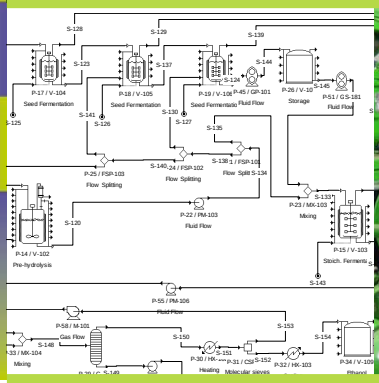
Biogas and Bioethanol Production in Organic Farming

Risø-PhD-Report



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Biogas and Bioethanol Production in Organic Farming

PhD Thesis

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Bioenergy and Biorefinery Program

Biosystems Division

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Preface

The presented PhD project was carried out at Bioenergy and Biorefinery Program, Biosystems Division, National Laboratory for Sustainable Energy – Technical University of Denmark (Risø DTU) from September 2007 to August 2010 under supervision of Head of Program Jens Ejbye Schmidt and co-supervision of Senior Scientist Anne Belinda Thomsen. The project also involves 3 months of research carried out during my external stay at Lawrence Berkeley National Laboratory, where I worked in the Joint BioEnergy Institute under supervision of Professor Harvey Blanch.

The thesis consists of two parts. First one is an introduction providing background information on organic farming, ethanol and anaerobic digestion processes, and concept of on-farm bioenergy production. Second part consists of the following papers:

Paper I: Oleskowicz-Popiel P., Thomsen M.H., Nielsen H.B., Schmidt J.E., Thomsen A.B.: Characterization of most relevant feedstock for biogas and bioethanol production in organic farming. Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, 25-28 May, 2008.

Paper II: Oleskowicz-Popiel P., Nielsen H.B., Thomsen A.B., Schmidt J.E.: Biogas and ethanol potentials in selected biomasses for organic farming. *Submitted*

Paper III: Oleskowicz-Popiel P., Schmidt J.E., Thomsen A.B.: Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomasses. *Submitted*

Paper IV: Oleskowicz-Popiel P., Lehtinen T.M., Schmidt J.E., Thomsen A.B.: Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol. *Submitted*

Paper V: Christensen A.D., Kadar Z., Oleskowicz-Popiel P., Thomsen M.H.: Production of bioethanol from organic whey using *Kluyveromyces marxianus*. Journal of Industrial Microbiology and Biotechnology, 2010, DOI 10.1007/s10295-010-0771-0.

Paper VI: Oleskowicz-Popiel P., Thomsen M.H., Thomsen A.B., Schmidt J.E.: A simulation model of combined biogas, bioethanol and protein fodder co-production in organic farming. International Journal of Chemical Reactor Engineering, 2009, vol.7, Article A71.

Paper VII: Oleskowicz-Popiel P., Schmidt J.E.: Techno-economic analysis of bioethanol and biogas production in organic farming. *Proceeding submitted to* 12th World Congress on Anaerobic Digestion, Guadalajara, Mexico, October 31st – November 4th, 2010.

Paper VIII: Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W.: Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. *Accepted*

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I would like also to thank Jens Bo Holm-Nielsen, my supervisor during my Master thesis and my previous boss at Aalborg University and University of Southern Denmark, for inspiring and introducing me to the green energy world and consequently inspiring me to start PhD studies in this area.

Finally, I would like to thank my mom and my dad for their never-ending believes in me and for support in all my decisions. I also would like thank my sister and her family for cheering as well as backing me up anytime I needed.

Last but not least, I would like to thank my girlfriend Magda for her infinite believe in me and for bringing even more happiness to my life.

Abstract

The consumer demand for environmentally friendly, chemical free and healthy products, as well as concern regarding industrial agriculture's effect on the environment has led to a significant growth of organic farming. On the other hand, organic farmers are becoming interested in direct on-farm energy production which would lead them to independency from fossil fuels and decrease the greenhouse gas emissions from the farm. In the presented work, the idea of biogas and bioenergy production at the organic farm is investigated. This thesis is devoted to evaluate such a possibility, starting from the characterization of raw materials, through optimizing new processes and solutions and finally evaluating the whole on-farm biorefinery concept with the help of a simulation software.

At first, different raw materials available at the Danish organic farm were selected and characterized for their methane and ethanol potentials, namely: fresh maize, ensiled maize, fresh rye, ensiled rye, dry rye, fresh clover, clover silage, dry clover, dried vetch, whey permeate and cattle manure. Anaerobic digestion batch experiments were carried out resulting in maize and rye silage demonstrating the highest methane potential. Additionally, continuous anaerobic digestion trials showed that methane yield in the co-digestion of cattle manure and maize silage resulted in a 36% increase of methane production with 33% from maize silage in the feedstock.

The ethanol potential of all the selected materials was estimated based on the sugar amount, resulting in the highest for dry rye and fresh maize. Furthermore, ensiling as a wet-storage and a biological pretreatment method for lignocellulosic ethanol production was investigated. Promising laboratory result were found, concluding that ensiling can maintain the "freshness" of the crop and prevent spoilage of wet-biomass while at the same time having a positive impact on ethanol fermentation process. Enzymatic convertibility tests showed that 51.5%, 36.5%, and 41.9% of the cellulose was converted by cellulytic enzymes in ensiled maize, rye, and clover grass, respectively. Noticeable amounts of ethanol were produced from only ensiled crops, the ethanol production was 33.9%, 28.5%, and 36.9% (by *K.marxianus*) and 30.6%, 28.1% and 34.5% (by *S.cerevisiae*); the yields significantly increased after hydrothermal pretreatment: 79.0%, 74.6%, and 80.2% (by *K.marxianus*) and 72.7%, 81.3% and 76.2% (by *S.cerevisiae*) of the theoretical ethanol yield based on the C6 sugar contents in untreated silage of maize, rye, and clover grass, respectively. It is concluded that ensiling has a high potential as a combined wet-storage and pretreatment method for investigated crops (maize, rye and clover). Additionally, trials with dry agricultural by-product were carried out. Ensiling of corn stover resulted in increased ethanol: 23.1 % compared to 16.4% of the theoretical in ensiled and non-ensiled corn stover, respectively.

Laboratory experiments on ethanol production from organic whey by *K.marxianus* were carried out. This process is planned to be part of the development of a concept for a decentralized biorefinery. It shows that no pasteurization or freezing of whey is necessary and it can be fermented with a high ethanol yield (~0.50 g EtOH/g lactose), and that during continuous fermentation using Ca-alginate-immobilized *K.marxianus*

high ethanol productivity is achieved: 2.5-4.5 g/L/h at dilution rate 0.2/h. This confirmed that *K.marxianus* is suitable for ethanol production from whey as a nutrientious and additional carbon source.

The final part of the work was devoted to evaluating the whole concept of the on-farm biorefinery. Within the presented work, a simulation model of on-farm bioenergy production was built. It was calculated that to supply a 100 ha organic farm with energy, 16.2 ha of rye and 14 milking cows is needed to produce ethanol from rye grains and whey. Alternatively, 5.7 ha clover grass, 2.5 ha maize and 13 cows are needed to produce the required biogas from clover silage, maize silage and cattle manure. After the further development of the simulation model, a techno-economic model was built for 5 different scenarios for bioenergy production at 1000 ha organic farm: scenarios Biogas I/II (10%/20% clover grass silage and cattle manure). Scenarios Bioethanol I/II (10%/20% rye grains and whey) and a combination of both (called: Combined). Combined scenario was characterized by the highest investment (3,330,000 USD) and the largest energy produced (29244 GJ/year). Biogas II was second best (26409 GJ/year) and it was characterized by lower investment cost (1,963,000 USD) when compared to the Biogas I which produced (19970 GJ/year) with an investment cost of (2,016,000 USD). Scenarios Bioethanol I and Bioethanol II represented the lowest investment costs (1,115,000 USD and 1,047,000 USD, respectively) and generated the least energy (4034 GJ/year and 5610 GJ/year, respectively). In all scenarios, there was enough fuel produced to supply the farm with self-produced energy.

Finally, an open access modeling tool of lignocellulosic ethanol biorefinery for broad biofuel community was built. Its purpose is to make it possible to analyze, explore and communicate the progress of biofuels production and to make it able to revise it by the academic and professional research community. Overall, it should help to bring the development of ligniocellulosic biorefineries closer to reality.

Resumé

Forbruger efterspørgsel på miljøvenlige, kemikaliefrie og sunde produkter, samt bekymring for den miljø påvirkning konventionelt landbrug medfører, har givet anledning til en betydelig vækst i økologisk landbrug. Samtidig har økologiske landmænd i højere grad fået interesse i udviklingen af energiproduktion direkte på bedriften, som kan føre til uafhængighed af fossile brændstoffer og reduktion af drivhusgasemissionen fra bedriften. I det fremlagte arbejde er idéen om biogas og bioenergi produktion i økologisk landbrug undersøgt. Med denne afhandling vurderes en sådan mulighed, startende med karakterisering af råvarer i økologisk landbrug, dernæst en optimering af nye processer og løsninger, og til sidst med en evaluering af det decentrale bioraffinaderi-koncept, ved hjælp af simulations software.

Først blev der udvalgt forskellige råvarer fra det økologiske landbrug i Danmark og disse blev karakteriseret i henhold til deres metan og ethanol potentialer. De valgte råvarerne var frisk majs, ensileret majs, frisk rug, ensileret rug, tørret rug, frisk kløver, kløver ensilage, tørret kløver, tørret vikke, valle samt kvæggylle. Batch eksperimenter af anaerob udrådning viste at majs og rug ensilage havde det største metan potentiale. Derudover viste forsøg med kontinuert anaerob udrådning, en stigning i metan udbyttet på 36% når gylle blev iblandet med 33% majsensilage.

Ethanol potentialet i alle de udvalgte råvarer blev evalueret på baggrund af sukker indhold, dette resulterede i højest potentiale for tørret rug og frisk majs. Derudover blev det undersøgt hvorvidt ensilering kan fungere som både opbevarings metode og som biologisk forbehandling til ethanol produktion fra lignocellulosisk biomasse. Resultaterne var lovende og det konkluderes at ensilering forebygger nedbrydning (forrådning) af biomassen og har samtidig en positiv indvirkning på ethanol fermentering. Enzymatisk konvertibilitet tests viste, at 51,5%, 36,5% og 41,9% af cellulose blev konverteret med cellulase enzymer i henholdsvis ensileres majs, rug og kløvergræs. Mærkbare mængder ethanol blev produceret direkte fra ensilerede afgrøder og udbyttet steg betydeligt efter hydrotermisk forbehandling. Ethanol produktion direkte efter ensilering var hhv. 33,9%, 28,5%, og 36,9% (ved *K.marxianus*) og 30,6%, 28,1% og 34,5% (ved *S.cerevisiae*) af det teoretiske udbytte baseret på C6 sukkerindhold i ensileret majs, -rug og –kløvergræs. Efter hydrotermisk forbehandling steg de udbyttet til hhv. 79,0%, 74,6% og 80,2% (ved *K.marxianus*) og 72,7%, 81,3% og 76,2% (ved *S.cerevisiae*). Det konkluderes at ensilering har et højt potentiale som kombineret opbevarings- og forbehandlings- metode for de undersøgte afgrøder (majs, rug og kløver). Desuden blev der foretaget studier på halm, som er et biprodukt i landbruget. Ensilering af majshalm resulterede i en øget ethanol production fra 16,4% til 23,1%, sammenlignet med ikke-ensileres majshalm.

Laboratorieundersøgelser af ethanol produktion fra økologisk valle med *K.marxianus* blev gennemført som en del-proces til udviklingen af et koncept for et decentralt bioraffinaderi. Forsøgene viser at ingen pasteurisering eller frysning af vallen er nødvendig før fermentering, og at det kan fermenteres med højt ethanol udbytte (~ 0,50 g EtOH/g laktose). Under kontinuert fermentering nåede udbyttet op på 2,5 -4,5 g/L/t

ved fortyndings hastighed 0,2 /time, ved hjælp af Ca-alginat-immobiliserede *K.marxianus.* Dette bekræftede, at *K.marxianus* er egnet til ethanol produktion med valle som næringsstof tilskud og ekstra kulstofkilde.

Den sidste del af arbejdet var helliget til at vurdere hele konceptet for decentralt bioraffinaderier direkte på bedriften. Dette arbejde bestod i at opbygge en simulationsmodel for forskellige scenarier ved hjælp af computer software. Herigennem blev det beregnet, at for at forsyne en 100ha økologisk gård med energi, skal der eksempelvis bruges 16.2ha rug og 14 malkekøer hvorfra der produceres ethanol fra rug kerner og valle. Alternativt kan der bruges 5.7ha kløvergræs, 2,5ha majs og 13 kreaturer hvorfra der producere biogas fra blandingen af kløverensilage, majsensilage og husdyrgødning. Efter yderligere udvikling af simuleringsmodellen, blev der opstillet en teknisk-økonomisk model som beskrev 5 forskellige scenarier for bioenergi produktionen på en 1000ha økologisk gård. De fem scenarier bestod i Biogas I og II (hhv. 10% og 20% kløvergræsensilage og husdyrgødning), Bioethanol I og II (hhv. 10% / 20% rug kerner og valle) samt kombinationen af biogas og bioethanol (kaldet: Kombineret). Kombineret scenariet gav den største investering (3.330.000 USD), men samtidig mest produceret energi (29.244 GJ/år). Biogas II var næstbedst på energi produktion (26.409 GJ/år), og gav samtidig lavere investeringsomkostninger (1.963.000 USD) i forhold til Biogas I som gav (19.970 GJ/år) for en investering på (2.016.000 USD). Scenarierne Bioethanol I og Bioethanol II gav de laveste investeringsomkostninger (hhv. 1.115.000 og 1.047.000 USD), men genererede også mindst energi (hhv. 4.034 og 5610 GJ/år). I alle scenarier blev der produceret nok brændstof til at forsyne bedrifterne med energi. Slutteligt blev der konstrueret et software baseret modelværktøj med åben adgang, til modellering af ethanol bioraffinaderier fra lignocellulosisk biomasse. Modellens formål er at gøre det muligt at analysere, udforske og kommunikere udviklingen indenfor produktion af biobrændstoffer, og dermed bidrage til at bringe udviklingen af ligniocellulosiske bioraffinaderier tættere på reel implementering i samfundet.

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1. Introduction

There is a concept that the two most important trends on which human society is/will be focused on in the near future are: “a transition toward a modern society based on sustainable resources” and “a technological revolution resulting from advances in understanding (…) living systems” (Lynd et al., 1999).

The organic movement fits well into the vision of such a modern society. Organic farming is becoming more popular and it is gaining more and more customers (Macilwain, 2004). The consumer demand for environmentally friendly, chemical free and healthy products, as well as concern regarding industrial agriculture’s effect on the environment has led to a significant growth of organic farming, especially in Europe and North America (Rigby et al., 2001; Hermansen et al., 2004).

A new concept within the organic movement is the idea of on-farm “green” energy production. Direct on-farm energy production can help to solve two major problems: firstly- the dependency on fossil fuels by the production of fuel and electricity generation from local raw materials; and secondly - reducing GHG emissions by using renewable resources. There is no doubt that global reserves of fossil fuels are depleting and that “peak oil” already has or it is going to be reached during the coming decades. Consequently, within a few decades, the world will begin to run short of its oil supply (Kerr, 2005). Additionally, according to the IPCC report from 2007 (IPCC, 2007): “green-house-gases (GHG) emission due to human activities has grown since pre-industrial times. (…) Carbon dioxide is the most important anthropogenic GHG; its annual emission grew by about 80% between 1970 and 2004”. Transport is responsible for 13.1% and energy supply for 25.9% within the total GHG emissions (data from 2004), amounting to 39% (IPCC, 2007).

In order to reduce carbon dioxide emissions, the development of a low energy input agricultural system would help (Daalgard et al., 2000). One possibility could be direct on-farm energy production at the organic farming system. Similar to ecosystems, where diversity of organisms brings stability, the energy supply should be diverse where different technologies co-exist according to surroundings (Logan, 2006). Several modern and environmentally friendly technologies could be used, such as wind, solar, geothermal. This work focuses on energy from biomass, particularly on two technologies: ethanol fermentation and anaerobic digestion. Both bioethanol and biogas could be directly produced on a farm and support organic agriculture with self-produced “green” energy. To establish on-farm energy production, identification and consequently biogas and bioethanol potential of possible raw materials available on the organic farm is necessary. Biomethane potentials studies were discussed in (Angelidaki and Sanders, 2004; Hansen et al., 2004; Angelidaki et al., 2009; Cropgen, 2010) but no raw materials originating from organic farming were identified. Ethanol, on the other hand, is produced only from sugars present in biomass; full potential is measured by total sugars determination (Foyle et al., 2007). In the case of lignocellulosic materials, a pretreatment step is needed (Schmidt and Thomsen, 1998; Yang and Wyman, 2008) prior to practical ethanol potential. Due to variety of pretreatment method, the

potential will differ depending on the applied techniques; moreover, the chosen process always depends on the type of raw material and there is no “one suit all” technology which can be applied. A pretreatment step is crucial for second generation (lignocellulosic) ethanol production (Aden and Foust, 2009) and an optimal, low-tech and low energy demanding process for small scale plant is still needed. Finally, after choosing suitable raw materials and technologies, techno-economic analysis is often a great help before establishing such an on-farm biorefinery (Wingren et al., 2003; Aden and Foust, 2009).

1.1 Outline of the thesis

The main objective of the thesis is to evaluate on-farm bioenergy production in organic agriculture. **Chapter 2** gives an overview of the organic farming agriculture movement and its main principles; furthermore presenting an idea for biomass based renewable energy production to be implemented at the organic farm. **Chapter 3** identifies available raw materials and focuses on two possible technologies: bioethanol and biogas production with new process concepts. Several raw materials available at the organic farm are evaluated for their biogas and bioethanol potentials in **Papers I** and **II**. Evaluation of an ensiling process which could be also a new, low energy demanding pretreatment method for ethanol production is investigated and described in **Papers III** and **IV**. Additionally, **Chapter 3** describes the possible integration of two processes (bioethanol and biogas) in the form of a farm-scale biorefinery. Laboratory trials on the production of bioethanol were carried out and results are presented in **Paper V**. **Chapter 4** goes more into detail of the organic farm biorefinery concept, where it is evaluated from a technological and economical point of view. The design and evaluation of the entire on-farm biorefinery was developed and it is described in **Paper VI** and the results from techno-economic analysis are shown in **Paper VII**. **Paper VIII** presents a process model for lignocellulosic ethanol biorefinery, which is an open tool for biofuel community to help the development of economical and environmentally sustainable biorefineries. At the end, the concluding remarks are drawn in **Chapter 5** and future perspective are presented in **Chapter 6**.

2. Organic farming and BioConcens concept

The definition of organic agriculture formed by the International Federation of Organic Agriculture Movements (IFOAM, 2010) says: "Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved." Following that, the four main principles of organic farming were formed, namely: the principle of health, ecology, fairness, and care (IFOAM, 2010). According to IFOAM, the principles are defined as follows:

- Principle of health – *organic agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible*; in other words, the health of individuals cannot be separated from the health of ecosystems, health is the wholeness and integrity of living systems;
- Principle of ecology – *organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them*; that principle connects organic agriculture with living ecosystems, the production should be based on ecological process and recycling; organic farming should fit the cycles and ecological balances in nature
- Principle of fairness – *organic agriculture should be build on relationships that ensure fairness with regard to the common environment and life opportunities*; it underlines that fairness should be ensured at all levels and to all parties (farmers, workers, consumers, etc.); the principle also insists on providing animals with conditions according to their physiology, natural behavior and well-being;
- Principle of care – *organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment*; increasing efficiency and productivity should not risk health or well-being, therefore new technologies must be assessed; organic farming should prevent significant risk by adopting appropriate technologies and rejecting unpredictable ones.

All of this should help to build more sustainable agriculture production. From these core concepts, a new one has grown: the modern trend in organic farming to become self-sufficient in energy supply. The European Directive does suggest lowering the environmental impact from food production, but it does not directly specify the required usage of renewable energy (EC 2007). On the other hand, sustainable energy sources (as locally produced or recycled organic materials) are of interest for organic farmers. Wood et al. (2006) indicated that the transition to organic farming could reduce greenhouse gas emission and energy use. Gundogmus (2006) compared energy use in conventional and organic farming. Using the example of apricot production in Turkey, it is showed that the total energy requirement is lower using organic farming when compared to the conventional one. This is mostly due higher energy efficiency in organic farming and no use of mineral fertilizer (it has the highest energy input use). The total energy input use was 38% lower for organic production; comprehending the lower yields in organic

farming. The benefit-cost ratios were nearly the same on both production systems (Gundogmus, 2006). Dalgaard et al. (2001) presented a model to compare fossil energy use in organic and conventional farming. In general, there is lower energy consumption in organic farming but also lower yields. Self energy production is the natural next step in development of organic farming.

BioConcens project (its full name: Biomass and bioenergy production in organic agriculture – consequences for soil fertility, environment, spread of animal parasites and socio-economy), focuses on bioenergy production form local biological resources, and at the same time analyzing the effect of bioenergy production on soil fertility, greenhouse gas emissions, survival of parasites and weed seeds, and socio-economy.

"This interdisciplinary project aims at developing new methods and processes for the co-production of bioethanol, biogas and animal feed based on resources from organic agriculture and associated food processing and suggests the outline of a medium-sized plant for co-production of biogas, bioethanol, and animal feed. The project also designs and tests a new cropping system for biomass production to be used for bioenergy, while at the same time safeguarding soil quality. The project analyzes the effects of remains from bioenergy production on soil fertility, greenhouse gas emissions, survival of parasites and weed seeds in the manure as affected by bioenergy production. Corporate and socio-economic analysis of the co-production of biogas and bioethanol at different scales is carried out" (http://www.bioconcens.elr.dk).

At the time of writing, this project is still on-going and the final results and conclusion are not yet available. Dalgaard et al. (2009) discussed synergies between the expansion of biogas production and organic farming, concluding that a 150% increase in organic farming in combination with bioenergy crop production is possible and would contribute to the vision of independency from fossil fuel in Denmark. Carter et al. (2009) measured and reported the amount of N_2O and CH_4 emissions when the residues from bioenergy production are recycled as organic fertilizer for energy maize cultivation. The effect on soil fertility when waste streams from bioethanol and biogas processes are recycled on the fields as fertilizers was studied in (Johansen et al., 2009) demonstrating almost no difference on soil quality between degassed and fresh manure. The strip intercropping (Haugaard-Nielsen et al., 2007) method was applied and studied in test fields for energy crop production to enhance soil fertility (Haugaard-Nielsen et al., 2009). Pugesgaard et al. (2010) evaluated the impact on the environment when biogas is produced at the organic farm. The initial results focusing on energy balance, nitrogen losses and greenhouse gasses emission on the organic farm with integrated bioenergy production were presented in (Pugesgaard et al., 2008). More details and the full list of publications can be read on the project's website (http://www.bioconcens.elr.dk).

The presented work in this thesis is focused on the "technological" part of the project. In the depicted concept, the bioenergy is produced from animal manure originating from dairy farms, a by-product from cheese production (agro/food industry) – whey permeate, and energy crops cultivated at the farm. The effluents from the bioenergy plant could serve either as natural fertilizer or protein feed, depending on the applied

technology. The bioenergy plant could be designed either for a single or several combined organic farms. Depending on the scale, the generated energy could supply only an organic farm or serve broader community**.** The whole scheme and the concept of the project are presented on the Figure 1.

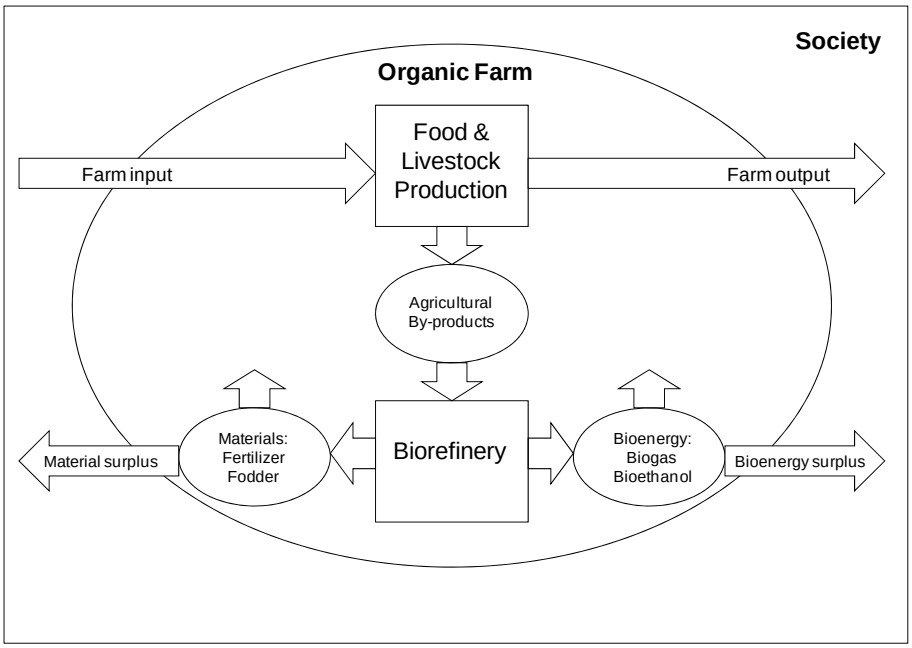

Figure 1. On-farm bioenergy production in BioConcens project

In our investigation, we used a model organic farm which is based on a statistical data on organic farms in Denmark in 2006. The organic farm consisted of: whole crop production (maize, grass/clover – silage in rotation, permanent grass), cash crops, grain production (spring barely, spring wheat, oats, winter wheat, winter rye, and triticale) and set aside and fallow land. Its distribution is presented in Figure 2. Detailed description can be found in (Pugesgaard et al., 2010).This baseline was used during the further evaluation of producing bioenergy at the organic farm.

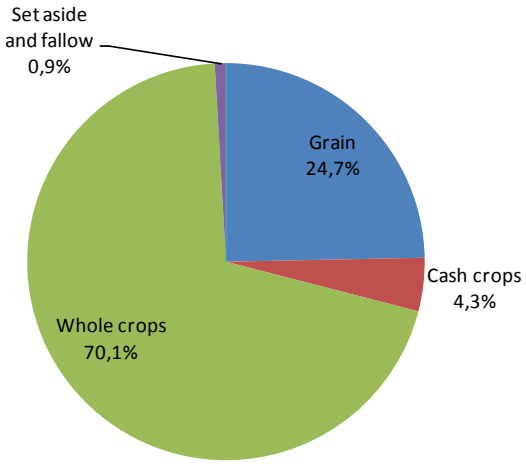

Figure 2. The crop distribution for the baseline in an exemplary organic farm in Denmark (based on Pugesgaard et al., 2010)

3. Biomass and bioenergy

Nowadays, world energy supply is dominated by fossil fuels (80% world's primary energy mix), biomass usage accounts for (11% world's primary energy mix), however part of it is utilized for simple cooking with very low efficiency. Modern bioenergy from biomass– commercial energy production for industrial purposes, power generation and transportation – is at the level of 7% (WEO, 2008). Figure 3 presents current and future energy trends and share of biomass in world energy supply.

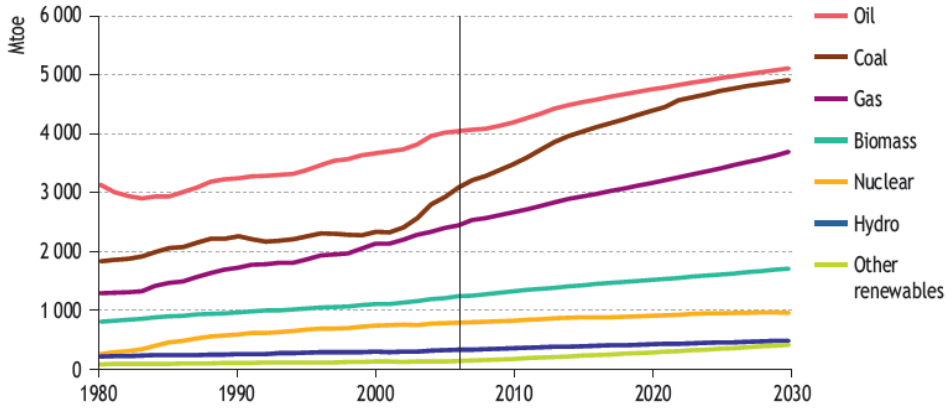

Figure 3. World primary energy demand by fuel (reference scenario) (WEO, 2008)

According to the International Energy Agency the world energy demand will expand by 45% from 2006 till 2030 and it will reach 17 Mtoe. It is estimated that fossil fuels will continue to dominate global energy use; the total energy demand for Europe in 2030 is estimated for almost 2000 Mtoe.

The largest increase in renewable energy use, in the coming years, will take place in the EU countries, driven by strong governmental support - the European Union strategy to lower the CO_2 emission, strengthen the security of energy supply and create diverse, efficient and sustainable energy mix. European Commission suggested the share of renewable energy should be in the range of 20% by 2020 and 50% and more by 2040-2050 (EC, 2006).

Diversifying energy sources would increase the security of supply. Building the new energy structure, based on different renewable resources should be the main target, where biomass, wind, solar, and hydro become an integrated part of the overall energy strategies, with an important sustainable role for bioenergy and biorefineries to play. (Biofuels Progress Report, Holm-Nielsen et al., 2007)

Development and implementation of improved growing systems for the purpose of biomass production for biorefinery utilisation will get more and more important, due to increasing demands for biofuels and a variety of biorefinery products. The commitment of making this kind of shift in using sustainable resources at much larger scale is growing already and will grow in the coming decades. Such a tendency is common all over the world: in rapid developing countries, like in China or India as well as in developed

regions. On the other hand, in many poorly developed countries in Africa and Asia, the biomass as an energy source is the only way to provide the heat and electricity to the society. The question is how will nature be influenced, and will the environment be harmed by increasing biomass production for the worldwide energy sectors. The greatest challenge will be to make the paradigm shift from fossil fuels to renewable resources in a sustainable manner (Holm-Nielsen et al., 2007). Johansson et al., (2010) raised the question whether agriculture does have a capacity to provide us with both food and fuel. Both biogas and bioethanol can be produced from residues but how much residues can be removed from fields without a risk of soil degradation. Johansson et al. (2010) claimed that with present rate of population increase, the challenge will be to assure food security. However, it was concluded that the calculated global potential of biogas could supply up to one-fourth of present motor fossil fuels. At present the global food production is sufficient to feed the world population, famine is rather a matter of its distribution, however there is a concern that this situation might change with growing world's population. There are many advantages from utilizing bioenergy, but there is also a great challenge, concern and responsibility, that cultivation of energy crops might reduce land availability for feed and food production (Holm-Nielsen et al., 2007).

It is estimated that around three-fourths of the biomass which is used for production of food, feed, industrial round wood and traditional wood fuel is lost at some point in processing, harvesting and transport (Smeets et al., 2007). Part of recovered biomass could be easily applied for bioenergy. Moreover, higher efficiency of production of food/feed, industrial round wood and traditional wood fuel means that there would be more available biomass for modern bioenergy production (Holm-Nielsen et al., 2007).

Crop residues might be significant source for bioenergy. However, removal of large quantities of residues from cropland has to be consistent with research-based guidelines in order to do it in a sustainable manner. In some cases removing any residues can cause loss of soil carbon, whereas on other soils some level of removal can be sustainable and even beneficial. Residue removal should not result in increased artificial fertilizer application, in this case the environmental and economy effects can be negative (Perlack et al., 2005).

3.1. Raw materials

Plant biomass can be considered as one of the most sustainable resource for organic fuels, chemicals, and materials. Growing plants consume CO_2 - therefore biomass-based products can be included in photosynthesis carbon cycle reaching almost CO_2-neutral lifecycle (Figure 4). Moreover, the biological processes are mostly carried out in aqueous environment and the effluents are non-toxic to the environment and easy to discharge. In some cases, the effluents can be even valuable by-products (Demirbas, 2006).

