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# Developments in crops and management systems to improve lignocellulosic feedstock production

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**Abstract:** There is an urgent need to develop viable, renewable, sustainable energy systems that can reduce global dependence on fossil fuel sources of energy. Biofuels such as ethanol are being utilized as blends in surface transportation fuels and have the potential to improve sustainability and reduce greenhouse gas emissions in the short term. Bioethanol, the most widely used liquid biofuel, is currently produced by converting sugars or starches from feed crops into ethanol. Use of this fuel source displaces and draws water consumption away from agricultural crops, increases soil erosion by shifting land from perennial grasses to annual crops, and increases use of fertilizers and insecticides. In contrast, bioethanol made from lignocellulosic biomass feedstocks does not have these limitations and in addition, offers a larger resource base: the amount of cellulosic material available for potential use vastly outweighs the amount of available starch-based feedstock. Therefore, bioethanol from lignocellulosic biomass has attracted considerable interest from biofuel developers. This review is an update of some developments to optimize cellulose extraction from feedstock crops and to improve crop yields and logistics. It concludes that agricultural and forestry systems that incorporate lignocellulosic biomass crops can be designed for improved ecological function and energy use efficiency. Development of crops that have both desirable cell-wall traits and high biomass productivity under sustainable low-input conditions can significantly enhance the economics and efficiency of the conversion process. Optimizing the logistics of moving feedstock from field or forest to bio-refinery can significantly reduce costs of using lignocellulosic feedstocks. © 2013 Society of Chemical Industry and John Wiley & Sons, Ltd

**Keywords:** biofuels; lignocellulosic; feedstock; production system

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## Introduction

Four of the great challenges facing humanity during the twenty-first century will be energy supply, fresh water supply, climate change, and global food security, and each of these will be influenced by our choice of biofuels. Global energy demand is rising steeply in both developed and developing nations and, although new sources of oil such as the Bakken and Eagle Ford shales in the USA are currently easing American energy concerns, they will be short-lived. With each new oil discovery, the extraction technology becomes increasingly complicated, expensive, and fraught with environmental issues such as high water use and increased greenhouse gases (GHGs). The era of cheap oil is over. Climate change has the potential to substantially alter global habitats and crop-growing conditions, and reducing CO<sub>2</sub> emissions from energy use is a real and urgent necessity. Thus, we focus on biofuels derived from non-food biomass, and largely from lignocellulosic materials that can be used in solid (pelleted material, torrefied biomass, etc.), liquid (ethanol or other alcohols, bio-oils, etc.) or gaseous (e.g. syngas) forms. Our first efforts at the production of biomass for biofuels involved feed grain crops but concerns about global food prices and supply have reoriented efforts toward developing biofuel crops that will grow on land not generally used for feed grains or to use plant-based residues not otherwise used. Currently, biofuels supply about 10% of the world's energy, with the bulk of this being low grade biomass used for cooking in developing countries. However, there are some examples of very successful biofuel development: Brazil has produced up to almost 50% of its liquid fuels on roughly 1% of its agricultural land and, through developing the resource, has positioned itself extremely well in a world where fossil fuels are relied on less and less. In the USA, total renewable fuels are forecast to be 16.55 billion gallons by 2013 (9.6% of total fuel) and advanced biofuels, including cellulosic, are forecast to be 2.75 billion gallons (1.6% of total fuel). Overall, the use of biofuels, especially the new forms that compete minimally with food production, is likely to rise, and the timeline of this transition suggests that a minimum of 10 million barrels per day of alternative fuels will be needed within a decade of the peak in production of conventional crude oil.<sup>1</sup> There is, therefore, an urgent need to develop viable, renewable, and sustainable energy systems that can displace global dependence on fossil fuel sources of energy. Although some sectors can manage with other energy sources, such as solar and wind-produced electricity, sectors such as the aerospace industry, are absolutely dependent on

energy-dense liquid fuels. Bioethanol, the most widely used liquid biofuel, can be utilized as a surface transportation fuel with little change to current technologies and has the potential to improve sustainability and reduce greenhouse gas emissions in the short term. Bioethanol is currently produced by first-generation technologies converting sugars directly from crops like sugarcane or sugarbeets, or indirectly through starch from corn, sorghum, or wheat. Domestic bioethanol production not only decreases dependence on fossil fuels, the addition of ethanol to gasoline also increases the fuel octane rating and results in cleaner, more complete combustion and lower GHG emissions. Combustion of 10% ethanol-blended gasoline results in production of 30% less carbon monoxide (CO), 10% less carbon dioxide (CO<sub>2</sub>), and 7% less NO<sub>x</sub>/SO<sub>x</sub>.<sup>2</sup>

## Generating biofuel from lignocellulosic biomass

First-generation bioethanol technologies, however, have several conspicuous limitations. Most notably is their total reliance on cultivated biomass and the diversion of feedgrains, such as corn to biofuel production.<sup>3</sup> In addition, there are environmental limitations including drawing water consumption away from land used to produce food crops, increased soil erosion through the shifting of land from perennial grasses to annual crops such as corn, and greater reliance on nitrogen and phosphate fertilizers, insecticides and herbicides.<sup>4–8</sup> The strongest argument against first-generation technologies, however, comes with the reality of their limited supply and competition with food. Even if all soybean and corn production in the USA were dedicated to biofuel production, only 12% of gasoline demand and 6% of diesel demand would be displaced.<sup>9</sup>

First-generation technologies are therefore not a solution to the world's long-term energy needs. Adopting present processing technologies to utilize a feedstock that does not require heavy cultivation and diversion of agricultural lands and foodstuffs could, however, contribute to a long-term solution to bioenergy generation and sustainable supply. Second-generation biofuels are made from lignocellulosic biomass feedstocks using advanced technological processes that convert cellulose, found in plant structural elements, to ethanol.<sup>10</sup> The amount of cellulosic material available for potential use vastly outweighs the amount of available starch-based substrate. A conservative estimate is that presently, there are approximately 400 million tons of biomass available and this number could grow to about 600 million by 2020.<sup>11</sup> The lignocellulosic feedstocks in Canada are cereal residues in agricultural regions and

wood residues in forest regions. The annual availability of wood infected by pine beetle in Canada is between 9.3 and 12.3 million tonnes, which has the potential to generate 2.8–3.6 billion liters of ethanol per year.<sup>12</sup> Every year, more than 40 million tonnes of lignocellulosic biomass, that is produced worldwide, is thrown away. There is no competition with food when these materials are converted to biofuels.<sup>13</sup> In the longer term (by 2050) there is the potential worldwide to produce 130–410 EJ/year of energy, equivalent to 33 to 100% of present energy production, by using only abandoned agricultural lands, low-productivity lands and 'rest lands'.<sup>14</sup> The cost of pre-processing cellulosic material to generate free glucose, however, is much higher than that for conventional feedstock, as both mechanical and thermochemical treatments are often required. However, conversion costs have been falling in recent years and pilot plants for the production of lignocellulosic fuels are now being developed. Lignocellulose biomass can also be used in the generation of heat and electricity through direct combustion.

Emerging demands for biofuels and bioenergy derived from biomass are creating new opportunities for redesigning agricultural and forestry systems for improved ecological function and efficient energy use. Technologies to optimize plant cell wall characteristics and reduce energy requirements for polysaccharide extraction, breeding, and agronomic management of feedstock production systems to increase productivity, and efficient harvesting techniques can all significantly enhance the economics and efficiency of second-generation biofuels and make them more cost effective as fossil fuel replacements. What follows is an update of some developments to optimize cellulose extraction from feedstock crops and to improve crop yields and harvesting technologies.

