Bioresource Technology 101 (2010) 8224–8231

Contents lists available at ScienceDirect

Bioresource Technology

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

Effect of harvesting date on the composition and saccharification of *Miscanthus x giganteus*

T. Le Ngoc Huyen [a], C. Rémond [b,c], R.M. Dheilly [a], B. Chabbert [b,c,*]

[a] Equipe IMaP, Laboratoire LTI (EA 3899), 41 Avenue Paul Claudel 80480 Dury, France
[b] University of Reims Champagne Ardenne, UMR614, Fractionnement des AgroRessources et Environnement, F-51686 Reims, France
[c] INRA, UMR614, Fractionnement des AgroRessources et Environnement, F-51686 Reims, France

ARTICLE INFO

Article history:
Received 20 January 2010
Received in revised form 18 May 2010
Accepted 25 May 2010
Available online 23 June 2010

Keywords:
Miscanthus x giganteus
Lignocellulose
Cellulases
Xylanase
Ammonia

ABSTRACT

The chemical composition of the whole aerial biomass and isolated organs of *Miscanthus x giganteus* was examined for saccharification into fermentable sugars at early and late harvesting dates. Delayed harvest was mainly related to increased amounts of cell wall and ester-linked phenolic acids. Addition of an enzyme cocktail (cellulases, β-glucosidase and xylanase) resulted in similar enzyme digestibilities at the two harvesting dates, ranging from 11–13% and 8–9% of the cellulose and arabinoxylan, respectively. However, the internodes, leaves and sheaths varied in cell wall content and composition and gave rise to different saccharification yields with internodes being the most recalcitrant organs. Non-cell wall fraction was estimated as the amount of material extracted by neutral detergent solution, and accounted for 23% of the whole aerial biomass harvested at an early date. However, saccharification yields from the miscanthus biomass did not change after soluble fraction removal. An ammonia pretreatment improved enzyme efficiency on early-harvested miscanthus, to a greater extent than on late-harvested biomass. This trend was confirmed for two different years of harvesting.

1. Introduction

Miscanthus (*Miscanthus x giganteus)* is a lignocellulosic crop that has been extensively investigated as a possible raw material for paper, energy, building materials, geotextiles and substrates in nurseries and greenhouses (Lewandowski et al., 2000). Given the non-competition of miscanthus with food and animal feed, this species has attracted considerable attention in Europe and the US as a possible energy crop, either as fuel for electricity generation or more recently for conversion into biofuel (Murnen et al., 2007). Miscanthus is a perennial rhizomatous grass and possesses numerous favorable characteristics. It exhibits high rates of photosynthesis, due to its C4 photosynthetic pathway, and has a high carbon dioxide fixation rate. Miscanthus can therefore grow very rapidly, and up to 4 m in height, producing high annual biomass yields of 20–26 tons dry weight per hectare (Lewandowski et al., 2000). In addition, the rhizomes provide a nutrients reservoir to satisfy the annual requirements for new shoots formation, as the biomass is harvested every year. Delaying the miscanthus harvest until early spring could have a beneficial impact on soil quality (Nick and Emmanuel, 2000).

Owing to its high cellulose content and high biomass yield, miscanthus has been described as a candidate energy resource for the production of high levels of fermentable glucose into ethanol. Likewise, saccharification of the lignocellulose biomass has long been recognized as a potential low-cost source of mixed sugars for fermentation to fuel ethanol or chemicals (Sun and Cheng, 2002). Extensive research has been carried out during the last two decades on the bioconversion of lignocellulosic materials into ethanol (Carroll and Somerville, 2009). This bioconversion requires the hydrolysis of cellulose into glucose which is subsequently fermented into ethanol by yeast or bacteria. Biological degradation of lignocellulose using polysaccharide-active enzymes would be advantageous in many respects. Unlike acid or alkaline hydrolysis, enzymatic degradation using cellulases and hemicellulases is usually conducted under mild conditions that do not corrode the system or generate components that would inhibit monosaccharide fermentation (Himmel et al., 2007).