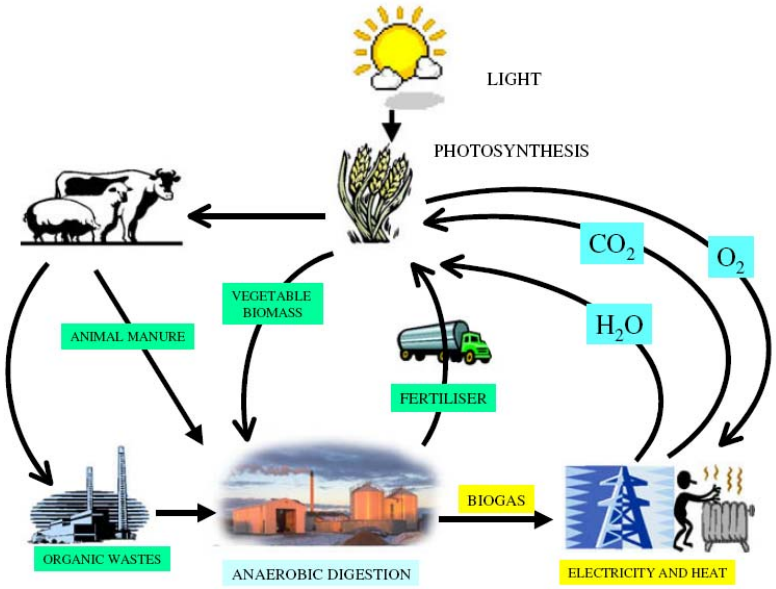

Figure 4. Schematic representation of the sustainable cycle of bioenergy (anaerobic co-digestion of animal manure and energy crops) (Al Seadi, 2002; Holm-Nielsen et al., 2009)

Biomass resources occur in variety of ways, such as woody and herbaceous species, wood wastes, bagasse, agriculture and industrial residues, waste paper, municipal solid wastes, sawdust, biosolids, grass, waste from food processing, animal wastes, aquatic plants and algae, and so on (Demirbas, 2008). Different global energy scenarios indicate that biomass could supply up to 30% of the energy needs by 2100 (Hamelinck and Faaij, 2006).

Through photosynthesis process, plants convert carbon dioxide and water to metabolite chemicals. Primary metabolites are carbohydrates (simple sugars, cellulose, hemicelluloses, starch etc.) and lignin – all together called lignocelluloses. Cellulose and hemicelluloses are two principle polymers and from those ethanol can be produced. Cellulose is β (1,4) linked polymer of glucose, it has high degree of polymerization and cristallinity. Hemicellulose is highly branched polymer built up from hexoses and pentoses (Carpita and Gibeaut, 1993). Lignin, the third component, is a complex hydrophobic cross-linked aromatic polymer which serves as “glue” for lignocellulosic structure. Secondary metabolites are mainly gums, resins, rubber, waxes terpenes, tepenoids, steroids, plant acids etc (Clark, 2007; Naik et al., 2010).

Energy carries from biomass can be produced in a variety of ways including liquid fuels such as ethanol, methanol, biodiesel, Fisher-Tropsch diesel, and gaseous fuels such as hydrogen and methane. There are also several ways to convert biomass into fuel, mainly thermo-chemical (combustion, gasification, pyrolysis, liquefaction) and biochemical (anaerobic digestion, fermentation).

Choice of raw material is crucial. Lignocellulosic biomass is the most abundant organic material on Earth and that is why is very interesting for bioenergy production (Wyman, 1996). In **Papers I** and **II** composition of several raw materials available at the organic farm which are suitable for biogas or bioethanol production is shown. Compositional

analysis is necessary to estimate overall efficiency of the process weather it is biogas or bioethanol: for the first one overall amount of organic matter (VS) is crucial. Very often it is a basic characterisation of inoculums and substrates i.e. (Lehtomaki et al., 2007). On the other hand, for ethanol fermentation the sugar concentration of ligocellulosic materials is the most important (Foyle et al., 2007). Example of such a characterisation is presented in (Petersson et al., 2007; Xu et al., 2010). Total and volatile solids of raw materials are shown on Figure 5; composition of investigated energy crops is summed up on the Figure 6.

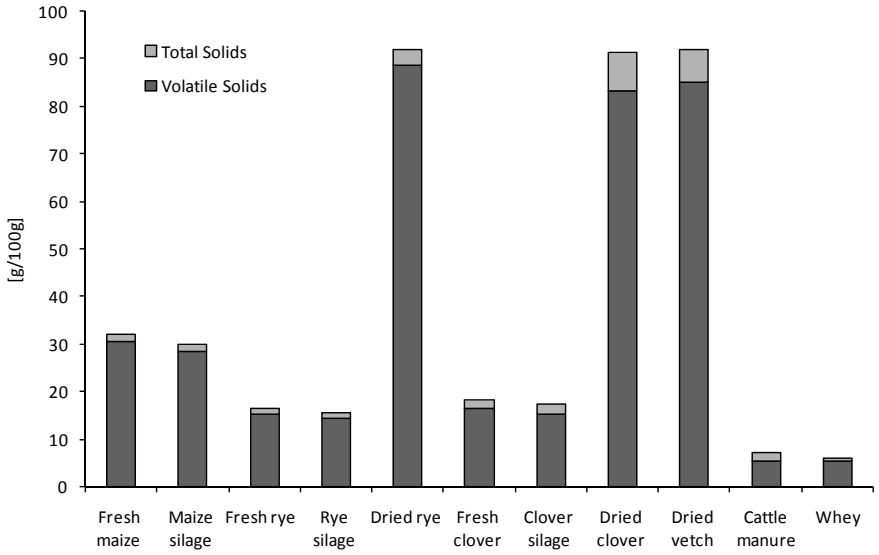

Figure 5. Total and volatile solids of the raw materials

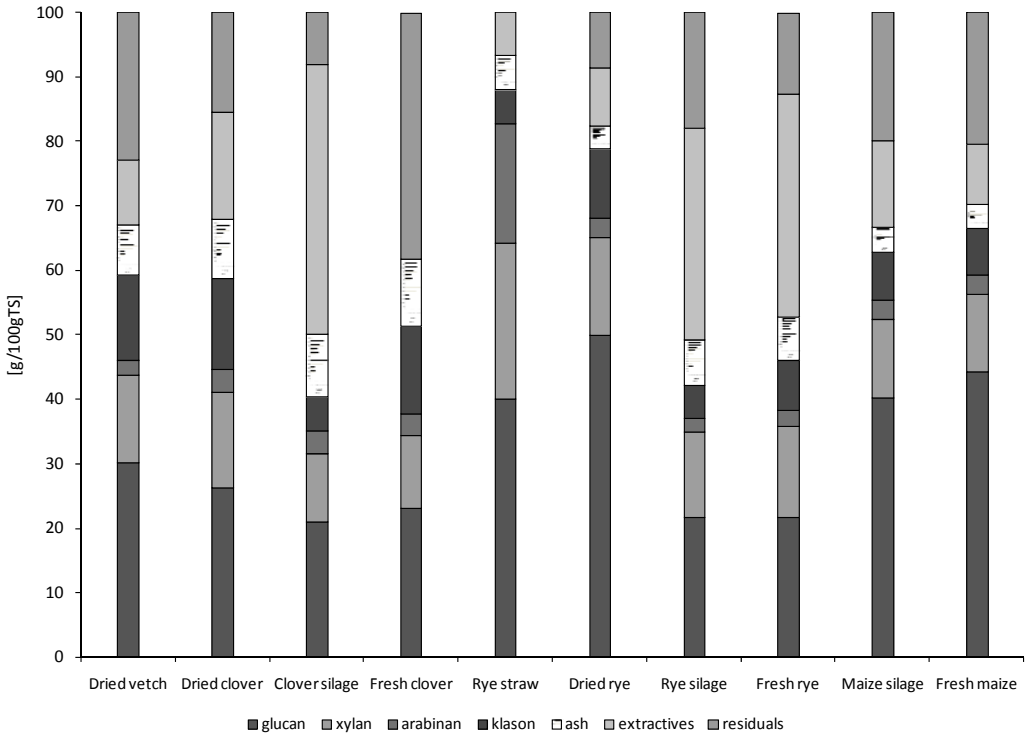

Figure 6. Composition of investigated organic energy crops

Each of the characterized biomass differs in amount of water, primary and secondary metabolites depending on the time of harvest, method of storage as well as type of plant. The dry part of the biomass is referred as TS (total solids) or DM (dry matter). In fresh green biomass samples: waxes, tars, colorants can be found – those are referred as extractives. The main organic compounds in biomass can be classified as cellulose, hemicelluloses and lignin. Cellulose is built from glucose monomers (C6) whereas hemicelluloses from xylose (C5) sugars. Additionally in fresh green biomass samples or in silage samples sugars like fructose can be found.

3.2. Ethanol

One of the best substitutions for fossil fuels could be bioethanol (Mandil, 2004), in 2009 74 billion liters of bioethanol were produced worldwide: 90% of comes both from US (from starch) and Brazil (sugar cane) (RFA, 2010). Ethanol as fuel has great property such as high octane number however the energy density is lower compared to gasoline. Different mixtures of ethanol and gasoline are available, the most popular in Brazil is E85 (contains 85% of ethanol) but it requires so called flex-fuel car. More common blends elsewhere E10 or E5 are suitable for unmodified cars. Detailed properties of ethanol containing fuels can be found in (Hsieh et al., 2002). Bioethanol also fits to the existing infrastructure and it can easily replace gasoline, which is very strong advantage of that fuel.

3.2.1. Ethanol potential

Ethanol produced via microbial fermentation can be produced from fermentable sugars: C6-glucose derived from starch or cellulose (from ligenocellulosic biomass) or from C5-xylose derived from hemicelluloses. So called, 1st generation ethanol based on starch is developed and mature technology, whereas 2nd generation ethanol (produced from lignocellulosic materials) is during the development (Larsen et al., 2008).

To estimate overall efficiency of the process of ethanol production from lignocellulosic materials, composition of substrate is necessary, mainly sugars concentration (Foyle et al., 2007). In **Paper II**, bioethanol potential of four different crops (maize, rye, clover grass and vetch) available on the organic farm was estimated. The study considered crops in diverse conditions (fresh, ensiled or dried) depending on type of the crop and common practice of storing it. Theoretical yields based on C6 and/or C5 sugar content were summarized. Results were presented in volume of ethanol produced per mass of raw material as well as energy content of produced fuel per area necessary to cultivate it. On the Figure 7, the theoretical ethanol potential, based on C5 and C6 sugars is shown. The numbers represent maximum ethanol which could be achieved through fermentation process, however after applied pretreatment method; the amount of produced ethanol would be lower. Xu et al. (2010) investigated hydrothermal method on maize silage, achieving from 55% to 77% of the theoretical one, in (Oleskowicz-Popiel et al. 2008) after wet-oxidation method, 82% of theoretical ethanol was produced from maize silage. Petersson et al. (2007) studied, among other materials, winter rye resulting in yield of 66% of the theoretical after wet-oxidation pretreatment method whereas Martin et al. (2008) produced around 87% of the theoretical ethanol from wet-oxidized

clover-ryegrass mixtures. Other authors showed results ranging from 60-90% of theoretical ethanol (Linde et al., 2008; Wyman et al., 2009; Carrasco et al., 2010). Obtained yields depend not only on the type of raw materials but also pretreatment method, concentration of enzymes and microorganisms, types of organisms and overall process conditions. Due to the fact that different lignocellulosic materials have different physico-chemical characteristics, almost each type of biomass has special optimal pretreatment conditions; the only way to compare full ethanol potential of several raw materials is through compositional sugar analysis.

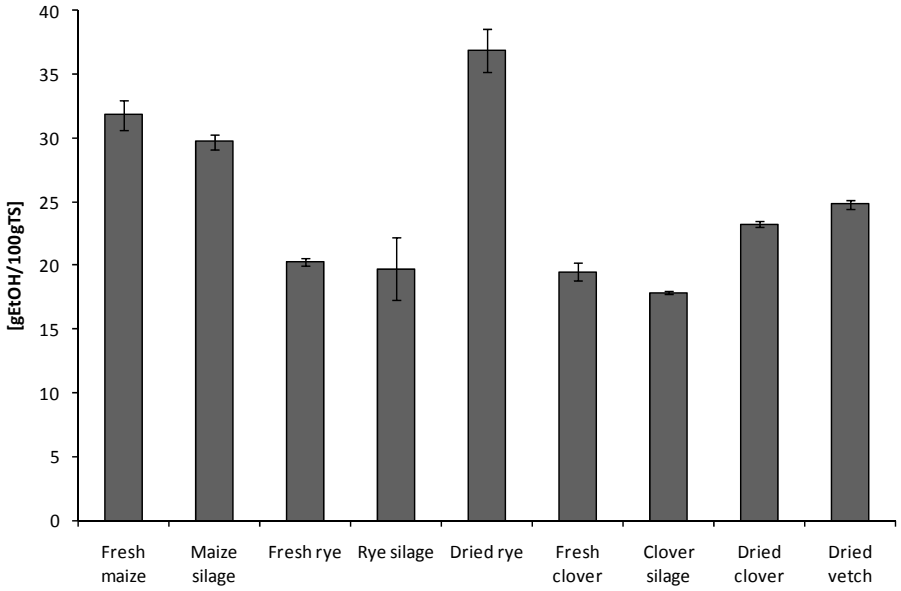

Figure 7. Theoretical ethanol potential in investigated raw materials (**Paper II)**

3.2.2. Pretreatment

Pretreatment refers to "disruption of the naturally resistant carbohydrate-lignin shield that limits the accessibility of enzymes to cellulose and hemicelluloses" (Yang and Wyman, 2008). One of the main technological challenges in lignocellulosic ethanol is to develop optimal pretreatment process (Chandra et al., 2007; Yang and Wyman, 2008). Over the years, several methods have been developed and are reviewed in (Wyman, 1996; Olsson et al., 2005; Alvira et al., 2010), to mention the most successful:

- Dilute acid pretreatment – dilute sulfuric acid pretreatment (Torget et al., 1991), besides achieving very high yields it has several disadvantages such as very corrosive environment and reaction degradation products such as furfural and acetic acid, which are strong inhibitors for microorganisms;
- Ammonia explosion – (Chou, 1986) the main advantage is low process temperature and low inhibitors formation, however it has high cost of ammonia and it is not suitable for woody substrates;
- Steam explosion – (Saddler et al., 1983) pretreatment is performed without presence of chemicals but with moisture. Hydrolysis is catalyzed by organic acids liberated from the biomass;

- Hydrothermal treatment – (Bonn et al., 1983) this technique involves cooking the biomass in water in high temperature.

Several other techniques have been investigating (Bjerre et al., 1996; Rossgard et al., 2007; Yang and Wyman, 2007, Galbe and Zacchi, 2007), where some of them were successfully applied in pilot scale for bioethanol production i.e. (Thomsen et al., 2006).

There is growing interest for a small farm-scale production renewable energy (Ahlgren et al., 2008). Especially organic farmers are interested and forced in improving their sustainability by using "green" energy and at the same time to make their farms self sufficient in terms of energy supply. As a result of this, there is a need for new and low-tech processes for bioethanol production with pretreatment techniques that will not interfere with organic farming principles and requirements (IFOAM) and at the same time are efficient.

Silage pretreatment - wet storage method can be one solution; it can be used to both preserve and pretreat biomass feedstock (Ren et al., 2006). Originally, ensiling is method for forage storing and preserving (Charmley 2001), which for long time has been used all over the world (Weinberg and Ashbell, 2003). The purpose of silage making is to store and preserve crops with minimum loss of nutrients i.e. feed value. Ensiled material, often referred to as silage, consists of the whole harvested plant (stem, leaves, and grain) and grasses which is used for animal feed. In a correct ensiling, lactic acid bacteria dominate the fermentation process; the low pH caused by fermentation of part of free sugars preserve the feedstock from further degradation by inhibiting fungus microbes, in that way effectively minimizing the degradation of sugars in a crop (Thompson et al., 2005). In conventional silage process without additives, half of the hemicelluloses content can be degraded but less than 5% of cellulose (Ren et al., 2006). (Yahaya et al., 2001) investigated polysaccharide degradation in orchardgrass and lucerene during ensiling, noticing 17.2-19.8% hemicellulose degradation and only 0.5-3-3% of cellulose. Similar conclusions, that hemicellulose is easier hydrolyzed than cellulose during that process, were found by (Kawamura et al., 2001).

Silage crops have been already widely used for biogas production (Zubr, 1986; Amon et al., 2007; Vervaeren et al., 2010) they were also used in ethanol trials but after wet-oxidation, hydro-thermal or steam pretreatment (Thomsen et al., 2008; Xu et al., 2010; Sipos et al., 2010). Investigation concerning ensiling as a stand-alone method for wet storage and pretreatment process for production of 2nd generation ethanol was presented in **Papers III** and **IV**.

Fresh maize, rye and clover grass were ensiled and the influence of the silage process was described and discussed in **Paper III**. The ensiling method in laboratory conditions was described in Materials and Methods in **Paper III**. Ensiled samples were compared to fresh crops. Enzymatic convertibility tests and fermentation trials were carried out on all investigated biomass. Very promising results were achieved and silage process has been proved to be efficient wet-storage method which additionally could serve as sterilization and mild pretreatment method for second generation ethanol.

Paper IV goes with one step further, dry lignocellulosic by-product – corn stover – is moisten and stored in a silage form (described in Materials and Methods section, **Paper IV**). Noticeable positive influence of the ensiling proved once again that it can be successful storage method for lignocellulosic materials. Ren et al., (2006) investigated ensiling of corn stover as long term feedstock preservation method concluding that it can guarantee stable 6 month biomass preservation.

Previous authors (Chen et al., 2007; Digman et al., 2010) already indicated that ensiling could be applicable in bioethanol industry. Chen et al. (2007) wrote that the ensiling significantly increased the conversion of cellulose and hemicelluloses to sugars during subsequent enzymatic hydrolysis. It was concluded that it is not as efficient as chemical pretreatment but it is low-cost and energy conserving technique. Digman et al. (2010) evaluated ensiling with and without chemical addition as a wet storage for switch grass and reed canary grass prior to conversion into ethanol. It was found out that addition of sulfuric acid was more effective compare to lime addition. Both investigations indicated that ensiling is very promising method for wet storage of lignocellulosic biomass and it increase overall ethanol yield. It is with accordance with results presented in **Paper III** and **IV**. Deeper understanding of the process and its optimization from feed preservation method towards lignocellulosic pretreatment for second generation bioethanol would be necessary.

3.2.3 Ethanol fermentation

The production of ethanol consists of several different steps (Aden et al., 2002). After choice of raw material, the next step is hydrolysis, which purpose is to split sugars from cellulose and hemicelluloses into monomeric sugars. It includes pretreatment (which is described above in point 3.2.2) and enzymatic hydrolysis. During enzymatic hydrolysis the polymer of cellulose is reduced to simple sugars. Typically cellulase enzymes are classified as follows (Petersson, 2005): endo-β-glucanases (cleave the polymer randomly), exo-β-glucanases (cleave off units of cellobiose), exo-β-glucosidase (cleave off glucose) and β-glucosidases (cut cellobiose into two units of glucose). The cellulase enzymes are rather costly and the enzyme loading should be minimized, but not to increase the time needed to complete hydrolysis (Olsson et al., 2005). In most cases cellulase enzyme are produced by *Trichoderma reesei* and *Aspergillus niger* (Hendy et al., 1984; Lo et al., 2010).

Recent review articles in that field describe current advances, opportunities and obstacles in successful enzymatic hydrolysis process (Meyer et al., 2009; Alvira et al., 2010; Talebnia et al., 2010). Several factors influence the results from enzymatic hydrolysis test (Alvira et al., 2010): cellulose cristallinity, degree of polymerization, available surface area, lignin barrier, hemicelluloses content, feedstock particle size, porosity and cell wall thickness. Enzymatic convertibility test can serve as first indicator on digestibility of raw material to produce biofuel, it can also give an idea about pretreatment severity necessary to open lignocellulosic structure.

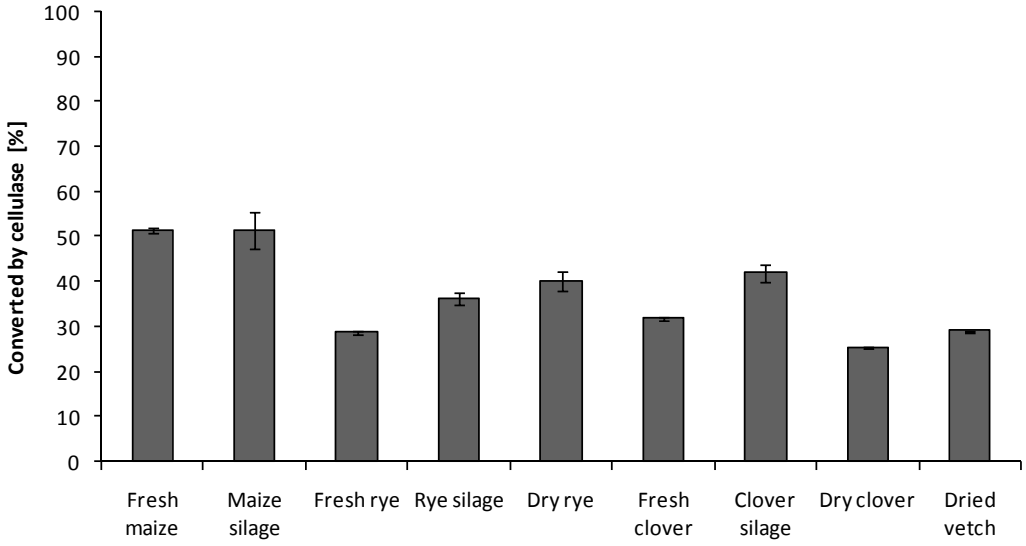

Figure 8. Results from enzymatic convertibility test of raw materials presented in Paper III and IV

Cellulase dosage of 10-30 FPU/ g cellulose is often used in laboratory trials because it gives high glucose yields in reasonable short time (Talebnia et al., 2010). The loading depends on substrate, pretreatment method; in laboratory experiments often exceed the loading applied in pilot or demonstration scale. Results from enzymatic convertibility tests presented in **Papers III** and **IV** and summarized on the Figure 8 aimed to characterized different raw materials for their digestibility for ethanol production.

In the fermentation process the monomeric sugars revealed during enzymatic hydrolysis, are converted into ethanol by microorganisms. Talebnia et al. (2010) reviewed different microorganisms used or studied to produce ethanol. The most common and at the same best performing are typical Baker's yeast – *Saccharomyces cerevisiae*. Those are also the organisms used in some of the test presented in **Paper III**. The main advantage of *S.cerevisiae* is its robustness, it is well suited for diverse agricultural raw materials where possible inhibitors can occur (Klinke et al., 2003), it also gives a high ethanol yield and high ethanol productivity. The main disadvantage would be disability of fermenting C5 sugars (arabionose and xylose), which also occur in lignocellulosic feedstock. Second strain applied in the experiments was thermo-tolerant yeast *Kluyveromyces marxianus* (**Paper III**, **IV** and **V**). The advantage was higher processing temperature (40°C), which were closer to optimum for cellulose hydrolysis (50°C) (Kadar et al., 2004); consequently higher rates of hydrolysis were expected.

In order to evaluate ensiling method as a wet-storage or pretreatment, batch fermentation trials were performed. In **Paper III**, two kinds of yeast were applied. Higher ethanol production was observed for the thermophilic yeast, which is explained by the higher process temperature (40°C compared to 32°C for Baker's yeast) being close to the optimal hydrolysis temperature for cellulase enzymes (50°C) - consequently more glucose was available to be converted into ethanol.

Part of the concept for the decentralized biorefinery at the organic farm (within BioConcens project) is to produce ethanol from whey. Whey, which is by-product from cheese industry, contains lactose – a disaccharide consisting of glucose and galactose. It cannot be fermented by *Saccharomyces cerevisiae*, which is commonly used in alcohol fermentation, because this strain of yeast lacks β-galactosidase activity. *K.marxianus* is capable of fermenting lactose to ethanol directly. *K.marxianus* was studied extensively and was the best choice for this raw material (Wang et al., 1987). Laboratory trials on that subject are described in **Paper V**. Main conclusion were that even without pasteurization or freezing of whey, *K.marxianus* successfully competed with lactic acid bacteria, producing high ethanol yield (0.50 g ethanol/ g lactose). Additionally, during continuous trials high ethanol productivity was achieved (2.5-4.5 g/L/h).

3.3. Biogas

3.3.1. Process principles

Anaerobic digestion is a biological process where most organic matter (carbohydrates, lipids, proteins) except for lignin components, in the absence of oxygen, is degraded into methane and carbon dioxide. The process consists of series of reactions and it is a natural process which takes places in several anaerobic environments. In anaerobic digestion processes can be divided into (Gujer and Zehnder, 1983; Angelidaki et al., 2002), the schematic view is shown on Figure 9:

- Hydrolysis - the fermentative bacteria hydrolyze biopolymers such as proteins, carbohydrates and lipids into oligo- and monomers by extracellular enzymes. The proteolytic bacteria produces proteases to hydrolyze proteins, the cellulytic and xylanolytic bacteria produces cellulases and xylanases to degrade carbohydrates and lipolytic bacteria produces lipases to hydrolyze lipids.
- Fermentation - during this process organic material will be transformed to methanogenic substrates (hydrogen, carbon dioxide and acetate) and lower fatty acids and alcohols. The main process is acetogenesis, where volatile fatty acids (VFA) and alcohols produced during fermentation step are oxidized to acetate – this reaction is catalyzed by acetogenic bacteria.
- Methane formation – the methanogenic bacteria are divided into two groups: the aceticlastic methane bacteria, which degrade acetate; and the hydrogen consuming methanogens. Methanogenesis is an energy producing process and it is regarded as the motive force for anaerobic digestion.

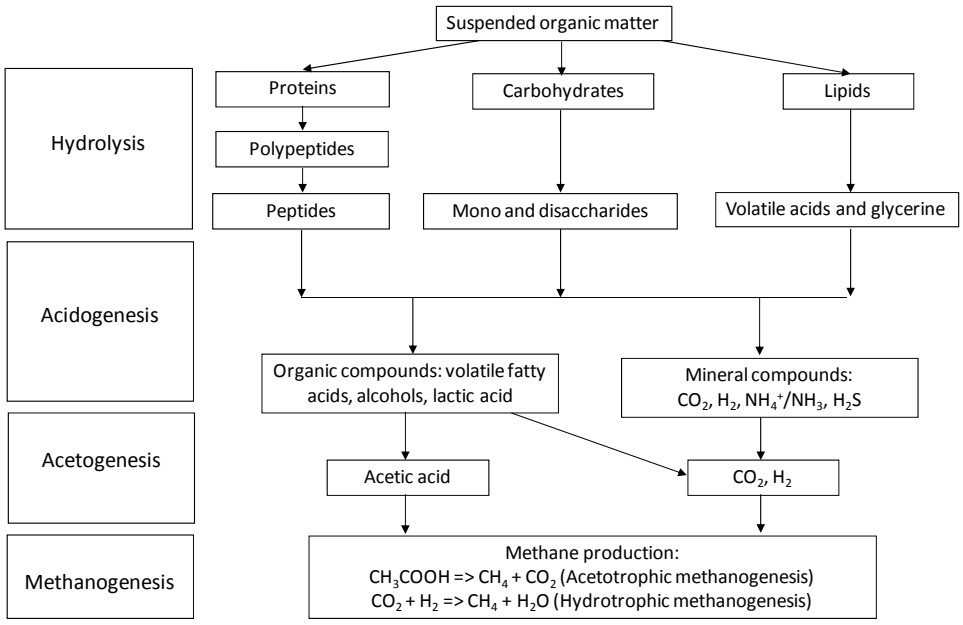

Figure 9. Schematic view of anaerobic digestion process (adapted from (Gujer and Zehnder, 1983; Angelidaki et al., 2002))

Anaerobic digestion, not only provides renewable energy source (biomethane) but it also deliver highly efficient natural fertilizer (Angelidaki et al., 2003). Möller and Stinner (2009) investigated effects of different manuring systems. They concluded that biogas digestion of field residues resulted in a win-win situation. Besides additional energy, there is a lower nitrate leaching and lower nitrous oxide emission, the disadvantage is higher ammonia volatilization compared to undigested manures. Anaerobic treatment also minimizes the survival of pathogens which is important in applying it as fertilizer.

Such benefits are very suitable for organic farmers, which are very concern about soil fertility and nutrients recycling. The greenhouse gas emission reduction and sustainable development of energy supply makes this technology one of the most promising for on-farm application (Svensson et al., 2005; 2006). Biogas as renewable energy source will play vital role in the future, it can replace fossil fuels for heat and electricity generation as well as vehicle fuel. If upgraded, if can be injected into natural gas grid, moreover biomethane can be a feedstock for producing other chemicals and materials (Holm-Nielsen et al., 2009; Weiland, 2010).

3.3.2. Biogas potential

Substrates type and its composition directly influence the biogas yield. The input to the process can be measured in chemical oxygen demand (COD) or total/volatile solids (TS/VS) values. It is crucial to determine the degradable and inert fraction of the feedstock. Animal manure, which in most cases is principle compound of feed, has low methane yield per COD or VS compared to other applied raw materials (Møller et al., 2004). Lignin is one of the non-degradable compounds. On the other hand, many industrial organic wastes contain significant amount of easily degradable compounds. In

Paper I and **II** biogas potentials of diverse crops available at the organic farm were presented (summarized on Figure 10).

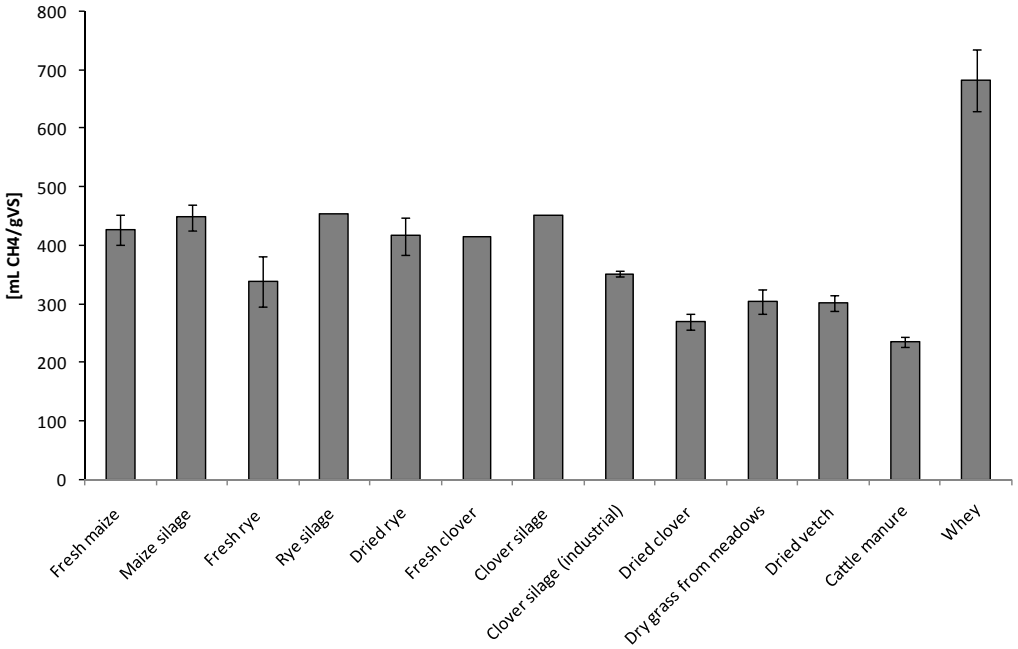

Figure 10. Methane potential of different raw materials available at the organic farm

Estimation of methane potentials differs from estimation of ethanol potentials (described previously). During anaerobic digestion most of the organic compounds are degraded. One way would be to complete characterize raw materials and calculate methane potential based on proteins, carbohydrates and lipids concentrations. This is however complicated and expensive. Rough estimation can be based on COD or VS content but it does not give precise results. The most common procedure is practical methane yield determined during batch laboratory experiments. Determining methane potential created several doubts how the test should be performed to obtain reliable and comparable results. In (Angelidaki and Sanders, 2004) attempt to systematize and unify the methane potentials was taken, where review of different methods is presented. Hansen et al. (2004) identified optimal process conditions for determining methane potential from organic solid wastes. It included ratio inoculum to sample, number of replicates, the origin of inoculum, process time and method for measurement of produced methane. Angelidaki et al. (2009) suggested common method for biomethane potential of solid organic wastes and energy crops, it was recommended to:

- Characterize substrate for total solids, volatile solids, chemical oxygen demand, nitrogen and phosphorus content, additionally content of lignin, cellulose and hemicelluloses could be determined;
- “Fresh” and “degassed” inoculum pre-incubated in the same temperature as process temperature, appropriate volume of inoculum according to its activity;
- Necessary nutrients/micronutrients/vitamins should be supplied unless they are present in inoculum or substrate;
- Blank assay should be always carried out;

- The number of replicates should be at least three for each dilution;
- Some mixing should be applied (e.g. turn up down once a day);
- For new substrates with unknown degradation characteristics, a number of different dilutions of the substrate (with water) are required;

Detailed description of assay experimental set up, guidelines and advices for can be found in (Angelidaki et al., 2009).