## Developments in cell wall degradation

Cellulose and hemicellulose, the main polysaccharides in lignocellulosic biomass are tightly bound to lignin in the plant cell wall, which hinders their availability for bioconversion to bioethanol.<sup>15</sup> Overcoming cell wall chemical and structural properties to extract the desired carbohydrates is currently an energy-intensive process. Reducing the energy requirements through the development of mechanisms for easier cell wall degradation is critical for the advancement of biofuel production from lignocellulosic biomass.

Separating lignin and cellulose currently requires heat and acid to remove the lignin and reducing or modifying

the initial lignin content of the biomass could partially replace this treatment.<sup>15,16</sup> In recent years, genetic modification of the lignin biosynthesis pathway has received a lot of attention.<sup>16</sup> Transgenic modification of this pathway to alter lignin composition in trees improved pulping efficiency.<sup>17</sup> It was observed that transforming poplar plants with antisense constructs of the lignin synthesis gene coding for 4-coumarate-CoA ligase (*Pt4CL*) resulted in significantly decreased (40%) lignin content.<sup>18</sup> However, negative effects of plant susceptibility to pathogens or harsh environmental conditions have been anticipated.<sup>19</sup> In crop plants, down-regulation of lignin synthesis genes improves saccharification efficiency, potentially eliminating the need for acid pre-treatment.<sup>20</sup> Although extensive research has been conducted in the area of genetic modification of lignin biosynthesis in trees and other dicots, it is still not clear how much one can extrapolate from dicots to grasses.<sup>21</sup> Another promising approach would be to engineer various cellulase/ligninase enzymes into crop biomass in order to deconstruct the biomass before bioprocessing and allow it to be more readily hydrolyzed to produce ethanol.<sup>21,22</sup> Various groups of cellulases, for example endoglucanases, exoglucanases and  $\beta$ -glucosidases, have been identified and successfully introduced into plants through genetic engineering to facilitate cellulose degradation. This approach resulted in the expression of bacterial endoglucanase E1 in model plant species, such as *Arabidopsis* and tobacco.<sup>23</sup> Specifically, a thermostable endo-1,4- $\beta$ -D-glucanase E1 from *Acidothermus cellulolyticus* was targeted to the apoplast of transgenic *Arabidopsis*.<sup>24</sup> The enzyme has a high temperature (81°C) optimum and activity is reduced at ambient temperatures. This suggests that the production of such enzymes in plants is possible by virtue of their limited enzymatic activity at temperatures compatible with plant growth. Based on these results, endoglucanase was introduced into maize and found to be active.<sup>25</sup> Conversion of rice and maize biomass to ethanol was improved by supplementing the process with thermostable endoglucanase expressed in transgenic rice.<sup>26</sup> Engineering *Festuca* with a fungal ferulic acid esterase targeted to the vacuole resulted in increased digestibility and reduced levels of cell wall esterified phenolics.<sup>27</sup> As heterologous expression of lignase/endoglucanase is feasible in crop plants, transformation of feedstocks with similar thermostable cellulases will also be a useful first step in developing these crops for cellulosic ethanol production. There will be a need to ensure that these enzymes are not degraded by high temperatures during biomass pretreatment processes.

Despite successes with engineering cellulose-degrading activities into crop plants, it is notable that besides cellulose, lignocellulosic biomass contains large amounts of hemicelluloses with high contents of five-carbon sugars. The most common structural polymer found in the hemicelluloses is a  $\beta$ -1,4-linked xylose polymer. These polymers are recalcitrant to hydrolysis by current technologies; however, hemicellulase enzymes introduced through genetic engineering can increase overall conversion of hemicellulose by xylanases in a synergistic fashion. Complete hydrolysis of hemicelluloses could lead to a dramatic improvement in the fermentative and extraction processes, which could further improve the bioenergy potential of lignocellulosic biomass.<sup>15,28</sup> Recently, a synthetic, modified, codon optimized xylanase gene (*XynZ*) from *Clostridium thermocellum* was successfully expressed in transgenic tobacco plants.<sup>29</sup> Further increase in performance of these enzymes and improvement to resulting sugar yields are vital to improving the efficiency of the lignocellulosic biofuel/bioproduct industry.

Transgenic technologies can also play an important role in enhancing yield and stress tolerance in biofuel crops.<sup>21,30</sup> Gene expression can be targeted to the apoplast and vacuole through a specific signal peptide sequence such as *Pr1a*, from tobacco and potato proteinase inhibitors. Identifying factors that facilitate tolerance and survival during exposure to drought, freezing, and other abiotic and biotic stresses will be vital. Therefore, feedstocks such as perennial grasses and relevant tree species could be transformed for more effective weed, disease, and insect control.<sup>31</sup>

At the cellular level, a new generation of energy crops will be characterized by a cellulose and hemicellulose content that is more accessible and energy efficient to extract. These crops must also have high biomass production and produce an optimized amount of fuel per unit of biomass while maintaining crop production system sustainability with minimal water and fertilizer inputs.

## Perennial grass production systems

Interest in using perennial grass species as energy crops is fairly recent and relatively little breeding has therefore been done for this purpose. Breeding programs, conducted since 1936, focused on improvements for forage purposes such as better nutritive value which included higher digestibility, higher concentration of various minerals, and lower fiber characteristics which are not always useful for bioenergy. Improved forage yield has also been a common goal, which would be an advantage for bioenergy

production; however, progress has been slow, due to the outcrossing nature and genetic complexity of many of the species. Similarly, agronomic management of perennial grass species has traditionally centered on forage quality and productivity. In recent years, however, a concerted effort has been made to evaluate the genetic by environment interaction of leading potential biomass species in a range of field trials across the USA.<sup>32</sup> Yield gains realized through improved crop management for biomass have frequently been of the same magnitude as those targeted through breeding programs.<sup>33–35</sup> Beyond biomass, the traits targeted in improved grass populations for biofuel production will depend on the technology pathway used to convert biomass to fuel.<sup>36</sup> For example, lignin composition and concentration and cellulose and hemicellulose concentration are related to ethanol yield in a fermentation system.<sup>37</sup>

Warm season ( $C_4$ ) grass species, including switchgrass (*Panicum virgatum* L.), Miscanthus (*Miscanthus*  $\times$  *giganteus* Greef et Deu. and other species) and prairie cordgrass (*Spartina pectinata* Bosc ex Link.), have received the most attention for biomass improvement through breeding. Reviews of warm season grasses as biofuel feedstocks, including information on genetic improvement, have recently been published.<sup>36–40</sup> A number of cool season grasses have also received interest, including reed canarygrass (*Phalaris arundinacea* L.).<sup>41</sup> Below we highlight key developments in the breeding and agronomic management of leading warm season grasses used for biomass production.

