Biomass quality of miscanthus genotypes for different bioconversion routes

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List of Acronyms

BP	British petroleum
COP	Conference of the parties
Mtoe	Million tons oil equivalent
EU	European Union
€	Euro
%	Percent
IEA	International energy agency
UN	United Nations
EC	European Commission
GHG	Greenhouse gas
1G	First generation
2G	Second generation
3G	Third generation
ha	Hectare
IEEP	Institute for European environmental policy
CO_2	Carbon dioxide
K	Potassium
Cl	Chloride
Si	Silicon
N	Nitrogen
NaOH	Sodium hydroxide

H_2SO_4	Sulfuric acid
NOx	Nitrogen oxide
Kg	Kilogram
P	Phosphorus
EMI	European miscanthus improvement project
OECD	Organization for economic co-operation and development
HNO_3	Nitric acid
M.	*Miscanthus*
sin.	*sinensis*
sac.	*sacchariflorus*
H.	Hybrid
gig.	*giganteus*
cm	Centimeter
m	Meter
LCA	Life cycle assessment
GWP	Global warming potential
S	Sulfur
Ca	Calcium
K_2SO_4	Potassium sulfate
KCl	Potassium chloride
QTL	Quantitative trait locus

1. Introduction

Over the last decades, global population growth and industrialization have considerably increased energy consumption, with the Earth's available natural resources being exploited to fulfil this demand. The global energy mix shows that fossil fuels are the major contributor to primary energy supply (Figure 1).

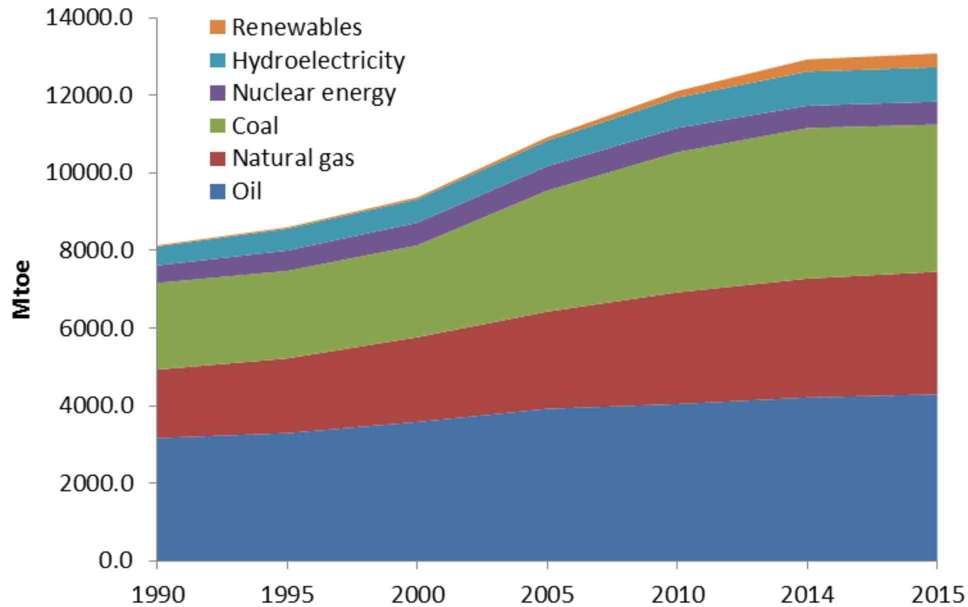

Figure 1: Global energy consumption by fuel types in million tonnes oil equivalent (Mtoe). Data from BP energy outlook statistics, 2016

However, the depletion of natural resources and global warming has raised concerns over the use of fossil fuels. Despite the climate change as a result of fossil fuel consumption, the other immediate effect of limited natural resources will be energy insecurity. Therefore, development of energy self-sufficiency will play a crucial role to continue with future economic growth. For example in the EU-28, up to 53% of energy needs are currently being fulfilled through imports, which cost more than € 1 billion per day (SWD, 2014). To cope with climate change and energy security challenges, exploration and utilization of sustainable and renewable energy resources is required. A wide range of renewable energy resources are presently being exploited such as wind, solar, hydro power, geothermal and biomass and waste based bioenergy. To promote the use of renewable energy resources, various policy initiatives have been taken at both global and EU level (IEA, 2015). The recent UN climate summit in Paris (COP21) is a prime example of global policy initiatives. The main aim of this summit was to channel global efforts to tackle climate change through the introduction of low- carbon fuels and improvement of the efficiency of energy systems. These pledges are expected to shift global energy mix by increasing the share of non-fossil fuels from 19% to 25% by 2040 (IEA, 2015). In addition to COP21, the EU has promoted alternative fuels through several policy instruments such as the renewable energy directive (2009/28/EC), which aims to reduce GHG emissions by 50% by 2017 and 60% by 2018 in comparison to fossil fuels. Under this directive, national targets were set to increase the share of renewable energy to 20% (Figure 2) and 10% of energy consumption for transportation from renewable resources by 2020 (Bringezu et al. 2009; Eurostat, 2016). In 2012, bioenergy contributed 62% of renewable consumption (SWD, 2014). It indicates that biomass-based energy production will play a significant role in achieving the future targets. The contribution of biomass is continually increasing and is expected to reach 139.5 Mtoe by 2020 (SWD, 2014).

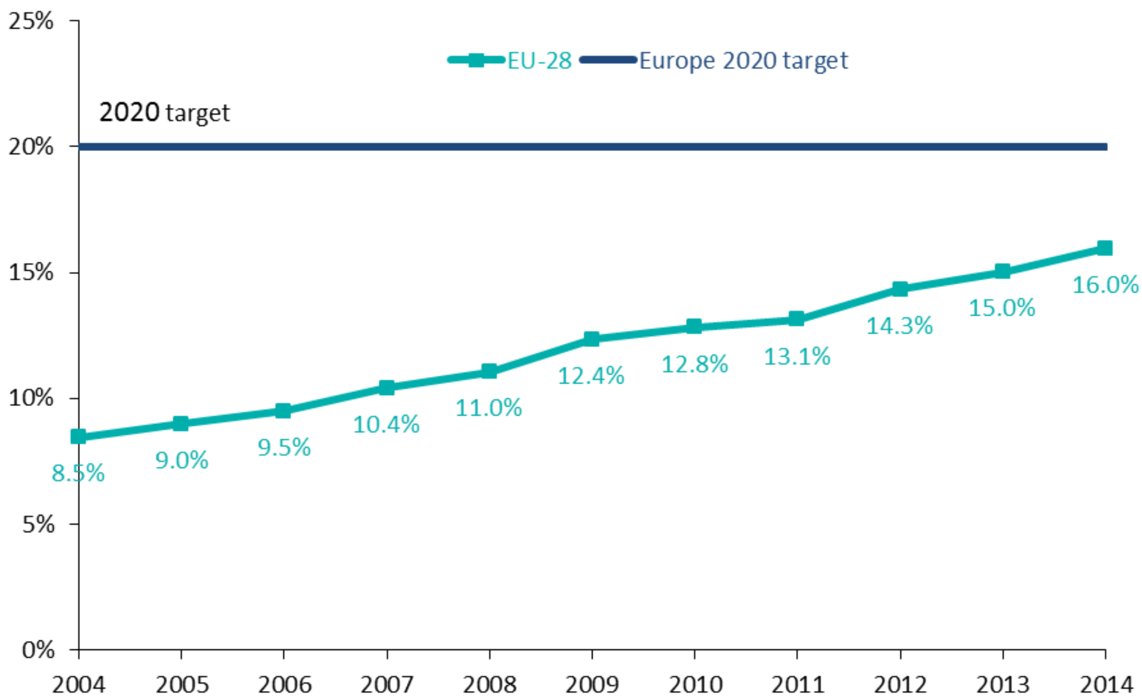

Figure 2: Development of renewable energy share from 2004 to 2014 in EU-28. Data from Eurostat, 2016

The policy initiatives and availability of diverse biomass resources indicates that biofuels industry is developing over the time and progressing towards advanced low carbon biofuels production, which in future will potentially contribute in each sector and help to achieve sustainability goals. For example, recently advanced biofuel has been tested to replace the conventional jet fuel in aviation and has shown that it could potentially reduce the CO_2 emissions up to 80% in comparison to conventional jet fuel (Kousoulidou and Lonza, 2016). It indicates that biomass based energy production will play a pivotal role in future to counter the environmental challenges by reducing the GHG emissions as well as contributing towards energy security. However, the efficiency in technical and economic terms needs to be improved further, which can be achieved by exploiting the biomass components for different purposes along with bioenergy use. For example use of process residues for bio-based products and biomaterials.

To achieve this and to improve the management and efficient use of available biomass based resources, European Union has formulated a bio-economy strategy and defined bio-economy as a comprehensive approach to continue the sustainable economic growth and addressing the inter-connected challenges such as food security, depleting natural resources, dependency on fossil fuels and global warming (EC, 2012). The biomass resources can be sustainably processed into a wide range of bio-based products (biochemical, food, feed, biomaterials) and bio-power (biofuels, heat) (de Jong and Jungmeier, 2015). To promote and to execute this integrated approach, European bio-economy strategy focusses on technological advances, development of new markets and improving the interaction between policymakers and the stakeholders (EC, 2012). This will lead to expansion of bio-economy and subsequently increase the demand of biomass resources (Lewandowski, 2015).

The multi-functionality of biomass makes it a preferable choice among other renewable resources (e.g. wind or solar). For example, it can be utilized for biofuels (e.g. liquid fuels) as well as for solid fuels (e.g. pellets). European commission has defined the biofuels as gaseous or liquid transport fuels manufactured through conversion of biomass (Balat, 2007). The emphasis on increasing the share of biofuels in the transportation sector with the aim of achieving sustainable biofuel production, led over the years to the development of first generation biofuels (1G) through to third generation biofuels (3G). The classification criteria for biofuels include type of feedstock, bioenergy route adopted, bioconversion technology used, properties of biofuel produced, global warming potential impact and both direct and indirect land- use change.

The 1G biofuels involve agricultural food crops containing simple carbohydrates (e.g. glucose) and starch such as ethanol production from maize grains (Mohr and Raman, 2013) and biodiesel from vegetable oils. First generation biofuels were introduced with the aim of replacing conventional fuels to cope with emerging climate-change challenges. However, the type of feedstock used for 1G biofuels production raised several sustainability concerns, mainly land-use issues and food security especially high food prices and low environmental value (Mohr and Raman, 2013). For example, in the United States, the extensive use of soybean and maize for biofuels production contributed to significant price hike (Koizumi, 2015). In addition to the ethical issues linked to the use of food crops for biofuels, the GHG mitigation potential of 1G biofuels is also questionable. At EU level (aggregate), depending on type of feedstock, the production and use of 1G biofuels does not always meet the GHG savings criteria laid out by the European Commission under the Renewable Energy Directive (Humpenöder et al. 2013). This indicates that the introduction of 1G biofuels failed to fully achieve sustainability objectives. As a result, the biofuel production industry is evolving from 1G to 2G.

So-called ‘2G’ biofuels are obtained from lignocellulose feedstocks such as agricultural and forestry residues, dedicated non-food crops (energy grasses, short rotation coppice) and municipal waste (Koizumi, 2015). The 2G biofuels have attracted much interest because a variety of feedstocks can be utilized without compromising environmental aspects. There are five main reasons for choosing 2G over 1G biofuels (OECD/IEA, 2008; Mohr and Raman, 2013). These are:

- No direct competition with food production, if agriculture/forestry residues are used or marginal lands are exploited for feedstock production;
- High GHG mitigation potential in comparison to 1G biofuels (depending on the feedstock used);
- More sustainably produced feedstocks, for example, the low input requirements of perennial energy crops and efficient use of available resources make such feedstocks favourable for sustainable biomass production;
- Expansion of 1G biofuels can potentially lead to mono-cropping such as growing of only maize for ethanol production because of high commercial value, whereas 2G biofuels exploit a wide range of biomass resources, therefore it can contribute towards agricultural diversification. In addition, the establishment of perennial energy crops can potentially contribute towards improvement in biodiversity (Dauber et al. 2015) and soil carbon reserves;
- Prevention of deforestation in case of 2G biofuels because the use of agricultural and forest residues don’t cause deforestation but it can effect soil quality if outsourced more than the set sustainable limits. For example, in case of wheat straw up to 40% of residue can be taken away without any negative effect on soil depending on site conditions (Scarlat et al. 2010).

Biofuels produced from autotrophic organisms such as algae are termed as 3G biofuels. Despite the fact that these are still in very early phase of development, they hold huge potential for future because they have a high photosynthetic rate and do not require arable land as conventional crops do (Milano et al. 2016). Macro- and micro- algae can be converted to ethanol through the fermentation process, to bio-oils by pyrolysis process or to combustible gases through gasification (Suganya et al. 2016). Autotrophic organisms will diversify feedstock resources, but cannot fulfil market demand on their own. Biofuel industry is not focusing on 3G because there are still huge technical difficulties to overcome until industrial production is possible and economically viable. Currently biofuel sector is aiming at optimization of technical challenges and scaling up of 2G biofuel production, which is not fully achieved yet. Therefore, 2G biofuels will play the leading role in meeting immediate biomass based energy demands.

Currently, as a part of 2G biofuels production, the focus is to ensure continue future biomass supply and utilize these biomass resources in a sustainable way. Presently the major 2G feedstock is wheat straw, which is being utilized for ethanol production. However, for 2G biofuels, other lignocellulose feedstocks such as dedicated energy grasses are being tested to evaluate the yield and biomass quality for a specific end use. Among dedicated energy grasses, miscanthus is the main energy grass in EU as switchgrass in USA. However, at research level, switchgrass field trials are being carried out under European conditions to compare it with miscanthus (Shield et al. 2012; Giannoulis et al. 2014). The cultivation area for miscanthus is 38,300 ha in Europe, which is even more than short rotation coppice (Elbersen et al. 2012). In addition, miscanthus is seen as a potential energy grass, which can be grown to exploit the available unused/under used, contaminated (Nsanganwimana et al. 2015) and marginal lands (Mi et al. 2014). In EU, the dedicated energy grasses are being tested on contaminated, unused/underused and marginal lands to achieve sustainable biomass production for bioenergy (Lord, 2015; Nsanganwimana et al. 2015). According to the IEEP (Institute for European Environmental Policy) report, at EU level the available land area (contaminated, unused/underused and marginal lands) is in range of 1 to 1.5 million ha, which can be potentially exploited for dedicated perennial energy crops to produce feedstock for bioenergy. It could generate about 7.7 to 16.7 million dry tonnes of biomass per annum (Allen et al. 2012). Based on the future biomass demand, the biomass production from energy crops need to be increased from 40 million dry tonnes in 2012 to 84 million tonnes dry matter by 2020 (Scarlat et al. 2015). The use of this potentially available land area to grow perennial energy grasses will contribute from 10 to 20% of the projected increase by 2020.

This growing demand of biomass should be achieved through sustainable biomass production. However, the expanding bio-economy not only requires sustainable biomass production but also high quality biomass to carry out sustainable bioconversion and processing of biomass. For a specific bioconversion route, users such as industries involved in biomass processing, require biomass with certain quality characteristics.

The quality of biomass and sustainability is largely dependent on management practices, which are adopted during biomass production. Sustainability here refers to production of biomass at low cost with minimum environmental implications and without compromising food security. Adoption of appropriate crop management practices offers an opportunity to deliver good quality biomass in a sustainable way. In addition, it will help to meet the user demand and follow the set quality standards such as ENplus wood pellets for combustion at EU level. Therefore, quality of biomass needs to be optimized as much as possible at field level to meet the users demand for a specific bioconversion route. Each bioconversion route requires a specific biomass quality characteristics, therefore biomass quality optimization strategies should be adopted according to end use. In addition, for a specific production chain, user needs to be consulted for optimization of biomass quality. It will help to counter the specific challenges, which come up during processing of biomass. The close collaboration between farmer and industry can help to improve the efficiency of whole production chain for a specific end use. Therefore, to fulfil the biomass user demand, biomass should be produced in sufficient quantities in a sustainable way with high biomass qualities for the intended production chain.

Currently, there are 4 main bioconversion routes, which are adopted to convert 2G biomass to bioenergy and bio-based products. It includes 1) biochemical conversion; 2) thermochemical conversion; 3) chemical conversion and 4) mechanical conversion (de Jong and Jungmeier, 2015). The thermochemical and biochemical conversion are most widely used bioconversion routes (Demirbaş, 2001). Thermochemical conversion can be carried out through 4 different processes 2a) liquefaction to produce heavy oil; 2b) pyrolysis to produce bio-oils; 2c) gasification for FT (Fischer Tropsch) oil; 2d) combustion to produce combustible gases such as H_2. The biochemical conversion route involves combination of chemical and biological processes such as chemical pre-treatment of biomass followed by enzymatic digestion and fermentation (Naik et al. 2010). For each of the bioconversion routes biomass quality needs to be optimized, which is not yet fully achieved.

In Europe, combustion is the most prevalent bioconversion process being used to produce biomass based heat and electricity. By 2020, out of 139.5 Mtoe biomass based energy production, 110.4 Mtoe will be heat and electricity (SWD, 2014). For combustion, a wide range of biomass resources can be exploited such as wood, agricultural residues, municipal waste or perennial dedicated energy crops. However, thermal bioconversion efficiency and the emissions depend on composition of biomass. Therefore, it is important to select a feedstock, which can be converted efficiently with low emissions. In Europe, dedicated energy grass, miscanthus, is mainly being used for direct combustion. *Miscanthus* is the main energy grass in Europe because of better adoptability, high yield potential and efficient use of soil resources such as water and nutrients (Van der Weijde et al. 2013). Presently, *M. x giganteus* is the only commercially grown genotype. However, a wide range of genotypes are being tested under the European conditions to select the most promising ones. The genetic diversity of miscanthus can be exploited to optimize the biomass quality for a specific end use. The low input requirements and efficient translocation of nutrients back to rhizomes before harvest leads to low mineral and ash content in the harvested biomass (Christian et al. 2008), which makes miscanthus a preferable choice for combustion. However, the main challenge especially with direct combustion is high emissions and combustion relevant problems such as corrosion, fouling and low ash melting point which, subsequently decreases the conversion efficiency and increases the maintenance cost as well (Arvelakis and Koukios, 2013). Therefore, the biomass with low mineral, ash and moisture content is preferred because it can already improve the conversion efficiency and decrease the emissions. The high ash and moisture content have negative effect on heating value of miscanthus biomass. The comparatively high K and Cl content in miscanthus biomass leads to low ash fusion temperature. The K and Cl participate in most of the corrosion related reactions and form deposits on heater tubes, which reduce the heat transfer and overall efficiency of the process (Aho, 2001). Miscanthus, being the herbaceous biomass has higher Cl content in comparison to wood (Blomberg, 2012). Because both elements (K and Cl) complement each other in nutrients uptake dynamics (Gielen et al. 2016), therefore the content of K is also higher in ash of miscanthus biomass in comparison to wood (Aho, 2001). The high K and Cl in miscanthus biomass could be the main reason for low ash fusion temperature in comparison to wood pellets. Other than high Cl and K content, miscanthus also has comparatively high Si content in harvested biomass. The Si content in biomass is important in terms of biomass quality because up to 74% of miscanthus biomass ash is composed of silica (Ryu et al. 2006). However, despite the high Si content in miscanthus ash compared to wood (Ryu et al. 2006), the effect of Si alone is not clear on ash melting behaviour during combustion process (Baxter et al. 2014).

The above mentioned biomass combustion quality relevant challenges can be countered through different ways such as technical improvements in boilers, use of chemical additives (Wang et al. 2012) or on field crop management practices (Baxter et al. 2012). On biomass production side, quality can be optimized through on field quality management practices such as selection of genotypes, appropriate harvesting time, rate of fertilization especially N fertilization (Baxter et al. 2012) and cutting the biomass and leaving it on field to allow leaching of minerals (Yu et al. 2014) and drying (Meehan et al. 2014). Genotype selection (Hodgson et al. 2011), harvesting time (Wahid et al. 2015; van der Weijde et al. 2016) and other management practices are adjusted according to the end use of biomass (Hodgson et al. 2011; Kludze et al. 2013).

Considering the EU 2020 target to reach the 10% share of renewable in transportation sector, lignocellulose based biofuels production needs to be increased. Therefore, testing new feedstock such as miscanthus biomass and optimization of pre-treatment process for ethanol production will play a key role to achieve the EU 2020 target. Currently, straw based ethanol production has been carried out successfully at pilot scale (Larsen et al. 2012). However, straw based ethanol production poses some limitations. For example, as straw is an agricultural residue, can only be taken away in limited quantities, therefore continue supply would be difficult (Scarlat et al. 2010). In addition, due to low density collection of wheat straw in sufficient quantities for commercial scale ethanol production is a challenge (Sharma et al. 2013). Therefore, other lignocellulosic biomass resources such as miscanthus will contribute towards continue supply of feedstock for commercial scale lignocellulosic ethanol production. In miscanthus based ethanol production, the major challenge is to digest the biomass through appropriate pre-treatment conditions. The process involves the biochemical conversion, where miscanthus is pre-treated through addition of chemicals such as NaOH (Cha et al. 2015) or H_2SO_4 under high temperature and pressure (Brosse et al. 2010) followed by enzymatic hydrolysis. The main aim of pre-treatment of biomass is to remove the lignin and make the cellulose accessible, which subsequently can be converted to ethanol (Chiaramonti et al. 2012). The high lignin content in substrate is major challenge for digestion of biomass and it requires severe pre-treatment conditions such as high concentrations of chemicals and high temperature and pressure. However, the severe pre-treatment conditions can also lead to inhibitor formation as well as increase the process cost. The inhibitor formation affects the subsequent conversion of cellulose to ethanol and reduces the overall efficiency (Guo et al. 2012). Therefore, pre-treatment process needs to be optimized in a way that efficient biomass pre-treatment with low inhibitor formation could be achieved in a cost effective way. Miscanthus has high lignin content compared to wheat straw but still high dry matter yield potential makes it an attractive option. In addition, miscanthus biomass can be exploited for developing multiple value chains because all the process residues such as lignin and other inhibitors can be processed to biomaterials and biochemicals (Chen and Zhang, 2015). However, for efficient digestion of miscanthus biomass, appropriate pre-treatment conditions are required.

The above mentioned biomass quality aspects and challenges for combustion and ethanol production indicate that the end user such as industry, require specific quality characteristics of biomass for each bioconversion route to carry out the process efficiently. Therefore, in current study, the focus is to evaluate the biomass quality relevant for thermochemical conversion mainly combustion and biochemical conversion (chemical pre-treatment followed by enzymatic hydrolysis) and the options to optimize biomass quality for each bioconversion route to fit the user demand.

To realise the above mentioned aim of this study, 15 miscanthus genotypes were compared for combustion whereas, for ethanol production one genotype *M. x giganteus* was tested. Two field trials were used: 1) long term field trial with 15 miscanthus genotypes (four *M. x giganteus*, one *M. sacchariflorus*, five *M. sinensis* hybrids and five *M. sinensis* genotypes) was established as randomized block design with three replications; 2) field trial with *M. x giganteus* and switchgrass was established as a randomized split plot design with different crops as main plots, divided into three subplots (180 m^2 each) with different N levels (0, 40, and 80 kg $ha^{-1}a^{-1}$). Each variant had 4 replicates. Switchgrass and wheat straw were used as reference crops for comparison. The biomass samples collected from these field trials were analysed in laboratory to test the biomass quality parameters for combustion (mineral analysis, silicon, chloride, ash, moisture and ash melting behaviour) and ethanol production (fiber analysis, acid/base based pre-treatment). The results generated through these experiments were used for preparation of five publications. Based on these publications, thesis work is divided into two main chapters:

Chapter-1, deals with biomass quality for combustion for different miscanthus genotypes. In addition, it also deals with management practices and their impact on biomass quality. This part is comprised of the following three publications:

1. Iqbal, Y. and I. Lewandowski. 2014. "Inter-Annual Variation in Biomass Combustion Quality Traits Over Five Years in Fifteen *Miscanthus* Genotypes in South Germany." *Fuel Processing Technology* 121: 47-55.
2. Iqbal, Y. and I. Lewandowski. 2016. "Biomass Composition and Ash Melting Behaviour of Selected *Miscanthus* Genotypes in Southern Germany." *Fuel* 180: 606-612.
3. Iqbal, Y., M. Gauder, W. Claupein, S. Graeff-Hönninger, and I. Lewandowski. 2015. "Yield and Quality Development Comparison between *Miscanthus* and Switchgrass Over a Period of 10 Years." *Energy* 89: 268-276.

Chapter-2, consists of two publications, which cover the aspects relevant to pre-treatment process and provide an insight about the measures to optimize the thermochemical process. Following are the publications which included in this chapter:

4. Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2015. "Comparing the Performance of *Miscanthus x Giganteus* and Wheat Straw Biomass in Sulfuric Acid Based Pretreatment." *Bioresource Technology* 180: 360-364.
5. Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2016. "Efficiency of Single Stage- and Two Stage Pretreatment in Biomass with Different Lignin Content." *Bioresource Technology* 211: 787-791.

2. Chapter-1

Three publications included in this chapter deal mainly with productivity and biomass quality of different miscanthus genotypes and switchgrass as well. The overall focus of this chapter is to compare yield and biomass combustion quality of different miscanthus genotypes including switchgrass. In addition, the effect of management practices such as harvesting time, N fertilization on biomass quality and optimization of biomass quality to fit the user demand through these management practices. The first publication deals with consistency of biomass quality characteristics of different miscanthus genotypes over the years. The main factors of interest are effect of genotype selection, climatic conditions especially rainfall and time of harvesting. In second publication, the effect of inorganic constituents of biomass especially K, Cl, Si contents was evaluated and information about biomass composition was exploited to explain the ash melting behavior of different miscanthus genotypes. The main focus of third publication is to compare the long term productivity and biomass quality of miscanthus and switchgrass under different management practices especially different N fertilization levels. In addition, it highlights the emissions especially NOx and the N offtake through harvesting of biomass. This chapter comprised of three sub-chapters which include all the relevant publications.

2.1. Inter-Annual Variation in Biomass Combustion Quality Traits Over Five Years in Fifteen *Miscanthus* Genotypes in South Germany

Publication-1

Iqbal, Y. and I. Lewandowski. 2014. "Inter-Annual Variation in Biomass Combustion Quality Traits Over Five Years in Fifteen *Miscanthus* Genotypes in South Germany." *Fuel Processing Technology* 121: 47-55.

In this study, inter-annual variation in biomass yield and composition between 2004 and 2010 was studied in a multi-genotype trial planted in South Germany. The main factors of interest in the inter-annual variation were climatic conditions (rainfall and temperature) and different harvest dates (January / February / March / April). The multivariate regression analysis showed that the interactions of harvest date–aging and harvest date–rainfall have significant effects on the stability of biomass quality characteristics over the productive growth period. In *M. sacchariflorus* the harvest date–aging interaction improved the combustion quality by reducing the Mg concentration by 29% and ash by 18%, whereas the harvest date–rainfall interaction contributed by decreasing the concentrations of Ca, Si and N by 8%, 4% and 6%, respectively. The *M. x giganteus* genotypes showed more consistency in mineral concentrations, especially K and Cl, and dry matter yield in comparison to *M. sinensis* over the productive growth period. In some years, such as 2004 and 2005, instability in N concentration exceeded the limit (0.5%) set by the standards which already exist for wood pellets (Pellet Norm EN 14961-2:2012 A2). This can reduce the efficiency of the combustion process and increase emissions.

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Inter-annual variation in biomass combustion quality traits over five years in fifteen *Miscanthus* genotypes in south Germany

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ABSTRACT

In recent years, miscanthus has emerged as a very promising alternative energy crop predominantly due to its low input requirements and high dry matter yield potential. In this study, inter-annual variation in biomass yield and composition between 2004 and 2010 was studied in a multi-genotype trial planted in south Germany. The main factors of interest in the inter-annual variation were climatic conditions (rainfall and temperature) and different harvest dates (January/February/March/April). The multivariate regression analysis showed that the interactions of harvest date–aging and harvest date–rainfall have significant effects on the stability of biomass quality characteristics over the productive growth period. In *M. sacchariflorus* the harvest date–aging interaction improved the combustion quality by reducing the Mg concentration by 29% and ash by 18%, whereas the harvest date–rainfall interaction contributed by decreasing the concentrations of Ca, Si and N by 8%, 4% and 6%, respectively. The *M. x giganteus* genotypes showed more consistency in mineral concentrations, especially K and Cl, and dry matter yield in comparison to *M. sinensis* over the productive growth period.

1. Introduction

The increasing demand for sustainably produced biomass for energy use has raised interest in perennial crops such as short rotation coppice (willow or poplar) and energy grasses such as switchgrass, reed canary grass and miscanthus. These crops combine high resource-use efficiency with low input requirements [1] and a higher carbon sequestration capacity [2]. Of these, miscanthus in particular has emerged as a very promising energy crop in Europe owing to its high biomass yield potential and water and nitrogen-resource efficiency [3,4].

Miscanthus is a warm season C_4 perennial rhizomatous grass, which originates from South East Asia and grows in diverse climatic regions from the arctic to the tropics [5]. The cropping area for *M. x giganteus* has expanded to 2000 ha in Germany alone [6]. In Europe, miscanthus biomass is currently used mainly for direct combustion. The major problem for the combustion of miscanthus biomass is its ash-melting behavior, which can lead to corrosion, fouling and slagging at comparatively low temperatures [7]. There are number of factors which contribute to the ash-melting behavior during the combustion process, such as inorganic constituents of the biomass and ash concentration. The combustion quality of biomass is determined by a) composites that affect the heating value of the biomass e.g. ash, moisture and lignin; b) composites that lead to harmful emissions e.g. nitrogen (N), sulfur (S), chloride (Cl) and heavy metals; c) composites that have an impact on ash fouling, slagging and corrosion e.g. chloride (Cl), potassium (K), phosphorus (P), magnesium (Mg), silicon (Si), calcium (Ca) and sodium (Na). High concentrations of chloride and potassium in the dry matter lead to ash melting and cause mechanical problems in the combustion processes such as corrosion and fouling [7,8]. Ash and moisture concentrations are relevant for the combustion process mainly due to their simultaneous effect on operating costs and heating value. High concentrations of nitrogen and sulfur in the plant biomass contribute to high emissions of primary pollutants such as nitrogen oxides (NO_x) and sulfur oxides (SO_x). The mineral composition and ash and moisture concentrations are mainly affected by fertilization, harvesting time [9] and crop age [10].

However, in miscanthus, the proportion of inorganic constituents in the harvested biomass is comparatively low. This is because miscanthus has a lower fertilizer demand than other crops [3,11] and remobilizes nutrients, transporting them back to rhizomes at the end of the growing season. Also, for quality reasons, miscanthus biomass is harvested after winter to let it dry out and allow precipitation to wash out ash, potassium and, in particular, chloride [4]. Presently, delayed harvest is being used effectively as a tool to improve the combustion quality characteristics, although it is at the expense of dry matter yield [9,12]. Delaying harvest from autumn (August) to spring (April) improved the combustion quality of industrial hemp (*Cannabis sativa* L.) by decreasing the N, K, Cl, Mg, moisture and ash concentrations [13]. Delayed harvest contributes to the improvement of combustion quality through high leaf drop-off because stems have better combustion qualities than leaves [9]. In another study, a large decrease in leaf-to-stem ratio

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in miscanthus was observed from January to March [14]. Therefore it is crucial to find the optimal harvesting time to deliver the highest quality biomass for combustion.

There are a number of factors responsible for the inter-annual variation in chemical composition of miscanthus, for example genetic differences [15], harvesting time [16] and climatic aspects, mainly temperature and rainfall [17]. In addition, crop age can also lead to inter-annual variation in mineral composition and ash content. For example Nassi O Di Nasso et al. [10] observed variation in ash content in giant reed (*Arundo donax* L.), over the productive growth period. There are several reports on anatomical and morphological differences among the miscanthus genotypes which also contribute to variation in the chemical constituents of the biomass [18,19].

Many studies have been conducted on miscanthus to evaluate its economic viability [1,20], productivity and combustion quality in relation to management practices [17,19], whereas there are no studies on stability of combustion quality characteristics for different miscanthus genotypes. However, research shows that fluctuations in biomass composition could affect the combustion behavior and efficiency of combustion units [21]. Stability in biomass quality characteristics is necessary mainly because: a) in future farmers may be paid according to biomass quality rather than quantity alone and a set of standards for biomass quality characteristics is currently being developed. Moreover, stability will enable the farmers to deliver biomass with a defined quality as required by the market in the coming years. Therefore, quality management over the productive growth period is important for farmers; b) biomass quality is not only relevant for the efficiency of the combustion units but also for the emissions. These may increase when biomass composition fluctuates. In addition, stability in quality characteristics would enable combustion unit owners to better plan their operations and to execute the running of their combustion units more efficiently over a longer period.

In this study, comparative investigations were made on the chemical composition of biomass from several miscanthus genotypes. In addition, the stability in combustion quality characteristics and the influence of various factors on the quality characteristics of each genotype over the productive growth period were evaluated. Data were assessed from a trial planted in 1997 for the EU project EMI (European Miscanthus Improvement) at the experimental farm, Ihinger Hof, in south Germany. Samples of 15 miscanthus genotypes were obtained from these field trials from 1997 to 2010 and their ash and moisture as well as mineral concentrations (N, P, K, Cl, Na, Mg, Ca, and Si) were analyzed. The stability of these quality characteristics over the productive growth period was assessed by consistency index/co-efficient of variation.

