Fodder Legumes for Green Biorefineries: A Perspective for Sustainable Agricultural Production Systems

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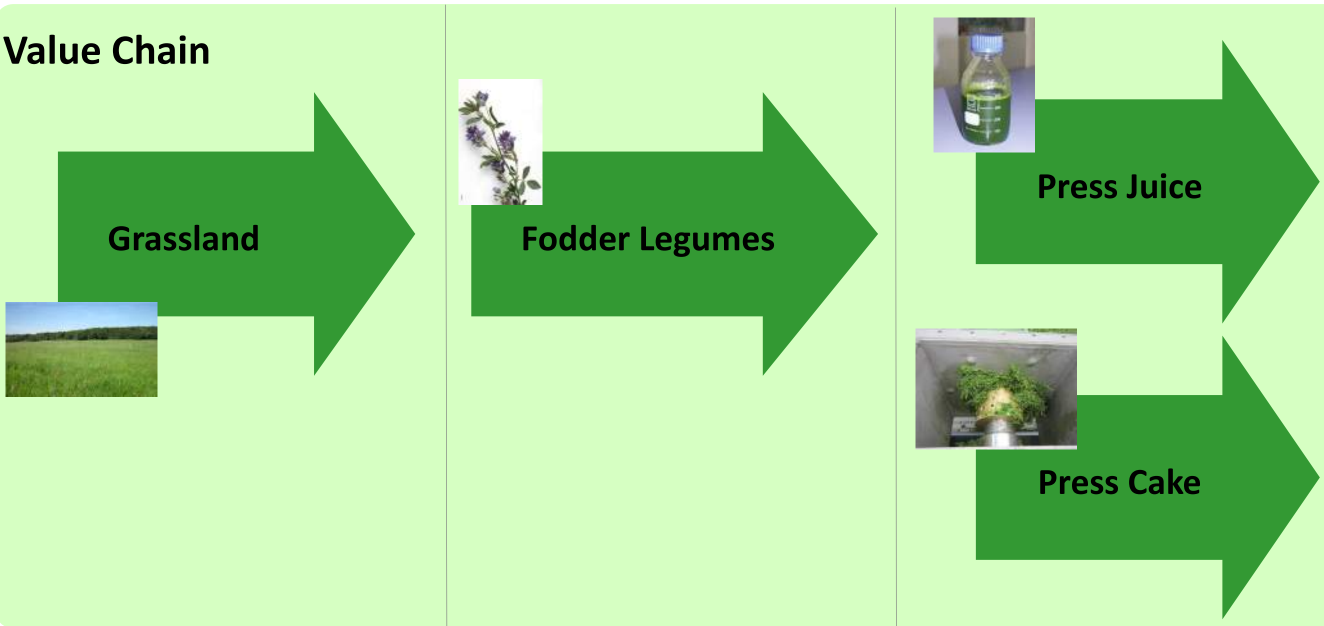
Graphical abstract

Developing a sustainable value chain for Green Biorefineries

Value Chain



Grassland

Fodder Legumes

Press Juice

Press Cake

Tasks

Analysis of biomass potential to assure sufficient feedstock supply for Green Biorefineries

Developing a sustainable production system for the Green Biorefinery feedstock

Quality analysis of press cake and juice under various conditions; Economic evaluation of producing them on a farm

Methods

Evaluation of statistical data on grassland potentials; Literature review

Field trials on temporary and permanent grasslands; Statistical evaluation of biomass quantity and quality for different sites, crops, and cutting dates; Literature review

Chemical analysis of biomass; Fermentation experiments with press juice; Silage experiments with press cake; Cost-benefit analysis; Literature review

Results

Sufficient amounts of feedstock from grasslands are available

Green Biorefinery approach widens cultivation and processing spectrum for fodder legumes; Decentralised processing is needed

Press juice and cake are valuable intermediate products in the Green Biorefinery approach; Producing them on a farm can be economically viable

Conclusion

Green Biorefinery as new utilization concept to use grasslands more efficient

Fodder legumes as feedstock for Green Biorefineries can support all three pillars of sustainability

The analysed value chain is a promising perspective for an efficient and sustainable feedstock processing in the future

Summary

Peak oil is forcing our society to shift from fossil to renewable resources. However, such renewable resources are also scarce, and they too must be used in the most efficient and sustainable way possible. Biorefining is a concept that represents both resource efficiency and sustainability. This approach initiates a cascade use, which means food and feed production before material use, and an energy-related use at the end of the value-added chain. However, sustainability should already start in the fields, on the agricultural side, where the industrially-used biomass is produced. Therefore, the aim of my doctoral thesis is to analyse the sustainable feedstock supply for biorefineries. In contrast to most studies on biorefineries, I focus on the sustainable *provision* of feedstock and not on the bioengineering *processing* of whatever feedstock is available.

Grasslands provide a high biomass potential. They are often inefficiently used, so a new utilisation concept based on the biorefining approach can increase the added value from grasslands. Fodder legumes from temporary and permanent grasslands were chosen for this study. Previous research shows that they are a promising feedstock for industrial uses, and their positive environmental impact is an important byproduct to promote sustainable agricultural production systems.

Green Biorefineries are a class of biorefineries that use fresh green biomass, such as grasses or fodder legumes, as feedstock. After fractionation, an organic solution (press juice) forms; this is used for the production of organic acids, chemicals and extracts, as well as fertilisers. A fibre component (press cake) is also created to produce feed, biomaterials and biogas. This thesis examines a specific value chain, using alfalfa and clover/grass as feedstock and generating lactic acid and one type of cattle feed from it. The research question is if biomass production needs to be adapted for the utilisation of fodder legumes in the Green Biorefinery approach. I have attempted to give a holistic analysis of cultivation, processing and utilisation of two specific grassland crops. Field trials with alfalfa and clover/grass at different study sites were carried out to obtain information on biomass quality and quantity depending on the crop, study site and harvest time. The fresh biomass was fractionated with a screw press and the composition of press juices and cakes was analysed. Fermentation experiments took place to determine the usability of press juices for lactic acid production. The harvest time is not of high importance for the quality of press juices as a fermentation medium. For permanent grasslands, late cuts, often needed for reasons of nature conservation, are possible without a major influence on feedstock quality. The press cakes were silaged for feed-value determination.

Following evidence that both intermediate products are suitable feedstocks in the Green Biorefinery approach, I developed a cost-benefit analysis, comparing different production scenarios on a farm. Two standard crop rotations for Brandenburg, producing either only market crops or market crops and fodder legumes for ruminant feed production, were compared to a system that uses the cultivated fodder legumes for the Green Biorefinery value chain instead of only feed production. Timely processing of the raw material is important to maintain quality for industrial uses, so on-site processing at the farm is assumed in Green Biorefinery scenario. As a result, more added value stays in the rural area. Two farm sizes, common for many European regions, were chosen to examine the influence of scale. The cost site of farmers has also been analysed in detail to assess which farm characteristics make production of press juices for biochemical industries viable. Results show that for large farm sizes in particular, the potential profits are high. Additionally, the wider spectrum of marketable products generates new sources of income for farmers.

The holistic analysis of the supply chain provides evidence that the cultivation processes for fodder legumes do not need to be adapted for use in Green Biorefineries. In fact, the new utilisation approach even widens the cultivation and processing spectrum and can increase economic viability of fodder legume production in conventional farming.

Zusammenfassung

Nachwachsende Rohstoffe ersetzen zunehmend fossile Energieträger. Die effiziente Verwertung dieser Ressourcen ist essentiell, um eine nachhaltige Rohstoffnutzung zu gewährleisten. Die Bioraffinerie ist ein Konzept, das darauf abzielt Ressourcen möglichst effizient und nachhaltig zu nutzen. Biomasse wird hierbei mit möglichst hoher Wertschöpfung verarbeitet und es entstehen sowohl Lebens- und Futtermittel, als auch Chemikalien und Energieträger daraus. Doch auch die Rohstoffe, die im Bioraffinerieprozess verarbeitet werden, müssen nachhaltig produziert sein. Das Ziel dieser Doktorarbeit ist es, die nachhaltige Bereitstellung von Rohstoffen für die Bioraffinerie zu untersuchen.

Als Rohstoff habe ich Futterleguminosen vom Grünland gewählt. Grünlandflächen sind in Deutschland und weiten Teilen Europas durch Umbruch oder aber Nutzungsaufgabe bedroht. Dies zeigt, dass sie nicht effizient genutzt werden. Neue Nutzungsansätze, die sich durch Bioaffinerien ergeben, könnten den Rückgang des Grünlands aufhalten. Sowohl Dauergrünland, als auch temporäres Grünland wird in dieser Arbeit untersucht. Futterleguminosen wurden gewählt, da frühere Studien sie als vielversprechenden Rohstoff für die industrielle Nutzung ausgewiesen haben und sie einen sehr positiven ökologischen Einfluss auf Agrarökosysteme haben können.

Eine Form der Bioraffinerie ist die Grüne Bioraffinerie, die als Rohstoff frische grüne Biomasse, wie zum Beispiel Gräser verarbeitet. Die Biomasse wird in einen Faseranteil (Presskuchen) und eine organische Lösung (Presssaft) fraktioniert. Der Presskuchen kann zur Herstellung von Futtermitteln, Biomaterialien oder auch als Biogassubstrat verwendet werden. Der Presssaft ist vielfältig in der biochemischen Industrie einsetzbar, z.B. zur Produktion organischer Säuren, Kosmetika und Pharmazeutika. Diese Doktorarbeit untersucht eine spezifische Wertschöpfungskette der Grünen Bioraffinerie, bei der Luzerne und Kleegras als Rohstoffe verwendet werden und aus ihnen Milchsäure sowie ein Futtermittel für Wiederkäuer produziert wird. Die Forschungsfragen in diesem Zusammenhang sind, (1) ob der Anbau der Futterleguminosen der Nutzung als Rohstoff in der Bioraffinerie angepasst werden muss; (2) wie die Biomasse für eine effiziente Rohstoffausbeute verarbeitet werden muss; und (3) ob der Anbau von Futterleguminosen für die Grüne Bioraffinerie wirtschaftlich rentabel umsetzbar ist. Ich habe mit dieser Arbeit somit eine ganzheitliche Untersuchung umgesetzt, die den Anbau, die Verarbeitung und letztlich auch die Produktverwertung untersucht.

Zur Beantwortung der Forschungsfragen wurden Feldversuche durchgeführt, um Informationen zu Biomassequalität und –quantität in Abhängigkeit der Kulturpflanze, des Untersuchungsstandortes sowie des Erntezeitpunktes zu erlangen. Die geerntete Biomasse wurde mit einer Schneckenpresse in Presssaft und –kuchen separiert und deren Inhaltsstoffe analysiert. In Fermentierungsexperimenten wurde die Güte der Presssäfte als Rohstoff in der Milchsäureherstellung untersucht. Die Herstellung von Milchsäure erfordert eine schnelle Verarbeitung der Biomasse, sodass diese direkt im landwirtschaftlichen Betrieb stattfinden sollte. Dies führt zu einer höheren Wertschöpfung direkt am Ort der Produktion und kann die Wirtschaft im ländlichen Raum stärken. Die Qualität des Presskuchens als Futtermittel wurde mithilfe von Silierversuchen bestimmt. Die Untersuchungen in dieser Doktorarbeit zeigten, dass der Erntezeitpunkt für die Rohstoffqualität in der industriellen Nutzung eine untergeordnete Rolle spielt. Somit werden späte Erntezeitpunkte möglich, die vor allem für extensiv genutztes Dauergrünland wichtig sind, um die Biodiversität zu erhalten.

Nachdem der Nachweis erbracht war, dass sowohl der Presssaft als auch der Presskuchen wertvolle Zwischenprodukte der Grünen Bioraffinerie sind, habe ich eine Kosten-Nutzen Analyse aufgestellt. Ziel war es herauszufinden, ob Landwirte von der Bereitstellung dieser Zwischenprodukte wirtschaftlich profitieren können. Es stellte sich heraus, dass vor allem große landwirtschaftliche Betriebe einen wirtschaftlichen Nutzen daraus ziehen können. Der Anbau von Futterleguminosen könnte in diesen Betrieben eine stark positive Umweltwirkung haben, da z.B. der Einsatz von Mineraldüngern reduziert werden kann und Bodenstrukturen verbessert werden.

Zusammenfassend eröffnet die Grüne Bioraffinerie neue Nutzungsansätze für Futterleguminosen und somit Grünlandstandorte und kann genutzt werden, um die Nachhaltigkeit in agrarischen Produktionssystemen zu steigern.

1. General Introduction

1.1 Motivation

The "food or fuel" discussion that arose from the production of bioenergy from wheat and maize shows how important an efficient and sustainable use of resources is. Dale et al. (2011) contend that the production of bioenergy has to be sustainable to be successful. This statement is fully transferable to any other biomass use. Sustainable agricultural production systems are by definition "capable of maintaining their productivity and utility indefinitely", but must be "resource-conserving, environmentally compatible, socially supportive and commercially competitive" (Ikerd, 1990). Ikerd (1990) stresses that all three pillars of sustainability (environmental, social and economic) are necessary and that it does not suffice to serve only one or two pillars. The importance of resource efficiency is addressed in national and international Sustainable Development Strategies, which emphasise this point in light of resource scarcity and the societal demand for sustainable resource utilisation (Bundesregierung Deutschland, 2002; Council of the European Union, 2006).

Biorefinery is described as one concept that addresses resource efficiency and sustainability (de Jong et al., 2009). Picking up the idea of oil refineries, the aim is to maximise outputs in the processing of feedstocks, in this case biomass and residuals (Lin et al., 2013). This approach reduces waste to a minimum and fosters a cascade use. This means food and feed production before material use and an energy-related use at the end of the value-added chain. The material use of biomass is already more sustainable than the energy use, since the added value is increased by a factor of four to nine (Carus et al., 2014). However, sustainability starts even earlier, on agriculture fields where the industrially-used biomass is produced. However, the point is not to increase pressure on agricultural land by raising demand for biomass, but instead should support sustainable production systems.

The aim of my doctoral thesis is to analyse the sustainable feedstock supply for biorefineries. Put another way, the approach may be thought of as a concept to connect biorefineries with a sustainable supply of feedstock. As a preliminary overview points out, fodder legumes from temporary and permanent grasslands fit well in exemplifying this approach. These legumes are not in direct competition to food production and, furthermore, the positive environmental impact of legumes on crop rotations is an important byproduct (see Section 1.2).

1.2 The sustainability of fodder legumes

1.2.1 Non-commodity values

Non-commodity values are all positive effects that are weakly (or not at all) jointly produced, positive externalities of agriculture for which no market exists (OECD, 2003). Legumes convert and use atmospheric nitrogen by means of nodule bacteria so that, in general, mineral nitrogen fertilisers are not necessary (National Research Council, 2002). Legumes previously were an essential element of crop rotations before mineral fertilisers became available at reasonable prices. However, their impact on the agricultural production system is more diverse than just delivering nitrogen. The perennial cultivation of fodder legumes promotes the accumulation of carbon in soils (Jensen et al., 2012) and impedes the spread of pests and diseases in cereal cultivars (Malézieux et al., 2009). The well-branched root system of the perennial plants increases the water infiltration capacity, reducing erosion risk in heavy rain events (Freyer, 2003). In addition, nutrient leaching will only rarely appear, because the root system takes up nutrients before they are transferred to the groundwater or into other ecosystems (Robertson et al., 2011). Moreover, the root system takes up nutrients, i.e. phosphorus, from the deep soil layers (Kahnt, 2008). These nutrients can be used by the plant or are stored for following crops, subsequently reducing the demand for mineral fertilisers throughout the whole crop rotation (Parajuli et al., 2015). Along with these benefits, soil fertility is increased; as a result, grain crop yields and grain quality for the succeeding crops are improved (Gooding et al., 2007; Grzebisz et al., 2001; Hejcman et al., 2012).

Unfortunately, cultivation figures do not yet reflect these benefits of legume cultivation.

1.2.2 Commodity values

Today, typical indigenous fodder legumes, like alfalfa and clover, have been replaced in animal nutrition by soy meal from Latin and South America, and are therefore no longer cultivated in conventional farming systems in Germany. Cultivation figures dropped from 1 million hectares in the 1950s in the Federal Republic of Germany to 274,000 hectares in 2013 in post-reunification Germany (DESTATIS, 2014). Economically viable production in conventional farming does not seem to be assured.

Politicians have recognised the problem, and strategies for legume support are already in existence or are under development (BMELV, 2012; Committee on Agriculture and Rural Development, 2011; Schreuder and De Visser, 2014). However, these strategies will only make an impact when use options and markets for these crops exist. New utilisation concepts generating products with a higher added value are needed.

Green Biorefineries are a class of biorefineries that use fresh green biomass, like grasses or fodder legumes as feedstock, as the main input of production. After fractionation, an organic solution (press juice) is produced; this is used in the subsequent production of, e.g. organic acids, chemicals and extracts, as well as fertilisers. Another product is the fibre component (press cake), which is used to produce feed, biomaterials and biogas (de Jong et al., 2009; Kamm et al., 2010a). Such industrial utilisation requires that sufficient amounts of raw material can be provided. Figures suggest that the potential for green biomass production is enormous. In fact, 31.5% of German agricultural land was covered with grasslands (permanent and temporary) in 2013 (DESTATIS, 2014). This large volume in combination with an inefficient utilisation concept in Germany (DAFA, 2015) make the delivery of adequate amounts of feedstock for industrialised processes in the biorefinery approach possible. This thesis examines a specific value chain, using alfalfa and clover/grass as feedstock, and generating lactic acid and a type of cattle feed from it.

Both, lactic acid and cattle feed are valuable commodity values. Lactic acid is the basic input for the production of polylactic acid (PLA), a biologically based plastic that can be biodegradable (European Bioplastics, 2014). The demand for lactic acid was estimated to be 714,000 tons in 2013 and is expected to further increase at an annual rate of 15.5% until 2020, which is chiefly based on the demand for bioplastic (Abdel-Rahman et al., 2013; SpecialChem, 2014). Hence, there is a market for lactic acid with positive future prospects. Next, feed production is the traditional usage of fodder legumes because of the high nutritional value. To study the potential of re-establishing this use option, it is integrated in the study.

The viability of fodder legume production in conventional farming for the Green Biorefinery approach also needs to be examined. Cost-benefit models are one approach to comparing different current state-of-the-art production systems with the proposed new approach. In my doctoral thesis I start off from that point and develop a sustainable crop rotation system to produce leguminous biomass for the biorefinery approach. This thesis proves that fodder legume production can be viable when new utilisation concepts are introduced.

1.3 State-of-the-art in Green Biorefineries

In Austria, a Green Biorefinery project was initiated to develop an integrated system for green biomass utilisation. The project analysed the processes needed to generate proteins, lactic acid and fibre components, and assessed the economic viability over the entire value chain (Koschuh et al., 2003; Kromus et al., 2004). A pilot plant planned in Havelland (Germany) will produce a protein concentrate to substitute for imported soy meal. In addition, a fermentation medium accrues as well as white proteins used for cosmetics (Kamm et al., 2010b). In both projects, biomass from permanent grasslands is used in the Green Biorefinery approach in an effort to preserve underutilised sites from conversion into arable land or total abandonement.

Another field of application is to increase the product range when grass is a resource readily available in a region. In Ireland, a blueprint for a Green Biorefinery was developed to produce protein and fibre products as well as fertilisers and biogas (O'Keeffe et al., 2011). In Denmark, extensive research in the field of Green Biorefineries has been done to reduce eutrophication from residual green juices appearing in the green crop drying industry (Andersen and Kiel, 2000). Here, press juices were used for the production of L-lysine, a high-value non-ruminant feed (Thomsen and Kiel, 2008).

Hence, a Green Biorefinery processes feedstocks that were formerly used (literally) as feed. The research question is if biomass production needs to be adapted for industrial use, because other plant metabolites have increased in importance. There is no evidence in the literature regarding a project that investigates the way biomass is used in detail. Different plant species have been compared (Koschuh et al., 2003; O'Keeffe et al., 2011) but the effects of cultivation methods and harvest time on delivering the most valuable feedstock for specific industrial products were not taken into account. As a corollary, therefore, one key question that has to be answered within this thesis is if the cultivation requirements for feed production are fully transferable to those for industrial uses.

1.4 Hypothesis and research objectives

Green biomass feedstocks are diverse, and so are the potential processing and utilisation steps in Green Biorefineries. Due to this hypothesis, use concepts have to be adapted depending on biomass quality and quantity, processing options and finally the potential market. To secure a sustainable value chain, a comprehensive analysis of the cultivation, processing and utilisation of a specific grassland crop for utilisation in a Green Biorefinery is needed.

Therefore, the research objectives of this thesis are:

1. To analyse the availability of biomasses for utilisation within Green Biorefineries.
2. To develop a sustainable value chain for Green Biorefineries.
3. To examine if alfalfa and clover/grass can be a worthwhile feedstock for Green Biorefineries.
    - 3.1. To analyse how large the differences in biomass composition and quantity are depending on the crop, cultivation site and cutting date.

Following evidence that they are worthwhile feedstocks for Green Biorefineries,

4. To investigate whether alfalfa and the clover/grass mixture can be produced in an economically sound manner.

1.5 Research methods

The methods used in this thesis are explained in detail in the published articles incorporated into the text as Chapters 3, 4 and 5. However, Figure 1 provides an overview of the various methods used in the different areas of focus of this thesis.

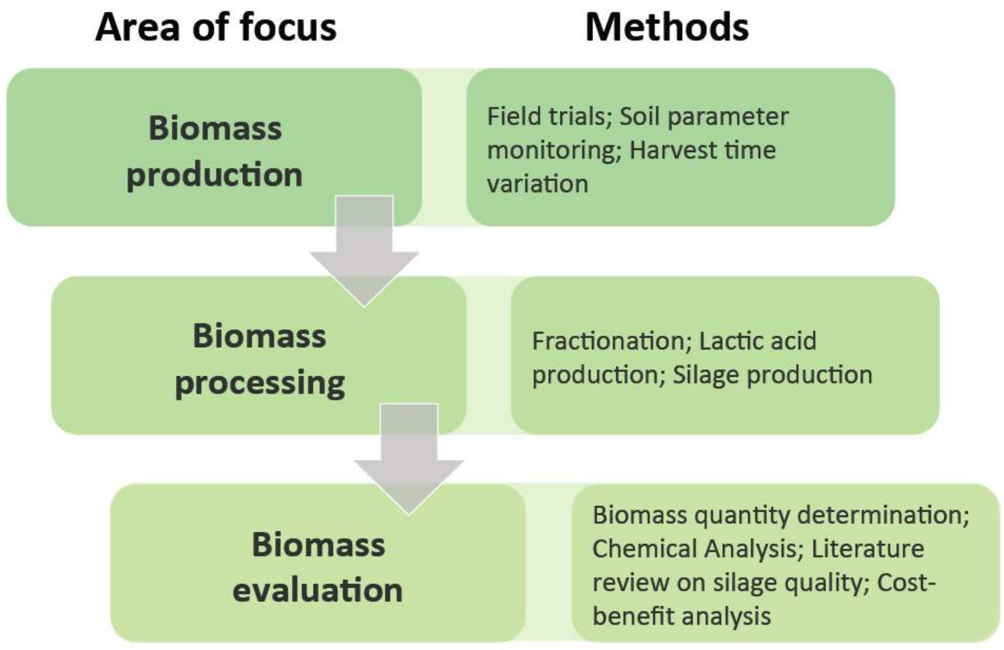


Figure 1 - *Areas of focus and applicable methods*

Field trials with alfalfa and clover/grass were carried out at different study sites to obtain information on biomass quality and quantity depending on the crop, study site and harvest time. Afterwards, the biomass was fractionated, varying the pressing process in an effort to analyse differences in the composition of both resulting compounds (juice and cake). The juice was utilised as fermentation medium in lactic acid production and the feed potential of the press cake was determined. After providing evidence that both lactic acid and feed can be produced from the biomass under review, the development of a cost-benefit model allowed me to analyse the economic profitability of the approach. The analyses thus build on one another. The core value of the thesis is the resulting comprehensive perspective on the entire value chain of fodder legumes in the Green Biorefinery approach.