## Switchgrass

Switchgrass is native to the prairie region of North America and has a number of characteristics which are desirable for use as a bioenergy feedstock, including high productivity, persistence, and wide adaptation.<sup>36,42</sup> Switchgrass has been evaluated for use as a bioenergy crop in the USA for more than 30 years and its history as the 'model' bioenergy crop by the US Department of Energy has been reviewed.<sup>43</sup> There are two distinct ecotypes of switchgrass, upland (mainly octaploid  $2n = 8x = 72$ ) and lowland (mainly tetraploid  $2n = 4x = 36$ ). The two ecotypes can be distinguished by cytoplasmic (chloroplast DNA) differences and nuclear DNA differences.<sup>44,45</sup>

Cultivars and existing native populations have not been found to be highly differentiated and are genetically diverse providing useful germplasm for selection for biomass yield.<sup>46</sup> Narrow sense heritabilities of biomass yield have been estimated to be low to medium.<sup>47,48</sup> Yield

components such as tiller density, tiller mass, and phytomer mass have been positively associated with biomass yield<sup>49,50</sup> and these components may have higher heritability than biomass yield. Development of hybrids to exploit heterosis has recently been investigated and a method for hybrid production proposed.<sup>51</sup> Tetraploid populations (largely lowland) were identified as different heterotic groups with F<sub>1</sub> hybrid populations showing 30–38% high parent heterosis.<sup>52</sup> F<sub>1</sub> hybrids of the lowland ‘Kanlow’ by upland ‘Summer’ are now in field trials across the USA, in preparation for expected commercial release.<sup>53</sup> Genomic tool development is not as advanced in switchgrass as in some other crop species, but a transformation project involving down-regulation of lignin pathway genes in switchgrass has been initiated.<sup>54</sup>

Management of switchgrass for biomass is dependent on location and cultivar selection, with general divisions evident between upland and lowland ecotypes. Generally, lowland ecotypes are best suited for warmer, wetter growth environments and have a longer growing period than upland ecotypes.<sup>55</sup> They have different morphologies, with larger, taller and fewer stems being characteristic of lowland ecotypes, often leading to higher biomass yields.<sup>34,50</sup> Further, the ecotypes have been shown to differ in their susceptibility to foliar diseases, such as rust (*Puccinia* sp.), necessitating different pest protection strategies depending on cultivar selection.<sup>56–58</sup> Ecotypes are also differentially affected by herbicides,<sup>59</sup> harvest management,<sup>60</sup> and resource availability<sup>55</sup> leading to changes in both biomass yield and biomass composition.<sup>61,62</sup> Numerous studies have evaluated nitrogen fertilizer application and harvest management in switchgrass, identifying these management practices as critical to not only crop productivity but also to the long-term stand persistence and GHG emission or sequestration. Within ecoregions, best management practices for nitrogen fertilizer and harvest time have been developed. In warmer, wetter climates it will likely be possible to harvest twice per year if nutrients are not limiting, but in temperate areas a single, late harvest leads to better fuel quality and more consistent yields.<sup>63</sup> A single harvest after senescence minimizes nitrogen removal in plant biomass, thus reducing the amount of fertilizer needed for optimal crop growth.<sup>64</sup>

## Miscanthus

*Miscanthus* is a C<sub>4</sub> grass native to East Asia, for which the main commercial focus has been on a single, high yielding sterile hybrid, *Miscanthus* × *giganteus*, a spontaneous allotriploid hybrid of *M. sinensis* and *M. sacchariflorus*

with 57 chromosomes.<sup>65,66</sup> There is genetic diversity available from a large gene pool of different species within *Miscanthus* and related species and a high genotypic variation for cell wall composition among *Miscanthus* genotypes has been found.<sup>67</sup> There are also major differences in biomass yield and associated traits among species and among ecotypes within species.<sup>37</sup> Breeding goals include both the development of new sterile hybrids which outyield the existing *M.* × *giganteus* hybrid, as well as the development of adapted, high yielding seed-propagated cultivars of *M. sinensis* or *M. sacchariflorus*, which would reduce establishment costs, although seeded varieties must be carefully evaluated for invasive potential.<sup>68,69</sup>

Variation exists among and within species for first-year overwintering ability in northern Europe, with genotypes of *M. sinensis* and species hybrids being superior.<sup>70,71</sup> Low-density marker maps have been generated in *M. sinensis* and potential quantitative trait loci (QTL) identified for a number of traits associated with biomass production.<sup>72</sup> Chloroplast DNA marker loci containing single nucleotide polymorphisms (SNPs) were identified, which can be used to differentiate *Miscanthus* species and identify cytoplasmic gene pools.<sup>73</sup>

*Miscanthus* has only been grown for biomass in North America for a short time. The first published field trials of *Miscanthus* in the USA compared it to switchgrass in Illinois, and found it to yield at least twice as much biomass.<sup>74</sup> Recent years have seen an explosion of *Miscanthus* research in the USA, following decades of experience with it in Europe.<sup>75,76</sup> The comparative yield advantage of *M.* × *giganteus* increases as climates become cooler, making it likely the most productive bioenergy crop available for cool, temperate regions.<sup>77,78</sup> So far, the biggest barriers to *Miscanthus* adoption revolve around propagation, planting, and winter survival of the sterile clone *M.* × *giganteus*. Propagule (rhizome) costs are high, frontloading *M.* × *giganteus* production costs; however, if *M.* × *giganteus* rhizome costs in the USA drop to prices seen in Europe, it is cost-competitive with other biomass feedstocks, or cheaper.<sup>79</sup> The crop is especially sensitive during the first winter after planting and becomes increasingly resilient as stands mature. Because vegetative planting material is so expensive, great care is typically given to establishing stands, with inputs similar to those seen in annual grain crops.<sup>80</sup> Following the establishment year, inputs are very low, giving it favourable economic and greenhouse gas budgets.<sup>81</sup> Like switchgrass, no clear recommendation exists for nitrogen fertility in *M.* × *giganteus* given its inconsistent response to N fertilizer.<sup>82</sup> Generally, nutrient removal is quite low if the crop is harvested after senescence.<sup>64</sup>

## Prairie cordgrass

Prairie cordgrass is native to North America and is of interest for biomass production because of its high productivity and adaptation to marginal land that is subject to salinity and water fluctuations.<sup>83</sup> Breeding and genetic studies for this species are limited. Populations are either tetraploid ( $2n = 4x = 40$ ) or octoploid ( $2n = 8x = 80$ ) and recently a sterile hexaploid plant was identified. High variation in biomass among seven natural populations in South Dakota, USA suggested that promising strains could be selected directly from these populations.<sup>46</sup>

Very little scientific research has been conducted on prairie cordgrass management for biomass. Research has shown that the crop does not easily produce or grow from seed, and vegetative propagation, similar to *M. x giganteus*, may prove a more reliable means of establishment.<sup>84</sup> Fertility and harvest requirements are not yet clear.

Management of perennial grasses for bioenergy will require the same thoughtful and logical development as was given to annual crops during the first Green Revolution. The heterogeneous nature of landscapes will require site-specific research and more management-intensive cropping practices, i.e., the inputs of resources such as fertilizer and fossil fuels may be reduced, but the need for informed, involved land managers will increase as we seek to harvest both fuel and food from crop land.

## Woody production systems

### Feedstock development

In the 2006 US Department of Energy's roadmap publication entitled *Breaking the Biological Barriers to Cellulosic Ethanol*, several forest trees species were identified as potential feedstock sources for cellulosic ethanol production. These included hybrid poplars, willows, silver maple, black locust, sycamore, sweetgum, and eucalyptus. In the Northern Hemisphere, poplar has been given the most attention and is the target of large breeding programs and plantations for solid wood and pulp and paper production. Hybrid poplars can be grown in many regions of the USA and Canada but to date the amount of land in industrial plantations is still quite limited. This could change if genetic improvement and/or conversion technologies develop sufficiently to produce a sustainable and economic feedstock. Three general approaches to feedstock improvement are being pursued: (i) Traditional hybrid breeding, (ii) Genomics-assisted breeding and (iii) Transgenic modification.<sup>85</sup> Traditional hybrid breeding involves making inter-specific crosses and selecting superior  $F_1$  individuals.