Various hypotheses were developed for this study. It was hypothesized that: a) the concentration of leachable elements would decrease with delayed harvest, as observed by Lewandowski et al. [4], because delayed harvest increases the proportion of standing dead biomass with more broken cuticles and the leaching rate increases with the amount of broken cuticles [22,23]; b) there is a negative correlation between the amount of the rainfall and the concentration of leachable elements; c) the concentration of leachable elements would be lower in thin-stemmed genotypes because thin stems facilitate leaching [24]; d) the concentration of leachable elements would be lower in early-senescing genotypes because leaching processes can occur earlier in the productive growth period once the stems have died off [4].

2. Materials and methods

2.1. Field trial and climatic conditions

As a part of the European Miscanthus Improvement (EMI) project, a field trial with 15 miscanthus genotypes was planted in 1997 at the University of Hohenheim's experimental farm, Ihinger Hof (48°40′ N: 09°00′ E) in south Germany. The clay-rich soil of the research field belongs to the soil order Vertisol (FAO, U.S. soil taxonomy). The soil sampled from the ground surface to a depth of 40 cm consists of 10.1% sand, 49.3% silt and 40.6% clay. Sampling was prohibited below 60 cm due to an underlying stony zone, but roots can still penetrate down to a depth of 80 cm. The soil pH ranged from 7.2 to 7.7. The macronutrients (N, P and K) and the humus content of the soil decreased with depth. The soil profile along with physical and chemical properties is described by Clifton-Brown and Lewandowski [25].

Rainfall and temperature data (see Table 1) were collected by a meteorological station situated 100 m away from experimental site.

2.2. Experimental design

Fifteen miscanthus genotypes were planted in May 1997 at Ihinger Hof. These were divided into 4 genotype groups. The first group consisted of four *M. x giganteus* genotypes (Gig-1 to Gig-4) of different origins. The second group consisted of one *M. sacchariflorus* genotype only. Group 3 contained five *M. sinensis* hybrids (Sin-H6 to Sin-H10) developed from crosses within *M. sinensis* and with *M. sacchariflorus* and the fourth group consisted of five *M. sinensis* genotypes (Sin-11 to Sin-15) selected in Japan (Table 2). These genotypes were micropropagated by two laboratories: TINPLANT, Germany, and the Danish Institute of Agriculture Science (DIAS), Denmark. Three plots of each genotype were established in three randomized blocks at a planting density of 2 plants m^{-2} (66 × 75 cm row spacing). Establishment of the field trial is described in detail by Clifton-Brown and Lewandowski [25].

2.3. Management of the trial

Before plantation, all the plots received modest inputs of NPK (60 kg N, 44 kg P and 110 kg ha^{-1} K). Subsequently, drip irrigation was performed by pumping water to the plots through a system of pipes during the period 28 May to 12 September 1997. The dose of N fertilization remained unchanged from 1997 to 2010 at 60 kg N ha^{-1} a^{-1}. But from 2004 to 2010, P and K were applied at yearly rates of 50 kg P and 144 kg K ha^{-1}. For the first two years, weeds were eradicated by hand weeding and mechanical hoeing. For the period 2004 to 2010, no weed control was performed.

2.4. Measurements

Plant height and number of shoots were measured by selecting 5 plants per plot. Flowering date and stem diameter for each genotype were monitored in the year 2010 [26]. Nine measurements of stem diameter were taken per genotype (three measurements per plot).

Table 1
Climatic data for the research farm, Ihinger Hof, in the years 2004 to 2010.

Year	Harvest dates	Annual precipitation (mm)	Mean annual temperature (°C)	Min-winter temperature (°C)
1997	11.11	661	8.5	−5.6
1998	23.11	663.1	8.7	−1.9
1999	21.11	813.9	9.2	−4.2
2000	1.02	708.4	9.7	−2.6
2001	26.02	773.5	8.8	−4.8
2002	18.02	936.8	9.4	−4.8
2003	24.02	539.4	9.7	−5.9
2004	09.02	672.9	9.0	−3.4
2005	15.01	604.4	8.8	−4.9
2006	11.01	624	9.4	−6.4
2007	19.03	681.2	9.6	−2.4
2008	01.04	793.9	9.2	−2.4
2009	03.03	812.7	9.3	−5.8
2010	29.03	701.86	8.1	−5.5
mean		713.4	9.1	

Table 2
Descriptions of miscanthus genotypes used in field trial at Ihinger Hof research station, according to Clifton-Brown and Lewandowski [25].

Group	Identification (ID).	Name	Abbreviation	Ploidy	Description
1	1	*M. x giganteus*	Gig-1	3n	1–4 *M. x giganteus* of different origins
	2	*M. x giganteus*	Gig-2	3n	
	3	*M. x giganteus*	Gig-3	3n	
	4	*M. x giganteus*	Gig-4	3n	
2	5	*M. sacchariflorus*	Sac-5	4n	Germany
3	6	*M. sinensis* hybrid	Sin-H6	3n	Progeny selected from open pollinated cross of *M. sinensis* genotypes.
	7	*M. sinensis* hybrid	Sin-H7	2n	Derived from two different *M. sinensis* parents in Europe
	8	*M. sinensis* hybrid	Sin-H8	Aneuploid	Derived by *M. sacchariflorus* and *M. sinensis* in Europe
	9	*M. sinensis* hybrid	Sin-H9	2n	Hybrid gained from a cross of two *M. sinensis* parents
	10	*M. sinensis* hybrid	Sin-H10	2n	Derived by *M. sacchariflorus* and *M. sinensis* in Europe
4	11	*M. sinensis*	Sin-11	2n	11, 12 selected 1988
	12	*M. sinensis*	Sin-12	2n	11–15 collected in Japan
	13	*M. sinensis*	Sin-13	2n	13–15 selected 1990
	14	*M. sinensis*	Sin-14	2n	
	15	*M. sinensis*	Sin-15	2n	

2.5. Sample collection and sample processing

The miscanthus genotypes were harvested twice a year, first harvest in November and 2nd harvest was performed between January–April over the whole productive growth period starting from 1997. However for this study, samples from 2004 to 2010 only were analyzed. At each harvest date plants were cut at an approximate stubble length of 5 cm from an area of 0.5–1.5 m^2 using manual cutters. Stems and leaves were separated at the ligule. The harvested biomass was dried in a circulating air drying oven at 60 °C for 48 h to estimate the dry matter yield. Samples were then chopped and milled to pass through a 1 mm sieve. The samples collected were used for the quality analysis.

2.6. Chemical analysis of the plant biomass

The *ash* concentration was assessed by monitoring the loss of ignition at 550 °C for 4 h.

For mineral analysis, half a gram of each sample dried at 105 °C was diluted in 8 ml HNO_3 (65%) and stirred 2–3 times within the following 30 min. The color development in the suspension was halted by adding 4 ml of clear liquid hydrogen peroxide. The samples were then digested in a microwave (MARS 5, CEM) at the specified temperature (120 °C–180 °C) and pressure (24.16 bar) for 40 min. Afterwards, the volume of each suspension was made up to 100 ml with distilled water in volumetric flasks and subsequently the extract was obtained by filtration with Whatman filter paper. The extracts of the biomass samples were analyzed for *calcium*, *potassium* and *sodium* using a Flame Photometer (ELEX 6361, Eppendorf).

Then from each sample 1 ml was taken and 9 ml lanthanum solution was added. These were mixed thoroughly and analyzed for *magnesium* concentration using an Atomic Absorption Spectrometer (220 FS, Varian). The *phosphorus* concentration was determined by adding (ammoniumvanadate + ammoniummolybdate + HNO_3) to the extract and measuring spectrophotometrically (Spectrophotometer PM 6, Zeiss).

Analysis of the *chloride* concentration in each biomass sample was performed by the Landesanstalt für Landwirschaftliche Chemie, University of Hohenheim, using High Performance Liquid Chromatography (HPLC) [8].

The *silicon* concentration was analyzed by the Landesanstalt für Landwirschaftliche Chemie, using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). For the total *nitrogen* measurement in the biomass samples, analysis was carried out according to the Dumas principle (Leco St. Joseph, MI).

2.7. Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS 9.2). To evaluate the relationship between quality characteristics, a correlation analysis was performed using the correlation procedure in SAS. The correlation coefficients were also determined for the relationship between qualitative and quantitative characteristics of the plant biomass. To meet the assumptions of a linear model, raw values were transformed. However, figures were plotted without data transformation.

To compare the variability of the data series over the years, the coefficients of variation were calculated. The coefficient of variation is the ratio of standard deviation to the mean. A large coefficient of variation indicates a more variable data series, i.e. the group is less stable and less uniform. A small coefficient of variation indicates a less variable, i.e. more stable and uniform group.

The variables with significant effect on the corresponding element were selected for each regression model. Regression models for quality characteristics were set up using regression analyses. To evaluate the effect of harvest date, aging and rainfall on chloride concentrations of each genotype the following regression model was developed:

$$x_{ij} = \mu + \alpha_i + \gamma_i + ß_i + \delta_i + e_{ij} \quad (1)$$

where x_{ij} represents the chloride concentration for j-th replicate of genotype i, μ is the general mean of each model, α is the effect of genotype i, γ is the effect of harvest date on mineral content of genotype i, $ß$ is the effect of aging for genotype i, δ is the effect of rainfall and e_{ij} the ij-th residual assumed to follow a normal distribution with zero mean.

For the second model, variables with significant effects (genotype, harvest date and aging) were selected to quantify their effect on potassium, magnesium and ash concentrations. The model was written as:

$$y_{ij} = \mu + \alpha_i + \gamma_i + ß_j + e_{ij} \quad (2)$$

where y_{ij} represents potassium, magnesium or ash concentrations respectively in j-th replicate of genotype i; other variables (μ, α_i, γ_i, $ß_j$, e_{ij}) are as for model (1).

To evaluate the significant effect of harvest date and rainfall on nitrogen, silicon and calcium, the third model was written as:

$$z_{ij} = \mu + \alpha_i + \gamma_i + \delta_i + e_{ij} \quad (3)$$

where z_{ij} represents nitrogen, silicon or calcium concentration respectively in the j-th replicate of genotype i; other variables (μ, α_i, γ_i, δ_i, e_{ij}) as for model (1).

The corresponding coefficients of harvest date, aging and rainfall are presented in Table 3.

Table 3
Coefficients of variables for best fitting regression models.

Model	Element	Coefficient		
		Harvest date	Rainfall	Aging
Model (1)	Cl	−0.0170	−0.0058	−0.0113
Model (2)	K	−0.0144		−0.0193
	Mg	−0.0022		−0.0002
	Ash	−0.0230		−0.0640
Model (3)	N	−0.0035	−0.0004	
	Si	−0.0220	−0.0039	
	Ca	−0.0025	−0.0004	

3. Results

3.1. Genotypic variation in yield, mineral, ash and moisture contents

Significant genotypic variation in yield and constituents of the plant biomass was observed between miscanthus genotypes (Figs. 1 & 2). The mineral composition varied, with particularly wide ranges for K, Cl, Ca, Mg and Si concentrations. *M. x giganteus* genotypes, Gig-2 and Gig-4 showed the highest mean K concentrations and highest dry matter yield of all genotypes. The lowest mean N concentration was observed in Gig-1 and Gig-3.

However, the lowest mean K and Cl concentrations were observed in Sin-15 along with the highest Si concentrations and lowest dry matter yield of all genotypes (Figs. 1 & 2). In addition, *M. sinensis* genotypes had the lowest mean moisture content of all genotypes. Generally, the *M. sinensis* genotypes had relatively low mineral concentrations in comparison to the other genotypes.

The *M. sinensis* hybrids had the highest mean N concentrations over the productive growth period (Fig. 2). Moreover, the Cl concentration was highest for Sin-H7 and Sin-H9, whereas the ash concentration was highest in the biomass of Sin-H6, Sin-H7 and Sin-H9 of all genotypes. A significant variation was recorded in terms of biomass yield and mineral composition between *M. sinensis* hybrids. For example, the K concentration was higher in Sin-H7 than in Gig-1 and Gig-3, whereas it was significantly lower in Sin-H8 compared to all *M. x giganteus* genotypes (Fig. 2).

M. sacchariflorus had the lowest Si, Ca, Mg and ash concentrations of all genotypes (Fig. 2). In addition, the concentrations of K, Cl and moisture content were also lower for *M. sacchariflorus* than *M. x giganteus* genotypes.

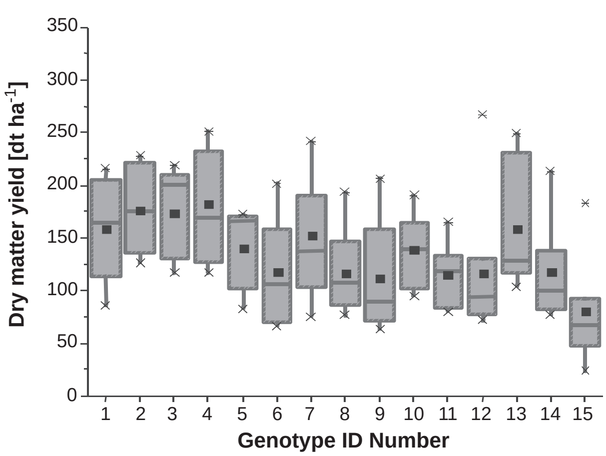

Fig. 1. Box plots of dry matter yield for each genotype in the years 2004 to 2010, with the mean value calculated (indicated by the black square) over the productive growth period. Vertical lines represent the minimum and maximum values, boxes indicate the upper and lower quartiles and the horizontal lines in the box show the median for each genotype from 2004 to 2010. Stars on both sides of the boxes indicate the outlier values (data taken from Gauder et al. [26]). (1 t = 10 dt).

3.2. Inter-annual variation in yield, mineral, ash and moisture contents

Considering the average of all the genotypes, significant increases in biomass production as well as significant variation in mineral concentrations were found from 2004 to 2009 (Figs. 3 & 4). In all genotypes the concentrations of Si, Ca and Mg increased from 2004 to 2006, then decreased in 2007, and remained stable from 2008 to 2010 (Fig. 4). However, the concentrations of K and Cl decreased in all genotypes from 2004 to 2007, followed by an increase in 2008 (Fig. 4). In the years 2004 and 2008–2010 the concentrations of ash did not change significantly in all genotypes. The highest concentration of ash was recorded in 2005 (Fig. 4). The concentrations of K and Cl were lowest in all genotypes in 2007. The lowest moisture content was recorded in the years 2007 and 2008 in all genotypes, whereas the highest content was observed in 2006 (Fig. 4). The average dry matter yield was highest in the year 2009in all genotypes (Fig. 3).

3.3. Quality characteristic stability of each genotype

Stability in quality characteristics was assessed by calculating coefficients of variation for each genotype. High stability is indicated by low coefficients of variation. The stability in N concentration was highest for Sin-13. Sin-H10 proved to be highly instable for N, K and Cl concentrations, whereas Sin-H7 showed stability in Si, Ca, Mg and ash concentrations (Table 4). *M. sacchariflorus* had the highest Si and Ca coefficient of variation values in comparison to other genotypes. *M. x giganteus* genotypes showed comparatively high stability in Cl concentrations and dry matter yield over the productive growth period, whereas *M. sinensis* showed a large variation in Cl concentrations and dry matter yield. There was a large variation in moisture content over the productive growth period in all genotypes. By contrast, the ash concentration remained consistent compared to other quality characteristics in all genotypes. However, the consistency in ash concentration was highest in *M. sinensis* and hybrids in comparison to other genotypes (Table 4). Among the *M. sinensis* hybrids, consistency in quality characteristics was highest for Sin-H7, which also had the highest dry matter yield in this group (Table 4).

3.4. Quality characteristics in relation to harvest date, rainfall and aging effect

Multiple regression models were used to evaluate the harvest date effect and the interactions of harvest date–aging, harvest date–rainfall and harvest date–aging–rainfall for selected quality characteristics of all genotypes in each model. Only those factors were included which had significant impact on respective quality characteristics. The mixed model analysis revealed that genotype and harvest date strongly influenced all combustion quality characteristics. For all genotypes, rainfall had a significant effect on Cl, N, Ca and Si concentrations, while the aging effect was significant only for Cl, K, Mg and ash.

The largest part of the total variability in quality characteristics for all genotypes can be explained by the effect of harvest date alone. A small part of the variability in K, Mg and ash is explained by the aging effect, whereas variation in N, Si, Ca and Cl concentrations can be explained to some extent by the effect of rainfall. In the 14th ratoon year, harvesting of the biomass in April decreased the K concentrations in Sin-15 by 72%, the highest among all genotypes. In the same time period, harvest date–aging interaction decreased the Mg concentration by 29% and ash by 18% in *M. sacchariflorus*, whereas harvest date–rainfall interaction decreased the Ca concentration by 8%, Si concentration by 4% and N concentration by 6% (Table 5). The regression model demonstrates that the harvest date–aging interaction decreased the K concentration by 35% in Sin-H8, which was the highest among the *M. sinensis* hybrids (Table 5). The quality data from the year 2005 was taken as baseline data because rainfall was lowest during this period. For aging effect, the data from the year 2004 was taken as baseline data.

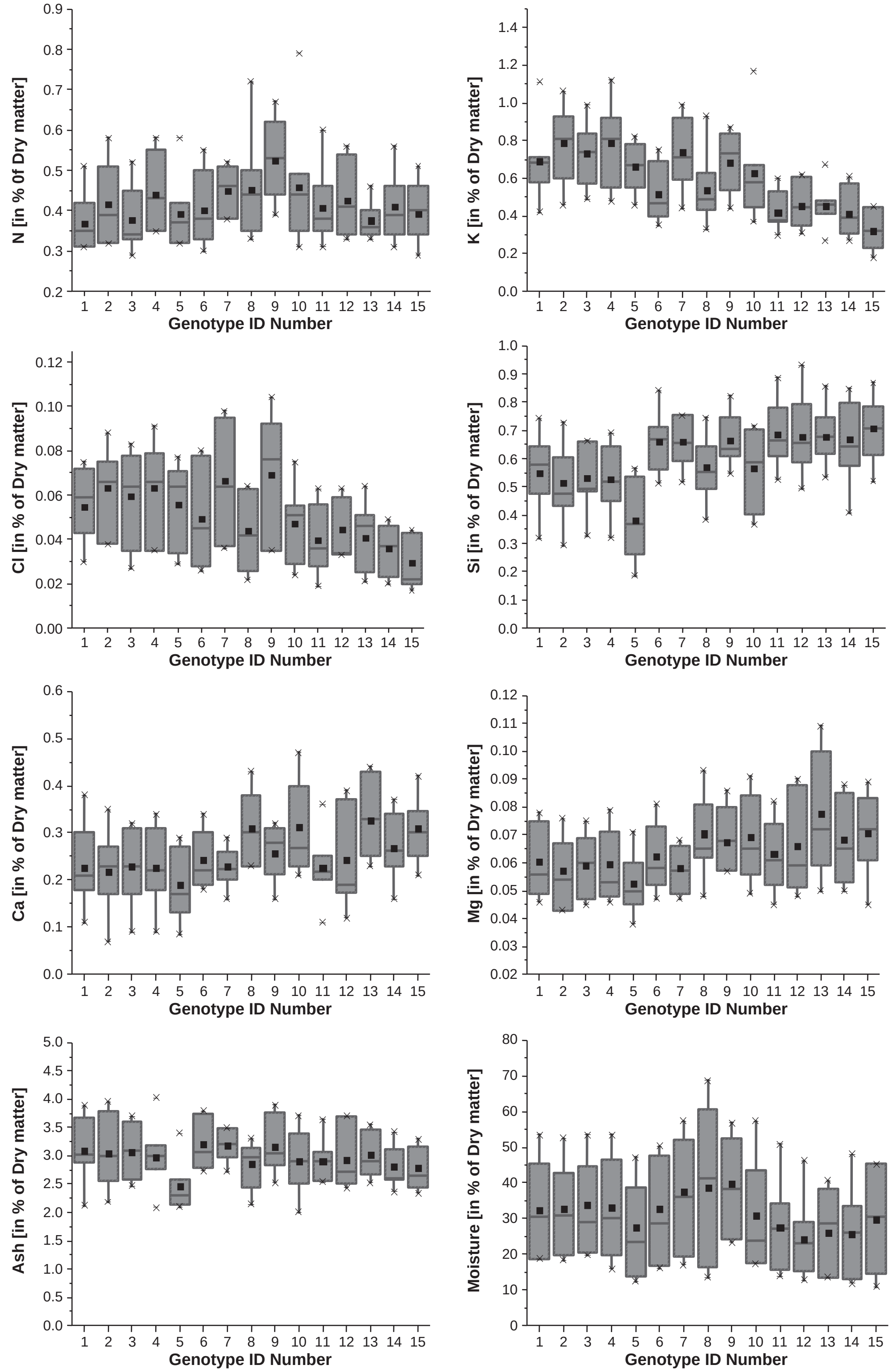

Fig. 2. Box plots for the mineral concentrations, ash and moisture contents in the dry matter of different genotypes in the years 2004 to 2010, with the mean values (indicated by the black square) calculated over the productive growth period. Vertical lines represent the minimum and maximum values, boxes indicate the upper and lower quartiles, stars on both sides of the boxes indicate the outlier values and the horizontal lines in the boxes show the median for each genotype from 2004 to 2010. (100 mg/kg = 0.01%).

The regression analysis shows that the effect of harvest date was significant for K, Cl, Mg and ash. There was a trend for mineral (K, Cl, Mg) and ash concentrations to decrease with delay in harvest. When harvesting was delayed from January to February, a significant decrease was observed in all genotypes only for Mg concentrations, with the highest decrease of 7% in *M. sacchariflorus* (Table 6). By further delaying harvesting from February to March, the concentrations of K decreased by 25% and the concentration of Cl by 27% for Sin-15. For the same genotype, when harvest date was delayed again from March to April the concentration of K decreased by 15% and the concentration of Cl

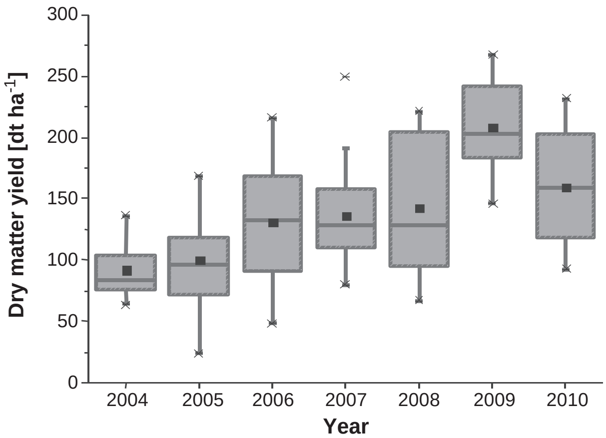

Fig. 3. Box plots of dry matter yield in the years 2004 to 2010, with the mean value (indicated by the black square) calculated for all genotypes. Vertical lines represent the minimum and maximum values, boxes indicate the upper and lower quartiles and the horizontal lines in the box show the median for each genotype from 2004 to 2010. Stars on both sides of the boxes indicate the outlier values (data taken from Gauder et al. [26]). (1 t = 10 dt).

by 12% (Table 6). In total, the concentrations of K and Cl decreased more rapidly for *M. sinensis* compared to the other genotypes. However, delaying harvest until April contributed little to further quality improvement of the biomass. The data from the earliest harvest date (January) were taken as baseline data.

4. Discussion

Supply of biomass with stable quality characteristics is critical for running biomass combustion units, mainly to avoid mechanical problems during combustion and to reduce long-term operational costs. In the future, stability in biomass quality may also become relevant for farmers if the market requires biomass of defined quality. Quality standards already exist for wood pellets and will probably be developed for other solid biofuels. If the criteria of the Pellet Norm EN 14961-2:2012 A2 are applied to the results of this study, the N concentrations of all genotypes would exceed the standard limit of 0.5% in DM in the years 2004 and 2005. In all other years the N values would fall below the given limit. For the farmer it will be important to know how miscanthus fields can be managed to meet the quality standards over the whole productive growth period.

The results of this study lead to the conclusion that the variation in biomass constituents can be divided into two main categories: a) controllable variation, predominantly: time of harvesting and appropriate genotype selection; b) uncontrollable variation, predominantly: weather conditions and aging effects. In each category the results reveal a different response of quality characteristics to these factors. For example, the concentration of leachable elements (K, Cl, Mg) and also the stability of these quality parameters was strongly affected by harvesting time alone, whereas the concentration of non-leachable elements (N, Si, Ca) was influenced by the interaction of harvest time and rainfall.

In the following sections, the concentrations and stability of *leachable elements* and the underlying mechanisms will be discussed first. The concentrations of leachable elements are mainly dependent on leaching processes. However, the concentration of some of these elements is also dependent on plant internal relocation mechanisms. This is true for K [3,27] and Si [28].

Indeed, it was observed that the concentrations of leachable elements in the biomass were predominantly affected by stem thickness and time of harvesting. Up to 27% of the variation in leachable elements can be explained by harvest time, the rest being due to the genotype effect and the interaction of harvest time with the uncontrollable variable, aging.

Contrary to the expectations, there was no general correlation between leachable elements and amount of rainfall. Correlations with annual rainfall were only significant for Cl. The only exception was the year 2007 when the Cl concentration was lowest despite low rainfall. However, even for Cl there was no correlation with the rainfall in the months critical to leaching, i.e. December to February.

According to the authors' own observations in recent years, early snowfall is a strong factor in instability. Early snow falls on the plants while they still have a high proportion of leaves, therefore leading to lodging. This has the effect of increasing the moisture content of the biomass and a higher chance it being contaminated by soil. Nazli and Lewandowski [29] observed that the effect of lodging was more prominent in thin-stemmed genotypes. Similarly, in the present study the variation due to harvest time was higher in thin-stemmed genotypes with low lignin content (unpublished data) than in thick-stemmed genotypes. This was true for all years.

Stem diameter showed a positive correlation with K and Cl concentrations. In addition, the low K and Cl concentrations in thin-stemmed genotypes indicate that stem thickness played a key role in decreasing the concentrations of leachable elements. Smaller stem diameter increases the leaching rate [24]. This led to lower concentrations of leachable elements and therefore higher combustion quality of biomass from thin-stemmed genotypes. However, the thin-stemmed genotypes have lower dry matter yield. Also, it was observed that the variability was high for Cl concentrations in thin-stemmed genotypes. This should not be overlooked because the extent of variability in these values exceeded the standard limits (Pellet Norm EN 14961-2:2012 B). Thus in this study stability is not seen as a single parameter but in the context of variability in relation to standard limits.

The statistical analysis showed a significant aging effect. As a steady increase in dry matter yield was observed from the years 2004 to 2009 (Fig. 3), it was hypothesized that plant age can influence quality characteristics through the dilution effect. The dilution effect is the removal of nutrients with the increase in dry matter yield over the productive growth period. In an attempt to explain the aging effect, a correlation analysis was performed between dry matter yield and leachable elements. It was assumed that an increase in dry matter yield would decrease the concentrations of leachable elements through the dilution effect. However, the results did not show this pattern and therefore this assumption could not be confirmed.

For *unleachable elements* it was found that the interaction of controllable variables and uncontrollable variables had a pronounced effect on stability. Up to 8% of the variation in unleachable elements was due to harvest date–rainfall interaction. The longer the harvest is delayed, the more stem breakage and leaf loss occur [16,30]. According to the weather data, the rainfall in the period October to December in the years 2004 to 2010 varied from 14 to 30% of the annual rainfall. Thus, it can be assumed that rainfall contributed to instability by affecting the percentage of leaf detachment and stem damage per plot for harvesting dates later than December. In addition, the negative correlation between rainfall and leaf-to-stem ratio (data not shown here) also indicates that higher rainfall contributed to a decrease in leaf-to-stem ratio in years with delayed harvest.

The concentration of unleachable elements in the harvested biomass is mainly dependent on the leaf-to-stem ratio and relocation mechanism [3]. In the current study, the concentration of unleachable elements was high in leaves compared to stems (data not shown here). Therefore, the variability in Si concentrations can be explained by

Fig. 4. Box plots for mineral concentrations, ash and moisture contents in the dry matter of different miscanthus genotypes for the years 2004 to 2010, with the mean value calculated (indicated by the black square) for all genotypes. Vertical lines represent the minimum and maximum values, boxes indicate the upper and lower quartiles, stars on both sides of the boxes indicate the outlier values and the horizontal lines in the boxes show the median for all genotypes in each year. (100 mg/kg = 0.01%).

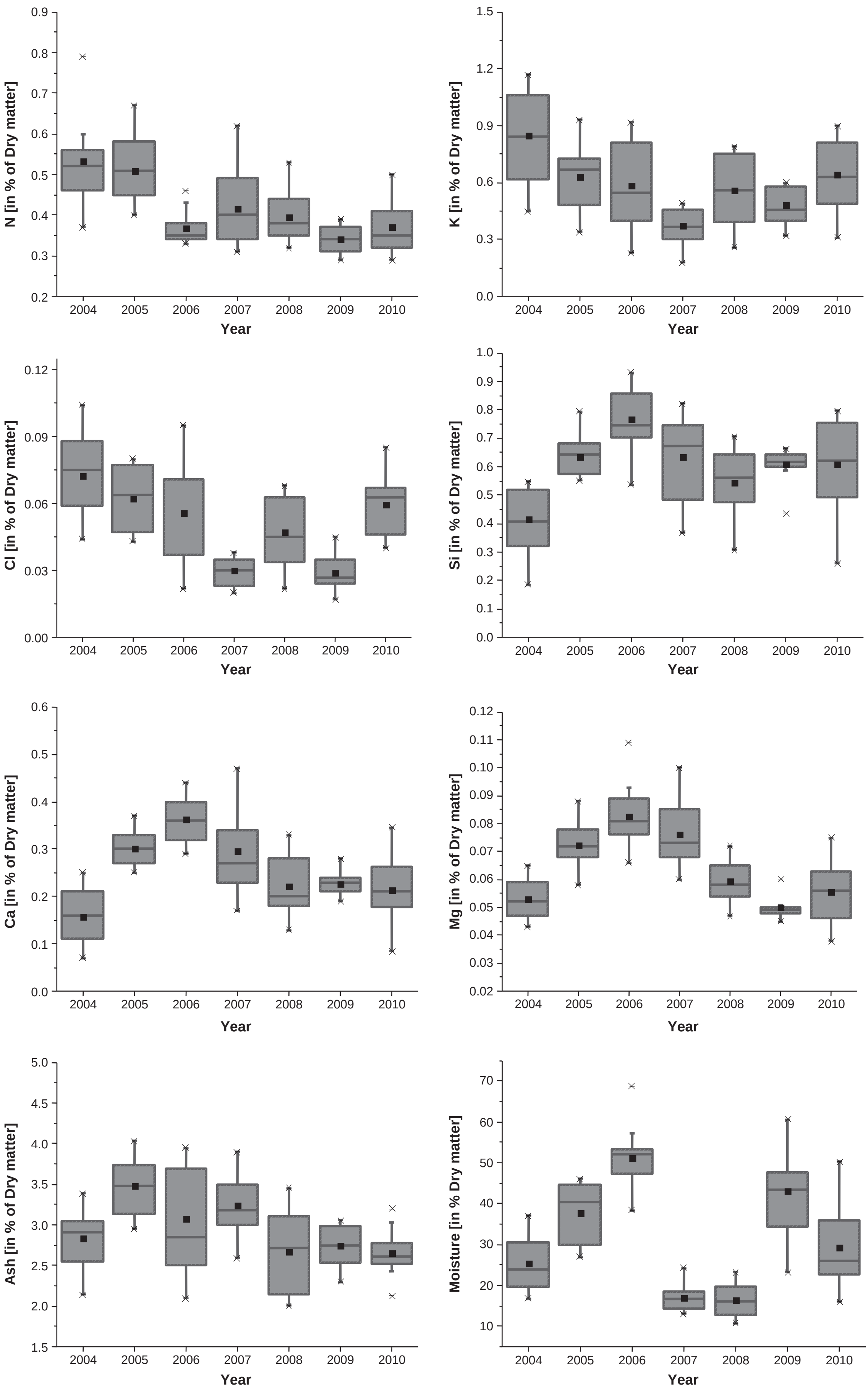
N [in % of Dry matter]
K [in % of Dry matter]
Cl [in % of Dry matter]
Si [in % of Dry matter]
Ca [in % of Dry matter]
Mg [in % of Dry matter]
Ash [in % of Dry matter]
Moisture [in % Dry matter]
2004
2005
2006
2007
2008
2009
2010
Year

Table 4
Coefficients of variation for quality characteristics of different *Miscanthus* genotypes.

