Improving Biomass Quality in Miscanthus

Subjects: Others

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Lignocellulosic crops are attractive bioresources for energy and chemicals production within a sustainable, carbon circular society. Miscanthus is one of the perennial grasses that exhibits great potential as a dedicated feedstock for conversion to biobased products in integrated biorefineries. The current biorefinery strategies are primarily focused on polysaccharide valorization and require severe pretreatments to overcome the lignin barrier. The need for such pretreatments represents an economic burden and impacts the overall sustainability of the biorefinery. Hence, increasing its efficiency has been a topic of great interest. Inversely, though pretreatment will remain an essential step, there is room to reduce its severity by optimizing the biomass composition rendering it more exploitable. Extensive studies have examined the miscanthus cell wall structures in great detail, and pinpointed those components that affect biomass digestibility under various pretreatments. Although lignin content has been identified as the most important factor limiting cell wall deconstruction, the effect of polysaccharides and interaction between the different constituents play an important role as well. The natural variation that is available within different miscanthus species and increased understanding of biosynthetic cell wall pathways have specified the potential to create novel accessions with improved digestibility through breeding or genetic modification.

Keywords: miscanthus ; lignocellulosic biomass ; saccharification ; cell wall ; pretreatment ; breeding ; cellulose ; hemicellulose ; lignin ; biomass quality

1. Introduction

Increased carbon dioxide levels are the foremost cause of anthropogenic climate change leading to global warming [1]. The goal of keeping the global average temperature well below a 2 °C increase from the pre-industrial levels has been set in the Paris Agreement, in order to halt global warming. To reach this goal, it is important to develop methods or set measures to reduce CO_2 levels [2][3]. The majority of CO_2 emissions are associated with the production of energy and synthetic polymers from fossil resources as oil, coal and natural gas [4][5]. Therefore, there is an increasing demand for less polluting alternatives for the production of energy and chemicals. While there are several renewable sources for energy production, such as solar and wind power, only biomass can serve both purposes. Although skepticism exists about the capacity of biomass usage regarding mitigation of CO_2 emissions [6], thorough assessments have shown that biomass has the potential to offset greenhouse gas emissions when properly used, and could be an integral part of a wider strategy in order to meet global climate goals [7][8]. Transition towards a biobased economy requires change across the whole production chain, with particular importance for advancements regarding efficient biomass production, conversion into different products and utilization [9][10].

Biomass itself is an attractive renewable energy source due to its potential to be carbon neutral and its global abundance. Carbon neutrality depends on the basic principle that the amount of CO_2 released upon combustion and or conversion is equal to the amount fixed by the crop during its lifetime [11]. As such, replacement of traditional fossil energy sources that heavily contribute to elevated levels of CO_2 with biomass energy can alleviate the associated effect on global warming [12]. Additionally, biomass provides an essentially unlimited source of natural and renewable building blocks for utilization as alternatives to oil-derived chemicals and materials [13].

Lignocellulosic biomass, retrieved from agricultural and forest side-streams or dedicated feedstocks is expected to become an essential resource for the production of energy, chemicals and materials in the near future. Dedicated biomass crops are needed next to agricultural and forest streams, because the contribution of the latter alone would be insufficient for meeting the energy demands [14][15][16][17]. Perennial C4 grasses have been considered as especially promising feedstocks due to their more efficient photosynthetic capacity relative to C3 plants. This is in most cases associated with higher biomass yield potential and increased nitrogen and water use efficiencies [18]. Moreover, their perennial nature also contributes to higher nutrient use efficiency in comparison to annual crops [19]. These features enable perennial grasses, like switchgrass and miscanthus, to achieve substantial yields even when cultivated on marginal and degraded lands [20][21][22]. Limiting cultivation to marginal lands avoids competition for arable land with food crops and will therefore not present a threat to food prices and security or induce land use change; both were points of concern and criticism accompanying the use of edible parts of food crops for the production of first generation biofuels [21][23][24]. Additionally, due to the perennial grasses' capacity to sequestrate CO_2 [25][26] and thereby tilt the carbon balance more favorably, it can be assumed that the detrimental effects of large scale use of forestry biomass on net CO_2 emissions [27][28] would not apply to these crops. Therefore, cultivation of perennial grasses can be seen as a sustainable alternative without any obvious negative societal impacts. From the available candidate biomass crops, miscanthus is seen as one of the most promising as it is able to utilize external resources even more efficiently than other C4 grasses [19][29].