Some very successful hybrids include *Populus x canadensis* (*deltoides x nigra*), *P. x generosa* (*trichocarpa x deltoides*), *P. x tomentosa* (*alba x tremula*) and *P. x wettsteinii* (*tremula x tremuloides*). These hybrids are now being further improved through genomics-assisted breeding<sup>85</sup> and transgenic modification.<sup>86</sup> Genomics-assisted breeding involves making selections based on the genotype of an individual rather than its phenotype. In practice, a combined genotypic and phenotypic selection strategy is often employed. Discovery of genetic markers by phenotypic trait relationships is the first step and two general approaches have been used: (i) QTL mapping<sup>87</sup> and (ii) association mapping.<sup>88</sup> The earlier QTL approach lacked high-resolution mapping of markers to genes affecting phenotypes and did not lead to application in breeding programs. The association mapping approach has delivered high-resolution marker x trait relationships and is currently being implemented into breeding programs, including programs seeking to develop poplar hybrids as biofuel feedstocks. The final genetic improvement approach is to produce genetically modified varieties through transgenic technology. Genetic transformation technology was first developed in a few poplar varieties back in the 1980s using the *Agrobacterium* system and has been used to develop transgenic varieties of many kinds, including those with genes modified to produce altered wood chemistry properties.<sup>89</sup> The transgenic approach holds great potential but is currently limited in application in the USA and Canada due to regulatory restrictions on deployment of genetically modified (GM) trees. Furthermore, many growers desire FSC (Forest Stewardship Council) certification and would not deploy GM trees even if they were available.

### Production systems

Woody perennial production systems are expected to have positive effects on soil properties, biodiversity, energy balance, GHG mitigation, and carbon footprint compared to arable crops.<sup>90</sup> Fast-growing willows can be harvested in three to ten year cycles,<sup>91</sup> and hybrid poplars are cut from twelve to twenty-five years after planting,<sup>92</sup> although shorter rotation coppicing systems also exist. Both crops have excellent potential for simultaneous heat and power generation through burning of wood pellets/biomass, but are not yet good candidates for bioethanol production due to the challenge of efficiently converting woody feedstocks into liquid biofuel.

Short rotation plantations of woody feedstocks are often established on unimproved or abandoned farmland, due to the relative ease of clearing and cultivating land that was previously devoted to agriculture. The plantation

may be the only crop in the field, or it may be grown with an intercrop. Tree-based intercropping systems feature widely spaced tree rows (10 to 15 m apart) with annual crops growing between established tree rows. Such systems diversify the rural landscape and provide economic returns to producers whilst trees are becoming established. They are also expected to store more carbon than conventional cropping systems through two mechanisms: (i) by increasing carbon storage in the biomass of planted trees,<sup>93</sup> and (ii) by adding inputs of lignin-rich litter that is slowly decomposed and thus stabilized as soil organic carbon, which is consistent with the goals of soil carbon sequestration.<sup>94</sup> After 22 years, there was 12% more soil organic carbon in a tree-based intercropping system than an adjacent conventional agroecosystem in Guelph, Ontario (Canada); the annual crop rotation in both systems was a corn-soybean-cereal rotation.

Woody feedstocks are well adapted for cool, temperate climates. Twelve clones of fast-growing trees established on abandoned farmland in southern Quebec, Canada, accumulated biomass at rates of 66 to 72 t dry matter ha<sup>-1</sup> (hybrid poplar) and 62 to 68 t dry matter ha<sup>-1</sup> (willow) after four growing seasons.<sup>95</sup> In plantations, these woody perennials grow optimally when given sufficient space to avoid interspecific competition (around 40 000 willow ha<sup>-1</sup> and 2000 hybrid poplars ha<sup>-1</sup>), adequate NPK fertilization, as well as weed and insect control.<sup>91,92,96</sup> Clone selection is critical, as wood density, fiber content, fiber length, and other feedstock characteristics important for heat and power generation are strongly controlled by genetic traits. The estimated clonal repeatability for wood density and fiber length were much greater than for growth traits (diameter at breast height, tree height).<sup>97</sup>

The response of woody perennials to climate change must be considered, due to the fact that some trees will grow for more than a decade before biomass is harvested. Under elevated CO<sub>2</sub> concentrations, there is significant increase in biomass accumulation and lignin deposition in hybrid poplar wood.<sup>97</sup> Yet, elevated CO<sub>2</sub> and N fertilization did not affect the calorific value of wood, which was 19.3 MJ kg<sup>-1</sup>. Judicious use of N fertilizer enhances the energy production per land area because the yields of woody biomass are enhanced by 50% or more, compared to trees grown on unfertilized land.<sup>98</sup> Future climate scenarios also suggest warmer, drier growing conditions, so the development of new genotypes with high water use efficiency and watershed-scale management plans that consider the land and water requirements of woody feedstocks is recommended.<sup>90</sup>

The development of dedicated woody feedstocks for cellulosic ethanol is still in its early days, although significant

research investments are being made in both the USA and Canada. Woody feedstocks may offer important benefits for the environment and contribute significantly as an alternative feedstock for energy production. Further work is needed to develop policies that effectively manage short rotation plantations in the context of climate change and consider the hydrological implications of including woody feedstocks at the landscape scale. The whole life cycle of woody feedstock production (clone and site selection, management and production decisions, harvesting, transport, and energy transformation) needs to be within a regulatory framework where sustainability is a central driver.

## Sustainable solutions for feedstock production systems

Feedstock production systems must demonstrate positive net energy balances and be able to grow on land that is marginal for food production. In addition, they must use minimal amounts of water and where possible, increase soil organic matter levels and stabilize soils against erosion. Reducing energy consumption through use of conservation tillage rather than conventional tillage and utilizing crops, possibly as part of a rotation, that have low water demands may be useful in some regions. For example, sorghum can be used as a bioenergy crop in arid and semi-arid lands as the water required for its production is much lower than switchgrass and *Miscanthus*.<sup>99,100</sup> Rocateli<sup>101</sup> studied grain sorghum (GS), high biomass forage sorghum (FS), photoperiod-sensitive forage sorghum (PS) under conventional and conservation tillage conditions and found that PS under conservation tillage produced the highest biomass and was the recommended bioenergy crop. From the perspective of this review, the material remaining after the seeds were removed, as a food or feed material, would be of interest as a biofuel feedstock.

Other factors can also improve plant growth while reducing inputs and thereby contribute to the development of low input, sustainable lignocellulosic production systems.

## Plant-growth-promoting rhizobacteria (PGPR)

PGPR include bacteria in the soil near plant roots, on the surface of plant root systems, in spaces between root cells or inside specialized cells of root nodules.<sup>102</sup> PGPR increase plant growth through a broad range



of mechanisms such as production of phytohormones (directly stimulating aspects of plant development and growth) or metal-chelating siderophores (making plant nutrients, such as iron, more available) and disease suppression through antibiosis.<sup>103</sup> Although a number of PGPR mechanisms are now understood, there is still much to discover regarding how bacteria-plant associations affect plant growth. At a time when we are looking to plants to provide biofuels and other novel bioproducts,<sup>104</sup> while still feeding the world's growing population, understanding mechanisms that can serve to increase overall plant productivity is increasingly imperative.

### PGPR-to-plant signals compounds

Lipo-chitoooligosaccharides (LCOs), a group of N<sub>2</sub> fixing PGPR, can alter the course of growth and development in a range of plants.<sup>105–111</sup> Enhanced germination and seedling growth, along with the mitogenic nature of LCOs, suggest accelerated meristem activity. LCOs, isolated from *B. japonicum*, accelerated seed germination, seedling emergence, root growth and development in soybean and non-leguminous plants,<sup>105,106</sup> and these effects were greater when the plants were under some level of stress. LCOs stimulated root growth in *Medicago truncatula*,<sup>110</sup> accelerated flowering (a typical response to stress) and increased yield when sprayed on tomatoes.<sup>111</sup> Foliar application of LCOs also induced resistance of soybean plants to powdery mildew.<sup>112</sup> Given that LCOs induce defence responses in *Medicago* cell cultures and roots,<sup>113</sup> that LCOs show structural similarity to chitin (they have a chitin backbone), and that chitin induces defence responses in plants, it is reasonable to hypothesize that LCOs induce aspects of plant defence responses similar to chitin. These defense mechanisms can aid biofuel feedstock crops in resisting both biotic (pathogen) and abiotic (cold, drought, etc.) stresses, leading to greater yields.