3.3.3. Continuous trials

Continuous trials with cattle manure and maize silage are presented in **Paper II**. In co-digestion process manure provides buffering capacity and nutrients while an energy crop with high carbon content balances carbon to nitrogen ratio of the feedstock. Such a combination has been proved to result in higher methane yields (i.e. Parawira et al., 2008). Our trials showed that methane yield in co-digestion of cattle manure and maize silage resulted in 267 mL CH_4/gVS_{added}. With 33% of maize silage in the feedstock, 36% increase of methane production was read. Methane yield originated from maize silage varied between 304 and 384 mL CH_4/gVS. Lehtomäki et al. (2007) investigated co-digestion of cow manure with sugar beet tops, grass silage and oat straw achieving methane yield of 229, 268 or 213 mL CH_4/gVS_{added}, respectively, where feed contained 30% of crop. Further increase of crop in the feedstock decreased methane yield. Comino et al. (2010) after mechanical pretreatment (chopped to a size of 2mm) of silage crop mix, increased till 70% VS crop portion in the feedstock. It resulted in 109% higher specific methane yield compared to start-up phase (only with manure). Further increase of crop percentage in the feed decreased methane production. Lindorfer et al. (2008), on the other hand, claimed that up to 96.5% VS of energy crop ratio is possible in a feedstock without any decrease, however longer adaptation time of the microbial population to the feedstock is required. Apart from high methane yield, digestion of only energy crops might have disadvantages, Lebuhn et al. (2008) studied mono-digestion of maize silage, it was found out that long term trace elements (cobalt, molybdenum, selenium) have to be supplemented.

3.4. Ethanol and biogas co-production

Process integration can lead to more intensive and cost-effective on-farm energy production. “Integration opportunities may provide the ways for a qualitative and quantitative improvement of the process so that not only techno-economical, but also environmental criteria can be met” (Cardona and Sanchez, 2007). One of the concepts for process integration within BioConcens project was to co-produce ethanol and biogas from germinated grains, whey and optionally clover grass silage.

Malting, normally used in brewing of beer, develops enzymes that are required to hydrolyse the complex starch in grain into simple fermentable sugars. Natural enzymes from cereals were used for hydrolysis of starch to glucose in accordance with technology in brewing technology. Enzyme production during germination was extensively studied on barley (Briggs et al., 1981). Biorefinery concept where bioethanol is produced from

germinated grains and whey and rich in protein effluent is as animal feed and remaining process water is treated in upflow bioreactor to produce biogas is presented on the Figure 11.

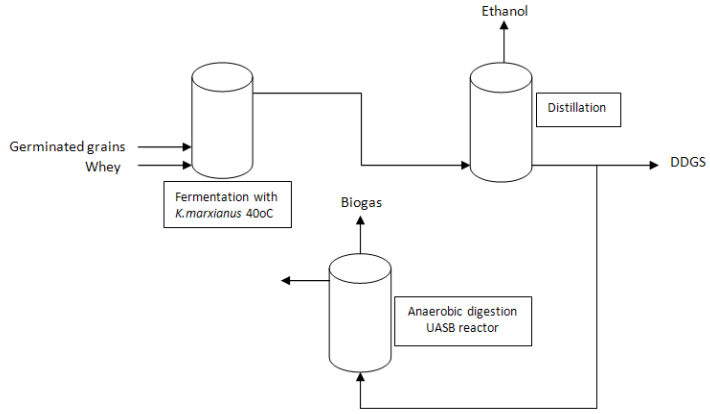

Figure 11. Concept for co-production of ethanol and biogas from germinated grains and whey

The effluent was separated into two streams: the solid part to be used as rich in protein animal feed, the liquid part should be further processed in UASB reactor (up-flow anaerobic sludge blanket reactor) to produce biogas from remaining organic compounds. Biogas production from whey was studied broadly (Hwang and Hansen, 1992; Kalyuzhnyi et al., 1997; Kato et la., 1997). Ergüder et al. (2001) concluded that undiluted cheese whey could be treated anaerobically at relatively short retention time (2.06-4.95 days) without any significant stability problems. Alternatively whey could be treated by co-digestion with manure in CSTR reactor. Gelegenis et al. (2007) achieved stable biogas production with whey fraction until 50%, above that the reactor turned to be unstable. From initial experiments following results were obtained:

- From mixture of: 14g (73%TS) of germinated grains and 86g (6.5%TS) of whey: 2.9g EtOH and 4988 mL methane was achieved
- From mixture of: 7g (73%TS) of germinated grains, 73g (6.5%TS) of whey and 20g (18%TS) clover grass silage: 2.2g EtOH and 4641 mL methane was achieved

Moreover, the biogas potential of each specific compound of effluent was measured and it is shown in Table 1.

Table 1. Methane potential of investigated feedstock

Feedstock	[mLCH_4/gTS]
Whey	~700
Fresh clover silage	~440
Effluent clover silage	~400
Grains	~600

Further experiments and development of this biorefinery concept continues.

4. Biorefinery modeling

4.1. Principles

Modeling and simulation of chemical and bioprocesses helps to identify possible improvements as well as to identify potential difficulties. During the development of the process, to some extent simulation can act as a substitute for the experimental part (Heinzle et al., 2006). The principle steps in the process modeling are presented in the Figure 12.

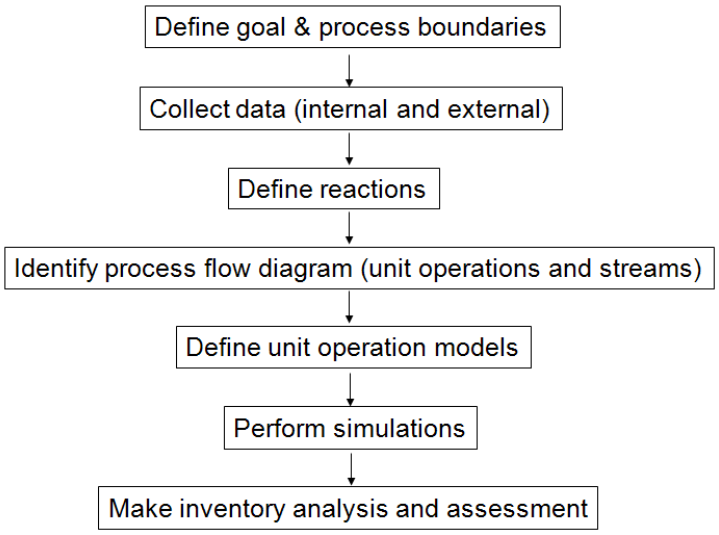

Figure 12. Basics steps in process modeling (adapted from Heinzle et al., 2006)

The definition of the goal, the model boundaries, the raw materials and the final product specification is the first step in building a model. After that, the necessary data must be collected either from your own experiments (preferred) or external sources (often used to fill the own data gaps and also to validate your own experiments). Following this, it is necessary to define the reactions in a process and it parameters such as yields, reaction (fermentation) time, product concentration, by-product formation, etc. In the next step, the process flow diagram, unit operation and process streams are defined, and finally, the simulation is performed and an analysis of the results made. Usually, before achieving final results several of those steps are repeated and improved. (Heinzle et al., 2006; Towler and Sinnott, 2008)

4.2. On-farm energy production

Biomass is a key parameter in an agriculture environment for energy production (Jørgensen et al., 2005); two of the very promising technologies which could be applied directly on the organic farm are: anaerobic digestion for production of biogas and ethanol fermentation (Frederiksson et al., 2006). Throughout this study process models for a single organic farm (around 100 ha) (**Paper VI**) and several organic farms (around 1000 ha) were developed (**Paper VII**). This biorefinery consists of two processes: ethanol fermentation and anaerobic digestion (Figure 13).

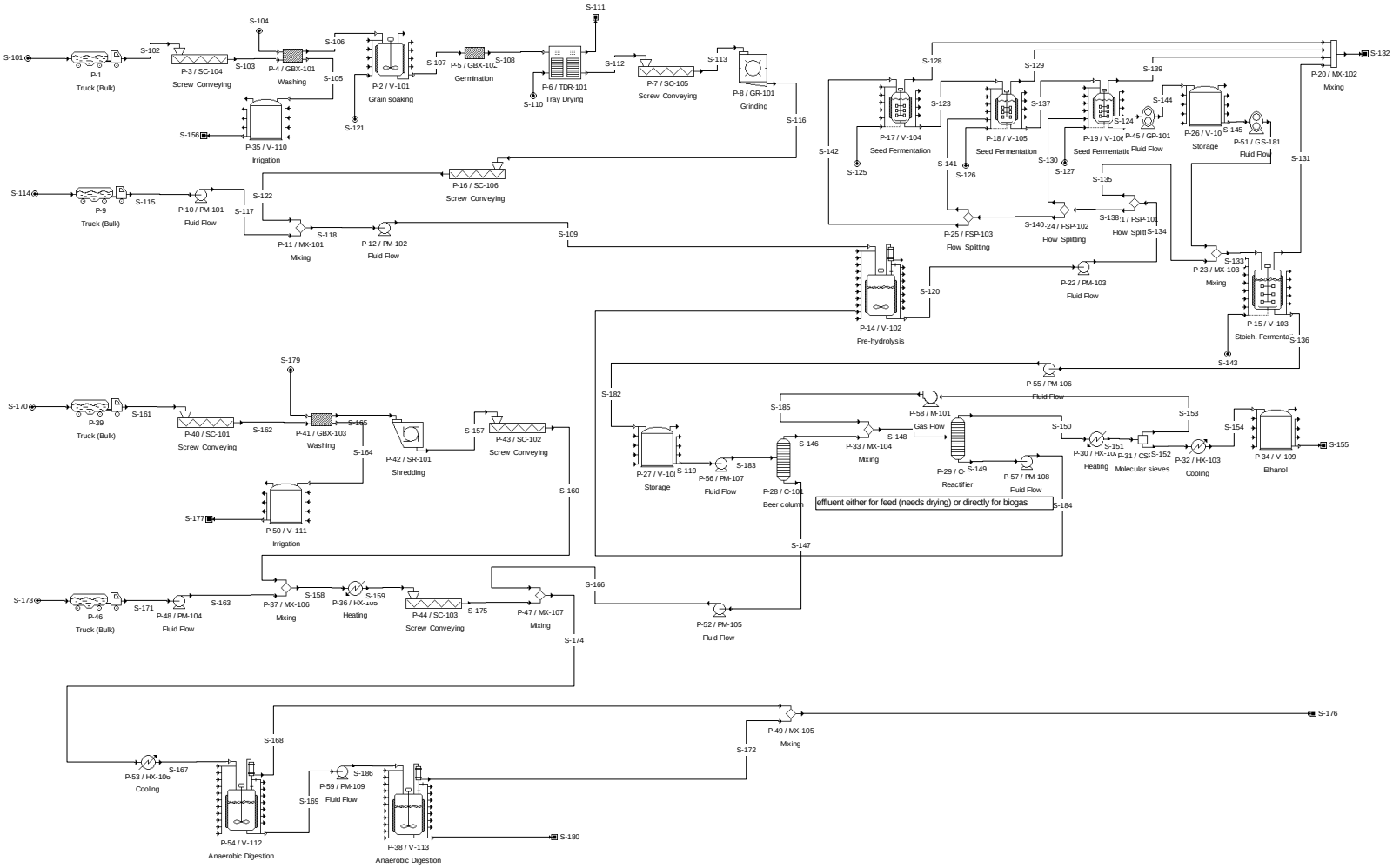

Figure 13. The on-farm biorefinery for co-production of ethanol and biogas (flow sheet from SuperPro Designer)

Ethanol is produced from rye grains and whey. Rye grains are soaked with water prior to germination to achieve moisture of 40-45%. Germination takes 24 hours at room temperature (25°C), during that process, natural amylases are produced. After germination, the grains are dried at 35°C, grinded and then mixed with whey. Germinated grains contain sugars, whereas whey supplies the process with nutrients, process water and an additional carbon source (lactose). To activate the enzymes, the input stream is pre-hydrolyzed at 50°C. The fermentation is carried out by *Kluyverimyces marxianus* at 40°C with a hydraulic retention time of 40 hours. Inoculum can be either produced in 3 step seed fermentors directly on the farm or bought from outside. Finally, the ethanol is purified in a two step distillation and the remaining water removed in molecular sieve. A final ethanol concentration of 99.6% is achieved.

Biogas is produced from cattle manure, clover grass and maize silages (**Paper VI**) or cattle manure and clover grass silage (**Paper VII**). Crops, after harvesting, are stored in silage form (ensiling process was not included in a model); both are shredded before being added to the fermentor where they are mixed with cattle manure in a ratio depending on the scenario (scenarios are described in detail in **Paper VI** and **VII)**. The anaerobic digestion process is performed in two stage continuous mode at thermophilic conditions (55°C) with a hydraulic retention time of 20 days with each reactor. During the process, biogas with a methane content of 60-65% is collected. The effluent from the anaerobic digestion (digestate) is commonly used as fertilizer. It contains undigested lignocellulosic leftovers (valuable carbon source for soil) and significant amounts of nitrogen, phosphorous and potassium - all originating from manure.

Several authors studied the possibility of energy production on a farm scale level. Table 2 summarizes those works showing raw materials, technology applied and presents main conclusions. It is suggested that the popular technologies - biogas and rape methyl ester - could be produced directly on the farm (Svesson et al., 2005; Svesson et al., 2006; Frederiksson et al., 2006; Hansson et al., 2007; Monreal et al., 2007;) Ahlgren et al (2008; 2009) suggested Fisher-Tropsh fuel or thermo-chemical gasification products. All the authors mentioned their concerns about the expensive downstream processing to clean the fuel (e.g. biogas upgrading or ethanol distillation) or the necessary engines modification to adjust to new fuels.

Table 2. Different on-farm bioenergy production concepts

Raw materials	Technology/ Fuel	Main Conclusions	Reference
Short rotation coppice (SRC)	-	5% of agricultural land could produce energy equaling 30-58% of the energy input for organic farming; Utilization of wastewater and sewage sludge to close the gap between agriculture and the cities; SRC crops reduce nitrate leaching (protection of water quality); SRC fields could be an outdoor areas for e.g. pig and poultry;	Jørgensen et al., 2005
Sugar beet tops, wheat straw, ley crops	Biogas	Technology: single stage fed-batch high-solids digestion; The results indicated the importance of choosing a substrate with a high methane yield and high N content; Positive effect of scale was observed, Positive effect of economy of numbers was discussed (significantly decreasing cost);	Svesson et al., 2005; 2006
Winter rapeseed	Rape methyl ester (RME)	Favorable energy balance; High land use and the emission associated with cultivation; Well-known technology and easy to implement on the farm scale; 72% reduction in GHG when compared to diesel;	Frederiksson et al., 2006
Winter wheat (grains)	Ethanol	Energy consuming process; Low area needed for cultivation; Technology is available but it is more optimal for large scale; 60% reduction in GHG compared to diesel;	
Silage	Biogas	Low need for arable land (small emission of GHG); Advantageous recycling of plant nutrients; Small scale technology for biogas upgrading is not optimal; 58% reduction in GHG compared to diesel;	
		Fuels produced outside the organic farm in industrial scale plants;	Hansson et al., 2007

		Systems based on the production of one raw material but with access to different fuels are economically favorable;	
Rapeseed	Rape methyl ester	8.5% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 8.3; No engine modification needed; High price of the organically produced rapeseed;	
Wheat	Ethanol	5.5% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 2.6; Low cost in large production facilities;	
Ley	Biogas	3.8% of the farm land is needed to achieve self-sufficiency in motor fuel; The total energy efficiency (energy in the fuel/total allocated energy use): 4.4; Raw materials available in large amounts; Cost of transport, storing and cleaning the gas is high; Significant modification in engine is needed if run only on gas;	
Animal manure/ straw and sorted municipal wastes	Biogas/ Gasification	5 different projects running for on-farm renewable energy production and GHG mitigation: Effective use of produced electricity with surplus fed to the grid, produced heat used for digester heating only; Planned nutrients recovery and concentration from AD effluent to produce 'nutrient-rich bio-fertilizer' was planned;	Monreal et al., 2007
Straw, short rotation willow coppice (Salix)	Fisher-Tropsh diesel (FTD)	To achieve self-sufficiency, 108 ha of Salix and 261 ha of straw collected (no land set aside) is needed out of 1000ha; Energy balance 8.9 and 9.6 from Salix or straw; Additionally, large amount of by-products is produced;	Ahlgren et al., 2008

	Dimethyl ether (DME)	To achieve self-sufficiency, 38 ha of Salix and 70 ha of straw collected (no land set aside) is needed out of 1000ha; Energy balance 10.1 and 10.0 from Salix or straw;	
		Fuel produced outside of the farm, utilized in fuel cell powered tractors; Studied technologies are not yet on a commercial scale and available at reasonable costs	Ahlgren et al., 2009
Straw	Hydrogen - thermochemical gasification	To achieve farm self-sufficiency, no land is needed to be set aside, but straw collected from 43 ha (out of 1000 ha); Energy balance 16.3; 97% reduction in GHG when compared to diesel;	
Straw	Methanol - thermochemical gasification	To achieve farm self-sufficiency, no land is needed to be set aside, but straw collected from 53 ha (out of 1000 ha); Energy balance 19.5; 97% reduction in GHG when compared to diesel;	
Salix	Hydrogen - thermochemical gasification	To achieve farm self-sufficiency, 16ha (out of 1000 ha) is required; Energy balance 14.2; 92% reduction in GHG when compared to diesel;	
Salix	Methanol - thermochemical gasification	To achieve farm self-sufficiency, 20ha (out of 1000 ha) is required; Energy balance 15.6; 91% reduction in GHG when compared to diesel;	
Ley	Hydrogen – biogas production	To achieve farm self-sufficiency, no land is needed to be set aside, but green manure harvested from 43ha (out of 1000 ha); Energy balance 6.1; 90% reduction in GHG when compared to diesel;	

In **Paper VI**, the scenario for energy sufficiency at the 100 ha organic farm was discussed. Two scenarios were considered: biogas and bioethanol production. The organic farm energy requirement was estimated at 180 GJ. According to (Frederiksson et al., 2006; Hansson et al., 2007) to produce 1 MJ of biogas, 216 kJ is needed, and to produce 1 MJ of ethanol, 228 kJ. The overall organic farm energy requirement accounted those values. The efficiency of CHP unit was estimated at 38%. Based on those assumptions it was concluded that 16.2% of the farm land area is need to produced ethanol from rye grains, or 8.2% of the farm area to generate biogas from maize and clover grass silages to achieve.

Hansson et al., (2007) indicated that only 5.5% of the farm area is need to produce a sufficient amount of ethanol and 3.8% for biogas to substitute motor fuel. To supply the organic farm with Fisher-Tropsh diesel or dimethyl ether, according to Ahlgren et al. (2009) 3.8 – 10.8% of farm land is necessary (Salix plantation). If fuel cell technologies are applied and a thermo-gasification product (such as hydrogen or methanol) or hydrogen produced from biogas, much less farm land is necessary to fulfill tractive power demands. It was indicated that less than 5% of the farm would be required. However, all of those technologies are still under development and the study considered only hypothetical production.

In **Paper VII**, scenarios to produce renewable energy at the farm were simulated. The diagrams describing them are presented on Figure 14. Five cases were designed to meet possible potentials of an organic farm: production of biogas from clover grass silage and cattle manure (two scenarios), production of bioethanol from rye grains and whey (two scenarios), and the combination of those two to produce on-farm biogas and bioethanol.

The crop distribution, number of animals and amount of manure in the baseline (Figure 2) are based on the statistical data on organic farmers in Denmark in 2006. The area for each crop, number of animals and amount of manure are means of full time organic farmers on sandy soil, being either dairy farmers or cash crop farmers. This farm type represents 61 % of organic farmers in Denmark. The data used origins from the Single Payment applications of Danish farmers. In 20% of the scenarios, the number of dairy cows are reduced in order to make room for a larger bioenergy production. Therefore, less manure is available. (Pugesgaard et al., in preparation)

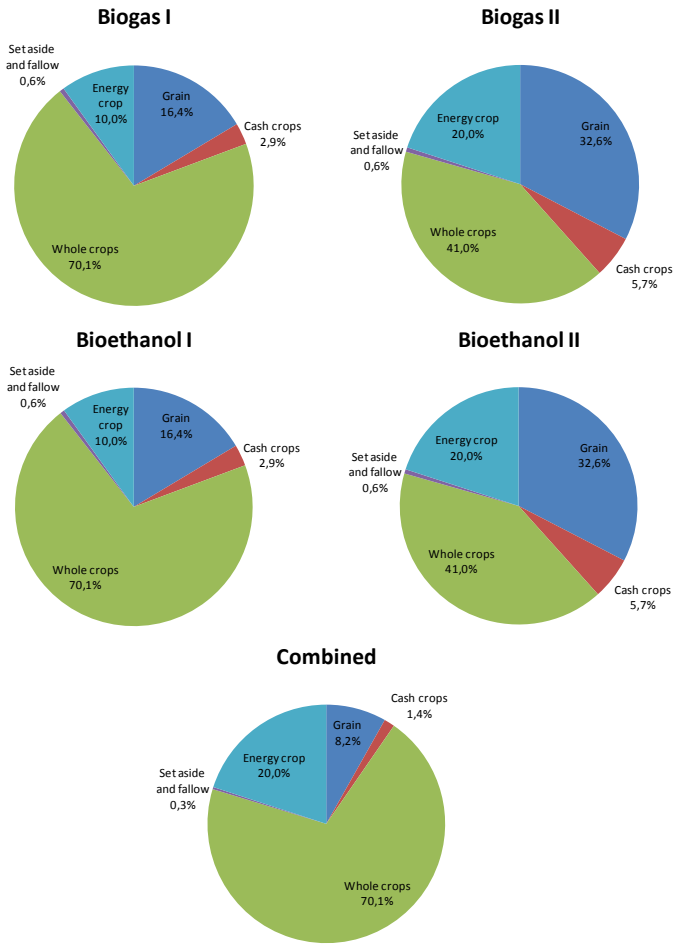

Figure 14. Five scenarios for bioenergy production at 1000ha organic farm

The main results from the study are shown in the Figure 15. The combined scenario was characterized by the highest investment but also by the largest energy produced (29244 GJ/year). Scenario Biogas II was second best in terms of the amount of produced energy (26409 GJ/year) and it was characterized by slightly lower investment cost compared to the scenario Biogas I, which was also less effective in terms of energy (19970 GJ/year). Although, Bioethanol I and Bioethanol II presented the lowest investment costs, they also generated the least energy (4034 GJ/year – Bioethanol I and 5610 GJ/year – Bioethanol II). Scenario Bioethanol I indicated a slightly higher total capital investment compared to the scenario Bioethanol II. Bioethanol scenarios include downstream processing (distillation) which increase the total fuel production cost, in case of the Biogas scenario, upgrading biogas to natural gas quality was found not to be necessary.

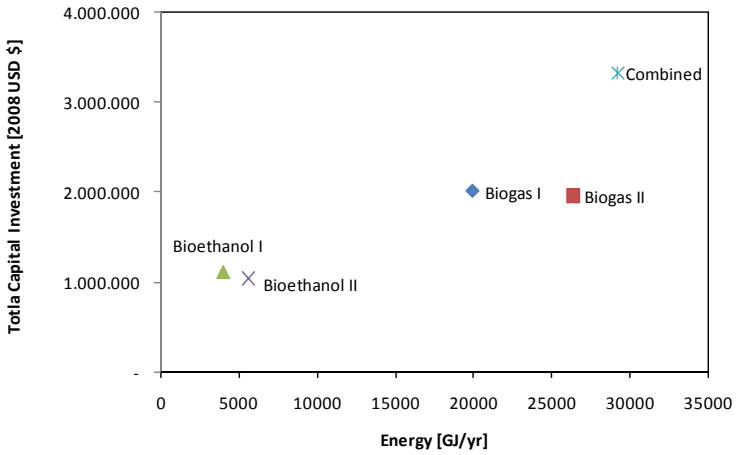

Figure 15**.** Relationship between total capital investment of each scenario and produced energy (in GJ/yr)

The achieved results for the Biogas scenarios correspond to what was calculated in a Danish report for the development of on-farm organic biogas plant (Tersbøl and Jørgensen, 2009). The Ethanol scenarios built were based on large scale ethanol industry (Wooley et al., 1999; Aden et al., 2002; Klein-Marcuschamer et al., 2010). Therefore, it is believed that those are also reliable. All the assumptions in building this model were described in **Paper VII**. That work presented an engineering tool which could be used in organic farming community to design and evaluate economic feasibility of an on-farm organic biorefinery. A large range of scenarios with different process configurations suitable for specific organic farm could be simulated and best one chosen.

4.3. Lignocellulosic biorefinery

The biorefinery refines and converts biological raw materials (biomass) into multiple valuable products (Kamm and Kamm, 2004). Similarly to the pertroleum refinery, the biorefinery should produce several different industrial products such as: transportation fuels, commodity chemicals and materials as well as high-value, low-volume speciality chemicals. At the moment, energy is a precursor and driver in this development, but over time other more sophisticated products will be developed (Clark and Deswarte, 2008). An example of the basic scheme of biorefinery is shown in Figure 16.

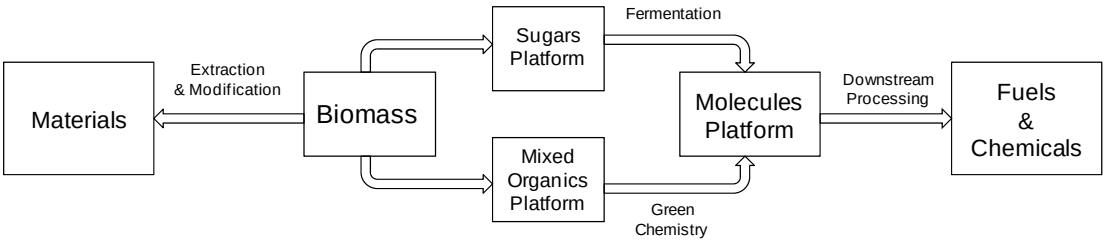

Figure 16. Biorefinery (adapted from Clark, 2007)

Biomass (seen as a platform) can be split into sugars and mixed organics platforms from where, through biological or chemical routes, a wide range of molecules are created. Apart from that, directly from biomass, valuable products can be fractionated through extraction and modification or themochemical processes as well as traditional chemical methods. By further processing, it is turned into fuels and high value products.

The principal rule of biorefinery should be to maximize the value of the biomass and minimize waste. In other words, all the streams should be utilized and converted into useful components. Costs will be cut down if the used fraction of biomass is increased.

Usually biorefinery products are diluted in complex aqueous solutions (e.g. ethanol in the fermentation broth). It is desired to make downstream processing, which typically is an expensive and wasteful stage of the process, a clean and low energy technique that could convert multicomponent systems into valuable clean products.

Detailed schemes on possible products from biorefinery is shown in Figure 17. Building blocks based on single to six carbon chemical compounds, aromatics or direct polymers are possible. Generally, a wide range of products for industry, transportation, housing, health purposes etc. may be produced. Modern biorefineries should follow market needs and be easily adjustable to produce, besides bulk chemicals and energy which would be the core of the plant, low-volume high-value chemicals filling market gaps. Additionally, biorefineries should be able to use various types of feedstocks - that way it can adapt towards changes in demand and supply for feed, food and industrial commodities (Kamm and Kamm, 2004).

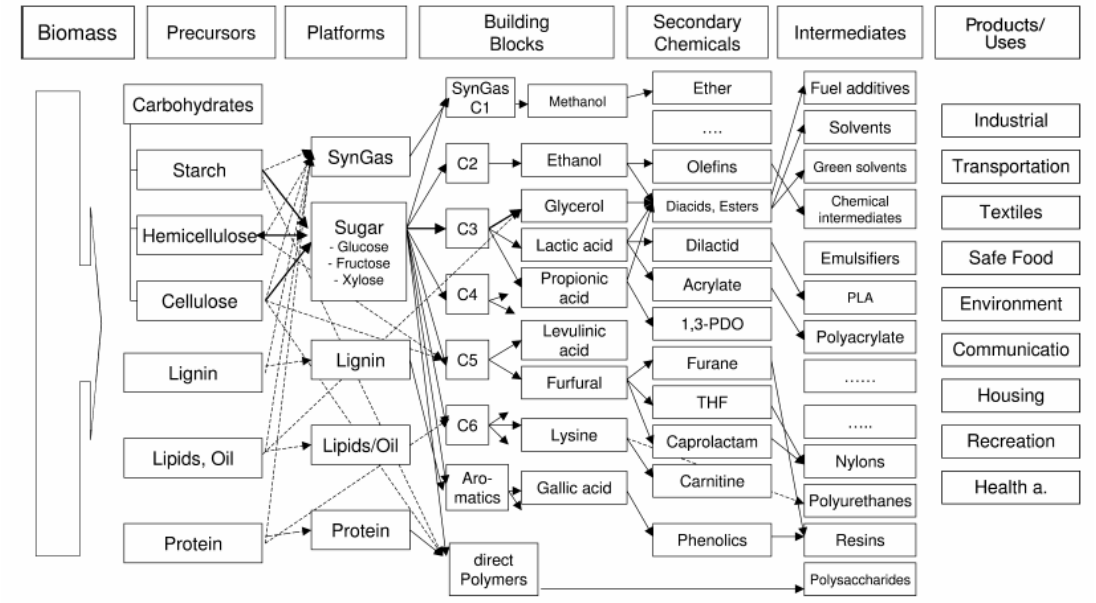

Figure 17. Model of a biobased products flow-chart for biomass feedstock (Werpy and Petersen, 2004)

4.3.1. Techno-economic models

Several techno-economic models about the potential of biofuels were published (Wingren et al., 2003; Aden and Foust, 2009; Sotoft et al., 2010). Many of them are limited to a set of scenarios and naturally cannot meet all the possible options which would be beneficial for broad biofuels community. The created techno-economic model of a lignocellulosic ethanol biorefinery presented in **Paper VIII** comes towards those needs. The model is deposited online and is available for download and evaluation; this tool can be revised by the academic and professional research community.

Scheme of lignocellulosic ethanol biorefinery is shown in the Figure 18, a detailed description of the whole process is available in **Paper VIII**. Apart from the base case, several scenarios were modeled: reducing acetate content of the biomass feedstock, increasing cellulolytic enzyme activity, reducing lignin content of the biomass content, increasing the rate of xylose-fermentation by yeast, and increasing the tolerance of yeast to acetic acid and ethanol. The total capital investment cost for all investigated scenarios (for modeled facility, which treats 2000 tons/day of wet biomass (app. 85% dry matter)) vary between 315 and 370 MM USD$.

The aim of the study was to develop a dynamic modeling tool through which different research groups, focusing on several stages in the biorefinrey process, could communicate. In that way, full techno-economic model would be created, bringing the economical and environmentally sustainable bioproducts closer (in this case liquid biofuels).