1.6 Thesis structure and declaration of own involvement in the publications

This thesis is structured into six main chapters. After the general introduction with background information on the topic, the decisive research articles that emerged out of my doctoral studies are included (presented as Chapters 2 to 5).

Chapter 2: “Biorefineries: relocating biomass refineries to the rural areas”. This article has been published as Papendiek et al. (2012) in *Landscape Online*.

Chapter 3: “Cultivation and fractionation of leguminous biomass for lactic acid production”. This article has been published as Papendiek and Venus (2014) in *Chemical and Biochemical Engineering Quarterly*.

Chapter 4: “Fodder legumes for lactic acid production – the influence of cutting date and site on biomass and bacterial nutrient yields”. The article is under review in *Legume Research*.

Chapter 5: “Assessing the economic profitability of fodder legume production for Green Biorefineries – a cost-benefit analysis to evaluate farmers’ profitability”. This article has been accepted for publication in the *Journal of Cleaner Production*.

The final chapter, Chapter 6, deals with general findings of the thesis and concludes the discussion. I am the first author of the articles in Chapter 2 to 5 and have performed the main work described in these chapters. Due to co-authorship, they are written in first person plural. The content of some chapters in the articles overlaps, especially in the section “Introductions” and “Methods” because of the cumulative approach of this thesis.

I want to acknowledge that during the work on my *Diplom* thesis (Papendiek, 2010), numerous questions arose that could not be covered there. These questions were therefore kept in mind, developed further and used as the basis for this doctoral thesis. All findings reported in this document are original and result from work done independently during the completion of the thesis.

i. The first article “Biorefineries: relocating biomass refineries to the rural areas” (Papendiek et al., 2012) is the sketch of ideas for this thesis. Competition between biomass use options and the benefits of chemical before energetic use are described. We argue that appropriate feedstocks for the Biorefinery could include underexploited grasslands or crops on arable land that improve sustainability in agricultural production, like fodder legumes. Initial ideas on how to process leguminous biomass are given. In addition, the article shows that fresh green biomass should be already refined on site (at the farm) to increase efficiency and to keep a high added-value on the farm. My contributions to this paper were the following: original idea, literature review, main author of all chapters.
ii. In the article “Cultivation and fractionation of leguminous biomass for lactic acid production” (Papendiek and Venus, 2014), the methods used in the field trials and the biomass processing are explained in detail. In this study, we analysed optimal cultivation and fractionation processes for generating a fermentation medium from legumes for lactic acid production by *Bacillus coagulans*. We then compared the contents of press juices from alfalfa cultivated on arable land at three different sites and from a clover/grass mixture on a grassland site taken on different sampling dates. In addition, we examined fresh biomass yields from the different biomass samples. Fermentation analysis of the different samples revealed that press juices can supplement the main parts of nutrients for lactic acid bacteria, producing economically attractive amounts of lactic acid. My contributions to this paper were the following: original idea, literature review, and main author of all chapters with the exception of the method section on analytical determination and batch fermentation as well the corresponding parts in the “Results and discussion” section.
iii. “Fodder legumes for lactic acid production - the influence of cutting date and site on biomass and bacterial nutrient yields” (Papendiek et al., 2015a) is the third article in the thesis. It analyses and discusses the results of the field trials. We statistically evaluated which indicators in plant cultivation are most important for biomass and

bacterial nutrient yields. Alfalfa produced significantly higher dry matter yields under the same environmental and cultivation conditions. The cutting time had no major influence on the quantity and impact of bacterial nutrients for lactic acid formation. The year of cultivation was not relevant either. This implies that press juices from the entire vegetation period may be used in production. My contributions to this paper were the following: original idea, literature review, and main author of all chapter, except parts from the "Site situation and methods" section (Sections 2.1, 2.4) and the "Results" section explaining the plant physiology.

iv. The final article "Assessing the economic profitability of fodder legume production for Green Biorefineries – a cost-benefit analysis to assess farmers' profitability" (Papendiek et al., 2015b) is based on the previously obtained results published in "Fodder legumes for lactic acid production - the influence of cutting date and site on biomass and bacterial nutrient yields". We compiled a cost-benefit analysis to assess the feasibility of legume cultivation for biorefinery use in practice. The production of feedstocks for Green Biorefineries, depending on prices paid for the legume juice, shows a high profit potential. My contributions to this paper were the following: original idea, literature review; the cost-benefit decision model was designed together with my co-authors Valentina Tartiu and Piergiuseppe Morone. I am the main author of all chapters.

2. Biorefineries: relocating biomass refineries to the rural area

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This chapter has been published as:

Papendiek, F., Ende, H.-P., Steinhardt, U., Wiggering, H., 2012. Biorefineries: Relocating Biomass Refineries to the Rural Area. *Landscape Online,* 27: 1-9.

Abstract

The field for application of biomass is rising. The demand for food and feeding stuff rises while at the same time energy, chemicals and other materials also need to be produced from biomass because of decreasing fossil resources. However, the biorefinery ideas and concepts can help to use the limited renewable raw materials more efficiently than today. With biorefineries, valuable products, such as platform chemicals, can be produced from agricultural feedstock, which can subsequently be further processed into a variety of substances by the chemical industry. Due to the role they play as producers of biomass, rural areas will grow in importance in the decades to come. Parts of the biorefinery process can be relocated to the rural areas to bring a high added value to these regions. By refining biomass at the place of production, new economic opportunities may arise for agriculturists and the industry gets high-grade pre-products. Additionally, an on-farm refining can increase the quality of the products because of the instant processing. To reduce competition with food production and to find new possibilities of utilisation for these habitats, the focus for new agricultural biomass should be on grasslands. But also croplands can provide more renewable raw materials without endangering a sustainable agriculture, e.g. by implementing legumes in the crop rotation. To decide if a region can provide adequate amounts of raw material for a biorefinery, new raw material assessment procedures have to be developed. In doing so, involvement of farmers is inevitable to generate a reliable study of the biomass potentials.

2.1 Introduction

Biomass constitutes a regenerative alternative to fossil resources, which can be used both for energy production and as raw material for further products. By 2020, the share of renewable energies in electricity generation in Germany should rise to 30%. According to the national Biomass Action Plan (BMELV and BMU, 2009), the bulk of the energy is to be supplied by biomass. Although biomass is just one of many possibilities for producing energy from renewable raw materials, these goals induce that political support instruments are heavily geared towards the use of biomass for energy production. At the same time this demand for biomass is competing with other biomass consuming sectors. The chemical industries depend on carbon compounds. Their substitute for fossil resources is biomass. The added value for the use of biomass for materials is five to ten times higher than for the energetic use (Carus et al., 2010). The chemical industries aim at achieving a 30% share of production from renewable raw materials by 2025; the current rate stands at approximately 13% (European Technology Platform for Sustainable Chemistry (SusChem), 2005). The "German Action Plan for the material use of renewable raw materials" (FNR, 2009), published by the German government in 2009, illustrates that political support programmes will in future also embrace the use of biomass for materials, including biorefineries, to advance the efficient use of regenerative raw materials. Biorefinery is the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat) (de Jong et al., 2009). Important chemicals from biomass, produced by biorefineries, are e.g. lactic acid, succinic acid or fumaric acid, building the base for bioplastics and polyester (Kamm and Kamm, 2007). The biorefinery concept ensures that as much of the feedstock as possible is exploited (van Ree and Annevelink, 2007).

The rising demand for biomass results in a shortage of resources. Statistics on cultivation and numbers of livestock on the farms cannot give reliable information on the resource potential of a region. As long as the quality and the current use of the agricultural area are not known, the assessment of potentials is doubtful. New ideas and concepts are required to handle the diversified biomass use and new methods are needed to compare all potential uses of biomass in a region and to find the most efficient type of use.

2.2 The rural area

In recent decades, the rural regions of Germany have undergone major changes. The farms situated in these regions dominate the landscape. Reforms of agricultural policy (BMELV, 2006), like the abandonment of set-aside land, lead to structural changes in agriculture and hence in the landscape.

The rising demand for renewable raw materials induces that rural areas will increasingly play a major economic as well as societal role in the future. New structures should be developed to enable agriculturists to benefit from this potential and to ensure their future existence, preferably without having subsidised production on their farms. Refining raw materials at the place of production, based on the concept of biorefinery, is one opportunity. Within the discussion about the so called "ecosystem services" this may turn out for agriculturists to supply specific "agrosystem services" to operationalize this approach and to add another component to this discussion.

2.2.1 Consequences of structural change

Decreasing numbers of livestock and the rapid increase of rape and maize monocultures are recent examples that have led to major changes in rural areas and the landscape (Amt für Statistik Berlin-Brandenburg, 2010a; Amt für Statistik Berlin-Brandenburg, 2010b). Some of these changes have had serious consequences. The number of livestock using grassland areas has decreased dramatically. In Brandenburg, the number of cattle has declined by 50% since 1990 (Amt für Statistik Berlin-Brandenburg, 2010b). By contrast, agriculturally used areas decreased only slightly in the same period (Amt für Statistik Berlin-Brandenburg, 2010a). This has led to a considerable abatement of grassland utilisation. In order to retain this important part of the cultural landscape, support programmes for the preservation of these areas were initiated by the Brandenburg state government (MUGV, 2010). The biomass yielded from these preserved grasslands remains unused. However, several projects (PROGRASS, GNUT) analyse if an increasing utilisation of these habitats for energy production in biogas or incineration plants would be marketable (PROGRASS, 2011; Thüringer Landesanstalt für Landwirtschaft, 2009). Biomass from these grasslands also could be a suitable resource for biorefineries. Thus, before an energetic use, the biomass could be used for material production to reach a higher added value.

In 2004, the Renewable Energy Sources Act (Erneuerbare-Energien-Gesetz, EEG) (Deutscher Bundestag, 2009) was amended and the support of power generation from renewables was pushed. Whilst the quantity of agriculturally used areas in Germany remained more or less constant, from 2004 the cultivation of energy crops skyrocketed, leading to a major reduction of the area cultivated for industrial crops and feeding stuff but also for food (FNR, 2011).

The "National Strategy on Biological Diversity" aspires to prevent further impairments to the landscape in Germany (BMU, 2007). The landscape in Germany, however, has changed drastically in places, owing to the support programmes in the Renewable Energy Sources Act. Monocultures of energy crops, such as rape and maize, were created to supply the bioethanol and biogas plants with adequate quantities of feedstock.

An increasing number of utilisation options must be supplied by the existing agricultural areas in Germany. Agriculturists often base their crop rotation planning on perspectives for the highest profit for their farms. Since the mentioned Renewable Energy Sources Act supports the use of biomass for energy production, there is a distorted tendency towards such use to the detriment of other uses, such as for materials, which may be outcompeted.

2.3 Potential of the biorefinery concept

The multiple use of raw materials, as envisaged for biorefineries, leads to a higher product yield than in other biomass processing plants, enhancing the plants' efficiency. An advancement of this concept is to locate parts of the biorefinery in rural areas and to refine the raw materials on the farm. This advanced concept aims to make optimum use of all exploitable parts of the raw materials cultivated on farms to ensure sustainability and cost effectiveness of their use.

The most difficult problem with biomass is its large volume and heavy weight in relation to the quantity of usable contents. By producing extracted juice from green biomass on the farm (see chap. 3.2.1), the quantity to be transported to the processing plant is reduced by more than 50% (Venus, 2006). Transportation costs for the raw materials are reduced and the delivery of pre-products, in form of e.g. press juices, starch or molasses reduces the number of feedstocks for the biorefinery plant and therewith the storage and processing expenditure.

Building small processing plants in the rural areas which process the pre-products from the regional farms can reduce the transportation costs of the raw materials and intermediate products even more and can enable the quality of the industrial feedstock to be enhanced.

Locating parts of the biorefinery to the heart of rural areas can be achieved if an all-year supply of plant raw material can be supplied within a suitable radius around the farm. Due to the decentralised location, the industrial plants buy products created by the regional farms. By this a stable regional sales market would develop for the preliminary products generated by farmers. Industry and agriculture may become linked, opening up new entrepreneurial structures for agriculturists. By investing in the refining of biomass feedstock, agriculturists could evolve into suppliers of high-grade products to industry, enabling them to tap completely new markets.

2.3.1 Pre-products for the chemical industry

The chemical industry tries to turn “green”. The production is more and more based on renewable raw materials, the processing is configured to be more efficient and eco-friendly while the products are preferably biodegradable and intoxic. With the biorefinery concept the chemical industry can produce a variety of chemicals and materials, as well as energy with lowest consumption of resources possible. The industrial plants require a sufficient quantity of high-grade raw materials to manufacture the products. The increasing orientation of the industry towards renewable raw materials presents new challenges to farms as the main producers with regard to altered product requirements. The quality standards for food, energy sources and industrial feedstock differ considerably. Consequently, the raw materials produced must be adapted to the requirements of the respective buyers.

The chemical industry primarily requires an all-year supply of agricultural products of consistently high quality at reasonable prices (Peters, 2010). Ensuring the year-round availability of domestic renewable raw materials constitutes a problem. For Central Europe the usual harvest period is from May to October. Sufficient quantities of raw material must be produced within these six months to be able to provide the chemical industry with an all-year supply. The demands for a consistently high quality can be met by adjusting the sowing, maintenance, harvest and processing of the raw materials to the use for materials. Above all, it is vital to preserve biomass rapidly to prevent the onset of uncontrolled fermentation processes (Thomsen et al., 2004). Degradation of the valuable contents can be virtually prevented altogether if the plant material was preserved within a period of 24 hours. This is crucial not only for the quality required by the industry, but also for the consistency of this quality. For the finished product, it must be irrelevant when and where the feedstock was produced and processed. The refining on the farm allows a very fast processing and can therewith deliver high quality pre-products to the decentral biorefinery plants, which produce the basic materials for the chemical industries.

2.3.2 Biomass refining on the farm

Since the demand for food and feeding stuff will continue to rise in future and the provision of these goods should take priority over the use of biomass for materials and energy production, it is necessary to exploit new raw material potential in addition to making the exploitation of existing agricultural feedstock more efficient. In the best case, the use of raw materials does not compete with food production. For this reason, in the area of agriculture, the use of biomass for materials and energy production should concentrate on the incrementally unused grassland locations. Nevertheless, green biomass from cropland also can be used for an on-farm refining. The examples presented below show potential possibilities to step in refining. The market potential for the products yielded from refining is high; hence the intermediate products also have a suitable profit potential. In these examples, part of the refining process can be relocated to the farm at relatively low cost from the investor's perspective and with regard to acquiring the technical expertise.

Extracted juice from green biomass

A culture medium for lactic acid bacteria is required to produce highly concentrated lactic acids from raw materials containing starch. A juice extracted from legumes has proven to be highly suitable for this (Leiß et al., 2010). Lactic acid is a primary platform chemical used not only in the food industry, but also as a parent substance for the synthesis of chemicals (for instance lactic acid ester) and in the production of polylactic acid (PLA). Plastic made from polylactic acid (polylactate) is a plastic for the future because it is biobased, biodegradable, or both (European Bioplastics, 2012a). Scenarios figure out that the market for PLA will grow further; the global bioplastics production capacity will more than double from 2010 to 2015 (European Bioplastics, 2012b). A culture medium for the production of lactic acid can be created on the farm by squeezing fresh green biomass. Nitrogen and inorganic salts, contained especially in legumes, foster cell growth in lactic acid bacteria, and are essential for the optimum exploitation of lactic acids (Venus, 2010). The juice yielded can either be used fresh, i.e. within 24 hours, as a culture medium or be preserved by adding lactic acid bacteria which create lactic acid (Thomsen et al., 2004). This process shows that lactic acid can also be produced directly from green biomass. For high lactic acid production in this case, the green biomass must be ensilaged immediately after harvesting. A wide range of green biomass types other than legumes can also be used in this method of processing (Kromus et al., 2003). The dry matter of the silage juice extracted consists to an average of 30% of lactic acid (Kromus et al., 2003). However, lactic acid produced in this way is not suitable for the manufacturing of bioplastics (Venus, 2010). Nevertheless, there are extensive areas of application: The market for biologically degradable solvents and cleaning agents is steadily growing; these products are easy to be manufactured by reacting lactic acid with ethanol (Kromus et al., 2003). It is also possible to use the substance as preservative and to produce food and feeding stuff after ensilaging.

The production and preservation of such extracted juices can enable farms to make additional gains if grassland locations and cultivated legumes have so far been used inadequately or not at all. The price for the screw press to produce a press juice highly depends on the possible throughput rate. So farmers can choose the optimal size of the press for their accruing amount of green biomass for the biorefinery process. The lactic acid bacteria the farmers need for the conservation can be equated with those used for the ensilage process and are available at reasonable prices.

Proteins from legumes

Plant proteins can be used for many applications. As food, they are a good alternative to animal proteins. Amongst other things, they are used in industry to manufacture adhesives, emulsifiers and cosmetics. A large proportion of the plant proteins produced, however, is used in the animal feed industry. They are mainly produced from legumes because they are not only rich in nitrogen, but their protein content also exceeds that of many other types of plants (Aufhammer, 1998).

The green food proteins can be produced on the farm completely. The proteins are separated by either heating the previously produced press juice or by varying the pH value in the proteinaceous solution (Bonk, 1999). Once the proteins manufacturing the feed have been separated, the deproteinised juice extracted from the legumes can be used as culture medium for bacteria (Leiß et al., 2010). Both grain and feed legumes are equally suitable for recovering proteins. Drying the proteins yielded is a very energy-intensive work step involving high investment costs by the farm (Edwards et al., 1975). These costs can be cut if the protein sludge is supplied directly to fattening plants in the vicinity in a moist state (Schönicke, 2010). Although it only remains usable for a few days, both parties will benefit from the lower production costs and the resulting lower price. Even if the farmers don't have to buy a dryer for the protein production, the investment is much higher than for the production of press juices and only pays off if on a farm or by a cooperation of farms high amounts of legumes can be produced and processed.

2.4 Raw material assessment

Usually, all the different users of biomass create individual raw material assessments to see if a region has the potential to supply suitable biomass for their use option. Often it is not considered that the various biomass potentials overlap reducing the single use options or even making some of them unavailable. First studies showed that analysing statistics on cultivation and livestock in agriculture, which is common for the raw material assessment, cannot give reliable information on biomass potentials (Papendiek, 2010; Papendiek et al., 2011). Participation of farmers is essential to get this information from agriculture. From the actual point of view farmers use their raw materials already quite efficiently (Papendiek et al., 2011). It is to clarify if different cultivation systems, e.g. a catch-crop cultivation (see chapter 5), can produce additional amounts of biomass for the energetic and material use. Studies have to follow to see if an integration of legumes in crop rotations of the conventional agriculture can produce high quality biomass for the biorefinery. This can diversify the crop rotation to valorize the soil quality and reduce the amount of fertilisers needed. On grasslands it has to be clarified if areas under nature conservation can provide suitable biomass for the biorefinery when the maintenance of these areas is intensified. Sowing of about 50% legumes on the grassland locations could optimize the quality of the biomass for an industrial use. Adequate analyses have to show if this and an extensive use can preserve or even increase the spectrum of species on grasslands. The impact of political parameters should also be included in a raw material assessment to take prospective developments into account. An impact assessment tool can be used to create political scenarios and compare the development of biomass use under the different conditions (Helming et al., 2008).

2.5 Discussion

The rising demand for raw materials can only be met by using previously untapped raw material sources and by implementing a cascade use of the raw materials. According to the definition of biorefinery (de Jong et al., 2009) this means to use the biomass for food and feeding stuff in the first instance, and then for materials or chemicals and, finally, as an energy source. Under-utilised grassland locations are ideal as a raw material source for the use of biomass for materials and energy production, since there is no competition with the production of food and feeding stuff on these sites. Due to the measures introduced, many of the grassland areas have been set aside for nature conservation. Today, therefore, economic use of such areas is only possible to a limited extent (Hertwig and Pickert, 2004). The PROGRASS project examines whether biomass from designated FFH habitats can be used for energy production and at the same time biodiversity in those areas can be preserved or even increased (PROGRASS, 2011). The use for materials and chemicals should also be examined because of the higher added value.

The rising demand for raw materials must not lead to the exploitation of reserves. Agricultural areas should still be managed sustainably. For example, it should be assumed that up to 80% of straw has to remain on the field on some sites to protect the soil (Boelcke, 2003). Carbon can only partly be fed back into the soil via biogas substrates, but primarily via manure and straw (Arthurson, 2009; Wragge et al., 2010). Even if the prices for the raw material increase, this must not lead to a reduced amount of straw being left on the fields. One option to remain higher yields and therewith more biomass from a constant area, is to integrate catch crops in the crop rotation. Catch crops here are meant as fast growing plants, e.g. legumes cultivated in between of two main crops. This cultivation system approach is to reduce erosion and nutrient leaching risks (Freyer, 2003). The yielded legumes provide important raw materials for the biorefinery and can support a sustainable management of the agricultural areas, optimizing the water availability and nutrient supply in soils (Kahnt, 2008). Therewith, several services are facilitated, offering a multiple dividend. According to the discussion on ecosystem services within agricultural landscapes these may be called “agrosystem services”, just to point out the coupled product of agricultural production.

When the biomass refining starts on the farm, the residual materials (press cake) left over after refining can be used as animal feed or a source of energy, and subsequently as fertiliser. In other words, nutrients and carbon are directly used on the farm and returned to the soil, which is a vital aspect in the sustainable management of agricultural areas (Kahnt, 2008).

Since in the biorefinery concept the use of biomass for energy production is placed at the end of the value-added chain, the substances contained in the plants are the key criterion for cultivation rather than their energy density. The cultivation of maize and rape in monocultures for use in energy production can therefore be reduced, and the diversity of the landscape due to production schemes can grow again. Biomass used as renewable raw material is generally produced in rural regions. For this reason, the added value should also commence there. Since the numbers of livestock have declined dramatically, farms have evolved into exclusive suppliers of primary raw materials. In the future, biorefineries bring new methods of refining raw materials to farms, which would promote the development of the rural areas. These first refineries would contribute to meeting the industry's demands on regenerative raw materials to the greatest extent possible. By working together, industry and agriculture can prevent errors in the cultivation, processing and transportation of renewable raw materials or the products gained from them, ensuring there are no fluctuations or reductions in quality, endangering the cost effectiveness of processing. The prices realised for the products yielded are expected to be higher and more stable than for primary raw materials. Regional and supraregional economic cycles can be established between agriculture and industry (e.g. discussion about "innovation partnerships" within the EU agriculture policy), leading to the promotion of investments in the regions and an increase in their economic strength.

A similar approach to the on-farm refining is the development of regional biomass processing centres (RBPC). The problem of large biorefinery plants is that they also entail increased costs of biomass transportation and storage, high transaction costs of contracting with large numbers of farmers for biomass supply, potential market power issues and local environmental impacts (Carolan et al., 2007). The RBPC is conceptualized as a flexible processing facility capable of pre-treating and converting biomass into appropriate feedstocks for a variety of final products such as fuels, chemicals, electricity and animal feeds (Carolan et al., 2007). They reduce the conflict between plant size and transportation costs. These centres are concepted for lignocellulosic resources and are proven to be economic for this kind of biomass. For dry biomass these centres are more capable than an on-farm refining. The only possible refining on farms would be the comminution but the transport costs wouldn't decrease noticeable because of this process and the quality for upcoming products wouldn't rise. Then again for fresh biomass this quick and local refining is very important for these aspects and increases the profitability of green biomass use.

