

Characterization of combined organic–inorganic acid-pretreated cassava stem

A. P. Chandrasekaran¹ · S. Sivamani¹  · V. Ranjithkumar²

Received: 8 January 2016/Revised: 18 November 2016/Accepted: 21 December 2016/Published online: 9 January 2017
© Islamic Azad University (IAU) 2017

Abstract A number of previous studies established that the autoclave-mediated pretreatment enabled the efficient way of producing fermentable sugars from lignocellulosic residues. Hence, our emphasis was on studying the surface morphology of cassava stem to reveal its complex internal structure. In this study, combined organic (oxalic)–inorganic (sulfuric) acid was utilized for the pretreatment of cassava stem at 121 °C and 1 bar of pressure for 15 min. For the pretreatment, mixture containing 10 mL of 1% (w/v) of oxalic acid and 1% (w/v) sulfuric acid (5 mL each) was added to 1 g cassava stem and autoclaved. Pretreated samples were characterized by Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and ultraviolet spectroscopy (UVS). FTIR spectral studies confirmed the removal of hemicellulose and lignin from the pretreated cassava stem (PCS) when compared with untreated cassava stem. SEM micrographs revealed the decimation in the surface of cassava stem after pretreatment. XRD motifs shown that crystallinity index of PCS decreased from 63 to 52%. Thus, this study established the structural modifications to unlock its valuable components for further applications.

Keywords Cassava stem · SEM · FTIR · XRD · UVS · Pretreatment

Introduction

The excessive utilization of fossil fuels, increasing global population and inexorable depletion of the global fuel supply are triggering the researchers to focus on renewable energy resources (Varga et al. 2004; Han et al. 2011). Sustainable conversion of biomass into a spectrum of biofuels (bioethanol, biodiesel, power) and bio-based products (food, chemicals, materials) is now the mainstream. Advancement from fossil fuel to renewable energy economy will reduce the dependency on fuel imports and reduces the deposition of greenhouse gases (GHGs) in ozone layer (Caldeira 2003; Farrell 2006; Ragauskas 2006). The biorefinery concept foresees the separation of biomass into value-added products (VAPs) using appropriate biochemical processes.

Cassava (*Manihot esculenta*) is a perennial plant and belongs to the family Euphorbiaceae. Its cultivation has spread throughout the world for its starchy storage roots. India is an agricultural country and stands tenth in the production of cassava globally; major producers are Nigeria, Brazil, Indonesia and Thailand (Varmudy 2001). Cassava shrub is a woody plant with erect stems and grows up to 4 m in height (Plant Village 2014). The above ground biomass of cassava is not consumed for commercial purposes and left as an agricultural waste (Ahamefule 2005). Cassava stem mainly consists of lignocellulose which could be potentially used as a feedstock for various products. It is the fourth largest agricultural waste which generates 120 tonnes/ha/yr for electricity production (Preston 2001). The exploitation of cassava stem can considerably increase the VAPs production. Cassava stem is used as firewood and leave our environment polluted. However, its high cellulose and hemicellulose content allured the researchers to use the biomass in a utilitarian prospect. But

Editorial responsibility: J. Aravind, M.Tech.

✉ S. Sivamani
sivmansomel@gmail.com

¹ Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu 641 049, India

² Department of Chemistry, Government College of Technology, Coimbatore, Tamil Nadu 641 013, India

the conversion of cassava stem into VAPs is more challenging because of its complex lignocellulosic structure (Preetha et al. 2011).

The efficient recalcitrance of plant cell wall to unlock its fermentable sugars is challengeable. Pretreatment increases the accessible surface area in certain ways: (1) Hemicellulose components are solubilized (Donaldson 1988); (2) lignin structure also disrupted which curtails shielding effects (Donaldson et al. 1988; Ramos et al. 1999); and (3) hunks and cracks are developed (Donaldson et al. 1988). The main aim of the pretreatment method is to deconstruct the lignocellulosic material in order to improve its digestibility, ameliorate the enzyme hydrolysis and enhance the yield of VAPs (Mtui 2009). Several methods have been inspected for pretreatment of lignocellulosic material. It includes physical (size reduction, hydrothermal), chemical (acid, alkali, solvents, ionic liquids, ozone), physicochemical (ammonia fiber explosion, liquid hot water, autoclave, steam explosion, CO₂ explosion) and biological pretreatment (Mosier et al. 2005; Nguyen et al. 2010; Romani et al. 2010; Singh and Wahid 2015). Nonetheless, dilute acid pretreatment is a propitious method for agricultural residues (Monavari et al. 2009). Dilute acids are used to remove hemicellulose segment from lignocellulosic convoluted matrix and unmasking cellulose content for further process (Schell et al. 2003).