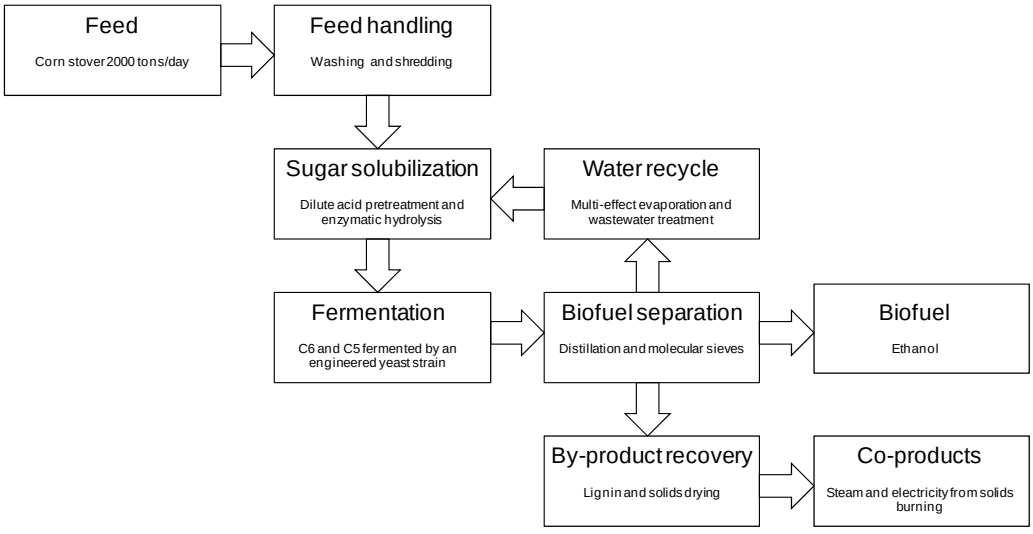

Figure 18. Scheme of lignocellulosic ethanol biorefinery (adapted from **Paper VIII**)

In **Papers VII** and **VIII**, models for two concepts of the biorefineries were discussed: the small farm scale, dedicated to develop self-sufficiency in the energy supply for an organic farm and the large industrial scale which aims for the bulk production of biofuels (bioproducts). The optimal size of biorefinery is not known yet, however, Clark and Deswarte (2008) believe that it should be a combination of large scale facilities - which would be advantageous for both economy of scale, and small scale plants - which could keep the transport cost to minimum by preprocessing and concentrating biomass or intermediate products (Clark and Deswarte, 2008). According to (Realff and Abbas, 2004) the goal is to find the balance between the increasing cost of transportation low-yield material and the reduction in costs in the increasing the scale of the process. Hess et al. (2003) indicated that to develop a sustainable biorefinery it is crucial to reduce the cost of collection, transportation and storage of biomass; it can be done through densification of raw material. Local small scale pretreatment/preprocessing units will play a significant role in a successful, economically feasible biomass based refinery. Realff and Abbas (2004) discussed that in some cases beneficial can be intermediate concentration of the biomass into a liquid form and then transport to a larger biorefinery. This will also allow farmers to play an important role in the supply chain, not only in biomass cultivation but also in the processing part.

5. Concluding remarks

In the work presented in this thesis, the possibility of biogas and bioethanol production in organic farming was investigated. The idea was evaluated from choosing and estimating the potential of raw materials through continuous trials on biogas and ethanol production, the investigation of simplified pretreatment method for lignocellulosic materials, and finally, the simulation of on-farm biorefinery concept and development of an open modeling tool for a lignocellulosic ethanol biorefinery.

The methane and ethanol potential from selected raw materials (maize, rye, clover, vetch, cattle manure and whey) were measured and evaluated. All the investigated substrates were suitable for energy production, either though anaerobic digestion or yeast fermentation. Maize and rye were characterized by the highest potential, both for methane production. During continuous trials, a significant boost of methane production was read after the increased ratio of maize silage to cattle manure in feed, and no inhibition of the process was noticed. The ensiling method was assessed for its suitability for lignocellulosic ethanol production. Maize, rye, clover and corn stover were ensiled in laboratory conditions and used as substrates in yeast fermentation. Very promising results were achieved, concluding that ensiling is a very efficient wet-storage method or even biological pretreatment method for second generation ethanol. Moreover, ethanol production by *K.marxianus* from organic whey in continuous process resulted in high ethanol productivity where neither sterilization nor pasteurization was needed. The experiments proved that whey is a suitable medium for ethanol production and successfully could be used in the on-farm biorefinery concept. Based on the results from laboratory experiments and additional literature data, simulation models for on-farm bioenergy production was built. The first results validated that there is enough land on the farm to supply it with self-produced energy. Further investigation led to the development of a techno-economic model where five scenarios were evaluated for on-farm energy generation. The aim of the presented modeling tools, both for small or industrial scale biorefineries, were to build a platform for differently sized biorefineries. The simulation models can be edited and adjusted to the specific needs. This way, the on-farm bioenergy production as well as large scale lignocelluloisc biorefineries can be brought closer to the reality.

6. Future outlook

The development of on-farm biorefineries requires a further update and optimization of the processes involved and building and adjusting simulation models, which can help the successful establishment of such a facility if required for particular needs. Several configurations should be modeled and the most suitable chosen. These configurations might differ from each other depending on the specific farm requirements. New process solutions such as ensiling method (presented in this thesis) and modern fuels (Ahlgren et al., 2009) or new generation fuels which are still in a lab phase (Steen et al., 2010) might be the answers of the future. Additionally, constructing more facilities, more on-farm biorefinery plants, will surely lead to a price drop, similar to the one experienced during development of the biogas plant in Denmark in 80s and 90s (Mæng et al., 1999).

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List of papers

Paper I: Oleskowicz-Popiel P., Thomsen M.H., Nielsen H.B., Schmidt J.E., Thomsen A.B.: Characterization of most relevant feedstock for biogas and bioethanol production in organic farming. Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, 25-28 May, 2008.

Paper II: Oleskowicz-Popiel P., Nielsen H.B., Thomsen A.B., Schmidt J.E.: Biogas and ethanol potentials in selected biomasses for organic farming. *Submitted*

Paper III: Oleskowicz-Popiel P., Schmidt J.E., Thomsen A.B.: Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomasses. *Submitted*

Paper IV: Oleskowicz-Popiel P., Lehtinen T.M., Schmidt J.E., Thomsen A.B.: Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol. *Submitted*

Paper V: Christensen A.D., Kadar Z., Oleskowicz-Popiel P., Thomsen M.H.: Production of bioethanol from organic whey using *Kluyveromyces marxianus*. Journal of Industrial Microbiology and Biotechnology, 2010, DOI 10.1007/s10295-010-0771-0.

Paper VI: Oleskowicz-Popiel P., Thomsen M.H., Thomsen A.B., Schmidt J.E.: A simulation model of combined biogas, bioethanol and protein fodder co-production in organic farming. International Journal of Chemical Reactor Engineering, 2009, vol.7, Article A71.

Paper VII: Oleskowicz-Popiel P., Schmidt J.E.: Techno-economic analysis of bioethanol and biogas production in organic farming. *Proceeding submitted to* 12th World Congress on Anaerobic Digestion, Guadalajara, Mexico, October 31st – November 4th, 2010.

Paper VIII: Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W.: Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. *Accepted*

Paper I

Oleskowicz-Popiel P., Thomsen M.H., Nielsen H.B., Schmidt J.E., Thomsen A.B.: Characterization of most relevant feedstock for biogas and bioethanol production in organic farming. Vth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunisia, 25-28 May, 2008.

Characterisation of most relevant feedstock for biogas and bioethanol production in organic farming

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Organic agriculture, organic farming, biogas, bioethanol

Abstract

Bioenergy production from local bioresources has a great potential. It is important to reduce dependency on fossil fuels and decrease green house gas emission in organic agriculture (OA). Both biogas and bioethanol can be produced in OA and significantly contribute to the sustainability of organic farms.

Soil fertility is the basis for OA: it has been of concern that the fertility might decline if most of the organic residues were converted into energy and only effluent from anaerobic digestion process was recycled. However, by intelligent management of organic residues and crop rotation it is possible to avoid decrease of soil fertility and at the same time produce renewable energy.

The presented study is part of the BioConcens project (http://www.bioconcens.elr.dk/uk/). This study is focused on characterisation of relevant feedstock for co-production of biogas and bioethanol within organic farming.

Clover grass silage, dry clover grass and dried grass from meadows were selected. Theoretical biogas and bioethanol yields were calculated. Biogas potential batch tests were performed for each substrate individually and the obtained yield in the lab was compared to the theoretical one. It is expected that the on-farm production of the bioenergy would improve not only sustainability of such a farm but as well economics. Further investigations will be carried out.

INTRODUCTION

The production of biofuels in organic agriculture (OA) can reduce its dependency of fossil fuels and decrease greenhouse-gas emission; consequently it might increase the sustainability of organic farming. Biorefinery concept based on co-production of biogas, bioethanol and protein fodder in organic farming is developed within BioConcens project (Biomass and bioenergy production in organic farming – consequences for soil fertility, environment, spread of animal parasites and socio-economy; http://www.bioconcens.elr.dk). The project is part of research programme called: "International research cooperation and organic integrity", which was commenced for a period 2006-2010. It is coordinated by DARCOF (The Danish Research Centre for Organic Farming). The whole programme, with acronym DARCOF III, consists of 15 projects (http://www.darcof.dk/research/darcofiii/index.html).

Anaerobic digestion based on animal manure and energy crops is well known technology. Due to utilisation of manure, the methane emission from livestock production is diminished. Production of heat and electricity from biogas will reduce fossil fuels usage; consequently GHG emission will be decreased. Furthermore, anaerobic digestion process improves nutrients utilisation and diminishes odour problem (Braun and Wellinger). Even though biogas technology is known as low land use and relatively low cultivation and soil-related emission, large storage facilities are required for biogas, moreover, compression of biogas is required for use as fuel (Frederiksson et al., 2006).

Biogas can be produced from any organic-carbon rich by-product. Commonly, grown in OA clover grass has a great potential as a raw material for anaerobic digestion (Jørgensen et al., 2005). Co-fermentation of clover grass with animal manure or whey permeate can be one of the examples for efficient biogas production in OA.

Bioethanol from starch can be a substitute for diesel or gasoline (Fredriksson et al., 2006), and

protein rich effluents from whey-based fermentations will be valuable product for organic pig production.
Biogas can be further utilized to produce heat electricity for organic farms (Persson et al., 2006).
Bioenergy from organic sources should not negatively influence the carbon and nutrients cycle, therefore intelligent management of organic residues and crop rotation is necessary. BioConcens project aims of design and evaluating a combined concept for biomass and bioenergy production in OA, while considering soil fertility. It is expected that the on-farm production of the bioenergy would improve not only sustainability of such a farm but as well economics.
The aim of the study was to investigate which kind of the feedstock would be favourable either to produce biogas or bioethanol to supply organic farm with necessary heat and power.

METHODS

Raw materials

Three different biomasses were selected:

- dry grass from meadows – the grass was harvested in 27-06-2007 from a meadow, in which in 2004 the hudegræs and Timotek was sown, no further changes were done in the meadow since that time
- dry clover grass (mixture of white clover grass, red clover grass separated on the field – Ø-45, **www.dlf.dk**),
- clover grass silage - mixture of white and red clover grass and rye grass, cut in November 2007 and ensilaged in bales – Ø-45, **www.dlf.dk**)

The total solids and volatile solids of the selected raw materials are shown in Table 1.

Table 1 Total solids (TS) and volatile solids (VS) of the selected organic raw materials

	TS [g/100g raw material]	VS [g/100g raw material]
Dry grass from meadows	89.1	83.0
Dry clover grass	91.3	83.3
Clover grass silage	71.3	64.0

Strong acid hydrolysis

The strong acid hydrolysis is an analytical method to determine full content of main sugars in the biomass. The biomasses (0.16 g DM) were treated with 1.5mL of H_2SO_4 (72%) at 30^{o}C for one hour, and then 42 mL of water was added and the samples were autoclaved (121^{o}C) for one hour. The acid hydrolyzate was filtered and the glucose, xylose, and arabinose were quantified by HPLC (Biorad HPX-87H). Klason lignin was calculated as the ash free residue after hydrolysis. The ash content was determined by heating for 3 h in an oven at 550°C.

Theoretical ethanol yield

The theoretical bioethanol potential was calculated based on the total amount of glucan in biomass from the formula:

$$Y_{EtOH}^{T} = 0.51 \cdot m_{Glucose} \text{ [g/100gTS]}$$

$$m_{Glucose} = 1.11 \cdot m_{Glucan},$$

Anaerobic digestion batch trials

A procedure for measuring the biogas potential for organic raw materials was developed. In order to optimize the process, two different concentration of the energy crops were prepared: 2.0; and 4.0 organic matter per 100g of the solution [gVS/100g] (where VS – volatile solids). Inoculum used in the experiment was effluent (digestate) from one of the Snertinge biogas plant (Denmark). 100g of the mixture of energy crop and inoculum was placed in into 500mL flasks; the bottles were flushed with nitrogen to remove the oxygen from the headspace and closed tightly in order to keep anaerobic conditions. Batch fermentation trials were performed in triplicates. The anaerobic digestion had been running in thermophilic conditions (53^{o}C) for around 40 days. The methane concentration in the headspace of the bottles was weekly measured with a gas chromatography (GC).

RESULTS AND DISCUSSION

Theoretical ethanol yield of selected lignocellulosic raw materials

The theoretical ethanol yield was calculated based on the total glucose content. The strong acid hydrolysis was performed to determine the total content of sugars in lignocellulosic biomass. The three main sugars (glucan, xylan and arabinan) were measured by HPLC (high pressure liquid chromatography). The most important is the polymer of glucose – glucan. Results are shown on the figure 1, similar were obtained by Neureiter et al., (2004). In typical yeast based ethanol fermentation only glucose is converted into ethanol. The rest of the sugars remain in the process effluent, which can be either used as a animal feed or further fermented to methane through anaerobic digestion process.

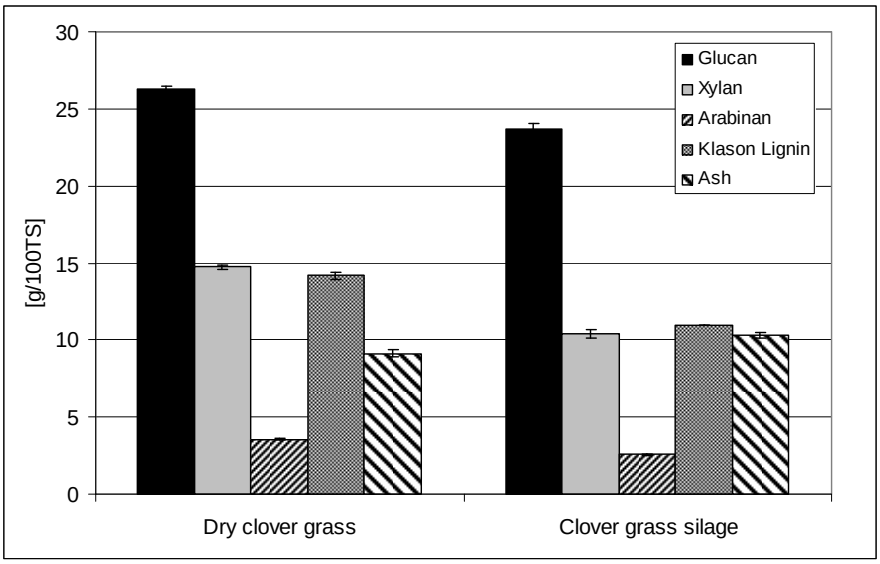

Figure 1 Sugars, Klason lignin and ash concentration in clover grass (dry and silage).

The theoretical ethanol yield based on glucose content was calculated and it is presented in table below.

The theoretical ethanol yield was calculated and it was found to be 14.9 and 13.4 [g/100TS] for dry and silage clover grass, respectively.

The dry clover grass contains more glucan compared to the silage form, which consequently gives higher theoretical ethanol yield. During the ensilaging process of the clover grass, the lactic acid bacteria are utilizing glucose to lactic acid, which cannot be further fermented into ethanol by yeast strains, therefore the lower ethanol yield. Future work will include measurements of the

sugars and calculation of the theoretical ethanol yield for following organic raw materials: maize (fresh and silage), vetch and rye, and whey permeate.
Enzymatic hydrolysis of the investigated raw materials including both, cellulase and amylase enzymes (to reveal glucose monomers from cellulose and starch, respectively) would be necessary to investigate the practical ethanol yield.

Biogas potential of selected raw materials

Practical biogas potential was measured for dry grass from meadows, dry clover grass and clover grass silage. Similar range of biogas yield was achieved by Amon et al., (2007), where potential of energy crops was investigated. The biogas potential of energy crops depends on time and place of harvest, ensilage method, etc., therefore it can differ even between the same species but harvested in different years.
It was concluded that the most optimal concentration to estimate the biogas potential of the energy crop was 2.0 gVS/100g solution. With the content of 4gVS/100g, the organic overloading was observed. This process inhibition occurs when more substrate is fed to the bioreactor than microorganisms can degrade (Angelidaki, 2002). The biogas production was significantly inhibited during the first 4 days of the process and after 38 days reached only around 200mLCH_4/gTS for all three substrates, therefore full biogas potentials could not be measured at that concentration. Further measurements of the biogas potentials of raw materials will be carried out only at 2.0 gVS/100g solution.

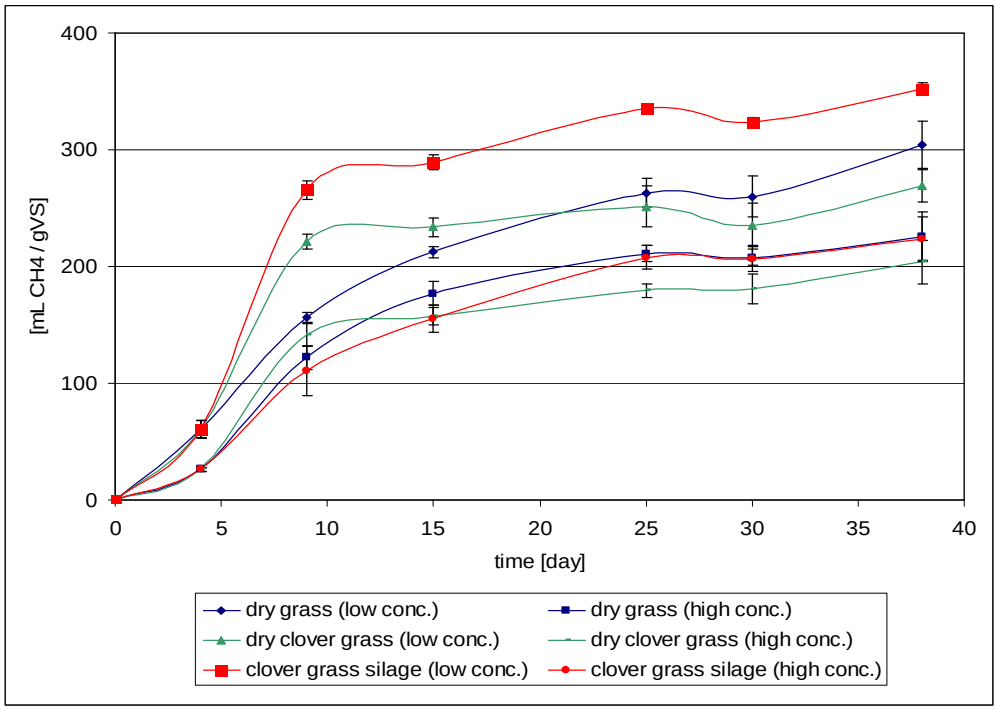

Figure 2 Biogas potential of dry grass from meadows, dry clover grass, and clover grass silage in two different concentrations: low (2gVS/100g) and high (4gVS/100g).

Table 2. Biogas potential of selected raw materials

	[mLCH_4/gTS]	[mLCH_4/gVS]
Dry grass from meadows	326 ± 21	304 ± 21
Dry clover grass	295 ± 14	269 ± 14

Clover grass silage	392 ± 5	352 ± 5

Table above summarizes the biogas potential of the investigated biomass. The biogas potential is expressed as mL of methane per 1g of total (dry matter) or volatile (organic matter) solids.

Most of the biogas was produced during the first 10-15 days of the incubation. Clover grass silage seems to be the most promising feedstock from the presented ones. Energy crop in form of silage might be more effective for the biogas production, due to easier access for microorganisms to the valuable organic compounds.
Further measurements of the biogas potential of other organic raw materials originating from organic farming (such as: maize (fresh and silage), vetch and rye, and whey permeate) is planned. Moreover, scale-up to 5L bioreactors for further optimization of the process will be performed for the most promising feedstock among selected ones.

CONCLUSIONS

From these initial results following conclusions can be drawn:

- The theoretical ethanol yield of the dry clover grass was slightly higher compared to clover grass silage (14.9 and 13.4 g/100TS, respectively). It was caused by converting part of the sugars into lactic acid during the ensilaging process which cannot be formed into ethanol;
- The highest biogas yield from the investigated raw materials was achieved from clover grass silage (394mL CH_4/gTS). The energy crop in form of silage can be easier degraded to the biogas during anaerobic digestion process than dry lignocellulosic material;

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Paper II

Oleskowicz-Popiel P., Nielsen H.B., Thomsen A.B., Schmidt J.E.: Biogas and ethanol potentials in selected biomasses for organic farming. *Submitted*

Biogas and ethanol potentials in selected biomasses for organic farming

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Abstract

The production of bioenergy in organic agriculture (OA) can reduce its dependency on fossil fuels and decrease greenhouse-gases (GHG) emissions; consequently increasing the sustainability of organic farming. Different biomasses were characterized biologically and chemically for their biogas and bioethanol potential at organic farms in Denmark.

Batch experiments indicated methane yield [GJ/ha]: 260.7 (fresh maize), 272.7 (maize silage), 127.1 (fresh rye), 169.7 (rye silage), 161.7 (dried rye), 110.5 (fresh clover), 117.8 (clover silage), 72.3 (dried clover), and 41.1 (dried vetch). Theoretical ethanol yield [GJ/ha] showed: 119.8 (fresh maize), 109.2 (maize silage), 36.9 (fresh rye), 39.9 (rye silage), 84.7 (dried rye), 28.9 (fresh clover), 24.4 (clover silage), 32.7 (dried clover), and 18.8 (dried vetch). The continuous biogas trials with maize silage resulted in 86% of yield of the batch experiments. All of the raw materials were suitable for biorefinery at the organic farm.

Keywords

Sustainable agriculture, organic farming, anaerobic digestion, bioethanol

1. Introduction

There are growing numbers of organic farms throughout Europe (Hermansen et al., 2004). The principle philosophy of organic agriculture is to focus on mechanisms to improve the richness and stability of the soil by restoring its organic matter and avoiding synthetic fertilizers, pesticides and herbicides (Macilwain, 2004). From those core concepts, another has grown: one of the modern efforts in organic farming is to increase the usage of renewable resources in production and processing systems (IFOAM), which in the recommendations for Danish organic farmers is directly connected to the limited usage of fossil fuels and switch to renewable energy (Økologisk Landsforening). However, there is a great concern among organic farmers that using organic sources such as straw for energy production will diminish the amount of carbon and nutrients recycled to the soil and thereby reduce its fertility. Still, a vast amount of organic matter (i.e. lignin substances) remains after both ethanol and biogas production and it was proven that returning such effluents to the soil had a positive effect on the nutrient balance on the farm (Holm-Nielsen et al., 1997; Tidåker P. et al., 2006) which is essential for organic agriculture systems (Haas et al., 2002).

Biomass is stored solar energy and a CO_2 neutral resource, which can be used for direct energy production from combustion or transformed through biological conversion methods into energy carriers such as ethanol and biogas. There are many varieties of these renewable resources in agricultural farming, which could serve as feedstock in a bioethanol or biogas production (Thomsen, 2005; Petersson et al., 2007). The biomass conversion can take place directly at the farm (Svensson et al., 2005) or outside the farm (Ahlgren et al., 2008). Both fermentations of biogas and bioethanol are technological simple and should relatively easy be implemented in an organic farm.

Ethanol can be produced from sugar, starch or any lignocellulosic biomass available at the farm (Jacques et al., 1999; Thomsen and Haugaard-Nielsen, 2008), and can substitute fossil fuels needed to power the agricultural machinery (Mandil eds., 2004). Ethanol fermentation is a biological process based on the conversion of sugars into ethanol (and carbon dioxide) by means of microorganism. In the absence of oxygen, it is possible to produce ethanol from glucose, fructose and sucrose by simple yeast fermentation. The difficulties occur when generating ethanol from lignocellulosic feedstock. In this case a pretreatment step is necessary to open up the lignocellulosic structure and further hydrolyse it into fermentable sugars (Thomsen et al., 2006; Schmidt and Thomsen, 1998; Yang and Wyman, 2008). Throughout pretreatment, the lignocellulosic biomass is fractionated into polymers of a solid cellulose, soluble hemicelluloses and lignin being partly soluble, partly insoluble. In order to estimate full ethanol potential, the amount of sugars in raw biomass has to be determined and the

theoretical potential calculated. Practical ethanol potential can vary depending on pretreatment method and the choice of microorganisms. Moreover, diverse crops (fresh, dry or ensiled) have different optimal conditions for pretreatment.

An alternative option for the on-farm bioenergy production is biogas production through anaerobic digestion, which can be based on animal manure and a wide range of biomass resources such as: leaves, grasses, energy crops, and any other organic residues available at the farm (Börjesson and Berglund, 2007). Anaerobic digestion is a biological process where most organic matter (carbohydrates, lipids, proteins) except for lignin components, in the absence of oxygen, is degraded into methane and carbon dioxide. The process principles are well described by Angelidaki et al., (2002). Anaerobic digestion is an effective way to provide not only energy, but to reduce greenhouse gas emissions due to controlled conversion of animal manure to a renewable energy carrier (methane) replacing fossil fuels and preventing CO_2 production from landfilling. The produced biogas can be used for the generation of heat and electricity as well as fuel for farm equipment (Börjesson and Berglund, 2006; Börjesson and Mattiasson, 2007). The effluent (digestate) from the process rich in N, P, K, as well as Ca, Mg and micro-nutrients, can be applied as organic fertilizer (in organic farming, the use of synthetic fertilizer has to be excluded or strictly limited (IFOAM)). The biomethane potential, in opposite to ethanol potential, is estimated through batch experiments (Angelidaki et al, 2009).

The objective of this study was to estimate the bioenergy potential of several different energy crops available at the Danish organic farm. This is initial study to get general overview of biomass potential for energy production at the organic farm. We chose two conversion technologies as being the most feasible to set up at an organic farm: ethanol fermentation and biogas production through anaerobic digestion. The farm scale biogas/bioethanol plant could be established to process agricultural by-products from one single farm or two/three neighbouring farms (Svensson et al. 2005). The selected feedstock were characterised by their practical methane yields and theoretical ethanol yields.

2. Materials and Methods

2.1. Raw Materials

Eleven different raw materials available at the Danish organic farm were compared: fresh and silage maize (the whole crop); fresh, silage and dried clover grass (the whole crop); fresh, silage (the whole crop, harvested premature) and dried (the whole crop, harvested mature) rye; dried vetch (the whole crop), whey permeate and

cattle manure. The crops were harvested at the test fields of Risø DTU, the National Laboratory for Sustainable Energy - Technical University of Denmark. Table 1 shows details about each crop.

Fresh crops were kept in a freezer from the harvesting time until the experiments were performed. Dried crops were stored at room temperature in a storage room. To prepare silage crops, fresh crops were mixed with Biomax Si forage additive containing Lactic Acid bacteria (to achieve stable silage production), packed in plastic bags and kept in a laboratory under anaerobic conditions at room temperature for about 30 days after that the silage crops were stored in the freezer prior to analysis. Whey permeate was collected from the Thiese Dairy Farm (Jutland, Denmark) and was kept in a fridge at 5^{o}C. Cattle manure, collected from the Danish organic farm (owned by Gårdejer Johan Frederiksen, Smedevej 18, Kyndeløse, 4070 Kirke Hyllinge, Denmark) , was stored in a freezer prior to analysis.

2.2. Total and volatile solids

Total solids (TS) and volatile solids (VS) were measured according to standard methods (Greenberg et al., 1998), to measure TS the samples were heated over night at 105°C, for VS measurement sample were ashed at 550°C for 3 hours.

2.3. Sugar content in biomass

After 24 hours extraction in boiling ethanol (to remove non-structural components such as waxes, fats and colouring matters) the sugar content in biomass was determined according to NREL method (Sluiter et al., 2005) by “strong acid hydrolysis” procedure which is an analytical method to determine the full content of the main sugars within the biomass. The biomasses (0.16 g DM) were treated with 1.5mL of H_2SO_4 (72%) at 30^{o}C for one hour, and then diluted with water and autoclaved (121^{o}C) for one hour. The acid hydrolyzate was filtered, and the glucan, xylan, and arabinan were quantified by HPLC (Biorad HPX-87H).

2.4. Theoretical ethanol potential

The theoretical ethanol potential was estimated as the amount of ethanol Y_{EtOH} which can be produced from the amount (determined as described above) of glucose (C6 sugar) or glucose and xylose (C5 sugar) in the biomass, from the formula:

$$Y_{EtOH}^{T} = 0.51 \cdot m_{Sugar}\ [g/100gTS]$$

2.5. Biogas batch fermentations

The biogas trials were made batch wise, testing 100g of the mixture of raw biomass and inoculum, placed into 500mL flasks; the bottles were flushed with nitrogen to remove oxygen from the headspace and closed tightly in order to keep anaerobic conditions. Each trial was carried out in 2 different concentrations: 1.0 and 2.0 gVS (volatile solids) of organic matter per 100g [gVS/100g]. Inoculum used in the experiment was an effluent (digestate) from the thermophilic Snertinge Biogas Plant (Denmark), treating cattle/pig manure (approximately 70-80% of TS) and various kinds of industrial waste. The anaerobic digestion took place in thermophilic conditions (55°C) for approximately 40 days. The methane concentration in the headspace of the bottles was measured on a weekly basis by a gas chromatography (GC) equipped with a flame ionization detector. Batch fermentation trials were performed in triplicates.

2.6. Continuous anaerobic digestion trials

Two 4.5 litre, continuously stirred, tank reactors (CSTR) with a 3.0 litre working volume were inoculated with effluent from a full-scale biogas plant (Snertinge, Denmark). Both reactors were equipped with a propeller, enabling efficient stirring of the reactor content, and a stable operational temperature (52°C) was ensured by circulating the heated water from a water bath through a reactor jacket (Nielsen et al., 2008). Biogas was collected in 10 litre gas bags. The reactors were fed 5 times a week (Monday to Friday). CH_4 and CO_2 production from the reactors were determined by gas chromatography equipped with a flame ionization detector.

During start up, the feedstock only consisted of organic cattle manure with a TS and VS percentage of 7.3±0.1 and 5.4±0.1, respectively. The loadings were gradually increased each time over a period of seven days: first increase from 0-17.5%, second one 17.5-25.8% and finally from 25.8 to 33.0% of VS fed to the reactor. The full loading resulted in 3.0 gVS per litre per day and a hydraulic retention time (HRT) of 18 days. This loading mode was maintained in a control reactor for the rest of the experiment. When a stable biogas production of two consecutive weeks was obtained in a test reactor, the loading in that reactor was increased by adding macerated whole crop maize silage. Each loading increase was maintained for at least one HRT and two weeks of stable biogas production.

3. Results and Discussion

3.1. Raw materials composition

An overview of all investigated raw materials used to produce bioenergy at the organic farm, from a simple total and volatile solids analysis, is shown in Table 2. Fresh and silage crops were characterized by high water content (around 70% for maize and more than 80% for rye and clover). Dried crops had typically up to 10% water content. Ratio between total solids and organic matter did not significantly changed during the ensilaging or drying processes.

In biogas production, the total organic content (volatile solids) is a good indicator of the theoretical methane potential. In ethanol production, only sugars can serve as substrate and only C6 sugars can be efficiently fermented by non-GMO microorganisms, meaning that C6 sugars are the substrates on which attention should be focused on in organic farming. Since glucose derives from cellulose and starch (in total named glucan) those components are considered as substrates for ethanol production. In other words, the ethanol yield is limited to the concentration of glucose in feedstock. The sugar concentrations of the selected biomasses are presented in Figure 1. The sugar analysis shows also the contents of C5 sugars represented as the hemicelluloses polymers xylan and arabinan.

Maize (both fresh and silage) and dried rye were characterized by the highest glucan concentration (44.3, 40.3 and 49.9 g/100gTS, respectively), followed by dried vetch (30.1 g/100gTS) and dried clover (26.3 g/100gTS) (Figure 1). Rye (fresh – 21.7 g/100gTS and silage – 21.8 g/100gTS) and clover (fresh – 23.2 g/100gTS and silage – 21.0 g/100gTS) had the lowest glucan content in the investigated biomasses. A slightly lower content of sugars in ensiled materials when compared to fresh green biomasses could be explained by the partial conversion of free sugars into a lactic acid. It is important to notice significantly higher amount of sugars per gram of total solids in dried crops, compared to fresh and ensiled.

Cattle manure and whey had noticeably lower TS/VS values and were considered mainly as nutrients and water process sources. Manure and whey can be significant contributors of macro and micro nutrients such as (N, P, K, Ca, Mg, Fe, Zn, Mn) and (Co, B, Cd, Cr, Co, I, Mo, Ni, V), respectively, to be supplemented in a microbial fermentation. Both manure and whey can be used for biogas production. The content of lactose (a dimer of mannose and glucose) makes whey suitable feedstock for the bioethanol fermentation.

