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Industrial hemp as a potential bioenergy crop in comparison with kenaf, switchgrass and biomass sorghum

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ABSTRACT

This study takes combined field trial, lab experiment, and economic analysis approaches to evaluate the potential of industrial hemp in comparison with kenaf, switchgrass and biomass sorghum. Agronomy data suggest that the per hectare yield (5437 kg) of industrial hemp stem alone was at a similar level with switchgrass and sorghum; while the hemp plants require reduced inputs. Field trial also showed that ~1230 kg/ha hemp grain can be harvested in addition to stems. Results show a predicted ethanol yield of ~82 gallons/dry ton hemp stems, which is comparable to the other three tested feedstocks. A comparative cost analysis indicates that industrial hemp could generate higher per hectare gross profit than the other crops if both hemp grains and biofuels from hemp stem were counted. These combined evaluation results demonstrate that industrial hemp has great potential to become a promising regional commodity crop for producing both biofuels and value-added products.

1. Introduction

Biomass conversion to biofuels and chemicals has generated a lot of interests due to the increasing demand for establishing a secure and sustainable energy supply that can be incorporated to the existing fuel system (Shi et al., 2011a). Traditionally, biofuels have been produced based on starchy or sugar crops such as corn, wheat, sugar beets, and sugar cane. Bioethanol derived from lignocellulosic biomass is considered as a promising renewable fuel because of the vast availability and low cost of the feedstocks (Chundawat et al., 2011). However, the major challenges of biofuels production from lignocellulosic biomass include a stable and consistent feedstock supply, development of efficient pretreatment technologies to remove lignin and facilitate enzyme access to the cellulose for sugar release, effective fermentation of sugars and valorization of lignin to value added chemicals (Yang and Wyman, 2008).

Industrial hemp (*Cannabis sativa* L.) has a long history being known and used by humans for a variety of applications, including fibers for cloths and building composites, seed as a source of essential oil and food, and secondary metabolites from hemp for pharmaceutical applications (Linger et al., 2002). In the United States, hemp farming goes back to the eighteenth century; however, industrial hemp became a controversial crop due to its genetic closeness to tetrahydrocannabinol (THC)-producing plants, and was stymied in the 1930s. Growing interests in the commercial cultivation of industrial hemp in the United States resurged since the 1990s. There are multiple, harvestable components of the hemp plant that can be used in diverse ways. Based on a recent report, the current annual sales of hemp based product in the U.S. alone is about $600 million dollars (Johnson, 2017). In the omnibus farm bill debate, the 113th congress made significant changes to the U.S. policies towards industrial hemp. The Agricultural Act ("farm bill", P.L. 113-79) was passed in 2014, which allows certain research institutions and state departments of agriculture to grow industrial hemp. The continuous introduction and clarification on industrial hemp at legislation level promoted industrial hemp related research and allowed for a kaleidoscopic realm of possibilities to be discovered.

The conversion of lignocellulosic biomass to biofuels usually undergoes three steps: (i) pretreatment to open the rigid structure of plant cell walls; (ii) enzymatic saccharification to breakdown solid cellulose into sugars; and (iii) fermentation to produce biofuels or chemicals (Kamireddy et al., 2013). Several pretreatment techniques have been studied over the years, with dilute acid, alkali, hot water, and steam

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explosion, being the most extensively inverstigated (Wyman et al., 2011). However, the efficacy of a pretreatment method largely depends on the selection of biomass feedstock; at the same time, the selection of a pretreatment technology greatly influences biomass decomposition and sugar release (Behling et al., 2016; Yang and Wyman, 2008). Alkali pretreatment using dilute NaOH or lime generally requires lower temperature and pressure, and less residence time compared to other pretreatment methods (Sun and Cheng, 2002). During an alkali pretreatment process, the ester bonds cross-linking between lignin and xylan are typically cleaved, thus increasing the accessibility of cellulose and hemicellulose enriched fractions to enzymatic digestion (Xu et al., 2010). In contrast, dilute sulfuric acid pretreatment solubilizes hemicelluloses, relocates lignin, and thereby disrupts the lignocellulosic composite material linked by covalent bonds, hydrogen bonds, and van der Waals forces (Mosier et al., 2005). Dilute sulfuric acid pretreatment has been shown as a leading pretreatment process that has been implemented at commercial scale (Shi et al., 2011b). The intensity of deconstruction during a pretreatment process depends on the characteristics of the biomass feedstock as well as the pretreatment conditions and the targeted end-products (Yang and Wyman, 2008).

In addition to the existing applications of hemp for fiber, oil and nutraceutical products, one potential application of industrial hemp is for biofuels production. Ethanol production from industrial hemp using a combined dilute acid/stream pretreatment technique was investigated previously (Kuglarz et al., 2014). Results show that pretreatment with 1% sulfuric acid at 180 °C for 10 min led to the highest glucose yield (73–74%) and ethanol yield of 75–79% (0.38–0.40 g-ethanol/g-glucose). In a follow-up study, an ethanol yield of 149 kg of ethanol/dry ton hemp was reported using alkaline oxidative pretreatment (Kuglarz et al., 2016). In another study, hemp hurds were fractionated by organosolv pretreatment for lignin degradation and sugar formation. More than 75% of total cellulose and 75% of total lignin were removed under the following experimental conditions: 165 °C, 3% H_2SO_4, 20 min reaction time, and 45% methanol (Gandolfi et al., 2014). Furthermore, due to its capacity to grow on heavy metal contaminated soil, industrial hemp has shown potential in bioremediation of heavy metals in addition to biofuel production (Kyzas et al., 2015).