Numerous studies have been reported about the pretreatment, hydrolysis and fermentation of the cassava stem for the production of bioethanol (Han et al. 2011; Nuwamanya et al. 2012; Sovorawet and Kongkiattikajorn 2012). But none of the studies has investigated the structural, chemical and functional group analyses of the cassava stem. We present the first report on the characterization analysis of the pretreated cassava stem. The aim of the current study was to characterize the before- and after-pretreated cassava stem by FTIR, SEM, XRD and UVS. Further optimization process is required for the pretreated cassava stem to unveil its VAPs potential.

Materials and methods

Substrate

Cassava stem was kindly provided by the cassava-processing industry from Namakkal District, Tamil Nadu, India. It was crushed into small pieces in jaw crusher (Almech enterprises, Coimbatore, India) and air-dried at 40 °C in hot air oven (Narang scientific works private limited, New Delhi, India) to constant weight. The crushed stems were milled in a domestic mixer grinder (Preethi kitchen appliances private limited, Chennai, India) and screened through 250 microns mesh size in a gyratory sieve

shaker (Lawrence & Mayo India private limited, Mumbai, India). Finally, the materials were packed in an airtight polybag and stored at 4 °C until use. All the chemicals and reagents used in this work were purchased from Sigma-Aldrich and of analytical grade.

Combined organic–inorganic acid pretreatment of cassava stem

In this work, combined organic–inorganic acid mixture was used for the pretreatment of cassava stem in autoclave. Ten milliliters of acid mixture (5 mL each of 1% (w/v) oxalic acid and 1% (w/v) sulfuric acid) was mixed with 1 g of cassava stem and autoclaved at 121 °C (1 bar) for 15 min. Then, sample was centrifuged at 5000 rpm for 5 min and the supernatant made up to 100 mL. The residue solid sample was dried in hot air oven at 100°C for overnight and used for further characterization studies by FTIR, SEM, XRD and UVS. The supernatant was analyzed for total soluble sugars (Dubois et al. 1956).

Characterization studies

FTIR

FTIR is a promising technique to evaluate structural variations in samples due to the pretreatment process. The structural changes in before- and after-pretreatment of samples were investigated by ALPHA FTIR spectrophotometer (Bruker, USA). The FTIR spectrum of the samples was recorded in the transmittance mode in the range of 4000–500 cm⁻¹.

SEM

SEM photographs of samples before and after pretreatment were taken with a scanning electron microscope (JSM 6390, JEOL, Japan). The surface morphology was examined with an accelerating voltage of 10 kV and magnification of 1,500×.

XRD

The structural analysis of the samples was evaluated by XRD (X'Pert PRO from PANalytical, the Netherlands) with a scanning rate of 5° per minute. The XRD patterns were obtained over the angular range $2\theta = 10\text{--}80^\circ$.

UVS

The UV spectroscopic analyses of the samples were performed with SPECORD 210 plus from Analytik Jena, Germany. It was done with the wavelength range of

200–400 nm with a slit width of 1 nm and a scan speed of 5 nm/s. UV spectra are often required to elucidation of the structure of organic molecules.

Results and discussion

Physical appearance

Figure 1a and b shows the physical appearance of cassava stem as raw material and pretreated cassava stem, respectively. As it indicates, the cassava stem before pretreatment was light brown in color and after-pretreatment sample was dark brownish color. During autoclaving pretreatment process, the lignocellulosic material was heated with high pressure (1 bar) and temperature of 121 °C. Subsequently, the biomass was disintegrated its components and resulted in lignin transformation (in the form of cellulignin; Tabil et al. 2011; Punsuvon 2013). Taken together, because of high temperature, the phenolic compound (lignin) was subjected to dissociate from the matrix which changes the physical appearances of the after-pretreated cassava stem. This confirmed the pretreatment was done in the sample, and further characterization analyses confirm the effectiveness of pretreatment.