2.6 Conclusions

Biorefineries allow more efficient use of biomass to save resources. The opportunities of an implementation in the rural area are diverse. The cost effectiveness of the biorefinery concept depends to a great extent on the costs involved in transporting the biomass. If a high part of the biorefinery process is implemented decentrally, transport distances are minimal, making biomass processing even more efficient. The highly concentrated chemicals produced in the decentral plants can be transported over longer distances to the central industry sites. The production of pre-products on the farm and in decentral plants results in a better quality of the industrial products and supports the sustainable development of rural areas. Refining biomass can constitute additional incomes for agriculturists, as is already the case in the use of biomass for energy production. The potential for establishing biorefineries should accordingly be linked directly to agriculturists' production-related decisions. All considerations of the potential availability of space for the cultivation of the relevant biomass are misleading. The refining of previously unused or only partially used raw materials for materials can facilitate stable incomes on a promising market through the close link to biorefineries, and hence to industry. After the refining of raw materials, substances are left that can be used to close material cycles on the farm. However, the potential of relocating biomass refineries to the rural areas is not sufficiently studied yet. It is not clarified whether the participation of agriculturists will help evolve a new method to describe raw material potentials more reliably. It still has to be investigated if on-farm refining can really pay off for the agriculturists and if it is possible to cultivate and refine enough high-grade biomass for the future demand of the various industries. One critical aspect is the fact that in legumes breeding towards higher and more stable yields only little progress has been made in recent decades. Moreover, it is still an open question whether grassland sites that are under nature conservation can be used for the production of biorefinery raw material while keeping or increasing biodiversity on these sites.

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3. Cultivation and fractionation of leguminous biomass for lactic acid production

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Abstract

Chemical industries are set to increase the proportion of renewable feedstock in their production in the decades ahead. Green Biorefineries that divide fresh green biomass into cakes and juice deliver valuable products for various industrial uses. Press juice can be used to produce lactic acid (LA), a promising building block for the future. In this study, optimal cultivation and fractionation processes for generating a fermentation medium from legumes for lactic acid production by Bacillus coagulans are analyzed. The contents of press juices from alfalfa cultivated on arable land at three different sites and from a clover-grass mixture on a grassland site taken on different sampling dates are compared. In addition, fresh biomass yields from the different biomass samples are examined. This paper focuses on the methods applied, and provides initial results. Yield differences of up to 40 % and 60 % were recorded between different study sites and sampling dates, respectively. Fermentation analysis of the different samples revealed that press juices can supplement the main parts of nutrients for lactic acid bacteria, producing economically interesting amounts of lactic acid. These findings could increase the use of lactic acid in chemical industries and bring about a shift towards a higher proportion of renewables, namely legumes, in the processing chain.

3.1 Introduction

In the decades ahead, biomass is expected to gradually replace fossil resources. The chemical industries will increase the proportion of renewable resources in their production accordingly. It is estimated that from 2012 until 2017 the total chemical sales of biotechnological products will double worldwide (Festel, 2011). To achieve this, products of a constantly high quality are required, which the concept of biorefinery aims to assure. "Biorefinery is the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)" (de Jong et al., 2009). A special type is the Green Biorefinery, where the focus is on using fresh or ensiled wet biomass as feedstock (FNR, 2012). Fractionation of this green biomass into press juice and press cakes is essential for the Green Biorefinery, and the new resulting feedstock constitutes valuable products for various industrial uses.

Green Biorefinery facilitates the multiple use of fresh green biomass (Kamm et al., 2010). Press cakes can be used as solid fuel (Thomsen et al., 2004) or fibrous composite materials (Biowert Industrie GmbH, 2013). In addition, fodder pellets can be produced if raw materials with high protein content, such as legumes, are processed (Kamm et al., 2010). Legumes, i.e. alfalfa and clover, are typical fodder plants (Kamm et al., 2010). Originally, press juices were the residue from green fodder pellet production. They were used as fertilizers on fields, but caused eutrophication (Andersen and Kiel, 2000). Being aware of the valuable contents of press juice, a number of approaches for industrial use were developed. Thomsen *et al.* (2004) explored a method for producing a feed concentrate from residual juices that is also suitable for non-ruminants. Chemical industries can use press juices as a universal fermentation medium (Andersen and Kiel, 2000). Either fresh or silage press juices can be used as such an inexpensive nutrient source. The juices, featuring a variety of nitrogen-containing compounds and inorganic salts, can act as a substitute for synthetic nutrients (Leiss et al., 2010; Venus, 2006). Already existing processes, like the microbial PHA-production, can become more efficient due to lower feedstock costs (Koller et al., 2005). Numerous investigations have been made into alfalfa press juices. These studies demonstrate that alfalfa press juices can act as a substitute for MRS, a synthetic nutrient, in lactic acid production, cutting processing costs (Kamm and Kamm, 2007; Leiss et al., 2010; Venus, 2006). Lactic acid is a chemical product that can be processed into many different products (Kamm and Kamm, 2007), including L-Lysin (Sieker et al., 2010), pollution-free solvents, cosmetics and bioplastics (Venus and Richter, 2007). Bio-based lactic acid (LA) is thought to be an important bulk chemical for the future (de Jong et al., 2012). A carbohydrate-containing raw material, lactic acid bacteria and a fermentation medium, such as alfalfa press juice, are required to produce lactic acid. It is assumed that the demand for press juices will increase in line with the growing market for bioplastics. In this study, therefore, the focus is on the optimal cultivation and fractionation of legumes to generate a high-value fermentation medium for lactic acid production.

Since it is vital that industries are supplied with high-quality preproducts (Peters, 2010), the composition of biomass contents must be stable and impartial to variation. Pilot plants that work with green biomass analyze the advantages and drawbacks of different feedstock. The main challenge in Green Biorefinery is the seasonal availability of the feedstock while it is not storable (Kromus et al., 2004). Uncontrolled fermentation processes start directly after harvesting (Thomsen et al., 2004). Therefore, the whole plant or the cake and juice need to be preserved, i.e. with lactic acid bacteria (Thomsen et al., 2004). Most of the planned or existing Green Biorefinery plants in Europe focus on biomass from grassland. The reason for this is that pressure on arable land is already high, and the demand for forage grassland has continued to decline, creating new potential for exploitation (Biowert Industrie GmbH, 2013; King et al., 2012a; Kromus et al., 2004; O'Keeffe et al., 2011). This study investigates leguminous biomass from both grassland and arable land. Arable land is included in the study due to the positive effect of legume cultivation on the soil fertility and the importance of legumes in crop rotations (Nemecek et al., 2008). Green Biorefinery pilot and demonstration plants show how the concept can be implemented successfully on the industrial scale (Kamm et al., 2010; Kromus et al., 2004; Thomsen et al., 2004; Venus and Richter, 2007). However, no systematic analysis of biomass cultivation for Green Biorefineries has been published to date, and only little information is available about fractionation (King et al., 2012b). Typically, screw presses are used for the fractionation process (King et al., 2012b). The first qualitative measurements on the fractionation process into cakes and juice were undertaken and published by King *et al.* (2012b). In our study, the contents of press juices from alfalfa cultivated on arable land and from a clover-grass mixture cultivated on grassland on different sampling dates are analyzed. The results enable assessment of the best harvest time for the production of a fermentation medium for lactic acid. In addition, valuable results on decisive characteristics of biomass for the pressing process and for calculating biomass potentials are obtained. Besides reporting some results generated from the study, this paper focuses on the methods that were used.

3.2 Methods

3.2.1 Cultivation and harvest

Field trials were established on four different sites in north Brandenburg (Germany). We cultivated alfalfa on arable land at three different sites. The fourth field was a clover-grass mixture cultivated on grassland. The climate in Brandenburg is continental; the average annual rainfall over the past 20 years was about 550 mm for all sites, and the average annual temperature was between 9.1 °C at Müncheberg/Steinbeck and 9.6 °C at Paulinenaue (Weather Data, 1991-2011; Weather Data, 1992-2011). Table 1 gives information on soil conditions, pretreatment and fertilizer input on the study sites. In Table 2, weather conditions for the sampling dates are given. Further details about the sites are given below.

Table 1 – *Sampling dates in 2012*

	Early	Middle	Late
1st cut	May 22	June 4	June 6
2nd cut	July 10	July 23	July 31
3rd cut	August 28	September 10	September 18

In the study region, fodder legumes are typically cut three times within the harvest period. In order to determine the optimal harvest time for industrial use, three sampling dates (early, middle, late) for each cut were set. These sampling dates, which were dependent on weather conditions and took into account weekends, were approximately at 10-day intervals (Table 3). Samples of alfalfa and clover-grass were harvested and chaffed at the study site, and then transported for analysis. At Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), the fresh biomass was pressed using a screw press Cv (VETTER Maschinenfabrik GmbH & Co.KG, Kassel/Germany) (Fig. 1). The press had a 7.5 kW engine and a flow rate of 600 to 800 kg h^{-1} depending on the feedstock. The sieve size was ø 1.1 mm. Maximum temperature of the juice outflow was 55 °C. The gap width was 25 and 35 mm, respectively, for the last two sampling dates (Fig. 2). The biomass was pressed such that there were sufficient soluble components in the press cake for potential use as feed.

Table 2 – *Weather conditions during the 2012 harvest period*

Study site	Date	Daily average temperature in °C (2 m above surface)	Precipitation in mm on the day of harvest and the day before
Müncheberg/Steinbeck	May 22	22.6	0.0
Müncheberg/Steinbeck	June 4	11.4	4.3
Müncheberg/Steinbeck	June11	16.6	0.0
Müncheberg/Steinbeck	July 10	19.1	0.0
Müncheberg/Steinbeck	July 23	16.7	0.0
Müncheberg/Steinbeck	July 31	16.1	0.0
Müncheberg/Steinbeck	Aug 28	15.9	0.5
Müncheberg/Steinbeck	Sept10	20.0	0.1
Müncheberg/Steinbeck	Sept 18	16.9	0.0
Paulinenaue	May 22	22.9	3.0
Paulinenaue	June 4	11.8	10.7
Paulinenaue	June11	17.5	0.2
Paulinenaue	July 10	19.0	4.2
Paulinenaue	July 23	18.2	0.0
Paulinenaue	July 31	16.9	0.7
Paulinenaue	Aug 28	15.3	0.5
Paulinenaue	Sept 10	18.2	0.0
Paulinenaue	Sept 18	15.5	1.0

Figure 1 – *Screw press*

Figure 2 – *Screw press gap width*

Samples of the fresh biomass, press cakes and press juice were taken for analytical purposes. Biomass and its press juice decay very quickly after harvesting, due to uncontrolled fermentation processes (Thomsen et al., 2004). Therefore, in this study, the raw material was always pressed and conserved close to where it was cultivated just a few hours after harvesting.

Table 3 – *Composition of liquid phases in the press juice at the Müncheberg site*

	Sampling date	Dry matter 105 °C (%)	Disaccharide (g L^{-1})	Glucose (g L^{-1})	Fructose (g L^{-1})	Nitrogen (g L^{-1})	Crude Protein (g L^{-1})	Phosphorus (g L^{-1})
1st cut	May 22	7.26	3.7	9.44	9.01	2.69	16.81	0.41
	June 4	5.88	5.4	7.94	5.77	1.88	11.75	0.3
	June 11	8.21	4.6	11.71	6.7	2.48	15.5	0.48
2nd cut	July 10	7.37	2.72	5.32	6.6	3.82	23.88	0.47
	July 23	11.54	2.22	4.67	5.83	4.21	26.31	0.53
	July 31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3rd cut	Aug 28	9.26	4.55	12.4	8.04	4.22	26.38	0.38
	Sept 10	8.4	3.22	11.0	10.2	3.68	23.0	0.18
	Sept 18	6.61	2.86	10.1	7.2	2.96	18.49	0.12

n.d. not detected

Site 1 – Leibniz Centre for Agricultural Landscape Research (ZALF) Müncheberg field station (coordinates: 52.516045, 14.124929): a fully randomized block design was created for the alfalfa site at Müncheberg field station. One hectare was divided to enable each cut to be harvested on three sampling dates (Table 3).

Yields were determined using a HALDRUP F-55 grass harvester, and afterwards harvested using a Maral 125 forage harvester. Biomass was directly chaffed into 5 to 10 cm pieces in the harvesting process, and transported to ATB laboratories for pressing and analysis.

A problem arose on July 31, 2012 when the forage harvester broke down, making it impossible to harvest the biomass. For this reason, only one sample of a few kilograms of fresh biomass was taken and analyzed, as opposed to a representative sample.

Site 2 - Steinbeck (coordinates: 52.713809, 13.909371): a farmer from Steinbeck allowed us to use part of his alfalfa fields for our study. The 400 m^2 area was divided into three plots. Alfalfa was in the fourth year of cultivation and plants were heavily overgrown with dandelion. In order to determine yield, the farmer's decision to harvest and the sampling date in the study had to be synchronized.

Samples in Steinbeck were only harvested but not chaffed on the first sampling dates. Problems arising when pressing long material necessitated chaffing. For this reason, we transported all samples taken from Steinbeck after June 4, 2012 to Müncheberg, where they were chaffed using a Maral 125.

Due to dandelion overgrowth, yields on the alfalfa fields in Steinbeck were so low that the farmer ploughed up the field after we had completed the second cut (July 31, 2012). For this reason, no samples or data are available for the final three cuts in Steinbeck.

Sites 3 and 4 – Leibniz Centre for Agricultural Landscape Research (ZALF) Paulinenaue field station (coordinates 52.683381, 12.685897): At the research station in Paulinenaue, one hectare of arable land was tilled with alfalfa and another hectare of existing perennial grassland site was resown with a perennial ryegrass and white clover mixture. On the first sampling date, alfalfa and the clover-grass mixture were harvested using a Splendimo 240 disc mower. Yields were determined in the same way as on the other sampling dates. The biomass was not chaffed but cut with a disc spacing of 8 to 10 cm, pressed into bales and transported to the laboratories. On the next two sampling dates (June 4, 2012; June 11, 2012), the biomass was also hackled and pressed into bales but not chaffed. However, the disc mower was unable to produce chaffs (average chop length of 20 cm) that were comparable to those produced by the forage harvester. This reduced the quality available for pressing. Consequently, from the second cut biomass was chaffed using a Claas Jaguar forage harvester. The loose material with an average chop length of 2 cm was transported to the laboratories.

Biomass samples from all of the study sites arrived in Potsdam at least 5 hours after harvesting and were pressed and conserved at least after 8 hours. As an exception, however, on the third sampling date of the first cut (June 11, 2012), the press broke down after pressing samples from Paulinenaue. The two remaining series of biomass samples from Müncheberg and Steinbeck were pressed later on, but immediately conserved in the freezer at -20 °C. It must be said that a number of irregularities occurred in the sampling due to the lack of an established sampling procedure; for the time being, these will have to be accepted.

3.2.2 Analytical determination

After pressing, a sample of each press juice was conserved in a freezer at -20 °C. In order to determine the dry matter (DM) value, a sample of each press juice was dried to a constant weight at 105 °C.

Organic acid and sugar concentrations were measured by HPLC using an ultimate 3000 from the company DIONEX (column: Eurokat H (300 x 8 mm, 10 µm), company KNAUER; mobile phase: 0.005 mol L^{-1} sulfuric acid; flow rate: 0.8 mL min^{-1}; sample volume: 10 µL). The single components were detected using an RI-71 detector (SHODEX) with a minimum detection limit of 0.01 g L^{-1} and a maximum limit of 5 g L^{-1}.

Total Kjeldahl nitrogen was analyzed using standard-method Vapodest apparatus from Gerhardt by digestion using a selenium catalyst. In order to calculate the content of raw protein, nitrogen values were multiplied by a factor of 6.25. The colorimetric technique was used to measure total phosphorus by applying the molybdenum blue method (according to DIN EN ISO 15681).

3.2.3 Batch fermentation

Batch fermentations with the strain *Bacillus coagulans* (internal ATB no. A107) were carried out in a 3-litre stirred tank reactor under the condition of both temperature (52 °C) and pH value (6.0) control. The medium used was a mixture of glucose stock solution (600 mL) and different press juices (1400 mL) with no other nutrients. The volume of a common inoculum was divided into three parallel cultivations to ensure the same bacteria activity at the beginning of the experiments. The preculture was used after 15 hours shake flask cultivation with a cell density of $2.7 \cdot 10^7$ CFU mL^{-1}. Aliquots of the fermentation liquid were taken periodically to determine the concentration of lactate and sugars, which were measured after biomass separation and dilution by HPLC as described above.

3.3 Results and Discussion

3.3.1 Influence of study site and harvest date on biomass yield

Analysis of all samples revealed major differences in biomass yield between study sites and sampling dates. Fig. 3 shows the total of fresh biomass yields from the first, second, and third cut for the study sites, with the exception of Steinbeck, which was no longer included in the study. Due to dandelion overgrowth, the site generated low yields on each sampling date (3 and 7 tons of fresh biomass per hectare) and was ploughed up after the second cut in 2012. Dandelion overgrowth is a typical progression for three- to four-year-old alfalfa if no herbicides are used (Kreil et al., 1983). Very few pesticides are available for alfalfa because the development and approval process is expensive and less rewarding for minor crops such as legumes (DAFA, 2012).

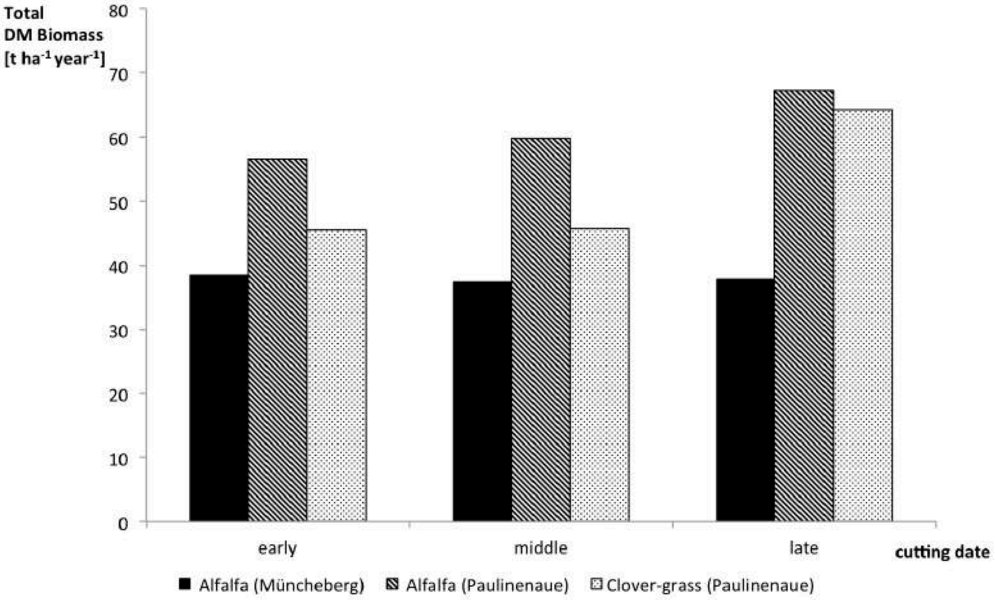

Figure 3 – *Total biomass production at the Müncheberg and Paulinenaue study sites in 2012*

Alfalfa developed better in Müncheberg than in Steinbeck, in spite of poorer soil conditions (Fig. 3). Total yields of nearly 40 t ha^{-1} are an adequate yield for the sandy soils that are not well suited for alfalfa cultivation. Kreil *et al.* (1983) analyzed yield data for alfalfa in the former GDR, and for sands with a clay layer in deeper soils, as they occur in Müncheberg, found a typical yield span between 37 and 48 t ha^{-1}. Müncheberg exhibited no significant differences in total fresh biomass yields between the early, middle and late sampling dates (Fig. 3). However, there were significant yield ranges between the sampling dates of one cut. The range was particularly high in the first and second cuts (Fig. 4).

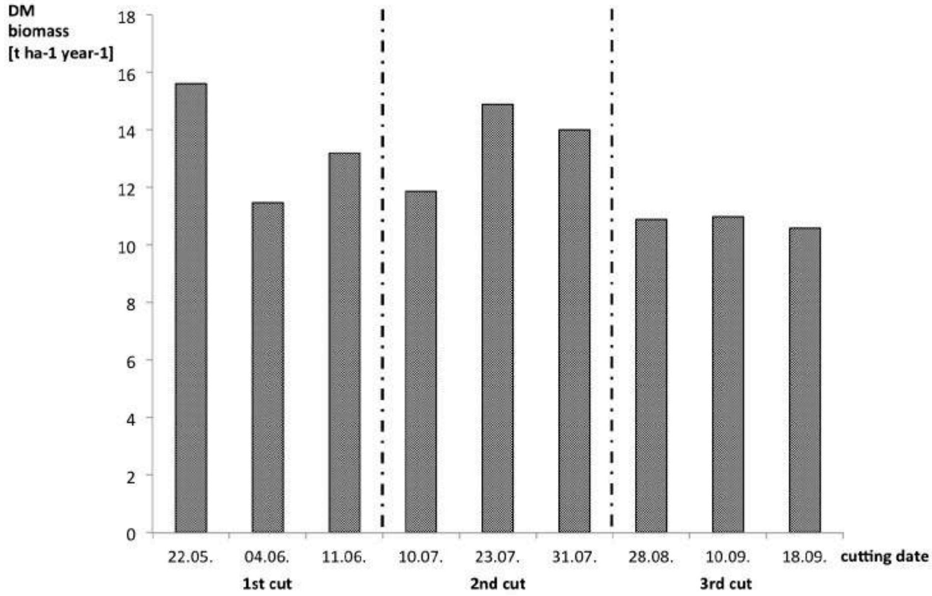

Figure 4 – *Biomass production on the individual harvest dates at the Müncheberg study site in 2012*

The study site at Paulinenaue exhibited much higher yields than in Müncheberg for alfalfa (Fig. 3). The sand humus gleys at Paulinenaue are very well suited for alfalfa cultivation and therefore can deliver yields of more than 60 t ha^{-1} fresh biomass (Kreil et al., 1983). Yield ranges within one cut were even higher than in Müncheberg. For alfalfa in Paulinenaue, the highest margins were 36 % in the first cut. The clover-grass mixture at Paulinenaue also delivered high yields but exhibited ranges of 60 % in the first cut, 40 % in the second and even 18 % in the third. Comparable yield data for clover-grass are generally given as dry matter. In 2011, the average yield for pastures and meadows, including clover-grass, was 6.5 t ha^{-1} in Germany (DESTATIS, 2012) compared to the annual dry matter yields of 9.7 to 13.6 t ha^{-1} recorded at Paulinenaue in this study. Accordingly, the study site delivered above-average yields for grassland for all harvest periods. It is difficult to find current and comparable yield data for legumes, especially for fodder legumes cultivated conventionally on arable land. The reason for this is that protein plants are relatively rare in many European Union Member States (European Union, 2011) due to cheap protein feed such as soybean meal imported mainly from Latin America (Khatun, 2012). Comparing the alfalfa yield data from Kreil *et al*. (1983) and the findings of this study, yields did not increase over the last 30 years. A main reason is the breeding arrear (DAFA, 2012). Legumes are therefore underrepresented in crop rotations. The positive effect they have on succeeding crops (Freyer, 2003) and their high nutritional value (Berendonk et al., 2011) therefore remain unexploited, which, in the context of our study, turns out to be a lost advantage. The multiple use of press cakes and juice generated by biorefineries could revitalize the cultivation of legumes in European agricultural systems and the unexploited breeding potential can even increase the economic potential of legumes.

The yield data recorded in the study show that the study site and sampling date play a major role. Although alfalfa is a perennial plant, it only delivers acceptable yields for two to three years after sowing (Berendonk et al., 2011). Nonetheless, even marginal sites, such as at Müncheberg, which has a low soil quality index, can produce good yields over the year. For better soils, the harvest date plays an even greater role. A much higher yield range between harvest dates occurred in Paulinenaue than in Müncheberg. The field station in Paulinenaue is a fen site where ground frost appears well into spring, thus delaying the harvest. This influences calculations on biomass potential, since this high variance is often not taken into consideration.

3.3.2 Influence of study site and harvest date on the composition of press juices

In this study, the task was to test whether protein components from juice can be used as a source of nitrogen and other nutrients for lactic acid production for further polymerization towards PLA. The objective was to substitute expensive yeast and meat extracts as well as peptone with proteins contained in the green juice.

Figure 5 shows the total dry matter (DM) content and the content of individual press juice components with regard to later application for fermentation processes, in particular to produce lactic acid. Data for the Müncheberg site is given. Carbohydrates contained in pressed juices for different sampling dates are shown. Green juice is unable to act as a source of carbon for lactic acid production due to the small quantities of total sugars (water soluble carbohydrates (WSC) content is usually less than 30 g L^{-1}). In a fermentation process that uses juice or its components containing nitrogen, the carbon source must be an external raw material such as starchy biomass, lignocellulosic feedstock or residues in order to enhance fermentable sugars and the efficiency of the entire process.

