Biomass and Bioenergy 111 (2018) 52–59

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

A biorefinery approach for fractionation of *Miscanthus* lignocellulose using subcritical water extraction and a modified organosolv process

Arielle Muniz Kubota[1], Raitis Kalnins[2], Tim W. Overton[*]

School of Chemical Engineering, University of Birmingham, Birmingham, B15 2TT, UK

ARTICLE INFO

Keywords:
Subcritical water
Principal component analysis
Delignification
Biofuel
Biorefinery

ABSTRACT

Using a biorefinery approach, biomass polymers such as lignin and carbohydrates can be selectively purified from lignocellulosic feedstocks with the aim of generating not only lignocellulosic bioethanol but also high value bio-based compounds. Furthermore, the efficient use of the entire biomass can increase overall feedstock value and significantly contribute to process cost-effectiveness. Therefore, the aim of this work was to fractionate the main compounds of the energy crop *Miscanthus x giganteus* (MxG) using 'green' solvents in order to obtain cellulose-enriched fibres as well as non-toxic streams rich in hemicellulose and lignin. Two processing routes were compared: a direct 1-step modified organosolv method for simultaneous lignin and hemicellulose removal; and a 3-step sequential process using subcritical water extraction for recovery of first extractives then hemicellulose, followed by modified organosolv lignin extraction. Both methods successfully generated cellulose-enriched fibres; from a complex mixture of compounds present in MxG, it was possible to obtain fibres comprising 78% cellulose without the use of commonly-applied toxic solvents that can potentially limit end uses for processed biomass and/or need additional neutralization steps. Fibres generated by the direct and sequential processes were very similar in composition; however, physicochemical analysis of the fibres using scanning electron microscopy, Fourier-transform infrared spectroscopy and principal component analysis confirmed structural differences resulting from the two processing routes, which were demonstrated to have an impact on downstream processing.

1. Introduction

The shift from a petroleum based economy towards one supported by renewable resources is not only environmentally beneficial, but it is also believed to be a way of achieving a sustainable economy and energy independence [1]. One potential renewable resource of current interest is lignocellulosic biomass, for example biomass comprising rapidly-growing plants or waste lignocellulosic biomass generated as a byproduct of agriculture and food processing [2–4]. In the former category, *Miscanthus x giganteus* (MxG) has been identified as an attractive source of biomass due to its potential for high yields even with few inputs (nutrients, irrigation), high photosynthetic efficiency, low cost, and adaptability to low-quality land [5].

The biorefinery concept describes the utilisation of biomass to generate a range of products, for example fuels, platform chemicals and high-value chemicals, in a manner similar to the refinery of petrochemicals [2]. Interest in the biorefinery concept as part of a bio-based economy is increasing with technological advances in agriculture, biotechnology and chemistry, as well as societal drivers [2,6]. Moreover, it is believed that the successful implementation of an integrated biorefinery platform with the co-production of valuable products can make 2nd generation bioethanol cost-effective [7,8]. In this process, ethanol is generated from the fermentation of monosaccharides extracted and depolymerised from the cellulose and hemicellulose fractions of lignocellulosic biomass. However, due to the highly recalcitrant structure of lignocellulose, extraction and depolymerisation of monosaccharides is a difficult process, often with low monosaccharide yield due to decomposition of released monosaccharides under harsh reaction conditions. Moreover, available technologies for lignocellulosic fractionation are expensive, and frequently use toxic solvents to access biomass components, presenting an environmental concern [9].

In addition, it is widely reported that lignocellulose treatments to liberate monosaccharides result in the formation of fermentation inhibitors, which inhibit the production of ethanol from monosaccharides

Abbreviations: MxG, *Miscanthus x giganteus*; SWE, Subcritical water extraction; PCA, Principle component analysis

[*] Corresponding author.
E-mail addresses: arielle.mbarros@gmail.com (A. Muniz Kubota), trejdevins@gmail.com (R. Kalnins), t.w.overton@bham.ac.uk (T.W. Overton).
[1] Current address: Interdisciplinary Centre of Energy Planning (Nipe), University of Campinas, 13083-970, Brazil.
[2] Current address: Eco Baltia Group, Maskavas st. 240-3, Riga, Latvia, LV-1063.

https://doi.org/10.1016/j.biombioe.2018.01.019
Received 15 August 2017; Received in revised form 22 January 2018; Accepted 26 January 2018

[10]. Thus, prevention of inhibitor formation during lignocellulosic processing to monosaccharides would potentially improve fermentative production of bioethanol.

An additional aim of biorefinery is similar in principle to chemical refineries: separation and purification of multiple commercially viable streams from a single feedstock. As well as hexose and pentose monosaccharides, useful for production of bioethanol via fermentation, potential streams from the biorefinery of lignocelluose include xylooligosaccharides (an emerging potential prebiotic [11]), and a variety of platform chemicals such as furan compounds, organic acids and phenolic compounds [12].

A major current issue with biorefineries using plant biomass as a feedstock is the use of harmful chemicals [13]. The use of 'green' solvents for lignocellulosic biomass processing is not only environmentally beneficial but it also holds the potential to generate non-toxic streams that could enhance the potential uses of biomass fractions for conversion into high-value products particularly for food and pharmaceutical applications [14]. Therefore, the use of subcritical water extraction (SWE) for hemicellulose extraction as a 'green' solvent is a potentially advantageous option that does not require additional catalysts, neutralization steps following processing or corrosion-resistant reactors [15,16]. SWE has previously been used for extraction of a wide range of different compounds in the biotechnology, food and pharmaceutical areas (reviewed by Ref. [17]). Lignin extraction can also be performed using 'green' solvents in a modified organosolv method using non-toxic solvents such as ethanol that can be recovered and re-used in the process [18] and alternative catalyst to replace bases (eg NaOH, KOH, ammonia) or mineral acids (H_2SO_4, HCl, H_3PO_4) used in delignification [13].

