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A Morphological Study of Organasolv Lignin Aggregates from *Miscanthus x giganteus* at Different Ethanol Concentration

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ABSTRACT

Understanding the interaction of lignin with a solvent containing two components with different concentrations, ethanol and water present a significant knowledge contribution prior to modification and converting lignin into useful renewable materials. Soluble lignin extract at different ethanol concentrations (50%, 25% and 10%) were examined for morphological characterisation via light microscopy. 10 recorded images from light microscopy were processed via imageJ software (1.50v) to characterise the size and shape factors such as circularity and circle equivalent diameter. Results showed that reduction of 50% ethanol concentration to 25% and 10% of soluble lignin extract resolved population of large particle into sub-population of small particle lignin aggregates. The big lumps of lignin aggregates broke into smaller and rounder pieces of particles. The reduction of particle size of lignin aggregates in the soluble lignin extract at low ethanol concentration was pivotal in preparing a feedstock for lignin modification process.

KEYWORDS

Lignin, Aggregates, Miscanthus, Morphological, Modification

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INTRODUCTION

Lignin is an abundant naturally occurring biopolymer currently produced as by-product from the pulping and paper industry, where the process generates lignin in the form of lignosulphonates. While there are many applications for lignin there are all low value and attempts to add value to lignin are hindered by its complex physico-chemical nature and the presence of sulphur. Lignin isolated via different methods can vary widely in terms of chemical composition and molecular structure. The differences also affect the physical properties such as solubility and molecular weight (Bruijnincx *et al.*, 2016). Lignin had random polymer globule structure with non-linearly and random cross-linked with other constituents, and the structure of lignin is not as the other two main components of lignocellulosic biomass, cellulose and hemicellulose which has a more linear shape structure (Chen, 2014).

The interaction of solute and solvent is pivotal in determining the properties of lignin and influences the behaviour of lignin aggregates. In addition, the solvent concentration had an influence on the lignin purity and recovery as well the other physical, chemical and structural properties of precipitated lignin and supernatant derived from soluble lignin fraction. The relationship between the solvent concentration and the resultant lignin macromolecules' is complex. The complex behaviour of lignin aggregates may result from the interaction of lignin with a solvent containing two components with different concentration (Da Silva *et al.*, 2002; Maitra and Bagchi, 2008). Understanding of these associations present a significant knowledge contribution, specifically for elucidation of unexpected behaviour in modifying and converting lignin into useful renewable materials. Therefore, in the context of the growing interest in developing valued added uses of lignin, this study focused on characterising lignin via sub-critical water; with particular emphasis on the formation of lignin aggregates at different ethanol concentration.

MATERIALS AND METHODS

Materials

Miscanthus x giganteus (*MxG*), a lignocellulosic biomass, was grown and harvested in Aberystwyth, Wales, United Kingdom and provided by the Institute of Biological, Environmental and Rural Sciences (IBERS, UK) and Phytatec (UK) Ltd. The biomass was stored dry and in the dark. Absolute ethanol (Fisher Scientific, UK), nitrogen (compressed oxygen free nitrogen, BOC, UK) and carbon dioxide (vapour withdrawal, BOC, UK) used had ≥ 99.8% purity.

Sequential Lignin Extraction

The steps taken in extracting lignin in this work are outlined in Figure 1. Prior to hydrolysis, *MxG* was mixed in water, then warmed up to 50°C to soften the grass structure. The mixture was soaked for 20 minutes to rehydrate the grass and the mixture was then ground for 3 minutes in a blender to reduce the particle size of material. The grinding conditions of amount of biomass, temperature, soaking time, grinding time and solid:liquid ratio were previously optimised to give an average particle size of 500µm (Roque, 2013). Sequentially processed *MxG* from 120°C and 180°C was only mixed in water solution and ethanol-water solution, respectively by warming up to 50°C and soaking time 5 minutes prior to the sequential hydrolysis step.