After strain selection, based on previous research, the aim was to investigate the suitability of complex agricultural materials such as alfalfa and clover-grass juice for the fermentation process. As noted above, green juice cannot be used as a carbon source in lactic acid fermentation. Therefore, sugar (glucose monohydrate) was used as the main carbon source. However, green juice contains a series of nitrogen-containing compounds and inorganic salts, which are essential for cell growth and a number of mineral salt components that microorganisms require for growth. Depending on the type and concentration of individual impurities (e.g. metal ions), these mineral salt components can cause problems in lactic acid purification when high-purity lactic acid is to be produced.

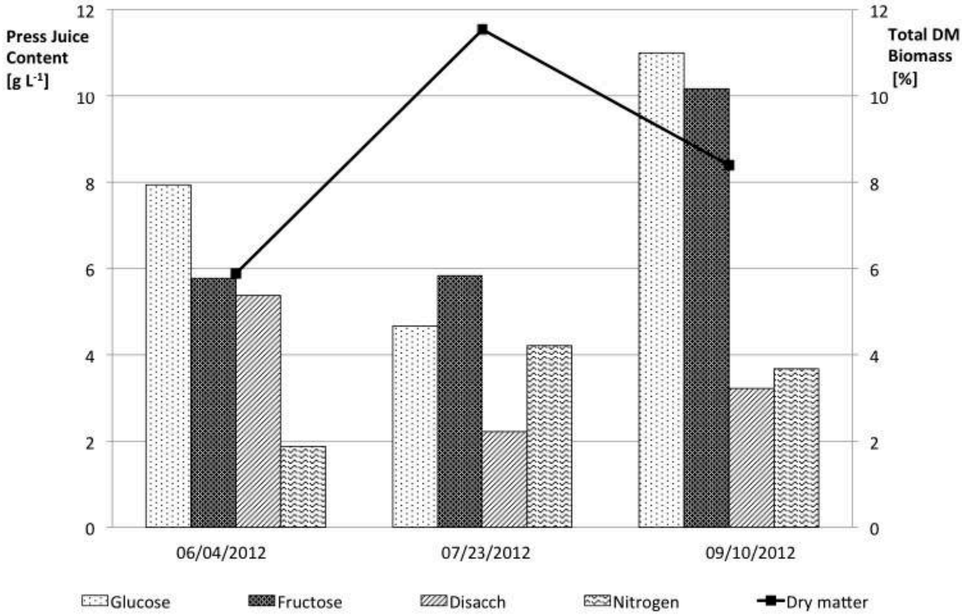

Figure 5 – *Characteristic parameters of alfalfa press juices at Müncheberg*

For the first evaluation of differences between the three cuts on the typical (middle) harvest date, three juices from Müncheberg were used as a nutrient supplement for a glucose-based broth. For the sugar and nitrogen content on the three typical cut dates (June 4; July 23; Sept 10), the tendency of the juice composition could not be correlated clearly with the dates, since the weather conditions were not really comparable (e.g. higher water content for the harvest date in June due to heavy rain). Nonetheless, the different time courses of lactic acid concentration and substrate consumption (Fig. 6) reveal a number of basic findings. The results for the green juice with the lowest nitrogen content (sample 06/04/2012) indicate speedier product formation but incomplete sugar consumption. The maximum volumetric lactate productivity was achieved after 20 hours (2.5 g L^{-1} h^{-1} together with a yield of produced lactate out of sugars $Y_{P/S}$ 0.66 g g^{-1}), whereas the sugar conversion at the end of the fermentation was calculated at 87.8 %. Due to the relatively high concentration of disaccharides, it is difficult to distinguish between magnitude of influence (e.g. inadequate nitrogen content and/or delay of sugars besides glucose and fructose). There is also a delay in the performance of samples with a higher nitrogen concentration (07/23/2012 and 09/10/2012). However, after passing the logarithmic phase, the final titer of lactate results in a higher level of about 80 g L^{-1}, together with nearly fully consumed sugars. The maximum volumetric productivity reached 2.65 g L^{-1} h^{-1} after 28 hours for the juice containing 4.21 g L^{-1} nitrogen, and 2.8 g L^{-1} h^{-1} after 24 hours for the juice containing 3.68 g L^{-1} L nitrogen, respectively. There is no negative impact on the fermentation process in general, and the final lactate concentration is in the same range as for all batch runs. Considering the partially different sugar concentration, lactic acid yield (product concentration in relation to the substrate concentration used) exhibits slightly higher values ($Y_{P/S}$ 0.74 and 0.71 g g^{-1}) for experiments conducted using press juice containing higher levels of nitrogen. At present, it can be summarized that green juice supplements can act as a substitute for most standard nutrients (mineral salts, complex nitrogen sources such as yeast extract, peptones) for lactic acid bacteria. These first runs have been done without replications due to the limit of raw material samples. With regard to the significance of the differences between the results, it should be stated that the fermentations were based on the same preculture at a time. From long-term experiences, the activity and behaviour of the inoculum is known as a key factor for the comparison of several process parameters. In this context, the composition of the fermentation broth (i.e. nitrogen content) can be assumed as the main influence on the product formation and sugar consumption, respectively.

The results concerning the composition of press juices can be compared to those determined by Kromus *et al.* (2004). Crude protein content is similar to that in press juices from Müncheberg; WSC with 12 % of the DM are much less concentrated than in the samples taken in this study. The results generated by King *et al.* (2012b) cannot be compared properly with the ones in this study because they used silage rather than fresh biomass. When compared, however, fresh press juices from alfalfa achieve far higher crude protein contents in most samples than the 100 to 155 g kg^{-1} DM yielded in grassland analyzed by King *et al.* (2012b), as well as larger amounts of WSC (Table 3). In this study, the pressure applied in the pressing process was set so that additional juice would occur in press cakes, enabling them to be used as a feed source. In contrast, King *et al.* (2012b) sought to gain the maximum quantity of juices.

The positive result for using different green juices in lactic acid fermentation correlates well with previous observation (Leiss et al., 2010; Vodnar et al., 2010). For more scientific conclusions much more experiments and observations on the cultivation and fractionation of leguminous biomass for lactic acid production are needed. In the following research years, the results of this study have to be verified. In addition, experiments on the quantity and composition of press juice caused by different chop length and gap widths in the press must be analyzed and an economic evaluation of legume cultivation and fractionation conducted.

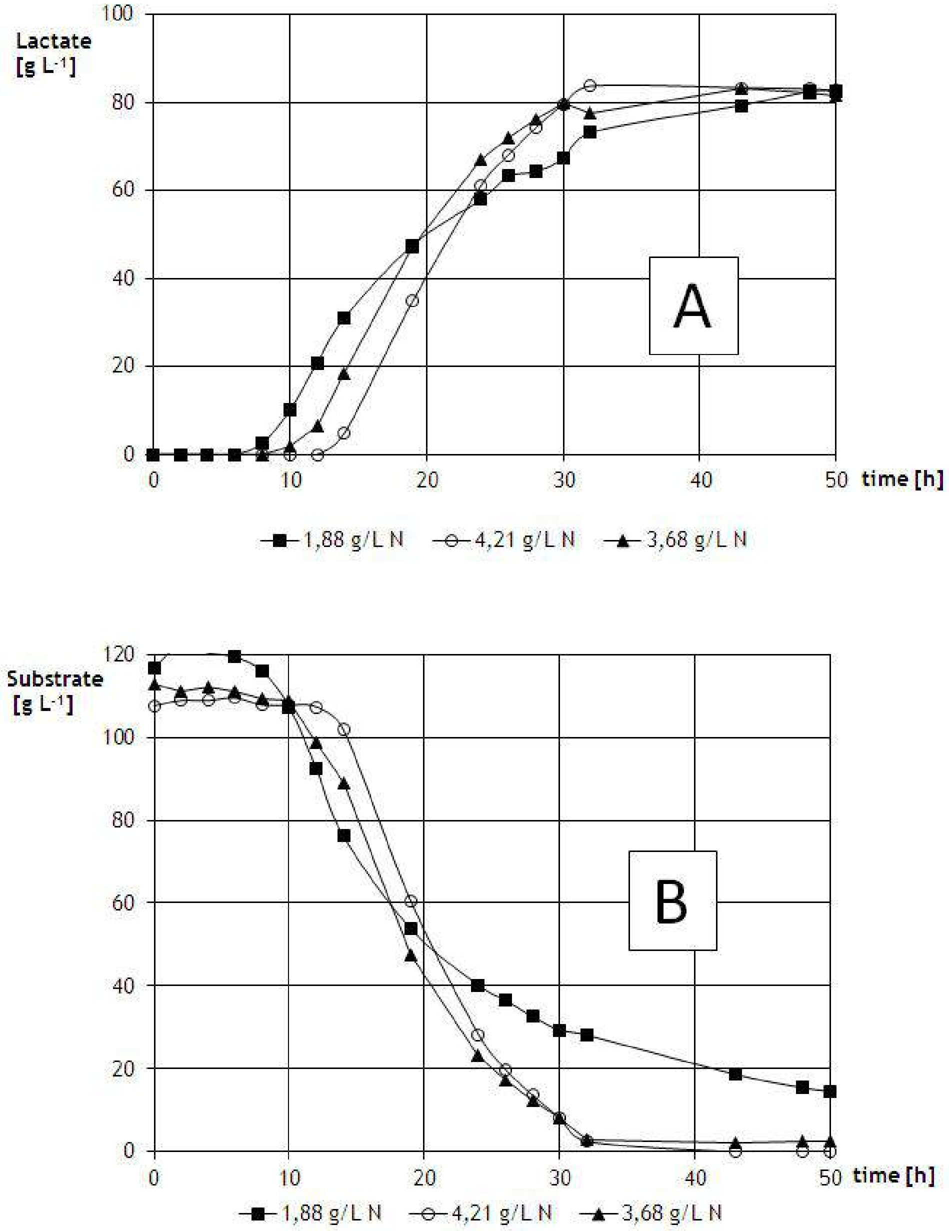

Figure 6 – *Product formation (A) and substrate consumption (B) with different nitrogen concentrations at the Müncheberg site*

3.3.3 Influence of chop length and weather conditions on pressing process

Biomass from the Müncheberg and Paulinenaue sites were sent to the laboratories with different chop lengths, depending on chaffing methods and machines. Unchaffed material from Steinbeck as well as the first samples from Paulinenaue delivered low amounts of juice. The fibers wrapped around the screw and material was scraped instead of pressed. In general, the shortest chop length of 2 cm generated the best results in the press with regard to quantities of press juice and throughput speed. However, the chop length had no significant impact on the composition of press juices.

The second sampling date was already postponed from June 1 to 4, due to unfavourable weather conditions but still the biomass had to be harvested and chaffed wet (Table 2). Whenever the biomass was harvested and chaffed wet, the short chop length increased the risk of the press becoming clogged. The gap width therefore must always be adjusted to the specific biomass characteristics.

3.4 Conclusions

In this study on fermentation media generation for lactic acid production from leguminous press juices, the influence of different parameters such as study site and harvest date on their use as substitutes in chemical industries was analyzed. New methods were established to obtain sound information on the first link in the supply chain for industries based on renewable resources. Initial results given in this paper reveal important differences for feedstock. These recent findings can influence calculations of biomass potential and also change processing methods in the chemical industries, generating an increased demand for legumes.

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4. Fodder legumes for lactic acid production – the influence of cutting date and site on biomass and bacterial nutrient yields

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Abstract

Lactic acid (2-hydroxypropionic acid) is one of the most promising platform chemicals for biotechnological production. Press juices from fodder legumes can act as a substitute for expensive bacterial nutrients such as yeast, meat extracts and peptone, which are required in the lactic acid fermentation process. Field trials were established at two separate sites in Brandenburg (Germany). Alfalfa *(Medicago sativa)* was cultivated on arable land and a clover/grass mixture *(Lolio-Cynosuretum)* on permanent grassland. The biomass from each plot was chaffed at the study site and then pressed to obtain press juice and cake. We evaluated statistically which indicators in plant cultivation are most important for biomass and bacterial nutrient yields. Alfalfa produced significantly higher dry matter yields under the same environmental and cultivation conditions. The cutting time had no major influence on the quantity and impact of bacterial nutrients for lactic acid formation. The year of cultivation was not relevant either. This implies that press juices from the entire vegetation period may be used in production. In order to improve the efficiency of biomass use, the residual press cake was also analysed. Analysis revealed that this feedstock can be used for ruminant feed production. Hence, both press juice and press cake are valuable feedstocks.

4.1 Introduction

Biomass has become an increasingly important renewable resource in the non-food sector, and is used in a growing range of contexts. Several scenarios exist concerning potential future uses. For example, Festel et al. (2012) suggested that the market share of bio-based chemicals may increase to 15.4 % of total chemical sales by 2017. Lactic acid (2-hydroxypropionic acid) is one of the most promising platform chemicals for biotechnological production. The food, cosmetic, pharmaceutical and chemical industries use lactic acid in a variety of applications, including for pH regulation, as an antimicrobial agent and as a green solvent and cleaning agent (Castillo Martinez et al., 2013). Lactic acid has also attracted attention as a monomer for use in the production of poly(lactic acid), a biodegradable plastic that has the potential to act as a substitute for many petroleum-based plastics in the future (Jim Jem et al., 2010; Madhavan Nampoothiri et al., 2010). As such, lactic acid is not only an industrially relevant platform chemical, but also an important product for the bio-based economy. The demand for lactic acid was estimated to be 714,000 t in 2013 (SpecialChem, 2014). Demand is expected to grow at an annual rate of 15.5 % between 2014 and 2020, due primarily to the demand for bio-plastic (Abdel-Rahman et al., 2013; SpecialChem, 2014). However, in order to make lactic acid more than a niche product, feedstock costs need to be reduced (Abdel-Rahman, 2013). Press juices from fodder legumes can act as a substitute for expensive bacterial nutrients such as yeast, meat extracts and peptone, which are required in the lactic acid fermentation process (Papendiek and Venus, 2014). The quantities of bacterial nutrients for lactic acid formation differ in fodder legumes, depending on the cultivation site and the time they remain on the field. For this reason, these aspects need to be analysed systematically before press juices can be used on an industrial scale, enabling optimised production schemes to be developed. To this end, we evaluated statistically which indicators in plant cultivation are most important for biomass and nutrient yields.

Fodder legumes were selected for the study due to their high protein and nitrogen content, which makes them interesting for industrial uses, especially lactic acid production. An added advantage is that legumes have a beneficial impact on cropping systems. They provide so-called agrosystem services (Wiggering et al., 2015), supporting the implementation of sustainable agricultural production (Jensen et al., 2012). The symbiosis between legumes and nodule bacteria delivers some hundred kg nitrogen per ha and year by making atmospheric nitrogen available to plants (Peoples et al., 2001; Wurth, 2006). Legumes also improve the macro-porosity and water infiltration of soils. This is particularly the case in alfalfa, which has a deep root system (McCallum et al., 2004). An increased application of fodder legumes in agricultural production systems for use in industry may therefore alleviate problems such as food insecurity, the excessive intensification of agricultural production and yield stagnation.

In this study, parts of fodder legumes were still used as feed. The biomass was partitioned into a cake (feed) and juice (industrial feedstock), based on the "Green Biorefinery" concept (Andersen and Kiel, 2000). This partition increases the efficiency of exploiting raw materials. Since we sought a more systemic approach, rather than focusing on one single production goal, we followed Kromus et al. (2004) and decided against seeking to achieve the highest juice yield in our study. Instead, we aimed to achieve a greater production of wet cake for animal nutrition. Taking this into account, points of criticism on the use of agricultural feedstock in non-food areas, as voiced in the food or fuel debate, have to be relativised.

Consequently, the main objective of this paper is to provide a systematic analysis of the impact of the site and cutting date on the quality of legumes as feedstock for lactic acid production or other industrial uses. Thus, biomass and bacterial nutrient yields were measured, enabling advice to be given concerning cultivation sites and cutting dates.

4.2 Site situation and methods

In order to substantiate the approach adopted, field trials were established as case studies at two different sites in Brandenburg (Germany) (Papendiek and Venus, 2014). To this end, alfalfa *(Medicago sativa)* was cultivated on arable land at field stations of the Leibniz-Centre for Agricultural Landscape Research (ZALF) in Muencheberg (coordinates: 52.516045, 14.124929) and Paulinenaue (coordinates 52.683381, 12.685897). In addition to planting alfalfa on arable land, a clover/grass mixture *(Lolio-Cynosuretum)* was cultivated on permanent grassland at Paulinenaue. There were different site prerequisites regarding soil conditions, cultivation and fertiliser input at the study sites (Table 1) and specific soil properties (Table 2). Both sites have a humid continental climate, characterised by low precipitation, cold winter and warm summer periods (Figure 1).

Table 1 - *Information on soil conditions, cultivation and fertiliser input at the study sites*

	Müncheberg (alfalfa)	Paulinenaue (alfalfa)	Paulinenaue (clover/grass)
Tillage	Ploughed in summer 2011	Ploughed in summer 2011	Reseeding in summer 2011
Variety /seeding year	Planet/2011	Plato/2011	Country Öko 2201 (70% perennial ryegrass, 30% white clover)/2011
Seeding rate	20 kg/ha	15 kg/ha	20 kg/ha
Soil type	Slightly loamy sands	Sand humus gleys	Sand humus gleys
Soil quality or grassland index	25	27	35
Fertilisers	2 t/ha lime fertiliser in autumn 2011, 2 t/ha lime fertiliser and 0.6 t/ha basic fertiliser (60 kg/ha P, 150 kg/ha K, 12 kg/ha Mg, 30 kg/ha S) in spring 2012 80 kg/ha nitrogen (230 l/ha NTS 27/3 nitrogen fertiliser solution with sulphur)	25 kg/ha phosphate 160 kg/ha potassium	25 kg/ha phosphate 160 kg/ha potassium

Table 2 – *Soil properties at the study sites*

	Muencheberg	Paulinenaue
C_{total} %	0.5	35.5
P_{DL} mg/l	11.0	3.7
K_{DL} mg/l	10.0	13.3
Mg_{CaCl2} mg/l	5.0	n.d.
Ca_{total}	n.d.	2911.0

n.d. not detected

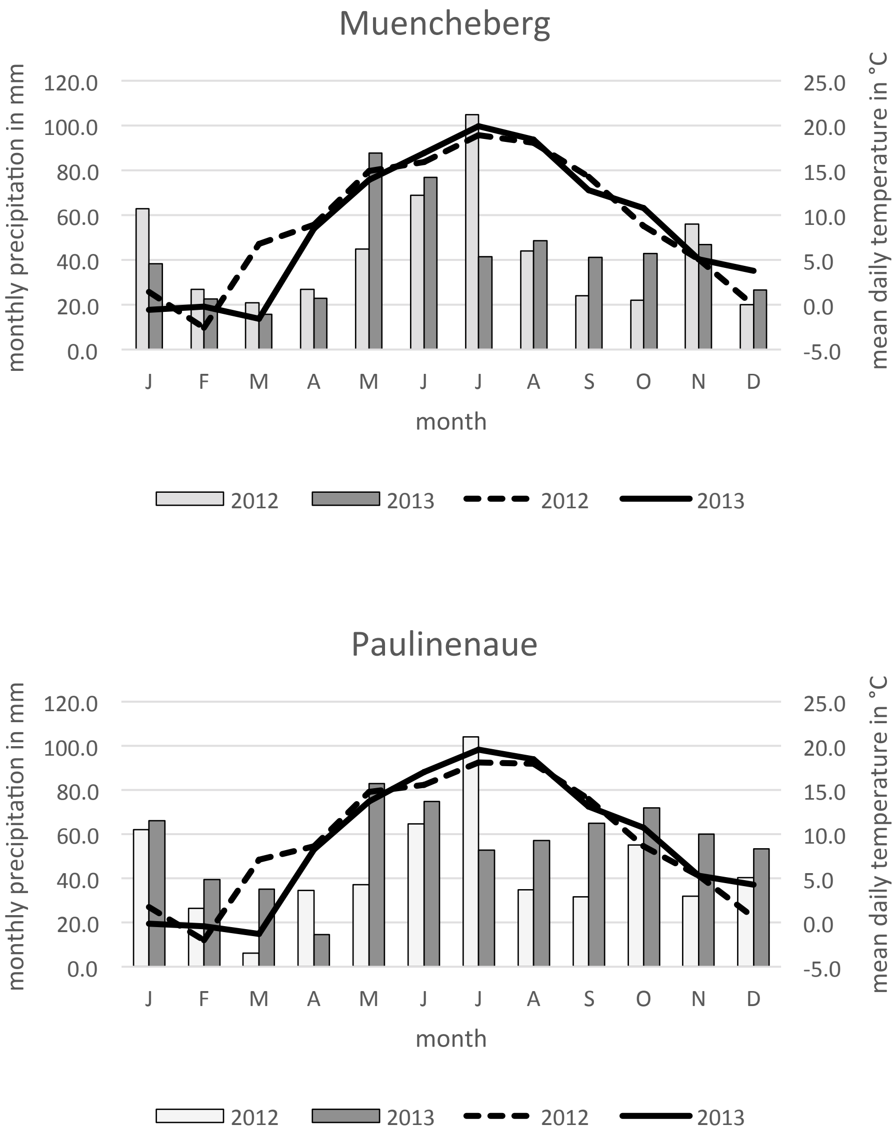

Figure 1 – *Weather data at Muencheberg and Paulinenaue field station*

4.2.1 Site situation

The soil in Muencheberg is characterised as Cambic Arenosol, referring to a slightly sandy soil, with deep-seated ground water and sparse stratum water. The topsoil has a low usable field capacity of about 12 % by volume. Due to a latent water deficit, the site is characterised by yield insecurity. At Paulinenaue, the study site is located on a Eutric Histosol, a lime fen with a generally good supply of water, owing to subsurface groundwater.

4.2.2 Harvest and pretreatment of yield

The field experiments were conducted in summer 2011; they were designed fully randomised with four replications. The gross size of each plot was 15.0 m x 15.0 m. An area of 22.50 m² (15.00 m x 1.50 m) was harvested from each plot to determine the biomass yield. The field experiment included three alternative cutting dates for each of the three obligatory cuts (Table 3).

Table 3 – *Sampling dates in 2012/2013*

	Early	Middle	Late
1st cut	22 May	4 June/3 June	11 June
2nd cut	10 July	23 July/22 July	31 July
3rd cut	28 Aug/29 Aug	10 Sept/9 Sept	18 September

The remaining biomass from each plot was chaffed at the study site and then taken to the next available screw press (Cv VETTER Maschinenfabrik GmbH & Co.KG, Kassel/Germany) at Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB) (Papendiek and Venus, 2014). Samples of the fresh biomass, press juice and cake were taken for analytical purposes.

4.2.3 Analytical determination of press juices

After pressing, a sample of each press juice was conserved in a freezer at -20°C in order to determine the dry matter (DM) content, and to measure organic acid and sugar concentrations (Papendiek and Venus, 2014). Nitrogen values were multiplied by a factor of 6.25 in order to determine the content of raw protein (Papendiek and Venus, 2014).

4.2.4 Batch fermentation

Batch cultivations were carried out in several lab-scale (3 litre) stirred tank reactors (BIOSTAT® B/MD, B. Braun Biotech International GmbH/Germany and BIOSTAT®B plus, Sartorius Stedim/Germany, each equipped with a digital control unit DCU) under temperature and pH value control. In order to test the green material as a bacteria nutrient supplement rather than as any other expensive nitrogen sources, a mixture of glucose stock solution and different press juices was inoculated with a pre-selected strain of *Bacillus coagulans*. Aliquots of the fermentation liquid were taken repeatedly to measure the concentration of lactate and sugars, which were determined after biomass separation and dilution by HPLC.