FTIR

The FTIR spectra of before and after pretreatment of cassava stem samples are shown in Fig. 2. Table 1 summarizes the absorption bands obtained in the mid-infrared (IR) region. Usually, all samples exhibited two transmittance regions: the first one at low wavelengths in the range of 750–1750 cm^{-1} and the second one at higher wavelengths in the range of 2750–3500 cm^{-1} . Those intensity bands,

which display the general components or functional groups of all the cellulose, hemicelluloses and lignin (as shown in Table 1), were changed significantly after pretreatment. Wide and intense bands of O–H stretching were observed at 3140–3150 cm^{-1} for before- and after-pretreatment of samples (Morán et al. 2008; Kuila et al. 2011; Ling Hii et al. 2014). It is due to the absorption of water molecules by the samples from the atmosphere. The spectral bands were noticed in the spectra of both the samples in the domain at 1650 and 1653 cm^{-1} for cassava stem before and after pretreatment, respectively, because of O–H stretch due to absorbed water (Le Troedec et al. 2008), 1515–1520 cm^{-1} due to aromatic skeletal lignin (Li et al. 2010a), 1380–1385 cm^{-1} due to C–H bending (Kumar et al. 2014) and 1310–1314 cm^{-1} due to C–O stretch. It

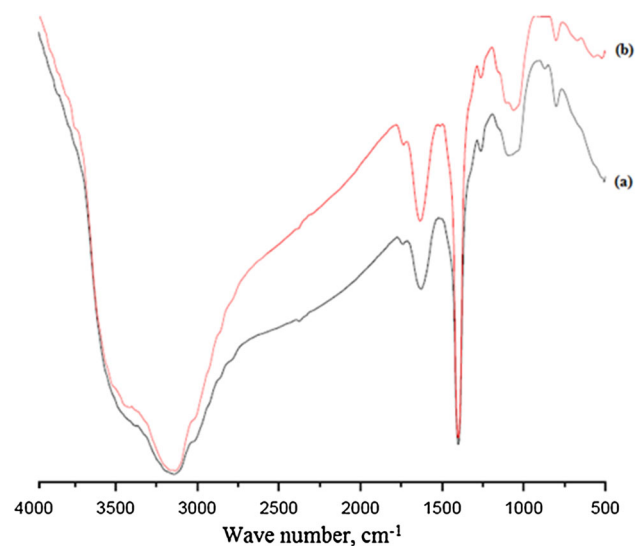


Fig. 2 FTIR spectra of cassava stem (a) before pretreatment and (b) after pretreatment

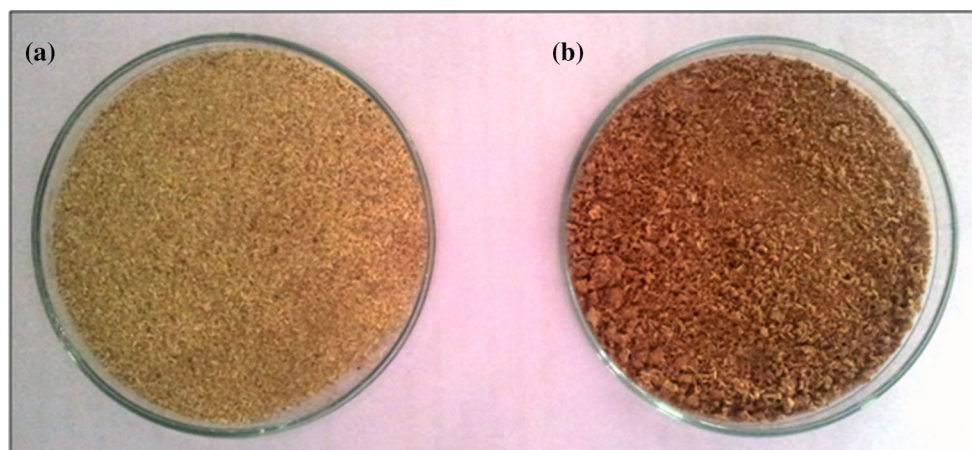


Fig. 1 Physical appearance of cassava stem (a) before pretreatment and (b) after pretreatment (1 g of cassava stem pretreated with 10 mL of acid mixture for 15 min at 121 °C)