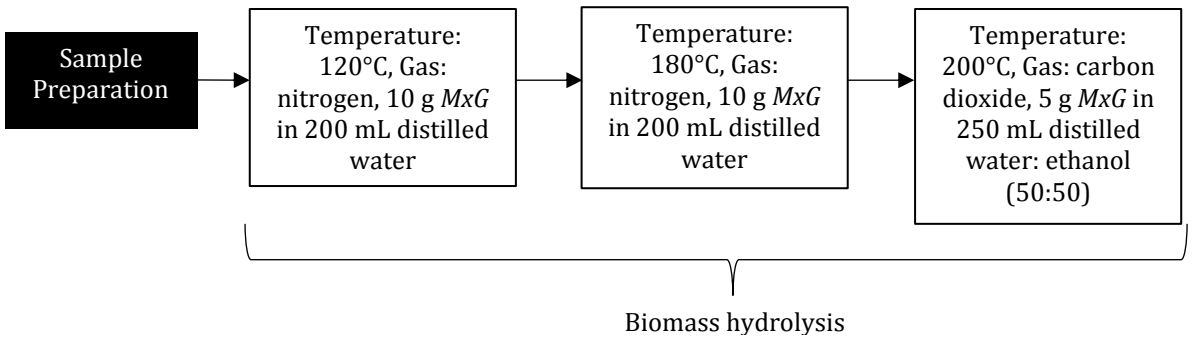

Figure 1. Flow chart of biomass hydrolysis.

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The *MxG* was treated through a three-stage temperature profile sequential extraction in order to differentially separate extractives, hemicellulose, cellulose and lignin. The first step applies SCW at 120°C with an equilibrium time of 30 minutes and 50 bar of nitrogen gas pressure to remove extractives such as starch, protein, glucose, lipids and pectin which could interfere with the isolation and later analytical steps. The second step used a SCW at regime of 180°C, for a reaction time of 30 minutes under 50 bar of nitrogen gas to attempt to hydrolyse hemicelluloses prior to delignification. The final step involves extraction of lignin via a SCW with associated modifiers using a water:ethanol (50:50 v/v) mixture at 200°C, a reaction time of 60 minutes and 50 bar of carbon dioxide gas.

Biomass Hydrolysis

The *MxG* slurry was transferred to a 500 mL stirred pressure vessel (Alloy C276 Parr, USA). The reactor was closed and pressurised with desired gas to 50 bar. The set point temperature was increased to the set temperature and it was kept stable during the reaction time by a controller (4386 Parr, USA). After the reaction, the reactor temperature was decreased by operating a cooling system which comprises of a cooling coil inside the pressure vessel where a coolant flows at an initial -7°C. When the temperature fell below 50°C, the reactor was depressurised slowly to atmospheric pressure before the reactor was opened. Finally, the solid fibres and solution were separated using a laboratory sieve (BS410-1 size 45 µm, Endecotts Ltd, England) for SE carried out at 120°C and 180°C. The 50% ethanol concentration of soluble lignin extract or filtrate for biomass hydrolysis at 200°C was recovered by vacuum filtration through a Pyrex sintered disc porosity 2. The 50% ethanol concentration soluble lignin extract from biomass hydrolysis was placed in a freezer at -20°C for 2 hours, after which the ethanol concentration was adjusted to either; 10% and 25% by adding distilled water.

Light Microscopy Analysis

0.01 mL of soluble lignin extract at different ethanol concentration were examined under the light microscopy (Olympus BX50, Japan) at 100x magnification equipped with a digital camera (Motic MC35X, China).

ImageJ Analysis

Using images captured by the light microscope, particle size analysis was conducted by ImageJ freeware (1.50v). Prior to particle size analysis, 10 recorded images were calibrated, despeckled to reduce visual noise and converted to gray scale. Image analysis captured a 2-dimensional image of a 3-dimensional particle and the projected area of lignin macromolecules enclosed by the outer contour of a particle from 2-dimensional image was analysed based on assumption that center of mass area (similar to centroid but brightness weighted) (Olson, 2011; Willen, 2008)(Equation 1).

$$\mathrm{A} = \pi\, r^2 \tag{1}$$

Where:

A = projected area, µm^2
r = radius of circle, µm

The circle equivalent diameter which is the diameter of a circle with the same area as the 2-dimensional image of the particle was calculated using Equation 2 (Olson, 2011).

$$D_{CE} = \sqrt{\frac{4A}{\pi}} \tag{2}$$

Where:

D_{CE} = circle equivalent diameter, µm
A = projected area, µm^2

The circularity, a dimensional value was determined based on the projected area and perimeter of particle (Equation 3). The circularity value is indicative of the importance of particle shape in the overall particle behaviour and interaction in terms of particle form and roughness, and therefore the reactivity of lignin macromolecules (Liu *et al.*, 2015). Lower circularity value (C≤1) indicates that the lignin macromolecules was away from a perfectly round and smooth circle that a particle becomes (Olson, 2011).

$$\mathrm{C} = \sqrt{\frac{4\pi A}{P^2}} \tag{3}$$

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Where:

C = circularity
A = projected area, μm^2
P = perimeter, μm

Statistical Analysis

SPSS software (Version 22) was used for statistical analysis. One-way analysis of variance was carried out at $\alpha = 0.05$ to analyse the significance difference of imageJ analysis.

RESULTS AND DISCUSSIONS

Light Microscopy Analysis

When reducing the ethanol concentration of soluble lignin extract, it resolved population of large particle into sub-population of small particle. Population of large particle aggregates at 50% ethanol concentration was verified with the morphology characterisation shown in Figure 2. Reduction of 50% ethanol concentration of soluble lignin extract to 25%, population of large particle began to dissociate gently into sub-population of small particle aggregates. Figure 3 presented the sub-population of lignin aggregates at 25% ethanol concentration. Figure 4 proved that the sub-population of small particles within population at 10% ethanol concentration of soluble lignin extract reduced and particles may be tending towards re-aggregation.

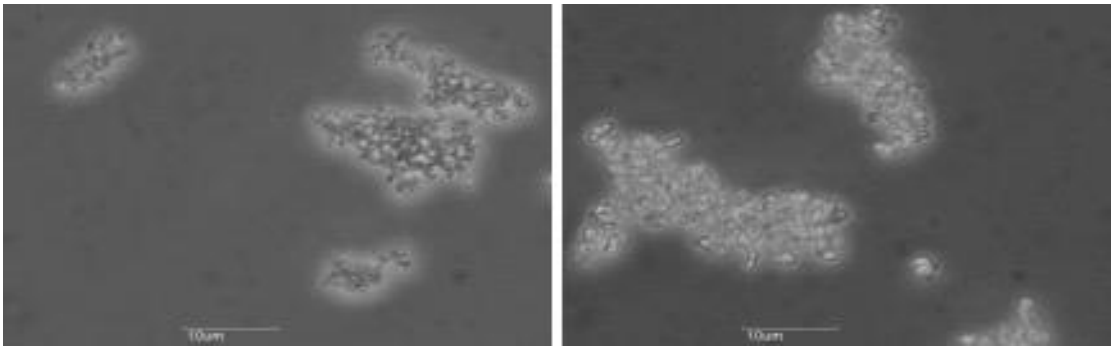

Figure 2: LM images of soluble lignin extract at 50% ethanol concentration

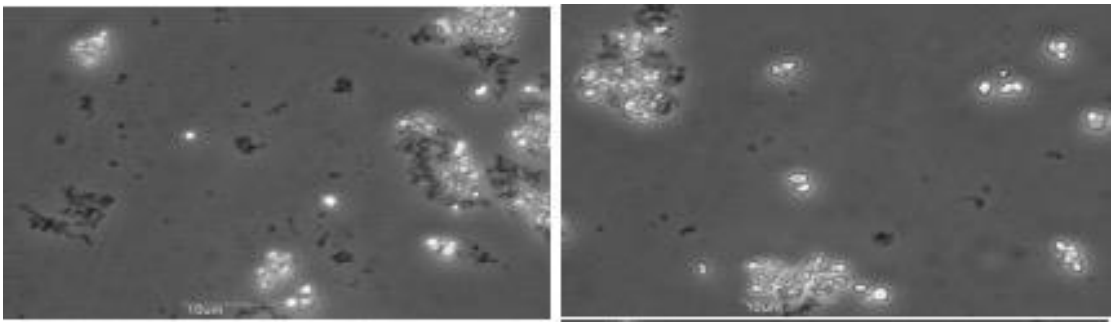

Figure 3: LM images of soluble lignin extract at 25% ethanol concentration.

