Energy crops for ethanol: a processing perspective

Bruce S. Dien[1], Gautam Sarath[2], Jeffrey Pedersen[2], Kenneth P. Vogel[2], Hans-Joachim G. Jung[3], Scott Sattler[2], Michael D. Casler[3], Robert B. Michell[2], and Michael A. Cotta[1]

[1]National Center for Agricultural Utilization Research, Agricultural Research Service-USDA[1], Peoria, IL, USA. Email: Bruce.Dien@ars.usda.gov
[2]Grain, Forage and Bioenergy Research Unit, ARS-USDA, Lincoln, NE, USA
[3]Plant Science Research Unit, ARS-USDA, St. Paul, MN, USA
[4]U.S. Dairy Forage Research Center, ARS-USDA, Madison, WI, USA

Abstract

Global production of bioethanol for fuel is over 13 billions gal per year. Continued expansion of ethanol production will necessitate developing lignocellulose as an alternative to today's use of starch and sugar producing crops. Dedicated energy crops are one such option. In the U.S., it has been estimated that enough perennial crops can be grown to supply 9–23 billion gal of ethanol/yr – assuming a yield of 60 gal/ton. However, further research is needed to understand the roles that agronomic practices and genetics play in affecting realizable ethanol yields. Biochemical conversion of biomass following thermo-chemical pretreatment is currently the leading technology for producing ethanol from these feedstocks. We compared a warm season grass (switchgrass), cool season grass (reed canary grass), and legume (alfalfa stems) for sugar production. To introduce further variation in this sample set, each species was harvested at 2 or 3 different maturities. Both species and maturity significantly affected carbohydrate content, composition, and sugar yields, indicating that looking beyond biomass yield may be important for determining feedstock suitability. We also evaluated the influence of plant genetics on ethanol yield. Over 100 samples of switchgrass were evaluated for ethanol yield by applying a low severity pretreatment assay. Xylose yields were positively correlated with xylan content as expected. However, ethanol yield could not be predicted by glucan content and was negatively correlated with acid detergent lignin content. More recently, a set of brown midrib lignin mutants of sorghum was likewise assayed for ethanol yield, and in this case lignin composition was also found to negatively impact glucose yield. These results will be discussed in the context of what can be done to further enhance the quality of energy crops for conversion to ethanol.

Media summary

Perennial herbaceous crops are a huge potential resource for producing fuel ethanol, but further research is needed to coordinate agricultural and breeding practices with biochemical processing demands.

Key Words

Bioethanol, Energy Crops, Switchgrass, Sorghum

Introduction

The U.S. is the largest ethanol producer in the world, with annual production of over 6.5 billion gal/yr (2007) (www.ethanolrfa.org). Recently, the Federal Government enacted a renewable energy standard that targets 36 billion gallons/yr by 2022 of which 60% is mandated from cellulosic feedstocks. While this standard also includes biodiesel, it is expected that ethanol will make up the great majority of this volume. Lignocellulose sources available for conversion include agricultural residues (e.g. corn stover),

---

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable

forest industry wastes, and dedicated energy crops. Dedicated energy crops can include such crops as switchgrass or hybrid popular trees. A recent joint study published by the USDA and DOE concluded that up to 1 billion tons/yr of lignocellulose could be made available for producing ethanol (Perlack et al., 2005).

This paper concerns the use of herbaceous energy crops. Herbaceous perennial crops have many advantages to recommend them as energy crops for conversion to bioethanol. These include:

- An estimated return of 5 KJ per each KJ of inputted energy (Schmer et al., 2008)
- An estimated 97% reduction in green house gas emissions relative to gasoline (ibid)
- Can be cultivated on marginal farmland unsuited for row crop production
- Will target depressed rural areas for economic development.

Despite their great promise, relatively little process research has been published on converting this type of biomass into ethanol (Dien et al. 2005). In this paper, three examples will be given which demonstrate the beneficial role that can be expected from developing plants specifically for ease of conversion to ethanol. Some of the work reviewed in this proceeding was previously published (Perlack et al., 2005).

Methods

All biomass samples were field harvested, dried at 50ºC, and ground in a Wiley Mill. Moisture contents were measured by drying at 105ºC. Samples were pretreated at 15% w/w solids with 1.5 to 2.5% w/v H_2SO_4 by heating at 121ºC for an hour in an autoclave. Resulting hydrolysates were either analyzed by enzymatic digestion or simultaneous saccharification and fermentation (SSF). Hydrolysates analyzed for enzymatic digestion were treated as previously described (Dien et al., 2006). Briefly, hydrolysates were neutralized with 4 N KOH, diluted to a final cellulose concentration of 10 g/l with dH_2O, further buffered with sodium citrate (50 mM, pH 4.8), stabilized with thymol, and digested using commercial cellulases (Celluclast 1.5L + Novo188, Novozymes, Denmark). Digestions were conducted at 45ºC and 150 rpm. Switchgrass samples evaluated by SSF were neutralized with $Ca(OH)_2$ and prepared for fermentation by adding sodium citrate buffer (50 mM, pH = 4.8), yeast extract (10 g/l), and peptone (20 g/l). The commercial cellulases used were GC220 (Genencor) and Novo188. Thus prepared, hydrolysates were inoculated to a final O.D. (600nm) of 0.5 with *Saccharomyces* D5A and incubated at 35ºC with gentle agitation (100 rpm). To prevent excess respiration and evaporation, fermentation vessels were sealed except for a 22 g needle used to exhaust CO_2. Finally, sorghum hydrolysates were washed 3x with sterile water following the dilute acid treatment and otherwise prepared for fermentation as previously described. The wash steps were included because sorghum samples had high concentrations of soluble sugars and starch, which would otherwise have interfered with the analysis. All digestion and fermentation assays were stopped after 72 hr, at which point they were sampled for sugars and ethanol. An HPLC system equipped with a refractive index detector was used for analysis.