Genotype	N	K	Cl	Si	Ca	Mg	Ash	Moisture	Dry matter yield
Gig-1	0.19	0.28	0.29	0.23	0.36	0.21	0.18	0.42	0.29
Gig-2	0.22	0.24	0.27	0.26	0.37	0.21	0.19	0.38	0.25
Gig-3	0.20	0.21	0.30	0.21	0.34	0.18	0.14	0.37	0.23
Gig-4	0.20	0.26	0.27	0.23	0.35	0.20	0.18	0.42	0.26
Sac-5	0.21	0.19	0.33	0.34	0.37	0.19	0.18	0.44	0.25
Sin-H6	0.21	0.27	0.38	0.15	0.23	0.18	0.13	0.40	0.39
Sin-H7	0.13	0.25	0.34	0.12	0.18	0.13	0.08	0.38	0.35
Sin-H8	0.27	0.33	0.32	0.19	0.23	0.20	0.13	0.54	0.32
Sin-H9	0.18	0.23	0.32	0.13	0.21	0.16	0.15	0.30	0.43
Sin-H10	0.32	0.38	0.39	0.22	0.29	0.20	0.19	0.45	0.23
Sin-11	0.22	0.23	0.35	0.16	0.30	0.19	0.12	0.43	0.24
Sin-12	0.20	0.25	0.35	0.20	0.40	0.25	0.18	0.43	0.55
Sin-13	0.11	0.24	0.33	0.14	0.24	0.25	0.13	0.39	0.34
Sin-14	0.19	0.26	0.34	0.21	0.24	0.20	0.12	0.47	0.37
Sin-15	0.18	0.27	0.35	0.15	0.20	0.19	0.13	0.47	0.58

variation in leaf-to-stem ratio. For example, in 2005 *M. sacchariflorus* had a high leaf-to-stem ratio but in 2007 it was on the low side and during that period Si concentration also followed the same pattern. Furthermore, the stem damage due to delayed harvest and rainfall can subsequently lead to the relocation mechanism ceasing. The relocation mechanism, especially for N, continues throughout winter [31] until frost kills the aboveground stems. This mechanism is very important from a quality viewpoint because miscanthus, being a perennial crop, translocates macronutrients, in particular N, into rhizomes before harvest [3,31], thus decreasing their concentrations in the harvested biomass. The relocation mechanism depends on the time of the first frost and the mean annual temperature. Therefore, the variability in N concentration can be explained by the effect of the weather on the relocation mechanism. For example, in 2004 and 2005 the concentration of N was high mainly due to the low autumn temperatures (Nov–Dec) combined with early frosts, which left less time for relocation to the rhizomes. Thus the combined effect of harvest date and weather events such as rainfall, first frost and temperature caused instability in unleachable elements through a change in leaf-to-stem ratio and the effect on the relocation mechanism.

From the results discussed above, it can be concluded that appropriate harvest time is the most important factor for stability in combustion-relevant quality characteristics, followed by selection of suitable genotype [8,12,32–34]. In this study, harvesting in March rather than January improved the biomass quality significantly for all genotypes without much compromise on yield. Further delay in harvesting had a non-significant effect on biomass quality improvement but there was a significant loss in biomass yield. Therefore, March is the appropriate harvest time for all genotypes to deliver optimal and stable biomass quality and yield in partially continental climates. It would be interesting to know whether higher rainfall over winter would allow an earlier harvest with higher yields and improved biomass qualities. Therefore, further research will be performed on quantifying the relation between rainfall, yield and quality.

Table 5
Combined effect of significant variables (harvest date, rainfall and aging) on mineral and ash concentration of the dry biomass.

Genotype	Decrease in mineral concentration (%)					
	Harvest date–rainfall interaction			Harvest date–aging interaction		
	N	Si	Ca	K	Mg	ash
Gig-1 to Gig-4	6	3	7	24	26	14
Sac-5	6	4	8	28	29	18
Sin-H6	6	2	6	37	24	13
Sin-H7	5	2	6	24	26	13
Sin-H8	5	3	4	35	21	15
Sin-H9	4	2	5	27	22	13
Sin-H10	5	3	4	29	21	15
Sin-H11	6	2	6	47	24	15
Sin-H12	5	2	6	43	22	15
Sin-H13	6	2	4	42	19	14
Sin-H14	6	2	5	45	22	15
Sin-H15	6	2	4	72	21	15

Table 6
Effect of delayed harvest on mineral and ash concentration of the dry biomass (parameters with significant impact are shown).

Genotype[a]	Delay in harvest dates	Decrease in mineral concentration (%)			
		K	Cl	Mg	Ash
Gig-1 to Gig-4	Jan to Feb	1	1	6	1
	Feb to Mar	8	12	15	3
	Mar to Apr	3	4	5	1
Sac-5	Jan to Feb	1	1	7	1
	Feb to Mar	10	13	16	5
	Mar to Apr	3	4	5	1
Sin-H7	Jan to Feb	1	1	6	1
	Feb to Mar	9	10	14	3
	Mar to Apr	3	3	5	1
Sin-H10	Jan to Feb	1	2	5	1
	Feb to Mar	11	16	11	4
	Mar to Apr	4	5	3	1
Sin-13	Jan to Feb	1	2	4	1
	Feb to Mar	15	19	10	4
	Mar to Apr	6	6	3	1
Sin-15	Jan to Feb	2	3	5	1
	Feb to Mar	25	27	11	4
	Mar to Apr	15	12	3	1

[a] Genotypes with high dry matter yield except for Sin-15 were selected (Sin-15 had lowest yield).

5. Conclusions

Both leachable and unleachable elements relevant for biomass combustion quality are most strongly influenced by harvest time and selection of miscanthus genotype. Weather and aging have smaller effects.

Farmers can control variation through appropriate harvesting time and genotype selection as part of on-field quality management. This study suggests March as an appropriate harvesting time for all genotypes in partially continental climates for optimal biomass quality and yield. However, every year harvesting time should be decided based on the prevailing weather conditions. The results of this study suggest that *M. sacchariflorus* can be selected for the production of solid biofuels on account of its comparatively high yield potential and more favorable biomass qualities, in particular low ash, Ca, Si, Mg concentrations.

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2.2. Biomass Composition and Ash Melting Behaviour of Selected Miscanthus Genotypes in Southern Germany.

Publication-2

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This study aims to monitor the ash melting behaviour of biomass over a period of eight years from 15 miscanthus genotypes grown at Ihinger Hof research station, near Stuttgart, Germany and explain the relationship between biomass composition and ash melting behaviour. To do this, biomass ash samples were prepared and subjected to different heating treatments (800-1100 °C) and categorized into 4 ash-fusion classes based on microscopic observations. The outcome of the study reveals that the sintering tendencies were lower for the *M. sinensis* genotypes than for all other genotypes but these genotypes are low yielding. Furthermore, *M. sinensis* hybrids and *M. sacchariflorus* performed better than *M. x giganteus* genotypes at higher temperatures. The correlation analysis between biomass composition and ash melting behaviour suggests that ash, K and Cl contents play a key role in inducing ash-related problems.

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Biomass composition and ash melting behaviour of selected miscanthus genotypes in Southern Germany

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HIGHLIGHTS

- Contents of ash, K and Cl are good indicators of biomass combustion quality.
- Optimization of biomass composition through crop management strategies can be a good tool to improve the ash melting behaviour.
- Sintering tendencies were lower for the thin stemmed *M. sinensis* genotypes than for all other genotypes.

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ABSTRACT

Optimization of biomass composition can lead to reduction of both ash-related problems such as slagging, fouling and corrosion as well as emissions (NO_x, SO_x) when combusted. This study aims to monitor the ash melting behaviour of biomass over a period of eight years from 15 miscanthus genotypes grown at Ihinger Hof research station, near Stuttgart, Germany and explain the relationship between biomass composition and ash melting behaviour. To do this, biomass ash samples were prepared and subjected to different heating treatments (800–1100 °C) and categorized into 4 ash-fusion classes based on microscopic observations. The outcome of the study reveals that the sintering tendencies were lower for the *Miscanthus sinensis* genotypes than for all other genotypes but these genotypes are low yielding. Furthermore, *M. sinensis* hybrids and *Miscanthus sacchariflorus* performed better than *Miscanthus* × *giganteus* genotypes at higher temperatures. The correlation analysis between biomass composition and ash melting behaviour suggests that ash, K and Cl contents play a key role in inducing ash-related problems.

1. Introduction

The depletion of fossil fuels together with their atmospheric carbon footprint and global climate change necessitate the use of sustainable energy resources. Lignocellulosic biomass such as crop residues, dedicated energy crops have the potential to deliver bioenergy without any direct competition with food [1]. Therefore, such feedstocks can be used to supply alternative fuels for heat and electricity generation through combustion or for the production of 2nd generation ethanol and biogas. In Europe, the rhizomatous C4 grass miscanthus has emerged as a strong candidate for biomass-based energy production due to its potential to deliver high biomass yields under low input conditions. In addition, the long-term productivity of the perennial crop miscanthus makes it one of the preferred choices among energy crops mainly because soil carbon is stored and nutrients are recycled efficiently within the plant system [2]. According to the statistics from the years 2006–2008, the cultivation area for miscanthus in Europe is 38,300 ha [3]. This is already more than the 35,018 ha under short rotation coppice (willow, poplar) [3]. Miscanthus is mainly used to provide solid fuel to power plants with many smaller-scale areas supplying biomass for domestic heating plants.

Various miscanthus genotypes have been tested in Europe to select those best suited for biomass production in terms of yield [4,5] and fuel quality [2]. Currently, power is mainly produced from miscanthus through direct combustion of its biomass. However, the efficiency of this power generation depends on the composition of the solid fuel being combusted. During the bioconversion processes, high moisture content of the harvested biomass leads to higher energy input for drying prior to combustion and low heating value. Similarly, high ash content and/or low ash-fusion temperature pose technical issues through deposition, sintering, fouling, slagging and corrosion. The latter can damage

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boilers and increase maintenance costs. The ash-forming elements potassium (K), phosphorus (P), chloride (Cl), silicon (Si), calcium (Ca) and sulphur (S) contribute to the above-mentioned ash-related mechanical problems. The main quality problems caused by combustion of poor quality biomass include:

– corrosion and fouling (K, Cl, ash)
– low ash melting point (K, S, Cl, Si)
– emissions (N, S)

The content of Ca and Mg has positive effect on ash melting behaviour. Any increase in Ca and Mg content will lead to improved ash melting behaviour [15].

To evaluate the effect of chemical composition of biomass, several indices have been developed to estimate the rate of fouling and slagging in biomass-based combustion. However, the most widely used are alkali indices and an ash composition index [6]. These indices provide useful information to describe the relationship between fuel composition and ash melting behaviour. The value of alkali index higher than one is an indication of increased fouling rate during the combustion process [7].

The importance of alkali index as an indicator of fouling rate signifies that K and Na contents are highly critical. This is mainly due to their tendency to react at relatively low temperature during the combustion process [8].

As Na content is very low in miscanthus biomass, K is of main relevance in ash melting behaviour. At elevated temperatures, biomass with high K, Cl, Si and S contents lead to formation of low-fusion-temperature silicates and sulphates, which decrease the efficiency of the thermal bioconversion mechanism through bed sintering and deposition on heater tubes [7,9]. Therefore, for biomass-based energy production to be economical and environmentally benign, it is important to address the above-mentioned mechanical problems. There are various possibilities for improving the efficiency of power plants. These include ensuring the supply of good quality biomass over the years through different crop management practices such as selection of appropriate genotypes. Apart from crop management practices technical upgrading of boilers could also help to counter the combustion related problems.

Through the appropriate combination of management practices, the composition of solid fuel – especially alkali metal and chloride contents – can be optimized. For example, leaching of minerals especially Cl by rain [10], appropriate harvesting time and fertilization application can all contribute significantly towards improvement of ash melting behaviour [11]. However, in current study, the focus is mainly on selection of genotypes with the potential to deliver high biomass quality for combustion over the years. It is important to state that technical upgrading of power plants does not fall into the remit of this study. This paper aims to monitor the ash melting behaviour of the biomass from selected miscanthus genotypes over the years and identify the factors responsible. As the data about biomass composition is already published, therefore in current study the effect of biomass composition on ash melting behaviour will only be evaluated.

Biomass samples from 15 miscanthus genotypes harvested in the years 2004–2011 were subjected to mineral analysis, which is already published [2]. Based on published information about dry matter yield and biomass composition, ash melting behaviour was performed for all genotypes for selected years (2004, 2008 and 2011) and selected genotypes for all years (2004–2011). Biomass ash samples from each genotype were prepared at low temperature (550 °C) and then heated to temperatures between 800 and 1100 °C to monitor the ash melting behaviour, the ash samples were then categorized by microscopic analysis into 4 ash-fusion classes [10]. The data was collected and analysed by following Proc Mixed procedure. In addition, correlation analysis was performed between biomass composition and ash melting behaviour.

2. Material and methods

2.1. Biomass samples

The miscanthus biomass samples used in this experiment were collected from a field trial established in 1997 under the European Miscanthus Improvement (EMI) project at Ihinger Hof (48°40′N: 09°00′E) in south Germany. The 15 miscanthus genotypes planted in this project are: 4 *Miscanthus × giganteus* (Gig), 1 *Miscanthus sacchariflorus* (Sac), 5 *Miscanthus sinensis* (Sin) and 5 *M. sinensis* hybrids (Sin-H). During the years 2004–2011, N fertilization was applied at the rate of 60 kg N ha^{-1} a^{-1} (NH_4NO_3), P 50 kg ha^{-1} a^{-1} (P_2O_5), K (K_2SO_4) at the rate of 144 kg ha^{-1} a^{-1}. It is important to state that in practice, fertilization is not common but in this field trial nutrient fertilization was carried out because the aim of this field trial was to assess the yield potential and long term productivity of different miscanthus genotypes. The fertilization recommendations were made based on the nutrient removal rate through harvested biomass.

The soil pH ranged from 7.2 to 7.7. The N content of soil decreased along the soil profile from 0.20% (30 cm) to 0.04% (90 cm), P content decreased from 14.1 mg/100 g (30 cm) to 1.3 mg/100 g (90 cm) and K content decreased from 31.3 mg/100 g (30 cm) to 1.3 mg/100 g (90 cm). For further details on genotypes and field trial performance see [2].

Biomass samples were collected from an area of 0.5–1.5 m^2 each year (harvest within the period January to April) using manual cutters. The harvesting time (from early to late harvest) was varied with the aim to assess the response of different genotypes to different harvesting times in terms of yield and quality. Samples were dried, chopped, ground and then passed through a 1-mm sieve. The samples were used for mineral analysis [2] and preparation of ash samples to monitor ash melting behaviour. Based on dry matter yield and biomass composition analysis information [2], for current study biomass samples from the years 2004 to 2011 were divided into two groups. In the first group, all 15 genotypes were analysed for 3 selected years (2004, 2008 and 2011). Years were selected based on considerable annual variation in terms of dry matter yield and biomass composition. In the second group, the most promising genotypes (Gig-2, Sac-5, Sin-H7 and Sin-13) in terms of dry matter yield and with contrasting biomass quality (based on already published data [2] were selected for ash melting behaviour for all years (2004–2011). The sample handling, preparation and heating treatments were identical for both groups.

2.2. Laboratory analysis

Dried biomass samples were analysed in the laboratory for mineral (N, P, K, Na, Cl, Si, Ca, Mg) and ash content. The analysis methods are listed in Table 1.

2.3. Heating of the ash samples

Low-temperature ash samples were prepared by heating 10-g biomass samples in ceramic crucibles in an electric muffle furnace at 550 °C for 4 h. From each low-temperature ash sample approximately 100 mg was transferred to a separate ceramic combustion boat. These were placed in an electric muffle furnace, which was heated at an average rate of 10 °C min^{-1} until the required heating temperature was achieved. All the low-temperature ash samples were subjected to 4 different temperature treatments of 800 °C, 900 °C, 1000 °C and 1100 °C. After two hours, the combustion boats

Table 1
Combustion parameters and analytical methods adopted during laboratory analysis [10].

Parameter	Method/instrument used
N	Dumas principle (Elementar Analysensysteme GmbH, Hanau)
K, Ca, Na	Flame photometer (ELEX 6361, Eppendorf AG, Hamburg, Germany), after microwave (MARS 5, CEM) digestion at 120–180 °C and pressure (24.16 bar) for 40 min with HNO_3 (65%) and addition of H_2O_2
Cl	HPLC (high-performance liquid chromatography) (ICS 2000, Dionex Corporation, Sunnyvale, California, US)
Mg	Atomic absorption spectrometer (220 FS, Varian), after digestion, lanthanum solution was added to the extracts
Si	ICP-OES (Vista Pro, Varian Inc., Palo Alto, California, US)
P	Atomic absorption spectrometry (Spectrophotometer PM6 W, Carl Zeiss AG, Oberkochen, Germany), after microwave digestion with HNO_3 and addition of H_2O_2, extract was amended additionally by ammonium vanadate and ammonium molybdate
Ash	Muffle furnace at 550 °C for 4 h

were removed and placed into desiccators to allow them to cool before further analysis.

2.4. Ash-fusion classes

After cooling, the ash samples were analysed under a stereo microscope (Zeiss Stemi 2000-C, Carl Zeiss AG, Oberkochen, Germany) at magnifications up to 40×. The ash samples were classified into 4 ash-fusion classes [10] based on their macroscopic structure and appearance, grading them from 1 (no sintering) to 5 (completely molten). The ash-fusion classes with a description of microscopic observations are listed in Table 2.

2.5. Weather data

Weather data (2004–2011) was collected from a weather station 100 m away from the experimental site. During the period 2004–2011 the mean annual rainfall was 687.9 mm and the mean annual temperature was 9.03 °C.

2.6. Statistical analysis

The statistical analysis was conducted following the Proc Mixed procedure of Statistical Analysis System (SAS). Analysis of variance for all factors and their interactions was carried out using a mixed model. The model included 'Year', 'Heating treatment', 'Genotype', 'Ash', 'Calcium', 'Magnesium', 'Silicon', 'Chloride' and 'Potassium' as main effects. The interactions were also tested and Heating treatment ∗ Genotype interaction had significant effect on ash melting behaviour. To obtain the normality and homogeneity, the data were log-transformed before statistical analysis. The models were compared and selected based on Akaike's Information Criterion (AIC). The difference in ash melting behaviour between genotypes and between years was tested at a significance level of 5%. The effect of the biomass composition on ash melting behaviour was determined through the Proc CORR procedure of SAS. This refers to the composition of the biomass, not to the ash composition.

To evaluate the consistency of the different genotypes over the years the co-efficient of variation was calculated based on mean ash melting behaviour over the years and standard deviation. The data presented in the figures and tables are without log transformation.

3. Results

3.1. Ash melting behaviour

The statistical analysis showed that the year, the different heating treatments, the genotype effect and the interaction of Heating treatment ∗ Genotype had a significant influence on the ash melting behaviour (Table 3). In addition, the effect of biomass composition also had significant effect on ash melting behaviour.

In the first group of biomass samples, the ash melting behaviour of all genotypes was analysed, but for selected years only, with the aim of comparing the all 15 genotypes at different heating treatments (800 °C, 900 °C, 1000 °C, 1100 °C). For the selected years (2004, 2008, 2011), most of the genotypes showed no sintering at 800 °C, except for 2011, where partial sintering was recorded for biomass samples of Gig-2, Gig-3, Sac-5 and Sin-H10. In addition, the Sin-H7 and Sin-H9 samples showed partial sintering for 2004 and 2011 at this temperature (800 °C). The sintering tendencies for all ash samples were comparatively low for the year 2004 at heating treatment 900 °C (Table 4). For this year, the ash samples from Sin-H7 and Sin-H9 were molten at 900 °C, whereas for all other genotypes 'no sintering' (Sac-5, Sin-15) to 'partial sintering' (Gig-1 to Gig-4, Sin-H6, Sin-H8, Sin-H10, Sin-11, Sin-13) and strong sintering (Sin-12, Sin-14) was recorded (Table 4). For the years 2008 and 2011, all biomass samples from *M. sinensis* genotypes showed 'no sintering' to 'partial sintering' at heating treatment 900 °C. For the year 2004, at heating treatment 1000 °C, all samples were molten except for Sin-15. For 2008 and 2011, *M. sinensis* genotypes showed slight sintering to molten at 1000 °C. At heating treatment 1100 °C for the years 2004 and 2008, all samples were molten except for Sin-15 in the year 2008. For the year 2011, at 1100 °C all the samples were molten except for Sin-H6,

Table 3
Type 3 tests for the significance of the main effects and their interactions (Year, Heating treatment, Genotype, Heating treatment ∗ Genotype) for the ash melting behaviour (for all genotypes and selected years).

Effect	*F*-value	Pr > *F*
Year	36.18	<.0001
Heating treatment[a]	42.08	<.0001
Genotype	130.34	<.0001
Heating treatment ∗ Genotype	11.2	<.0001

[a] Heating treatments (800 °C, 900 °C, 100 °C, 1100 °C).

Table 2
Ash-fusion temperature and ash-fusion classes along with microscopic observations for each ash fusion class [10].

Ash-fusion temperature	Ash-fusion classes	Microscopic observations
Initial temperature	No sintering (1–2)	Particles are arranged in loose layers, spatula can move through without any resistance, shiny surfaces with tiny molten vesicles
Softening temperature	Partially sintered (2–3)	Particles start becoming compact through strong adhesive forces, still easy to disintegrate, produces crispy sound when spatula passes through, larger molten vesicles on the surface
Hemisphere temperature	Highly sintered (3–4)	Difficult to disintegrate, most of the area covered with larger molten vesicles. Organogenic material also visible in some parts
Flow temperature	Molten (4–5)	Particles are completely molten, manual disintegration is not possible, no organogenic material visible

Table 4
Ash-fusion classes of 15 miscanthus genotypes at different temperatures for the years 2004, 2008 and 2011.

Genotype	2004				2008				2011			
	800 °C	900 °C	1000 °C	1100 °C	800 °C	900 °C	1000 °C	1100 °C	800 °C	900 °C	1000 °C	1100 °C
Gig-1	1.3 ± 0.08	2.0 ± 0.50	4.2 ± 0.39	5.0 ± 0.00	1.2 ± 0.13	3.2 ± 0.25	4.4 ± 0.18	5.0 ± 0.06	1.9 ± 0.20	3.8 ± 0.07	4.2 ± 0.31	5.0 ± 0.02
Gig-2	1.3 ± 0.11	2.6 ± 0.52	4.2 ± 0.39	5.0 ± 0.00	1.1 ± 0.10	3.7 ± 0.39	4.4 ± 0.32	5.0 ± 0.03	2.1 ± 0.14	3.7 ± 0.21	4.0 ± 0.29	5.0 ± 0.00
Gig-3	1.5 ± 0.15	2.7 ± 1.13	4.8 ± 0.27	5.0 ± 0.00	1.3 ± 0.13	3.7 ± 0.18	4.3 ± 0.25	5.0 ± 0.01	2.2 ± 0.03	3.9 ± 0.04	4.5 ± 0.10	5.0 ± 0.00
Gig-4	1.5 ± 0.46	2.5 ± 1.12	4.4 ± 0.42	5.0 ± 0.02	1.6 ± 0.32	3.7 ± 0.37	4.9 ± 0.25	4.9 ± 0.10	1.9 ± 0.17	3.3 ± 0.35	3.9 ± 0.08	5.0 ± 0.00
Sac-5	1.3 ± 0.15	1.6 ± 0.32	4.5 ± 0.46	5.0 ± 0.00	1.3 ± 0.20	3.1 ± 1.40	4.1 ± 0.86	4.8 ± 0.29	2.1 ± 0.52	3.5 ± 0.32	4.2 ± 0.11	5.0 ± 0.00
Sin-H6	1.7 ± 0.28	2.8 ± 0.72	4.1 ± 0.20	4.9 ± 0.31	1.8 ± 0.32	2.6 ± 1.04	4.6 ± 0.31	4.9 ± 0.25	1.7 ± 0.22	2.8 ± 0.50	3.7 ± 0.86	3.8 ± 0.89
Sin-H7	2.3 ± 0.43	4.2 ± 0.10	4.9 ± 0.12	4.8 ± 0.21	2.3 ± 0.08	3.6 ± 0.05	4.4 ± 0.17	5.0 ± 0.00	2.9 ± 0.17	3.8 ± 0.10	4.3 ± 0.19	5.0 ± 0.00
Sin-H8	1.8 ± 0.56	2.9 ± 0.16	4.4 ± 0.02	4.9 ± 0.29	1.5 ± 0.02	2.6 ± 0.46	3.7 ± 0.35	5.0 ± 0.00	1.7 ± 0.07	3.0 ± 0.25	4.0 ± 0.24	4.9 ± 0.15
Sin-H9	2.7 ± 0.30	4.2 ± 0.24	5.0 ± 0.00	5.0 ± 0.00	1.7 ± 0.20	2.5 ± 0.50	3.8 ± 0.38	5.0 ± 0.12	2.3 ± 0.21	3.3 ± 0.66	4.0 ± 0.15	4.9 ± 0.10
Sin-H10	1.8 ± 0.52	2.3 ± 0.41	4.6 ± 0.17	5.0 ± 0.21	1.7 ± 0.14	3.1 ± 0.10	4.0 ± 0.03	5.0 ± 0.29	2.0 ± 0.32	2.7 ± 0.47	4.1 ± 0.04	5.0 ± 0.08
Sin-11	1.8 ± 0.31	2.9 ± 0.40	4.3 ± 0.27	4.2 ± 0.33	1.2 ± 0.10	1.1 ± 0.18	2.8 ± 0.23	4.8 ± 0.18	1.9 ± 0.07	2.2 ± 0.14	3.2 ± 0.79	4.1 ± 0.34
Sin-12	1.5 ± 0.28	3.1 ± 0.32	4.9 ± 0.20	4.9 ± 0.30	1.2 ± 0.17	1.3 ± 0.28	3.3 ± 0.70	4.9 ± 0.10	1.8 ± 0.15	2.2 ± 0.17	3.8 ± 0.44	4.0 ± 0.07
Sin-13	1.4 ± 0.10	2.6 ± 0.24	4.4 ± 0.39	5.0 ± 0.09	1.2 ± 0.06	1.4 ± 0.08	2.2 ± 0.32	5.0 ± 0.00	1.8 ± 0.13	2.9 ± 0.43	4.0 ± 0.98	4.8 ± 0.22
Sin-14	1.9 ± 0.14	3.1 ± 0.26	4.5 ± 0.42	5.0 ± 0.17	1.2 ± 0.02	1.3 ± 0.07	1.7 ± 0.23	4.8 ± 0.18	1.6 ± 0.13	2.1 ± 0.22	2.0 ± 0.64	3.1 ± 1.01
Sin-15	1.2 ± 0.12	1.9 ± 0.19	3.9 ± 0.55	4.7 ± 0.15	1.2 ± 0.16	1.7 ± 1.14	2.1 ± 1.62	3.2 ± 1.74	1.4 ± 0.03	1.5 ± 0.05	1.3 ± 0.36	2.1 ± 0.57

Sin-14 and Sin-15. Overall, sintering tendencies for the years 2008 and 2011 were comparatively lower for the *M. sinensis* genotypes than for all other genotypes (Table 4). Sin-15 belonged to the best performing genotypes at all heating treatments in terms of ash melting behaviour.

3.2. Ash melting behaviour over the years

To evaluate the ash melting behaviour over the years (2004–2011), the high yielding miscanthus genotypes were selected. Sac-5 has the lowest ash content in comparison to Gig-2, Sin-H7 and Sin-13 (Fig. 1). No visible sintering was observed at 800 °C except for Sin-H7, which also had the highest dry matter ash content. At 900 °C, strong sintering was observed in Gig-2 and Sin-H7, Sac-5, whereas Sin-13 showed only partial sintering. The effect of change in heating treatment from 900 °C to 1000 °C was lower in Sin-13 than in all other genotypes (Fig. 1). The change in temperature from 1000 °C to 1100 °C had a significant effect on ash melting behaviour of all genotypes. At 1100 °C the ashes of all the genotypes were molten.

The co-efficient of variation (CV) for selected genotypes was calculated over the years 2004–2011. At 1000 °C heating treatment, Gig-2 and Sin-H7 showed relatively consistent ash melting behaviour in comparison to other genotypes. Despite the inability to meet the Pellet Norm EN 14961-2: A1, all the genotypes except Sin-13 showed consistent ash melting behaviour over the years at 1100 °C (Table 5). The value of CV indicates that overall Sin-H7 showed consistent behaviour at all heating treatments, whereas Sin-13 showed inconsistent behaviour. The results indicate that at higher temperatures, the ash melting behaviour showed more consistency for all genotypes and CV value is lowest at 1100 °C for all genotypes.

Table 5
Co-efficient of variation calculated based on mean of ash melting behaviour over the years (2004–2011) and standard deviation for each temperature for selected genotypes.

Genotype	Co-efficient of variation (CV)			
	800 °C	900 °C	1000 °C	1100 °C
Gig-2	0.18	0.16	0.09	0.04
Sac-5	0.21	0.24	0.19	0.01
Sin-H7	0.09	0.06	0.05	0.03
Sin-13	0.28	0.33	0.35	0.27

3.3. Composition of solid fuel and ash melting behaviour

The results showed that the ash melting behaviour was significantly influenced by the composition of the solid fuel. The mineral content (especially Ca, Mg, Si, Cl and K), heating treatment, year and genotype had significant effect (at $p < 0.05$) on ash melting behaviour. However, the effect of ash content in the solid fuel was non-significant for ash melting behaviour under the different heating treatments (Table 6).

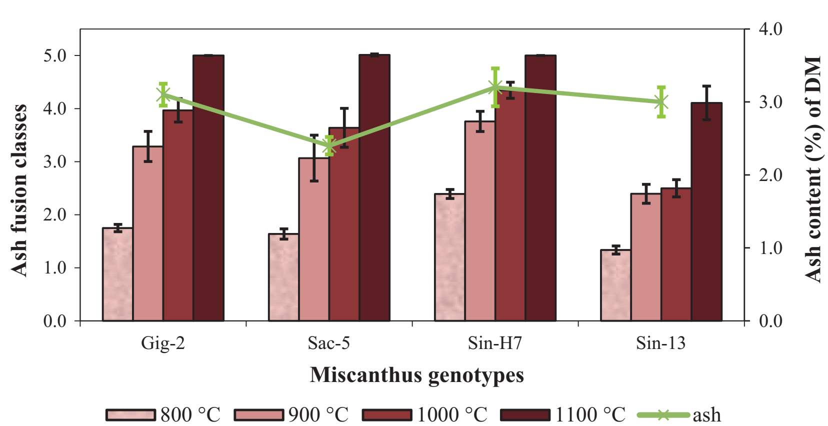

Fig. 1. Bars indicate the ash melting behaviour of selected genotypes evaluated over the study period (2004–2011) at 800 °C, 900 °C, 1000 °C and 1100 °C ash-fusion temperatures (error bars indicate the standard deviation calculated over the years). The line represents the ash content of each genotype.

Table 6
Type 3 tests to evaluate the main effects of biomass composition, ash content, heating treatment, year and genotype (for selected genotypes) on ash melting behaviour.

Effect	*F* value	Pr > *F*
Ash	1.28	0.2613
Calcium	109.93	<.0001
Magnesium	32.15	<.0001
Silicon	43.87	<.0001
Chloride	5.67	0.0274
Potassium	65.09	<.0001
Heating treatment	187.66	<.0001
Year	11.87	<.0001
Genotype	13.79	<.0001

The level of significance = $p < 0.05$.

The results indicate that ash sintering decreased with increase in Ca and Si content of the biomass samples with a correlation coefficient $r = -0.61$ (Ca) and $r = -0.61$ (Si). The ash sintering significantly increased with the increase in K and Cl content of the biomass samples. The correlation coefficients are $r = 0.68$ for K and $r = 0.66$ for Cl (Fig. 2).

