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Enhancing Biomass Utilization for Bioenergy — Crop Rotation Systems and Alternative Conversion Processes

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Additional information is available at the end of the chapter

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1. Introduction

With ever increasing global populations there is a rising demand for energy to support even modest changes in lifestyle. It has been recognized for some time now that with decreasing oil reserves on a global scale there is a need for alternative energy sources. Many of our needs for energy utilizing electricity can be met by alternatives to petroleum and coal-based power generation. Of particularly high potential is the efficient utilization of solar energy. According to Lewis and Nocera [1], the earth receives approximately 7000 times more energy from the sun than is utilized by all of mankind. There are several technologies that are being utilized, ranging from photovoltaic to focusing mirrors to super heat fluids for steam generation in the production of electricity. The continued development of these technologies, along with other types such as wind-driven turbines, geothermal, hydroelectric, and ocean wave motion for electricity production, will greatly lessen the demand on petroleum-based energy. However, a critical need is liquid fuels for transportation. The movement of people and goods over great distances is a vital part of the world economy.

Part of the answer may still lie in the utilization of solar energy; not in a direct manner to power vehicles (cars, trucks, trains, and airplanes), but what it has been doing for billions of years in providing energy to growing plants. Conversion of plant biomass to energy or the production of bio-based liquid fuels (biofuels) has received greater attention in the last couple of decades. Although there is a tremendous amount of potential energy stored in the total plant biomass as it goes through its normal life cycle, much of the current technology has focused on the utilization of grains (corn, cereals, and soybeans) or sugars from storage organs of specialty plants (sugar cane, sugar beets). This has allowed a rapid ramping up of liquid fuel production in the form of ethanol. The technology needed for this production was not something that required a lot of development, but was basically a matter of scale. After all the brewing industry

has been utilizing this process for centuries. For corn grain and cereals, it is a matter of converting starch to glucose, a simple enzymatic process followed by the fermentation of glucose by yeast to ethanol. In the case of sugar cane or sugar beets, the same technology was already being utilized to efficiently remove the sugar (sucrose) from plant biomass and easily convert to sugars fermentable with yeast [2]. Even for the production of plant-derived biodiesel, the grains from oil-producing crops are pressed to release oils in which the fatty acids can be methyl- or ethyl-esterified, producing a suitable diesel alternative. Biodiesel lags well behind other types of biofuel production systems and seems to be focused primarily on the utilization of waste products from the food industry[3].

With current scenarios, the ethanol industry will have to compete with increasing demands on grains for feed and food [2]. A concern has been the diversion of land from food production to energy production and rightly so with increasing world populations. With this in mind, much attention has been directed to the conversion of cellulosic biomass to liquid fuels. This subject has been highly reviewed in the past few years, addressing a wide range of concerns and potential advantages. It is clear that crop residues will play a key role in meeting the projected total biomass needed to provide the amount of liquid fuel to meet the goal of replacing 30% of U.S petroleum consumption by 2030 [2]. Dedicated biofuel crops such as switchgrass and fast-growing poplar also figure prominently into meeting this goal. It is envisioned that the dedicated energy crops could be grown on marginal lands poorly suited for the high capacity needs of feed and food [4]. Recently Schmer et.al.,2008 [5]demonstrated that switchgrass grown in areas considered to be margin cropland could be an effective source of biomass for biofuels. It has been proposed that establishment of low input man made prairies could be an economical way of producing biomass for biofuels [6]. Although this could be a way to supply some of the required biomass it may fall well short of the amount needed per acre to make it a practical enterprise for harvest and transportation. Well-managed switchgrass plots on marginal croplands supplied higher estimated ethanol yields per acre (93% greater than poor management) [5]. Genetic improvement is a critical component to establish switchgrass as a major biomass source that can meet the demands for more biofuels [7]. It should be kept in mind that biofuel programs must fit into an agricultural system that maximizes the production potential of each acre of farmland while protecting the environment. In this respect switchgrass on marginal croplands could also provide a nutrient sink for nitrogen waste from animal production. Switchgrass needs little nitrogen input but as with any crop production increases with the application of nitrogen [5]. Well-managed switchgrass plots could extend the useful life of croplands no longer fit for typical row crop production. Perennial grasses such as switchgrass can provide runoff protection as buffer strips along streams and rivers to keep nutrients out of waterways and lakes, thus providing dual benefits.

Although there have been a wide range of crop residues proposed to contribute to the total biomass needed for biofuel production, corn stover would be the largest contributor. It has been estimated that corn stover would contribute as much as 20% of the total biomass requirement [2]. One of the concerns of removing crop residues is the long-term impact upon soils. Removing large portions of the residues leaves the soil surface vulnerable to wind and water erosion. Guidelines have been proposed for leaving sufficient biomass on the fields to

keep this from becoming too much of a problem [8]. In addition, removing large portions of the biomass leads to a depletion of the soil organic carbon levels [9]. If sufficient amounts were left in place to meet these demands, this in turn would limit the amount of biomass for biofuel production [10]. With anticipated small profit margins, especially in the early going, there will be a temptation to remove more of the biomass, leaving the soils vulnerable to erosion and risking soil organic carbon depletion. Once these soils have reached high depletion levels, productivity will be severely restricted and returning them to better productivity will be a monumental task. Switching these lands to crops such as switchgrass that can do well in marginal soils would help the biofuels industry, but some of the most productive farmland for food and feed would be lost. This would most certainly sharpen the debate over land use for biofuels vs. food. No matter the approach it is clear multiple scenarios will need to be investigated to meet biomass for biofuel needs in a sustainable manner. The driving force behind future directions should be one of maintaining our existing high production lands while capturing increased value from lands that should not be in continuous crop production. The challenge moving forward is to develop farming systems that are both economic and environmentally sustainable while meeting the increasing demands of food, feed, fiber, and now bioenergy. There is no doubt that crop residues, especially corn stocks, play a major part in making this vision a reality but as already pointed out it is walking a fine line between productivity and maintaining soil health.