Bai *et al.*<sup>114</sup> isolated a PGPR, *B. thuringiensis* NEB 17, from soybean root nodules and showed that it enhances soybean nodulation and N<sub>2</sub> fixation when co-inoculated with *B. japonicum*.<sup>115</sup> The liquid medium that was used to grow NEB17 for plant growth stimulating materials was shown to contain a 31 KDa peptide, now named Thuricin-17<sup>116,117</sup> which, when sprayed on leaves or applied to roots, stimulates growth of corn and soybean, in a manner similar to that caused by LCOs.<sup>118,119</sup> Thuricin-17 is not toxic to *B. japonicum* 532C.<sup>117</sup> Bacteriocins are bacteria-produced peptides that are either bactericidal or bacteriostatic to specific bacterial strains that compete most closely with the producer strains. Bacteriocins are often isolated from bacteria found in food, such as strains

of *Bacillus*.<sup>120</sup> However, some bacteriocins have been isolated from extracellular PGPR (ePGPR), such as *Bacillus thuringiensis* subsp. *kurstaki*,<sup>121</sup> *Pseudomonas* spp.<sup>122</sup> and the nodulating intracellular PGPR (iPGPR), such as *Rhizobium leguminosarum* 248.<sup>123</sup> It has been postulated that bacteriocins produced by PGPR provide a competitive advantage to the producer strains<sup>124</sup> and may enhance nodule occupancy when the producer strain is one of the rhizobia.<sup>123</sup> Clearly, there are some previously unknown mechanisms at play and these could be exploited in the development of low-input biofuel feedstock production systems.

### Nitrogen fixers

Micro-organisms capable of biological nitrogen fixation (BNF) are largely beneficial soil bacteria and include rhizobia and free-living diazotrophs. These N<sub>2</sub>-fixing bacteria are collectively considered to be PGPR and are often found near, on, or within plant roots.<sup>102,125,126</sup> The success of bioethanol production from sugarcane in Brazil, has been attributed to lower inputs of N fertilizer since up to 80% of the plant N is derived from biological N<sub>2</sub>-fixation by associated PGPR.<sup>127,128</sup> The diazotrophs isolated from sugarcane include *Azospirillum* and *Acetobacter* or *Gluconacetobacter* species, as well as endophytic diazotrophs of the genera *Herbaspirillum* and *Burkholderia*.<sup>129,130</sup> Members of the diazotrophic genus *Azospirillum* are important sources of N<sub>2</sub> fixation and N transfer to many plants.<sup>130</sup> *G. diazotrophicus*, the predominant diazotroph of sugarcane, has also been shown to colonize rice, wheat, maize, and *Arabidopsis thaliana*.<sup>131</sup> Inoculation of *Herbaspirillum seropedicae* onto rice seedlings increased N content by 30%,<sup>132</sup> while inoculation of *Azospirillum lipoferum* and *A. brasilense*, isolated from kallar grass, onto rice provided nearly 70% of fixed nitrogen.<sup>133</sup> Potential utilization of BNF in the growth of cellulosic feedstock crops would significantly reduce N fertilizer and thus energy requirements associated with their production.

### Mycorrhizae

Mycorrhizal fungi constitute a very ancient symbiosis between higher plants and fungi.<sup>134</sup> The relationship is so well developed that the fungi often cannot grow in the absence of the host plant.<sup>135</sup> The fungi improve the ability of plants to take up soil P and Zn by effectively increasing root surface area and their ability to take up low mobility nutrients such as P.<sup>136</sup> These fungi are present in almost all soils of the world but selection for enhanced types and effective inoculation strategies can improve crop yields.<sup>137</sup> Phosphorus

conservation is particularly important as peak extraction of this nutrient is forecast for as little as a few decades from now, which could place a strong limitation on biomass and food crop production systems. Thus, biofuel production systems and very effective mycorrhizal systems, able to make the most of this P, will be critical in the near future.

## Biochar

Biochar is black, carbon-rich material produced when organic matter is thermally cracked in an oxygen-limited or oxygen-free environment (pyrolysis). The particles of char produced this way contain primarily carbon and inorganic matter (ash), are highly porous<sup>138</sup> and retain nutrients and water that might otherwise be lost from the root zone.<sup>139,140</sup> Biochar is valuable when used as a fuel, carbon sink, or soil amendment<sup>141,142</sup> and a very promising technology for using it to make organic slow release nitrogenous fertilizer has been developed and patented.<sup>143</sup>

Biochar has increased soil pH and nutrient availability leading to crop yield improvements that persist for several years after a single application.<sup>144–149</sup> Nutrients contained in and applied with biochar materials can be responsible for short-term increases in crop growth.<sup>145</sup> The long-term improvement in soil fertility arises from the fact that the biomass thermal cracking process (pyrolysis) generates stable compounds consisting of single and condensed ring aromatic carbon with a high surface area per unit mass.<sup>150</sup> This surface becomes oxidized and cation exchange capacity (CEC) develops over time and can lead to greater nutrient retention in 'aged' as opposed to 'fresh' biochar.<sup>154–157</sup> The resulting high CEC presumably captures positively charged plant nutrients such as  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  which are retained on the biochar surface and not lost through volatilization ( $\text{NH}_4^+ \rightarrow \text{NH}_3$ ) or leaching ( $\text{K}^+$ ,  $\text{Ca}^{2+}$  and possibly  $\text{Mg}^{2+}$ ).<sup>155</sup> The binding of  $\text{NH}_4^+$  to the biochar surface is of particular interest because this can slow the rate of nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ) and hence the loss of  $\text{N}_2\text{O}$  and  $\text{N}_2$  via denitrification. Biochar also binds  $\text{PO}_4^{3-}$  by surface adsorption,<sup>156</sup> thus providing a mechanism for better management of this key plant nutrient. Thus, biochar-amended soils may require less fertilizer to achieve target crop yields, leading to, for instance, less contamination of surface and ground water by  $\text{PO}_4^{3-}$  and  $\text{NO}_3^-$ <sup>157,158</sup> and less production of the greenhouse gas  $\text{N}_2\text{O}$ .<sup>159,160</sup>

Good quality biochar is very porous, contains less inorganic matter and can hold several times its weight in water.<sup>139,140</sup> Thus a field with  $10 \text{ t ha}^{-1}$  of added biochar might retain an additional  $30 \text{ t ha}^{-1}$  of water following a rainfall or irrigation event. This could be extremely important to crops growing in water-limited areas and

could also enhance the retention of N, S and P in soil and reduce fertilizer requirements.

The mean residence time (MRT) of microbially processed soil organic carbon is as short as 30 years.<sup>161</sup> In contrast, biochar creates a carbon pool with high stability.<sup>162</sup> MRTs of 6850 and 4035 years have been reported for biochar in the Amazonia Dark Earth region<sup>163,164</sup> and 364 years for purposefully applied biochar in the field, normalized for a mean annual temperature of  $10^\circ\text{C}$ .<sup>149</sup> Thus, biochar is very effective in the long-term sequestration of carbon into soils.