4. Discussion

Ash melting behaviour is one of the main criteria in the evaluation of biomass combustion quality. Low ash melting temperature can lead to severe technical problems, which subsequently reduce the efficiency of heat transfer. There are various thermal bioconversion routes for the production of energy through combustion of biomass at a specific temperature. The temperature range for the different thermal bioconversion processes is 900–1600 °C [8]. Therefore, biomass with a low ash melting temperature leads to several mechanical and technical problems and reduces the conversion efficiency of boilers. In this study, the ash melting behaviour of biomass harvested from selected genotypes over a period of years was determined at different temperatures (800–1100 °C) with the aim of developing a link between ash melting

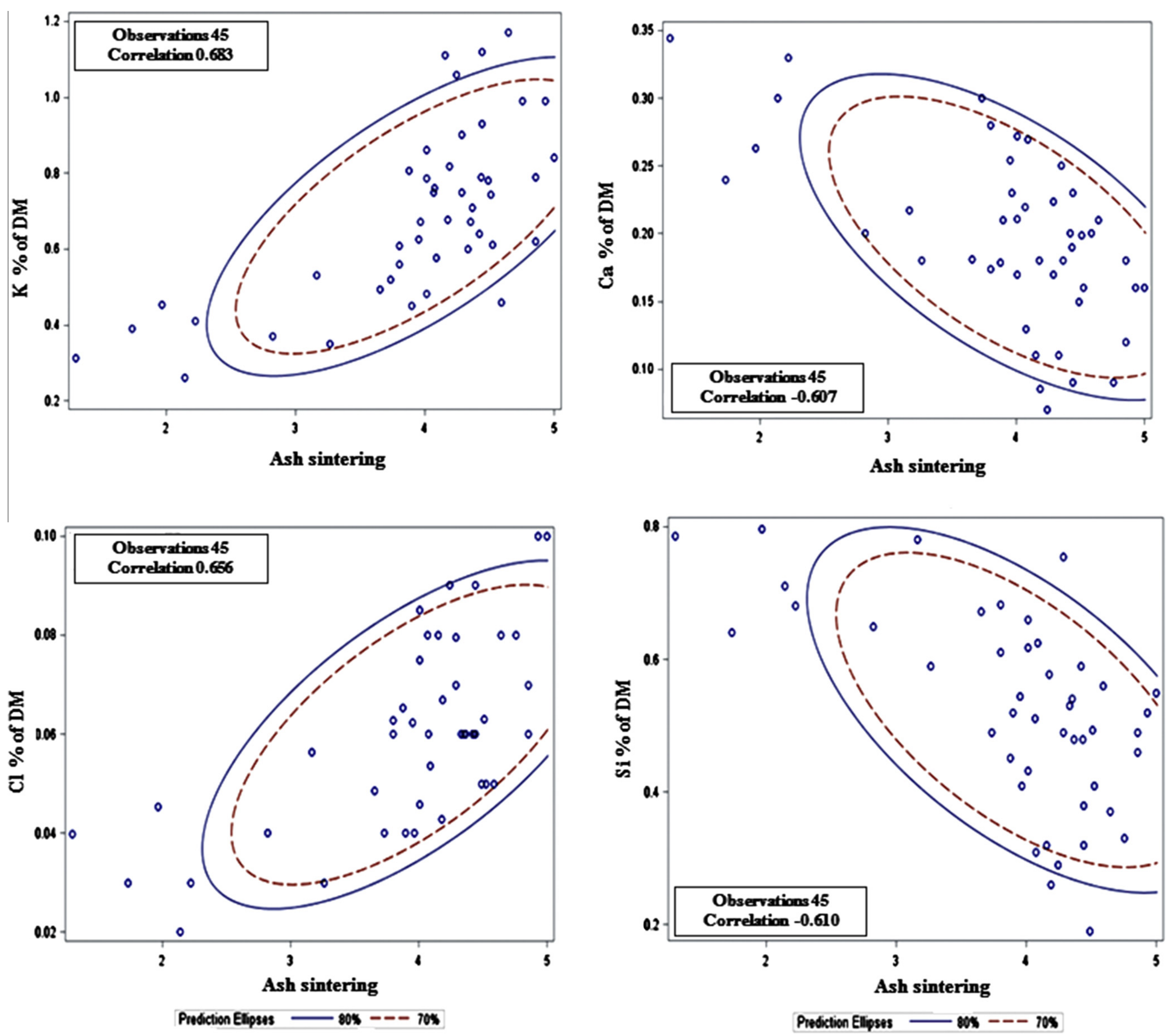

Fig. 2. Scatter plots for all genotypes and for selected years (2004, 2008, 2011) indicate the correlation between mineral content (K, Ca, Cl, Si) of biomass samples and ash sintering at 1000 °C heating treatment. All correlation coefficients were highly significant at $p < 0.05$. Prediction ellipses (70%, 80%) were created as a part of correlation analysis.

behaviour and biomass composition. The temperature range (800–1100 °C) was selected based on pre-trial results tested for *M. × giganteus* genotype.

If the Pellet Norm EN 14961-2: A1 is applied to the results of this study, the ash melting behaviour of all genotypes falls outside the permitted limits. The criteria set by Pellet Norm EN 14961-2: A1 is that ash melting must not occur below 1200 °C. For all the genotypes (except for some *M. sinensis* types), complete ash melting was observed at 1100 °C. The main reason for the relatively low ash melting temperature in miscanthus biomass compared to wood pellets is the high ash [12] and mineral content especially Cl, because Cl is higher in energy crops than wood [13]. The main barrier to exploiting biomass available from energy crops for combustion purposes is the high K and Cl content. Therefore, the following section focuses on the effects of K and Cl during the thermal bioconversion process and the evaluation of the impact of genotypes and crop-management factors on biomass composition, in particular K and Cl content. The quantity of biomass constituents – especially K and Cl, which lead to low ash-fusion temperature and deposition during combustion – plays a key role in defining the technical sustainability of combustion boilers. During the thermal bioconversion processes, the Cl in biomass is converted into two forms; (a) gases such as Cl_2 and HCl; (b) alkali chlorides such as KCl and NaCl [13]. The gaseous Cl in flue gas condenses and leads to deposition. At high temperatures, in addition to gaseous Cl, aerosols composed of alkali metals and heavy metal salts are formed which lead to both emissions and depositions. Therefore, gaseous-phase species in combination with alkali chloride depositions are one of the main causes of corrosion [14]. Deposition can occur in form of slagging or fouling, leading to deterioration of boilers and increasing operational costs [8,15]. The intensity of the corrosion mechanism depends on the Cl content of biomass. The higher the Cl content the greater the mobility of alkali metals especially K, because Cl facilitates reactions with K during combustion. Therefore, the genotypes with high K and Cl (*M. × giganteus*) [2] showed strong sintering and subsequently low ash melting temperature compared to genotypes with low Cl and K content (*M. sinensis*) [2].

The results of this study had shown that the K and Cl content in biomass is positively correlated with ash melting behaviour whereas Si content is negatively correlated with ash melting behaviour. This could be because Si content could inhibit the formation of alkali chlorides during combustion process and release the Cl in the form of HCl. For example, to carry out efficient combustion process, aluminium silicates are used as additives to trap alkalis [9,16]. The mechanism of alkali trapping (mainly K here) and removal of Cl through addition of aluminium containing additives follows the following reaction [9,16]:

$$Al_2O_3 \cdot 2SiO_2 + 2KCl + H_2O \rightarrow K_2O \cdot Al_2O_3 \cdot 2SiO_2 + 2HCl \quad (1)$$

In the following section, the effect of fertilization, stem thickness, rainfall and harvesting time on biomass composition will be discussed. The fertilization effect, stem thickness, rainfall and harvesting time effect is not tested in this study, however in already published study [2] for the same site, same genotypes, the effect of aforementioned on biomass composition was evaluated. In current study, the information from already published study [2] about biomass composition is used to explain the ash melting behaviour.

In this study, K fertilization was applied every year in the form of potassium sulphate [2]. Therefore, the high K content of the harvested biomass could also be due to the fertilization. In another study, it was found that high K fertilizer doses led to increased K content in the harvested biomass [17]. In addition, the strong positive correlation coefficient for K and Cl indicates that these elements complement each other in nutrient uptake dynamics, where Cl operates as a counter ion for the transportation of the K cation [18]. Therefore, the high K content in biomass can lead to high Cl content.

The difference in Cl and K content between genotypes can be explained by morphological characteristics especially stem thickness. The thick-stemmed genotypes (*M. × giganteus)* had high K and Cl contents compared to the thin-stemmed genotypes (*M. sinensis*). The results of already published study about biomass composition of same miscanthus genotypes [2], where it was found that the content of Cl and K also depends on time of harvesting and weather conditions especially rainfall. All three factors (stem thickness, harvesting time, rainfall) interact with each other affecting the rate of leaching, in particular of K and Cl [2].

Harvesting time plays a vital role because a delayed harvest allows cuticles to be broken and they are exposed for a longer time, increasing the chances of leaching of minerals especially Cl through rainfall. The biomass quality improvement through delayed harvest is quantified in already published study [2]. The delayed harvest also contributes towards biomass quality improvement through loss of leaves. However, the biomass quality improvement through delayed harvest also depends on crop morphology such as stem thickness and leaf to stem ratio which varies from genotype to genotype. This partially explains the variation in ash melting behaviour among genotypes.

The proportion of broken cuticles depends on stem thickness. Therefore, less leaching is to be expected in thick-stemmed (less broken cuticles) than in thin-stemmed (more broken cuticles) genotypes. For example, in 2008 the biomass was harvested in April, which led to an improvement in ash melting behaviour at 900 °C and 1000 °C for thin-stemmed genotypes (*M. sinensis*). This is mainly due to better leaching of Cl and K facilitated by the thin stems [19]. For the year 2011, biomass was harvested early (January) but it did not show clear trend for thin stemmed genotypes (*M. sinensis*).

The other important morphological characteristic which is also dependent on harvesting time and can subsequently affect biomass quality is leaf-to-stem ratio. It's mainly because stems have better combustion quality compared to leaves [2]. The ash content of biomass could also affect the ash melting behaviour during combustion process. However, in this study the effect of ash content was not significant on the ash melting behaviour of the different genotypes. This could be because the difference in ash content between the genotypes was not significant except for *M. sacchariflorus*. The low ash content in *M. sacchariflorus* can be partially explained by the low leaf-to-stem ratio because the ash content of leaves is higher than that of stems [2]. Along with harvesting time, rainfall could also affect the leaf-to-stem ratio.

Therefore, the combined effect of harvest time and rainfall is more useful than considering rainfall or harvest time alone. For example, in 2004 despite high rainfall between December–March (time period critical for leaching), the ash melting behaviour did not improve much. It could be because during this year the biomass was harvested early (February). Therefore, delayed harvest in combination with sufficient rainfall can contribute towards biomass quality improvement.

From the above discussion and based on the already published data about biomass composition of miscanthus genotypes it can be deduced that the most relevant factors are harvest time, rainfall and genotype selection for ash melting behaviour. Harvesting time, especially delayed harvest, is important to improve biomass quality for combustion, but also leads to lower biomass yield [2]. The trade-offs between quality and yield under delayed harvest are not evaluated in this study.

The use of thin-stemmed genotypes and a delayed harvest regime can improve the ash melting behaviour of biomass. However, in this study, the mineral content (especially K and Cl) of miscanthus biomass was still high enough to induce low-temperature ash melting despite appropriate genotype selection and delayed harvest. Therefore, other measures are required for the efficient use of miscanthus biomass in thermal bioconversion, especially to keep the K and Cl contents low. There are various ways of countering high K and Cl content and thus reducing the alkali chloride depositions and corrosion mechanism. For example, the leaching pretreatment of biomass has been shown to improve the ash melting temperature and reduce slagging, fouling and corrosion [12]. In addition, leaving the biomass on the ground (swath or flat thin layer) after harvest could also help to improve the combustion quality through facilitating leaching process especially Cl [20]. Other options include the use of chemical additives to remove Cl during the thermal bioconversion process and the upgrading of boilers to reduce ash-related problems [15,21,9]. However, the aforementioned options to improve ash melting behaviour don't fall under the scope of this study, therefore not discussed in detail.

5. Conclusions

For miscanthus biomass, the combined contents of ash, K and Cl are good indicators for ash-related problems during energy production through combustion.

Based on the results of current study, thin-stemmed *M. sinensis* genotypes are most suitable for the combustion process. However, these genotypes are also the lowest yielding and an economic analysis is required to quantify the trade-offs between biomass quality and quantity.

The high yielding *M.* × *giganteus* genotype, which covers most of the current miscanthus plantation in Europe, can be used in coal co-firing or in combination with wood.

Despite the selection of optimal genotypes and appropriate harvest time, on-field quality management measures were not sufficient to guarantee a miscanthus biomass quality that meets the Pellet Norm EN 14961-2: A1 standards. Therefore, the use of miscanthus biomass on its own cannot be recommended without any post harvesting treatments (such as swathing or cutting the crop and leaving it flat on the field [20]) for power generation via combustion. In addition, data presented here is from one site, which was fertilized annually, therefore it could be different for other sites under different management practices.

However there is the possibility of using miscanthus biomass by blending it with other biomasses with low ash, K, and Cl contents. Further research is required to find the optimal blend of biomass with regard to ash melting behaviour.

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2.3. Yield and Quality Development Comparison between Miscanthus and Switchgrass Over a Period of 10 Years.

Publication-3

Iqbal, Y., M. Gauder, W. Claupein, S. Graeff-Hönninger, and I. Lewandowski. 2015. "Yield and Quality Development Comparison between Miscanthus and Switchgrass Over a Period of 10 Years." *Energy* 89: 268-276.

This study is based on data collected from a field trial between 2002 and 2012 and was carried out with the main objective of evaluating the yield and quality performance of miscanthus and switchgrass using different harvest dates and N fertilization regimes (0 kg, 40 kg, 80 kg). Over the whole plantation period (including three years of establishment period), the mean yield of miscanthus was 16.2 t DM ha^{-1} a^{-1}, while switchgrass yielded 10.2 t DM ha^{-1} a^{-1}. In miscanthus, each increase in fertilizer level increased the N content in the harvested biomass, whereas in switchgrass, no significant difference was recorded for 0 kg and 40 kg N levels. The effect of N fertilization on ash was significant but independent of the crop. Both miscanthus and switchgrass biomass samples from the late harvests had a significantly lower N content than those from the early harvests. A Life Cycle Assessment covering the conducted field work and inputs of this trial showed relatively low energy input and emissions connected to the cropping of miscanthus.

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Yield and quality development comparison between miscanthus and switchgrass over a period of 10 years

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Harvest date
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ABSTRACT

The establishment of perennial crops has emerged as a very viable option for biomass-based energy production mainly due to their comparative ecological advantages over annual energy crops. This study is based on data collected from a field trial between 2002 and 2012 and was carried out with the main objective of evaluating the yield and quality performance of miscanthus and switchgrass using different harvest dates and N fertilization regimes (0 kg, 40 kg, 80 kg). Over the whole plantation period (including three years of establishment period), the mean yield of miscanthus was 16.2 t DM ha^{-1} a^{-1}, while switchgrass yielded 10.2 t DM ha^{-1} a^{-1}. In miscanthus, each increase in fertilizer level increased the N content in the harvested biomass, whereas in switchgrass, no significant difference was recorded for 0 kg and 40 kg N levels. The effect of N fertilization on ash was significant but independent of the crop. Both miscanthus and switchgrass biomass samples from the late harvests had a significantly lower N content than those from the early harvests. A Life Cycle Assessment covering the conducted field work and inputs of this trial showed relatively low energy input and emissions connected to the cropping of miscanthus.

1. Introduction

In the last decade, policy support and biofuel-driven mandates have led to a significant increase in the production of dedicated energy crops in Europe and America. However, criticism of the production of food and feed crops grown especially for energy purposes, such as maize or rapeseed, has motivated the search for high-yielding non-food energy crops. In recent years, the establishment of perennial crops has emerged as a very viable option mainly due to their comparative ecological advantages over annual energy crops [27,28,32]. Among these, the C4 grasses miscanthus and switchgrass combine the potential to deliver high biomass yield and ability to grow under a wide range of climatic conditions [21].

Miscanthus is characterized by a high dry matter yield potential [6], and can be grown without any pest or weed control measures once the crop is established [24]. However, because there is presently only one commercially available clone, *Miscanthus x giganteus*, it has some limitations such as a lack of winter hardiness during the establishment period [21]. Additionally *M. x giganteus* needs to be propagated vegetatively resulting in high plantation costs [3]. Contrary to this, switchgrass can be established via seeds and the lower production costs make it a more practical option among the energy crops [26]. However, the biomass yield potential of switchgrass is considered to be lower than that of miscanthus [21].

Miscanthus and switchgrass can be cultivated on marginal soils (mainly low fertile) due to their low nutrient requirements and high net primary production potential [31]. The low nutrient requirements of miscanthus in particular [8] are accommodated by its well-developed rooting system [27] and the relocation of nutrients back to rhizomes at the end of the growth season. The annual dry matter yield production potential of the aforementioned C4 perennial crops is 10–20 t ha^{-1} in temperate climates [36]. Under optimal growth conditions however, the yield can be higher than 30 t ha^{-1} [27]. The productivity can be further improved by the optimized combination of management practices, such as the appropriate N fertilization rate [1] and choice of appropriate harvest time [24]. However, improvement in yield through various management practices can, over time, affect the composition of the produced biomass and subsequently the thermo-chemical conversion processes for energy production.

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Currently, the biomass produced from these crops is mainly used for direct combustion. However it can also be used for the production of so-called 2nd generation liquid fuel [3] and biogas [26].

The energy production through thermo-chemical conversion of biomass can be categorized into three main processes; a) direct combustion; b) gasification; c) pyrolysis. To carry out problem free thermo-chemical conversion, all aforementioned processes require feedstock with defined quality. For example, in direct combustion, high contents of Cl and K lead to deposition, slagging, fouling and corrosion problems [4]. In energy crops, the content of Cl is comparatively high, which makes it challenging to combust such biomass for bioenergy production [44]. In addition, the high ash, moisture and other inorganic constituents such as N also influence the combustion process along with environmental issues. High contents of N lead to NOx emissions and high ash contents increase the operational cost. Therefore, it is important to improve the biomass quality through different on field quality management practices such as selection of cop, fertilization and harvesting time. The composition of biomass is mainly dependent on climatic conditions, genetic background [17], location, and agronomic practices especially fertilization rate and harvest time [25]. Research has shown that on-field quality management can be performed by the adjustment of harvest date but with compromised yield. The timing of the harvest is mainly dependent on the end use of the biomass e.g. early harvest for liquid fuels [15] and late harvest for combustion [4]. The comparatively high N content in energy crops [41] has raised concerns over the use of biomass for direct combustion mainly due to chances of NO_x formation. The N content is higher in leaves than in stems. Therefore delayed harvest can contribute to lower NO_x formation by affecting the leaf-to-stem ratio and providing sufficient time for relocation mechanism. Moreover, along with biomass composition analysis and optimization of biomass compositions through on field quality management practices, it's also crucial to give an overview about the whole value chain from crop production till thermo-chemical conversion by performing LCA (life cycle assessments). These analyses can help to better estimate the environmental impacts of the production of different feedstock.

The aim of this study was to evaluate the effect of different 'on field management practices' such as N levels, crop age, harvest time including weather conditions (mainly rainfall and temperature) on dry matter yield and quality parameters of miscanthus and switchgrass at the same time on the same place. Hence, a comparison of both crops in terms of productivity and quality characteristics was a second focus. In addition a comparison of the N removal from the soil by both crops was calculated at different N fertilization levels to identify the optimal cropping system. To get a rough estimation of possible emissions linked to the cropping of these perennial grasses in this trial, an LCA was conducted for the miscanthus cropping system including all the management practices and inputs used. Biomass samples of both crops were collected from 2002 to 2012 from the field trial established in 2002 at the research station of the University of Hohenheim, Ihinger Hof, in south-west Germany. The harvested biomass was processed before calculation of dry matter yield, moisture, ash and N content.

Three hypotheses were developed: a) morphological characteristics especially number of shoots and plant height are good indicators of crop yield: b) high N fertilization levels can increase the N content in the harvested biomass, whereas delayed harvest decreases the N content: c) miscanthus is a better feedstock than switchgrass in terms of quantity and quality of biomass and ecological benefits under the climatic conditions of the experimental area.

2. Materials and methods

2.1. Site characteristics

The field trial was planted in May 2002 with the main objective of evaluating annual and perennial cropping systems under different fertilization regimes. For the current study, two of these crops – miscanthus and switchgrass – were selected. The experimental field plots are located at the University of Hohenheim research station, Ihinger Hof, south-west Germany (48.75°N and 8.92°E). According to the FAO classification, the soil of this site belongs to Haplic Luvisol with a predominantly silt clay texture and overlying loess loam. Some of the physical and chemical properties of the soil profile are given in Table 1.

The weather data for each year (2002–2012) were collected by a nearby meteorological station. During the period 2002–2012 the mean annual rainfall was 707.5 mm and the mean annual temperature was 9.2 °C. Weather data and harvest dates are presented in Table 2.

2.2. Experimental design and management practices

The field trial was established as a randomized split plot design with different crops as main plots, divided into three subplots (180 m^2 each) with different N levels (0, 40, and 80 kg $ha^{-1}a^{-1}$). Each variant had 4 replicates.

The clone *M. x giganteus* was planted as micro-propagated plantlets. The planting density was two plants m^{-2} with a row spacing of 66 × 75 cm. Switchgrass (*Panicum virgatum* L.) 'Kanlow' was established through seeds at the rate of 10 kg ha^{-1}.

Fertilizer application was carried out through ammonium-stabilized N-fertilizer Entec 26 (K+S Nitrogen GmbH, Mannheim, Germany) to tackle the problem of N losses. The fertilizer contains 7.5% nitrogen-N, 18.5% ammonia-N and 13% sulfur. Fertilizer was applied each year from April to May before the emergence of new shoots.

Weed control was carried out by herbicide application. In the first two years, the herbicide Basagran DP (BASF, Ludwigshafen, Germany) was applied at a rate of 999 g Bentazon and 699 g ha^{-1}Dichlorprop-P. In 2005 Clinic (Nufarm, Cologne, Germany) and in 2006 Durano (Monsanto, Antwerpen, Belgium) were applied at the same rate of 1080 g ha^{-1} Glyphosate.

2.3. Field data collection

Measurements of growth components for both crops were taken at random intervals over the years. These included number of shoots, height, stem diameter and leaf to stem ratio. Soil parameters, especially carbon and nitrogen contents, were determined regularly from 2005 to 2009 except for 2008.

2.4. Harvesting and sample preparation

Starting from 2002, harvesting was carried out every year between October (early harvest) and April (late harvest). For switchgrass, in some years, samples were collected twice in the same year, for instance, early harvest in June or August followed by final harvest in April. Final harvest for both crops was performed at the same time of the year. For each crop, the sampling area was 1 m^2 to 12 m^2. For each harvest the total fresh weight of the collected biomass samples was recorded. Then sub-samples were chopped, weighed and put in the oven to dry at 60 °C for 48 h to estimate the dry matter content. For the laboratory analysis, all the samples were milled using a mill with 1 mm sieve to ensure a uniform particle size.

Table 1
Physical and chemical soil properties determined in 2002 at research farm Ihinger Hof, with standard deviation for total nitrogen (TN) and total carbon (TC).

Crop	Depth (cm)	Bulk density (mg/cm^3)	pH (H^+)	TN (%)	TC (%)
Miscanthus	0–30	1.43	6.78	0.097 ± 0.024	0.990 ± 0.077
	30–60	1.50	7.12	0.046 ± 0.009	0.430 ± 0.056
	60–90	1.54	7.29	0.030 ± 0.006	0.418 ± 0.119
Switchgrass	0–30	1.39	6.53	0.104 ± 0.013	0.957 ± 0.089
	30–60	1.50	7.07	0.049 ± 0.002	0.428 ± 0.080
	60–90	1.54	7.40	0.039 ± 0.003	0.902 ± 0.584

Table 2
Weather conditions and harvest dates for each year during the growth season (April–October) at the research station.

Year	Harvest date	Growth season precipitation[a] (mm)	Mean annual temperature (°C)	Min. winter temperature[b] (°C)
2002	01.10.2002	542.9	9.4	−4.8
2003	11.12.2003	282.1	9.7	−5.9
2004	17.01.2005	345.8	9.0	−3.4
2005	10.01.2006	382.2	8.8	−4.9
2006	02.04.2007	409.0	9.4	−6.4
2007	01.04.2008	391.9	9.6	−2.4
2008	31.03.2009	522.2	9.2	−2.4
2009	24.03.2010	490.5	9.3	−5.8
2010	28.03.2011	414.5	8.1	−5.5
2011	20.03.2012	334.1	9.9	−2.4
2012	25.03.2013	390.4	9.3	−7.2
Mean		409.6	9.2	−4.7

[a] April–October.
[b] Mean of minimum day temperatures during the coldest month.

2.5. Ash and mineral analysis

For the assessment of ash, 1 g of each sub-sample was weighed out and put into the muffle furnace for 4 h at 550 °C. The samples were then removed, reweighed and the ash content was recorded. The total N content of the biomass samples was determined using the NIRs technique. Calibration samples were analysed following the Dumas principle (Leco St. Joseph, MI). The validation test indicated that the calibration model covered the normal variation in N content for biomass samples.

2.6. LCA (life cycle assessment) and measurement of soil-born emissions

The LCA of the miscanthus cropping system systems was conducted using the software GaBi4.4 (PE International, Germany) and covered the production system including all materials used in the processes. The system boundaries were set from "cradle to farm gate" which means that the process of production of machinery and inputs is incorporated in the balance. Documentation and evaluation ended at the farm gate, thus harvest and transport to the farm (2 km) was included in the calculations; however, further conversion of the biomass into the final-energy form was not included. The processes in each system were assumed to have been conducted with the most conventional techniques, while data inventory for these processes was based on data sets of PE International (Germany), Ecoinvent (Switzerland) and literature sources. The total amounts of inputs transformed to GWP (global warming potential) for N fertilized miscanthus are displayed in Table 3.

The resulting volatile emissions were transformed to CO_2 equivalents using the emission factors suggested by the [19]. Soil-born trace gas emissions are not included in the LCA inventory; hence, supplementary measurements were conducted using the static chamber method [18]. For the measurements, frames were permanently installed in 3 plots of each variant. Each week the frames were closed and the flux of CO_2, N_2O and CH_4 was monitored during 45 min. The measurements were conducted throughout the year (2010), with the interval of one week (one measurement interval per week) (for details see Ref. [14]).

2.7. Statistical analysis

Data analysis was performed by SAS 9.2 (SAS Institute Inc., Cary, NC, USA). All the parameters were analysed using a mixed model with block, crop, N-levels and years as fixed effects and the interaction of crop and block within the years as a random effect. This model evaluates the effect of different N levels and different interactions within and over the years. All effects were tested at a p value of 0.05. Regression and correlation analyses were performed to estimate the relationship between yield and growth components. The notation of the mixed model was as follow:

$$y_{ijrkt} = \mathbf{m} + \mathbf{b}_r + \mathbf{c}_i + \mathbf{n}_j + \mathbf{a}_k + \mathbf{ba}_{rk} + \mathbf{ca}_{ik} + \mathbf{na}_{jk} + \mathbf{cn}_{ij} + \mathbf{cna}_{ijk} + \mathbf{cb(a)}_{irk} + \mathbf{cnba}_{ijrkt}$$

where

$\mathbf{y}_{ijrkt}$ = t-th measurement in r-th replication in k-th year of i-th crop with j-th N level
$\mathbf{m}$ = general effect
$\mathbf{b}_r$ = main effect of r-th replication
$\mathbf{c}_i$ = main effect of i-th crop
$\mathbf{n}_j$ = main effect of j-th N level
$\mathbf{a}_k$ = main effect of k-th year
$\mathbf{ca}_{ik}$ = interaction of i-th crop with k-th year
$\mathbf{na}_{jk}$ = interaction of j-th N level with k-th year
$\mathbf{cn}_{ij}$ = interaction of i-th crop with j-th N level
$\mathbf{cna}_{ijk}$ = interaction of i-th crop with j-th N level and k-th year
$\mathbf{cb(a)}_{irk}$ = random deviation of i-th crop in r-th replication over the years
$\mathbf{cnba}_{ijrkt}$ = residual error term corresponding to $\mathbf{y}_{ijrkt}$

The crop effect was set as autocorrelated, with a heterogenic variance for each year. For quality analysis, with fewer years as input, the autocorrelation of the years was set as non-heterogenic.

Table 3
Life Cycle Inventory for 10 years of miscanthus cropping (with 80 kg N ha^{-1}) based on the inputs and management practices conducted in this trial (GWP refers to Global Warming Potential).

Process inputs	Amount in GWP [kg CO_2-eq.]	Percentage of inputs (%)
Ammonium nitrate	4838	72
Harvest with chopper	591	9
Potassium chloride	162	2
Diesel	156	2
Transport planting material	150	2
Harvest planting material	148	2
Tractor usage	119	2
Alachlor	111	2
Magnesium sulfate	93	1
Soil tillage plough	61	1
Seedbed preparation	56	1
Planting	53	1
Application of pesticides	50	1
Application of fertilizers	50	1
Mechanical weed management	34	1
Harvest pick up of stalks	8	0
Total GWP	**6680**	**100**

3. Results

3.1. Dry matter yield development over the years

The dry matter yield for the miscanthus and switchgrass harvested in early spring increased during the first four years, reaching a first peak in the fourth year. This relatively high yield level of the 4th year was not reached in the 5th year by either crop. However, in the 6th year, the dry matter yield of the switchgrass was the highest of all years, whereas the highest yield for miscanthus was recorded in the 8th year (Fig. 1). From 2007 (6th year) to 2012 (11th year), the dry matter yield for switchgrass decreased continually. However, for miscanthus, after the 8th year (highest yielding year), the dry matter yield remained relatively stable at over 16 t DM ha^{-1} a^{-1}. In every year after the 2nd plantation year the miscanthus yields exceeded the switchgrass yields significantly (Fig. 1). In the first two years of establishment, the yields of miscanthus and switchgrass (mean of the three N fertilization levels) were not significantly different; however, in each following year the yield of miscanthus was significantly higher than that of switchgrass. Over the whole plantation period (including the establishment period), the mean yield of the miscanthus was 16.2 t DM ha^{-1} a^{-1}, while that of the switchgrass was 10.2 t DM ha^{-1} a^{-1}.

The interaction of crop*N level was not significant (Table 4), however, the main effect of N fertilization was significant and led to yield increase for each crop. For miscanthus, at 0, 40 and 80 N levels the mean yields (including the establishment period) were 13.7, 16.6 and 18.3 t DM ha^{-1} a^{-1}, respectively, whereas, switchgrass yielded 7.2, 10.0 and 13.3 t DM ha^{-1} a^{-1}, respectively. Hence, the additional input of 40 kg N ha^{-1} a^{-1} led to a yield increase of approximately 3 t DM ha^{-1} a^{-1} for each crop in the tested range up to 80 kg N ha^{-1} a^{-1}.

Table 4
Type 3 tests of fixed effects on dry matter yield.

Effect	F-statistic	Pr > F
repetition	3.49	0.0339
crop	176.71	<0.0001
N level	46.03	<0.0001
year	209.86	<0.0001
rep * year	1.10	0.4907
crop * year	22.78	<0.0001
N level * year	5.58	<0.0001
crop * N level	1.33	0.2926
crop * N level* year	1.61	0.1033

3.1.1. Accumulative dry matter yield (2002–2012) at different N levels

The accumulative dry matter yield from 2002 to 2012 increased significantly for switchgrass with each increase in N fertilization level. However, for miscanthus, a significant increase in accumulative yield was only recorded when the N fertilization level increased from 0 to 80 kg. In addition, no significant increase in the dry matter yield of miscanthus was recorded with the change from 40 to 80 kg N fertilization. The switchgrass at 80 kg N had almost the same yield as the miscanthus at 0 kg N (Fig. 2).

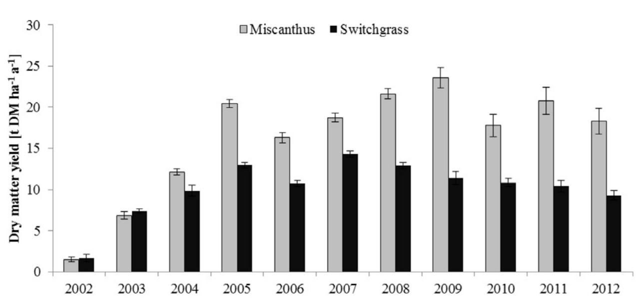

Fig. 1. Dry matter yield comparison between miscanthus and switchgrass for the plantation period 2002 to 2012 (Mean of three N levels, error bars indicate standard error).

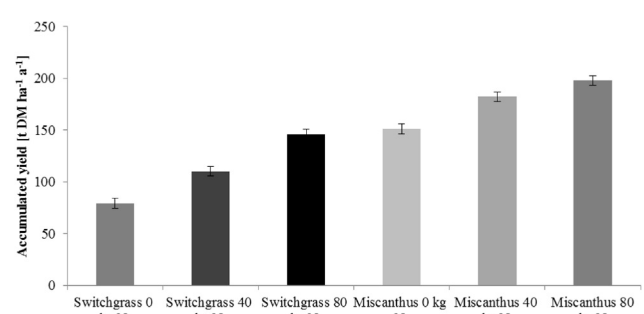

Fig. 2. Accumulative dry matter yield comparison between miscanthus and switchgrass for 11 years (2002–2012) under different N fertilization regimes (Error bars indicate standard error).

3.1.2. Growth parameters

Among the growth parameters, shoot density and plant height showed a positive correlation with dry matter yield for both crops. A significant positive correlation was found between dry matter yield and plant height for miscanthus and switchgrass, with coefficient of determination $r^2 = 0.65$ and $r^2 = 0.60$, respectively. The relationship between shoot density and dry matter yield was also significant for miscanthus and switchgrass with coefficients of determination $r^2 = 0.33$ and $r^2 = 0.28$, respectively. Correlations for dry matter yield with growth parameters are shown in Fig. 3.

3.2. Quality characteristics

3.2.1. N content over the years

The N content for whole plants varied significantly at different fertilization levels for both crops over the years. However, it was significantly lower in the miscanthus biomass compared to the switchgrass biomass at each fertilization level (Fig. 4). In miscanthus, an increase in fertilization level increased the N content in the harvested biomass, whereas in switchgrass no significant difference was recorded for 0 kg and 40 kg N levels. However, the increase in fertilization level from 0 kg to 80 kg N and 40 kg N to 80 kg N increased the N content in the harvested biomass of switchgrass (Fig. 4).

