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Novel method for the preparation of lignin-rich nanoparticles from lignocellulosic fibers

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ABSTRACT

Nanoparticle research in the field of biopolymers is significant from the perspective of efficient utilization of sustainable resources. The most abundant biopolymer in nature, cellulose, has been extensively studied. Lignin is the second most abundant natural renewable biopolymer that has immense potential and is undervalued largely due to structural and morphological heterogeneity. This limitation of lignin can be addressed by efficient methods; one of which could be conversion of lignocellulosic fibers to lignin nanoparticles. In this research work, we have prepared lignin-rich nanoparticles from lignocellulosic fibers of Indian ridge gourd (*Luffa cylindrica*) by the breakdown of the lignin-cellulose complex by specific enzymes. These nanoparticles are characterized by electron microscopy, X-Ray diffraction, thermal studies and spectroscopic measurements. Microscopy results indicate that the particle size is around 20–100 nm with a cuboidal shape. The X-ray diffraction data indicate reduced crystallinity upon enzymatic hydrolysis. The thermal and spectroscopic studies confirm that upon enzymatic hydrolysis, cellulosic content in nanoparticles is substantially reduced. The work highlights a method for obtaining unique cuboidal shaped lignin-rich nanoparticles that are uniform in size and shape. These lignin-rich nanoparticles have a potential for various applications across fields including automobile, pharmaceutical and polymer industries.

1. Introduction

Lignocellulose, the most abundant renewable biomass on earth, chiefly consists of cellulose, hemicelluloses, lignin and pectin as the major constituents with resins, waxes, proteins and extractives as minor constituents (Malherbe and Cloete, 2002). These different polymers are associated with each other in a complex hetero-matrix. They are linked with each other to different degrees and their composition varies depending on the type of biomass, species of plant, and even source of the biomass (Himmel et al., 2007). The complex hierarchal structure of lignocellulosic biomass is the main obstacle for fractionation of lignocellulosics to obtain value added products. There are many physicochemical, structural, and compositional factors that deter complete and efficient degradation of lignocellulosics. Over the years several types of hydrolysis process by mechanical, chemical or catalytic routes have been extensively explored (acid hydrolysis, alkaline hydrolysis, delignification via oxidation, organosolv pretreatment, and ionic liquids pretreatment) to breakdown the lignin-carbohydrate complexes in lignocellulosics (Kumar et al., 2009; Lee et al., 2014; Hendriks and Zeeman, 2009; Wyman et al., 2005). Pretreatment methods enhance the degradation of the polymeric structure of lignocellulosics. Of all the different methods of biomass pretreatment, chemical pretreatment has proven to be the most efficient and cost effective method so far. Physical pretreatment methods that include chipping, grinding, and milling and thermal methods are less efficient and consume more energy than chemical methods (Cybulska et al., 2012).

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In the recent years enzymatic methods in biomass degradation processes have shown an increased potential as researchers are focused on combining saccharification and fermentation processes (Hasunuma et al., 2013) to produce biofuels and bio-based chemicals to achieve cost effectiveness in biorefinery. Specific enzymes obtained from lignocellulolytic organisms (especially wood-degrading fungi and bacteria) have natural lignocellulose degradation ability as part of natural energy transfer and carbon cycle (Himmel et al., 2010). As potential industrial catalysts for biomass conversion, enzymes provide high specificity, low energy or chemical consumption, and low environment pollution. Recently, Liu and Hu showed that it is possible to obtain nearly 77% of cellulose by enzymatic hydrolysis of bamboo fibers (Liu and Hu, 2009).

In most of the biomass degradation methods the emphasis is to remove lignin and focus on getting a maximum yield for cellulose. Presence of lignin in lignocellulosic biomass is the main reason for recalcitrance during separation and purification process. Lignin is a phenolic polymer that provides a protective barrier for plant cell permeability and provides resistance against microbial attacks and thus prevents plant cell destruction (Yang et al., 2007). Although in most of the biomass degradation procedures, lignin is regarded as an undesired by-product, lignin has gained importance in the recent years as a promising source for a variety of products including composites and chemicals. Lignin is regarded as a raw material with a high recovery potential, accessible from renewable sources, with low costs and a negligible environmental pollution hazard (Matsushita et al., 2006; Popa et al., 2011). Besides the impressive properties of lignin, such as its antioxidant, antimicrobial attributes along with its CO_2 neutrality and reinforcing capability, it is an ideal candidate for the development of novel products across various industries (Nagarajan et al., 2013). The general perception is that lignin is amorphous and the structural heterogeneity which arises due to diverse polymerization limits the application of lignin in the category of high value product (Brenelli et al., 2015). These disadvantages may be overcome by the conversion of macro or microscopic lignin into nanostructures (Richter et al., 2016; Yang et al., 2016). As it is evident in many cases, there is a significant change in the physico-chemical properties of the materials as they are converted into nano forms (Editorial, 2009). Earlier reports have indicated that the lignin nanoparticles are biodegradable and are non-toxic to yeast and microalgae (Frangville et al., 2012). Recently, there have been few publications that highlight the potential use of lignin in nanoparticle formulations. As a material for applications in chemical sensors, lignin was doped with multi-walled carbon (Faria et al., 2012) and nanolignin particles have been prepared using a high-yield, scalable nano manufacturing approach with a controlled particle size to adapt for suitable nanoemulsions (Ago et al., 2016). Additionally, ion-responsive nanofibers (Gao et al., 2012) and lignin based nanocapsules have been prepared that can be applied for delivery of hydrophilic drug molecules (Yiamsawas et al., 2014).