Table 1 Analogy of spectral bands for cassava stem before and after pretreatment

Wave number, cm^{-1}		Functional group assignments
Before pretreatment	After pretreatment	
3145	3139	O–H stretch
1650	1653	O–H stretch due to adsorbed water
1515	1517	Aromatic skeletal lignin
1380	1385	C–H rock
1310	1312	C–O stretch
1105	1109	C–O–C glycosidic ether band
902	–	Amorphous cellulose/associated with the cellulosic β -glycosidic linkages

was believed that the distorted intensity indicates cleavage of lignin side chains. In case of cellulose, a small peak at 1135 cm^{-1} was observed for after-pretreatment cassava stem spectrum, while no peak was observed for before-pretreatment of cassava stem. This is due to the modification of C–O–C glycosidic ether bond in cassava stem before pretreatment (Kumar et al. 2014). This indicated the pretreatment in removing the hemicellulose, thus exposing more crystalline cellulose, and was also confirmed by the XRD. Furthermore, the pretreatment has also favorably transformed the cellulose in after-pretreatment sample with the absence of amorphous hemicellulose (780 cm^{-1}). This study was in line with the results in XRD as explained below.

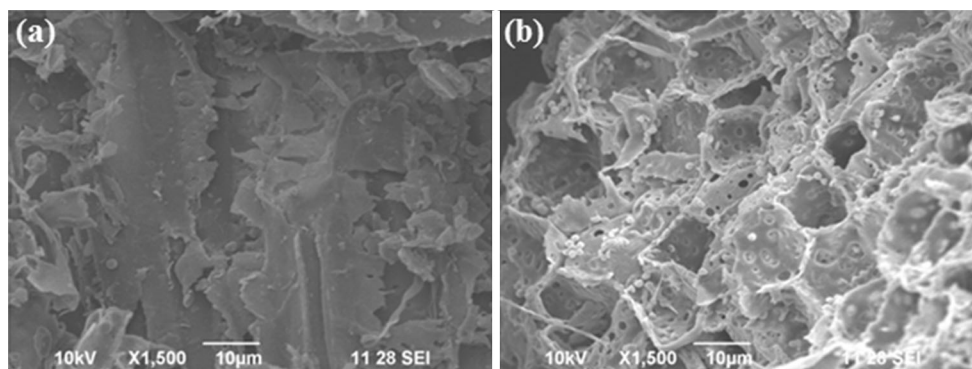
SEM

SEM micrographs divulged the morphological changes in before- and after-pretreatment of samples (Fig. 3). Generally, cassava stem has a complex structure. It comprised of major components include cellulose, hemicellulose and lignin. It is reported that cellulose is insoluble in water and dilute acid, while it is soluble in concentrated acids. But

severe degradation of the polymers by hydrolysis results in cellulose degradation. Thus, the cassava stem is treated with 5 mL each of 1% (w/v) oxalic acid and 1% sulfuric acid. It was observed that the sample before pretreatment was rigid and highly ordered due to complex lignin–hemicellulose matrix that binds to cellulose fibers (Ling Hii et al. 2014). After pretreatment, the complex structure was demolished because of combined acid pretreatment. Also, it is clearly showed that untreated cassava stem had an even and continuous surface. On the other hand, pores were present in the pretreated sample, which confirmed the removal of residual lignin. Increased porosity in the pretreated sample was due to the destruction of lignin–hemicellulose component. Therefore, the surface area of cellulose increased, suggesting improved enzymatic accessibility to cellulase.

XRD

The XRD patterns of before- and after-pretreatment of samples are given in Fig. 4. Both before- and after-pretreatment of cassava stems were displayed amorphous nature with different peaks. The amorphous pattern retained by the before-pretreated sample was attributed to the high amount of hemicellulose content. As a result, the enzymatic hydrolysis was debilitated as the cellulose was masked and protected by the lignin and hemicellulose seal. The crystallinity index (CI) was calculated by using amorphous subtraction method (Thygesen et al. 2005; Park et al. 2010). It was found that the CI of before- and after-pretreatment samples was 63 and 52%, respectively. These results depicted the effectiveness of pretreatment on cassava stem which eliminated amorphous hemicellulose and lignin. Also, it unmasked all crystalline cellulose available for further applications (Li et al. 2010b). Table 2 shows the crystallite sizes for before and after pretreatment of cassava stem. On comparing these values, the amorphous hemicellulose regions were curtailed by the effective pretreatment of combined acids.