3.2.2. N content at different harvest dates

The total mean N content decreased with the delay in harvest for both miscanthus and switchgrass. In miscanthus, the earliest harvest was performed in December, where the N content was highest, whereas the lowest N content was recorded in April. This was the case for all N fertilization levels. However at each harvest date the N content of the harvested biomass was higher with each

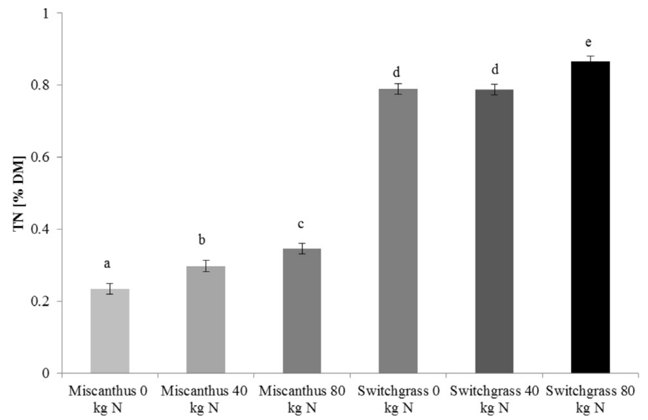

Fig. 4. Mean total N (TN) content of whole plants over three years (2005–2007) for miscanthus and switchgrass at different fertilization levels. The bars with the same superscript do not differ significantly from each other according to multiple t-test $\alpha = 0.05$).

higher N fertilization level (Fig. 5). In switchgrass, a sharp decrease in N content of the harvested biomass was recorded with the delay in harvest date from June to April. There was no significant effect of N fertilization levels on N content of the harvested biomass in the January and April harvests. Only in the August harvest was the N content of the harvested switchgrass biomass at the 0 kg N fertilization level significantly lower than the other two levels (Fig. 5).

3.2.3. Ash content

For both miscanthus and switchgrass, ash content could only be measured in three years (2006, 2007 and 2010). The miscanthus

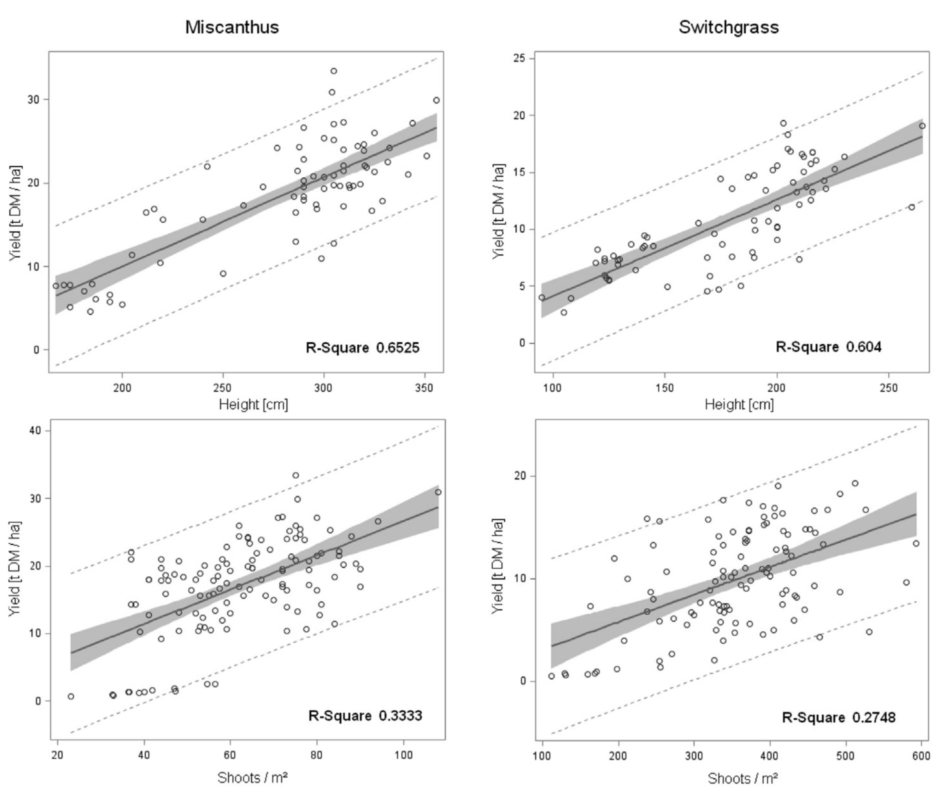

Fig. 3. Scatter plots for growth parameters (height, shoot density) and dry matter yield for miscanthus and switchgrass computed with 95% confidence limits and prediction limits and fitted regression line for each parameter.

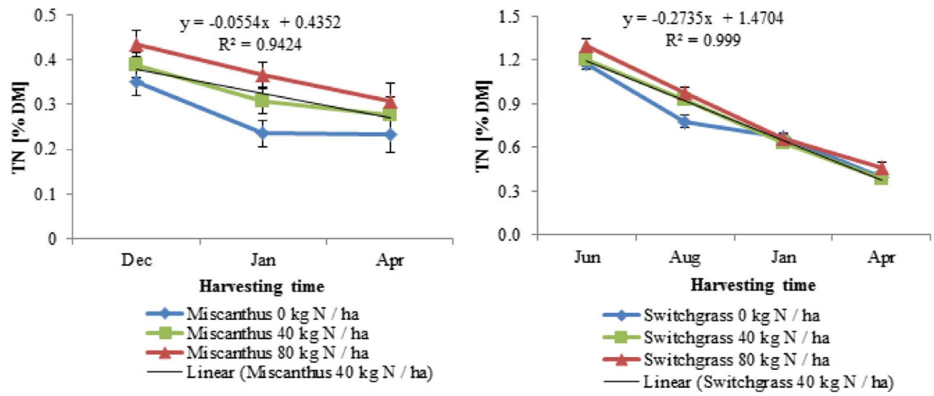

Fig. 5. Mean total N (TN) content of whole plants for miscanthus and switchgrass at different harvest dates and fertilization levels (error bars indicate standard errors).

biomass had significantly lower ash contents than the switchgrass. The effect of N-fertilization was significant but independent of the crop (Table 5). The difference was very small. However, the plots with the highest N fertilization rates had the highest mean ash content of 3.5% for both crops; whereas the medium N fertilization rate and the control both had mean ash contents of 3.4% which was significantly lower than for the other N fertilization treatments.

For both miscanthus and switchgrass, the ash content decreased in the surveyed years. The lowest ash content was recorded in 2010 for both crops, whereas ash contents in 2006 were significantly higher compared to other years (Fig. 6).

3.3. N removal

The increase in N fertilization increased the N removal from the soil mainly by increasing the biomass yield and N content of the harvested biomass in both crops. Despite the high biomass production, the mean N removal through the harvesting of miscanthus biomass was significantly lower than for switchgrass at each N fertilization level. The highest N removal was recorded for plots under the highest N fertilization levels for both crops (Table 6).

3.4. Volatile emissions and energy input

To appraise the environmental impact and the energy input needed for this cropping system, an LCA (life cycle assessment) was conducted for the high yielding crop, miscanthus. Volatile emissions connected to the production of miscanthus were calculated for the 0 kg and for the 80 kg N fertilization level. At first, an LCA was conducted based on management records of the already established long-term field trial. Hereafter, the soil-borne trace gas emissions, measured during a one year measurement campaign, were added. The total emissions calculated by the LCA accounted to 178 kg CO_2 equivalents in the 0 N fertilization regime and 668 kg CO_2 equivalents in the 80 kg N fertilization regime. The difference is mainly due to the high energy consumption during the process of fertilizer production and the emissions linked to this process. In addition, the energy input was doubled by the use of 80 kg N-fertilizer. Again, the main part of this energy consumption was linked to the production of fertilizer and not to the application of the fertilizer or to the transportation for high harvest volume. When adding the soil-born trace gas emissions, which are not included in the LCA approach, the total emissions sum up to 1088 kg CO_2 equivalents for the N fertilized miscanthus. While the negative gas fluxes slightly decreased the total emissions of non N fertilized miscanthus to 157 kg CO_2 equivalents (for details on the trace gas fluxes see Ref. [14]).

The energy input in non N fertilized miscanthus was mainly affected by the diesel consumption and for the N fertilized miscanthus, the main energy input was energy required during the production process of N fertilizer. The energy input was 10 GJ ha^{-1} a^{-1} (N fertilized) and 4 GJ ha^{-1} a^{-1} (non N fertilized), which was quite smaller than energy content of N fertilized and non N fertilized (324 and 235 GJ ha^{-1} a^{-1} respectively) biomass (Table 7).

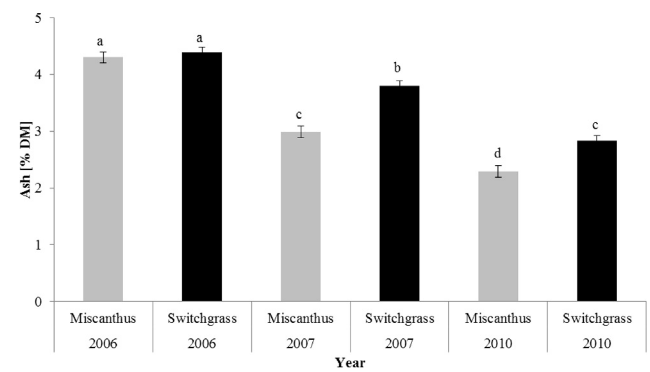

Fig. 6. Ash content comparison between miscanthus and switchgrass over three years (error bars indicate standard errors). The bars with the same superscript do not differ significantly from each other according to multiple t-test $\alpha = 0.05$).

Table 6
Mean N removal through harvesting of miscanthus and switchgrass under different N fertilization levels over three years.

	Miscanthus			Switchgrass		
N level [kg ha^{-1}]	0	40	80	0	40	80
Dry matter yield [t ha^{-1}]	16.4	20.3	22.5	7.9	11.5	15.4
Total N content [g kg^{-1}]	2.3	3	3.5	7.9	7.9	8.7
Mean N removal [kg ha^{-1}]	38	61	79	63	91	134

Table 5
Type 3 tests of fixed effects for ash content.

Effect	F-value	Pr > F
crop	24.42	0.0149
replication	1.82	0.3465
N level	4.37	0.0271
year	240.83	<0.0001
N level*crop	2.14	0.1441
year*crop	8.89	0.0104

Table 7
Mean annual volatile emissions and energy input of miscanthus cropping (mean of 10 years).

Cropping system	Volatile emissions (LCA)	Soil-born trace gases (measured)	Sum	Relative emissions	Energy input	Energy content of the harvested biomass
	[kg CO_2-eq.]	[kg CO_2-eq.]	[kg CO_2-eq.]	[kg CO_2-eq./t DM]	[GJ ha^{-1} a^{-1}]	[GJ ha^{-1} a^{-1}]
Miscanthus 0 kg N ha^{-1} a^{-1}	178	−22	157	12	4	235
Miscanthus 80 kg N ha^{-1} a^{-1}	668	420	1088	59	8	324

4. Discussion

Biomass-based energy production requires crops with a high yield output along with high biomass qualities. The yield comparison of the two crops, miscanthus and switchgrass, confirmed that dry matter production increased with increasing N input between 0 and 80 kg N ha^{-1}. However, it had a negative effect on the quality of the produced biomass by increasing the N and ash content.

Based on the current study, the dry matter yield development can be divided into two production phases: a) the establishment period (2002, 2003) where low crop yield was accompanied by low shoot density and lower plant height; b) post-establishment period (2004–2012) where crop yield was increased by the development of dense and tall shoots. The miscanthus developed thicker and taller stems than the switchgrass. Therefore, in the post-establishment period it delivered high dry matter yield. This indicates that morphological characteristics, in particular shoot diameter and plant height, are good indicators for the comparison of crop yield development [13,43]. The shoot density and development was affected positively by N fertilization in both crops. However, despite this positive effect of N fertilization, the miscanthus took longer than the switchgrass to reach the maximum yield, mainly as a result of the longer time period required to establish its extensive rhizomatic rooting system [39]. Once the rhizomatic rooting system is established, miscanthus uses water and nutrient resources very efficiently [8,40]. This accounts for the increased net primary production and higher yields in the post-establishment period.

However, in this study the switchgrass responded well to high N fertilization levels (80 kg N ha^{-1}). Therefore, it is possible that switchgrass yield could be further increased through even higher N fertilization. The effect of high N fertilization (40–80 kg N ha^{-1}) on miscanthus was insignificant. This indicates that under the given soil and weather conditions a fertilization rate of about 40 kg N ha^{-1} a^{-1} is sufficient to achieve the yield potential of miscanthus. Considering the lower N demand of miscanthus and the lower N content in its biomass in this study, it can be assumed that there will be lower NOx emissions in a combustion process when using miscanthus compared to switchgrass.

In miscanthus, the yield decreased by 20% in the three years following the peak yield (in the 8th year), whereas in switchgrass the yield decreased by 23% in the 5 years after the peak yield (in the 6th year). This comparison indicates that despite the yield decrease in both crops, the trend towards a continuous yield decrease after the peak yield set is earlier in switchgrass than in miscanthus. Another reason for the better performance of miscanthus was a higher post-peak mean yield compared to the pre-peak mean yield. This was mainly because the switchgrass yield continued to decrease with every year in the post-peak period, whereas the miscanthus yield remained constant at a level above 16 t ha^{-1}. For switchgrass, it can be speculated that the yield decreased due to stand age effects [2] such as invasion of weeds and decline in soil fertility over the years. In this study, based on the authors' own observations, in the switchgrass plots the invasion of weeds increased over the years in the post-peak yield period (after the 6th year) and contributed to the suppression of crop growth. This indicates that switchgrass becomes less able to resist weed competition after the 6th year on this site. Therefore, it could be more productive to replant switchgrass after the 7th to 9th plantation year. Another explanation for the continual decrease in yield could be that the switchgrass had used up some of the limiting soil nutrients to reach the peak yield [2], although in current study macronutrients were held in optimum range.

However for the miscanthus, the observed three-year period of post-peak yield is not sufficient to determine a clear trend for the aging effect. Hence, measurements from the coming years will be necessary for its analysis. In another field experiment carried out under very similar climatic conditions at the same location, miscanthus reached peak yield in the 13th year [13,20]. Therefore, it can be concluded that miscanthus can deliver high yields over longer time periods without any significant effect of aging, depending on the site conditions. This higher productivity over a longer time compared to switchgrass could be a result of miscanthus' efficient translocation system [12], which ensures a sufficient nutrient supply for the next growth season. The long-term productivity of miscanthus also accommodates its higher establishment costs. The largest part of this is the cost of rhizomes, which can amount to about 3200 € ha^{-1} in some EU countries (Hungry, Italy, Lithuania, Poland UK) compared to switchgrass seed costs of 540 € ha^{-1} [38]. If calculated over a longer time period, the high establishment costs could be compensated by the long-term high productivity and this could enable miscanthus to compete with leading energy crops.

Statistically there was no significant correlation between the mean climatic conditions during the growth period and the yields of the following harvest. However, the yield variations in the post-establishment period are probably a result of specific weather conditions during critical growing periods and variable harvest times between the years [20].

In future, the development of European Union quality standards with particular focus on NOx emissions could limit biomass-based fuel production, especially for direct combustion. The comparison of the combustion quality of miscanthus and switchgrass with commercial standards for herbaceous biomass pellets (EN-14961-A2) [11] indicates that the N and ash content of both crops fall within the defined limits. However, unlike other herbaceous biomass, the miscanthus N content even falls within the limits set for wood pellets (EN-1496-2-A2), whereas switchgrass only falls within these limits when harvested in April. This indicates that miscanthus has better biomass qualities compared to other herbaceous crops including switchgrass and can even compete with wood pellets.

It was hypothesized that delayed harvest and different N levels can affect the N content of the harvested biomass. This hypothesis was proved correct, because the low N fertilization levels and delayed harvest indeed significantly decreased the N content in the harvested biomass. This trend of low N content due to delayed harvest has also been reported in the literature for miscanthus and switchgrass [16]. The low N content in the harvested biomass can lead subsequently to low NOx emissions during thermo-chemical conversion processes. For ash content, the harvest date effect was not evaluated in this study. The delayed harvest can contribute to low N and ash content in two ways: a) by providing ample time to translocate the N [39] and other main ash constituents such as Si to the rhizomes: b) longer time for the detachment of leaves, as these contain a larger proportion of N and ash than stems [4]. Kludze et al. [25] also reported that in delayed harvest the largest part of yield losses is due to the detachment of leaves. This indicates that the proportion of leaves in the harvested biomass plays a key role in determining the quality of the harvested biomass. Therefore, the low ash and N content in miscanthus can be explained through low leaf-to-stem ratio compared to switchgrass (data not shown). The significantly low ash content of miscanthus biomass can also be explained through its natural ability to store Si in rhizomes [37] because Si constitutes up to 74% of miscanthus ash [35]. The epidermis, cortex and the vascular cylinder are the three main Si deposition zones in miscanthus rhizomes [37].

Despite the yield decrease, overall the biomass quality improved for both crops over the measured years (2005, 2006, 2007, 2010). This could be due to the development of large rhizomes, in particular in the case of miscanthus, which increased the relocation capacity of the crops, and subsequently decreased the inorganic constituents of the harvested biomass [9]. However, the decreasing ash content during the post-establishment period (ash data collected in 2006, 2007, 2010) could also be an effect of the climatic conditions during pre-harvest time. Further examination is required to establish whether this effect can be linked to crop age.

Although the yield under the prevailing climatic conditions of this study was low, switchgrass can still be considered a sustainable perennial crop for biofuel production mainly on account of its ability to grow under marginal soils accompanied by nutrient translocation before harvest [42]. However, in this study the significantly lower N and ash content in the harvested miscanthus biomass reveals that the recycling of nutrients through leaf fall and the translocation mechanism [12] is more efficient in miscanthus than in switchgrass. There are many factors which can affect the translocation mechanism, such as harvest time and plant species [42], but these were not evaluated in this study. As a result of the efficient translocation mechanism of miscanthus, it would be worthwhile in future to test miscanthus on soils with low nutrient content and low input use. In this way, it could help to minimize the competition between food and feed crops for arable soils while providing high dry matter yield for biofuel production. When comparing the total energy input into each cropping system with the energy content stored in the harvested biomass, a clear positive balance was achieved in the investigated cropping system. For miscanthus energy inputs exceeded energy gains in the first year, since no biomass was harvested; however in the second year the energy balance was clearly positive for miscanthus since only 2–8 % of the harvested energy was consumed before. It has to be kept in mind that for the total energy balance of a bioenergy pathway, the conversion process is of decisive importance since total conversion efficiencies differ highly (for details on transformation efficiencies see Refs. [5,10,30].

The conversion to heat or to heat and electricity in a Combined Heat and Power plant (CHP) is one of the favorable pathways of energetic use of miscanthus and switchgrass. One ton of miscanthus feedstock yields about 10 GJ heat energy, when burned in a boiler [30]. This leads to a potential mean heat production of about 175 GJ $ha^{-1}a^{-1}$ from fertilized miscanthus. Alternatively, gasification, Fischer-Tropsch synthesis, or ethanol production are alternative pathways for feedstocks like miscanthus and switchgrass (e.g. Refs. [26,33].

The global warming potential for one ha of miscanthus was estimated to about 1 t CO_2-equivalents. This estimation lies below other calculations made by Refs. [30]; who estimated almost 2 t CO_2-equivalents for the same area. However, when crediting carbon sequestration to the LCA, Parajuli et al. [30] estimated an even negative GWP for miscanthus cropping at a field scale.

The emissions of nitrogen fertilizer which affect global warming accounted to 72% of total emissions in the conducted LCA. This highlights the importance of nitrogen (N) fertilization on energy balance and GHG emissions of energy cropping. This finding is proved by many LCAs of agricultural systems [7,23,29]. Nitrogen efficiency is therefore one major factor to convey favorable energy balances of energy cropping. Perennial crops show advantageous N efficiencies compared to annual crops [22,34].

In this study miscanthus was more efficient than switchgrass in N use and there was less N removal through the harvested biomass and therefore, there will be less chances of NOx emissions. Nitrogen is one of the limiting factors in crop production and also linked with ecologically critical aspects. Efficient N use and ability to store N in rhizomes leads to lower GHG emissions, lower energy input, less leaching and less eutrophication.

5. Conclusions

This study revealed that morphological characteristics - especially shoot diameter and plant height - are good indicators for the prediction of crop yield development in the energy grasses miscanthus and switchgrass. Miscanthus plantations appear to provide higher yields over a longer period of time than switchgrass plantations, for which a turnover after 7–9 years is recommended under the given conditions. Miscanthus plantations are productively sustainable for at least 15 years under these conditions. In contrast to switchgrass, N fertilization beyond 40 kg ha^{-1} did not significantly increase yield in miscanthus. Thus it can be concluded that the N demand for switchgrass on a per-hectare and per-ton basis is higher than that for miscanthus. Hypothesis a) was proven, since it was shown that shoot height and shoot number were significantly correlated with crop yield for both crops.

Due to the higher yields and longer productive period, miscanthus could compensate for the higher establishment costs compared to switchgrass. Like it was assumed in hypothis c), analysis of quality characteristics showed that miscanthus had a lower N and ash content in the harvested biomass. For both crops increased N fertilization rates led to a significant increase in ash and N contents. Delayed harvest time led to a decrease in N content of the biomass in both crops, which proved hypothesis b).

Summarizing the findings of this study, it is apparent that miscanthus performs better with regard to quality and quantity aspects of biomass production at the given site. However, switchgrass combines low establishment costs with a greater frost tolerance and is therefore more suitable for certain marginal sites than miscanthus.

The conducted LCA showed that the production of miscanthus as a feedstock for bioenergy plants was connected with relatively low volatile emissions. Since the emissions were mainly affected by N fertilization, further improvements regarding amount, time and form of fertilization seem a promising future option to optimize the cropping system. Based on this study, from production till thermochemical conversion, it can be concluded that miscanthus feedstock was better suitable for combustion in terms of feedstock supply, quality, conversion efficiency and ecological benefits.

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3. Chapter-2

This chapter includes two research publications, which mainly deal with pre-treatment process to improve the digestibility of biomass for subsequent processing such as ethanol production. The overall aim of this chapter is to optimize the pre-treatment conditions for miscanthus biomass to improve the bioconversion efficiency of biomass for ethanol production. The first publication encompasses on miscanthus and wheat straw performance under sulfuric acid pre-treatment. It compares the formation of inhibitors under different pre-treatment conditions from mild to severe. In addition, the influence of fiber composition on biomass digestibility is also evaluated and compared for both substrates. In 2^{nd} publication, single stage pre-treatment and two stage pre-treatment process is compared for both miscanthus and wheat straw. Here, wheat straw is used as reference feedstock. This chapter further divides into two sub-chapters comprised of two publications.

3.1. Comparing the Performance of *Miscanthus X Giganteus* and Wheat Straw Biomass in Sulfuric Acid Based Pretreatment.

Publication-4

Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2015. "Comparing the Performance of Miscanthus X Giganteus and Wheat Straw Biomass in Sulfuric Acid Based Pretreatment." *Bioresource Technology* 180: 360-364.

The objective of this study was to assess and compare the suitability of *M. x giganteus* and wheat straw biomass in dilute acid catalyzed pretreatment. Miscanthus and wheat straw were treated in a dilute sulfuric acid/steam explosion pretreatment. As a result of combining dilute sulfuric acid- and steam explosion pretreatment the hemicellulose hydrolysis yields (96% in wheat straw and 90% in miscanthus) in both substrates were higher than reported in literature. The combined severity factor (=CSF) for optimal hemicellulose hydrolysis was 1.9 and 1.5 in for miscanthus and wheat straw respectively. Because of the higher CSF value more furfural, furfuryl alcohol, 5-hydroxymethylfurfural and acetic acid was formed in miscanthus than in wheat straw pretreatment.

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Short Communication

Comparing the performance of *Miscanthus x giganteus* and wheat straw biomass in sulfuric acid based pretreatment

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HIGHLIGHTS

- Miscanthus required more energy/acid input in hydrolysis than wheat straw.
- Combining dilute sulfuric acid and steam explosion resulted in high C5 sugar yield.
- ADL lignin in miscanthus prevented efficient breakdown of fiber recalcitrance.

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ABSTRACT

The objective of this study was to assess and compare the suitability of *Miscanthus x giganteus* and wheat straw biomass in dilute acid catalyzed pretreatment. Miscanthus and wheat straw were treated in a dilute sulfuric acid/steam explosion pretreatment. As a result of combining dilute sulfuric acid- and steam explosion pretreatment the hemicellulose hydrolysis yields (96% in wheat straw and 90% in miscanthus) in both substrates were higher than reported in literature. The combined severity factor (=CSF) for optimal hemicellulose hydrolysis was 1.9 and 1.5 in for miscanthus and wheat straw respectively. Because of the higher CSF value more furfural, furfuryl alcohol, 5-hydroxymethylfurfural and acetic acid was formed in miscanthus than in wheat straw pretreatment.

1. Introduction

An increase of global bioethanol production require the use of other resources than grain only to avoid a situation of competition between food- and bioenergy production. Lignocellulosic materials can replace grain in bioethanol production only if an efficient bioconversion is ensured. Two promising lignocellulosic materials for ethanol production in Europe are wheat straw and *Miscanthus x giganteus*. Wheat straw as an agricultural lignocellulosic byproduct is already being used for the production of biofuels for example in pilot scale plants (Talebnia et al., 2010). However, the use of wheat straw for biofuel production has some limitations. For example, an unfavorable volume to weight ratio and low yield per hectare can lead to high transport costs. Also, a concurrence situation caused by high demands in animal husbandry, for soil conservation and bioenergy production leads to increasing prices (Barzegar et al., 2002). *Miscanthus x giganteus*, a dedicated energy crop with high dry matter yield and the ability to grow under diverse climates, has emerged as an attractive alternative to wheat straw (Gauder et al., 2012). Khanna et al. (2008) reported for the USA that the biomass production costs of *Miscanthus x giganteus, in the following text called miscanthus*, are lower than those of short rotation woody crops such as willow and poplar. However, for ethanol production not only biomass production and transport costs, but also the conversion efficiency is an important parameter for determining feedstock suitability. In bioconversion process the application of an appropriate pretreatment method is probably the most crucial step because it has a large impact on the efficiency of the overall bioconversion process (Wyman et al., 2005). For an efficient bioconversion process all carbohydrates (from cellulose and hemicellulose), not only glucose (from cellulose), have to be used. Following glucose, the xylose carbohydrate fraction of hemicellulose is the second largest fraction and can be used for production of biofuel or food additives (xylitol) (Demeke et al., 2013). The separate use of both, glucose and xylose, makes the bioconversion process more economical. Pretreatment methods include physical, chemical and biological processes (Talebnia et al., 2010). Among chemical pretreatment methods dilute sulfuric acid is most often used. In combination with steam explosion, a physical method, it

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is the most common steam pretreatment for woody biomass (Wyman et al., 2005; Zhu and Pan, 2010). The use of dilute sulfuric acid leads to efficient hydrolysis of hemicellulose and subsequently high sugar yields but it also has some disadvantages such as sugar losses due to formation of fermentation and enzyme inhibitors, such as furfural, 5-hydroxymethylfurfural and acetic acid (Palmqvist and Hahn-Hägerdal, 2000). The loss of sugars and formation of inhibitors is caused by different chemical characteristics of pentose and hexose sugars, both part of the hemicellulose and cellulose fraction. Therefore hemicellulose- and cellulose fraction should be separated to avoid sugar losses (Nguyen et al., 2000). The aim of the current work is a direct comparison of miscanthus and wheat straw biomass performance in diluted acid catalyzed pretreatment. The main parameters assessed in this study are: (a) hemicellulose hydrolysis sugar yields in both substrates, (b) energy (reaction temperature and time)/acid consumption, (c) amount of inhibitor formation.

2. Methods

2.1. Substrate collection

Wheat (*Triticum aestivum L.*) straw samples were supplied by Meiereihof University of Hohenheim (Baden-Württemberg) in 2012. *Miscanthus x giganteus* was harvested in October 2013 from a field trial established in 1992 at the University of Hohenheim. All samples were chopped to a particle size of 1 cm, oven-dried at 60 °C, for 2 days, and then stored in plastic bags at room temperature. All chemicals used in the experiments are technical grade (Distributor: Brenntag AG) and all chemicals used in analysis are research grade (distributers: Merck KGaA; Carl Roth GmbH+ Co.KG; Fluka (Sigma–Aldrich)).

2.2. Experimental set-up and operation

A 10 l steam jacketed and steam injected batch paddle type mixed reactor was used for all treatments. The reactor was heated by high pressure saturated steam at 7 bar. The steam was supplied directly (injected) and indirectly (jacketed) to the reactor, and during the experiments a combination of direct and indirect supply was used to allow fast heating to different target temperatures and to hold the desired reaction temperature. Every time the reactor took 3 min to reach the desired temperature by combination of direct and indirect heating and regulation of the steam flow. The reaction temperature was maintained during the experiment through indirect steam supply. Reaction temperature and air pressure was monitored through a thermometer, and a pressure gauge. The reactor vessel was loaded from top and emptied by a valve at the bottom of the reaction vessel. The reactor has a mechanical stirrer, which was used to ensure proper mixing and exposure of substrate to the pretreatment conditions. The desired amount of sulfuric acid was mixed with the appropriate amount of tap water to reach 20% dry matter in combination with the biomass and transferred into the reactor. Afterwards 1 kg dry matter of miscanthus or wheat straw was added. The required pretreatment conditions were applied and at the end of the reaction time the reactor was emptied by steam explosion into a collection bucket. All experiments were at least done in triplicate.

2.3. Sample collection

The Laboratory Analytical Procedure (LAP): "*Summative Mass Closure*" from the US National Renewable Energy Laboratory (NREL 2014 Technical Report NREL/TP-510-48825) was used for sample collection and analysis: The LAPs was used as follows. The slurry was vacuum filtrated to separate solid and liquid fraction. The solids were repeatedly washed with deionized water and vacuum filtrated until glucose remained in wash was less than 0.05 g/l (determined by HPLC). The liquor (liquid fraction of biomass slurry) and wash water was used for sugar and inhibitor analysis. Samples from liquor, wash water and solids were stored at 4 °C. A subsample of the washed solids was taken and dried at 105 °C for 48 h to estimate dry matter content.

2.4. Fiber analysis

Substrate parameters were determined by fiber analysis, a method which is optimized for determination of carbohydrates. The fiber analysis was done through estimation of NDF (Neutral-detergent-fiber according to VDLUFA, 6.5.1 (Van Soest and Wine, 1967), ADF (Acid-detergent-fiber according to VDLUFA, 6.5.2 (Van Soest and Wine, 1967) and ADL (Acid-detergent-lignin according to VDLUFA, 6.5.3 (Van Soest and Wine, 1967). For each sample 500 mg weight was taken and packed into air tight filter bags. For NDF, samples were digested in NDF solution with addition of alpha amylase. Digestion was carried out for 75 min in the fully automatic fiber analyzer *ANKOM 2000*. ADF was estimated by digesting the samples in the ADF solution for 1 h by using fully automatic fiber analyzer *ANKOM 2000*. For ADL analysis, samples were placed into ADL solution and rotated for 3 h in Daisy incubator (ANKOM Technology). ADL lignin represents not the whole amount but only the acid insoluble fraction of lignin in fiber.

2.4.1. Sugar, acid and inhibitor analysis

The concentrations of glucose, xylose, arabinose, acetic acid, sulfuric acid, furfural, furfuryl alcohol and 5-hyddroxymethylfufural in liquor and wash water from pretreatment were measured by HPLC according to the Laboratory Analytical Procedure (LAP): "*Determination of Sugars, Byproducts and Degradation Products in Liquid Fraction Process Samples*" from the National Renewable Energy Laboratory (NREL 2014 NREL/TP-510-42623). A HPLC system from Bischoff was used with an Rezex™ *ROA Organic acid H*$^+$ column at 75 °C column temperature and 0.005 N H_2SO_4 as eluent, at 0.6 ml/min. Detector was Shodex RI – 101 at 50 °C detector temperature.

2.5. Data analysis

2.5.1. Hemicellulose yield and inhibitors

Hemicellulose sugar (arabinose; xylose) and glucose yield in sulfuric acid pretreatment was calculated as follows:

$$\text{Sugar yield}\,(\%) = \frac{\text{Sugar liquid}}{\text{Sugar substrate}} \times 100 \qquad (1)$$

"*Sugar liquid*" is the sum of arabinose and xylose (hemicellulose sugar yield) or glucose in the liquor and wash water in gram. It was calculated from individual sugar concentration (g/l) in liquor and wash water, and the amount of liquor and wash water in liter. "*Sugar substrate*" is the amount of glucose equivalents in cellulose, and of xylose/arabinose equivalents in hemicellulose in gram. It is calculated from fiber analysis (see Table 1) and the amount of substrate used in treatment. The amounts of inhibitors (acetic acid, sulfuric acid, furfural, furfuryl alcohol and 5-hyddroxymethylfufural) were measured as concentration in g/l in the liquor.

2.5.2. Combined severity factor (=CSF)

The CSF expresses the severity of the pretreatment by combining the effect of reaction temperature, acid concentration and reac-

Table 1
Water content and fiber composition of miscanthus and wheat straw (standard deviation), inhibitors (furfural, furfuryl alcohol, 5-HMF, acetic acid) produced per gram sugar (glucose, xylose, arabinose) after pretreatment and hemicellulose sugar yield (standard deviation) with required pretreatment parameter (temperature/reaction time/sulfuric acid conc.).