## Municipal solid waste as a source of bioethanol production

Municipal solid waste (MSW) consists of combustible and non-combustible wastes that come from household, municipal, commercial and industrial sites.<sup>165</sup> In the UK, 500 kg of waste per capita are produced each year amounting to 30 million tonnes of MSW annually.<sup>166</sup> These produce large amounts of GHGs at dumping sites and represent wasted energy.<sup>165</sup> Many countries including the US have increased their efforts to use MSW via recycling, thermo-chemical and biological conversions.

## Logistic challenges in using lignocellulosic feedstocks

The biomass-to-energy industry has been developing over the past 30 years<sup>167</sup> and during this period many challenges had to be overcome.<sup>168</sup> As the industry grows and matures, many other challenges arise, including logistics. Logistics is defined as 'the art and science of obtaining, producing, and distributing material and product in the proper place and in proper quantities'<sup>169</sup> and plays a vital role in achieving operational excellence in all the types of industries, including bioenergy. To fully understand the importance of logistics in biomass, first it is required to understand the different processes that are involved (Fig. 1).

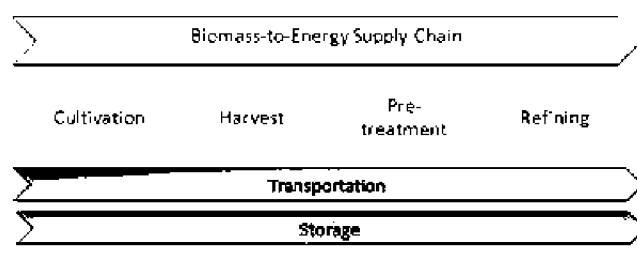


Figure 1. Biomass-to-energy supply chain

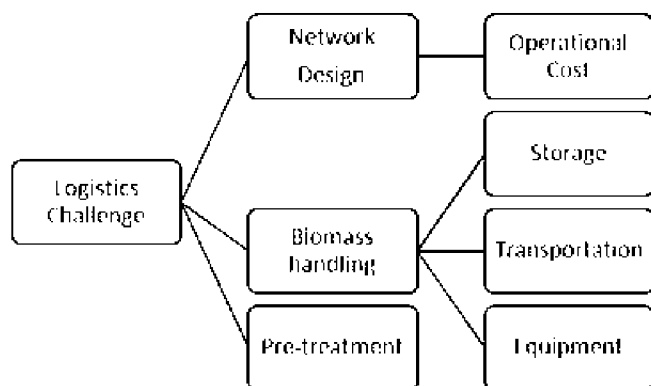


Figure 2. Logistic challenges

The biomass supply chain begins at cultivation and includes land use and crop selection which can significantly impact the expected yield and overall energy efficiency.<sup>170</sup> Additionally, site selection will impact the operational cost since the proximity of the feedstock to processing plants has a direct impact on transportation cost. The second step of the biomass-to-energy supply chain is harvest; it must consider the direct relationships between cultivation, storage, transportation and even conversion which are all significant drivers in logistics cost. Harvesting will be discussed in the next section. Following harvest is pre-treatment where the first steps of biomass conversion occurs and where the feedstock is transformed for further downstream processing. During the pre-treatment process, typically only including physical processes, the properties of the biomass are modified using drying, densification and fractionation. From a logistic point of view, pre-treatment plays a key role in the supply chain; it helps to improve the storage and transportation process by increasing bulk density as well as improves the efficiency at the refining stage.

As many studies have described,<sup>170–176</sup> logistics is a major area of focus for development of the biomass-to-energy value chain and is critical when considering the wide spatial distribution of potential biomass, variable moisture content, low bulk density, and short harvesting window. The biggest challenges that need to be addressed in the short term include pre-treatment processing,<sup>170–178</sup> biomass handling (transportation and storage),<sup>170–172</sup> and network design<sup>173–175</sup> (Fig. 2).

## Pre-treatment

### Densification

Densification is the process that uses compression or compaction on the biomass to remove inter- and intra-particle

voids.<sup>176</sup> From the perspective of logistics, densification overcomes the low bulk density of the feedstock, which is a major barrier for developing biomass as an energy source.<sup>177</sup> Increasing the density of the feedstock directly impacts storage and transportation that are mainly based on the volume of biomass to be handled.<sup>170</sup> Densification allows for feedstock uniformity,<sup>177</sup> improving the handling efficiency, and process throughput (i.e. optimizing upload time, storage handling efficiency, etc.).

## Biomass handling

Biomass handling is divided into two elements transportation and storage.

### Transportation

Transportation accounts for as much as 35–60% of the biomass logistics cost.<sup>175</sup> Due to this major cost, optimizing transportation requirements and even small improvements in competitiveness can result in significant reductions in overall operation costs. The type of feedstock and method of pre-treatment will define the biomass bulk density and the maximum handling capacity using different transportation modules (truck, rail, ship, etc.)<sup>178</sup> and the steps required within the system, before reaching its destination. For example, truck loading and unloading operation cycle time can play an important role in transportation efficiency.<sup>171</sup> Sokhansanj and Hess<sup>171</sup> described that the loading of a 36-bale truck may take 30–40 min. By increasing the bulk density of the biomass, the handling efficiencies can be improved but the cost to reformat the biomass must be included in the final consideration. Other factors such as trip distance, truck-carrying capacity and fossil fuel consumption can have a negative impact on local transport costs and logistics operations.<sup>170</sup> Ultimately, transport costs increase when the biomass feedstock is dispersed over large areas requiring significant road transport, but pre-treatment can be used to reduce these costs.

### Storage

Biomass storage and quality is directly impacted by its moisture content which can in turn directly impact the energy efficiency. For long-term storage of most bioenergy feedstock, the moisture should be below 17.5% on a dry weight basis. However, safe moisture content will depend on the selected feedstock.<sup>179</sup> Biomass with a moisture content between 40 and 60% is difficult to manage during storage; wet crops (<60% moisture) are more susceptible to microbial degradation and losses due to liquid effluent

production during storage.<sup>179,180</sup> Losses during wet storage are higher than dry storage and drying biomass below 20% moisture is required to avoid large losses during storage. Storage infrastructure can be a stronger determinant of biomass loss than moisture content for hay collected below 40% moisture<sup>180</sup> Additionally, proper slotting efficiency (optimizing biomass storage according to its shape) is important and circular biomass bales are not the best option during storage; square bales have a better storage performance.<sup>171</sup>

## Network design

With many feedstock harvest locations the logistic operations tend to be a complex system that require a well-designed network and a robust transportation system to supply the bio-refinery. Based on biomass seasonality and network design, the feedstock may need to be stored in satellite locations for a period of time before its transformation into an energy source. The transportation cost will increase as a consequence of increasing distance and increased steps required before reaching the refining step. A poor network design will have a direct impact on the bio-refinery operational cost and consequently its financial performance.<sup>172</sup>

Most optimization models use the integrated biomass supply and logistic (IBSAL) software as the primary optimization tool.<sup>174,175</sup> IBSAL is a time-dependent simulation developed by the US Department of Energy as a tool for analysing and optimizing complex biomass supply systems.<sup>8</sup> IBSAL is a powerful tool for evaluating the supply chain from field to bio-refinery.<sup>174</sup>

Another technological tool that has been used for optimizing the biomass supply chain are geographical information systems (GIS), which enable the mapping of actual road networks.<sup>172</sup> Figure 3 is an example of how GIS can be used to define an optimal location for a large-scale