Despite the existing studies related to the biofuels potential of industrial hemp, its technical and economic feasibility still remains unclear (Johnson, 2017). It is necessary to understand whether industrial hemp can yield biofuel quantities comparable to the other biomass feedstocks and whether it is economically profitable to grow industrial hemp for biofuels and bioproducts. In order to answer these questions, this study aims to evaluate the potential of industrial hemp as a biofuel crop using a combined agronomic, experimental and economic analysis approach in comparison with kenaf, switchgrass and biomass sorghum. Specific objectives are to: 1) compare the composition and heating value of industrial hemp with other biomass feedstocks; 2) compare the recalcitrance of the four feedstocks upon dilute sulfuric acid or alkali pretreatment and their sugar yields from subsequent enzymatic hydrolysis; 3) compare both theoretical and predicted ethanol yields from all four feedstocks and 4) conduct an economic analysis by integrating agronomy and experimental data to evaluate the economics of industrial hemp as potential biofuels feedstock as compared to the other biomass feedstocks. Results from the first-of-a-kind evaluation demonstrate the great potential of using industrial hemp as a promising regional commodity crop for producing both biofuels and value-added products.

2. Materials and methods

2.1. Feedstocks

Industrial hemp (*Cannabis sativa* L., cv 'Futura 75') and kenaf (*Hibiscus cannabinus*, cv 'Whitten') for this study were seeded at a site with a Maury silt loam (Fine, mixed, active, mesic Typic Paleudalfs; 4.2% organic matter, pH = 6.3) at a research farm, University of Kentucky in June 2015. The research area was prepared by conventional tillage. Nitrogen was applied pre-plant at 55 kg N/ha via urea (46-0-0). No other nutrients, pesticides, or any other inputs were applied throughout the trial. Plots were seeded with an amended plot drill (Almaco; Nevada, Iowa) with 2.4 m effective width in 20 cm rows. Hemp and kenaf were seeded at rates of 66 and 44 kg pure live seed ha^{-1}, respectively. The above ground portions of the plants (at approximately 6 cm above the soil surface) were collected by a hand-held sickle mower 110 days after seeding on 29 Sep 2015. All plant material was dried for 7 days in a forced-air dryer at ambient temperatures and transported to the laboratory for analyses. The yields of cellulosic biomass portion (stem) for hemp and kenaf were 5347 kg/ha and 8227 kg/ha, respectively on dry basis; while yield of the grain portion of hemp was 1230 kg/ha on dry basis. A subsample from the stem was collected, ground to pass a 2 mm sieve using a model 4 Wiley mill, and stored in Ziploc bags at room temperature for subsequent experiments. Switchgrass (*Panicum virgatum*, Alamo) and sorghum (*Sorghum bicolor*, forage variety ES5200) samples were provided by the Bioenergy Feedstock Library, Idaho National Laboratory, Idaho Falls, ID.

2.2. Pretreatment

For dilute alkali pretreatment, 2 g of biomass was mixed with 18 mL of 2 wt% NaOH solution in a tubular reactor (made of stainless steel SS316, 6″ in length and ¾″ in outer diameter). The reactors were then capped and the premixed slurry was soaked at room temperature for 4 h. Pretreatment was conducted at 140 ± 2 °C for 1 h in a temperature controlled oil bath. As a comparison, dilute sulfuric acid pretreatment was carried out at 160 ± 2 °C for 30 min on the four feedstocks. The reaction was performed by adding 18 mL of 1 wt% of H_2SO_4 to 2 g biomass sample, in the same experimental setup as mentioned above. The pretreatment conditions for dilute alkali and acid pretreatment were selected based on previous reports (Shi et al., 2011b; Xu et al., 2010). After pretreatment, the pretreated biomass was washed 4 times with 40 mL deionized (DI) water for each wash and the solids were separated from the liquid by centrifugation at 4000*g*. The liquid from first wash was collected for sugar analysis. The washed solids were stored at 4 °C for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the four untreated and pretreated biomass were carried out by following the NREL laboratory analytical procedure (Selig et al., 2008). The cellulase (CTec2, Novozymes Inc.) and hemicellulase (HTec2, Novozymes Inc.) enzymes were premixed at a 9:1 v/v ratio. The saccharification was performed at 50 °C for 72 h at an enzyme loading of 10 mg enzyme protein/g starting biomass in an orbital shaker (Thermo Forma 435, Thermo Fisher Scientific Inc., Waltham, MA, US). Liquid samples were taken at 2, 6, 12, 18, 24 and 72 h, centrifuged at 4000 rpm for 10 min and the supernatant was analyzed by high performance liquid chromatography HPLC, (Ultimate 3000, Dionex Corporation, Sunnyvale, CA, US)) equipped with a refractive index detector and Bio-Rad Aminex HPX-87H column and guard column assembly. Product separation was obtained at 50 °C with 5 mM H_2SO_4 as mobile phase at a flow rate of 0.4 mL/min to measure fermentable sugar (glucose and xylose) contents.

2.4. Analytical methods

Acid soluble lignin (ASL), acid insoluble lignin (AIL), and carbohydrate content in the untreated feedstocks were determined using the procedure described by NREL (Sluiter et al., 2008). Monomeric sugars including glucose, xylose and arabinose in the untreated biomass and pretreatment and enzymatic hydrolysates were determined via HPLC. The calorific value of biomass was measured using a LECO AC600 bomb