Parameter	Miscanthus	Wheat straw
Water (% of FM)	6.9 ± 0.83	9.6 ± 0.62
Cellulose (% of DM)	51.3 ± 0.25	43.4 ± 0.30
Hemicellulose (% of DM)	25.2 ± 0.58	27.0 ± 0.23
ADL lignin (% of DM)	11.8 ± 0.83	5.5 ± 0.16
Inhibitor (ml)/sugar (g)	0.198:1	0.083:1
Hemicellulose sugar yield	89.9 ± 4.4%	96.2 ± 4.7%
Required pretreatment parameter	150 °C; 10 min; 1.44% w/w acid	140 °C; 10 min; 1.13% w/w acid

tion time (Aguilar et al., 2002). In this study this concept was applied as follows:

$$CSF = log_{10}(Ro) - pH \quad (2)$$

$$Ro = t_r * \exp((T_r - 100)/14.75) \quad (3)$$

Where t_r is the reaction time in minutes which was kept between 5 and 15 min and T_r is the reaction temperature in degrees Celsius, which was kept between 130 and 160 °C. The pH value is calculated from the sulfuric acid concentration of the hydrolyzate samples taken after pretreatment (as described in Section 2.2) and analyzed by HPLC.

2.5.3. Statistic

The arithmetic means, standard deviation and standard error of the means were calculated. To compare the means, *t*-test with a 95% confidence interval was applied. Calculation and visualization was done with Matlab.

3. Results and discussion

3.1. Pretreatment xylose and arabinose yield

Fig. 1 shows the hydrolysis of hemicellulose during pretreatment in wheat straw and miscanthus. Wheat straw hemicellulose hydrolysis had its peak, reaching 96.2 ± 4.7% of sugar yield, at a CSF of 1.5 with 140 °C reaction temperature, 10 min reaction time and 1.13% w/w sulfuric acid. Also some cellulose hydrolysis occurred but, as discussed by Lee et al. (1999), due to slower hydrolysis of cellulose at low CS factors glucose yield was low (8.6% yield at CSF 1.5 data not shown). The hydrolysis of miscanthus hemicellulose reached its peak at a CSF of 1.9 with 89.9 ± 4.4% sugar yield. The reaction temperature for CSF 1.9 was 150 °C, reaction time 10 min and 1.44% w/w sulfuric acid. Glucose yield also showed a similar trend as in wheat straw, but with slightly lower yields (6% yield at CSF 1.9 data not shown). The results for sugar yields in this study were higher than as reported in literature (Kootstra et al., 2009; Satari Baboukani et al., 2012; Guo et al., 2008, 2013). Possible reasons for higher yields in current work were the fast preheating speed (17–27 °C/min)/short reaction times and the combined dilute acid and steam explosion pretreatment. Short reaction and preheating times minimize sugar losses through sugar degradation (Lee et al., 1999; Tucker et al., 2003). The combination of dilute acid and steam explosion pretreatment enhanced sugar yield and enzymatic hydrolysis in rice straw and corn stover compared to a treatment without that combination (Lloyd and Wyman, 2005; Chen et al., 2011).

3.2. Pretreatment energy and acid consumption

In addition to carbohydrate yield also energy (reaction temperature and time) and acid consumption have to be taken into account when assessing the performance of both biomasses in pretreatment process. Zhu and Pan (2010) stated that the technical performance of any pretreatment technology needs to be evaluated based on total fermentable sugar production as well as on

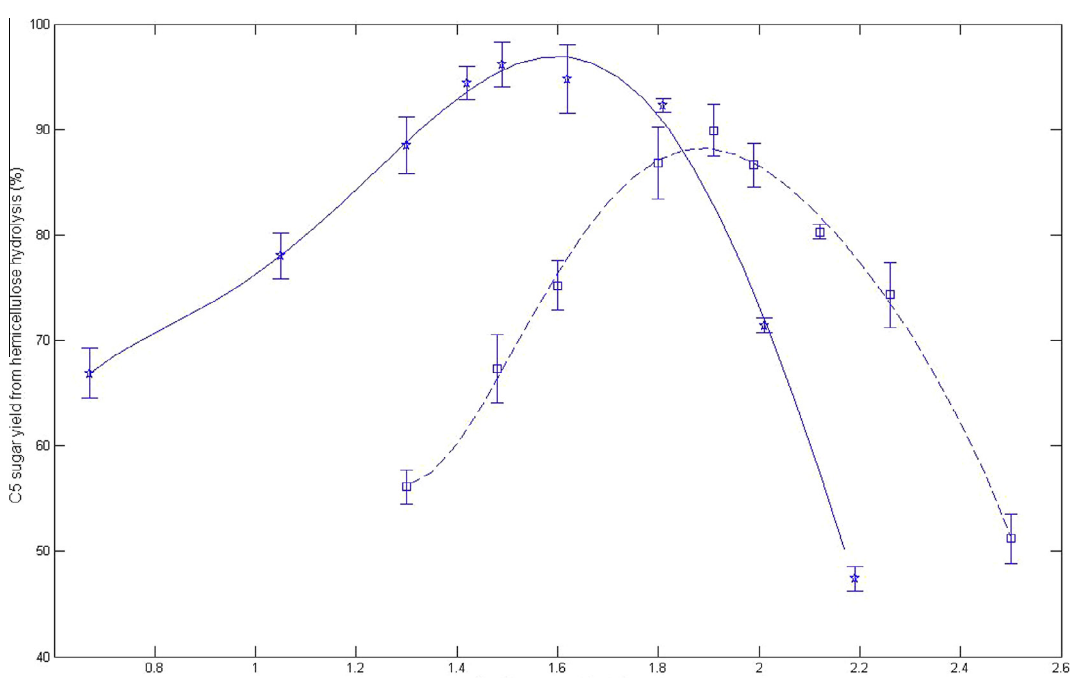

Fig. 1. Effect of pretreatment severity on hemicellulose hydrolysis sugar yield (xylose + arabinose) in wheat straw ("star" and "continuous line") and miscanthus ("square" and "broken line") with standard error of the mean.

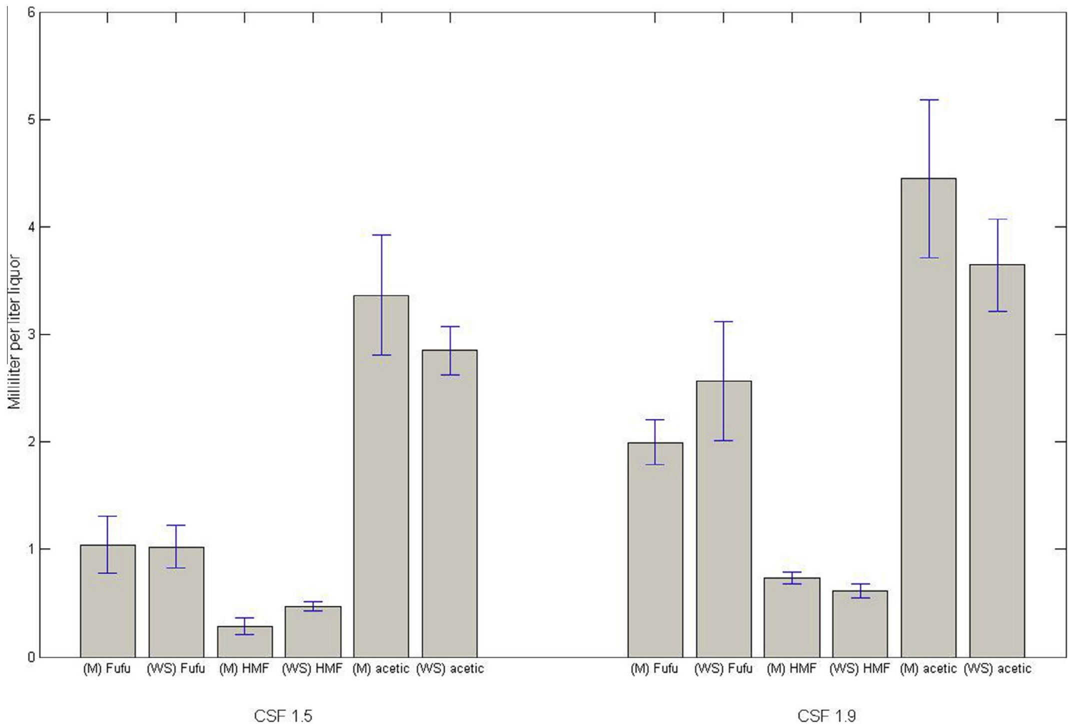

Fig. 2. Effect of CSF 1.5 and CSF 1.9 on formation of furfural/furfuryl alcohol (=Fufu), 5-hydroxymethylfufural (=HMF) and acetic acid (=acetic) in wheat straw (=WS) and miscanthus (=M) in liquor with standard error of the mean.

energy consumed per unit sugar produced. In the current study the necessary CSF for optimal hemicellulose hydrolysis was higher for miscanthus than for wheat straw. The more energy intensive hemicellulose hydrolysis was probably caused by higher content of lignin in general and, especially by not acid dissolvable ADL-lignin, in miscanthus protecting hemicellulose from hydrolysis (Studer et al., 2011). Although the hemicellulose hydrolysis was more energy intensive in miscanthus, the energy/acid consumptions, as displayed in Table 1, was only slightly higher than for wheat straw. Therefore an advantage of wheat straw over miscanthus can hardly be recognized.

3.3. Inhibitors

Although there is also a fraction of inhibitors derived from lignin degradation it was not considered in this study because here the focus is on hemicellulose and cellulose hydrolysis and degradation. From hemicellulose degradation **furfural** and from furfural degradation furfuryl alcohol were considered most important and are displayed in Fig. 2. In miscanthus optimum hemicellulose hydrolysis level (CSF 1.9), 1.99 ml/l furan derivatives from hemicellulose (furfural, furfuryl alcohol) were formed. This was significantly higher compared to 1.02 ml/l in wheat straw at CSF 1.5. The more severe condition for optimal hemicellulose hydrolysis in miscanthus facilitated inhibitor formation (Palmqvist and Hahn-Hägerdal, 2000). The amount of **5-hydroxymethylfurfural** (5-HMF) from cellulose degradation was low compared to furfural from hemicellulose in both substrates (Fig. 2). Also, a trend but no significant increase of 5-HMF under more severe conditions was observed. Similar amounts of furan derivates were described in literature (Guo et al., 2008, 2013; Kootstra et al., 2009). One fact should be highlighted. In Fig. 2 it can be seen that higher furfural/furfuryl alcohol concentrations occurred in wheat straw under CSF 1.9 than in miscanthus. Xylose and arabinose were faster hydrolyzed as a result of higher energy input (CSF 1.9) in wheat straw than in miscanthus, making them accessible for degradation for a longer period. As a result more degradation of C5 sugars occurred in wheat straw at CSF 1.9. In miscanthus slower hydrolysis (because more energy intensive) reduced the amount and time in which xylose and arabinose were in a free, unprotected state in the liquid phase (Nguyen et al., 2000). **Acetic acid** is known to be a fermentation inhibitor, especially in synergy with other inhibitors (Palmqvist and Hahn-Hägerdal, 2000). In the current study a general but non-significant trend for lower acetic acid concentrations in wheat straw than in miscanthus was observed. Under more severe pretreatment conditions (CSF 1.9) significantly more acetic acid was formed in wheat straw compared to less severe conditions (CSF 1.5). The same trend was observed in miscanthus but non-significant. The amount of acetic acid which can be formed during pretreatment depends on the degree of acetylation of hemicellulose (Guo et al., 2008). Fiber analysis showed that wheat straw had more hemicellulose (27.0%) than miscanthus (25.2%), but more acetic acid was formed in miscanthus (Table 1, Fig. 2). It is possible that hemicellulose in miscanthus was more acetylated than in wheat straw. Guo et al. (2008) found similar acetic acid concentrations after pretreatment of miscanthus biomass. In comparison to rice straw Guo et al. (2013) found that miscanthus hemicellulose was even more acetylated and therefore more acetic acid was produced. The higher amounts of inhibitors in miscanthus (Table 1) can lead to higher costs required for detoxification. But in current work no difference was recorded in the amount of wash water required for detoxification of miscanthus and wheat straw.

4. Conclusion

High hemicellulose derived carbohydrate yields from biomass of miscanthus and wheat straw can be achieved by combining sulfuric acid- /steam explosion pretreatment, using fast preheating- and short reaction times. Miscanthus requires slightly higher energy and acid than wheat straw. However, this resulted not in a significant advantage of wheat straw over miscanthus biomass. For assessing the overall comparative suitability of miscanthus and wheat straw biomass for ethanol production and for assessing the ethanol production costs, the results for pretreatment have to be analyzed within the context of the complete value chain, including biomass production, transportation requirements and enzymatic hydrolysis.

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3.2. Efficiency of Single Stage- and Two Stage Pretreatment in Biomass with Different Lignin Content.

Publication-5

Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2016. "Efficiency of Single Stage- and Two Stage Pretreatment in Biomass with Different Lignin Content." *Bioresource Technology* 211: 787-791.

In current study the enzymatic glucose yields of miscanthus and wheat straw were compared after single stage- and two stage pretreatment with dilute sulfuric acid at different pretreatment severities. Glucose yields after two stage pretreatment were higher than after single stage pretreatment in miscanthus. Whereas wheat straw had higher glucose yields after single stage pretreatment. The study shows that two stage pretreatment has a negative effect on glucose yield in biomass with low not-acid degradable lignin content and a positive one in biomass with high not-acid-degradable lignin content. The not-acid-degradable lignin fraction offers a higher degree of protection of the whole lignin structure against chemical attacks by mineral acids. More severe pretreatment conditions were needed to achieve a sufficient breakup of the lignin structure. But more severe conditions enhance resin formation, leading to lower enzyme activity and reduced carbohydrate yields.

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Short Communication

Efficiency of single stage- and two stage pretreatment in biomass with different lignin content

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HIGHLIGHTS

- Single stage pretreatment had higher enzymatic glucose yields in wheat straw.
- Two stage pretreatment had higher enzymatic glucose yields in miscanthus.
- Lignin content was key factor affecting single/two stage pretreatment performance.

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ABSTRACT

In current study the enzymatic glucose yields of miscanthus and wheat straw were compared after single stage- and two stage pretreatment with dilute sulfuric acid at different pretreatment severities. Glucose yields after two stage pretreatment were higher than after single stage pretreatment in miscanthus. Whereas wheat straw had higher glucose yields after single stage pretreatment. The study shows that two stage pretreatment has a negative effect on glucose yield in biomass with low not-acid-degradable lignin content and a positive one in biomass with high not-acid-degradable lignin content. The not-acid-degradable lignin fraction offers a higher degree of protection of the whole lignin structure against chemical attacks by mineral acids. More severe pretreatment conditions were needed to achieve a sufficient breakup of the lignin structure. But more severe conditions enhance resin formation, leading to lower enzyme activity and reduced carbohydrate yields.

1. Introduction

In recent years, the focus of biofuels research shifted towards their production from lignocellulose biomass. The main challenge in this field is the optimization of an economic hydrolysis process to increase the sugar release for subsequent fermentation process. There are different hydrolyses options and acid/base – single or two stage pretreatment plays a major role (Demirbas, 2009; Talebnia et al., 2010). Dilute acid hydrolysis is an essential step in hemicellulose fractionation and has been widely investigated (Wyman et al., 2005). The two stage pretreatment process is important because it offers the opportunity to separate hexose and pentose. Nguyen et al. (2000) reported that two stage dilute acid pretreatment in softwoods improved overall sugar yield by 10% and reduced net enzyme requirement by 50% in comparison to single stage pretreatment. However, acid hydrolysis needs to be optimized for each feedstock in a way that sugar recovery is maximized and production of inhibitory compounds is minimized. Considering the variations in feedstock composition especially in lignin content, there is a high possibility that some substrates are more suited for two stage dilute acid pretreatment than others. Therefore, in current study two different feedstocks were selected, wheat straw was used as a substrate with low lignin content, and *Miscanthus x giganteus* as a substrate with comparatively high lignin content. The influence of different lignin content on enzymatic hydrolysis, after two stage and single stage pretreatment was examined.

2. Materials and methods

2.1. Substrate collection

Wheat (*Triticum aestivum* L.) straw samples were supplied by Meiereihof, University of Hohenheim (Baden-Württemberg) in 2012. *Miscanthus x giganteus* was harvested in October 2013 from

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a field trial established in 1992 at the University of Hohenheim. All samples were chopped to a particle size of 1 cm, oven-dried at 60 °C, for two days, and then stored in plastic bags at room temperature. All chemicals used in the experiments are technical grade (Distributor: Brenntag AG) and all chemicals used in analysis are research grade (distributers: Merck KGaA; Carl Roth GmbH + Co. KG; Fluka (Sigma–Aldrich)).

2.2. Fiber analysis

Substrate parameters were determined by fiber analysis, a method which is optimized for determination of carbohydrates. The fiber analysis was done through estimation of NDF (neutral-detergent-fiber), ADF (acid-detergent-fiber) and ADL (the not-acid-degradable lignin fraction) according to VDLUFA, 6.5.3 (Van Soest and Wine, 1967). For each sample 500 mg weight was taken and packed into air tight filter bags. For NDF, samples were digested in NDF solution with addition of alpha amylase. Digestion was carried out for 75 min in the fully automatic fiber analyzer *ANKOM 2000*. ADF was estimated by digesting the samples in the ADF solution for one hour by using fully automatic fiber analyzer *ANKOM 2000*. For ADL analysis, samples were placed into ADL solution and rotated for three hours in Daisy incubator (ANKOM Technology).

2.3. Experimental set-up and operation

Samples were pretreated in a 1st stage (St 1) with sulfuric acid and glucose yields were assessed from the liquid fraction. The solid fraction went into the 2nd stage (St 2) pretreatment with sulfuric acid and again the glucose yield of the liquid fraction was determined. Samples from 1st stage (=single stage pretreatment) and 2nd stage (=two stage pretreatment) were hydrolyzed by enzymes and glucose yields were measured.

2.3.1. Pretreatment

A 10 L steam jacketed and steam injected batch paddle type mixed reactor was used for all treatments. The reactor was heated by high pressure saturated steam at 7 bar. The steam was supplied directly (injected) and indirectly (jacketed) to the reactor, and during the experiments a combination of direct and indirect supply was used to allow fast heating to different target temperatures and to hold the desired reaction temperature. Every time the reactor took 3 min to reach the desired temperature by combination of direct and indirect heating and regulation of the steam flow. The desired amount of sulfuric acid was mixed with the appropriate amount of tap water to reach 20% DM in combination with the biomass and transferred into the reactor. Afterwards, 1 kg dry matter of miscanthus or wheat straw was added. The pretreatment conditions were applied and at the end of the reaction time the reactor was emptied by steam explosion into a collection bucket. All experiments were done in triplicate.

2.4. Sample collection

2.4.1. Sample collection from pretreatment and enzymatic hydrolysis

The Laboratory Analytical Procedure (LAP): "Summative Mass Closure" from the US National Renewable Energy Laboratory (NREL 2014 Technical Report NREL/TP-510-48825) was used for sample collection and analysis for samples from pretreatment and enzymatic hydrolysis. The LAP was used as follows: The slurry from 1st/2nd stage pretreatment/enzymatic hydrolysis was vacuum filtrated to separate solid and liquid fraction. The solids were repeatedly washed with deionized water and vacuum filtrated until glucose remained in wash water was less than 0.05 g/L (determined by HPLC). The liquor (liquid fraction of biomass slurry) and wash water was used for sugar analysis. Liquor, wash water and solids samples were stored at 4 °C. A subsample of the washed solids was taken and dried at 105 °C for 48 h to estimate dry matter content.

2.4.2. Enzymatic hydrolysis and sample collection (enzymatic cellulose hydrolysis)

All enzymatic hydrolyses were done in triplicate and performed on 60 g of pretreated solids (dry weight) in a stainless steel vessel incubated in a water bath.

The samples were diluted with tap water to reach 7% dry matter in slurry and buffered in Na-citrate at pH 4.5. The samples were hydrolyzed by a noncommercial cellulase and a xylanase (to remove the remaining hemicellulose) from Erbslöh for 40 h at 50 °C. According to the distributor Erbslöh the cellulase had a specific enzyme activity (measured by Somogyi-Nelson test) of 409.2 carboxymethyl cellulose-U/g and a protein content of 15.2% and the xylanase 38.4 xylan-U/g with a protein content of 7.1%. Therefore the used amount of enzyme in current study had an EA of 10.6 Units (cellulase) and 0.46 Units (xylanase).

2.5. Glucose analysis and yield

The concentrations of glucose, in liquor and wash water from pretreatment and enzymatic hydrolysis were measured by HPLC according to the Laboratory Analytical Procedure (LAP): "Determination of Sugars, Byproducts and Degradation Products in Liquid Fraction Process Samples" from the National Renewable Energy Laboratory (NREL 2014 NREL/TP-510-42623). A HPLC system from Bischoff was used with an Rezex™ ROA Organic acid H^+ column at 75 °C column temperature and 0.005 N H_2SO_4 as eluent, at 0.6 ml/min. Detector was Shodex RI −101 at 50 °C detector temperature.

Glucose yield from pretreatment and enzymatic hydrolysis was calculated as:

$$\%\text{Glucose per DM biomass} = (\text{sugar from liquid}/\text{DM biomass}) * 100 \quad (1)$$

where "sugar from liquid" is the sum of glucose in liquor and wash water in gram. It was calculated from individual sugar concentration (g^*L^{-1}) in liquor and wash water, measured by HPLC, and the amount of liquor and wash water in milliliter. "Dry matter biomass" is the amount of dry matter biomass used in enzymatic hydrolysis.

2.6. Combined severity factor

Combined severity factor (=CSF). The CSF expresses the severity of the pretreatment by combining the effect of reaction temperature, acid concentration and reaction time (Aguilar et al., 2002). The concept was used as follows:

$$\text{CSF} = \log 10(\text{Ro}) - \text{pH} \quad (2)$$

$$\text{Ro} = tr * \exp((Tr - 100)/14.75) \quad (3)$$

where *tr* is the reaction time in minutes which was kept between 5 and 15 min and *Tr* is the reaction temperature in degrees Celsius, which was kept between 130 °C and 160 °C. The pH value is calculated from the sulfuric acid concentration of the hydrolysate samples, taken after pretreatment.

2.6.1. Statistic

The arithmetic means, standard deviation and standard error of the means were calculated. To compare the means, *t*-test with a 95% confidence interval was applied. Calculation and visualization was done with Matlab (see Table 1).

Table 1
Fiber composition of miscanthus and wheat straw (standard deviation).

Parameter	Miscanthus	Wheat straw
Cellulose [% of DM]	51.3 ± 0.25	43.4 ± 0.30
Hemicellulose [% of DM]	25.2 ± 0.58	27.0 ± 0.23
ADL Lignin [% of DM]	11.8 ± 0.83	5.5 ± 0.16

Table 2
Enzymatic glucose yields after single stage and two stage pretreatment.

Pretreatment (and CSF)	% Glucose per DM biomass Miscanthus	% Glucose per DM biomass Wheat straw
Single stage	10.0 ± 0.30	24.7 ± 0.28
Two stage (2nd stage CSF 2.2)	10.4 ± 0.33	12.1 ± 0.23
Two stage (2nd stage CSF 2.45)	6.8 ± 0.57	10.9 ± 0.41
Two stage (2nd stage CSF 2.7)	7.3 ± 0.41	10.4 ± 0.59

3. Results and discussion

The fiber analyses showed that cellulose content in miscanthus was higher than in wheat straw, whereas hemicelluloses content was on equal levels (Table 2). The not-acid-degradable lignin content (=ADL lignin) was twice as much in miscanthus than in wheat straw.

3.1. Cellulose hydrolysis in 1st and 2nd stage (sulfuric acid) pretreatment

1st stage pretreatment: The glucose yields from chemical cellulose hydrolysis in 1st and 2nd stage pretreatment were displayed in Fig. 1. As a part of current work which is already reported (Kärcher et al., 2015), the optimal conditions for hemicellulose hydrolysis for wheat straw were achieved at CSF 1.5. The glucose yield per DM biomass at CSF 1.5 for wheat straw was 8.9%. At CSF 1.9, the optimal hemicellulose hydrolysis condition in miscanthus (Kärcher et al., 2015), the glucose yield was less than 6%.

Because there are glucose side chains in hemicellulose structure it was not possible to determine how much cellulose hydrolysis and hemicellulose hydrolysis contributed each to 1st stage chemical glucose yield (Dussan et al., 2015).

2nd stage pretreatment: The chemical cellulose hydrolysis in 2nd stage pretreatment was significant higher in wheat straw than in miscanthus (Fig. 1).

According to Studer et al. lignocellulose substrate with high lignin content (Miscanthus) is more resistant to breakup of the lignin structure and offers a higher degree of protection against cellulose hydrolysis (Studer et al., 2011). Because of that more severe pretreatment conditions were needed to achieve a sufficient breakup of the lignin structure in miscanthus than in wheat straw.

3.2. Enzymatic cellulose hydrolysis after single- and two stage pretreatment

Samples of single stage pretreated (CSF 1.5 in wheat straw and 1.9 in miscanthus) and two stage pretreated (CSF 2.2 = 150 °C, 15 min reaction time, 1.9 vol.% sulfuric acid; CSF 2.45 = 160 °C, 15 min reaction time, 1.9 vol.% sulfuric acid; CSF 2.7 = 160 °C, 25 min reaction time, 1.8 vol.% sulfuric acid) miscanthus and wheat straw samples were enzymatically hydrolyzed. The enzymatic hydrolysis started quick in all samples and no differences between miscanthus and straw were observed in enzyme kinetics (Fig. 2 shows the kinetic of both substrates after single stage pretreatment).

But there were differences in glucose yield from enzyme hydrolysis as can be seen in Table 2. The data in Table 2 showing little influence of the CSF on enzymatic glucose yield after two stage pretreatment. Only pretreatment at CSF 2.2 (the "mildest" pretreatment condition in 2nd stage) had slightly higher glucose yields in both substrates. When comparing both substrates the glucose yields in wheat straw were all significant higher than in miscanthus, like in chemical cellulose hydrolysis. But the most interesting result was that in wheat straw, single stage pretreatment resulted in significant higher enzymatic glucose yields than

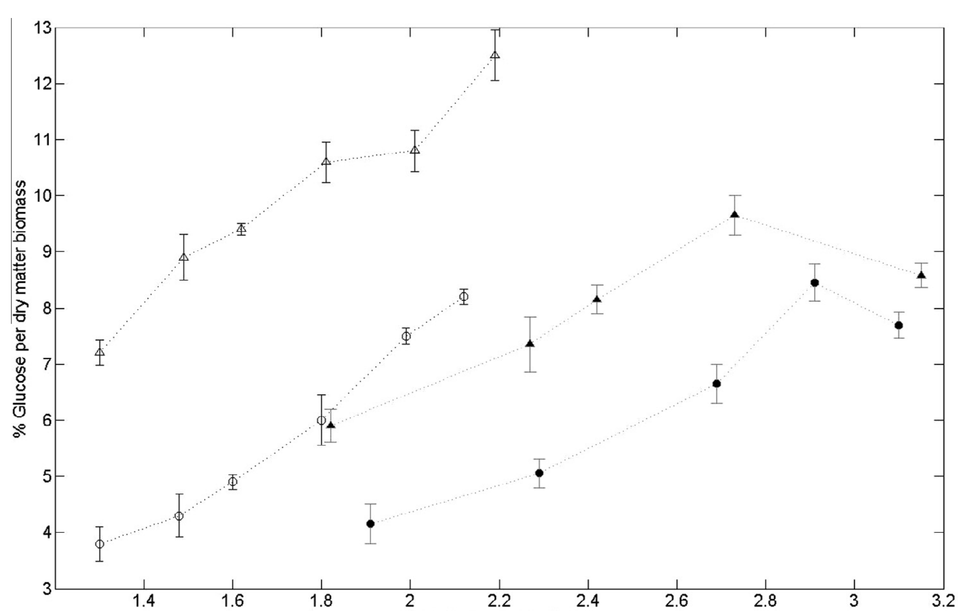

Fig. 1. Glucose yields of chemical cellulose hydrolysis with standard error of the mean of 1st stage sulfuric acid pretreatment in wheat straw (open "triangles"), miscanthus (open "circles") and 2nd stage sulfuric acid pretreatment in wheat straw (filled "triangles") and miscanthus (filled "circles") under different combined severities (CSF).

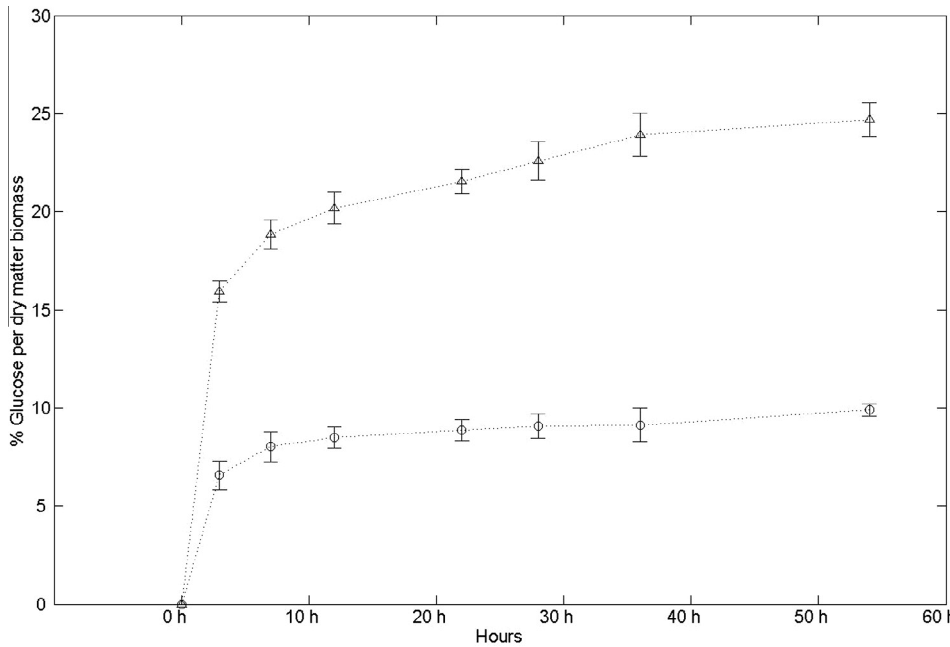

Fig. 2. Enzymatic glucose yields with standard error of the mean after 1st stage sulfuric acid pretreatment in wheat straw (open "triangles") with CSF 1.5 and miscanthus (open "circles") with CSF 1.8 and at different treatment times.

two stage pretreatment. Whereas in miscanthus two stage pretreatment (CSF 2.2) had higher glucose yields than single stage pretreatment (Table 2).

Nguyen et al. (2000) also reported enhanced enzymatic yields after two stage pretreatment in substrates with high lignin content (fir and pine wood chips) like other research groups (Studer et al., 2011; Zhu and Pan, 2010).

The major difference of both substrates used in current study was a higher ADL lignin content in miscanthus. The difference in ADL lignin content seems to be a key factor which influenced enzymatic cellulose hydrolysis in current study. Mineral acids can degrade lignin structure and alter dissolved lignin molecules, leading to resinification which decreases the effectiveness of enzyme hydrolysis (Palmqvist and Hahn-Hägerdal, 2000). But ADL lignin offers a higher degree of protection against chemical attacks by mineral acids and subsequently a higher amount of the lignin structure will stay intact. This means that:

1. (*Wheat straw*) Lignin structure was broken up in 1st stage more easily due to low ADL lignin content. The less severe conditions in first stage prevent resin formation of the dissolved lignin. Enzymatic hydrolysis benefits from the brake up of the lignin structure and the absence of resin formation, which is known to inhibit enzymatic activity (Palmqvist and Hahn-Hägerdal, 2000). During the more severe 2nd pretreatment stage the disruption of the lignin structure is enhanced but the resin formation, by chemical alteration of the dissolved lignin complexes, too. This led to a stronger inhibition of the enzyme activity, resulting in lower glucose yields in wheat straw after two stage pretreatment than after single stage pretreatment.
2. (*Miscanthus*) Lignocellulose substrate with a high degree of ADL lignin is more resistant to breakup of the lignin structure under mild pretreatment conditions. A larger amount of the lignin structure keeps intact, in comparison with wheat straw. This prevents access of the enzymes to the cellulose fibers, resulting in low enzymatic hydrolysis yield. More severe pretreatment conditions, like in 2nd stage, were needed to achieve a sufficient breakup of the lignin structure to improve enzymatic cellulose hydrolysis. This led to higher glucose yields after two stage pretreatment. But, as mentioned previous, more severe pretreatment conditions also enhanced resin formation which led to enzyme inhibition and resulted in lower yields in miscanthus than in wheat straw.

4. Conclusions

The results of this study show that amount and composition of lignin in lignocellulose substrate influence the effectiveness of the pretreatment method. In acid based pretreatment the decision which pretreatment method is most effective should be based on the amount of ADL lignin in substrate. A high amount of ADL- lignin, like in miscanthus in current study, profits from two stage pretreatment. In lignocellulose substrate, like wheat straw, with low amount of ADL lignin a single stage pretreatment is more effective than a two stage dilute acid pretreatment in terms of process costs and carbohydrate yields.