2. The role of crop rotations

At one time crop rotations utilizing nitrogen fixing legumes were much more prevalent on the landscape due to the cost and availability of commercial fertilizers. With the availability of commercial fertilizers there was no longer a need for utilizing legume forages that are particularly good at fixing nitrogen to be used for subsequent crop production. In the most productive regions in the United States particularly the Midwest Breadbasket there is economic pressure to produce monocultures of crops such as corn. This is made possible due to the relatively cheap source of commercial nitrogen-based fertilizer [11] and to the development of pesticides and herbicides. The Haber-Bosch process to produce ammonia requires large amounts of energy and appropriate catalysts to complete the transformation of hydrogen and nitrogen into ammonia. The commercialization of this process has been referred to as the detonator for the world population explosion because lands could now produce much higher levels of food to support increased populations [12]. Although this has allowed increased grain production the cost of nitrogen fertilizers has increased nearly 8 to 14 fold from a low in early 1970s to 2013 (USDA-REE statistics, <http://www.ers.usda.gov/dataproducts/fertilizer-use-and-price.aspx#.VDwPcOe9i-Q>). Much of the increased cost of nitrogen based commercial fertilizers has been driven by rising energy costs not only for production of anhydrous ammonia but also for transportation. As fossil based fuels continue to become in greater demand and at some point become limiting the price of fertilizers will continue to go up (See fertilizer price trends USDA-REE statistics) putting greater pressure on the value of crops produced on each acre of land. An alternative is to find other methods of increasing soil fertility. In farming

regions where animal production is an integral part of the farming system, animal waste provides a valuable nutrient source (e.g., dairy production). Although a good source of nitrogen based nutrients for crops, good management is critical to maintaining nutrient availability for crop production and preventing excessive soil erosion.

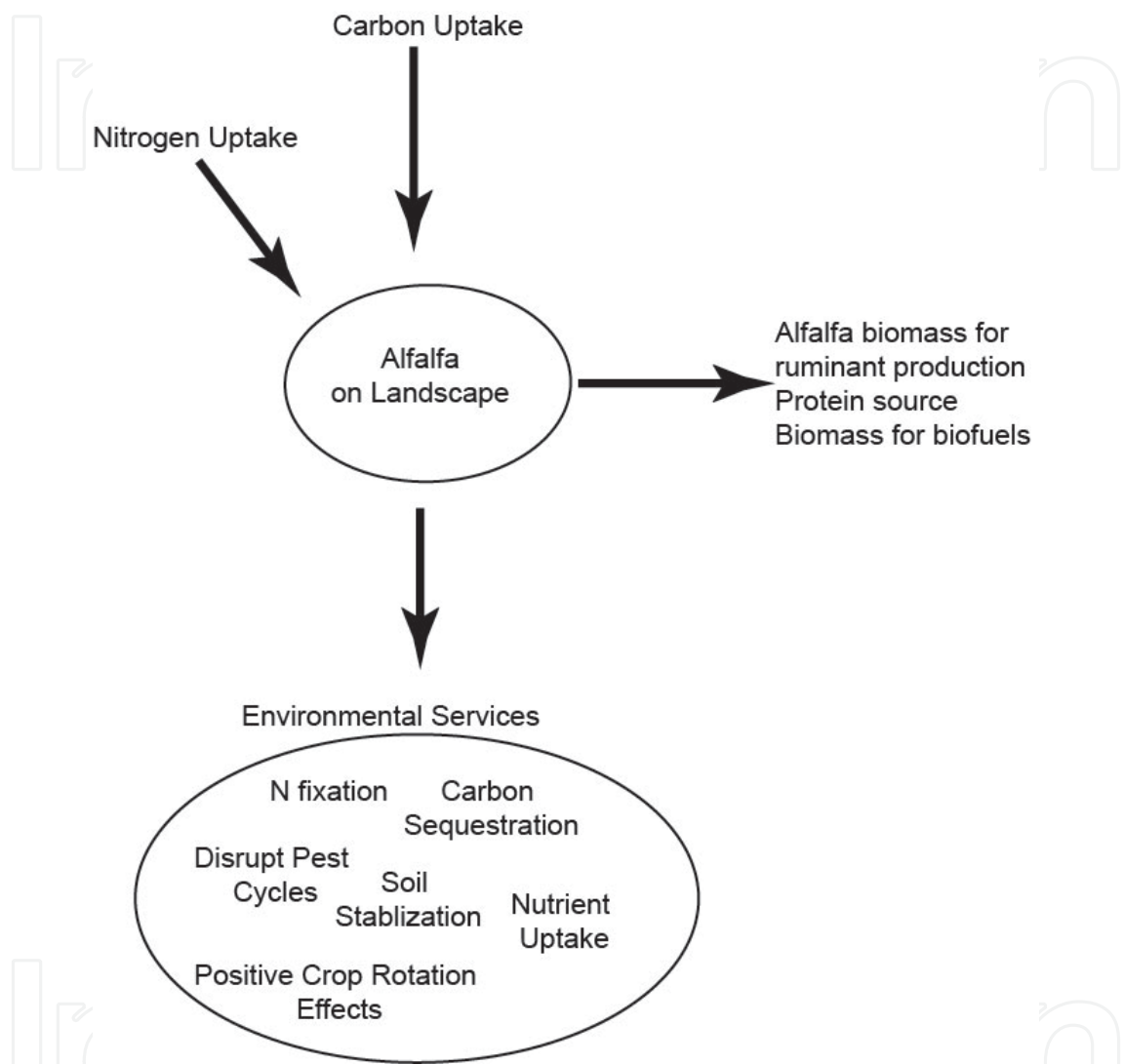


Figure 1. Diagram of alfalfa production with environmental and economic impacts. Alfalfa as a rich source of protein in its leaves can have multiple uses in terms of uses as animal feedstuff. The high fiber stem fraction could be used for bioenergy production. There are also many benefits to the environment by including alfalfa into crop rotations to allow sustainable production systems.

Production of forage legumes in rotation with row crops provides opportunities for increasing nitrogen for crop production while stabilizing and improving the environment (Figure 1). In 2010, a workshop (organized by National Alfalfa & Forage Alliance, Pioneer, USDA-Agricultural Research Service, and the National Corn Growers Association) was held to discuss the feasibility and benefits of establishing alfalfa-corn rotations to meet food and feed demands, as well as providing biomass for biofuel production (proceedings available online:

falfa-forage.org). Workshop attendees evaluated the feasibility of using crop rotations to maintain soil fertility while providing sufficient biomass for biofuel production. Jung reported [13] alfalfa (*Medicago sativa* L.) is a deep-rooted perennial legume forage typically used as a feed source for ruminant animal production. Because of its high capacity to fix nitrogen, there is no need for the addition of nitrogen fertilizer for its own growth. Nitrogen stored in the roots after two years of growth would be sufficient to supply approximately 75% of the next two years of corn production [13]. This result would have several positive environmental impacts: 1) decreased greenhouse gas emissions from reduced dependence upon commercial fertilizers; 2) reduced soil erosion; 3) reduced nutrient run-off; and 4) improved carbon sequestration [13]. A potential advantage of such a rotation system would be the accumulation of soil organic carbon if proper soil/plant management was put into place [14] (Figure 1). However, Baker [15] cautions that assessing changes in soil organic carbon is not easy in a rotation system due to the relatively short duration of the alfalfa in its rotation sequence especially in the early years of adaption of such a farming system. Having the organic matter incorporated into the soil already in the form of extensive root systems eliminates the need for soil tillage to assist in moving organic matter in crop residue to the soil biome.