4.2.5 Analysis of press cakes

Samples of the fresh press cake were adducted for silage production on 18 September 2013 in order to obtain information about the ensiling capability and feed quality. Twelve samples were taken from each study site and poured into 1.5 litre preserving jars. These jars were compacted to a pore volume of 4 litres per kg and airtight sealed. Six samples from each study site were opened after five days to determine the dry matter content, pH value and sugar (glucose, fructose, sucrose) content. The remaining jars were opened after 49 days to analyse the dry matter content, pH value, fermentation acids, alcohols and diols. Additional feed quality analyses were conducted by the Bavarian State Research Institute for Agriculture. Frozen press cake samples and silage samples were analysed using the near-infrared (NIR) method; fermentability indicators were determined using wet chemical methods (Hönig, 2014).

4.2.6 Statistical analysis

R software, version 3.0.2, was used for the statistical analysis (R Development Core Team). Variance analysis was performed using the ANOVA tool in order to determine which site factor, or combination of site factors, significantly influences yields at a level of significance of 95 %. In addition, a two-sided Welsh t-test was applied to identify statistical correlation between single site factors and measured biomass as well as bacterial nutrient yields.

4.3 Results and discussion

Alfalfa is one of the most widely grown legumes in the North German Lowlands. It has adapted to the comparatively low levels of precipitation in this area (Figure 1). Deep ingrained roots enable alfalfa plants to survive dry periods. Nevertheless, a wide range of yield is commonplace, dependent on the prevailing soil conditions (Chmelíková et al., 2013). Sustained dry periods reduce biomass yields, especially on light sandy soils with a low groundwater table, where sandy and gravelly substrate dominates in deep soil layers. As a result, alfalfa yields can vary tremendously on the small-scale heterogenic soils in Northern Germany. Statistical analysis reveals significantly higher dry matter yields at Paulinenaue field station than in Muencheberg (p-value = 0.02513). However, the cultivation of alfalfa exhibited suitable biomass yields in 2012 for both sites Muencheberg and Paulinenaue (Figure 2) (Hochberg, 2011; Kreil et al., 1983; Papendiek and Venus, 2014).

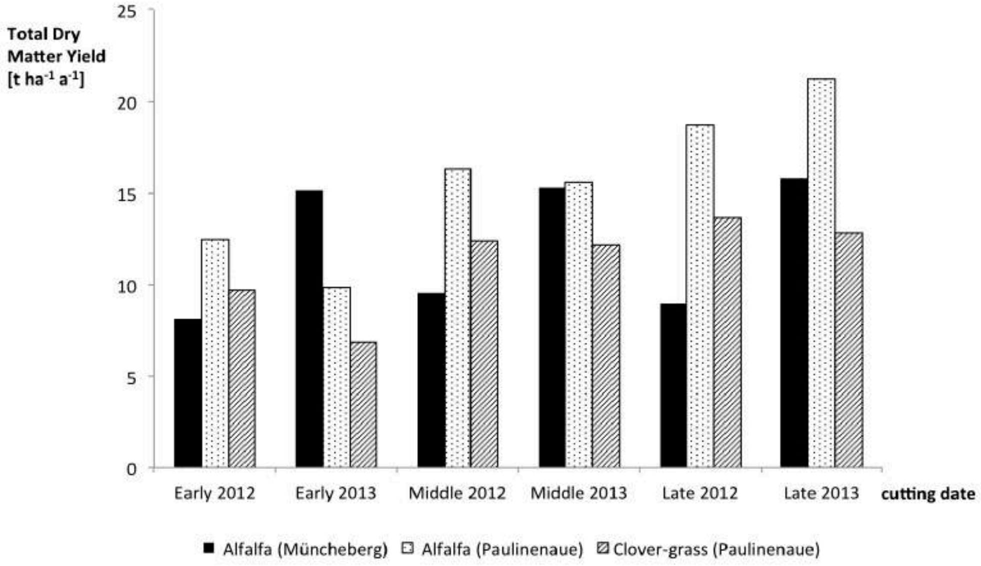

Figure 2 –*Aggregated dry matter yields in t/ha at the different sites and on the various cutting dates*

Alfalfa yields at Paulinenaue field station were very high and constant in 2012 and 2013. Dry matter yields from Muencheberg field station were exceptionally high in 2013 (p-value = 0.00161), with each cut yielding around twice the quantity yielded in 2012. The reason for this is that alfalfa was established at the site in the second season. This demonstrated that perennial cultivation is clearly beneficial, and the full effect of lime fertilisation (Table 1) was unfolded, increasing the pH value of the soil. It must be said, however, that the weather conditions were conducive to this development (Figure 1). Although the growing period started one month later, reducing yields from the early and middle first cut, especially at Paulinenaue field station (Figure 3), higher precipitation and mean daily temperatures later on in the 2013 growing season led to high yields for the second and third cut on all three cutting dates. As such, the cutting date (early, middle, late) played a significant role in 2013. Although this correlation cannot be exploited in the evaluation of cutting dates, one of the findings gained is that if the harvest time is adapted to weather conditions, a sufficient quantity of biomass can be generated. The temporary drought in July 2013 (Figure 1) had no negative impact on yields, although first drought stress characteristics were discernible on the leaves.

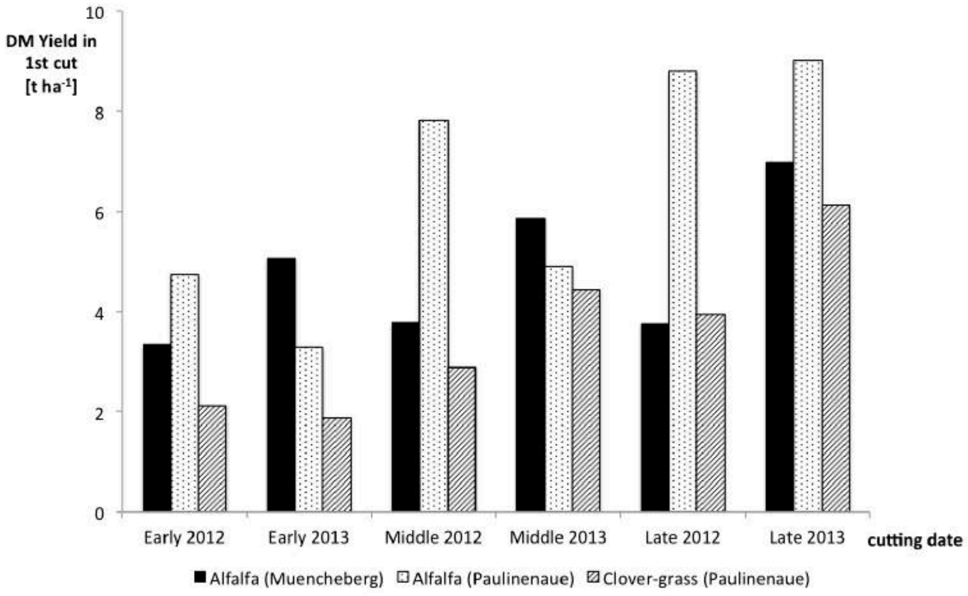

Figure 3 - *Dry matter yields in t/ha at the different sites and on the various cutting dates within the first cut*

It is also very common to grow a ryegrass/white clover mixture on North German Lowlands featuring permanent grassland (Hochberg, 2011). This mixture was also applied at Paulinenaue field station. Grass/clover mixtures are used to reduce the risk of total yield failure and to generally increase yields (Lütke Entrup, 2011). Since our field trial was situated on permanent grassland, the plants were already established. In addition, water supplies were ensured irrespective of weather conditions, due to a high groundwater level. Dry matter yields for the clover/grass mixture varied considerably over the year (Papendiek and Venus, 2014). In 2012, 9 to 13 t ha^{-1} a^{-1} was harvested compared to 9 -12 t ha^{-1} a^{-1} reported by Hochberg (2011) for a *Lolio Cynosuretum* mixture (2011). The lowest biomass yields were harvested during the early first cut in both years (Figure 3). This date was too early for the grassland site in this lowland field. The highest yield in one cut (6 t ha^{-1}) was generated in the late first cut in 2013. Altogether, the site produced average to high biomass yields. However, alfalfa produced significantly higher dry matter yields under the same environmental and cultivation conditions (p-value = 0.003454).

Fresh matter yields for clover/grass varied considerably corresponding to dry matter yields. For alfalfa, the year of cultivation was significant (p-value = 0.0003653) due to delayed fertiliser effects and the different weather conditions. At Muencheberg field station, a heavy rain event on 3 June 2013, just before harvest, increased the fresh biomass yield for alfalfa enormously. The yield was approximately ten t ha^{-1} higher than for the early and late cut at that site. The astonishing water infiltration capacity of alfalfa benefited from this heavy rain event (Figure 4).

Figure 4 – *Heavy rain event on an alfalfa site and a directly adjacent field*

We harvested alfalfa using our normal harvesting machines without compacting the soil; on the bordering fallow land, puddles appeared, making it difficult for us to enter the field.

In addition to conducting investigations into biomass yield, we statistically analysed which site factors may have a significantly relevant effect on the quantities of bacterial nutrients in press juices. The site (p-value = 0.001335) had a significant influence on the raw protein content in press juice. Alfalfa at Muencheberg field station produced the highest raw protein content (up to 26 g l^{-1}); at Paulinenaue field station, the maximum yields for alfalfa and clover/grass were 19 g l^{-1} and 16 g l^{-1}, respectively. The yield differences in crops were insignificant. As the so-called ‘queen of fodder plants’, alfalfa contains very large quantities of protein (Linnemann and Dijkstra, 2002); the clover/grass mixture can also produce similar quantities (Elgersma et al., 2014). The crop was also relevant for lactate formation (p-value = 0.0008796), particularly with higher yields for alfalfa, especially in 2013 (p-value = 0.02053). In this case, the site had no influence on yield. Hence, the smaller raw protein quantities produced at Paulinenaue field station are sufficient for lactate formation; by comparison, the very low content (7 g l^{-1} on average) for clover/grass in 2013 is too low. Although green juices differ in terms of their nitrogen and protein concentration, no strong correlations with lactic acid formation are documented in the literature. Only a few C/N ratios have been reported by (Maas et al., 2008; Sun et al., 2012), in particular for *Rhizopus oryzae*. In general, the addition of nutrients and higher nutrient concentrations have a positive effect on lactic acid production (Hofvendahl and Hahn-Hagerdal, 2000). However, yeast extract exceeding 20 g l^{-1} generated no significant increase in growth (Nancib et al., 2001) and too much nitrogen, as ammonia could be toxic. Thus, the type of nitrogen in the form of amino acids, peptides, proteins, and so on, is the critical parameter that influences the lactic acid performance of individual strains. For water-soluble sugars (WSC), only the crop was correlated to yield (p-value = 0.01392). The median for alfalfa was 20 g l^{-1} in press juice and only 12 g l^{-1} for the clover mixture, probably due to the grass content. The cutting time had no significant influence on the quantity and impact of these bacterial nutrients for lactic acid formation. The year of cultivation was not relevant either. This implies that press juices from the entire vegetation period can be used in the biorefinery process. Hence, the potential harvest window for obtaining sufficient quantities of nutrients for the industrial use of biomass is very wide. Weather conditions seem to be buffered. According to Jeroch et al. (2008), this correlates with findings generated from feed science, where legumes were found to be plants that retain a high level of feed quality on the field (Jeroch et al., 2008). In sum, the concentration of proteins and soluble sugars is higher in alfalfa. Since alfalfa also delivers higher and more constant biomass yields, this crop should be preferred over the clover/grass mixture as feedstock for lactic acid production. However, it also makes sense to cultivate clover/grass mixtures on permanent grassland if arable land is required for food production or if grassland would otherwise become fallow. Grassland in Germany is at risk of being abandoned. New use options, such as cultivating legume biomass for lactic acid production, may therefore be welcome (DAFA, 2015).

This study demonstrates that the impact of site and crop on biomass and bacterial nutrient yields is more significant than the variation in cutting dates for each cut. It can only be concluded that the early variation cannot be recommended because biomass yields are low, especially for the first cut, and the concentration of bacterial nutrients is no higher than in the other variations. As a rule of thumb, soils with a higher soil value produce higher biomass yields. However, even sites with a low soil value, such as Muencheberg field station, generate valuable biomass yields when fertilised properly. The composition of bacterial nutrients was even more valuable at this site. These poorer soils may benefit most from cultivating fodder legumes perennially, since fertility is increased due to the formation of organic matter and soil loosening (Kautz et al., 2010).

The results of this study can also be used to assess the feasibility of fodder legumes as feedstock for other industrial uses apart from lactic acid production (Pleissner and Venus, 2014). In general, green press juices contain a variety of nitrogen compounds and inorganic salts, and can act as a substitute for synthetic compounds in existing industrial processes. The microbial production of polyhydroxyalkanoates, for example, may be even more efficient if press juices are used as the nutrient source (Davis et al., 2013; Koller et al., 2005). Originally, the green press juice was a residue from fodder pellet production. Since it contains valuable substances, however, utilisation approaches have been developed, such as a feed concentrate suitable for non-ruminants, developed by Thomsen et al. (2004).

This study is also based on the assumption that fodder legumes, used as feed, can become an additional use option as industrial feedstock. To achieve this, the yield of both cake and juice needs to be adequate for exploitation. In order to measure raw material exploitation, we carefully examined the quantities of juice and cake generated in the pressing process. To achieve this, the gap width of the press was changed on 10 July 2013. Using a 3 cm gap, we obtained an average of around 40 % juice and 60 % cake from the inserted biomass (Venus, 2006). A gap width of 3.5 cm compared to 3 cm decreased juice yields by about 10 % for both alfalfa and clover/grass. No further reduction of the gap was possible because the pressure increased to such an extent that the engine overheated. A larger gap reduced the juice yield considerably because no pressure built up. Screw presses that are perfectly adapted to the feedstock and the throughput volume could probably increase the juice yield further. In this study, however, our aim was to keep the press cake wet for use as feed. Analysis of the press cake revealed that this feedstock can be used for ruminant feed production. We obtained good results after ensiling the press cake (Hönig, 2014). Feeding experiments were conducted, but a literature review showed that our press cakes could be a good alternative feed for dry cows, which have a low energy demand (Hönig, 2014). Hence, the production of a press cake and juice enables raw materials to be exploited more efficiently. Last but not least, these products may represent an additional source of income for farmers.

4.4 Conclusions

The study shows that the cultivation site has a high impact on biomass and nutrient yields. The point of harvest has less impact than we thought, which means that the potential harvest window is very wide. This is a clear benefit for industrial uses because it makes it easier to provide high-quality press juices. Both press juice and press cake are valuable feedstock, hence the Green Biorefinery approach clearly improves the efficiency of biomass use.

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5. Assessing the economic profitability of fodder legume production for Green Biorefineries – a cost-benefit analysis to evaluate farmers' profitability

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Abstract

Fodder legumes play a major role in developing sustainable agricultural production systems and contain a range of compounds, which can be utilized to produce a wide spectrum of materials currently manufactured from petroleum-based sources. Hence, if associated with Green Biorefinery technology, the use of fodder legumes brings about significant advantages in terms of overall environmental sustainability. Since fodder legume production in Europe is currently very low, the objective of this study is to assess if a new value chain generated by Green Biorefineries can make fodder legume production profitable for farmers, and therewith increase cultivation numbers. We conducted a financial cost-benefit analysis of producing biomass from agricultural land in the federal state of Brandenburg (Germany) in three different production scenarios at two farm size levels. Costs, benefits, expected profits and risks between the scenarios were quantified. Fodder legume production for traditional fodder production was already able to increase the internal rate of return, while the production of feedstocks for Green Biorefineries, depending on prices paid for the legume juice showed an even higher profit potential. Therefore, in future agricultural production systems, fodder legumes should be part of crop rotations again.

5.1 Introduction

A growing demand for agricultural sustainability and, more broadly, environmental sustainability, has brought to the attention of researchers and policy makers the need to reconsider farming production systems. In this regard, legumes and specifically fodder legumes play a major role in contributing to the development of sustainable agricultural production systems by i.e. accumulating nitrogen in the soil. Moreover, if associated with Green Biorefineries,[I] the use of fodder legumes brings about other significant advantages in terms of overall environmental sustainability as Green Biorefineries, like any biorefinery create a wide range of substitutes for fossil-based products, generating marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat) from biomass (de Jong et al., 2009).

In spite of all these desirable features, fodder legume production in Europe is currently very low, being the outcome of a secular decline (Stoddard, 2013). The reasons for the sharp decrease in its production over the last 100 years are to be partly found in the low cost of mineral nitrogen fertilizers and in the substitution of domestic protein feed with imported protein soy (Stoddard, 2013). Even though prices for mineral fertilizers are increasing (Jenkinson, 2001), making fodder legume production a viable economic option, these remain under-represented in the farmers' choices. This phenomenon calls for further understanding, having the objective of assessing if a new value chain generated by Green Biorefineries can make fodder legume production more profitable for farmers, and therewith increase sustainability in agricultural systems.

The aim of this paper is twofold, namely: (i) to quantify how profitable fodder legume production is, compared to more common market crop systems in a sustainable agricultural production system; and (ii) to assess the impact of Green Biorefineries on this profitability. To this aim, we shall present and compare the following three scenarios, which involve crop rotations with: (a) only market crop production, (b) legumes for fodder production and (c) legumes as Green Biorefinery feedstock.

The remainder of the paper is structured as follows: in section 2 a brief overview on fodder legumes and Green Biorefineries is provided, while the experiment design and a detailed description of the three proposed scenarios are depicted in section 3 as well as the method

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[I] In Green Biorefineries, wet, 'green biomass', such as fodder legumes or grass, is used as feedstock. The biomass is processed into press cakes and juice, which can be then utilized for a wide range of applications (de Jong et al., 2009; Kamm et al 2010).

used in the cost benefit analysis. Results of the CBA are illustrated in section 4 and section 5 gives some conclusions on these results. In section 6 the results are discusses in order to support farmers in the decision making process.

5.2 Motivation: Fodder Legumes and Green Biorefineries as means for economic profits and environmental sustainability

The focus of our study is on fodder legumes; we shall now briefly describe the impact this feedstock has on environmental sustainability, and how such impact could be magnified when associated with Green Biorefineries.

First and foremost, fodder legumes have a potential to mitigate the adverse effects of agricultural production on the environment through:

(i) their positive impact on soil structure and composition, i.e. improving water storage capacity and increasing organic matter content (Kahnt, 2008; Kautz et al., 2010);

(ii) their unique ability to fix atmospheric N2 and therefore to have no requirement for N-fertilizers (National Research Council, 2002);

(iii) their diversifying effect in cereal-rich cropping systems reducing the requirement for pesticides (LEGATO, 2014);

Indeed, agricultural systems using nitrogen from legumes are potentially more sustainable than others when ecological integrity, food security and fossil energy input are compared (Crews and Peoples, 2004; Pimentel et al., 2005). Moreover, a growing number of authors argue that legume production could increase farmers' profits by increasing income stability and reducing production costs because of lower pesticide demand (for instance (Malézieux et al., 2009; Peeters et al., 2006).

Along with these benefits, grain crop yields and grain quality are improved by the preceding legume crop (Gooding et al., 2007; Grzebisz et al., 2001; Hejcman et al., 2012) with yield benefits of 10% to 20% for the succeeding crop (Freyer, 2003; Kirkegaard et al., 2008).

Combining fodder legumes production with Green Biorefineries might yield additional benefits. The arising press cake can be used to produce, for example, solid fuels (Thomsen et al., 2004), fibrous composite materials (Biowert Industrie GmbH, 2013) or feed (Bryant et al., 1983; Lu et al., 1979). The press juice, on the other hand, is a valuable fermentation medium for the biochemicals industry (Andersen and Kiel, 2000; Kamm et al., 2010). Fermentation experiments showed that press juices from fodder legumes are a very good substitute for synthetic compounds in existing processes like the polyhydroxyalkanoates production (Davis et al., 2013; Koller et al., 2005).

More in general, the Green Biorefinery technology matches future developments in non-food industries that will undoubtedly lead to an increase in the amount of renewable raw materials required as feedstock. The reasons for this expected development are that fossil resources are limited and that there is a shift in consumer demand towards eco-friendlier, more sustainably-produced products (European Technology Platform for Sustainable Chemistry (SusChem), 2005). As a viable example of such trend we can refer to lactic acid (2-hydroxypropionic acid), a promising platform chemical that can be produced from a carbon source (i.e. cereals) by using press juices as fermentation medium. Food, cosmetic, pharmaceutical and biochemical industries use lactic acid in many applications (Castillo Martinez et al., 2013). Furthermore, lactic acid is applied in the production of poly (lactic acid) (PLA), which is a bioplastic that has the potential to substitute ample amounts of petroleum-based plastics in the future (Jim Jem et al., 2010; Madhavan Nampoothiri et al., 2010). There are moves afoot within the European Union to drastically reduce plastic bag utilization (Council of the European Union, 2014) and bioplastic is an alternative especially for lightweight plastic carrier bags that are endangering the environment. Already today, bioplastics play an important role in the field of packaging, agriculture, gastronomy and automotive (European Bioplastics, 2012). In 2013, the demand for lactic acid was estimated at 714,000 t and it is expected to further increase at an annual rate of 15.5% between 2014 and 2020, mainly as a result of the growing demand for bioplastics (Abdel-Rahman et al., 2013; SpecialChem, 2014).

Pooling these together, fodder legumes do not bring about only improvements in terms of environmental sustainability, but might rather generate significant profit increase in the farm sector. In what follows, the hypothesis of profit increase will be tested through a cost-benefit-analysis (CBA) mainly based on field data collected in the Federal State of Brandenburg (Germany). As a matter of fact, in 2013, fodder legumes represented only 2.9% of the arable land in the Federal State of Brandenburg (State Statistical Institute Berlin-Brandenburg, 2014), a figure highly comparable with that of Germany which is now equal to 2.3% (DESTATIS, 2014), marking a sharp drop from the 1955 level of 9.7% (Bauer et al., 1956). Many countries in Europe are facing a similar strong decline in cultivation numbers (e.g. Poland and Denmark), even though the positive effects on agricultural production systems are known (Stoddard, 2013).

5.3 Methods

5.3.1 Case study and scenario definition

In order to enhance understanding of the contribution of fodder legumes to the development of sustainable farming production systems, we based our cost-benefit analysis on data gathered from experimental sites situated in Germany.

Field trials were conducted in two different sites in north Brandenburg (Germany) (Papendiek and Venus, 2014). We cultivated alfalfa (*Medicago sativa)* on one hectare of arable land at Leibniz Centre for Agricultural Landscape Research (ZALF) Muencheberg field station (coordinates: 52.516045, 14.124929) and at Paulinenaue field station (coordinates 52.683381, 12.685897). The sites are typical for glacial shaped landscapes and have continental-influenced humid climate. They are characterized by respectively low precipitation, cold winter and warm summer periods. The field experiment was established in summer 2011. The biomass was harvested, as it is typical in this region, three times per year. The biomass was chaffed at the study sites, and then transported to the Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), where a wet fractionation took place. This is a process where fresh plant material with a low dry matter content is mechanically forced, in this case pressed, to produce a juice fraction and a solid residue (Venus, 2006). The fresh biomass was pressed using a screw press Cv (VETTER Maschinenfabrik GmbH & Co.KG, Kassel/Germany). Samples of the fresh biomass, press cakes and press juice were analyzed. Dry matter, raw protein as well as sugar content were determined. The press juice was processed as fermentation medium in a batch fermentation together with a glucose solution to produce lactic acid (see chapter 4.3) (Papendiek and Venus, 2014). The press cake was silaged and nutrition content as well as feed value were identified (Hönig, 2014). The performance of the different biomass batches in the pressing process was analyzed to estimate the average juice and cake yields (Papendiek et al., 2015).

As outlined in the Introduction, we performed a CBA based on the gathered data comparing three scenarios, which where replicated for a typical small farm size in Brandenburg (set equal to 210 ha) and a medium farm size (set equal to 420 ha). 88% of agricultural area in the Federal State of Brandenburg is farmed at farm sizes from 200 ha to more than 1000 ha (Amt für Statistik Berlin-Brandenburg, 2014). This proportion is typical for states of former Eastern Germany (Statistisches Landesamt Sachsen-Anhalt, 2014). In other European Countries the field sizes set in our study are also typical, as in Denmark, France or Spain or count already to the big farm sizes, i.e. in Poland or Greece (Eurostat Statistics Explained, 2014). Since farm sizes of more than 600 ha are rare in Europe, we focussed in our model on small and medium sizes. The field size cycling the two developed crop rotations was set at 10 ha to keep the risk of erosion low. The farm-field-distance was constant and equal to 10 km for all three scenarios. Base investments for cultivation and processing, like rental fee and acquisition costs for machines, were not taken into account for all three scenarios, except for the additional investment for the presses in scenario (c). The presses were bought not hired because price variations for hiring in different regions are big. The purchase of presses can only cause an underestimation of economic profitability in scenario (c) and is therefore accepted in the context of our research question.

