Lignin carbohydrate complexes in biomaterials biology essay

Business, Industries

Stellenbosch University has received national and international acknowledgment in research on the production of liquid bio-fuels from lignocellulose.

The Forest and Wood Science section has huge expertness in the word picture, processing and merchandise public presentation testing of wood and wood based merchandises. The section therefore plays a important function in the development of protocols and advanced analytical techniques for word picture of natural stuffs, intermediate and concluding merchandises in all types of processing. Current research in the section as hosted by the bio-fuels research chair involves the isolation and word picture of lignin-carbohydrate composites (LCCs) . The being of covalent bonds between lignin and saccharides is of considerable involvement in connexion with a figure of issues in wood chemical science, such as the reactions taking topographic point during the formation of wood, the natural molecular weight distribution of lignin and saccharides, swelling and handiness belongingss and the responsiveness of wood during its processing, e.

g. , during chemical pulping or bio-processing into ethyl alcohol. The research is much more cardinal in nature and yet more advanced as among other things, it seeks to understand the alterations that take topographic point non merely in the lignin saccharide bonds but besides in lignin-lignin bonds as affected by chemical or bio-processing of biomass. Recently, the Paper Manufacturers ‘ Association of South Africa (PAMSA) has recognized a critical demand for both engineering and human resources development to guarantee the economic sustainability of this of import industry in South

Africa, to better the industry ‘ s international fight, and to back up developing participants in the local industry.

In peculiar, the scarceness of skilled proficient staff has become a important menace to the SA industry, with a deficit of procedure applied scientists with research experience in pulp-and-paper processing being the most pressing. Such proficient staff will go cardinal procedure pioneers and job convergent thinkers in the industry. At the same clip, new engineerings are required by the industry to better current procedures and develop new industries around mush Millss to better economic value added to the feedstock. Key countries of engineering and procedure development required are fiber processing and bio-refining, recycling of fibres, fiber technology and betterment in the overall energy ingestion and associated environmental impact of mush Millss. These aims can merely be met through research capacity edifice at universities ; a vision shared by PAMSA. The proposed survey signifiers portion of the biomass procedure development plan, which considered the application of world-leading engineerings for bio-ethanol and mush production.

The work done will lend critical constituents of biomass word picture before, during and after treating. The proposed survey identifies critical mechanisms in the development of biomass to ethanol and pulping procedures. Aspects of chemical word picture of the substrate are integrated into research on pre-extraction of hemicelluloses, pulping and or biomass pre-treatment and enzymatic hydrolysis procedure development, to show the importance of advanced chemical word picture of substrates for procedure development.

The proposed work plays a prima function in beef uping the integrating of research activities in assorted subdivisions of the cellulosic-ethanol and pulping value concatenation.

Scope of the survey

This work is aimed in developing protocols and advanced analytical techniques for word picture of woody biomass natural stuffs, intermediate and concluding merchandises in all types of processing, which will concentrate in peculiar on the isolation and word picture of lignin-carbohydrate composites (LCCs) in order to obtain relevant information on biomass formation and on the delignification procedure from an LCC position.

Thesis statement

Investigate alterations in lignin saccharide composites when the biomaterials are subjected to pre-treatment and subsequently to mush samples. This will give cognition about the ultra-structure of wood for doing chemical mush as the survey was developed for softwood biomass stuffs.

The method for the isolation of lignin saccharide composites has been developed for deals (Lawoko et al. , 2003) . In this survey the application of the method will be employed together with an inorganic method to hold comparable consequences based on different methods.

Theoretical background

Linkages between lignin and saccharides have been suggested to be a major obstruction to finish delignification of biomass feedstocks.