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4. General discussion

In general discussion chapter, the results from previous chapters will be discussed in broader context to assess the challenges linked with efficient bioconversion of miscanthus. In addition, the measures to optimize the biomass quality for both combustion and ethanol along the production chain from biomass production till processing of biomass (Figure 3) will be discussed.

The general production chain, which is considered here, is comprised of biomass production, harvesting and logistics, pre-treatment and end use (Figure 3). The measures for optimization of biomass quality will be explored at each step and recommendations about the most appropriate measures for biomass quality optimization will be made along the production chain. The production chain along with measures to optimize biomass quality, is presented below in the form of triangle (Figure 3). The opportunity to optimize biomass quality decreases along the production chain with biomass production at the top of triangle because of huge potential and end use at the bottom with lowest. In addition, the production chain is sorted based on the impact of each part of production chain towards biomass quality optimization with least environmental implications and in a cost effective way. In the following text, each part of the production chain will be discussed to come up with recommendations.

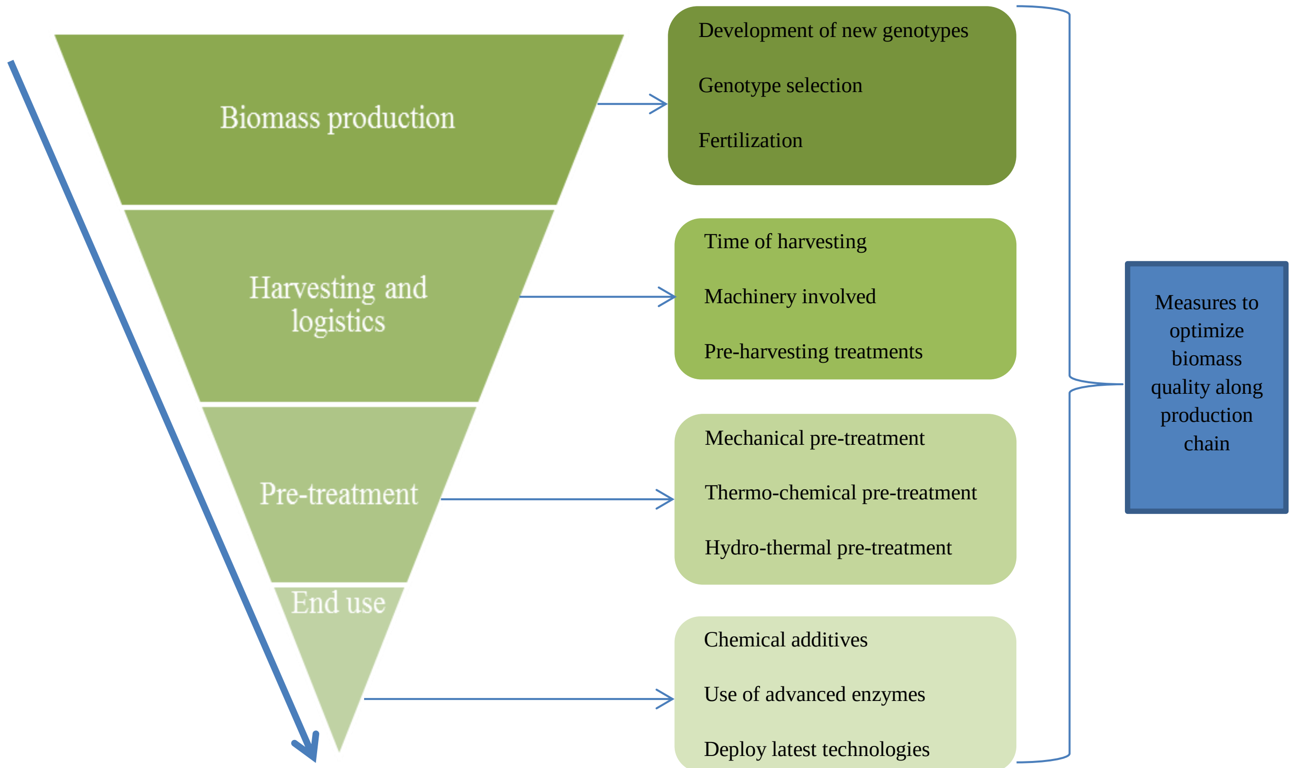

Figure 3: A general production chain adopted for both combustion and ethanol production- from biomass production to end use and the measures to optimize biomass quality along the production chain

4.1. Measures to optimize biomass quality during biomass production

Among the 2G feedstocks, the biomass production through establishment of dedicated perennial energy grasses such as miscanthus will play a key role in future for biomass based energy production (Saini et al. 2015). The per unit energy yield depends largely on biomass yield and quality. Therefore, to achieve high energy yield per unit, high amount of biomass with good quality characteristics relevant for a specific end use, is required. The crop management practices adopted during biomass production determine the yield, biomass quality (Baxter et al. 2014), overall cost of production and environmental performance of the crop (Smeets et al. 2009). In the following text, the measures to optimize biomass quality for both combustion and ethanol at first step of production chain, will be covered.

The main factors relevant for miscanthus biomass production, which offer opportunity to optimize biomass quality for both combustion and ethanol production at this step of production chain include development of new genotypes, selection of appropriate genotype and fertilization. The outcomes of the chapter-1 had shown that the selection of genotype defines the biomass yield and quality characteristics. In addition, it also affects the stability in yield and quality characteristics over the years.

There is large variation among genotypes for biomass yield and quality characteristics for both combustion (Clifton-Brown et al. 2001) and ethanol production (Hodgson et al. 2010). The composition of biomass, which determines the quality of feedstock for a specific end use depends primarily on genetic makeup (Clifton-Brown et al. 2001; Atienza et al. 2003; Lewandowski et al. 2003; Hodgson et al. 2010). For combustion, genotypes with high lignin and low mineral, ash and moisture content are preferred, whereas for ethanol production high cellulose, hemicellulose and low ash and lignin content is favoured. In this study, only *M. x giganteus* was tested for ethanol production, no genotype comparison was performed. However, by making the literature based comparison of fiber composition of different miscanthus genotypes, recommendations will be made for ethanol production as well.

In current study, a large variation is recorded among miscanthus genotypes for combustion quality characteristics. The major challenge for the genotypes with better biomass qualities for combustion is low yield. The combustion quality standards specific for herbaceous biomass are not yet defined and currently wood pellet standards are used for non-woody biomass as well. In wood pellet standards such as ENplus, the limit for Cl (0.02-0.03%) is set but there is no threshold value defined for K content because in woody biomass K content is generally low (Boston, 2013). Therefore, it is recommended to define combustion quality standards specific for non-woody biomass, which will make the comparison easier and will help to optimize the biomass quality. In case of ethanol, no such quality standards exist regarding biomass composition.

In figure 4, Cl content is presented for different miscanthus genotypes and compared with ENplus-B wood pellet standards. The comparison shows that all of the genotypes investigated in this study except for *M. sinensis* are well above the threshold level set for wood pellets ENplus-B. However, when genotype impact was evaluated based on the outcome of the first publication, it had shown that up to 20% of Cl content can be reduced through selection of appropriate genotype. However, this quality improvement through selection of genotype could compromise yield up to 34% (own data). For bioenergy purposes, large quantities of biomass is required, therefore the genotypes with high biomass quality characteristics but low yield will not serve the purpose. Based on the data published by Hodgson et al. (2010), where cell wall composition (lignin, cellulose, hemicellulose) was compared for different miscanthus genotypes, calculations were made to evaluate the genotype impact. The outcome shows that up to 16% of variation in cell wall composition is attributed to genotype selection. This variation can be exploited to improve the overall efficiency of conversion process by decreasing the lignin content and subsequently lowering the energy and chemical inputs required for pre-treatment. In this study, no quantification is made about improvement in final ethanol yield. The final ethanol yield depends on biomass composition, pre-treatment conditions and the use of technology for processing of biomass.

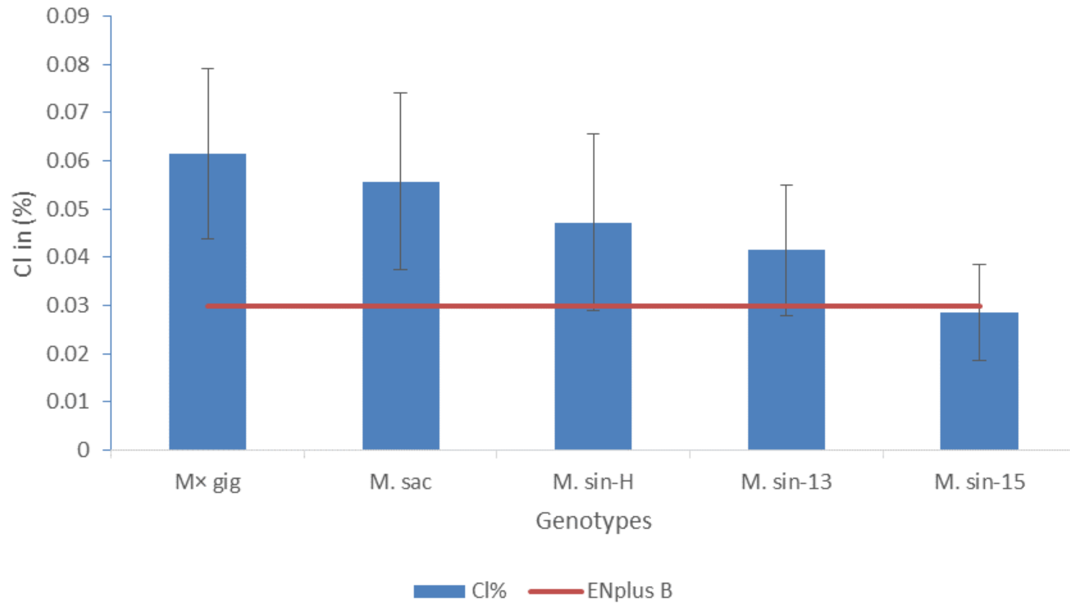

Figure 4: Mean Cl content of different miscanthus genotypes, red line represents the threshold limit set for Cl content according to ENplus-B wood pellet standards (own data)

During combustion process, inorganic constituents of biomass such as K and Cl interact with each other and define ash melting behavior (Baxter et al. 2014) whereas lignin, ash and moisture content defines the heating value of biomass. In this study (chapter-1), the impact of mineral composition on ash melting behavior was evaluated for different genotypes. Based on the outcome of the chapter-1, it can be concluded that the ash content of biomass does not have significant impact on ash melting behavior, however the composition of ash plays major role especially K and Cl content. Therefore, one aim to optimize the biomass composition for combustion is to keep K and Cl low in the biomass to carry out efficient processing of biomass. As already described, the composition of biomass depends on genotype selection, therefore the impact of genotype on ash melting behavior was also evaluated based on the outcomes of the study. It shows that ash melting behavior can be improved up to 40% through appropriate selection of genotype. In practical terms, if the ash deformation temperature is 800 °C, through appropriate genotype selection, it can be improved up to 1100 °C. It indicates that appropriate selection of genotype will help to counter the combustion relevant problems during bioconversion processing by improving the ash fusion temperature. The reduction in Cl content through genotype selection was only 20%, it is mainly because in case of ash melting behavior a number of elements (such as P, K, Cl, Si, Ca, Mg) interact with each other to define the ash melting behavior. Therefore, the impact of genotype selection is high for ash melting behavior compared to Cl content alone.

According to ENplus-B wood pellet standards, the threshold value set for ash deformation is ≥ 1100 °C. Based on the outcomes of the Chapter-1, it can be said that all the genotypes showed ash deformation well below the threshold temperature defined by wood pellets except for *M. sinensis*. Considering the low K, Cl content and high ash fusion temperature, *M. sinensis* fits best for combustion, however because of low yield, it cannot be recommended. However, there are many interesting traits such as high yield potential, lignin content of *M. x giganteus*, low ash content of *M. sacchariflorus* and low mineral especially K and Cl content of *M. sinensis* which can be exploited to develop new genotypes for combustion. In *M. sinensis*, quantitative trait analysis was performed and 4 QTLs were found which control the K and Cl content in biomass (Atienza et al. 2003). Therefore, such information can be used as baseline to develop genotypes with low mineral and ash content for combustion purposes through breeding.

Combustion quality characteristics are not relevant for ethanol production except for lignin and ash content. Considering the importance of genotype selection in optimization of biomass quality for both combustion and ethanol, it is important to look into mechanisms behind, which potentially contribute towards improvement in quality characteristics. In addition, based on these mechanisms, recommendations can be made for breeders to develop new genotypes.

The research shows that yield and biomass composition is mainly determined by phenological (Robson et al. 2012) and morphological characteristics of each genotype (Jørgensen, 1997; Jezowski et al. 2011). The phenological characteristics mainly refer to time of flowering and senescence, whereas morphological characteristics cover stem thickness and leaf to stem ratio. These phenological and morphological characteristics can be adjusted through breeding to optimize biomass quality and yield for a specific end use. For combustion, the phenological characteristics such as time of flowering and senescence influence the biomass composition (Robson et al. 2012) through affecting relocation mechanism whereas for ethanol production these processes can affect the lignin content. The efficiency of relocation mechanism depends on ripening processes such as flowering and senescence, which subsequently affects the biomass quality. That's why, *M. sinensis* genotypes have better combustion characteristics because being early ripening, these genotypes complete the relocation of nutrients back to rhizomes efficiently which lead to decrease in mineral, ash and moisture content in the harvested biomass. In addition, biomass is also lignified which increases the heating value of biomass (Hodgson et al. 2010). However, for ethanol production completion of ripening process could lead to decrease in carbohydrates in stem and increase in lignin content, which is not suitable for ethanol production. The decrease in carbohydrate content is mainly because of translocation back to rhizomes before harvest (Kiesel and Lewandowski, 2016). It indicates that early ripening characteristic of genotype can negatively affect the suitability of feedstock for ethanol production. However, there is not sufficient literature evidence to support this argument. Therefore, it is recommended to consider this aspect in future investigation relevant to genotype selection for ethanol production.

The phenological characteristic of genotype does not affect the biomass composition alone, rather morphological characteristics also play their role in determining the biomass yield and quality for a specific end use. The morphological characteristics vary from genotype to genotype. The stem thickness and leaf to stem ratio are the most important from biomass quality perspective. The morphological characteristics influence the biomass composition by affecting the leaf fall and leaching process.

Based on the outcomes of the chapter-1, it is concluded that at this location, active relocation takes place maximum until January-February depending on genotype and weather conditions. Therefore, once the stems are dead and active relocation stops, then most of the changes in biomass composition take place through leaf fall or leaching. The leaf fall is mainly relevant for ash and N content (Baxter et al. 2012) whereas leaching influences ash, K and Cl content (Jørgensen, 1997). The process of leaching depends on stem thickness and this is one of the main contributing factors towards better combustion quality characteristics of *M. sinensis* genotypes because thin stems facilitate the leaching process. The process of leaching is dependent not only stem thickness but also on prevailing weather conditions especially rainfall and wind speed. Wind and rainfall break the cuticles and expose the stems for leaching of minerals especially Cl (Tonn et al. 2012). Weather conditions cannot be controlled, however morphology of plant depends on genotype selection and can be improved through breeding for combustion purposes.

For ethanol production, leaching process is not relevant but stem thickness and leaf to stem ratio have the potential to influence the fiber composition of harvested biomass. The thick stemmed genotypes such as *M. x giganteus* and *M. sacchariflorus* have high cellulose content but also tend to accumulate more lignin, which is not favourable for bioconversion (Hodgson et al. 2010). The efficiency of ethanol production process depends on cellulose, hemicellulose and lignin content of biomass. The subsequent processing such as pre-treatment of biomass largely depends on lignin content of biomass. Therefore, at first step of ethanol production chain, selection of genotypes with low lignin content will require less-severe pre-treatment conditions which increase the efficiency of overall process (Hodgson et al. 2010) by decreasing the energy and chemical inputs. Considering the high dry mater yield, cellulose and hemicellulose content, *M. x giganteus* and *M. sacchariflorus* are interesting for ethanol but to make it more efficient, it is important to decrease the lignin content through breeding. The other relevant aspect for ethanol production in morphological characteristics which can be considered for breeding is leaf to stem ratio. In leaves the lignin content is lower than stems (Dierking et al. 2016), therefore the genotypes with high leaf proportion can be recommendation for ethanol production.

Along with good yield and biomass quality for a specific end use, other important aspect relevant for development of new genotypes/selection of genotypes is stability in yield and quality characteristics over the years. In future, once the quality standards specific for non-woody biomass especially for combustion are developed, then stability in biomass quality characteristics becomes highly relevant. For ethanol production, there are no such quality standards, however still stability in quality characteristics is important because any variation in biomass composition could affect the subsequent processing of biomass. Miscanthus is a perennial crop and genotype selection is made at the beginning, for next 15-20 years depending on productivity in terms of yield. Therefore, quality and yield could vary from year to year, if the selected genotype is not capable to withstand weather changes over the years. Considering the market demand and to meet the quality standards, it is important to deliver stable biomass yield and quality every year by adjusting the crop management practices according to prevailing yearly conditions. It is mainly because variation in quality characteristics could go beyond the set quality standards which will not fulfill the user demand. Based on the outcomes of this study, *M. x giganteus* and *M. sacchariflorus* proved to be very stable over the years in terms of yield and mineral composition especially Cl content. This shows that these genotypes are better adopted and has the potential to withstand changing weather conditions over the growth period. Based on the outcome of chapter-1, it can be concluded that, the reason for instability in yield and quality characteristics of *M. sinensis* genotypes is poor mechanical strength of stems which undermine the ability to withstand severe weather conditions. The main factors which are relevant for stability of biomass yield and quality include; a) time of harvesting every year; b) genotype selection; c) crop management practices adopted every year; d) weather conditions. Therefore, it is necessary to consider stability factors and select such genotypes, which have potential to deliver stable yield and quality over the years. It will help the farmers to meet the quality standards every year and get good market price. In addition, it is important to consider the stability factors for development of new genotypes for future breeding programs as well. The relevant trait to improve the stability in terms of quality and yield is stem thickness.

Based on the above discussion, recommendations are summarized into Table 1 for breeders to improve biomass yield and quality through developing new genotypes both for combustion and ethanol production (Table 1):

Table 1: Suitability of genotypes for combustion and ethanol production and recommendations for breeders to develop new genotypes by combining traits of interest for a specific end use

Genotypes	Suitable bioconversion route		Recommendations for breeders
	Combustion	Ethanol production	
M. x gig & *M. sac*		✓	- Identify the QTLs relevant to lignin content and develop genotypes with low lignin content - improve the relocation mechanism for minerals to enable the early harvest by combining the traits of early senescence - Improve the leaf to stem ratio because leaves carry low lignin content
M. sin-hybrid & *M. sin*	✓		- improve biomass yield through selecting taller and thick stems traits - enhance the lignin content - decrease the leaf to stem ratio - improve the morphological traits to achieve the stability in quality and yield over the years

In biomass production, after selection of appropriate genotypes, the other important aspect which can play role in optimization of biomass yield and quality is fertilization. The rate and type of fertilization have significant impact on biomass yield and quality. For example, on one hand optimal rate of fertilization needs to be maintained to achieve high yield but it can also lead to changes in biomass composition, which subsequently affect the combustion quality characteristics (Baxter et al. 2012) and bioconversion for ethanol production (Dierking et al. 2016). Research shows that beside ash and N content in harvested biomass, N fertilization influences the fiber composition as well (Dierking et al. 2016). The other important aspect relevant for both combustion and ethanol production in context of fertilization is energy yield per hectare. Any decrease in yield due to low fertilization will be translated to low energy yield per hectare. Therefore, fertilization needs to be adopted considering both biomass yield and quality.

The fertilization is mainly relevant in case of N and K applications. High N fertilization application increases the N content in the harvested biomass and leads to more NOx emissions during combustion process. Miscanthus is a resource efficient crop, therefore has the potential to deliver high yield under low input conditions (Cadoux et al. 2012). It offers opportunity to decrease the fertilizer input to optimize biomass quality without compromising yield. In fertilization, N fertilization is very important because it constituted up to 72% of the emissions in the conducted LCA described in chapter-1. Therefore, in case of high N fertilization, it not only affects the biomass quality but also increases the cost of biomass production and decreases the environmental performance of the crop (Smeets et al. 2009; Murphy et al. 2016). Based on the outcomes of the third publication, it can be concluded that at this location 40 kg ha^{-1} N fertilization is sufficient to achieve good yield and quality biomass under late harvest regimes. This recommendation is made based on yield, quality aspects and GWP (global warming potential). At 40 kg ha^{-1} N fertilization, the N content in the harvested biomass was still well below the threshold level set by ENplus-B wood pellets. The reduction in N fertilization inputs has the potential to optimize the biomass quality and subsequently decreases the NOx emissions during processing of biomass. The outcome of the study indicates that the N content in the harvested biomass can be reduced up to 10% through decreasing fertilization from 80 to 40 kg N ha^{-1}.

Apart from rate of fertilization, the type of fertilization also influences the biomass composition depending on soil type. It is mainly relevant for K fertilization application. In case of K supply, fertilizers are usually applied in the form of potassium chloride, potassium sulfate, potassium carbonate or potassium nitrate. In current study, K fertilization was applied in the form of potassium sulfate because of low sulfur content in soil. Therefore, despite application of potassium sulfate, the sulfur content in harvested biomass was still very low and within the set limits for wood pellets. However, it should be decided based on the soil type. For ethanol production, type of fertilization is not very relevant. Miscanthus being perennial crop adds up organic matter every year through leaf fall and does not require intensive fertilization (Cadoux et al. 2012). Therefore rate of fertilization and type of fertilization should be decided based on offtake of nutrients and soil nutrient status.

4.2. Measures to optimize the biomass quality and yield during harvesting and logistics of biomass

The 2nd step of value chain is harvest, handling, transport and storage of biomass, which also offers great opportunity to optimize biomass quality. In harvesting of biomass, the time of harvesting plays key role and it depends on the end use of biomass and prevailing weather conditions. However, time of harvesting affects not only biomass quality but also yield. For combustion delayed harvest is preferred, whereas early harvest is suitable for ethanol production. The delayed harvest leads to yield losses through stem damages and leaf fall. Many studies indicate that the delay in harvest time improves the biomass combustion quality but it is on expense of dry matter yield (Clifton-Brown et al., 2001; Lewandowski and Heinz, 2003; Kludze et al., 2012; Bilandzija et al. 2016) and the yield losses can be up to 29% (Stéphanie and Maryse, 2015). For combustion, low leaf content is preferred because leaves carry high N and ash content (Baxter et al. 2012). Therefore to reduce emissions and to increase the overall efficiency of the process, there is need to use biomass with low leaf proportion. For harvesting time, the content of carbon, hydrogen and oxygen need to be considered because these elements affect the combustion process and play key role in defining the final energy content of biomass. Delay in harvest helps to achieve the right proportion of hydrogen, carbon and oxygen in the harvested biomass to carry out the combustion process efficiently (Bilandzija et al. 2016).

In current study, March is the appropriate harvesting time for combustion depending on prevailing weather conditions. Through delay in harvest for combustion, biomass quality can be improved up to 18% depending on genotype selection. Here, K and Cl are considered as main parameters for combustion. The main aim of the delayed harvest is to provide ample time for relocation of nutrients back to rhizomes and as well as to allow the nutrients to leach down. For leaching of minerals rainfall is required. Therefore if there is sufficient rainfall then biomass can be harvested even earlier in January or February depending on prevailing conditions. The early harvest of biomass can subsequently help to reduce the yield losses. The time of harvesting can be different for each genotype. The genotypes such as *M. sinensis* which complete the relocation mechanism earlier can be harvested earlier (January- February), whereas for the genotypes (*M. x giganteus* and *M. sacchariflorus*) requiring longer time to complete their growth cycle, delayed harvest is suitable (e.g. March). In this study, the effect of delayed harvest on different quality characteristics for combustion was quantified through using different statistical models. The results showed that the impact of delayed harvesting in terms of biomass combustion quality improvement for *M. sinensis* type genotypes was greater than the *M. x giganteus* and *M. sacchariflorus* genotypes (Figure 5)

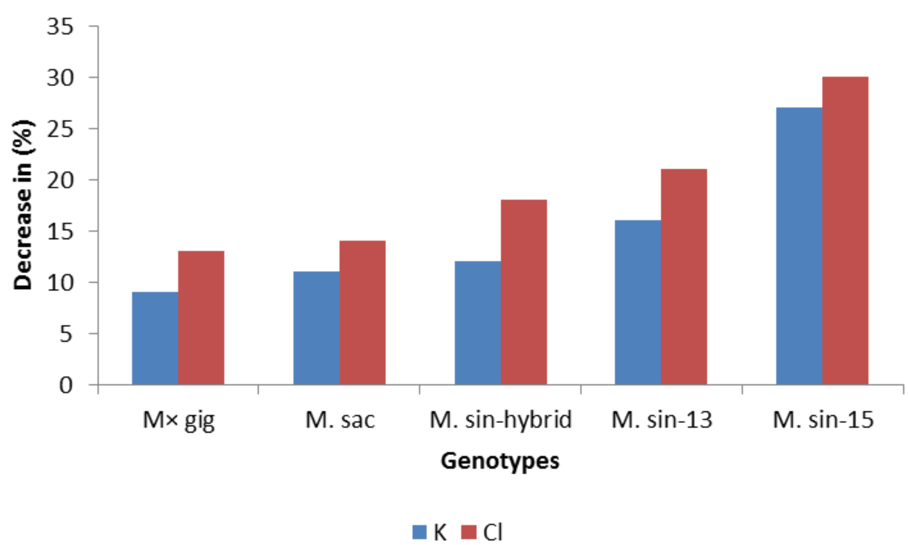

Figure 5: Decrease in K and Cl content for different miscanthus genotypes when harvesting was delayed from January to March (own data)

The variable response of genotypes to delayed harvesting indicates that genotype selection interacts with harvesting time to affect the biomass quality. Despite quantification of impact of delayed harvest on specific combustion relevant elements such as K or Cl in this study, the mechanisms behind need more in depth investigations. Therefore further research is needed by taking into account all the relevant factors through more sophisticated modelling tools. It can be done by selecting the peak yield period as baseline and then compare improvement in biomass quality for combustion and yield losses till March.

For ethanol production, early harvest is preferred. The peak yield of miscanthus under these conditions is September-October, during this time period biomass has high moisture content but low lignin content which is suitable for bioconversion for ethanol production. In current study, impact of delayed harvesting was calculated for combustion from January to March, whereas from fiber composition perspective, September-October is more relevant because in January biomass is already lignified. Therefore, in this study the impact of delayed harvest on ethanol production was not quantified. Based on the published data about different miscanthus genotypes (Hodgson et al. 2010), the impact of harvesting time on fiber composition was quantified when harvesting was delayed from autumn to winter. The outcome indicates that early harvest during peak yield period improves the biomass quality up to 12% in comparison to winter harvest. In this case, lignin content was considered as leading parameter. Therefore, it is important to harvest biomass early during peak yield period for ethanol production. However, it affects the relocation mechanism and increases the offtake of nutrients through harvest (Strullu et al. 2011) which lead to high fertilizer inputs for next growth year. One of the studies shows that early harvest could only remobilize 42% of peak nitrogen content back to rhizomes whereas under late harvest regime remobilization of peak N was recorded up to 71% (Strullu et al. 2011). In addition, the leaf fall during late harvest is also good source of soil nutrients, adds up N up to 15.5 ± 3.5 kg N ha^{-1} whereas this addition is negligible when biomass is harvested early (Karlen, 2014). It shows that early harvest increases the demand of fertilizer inputs especially N fertilization significantly. However, by promoting the recycling of nutrients through application of digestates released at the end of ethanol process the nutrients off take in case of early harvest can be compensated.

Apart from harvesting time, type of harvesting system can affect the yield as well as biomass quality. Currently, two types of harvesting systems are being used for miscanthus; a) harvesting of biomass and chipping it simultaneously; b) mow and bale system (Meehan et al. 2013). Based on the net energy yield in terms of harvested biomass, harvesting of biomass and chipping is more efficient compared with mow and bale system (Meehan et al. 2013). It indicates that in case of harvesting and chipping the biomass simultaneously, the yield losses are low compared to other system but it can vary with transportation distance. The adoption of harvesting system affects yield, quality, cost of production and environmental performance of whole production chain. Currently, there are no miscanthus specific harvesters. However, development of large miscanthus specific harvesting machinery as in case of straw collection will help to control the yield losses, deliver stable quality by avoiding contamination such as dirt (affects ash content) and will improve overall performance of whole production chain. Among the above discussed forage based harvesting systems, cutting of biomass and chipping it simultaneously can be recommended for miscanthus because of low yield losses and low cost (Smeets et al. 2009).

After harvesting, handling and storage of biomass also affects both biomass quality and yield. Inappropriate handling during harvesting and storage of biomass leads to contamination such as soil contamination, which subsequently affects the processing of biomass both for combustion and ethanol production. At field level soil contamination can lead to increase in ash content, therefore it is important to avoid any dirt. For every bioconversion route, low ash content is preferred.

Handling also involves the pre-treatment of biomass at field level especially with the aim to improve the biomass quality before transporting to processing unit. This is done mainly for combustion. The pre-treatment could be cutting the biomass and leaving it on ground to let it dry and allow the minerals to leach down. However, it is relevant only for combustion quality characteristics especially for Cl because Cl is main challenge during processing of biomass for combustion and it is not easy to get rid of Cl during combustion process. Research shows that leaving the biomass in field after harvesting improves the biomass quality significantly with low Cl and ash content (Meehan et al. 2014). This is especially performed in UK, where the moisture content in harvested biomass is still too high even when harvested in March.

The other important component of biomass logistics is storage. The storage method depends on end use of biomass, potential losses of biomass and cost. There are different options to store biomass; a) open air storage either covered with plastic sheet/organic material; b) storage in farm buildings (Smeets et al. 2009); c) ensiling (Oleskowicz-Popiel et al. 2011). The simplest and cost effective way of biomass storage is open air storage with plastic sheeting (Smeets et al. 2009). However, this method can lead to high moisture and ash content, loss of biomass and decrease in heating value (Yue et al. 2014). The high moisture and ash content directly affects the combustion quality but it can be controlled through better storage facilities such as storing biomass in warehouses with drying capability. This method has the potential to minimize the yield losses (Cundiff et al. 1997). However, building such warehouses will require high investments.

For ethanol production, storage method also plays very important role. Ensiling of biomass is very established method for forages which can be applied for miscanthus as well. This method offers opportunity to store biomass with high moisture content. In addition, it minimizes the yield losses and improves the enzymatic digestibility of biomass. The combination of ensiling and low temperature pre-treatment of biomass had improved the efficiency of overall process for ethanol production and decreased the cost and energy input (Chen et al. 2007). In grass variety *Festulolium* Hykor, ensiling had improved the cellulose convertibility, which subsequently led to high ethanol yields (Ambye-Jensen et al. 2013). Therefore, in miscanthus this method can be adopted. This will not only offer a good solution for biomass storage but also improve the subsequent processing of biomass. The ideal characteristics of ensiling are presence of high soluble carbohydrates, with high moisture content (50-70%) and chopped biomass which can easily be compacted (FAO, 2002). Miscanthus does not exactly fit to the criteria defined for ideal ensiling crop, however fine chopping and use of additives can improve the quality of miscanthus biomass ensiling. The other option to improve the biomass quality for miscanthus ensiling is to combine with maize because maize can supply the easily soluble sugars for microorganisms which subsequently will help in fermentation of miscanthus biomass. The adoption of ensiling as a storage process will reduce the chemical and energy input required during pre-treatment of biomass, which will improve the overall performance of production chain in economic and environmental terms.

4.3. Pre-treatment relevant for each production chain

After biomass production, harvesting and logistics, the other important aspect at processing side of biomass is pre-treatment. Pre-treatment is relevant for both value chains combustion and ethanol production.

Despite, biomass quality optimization strategies at field level if the mineral content is still high enough to affect the efficient bioconversion of biomass, then this requires pre-treatment of biomass. There are different pre-treatment options to decrease the mineral, ash and moisture content of harvested biomass. The simplest form of pre-treatment is washing of biomass to remove any soil contamination and facilitate the leaching of minerals and ash, which improves the biomass combustion quality. For example, biomass harvested from grasslands was subjected to washing pre-treatment, which decreased the mineral and ash content through leaching and subsequently improved the ash melting behavior for combustion processing (Tonn et al. 2012). However, the effect of pre-treatment is different for each element because some of the elements can get dissolved in water easily and leach down such as K and Cl whereas other elements such as Ca have low solubility in water. Therefore, for elements, which are not easily soluble in water require severe pre-treatments such as hot water pre-treatment or acidic pre-treatment (Gudka et al. 2016). In one of the studies, fruit tree residues were treated to remove the K and Cl content to improve the combustion quality characteristics. The hydrothermal treatment (180 ºC), followed by water washing, reduced the K content up to 92% and Cl up to 72% (Yan et al. 2015). The efficiency of pre-treatment also varies from feedstock to feedstock. In herbaceous biomass such as miscanthus, most of the inorganic constituents are easily soluble in water compared to wood, therefore simple washing can be sufficient. As already described that main challenges for miscanthus based combustion are high K and Cl, therefore, main focus is on removal of K and Cl. Research shows that for miscanthus K content can be removed up to 62% and Cl content up to 100% by soaking in water at room temperature for 20 hours (Saddawi et al. 2012). The removal of K and Cl content improves the ash composition of biomass, which subsequently leads to high ash melting temperature (Saddawi et al. 2012). The pre-treatment of biomass is important because poor quality biomass can lead to more emissions and corrosion related problems during combustion process. Considering the above mentioned improvement in biomass quality through hydrothermal pre-treatment shows that, if biomass is not meeting the set standards as in case of *M. x giganteus* and *M. sacchariflorus*, biomass composition at this level of production chain can be optimized to carry out problem free processing. However, after application of these pre-treatments, it is important to evaluate the economic and environmental performance of whole production chain, which is not fully done yet.

