Development of Alfalfa for Ethanol Production and Other Bioproducts

by Deborah Samac, Hans Jung, and JoAnn Lamb; USDA-ARS-Plant Science Research, St. Paul, Minnesota

Considerable potential exists for alfalfa as a feedstock for production of fuels, feed and industrial materials. However, unlike other major crops such as corn and soybean, which are commonly refined for production of fuel and industrial materials, refining of alfalfa remains undeveloped. Instead, alfalfa is a primary feed for dairy and beef cattle, horses, sheep and other livestock. If alfalfa is fully developed as a feedstock for biorefining, a major shift may occur in how alfalfa is produced and used for feeding farm animals.

Alfalfa's advantages. Many attributes make alfalfa attractive for production of biofuels and for biorefining.

1) Technologies for cultivation, harvesting and storing are well established; harvesting machinery is widely available; and farmers are familiar with its production.
2) A well-developed industry exists for cultivar development, seed production, processing and distribution.
3) High biomass potential is based on underground, typically unobserved traits.
   a. Alfalfa develops an extensive, well-branched root system capable of deep soil penetration, allowing plants to access water and nutrients not available to more shallowly rooted annual plants. This enables established plants to produce adequate yields under less than optimal rainfall.
   b. Alfalfa roots engage in a symbiotic relationship with a soil bacterium; this results in 'fixing' apx. 135 lb N/ac per year, eliminating need for applied nitrogen fertilizers.
   c. Alfalfa can reduce the nitrate concentrations in soil and drainage water, and prevent soil erosion.
4) Energy costs of production are low.
5) Alfalfa is amenable to genetic transformation which has been used to alter alfalfa for valuable co-product production.

The key advantage of alfalfa as a biofuel feedstock compared to other perennial forages is the ability to easily separate leaves and stems to produce a co-product. Leaves can be separated from stems mechanically. Leaf meal can be sold separately as a high protein feed and stems utilized for gasification and conversion to electricity or fermentation to ethanol.

Different management. Currently, alfalfa for feed is harvested frequently at early maturity with high leaf to stem ratio, producing high protein, easily digestible hay. Maximum forage yield, occurring at later maturity stages, is usually sacrificed to produce high quality hay. For competitive use as a biofuel feedstock, research is needed to develop alfalfa germplasm and management strategies that yield more biomass (both leaf and stem) with minimal production costs.

As alfalfa plant densities increase, total forage yield increases, but yield of individual stems and number of stems per plant decreases. Higher plant densities produce finer stems and have more leaf drop due to shading. Different management practices to produce it as a biofuel feedstock may include decreasing plant density to apx. 45% of conventional alfalfa stands and delaying harvest until later maturity stages. Recent research shows that reduced plant density decreased plant-to-plant competition for light, water and nutrients, minimizing leaf drop caused by shading. Delaying harvest until late flower to green pod maturity stages increased stem yield and maximized total forage yield compared to conventional management practices (Figure. 1).

Chemical composition. Utility of any biomass crop as an ethanol production feedstock depends largely on its chemical composition, both in amount of potentially fermentable carbohydrates and presence of compounds limiting yield of these carbohydrates. Current commercial yeast strains only utilize glucose as a substrate for ethanol production. Glucose can be derived from cellulose in cell walls of biomass species. Reduced lignin content of biomass should result in high concentrations of cell wall polysaccharides, increasing the potential amount of fermentable sugars. Composition of biomass crops is very diverse and varies due to species, genetics, maturity, and growth environment.

A survey of 190 alfalfa plant introductions in the US germplasm collection found leaves averaged 28% crude protein (CP) compared to 9% CP in stems. In contrast, the neutral detergent fiber (NDF) concentration of stem's far exceeded leaves (66 and 23% NDF, respectively). These differences reflect the stems role in providing an upright growth form and supporting leaf mass. Alfalfa stems develop extensive xylem tissue (wood) with thick cell walls comprised of cellulose, hemicellulose, pectin, and lignin. Representative composition of alfalfa stems is shown in Table 1. Both leaves and stems have low concentrations of simple sugars and starch.

Leaf meal's value. Since alfalfa leaves contain apx. 30% CP, they have greater value as an animal feedstuff than for conversion to ethanol. Based simply on protein concentration, alfalfa leaf meal has a value of ~$125/ton. This far exceeds the target feedstock value of ~$30/ton in a functioning corn stover-to-ethanol production system. In an extensive series of studies involving lactating dairy cows and fattening beef cattle, alfalfa leaf meal was an acceptable protein feed supplement to soybean meal. Besides providing protein for beef steer growth, it also reduced the incidence of liver abscesses at slaughter, increasing the cattle market value. Furthermore, it could replace alfalfa hay in the diet of lactating dairy cows as a proteinand fiber source to support normal milk production. Suckling beef calves gained weight more rapidly when creep-fed alfalfa leaf meal vs. a soybean meal-based supplement. It is clear that alfalfa leaf meal could provide a valuable co-product for an alfalfa-to-ethanol production system.

Table 1. *Composition of immature (bud stage) and mature (full flower) alfalfa stems. Data from Dien et al., 2005.*

Component	Immature	Mature
	----------- % of DM -----------	
Protein	12.7	8.8
Lipid	0.9	0.7
Ash	8.1	5.8
Soluble carbohydrates	5.5	4.9
Starch	0.3	0.2
Cellulose	27.5	30.6
Hemicellulose	10.5	12.2
Pectin	12.5	11.9
Lignin	15.8	17.5

Separating leaves and stems. Two methods have been developed for capturing the protein-rich fraction from alfalfa and separating it from the more fiber-rich fraction. With hay, leaves can be separated from denser stems using shaking screens. Fresh material can be dried using a rotary drum drier and leaves separated aerodynamically due to lower mass and faster drying time than stems. Wet fractionation involves mechanical maceration of fresh total herbage followed by expression of protein-rich juice. Apx. 20-30% of DM herbage can be captured in juice. Wet fractionation has been successful in small-scale experiments to refine alfalfa into a high value protein fraction and a fiber fraction that was further refined and fermented to produce ethanol, lactic acid and wood adhesive. Fiber can also be processed into animal feed. Wet fractionation has the advantage of minimizing leaf loss and is less weather dependent than field drying. Dried material is lighter to transport and is easily stored for later processing and refining.

Fermentability. Ethanol production depends on fermentation of simple sugars by microorganisms. Yield of potentially fermentable sugars from the conversion process is the critical response variable in assessing the value of alfalfa as an ethanol production feedstock. Potentially fermentable sugar yield is a function of both carbohydrate composition and concentration, and the efficiency with which the cell wall complex sugars are converted to simple sugars through processing. Alfalfa stem processing will likely include pre-treatments to aid in breaking down the cellulose into sugars that the microorganisms can convert into ethanol. Pre-treatments could include additions of acidic solutions, ammonia or liquid hot water to increase the conversion efficiency of the stem fiber to ethanol.

The future. Although commercial biorefining of alfalfa remains undeveloped, alfalfa has tremendous potential as a feedstock for production of ethanol and other products. A biomass-type alfalfa variety is being developed that has more upright growth and performs well in a reduced frequency harvest system, maximizing yield of leaves and stems while lowering production costs. Incorporation of enhanced compositional traits (i.e. more cellulose, less lignin, valuable transgenic protein products) into this alfalfa biomass type through traditional breeding and using biotechnology tools will add to the value alfalfa brings to biofuels and bioproduct systems.