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Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass

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Abstract

Alfalfa stems, reed canarygrass, and switchgrass; perennial herbaceous species that have potential as biomass energy crops in temperate regions; were evaluated for their bioconversion potential as energy crops. Each forage species was harvested at two or three maturity stages and analyzed for carbohydrates, lignin, protein, lipid, organic acids, and mineral composition. The biomass samples were also evaluated for sugar yields following pretreatment with dilute sulfuric followed by enzymatic saccharification using a commercial cellulase preparation. Total carbohydrate content of the plants varied from 518 to 655 $g\,kg^{-1}$ dry matter (DM) and cellulose concentration from 209 to 322 $g\,kg^{-1}$ DM. Carbohydrate and lignin contents were lower for samples from early maturity samples compared to samples from late maturity harvests. Several important trends were observed in regards to the efficiency of sugar recovery following treatments with dilute acid and cellulase. First, a significant amount of the available carbohydrates were in the form of soluble sugars and storage carbohydrates (4.3–16.3% wt/wt). Recovery of soluble sugars following dilute acid pretreatment was problematic, especially that of fructose. Fructose was found to be extremely labile to the dilute acid pretreatments. Second, the efficiency at which available glucose was recovered was inversely correlated to maturity and lignin content. However, total glucose yields were higher for the later maturities because of higher cellulose contents compared to the earlier maturity samples. Finally, cell wall polysaccharides, as determined by the widely applied detergent fiber system were found to be inaccurate. The detergent fiber method consistently overestimated cellulose and hemicellulose and underestimated lignin by substantial amounts.
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Keywords: *Medicago sativa* L.; *Phalaris arundinacea* L.; *Panicum virgatum* L.; Bioethanol; Biomass; Bioenergy

1. Introduction

Biomass can be converted into energy by thermochemical processes, including combustion, pyrolysis, and gasification [1], or by fermentation of carbohydrates to produce methane and ethanol [1,2]. Sources of lignocellulosic biomass include wood, paper waste, crop residues, and herbaceous energy crops. Perennial herbaceous energy crops have much to recommend them as a feedstock because once established they do not require annual reseeding, they require lower energy inputs (i.e., fertilizer and pesticides) than annual crops, and they can often be grown on more marginal cropland [3–5]. They also have environmental benefits including reduced soil erosion, enhanced carbon sequestration, and providing wildlife habitat [4,6–9]. Both the US and EU have supported research on herbaceous energy crops since the mid-1980s. Thirty-five herbaceous perennial species were screened by the US

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[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Department of Energy and switchgrass (*Panicum virgatum* L.) was selected for intensive study [10,11]. The EU investigated 20 perennial grasses and selected 4 as the most promising: miscanthus (*Miscanthus* spp. Anderss.), reed canarygrass (*Phalaris arundinacea* L.), giant reed (*Arundo donax* L.), and switchgrass [12]. Alfalfa (*Medicago sativa* L.) has also been considered for use as an energy crop in the US [13].

Three forage crops were selected for this study: alfalfa (only stems), reed canarygrass, and switchgrass. Selection of the three forage crops evaluated in this study was based upon high yield potential and other agronomic considerations. All of these species are broadly adapted to a range of environmental regions, but each species is also uniquely suited to special situations. For example, reed canarygrass is a cool-season grass that is very tolerant of flooding and its productivity is very responsive to high levels of nitrogen fertilization, making it a useful crop for disposal of manure from livestock operations [14]. In contrast, switchgrass is a warm-season grass that requires higher growth temperatures for maximum productivity, but this species is extremely drought tolerant and productive with minimal fertilizer inputs [10]. Alfalfa's unique traits include the fact that this legume does not need nitrogen fertilizer and the leaves are a valuable supplemental protein feed for livestock, providing another revenue stream from the use of this species as a biomass crop [15]. Of the three forage species evaluated in the current study, alfalfa may be best suited for use on land suitable for row cropping because alfalfa's productivity declines after 3–5 years and alfalfa can provide the majority of the nitrogen fertilizer requirements for 2 years of maize (*Zea mays* L.) production after the alfalfa stand is plowed down. Switchgrass and reed canarygrass remain productive for longer periods of time and are more suited to marginal cropland because these perennial grasses are more effective at controlling erosion and nutrient leaching. Clearly, choice of biomass crops must include their applicability to farming systems and characteristics of the land base available.

The efficiency of conversion of biomass to ethanol depends upon feedstock characteristics and composition, pretreatment processes, and the fermentation technologies that are utilized [1,2,16]. Feedstock quality for herbaceous energy crops has been extensively studied for use as livestock feed but not for ethanol conversion. Legumes, grasses with the C_3 photosynthesis system, and grasses with the C_4 photosynthesis system differ in plant anatomical characteristics which affect their chemical composition and utilization by ruminant animals [17]. Other important factors that are known to strongly impact chemical composition and digestion by ruminant animals include forage genotype, maturity, and growth environment, as well as, interaction among these factors [18]. This study focused on the influence of plant-type and maturity. The forages selected for this study include a legume (alfalfa), C_3 grass (reed canarygrass), and C_4 grass (switchgrass) each of which was harvested at two or three maturities. Biomass samples were characterized for total chemical composition, including carbohydrates, protein, lipids, Klason lignin, ash, etc. Next, recoverable sugar yields were evaluated by measuring monosaccharides released from the cell-wall matrix following treatment with dilute sulfuric-acid (at 121 and 150 °C) and enzymatic saccharification with a commercial cellulase. Finally, the compositional and yield data were combined to calculate the relative amount of recoverable sugars for each sample. The results showed clear distinctions among the samples based upon both plant-type and harvest maturity.

2. Materials and methods

2.1. Plant material

Herbaceous biomass crop samples were grown and harvested in 2003. The two alfalfa samples were created by harvesting and bulking numerous individual plants from several genetic nurseries at Rosemount and Becker, MN. These nurseries were established in 2001 and consisted of mature plants derived from intercrossing commercial alfalfa varieties. The reed canarygrass plant material was derived from a low-alkaloid population selected for improved establishment capacity that was planted at Arlington, WI. Switchgrass samples were collected from an established stand of the variety Cave-in-Rock located at Mead, NE. All field plots were fertilized for high productivity under local soil conditions. Plant materials were harvested at a 10 cm stubble height. The specific maturity stages and morphological description of the samples are detailed in Table 1. Following harvest, the biomass was air dried on greenhouse benches (switchgrass) or in forced-air ovens at 60 °C (alfalfa and reed canarygrass). The dried

Table 1
Description of biomass samples used for pretreatment experiments

Species Maturity[a]	Sample description
Alfalfa (*Medicago sativa* L.)	
Bud (KF3)	Stems, flower buds present, no open flowers
Full flower (KF6)	Stems, open flowers on all stem shoots
Reed canarygrass (*Phalaris arundinacea* L.)	
Vegetative (V3)	Leaf blades and sheaths, no stem elongation
Ripe seed (S5)	Whole herbage, ripe seed
Switchgrass (*Panicum virgatum* L.)	
Pre-boot (E3)	Leaf blades and sheaths, elongated stems
Anthesis (R4)	Whole herbage, flower panicle on stems open
Post-frost (S5+)	Whole herbage, ripe seed, senescent, post-frost

[a]Alfalfa maturity stage designations follow [19]. Maturity stage system for grasses is based on [20].