**Fig. 3** SEM image showing the structural morphology of cassava stem (a) before and (b) after pretreatment

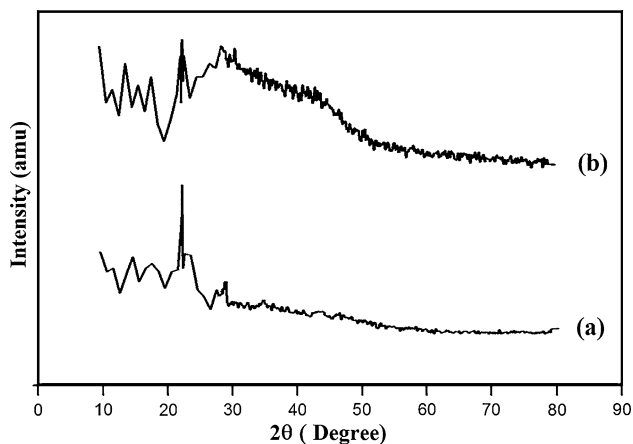


Fig. 4 XRD spectra of cassava stem (a) before pretreatment and (b) after pretreatment

Table 2 Crystallite sizes for before and after pretreatment of cassava stem

Before pretreatment		After pretreatment	
2θ	Crystallite size (nm)	2θ	Crystallite size (nm)
15.0	5.0	13.9	6.1
17.9	3.3	16	7.3
23	2.2	17.9	7.3
28	6.3	23	6.2
30.5	6.3	28.9	8.2
32.9	6.2	32.7	4.0
35	6.4	36.9	3.5
38.8	10.4	40.2	8.4
41.25	8.5	42.35	5.3
43.9	4.5	45	10.7
53	8.2	54.1	6.86
69.1	7.4	57.8	4.8

UVS

UV–Vis spectroscopy was used to determine the presence and concentration of any component in the given sample. These results are plotted for comparison purposes and to discuss the differences between the before- and after-pretreatment of samples. Usually, lignin displayed absorption maximum around 274 nm in UV spectrum (Fig. 5). Our sample also showed absorption peak around 270 nm for both before- and after-pretreated samples. As confirmed with FTIR and XRD results, the pretreatment has occurred in the sample which indicated the successful removal of hemicellulose from the microfibrils and macrofibrils of cellulose. These findings are in line with the UVS since both before- and after-pretreatment of cassava stems have retained the lignin. As shown in the spectra, both before- and after-pretreated fractions exhibited basic UV spectra

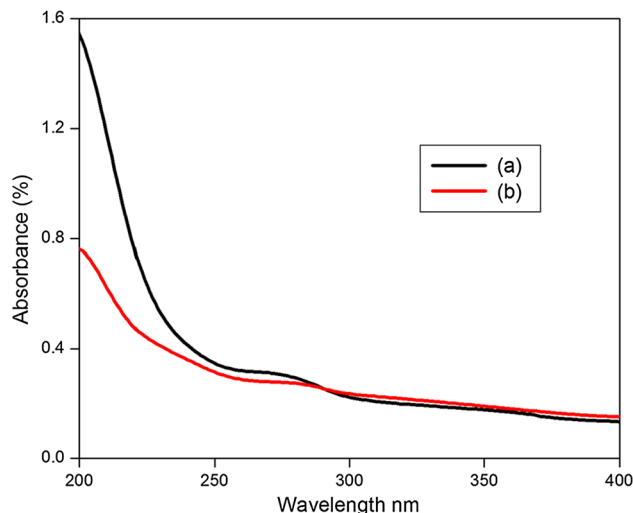


Fig. 5 UV spectra of cassava stem (a) before pretreatment and (b) after pretreatment

that have the first maximum absorption coefficient at 270 and 278 nm, respectively.

