ul in each test tube) 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 concentration of 0.50mg.ml^{-1}, 2.00mg.ml^{-1}, 4.00mg.ml^{-1}, 6.00mg.ml^{-1} and 8.00mg.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 materials (Miller, 1959).

Results and discussion

Increasing volumes of accumulated solid waste that are not effectively managed is a major problem for cities in many countries. Not only is valuable land occupied with these waste products, but air pollution as a result of the degradation of the organic component of solid waste is a threat to the health of local populations. The residents around the Lagos Lagoon are extensively exposed to massive amounts of sawdust. Spontaneous combustion of these flammable materials has already caused disruptions in local suburbs and although the accumulated sawdust has been used as briquettes/pellets its potential as a feedstock for the synthesis of chemicals and pharmaceuticals have not been realized (Owayemi *et al*., 2016; Okedere *et al*., 2017). In order to utilize sawdust as a raw chemical material it is essential that the cellulose component of this biopolymer is to be hydrolyzed into fermentable

sugars such as glucose that could be used during chemical and biological synthetic procedures (fermentation) as a starting material for the production of bio-products. *A. niger* cellulase is a multi-component enzyme system which activity on various cellulose substrates such as sugar cane bagasse, ground nut shells and corn cobs have been investigated and described (Reddy *et al.,* 2015).

The saccharification of sawdust from the Lagos Lagoon with *T. viride* cellulase have been described as well as the successive fermentation of the resulting sugars into bio-ethanol (Ndukwe *et al.*, 2013; Ndukwe *et al.*, 2018). Delignification is a well-known procedure to remove lignin from cellulose whereby the cellulose is more susceptible for cellulase catalized conversion into sugars (Jafari *et al.*, 2015).

Figs 1-5 represent the relative sugar formation during *A. niger* cellulase catalized degradation of delignified and non-delignified sawdust cellulose from five different trees along the Lagos Lagoon at four different incubation temperatures. The amount of free sugars associated with the non-delignified and delignified cellulose prior to cellulase catalyzed bio-conversion is also reflected on the various graphs. Fig. 1 represents the sugar formation of lignified and delignified cellulose from *Ipomoea asarifolia* by *A. niger* cellulase during saccharification at different incubation temperatures.

The amount of free sugars released from the non-delignified samples before cellulase catalyzed bioconversion varied between of 1.9mg.ml^{-1} and 2.1mg.ml^{-1} while the concentration of free sugars released from delignified cellulose varied between 2.3 and 2.6mg.ml^{-1} at all incubation temperatures. Sugar released during the saccharification of the non-delignified sawdust varied between 3.9mg.ml^{-1} and 4.3mg.ml^{-1} obtained at temperatures of 40°C and 50°C, respectively. During saccharification of the delignified cellulose the highest sugar concentration of 5.9mg.ml^{-1} was obtained at an incubation of 50°C whilst the highest sugar concentration obtained from this delignified cellulose was 37% higher than the maximum sugar obtained from the non-delignified cellulose (4,3mg.ml^{-1}).

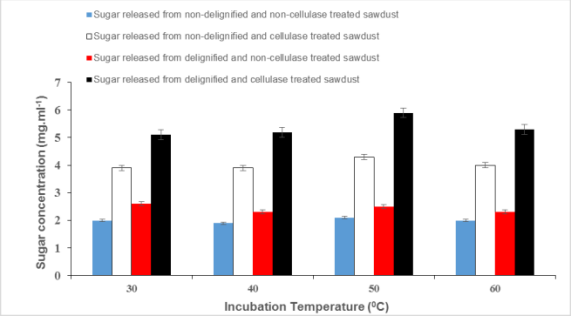

Fig. 1. Saccharification of non-delignified and delignified sawdust from *Ipomoea asarifolia* with *A. niger* cellulase.

The concentration of free sugars released from non-delignified and non-cellulase treated *Hallea ciliate* cellulose (Fig. 2) varies between 1.3 and 1.6mg.ml^{-1} when exposed to the different incubation temperatures while the concentration of free sugars released from the delignified cellulose that was not degraded by cellulase was calculated at values between 3.7 and 4.1mg.ml^{-1}. When non-delignified cellulose from this cellulose material was exposed to *A. niger* cellulase a sugar concentration between 2.7 and 3.0mg.ml^{-1} was obtained while an optimum sugar concentration of 7.2mg.ml^{-1} was recorded during cellulase catalyzed degradation of delignified cellulose at 50°C. This optimum amount of sugar obtained during degradation of delignified cellulose at 50°C was 10% more than the maximum amount of sugar released during cellulase catalyzed saccharification at the other incubation temperatures and 140% more than the maximum amount of sugar released during cellulase action on the non-delignified cellulose at 30°C and 60°C.

The amount of free sugars released from the delignified material at all four incubation temperatures were almost similar at a concentration of 4.0mg.ml^{-1} and higher than the amount of sugar released form the non-delignified material. Fig. 3 represents the amount of free sugar associated with non-delignified and delignified cellulose from *Sacoglottis gabonensis* as well as sugar released

during saccharification of non-delignified and delignified sawdust from this tree with *A. niger* cellulase. Free sugars released from non-delignified cellulose and not exposed to cellulase action varied between 1.5 to 1.7mg.ml^{-1} at the different incubation temperatures whilst the free sugar concentration was released from the delignified cellulose at concentrations between 3.3 and 3.5mg.ml^{-1}.