2. Miscanthus for Industrial Use: Advantages, Challenges and Applications

Miscanthus is a genus of rhizomatous perennial grasses originating from Eastern Asia, which comprises around 12 different species [30][31]. The species have been adapted to a broad range of different climate conditions and hold substantial amounts of genetic diversity for key traits [32][33]. Interest in miscanthus has been, for a large part, due to excellent biomass yields that are provided on a yearly basis and could be achieved without the need of additional irrigation in Northern Europe [34]. Such yield potential is achieved due to its ability to maintain photosynthetic capacity at moderate temperatures [35][36]. Furthermore, low input requirements due to its high levels of water [29][37][38] and nutrient use efficiency [39][40] are also highly favorable characteristics of miscanthus species.

Initially, most research has focused on *M. x giganteus*, an interspecific sterile hybrid between *M. sinensis* and *M. sacchariflorus*. Cultivation of *M. x giganteus* is possible in areas where temperatures remain sufficiently high during winter, and high yields (18.7–36.8 t/ha) have been achieved [41]. Moreover, substantial yields (13–21 t/ha) were reported when cultivated on marginal soils [42][43][44]. The potential for phytoremediation of heavy metal-contaminated soils [45][46] and its ability to act as a carbon sink during its cultivational lifespan [25][47][48] clearly add to why *M. x giganteus* is considered as one of the most promising biomass crops. However, costly rhizome propagation [49], vulnerability to potential pests and diseases due to the absence of genetic variability [50][51][52][53] and lack of cold tolerance leading to severe losses in the first winter after establishment [41][54][55][56] are notable drawbacks of this specific accession.

Despite being a highly promising energy crop, a major drawback surrounding *M. x giganteus* biomass is the resistance of its cell wall against deconstruction, making it recalcitrant towards targeted conversion and valorization through biorefinery. The recalcitrance of the cell wall is directly related to the composition, structure and architecture of the molecules it contains. *M. sinensis* and *M. sacchariflorus* genotypes with lower recalcitrance, performing up to 50% better, have been identified [57].

A large number of applications have been described for miscanthus biomass, with the suitability for a given application ultimately being determined by the cell wall composition. Especially the composition of the secondary cell wall, consisting of cellulose, hemicellulose and lignin, is of importance, as it accounts for >90% of the dry matter of the plant biomass.

Some applications aim to use the whole biomass fraction, such as energy generation through combustion or fast pyrolysis [58][59] or the production of biomaterials such as composite polymers, concrete or fiber boards [60]. Other applications only target a specific fraction of the cell wall for conversion into high value products. Polysaccharide-driven biorefineries are the most well-known example in this context, striving to hydrolyze cell wall polysaccharides into constituent monosaccharides to be fermented to ethanol or methane [61][62] or converted to platform chemicals such as furfural or 5-hydroxymethylfurfural [63]. Alternatively, cellulose could also be used for manufacturing nanocrystals [64]. Although the (hemi)cellulosic parts are still mainly targeted in most lignocellulosic refinery processes, and lignin is therefore generally considered an inconvenient barrier against the conversion of the biomass polysaccharides, lignin valorization is expected to become increasingly important, with it being the most abundant natural resource of aromatic building blocks [65][66].

Ideally, each biomass component could be efficiently separated and isolated for further processing [67][68]. However, this requires pretreatment of the lignocellulosic biomass, since it is recalcitrant to this fractionation and degradation. The required pretreatment stringency remains the first and foremost bottleneck for the design of a green and economically feasible production chain [69][70], requiring both high biomass digestibility and pretreatment efficiency. A useful measure to this end is enzymatic saccharification, since it allows evaluation of the amount of released monosaccharides.