However native lignocellulosic biomasses, because of their complex structures, are generally recalcitrant to enzymatic conversion (Himmel et al., 2007). These materials are natural composites consisting of three main polymer components: cellulose, hemicelluloses and lignin, as well as other minor components, such as

Abbreviations: NDS, neutral detergent solution; NDF, neutral detergent fibre; KL, Klason lignin; PCA, *p*-coumaric acid; FA, ferulic acid; S, syringyl; G, guaiacyl; IN, internode; SAA, soaking in aqueous ammonia; AFEX, ammonia fibre explosion.

* Corresponding author. Address: INRA, UMR614, Fractionnement des AgroRessources et Environnement, 2 Esplanade R. Garros, BP 224, F-51686 Reims, France. Tel.: +33 3 26 77 35 94; fax: +33 3 26 77 35 99.
E-mail address: chabbert@reims.inra.fr (B. Chabbert).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.05.087

extractives, pectins or proteins and hydroxycinnamic acids (Fengel and Wegener, 1984). All these components are interconnected through non-covalent and covalent bonds into a highly organized network that may restrict enzyme accessibility and thereby reduce the efficiency of deconstructing enzymes (Sun and Cheng, 2002). Lignin is also reported as a main limiting factor of cellulase action (Berlin et al., 2006). Thus, a physicochemical pretreatment is an essential prerequisite to overcome these limitations and to render the biomass more accessible to enzyme action. The aim of such a treatment is to remove the lignin and hemicelluloses, reduce the crystalline structure of cellulose and increase the porosity of the materials. Various pretreatment options, including concentrated acid, dilute acid, alkaline, SO_2, hydrogen peroxide, steam explosion, ammonia fibre explosion (AFEX), wet-oxidation, lime, liquid hot water, CO_2 explosion and organic solvent treatments, are available (Kumar et al., 2009). Among these, ammonia has a number of desirable characteristics. It is an effective swelling reagent for lignocellulosic materials and shows high selectivity for reactions with lignin rather than with carbohydrates (Kim and Lee, 2005). The ammonia pretreatment of grass species, as compared to woody species, would require less severe conditions owing to the higher alkali solubility of grass lignin and the occurrence of ester-bound hydroxycinnamic acids that cross link lignin and hemicelluloses (Buranov and Mazza, 2008; Grabber et al., 2004).

A robust strategy to substitute fossil fuels by lignocellulosic biomass would be based on perennial grass species (Nick and Emmanuel, 2000). However, sustainable lignocellulosic bioconversion into ethanol would imply not only a reduction in the consumption of chemicals and/or energy required in the physicochemical pretreatment but also identification of the best cropping scenario to obtain high biomass yields while preserving soil and environmental quality. In this context, the miscanthus harvesting regime had received little attention with respect to environmental impact and biomass quality (Lewandowski and Heinz, 2003), and in most such studies, miscanthus was considered for combustion rather than for bioethanol production (Christian et al., 2008). A deeper insight into the limitations of the cell wall to bioconversion might be beneficial for designing improved biomass ideotypes and cropping scenarios. Although literature data regarding the chemical and enzymatic breakdown of miscanthus are available, there are few reports of the chemical composition, especially lignin and phenolic acids, of the cell wall components of this species. Moreover, the variations in chemical characteristics of miscanthus according to the harvesting period or the chemical heterogeneity of organs with regard to the cell walls are rarely mentioned. Thus, the main aim of this study was to determine the impact of harvesting date (early and late harvest) on the chemical features of the whole aerial biomass and selected organs of *Miscanthus x giganteus* while focusing on the cell wall components. The sensitivity of the corresponding miscanthus biomass to enzymatic bioconversion was assessed using cellulases and a hemicellulase. Improvement of the enzymatic degradation of miscanthus was further addressed by applying an aqueous ammonia pretreatment.