Previous work aiming to reduce MxG recalcitrance have been focused on lignin removal rather than biomass fractionation [19] and the use of mineral acids [19,20] and hydrogen peroxide [21] for extractions. Moreover, physicochemical evaluation of MxG fibres has been focused on visual evaluation of FTIR spectra rather than the use of a statistical analysis such as PCA [20]. Therefore, the aim of this work was to evaluate two different routes to obtain purified cellulose fibres from MxG: a single-step modified organosolv approach; and a three-step SWE/modified organosolv approach designed to sequentially remove biomass extractives, hemicellulose and lignin from cellulose fibres (Fig. 1). Moreover, a physicochemical evaluation of the effect of these processing routes in the obtained fibre is presented using SEM, FTIR and PCA. Thus, this work proposes environmentally-friendly processes in a biorefinery approach as an attempt to fractionate lignocellulosic biomass and to obtain purified streams of hemicellulose and lignin and cellulose-enriched fibres that can be further processed into a variety of products including biochemicals and bioethanol.

2. Materials and methods

2.1. Materials

Air-dried *Miscanthus x giganteus* (MxG) was cultivated in Wales (UK), harvested in 2013, and kindly provided by Phytatec (Aberystwyth, UK). MxG used in this work contained (as percentage of dry weight): 11.5% of extractives, 22.6% of Klason lignin, and 18.3% of hemicellulose, all determined using NREL methods [22,23].

2.2. Extraction methods

2.2.1. Extractives SWE

0.01 kg (wet weight) of MxG was soaked in 0.2 L of distilled water at 50 °C for 20 min. The suspension was then ground in a domestic blender for 3 min and placed in a 0.5 L high-pressure reactor (Parr, alloy C276). The reactor was purged and pressurized to 5.0×10^6 Pa using N_2 and a heating jacket was set to 120 °C. The extraction lasted for 30 min (all residence times reported in this work starts when target temperature was achieved, i.e., heating time was not taken into consideration. Heating time varied according to the target temperature and was from 12 to 27 min). At the end of the extraction, the reactor was cooled in an ice bath. Remaining fibres were filtered and dried completely at 65 °C. The fibres resulting from this procedure were called 120 °C fibres.

2.2.2. Hemicellulose SWE

0.01 kg of dried 120 °C fibres were placed in the same reactor as above and mixed with 0.2 L of distilled water. The reactor was purged and pressurized to 5.0×10^6 Pa with N_2 and a heating jacket was set to 180 °C for 30 min. After cooling the reactor in an ice bath, remaining fibres were filtered, dried completely at 65 °C and named 180 °C fibres. Temperatures for both extractives and hemicellulose SWE steps were chosen after preliminary tests.

2.2.3. Modified organosolv lignin extraction

The lignin extraction step was performed using a modified organosolv method adapted from Roque [24] in which mineral acids were replaced by pressurized CO_2 as catalyst. 0.25 L of 50% (v/v) ethanol in distilled water (50 °C) was mixed with 0.005 kg of starting material (MxG, for direct delignification; 180 °C fibres for sequential extraction) and then allowed to soak for 20 min before being placed in the 0.5 L reactor. In the case of direct extraction, the suspension was ground in a domestic blender for 3 min before being placed in the reactor. The reactor was purged and pressurized to 5.0×10^6 Pa using CO_2 and set to 200 °C. The reaction lasted 60 min, and then the reactor was placed into an ice bath. Remaining fibres were filtered, air dried for 48 h and then dried completely at 65 °C. Cellulose-enriched fibres obtained after lignin extraction were named DEL in the direct route and SEQ in the sequential extraction route (Fig. 1).

2.3. Quantitative/qualitative analysis

2.3.1. Extractives determination

The extractives content of the starting MxG material was determined using the National Renewable Energy Laboratory (NREL) protocol. This is a 2-step extraction procedure in a Soxhlet apparatus using first water (HPLC grade) as solvent for two consecutive days for 8 h per day, and then ethanol as solvent for the same period of time [20]. Fibres were weighed before and after the extractions and the extractives compounds were calculated as the mass difference.

2.3.2. Lignin quantification

Lignin quantification was performed using the National Renewable Energy Laboratory (NREL) protocol [23] for Klason Lignin quantification using the Klason Lignin method.

2.3.3. High Performance Anion Exchange Chromatography (HPAEC)

Glucose (99.5%), arabinose (98%), xylose (99%), fructose (99%), cellobiose (98%), 5-hydroxymethyl-2-furaldehyde (HMF) (99%), erythrose (75%), and Avicel were purchased from Sigma Aldrich. Cellotetraose (95%) and cellohexaose (90%) were purchased from Megazyme, and galactose (99%) was purchased from Acros Organics. Sugar analysis in liquid samples were performed by High Performance Anion Exchange Chromatography coupled with Pulse-Amperometric Detection (HPAEC-PAD) from Dionex/Thermo (ICS-5000) using a guard CarboPac™ PA1 column (4 × 50 mm) and an analytical CarboPac™ PA1 column (4 × 250 mm). Oven and detector compartments were kept at 30 °C and 25 °C, respectively. Flow rate was 0.001 L/min and sample volume injected was 10×10^{-6} L, Milli-Q® water was used as solvent A and in the preparation of the other solvents. 0.2 M NaOH and 1 M NaOAc were used as solvent B and C respectively.

The method started with an isocratic step using 0.021 M of B during 20 min. At 20 min, B was increased to 0.080 M. Then, from 20 to 60 min, solvent C was introduced from 0 to 20 mM and B was kept at 0.080 M. A washing step was performed from 60 min in which B and C