Results and Discussion

I. Evaluating switchgrass, alfalfa stems, and reed canarygrass as potential energy crops
Switchgrass (*Panicum virgatum L.*), alfalfa (*Medicago sativa L.*), and reed canary grass (*Phalaris arundinacea L.*) have all been identified as potential energy crops because of their high production yields. The selection of plants was also chosen to maximize sample diversity: the set represents cool and warm season grasses and a legume. Each species was harvested at multiple maturities to further vary plant composition. Only the stems were evaluated for alfalfa because the leaves are high in protein and have value as an animal feed. A complete listing of the samples can be found in Table 1.

As evidenced by the data plotted in Figure 1A, ethanol yields varied with both species and maturity. The alfalfa stems gave significantly lower yields than either grass. This might be because the leaves were not included with the alfalfa sample (e.g. stems are harder to digest than leaves) or because legume type cell walls are harder to digest than those of grasses. The little information available from literature suggests both explanations are probably relevant (discussed in Dien et al., 2006). The alfalfa stems were also found to have a higher buffering capacity than the grasses, which meant more mineral acid was needed for the pretreatment (data not shown). Pectin, which is present in legumes but not grasses, is most likely to be responsible for the enhanced buffering capacity of the alfalfa stems.

The efficiency of sugar extraction, following enzymatic digestion, was also found to decrease uniformly with maturity. It is possible lignification is the cause; as plants mature, the average cell wall becomes more lignified. Lignification has been previously determined to have a detrimental effect on sugar yields. This hypothesis was tested by plotting the % of recovered glucose vs. lignin content. As shown by Figure 1B, the two had a strong negative correlation ($r = 0.85$). These results clearly illustrate that cell wall properties do effect biochemical conversion to sugars.

II. Evaluating spread in relative ethanol yields for a diverse population of switchgrass samples

To better understand the influence of biomass composition on conversion efficiency, a large sample set of switchgrass was screened for ethanol yield. For this analysis, a simple assay was developed for measuring relative ethanol yield suitable for use on a large population. The assay incorporated a low-severity dilute-acid pretreatment, where the lower severity was thought to give the assay a greater sensitivity to differences in plant cell walls. A commercial cellulase enzyme was used to convert cellulose to glucose and the yeast *Saccharomyces* was used to ferment it to ethanol; final xylose concentration was measured directly from the fermentation because this yeast is unable to ferment it. The sample set was extremely diverse, including samples from different field trials as well as separate plant components (e.g. stems and leaves). Ethanol yields (Figure 2A) varied widely among the set and were negatively correlated, albeit weakly, with lignin and not correlated with initial cellulose content (data not shown). In contrast, xylan yield was positively correlated with beginning xylan content ($r = 0.70$). Therefore, these results demonstrate that cell wall composition plays an important role in determining ethanol yield at low severity. And, while it is true greater biomass recalcitrance can be in part compensated by applying a higher severity pretreatments, it is expected that using less harsh conditions will be commercially advantageous.

III. Improving traits of energy crops for conversion to ethanol using forage sorghum as a model

A better understanding of the role of chemical composition and cell wall structure in determining ethanol yield may allow for crops to be bred for easier conversion. As previously observed, lignin content appears to negatively impact ethanol yield. We were able to obtain a set of sorghum samples that were isogenic other than for mutations in lignin synthesis (Pedersen et al., 2006). Although forage sorghum is an annual, it also has potential as an energy crop because of its high yield potential and, furthermore, serves as a useful model for other C4 grasses. The set of 12 samples varied widely in lignin content.

Each sample was evaluated for ethanol yield following a low severity pretreatment. The assay was similar to that applied for the switchgrass, except they were washed following pretreatment to avoid interference from soluble sugars and starch. Xylose released from the pretreatment was measured following the dilute-acid pretreatment and prior to the washing step. It was found that the yield of xylose was uniformly high and unaffected by lignin content (data not shown). In contrast, the ethanol yield from cellulose was strongly retarded by the presence of higher lignin content (Fig 2B). This result suggests that engineering of plant lignification would be a beneficial target for improving energy crops.