Accumulation of fixed nitrogen in alfalfa is substantial (152 kg N ha⁻¹ over a range of environments and soil types) [16]. This decreases the need for application of commercial fertilizer that is dependent upon fossil fuels in the form of methane for production. As a perennial legume, alfalfa's early spring growth as well as late fall growth provides cover for soils when row crops would be planted and after harvest when soils are most vulnerable to erosion. This does not remove the need for good management practices during the corn production part of the cycle; the severity is greatly reduced over a continual corn or corn-soybean rotation. According to Vadas et.al., [17] alfalfa-corn rotations for bioenergy production can have significant advantages mostly in terms of efficiency of energy production and decreased soil erosion and less nitrogen leaching compared to continuous corn. The bottom line was continuous corn had the greatest production costs but also had the greatest profit potential. This is not assigning a cost to the soil erosion. Scientists at the U.S. Dairy Forage Research Center in conjunction with University of Wisconsin-Madison researchers Grabber, Renz, and Lauer have shown that inter-seeding alfalfa with corn can double the first-year yields from the alfalfa [18]. Such a practice would insure cover-crop availability once the corn is harvested and would provide a jumpstart on the production of alfalfa the following spring [19]. The use of alfalfa as a cover crop would appear to have some drag on total corn production during the establishment year but alfalfa production would to significantly increased during the first full year of production. Most importantly the soil would be better protected during the last year of corn production and during the alfalfa establishment decreasing soil erosion potential during alfalfa establishment. Additionally since alfalfa is a deep-rooted perennial it can recover nitrogen that has leached beyond the limited root zone of corn, helping prevent further leaching and contamination of ground water.

In the early 90s (1993 to 2000) a pilot program was initiated to test the feasibility of alfalfa-corn rotation for energy production [13]. The alliance involved the University of Minnesota, USDA-Agricultural Research Service, Minnesota Valley Alfalfa Producers, and the DOE. The

proposed system utilized dry baled alfalfa from which stems were mechanically separated from the leaves creating two feedstock components; one being the high fiber stems for energy production and the other leaf meal as a high protein fraction. Feeding trials with the alfalfa leaf meal found that it could successfully replace other protein sources such as soybean meal in diets of calves, dairy cows, and feedlot steers [13]. Although the early work indicated feasibility and advantages of alfalfa-corn rotations in a bioenergy production system the project fell apart before it could move to the next stages of testing and the project abandoned. However, these initial results indicated an existing infrastructure for handling alfalfa that could be easily adapted to a biofuel production program.

There is no doubt that rotation of corn and alfalfa would have significant environmental benefits over continuous corn. What is the economic and environmental impact upon available biomass for biofuels and the need for feed and food? Alfalfa leaves can contain as much as 30% or more protein as a fraction of the total dry matter. Typically during plant development, the stem becomes an increasing proportion of the total biomass; being lower in protein, the total plant protein decreases [20]. Harvesting schemes currently in place requires cutting the alfalfa at early-bud stage of development to keep the fiber content as low as possible and the protein content as high as possible. The down side to this harvesting practice is the need for frequent trips over the field to catch plant development at the early-bud stage. This may be reasonable for feed production for ruminant animals, but does not lend itself to practices that would be widely adopted in corn-alfalfa rotations. However, due to the high protein content of the leaves, separation of leaves from stems results in a rich source of protein for a potentially wide range of uses (Figure 2).

Earlier work using a dry fractionation system to separate leaves from stems resulted in an alfalfa leaf meal (pellets) with an estimated value of \$200/ton [21]. However, there are few, if any, existing processing plants in North America today to determine if the value would be more or less than this predicted value [22]. A newly proposed system for harvesting alfalfa separates the leaves from the stems as they are harvested in the field, producing two components.

One fraction is rich in protein (leaves) and the other is rich in fiber (stems) [23]. The leaf fraction could be used in a wide range of applications including direct ensiling for high-protein feed, or dehydrated as alfalfa meal or other value-added products requiring high-protein materials [22]. The stems could be used as a source of biomass for biofuel production or for feed depending upon the needs of fiber in the ruminants diet. Because the alfalfa leaf does not change appreciably in protein content over the development of the plant, harvest can be delayed to allow greater amounts of total biomass accumulation [24]. According to Shinnars, the advantages of field harvesting and fractionation include 1) production of a high-value protein fraction that avoids losses due to weather, 2) fractionation occurs at harvest so no further processing steps or equipment are needed, 3) capital costs of fractionation equipment are low, 4) fractionation occurs on the farm so only the desired fractions need leave the farm, and 5) ruminant feeds can be recombined to produce high-quality rations[22]. This system would provide an alternative to the harvesting/marketing system that is available today for

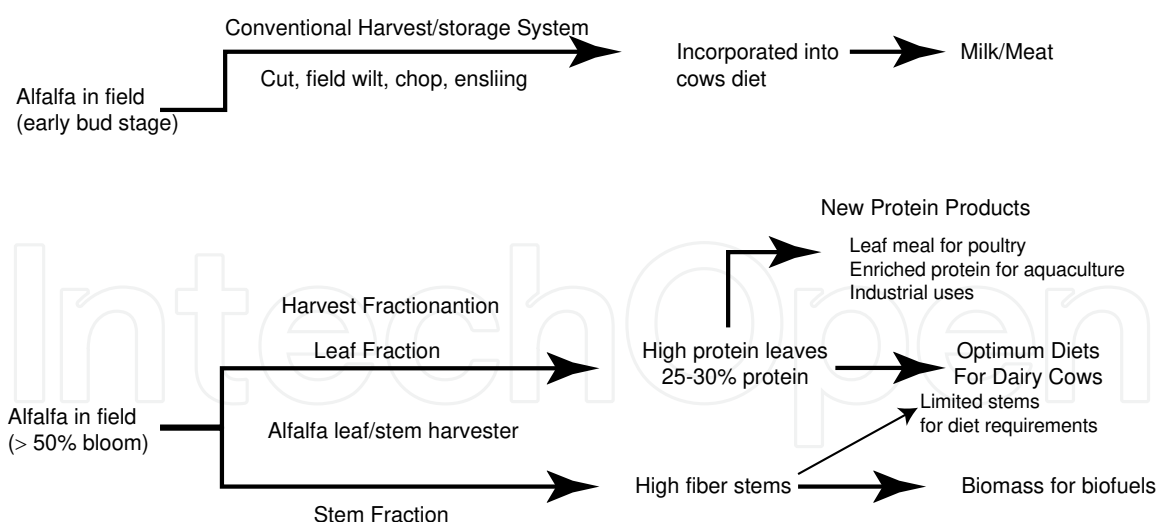


Figure 2. A comparison of the conventional harvest system for alfalfa compared to the proposed system of harvest and fractionation of leaves and stems into two component streams. This harvest system creates a high protein fraction and a high fiber fraction that allows better utilization of materials grown to fit specific needs whether it is animal feed or high fiber material for biofuels production. It is envisioned that the high protein leaf fraction could be utilized for a wide range of different animal production systems from dairy cows to poultry to enriched protein for aquaculture. The stems would be used for meeting fiber needs of ruminants (less than what would actually be produced per acre) to providing a feedstock for biofuels.

alfalfa and may provide the farmer with a cash crop incentive to produce more alfalfa in conjunction with corn (See Figure 3).