Sustainability of the agricultural production system was set as fixed condition in our model so that tight crop rotations and big erosion causing field sizes are not appearing. This is crucial because the approach of the study is to demonstrate if under environmental sound conditions legume production is economically profitable. We also determine that pressing of the green biomass in scenario (c) is always carried out on-farm so that farmers profit from the additional working step and more added value stays in the rural area.

a) State-of-the-art scenario without fodder production

This scenario describes a today's typical crop rotation on arable land. The farm produces market crops and there is no livestock. The rotation consists of 3 crops, namely: winter barley (WB), winter rape (WRA) and winter rye (WR). Both corn and straw of cereals are marketed. Rape straw stays on the field for carbon return, while 70% of rye and 80% of barley straw are taken out of the system (Tab. A1). Fertilizers and pesticides are applied according to the specific demand of each crop (Tab. A2).[II]

b) State-of-the-art-scenario with fodder production

The state-of-the-art scenario with fodder production describes a crop rotation with market crop and feed production. It is a 7-year rotation that consists of winter rye (WR), alfalfa (AL) cultivated for 3 years, winter barley (WB), winter rye (WR) and, finally, winter rape (WRA). As in the first scenario, cereals, cereal straw and rapeseed are marketed. Rape straw is left on the field, while 60% to 75% of cereal straws are taken out of the system (Tab. A1).

[II] Specific costs for the state of the art scenario without fodder production are given in Table A3.

Alfalfa as perennial fodder legume is cultivated as feed for cattle on the farm hence it is not marketed. Alfalfa is harvested three times a year with a dry matter content of about 30%. Directly after harvest the biomass is chaffed and compacted in a concrete silo for silage production. Pesticides are not used in alfalfa cultivation. Nitrogen fertilizers are only needed in small quantities in the initial phase of the perennial crop (Tab. A2). The effect of soil quality improvement caused by legumes is measured in the CBA having in mind the beneficial effect on the succeeding crop. The succeeding winter barley does not need any additional nitrogen input, but still achieves corn yields which are 13% higher. The positive impacts on the whole crop rotation (such as higher yields and lower pesticide demand) cannot be taken into account because of the lack of reliable data necessary to be included in the cost benefit analysis (CBA). Therefore, all crops except of the barley directly succeeding alfalfa are assessed in the CBA as they are in the first scenario.[III]

c) Green Biorefinery scenario

In the Green Biorefinery scenario the crop rotation is the same as in scenario (b). Cultivation and harvest of all crops are also identical. Cereals, cereal straw and rapeseed are marketed. However, in the Green Biorefinery scenario fodder legumes are processed differently. The biomass is not silaged directly after harvest but pressed in a screw press to divide it into press cake and juice. Juice yield is 40% of the fresh biomass yield. The juice is sold to lactic acid producing plants as fermentation medium.[IV] The remaining press cake is silaged and finally used as feed for cattle, preferably dry cows.

Due to the scarcity of reliable information regarding the juice price, we consider this variable as 'uncertain' in our cost-benefit analysis model. More specifically, juice price is estimated under the assumption that the obtained press juice fully substitutes semisynthetic fermentation media used in biochemical industries (Papendiek and Venus, 2014). Green juice contains proteins, free amino acids and inorganic salts which are essential for microbial growth and can therefore be used as fermentation medium for lactic acid bacteria instead of the standard medium MRS (according to De Man, Rogosa and Sharpe) (De Man et al., 1960). The water content and natural variations in the biomass have no major influence on the quality of bacterial nutrients for lactic acid formation (Papendiek et al., 2015). The price of the MRS bouillon that is substituted is around 5000 € t^{-1} (AppliChem GmbH, 2014). The focus of this study is on the costs for a farm, taking

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[III] Specific costs for the state-of-the-art scenario with fodder production are shown in Table A4.

[IV] Specific costs for the Green Biorefinery scenario are shown in Table A5.

into consideration the cultivation, harvest and first processing (pressing) of the biomass to produce a juice utilizable as MRS substitute. For the screw press, additional investment costs have to be taken into account. A screw press from VETTER Maschinenfabrik GmbH & Co.KG, with a throughput of 5 t/h, costs 200.000 €. Five of those presses are needed to press the biomass of a small/medium farm (pressing, on average, the biomass of a 10ha plot within a working day). Depreciation of screw presses was calculated using the straight-line method[V] with an assumed useful life which covers the whole project lifetime (21 years) and no salvage value for the machinery at the end of the project lifespan.[VI] Also the maintenance of the machines has to be considered, with an annual average cost per press of 12.500 € for new sieves and 25.000 € every third year for a new screw. The press is delivered with a hopper and a wheel loader to fill the hopper with the chaffed biomass material is available on the farm for silage production. (Anderson et al., 2013)

For an estimation of the costs in the succeeding engineering process, we refer to Thomsen (2005b) who was doing a cost estimation of the equipment needed for the provision of press juices. These calculations go far beyond the on-farm processing since they include costs like fermentation, stabilization, long-term storage and transportation. The value is used to estimate the costs for the engineering part of MRS substitute production. Thomsen states these costs to be 17 € t^{-1} for a green pellet factory to sell it to a processing plant (2005a). We have to assume higher costs for our scenario because in the harvest season the fresh press juices (40 to 70 m^3) must be picked up daily. We determine the processing plant and not the farmer bears these costs. Accordingly and since the price for the substitute should be much lower to be an attractive alternative, the maximum price is assessed to be not more than 50% of the MRS medium and therefore is set at 2500 € t^{-1}, the most likely price at 1000 € t^{-1} and the minimum price at 500 € t^{-1}.

[V] The annual cost of screw presses was calculated using the following equation for the straight-line depreciation method plus the opportunity cost of capital represented by the average value times the interest rate: $((Total\ Investment - Salvage\ Value))\ /\ (Useful\ Life) + ((Total\ Investment + Salvage\ Value) \times (Interest\ Rate))\ /\ 2]$.

[VI] No salvage value is a common assumption in this type of studies (e.g. Anderson et al. 2013), especially when the project life is above 15 years it is assumed that dismantling costs would cover the residual value of the machinery. Moreover, it corresponds to the 'worst scenario' in terms of costs. Hence, if the Green Biorefinery scenario will prove to be profitable under such assumption it will be even more so under less stringent costs assumptions.

5.3.2 Cost-Benefit Analysis

In order to conduct the cost-benefit analysis of biomass production for the three different scenarios described in section 3, a cost-benefit decision model was built using Microsoft Office Excel 2013. The model includes a systematic categorization of the costs and benefits associated with different crop production systems on a typical soil type in Brandenburg. The project lifetime is 21 years – corresponding to seven rotation cycles for scenario (a) and 3 rotation cycles for scenarios (b) and (c).

More specifically, the following categories of costs have been considered: total land preparation costs (€/ha), total growing costs (€/ha), total harvest, transport & processing costs (€/ha), total production costs (€/ha). Benefits, on the other hand, have been calculated based on the prices paid after harvest.

As mentioned in section 3, analyses were carried out for farm sizes of 210 ha and 420 ha; this allowed us to compare the three scenarios both for small and medium sized farms and to assess at what farm size investments for the Green Biorefinery scenario pay off. For all scenarios it was assumed that for each new crop within the rotation the working steps were: ploughing, rolling and ciscel ploughing. The constant working steps for all scenarios, their costs, timing and needed machinery are outlined in Table A6. We integrated in the CBA labour, machinery and diesel as costs associated with all working steps. The appropriate figures were obtained from a tool used in Germany by farmers to calculate their costs for the upcoming year (KTBL - Board of Trustees for Technique and Engineering in Agriculture, 2014). Figure A 1 shows how the KTBL database is set up. The selected machinery for the specific working steps is given in Table A3 to A5.[VII] Diesel costs were calculated by multiplying diesel demand (given in the KTBL database) with the current diesel costs. Analogous the labour costs are the coefficient from labour demand and average wages of 9.50 € h^{-1}. In addition to labour, machinery and diesel costs, for every crop, seeds as well as fertilizers and pesticides costs were integrated in our cost-benefit model. Costs for these specific working steps of each scenario were cumulated for all crops over the study lifetime of 21 years (Tab. A3 to A5).

[VII] The used machinery was recommended by the KTBL tool for the specific working steps. For an economically sound CBA it was necessary to keep the number of different machines used in the scenarios to a minimum to ensure optimal use of machine capacity. However, due to fixed machinery combinations given in the KTBL tool, this was not always possible.

Benefits were calculated from prices paid for cereals and rape after harvest (Bauernzeitung for Brandenburg Mecklenburg-Western Pomerania and Saxony-Anhalt, 2014b). Due to their volatility over time, prices were kept constant – over the lifetime of the simulation – at their 2013 values. Cereal and rape yields were taken from the Landesbauernverband of Brandenburg, an association of farmers in the Federal State, for the current harvest year of 2013 (LBV Brandenburg e.V., 2014). For alfalfa, the yields were available from the field trials. However, prices were not directly accessible for legumes. They are in general used as feed on the producing farm and have therefore no market price. We assumed from literature that legume silage can partly substitute maize silage (Bulang et al., 2006). Prices for maize silage were available (Bauernzeitung for Brandenburg Mecklenburg-Western Pomerania and Saxony-Anhalt, 2014a) so the ratio between the NEL (net energy content for lactation) amount in maize (Engling et al., 2009) and alfalfa silage was calculated. Therewith a corrected price was available to determine the costs saved for maize silage purchase.

In the case of the third scenario, since there is no reliable market yet for press juice and cake from alfalfa, prices were estimated based on available data (see sections 3.1 and 4.3). Moreover, in order to account for the significant fluctuations possible in the case of juice price, we associated a triangular distribution based on the minimum price, the most likely price and the maximum one (we shall come back to this in section 4.3).

In order to evaluate the financial performance of the three considered scenarios, we took into account two indicators (Karellas et al., 2010), namely:

(i) net present value (NPV) – i.e., the difference between the present value of the future after-tax cash flows from an investment and the amount of the initial investment. Present value of the expected cash flows is computed by discounting them at the required rate of return.

(ii) the internal rate of return (IRR) – i.e., the average annual return earned through the life of an investment calculated as the discount rate that reduces to zero the after-tax net present value.

These two indicators provide different information on the overall profitability of the investment and might be traded off one against the other. For instance, an investor/farmer might prefer a project that has a low IRR and a high NPV over a project with a very high IRR and low NPV. At the same time, an investor might be concerned about a large project that has a high value of NPV but an IRR just above the cost of capital.

We took into consideration a discount rate of 5% and VAT equals to 10.7 % (steuerberaten.de Steuerberatungsgesellschaft mbH, 2014). Choosing the right discount rate is not a trivial task. In fact, the discount rate is effectively a desired return, or the return that an investor would expect to receive on some other typical investment projects of equal risk. The discount rate typically includes: (1) the *rate of time preference* (most people prefer consumption undertaken now rather than later; thus, a euro available now is more highly valued than one received later); (2) *uncertainty and risk* associated with the project (there is necessarily some degree of uncertainty and risk as to whether a future euro will actually be received; therefore its value is lessened in proportion to the expected size of this uncertainty/risk factor).

There is no single rate of return that is appropriate for every project. Many economists use discount rates in the range of 8% - 12%. However, the higher the discount rate is, the lower is the value associated with the future (and future generations). Moreover, many national public institutions have recently lowered the interest rate associated with social cost-benefit analysis. This revision is mainly justified in light of the big changes in macroeconomic conditions, including the low interest rates, the deflation threatening the Eurozone, and the need of a more significant long-term planning in public projects appraisal (Cruz Rambaud and Munoz Torrecillas, 2005). In light of these changes, we decided to set the discount rate at 5%.

As for the VAT level, this corresponds to the rate applied under the farmer's flat rate scheme to suppliers of typical agricultural goods and services, as well as to specific suppliers by sawmills.

5.4 Results

This study aims at quantifying and comparing costs, benefits, expected profits and risks among the three scenarios depicted in section 3. Thus, Tables A3 to A5 show the costs in the different scenarios for the needed working steps. At first sight, we can notice that the crop rotation in the two scenarios with legume cultivation led to a reduction in the use of fertilizers (29%) and pesticides (45%) with respect to the market crop rotation in the first scenario. However, costs for harvest and conservation (i.e. silage production) in the two legume scenarios are more than twice that of the market crop scenario. We shall now look in more detail at the key results of the CBA for the three considered scenarios at different farm size levels.

5.4.1 State-of-the-art scenario without fodder production

The cost-benefit analysis shows that farm size influences neither the cost-benefit ratio nor the internal rate of return, with CBR equal to 0.79 and IRR being equal to 26% in both cases (Tab. 1). This finding suggests the absence of economies of scale and shows an overall profitability of the investment.

Table 1 – *Cost-Benefit Analysis of the state-of-the-art scenario without fodder production*

	Farm size – 210 ha	Farm size – 420 ha
Costs (€)	1075634.81	2151269.63
Benefits (€)	1353294.62	2706589.24
Net benefits (€)	277659.81	555319.61
Net present value (€)	137183.42	274366.84
Cost-benefit ratio	0.79	0.79
Internal rate of return	26%	26%

Assuming a 5% discount rate and 10.7 % taxes

If we look at costs and benefits associated with individual crops, we can get a more fine-grain picture. In this market crop rotation, rape is the most profitable crop. Benefits are more than 25% higher than for rye even though only the seed and not the rape straw is marketed (Fig. 1, 2 and Tab. A7). Straw recovery from cereals however is profitable with about 100 € profit per ha for the farmer. The highest fertilizer and pesticide costs in the crop rotation of rape are compensated by comparably low seed prices and a high market price for rapeseed. Rye shows a profit loss per ha of 18% compared to barley and 38% compared to rape. In Brandenburg, however, rye is a very important crop with stable yields even in years with low precipitation and production costs are the lowest of the whole crop rotation.

5.4.2 State-of-the-art scenario with fodder production

Findings for the second scenario show an overall increased profitability of the investment, which is displayed with an IRR of 41% compared to 26% in scenario (a) and a CBR dropping from 0.79 to 0.72 (see Tab. 1 and 2).

The increased IRR is likely to be linked to the fact that farmers can save costs for maize silage purchase for cattle when legumes are cultivated. If the alfalfa silage was sold and not used on the farm, the benefits would be lower because transport costs need to be taken into account. Also in this case findings suggest the absence of economies of scale.

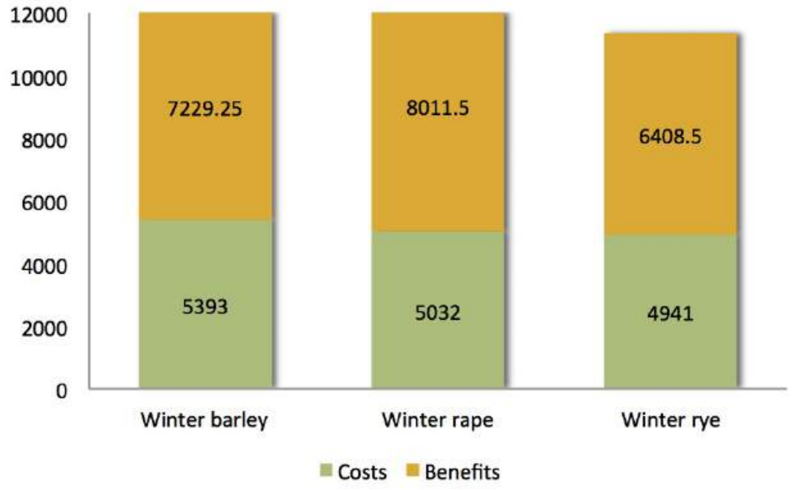
Table 2 – *Cost-Benefit Analysis of the state-of-the-art scenario with fodder production*

	Farm size – 210 ha	Farm size – 420 ha
Costs (€)	488682.68	977365.36
Benefits (€)	718029.60	1436059.20
Net benefits (€)	229346.92	458693.84
Net present value (€)	124992.04	249984.09
Cost-benefit ratio	0.72	0.72
Internal rate of return	41%	41%

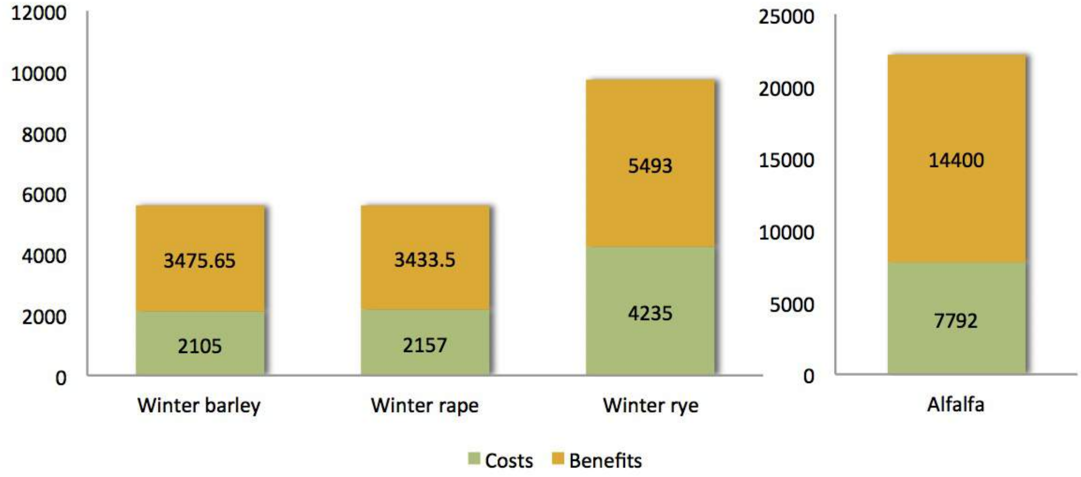
Assuming a 5% discount rate and 10.7 % taxes

Again, we can get more insights by looking at costs and benefits associated with individual crops (Fig. 1, 2 and Tab. A7). Rape and rye display costs and benefits which are identical to those observed in the first scenario. Barley after alfalfa becomes more profitable since fertilizer costs are saved and a yield increase of 13% is estimated due to the preceding crop effect of alfalfa. In this scenario farmers profits from barley exceed the profits from rape per hectare by 45% and are nearly 60% higher than in the first scenario. Alfalfa cultivated for 3 years has similar preparation and growth costs like the other crops in one year. However, harvest, transport and processing costs are much higher even for one year of alfalfa production because harvest takes place three times a year. The price for alfalfa deduced from the current maize silage price is 28% lower than the maize silage price. Still, annual profits for the farmer are higher than for the other crops in the rotation. This finding suggests that introducing the cultivation of legumes makes this second scenario more profitable when compared with a rotation scheme, which considers only market crop production.

Scenario A - State-of-the-art scenario without fodder production



Scenario B - State-of-the-art scenario with fodder production



Scenario C - Green Biorefinery scenario

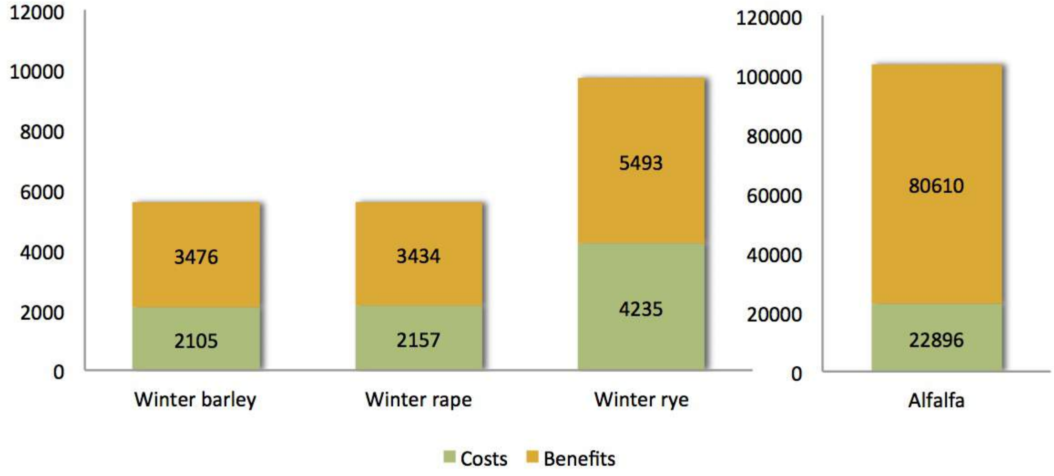


Figure 1 – *Total costs and benefits over project lifetime of 21 years for the crops*

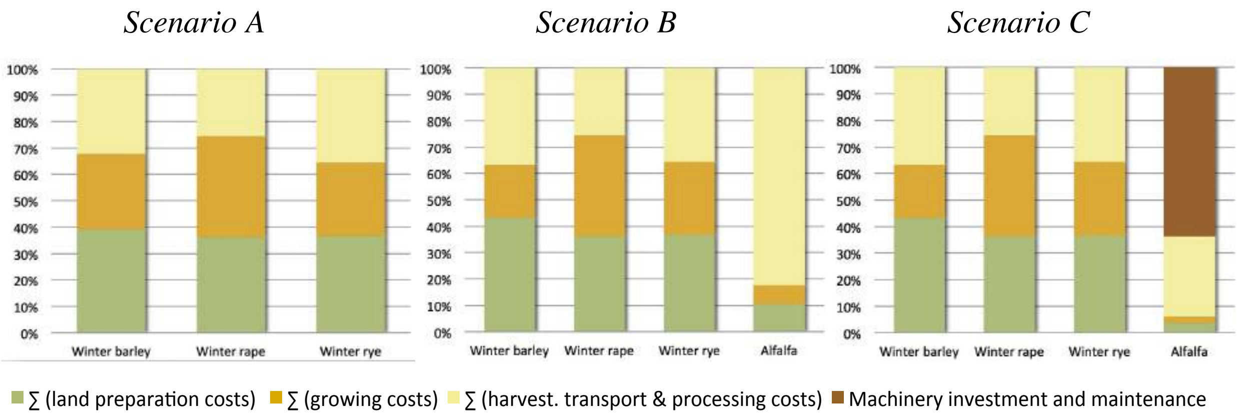


Figure 2 – *Total costs breakdown*

5.4.3 Green Biorefinery scenario

In the Green Biorefinery scenario the crop rotation is the same as in the state-of-the-art scenario with fodder production. Therefore, costs and benefits for cereals and rape are the same as in the previous scenario (Tab. 2).

The additional investments in screw presses for the production of press juice as feedstock for biochemical industries make the farm size and potential prices for press juice and cake important parameters to determine the profitability of alfalfa cultivation. As discussed in section 3, we associated a triangular distribution for the juice price with a minimum price of 500 € t^{-1}, the most likely price of 1000 € t^{-1} and a maximum price of 2500 € t^{-1} (Fig 3).

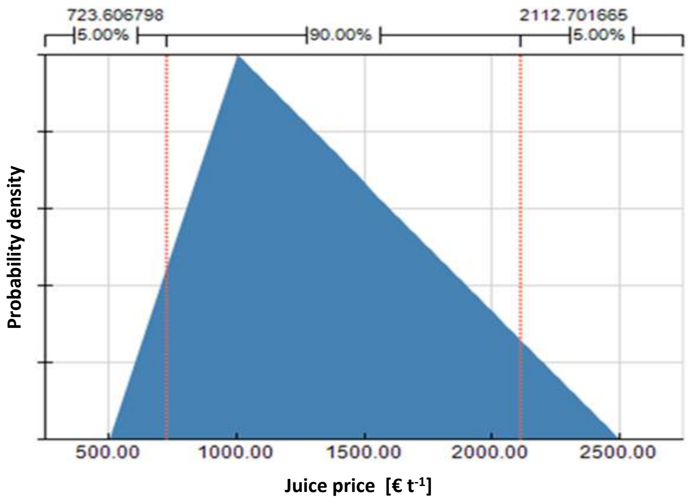


Figure 3 – *Triangular distribution of juice price*

Looking at these results, we notice that there is a probability of 5% for the juice price to belong to the interval [500.00; 723.60), a 90% probability for the juice price to be part of the interval [723.60; 2112.70), and a 5% probability to be included in the interval [2112.70; 2500.00].