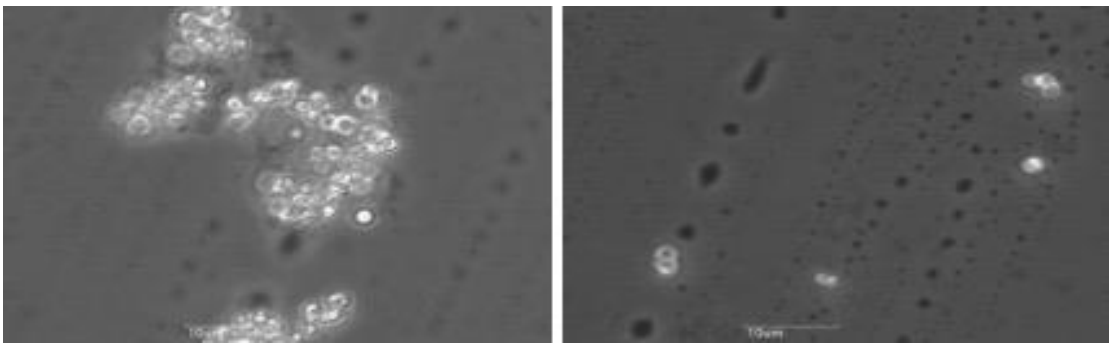

Figure 4: LM images of soluble lignin extract at 10% ethanol concentration.

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Theoretically, an increase of ethanol concentration resulted in more dispersion or aggregation of particles in the ethanol-water mixture (Claverie *et al.*, 2010; Dan *et al.*, 2009). As dispersion increases, the particle size of molecules decreases (Bergeret and Gallezot, 2008; Dan *et al.*, 2009). High dispersion of lignin macromolecules also has a broad particle size distribution. As might have been expected, the findings in this study were contradictory with the theory. The reason for this rather contradictory result is still not entirely clear, but interestingly, the preliminary study of light microscopy images has proven that aggregation of lignin occurs at low ethanol concentration. Furthermore, formation of lignin aggregates at low ethanol concentration produced rounder or sphere particles as have been discussed in the next section. A possible explanation of contradict lignin aggregates behaviour may be that lignin is an amphiphilic polymer that contains both hydrophobic and hydrophilic segments besides possesses self-assembly behaviour (Xiong *et al.*, 2017). The hydrophilic segments of lignin macromolecules had an affinity to ethanol, whereas the hydrophobic segments of dissociated lignin aggregate into spherical or rounder shape in the ethanol-water mixture (Li *et al.*, 2016; Qian *et al.*, 2014; Rao *et al.*, 2017; Xiong *et al.*, 2017).

ImageJ Analysis

Image analysis via light microscopy is suggested to characterise individual characteristics of the particles from a 2-dimensional image, from which it determines different size and shape factors such as circularity and circle equivalent diameter. The imaging analysis inspected visually as data in pictorial form could be used as indicator to verify the particle size of particles (Gao *et al.*, 2005; North, 2006; Vaux, 2012; Wilt *et al.*, 2009). As a function of ethanol concentration, soluble lignin extract images captured by light microscopy were further analysed using ImageJ. Average circle equivalent diameter and average circularity versus ethanol concentration was presented in Figure 5. Overall, the average circle equivalent diameter and average circularity appeared to be significantly different ($p<0.05$) of all ethanol concentration studied. Figure 5 showed that there has been a sharp drop in the average circle equivalent diameter from 50% (7.62 μm) to 25% ethanol concentration (2.90 μm), and a slight increase at 10% ethanol concentration (3.02 μm). The average circularity showed a gradual increase from 50% (0.62 μm) to 25 (0.81μm) and 10% ethanol concentration (0.86 μm). In general, when the ethanol concentration was reduced from 50% to 25% and 10% ethanol concentration of the soluble lignin extract, the big lumps of lignin macromolecules broke into smaller and rounder pieces of particles. Therefore, small particles result in an increase in the reaction rate in downstream processes due to the greater surface area with more exposed particles (Khadka *et al.*, 2014).