Covalent lignin-carbohydrate linkages exist in lignocellulose from wood and groups of herbaceous workss although ambiguity in the types, frequence and measure exist. Xylan-linked lignin is more immune to oxidative reactions and tends to stay in the biomass, while galactan-linked lignin tends to fade out during oxygen delignification. The decrease of reactive phenoplast and carboxyl groups during delignification is besides responsible for the low degree of subsequent delignification. In add-on, the location and handiness of residuary lignin may besides hold a important consequence on delignification. An apprehension of the construction and composing of lignin saccharide composites (LCCs) of Eucalyptus grandis and sugar cane bagasse prior to and after Kraft pulping could be developed through experimental informations aggregation.

In this manner, the pulping procedure conditions can be tailored to the specific chemical and structural composing of the Eucalyptus grandis or bagasse. This in bend can be modelled for other lignocellulosic biomass feedstocks such as bamboo, sorghum etc. In add-on, even more significantly, the consequence of hemicelluloses pre-extraction on LCCs bonds prior to and after pulping of Eucalyptus grandis can be established.

The information collected will be of great significance in the ulterior phases of the mush processing such as bleaching. The analytical techniques developed for isolation and analysis of LCCs will non merely be of import for Kraft pulping but for other biomass application processes such as the hydrolysis of biomass for bio-ethanol production and as such, this survey can function as a theoretical account for such applications.

Experimental clip program

The LCC isolation process chosen is non-modifying to the fibers and the fractional process constituents are obtained in high outputs unlike old processs.

In add-on, they are about free from taint. Ball milling is an built-in portion of this process. Therefore, optimal ball milling conditions have to be established in order to obtain integral LCC webs from biomaterials and their merchandises.

The optimisation phase could non be performed old twelvemonth as the ball factory was impeding due to its inaccessibility. The optimization measure is to be followed by chemical word picture of LCC constructions utilizing advanced analytical processs such as NMR, HPLC and GC-MS. Previous surveies show that glucomannan-rich LCC fraction (GlcMan-L-Xyl) is comparatively more stable than the other LCC types toward the terminal of Kraft cook. Table 1: Detailed activity clip program

Time period

Undertaking

March – May 2011Redo chemical composing of samples both Eucalyptus grandis and sugar cane baggaseBook FTIR to characterize the chemical bonds before the lignin saccharide composites (LCCs) are isolated. Submit first twelvemonth advancement study to Process Engineering Department and PAMSASubmit chapter one based on the chemical composing of the samplesMay – June 2011Establish conditions of ball milling samples from

natural stuffs to mush samplesAcetylation will been done to find the molecular mass distribution of the lignin samplesMay – August 2011Get down the isolation of LCCs from Eucalyptus grandisMake thioacidolysis to quantify the ?-O-4 bondsCharacterize the samples with FTIR after the LCCs have been isolatedAugust 2011Submit chapter two, based on the literature reappraisalAcetylation will been done to find the molecular mass distribution of the lignin samples after the LCCs have been isolatedAugust – September 2011Start isolation of LCCs from sugarcane baggase samplesCharacterize the samples after the LCC isolation phaseSeptember 2011Submit chapter three based on the methodological analysis of the surveyOctober 2011Submit chapter 4A based on the consequences and treatments of the survey for E. grandisWrite research paper based on the findings for the isolation of LCCs from hardwoodsOctober 2011Submit chapter 4B based on consequences and treatments for sugar cane bagasseNovember 2011 – January 2012Rework on isolation and word picture of LCCs for conclusive consequencesWrite tests (WPS144)February and March 2012Submission of thesis and corrections

Materials and Preliminary consequences

The Eucalyptus grandis that is used in this survey was supplied by Sappi Ltd, the sugar cane bagasse was from industrial bagasse. The mush and pre-treated stuffs were supplied by a PhD pupil (Ms PF Vena) at the University of Stellenbosch.

The procedure of change overing renewable lignocellulosic biomass to ethanol requires a figure of stairss, and pre-treatment is one of the most of

import. Pre-treatment normally involves a hydrolysis of the easy hydrolyzed hemi-cellulosic constituent of biomass utilizing some signifier of thermal/chemical/mechanical action that consequences in a merchandise that can be farther hydrolyzed by cellulase enzymes. The sugars produced can so be fermented to ethanol by fermentative micro-organisms.