Estimations of the price for press cakes are derived from analyses on the application of alfalfa press cake in ruminant feeding (Hönig, 2014). When estimating this price, we observed less fluctuations around an average price of 29 € t^{-1} (with the price ranging from 27 € t^{-1} to 32 € t^{-1}). Hence, due to the fact that press cake price span is lower, fluctuations are less of an issue than in juice price, and its influence on the overall profitability of the scenario is negligible (due to its relatively low *per-ton* price), we decided to perform the cost-benefit analysis keeping the press cake price constant at its average price of 29 € t^{-1}.

Obtained results are reported in Tables 3, A8, A9 and in Figures 4, 5 and 6. Specifically, in Table 3 we report results calculated at the centre of each price interval (minimum price, most likely price and maximum price), whereas in Tables A8 and A9 as well as in Figures 4 to 6 we show the IRR calculated for several price values of the three intervals.

Table 3 – *Cost-Benefit Analysis of the Green Biorefinery scenario*

	Farm size 210 ha			Farm size 420 ha		
	Minimum price	Most likely price	Maximum price	Minimum price	Most likely price	Maximum price
Costs (€)	5093358.22	5093358.22	5093358.22	5599216.44	5599216.44	5599216.44
Benefits (€)	3507672.30	6810879.30	11150859,3	7015344.60	13621758.60	22301718.60
Net benefits (€)	-1585685.92	1717521.08	6057501.08	1416128.15	8022542.15	16702502.15
Net present value (€)	-1304714.27	804456.76	3575630.38	589892.98	4808235.04	10350582.27
Cost-benefit ratio	1.59	0.81	0.5	0.87	0.44	0.27
Internal rate of return		15%	41%	12%	49%	87%

Assuming a 5% discount rate and 10.7 % taxes
Min price: juice = 615 €/t; Most likely price: juice= 1300€/t; Max price juice =2200 €/t whereas cake price is constant and equals 29€/t.

In the first case (Fig. 4) the juice price takes values in the interval [500; 723.6) and a small farmer (210 ha) never obtains a positive IRR. Hence, the Green Biorefinery option is never convenient for a small farmer. The IRR is even too low to be calculated in a standard formula (Fig. 4, Tab. A8). As we increase the farm size to 420 ha, we always get a positive IRR. However, the estimated IRR is always below the IRR estimated for scenario (a) and (b). We can conclude that with a low juice price, the profitability of the Green Biorefinery scenario is not given when compared to the other two scenarios. This finding applies for both analysed farm sizes; however, we should recall that the probability that the real juice price fell within the minimum price interval is just 5%.

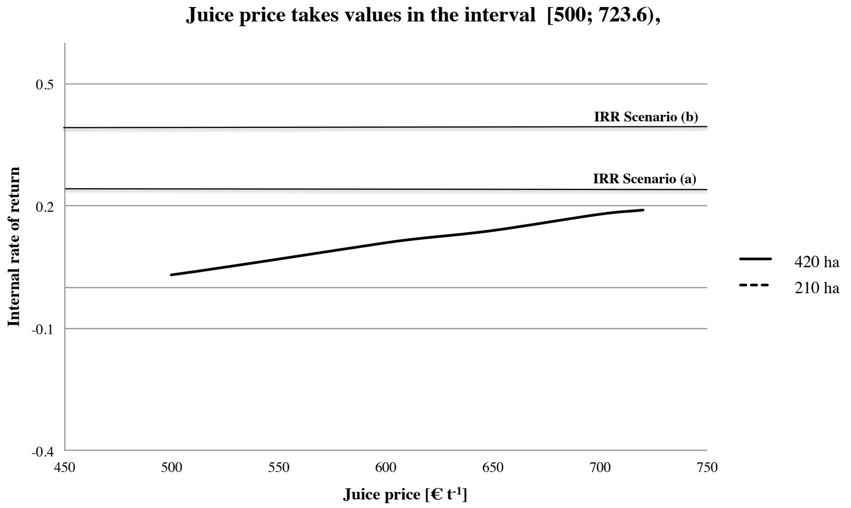


Figure 4 – *IRR for various juice prices (minimum price interval)*

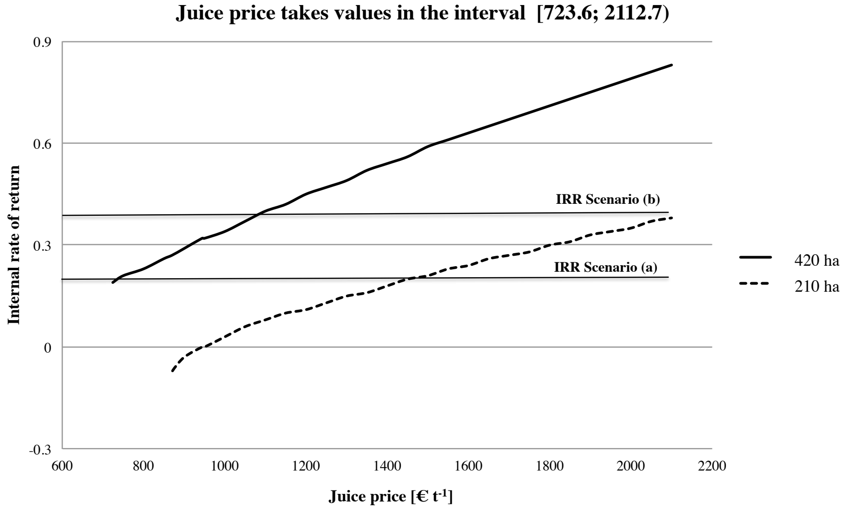


Figure 5 – *IRR for various juice prices (most likely price interval)*

The picture changes significantly if we consider the most likely price interval (Fig. 5). Specifically, for a small farm of 210 ha, any juice price higher than 944 € t^{-1} will assure the farmer a profit. On the contrary, any juice price below this threshold provides no economic incentive to the farmer to opt for the Green Biorefinery scenario. The Green Biorefinery scenario only displays an IRR above the one obtained in scenario (a) whenever the juice price exceeds 1650 € t^{-1}. Hence, the investment risk is still high. For a medium sized farm the estimated IRR is above the one obtained in scenario (a) whenever the juice price exceeds 850 € t^{-1}and exceeds the one obtained in scenario (b) any time the juice price is above 1125 € t^{-1}. As it seems, in the most likely price case, the Green Biorefinery scenario is performing relatively well and, for a medium sized farm, it is most likely to be superior to scenario (a). Also compared to scenario (b) the price span for press juices where the Green Biorefinery scenario can be more profitable is high. Recalling now that there is a probability of 90% for the juice price to belong to the interval, we can conclude that this is a rather relevant finding of our investigation.

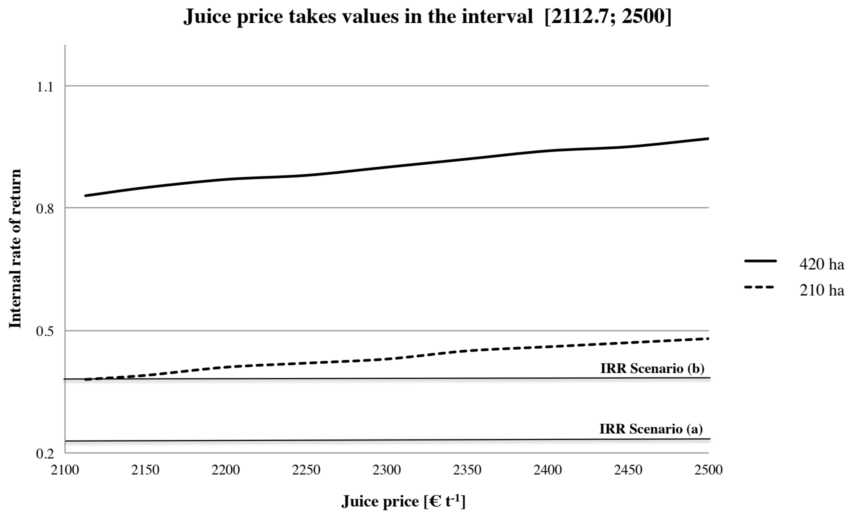


Figure 6 – *IRR for various juice prices (maximum price interval)*

We conclude our analysis looking at the third price interval (Fig. 6) – i.e., the maximum price to be included in the interval [2112.70; 2500.00]. In this case, the Green Biorefinery scenario is almost always dominating the other two scenarios, independently of the farm size. The IRR for a small farm at a juice price of 2200 € t^{-1} is the same as for scenario (b). For a small farm, the IRR ranges between 0.38 and 0.48 whereas for a medium size farm the IRR ranges between 0.83 and 0.97. These figures are indeed very high and should be interpreted with caution. In fact, when interpreting these results one should bear in mind that the probability that the juice price exceeds 2112.70 €/t are very slim. Moreover, and most importantly, we should recall that in our analysis some costs that are constant for all rotations (like the rental fee for the land or machinery that is needed in the cultivation process of all scenarios) were not included. The drawback of this approach is that the IRR in the scenarios is not comparable with reality, because of the exclusion of baseline costs. However, the IRR comparability between the three scenarios is still sound, because baseline costs in all scenarios would be the same.

5.5 Conclusions

As outlined in the introduction, the aim of this paper was to quantify how profitable fodder legume production is, compared to more common market crop systems and, subsequently, to assess the impact of Green Biorefineries on this profitability. To address these research questions we conducted a cost-benefit-analysis based on field data collected in the Federal State of Brandenburg (Germany). It should be mentioned that, although the case study refers to a very specific area, results are more generally applicable since there are strong morphologic similarities (e.g. fodder legumes share of overall arable land) among our case and Europe overall. Our study concentrated on small (210 ha) and medium farm (420 ha) sizes – big farms being explicitly excluded, as they are not that common in Europe – and compared three scenarios, which involve crop rotations with: (a) only market crop production, (b) legumes for fodder production, and (c) legumes as Green Biorefinery feedstock.

Our empirical investigation showed that including fodder legumes only for fodder production and soil improvement (i.e., scenario b) has a higher IRR than the pure market crop rotation (i.e., scenario a). This would be the case whenever a farmer produces fodder for her/his own cattle or purchases the fodder close by the farm. For higher distances, the potential profit for alfalfa silage would be lower, due to higher transportation costs.

Moreover, results reported in section 4 show how the production of fodder legumes becomes even more profitable when the Green Biorefinery scenario is considered: the additional investments associated with the Green Biorefinery scenario can pay off for a medium sized farm within three years for the most likely press juice price. However, for the minimum price at both farm sizes and the most likely price at a small farm size, the investments doesn't pay off within the project lifetime of 21 years. Indeed, a key issue associated with the robustness of our results is the lack of available data on press juice price as well as on possible significant fluctuations. To get around this problem we associated a triangular distribution based on the minimum price, the most likely price and the maximum one – this allowed us reducing the risk of misjudgement of the press juice price. According to the results of the CBA model, high profits were obtained for a farm size of 420 ha in Green Biorefinery scenario. However, also in the case of a small farm the Green Biorefinery scenario showed to be profitable, but with a high investment risk.

5.6 Discussion and limitations

We shall now discuss in this final section some potential drawbacks associated with legume production, which might hold back farmers from switching to fodder production, as well as some general limitations associated with our study. When interpreting our findings, one should be very careful and take such obstacles in due consideration.

We start considering lower yields and yield stability as well as the potential disadvantage associated with the loss of knowledge on legume cultivation among farmers. This, in turn, might increase the probability of mistakes in cultivation and therefore reduce farmers' willingness to switch to fodder production (Kuhlman and Linderhof, 2014). The main (not strictly economic) advantage of the market crop rotation (scenario a) is therefore that it is an established way of cultivation, a fact which results in high stability as well as high flexibility for farmers. On the contrary, the perenniality of fodder legumes impedes the direct annual serving of the market, which reduces profits especially in years with high cereal prices.

Farmers need to be aware of these drawbacks in legume cultivation; however, the CBA model in this study shows the potential which legume production does hold. Overall, on less fertile soils, the integration of legume cultivation seems to deliver gains, linked mainly to the soil improvement and to the increased yield potential of the succeeding crops (Adams et al., 1970). Beyond this cost-benefit type of reasoning, there are at least two, more general, arguments in favor of fodder legume production. First and foremost, as discussed in section 2, there are general environmental sustainability effects as fodder legumes have a potential to mitigate various adverse effects of agricultural production on the environment – considering that legumes are preferable when ecological integrity, food security and fossil energy input are taken into account. Moreover, fodder legumes make farmers less dependent on fertilizers and pesticides, reducing their production costs and their vulnerability to market fluctuations for production input. This latter point can become a crucial issue if prices for nitrogen fertilizer rise – a scenario which is likely to occur within the next decades due to the high energy demand in the production process (Vance, 2001).

On a more general level, a growing awareness to the intrinsic unsustainability of the current economic model has contributed to the emergence of the idea that modern society should move towards a greener society following an imminent paradigm shift from a fossil fuels economy to a biobased one. Such a major change entails a socio-technical transition, involving the co-evolution of social, economic and technological relationships (van den Bergh et al., 2011). This transition from an old and stable production paradigm to a new one is mostly characterised by uncertainty and higher levels of risks. Our study is nested within this broad framework, providing some preliminary insights into the technological and economic feasibility of Green Biorefinery for farmers, hence starting reducing the risk and uncertainty spectrum typically associated with radical changes.

As we show, the Green Biorefinery technology allows farmers to produce fodder legumes with profits. Moreover, for the press juices, results from field trials are promising and show that the quality of the output as fermentation medium is very stable over the year and comparable to MRS[VIII] (Papendiek et al., 2015). This could increase the cultivation figures linked to fodder legumes and lead to a more sustainable, less fossil-based agricultural production. Furthermore, an increase in cultivation figures for legumes will reduce the extent of the drawbacks associated with legume production: on the one hand, new breeding can increase yield and yield stability, while on the other hand the knowledge on cultivation can be acquired again.

Although promising, these results, from data collected at two study sites in the same federal state in Germany clearly need to be replicated and validated to allow drawing more general conclusions. Further crops need to be investigated so that farmers can cultivate the fodder legume most suitable for the specific soil and climate conditions.

For Green Biorefineries, there have been studies for example in Austria (Kromus et al., 2004), Denmark (Andersen and Kiel, 2000) and Ireland (O'Keeffe et al., 2009) focusing on the bioengineering processing of the biomass and potential products.

We are aware that we neglect the engineering part and that the cost-benefit analysis is only based on one part of the costs, namely the farmers site. Field data on press juice prices would make the study far more robust. However, by means of triangular distributions, we tried to account for price uncertainties in the market. The added value of this study is that farmers can estimate what price must to be paid for the press juice to be profitable for their farm features.

A further issue to be considered refers to the potential size of the market for press juices. In fact, as discussed in this paper, the on-farm produced alfalfa press juice can be used as fermentation medium in the production of lactic acid (Papendiek and Venus, 2014). However, farmers could also find other buyers for the green press juices as there are probably many more fields of application for the juice, which have not been explored yet (e.g. proteins for the production of feed for non-ruminants bought as substitute for imported soy meal. See (Thomsen et al., 2004). Indeed, the potential size of the market for press juices is only roughly estimated in this study. In particular, examples for fields of application are named and for

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[VIII] MRS is a stable, well-known fermentation medium but costs are just too high to be an economically sound feedstock for the large-scaled production of lactic acid.

lactic acid a development forecast is given. However, a detailed demand forecast, based on data from industries, is needed to verify the assumptions.

Finally, it is worth reasoning on the relevance of processing the green biomass rapidly after harvest, preventing uncontrolled fermentation processes from taking place and keeping the quality of plant metabolites high (Thomsen et al., 2004). That speaks for a decentralized processing. Regional biomass processing centres (RBPCs), in our case producing lactic acid, would allow a quick processing and low transport distances (Carolan et al., 2007). However, the final PLA production will be probably organized in large and sophisticated factories. The very first processing (pressing) of the fresh biomass should be located on the farm because of the heavy weight and large volume. The pressing reduces the juice quantities that need to be transported by more than 50% (Venus, 2006). The impact of value chains that are adapted to the specific characteristics of fresh green biomass on the economic profitability and the sustainability of resource processing still needs to be explored.

All in all, this study is only the beginning of research on this topic. First experiments have been performed to find out if press juices from fodder legumes are a proper feedstock for biochemical processes and if an economically sound processing is possible. We provide evidence that alfalfa is an interesting alternative feedstock for industrial uses. However, more research is needed (e.g. on the mixture ratio of press juices and sugar source for the most efficient exploitation of the plants) and we hope this study will stimulate and pave the way to such new investigations.

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Annex

Table A1 – *straw recovery in the scenarios*

Scenario	crop	Straw recovery (%)
State-of-the-art scenario without fodder	WB	80
Production	WRA	-
	WR	76
State-of-the-art scenario with fodder production /	WR	61
Green Biorefinery scenario	AL	-
	WB	75
	WR	76
	WRA	-

† WB, winter barley; WRA, winter rape; WR, winter rye; AL, alfalfa

Table A2 – *fertilizer and pesticide demand in the scenarios*

scenario	crop	Fertilizers (kg ha^{-1}) †	Pesticides (low intensity) ‡
State-of-the-art scenario without fodder production	WB	120 - 180 N	Fungicide, herbicide, Plant growth regulator
	WRA	190 N, 50 S	Fungicide, herbicide, insecticide
	WR	130-170 N	Fungicide, herbicide, Plant growth regulator
State-of-the-art scenario with fodder production / Green Biorefinery scenario	WR	130-170 N	Fungicide, herbicide, Plant growth regulator
	AL yr 1	80 N, 200 Mg Lime	-
	AL yr 2	200 Mg Lime	-
	AL yr 3	200 Mg Lime	-
	WB	-	Fungicide, herbicide, Plant growth regulator
	WR	130-170 N	Fungicide, herbicide, Plant growth regulator
	WRA	190 N, 50 S	Fungicide, herbicide, insecticide

† Sources: BayWa Deutschland; for alfalfa fertilizer demands were taken from the field trials in Brandenburg
‡ Source: Bavarian State Research Centre for Agriculture; for alfalfa pesticide demands were taken from the field trials in Brandenburg

Table A3 – *costs for specific working steps over project lifetime of 21 years for state-of-the-art scenario without fodder production*

Working step	Unit	Costs (€) †	occurance	Used machinery †	Notes
Seeding	ha	2941.94	Annually	Rotary harrow + seed drill 2.5m, 67kW	
Fertilizing	ha	1619.21 to 1905.76	According to demand	Front loader, 1750 daN, mineral fertilizer shovel, 75m^3, 67 kW, three-way tipper tailer, 14t, 67kW	
Pesticide use	ha	3070.27	According to demand	Mounted pesticide sprayer 18m, 1500l; 67kW	
Corn threshing (incl. transport)	ha	3499.91	Annually	Complex 2 harvester, 8500l, 200kW, cutting system, 6m, double tractor each 18t, three-way tipper tailer, 83kW	
Straw processing (incl. transport)	ha	1285.98	Annually for cereals	Round baler, 1.5m, 275 kg/ bale, 67kW, double tractor each 8t, three-way tipper tailer, front loader, , 1750 daN, bale spike, 67kW	Rape straw stays on the field

† Sources: Used machinery and costs for machinery, labour and diesel. KTBL tool; Seed and pesticide prices, Bavarian State Research Centre for Agriculture; Fertilizer prices, Bauernzeitung for Brandenburg, Mecklenburg Western Pomerania and Saxony Anhalt

Table A4 – *costs for specific working steps over project lifetime of 21 years for state-of-the-art scenario with fodder production*

Working step	Unit	Costs (€)†	Occurrence	Used machinery †	Notes
Seeding	ha	2039.70	when crop changes	Rotary harrow + seed drill 2.5m, 67kW|seed drill 3m, 67kW	
Fertilizing	ha	1260.10 to 1358.35	According to demand	Front loader, 1750 daN, mineral fertilizer shovel, 75m^3, 67 kW, three-way tipper tailer, 14t, 67kW	
Pesticide use	ha	1689.45	According to demand	Mounted pesticide sprayer 18m, 1500l; 67kW	Not necessary for alfalfa
harvest (incl. transport)	ha	7844.78	Annually. for alfalfa 3 times a year	Complex 2 harvester, 8500l, 200kW, cutting system, 6m, double tractor each 18t, three-way tipper tailer, 83kW | Rear mower, 2.1m, 45kW, retrival with self-propelled forage harvester, swath deposit, 3m, 45kW; 250kW, double tractor each 14t, three-way tipper tailer, 67kW	Corn threshing for cereals and rape | mowing. swathing and chaffing for green biomass
Straw processing (incl. transport)	ha	842.81	Annually for cereals	Round baler, 1.5m, 275 kg/ bale, 67kW, double tractor each 8t, three-way tipper tailer, front loader, 1750 daN, bale spike, 67kW	Rape straw generally stays on the field
Silage production	ha	559.43	Directly after legume harvest	Wheel loader, 13.5t, 105kW, lightweight shovel, 4m^3	Compacting in concrete silo

† Sources: Used machinery and costs for machinery, labour and diesel, KTBL tool; Seed (except alfalfa) and pesticide prices, Bavarian State Research Centre for Agriculture; Seed prices alfalfa, Deutsche Saatgutveredelung AG; Fertilizer prices, Bauernzeitung for Brandenburg, Mecklenburg Western Pomerania and Saxony Anhalt

Table A5 – *costs for specific working steps over project lifetime of 21 years for the Green Biorefinery scenario*

Working step	Unit	Costs (€)†	Occurrence	Used machinery †	Notes
Seeding	ha	2039.70	when crop changes	Rotary harrow + seed drill 2.5m, 67kW|seed drill 3m, 67kW	
Fertilizing	ha	1260.10 to 1358.35	According to demand	Front loader, 1750 daN, mineral fertilizer shovel, 75m^3, 67 kW, three-way tipper tailer, 14t, 67kW	
Pesticide use	ha	1601.10	According to demand	Mounted pesticide sprayer 18m, 1500l; 67kW	Not necessary for alfalfa
harvest (incl. transport)	ha	7844.78	Annually. for alfalfa 3 times a year	Complex 2 harvester, 8500l, 200kW, cutting system, 6m, double tractor each 18t, three-way tipper tailer, 83kW |Rear mower, 2,1m, 45kW, retrival with self-propelled forage harvester, swath deposit, 3m, 45kW; 250kW, double tractor each 14t, three-way tipper tailer, 67kW	Corn threshing for cereals and rape | mowing. swathing and chaffing for green biomass
Press juice production	ha	734.48 ‡	3 times a year	5 Screw presses, each with a throughput of 5 t/h	
Straw processing (incl. transport)	ha	842.81	Annually for cereals	Round baler, 1.5m, 275 kg/ bale, 67kW, double tractor each 8t, three-way tipper tailer, front loader, 1750 daN, bale spike, 67kW	Rape straw stays on the field
Silage production	ha	345.43	Directly after alfalfa pressing	Wheel loader, 13.5t, 105kW, lightweight shovel, 4m^3	Compacting in concrete silo

† Sources: Used machinery and costs for machinery, labour and diesel, KTBL tool; Seed (except alfalfa) and pesticide prices, Bavarian State Research Centre for Agriculture; Seed prices alfalfa, Deutsche Saatgutveredelung AG; Fertilizer prices, Bauernzeitung for Brandenburg, Mecklenburg Western Pomerania and Saxony Anhalt

‡ Source: Costs for machinery, labour and diesel, VETTER Maschinenfabrik GmbH & Co,KG, Kassel/Germany

Table A6 – *Costs for constant working steps in all scenarios*

Working step	Unit	Costs (€)†	Occurrence	Used machinery †
Ploughing	ha	83.63	when crop changes	10 shares, 3.5m, 102kW
Rolling	ha	20.36	when crop changes	10.25m, 67kW
Glean ciscel ploughing flat	ha	29.61	when crop changes	ECODYN, 3m, 67kW