It is envisioned harvesting alfalfa using in field fractionation creates two product streams to enhance the total value of the alfalfa crop. Prototype machines have been built to effectively remove the leaves from stems creating two alfalfa components at harvest [23]. One of the real advantages of this type of harvest system is the ability to open the harvest window to avoid bad weather and to decrease the total number of harvests. A prototype leaf stripper was used to harvest alfalfa leaves and stems during the summer of 2013 to test the feasibility of creating high quality diets for dairy cows when harvesting late in plant development (full bloom stage). The idea is to decrease the number of harvests per season to limit production costs, but be able to recombine the two fractions in appropriate amounts of stems and leaves to meet the needs of a high producing dairy cow. Results of feeding trial indicated total milk production and quality of the milk remained the same and excess stems could be used for other applications such as biofuel production [25]. Although this was centered around a feeding trial it demonstrated the feasibility of having a viable harvest system that creates two value components from the alfalfa plant. Energy inputs into such a harvest system are less than what is required under the normal production scenarios [22]. Separation of leaves from the stems also allows additional in field processing to render the stems more digestible. Maceration breaks the stem material open allowing easier access of enzymes or microbes to enhance degradability/digestibility [26]. Processing the stems separately from the leaves does not risk the loss of protein from the leaf due to juicing this material during the maceration process. Hence the

high protein fraction is preserved and the high fiber fraction is processed in the field requiring less post harvest processing at the biofuel production sites.



Figure 3. Prototype alfalfa leaf stripper. A. Process of stripping the leaf fraction from alfalfa plants. In this prototype machine, harvesting stems was a separate activity from harvesting of the leaf fraction. The stem fraction was left standing in the field until leaves had been removed and then stems were cut and chopped for ensiling. Next generation harvesters would combine these two operations into a single pass over the field. B. Alfalfa stems with 80-90% of the leaves removed.

The genetic make up of alfalfa has been studied over the past 20 years to maximize quality and digestibility. A key component of this research in the past has been genetic selection for alfalfa germplasm that can withstand frequent cuttings as opposed to the accumulation of large amounts of biomass. Now there is interest to exploit the genetic potential to increase more biomass than is currently available for alfalfa. Efforts to genetically select for a biomass-type alfalfa that produces larger stems and more branching with greater total yields has been successful [13, 24, 27]. According to Lamb et al., [24, 27] alfalfa genetically selected for increased biomass production and managed to maximize yields resulted in a 40% increase in tons per acre. Revised management techniques amounted to decreased stand density providing more space for individual plant growth and development coupled with a delayed harvest i.e., switching from early bud stage to plants at 50% bloom or later. This provides the biomass alfalfa plant to accumulate higher amounts of total plant material, both leaves and stems. With the larger more robust stems lodging is minimized compared to the typical hay type alfalfa [13]. Coupled with a new harvesting technique of in-field fractionation, this could improve the amount of biomass for biofuels while still producing a high-protein fraction for value-added products. The theoretical ethanol yield for alfalfa stems would be 137 gal/acre compared to 174 gal/acre for corn stover assuming only half of the stover is removed to maintain soil health and long term productivity [13]. Including the grain for ethanol production (473 gal/acre), corn far outpaces the amount of ethanol potential from alfalfa. However, the estimated protein yield per acre would be 0.49 tons/acre for alfalfa leaves, zero for the corn stover and 0.34 tons/acre for corn grain [13]. In the face of growing world populations protein production will be of increasing concern. In terms of outright biomass production, the system of crop rotations between corn and alfalfa lags behind year after year of corn production. From an economic

perspective alfalfa-corn rotations provide several advantages in the corn production following alfalfa; 1) yield benefit of \$30 to 60/acre, 2) lower fertilizer nitrogen inputs required (2 year time frame) \$75 to 150/acre, and 3) no insecticide required the first of corn production \$15/acre [13]. This results in an accumulative savings potential of \$120 to 225/acre. The rotation system does provide for a more sustainable system, both from an environmental and economic standpoint, primarily from decreasing the application of commercial fertilizers by 75% over two years of production. These economic values do not take in to account the impact of carbon sequestration that would help offset aggressive removal of corn stover during that phase of the rotation cycle.

3. Alternatives for biofuel production

Current technologies rely primarily on the yeast-ethanol platform to create liquid fuels. The process has been well studied and continues to undergo development to utilize more of the cell wall sugars in addition to the cellulosic glucose. Much of the current biofuel industry is based on yeast fermentation of glucose that is derived from starch primarily from corn grain, although any cereal grain could be used. Brazil has adopted a slightly different approach and has based much of its ethanol production on sugarcane using yeast fermentation. These systems are not sustainable in the long run due to ever increasing populations with increasing demands for food. Capturing biomass for conversion to biofuels is a big part of the vision for decreasing dependence upon fossil fuels. Biomass to biofuels does not directly compete with production needs for food and feed and provides opportunities to maximize utilization of our landscape in ways that are sustainable and improves productivity. However, converting biomass to biofuels efficiently is a critical part of the story.

At this time ethanol production is the main form of biofuel product proposed for biomass[1-2]. This system utilizes yeast-based fermentation using primarily glucose as the substrate for ethanol production. The challenge in using corn stover or any other source of biomass in this process is the complexity of the plant cell wall. Cell walls are complex matrices composed of largely of cellulose microfibrils embedded in a matrix of structural polysaccharides. Once cell walls have reach their maximum size lignification occurs producing a hydrophobic polymer that drives the water from free spaces within the wall as it fills in these open areas (Figure 4) imparting additional strengthen to the wall. This process creates regions within the cell wall that are difficult to hydrolyze especially once the wall has been dried. A comparison of alfalfa stem cell wall composition with that of corn stover provides similar proportions of glucose on a kilogram of dry matter basis (Table 1). To render the glucose available for fermentation current technologies for ethanol production rely heavily on pretreatments to release sufficient amounts of the cellulosic portion of the wall for enzymatic conversion to glucose [28]. Pretreatments are designed to disrupt the cell wall matrix allowing cellulytic enzymes access to the cellulosic components while minimizing the formation of degradation products. Typically dilute acids combined with high temperatures are the most common form of biomass pretreatment [28]. In the case of grasses pretreatments effectively disrupt cross-linking of cell wall