If the pre-treatment measure is non terrible plenty, the end point residue is non as easy hydrolyzed by the cellulase enzyme. More terrible pre-treatment conditions result in the production of debasement merchandises that are toxic to the fermentative micro-organism. The stuffs used in the survey are Eucalyptus grandis and sugar cane bagasse with their corresponding chemically treated (alkalic and dilute acid pre-treatment, Kraft and soda AQ) stuffs.

Chemical analysis of sugar cane bagasse and E. grandis natural stuffs

The modified Klason lignin method was used (NREL) .

Samples of 0. 3 g were treated with 3 milliliters of 72 % H2SO4. After an hr of continues stirring at 30 & A ; deg ; C in a H2O bath, 81 milliliter of H2O was added to the mixture, which was station hydrolysed under 121 kPa for an hr. The merchandise was filtered and the indissoluble lignin (Klason lignin) was quantified by weight. The hydrolysate was analysed by high public presentation liquid chromatography (HPLC) . Table 2: Chemical composing of E. grandis and sugar cane bagasse.

Sample

Extractives (%)

Ash (%)

Lignin (%)

Pentosan (%)

Glucose (%)

Xylose (%)

Cellulose (%)

E. grandis2. 59 ± 1. 170. 18 ± 0.

0810. 89 ± 1. 9024. 7 ± 1. 6939. 3 ± 0. 1213.

8 ± 0. 2944. 38 ± 0. 20Sugarcane bagasse7. 0 ± 0. 051. 60 ± 0.

0316. 7 ± 0. 0526. 4 ± 0. 9646. 1 ± 0. 2213. 6 ± 0.

2149. 16 ± 0. 20The sugar cane bagasse and Eucalyptus grandis analysis is presented in Table 2. The sum of ash is lower than that mentioned by Pate (1982) , owing possibly to the influence of different factors on sugar cane cultivation and processing. It besides shows that 13.

6 % is composed of xylose and 46. 1 % is composed of glucose. This difference in cut downing and fermentable sugars could be ascribed to the presence in the hemicelluloses of arabinose, mannose and oligomers like celotriose, arising in uncomplete molecule hydrolysis. It could besides be ascribed to the presence of compounds like brain sugar, 4-o-methyglucuronic

acid and aldobiuronic acid, which were non detected by High Performance Liquid Chromatography (HPLC) . Similar differences were besides found by Roberto et Al. (1994) ; Fox et Al.

1984 ; Morjanoff and Gray (1987) . The hydrolysis of the hemicelluloses fraction during acerb pre-treatment involves solubilization and partial devastation of the cut downing sugar produced. As a effect, the sum of cut downing sugar recovered from the bagasse depends on intervention clip, temperature and acerb concentration. The clip intervals necessary for heating and chilling were non considered here. Several Kraft and soda AQ mush samples from Eucalyptus grandis and sugar cane bagasse were used in this survey.

The samples were alkalic and acerb pre- extracted. Eucalyptus grandis and sugar cane bagasse samples were subjected to following chemical interventions: Table 3: Pulping conditions and ensuing Kappa Numberss

Sample

Pulping status

Kappa figure

E. grandisKraft puling

2M NaOH at 40 & A ; deg ; C for 240 proceedingss& A ; lt ; 27

E. grandissoda AQ pulping

0. 3 (% v/v) at 140 & A ; deg ; C for 20 proceedingss& A ; lt ; 23

SCB car hydrolysis

Hot H2O~ 20. 9

SCB sodium carbonate AQ pulping

1. 5M NaOH at 65 & A ; deg ; C for 240 proceedingss~ 15.

5Kraft pulping was done on sugar cane bagasse, but the generated mush was non in a criterion of bring forthing documents. Therefore, the method was discarded and soda AQ was one of the pulping conditions for the biomaterial. Soda AQ pulping was done on dilute pre-extracted E. grandis samples while Kraft pulping was done on alkalic pre-extracted biomaterial.