When *A. niger* cellulase catalyzed hydrolysis of the non-delignified cellulose took place the produced sugar concentration was calculated at values between 3.1mg.ml^{-1} and 3.4mg.ml^{-1}. Sugar released during cellulase catalyzed degradation of the delignified cellulose resulted in maximum sugar concentration of 6.1mg.ml^{-1} at an incubation temperature of 50°C that was 74% higher than the optimum amount of sugar produced during saccharification of the non-delignified cellulose material a 40°C.

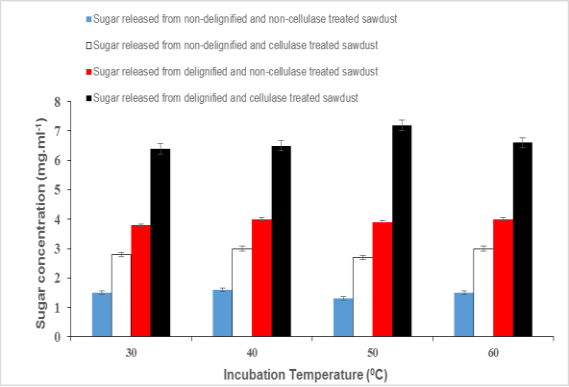

Fig. 2. Saccharification of non-delignified and delignified sawdust from *Hallea ciliate* with *A. niger* cellulase.

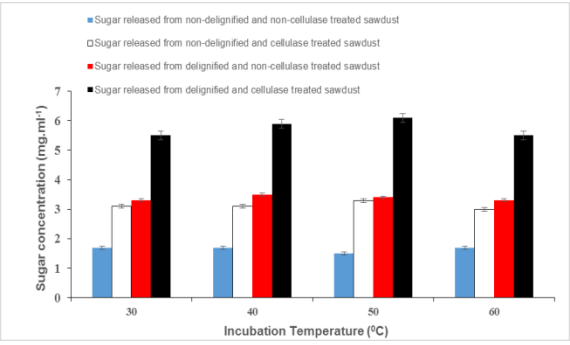

Fig. 3. Saccharification of non-delignified and delignified sawdust from *Sacoglottis gabonensis* with *A. niger* cellulase.

The amount of free sugars released as well as produced during *A. niger* cellulase action on the delignified and non-delignified cellulose from *Pycnanthus angolensis* cellulose is illustrated in fig. 4. Similar to the other cellulose materials was the lowest amount of free sugar released from the non-delignfied, material at concentrations between 1.4 and 1.7mg.ml^{-1}. Free sugar concentrations released from the delignified cellulose varies between 3.7mg.ml^{-1} and 4.7mg.ml^{-1} at the different incubation temperatures. When treated with *A. niger* enzyme cellulase the amount of sugar released from the non-delignified cellulose at the various incubation temperatures were very similar at concentrations between 2.9mg.ml^{-1} and 3.2mg.ml^{-1}.

Saccharification of delignified cellulose resulted in an optimum sugar concentration of 7.3mg.ml^{-1} when incubated at 50°C with the lowest concentration of 6.3mg.ml^{-1} when degraded at 60 °C. Maximum sugar concentration obtained during saccharification of delignified cellulose a 50°C was 128% higher than the maximum amount of sugar released when non-delignified cellulose was saccharified at 40°C. When treated with *A. niger* cellulase the delignified cellulose from *Terminatia superb* (Fig. 5) showed a relative high sugar concentration of 8.4mg.ml^{-1} released at the optimum incubation temperature of 50°C.

The lowest amount of sugar released from the saccharified delignified material was observed at 30°C producing a sugar concentration of 7.4mg.ml^{-1}. *A. niger* cellulase catalyzed degradation of the non-delignified sawdust resulted in the highest amount of sugar concentration of 3.1mg.ml^{-1} that was obtained at an incubation temperature of 40°C. This optimum sugar concentration obtained from the non-delignified cellulose was 220% less than the sugar concentration released when the delignified substance was bio-converted at 50°C. The relative amount of free sugars released from the non-delignified material varied between concentrations of 1.4mg.ml^{-1} and 1.6mg.ml^{-1} while free sugar formation from delignified cellulose was calculated at much higher concentrations between 5.7 and 6.9mg.ml^{-1}.

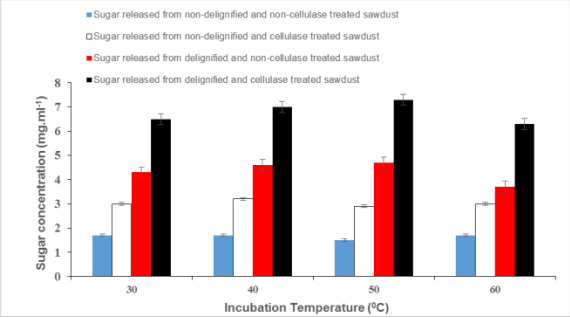

Fig. 4. Saccharification of non-delignified and delignified sawdust from *Pycnanthus angolensis* with *A. niger* cellulase.