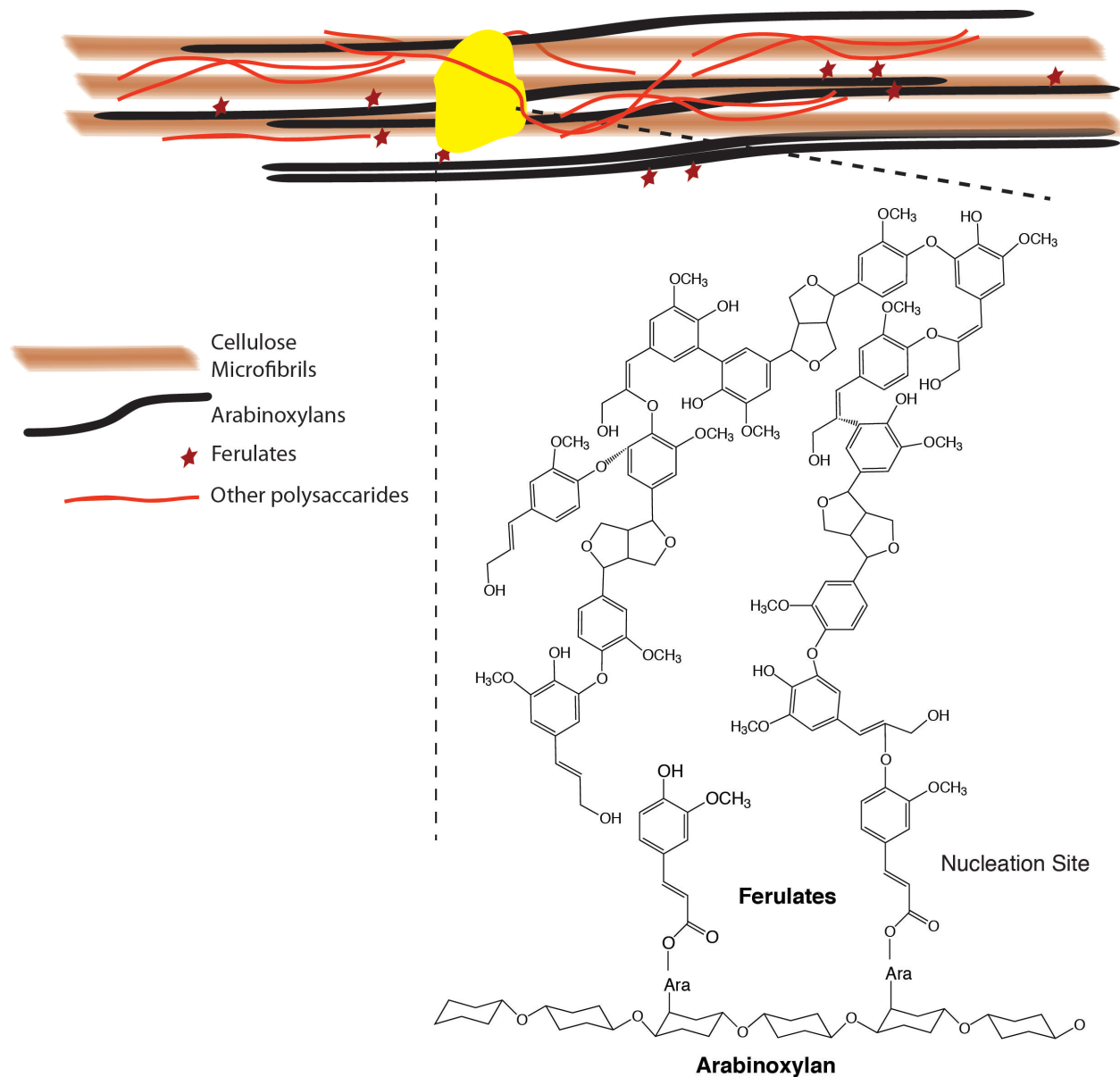


Figure 4. Cell wall model showing formation of lignin in grass wall matrix. Lignin in grasses is attached to ferulates that are shuttled out into the wall attached to arabinosyl side chains of arabinoxylans. This creates a tightly integrated wall matrix of lignin with wall structural polysaccharides. Similar cross-linking most likely occurs in dicot walls except the ferulates are not likely to be the most prominent anchor points to the wall carbohydrates. Treatment of walls with hot dilute acid solutions removes most of the non-cellulosic polysaccharides opening up the matrix to be more easily degraded by the addition of cellulosic enzyme cocktails.

arabinoxylans via ferulate dimers and to lignin via ferulate bridges (Figure 4) [29]. Acid treatments easily hydrolyzed arabinofuranose side chains of arabinoxylans, including those with attached ferulates allowing the wall to relax and expand for easier access by wall hydrolyzing enzymes. Treatment of alfalfa stems with low levels of acid during ensiling increased the amount of ethanol that could be produced [30]. However, best ethanol production was obtained after washing stem material after the acid treatment to remove degradation products that would interfere with yeast fermentation. A problem with acid hydrolysis of cell

Cell wall Component	Alfalfa Stem (N=153)	Corn	
		Stover (N=32)	Cob (N=56)
% Dry Matter			
Glucose	18-37	23-34	20-33
Other Hexoses	21-41	26-36	23-34
Xylose	5-13	15-23	18-33
Other Pentoses	6-15	18-27	22-35
Lignin	7-22	6-12	3-15

Table 1. Cell wall composition of alfalfa stems compared to corn stover and corncobs. Other hexoses include the C6 sugars galactose and mannose and other pentoses refers primarily to the C5 sugar arabinose. Data from [13] and [55].

walls especially at high temperatures is the production of furfurals that inhibit yeast. The advantage of coupling dilute acid with ensiling is avoiding the need for high temperatures. Instead utilizing the longer-term storage of the biomass to allow limited degradation of the polysaccharides while minimizing the formation furfurals and other degradation products[30]. There may be highly effective means of solubilizing the cell wall (e.g., complete acid hydrolysis of all cell wall polysaccharides to monomeric sugars), but such methods are prohibitively expensive or make it difficult to remove byproducts. To prevent unwanted microbial fermentation of the released sugar, yeast-based fermentation must be maintained in a sterile environment. Providing and maintaining a sterile environment must be factored into the sequence of events from pretreatment to fermentation; it can be achieved, but at an additional cost to the overall process. From a utilization of the total biomass standpoint yeast fermentation leaves a 20 to 40% of potentially fermentable carbohydrates behind (Table 1) simply because yeast cannot deal effectively with them. This leaves a good deal of potential energy forming material off the table.