3.2. Batch and continuous anaerobic digestion trials

To calculate the energy potential of different crops, the practical methane yield of all biomasses was measured. In Figure 2, the example of batch fermentation trials with dried rye (the whole crop) and dried vetch (the whole crop) is shown.

To assess the methane potential, the trials were run for approximately 40 days, and to see if there is any further degradation, the biomass trials were prolonged by up to 80 days. However, an insignificant increase in methane yield was observed after 40 days of anaerobic digestion. In all cases, around 80% of the methane yield was achieved within the first 20 days, which is the usual retention time for most Danish biogas plants.

The trials were carried out in two concentrations: 1.0 and 2.0 gVS of feedstock per 100 gram of the solution to find optimal substrate loading (Figure 2). In Figure 3, the presented methane yields of investigated biomass are from trials conducted at concentration 1.0 gVS/100gVS.

With the aim of a more detailed investigation on the influence of silage crops on anaerobic digestion, a continuous fermentation with cattle manure and maize silage was carried out. The trials were carried out for approximately 120 days, including 40 days for stabilization of the process and the hydraulic retention time was 18 days (Figure 4). The first load of 17.5% of maize silage was added on day 56 resulting in a methane yield increase of approximately 12% when compared to the control reactor (only fed with cattle manure) (from 205 till 231 $mLCH_4$/gVS). When further boosted with 25.8% and 33.0% VS maize silage the methane yield increased by 17% and 25%, respectively. Figure 4 - graph A shows methane production per gVS in control and test reactor, additionally the percentage of VS originated from maize silage is indicated. On the part B of the Figure 4, the methane production per g of feed added is presented. The increasing amount of VS added to the test reactor during the trial is shown there as well. On both graphs of the Figure 4, it is noticeable that addition of maize silage boosted significantly methane production. At the end of the trial, the methane yield in a reactor fed with maize silage was equal to 266$mLCH_4$ per gVS.

3.3. Biogas production

Ensiled crops are the most promising substrates for agriculture biogas production, as they gave the highest methane potential. Methane yield is even higher than in fresh crops, which means that a fraction of the lignocellulosic structure could have been broken down during silage process and the greater part of the biomass is digested. Dried clover and dried vetch has the lowest potential of all crops. Such biomass is not easily attacked

by microbes and potential pretreatment prior to the anaerobic digestion process could increase the yield. Cattle manure contains only solids which already have passed through the natural digestion process (cow's stomach), so there is no surprise that those are not easily decomposed during the anaerobic digestion. Whey, which contains a lot of proteins and sugars (lactose), is obviously easily fermentable. However, one should be aware of low TS/VS content in this biomass. Whey could definitely serves as process water for fermentation process.

During continuous anaerobic digestion with cattle manure and maize silage, methane yield originating from maize silage varied between 304 and 384 $mLCH_4$ per gVS, which is comparable to the yield obtained from biogas plants running on similar feedstock. No inhibition was observed in the presented trials; therefore, the further addition of this feed would be possible and a further increase of methane production would be expected. Although the overall yield was lower than the methane potential of maize silage measured in batch fermentation trials. It could be concluded that there is still space for improvement and higher methane yield could be achieved from this feedstock in a continuous anaerobic digestion process. Most likely, prolonging the hydraulic retention time would result in the improved digestibility of maize. In German and Austrian biogas plants, which are running on energy crops (Resch et al., 2008), it is common practice to prolong retention time, and in that way, almost maximum yield can be achieved (Braun, 2007). Optimum conditions between retention time and methane yield have yet to be found.

3.4. Methane and ethanol for organic farms

The presented results indicate that both biogas and bioethanol can be produced from all the examined raw materials available at the organic farm. Table 3 present the yields: the practical methane yield obtained during batch experiments and theoretical ethanol yield calculated from sugars content in the feedstock.

No experiments on practical ethanol yield were performed, to estimate full ethanol potential only sugar analysis is necessary. To perform ethanol fermentation trials, there is need of pretreatment method and choice of proper microorganisms. The results would be strongly influenced by these choices. Additionally, different raw materials require differently optimized pretreatment method. The scope of this paper was to show highest possible ethanol which could be produced from selected raw materials available at the organic farm.

In order to compare these two technologies (biogas and ethanol), yields were recalculated for MJ per kg of dry matter (TS) and for GJ per hectare. It obvious that the

methane yield is significantly higher compared to ethanol, primarily because ethanol yield is based only on sugars, whereas methane is produced from a variety of chemical compounds (sugars, proteins, fats). One could justify bioethanol fermentation, however, the production of liquid biofuel might have several advantages over gaseous one (liquid biofules are compatible with current vehicles and blendable with current fuels, and they can share established liquid fuel distribution infrastructure, etc. (Mandil eds., 2004)). Additionally, the effluent from ethanol fermentation could easily serve as either a substrate for anaerobic digestion process or a rich in protein and remaining sugars (if C5 are not to be fermented) valuable animal feed. Another option would be an improvement of ethanol production through the introduction of microorganisms which can also ferment C5 sugars (Hahn-Hagerdal et al., 2007). In this way, the ethanol yield would significantly increase (Table 3).

Maize silage is a very “efficient” crop for both processes, and it gives both high methane and high ethanol yield. Dried rye is also “energetic” and it results in the highest theoretical ethanol potential, but, as mentioned before, the investigated biomass was the whole crop containing matured and well developed rye seeds – the high starch content resulted in high theoretical ethanol yield. For the organic farm biorefinery, rye straw is most likely to be used, which have slightly lower ethanol potential: 23.1 gEtOH/100gTS for rye straw compared to 28.3 gEtOH/100gTS for the whole dried crop of rye. It is interesting to take note of both rye and clover silages’ potential: they give similar methane yield compared to maize silage but significantly lower theoretical ethanol yield. Obtained results are very important for designing on-farm organic biorefinery and selecting the proper feedstock for each of the process. If both fuels are to be produced, rye and clover grass silages’ would be preferred substrates for biogas production, whereas maize silage could be suitable feedstock for bioethanol production. Dried vetch gives average yields for both processes and it would be a sufficient co-substrate. Although low in energy content, vetch is an important crop for organic farming – similar to clover grass, it is a nitrogen-fixing plant, and might have a positive influence on soil and a potentially positive impact on the fermentation processes due to high nitrogen content. Comparing energy yield per hectare shows maize having the highest yield, followed by rye and then clover grass and vetch (Table 3).

It was shown that biorefinery for organic farm could use all investigated raw materials for energy production. It should be mentioned, that while considering the yields of the different biomass, one should not forget about the energy input for each crop and the influence on soil or water demand (EEA Technical report, 2007).

4. Conclusions

All raw materials are suitable for biorefinery at the organic farm. Maize silage and rye silage are characterized by highest biogas potential. During continuous biogas trials based on cattle manure and maize silage up to 86% of yield from maize silage (compared to batch trials) was achieved. Higher methane production per g of VS can be achieved by prolonging the hydraulic retention time of the fermentation.

For ethanol production, dried rye has the highest potential followed by fresh maize. The results indicated that both processes (ethanol fermentation and anaerobic digestion) could have a positive impact on the organic farm energy supply with reduced fossil fuels usage and consequently reduced GHG emission.

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List of figures:

Tables

Table 1. Harvest time and yield of the investigated crops

Crop		Yield [tTS/ha]	
Maize	*Zea mays* L. ssp.	16.1 ± ND	It was cultivated at the research farm of the University of Copenhagen, Faculty of Life Sciences. The cultivar was Companero.
Rye	*Secale cereale*	10.1 ± 0.8	It was harvested as green rye for silage (a precrop before sowing maize to avoid black soil (fallow) during autumn and winter. The cultivar was Carotop.
Clover grass mixture		7.4 ± 0.2	The seed mixture was ForageMax 42 (DLF Trifolium, Denmark, 2010)
Vetch	*Vicia villosa*	3.7 ± 0.2	The cultivar was Latigo.

Table 2. TS/VS of the raw materials

Crop	TS [g/100g]	VS [g/100g]	VS [%TS]
Fresh maize (the whole crop)	32.1 ± 3.1	30.6 ± 3.1	95.3 ± 3.1
Maize silage (the whole crop)	29.8 ± 3.2	28.3 ± 3.3	95.0 ± 3.3
Fresh rye (the whole crop)	16.5 ± 0.8	15.4 ± 0.8	93.3 ± 0.8
Rye silage (the whole crop)	15.6 ± 0.2	14.5 ± 0.2	93.0 ± 0.2
Dried rye (the whole crop)	91.9 ± 0.1	88.6 ± 0.1	96.4 ± 0.1
Fresh clover (the whole crop)	18.4 ± ND	16.6 ± ND	90.2 ± ND
Clover silage (the whole crop)	17.4 ± ND	15.4 ± ND	88.5 ± ND
Dried clover (the whole crop)	91.3 ± 0.2	83.3 ± 0.7	91.2 ± 0.7
Dried vetch (the whole crop)	92.0 ± 0.1	85.0 ± 0.1	92.3 ± 0.1
Cattle manure	7.3 ± 0.1	5.4 ± 0.1	74.0 ± 0.1
Whey	6.0 ± 0.0	5.5 ± 0.0	91.7 ± 0.0

Table 3. Bioethanol yield calculated from sugars content and biogas yield measured during batch experiments

Crop	Theoretical ethanol yield: C6 sugars			Theoretical ethanol yield: C5+C6 sugars			Practical methane yield		
	[gEtOH/ 100gTS]	[MJ/kgTS]	[GJ/ha]	[gEtOH/ 100gTS]	[MJ/kgTS]	[GJ/ha]	[mLCH4/ gTS]	[MJ/kgTS]	[GJ/ha]
Fresh maize (the whole crop)	25.1 ± 0.5	7.4 ± 0.2	119.8 ± 2.4	31.8 ± 1.2	9.5 ± 0.4	152.2 ± 5.7	407 ± 25	16.2 ± 1.0	260.7 ± 15.9
Maize silage (the whole crop)	22.8 ± 0.5	6.8 ± 0.2	109.2 ± 2.6	29.7 ± 0.6	8.8 ± 0.2	142.1 ± 3.0	426 ± 22	16.9 ± 0.9	272.7 ± 14.2
Fresh rye (the whole crop)	12.3 ± 0.1	3.7 ± 0.1	36.9 ± 3.3	20.3 ± 0.3	6.0 ± 0.1	60.9 ± 5.8	316 ± 40	12.6 ± 1.6	127.1 ±26.2
Rye silage (the whole crop)	12.3 ± 1.3	4.0 ± 0.4	39.9 ± 7.2	19.8 ± 2.4	5.9 ± 0.7	59.3 ± 12.0	422 ± 9	16.8 ± 0.4	169.7 ± 17.1
Dried rye (the whole crop)	28.3 ± 1.2	8.4 ± 0.4	84.7 ± 10.3	36.9 ± 1.7	11.0 ± 0.5	110.6 ± 13.8	402 ± 31	16.0 ± 1.2	161.7 ± 25.3
Fresh clover (the whole crop)	13.1 ± ND	3.9 ± ND	28.9 ± 0.8	19.5 ± ND	5.8 ± ND	42.9 ± 1.2	375 ± ND	14.9 ± ND	110.5 ± 3.0
Clover silage (the whole crop)	11.1 ± 0.4	3.3 ± 0.1	24.4 ± 0.7	17.9 ± 0.1	5.3 ± 0.1	39.3 ± 1.3	400 ± ND	15.9 ± ND	117.8 ± 3.2
Dried clover (the whole crop)	14.9 ± 0.1	4.4 ± 0.1	32.7 ± 1.2	23.2 ± 0.3	6.9 ± 0.1	51.1 ± 1.9	245 ± 12	9.8 ± 0.5	72.3 ± 5.6
Dried vetch (the whole crop)	17.1 ± 0.2	5.1 ± 0.1	18.8 ± 1.2	24.8 ± 0.4	7.4 ± 0.1	27.3 ± 1.9	279 ± 13	11.1 ± 0.5	41.1 ± 4.1
Cattle manure	-	-	-	-	-	-	174 ± 6	0.5 ± 0.0 [MJ/L]	-
Whey	~2.4 [g/100mL]	~1.4 [MJ/L]	-	-	-	-	625 ± 48	1.5 ± 0.1 [MJ/L]	-

HHV_{EtOH}=29.7 MJ/kg

HHV_{CH4}=55.5 MJ/kg=39.8 kJ/dm^3

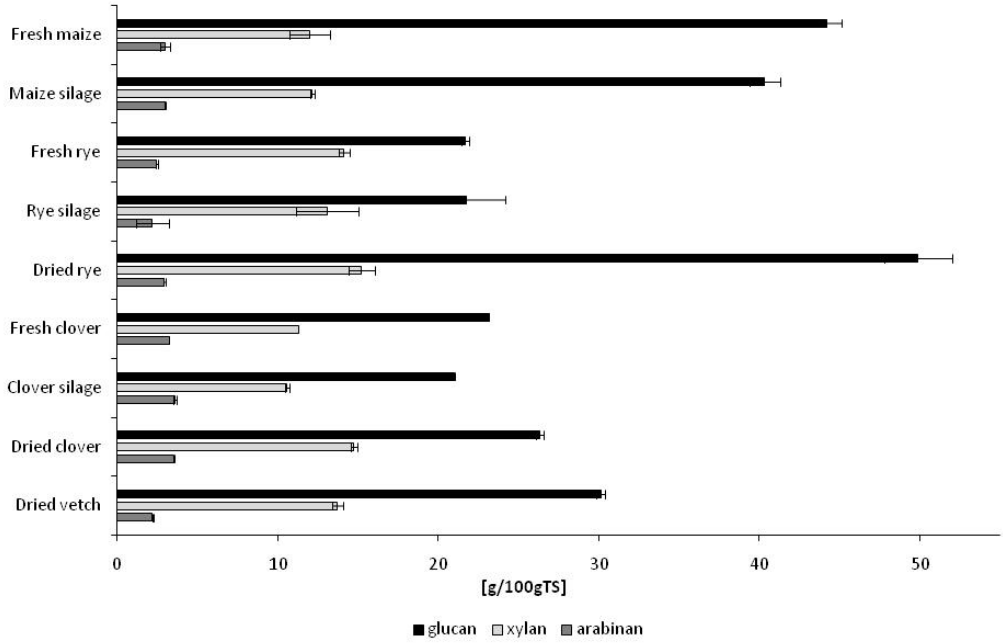
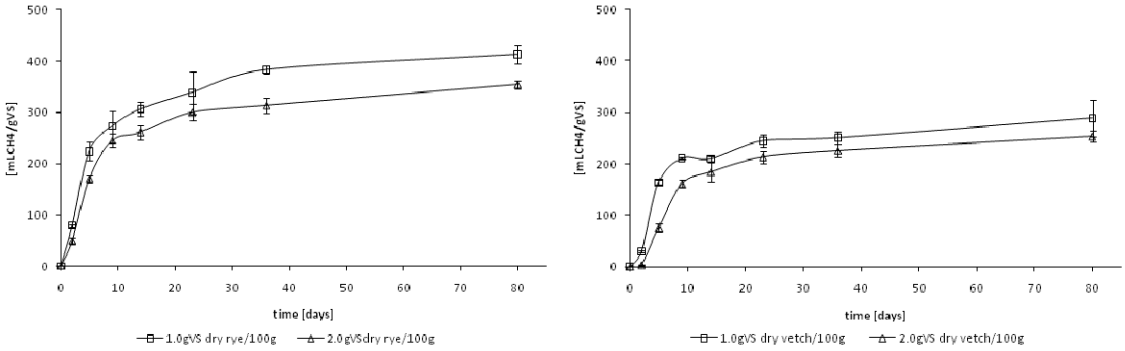

Fresh maize
Maize silage
Fresh rye
Rye silage
Dried rye
Fresh clover
Clover silage
Dried clover
Dried vetch
0
10
20
30
40
50
[g/100gTS]
glucan
xylan
arabinan

Figure 1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400
300
200
100
0
[mLCH4/gVS]
0
10
20
30
40
50
60
70
80
time [days]
1.0gVS dry rye/100g
2.0gVSdry rye/100g
500
400
300
200
100
0
[mLCH4/gVS]
0
10
20
30
40
50
60
70
80
time [days]
1.0gVS dry vetch/100g
2.0gVS dry vetch/100g

Figure 2.

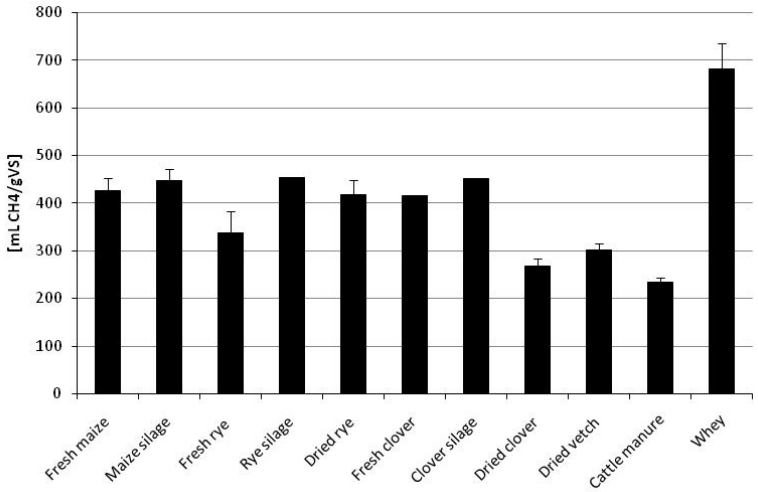
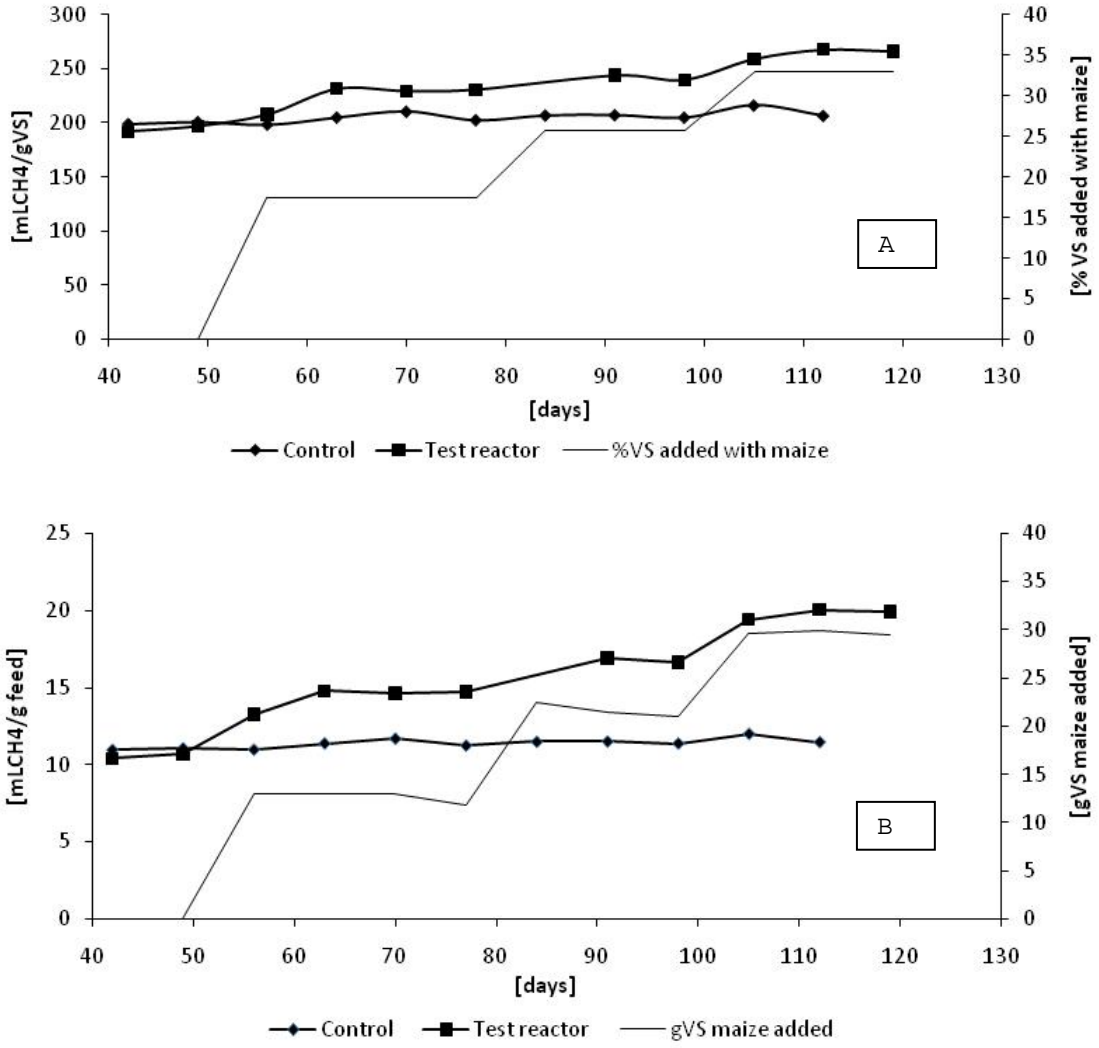

800
700
600
500
400
300
200
100
0
[mL CH4/gVS]
Fresh maize
Maize silage
Fresh rye
Rye silage
Dried rye
Fresh clover
Clover silage
Dried clover
Dried vetch
Cattle manure
Whey

Figure 3.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[% VS added with maize]
[days]
A
Control
Test reactor
%VS added with maize
[mLCH4/g feed]
[gVS maize added]
[days]
B
Control
Test reactor
gVS maize added

Figure 4.

Paper III

Oleskowicz-Popiel P., Schmidt J.E., Thomsen A.B.: Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomasses. *Submitted*

Ensiling – a wet-storage and a biological pretreatment method for bio-ethanol production from lignocellulosic biomass

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Short running title: Wet-storage and biological pretreatment for bioethanol production

Abstract

Ensiling (silage pretreatment) of humid biomass samples wrapped in plastic bales has been investigated as a wet-storage and low-cost alternative to traditional pretreatment techniques for bioethanol production from three lignocellulosic biomass samples i.e. maize, rye, and clover grass. During the silage process, lactic acid bacteria fermented free sugars to lactic acid, and consequently by lowing pH, inhibiting other microbes to degrade the polysaccharides; at the same time, microbes partly disrupt the lignocellulosic structure making it accessible for controlled enzyme attack. Following silage treatment, enzymatic convertibility tests showed that 51.5%, 36.5%, and 41.9% of the cellulose was converted by cellulytic enzymes in ensiled maize, rye, and clover grass, respectively. In addition, tests of SSF (simultaneous saccharification and fermentation) were carried out using combined enzymatic hydrolysis and fermentation with two different microorganisms *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, the ethanol production was 33.9%, 28.5%, and 36.9% (by *K.marxianus*) and 30.6%, 28.1% and 34.5% (by *S.cerevisiae*); the yields significantly increased after hydrothermal pretreatment: 79.0%, 74.6%, and 80.2% (by *K.marxianus*) and 72.7%, 81.3% and 76.2% (by *S.cerevisiae*) of the theoretical based on the C6 sugar contents in untreated silage of maize, rye, and clover grass, respectively.:

Key words

Pretreatment, silage, ensiling, ethanol, lignocellulose

Introduction

Bioethanol from lignocellulosic materials has a potential to become an important sustainable fuel in the coming years. It can substitute fossil fuels needed in the transportation sector (Mandil eds., 2004), and fuel ethanol is already produced in vast amounts (74 billion litres in 2009). In summary, almost 90% of all produced fuel ethanol comes from both Brazil (from sugar cane) and USA (mainly from corn) (RFA, 2010). Since sugar cane and maize are grown at these places for primarily ethanol production, this fuel ethanol is called 1st generation bioethanol (Zuurbier and van de Vooren, 2008). In many countries bioethanol is mandatory supplement of gasoline. In Denmark, to example, bioethanol should be added to all gasoline blends from the 1st of March 2010. The market for ethanol is expected to further increase in the future and more diverse crops and materials should be found for the security of safe supply.

Ethanol can be produced from fermentable sugars, *e.g.* C6-glucose derived from starch or cellulose from any lignocellulosic biomass, for example, agricultural waste or forest residues (Jacques et al., 1999; Thomsen and Haugaard-Nielsen, 2008). Bioethanol from lignocellulosic materials is called 2nd generation bioethanol. 1st generation bioethanol is a mature technology whereas 2nd generation bioethanol is still under development. The technological challenge now is to develop cheap and simple methods for production of 2nd generation bioethanol to avoid important food materials - corn and wheat - to be used for transportation fuels in a world with huge demand for food and feed for humans and animals.

A major task in 2nd generation bioethanol is the pretreatment step, which is necessary to disrupt and open up the tight lignocellulosic structure of cellulose, hemicelluloses and lignin, facilitating the further conversion into fermentable sugars (Yang and Wyman, 2008). During hydrothermal pretreatment, the lignocellulosic biomass is fractionated into a water soluble hemicellulose fraction and a solid fraction of cellulose and lignin, which is then accessible for hydrolytic enzymes. Following or simultaneous with an enzymatic hydrolysis the pretreated substrate can be converted into ethanol by microbial fermentation. There are several pretreatment methods; among these, the most established are hydrothermal pretreatments working at elevated temperature and pressure at high water demand: acid treatment (Torget et al., 1991), steam explosion hydrothermal (Lee et al., 1999; Rosgaard et al., 2007; Galbe and Zacchi, 2002) and wet oxidation - a reaction involving oxygen and water at elevated temperature and pressure (Bjerre et al. 1996; Klinke et al., 2002). However, due to the high needs for, energy, chemicals, corrosion resistant and high pressure reactors, etc., pretreatment is one of the most expensive steps in the 2nd generation ethanol production (Aden and Foust, 2009). Therefore, there is a huge interest to develop low-cost pretreatment methods.

Silage storing of green crops used, at the same time, as method for pretreatment is a new and interesting technique for bioethanol production (Chen et al., 2007). Ensiling is a method of moist forage preservation, which is widely used all over the world. It is a well established and mature technology in animal fodder industry and it is being used all over the world (Weinberg and Ashbell, 2003), which makes it easy to implement for other purposes. The aim of silage making is to store and

preserve the crop with minimum loss of nutrients i.e. feed value (Charmley 2001). Ensiled material, often referred to as silage, consists of the whole harvested plant (stem, leaves, and grain) and grasses which is used for animal feed. It is well known that ensiled grass has a higher energy content and more nutrient rich than hay (dried grass in bales). In a correct ensiling, lactic acid bacteria dominate the fermentation process; the low pH caused by fermentation of part of free sugars preserve the feedstock from further degradation by inhibiting fungus microbes, in that way effectively minimizing the degradation of sugars in a crop (Thompson et al., 2005). The whole-crop silage consists of degradable lignocellulosic material, hemicellulosic material and, in case of maize or rye, small amount of starch (Thomsen et al., 2008). Oleskowicz-Popiel et al. (2008) used wet-oxidized maize silage to produce bioethanol and achieved 82% of the theoretical ethanol yield during batch fermentation. Despite several experimental investigation on biological pretreatment methods (Carmona et al., 2009; Bak et al., 2009), very few reports consider ensiling as pretreatment step in the bioethanol production.

In this study, we have investigated the ensiling process as a wet-storage and a standalone pretreatment method for the 2nd generation bioethanol production in trials performed on maize, rye and clover grass silages. The effect on ensiling process were examined by enzymatic hydrolysis of polysaccharides with specials emphasis on the glucose yield, sugar recovery, and further conversion to ethanol by the ethanol producing strains *Saccharomyces cerevisiae* and *Kluyveromyces marxianus.*

Materials and Methods

Raw materials

Three different species were tested: maize (*Zea mays* L.ssp.), cultivar Companero; rye (*Secale cereal*), cultivar Carotop; and clover grass from a seed mixture ForageMax 42 (DLF Trifolium, Denmark). All crops were harvested at the test field of National Laboratory for Sustainable Energy, Technical University of Denmark (Risø DTU). All the raw materials were wet biomass of which the chemical composition of cellulose, hemicelluloses, lignin, starch and fructose is shown in Table I. Fresh crops have a water content of 83.5% for fresh rye, 81.6% for clover grass and 67.9% for maize whereas the ensiled crops had a 5-10% lower dry matter content compared to the fresh ones (Table I).

Chemical analysis

The cellulose and hemicellulose contents and composition hereof in raw and silage treated crops was measured based on NREL chemical analysis (Sluiter et al., 2005) by the method called “strong acid hydrolysis” hereby briefly described: the 0.16 g DM feedstock were treated with 1.5mL of H_2SO_4 (72%) at 30^{o}C for one hour with addition of standard stock solutions of glucose (cellulose), xylose and arabinose (hemicelluloses), then diluted with water corresponding to 4% H_2SO_4 and following autoclaved at 121^{o}C for one hour. The acid hydrolysate was filtered and the hydrolysed glucose, xylose, and arabinose were quantified by HPLC (Biorad HPX-87H) from which the content of

original glucan, xylan and arabinan was calculated. Starch was determined according to (Sluiter and Sluiter, 2005).

Ensiling process

Each of the fresh crops was cut manually into 2-3 cm pieces, mixed with Biomax Si (Chr. Hansen A/S, Denmark) forage additive containing lactic acid bacteria (strain of *Lactobacillus Plantarum,* to ensure stable silage production). 1 g of powder of forage additive was dissolved in 10 liters of water and sprayed over wet biomass samples in the amount equal to 40 mL of solution per 1 kg of biomass. Subsequently, the crops were packed anaerobically in plastic bags; the air was pressed out manually to maximal possible extent. Such packed materials were kept at room temperature for 30 days (Yahaya et al., 2001). After that all the samples were stored in a freezer prior to analysis. All the experiments were performed in duplicates.

Hydrothermal pretreatment process

The hydrothermal pretreatments were performed in a loop reactor designed and constructed at Risø DTU (Denmark) (Bjerre et al., 1997). Three materials (maize, rye and clover grass silages) were pretreated at moderate severity (10 minutes at 190^{o}C). 60 g ensiled material (corresponding to DM biomass) was mixed with water to achieve 6% DM concentration and was inserted to the loop reactor.

Enzymatic hydrolysis

Enzymatic hydrolysis, testing the convertibility of sugars to glucose by cellulytic enzymes, (Celluclast 1.5 and Novozym 188 (kindly provided by Novozymes, Denmark)) was carried out in a buffer system at 50^{o}C, pH 4.8, with 0.1g dry matter in 5 mL and an enzyme loading of 30FPU endoglucanase (Celluclast 1.5) supplemented with 20 v/v% beta-glucosidase (Novozym 188) per 1g dry matter. The amount and composition of sugars (glucose, xylose, and arabinose) released after 24 hours was determined by HPLC (Biorad HPX-87H).

Simultaneous Saccharification and Fermentation (SSF)

All the fresh and raw silage crops were chopped into small pieces (0.5-1 cm) prior to fermentation. Two kinds of microorganisms were applied: the mesophile *Saccharomyces cerevisiae* and the thermo-tolerant *Kluyveromyces marxianus* (DSMZ 7239). Dry commercial Baker's yeast (Malteserkors tørgær, Lallemand) was stored in fridge; 0.2 g of dry yeast per shake flask was used. *K.marxianus* strain was maintained at -85°C in the synthetic medium (mixture of 50 vol% glycerol and yeast, peptone, lactose solution, which contained per liter demineralized water: 5 g of bacto peptone, 5 g of yeast extract, 30 g of lactose, 2 g of NH_4Cl, 0.3 g of $MgSO_4 \cdot 7H_2O$ and 1 g of KH_2PO_4). Starter

culture of *K.marxianus* was grown for 24 hours at 40°C in 250 mL flask containing 150 mL synthetic medium, the same which was used for strain maintenance.

SSF tests were performed according to (Varga et al., 2004). Due to mechanical problems, each raw material content was lowered to 6 g DM and then mixed with 100 mL Millipore water in 250mL blue cap shake flasks. The flask were placed in a temperature controlled Lab-shaker (Lab-Therm LT-X, Holm & Halby, Denmark). Firstly, a pre-hydrolysis step for 24 hours at 50^{o}C with addition of Celluclast 1.5 and Novozyme 188 (Novozyme, Denmark) (15 FPU/gDM - dry matter) was carried out for liquefying the highly condensed solid dry matter. Afterwards, the temperature was lowered to 32^{o}C for *S.cerevisiae* and 40^{o}C for *K.marxianus* and a new enzyme loading of 20 FPU/g DM was applied. The flasks were equipped with yeast locks filled with glycerol allowing CO_2 to release to daily monitor ethanol production. Since no other products than CO_2 and ethanol were considered during the fermentation process, the mass of CO_2 in g produced is equal to 1.045 times mass of ethanol produced during fermentation process, thus the mass (ethanol) of ethanol was calculated by :

$$Mass_{ethanol} = 1.045 \times Mass_{CO2}[g]$$

The final ethanol concentration in the effluents was determined by HPLC (Biorad HPX-87H). The SSF experiments ran for about 6 days and were followed daily by weight loss and were performed in triplicate.