Production of bioethanol and methane are among the best studied applications for lignocellulose feedstocks, as they were initially identified as the most promising value chains [71]. Their production makes use of different ways of enzymatic saccharification, either through the application of enzymatic cocktails containing endo- and exo-glucanases and β-glucosidases of fungal origin or through exposure of biomass to hydrolytic bacteria [72][73][74][75]. After the saccharification step monosaccharides generated for bioethanol production are fermented, followed by distillation of the produced ethanol [70]. Alternatively, for methane production the monosaccharides are converted into organic acids and alcohols by acidogenic bacteria, which are subsequently converted into acetate, that serves as a substrate for methanogenic bacteria to produce methane and carbon dioxide [72][73][74]. Genetic studies in the field of biomass digestibility or pretreatment optimization for miscanthus or other lignocellulose grasses often use one of these approaches as a way to assess the performance differences among diverse accessions or pretreatment conditions.

3. Improving Biomass Quality in Miscanthus and Breeding Efforts

Interest in breeding of miscanthus is relatively recent, especially when compared to other crops [30]. It is a time-consuming and laborious process as, due to the perennial nature of the crop, agronomically relevant traits, such as plant yield and biomass quality, can only be evaluated in a representative matter after a growth period of at least 2–3 years [76]. Within the genus *Miscanthus*, a large variability for the different traits contributing to cell wall quality is present, enabling selection and breeding for reduced cell wall recalcitrance based on knowledge of cell wall composition. However, cell wall quality is not easily assessed or captured during the breeding process as it is determined by many different traits that are polygenetic in nature.

Breeding starts with the availability, generation and search of genotypes with promising quality properties. In general, such genotypes do not have the best overall agronomic characteristics and need to be crossed with advanced breeding material to combine quality with other desirable characteristics, such as high-yielding potential. In practice, this implies a recurrent cyclic approach of crossings and selection to improve quality and agronomic performance and requires appropriate screening tools. The highly diverse germplasm available in *Miscanthus* are attractive sources for desirable cell-wall properties. For instance, natural *M. sinensis* populations from different geographical origins include six distinct genetic clusters and thereby the existence of potential heterotic groups that have so far remained unutilized [33]. Alternatively, deliberately created mutants obtained through targeted genetic modification or undirected mutagenesis could become an additional beneficial source of variation for cell wall genes. To discover useful quality characteristics from selected mutated plants, advanced breeding material and/or wild germplasm, they have to be clonally propagated, through plant splitting or tissue culture to establish replicated field trials for evaluation. The best performing genotypes could either be tested in multi-location trials for their potential as a clonally propagated cultivar, used as parental lines for production of hybrid seeds or serve as a source of beneficial genes for recurrent selection breeding [77][78].

The mating system of fertile species like *M. sinensis*, being gametophytic self-incompatibility (SI), influences the actual breeding in different ways. The system, most likely based on two multiallelic genes as is commonly found among grasses [79], prevents self-pollination but on the other hand it enables the use of heterosis. The breeding program at Wageningen University focusses on the latter and aims to breed for seed-based *M. sinensis* experimental hybrids through pair-wise crossings in isolation among selected genotypes. The SI system limits the full potential of hybrid breeding, but mating between either full sibs or half sibs can circumvent this limitation [80]. Emphasis of the Wageningen breeding program is on selection of candidate clones/individuals for making biparental crosses and on subsequent testing of full-sib families, in particular [81]. The ultimate goal is the creation of hybrid families suitable for commercial use. To remake the original families, the parental clones are maintained. Other breeding programs use similar approaches but instead aim mainly for interspecific seed-based hybrids [78]. Alternatively, creation of new clonally propagated “giganteus” varieties (*M. sinensis* x *M. sacchariflorus*) is also ongoing [82][83].

The use of molecular tools has been explored in miscanthus and resulted in the identification of genetic markers that could potentially speed up the breeding process dramatically. Mapping populations have successfully identified numerous QTLs contributing to important traits such as biomass yield and quality [84][85][86][87]. Additionally, genome-wide association studies in an experimental *M. sinensis* population showed the potential of genomic prediction and selection [88][89]. The use of sufficiently large populations to find SNPs corresponding to traits of interest requires phenotyping of a large number of plants. Analytical protocols for analysis of the main cell wall components and structural sugars have been commonly used for many years. Additionally, there are many more protocols available that make it possible to obtain detailed insight into the composition and structure of these components. While these methods provide information that could be critical for further advancing selection, they often require specialized equipment and are not considered as high throughput. Many of these traits have been successfully analyzed using infrared and near-infrared spectroscopy techniques, that are capable to predict the content of many cell wall structures and can be considered as high-throughput alternatives once appropriate calibration models have been created [90][91].

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