Ethanol is not the only biofuel under consideration as a product for biomass. Alternative systems for the conversion of biomass to biofuel are the syngas platform (details of this system can be found on the National Renewable Energy Laboratory website: www.nrel.gov/biomass/biorefinery.html) and the carboxylate platform. The syngas platform requires large inputs of energy to produce effective amounts of a useful biofuel. The carboxylate platform requires undefined mixed bacterial cultures under anaerobic conditions [31] (Figure 5). One of the big advantages of this system is the flexibility of the undefined mixed bacterial cultures to handle a wide range of substrates going into the system. More importantly they do not require a sterile environment in which to function. Popular sources of mixed anaerobic cultures are sewage sludge digesters and marine sediments[31-32]. The carboxylate platform works by the process of anaerobic degradation of carbohydrates to produce volatile fatty acids primarily acetic (C2), propionic (C3), and butyric (C4) acids although other VFAs can be produced.

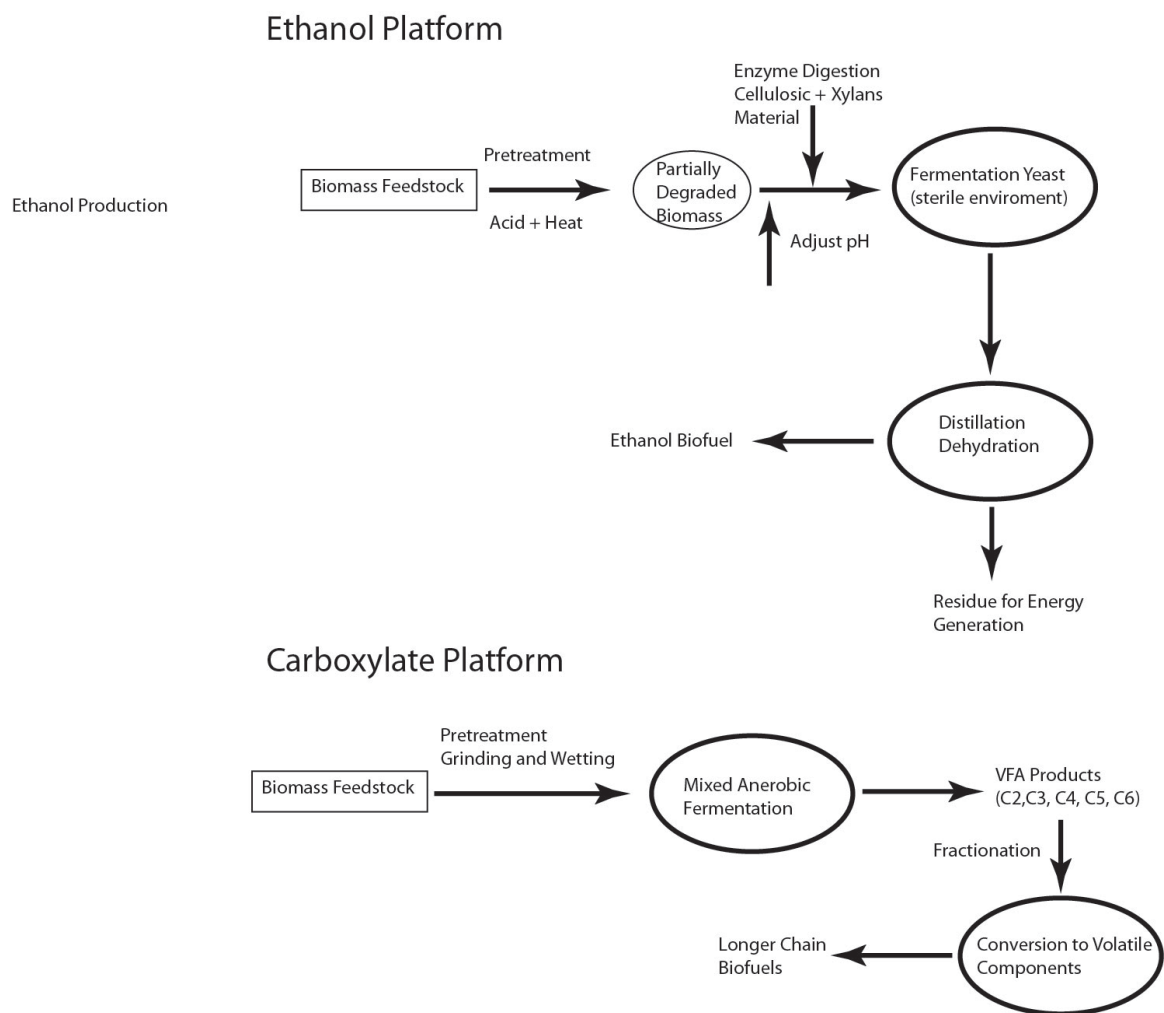


Figure 5. Schematic of biofuel production systems. Ethanol platform is the typical process proposed for conversion of plant biomass to liquid fuels. The carboxylate platform is an alternative method of producing liquid fuels using mixed anaerobic bacteria (ruminal microbes have advantages over typical systems utilizing sewage sludge or marine sediments) to produce VFAs for conversion to volatile fuel components. Cultures can be manipulated to produce higher ratios of C5, C6 and C7 VFAs for more energy dense molecules.

An advantage of the carboxylate platform is the general low inputs needed to obtain materials that can be modified to produce biofuels or bio-refinery products. Pre-treatments are minimized and may be confined to particle size reduction or mild chemical treatments providing the greatest advantages[31]. Most importantly the carboxylate platform does not require an antiseptic environment in which to operate, greatly simplifying handling of raw materials going into digesters. Significant work has been done on carboxylate platforms utilizing mixed cultures from sewage sludge treatments [31, 33]. Such systems have a great deal of flexibility when it comes to handling a wide range and complexity of crop residues or other carbon based materials from agricultural practices. These organic materials may be relatively abundant and of relative low value in their present form before fermentation to VFAs. A disadvantage of the sewage sludge inoculum is the general slow conversion rate and methanogens producing large amounts of methane[31]. In the case of manure or other organic waste digesters where time is

not a limiting factor this is quite acceptable and the methane can be easily captured and used as an energy source. With the right type of microbial mix, it is possible to produce longer-chain carboxylates caproate (C6) and caprylate (C8) from acetate in addition to the typical acetate, propionate, and butyrate through a process referred to as reverse β -oxidation[34]. The potential down side of this approach is the process tends to be slow and requires inhibition of methanogens to force the system to produce larger quantities of the longer-chain VFAs, e.g., n-caproate (C6) and n-caprylate (C8). Inhibition of methanogens can be efficiently achieved with compounds like bromoethane sulfonic acid, but this is relatively expensive and would be prohibitive on a large scale[31].