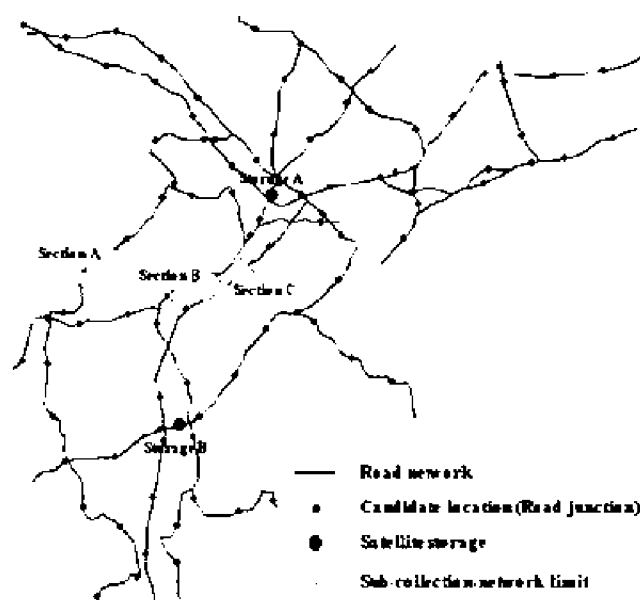


Figure 3. Road network definition by GIS model

bio-refinery and potential satellite storage locations based on biomass availability and network optimization.<sup>199</sup>

## Energy

Many studies have analyzed the energy consumption throughout the supply chain. Table 1 summarizes the different results from previous works mostly in wood feedstock. Dijkman and Benders<sup>181</sup> calculated the energy consumption of different processes in order to generate electricity from wood (willow and poplar; energy inputs for electricity from wood were given over a period of 20 years). The pre-treatment (drying and chipping) process is the most energy consuming in the supply chain because it is assumed that the initial moisture content is 50% and includes the process to reduce this moisture content to an optimal level of 15%. Transportation and fertilization are

**Table 1. Energy consumption throughout the supply chain. Dijkman and Benders<sup>181</sup> (a) Benoit *et al.*<sup>182</sup> calculated the energy consumed to generate 1 GJ of heat. (b) Valente *et al.*<sup>196</sup> Energy balance of a woody biomass supply chain (transportation assumed at 30 km). (c) Valente *et al.*<sup>197</sup> Primary energy input for 18 251 m<sup>3</sup> of woody biomass harvested, the transportation was assumed for 64 km**

Reference	Year	Feedstock	Unit	Energy Consumption					
				Cultivation	Harvest	Pre-treatment	Refining	Transport	Total
Dijkman & Benders	2010	Wood	MJ/ha	3,674	3,543	11,400	–		18,617
Benoit <i>et al.</i> (a)	2013	Eucalyptus	MJ/GJ	33.81	15.93		0.29	42.67	50
Benoit <i>et al.</i> (a)	2013	Eucalyptus	MJ/GJ	10.49	23.52		0.29	42.67	34
Valente <i>et al.</i> (b)	2011	Wood	K Wh/m <sup>3</sup>	10.46	4.22	17.8		11.92	32
Valente <i>et al.</i> (c)	2011	wood	MJ/m <sup>3</sup>	3.68	40.81	59.73		74.46	104

the two main drivers in the energy consumption for wood chips combustion; the model assumed transportation of 80 km for the wood chips in the calculation.<sup>182</sup>

## Biomass harvesting for lignocellulosic energy crops

Cheap and efficient harvesting methods, a critical part of the supply chain for using biomass as an energy source, depend on the type of feedstock to be used as a fuel source (wood, grasses, residues, or other materials). The first feedstocks used for biofuels to penetrate the market, such as crop grains and wood pellets, relied on harvesting technologies taken directly or modified from existing agricultural and forestry harvesting methods. Agricultural technologies included a mower used to cut the forage, a baler to densify and produce a transportable package and trucking for transportation. Additional equipment was required depending on the need to reduce the moisture content of the final product, including conditioners for crimping the material, a hay rake for windrowing or turning the grass, and a tedder to spread the grass. Traditional wood harvesting methods include a feller-buncher to cut and gather several trees at once, a skidder/forwarder to move logs from the forest to loading area, a loader/picker and truck/transport.<sup>183</sup> Additional equipment and or personnel may be required for delimbing and bucking the tree (cutting the log to size).

### Traditional harvesting technologies

#### Forestry

The challenge with using traditional harvesting methods is that the labor, energy consumption, and equipment costs do not always make sense from an economic or energy balance perspective when the end product is biofuels. Alternative harvesting methods and equipment are slowly being developed and are focused on four major feedstocks for future biofuel development: purpose-grown grasses (switchgrass, *Miscanthus*, etc.), short rotation plantation species (willow and hybrid poplar), crop residues (corn-sorghum stover/cereal straw), and forestry residues.<sup>184–186</sup> Three systems described below are new technologies that have reduced the cost and energy requirements of harvesting forest residues, willow plantations and corn stover.

### New harvesting technologies

#### Biobaler

The Biobaler (Anderson Group Co., Chesterville, Quebec) was developed in 2005 as a willow harvesting system based

on an agricultural baler. The original system was designed to harvest plantation willows using a reinforced large round baler.<sup>184</sup> A cutter head was attached to the front of the round baler, allowing simultaneous cutting and conditioning of the woody material before ejecting it into the baler chamber for compression and wrapping. Subsequent variations of the original cutter included horizontal saw blades, flail hammers, flail knives, and a flail shredder. The various cutters were designed to operate on rough terrain and allow harvesting in natural brush conditions, with the cutter selected depending on the wood material to be harvested. The Biobaler unit has a full width cutting size of 2.6 m, and a length of up to 5.5 m requiring at least a 180 HP tractor to operate. The harvested bales weigh 500 to 600 kg, are 1.4 m in diameter (1.2 m wide) and have a density of 220 kg/m<sup>3</sup>, with 50% moisture content. The Biobaler offers a versatile alternative to harvest wild brush and planted woody crops. The technology is helpful for land management and provides a method to harvest otherwise neglected biomass. The biobaler, has an ability to harvest woody material up to 10 cm in diameter, 7 m in height and produces between 8 and 20 tonnes per hour (15 to 40 bales). It has operated in a range of harvesting locations including plantations, abandoned and fallow land, field edges, along roads, near watercourses, and understory harvesting. Management of the trees can be controlled through the selection of the cutters for the front end of the biobaler, with some offering a clean cut (saw blades) and others producing rough edges (flail cutting). Rough edges on stumps can allow water to stagnate and slowly penetrate the root system, resulting in detrimental stump and root health. Such slow regrowth of the woody crop after harvest is an advantage in some undergrowth management systems (abandoned or fallow land, river bank brush and understory vegetation in forests).

#### Willow harvester

Many willow harvesting systems are based on modifications to existing forage or sugar cane harvesters.<sup>186</sup> In Europe, the most effective machines are the modified Claas Jaguar corn harvester (Harsewinkel, Germany) and the Bender Harvester (Uppsala, Sweden). In North America, a Case-New Holland forage harvester (FX-45; Burr Ridge, IL, USA) has been modified for willow harvesting. The Claas harvester is a front head implement with saw blades, two blades per row that cuts stems 5 to 10 cm from the ground. The Bender willow harvester uses a single long, chain-saw cutting chain to cut the willow stems and cuts and chips the willow biomass in one pass using a 140HP tractor and harvester. The modified North

American system uses a modified row-independent CNH forage harvester to successfully harvest and chip willow biomass.<sup>186</sup> The unit has been tested on both plantation willow and hybrid poplar. Harvesting rates have been reported of up to 2 ha h<sup>-1</sup> with stems up to 13 cm diameter.

These modified forage harvesters cut the plants into chips, which are then blown into a wagon towed by the harvester or into a wagon/truck that drives alongside the harvester. A stem bundling system has been developed in Europe but has limited application for bioenergy production due to increased costs of transport and handling. However, bundled stems have improved results when used for transplanting of willow or when the biomass requires longer storage times.<sup>186</sup> Overall the forage harvesting equipment has been improving with harvest rates from 22 to 45 tons per hour (0.5 to 2 ha h<sup>-1</sup>).