Table 4: Chemical composing of mush samples

Sample

Entire Lignin (%)

Ash (%)

Glucose (%)

Xylose (%)

Arabinose (%)

E.

grandisraw untrd mush5. 9110030. 33344.

9

E. grandisRM- Kraft mush3. 556930. 18289.

2

E. grandisdil acid- Kraft5. 8070220. 06518. 4

E. grandisRM- sodium carbonate AQ7. 827520. 07269.

5

E. grandisdil acid- sodium carbonate AQ3. 8761530. 132737

SCB RM – sodium carbonate AQ7. 5925820. 48251.

12. 9SCB alkaline – sodium carbonate AQ36. 996230. 24349.

98. 7SCB dil acid – sodium carbonate AQ mush3. 6038270.

2349. 45. 8(E. grandis = Eucalyptus grandis ; RM = natural stuff ; SCB = sugar cane bagasse ; dil = dilute ; untrd = untreated)The hemicelluloses are changing in outputs.

The acerb hydrolysis to find monosaccharide composing was analysed by HPLC. It is apparent that the entire composing of entire saccharides is really low as the tabular array indicates that the stuffs contained more xylose and glucose than arabionose. The analysis has to be re-done as the outputs are really low. The low output may be due to analytical mistake. Harmonizing to literature, the glucose yiled should be in the scope of 50-70 % . For acid and alkaline treated stuffs, xylose outputs should be in the scope of 75-90 and 60-75 % severally (Hamelinck et al. , 2005) . The addition in fermentable sugars is due to the high lignin content removed during chemical intervention.

Relative distribution of guaiacyl-type debasement has to be done in order to compare the samples with Kraft pulped samples. This is for the finding of differences in the formation of guaiacol from hemicelluloses. It is of import to make the analysis as it will besides help in finding the alterations that occur within the lignin-carbohydrate bonds.

FTIR spectrometry of natural ; pre-treated and mush samples of sugar cane bagasse and E.

grandis

FTIR spectra of samples were obtained straight from untreated, pre-treated and mush samples using diffuse coefficient of reflection infrared with Fourier transform technique (Perkin Elmer – Spectrum GX) . The spectra were normalised by the soaking up at 900 – 2000 cm-1, after baseline rectification. Figure 2: Normalised FTIR spectra of natural, pre-treated and mush samples of sugar cane bagasse and Eucalyptus grandis.

The effects of chemical intervention on samples (natural, pre-treated and mushs) were assessed by FTIR combined with PCA. The FTIR normalised spectra of different samples in the part of 800-2000cm-1 are presented in Figure. It was observed that there was important difference among samples.

The alterations were assessed by PCA. The PCA mark secret plan of the matrix incorporating spectral informations showed that the samples were significantly different which were clearly separated by PC1. This Personal computer described 69 % of fluctuation in the information set.

Chief component analysis of the FTIR spectra

Spectra of the samples were converted to stand out utilizing OMNIC package.

The normalised optical densities in the scope of 900 – 2000 cm-1 were submitted to principal component analysis (PCA) computations utilizing STATISTICA 10. Chief constituent analysis (PCA) groups samples harmonizing to their similarities and differences. It provides information on the mass spectral characteristics that are footing of chemical similarities and differences. It allows for easy differentiation of samples that have been subjected to a chemical intervention from natural, pre-treated and mush samples with high concentration of one constituent from samples with low concentration of a peculiar constituent. Harmonizing to PCA, constituent 1 differs significantly from component 2.

Component 1 and 2 are 69 and 14 % different severally. They have a sum square of 83 % (sum square – A statistical technique used in arrested development analysis. The amount of squares is a mathematical attack to finding the scattering of information points. In a arrested development analysis, the end is to find how good a information series can be fitted to a map which might assist to explicate how the information series was generated. The amount of squares is used as a mathematical manner to happen the map which best tantrums (varies least) from the information. In order to find the amount of squares the distance between each informations point and the line of best tantrum is squared and so all of the squares are summed up.