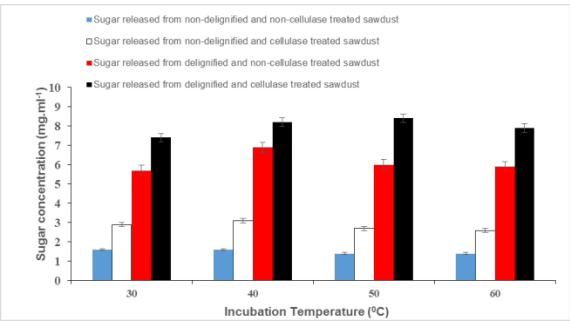

Fig. 5. Saccharification of non-delignified and delignified sawdust from *Terminalia superb* with *A. niger* cellulase.

The highest sugar concentration released during the bio-conversion of delignified and non-delignified sawdust from five different trees along the Lagos Lagoon in Nigeria with *A. niger* cellulase are reflected in Table 1. Also reflected in Table 1 is the activity of the cellulase enzyme on the various cellulose materials in terms of IU permg cellulase enzyme. These results indicate that optimum saccharification of non-delignified cellulose materials was obtained at a temperature of 40°C for cellulose obtained from *H. ciliate*, *P. angolensis* and *T. superb* while optimum bio-conversion for cellulose from *I. asarifolia* and *S. gabonensis* was observed at an incubation temperature of 50°C. Sawdust cellulose from *Ipomoea asarifolia* produced the highest sugar concentration of 4.3mg.ml^{-1} that was 43% more than the sugar released from *H. ciliate* at a concentration of 3.0mg.ml^{-1}. Sugar released from the other three cellulose materials was slightly higher than the lowest concentration at values of 3.1 and 3.2mg.ml^{-1}. Optimum *A. niger* cellulase catalyzed degradation of delignified materials was calculated at an incubation temperature of 50°C for cellulose obtained from all five different trees. Delignified cellulose from *T. superb* resulted in the highest sugar concentration of 8.4mg.ml^{-1} followed by sugars released from *P. angolensis, H. ciliate, S. gabonensis* and *I. asarifolia* (5.9mg.ml^{-1}) that was 42% lower than the highest sugar concentration of 8.4mg.ml^{-1}.

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

Name of tree	Delignified cellulose			
	Optimum temperature (°C)	Optimum sugar concentration (mg.ml^{-1})	Optimum temperature (°C)	Optimum sugar concentration (mg.ml^{-1})
Ipomoea asarifolia	50	4,3	50	5,9
Hallea ciliate	40	3,0	50	7,2
Sacoglotus gabonensis	50	3,3	50	6,1
Pycnanthus angolensis	40	3,2	50	7,3
Terminalia superb	40	3,1	50	8,4

Currently sawdust has been indicated as a feedstock for the production of bio-methane (Ali *et al.,* 2020), bio-butanol (Cebreiro *et al.,* 2019), bio-ethanol (Trevoral *et al.,* 2018) as well as other application such as for the bio-remediation of oil spills (Ismael *et al.,* 2019) and the synthesis of cellulose nanoparticles (Shabeen *et al.,* 2018). Sugarcane, starch (grain corn), or lignocellulosic (plant)-based feedstock can also be utilized during cellulase catalyzed degradation producing sugar, which could then be transformed by yeast through fermentation into bio-ethanol (Demain *et al.,* 2005). Cellulosic materials such as wood, grass, fibrous plant materials, and recycled waste (e.g. paper and cardboard) are also good substrates for the development of renewable fuels such as bio-ethanol.

Although sawdust is a major waste product produced in the wood industry and it occupies valuable land and renders a threat to the environment and to the health of many people its potential as a resource for bio-product development has not yet realized and more scientific initiatives will be applied in developing it as a bio-feedstock.

Conclusions

Operational sawmills along the Lagos Lagoon in Nigeria is a major industry that offers jobs to many people. The major waste product from these sawmills is sawdust which is a threat to the environment, water resources, ecology and human health as massive amounts of this wood product is accumulating on the banks of the Lagos Lagoon without being considered or explored as a potential feedstock for the chemical and pharmaceutical industries. A structural component of sawdust is cellulose, a glucose based biopolymer which can be hydrolysed into glucose a fermentable sugar through the action of cellulase enzymes. Sawdust from five different trees has been saccharified with the hydrolytic action of the *A. niger* cellulase and the extension of bioconversion into fermentable sugars has been increased by delignifying the various cellulose materials with the Kraft process and hydrogen peroxide treatment. All cellulose materials, delignified as well as non-delignified have been degraded into fermentable sugars with delignification caused an increase in saccharification relative to degradation of the non-delignified materials. The amount of produced sugars from the different cellulose materials were not the same and from this investigation it can be concluded that sawdust as an organic solid waste material could be investigated and developed as a renewable substance for bio-product development that would be environmental friendly with a less negative effect on the health of humans and the environment.

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