### Stover/combine harvester

Corn/sorghum and cereal grain stover harvesting can occur in at least three forms: a single pass with simultaneous grain and stover harvesting, a two-pass grain harvesting and baling/forage harvesting of the stover windrow, and a three-pass system with grain harvesting, mowing/raking and baling/forage harvesting.<sup>185,187–193</sup> All three options have been used with varying levels of acceptance, and all use the combine for grain harvest. The single pass system consists of a modification to the combine with a corn stem cutting or ear-snapping header to provide a second stream of stover materials in addition to the grain.<sup>194</sup> This material is processed by a forage harvester type system that blows the material into a wagon for transport off the field. A modification to the single pass system is the two-pass system where the combine harvests the grain and provides a windrow of the stover for baling or forage harvesting in a second pass. The two-pass system is typically used for cereal grain with the baling of wheat and barley straw but is also used for corn stover.<sup>185</sup> The three-pass system uses the standard combine for corn seed harvest, then a mower cuts the stalks and windrows the material for baling or forage harvesting in the third step. Increased energy advantages, of up to 55%, have been reported with the single pass system.<sup>195</sup> Biomass removal efficiency has ranged from 35 to 93% depending on the type of corn head and head height used.<sup>194</sup> However, increased costs of the machinery and increased moisture content can limit its use. Placement of the stover into a windrow allows reduction in moisture content of the stover before baling, but can increase soil contamination of the stover.

The incorporation of agricultural and forestry harvesting methods into new biomass harvesting systems has

improved harvest efficiency, which can greatly reduce the time, cost, energy and manpower required for harvest. Reducing the machinery requirements and number of passes within the field/forest result in reduced labour costs and lower harvesting costs, allowing a lower cost biomass feedstock for downstream processes.

## Summary

The development of an energy source that is sustainable over the long term and will reduce our dependence on fossil fuels is beneficial both to the environment and the economy. Lignocellulosic feedstocks grown on land that is marginal for agriculture, using carefully selected species and production systems, have the potential to provide biofuels that are energy efficient, cost effective and environmentally sound. Ongoing research and development on suitable biomass feedstock is multifaceted and at scales that range from the cellular to the plantation. Genetic engineering is improving access to the carbohydrates stored in plant cell walls and reducing the energy costs currently associated with breaking down lignin. Perennial feedstocks that have a high productivity, strong persistence and wide adaptation to a variety of climatic and soil conditions are being selected and developed. Proper utilization of municipal solid waste can lead to economical biofuel production. Production systems that produce a maximized amount of fuel per unit of biomass, while maintaining crop sustainability with minimal inputs, are being developed. Enhanced nitrogen fixation and phosphorus uptake by plants and increased soil water and nutrient holding capacities are being developed and have the potential to improve soil fertility and allow lignocellulosic production systems to produce biofuels sustainably over the long term. Logistics play a vital role in achieving operational excellence in bioenergy and is a major area of focus for development of the biomass-to-energy value chain. Logistics are critical when considering the wide spatial distribution of potential biomass, variable moisture content, low bulk density, and short harvesting window. The biggest challenges that need to be addressed in the short term include pre-treatment processing, harvesting, transportation and network design. By way of recommendations we feel that:

1. We should develop plant genotypes with lignocellulosic materials that are effectively and efficiently converted into liquid fuels.
2. There is also a need to identify and genetically improve biofuel feedstock crops that are best adapted to specific geographical areas.

3. There is a need to develop sustainable/low-input production methods that serve to enhance the yields of biofuel feedstock crops while also enhancing the energy balance and carbon life-cycle analysis of these crops.
4. Effective, low energy and safe harvest and storage practices need to be developed for biofuel feedstock crops.

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### Bruce Coulman

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Dr Ajay K. Dalai is a Tier 1 Canada Research Chair in Bioenergy and Environmentally Friendly Chemical Processing at the University of Saskatchewan. His research focus is the novel catalyst development for gas to liquid technologies, and biodiesel productions and applications. During his years as a

researcher, Dr Dalai has published 250 research papers. He is a fellow of CIC, CAE and EIC.



### Emily Heaton

Dr Emily Heaton is an Assistant Professor of Agronomy at Iowa State University. Her aim is to understand the growth and productivity of dedicated biomass crops, and how they can be managed to provide multiple ecosystem services. She specifically seeks to elucidate the reciprocal impact of environment on key physiological processes like

photosynthesis, biomass accumulation, water use and nutrient cycling.



### Donald L. Smith

Donald L. Smith – James McGill Professor at McGill University – conducts research on crop-microbe interactions, including biofuel feedstock crops. He has trained 60 graduate students, generated 8 patents, started a spin-off company, and commercialized technologies applied to millions of ha each

year. He leads BioFuelNet Canada (\$12 million per year).



### Mark Lefsrud

Mark Lefsrud is an assistant professor of Bioresource Engineering at McGill University. He is currently conducting research on growing plants and other micro-organisms in controlled environments (greenhouses and growth chambers), and harvesting and handling biomass for biofuel.



### David B. Levin

David B. Levin leads a multidisciplinary research group focused on bioengineering for biofuels and bioproducts at the University of Manitoba. He co-leads a Genome-Canada-funded project on microbial genomics for biofuels and co-products from biorefining processes, and is Prairie Platform leader within BioFuelNet, a pan-Canadian research network funded by the Network Centres of Excellence program.



### Camilo Perez Lee

Camilo Perez Lee is a Master's student in the field of Bioresource Engineering at McGill University. He is conducting research on harvest and post-harvest densification under the supervision of Dr Mark Lefsrud. Camilo's research focuses on the bioenergy supply chain planning and logistics from harvest-

ing to biofuel. He holds a BSc in the field of Industrial Engineering.



### Peggy G. Lemaux

Peggy G. Lemaux is a faculty member at the University of California, Berkeley, where efforts in the Lemaux laboratory have focused on engineering cereal crops, like sorghum, barley and wheat, to improve agronomic performance, nutritional profile, and suitability for biofuel use. Most recently, experi-

mentation has turned to engineering tobacco to produce drop-in hydrocarbon biofuels in leaves.



### Jaswinder Singh

Dr Jaswinder Singh researches enhancement of quality traits, stress tolerance and bioenergy capability of crops using genomic and biotechnological tools at McGill University. His current projects involve the assessment of diversity in cell walls and cell-wall synthesis genes in grasses;

and the exploration of cultivated and wild germplasm for novel genes suitable for the next generation of cereal crops.



### David B. Neale

Dr David B. Neale is an adjunct geneticist and professor at the Department Plant Science at UC Davis. His research interests concern the genomics of forest trees, complex traits, QTL and association studies, population genetics and adaptation, and marker-based breeding. The focus has been

on traits of practical value such as wood quality, growth, and disease resistance but will expand to genes determining adaptation and response to environmental stresses.



### Joann Whalen

Dr Joann Whalen is a soil ecologist at McGill University who investigates nutrient cycling in the soil-plant system. She is interested in biomass production systems that increase soil carbon sequestration and improve nutrient use efficiency, and evaluates system performance with life cycle assessment.



### Sharon P. Shoemaker

Dr Sharon P. Shoemaker is Executive Director of the California Institute of Food and Agricultural Research at UC Davis. She is the author of several patents on yeasts to convert biomass to ethanol and bacterial strains to produce new forms of cellulose. She is researching the application of cel-

lulases in biomass conversion, the integration of various unit operations in biomass conversion processes, and the development of new analytical methods for quantifying cellulose activity.