The theoretical ethanol potential was estimated as the amount of ethanol Y_{EtOH} which can be produced from the amount (determined as described above) of glucose (C6 sugar) (Figure 1) from the formula presented below.

$$Y^{T}_{EtOH} = 0.51 \times Mass_{glucose}[\frac{g}{100g}]$$

Results

Enzymatic hydrolysis

Prior to the ethanol fermentation an enzymatic hydrolysis is needed to convert the sugar polymers into simple sugars. The tested enzyme mixture was cellulases, beta-glucosidase and hemicellulases in enzyme cocktail of (80% Celluclast and 20% Novozym 188 (from Novozymes A/S). The enzymatic convertibility of the three biomass samples (maize, clover and rye) is shown in Figure 2. Fresh maize (51.4 %) and fresh clover (31.8.7%) are followed by fresh rye (28.6%). The enzymatic convertibility of ensiled materials (Figure 2B) was for all three biomass samples slightly higher than those of fresh crops. Small diference was noticed in maize silage (51.5%), in comparison, rye and clover silages showed significantly increased convertibility (36.5% and 41.9%, respectively). It is well known that lignocellulosic materials normally need severe pretreatment before conversion to ethanol, so these results are outstanding compared to existing literature. Even though no amylases were added in

the experiment, the Celluclast 1.5 (Novozyme A/S) enzyme cocktail is known to hydrolyze starch as well.

The results obtained from convertibility tests of hydrothermal pretreated and ensiled crops are seen on Figure 2C. The three hydrothermally pretreated ensiled materials showed following results of glucose yield: 50.9% for clover grass silage and 54.0% for both maize and rye silage materials.

On the Figure 3, the amount of glucose revealed by cellulase enzymes is compared to total C6 sugars amount in the sample and presented in g of generated glucose per g of total glucose in the raw material. Fresh crops are characterized by additional fructose content, therefore a bit higher sugar content, for fresh maize 33.2 g out of 64.7 g is released, for fresh rye 11.6 g out of 40.6 g and for fresh clover 13.5 g out of 42.6 g (Figure 3A). For only ensiled crops (Figure 3B): 31.0 g out of 60.2 g (51.5%) is released for maize silage, 9.4 g out of 25.8 g rye silage (36.5%), and 9.7 g out of 23.1 g (41.9%) for clover grass silage. These results differ from those of hydrothermal pretreated ensiled crops (Figure 4B): 28.4 g out of 52.6 g (54.0%) was hydrolysed from maize silage, 25.7 g out of 47.6 g (54.0%) from rye silage, and 22.0 g out of 43.2 g (50.9%) for clover grass. This could be explained by the higher sugars concentrations found in the solids of the hydrothermal pretreated materials.

Simultaneous Saccharification and Fermentation (SSF)

Fermentation trials with three investigated fresh and ensiled crops were conducted with two different microorganisms: *K.marxianus* (40^{o}C) and *S.cerevisiae* (32^{o}C). The reason of using two types of microbes was to observe differences in yields when ethanol fermentation were run at two different temperatures 32^{o}C for *S.cerevisiae* and 40^{o}C for *K.marxianus*, which could influence the ethanol productivity and also have an effect on contaminating microbes. Figure 4 and 5 shows that the thermophilic yeast *K.marxianus* (Figure 4A and 5A) performed slightly better compared to Baker's yeast (Figure 4B and 5B). The highest ethanol yield was achieved from fermenting fresh clover grass: 49.3% of theoretical after 120 hours with *K.marxianus* and 41.8% of theoretical after 116 hours with *S.cerevisiae* (Figure 4). The ethanol yields from clover grass silage were 36.9% and 34.5% of theoretical after 140 hours of fermentation, by *K.marxianus* and *S.cerevisiae,* respectively (Figure 5). Slightly lower results were found for fresh rye: 42.0% of theoretical after 116 hours of SSF with *K.marxianus* and 39.0% of theoretical after 116 hours SSF with *S.cerevisiae,* for ensiled rye following results were achieved: 28.5% and 28.1% of the theoretical by *K.marxianus* and *S.cerevisiae,* respectively (Figure 5). The same experiments for maize resulted in: 34% and 29.9% of the theoretical ethanol yield for fresh maize by *K.marxianus* and *S.cerevisiae,* respectively (Figure 4), ensiling of maize crop gave following: 33.9% and 30.6% of the theoretical ethanol yield for by *K.marxianus* and *S.cerevisiae,* respectively (Figure 5).

Figure 6 shows the ethanol production conducted at similar fermentation conditions using all three silages after hydrothermal pretreatment. An increase of the ethanol yield for all three crops was found: for rye and clover silages, 74.6% and 80.2% with *K. marxianus*, and 81.3% and 76.2% with *S.*

cerevisiae, respectively and for hydrothermal treated ensiled maize, the ethanol yield increased to 79.0% in fermentations by *K. marxianus* and 72.7% by *S.cerevisiae.*

Discussion

The results obtained from the fermentation trials clearly showed that the ensiling process can be used as a efficient wet-storage and pretreatment method where already part of the lignocellulose structure was disrupted making it easier for cellulytic enzymes to hydrolyse the polymeric sugar substrates into fermentable monomeric C6 sugars. High ethanol yields and no contamination were observed. Under anaerobic conditions, lactic acid bacteria convert water-soluble sugars into organic acids, mainly lactic acid. As a consequence of accumulating lactic acid, the pH drops below 5.0 (Weinberg and Ashbell, 2003) and conserve the raw material.

The high enzymatic convertibility of fresh crops (Figure 2A) might be caused by the presence of water-soluble sugars in the biomass, however fresh crops are very difficult to preserve, they will be fast degraded and consumed by natural existing miroorganisms. Excess of oxygen and presence of free sugars are very attractive to different microorganisms that naturally grow on biomass during aerobic conditions and consequently spoil the feedstock (Weinberg and Ashbell, 2003). This is why green fresh crops need to be stored in either dry or silage form.

Digman et al., (2010) investigated ensiling with and without chemical addition of switchgrass and reed canarygrass prior to conversion to ethanol. Followed by sulfuric acid pretreatment, the achieved ethanol conversion efficiency ranged between 22-83% and 16-46% for switchgrass and reed canarygrass, respectively. Ensiling followed by lime pretreatment resulted in slightly lower yields: 21-55% and 18-54% for switchgrass and reed canarygrass, respectively. Sipos et al., (2010) used steam pretreatment on dry or ensiled industrial hemp, though lower ethanol yields were achieved for ensiled hemp (71% of the theoretical) compared to dry hemp (74% of the theoretical).

Hydrothermal pretreatment, as expected, significantly increased ethanol production from ensiled crops. Although the conditions were not optimized for those materials promising results were achieved, different severities pretreatment conditions should be tested. Xu et al., (2010) evaluated potential of maize silage for ethanol production. Maize silage was hydrothermal pretreated at five different severities. Optimal conditions were found to be: 195°C for 7 min or 185°C for 15 min resulting in 78.0% or 77.2% of the theoretical, respectively. In our case, 79% of the theoretical was achieved after 10 min at 190°C by *K.marxianus*, lower value was obtained by *S.cerevisiae*: 72.7% of the theoretical.

Higher ethanol production was observed for the thermophilic yeast, which is explained by the higher process temperature (40^{o}C compared to 32^{o}C for Baker's yeast) being close to the optimal hydrolysis temperature for cellulase enzymes (50^{o}C) - consequently more glucose was available to be converted into ethanol.

The simultaneous saccharification and fermentation tests performed on fresh biomass samples indicated high convertibility of the ethanol, however fresh crops cannot be stored for long time. A

storage method is needed and ensiling could be one of the solutions. It is not realistic to produce bioethanol from fresh green biomass without a storage facility implemented due to the unavailability of fresh crops all year long. The harvested crops have to be stored either in dry or ensiled form. Enzymatic convertibility test indicated high glucose (ethanol) yields for the fresh crops. Silage is a way to preserve freshness of the raw materials making it available for fermentation purpose all year long until the next harvest time.

Using ensiling as wet-storage and pretreatment process for ethanol productions has many perspectives. Ensiled crops can be stored all year long; the low pH and shortage of oxygen preventing their contamination (Thompson et al., 2005), in that way, no further consumption of organic matter will occur. The results from our investigation clearly showed that no sterilization was needed prior to the incubation with enzymes and yeast which was very positive and unexpected. The results show that yeast fermentation is controlled by the low pH obtained by production of lactic acid. In that way the biomass soluble sugars serve as production for “antibiotics” i.e. lactic acid.

This investigated ensiling treatment, used as pretreatment and combined storage method followed by further mild and well optimized enzymatic hydrolysis (in order to increase sugar content in the solids) and simple yeast fermentation could be very promising.

Conclusions

This investigation showed that ensiling process can be used as a wet-storage and a biological pretreatment technique for crops like maize, rye or clover. Ensiling can keep the “freshness” of the crops and at the same time prevent contamination of wet biomass, noticeable amount ethanol was produced already from only ensiled crops. Followed by hydrothermal pretreatment high yields were achieved: production of 72.7 - 79% for maize, 74.6 - 81.3% for rye and 76.2 - 80.2% for clover of theoretical ethanol yield run by SSF with cellulytic enzymes and yeast strain of *S.cerevisiae* and *K.marxianus*. Silage treatment has a high potential as combined storage and pretreatment method for all investigated crops such.

Acknowledgment

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Tables

Table I. Composition of the raw materials: cellulose, hemicelluloses, lignin, DM (dry matter) and ash

Crop	Cellulose [g/100g]	Hemicellulose [g/100g]	Fructose [g/100g]	Starch [g/100g]	Lignin [g/100g]	Extractives/ residuals	DM [g/100g]	Ash [g/100g]
Fresh maize (the whole crop)	44.3 ± 0.9	15.0 ± 1.6	5.0	29.3 ± 5.8	7.4 ± ND	13.5	32.1 ± 3.1	1.5 ± 0.2
Maize silage (the whole crop)	40.3 ± 1.0	15.1 ± 0.2	0.0	30.5 ± 0.8	7.4 ± 0.7	13.5	29.8 ± 3.2	1.5 ± 0.2
Fresh rye (the whole crop)	21.7 ± 0.3	16.6 ± 0.4	15.5 ± 0.2	3.4 ± 0.0	7.8 ± 0.2	33.9	16.5 ± 0.8	1.1 ± 0.1
Rye silage (the whole crop)	21.8 ± 2.3	15.3 ± 3.0	0.0	3.4 ± 0.0	5.1 ± 3.0	32.9	15.6 ± 0.2	1.1 ± 0.1
Dried rye (the whole crop)	49.9 ± 2.2	18.2 ± 0.9	ND	19.8 ± 0.6	10.7 ± 0.6	0	91.9 ± 0.1	3.3 ± 0.1
Fresh clover (the whole crop)	23.2 ± 0.8	14.6 ± 0.4	19.3 ± 0.8	ND	13.5 ± ND	27.5	18.4 ± ND	1.8 ± ND
Clover silage (the whole crop)	21.0 ± 0.1	14.2 ± 0.3	0.0	ND	5.3 ± 0.1	41.8	17.4 ± ND	2.0 ± ND
Dried clover (the whole crop)	26.3 ± 0.3	18.3 ± 0.3	ND	ND	14.2 ± 0.1	33.2	91.3 ± 0.2	8.0 ± 0.1

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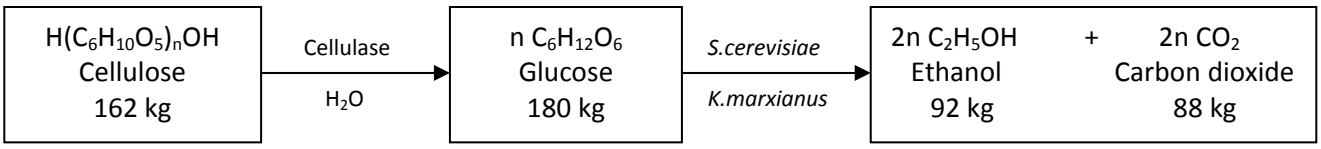

Figure 1. Scheme of ethanol production from cellulose biomass by yeast (*S.cerevisiae* and *K.marxianus*)

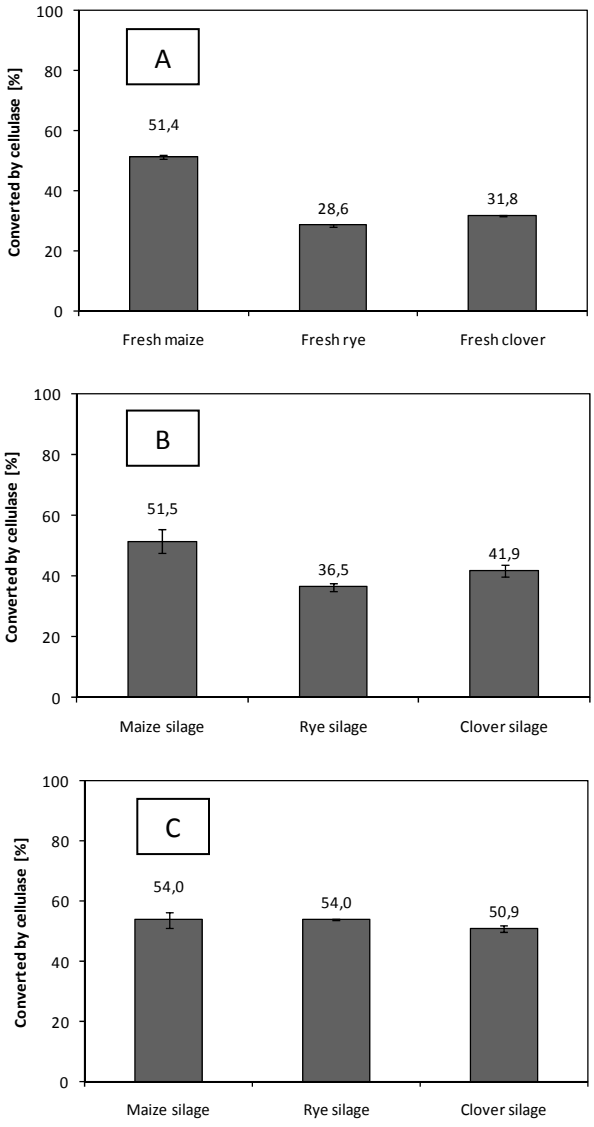

Figure 2. Enzymatic cellulose convertibility of ensiled biomass in acetate buffer (enzyme loading 30 FPU) for 24 hours presented in % of cellulose converted to glucose of fresh crops (A), ensiled crops (B) and hydrothermal pretreated ensiled crops (C)

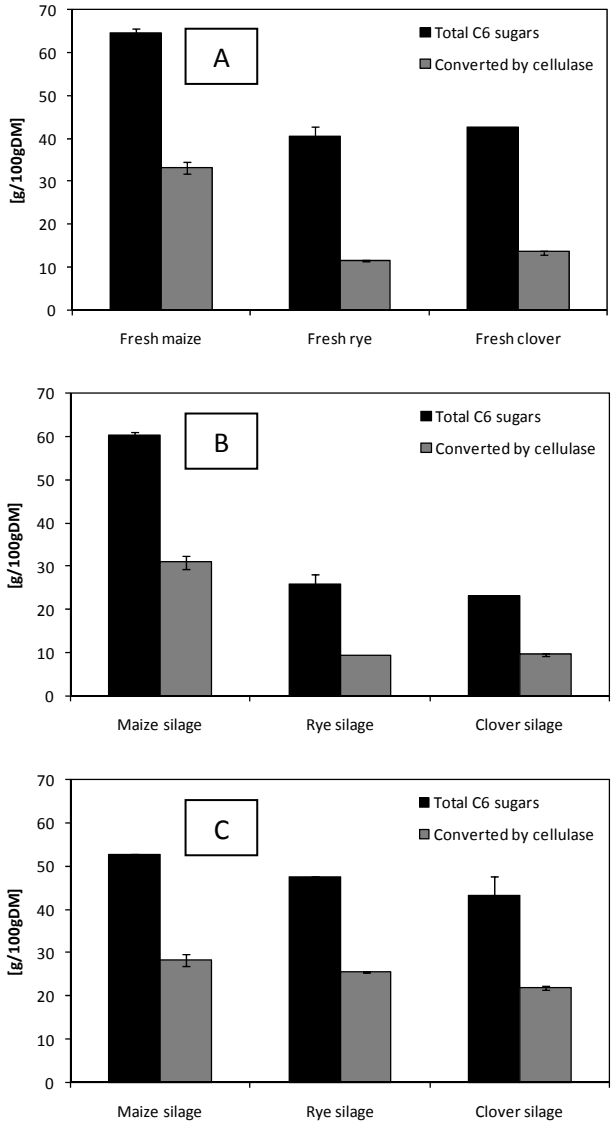

Figure 3. Enzymatic cellulose convertibility of ensilaged biomass in acetate buffer (enzyme loading 30 FPU) for 24 hours of fresh crops (A), ensiled crops (B) and hydrothermal pretreated ensiled crops (C) presented in g of glucose per 100 g of dry sample

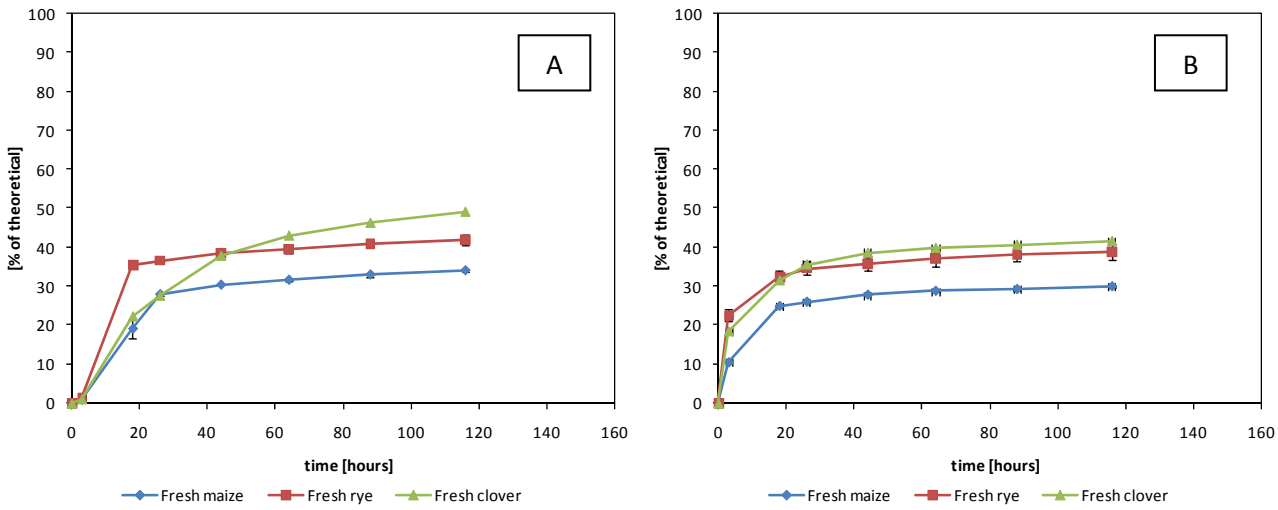

Figure 4. Ethanol production (expressed as percentage of theoretical ethanol yield) by: *Kluyveromyces marxianus* during simultaneous saccharification and fermentation of fresh crops (A) and *Saccharomyces cerevisiae* during simultaneous saccharification and fermentation of fresh crops (B)

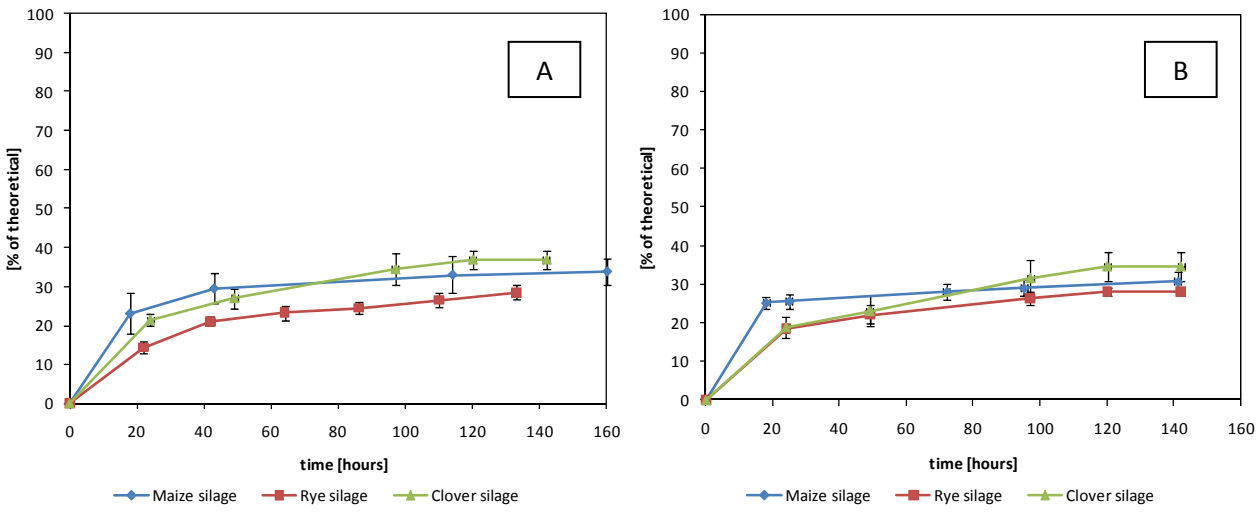

Figure 5. Ethanol production (expressed as percentage of theoretical ethanol yield) by: *Kluyveromyces marxianus* during simultaneous saccharification and fermentation of ensilaged crops (A) and *Saccharomyces cerevisiae* during simultaneous saccharification and fermentation of ensilaged crops (B)

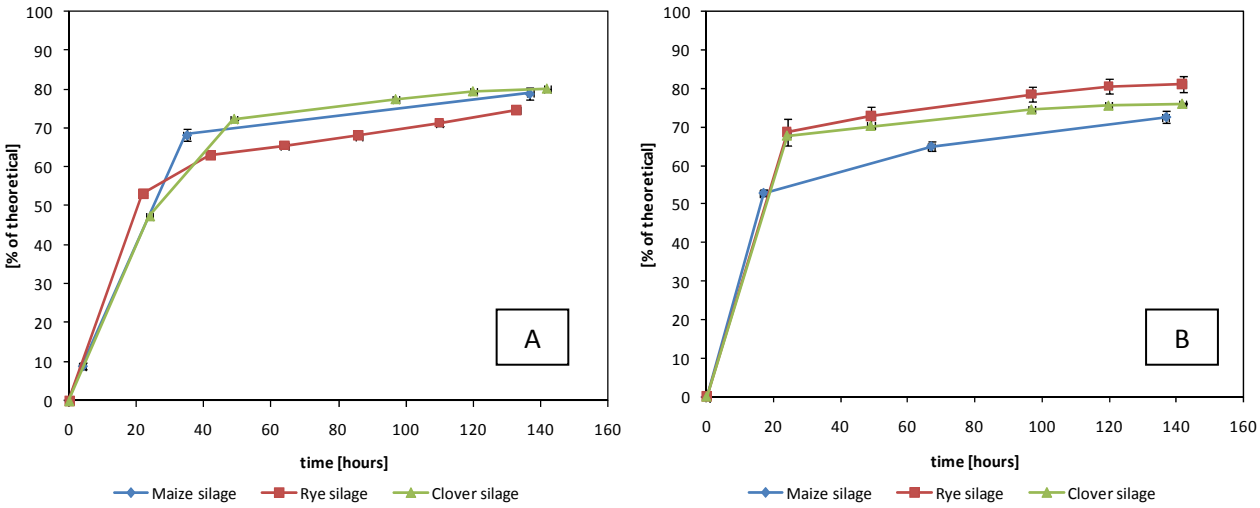

Figure 6. Ethanol production (expressed as percentage of theoretical ethanol yield) by: *Kluyveromyces marxianus* during simultaneous saccharification and fermentation of hydrothermal pretreated ensilaged crops (left) and *Saccharomyces cerevisiae* during simultaneous saccharification and fermentation of hydrothermal pretreated ensilaged crops (right)

Paper IV

Oleskowicz-Popiel P., Lehtinen T.M., Schmidt J.E., Thomsen A.B.: Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol. *Submitted*

Ensiling – wet-storage and pretreatment of corn stover to produce bioethanol

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Abstract
Ensiling method for storing and preserving crops has been widely used in feed industry and only more recently in the biofuels production sector; in the current paper, the latter is looked into with a focus on influences of ensiling to dry agricultural by-products such as corn stover. Microscopic images of raw and ensiled biomass were taken, showing a disruption of the lignocellulosic structure after ensiling process. Enzymatic convertibility tests as well as fermentation trials were carried out on the dry, ensiled and hydrothermal treated corn stover. The increase in ethanol yield after ensiling was noticed during the simultaneous saccharification and fermentation by *Kluyveromyces marxianus* strain (DSMZ 7239). For raw corn stover, the ethanol produced after 95 hours corresponded to 16.4% (±2.4) of the theoretical. For ensiled corn stover, the ethanol yield resulted in 23.1% (±0.9) of the theoretical. The highest ethanol yield was produced from hydrothermal treated ensiled corn stover: 66.4% (±4.1) of the theoretical. During ensiling process some disruption of the lignocellulosic structure occurred giving then a lift to the ethanol production in the following fermentation process. Thus we conclude that ensiling could serve as an efficient wet-storage method for agricultural by-products prior to ethanol fermentation.

Keywords: bioethanol, pretreatment, ensiling, silage, wet-storage

1. Introduction

There is a need for a new source of liquid transportation fuels to overcome environmental and economic problems; the global reserves of fossil fuels are depleting and the global oil consumption is reaching its peak in the coming decades [1]. The huge increase in oil demand in developing countries, especially in China and India [2], adds to the pressure on the world's oil supply. Furthermore, the environmental impact of CO_2 emissions on the global climate change [3], which in majority originates from petroleum combustion, further amplifies the need for a CO_2-neutral substitute for transportation fuels. Biomass represents one of the sustainable and suitable resources, which could be converted into liquid fuels: the lignocellulosic feedstock is available on a large scale and it is cost-competitive with petroleum [4].

Bioethanol can be produced from starchy or lignocellulosic materials; in the commercial scale it is currently produced only from sugar cane or starch, mostly in Brazil and US [5]. So far lignocellulosic bioethanol exists in pilot and demonstration scales [6, 7, 8] but its implementation in full commercial scale is being investigated [9, 10].

For ethanol production from lignocelluloses a pretreatment step is necessary. This process calls for a disruption in the lignocellulosic structure to make the cellulose and hemicellulose fractions available for enzymes and consequently for ethanol producing microorganisms [11]. The choice for a pretreatment technology is a key-issue for the whole process [12]. Several different methods have been developed over the years: acid treatment [13], steam explosion hydrothermal treatment [14, 15, 16] and wet-oxidation [17]. All those techniques require advanced technology and expensive equipment, which increases costs and makes bioethanol less competitive against fossil fuels.

In the ensiling process, already a widely used storing and crop-preserving method for fodder [18], lactic acid bacteria dominate creating lactic acid to lower the pH and inhibit further degradation of polysaccharides [18, 20]. Ren et al. [21] studied ensiling of corn stover as feedstock preservation for particleboard production. Increase of cellulose concentration was found during ensiling process, it was caused by decrease of biodegradable constituents and thus a total mass caused the increase in cellulose percentage; during ensiling process hemicelluloses is degraded selectively over cellulose. It can be fairly assumed that the ensiling process should make an efficient wet-storage and pretreatment for ethanol production from lignocellulosic biomass. Digman et al. [22] investigated an on-farm pretreatment method of perennial grasses. The raw materials, after addition of sulfuric acid or calcium hydroxide, were stored anaerobically from 0-180 days at moisture content of 40% or 60%. It was concluded that addition of chemicals suppress microbial activity during storage. After fermentation of wet biomass, the conversion of cellulose to ethanol varied between 22-83% for reed canary grass and 16-46% for switch grass after addition of sulfuric acid, slightly lower conversion were achieved after addition of lime. Sipos et al. [23], on the other hand, performed trials with steam pretreated ensiled industrial hemp, resulting in 71% of the theoretical maximum to produce ethanol.

In the current investigation we present the influence of ensiling on dry agricultural by-products such as corn stover. Enzymatic convertibility tests as well as fermentation trials were carried out. Additionally, microscopic images of raw and ensiled biomass were taken in order to observe any disruptions in the lignocellulosic structure.

2. Material and Methods

2.1. Dry matter and ash content

Dry matter (DM) and ash contents were measured according to a standard method [24]. After drying the samples overnight at 105°C, the DM values were determined and the samples incinerated at 550°C for three hours.

2.2. Ensiling

Dry corn stover was soaked in water to achieve a dry matter content of 25–30%, after which the forage additive Biomax Si (CHR Hansen, Denmark) containing lactic acid bacteria (strain of *Lactobacillus Plantarum*) was added. 1 g of Biomax Si powder was dissolved in 10 liters of water. 120 mL of solution was sprayed over 3 kg of wet biomass, which then was packed anaerobically in a plastic bag and kept in room temperature for 30 days. The experiment was done in duplicate, and the samples were stored in -18°C prior to further analysis.

2.3. Hydrothermal treatment

Some of the ensiled corn stover was hydrothermal treated at a loop reactor designed and constructed at Risø DTU [17]. 60 g of biomass (dry matter) was mixed with water at a concentration of 6% DM and pretreated in moderate severity at 190°C for 10 minutes. The trials were run in duplicate.

2.4. Strong acid hydrolysis

Strong acid hydrolysis [21] was applied to the biomass to disrupt lignocellulosic structure of plant biomass and yield their monosaccharide sugar components. 0.16 g DM was treated with 1.5 mL H_2SO_4 (72%) at 30°C for one hour, then diluted with 42 mL of added water, and autoclaved in 1 bar overpressure at 121°C for one hour. The concentrations of glucose, xylose and arabinose were quantified by HPLC (Biorad HPX-87H).

2.5. Enzymatic hydrolysis

The convertibility of cellulose to glucose by cellulytic enzymes (Celluclast 1.5 and Novozym 188 by Novozyme, Denmark) was tested. With a concentration of 0.1 per 5 mL and an enzyme loading of 30 FPU (FPU – filter paper unit – amount of cellulase enzymes needed to release exactly 2 mg glucose from 50 mg filter paper during 1 hour enzyme hydrolysis at 50°C), the test was carried out in a buffer system (pH 4.8) at 50°C. The amount of glucose released after 24 hours of hydrolysis was determined by HPLC (Biorad HPX -87H). The analysis was performed in triplicate.

2.6. Simultaneous Saccharification and Fermentation (SSF)

The SSF was performed in 250 mL blue-cap laboratory bottles according to [26]. Firstly, 100 mL of solution containing 6% DM was mixed out of biomass and demineralized water (R ≥ 18 µS cm^{-1}) and adjusted to pH 4.8. Secondly, enzymes were added to commence prehydrolyzation (loading: 15 FPU/gDM, enzyme cocktail of Celluclast 1.5 and Novozym 188, Novozymes, Denmark). The prehydrolysis step ran for 24 hours at 50°C. Afterwards, the solution was cooled down to 40°C, more enzymes added (loading: 20 FPU/gDM) and inoculated with a thermotolerant yeast, *Kluyveromyces marxianus*. The *K.marxianus* strain (DSMZ 7239) was maintained at -85°C in a synthetic medium (a mixture of 50 vol-% glycerol and yeast, peptone, lactose solution, which contained per liter demineralized water: 5 g of bacto peptone, 5 g of yeast extract, 30 g of lactose, 2 g of NH_4Cl, 0.3 g of $MgSO_4 \cdot 7H_2O$ and 1 g of KH_2PO_4). A starter culture of *K.marxianus* was grown for 24 hours at 40°C in a 250 mL flask containing 150 mL synthetic medium, the same that was used for strain maintenance.