† Source: KTBL tool

Table A7 – *Total costs and benefits over project lifetime of 21 years for the crops in all scenarios*

Costs (€ ha^{-1})	Winter barley	Winter rape	Winter rye	Alfalfa
Seeding	a) 1182.77 b)/c) 506.90	a) 885.76 b)/c) 379.61	a) 873.42 b)/c) 748.64	b)/c) 404.55
Ploughing	a) 585.41 b)/c) 250.89	a) 585.41 b)/c) 250.89	a) 585.41 b)/c) 501.78	b)/c) 250.89
Rolling	a) 142.52 b)/c) 61.08	a) 142.52 b)/c) 61.08	a) 142.52 b)/c) 122.16	b)/c) 61.08
Ciscel ploughing	a) 207.28 b)/c) 88.83	a) 207.28 b)/c) 88.83	a) 207.28 b)/c) 177.67	b)/c) 88.83
∑ (land preparation costs)	a) 2117.97 b)/c) 907.70	a) 1820.97 b)/c) 780.41	a) 1808.63 b)/c) 1550.25	b)/c) 805.35
Fertilizers	a) 451.55 to 623.48 b)/c) 0.00	a) 722.37 b)/c) 309.59	a) 445.28 to 559.90 b)/c) 381.67 to 479.92	b)/c) 568.84
Pesticides	a) 996.59 b)/c) 427.11	a) 1201.90 b)/c) 515.10	a) 871.78 b)/c) 747.24	b)/c) 0.00
∑ (growing costs)	a) 1448.14 to 1620.07 b)/c) 427.11	a) 1924.27 b)/c) 824.69	a) 1317.06 to 1431.68 b)/c) 1128.91 to 1227.16	b)/c) 568.84
Corn production	a) 1097.53 b)/c) 478.52	a) 1287.16 b)/c) 551.64	a) 1115.22 b)/c) 955.91	b)/c) 0.00
Straw production	a) 642.99 b)/c) 291.68	a) 0.00 b)/c) 0.00	a) 642.99 b)/c) 551.13	b)/c) 0.00
Legume production	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b)/c) 0.00	b)/c) 5858.72
Silage production	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b)/c) 0.00	b) 559.43 c) 345.43
Juice production	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b) 0.00 c) 734.48
∑ (harvest. transport & processing costs)	a) 1740.52 b)/c) 770.19	a) 1287.16 b)/c) 551.64	a) 1758.21 b)/c) 1507.04	b) 6418.15 c) 6938.63
∑ (production costs)	a) 5306.63 to 5478.57 b)/c) 2105.01	a) 5032.40 b)/c) 2156.74	a) 4883.90 to 4998.52 b)/c) 4186.20 to 4284.45	b) 7792.35 c) 8312.82
Machinery investment and Maintenance € ha^{-1}	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b)/c) 0.00	a)/b) 0.00 c),*) 14583.33 c),+) 7291.67
Benefits € ha^{-1}	a) 6776.00 to 7682.50 b)/c) 3267.30 to 3684.00	a) 7875.00 to 8148.00 b)/c) 3375.00 to 3492.00	a) 5852.00 to 6965.00 b)/c) 5016.00 to 5970.00	b) 14400.00 c) 80610.00

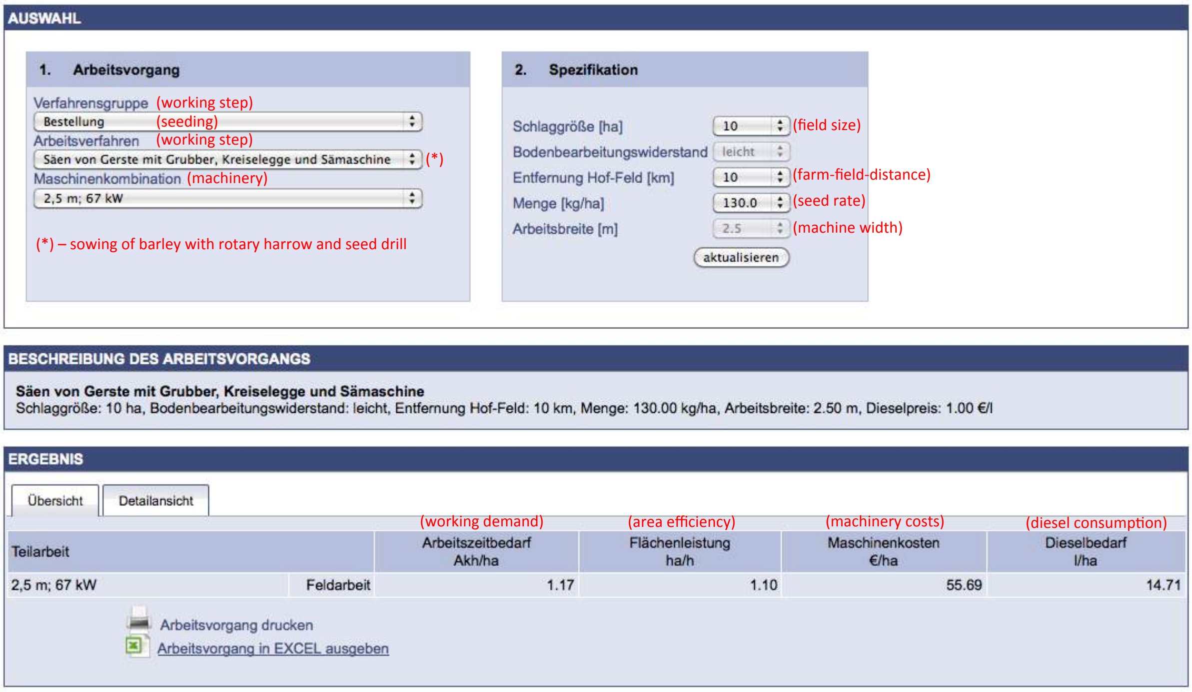
a) state-of-the-art scenario without fodder production
b) state-of-the-art scenario with fodder production
c) Green Biorefinery scenario for a juice price of 1300 € t^{-1} and cake price of 29 € t^{-1}
∗) 210 ha farm size
+) 420 ha farm size

Table A8 – *IRR for a farm size of 210 ha at various juice prices (cake price equals 29 €/t)*

Juice price € t^{-1}	**500**	720							
IRR	**n.a.**	n.a.							
Case 1: The juice price takes values in the interval [500.00; 723.60)									
Juice price € t^{-1}	**723.6**	860	865	870	900	**944**	1000	1050	1100
IRR	**n.a.**	n.a.	-0.09	-0.07	-0.03	**0.00**	0.03	0.06	0.08
Juice price € t^{-1}	1150	1200	1250	**1300**	1350	1400	1450	1500	1550
IRR	0.1	0.11	0.13	**0.15**	0.16	0.18	0.2	0.21	0.23
Juice price € t^{-1}	1600	**1650**	1700	1750	1800	1850	1900	1950	2000
IRR	0.24	**0.26**	0.27	0.28	0.3	0.31	0.33	0.34	0.35
Juice price € t^{-1}	2050	2100							
IRR	0.37	0.38							
Case 2: the juice price takes values in the interval [723.60; 2112.70)									
Juice price € t^{-1}	**2112.7**	2150	**2200**	2250	2300	2350	2400	2450	**2500**
IRR	**0.38**	0.39	**0.41**	0.42	0.43	0.45	0.46	0.47	**0.48**
Case 3: the juice price takes values in the interval [2112.70; 2500.00]									

Table A9 – *IRR for a farm size of 420 ha at various juice prices (cake price equals 29 €/t)*

Juice price € t^{-1}	**500**	550	600	**615**	650	700	720		
IRR	**0.03**	0.07	0.11	**0.12**	0.14	0.18	0.19		
Case 1: the juice price takes values in the interval [500.00; 723.6.0)									
Juice price € t^{-1}	**723.6**	750	800	850	900	950	1000	1050	1100
IRR	**0.19**	0.21	0.23	0.26	0.29	0.32	0.34	0.37	0.4
Juice price € t^{-1}	1150	1200	1250	**1300**	1350	1400	1450	1500	1550
IRR	0.42	0.45	0.47	**0.49**	0.52	0.54	0.56	0.59	0.61
Juice price € t^{-1}	1600	1650	1700	1750	1800	1850	1900	1950	2000
IRR	0.63	0.65	0.67	0.69	0.71	0.73	0.75	0.77	0.79
Juice price € t^{-1}	2050	2100							
IRR	0.81	0.83							
Case 2: the juice price takes values in the interval [723.60; 2112.70)									
Juice price € t^{-1}	**2112.7**	2150	**2200**	2250	2300	2350	2400	2450	**2500**
IRR	**0.83**	0.85	**0.87**	0.88	0.9	0.92	0.94	0.95	**0.97**
Case 3: the juice price takes values in the interval [2112.70; 2500.00]									


AUSWAHL

1. Arbeitsvorgang

Verfahrensgruppe (working step)
Bestellung (seeding)
Arbeitsverfahren (working step)
Säen von Gerste mit Grubber, Kreiselegge und Sämaschine (*)
Maschinenkombination (machinery)
2,5 m; 67 kW

(*) – sowing of barley with rotary harrow and seed drill

2. Spezifikation

Schlaggröße [ha]: 10 (field size)
Bodenbearbeitungswiderstand: leicht
Entfernung Hof-Feld [km]: 10 (farm-field-distance)
Menge [kg/ha]: 130.0 (seed rate)
Arbeitsbreite [m]: 2.5 (machine width)

BESCHREIBUNG DES ARBEITSVORGANGS

Säen von Gerste mit Grubber, Kreiselegge und Sämaschine
Schlaggröße: 10 ha, Bodenbearbeitungswiderstand: leicht, Entfernung Hof-Feld: 10 km, Menge: 130.00 kg/ha, Arbeitsbreite: 2.50 m, Dieselpreis: 1.00 €/l

ERGEBNIS

Teilarbeit		(working demand) Arbeitszeitbedarf Akh/ha	(area efficiency) Flächenleistung ha/h	(machinery costs) Maschinenkosten €/ha	(diesel consumption) Dieselbedarf l/ha
2,5 m; 67 kW	Feldarbeit	1.17	1.10	55.69	14.71

Figure A 1 – *KTBL database set up exemplary on the working step seeding*

6. General discussion and conclusions

6.1 Availability of biomasses for utilisation within Green Biorefineries

There is a great potential for the Green Biorefinery approach in Germany. More than 30% of the agricultural land in Germany is grasslands (see also Chapter 1.2.2) which are often inefficiently used agricultural production systems (DAFA, 2015; Koschuh et al., 2003). Since the proportion of natural conservation areas for grasslands is high compared to all agriculturally used land, these areas are very important for the conservation of nature (Becker et al., 2014). Therefore, the challenge for the use of grasslands is to combine provisioning services (i.e. feed) and non-provisioning services (i.e. biodiversity). Extensive utilisation is thus often necessary to retain the high natural value. Extensive in this context means that the biomass has to be grazed or harvested more or less regularly and a nutrient input either via manure from grazing or other fertilisers has to take place to avoid a complete lixiviation of soils (Becker et al., 2014; Isselstein et al., 2005). Without a nutrient input, the depletion of the species richness (which justifies the preservation of these sites in the first place) may be the result (Pechackova et al., 2010). The industrial use of biomass can promote new utilisation concepts for extensive grassland sites, balancing the maintenance of biodiversity with the efficient extensive use of these sites. One of these utilisation concepts is cutting the biomass for further processing in the Green Biorefinery process. Such cuttings need to be late for reasons of nature conservation. This thesis proves that late cuttings deliver suitable feedstocks for lactic acid production. In addition, when the biomass is harvested solely for reasons of nature conservation, fertiliser inputs are not common. Therefore, the Green Biorefinery concept could even support biodiversity on some natural conservation sites while allowing an industrial use of the harvested biomass.

However, the Green Biorefinery approach can also for intensively used sites release biomass capacities. The cascade use in this approach, separating the biomass into a cake and juice and using it not only for feed production but also for industrial products expands the utilisation options. Intensively used grasslands produce high amounts of fresh biomass with a high nutrient content. Dairy cattle require high amounts of protein and minerals, but there are still capacities to separate some of the nutrients and produce a press juice for industrial uses. The remaining press cake can be used as a valuable fodder for dairy cows during their dry period (Hönig, 2014).

6.2 Fodder legumes as valuable feedstock for Green Biorefineries

6.2.1 The feasibility aspect

Regarding the feasibility of using legumes in a Green Biorefinery approach, two aspects are of major importance – the dynamics of press juice content determining the quality of press juices for industrial uses and the dynamics of biomass quantity over the growing season.

Investigations performed in this thesis proved the suitability of alfalfa and clover/grass for the Green Biorefinery approach. Methods used to investigate this research objective are described in detail in Chapter 3, while the statistical analysis for the data from our field trials in 2012 and 2013 is given in Chapter 4. Results show that the harvest time is not of high importance for the quality of press juices as a fermentation medium. Farmers can therefore integrate both crops into their harvest schedule without overlapping with cereal harvest times. Another benefit of the broad harvest window that I discovered is that not all fodder legume fields need to be harvested at the same time, reducing the demand for industrial presses and respective labour force. As analysed in the cost-benefit analysis in Chapter 5, these factors are highly relevant for the viability of press juice production on the farm. However, biomass quantity is the limiting factor for the expanse of the harvest window. If plants are still too small or the dry matter content is already too high, juice quantities are negatively affected. Thus, harvest time has to be adapted to weather conditions and other external influences to generate sufficient quantities of biomass.

Regarding crop choice, results show that alfalfa performed better in direct comparison to clover/grass with regard to biomass quantity and quality. However, the results obtained for clover/grass also show a high potential for permanent grassland sites. Biomass quantities are still attractive for the demand in Green Biorefineries, since the quality as fermentation medium is only marginally lower. For extensive grasslands, biomass use in biochemical industries can be a chance for preservation. These areas are often endangered due to abandonment or conversion into arable land (DAFA, 2015). Late cuttings, often needed for reasons of nature conservation, are possible without a major influence on the press juice quality. Again, biomass quantities are the limiting factor for this use option. For intensive-use grasslands, the Green Biorefinery approach can further increase biomass use efficiency, producing feed and press juices for industrial uses. The restriction here is that in milk production systems using these highly productive sites, fodder with a very high energy content is needed. The press cake is a less energy-rich food, and therefore only utilisable for "dry" cows. A potential biorefinery scenario for these sites is to produce a high-energy feed from the first one or two cuttings and press the other cuttings before silaging. The economic profitability of such a production system needs to be evaluated. Such an evaluation was however beyond the scope of this thesis. The resulting higher proportion of fodder legumes in the permanent grassland site can improve the environmental sustainability, claimed for intensively used sites (DAFA, 2015).

6.2.2 The economic viability

A key task of this thesis was to find out if fodder legume production can become profitable for farmers again when a new purchaser, in the form of biochemical industries, appears. Therefore, I carried out a cost-benefit analysis, using data from field trials conducted in the course of this thesis. This thesis analyses the potential benefits – and risks – of such a new market for legumes. Such information is essential for farmers to assess whether they want to revive the production of fodder legumes on their farms. This is an important research field for biochemical industries as well, since they want to persuade farmers to supply this market.

The cost-benefit analysis described in Chapter 5 compares different production scenarios on a farm. Two standard crop rotations for Brandenburg, producing either only market crops or market crops and fodder legumes for ruminant feed production are compared to a system that uses the cultivated fodder legumes for the Green Biorefinery value chain (see Section 6.5) instead of only feed production. Two farm sizes, common for many European regions, were chosen to examine the influence of scale. The cost structure of the farms was analysed in detail to assess which farm characteristics make the production of press juices for biochemical industries viable. Results show that for large farm sizes in particular, the potential profits are high (Table 1).

Table 1 – *Internal rate of return for specific scenarios and farm sizes*

Scenario	Farm size 210 ha	Farm size 420 ha
State-of-the-art scenario without fodder production	26 %	26 %
State-of-the-art scenario with fodder production	41 % [a)] /18 % [b)]	41% [a)] /18% [b)]
Green Biorefinery scenario ★	15 % [a)] / >100 %	49 % [a)] / >100 %

[a)] alfalfa on temporary grassland sites; [b)] clover/grass on permanent grassland sites
★ for the most likely juice price of 1300 € t^{-1}

The cost-benefit analysis in Chapter 5 was carried out for alfalfa on arable land only. Results have not yet been published regarding a permanent grassland site. Compared to the second scenario in Chapter 5, using fodder legumes for feed production, the internal rate of return (IRR) for permanent grasslands is much lower, with 18% instead of 41% for temporary grassland in a crop rotation. The picture changes when looking at the third scenario, introducing Green Biorefineries. Here, profits are higher for permanent grassland sites for both sizes, since production costs are lower (Table 1). The calculated IRRs are not comparable with reality because of the exclusion of baseline costs. However, the IRR comparability between the three scenarios is still sound, because baseline costs in all scenarios would be the same.

6.3 Reliability of results and limitations

As a research topic, biorefining inspires great hopes of increasing resource efficiency and substituting major parts of fossil fuel-based industries. So far, research has taken place on the laboratory and pilot scale and is still rare. The work done for this thesis has been undertaken to fill a specific research gap in this subject area. Two crops were analysed to understand and reveal their role in a specific Green Biorefinery value chain. In Chapter 2 another potential value chain for Green Biorefineries is explained, namely protein production from legumes. I did not pursue the issue of protein production any further, because there were no accessible pilot plants to process and analyse our biomass samples under this aspect.

Giving one answer, several new research gaps have been identified. Nevertheless, in contrast to most studies on biorefineries, I analysed the sustainable provision of feedstock and not on the bioengineering processing of any feedstock available. Within this thesis, an environmental sound agricultural production system was generated. Subsequently, I analysed whether this system is technically feasible and economically viable.

The variety of investigations in the field trials was very limited in space and time. Potential technical failures and data losses were insufficiently included in the data-mining concept because of limited financial and time resources. In 2012, the first year of my field trials, I lost one sample because the forage harvester broke down. In the beginning of the field trials, I had an additional study site on a conventional farm close to Müncheberg. Unfortunately, this field was ploughed over within the first year of the field trials, due to dandelion overgrowth. The previous gathered data from this site were therefore not usable. A bigger data set, with field trials in different regions of Europe would have underpinned the findings of this thesis.

From the biomass delivered to the pilot plant, a composite sample was taken to analyse the nutrients. Accordingly, the standard deviation in the biomass was not determined, and the biomass quality of each cutting and site was assumed to be homogeneous.

With regard to press juice quantities, another limitation appeared. The experimental setup to examine press juice yields depending on the pressure in the press was only carried out once because of the high logistical expenditures required by all colleagues. Furthermore, experiments on the most efficient blend of glucose solution and press juice in the fermentation process have not been carried out. A sufficiently high quantity of press juices was used. However, investigations on that subject are essential to assess the demand for press juices in biochemical industries and to deduce from that if fodder legume cultivation could be revived on a large scale, triggering a major agricultural change.

The triangular distribution used to assess the potential price for the press juice is a very vague but common tool when data is scarce. There are currently only R&D projects working on lactic acid production from press juices, and therefore no reliable data on prices exists. The only way to generate a cost-benefit analysis was to deduce potential prices from the feedstock that is about to be substituted. This analysis, however, reveals important information for farmers, since they get an idea of what price must be paid for the press juice to be profitable based on their farm's characteristics.

6.4 Conclusions

The overall aim of this thesis was to encourage sustainability in the provision of available feedstock for biochemical industries and to increase resource efficiency. To meet these requirements, I analysed a specific value chain, attuned to sustainability issues (Figure 2).

Chapter 2 describes the idea of the thesis. The original idea to use green press juices as a fermentation medium in the lactic acid production process was retained. To complete the value chain, the usability of the solid residue from juice production as feed was analysed.

Perennially produced fodder legumes support non-commodity outputs within the perspective of environmentally sound, sustainable agricultural production systems (see Section 1.2.1). First, processing takes place on the farm to generate a high-value juice and to retain the residual press cake as feed for ruminants on the farm itself or a farm nearby.

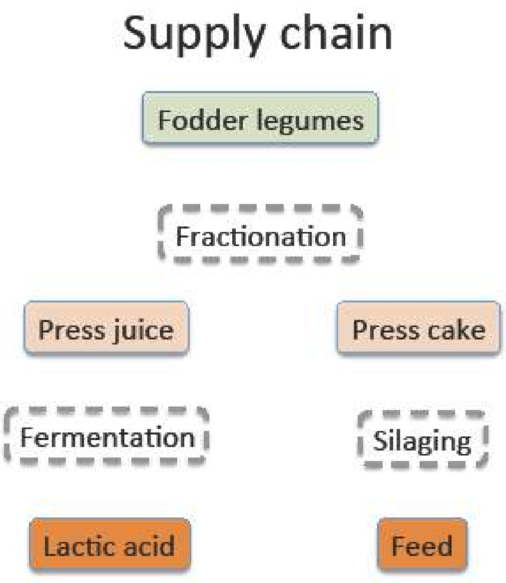


Figure 2 – *Sustainable supply chain for a Green Biorefinery*

The utilisation of fresh biomass requires prompt processing of the raw material. Therefore, decentralised approaches are needed to reduce transportation and storage costs. Regional biomass pre-processing centres (Carolan et al., 2007) may develop to keep the added value in the rural regions of biomass production. Press juices from regional farms can be processed in these regional centres to create lactic acid. The production of bioplastics would probably be centralised in a large sophisticated factory.

The accruing products can improve environmental sustainability. The derived feed can partly substitute for imported soy meal, and the lactic acid could be used for bioplastic production, an eco-friendly packaging alternative. The more sophisticated value chain helps a relevant proportion of the added value to stay in the rural area, which improves social sustainability. The economic viability of the value chain, as a crucial part of sustainability, was also explored in this thesis and the economic potential has been proved.

6.5 Outlook

Grains are the main component of lactic acid production. Therefore, to develop sustainable lactic acid production, it is necessary to search for the sustainable provision of this carbon source as well. My thesis only focuses on fodder legumes and excludes other needed feedstocks in the production process. However, the adaptation of crop rotations for the production of fodder legumes as described in Chapter 5 already increases sustainability in the cultivation process of cereals. Studies that analyse the industrially used carbon feedstock already in the cultivation process are needed to ensure sustainable chemical products and therewith to satisfy societal requirements.

Further development of the value chain to increase resource efficiency even more should be investigated. Using waste from the lactic acid production such as manure is one potential research field, as well as the use of leguminous biomass for energy after using it as feed or industrial feedstock.

Field trials for this thesis were carried out on arable land and highly productive permanent grassland sites. The late cuttings harvested in the study already had a high dry matter content and were comparable to some natural conservation sites. However, the investigation of real natural conservation sites would improve our general understanding of the suitability of different grass qualities and species compositions. Any successive research project should analyse if the biomass used for industrial purposes can promote nature conservation. Field trials with variations in fertiliser input, looking not only at biodiversity but also at the quality and quantity of biomass for industrial uses, would have to be performed.

Finally, other value chains, producing proteins, fibres or other products having a promising future, should be investigated as well.

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Declaration

I hereby declare that this Ph.D. thesis entitled „ Fodder Legumes for Green Biorefineries: A Perspective for Sustainable Agricultural Production Systems“ was carried out by me for the degree of Doctor rerum naturalium under the guidance and supervision of Prof. Dr. Hubert Wiggering, Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany.

I prepared this dissertation without illegal assistance. The work is original except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other degree.

The four main chapters (Chapters 2 to 5), which have been considered for publication in international, peer-reviewed journals, were all co-authored. I am the first author of the papers. My contributions to the chapters are highlighted in the introductory section.

Eidesstattliche Erklärung

Die geltende Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Potsdam ist mir bekannt.

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation selbständig angefertigt habe und hierbei alle verwendeten Hilfsmittel und Quellen angegeben habe.

Ich habe weder die vorliegende Arbeit noch eine in wesentlichen Teilen ähnliche Abhandlung bei einer anderen Hochschule als Dissertation eingereicht.

Potsdam, den 17.09.2015

Franka Papendiek