The line of best tantrum will minimise this value) . The other constituents have really small difference which is 17 % . The mark secret plan demonstrates that samples 1, 4, 5, 11, 15 and16 are really different from other samples. Samples 2, 3, 6, 7, 8, 9, 10, 12, 13, 14, 17, 18 and 19, have really small difference. Figure 3: Mark secret plan for natural, pre-treated and mush samples from different biomass stuffs. The spectra were antecedently normalised. The samples were grouped into 3 groups A, B and C.

The grouping was based on the lignin content that each sample contains. Samples in group A had low response when they were subjected to chemical intervention in losing and degrading lignin as they fall in the negative part of the axis. Group C consists of samples that had high loss and debasement of lignin whilst in group B there was no to small alteration in lignin content. Figure 4: Scatter secret plan of multiple variables against variable figure.

The tonss secret plan utilizing PC versus variable figure (wave figure converted to variable figure) gave a spectrum of informations set in Figure. This gave information on the moving ridge Numberss that contributed to the clear separation of samples as in conformity to PC1 values. Characteristic extremums of lignin (C= C of aromatic at 1595 cm-1, 1550 cm-1 and 1500 cm-1 ; C-O-C of aryl quintessence at 1225 cm-1 and 1263 cm-1, C-O-C of allyl quintessence at 1137 cm-1 and 1188 cm-1 ; C-H of aromatic ring at 820 cm-1, 850 cm-1 and 930 cm-1) dominated the spectrum. The extremums of cellulose and hemicelluloses (C-O of 1st intoxicant at the scope of 1038 cm-1, C-O of 2nd intoxicant at the scope of 1088 cm-1 seemed to be unaffected

by the chemical interventions in all the samples. Components 1 and 2 are really different in the part of lignin this is due to the fact that lignin was degraded when the samples were subjected to different chemical intervention processing methods (pre-treatment and pulping) .

Alternate method for the isolation of LCC

Figure 5: Conventional representation of an alternate method for the isolation of LCCs from E. grandis (Li et al. , 2011) . There is a possibility of look intoing the alterations in lignin saccharide composites when a stuff is subjected to pre-treatment. The hydrolysate can be analysed as the xylan will be isolated by dilute acid or alkaline pre-treatment method. The chief purpose is to hold merely lignin and saccharides on the stuff.

The intent of insulating cellulose is to minimise the figure of C atoms when they material is analysed by NMR and FTIR. The expected extremums are of xylose anchor. The method that was developed by Lawoko et al. , 2003 for the isolation of LCCs was for softwood species. The method is said non to be suited for hardwood biomass stuff. Harmonizing to literature, there have been many statements for the isolation of LCCs from hardwoods with enzymes.

The isolation of lignin by enzymatic hydrolysis is accompanied by minimum structural alterations. The literature besides says that, the presence of saccharides in readyings makes the analysis of lignin more complicated, but on the other manus it allows probe of lignin-carbohydrate (LC) linkages in mush (Minor 1986 ; Tamminen and Hortling 1999) . However, enzymatic

residuary lignin normally contains some protein contaminations, which can impact the word picture of lignin peculiarly by spectroscopic techniques. An attack utilizing a combination of enzymatic hydrolysis with mild acidolysis has late been suggested (Argyropoulos et al. 2002) . This method inherited both advantages and defects of enzymatic hydrolysis and acidolysis (JO“ O“ skelO“ inen et al. 2003) .

Isolation of enzymatic residuary lignin from deal Kraft mush is a well-established process bring forthing lignin readyings with high outputs and comparatively low enzyme drosss (Tamminen and Hortling 1999) . In contrast, the isolation of enzymatic residuary lignin from hardwood mush is non good developed. There are really few publications on the isolation of residuary lignin from hardwood mush, and all of them report comparatively low outputs of residuary lignin and the presence of instead high sums of protein taints (Tamminen et al. 1999 and Choi and Faix 1999) .