The blue-cap bottles were equipped with yeast locks filled with glycerol allowing CO_2 release. The ethanol production was then monitored in a stoichiometric ratio from the reduced weight of CO_2. The final concentration of ethanol was determined by HPLC (Biorad HPX 87H). All the experiments were run in triplicate.

The theoretical ethanol yield was calculated from the amount of ethanol available from the amount of glucose (determined and described above). For *K. marxianus*, the mass of ethanol equals 0.51 times mass of glucose in the raw material.

2.7. Microscopic analysis

Microscopic pictures were taken with a Hitachi TM1000 tabletop microscope, equipped with a tungsten filament and solid state backscattered electron detector and operated with an accelerating voltage of 15 keV. All the samples were dried at 50°C prior to analysis.

3. **Results and Discussion**

3.1. Characterization of materials

Corn stover in three forms was evaluated: raw, ensiled and ensiled after hydrothermal treatment; the results are presented in Table 1. Corn stover silage was characterized by the lowest dry matter content (28.2% compared to 91.4% for raw corn stover and 94.6% hydrothermal treated corn stover silage) as well as slightly lower glucan content (36.3 g/100gDM) compared to two other ones (40.1 g/100gDM and 39.8 g/100gDM for raw corn stover and hydrothermal treated corn stover silage, respectively). Raw and hydrothermal treated corn stover showed similar glucan contents; however, after hydrothermal treatment, lower xylan (16.8g/100gDM compared to 22.3g/100gDM) and arabinan (1.5g/100gDM compared to 3.5g/100gTS)) concentrations were noticed. During hydrothermal treatment, hemicellulose sugars are dissolved to the liquid phase thus decreasing xylan and arabinan concentrations in the solid plant matter. Accordingly, lignin concentration was found to have slightly increased in hydrothermal treatment (17.2g/100gDM compared to 15.7g/100gDM for raw corn stover and 15.9g/100gDM for

ensiled corn stover). A similar pattern was observed when comparing corn stover to corn stover silage: during ensiling some of the water-soluble sugars were consumed by lactic acid bacteria; therefore the lignin and ash concentrations were found to have slightly increased. Similar behavior was observed in [21], hemicellulose was degraded selectively over cellulose during ensiling process.

Theoretical ethanol yield, calculated on glucan content in biomasses, was the highest for raw corn stover (22.7gEtOH/100gDM). Yet, all the differently treated biomasses showed very similar values: 20.5gEtOH/100gDM for corn stover silage and 22.5gEtOH/100gDM for hydrothermal treated corn stover silage.

3.2. Degradation of lignocellulosic structure

To better investigate the degradation of the lignocellulosic structure during ensiling process, microscopic images of corn stover and corn stover silage were taken (see Figure 1). Maize silage, which in our previous experiments proved to be highly efficient raw material for bioethanol production (data submitted), was portrayed only for a comparison with corn stover and corn stover silage.

In the first row of Figure 1, the structure of corn stover (A-C) is shown in different magnitudes. Hard untouched biomass fibers are easy to notice. Different characteristics are visible in both silages (corn stover (D-F) and maize (G-I), second and third row, respectively). The hard lignocellulosic structure was obviously disrupted during ensiling process. It is assumed that this decomposition of fibers has a positive effect on the yield in enzymatic hydrolysis and consequently on the ethanol yield during fermentation.

3.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the three investigated biomasses was carried out with results presented in Figure 2. No difference was observed between the raw and ensiled corn stovers (28.7% and 28.3% for corn stover and corn stover silage, respectively). The highest convertibility was obtained for hydrothermal treated and ensiled material, which was equal to 57.4% for C6 sugars. In comparison, Varga et al [27] achieved conversion of cellulose to glucose of about 85% for wet-oxidized corn stover in following conditions: 195°C, 15min, 12 bar O_2 with addition of 2 g/L Na_2CO_3.

3.4. Fermentation

Simultaneous saccharification and fermentation was carried out on the three differently treated corn stovers (Figure 3). Even though no difference was noticed during enzymatic convertibility between corn stover and corn stover silage, an increase in ethanol yield was observed during the fermentation process. For raw corn stover, the ethanol produced after 95 hours equaled to 16.4% of theoretical yield. For ensiled corn stover, the ethanol yield was 23.1% of the theoretical after the same process time. The most ethanol was produced from hydrothermal treated: 66.4% of the theoretical.

Our previous experiments on silage crops (data submitted) indicated higher ethanol yields in trials conducted with a thermophilic yeast (*K.marxianus* DSMZ 7239) compared to the mesophilic

Baker's yeast (*S.cerevisiae*). Accordingly, all the experiments with corn stover were carried out with *K.marxianus*.

Xu [28] investigated different pretreatment conditions for corn stover, and the results were as follows: for biomass treated at 195°C at different reaction times, ethanol yield ranged from 61.2% to 71.2% of the theoretical. In our investigation of ensiling corn stover, a lower production was observed. However, a noticeable increase in produced ethanol from corn stover silage should not be disregarded.

Ensiling process could be applied as a wet-storage condition of dry agriculture by-products (such as corn stover) prior to second generation bioethanol production. Investigations on the lignocellulosic structure as well as identification of active enzymes in the process could be the next steps. Moreover, influence of different chemical compounds on the preservation sugars as well as the disruption of lignocellulosic structure would be of interest. Digman et al. [22] i.e. improved ensiling of perennial grasses by addition of sulfuric acid or calcium hydroxide and achieved very promising results from performed trials.

4. Conclusions

Promising effects of ensiling process were noticed from microscopic images, in which a disruption of lignocellulosic structure was observed in ensiled corn stover compared to raw corn stover. Even though no difference in enzymatic convertibility was noticed between corn stover and ensiled corn stover, a higher ethanol yield was achieved for corn stover silage compared to raw corn stover during simultaneous saccharification and fermentation by *K.marxianus*. During ensiling some disruption of the lignocellulosic structure occurred giving then a lift to the ethanol production in fermentation. Thus we conclude that the ensiling process could serve as an efficient wet-storage method for agricultural by-products prior to ethanol fermentation.

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Tables

Table 1. Chemical composition of corn stover, ensiled corn stover, and hydrothermal treated (190°C, 10 min) ensiled corn stover.

	Unit	Corn stover	Corn stover silage	Pretreated corn stover silage
Glucan	[g/100gDM]	40.1±0.6	36.3±1.3	39.8±2.0
Xylan	[g/100gDM]	22.3±0.2	20.9±0.3	16.8±ND
Arabinan	[g/100gDM]	3.5±0.0	2.9±0.1	1.5±1.5
Lignin	[g/100gDM]	15.7±1.0	15.9±0.4	17.2±2.4
DM	[%]	91.4±0.3	28.2±0.4	94.6±ND
Ash	[g/100gDM]	5.8±0.7	10.3±0.5	ND±ND
Th. ethanol yield	[g/100gDM]	22.7±0.3	20.5±0.7	22.5±1.1

List of figures

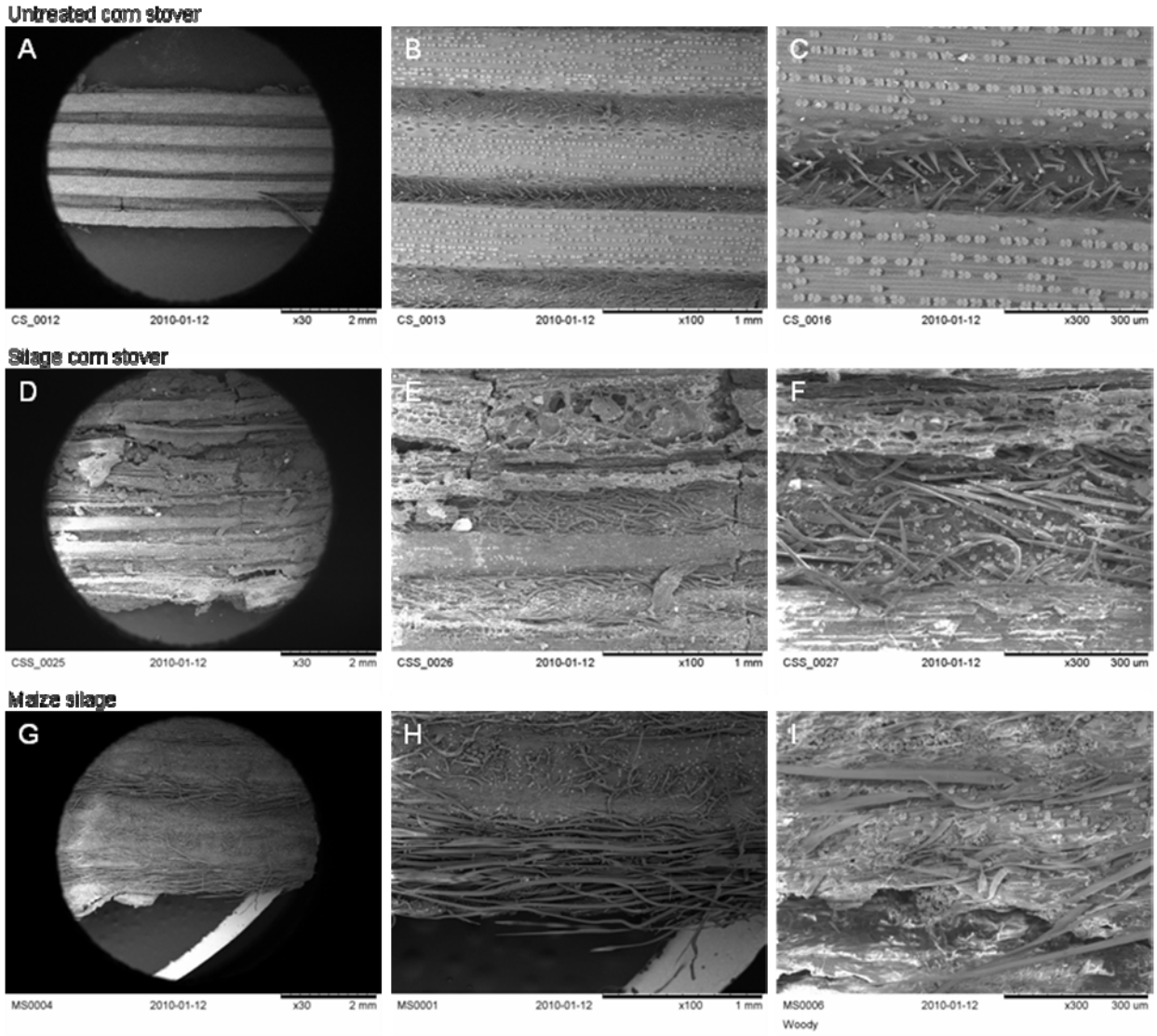

Fig. 1. Corn stover (A-C) and corn stover silage (D-F) used in the enzymatic hydrolysis and fermentation trials. Maize silage (G-I) used for a comparison.

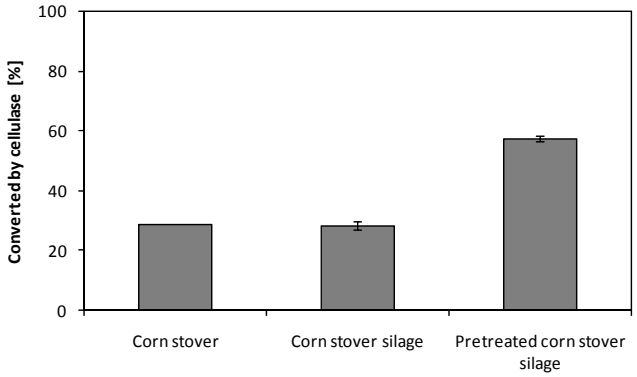

Fig. 2. Enzymatic hydrolysis of corn stover, ensiled corn stover and pretreated (190°C, 10 min) corn stover silage in acetate buffer for 24 hours with enzyme loading of 30 FPU.

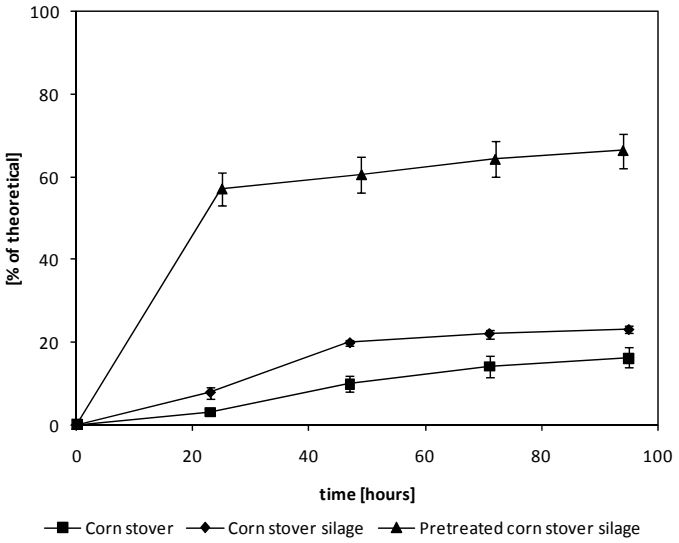

Fig. 3. Ethanol production (expressed in % of the theoretical ethanol yield) during simultaneous saccharification and fermentation by *K. marxianus* at 40°C.

Paper V

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ORIGINAL PAPER

Production of bioethanol from organic whey using *Kluyveromyces marxianus*

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Abstract Ethanol production by *K. marxianus* in whey from organic cheese production was examined in batch and continuous mode. The results showed that no pasteurization or freezing of the whey was necessary and that *K. marxianus* was able to compete with the lactic acid bacteria added during cheese production. The results also showed that, even though some lactic acid fermentation had taken place prior to ethanol fermentation, *K. marxianus* was able to take over and produce ethanol from the remaining lactose, since a significant amount of lactic acid was not produced (1–2 g/l). Batch fermentations showed high ethanol yield (~0.50 g ethanol/g lactose) at both 30°C and 40°C using low pH (4.5) or no pH control. Continuous fermentation of nonsterilized whey was performed using Ca-alginate-immobilized *K. marxianus*. High ethanol productivity (2.5–4.5 g/l/h) was achieved at dilution rate of 0.2/h, and it was concluded that *K. marxianus* is very suitable for industrial ethanol production from whey.

Keywords *Kluyveromyces marxianus* · Cheese whey · Ca-alginate · Immobilization · Continuous fermentation

Introduction

It is a fact that the Earth is running out of fossil raw material. It is also a fact that global warming is changing our climate and that these changes are caused by an increased concentration of CO_2 in the atmosphere. It is therefore of great interest to substitute fossil fuels with renewable natural resources. Bioethanol is a renewable CO_2 reduced fuel that can be produced from raw materials rich in monosaccharides (sugar canes and sugar beets) and from crops rich in starch (corn or wheat). The sustainability of bioethanol obtained from raw materials that can also be used as food or feed (so-called first-generation bioethanol) is questionable. Therefore, it would be more advantageous if bioethanol production could be based on alternative substrates such as lignocellulosic raw materials by using second-generation conversion technologies and other byproducts from agriculture, forestry, and the food industry. Whey is a byproduct from the dairy industry. It represents a disposal problem and is an important source of environmental pollution due to its enormous global production rate all over the world (to make 1 kg cheese, 9 kg whey is generated) [13]. Bioconversion to ethanol could be an alternative use for this feedstock. The major components of whey are lactose (5–6%), protein (0.8–1%), and fat (0.06%) [13]. Lactose is a disaccharide consisting of glucose and galactose. It cannot be fermented by *Saccharomyces cerevisiae*, which is commonly used in alcohol fermentation, because this strain of yeast lacks β-galactosidase activity; it can, however, ferment the hydrolysis products of lactose: glucose and galactose. Unfortunately, acid hydrolysis can form some byproducts that may inhibit the fermentation, and enzymatic hydrolysis will add expense to the process. Another option is to use a different yeast strain, *Kluyveromyces marxianus*, which is capable of fermenting lactose to ethanol directly. *K. marxianus* has been studied extensively for utilization of whey, e.g.: the effect of multiple substrates in ethanol fermentation from cheese whey [17], ethanol production from crude whey

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[19], batch fermentation [1, 18], fed-batch fermentation [1, 9, 15], continuous fermentations [4, 11, 12], studies on cheese whey powder [6, 11–14], immobilization of thermotolerant yeast on delignified cellulosic materials [7], and alginate-immobilized yeast cells [2, 3, 10]. It has been found that, when using alginate-immobilized cells, cell flush-out is avoided and also the production of ethanol is raised compared with ethanol production from free cells [3]. In literature, no experiments have been found that study alginate-immobilized cells of *K. marxianus* in continuous fermentation of cheese whey.

The aims of this study are to find the best way of utilizing whey and to design a process for conversion of organic whey into bioethanol by fermentation using *K. marxianus*. This process is planned to be part of developing a concept for a decentralized biorefinery concept to be used in the organic agricultural industry in Denmark, by integrating energy production (biogas and bioethanol) in organic farming to increase the sustainability and self-reliance of energy utilized in this industry. This can be done by better utilization of byproducts from the farm and/or farm units in combination with byproducts from related food industries such as whey from dairy. Figure 1 shows the concept of the proposed biorefinery, where intercrops and byproducts from the agro industry are used as substrates for on-farm energy production. By combining whey produced from organic cheese production with crops produced by sustainable methods this study shows how bioethanol can be produced in a sustainable way and organic farms and/or dairies can be converted into biorefineries.

Materials and methods

Yeast strain

K. marxianus strain DSMZ 7239 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The strain was maintained at −85°C in a mixture of 50% v/v glycerol and growth medium solution, which contained per litre of demineralized water: 5 g bacto peptone, 5 g yeast extract, 30 g lactose, 2 g NH_4Cl, 0.3 g $MgSO_4 \cdot 7H_2O$, and 1 g KH_2PO_4.

Yeast cultivation

Starter culture of *K. marxianus* DSMZ 7239 was grown in 250-ml cap flasks containing 150 ml culture medium. The medium for growth of yeasts was the same synthetic lactose medium which was used for strain maintenance. The medium was sterilized at 121°C for 20 min. The flasks were incubated in an orbital shaker at 100 rpm for 24 h at 30°C.

Raw material: whey

The cheese whey used in the experiments was provided by the Thise Mejeri organic dairy, Denmark. Four different types of whey were provided, which had been treated differently in the dairy. Type 1 was raw whey taken from the cheese manufacturing process and stored cold (2–5°C). Type 2 was whey that had been stored at room temperature, which causes the lactic acid bacteria (added during the

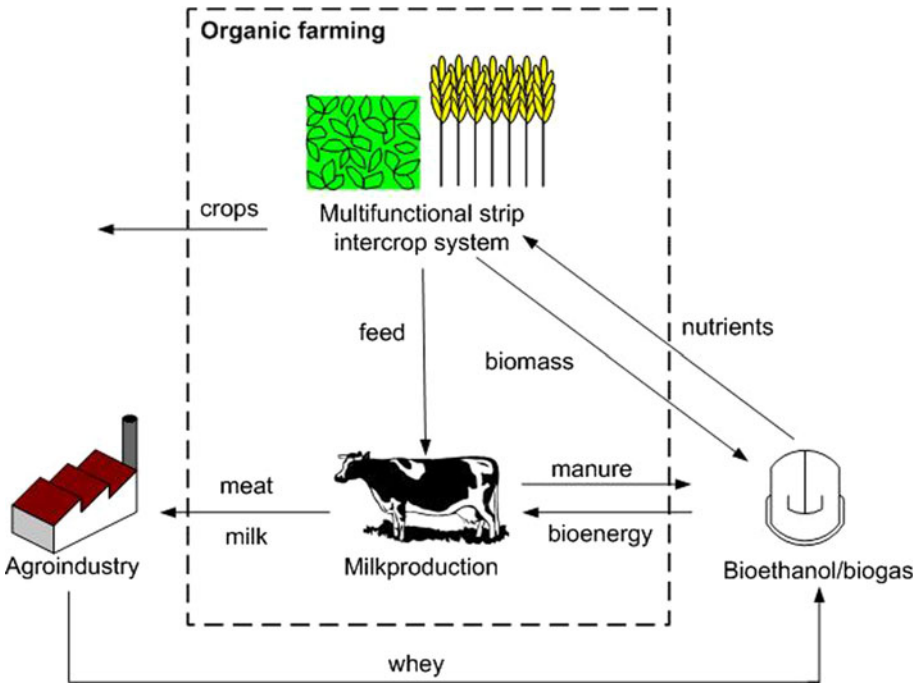

Fig. 1 Concept of bioenergy production in organic farming

cheese-making process) to convert the lactose to lactic acid. After arrival to the laboratory the lactic-acid-fermented whey was stored at 2–5°C. Type 3 was raw whey that had been pasteurized and kept frozen (−5°C), and type 4 was raw whey that had been pasteurized and stored cold (2–5°C). Table 1 gives an overview of how the four types of whey were treated in the dairy.

Screening of the four types of whey in flask fermentations

Inoculum (1 ml) was added to 100 ml whey in 250-ml shake flasks equipped with yeast locks. The flasks were incubated at 30°C at 100 rpm, and samples for lactose and ethanol analysis were taken once a day for 3 days.

Batch fermentation of whey

Two batch fermentations were performed in 2.5-l fermentor (Minifors, Infors HT, Switzerland) containing 2 l nonsterilized whey. In the first experiment 25 ml inoculum [1.25% (v/v)] was added to the whey. The temperature was controlled at 30°C, and pH was maintained at 4.5 by addition of 1 M HCl and 1 M NaOH throughout the fermentation (150 h). In the second batch experiment only 5 ml inoculum [0.25% (v/v)] was added, temperature was controlled at 40°C, and no pH control was applied. The fermentation time was 170 h. Agitation was 500 rpm in both experiments, and samples were withdrawn from the fermentor for analysis of lactose, ethanol, lactic acid, and acetic acid.

Continuous fermentation of whey with Ca-alginate-immobilized *K. marxianus*

Cells of *K. marxianus* were immobilized by suspending 2.6 g centrifuged washed wet cells in 250 ml 4% sodium-alginate gel. The yeast–alginate mixture was extruded as drops into a 4% calcium chloride solution kept on ice. For extrusion a pump and a Pasteur pipette were used, which resulted in uniform round beads of approximately 2 mm in diameter. The beads were washed with sterile 0.1% salt-water and stored in a sterile synthetic lactose medium at 4°C until use. The continuous fermentation was performed in a 300-ml fluidized bed reactor with an outside water flow for temperature control. Beads (100 ml) with immobilized cells were filled in the bottom of the reactor, and the reactor was filled to the overflow with whey. The temperature was kept at 32°C using water pumped from a temperature-controlled water bath to the outside of the reactor. Fermentation was initiated in batch mode for the first 3 h. After 3 h the substrate flow was turned on at low dilution rate ($\mu_{max}/10$). The dilution rates were based on the maximum specific growth rate (μ_{max}) of *K. marxianus* in this type of whey. The nonsterilized whey was kept on ice and pumped to the fermentor using a pump (Masterflex L/S 07534—04, USA). Samples were withdrawn five times in the first 48 h and every 24 h thereafter and analyzed for lactose and ethanol content. Productivity was calculated by multiplying the dilution rate by the actual ethanol concentration.

Table 1 Treatment in the dairy of the four types of whey used in the study

Whey	Pasteurization	Cooling	Freezing	Lactic acid fermentation
1	−	+	−	−
2	−	+	−	+
3	+	−	+	−
4	+	+	−	−

Analytical methods

Growth rate of *K. marxianus* was followed by measuring the optical density at 600 nm using a spectrophotometer (Spectrophotometer 6305; Buch & Holm A/S, Denmark).

The concentrations of lactose, glucose, ethanol, lactic, and acetic acid in the samples were determined by high-performance liquid chromatography (HPLC) (Shimadzu Corp., Kyoto, Japan) using a Rezex ROA column (Phenomenex, Torrance, CA, USA) at 63°C and 4 mM H_2SO_4 as eluent at flow rate of 0.6 ml/min, equipped with a refractive index detector (Shimadzu Corp.). Samples were pH-adjusted to 2.0–2.3 and filtered through a 0.45-μm membrane prior to injection into the vials.

Results and discussion

Screening of the four types of whey in flask fermentations

The whey was treated in four different ways at the dairy (Table 1) before being used for ethanol fermentation by *K. marxianus* in the laboratory. The chemical compositions of the four resulting whey types were analyzed with regards to sugars, ethanol, and organic acid content (Table 2). The composition of type 2 significantly differed from other types. This untreated whey sample, as expected, had much lower content of lactose and high content of lactic acid, due to the natural lactic acid fermentation taking place at these conditions. The composition of types 3 and 4, which had been pasteurized and frozen or kept cool, respectively, did not differ significant from type 1, which had just been kept cool, showing that

Table 2 Chemical composition of the four different types of whey

Concentration (g/l)	Whey 1	Whey 2	Whey 3	Whey 4
pH	5.67	3.46	6.85	6.62
Lactose	46.8	19.3	48.6	48.6
Glucose	0	0.22	0.16	0.15
Xylose	0.16	0.16	0.18	0.17
Acetic acid	0.13	0.25	0.28	0.02
Lactic acid	1.06	9.19	0.21	0.16
Formic acid	0.11	0.28	0.02	0.20
Ethanol	0	0.19	0	0

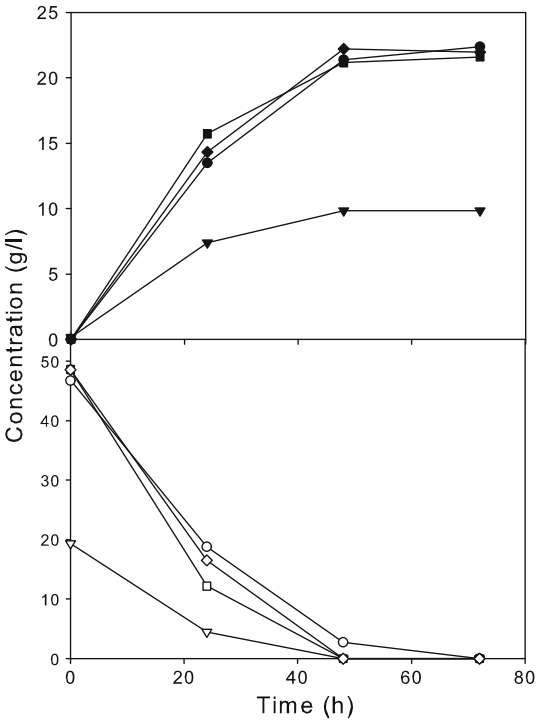

Fig. 2 Ethanol production (*closed symbols*) and lactose consumption (*open symbols*) in flask fermentations of the four different types of whey: type 1 (*circles*), type 2 (*inverted triangles*), type 3 (*squares*), and type 4 (*diamonds*)

pasteurization of the whey was not necessary in order to keep the lactose from being fermented. However, pasteurization might be necessary in order to prevent the lactic acid bacteria (present from the cheese production) from taking over during ethanol fermentation of the whey; this was examined in flask fermentations.

The four types of whey were fermented in flask fermentations with *K. marxianus* to examine the potential ethanol production by this strain and to choose the type of whey to use in subsequent experiments (Fig. 2).

The lactic-acid-fermented whey (type 2) gave the lowest ethanol concentration due to the lower lactose content. Also, ethanol was produced at a lower rate, which could be due to the lactic acid present in this substrate. However, the highest ethanol yield per gram of sugar was achieved in this experiment (0.51 g ethanol/g lactose), which can be explained by the low pH in the whey, which forces the yeast to use a lot of energy pumping H^+ ions out of the cell instead of using the energy on biomass formation. Consequently, this gives a higher ethanol yield, because more lactose is used for production of energy instead of formation of biomass. Similar ethanol yields were obtained in the other three types of whey: 0.48, 0.44, and 0.45 g ethanol/g lactose for types 1, 3, and 4, respectively. Figure 2 depicts that lactose was utilized and the ethanol concentration reached a steady level after 48 h. No lag phase was observed in any of the experiments.

Type 1 was chosen as the type of whey to use in subsequent experiments, since it gave the highest ethanol production as well as a slightly higher ethanol yield (excluding the lactic-acid-fermented whey). Furthermore, these experiments showed that the yeast had no problem competing with the live lactic acid bacteria present in the nonpasteurized whey, and it is advantageous that no pasteurization of the whey is needed before ethanol fermentation.

Batch fermentation of whey with *K. marxianus*

Two different batch experiments of whey (type 1) were performed, at (1) 30°C, pH 4.5 (Fig. 3) and (2) 40°C, without pH control (Fig. 4). Figure 3 illustrates the ethanol production and lactose utilization in the fermentation performed at 30°C and pH 4.5. The low pH was chosen to overcome bacterial contamination. Lactose utilization started within 24 h, and all lactose was utilized after 72 h. The ethanol concentration continued to increase until approximately 140 h, when a concentration of 20 g ethanol/l was achieved, corresponding to a yield of 0.47 g ethanol/g lactose (calculated based on the initial lactose content determined at the beginning of the fermentation). This value (43 g/l) is lower than that shown in Table 1 (46.8 g/l), due to inoculation causing dilution. Slight decrease in lactose content during storage was also observed due to the activity of microorganisms present in the whey, originating from the cheese-making process. No lag phase in ethanol production was observed in this experiment, and the large inoculum size (25 ml) and low pH efficiently controlled lactic acid bacteria, so that no lactic acid was produced.

Batch fermentations were also carried out at 40°C, since our future aim is to apply cheese whey together with different byproducts from organic farming in a biorefinery concept in a simultaneous saccharification and fermentation (SSF) process which is usually carried out at 40°C. Figure 4 shows the ethanol production and lactose

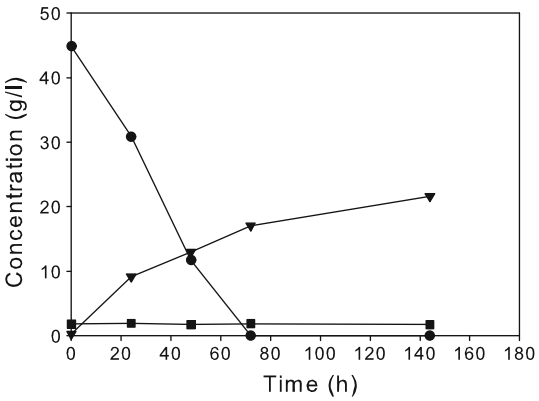

Fig. 3 Lactose consumption and ethanol and lactate production in batch fermentation of nonsterilized whey (type 1) performed at 30°C and pH 4.5 with 1.25% (v/v) inoculum: lactose (*closed circles*), ethanol (*closed inverted triangles*), and lactate (*closed squares*)

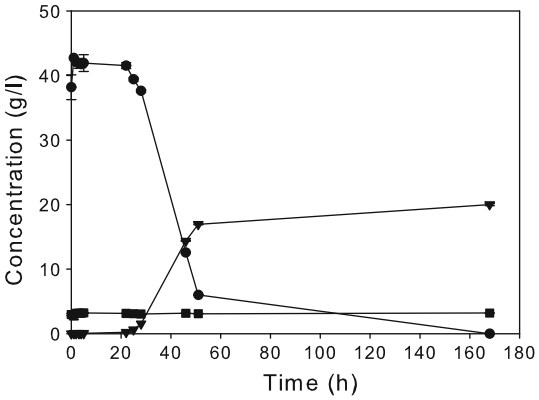

Fig. 4 Lactose consumption and ethanol and lactate production in batch fermentation of nonsterilized whey (type 1) performed at 40°C without pH control with 0.25% (v/v) inoculum: lactose (*closed circles*), ethanol (*closed inverted triangles*), and lactate (*closed squares*)

utilization in the fermentation performed at 40°C without pH control. In this experiment a lag phase of approximately 24 h was observed, which can be explained by the lower inoculation volume used (5 ml). It can be seen from the figure that the initial lactose concentration is slightly lower than in the first experiment (Fig. 3) and that the lactic acid concentration is slightly higher. This could indicate that lactic acid fermentation was initiated during start-up of the fermentor. However, even under these conditions the yeast was able to take over and efficiently convert lactose to ethanol, after the initial lag phase, and no lactic acid was produced during ethanol fermentation. Furthermore, the initial conversion rate was slightly faster at these conditions, which can be explained by the fact that 40°C is closer to the optimal growth temperature of *K. marxianus*, which has been found to be 36°C in our previous experiments (unpublished data). The final ethanol yield was 0.47 g ethanol/g lactose in this experiment (based on initial lactose content in the fermentation). Both experiments showed that *K. marxianus* was capable of adapting to a changing environment very quickly and was able to control the fermentation in the nonsterilized whey.