Conclusion

Cassava stem was effectively pretreated with combined organic (oxalic)–inorganic (sulfuric) acid. After pretreatment, the sample was subjected to functional group, surface morphology, topography and UV characterization studies using FTIR, SEM, XRD and UV spectroscopy, respectively. FTIR studies revealed the delignification in PCS. Surface morphology of PCS confirmed the destruction of lignocellulosic matrix in biomass. XRD studies interpreted the effectiveness of pretreatment through CI. UV studies confirmed the presence of phenolic compounds. This study gives an overview of how characterization analyses have been implemented to qualitatively assess the cassava stem for quintessential bioethanol traits such as cellulose, hemicellulose and lignin constituents. However, optimization of the pretreatment process conditions has been done to achieve more glucose yield. The current results demonstrated that the characterization of cassava stem (both raw and pretreated) by the above techniques revealed the complexity of the surface morphology. Hence, it can be concluded that the combined acid was an effective pretreatment method for cassava stem.

Acknowledgement The authors would like to sincerely thank the Proprietor, Suriya Sago Factory, Namakkal, India, for generously provided cassava stem. The authors would like to thank the Central Research Facility, Karunya University, Coimbatore, for providing SEM facilities; the Department of Chemistry, PSG College of Arts and Science, Coimbatore, for providing FTIR facilities for this research; and the Department of Nanotechnology, Sri Ramakrishna

Engineering College, Coimbatore, for providing XRD facilities. We would like to express our heartfelt thanks to the Management of Kumaraguru College of Technology, Coimbatore.