An alternative source of anaerobic microbes for the carboxylate platform for the conversion of plant biomass would be the cow's rumen. In comparison to waste stream anaerobic microbes, the rumen is a more specialized system having evolved to extract nutrient value out of a wide range of plant materials [35]. Although cell wall degradation and total feed utilization by dairy and beef cows can be improved, the microbial community in these ruminants has evolved to degrade fibrous plant material relatively quickly to supply needed nutrients to the animal [36]. The rumen is a mixed culture of anaerobic organisms effectively degrades carbohydrates, proteins, and fats present in feed mixtures to produce short-chain VFAs. The efficiency of this ruminal system appears to be much greater than what is in the typical waste stream systems[37]. The advantage of a ruminant-based carboxylate platform is the ability to degrade all the organic materials (polysaccharides, proteins, fats, and oils) with the exception of the lignin within short time periods of 24-72 hours. High producing ruminants like the dairy cow must be able to extract sufficient energy from feed materials within 48 hours to support her maintenance and milk production. Cow ruminant microbial communities have evolved over time to handle a diversity of substrates (i.e., easily degraded starch to more recalcitrant fiber materials). Ruminal microbial communities are quite complex with redundancy in the types of hydrolytic abilities that may come into play as substrates change coming into the cow [36]. Due to the relatively short incubation times slower growing acetogens (convert C3-C6 VFAs to acetate) and the methanogens (convert acetate to methane) do not have a chance to become well established. This in turn restricts methane production (8-15% of total energy) in this type of carboxylate platform avoiding the need to add specific methane inhibitors [36]. The small amount of methane that is produced could be captured and utilized as an energy input to maintain incubation temperatures.

Recently Weimer et.al., 2014 [38] demonstrated the ability of rumen microbial cultures to produce large amounts of valeric and caproic in short time periods of 48-72 hour incubations. It has been demonstrated that the addition of dilute amounts of ethanol to mixed culture fermentations in the carboxylate platform results in the extension of the short chain VFAs to medium length molecules thus capturing the fuel value of ethanol in a form that could be more easily recovered [34, 39]. What is unique and promising about the work of Weimer et.al., is the ability to speed up this process using ruminal mixed culture fermentations as opposed to the typical source of sewage digesters [38]. In addition they found that supplementing the mixture with ruminal derived *Clostridium kluyveri* an ethanol-utilizing bacteria resulted in production levels of 4.9-6.1 g/L of caproate in 48-72 hours using either switchgrass or alfalfa stems as the substrate. The level of caproate production seen by the Weimer group is similar to what

others have achieved [34, 40], but in a 10 to 30 times less time frame for incubation. Being able to generate longer VFAs increases the energy density in each molecule increasing the value of the material for liquid fuels. In addition, the longer chain VFAs are easier to extract from the fermentation media decreasing recovery costs[38-39]. For any biomass to biofuel production process a key element is being able to produce sufficient amounts of fuel molecules in short periods of time and with limited inputs. The carboxylate platform based on ruminal microbes supplemented with additional strains of more specialized bacteria (e.g., *Clostridium kluyveri*) appears to hold a great deal of promise for biomass conversion. Little sample preparation was needed to treat the switchgrass and alfalfa stems for biofuel production using the ruminal microbial system. The fermentation process described here could be combined with other platforms that produce ethanol. For example concept of consolidated bioprocessing (CBP) [36, 41] is considered as a possible avenue for the production of ethanol from biomass to avoid the need for the addition of expensive hydrolytic enzymes. In most cases the CBP system does not produce sufficient ethanol to be cost effective [41]. However, coupled with a ruminal microbial based carboxylate platform the limited ethanol production could be effectively utilized to produce longer chain VFAs increasing energy density of each molecule [38].

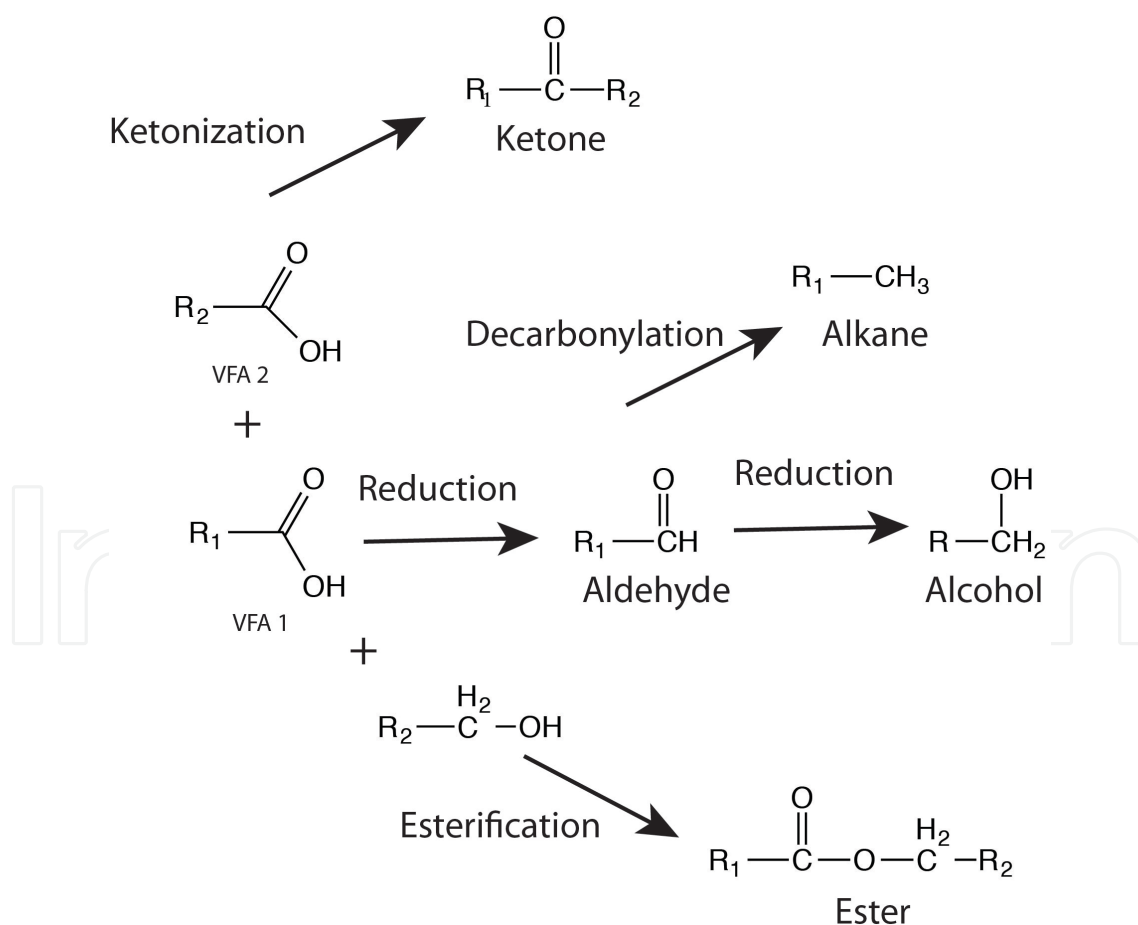


Figure 6. Multiple pathways for converting VFAs to volatile compounds that can serve as biofuels or as intermediates for the formation of additional organic compounds.