2. Methods

2.1. Plant material

Miscanthus x giganteus was planted in April 2006 and grown for two years without nitrogen amendment in experimental fields at Inra Estrées-Mons (France). The plant biomass was harvested in autumn (November 2007, early harvest) or in winter (late February 2008, late harvest). The dry matter yield was 21 t DM ha^{-1} and 15 t DM ha^{-1} at early and late harvest, respectively (Amourou et al., in press). In each case, plants were collected from three random blocks in the field. Two methods were used for sampling the miscanthus plants: (1) mechanical harvesting of all aerial parts, (2) manual isolation of the plant organs. In the latter case, the leaves and sheaths were isolated from the stovers then each stem was divided into internodes; the nodes were discarded. The internodes were numbered from the base of the stem and the internodes located at the basal (internode 2) and apical regions (internode 11) were selected for further investigations. Plant samples were dried at 40 °C in an air forced oven for three days and stored under dry conditions at room temperature until used. All samples (total biomass, selected internodes, mixed leaves, mixed sheaths) were ground using a Retsch mill equipped with a 0.5 mm sieve.

2.2. Chemical analysis

2.2.1. Cell wall preparation

All samples were extracted with hot water for 30 min then with neutral detergent solution (NDS) at 100 °C for 1 h using FIBERTEC M6, as previously described (Bertrand et al., 2009). The samples were then washed with water and acetone to eliminate traces of detergent. The remaining neutral detergent fibre (NDF) fraction, corresponding to the cell walls, was dried in an oven at 35 °C prior to chemical analysis of the cell wall components. All chemical analyses were performed in duplicate for each sample of NDF obtained from the three field-blocks.

2.2.2. Determination of acid-insoluble lignin (Klason lignin)

Samples of NDF from the plant samples were analyzed for lignin content according to the Klason procedure (Chabbert et al., 1994).

2.2.3. Lignin monomer composition

The monomer composition of the labile ether lignin fraction was determined by thioacidolysis that specifically disrupts the non-condensed intermonomer linkages (alkyl-aryl ether). The thioacidolysis reaction was performed in duplicate on each sample using 10 mg NDF and ethanethiol/BF_3etherate/dioxane reagent as detailed previously (Lapierre et al., 1986). One mL of tetracosane (CAS 646-31-1) (250 μg mL^{-1} $C_{24}H_{50}$) was added as an internal standard. After a 4 h reaction, the mixture was extracted three times with dichloromethane (CH_2Cl_2) (3 × 25 mL). Guaiacyl (G) and syringyl (S) thioethylated monomers were determined as their trimethylsilyl derivatives using a gas chromatograph, equipped with a fused silica capillary DB1 column (30 m × 0.3 mm) (J&W Scientific®) and flame ionization detector. The temperature gradient was 160–280 °C at 2 °C/min.

2.2.4. Characterization of cell wall phenolic acids

Ester-linked phenolic acids were released from 20 mg NDF using 10 mL of 2 M NaOH with constant stirring under a nitrogen flow at 35 °C. The alkaline filtrate was acidified to pH 1 with 6 M HCl, mixed with 1 mL of 3,4,5-trimethoxy-*trans*-cinnamic acid 250 μg mL^{-1} as internal standard and extracted three times with 25 mL diethyl oxide $(C_2H_5)_2O$. The organic phase was dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure (700 mbar) at 45 °C, dissolved in 1 mL of methanol/water (1/1, v/v) and filtered (0.45 μm) prior to high pressure liquid chromatography on a Spherisorb S5-0DS2, RP18, 4,6 × 250 mm (WATERS®) column. Gradient elution was as previously described (Beaugrand et al., 2004) using a combination of acetonitrile, methanol and 1% orthophosphoric acid in ultra-pure water. Ester-linked phenolic acids were quantified at 302 nm. The NDF residues of the whole aboveground biomass and ammonia-pretreated residues were also treated with 4 M NaOH (10 mL) at 170 °C for 2 h in order to extract both the ester- and ether-linked phenolic acids (Iiyama et al., 1994). Subsequent analyses were then performed as described above.