References

- Ahamefule FO (2005) Evaluation of pigeon pea-cassava peel based diets for goat production in South-Eastern Nigeria. Michael Okpara University of Agriculture, Umudike
- Caldeira K (2003) Climate sensitivity uncertainty and the need for energy without CO₂ emission. *Science* 299:2052–2054. doi:10.1126/science.1078938
- Donaldson L (1988) Ultrastructure of wood cellulose substrates during enzymatic hydrolysis. *Wood Sci Technol* 22:33–41. doi:10.1007/BF00353226
- Donaldson LA, Wong KKY, Mackie KL (1988) Ultrastructure of steam-exploded wood. *Wood Sci Technol* 22:103–114. doi:10.1007/BF00355846
- DuBois M, Gilles KA, Hamilton JK et al (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356. doi:10.1021/ac60111a017
- Farrell AE (2006) Ethanol can contribute to energy and environmental goals. *Science* 311:506–508. doi:10.1126/science.1121416
- Han M, Kim Y, Kim Y et al (2011) Bioethanol production from optimized pretreatment of cassava stem. *Korean J Chem Eng* 28:119–125. doi:10.1007/s11814-010-0330-4
- Kuila A, Mukhopadhyay M, Tuli DK, Banerjee R (2011) Accessibility of enzymatically delignified *Bambusa bambos* for efficient hydrolysis at minimum cellulase loading: an optimization study. *Enzyme Res* 2011:1–8. doi:10.4061/2011/805795
- Kumar A, Negi YS, Choudhary V, Bhardwaj NK (2014) Characterization of cellulose nanocrystals produced by acid-hydrolysis from sugarcane bagasse as agro-waste. *J Mater Phys Chem* 2:1–8. doi:10.12691/jmpc-2-1-1
- Le Troedec M, Sedan D, Peyratout C et al (2008) Influence of various chemical treatments on the composition and structure of hemp fibres. *Compos Part A Appl Sci Manuf* 39:514–522. doi:10.1016/j.compositesa.2007.12.001
- Li C, Knierim B, Manisseri C et al (2010a) Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. *Bioresour Technol* 101:4900–4906. doi:10.1016/j.biortech.2009.10.066
- Li M-F, Fan Y-M, Xu F et al (2010b) Cold sodium hydroxide/urea based pretreatment of bamboo for bioethanol production: characterization of the cellulose rich fraction. *Ind Crops Prod* 32:551–559. doi:10.1016/j.indcrop.2010.07.004
- Ling Hii K, Pin Yeap S, Mashitah MD (2014) Utilization of palm pressed pericarp fiber: pretreatment, optimization and characterization. *Environ Prog Sustain Energy* 33:238–249. doi:10.1002/ep.11757
- Monavari S, Galbe M, Zacchi G (2009) The influence of solid/liquid separation techniques on the sugar yield in two-step dilute acid hydrolysis of softwood followed by enzymatic hydrolysis. *Biotechnol Biofuels* 2:6. doi:10.1186/1754-6834-2-6
- Morán JI, Alvarez VA, Cyras VP, Vázquez A (2008) Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose* 15:149–159. doi:10.1007/s10570-007-9145-9
- Mosier N, Wyman C, Dale B et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* 96:673–686. doi:10.1016/j.biortech.2004.06.025
- Mtui GYS (2009) Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *Afr J Biotechnol* 8:1398–1415. doi:10.1073/pnas.1014862107/-DCSupplemental.
www.pnas.org/cgi/
- Nguyen T-AD, Kim K-R, Han SJ et al (2010) Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresour Technol* 101:7432–7438. doi:10.1016/j.biortech.2010.04.053
- Nuwamanya E, Chiwona-Karlton L, Kawuki RS et al (2012) Bioethanol production from non-food parts of cassava (*Manihot esculenta* Crantz). *Ambio*. 41:262–270. doi:10.1007/s13280-011-0183-z
- Park S, Baker JO, Himmel ME et al (2010) Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol Biofuels* 3:10. doi:10.1186/1754-6834-3-10
- Plant Village 2014. https://www.plantvillage.org/en/topics/cassava-manioc/infos/diseases_and_pests_description_uses_propagation. Accessed 2 Jan 2015
- Preetha B, Magesh A, Viruthagiri T (2011) Statistical optimization of process variables for direct fermentation of 226 white rose tapioca stem to ethanol by *Fusarium oxysporum*. *Int J Chem Tech Res* 3:837–845
- Preston TR (2001) Potential of cassava in integrated farming systems. In: International workshop on current research and development on use of cassava as animal feed. Khon Kaen
- Punsuvon V (2013) Optimization of delignification and enzyme hydrolysis of steam exploded oil palm trunk for ethanol production by response surface methodology. In: Chandel AK, Silva SS (eds) Sustainable degradation of lignocellulosic biomass—techniques, applications and commercialization. InTech. doi:10.5772/54691
- Ragauskas AJ (2006) The path forward for biofuels and biomaterials. *Science* 311:484–489. doi:10.1126/science.1114736
- Ramos LP, Mathias AL, Silva FT et al (1999) Characterization of residual lignin after SO₂-catalyzed steam explosion and enzymatic hydrolysis of *Eucalyptus viminalis* wood chips. *J Agric Food Chem* 47:2295–2302. doi:10.1021/jf9810665
- Romaní A, Garrote G, Alonso JL, Parajó JC (2010) Bioethanol production from hydrothermally pretreated *Eucalyptus globulus* wood. *Bioresour Technol* 101:8706–8712. doi:10.1016/j.biortech.2010.06.093
- Schell DJ, Farmer J, Newman M, McMillan JD (2003) Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor: investigation of yields, kinetics, and enzymatic digestibilities of solids. *Appl Biochem Biotechnol* 105–108:69–85. doi:10.1385/ABAB:105:1-3:69
- Singh L, Wahid ZA (2015) Methods for enhancing bio-hydrogen production from biological process: a review. *J Ind Eng Chem* 21:70–80. doi:10.1016/j.jiec.2014.05.035
- Sovorawet B, Kongkiattikajorn J (2012) Bioproduction of ethanol in SHF and SSF from cassava stalks. *KKU Res J* 17:565–572
- Tabil L, Adapa P, Kashaninej M (2011) Biomass feedstock pre-processing—part 1: pre-treatment. In: Bernardes MADS (ed) Biofuel's engineering process technology. InTech, Tiruchirappalli
- Thygesen A, Oddershede J, Lilholt H et al (2005) On the determination of crystallinity and cellulose content in plant fibres. *Cellulose* 12:563–576. doi:10.1007/s10570-005-9001-8
- Varga E, Klinke HB, Reczey K, Thomsen AB (2004) High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. *Biotechnol Bioeng* 88:567–574. doi:10.1002/bit.20222
- Varmudy V (2001) Marketing of vegetables in India. Daya Publishing House, New Delhi