Conclusions

In three separate cases, it was observed that sugar and ethanol yields varied widely with changes in plant cell wall composition. Reduced lignin content appears to have the strongest effect in improving glucose and ethanol yields. In contrast, lignin appears to have no discernable effect on determining the yield of xylose; at least when biomass is pretreated with dilute-acid. When a set of sorghum samples isogenic, except for lignin, were pretreated and fermented to ethanol, lignin content explained 82-85% of the variation in yields. Furthermore, the observed range in ethanol yields between high and low lignin plants was nearly 100%. These results suggest that lignin content and structure should be a primary target for breeding more easily processed biomass. However, because biomass production yield will still be the dominant factor in determining the cost of producing ethanol yield from lignocellulose, it is important that any added traits for making the biomass easier to convert to ethanol do so without reducing biomass yield.

References

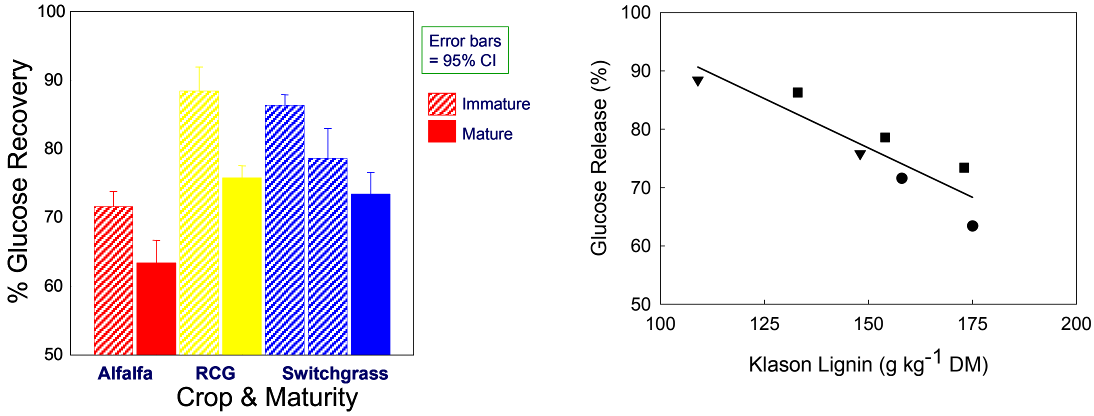
Dien BS, Iten L, Skory CD. 2005. Converting herbaceous energy crops to bioethanol: a review with emphasis on pretreatment processes. In Handbook of Industrial Biocatalysis pp 1–11. Taylor and Francis Group, Boca Raton, FL.

Dien BS, Jung HG, Vogel KP, Casler MD, Lamb JFS, Weimer PJ, Iten L, Mitchell RB and Sarath G. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioenergy 30 880–891.

Pedersen JF, Funnell DL, Toy JJ, Oliver AL, and Grant RJ. 2006. Registration of Seven Forage Sorghum Genetic Stocks Near-Isogenic for the Brown Midrib Genes *bmr*-6 and *bmr*-12. Crop Sci. 46:490.

Perlack RD, Wright LL, Turhollow AF, Graham RL, Strokes BJ and Erbach DC (2005) Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. Rep. DOE/GO-102995-2135. USDA and DOE, Washington, DC.

Schmer MR, Vogel KP, Michell RB, Perrin RK. 2008. Net energy of cellulosic ethanol from switchgrass. PNAS. 105(2):464-469.



Figures 1A and B: glucose yield results from different bioenergy crops harvested at varying maturities.

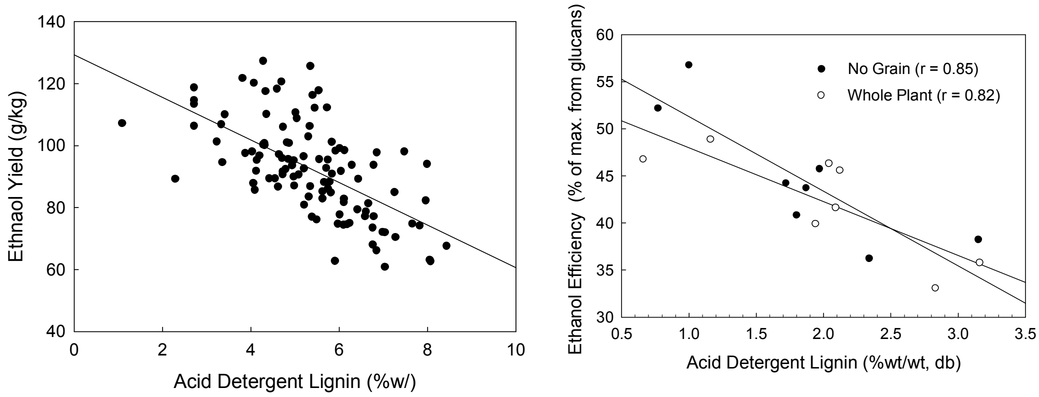


Figure 2 A and B: ethanol yield vs. ADL for switchgrass and ethanol efficiency vs. ADL sorghum.