When the enzyme isolation method is used on hardwoods, it fails to give quantitative recovery of lignin either present in LCCs or as lignin-free saccharides. An alternate method for the isolation of LCCs from hardwoods has late been developed by Li et al. , 2011. The method permits complete disintegration of wood and mush samples in the class of which subsequent fractional process into single LCCs is possible. The samples are subjected to ball milling to destruct the crystalline construction of cellulose without impacting the construction of lignin. Once the formless construction is obtained, the stuff is treated with assorted inorganic dissolvers. Table 5: Drumhead tabular array for methodological analysis of the survey

Undertaking

Remark

Status

Chemical composing analysis of E.

grandisand sugar cane bagasseThe sum of ash is lower than thatmentioned in literatureDoneFTIR analysis of biomaterialsThe stuffs show important differences when analysed by PCA. DoneIsolation of lignin saccharide composites from natural biomaterialsThis is of import to understand the composing of LCCs before a stuff is subjected to chemical interventionPendingIsolation of LCC from processed (pre-treatment and pulping) biomaterialThis serves for the apprehension of biomaterials how they change when they have been treatedPendingFTIR and NMR analysis of processed biomaterialThis is to find the chemical alterations within the biomaterial constructionPendingWord picture of biomaterial before and after processing by thioacidolysisThe method is for quantifying ?-O-4 linkagesPending

Decision

Harmonizing to FTIR-PCA analysis of the samples, they showed that lignin was removed when the biomass stuffs were subjected to different chemical processing. The lignin that remained will be farther analysed to find its association with hemicelluloses as the literature elaborates.

Literature cited

Argyropoulus, D.

S. , Sun, Y. and Palus , E. (2002) . Isolation of residuary lignin in high output and pureness. J. Pulp Pap. Sci.

28: 50-54. Browning, B. L. Methods of wood chemical science 2: 785. Choi, J.-W. and Faix, O.

(1999) . Probe on residuary lignins and residuary saccharides and the covalent bonds between them. In: 10th International symposium on wood and pulping chemical science, 1: 368-373.

Hamelinck, C. N. , new wave Hooijdonk, G. and Faaij, A. P. C.

(2005) . Ethyl alcohol from lignocellulosic biomass: techno-economic public presentation in short- , middle- and long-run, Biomass and Energy 28, 384-410. JO“ O“ skelO“ inen, A.-S. , Sun, Y. , Argyropoulos, D. S. , Tamminen, T.

and Hortling, B. (2003) . The consequence of isolation method on the chemical construction of residuary lignin. Wood Sci. Technol. 37: 91-102.

Lawoko, M. and Henrikson, G. (2003) . New method for quantitative readying of lignin-carbohydrate composite from uncolored deal Kraft mush: lignin-polysaccharide netwoks J.

Holzoforschung 57, 69-74. Li, J. , Martin-Sampedro, R. , Pedrazzi, C.

and Gellerstedt, G. (2011) . Fractionation and word picture of lignin-carbohydrate composites (LCCs) from eucalyptus fibers.

Holzforschung 65, 43-50. Minor, J. L.

(1986) . Chemical linkage of polyoses to residual lignin in loblolly pine Kraft mush. J. Wood Chem.

Technol. 6: 185-201. Pate, F. M. (1982) . Value of Treating Bagasse with Steam under Pressure for Cattle Feed.

Trop. Agric. , 59, 293. Tamminen, T. and Hortling, B. (1999) . Isolation and word picture of residuary lignin.

In: Advancement in Lignocellulosics Characterization. Ed. Argyropoulos, D. S.

Tappi Press, Atlanta: 1-42. Tamminen, T. , Hortling, B. , Ranua, M. , Luonteri, E.

, SuurnO“ kki, A. , Tenkanen M. and Buchert, J. (1999) . Enhanced bleachability of spruce kraft mush by mechanical and enzymatic interventions. In: 10th International symposium on wood and pulping chemical science, 1: 584-588.