Volatile fatty acids must be converted to a form that increases their volatility to be good energy molecules. The medium length VFAs can be recovered by extraction [42] to allow additional modifications. Conversion of VFAs can be accomplished in different ways depending upon the desirable end product and its potential use. Possible conversion practices could utilize pure cultures of specific bacteria, electrochemical and thermochemical process. Useful end products that could be used for energy, solvents, or other biorefinery intermediates include ketones, aldehydes, alcohols, and alkanes (Figure 6). Due to the flexibility in the type of end product there are several avenues available to reach the desired outcome. Conversion process can be accomplished in a multitude of different ways using a single or multiple steps to reach desired products. Products such as ketones from VFAs using catalytic coupling [43] or ketones and secondary alcohols as produced in the MixAlco process [33]. The formation of volatile esters can be formed as demonstrated by Lange et.al., [44], Levy et.al., [45] or using microbial systems [46]. Production of alkanes can be achieved by decarboxylation of using pure cultures of microbes [47] or the use of electrochemical process using the Kolbe and/or the Hoefer-Moest processes [48]. The conversion of VFAs especially the medium length (C4-C6) increases volatility and at the same time decreases miscibility with water improving extraction process to isolate the biofuel molecules. The added advantage of VFA production (C2-C6 or longer) coupled with conversion technologies is the flexibility to produce a wide range of molecules that can be used for higher energy density fuel molecules or as starting molecules for other organic materials.

Typically biomass to biofuel systems are envisioned with a centrally located processing plant to handle large amounts of biomass. Unlike the grain ethanol production systems in which the grain is of relatively high density in terms of potential energy per volume, biomass tends to be much bulkier unless it is pelletized to increase bulk density [49]. When one is considering the utilization of corn stover and/or alfalfa stems these materials can be field processed into relatively high-density bales to improve the efficiency of shipping [50]. This is just one step in the complete process of collecting and moving biomass to centralized points for conversion to biofuels [51]. The challenge is keeping the collection, improving bulk density, and transportation costs to minimal levels to help final economic returns and the minimizing the carbon footprint associated with biomass to biofuels [50]. Perhaps it would be feasible to consider on farm conversion at least for the initial steps of the conversion process. In this scenario the harvested plant material (corn stover, alfalfa stems, switchgrass, etc) would be stored on the farm more with an ensiling process compared to dry storage. This provides an opportunity to add enzymes or dilute chemicals to enhance the subsequent digestion of the materials. Size reduction could also be incorporated into the process and storing materials wet eliminates the need for rehydration for fermentation. It could be envisioned that small on farm digesters could be used to process the biomass materials to produce VFAs (select additions of pure cultures and ethanol to create products for special uses) that would be recovered and transported to conversion sites. Processing on farm eliminates the need for consolidating biomass for shipment to centralized processing plants and open opportunities for other types of storage that could enhance conversion efficiency. Recovery of the VFAs or conversion on site to intermediates followed by extraction results in a improvements in energy density and allows

materials to be shipped greater distances for further processing into molecules that provide the greatest benefit either as biofuels or as precursors for other organic based materials.

One of the challenges of any biomass conversion platform is dealing with the fermentation residual materials. Lignin is a primary component of the fermentation waste and in many schemes it is recovered and burned to supply energy for other steps in the complete process. With the carboxylate platform based upon mixed ruminal microbes, one of the by products could be the microbial protein as a value-added material. In the normal rumination process, formation of microbial protein is an important component to supply needed protein to the animal. In dairy production, microbial protein helps supply critical amino acids required for milk production, especially methionine and lysine that are often low or lacking in many forage-based diets [52]. Harvesting the microbial protein after biomass conversion to biofuels could provide an important protein supplement for dairy cow diets that is enriched in methionine and lysine. The microbial proteins would be insoluble along with the typical insoluble materials, i.e., lignin and other cell wall components. Recovery of these insoluble materials would be relatively straightforward. As an alternative the lignin-microbial-carbohydrate residue from the fermentation process could be used to replace phenolic-formaldehyde based adhesives[53]. Many of the ruminal microbes contain glycocalyx materials surrounding the individual cells that help them adhere to plant materials during digestion. The glycocalyx is a glycoprotein-polysaccharide complex that surrounds the cell membrane of some bacteria[54]. It has also been demonstrated that the lignin-microbial residues from ruminal fermentations, as proposed for the carboxylate platform, could be used to replace phenol-formaldehyde compounds as adhesives in the production of plywood composites[53]. Up to 70% of the typical phenol-formaldehyde formulation could be replaced by the more environmentally friendly residues that are byproducts of ruminal-based fermentations. Even if it would not be possible to replace all of the phenol-formaldehyde adhesive, decreasing significant amounts of this material would provide for healthier composites by decreasing the amount of formaldehyde outgassing that are a human health concern[53]. Key to the effectiveness of fermentation residues is creating the correct balance of lignin, the blend of rumen microbes and the types of glycocalyx material, and other minor phenolic materials in the plant materials.

4. Conclusion

This chapter is not meant to be a comprehensive assessment of biomass to biofuels, but rather a look at unconventional approaches that would enhance the sustainability of the entire process. To meet the goals of biofuel production by 2030 will require optimizing land use for food, feed, and bioenergy production. It should be approached from a standpoint of developing a viable biofuel production system that increases the amount of energy stored in the molecules making up the biofuels, i.e., longer-chain molecules, more energy per unit of fuel. To be sustainable into the future we must be willing to develop alternative systems that supply a range of biomaterials. Although the producing energy alternatives is of major concern at the present time we should be evaluating and developing bioenergy systems that allow flexibility not only in terms of feedstock going in, but the products coming out. Development of biomass

to biofuels systems should look at how we can maximize the value of the total process, that is, optimize land use, embrace farming systems that decrease or eliminate soil/nutrient losses, improve economics of production, utilization of value-added products, and total energy production versus inputs. The entire process must also be sustainable from an environmental standpoint and provide economic advantages to the producer. Our vision into the future should be one of maximizing the productivity of each acre of farmland while meeting the needs for feed, food, and energy along with improving the soil for future generations. Decisions made today should not be overly influenced solely by short term economic gains.

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