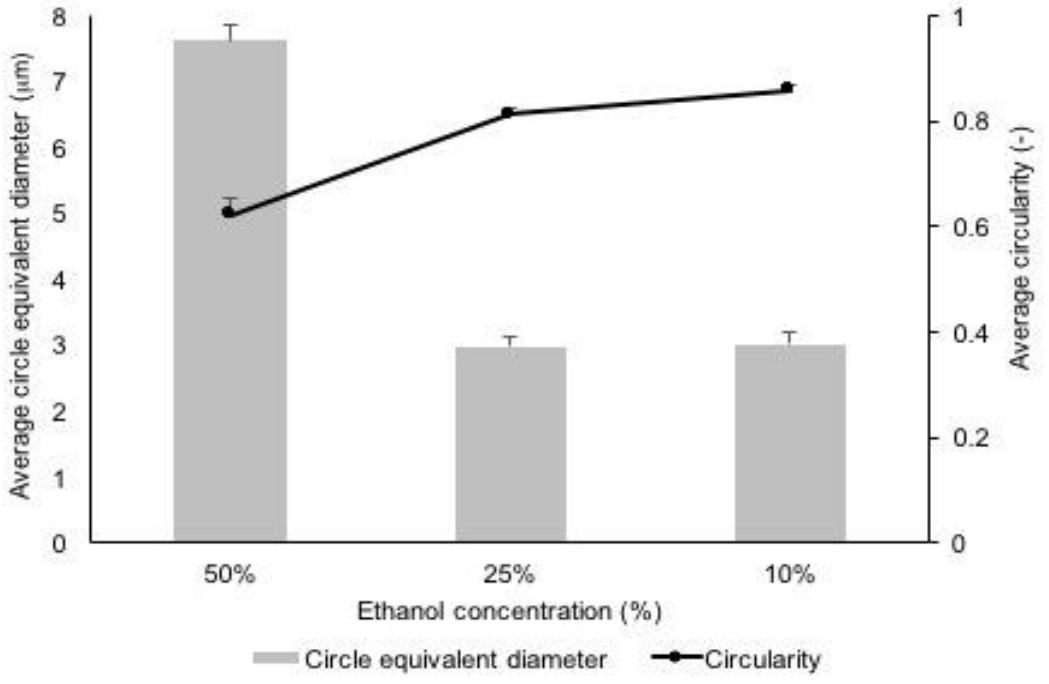

Figure 5: Average circle equivalent diameter and average circularity at different ethanol concentrations by ImageJ.

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CONCLUSIONS

The focus of this preliminary study was primarily tried to understand the behaviour of lignin aggregates in soluble lignin extract and the study has raised important question whether the effect of ethanol concentration influenced the behaviour of lignin aggregates. Basically, in this study, it is hypothesised that water had influence on hygroscopic solvents, i.e. different ethanol concentration that has different ability to attract and hold water molecules from surrounding environment. Solvation of ethanol and water creates non-covalent interactions, such as hydrogen, Van der Waals and hydrophobic bonding that have a strong tendency to form aggregates with other molecules. The results of preliminary study via light microscopy to study the effect of ethanol concentration showed that the ethanol concentration affected the behaviour of lignin aggregates in ethanol-water solution. Therefore, the study would have been more relevant if further study conducted to assess the effect of solute concentration in order to understand the driving forces behind the dispersion or aggregation of lignin. The good dispersion state of lignin macromolecules was also responsible for an excellent compatibility of lignin in polymer matrices of bio-composites.

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