For lignocellulosic ethanol production, pre-treatment is the first step. Even after on field quality optimization strategies, pre-treatment is required because the lignin content is still high enough, to hinder the bioconversion of biomass. Therefore, to release the cellulose, biomass need to be pre-treated. An effective pre-treatment process can be determined by following factors (Mosier et al. 2005);

- The pre-treatment process has the potential to digest biomass without performing any additional biomass handling step such as milling of feedstock to reduce particle size, which is energy intensive
- Can release the C6 sugars without affecting the C5 sugars
- Process has low energy demand and also does not lead to high costs
- Minimum formation of inhibitors because it can negatively affect the fermentation process.

The optimal pre-treatment process should have the potential to meet the above mentioned criteria. The main challenge for pre-treatment is to optimize the process in a cost effective way which leads to cellulose release with minimum inhibitor formation and sugar losses. Pre-treatment is the most important step in lignocellulose ethanol production, it constitutes up to 18% of total processing cost (Balat, 2011; Lewandowska et al. 2016). For lignocellulosic material there is no well-established cost effective pre-treatment method yet which could be used at large scale. It is mainly because in lignocellulose biomass, there are two main types of sugars; 1) hexose sugars; 2) pentose sugars. Both of these sugars have different characteristics, which require specific pre-treatment conditions for each type of sugar release. This makes the whole process more complicated because along with release of both type of sugars, inhibitor formation should also be kept at low level. The pre-treatment conditions suitable for one type of sugars can lead to loss of others, therefore appropriate pre-treatment conditions are required. In this study, two stage process was applied where at first stage the focus is on release of C5 sugars followed by second stage process to release the C6 sugars. The two step process offers the opportunity to separate both sugars which increases the overall efficiency of process by increasing the amount of fermentable sugars (Nguyen et al. 2000). It also has the potential to minimize the formation of inhibitors which is important for fermentation of sugars. For miscanthus, two step pre-treatment (sodium hydroxide based pre-treatment followed by dilute sulfuric acid pre-treatment) is recommended. Sodium hydroxide based pre-treatment had removed lignin up to 80%

(Lewandowska et al. 2016). However, if the biomass is ensiled during storage then another pre-treatment pathway is recommended. In that case, ensiling should be followed by hydrothermal pre-treatment and then subsequent enzymatic hydrolysis. The combination of ensiling and hydrothermal pre-treatment improved the conversion efficiency up to 78% (Lewandowska et al. 2016). Therefore, for miscanthus both aforementioned pre-treatment processes need to be further tested to reach the goal of efficient bioconversion of biomass in a cost effective way. The pre-treatment tests specifically miscanthus is on very early stages, most of the work is done on laboratory scale. Therefore, there is need to use larger sample size to pre-treat the biomass, which could be adopted later at industrial scale. The requirements of severe pre-treatment conditions will not only lead to high costs, but also needs high energy inputs and affects the environmental performance of whole process.

4.4. End use-measures to optimize production chain during processing of biomass

Biomass quality optimization at the end of production chain during processing of biomass is an expensive option compared to field level quality optimization strategies described above. There are different ways to optimize the biomass quality at this level. It can be performed through following approaches:

- Technological improvements required for both production chains combustion and ethanol production.
- Use of additives to control emissions and avoid any deposition during combustion process. For ethanol production, use of advanced enzymes to ferment both hexose and pentose sugars and has the potential to withstand degraded products formed during pre-treatment process.
- The other approach to optimize biomass quality at this step would be, use blends rather than using pure miscanthus.

Technological advances can play a role for both combustion and ethanol production. For combustion, development of boilers with non- sticky surfaces to avoid any deposition on heater tubes will be helpful to improve the efficiency of conversion process. Such boilers have the potential to resist any corrosion mechanism which otherwise could damage the boiler and reduce the heat transfer. It will allow the users to combust biomass even with poor combustion qualities as in case of *M. x giganteus* and *M. sacchariflorus*, however it requires more investment and installation of new systems. For miscanthus based ethanol production, there is already wheat straw based pilot scale plants to produce ethanol, same technology can be replicated for miscanthus.

Apart from technological advances, the other option at this level to improve biomass quality is use of additives to counter the problems during bioconversion process and increase the efficiency of process. For combustion, a wide range of chemical additives (S-based, Al-Si-based, P-based or Ca-based) are being used to avoid corrosion, slagging and fouling (Gudka et al. 2016). At commercial scale, aluminium silicate based additives are used during combustion process to form potassium based aluminium silicates. The potassium based aluminium silicates have higher melting temperature in comparison to potassium silicates (Wang et al. 2012). Therefore, combination of Si and Al content in biomass plays positive role during combustion process. In case of calcium based additives, Si will react with Ca to form calcium silicates and inhibit the formation of potassium silicates during combustion process. The potassium silicates have lower ash fusion temperature in comparison to calcium silicates, therefore formation of calcium silicates will subsequently increase the ash melting temperature (Wang et al. 2012). The S-based additives convert the KCl to K_2SO_4 through sulphation (Aho and Silvennoinen, 2004; Wang et al. 2012).

As Si content in miscanthus biomass is already high (Baxter et al. 2014), therefore Si-based additives are not suitable for miscanthus. For miscanthus, Ca-based and S-based can be recommended to remove K and Cl during combustion process. Calcium and sulphur based additives follow the following pathway to remove the K and Cl (Aho and Silvennoinen, 2004):

$$2KCl + SO_2 + 1/2\ O_2 + H_2O \rightarrow K_2SO_4 + 2HCl \quad (1)$$

$$CaO + 2HCl \rightarrow CaCl_2 + H_2O \quad (2)$$

These additives will enable us to use the genotypes investigated in this study despite poor biomass quality. However it will lead to increase in process cost and will also compromise the environmental performance of whole production chain. Therefore, it is recommended that focus should be on improvement of biomass qualities for a specific end use through quality management practices at field level during production and harvesting.

Other option to optimize the biomass quality is to combust miscanthus by blending it with some other biomass which could positively influence the ash melting behaviour. The best option is to combine miscanthus with woody biomass because wood has better biomass qualities for combustion. Therefore, it is recommended to test the miscanthus-wood blends and find the optimal combination, which could improve the overall efficiency of combustion process. In one of the study miscanthus was blended with peat, the increase in peat share lead to improvement in ash melting behaviour through release of K (Sommersacher et al. 2015).

For ethanol production, after pre-treatment, solid part is removed and hydrolysate is subjected to fermentation to produce ethanol. For fermentation, microorganisms which have the potential to ferment both C5 and C6 sugars and can also withstand inhibitor formation are required. Research shows that *Mucor rouxii*, filamentous fungi can potentially be used for fermentation of lignocellulose hydrolysate (Millati et al. 2008; Lewandowska et al. 2016). It not only has the potential to digest C5 and C6 sugars simultaneously but also can survive despite formation of degraded products hydrolysate during pre-treatment process (Karimi et al. 2006; Lewandowska et al. 2016). Therefore for miscanthus based ethanol production, *M. rouxii* can be recommended to carry out efficient bioconversion and improve the overall efficiency of the production chain (Lewandowska et al. 2016).

Based on the above discussion of whole production chains for both combustion and ethanol production, it can be concluded that high yielding genotypes such as *M. x giganteus* and *M. sacchariflorus* can be used for both purposes if certain measures are adopted along the whole production chain. The optimized production chains are presented below, at first the optimized production chain for combustion is presented along with recommendations at each step production chain to improve combustion quality (Figure 6). In this study focus was to optimize biomass quality as much as possible at field level. The recommendations about pre-treatment and end use are literature based. The biomass combustion quality optimization through pre-treatment or during processing of biomass is possible but in practical terms it is least desirable because of high costs and energy inputs.

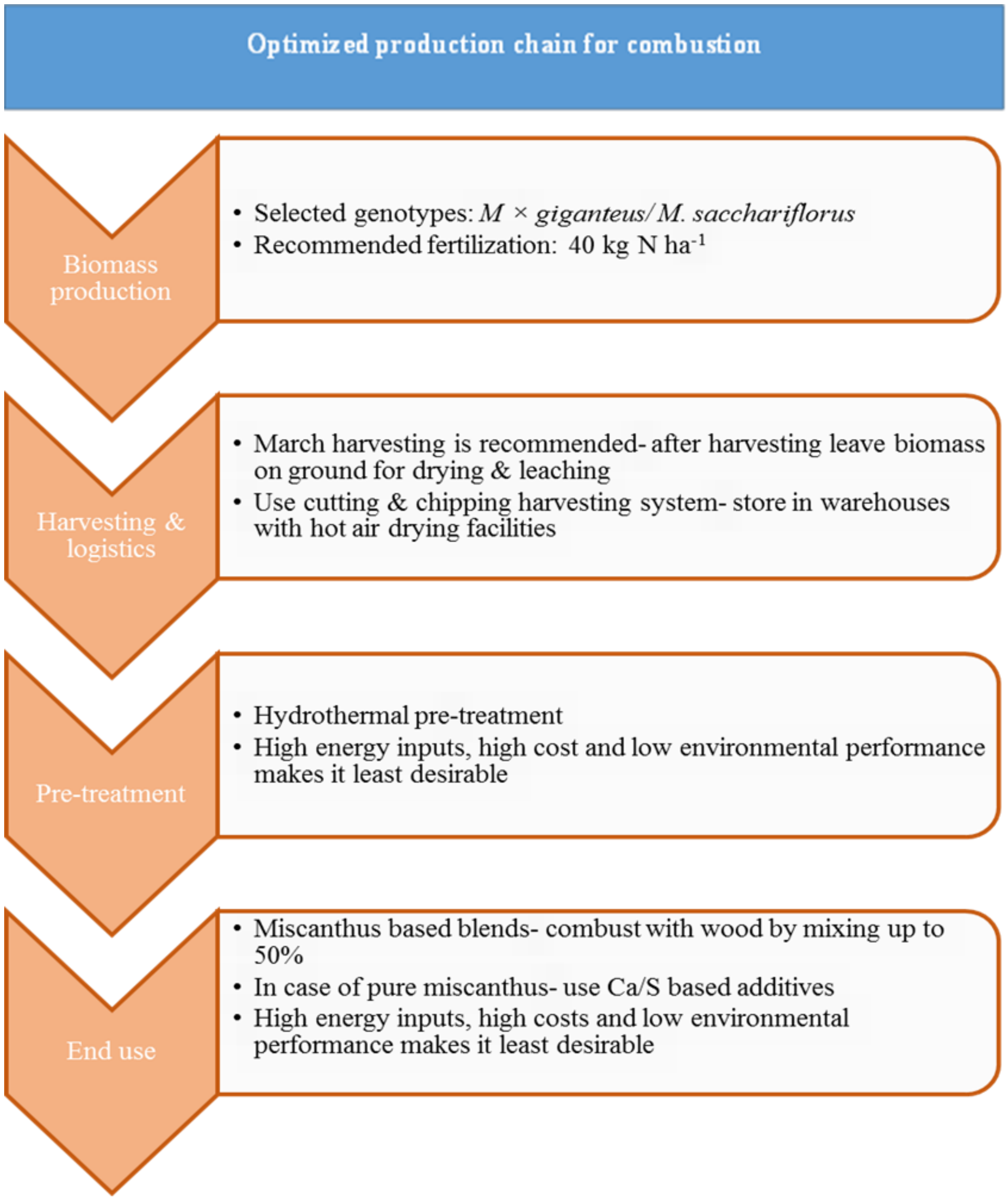

Figure 6: Optimized production chain for combustion from biomass production till end use

The following optimized production chain is for ethanol production. At each step, the most promising biomass quality optimization measures were recommended along the whole production chain from biomass production till end use (Figure 7). There are options to improve processing of biomass during pre-treatment and end use but it increases the overall cost of the whole bioconversion process. The improvement in fiber composition does not mean that it will be translated to final ethanol yield. It refers to mainly lignin, cellulose and hemicellulose content. The final ethanol yield depends on fiber composition, pre-treatment conditions and fermentation process. In this study, quantification about improvement in final ethanol yield is not performed.

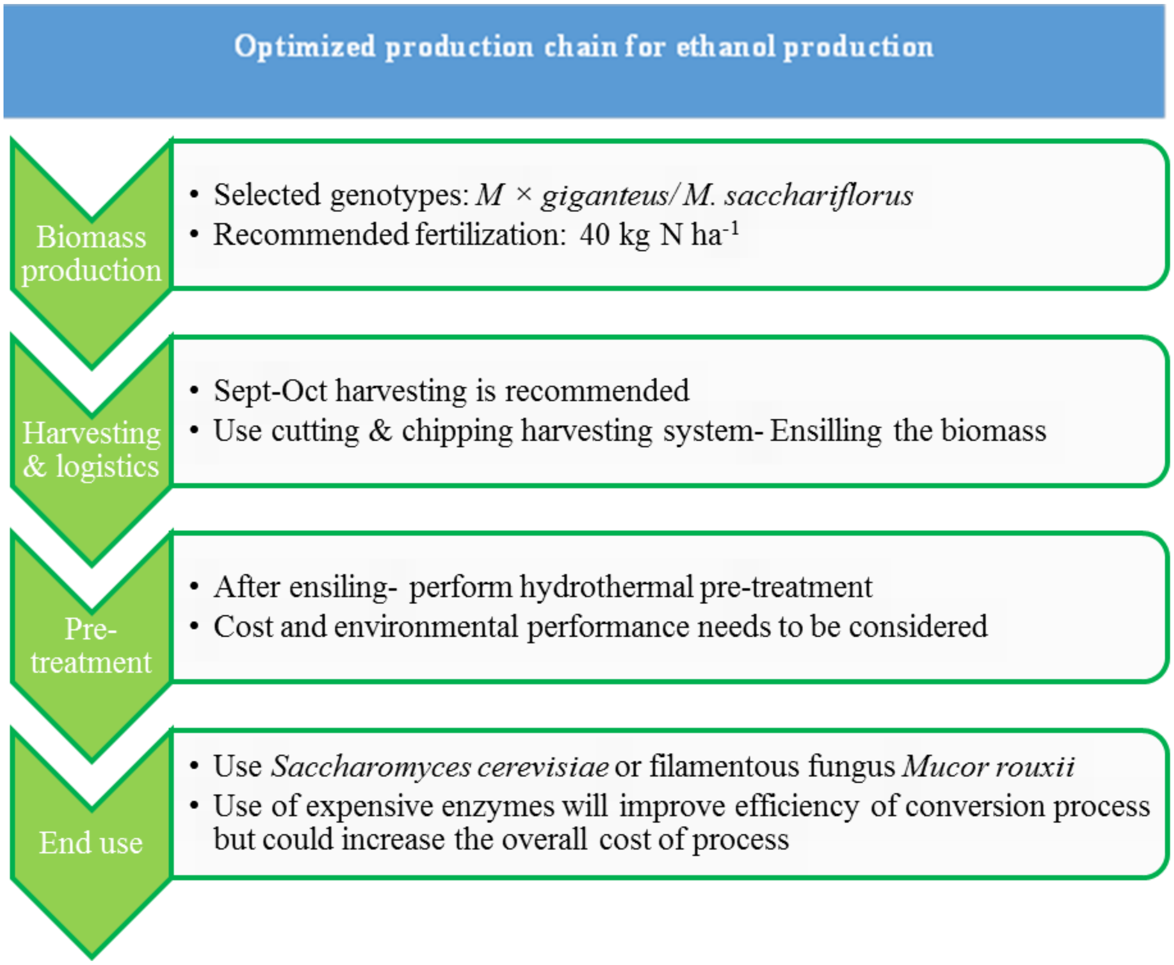

Figure 7: Optimized production chain for ethanol production from biomass production till end use

It is important to state here that for both production chains despite poor quality *M. x giganteus* and *M. sacchariflorus* genotypes were recommended. It is mainly because these genotypes are high yielding which significantly affects the energy yield per unit. However, the efficient bioconversion of these genotypes would only be possible if the recommendations at harvesting & logistics, pre-treatment and end use are followed. The recommendations at pre-treatment step and at end use can theoretically improve processing up to 100% but in practical terms, it is not possible because of high costs and environmental implications.

5. Summary

Currently, a wide range of biomass based resources (wood, agricultural residues, municipal waste, perennial dedicated energy crops) are being tested for different bioconversion routes such as combustion and ethanol production. In Europe, combustion is the most prevalent bioconversion route being adopted to produce heat and electricity. By 2020, in Europe out of 139 Mtoe biomass based energy production, 110.4 Mtoe will be heat and electricity. Along with combustion, EU (European Union) focuses on increasing the share of biofuels production to achieve the EU 2020 target to reach 10% share of renewables in the transportation sector. For both aforementioned bioconversion routes, large amount of feedstocks, produced in a sustainable way, are required. Miscanthus, being a perennial dedicated energy crop has the potential to deliver high yields by using the soil resources efficiently. However, the per unit energy yield depends not only on biomass yield but also quality of biomass relevant for a specific end use. For miscanthus based combustion, high lignin contents increase the energy yield of the biomass. The main challenges are high emissions (e.g. NOx) and combustion relevant problems such as corrosion, fouling and low ash melting temperature. Other than for combustion, the high lignin content is the main problem during miscanthus based ethanol production. Presently, *M. x giganteus* is the only commercially grown genotype, however a wide range of genotypes are being tested under the European conditions to select the most promising ones for both combustion and ethanol production. Therefore, the focus of this study is to evaluate the biomass quality of different miscanthus genotypes for combustion and ethanol production and relevant measures for each bioconversion route to optimize biomass quality at field level to fit the user demand.

To realise the aim of this study, two different field trials were used: 1) long term field trial with 15 miscanthus genotypes (four *M. x giganteus*, one *M. sacchariflorus*, five *M. sinensis* hybrids and five *M. sinensis* genotypes) was established as randomized block design with three replications; 2) field trial with *M. x giganteus* and switchgrass was established as a randomized split plot design with different crops as main plots, divided into three subplots with different N levels (0, 40, and 80 kg N $ha^{-1}a^{-1}$). The biomass samples collected from these field trials were processed and analysed in laboratory to test the biomass quality parameters for combustion (mineral analysis, silicon, chloride, ash, moisture and ash melting behaviour) and ethanol production (fiber analysis, acid/base based pre-treatment).

The outcomes of this study show that at biomass production level, crop management practices such as selection of appropriate genotypes, fertilization and time of harvesting determine the yield, biomass quality, overall cost of production and environmental performance of the crop for a specific bioconversion route (combustion, ethanol production). The ash melting behavior during combustion process can be improved through appropriate genotype selection from an ash deformation temperature of 800 °C up to 1100 °C. For ethanol production, fiber composition can be improved up to 16% through appropriate genotype selection by decreasing the lignin content and improving the cellulose content. This improvement will not be completely

translated to increase in ethanol yield. However, it can improve the overall efficiency of conversion process by decreased the lignin content and subsequently lowering the energy and chemical inputs required for pre-treatment. In this study, no quantification is made about improvement in final ethanol yield.

In fertilization, N fertilization is very important because it constituted up to 72% of the emissions in the conducted LCA described in chapter-1. Therefore, in case of high N fertilization, it not only affects the biomass quality but also increases the cost of biomass production and decreases the environmental performance of the crop. Based on the outcomes of this study, it can be concluded that at this location 40 kg N $ha^{-1}a^{-1}$ fertilization is sufficient to achieve good yield and quality biomass under late harvest regimes (March). At 40 kg N $ha^{-1}a^{-1}$ fertilization, the N content in the harvested biomass was still well below the threshold level set (0.3-1%) for biomass by the ENplus wood pellets.

The other important factor which offers opportunity to optimize biomass quality is time of harvesting. Through appropriate harvesting time, biomass combustion quality can be improved up to 30% through decreasing the mineral, chloride and ash content whereas for ethanol production, fiber composition can be improved up to 12% by decreasing the lignin content. In practical terms, the delay in harvest will help to meet the set quality standards and counter the relevant challenges for each bioconversion route. In current study, none of the biomasses harvested from the different miscanthus genotypes, except for *M. sinensis,* could meet the ENplus-B wood pellet standards. For combustion, early ripening thin stemmed genotypes such as *M. sinensis* are recommended under late harvest regime (March). However, the low yield of these genotypes is a major concern because low biomass quantity decreases the final energy yield. Considering the high dry matter yield, cellulose and hemicellulose content, *M. x giganteus* and *M. sacchariflorus* are recommended for ethanol production under early harvest regimes (September-October). However, the high lignin content of *M. x giganteus* and *M. sacchariflorus* reduces the efficiency of overall process. Therefore, in this study recommendations were given to breeders about development of new genotypes for combustion by combining interesting traits such as high yield and lignin content of *M. x giganteus*, low ash content of *M. sacchariflorus*, low mineral content especially K and Cl of *M. sinensis*, whereas for ethanol production low lignin content of *M. sinensis* can be combined with high yield of *M. x giganteus*.

This study suggests that optimization of biomass quality for a specific end use can be achieved through adoption of appropriate crop management practices such as selection of appropriate genotype and time of harvesting. This is the most cost-effective way with least environmental implications.

6. Zusammenfassung

Derzeit wird ein breites Spektrum biobasierter Rohstoffe – wie zum Beispiel Holz, Reststoffe aus der Landwirtschaft, Kommunalabfälle sowie mehrjährige Energiepflanzen – auf ihre Eignung für verschiedene Biokonversionspfade, wie beispielsweise Verbrennung oder Ethanolproduktion, getestet. In Europa ist der vorherrschende Konversionspfad die Verbrennung, mittels der Strom und Wärme produziert wird. Bis zum Jahr 2020 wird die Bioenergieproduktion in Europa 139 Mtoe betragen, davon werden 110,4 Mtoe Strom und Wärme sein. Zusammen mit der Verbrennung konzentriert sich die EU (Europäische Union) auf die Erhöhung des Anteils der Biokraftstoffe um ein Ziel der Europa 2020 Strategie zu erreichen, einen Anteil der Erneuerbaren Energien im Transportsektor von 10% zu erzielen. Für beide der zuvor dargestellten Biokonversionspfade werden große Mengen an nachhaltig produzierten Rohstoffen benötigt. Miscanthus, eine mehrjährige Energiepflanze, nutzt die Bodenressourcen sehr effizient und hat dabei ein hohes Ertragspotential. Der Energieertrag ist jedoch nicht nur vom Biomasseertrag abhängig, sondern auch von den Biomassequalitätsmerkmalen, die für den jeweiligen Nutzungspfad relevant sind. Bei der Verbrennung von Miscanthus erhöhen hohe Ligningehalte den Energieertrag der Biomasse. Die größten Herausforderungen sind hohe Emissionen (z.B.: NOx) und andere für die Verbrennung relevante Probleme wie zum Beispiel Korrosion, Verschmutzung und ein geringer Ascheschmelzpunkt der Biomasse. Im Gegensatz zur Verbrennung ist der hohe Ligningehalt ein großes Problem bei der Ethanolherstellung aus Miscanthus. Zurzeit ist *M. x giganteus* der einzig kommerziell angebaute Genotyp. Jedoch wird eine große Bandbreite an Genotypen unter europäischen Bedingungen getestet, um die vielversprechendsten sowohl für die Verbrennung als auch die Ethanolherstellung zu selektieren. Daher ist das Ziel dieser Studie die Biomassequalität verschiedener Miscanthusgenotypen für die Verbrennung und die Ethanolherstellung zu bestimmen. Des Weiteren sollen Maßnahmen, um die Biomassequalität auf dem Feld zu optimieren und an den Bedarf des jeweiligen Nutzers anzupassen, für beide Biokonversionspfade bewerten werden.

Dazu wurden zwei verschiedene Feldversuche genutzt: 1) ein langjähriger Feldversuch mit 15 Miscanthusgenotypen (vier *M. x giganteus*, ein *M. sacchariflorus*, fünf *M. sinensis* Hybriden und fünf *M. sinensis* Genotypen), die in einer randomisierten Blockanlage mit drei Wiederholungen etabliert wurden; 2) ein Feldversuch mit *M. x giganteus* und Rutenhirse in einer randomisierten Spaltanlage mit den verschiedenen Kulturarten als Großteilstücke, unterteilt in drei Kleinteilstücke mit verschiedenen Stickstoffdüngungsstufen (0, 40, und 80 kg N $ha^{-1}a^{-1}$). Die Biomasseproben von diesen Feldversuchen wurden aufbereitet und im Labor auf ihre Qualitätseigenschaften für die Verbrennung (Mineralstoffanalyse, Silizium, Chlor, Asche, Feuchtigkeit sowie das Ascheschmelzverhalten) und die Ethanolherstellung (Faseranalyse, Vorbehandlung mittels Säure) analysiert.

Die Ergebnisse dieser Studie zeigen, dass bei der Biomasseproduktion Kulturmaßnahmen wie die Auswahl geeigneter Genotypen, Düngung und Erntezeitpunkt entscheidend sind für den Ertrag, die Biomassequalität, die Produktionskosten und die Umweltleistung der Pflanze für einen bestimmten Konversionspfad (Verbrennung, Ethanolherstellung). Das Ascheschmelzverhalten während des Verbrennungsprozesses kann durch die Auswahl geeigneter Genotypen verbessert werden. Die Temperatur der Ascheschmelze erhöht sich dadurch von 800 °C auf 1100 °C. Bei der Ethanolherstellung kann die Faserzusammensetzung durch die Auswahl geeigneter Genotypen mit einem niedrigen Lignin- und einem höheren Zellulosegehalt um 16% verbessert werden. Diese Verbesserung kann zwar nicht vollständig in einen höheren Ethanolgehalt umgesetzt werden, jedoch verbessert sich die Gesamteffizienz des Konversionsprozesses durch den niedrigeren Ligningehalt und den dadurch niedrigeren Energie- sowie Chemikalienbedarf für die Vorbehandlung. Im Rahmen dieser Studie wurde die Verbesserung des Endethanolertrages nicht quantifiziert.

Hinsichtlich der Düngung ist insbesondere die Stickstoffdüngung sehr wichtig, da diese für bis zu 72% der in der LCA (Kapitel 1) ermittelten Emissionen verantwortlich ist. Eine hohe Stickstoffdüngung beeinflusst deshalb nicht nur die Biomassequalität, sondern erhöht auch die Produktionskosten der Biomasse und verschlechtert die Umweltleistung der Pflanze. Auf Grundlage der Ergebnisse dieser Studie kann gefolgert werden, dass an den gewählten Versuchsstandorten eine Stickstoffdüngung von 40 kg N $ha^{-1}a^{-1}$ ausreicht, um hohe Erträge und eine hohe Biomassequalität bei einer späten Ernte im März zu realisieren. Bei einer Düngung von 40 kg N $ha^{-1}a^{-1}$ lag der Stickstoffgehalt der Biomasse deutlich unter dem Grenzwert von 0,3-1% für Biomasse, welcher in der Norm ENplus für Holzpellets festgelegt ist.

Der zweite wichtige Einflussfaktor zur Verbesserung der Biomassequalität ist der Erntezeitpunkt. Durch einen angepassten Erntezeitpunkt kann die Biomassequalität für die Verbrennung durch die Senkung des Mineralstoff-, Chlor- und Aschegehaltes um bis zu 30% verbessert werden. Bei der Ethanolherstellung kann die Faserzusammensetzung durch einen niedrigeren Ligningehalt um bis zu 12% verbessert werden. In der Praxis hilft der spätere Erntetermin die gegebenen Qualitätsanforderungen zu erfüllen und die Herausforderungen des jeweiligen Konversionspfades zu bewältigen. In der vorliegenden Studie konnte keiner der verschiedenen Miscanthusgenotypen, mit Ausnahme von *M. sinensis*, den Holzpellet Standard ENplus-B erfüllen. Für die Verbrennung sind bei einer späten Ernte (März) früh reifende Genotypen mit dünnen Stängeln wie *M. sinensis* zu empfehlen. Ein größeres Problem stellt hierbei jedoch der niedrige Ertrag dieser Genotypen dar, da ein niedriger Biomasseertrag den Endenergieertrag senkt. Unter Berücksichtigung des hohen Trockenmasseertrages sowie des hohen Zellulose- und Hemizellulosegehaltes sind bei einer frühen Ernte (September-Oktober) *M. x giganteus* und *M. sacchariflorus* für die Ethanolherstellung zu empfehlen. Der hohe Ligningehalt von *M. x giganteus* und *M. sacchariflorus* reduziert jedoch die Effizienz des Gesamtprozesses. Aufgrund dessen wird im Rahmen dieser Studie Züchtern die Empfehlung gegeben, zur Entwicklung neuer Genotypen für die Verbrennung Eigenschaften wie den hohen Ertrag sowie den hohen Ligningehalt von *M. x giganteus*, den niedrigen Aschegehalt von *M. sacchariflorus* und den niedrigen Mineralstoffgehalt – insbesondere hinsichtlich Kalium und Chlor – von *M. sinensis* zu kombinieren. Bei der Züchtung neuer Genotypen für die

Ethanolherstellung sind hingegen insbesondere der niedrige Ligningehalt von *M. sinensis* und der hohe Ertrag von *M. x giganteus* interessant.

Die Ergebnisse dieser Studie legen nahe, dass die Optimierung der Biomassequalität für einen spezifischen Verwendungszweck durch die Anwendung geeigneter Kulturmaßnahmen wie die Auswahl angepasster Genotypen und den Erntetermin erreicht werden kann. Dies ist nicht nur die kostengünstigste Variante, sondern auch diejenige mit den geringsten Auswirkungen auf die Umwelt.

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Appendix

Overview of presentations and publications relevant to this dissertation

Publications

1. Iqbal, Y. and I. Lewandowski. 2014. "Inter-Annual Variation in Biomass Combustion Quality Traits Over Five Years in Fifteen Miscanthus Genotypes in South Germany." *Fuel Processing Technology* 121: 47-55.

2. Iqbal, Y. and I. Lewandowski. 2016. "Biomass Composition and Ash Melting Behaviour of Selected Miscanthus Genotypes in Southern Germany." *Fuel* 180: 606-612.

3. Iqbal, Y., M. Gauder, W. Claupein, S. Graeff-Hönninger, and I. Lewandowski. 2015. "Yield and Quality Development Comparison between Miscanthus and Switchgrass Over a Period of 10 Years." *Energy* 89: 268-276.
4. Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2015. "Comparing the Performance of Miscanthus X Giganteus and Wheat Straw Biomass in Sulfuric Acid Based Pretreatment." *Bioresource Technology* 180: 360-364.
5. Kärcher, M. A., Y. Iqbal, I. Lewandowski, and T. Senn. 2016. "Efficiency of Single Stage- and Two Stage Pretreatment in Biomass with Different Lignin Content." *Bioresource Technology* 211: 787-791.

6. Van der Weijde, RT Kiesel, A Iqbal, Y Fonteyne, S Muylle, H Mos, M Dolstra, O Roldán-Ruiz, I Lewandowski, I Trindade, LM. Evaluation of Miscanthus sinensis biomass quality as feedstock for conversion into different bioenergy products. 2016. Accepted in GCB Bioenergy doi: 10.1111/gcbb.12355.

Conference contributions

1. Iqbal Y. Lewandowski I (2012) Effect of harvesting date on yield and combustion quality of different miscanthus genotypes. SLU Alnarp, Sweden (9-10 November)

2. Iqbal Y. Lewandowski I (2015) Combustion quality of miscanthus genotypes. Miscanthus Tagung, Chartres, France (19-21 January)

3. Kiesel, A. Iqbal Y. Lewandowski I (2015) Optimisation of miscanthus giganteus pre-treatment for biogas production. 23rd European Biomass Conference and Exhibition, Vienna, Austria (1-4 June)

4. Iqbal Y. Lewandowski I (2015) Effect of nitrogen fertilization and harvest date on ash melting behavior of miscanthus and switchgrass biomass. 23rd European Biomass Conference and Exhibition, Vienna, Austria (1-4 June)

5. Iqbal Y. Lewandowski I (2015) Comparison of different miscanthus genotypes for ash melting behavior. Perennial Biomass Crops for a Resource Constrained World. University Hohenheim, Stuttgart, Germany (7-10 September)

6. Spöttle, M. Iqbal, Y and Lewandowski, I (2015) Maximising the yield of biomass from residues of agricultural crops –Preliminary outcomes. Fifth International Conference on Lignocellulosic Ethanol, Brussels, Belgium (15-17 September)

7. Iqbal, Y. Gauder, M. and Lewandowski, I. 2016. Perennial energy crops: Potential future biomass resources for bioenergy production. World Sustainable Energy Days (WSED). 24-26 February, Wels, Austria

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