From the foregoing it becomes evident that nanolignin plays an important role in expanding the applications of lignin. It is essential to explore, identify, optimize and characterize methods for efficient production of lignin nanoparticles. Nair et al. have recently reported preparation of nanolignin from Kraft lignin using high shear homogenization (Nair et al., 2014). Most of the earlier methods use Kraft lignin or indulin to prepare lignin nanoparticles. The method of preparation reported so far is usually precipitation or mechanical methods (Jiang et al., 2013). To the best knowledge of the authors, there has been no reported information on the studies of nano lignin by enzymatic hydrolysis particularly using the lignocellulosic materials so far. Probably this may be the **first** such attempt. Accordingly, this paper presents a new strategy for the preparation and characterization of lignin-rich nanoparticles from the Indian ridge gourd [*Luffa cylindrica,* hereafter called as LCF throughout the text], using enzymatic hydrolysis. This method highlights site specific breakdown of the lignocellulosic complex structure to yield uniform cuboidal nanoparticles of high lignin content. The formation of these nanoparticles was characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infra-Red (FTIR) Spectroscopy and Ultra-Violet (UV) Spectroscopy. This study opens up the scope for lignin based nanoparticles that can have impact in a wide range of fields including medicine, pharmaceuticals, automobile, chemicals and packaging industries.

2. Experimental aspects

2.1. Materials

The dried fruits of *Luffa cylindrica* were obtained from the local source. Laboratory grade reagents such as acetone, ethanol, toluene, sodium acetate, and acetic acid, sodium hydroxide and sulphuric acid were obtained from the local market. The enzymes, Multifect CX GC cellulase, Multifect Pectinase FE, Optimase CX 255L, were received from Genencor, Mumbai-India, as gifts samples. This company being private, more details of the enzyme are not available.

2.2. Methods

2.2.1. Preparation of lignin rich nanoparticles

The whole dried fruit of *Luffa cylindrica* was cryo-crushed by freezing in liquid nitrogen followed by milling. The fibers were then subjected to organo-solvent extraction process using Soxhlet apparatus for 6 h in a mixture of acetone, ethanol and toluene in the ratio of 1:1:4. The extracted powder was dried in an oven overnight at 100 °C.

Following chemo-mechanical treatment and drying, the fibers obtained from the milling process and chemically treated as mentioned above were soaked in acetate reaction buffer of pH 4.5 for 1–2 h. The soaked fibers were treated with the enzymes, Multifect CX GC Cellulase, Multifect Pectinase FE, Optimase CX 255L (10%, 15% and 15% respectively). The enzymatic reactions were carried out in small batches with 0.5 g–2.0 g of the fibers. The pH and temperature optimization was carried out in micro batches with 0.5 g of the fiber. The fiber and the enzyme mixture were incubated with shaking at 55 °C and pH of 4.5 for 24 h. The samples were prepared as necessary for subsequent analysis. Preparation of LCF nano particles and their characterization by different techniques are shown schematically (Scheme 1).

It may be noted that the enzymatic degradations mentioned above have been carried out on a micro scale as optimizations of conditions was the major emphasis to provide a proof of concept of obtaining lignin rich nanoparticles from enzymatic degradation. Hence, calculation of percentage yield or concentration of the fiber content could not be done at this stage. However, the authors are currently exploring scale up options, which poses significant challenges given the enzymatic sensitivity to substrate and product concentrations in the reaction mixture besides other scale up optimizations.

2.2.2. Characterization

2.2.2.1. Morphology studies. A field emission scanning electron microscope (FESEM) model FEI SEM Quanta-200, with accelerating voltage of 12.5 kV was used to study the fibers and the surface topography. The samples were mounted onto a carbon tape and coated with a thin layer of gold.

A FEI Tecnai T20 U-Twin transmission electron microscope was used to observe the lignocellulosic nanoparticles using an