Continuous fermentation with alginate-immobilized cells of *K. marxianus*

Continuous fermentation was carried out using alginate-immobilized cells. No pH control was applied, and the temperature was kept constant at 32°C. pH in the medium stayed between 4.26 and 4.76 throughout the fermentation. The continuous fermentation was initiated in batch mode (3 h), and the dilution rate was doubled two times until 0.2/h (approximately half the maximum specific growth rate of *K. marxianus*). Figure 5a shows the lactose consumption and ethanol/lactic acid/acetic acid production during the continuous fermentation.

During the first 3 h there was no flow of whey, and the fermentation ran in batch conditions (not shown in the figure). During the first 17 h the dilution rate was set to 0.04/h, which gave a flow rate of 0.2 ml/min. During the following 5 h the dilution rate was increased to 0.08/h, which resulted in flow rate of 0.4 ml/min. After 22 h of the experiment, the dilution rate was changed to 0.2/h and the flow rate to 1 ml/min. This dilution rate remained constant until the end of the experiments.

During the initial phase with low dilution rate (0.04–0.08/h) lactose was efficiently utilized and ethanol production of 17.6 g/l was achieved. The dilution rate was increased to 0.2/h after 22 h, and still very efficient ethanol production was observed. However, after 28–78 h at this dilution rate the lactose in the effluent started to increase and less efficient ethanol production was observed. Nevertheless, this was overcome by the microorganisms, and for the last 100 h of fermentation all lactose was utilized and high ethanol concentrations were measured. No lactic acid was produced during any stages of the fermentation, but towards the end of the fermentation (after approximately 200 h), as the ethanol productivity decreased slightly, some acetic acid was produced. This could be due to changes in the metabolism of *K. marxianus*. The average ethanol yield calculated at dilution rate of 0.2/h was 0.48 g ethanol/g lactose, and during the last stages of the fermentation a very high yield of 0.59 g ethanol/g lactose was measured. Figure 5b shows the productivity at different stages of the fermentation. At the highest dilution rate of 0.2/h the productivity varied between 2.5 and 4.5 g/l/h and stabilized around 4 g/l/h towards the end of the fermentation. Other studies have reported productivity of 0.7 g/l/h by *K. marxianus* in continuous fermentation of whey with free cells [13], 2.9 g/l/h in fed-batch fermentation on lactose medium [9], and 1.3 g/l/in batch fermentation of cane

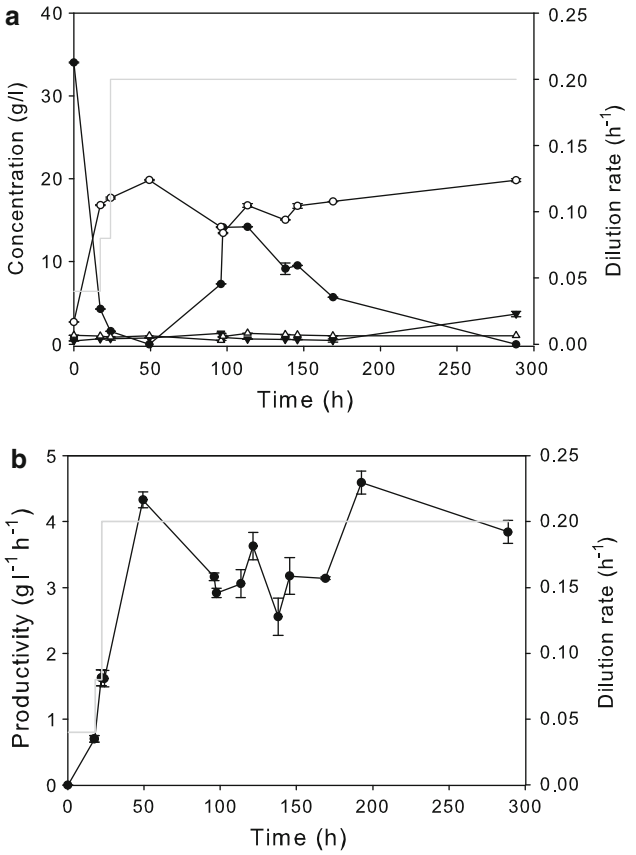

Fig. 5 **a** Lactose consumption and production of ethanol, acetic acid, and lactic acid at different dilution rates in continuous fermentation of nonsterilized whey (type 1) with Ca-alginate-immobilized *K. marxianus*: lactose (*closed circles*), ethanol (*open circles*), acetic acid (*closed inverted triangles*), lactic acid (*open triangles*), and dilution rate (*dash*). **b** Productivity at different dilution rates in continuous fermentation of nonsterilized whey (type 1) with Ca-alginate-immobilized *K. marxianus*: productivity (*closed circles*) and dilution rate (*dash*)

juice [8]. Studies have been carried out using engineered flocculating *S. cerevisiae* on lactose medium, reporting a productivity of up to 2 g/l/h in continuous fermentations [5]. In comparison with these previous studies it seems that immobilization of *K. marxianus* in Ca-alginate gel is a promising method for achieving high ethanol productivity. However, since these productivities were achieved at dilution rate of 0.2/h even higher productivity should be possible, since immobilized systems should be able to run close to or even above the maximum specific growth rate of the microorganism, which has been found to be 0.4/h for *K. marxianus* in this whey medium.

A more suitable immobilization method, e.g., flocculation, should be explored for industrial use, and the system should be optimized to be less fluctuating. Although high biomass loadings can be obtained by gel-entrapment immobilization methods (such as in Ca-alginate), this approach has received less attention in the fermentation industry because of several drawbacks such as diffusion limitations of nutrients, metabolites due to the gel matrix and the high cell densities in the gel beads, chemical and physical instability of the gel, and the nonregenerability of the beads, making this immobilization approach rather expensive [16]. Use of flocculating yeast is very attractive, due to its simplicity and low cost. However, flocculation is affected by numerous parameters, such as nutrient conditions, agitation, Ca^{2+} concentration, pH, fermentation temperature, yeast handling, and storage conditions.

Conclusions

Ethanol production by *K. marxianus* in different kinds of whey from organic cheese production was examined in batch and continuous mode. The results showed that pasteurization was not necessary prior to the process, which is a great advantage from an industrial point of view, where pasteurization/sterilization of the whey would add expense to the process. Batch fermentation of the nonsterilized whey showed high ethanol yields ($\sim$0.50 g ethanol/g lactose) at both 30°C and 40°C using low pH (4.5) or no pH control. Continuous fermentation of nonsterilized whey was performed using Ca-alginate-immobilized *K. marxianus*. High ethanol productivity (4.5 g/l/h) was achieved at dilution rate of 0.2/h, and *K. marxianus* was capable of maintaining high productivity at low pH in nonsterilized whey. *K. marxianus* was able to take over lactic acid bacteria present in the whey and was found to be a very robust microorganism capable of producing ethanol at high temperature and low pH in whey.

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Paper VI

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A Simulation Model of Combined Biogas, Bioethanol and Protein Fodder Co-Production in Organic Farming

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Abstract

In order to evaluate new strategies for the production of renewable energy within sustainable organic agriculture, a process-simulation model for a 100 ha organic farm was developed. Data used for the model was obtained from laboratory trials, literature data, consultancy with experts, and results from the BioConcens project (http://www.bioconcens.elr.dk). Different design approaches were evaluated in order to establish the most suitable configuration. Rye grains, clover grass silage, maize silage, whey and cattle manure were selected as raw materials for co-production of fuels, feed and fertilizer at the organic farm, based on the fact that crops grown in organic agriculture act as key carbon sources whereas manure and whey were applied primarily as the nutrient and water supply for the fermentations within the process (anaerobic digestion and simultaneous saccharification and fermentation, respectively). Results from batch and lab-scale fermentation trials provided basic input for the model. To cover the direct energy requirements on the farm, it was calculated that it requires approximately 16.2 ha of rye and 14 milking cows or 5.7 ha of clover grass, 2.5 ha of maize and 13 cows to supply a 100 ha organic farm with ethanol or biogas, respectively. This calculation was based on the assumption that the electrical efficiency of CHP (combined heat and power) unit was 38%. A variety of different scenarios can be simulated to mirror the farmer's needs.

KEYWORDS: biorefinery, ethanol, anaerobic digestion, sustainable agriculture

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Introduction

The increasing interest in organic products across Europe (Rigby and Caceres, 2001; Hermansen et al., 2004) raised the question as to how it is possible to increase the sustainability of organic farms. The aim of modern organic farming is to enhance the usage of renewable resources in production and processing systems (IFOAM, 2009) which, in the recommendation for Danish organic farmers, is directly connected to the limited usage of fossil fuels and their transfer to using renewable energy (Økologisk Landsforening, 2009). In many European countries, organic farming is often considered to be a potential solution to many environmental problems (Høgh-Jensen, 1998).

Biomass is a key parameter in an agriculture environment for energy production (Jørgensen et al., 2005); two of the very promising technologies which could be applied on the organic farm are: anaerobic digestion for production of biogas and ethanol fermentation (Frederiksson et al., 2006). A sustainable approach to producing biofuels should consider local production from local feedstock, and be adjusted to the socio-economic and environmental characteristic of the region where they are produced (Antizar-Ladislao and Turrion-Gomez, 2008).

Anaerobic digestion, based on animal manure and energy crops, does not only effectively produce energy, but it also reduces green-house gas emissions (Holm-Nielsen et al, 2009). Produced methane through digestion of manure is not released to the atmosphere but instead converted into heat and electricity (methane is 21 times "stronger" greenhouse gas than carbon dioxide). The utilization of biogas instead of fossil fuels further contributes to reduction of CO_2 emissions. In turn, the effluent (digestate) from the bioprocess can serve as a highly efficient and nutritious fertilizer (Ghafoori and Flynn, 2007). Sanchez et al. (2008) indicated the positive influence of anaerobic digestion in the stabilization of livestock farm waste and its application on agricultural land. Returning the key elements back to the soil insures the soil fertility - a basic requirement in organic farming principles (Haas et al., 2002). The main product – biogas (with methane variation 53-70% (Persson et al., 2006)) - can be either utilized in modified diesel engine, gas engine (Tippayawong et al., 2007); cleaned of CO_2 (up to 95-98% methane) and used as a car fuel (Börjesson and Mattiasson, 2007) or upgraded to natural gas quality and injected into the natural gas grid (Persson et al., 2006). When combined into the broader biorefinery concept, (in the co-production of energy, food/feed, and fertilizer) it significantly contributes to the overall biorefinery economy (Agler et al., 2008) and when compared to alternatively technologies (Svensson et al. (2005, 2006)), biogas production from crop residues at the farm level is considered to be one of the most promising.

Ethanol fermentation can be considered as the second promising technology opportunity - even though there might be some obstacles in developing an economically efficient farm scale ethanol plant (Frederiksson et al., 2006). Ethanol from starchy or lignocellulosic material could supply organic farmers with the liquid fuel necessary to run agricultural machinery. Ethanol from raw materials containing starch (1st generation) is a well established technology, whereas lignocellulosic bioethanol (2nd generation) is still under extensive research, with only pilot plant facilities are established (Thomsen and Haugaard-Nielsen, 2008). Second generation ethanol offers several advantages over the first: the whole crop can be converted into ethanol and non food raw materials are also utilized. However, the process needs a pre-treatment step (Thomsen et al., 2006) which is difficult to set up in a farm scale environment. Typical microorganisms applied in the ethanol fermentation can only convert C-6 sugars - combining the fermentation of C-6 and C-5 sugars would increase the overall ethanol yield, but this technology is not yet fully developed. Bioethanol production combined with protein recovery from the process effluent is a common solution in order to optimize the efficiency of the biorefinery process (Prasad et al., 2007).

In order to combine the biogas and bioethanol processes in one farm-scale biorefinery, the process engineering tool is required. The optimal configuration can reduce the energy production cost and increase the sustainability of the farms, and computer simulations have been successfully applied to understand and optimize the bioenergy production processes (Wooley et al., 1999; Kwiatkowski et al., 2006; Cardona and Sanchez, 2007; Ramirez et al., 2008). Different biorefinery concepts were considered: Pfeffer et al. (2007) investigated the usage of ethanol fermentation by-products for biogas production and its conversion into the heat and electricity necessary to cover the demands of the ethanol process. The process heat demand was significantly decreased by the process integration. An alternative solution was proposed by Sadhukhan et al. (2008), who analyzed a biorefinery integrated with value added production - in this case with the co-production of ethanol and arabinoxylans.

A variety of different effluents and process water re-circulations can be employed in a small scale biorefinery system. The main production focuses either on biogas or bioethanol. The choice of the fermentation technologies and the way to achieve effective system optimization depends on the feedstocks and its quality (Haas et al., 2006). The choice of the processes configuration, as well as the scale of the operation, will influence on the overall cost of the plant (Svensson et al. 2005 and 2006).

Throughout this study, the development of bioprocesses (biogas together with fertilizer production and bioethanol together with protein rich fodder production), and its potential cogeneration in a single unit, made it possible to design sustainable energy production for the single organic farm. A process-

simulation model for small organic farms (around 100 ha) was developed in order to evaluate new strategies for the production of renewable energy in sustainable agriculture. We developed a simulation model containing processing information such as: the composition of raw materials, the flow rates of the various streams, the description of the specific unit operations and the amount of generated products. The study suggests that the simulation models will help in handling different substrates, considering diverse configuration and taking into account different process conditions. The presented work is the first step in order to evaluate the optimal biorefinery configuration to enable organic farms to become energy self-sufficient in Danish conditions.

Methodology

The calculations in the presented study were performed using the SuperPro Designer® software (v.7.0 – academic version, Intelligen, Inc.). It was assumed that the total yearly energy requirements for an average organic farm (app. 130 ha) are equal to 60000 kWh (energy demand for a Danish organic farm, "Krogagergaard", (Ørnekildevej 22, Ørslevvester, 4173 Fjenneslev, County of Western Zealand, Denmark)) which would correspond to approximately 50000 kWh (or 180 GJ) per year for a 100 ha organic farm. In all the calculations, the physico-chemical properties of the compounds and the conversion factors were taken from the SuperPro Designer® database or from the handbooks of Perry and Green (1997) and Lide (1993-1994).

System boundaries

To simulate on-farm renewable energy production, an engineering tool was applied. Figure 1 shows the system boundaries of the simulated process.

The effluent serves as animal fodder rich in protein (from the ethanol process) and rich in N/P fertilizer (from the anaerobic digestion process). Effluent from the ethanol process can be divided and the liquid part directed to anaerobic digestion in order to increase the amount of produced fuel, the solid used for animal feeding. The dashed line represents the system boundaries.

The model includes feedstock handling and preparation, fermentation processes and fuel preparation. It does not contain animal production and crop cultivation/harvesting, feed preparation and fuels utilization.

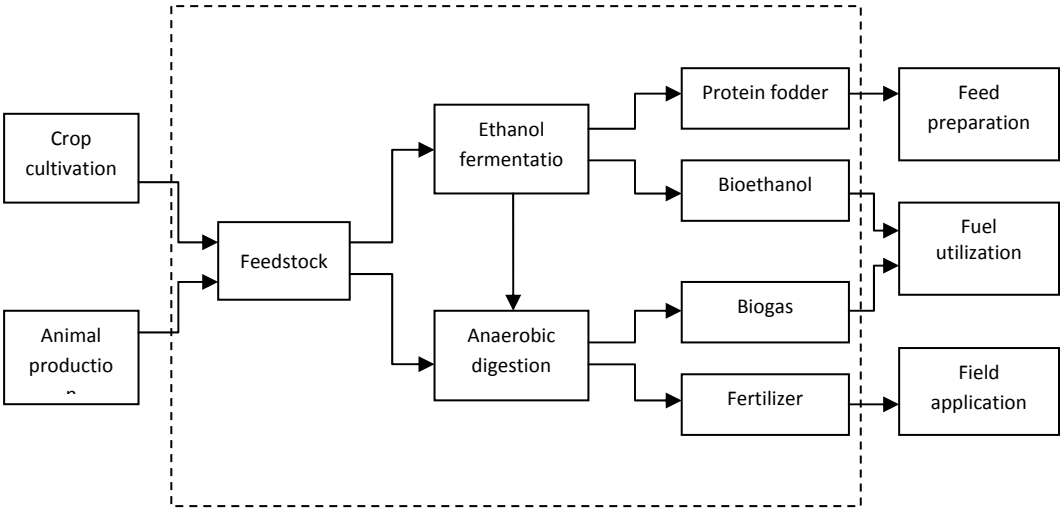

Figure 1. Simplified diagram of the process: The two fuels (bioethanol and biogas) are produced from selected raw materials.

Input data and process assumption

The process includes feedstock handling and preparation, simultaneous saccharification and fermentation for ethanol production, anaerobic digestion for biogas production and downstream processing. Data for the development of the model was obtained from laboratory trials on the biomass potential for ethanol and biogas production in organic farming (not yet published) and ethanol production from germinated grains and whey (not yet published) conducted at the laboratories of NRG-group (Biosystems Division, Risø DTU, Denmark), consultancy with experts, and results from the BioConcens project (http://www.bioconcens.elr.dk). Three different scenarios were evaluated: ethanol production for 100 ha organic farm, biogas production for 100 ha organic farm and the combination of these two processes and energy production for two farms.

Raw materials

In the presented study, the following substrates were chosen: maize silage, clover grass silage, rye grains, whey and cattle manure. Table 1 presents a brief characterization of the selected feedstock.

The total and volatile solids determination was conducted according to standard methods (Greenberg et al., 1998).

The sugar content in silage crops was measured after 24 hours extraction in boiling ethanol in order to remove non-structural components such as waxes, fats and coloring matters. The sugar content in biomass was determined by the "strong acid hydrolysis" procedure - an analytical method that determines the full content of main sugars in the biomass. The biomasses (0.16 g DM) were treated with 1.5mL of H_2SO_4 (72%) at 30^oC for one hour, and then diluted with water and autoclaved (121^oC) for one hour. The acid hydrolyzate was filtered and the sugars were quantified by HPLC (Biorad HPX-87H).

Protein content was taken from database at (Pedersen eds, 2007).

Table 1. Brief characterization of the selected substrates for separate and for co-production of ethanol, biogas and protein fodder

	TS [g/100g]	VS [g/100g]	Significant sugar for process simulation [g/100gTS]	Protein [g/100gTS]	Lactose [g/100gTS]
Biogas					
Maize silage	30.0	28.5	43.3 (glucose)	9.7	-
Clover grass silage	30.0	27.1	24.0 (glucose)	20.0	-
Bioethanol					
Cattle manure	5.0	3.5	8.0 (glucose eq.)	-	-
Rye grains	85.0	-	51.0 (starch)	-	-
Whey permeate	6.9	0.5	73.9 (lactose)	13.0	73.9

- not measured

Ethanol production

We planned that ethanol should be produced in a small scale fermentation process with a yearly production fulfilling the needs for small (100 ha) Danish organic farm. The energy demand was estimated for 180 GJ. To produce 1 MJ of ethanol, 228kJ is required (Frederiksson et al., 2006; Hansson et al., 2007). Based on these results, 63.5 GJ is needed to produce 180 GJ of ethanol - the total energy giving 243.5 GJ. In the calculations the electrical efficiency of the CPH unit was assumed to be 38% (Walla and Schneeberger, 2008). The necessary energy to produce is 640 GJ corresponds to 30330 dm^3 of ethanol (based on low heating

value LHV_{EtOH}=21.1 MJ/dm^3). The heat produced is considered to be an additional product.

Biogas production

The amount of biogas required to supply a 100 ha organic farm with energy was calculated in a similar manner as in the case of ethanol. To produce 1 MJ of methane, 216 kJ of energy is required (Frederiksson et al., 2006; Hansson et al., 2007). Therefore, to produce 180 GJ of CH_4, 38.9 GJ is required, the total energy amounting to approximately 218.8 GJ. The electrical efficiency of the CPH unit was assumed to be 38% (Walla and Schneeberger, 2008). The necessary energy to produce is 576 GJ corresponds to 16090 m^3 CH_4/year (based on low heating value LHV_{CH4}=35.8 MJ/m^3). The heat produced is considered to be an additional product.

Combined ethanol and biogas production

The third scenario combines both bioethanol and biogas production for two farms. The effluent from the ethanol fermentation is separated, the solid part taken for animal feed and the remaining liquid part transferred to the anaerobic digester. The results from this simulation were validated against laboratory trials conducted at Biosystems Division, Risø DTU, Denmark.

Land use and animal production assumptions

The land use was calculated on the assumptions for the yearly average yield for well prospering organic farms in Denmark - for rye grains (4.0 t dry matter/ha), clover grass (10.0 t dry matter/ha) and maize (11.0 t dry matter/ha) (Pederson et al., 2007). It must be remembered that these are only rough numbers and they should be specified for each organic farm as crop yield are dependent on many factors and may differ from year to year.

To estimate the size of a dairy farm in order to generate the required amount of whey for ethanol fermentation, the following assumptions were made. According to Thise – a diary organic farm - from 10 liters of milk, 1 kg of cheese and 9 kg of whey is generated. The Jersey cow (which is often used in organic farming) has a yearly production of 5000 liters of milk (www.thise.eu – Danish organic dairy farm, checked on July 2009).

In a biogas process, to calculate the number of cattle needed to generate required manure, it was assumed that one cow generates 60 kg of manure per day which gives 21900 kg of manure per year (Faculty of Agricultural Science, Aarhus University, 2007).

Results

Process model overview

The whole on-farm biorefinery concept includes many steps from feedstock handling to fuel purification. The model is based on the Danish organic farm energy demand. Table 2 shows rates substrates when the production is focused only on the one of the technologies. The detailed description of the processes in such a simulation is presented below.

Germination of grains

Rye grains, with an average water content of 15%, are soaked with water for 16 hours and stirred a tank to achieve a moisture rate of 40-43%, which is optimal for grain germination. Soaked grains are kept for 24 hours at 25^{o}C in an incubator which allows air flow. During this period, germination occurs and natural amylase enzymes are produced. Afterwards grains are dried at 35^{o}C on trays until the moisture content drop till around 20%.

Whey is collected in a storage tank and pumped and mixed with germinated and grinded grains. Germinated grains contain sugars, whereas whey supplies the process with nutrients, process water and an additional carbon source (lactose).

Fermentation

The fermentation process is carried out at 40^{o}C in a continuous reactor tank. To degrade starch, the inherent enzymes of the malt are used. The fermentation broth is inoculated with *Kluyveromyce marxianus* yeast. This simulation is based on laboratory trials which were conducted at our laboratories. The simulation results were validated against the one made during laboratory experiments. The hydraulic retention time is 40 hours. Half of the effluent is recirculated back to the fermenter.

Distillation

Distillation of the fermentation broth takes place in two distilling columns heated by steam. The ethanol concentration after the first column is approximately 68%, after the second, almost 95%. The distillate is transferred through an organic membrane for dewatering ethanol. In this way, it is possible to achieve 99.6% ethanol. The effluent from the first distillation step can be either used as animal

feed or separated, the liquid part pumped into a biogas reactor and remaining solid part used as animal feed.

Feedstock preparation for biogas production

Maize and clover grass silages are stored in silo prior to the fermentation process. They are transported by screw conveyors and shredded before being added to the reactor. Shredding is important procedure to avoid clogging and blocking the pumps and stirrers. Both substrates contain around 30% of water, typical for ensiled crops. Manure (with a water content of 95%) is pumped directly to the reactors. The storage of manure before the anaerobic digestion process should avoided or minimized to decrease the risk of uncontrolled fermentation.

Anaerobic digestion

The anaerobic digestion of maize silage, clover grass silage and cattle manure takes place in a concrete continuously stirred tank reactor (CSTR). The hydraulic retention time (HRT) is 20 days, which is usual HRT for Danish biogas plants using similar substrates. The process runs under thermophilic conditions (55^{o}C). The biogas potentials of separated substrates were measured at our laboratories (results not published yet). After the process, the effluent is pumped to the covered post-treatment tank and the remaining produced gas is recovered. Produced biogas contains approximately 65% methane - such a concentration is achievable in a well optimized biogas plant operating on maize silage, clover grass silage and cattle manure.

Additionally, the effluent from ethanol distillation can serve as an extra carbon source for anaerobic digestion. Biogas (ca. 65% methane) can be used either in a CHP unit or in farming machinery adjusted to run on biogas.

Fertilizer recovery

Effluent (digestate) from anaerobic digestion is commonly used by farmers as a natural fertilizer. It contains undigested lignocellulosic leftovers (valuable carbon source for soil) and significant amounts of nitrogen, phosphorous and potassium, all originating from manure. It can be applied in a form it exists in directly to agricultural land or it can be divided into solid and liquids phase, serving for soil conditioning and irrigation respectively. Additional unit procedures can be added to the simulation model to meet the farmers' requirements.

Energy use

The values of the required amount of renewable fuel to fulfill the organic farm's energy demands (with the assumption described in "Methodology" section) are depicted in table 2. The numbers are the results based on the simulation process model.

Table 2. Organic farm biorefinery substrates and products rates from the process model

Substrate/Product	Rate
Ethanol production	
Rye grains (15% water)	76 212 kg/year
Whey (93% water)	76 212 kg/year
Ethanol (99.6%)	30 503 L/year
Anaerobic Digestion	
Clover grass silage (70% water)	188 340 kg/year
Maize silage (70% water)	91 980 kg/year
Cattle manure (95% water)	283 824 kg/year
Biogas (65% methane)	26 710 m^3/year
Biogas (65% methane) (if part of the effluent from ethanol production would be included)	28 534 m^3/year

In table 3, the volume of the fermenters is specified based on the SuperPro Designer® simulation model.

Table 3. Fermenters specification based on the outcome from the simulation model

Description	Volume
Ethanol production	
Reactor working volume	1 276 L
Reactor total volume	1 418 L
Anaerobic Digestion	
Reactor working volume	30 429 L
Reactor total volume	40 572 L

Figure 2 shows concept for producing biogas, ethanol, animal feed and natural fertilizer.

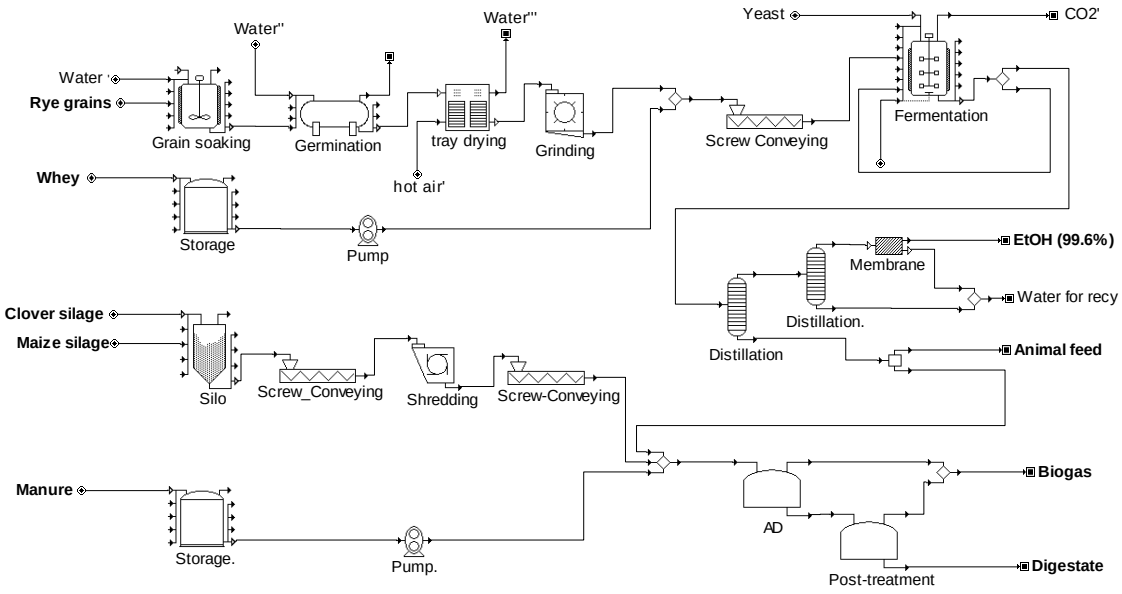

Figure 2. Simplified flow diagram of the organic farm scale biorefinery for the co-production of biogas and bioethanol

Land use

Based on the assumptions from "land use and animal production" section and the results in table 3, a rough estimation of land need and the size of animal production for both ethanol and biogas processes was performed.

The presented scenario requires approximately 16.2 ha of rye (16.2% of the farm land) and 14 milking cows for ethanol production. For biogas generations, the land requirements are 5.7 ha for clover grass and 2.5 ha for maize (in total 8.2% of the farm land). To generate required amount of cattle manure, 13 cows are necessary.

Discussion

Organic farming is often seen as "a living entity", operating self-regulating cycles with a tendency towards a closed system in a nutrients flow, remaining "responsive and adapted to its environment" (Woodward, 2002). In the development of the biorefinery for organic farms, the principles for organic agriculture must be remembered.

The ethanol process is based on fermentation from germinated grains without the application of industrial enzymes. To obey the rules for organic

farming, no genetically altered organisms, enzymes or chemicals produced by genetically altered organisms are used in this process set up. By steeping and germinating the grain, natural enzymes (amylases) are produced, enough to hydrolyze the starch material in the grain which, in turn, facilitates ethanol production. This method was first examined at the laboratory in a small scale (100g), and based on promising results (not yet published) the process model was built. What justifies the usage of starch material for ethanol production is the significant amount of grains on the farms which very often cannot be used either for human or animal consumption (bad quality, rotting, etc.). It is hard to estimate their amount, as it differs from year to year, but what remains constant is the fact that their use in ethanol production would be a good alternative to discharging them. In the future the lignocellulosic biomass should be primary source for on-farm bioethanol, using grain as the only additional carbon source. However, at the moment, the 2nd generation bioethanol technologies do not seem to be suitable for small scale production, due to their high cost of production and high energy usage, especially in organic farming as they require high pressure, temperature and chemical additives.

The mixture of rye grains and whey will be further optimized in laboratory trials to achieve the most efficient result. The ratio between substrates can also be adjusted to suit the personal needs of farm and the amount of available feedstock on the farm. In the presented process *Kluyveromyces marxianus* yeasts are applied. The choice of *K.marxianus* was derived from the usage of whey in the fermentation process. The major component – lactose - cannot be fermented by *S.cerevisiae*, because it lacks β-galactosidase activity. The distillation step takes place at the farm in order to obtain 99.6% ethanol, which could be used as fuel for agriculture machinery. In some cases, due to the ethanol properties as fuel, an ignition improver has to be added (Frederiksson et al., 2006) – this part is not included in the simulation. Ethanol can be stored in fuel tanks on farms, which must fulfill Danish regulation for fuel storage. The effluent from the initial distillation can be partly recycled as process water; the rest can be either recirculated to the biogas process or recovered as protein fodder.

The advantage of bioethnol over biogas is the simplicity of its storage. It can be stored directly on the farm and does not require any special equipment. Ethanol can be mixed with fossil fuels (in that case only minor changes of the engines are necessary) or used pure (somewhat more advanced adjustment is necessary but significantly smaller than converting a typical engine to run on gas) in conventional engines. Furthermore, liquid fuel can easily be sold as the current fuel infrastructure is set for liquid fuels.

Anaerobic digestion runs on silage crops and animal manure. Cattle manure serves mainly as a source of nutrients and microorganisms. Its strong buffer capacity creates favorable conditions for anaerobes. To boost the methane

production, an extra carbon source in the form of silage crops was added to the reactor. Similar to ethanol process, the combination of raw materials can be further optimized and adjusted to the specific organic farm. The effluent from anaerobic digestion (digestate) is proved to be a valuable micro- and macro-nutrients fertilizer (Braun and Wellinger, 2002). In organic farming, the use of industrials fertilizers is prohibited and the recirculation of animal and green manure to the soil is a common practice to keep the nutrients balance. Produced biogas can be burned in a modified diesel engine or gas turbine to produced heat and electricity. The second solution would be to up-grade it to natural gas quality and utilizes it as natural gas. This can be achieved in a simple absorption tower with a degasifying unit for the recirculation of process water.

Heat recirculation was not modeled in the SuperPro Designer® but it is an important factor to improve the economy of the biorefinery. In a case where biogas is utilized on the farm in a modified diesel engine or gas turbine, waste heat produced during the generation of electricity can be utilized to assure the fermentation processes temperatures. Excess heat could also be applied to the distillation process for ethanol production. It would significantly decrease the energy demand for such a biorefinery.

The yields of the crops used for our calculations were the average yield for organic farming in Denmark in 2007. When adjusting to the selected organic farm, the average of several years' crop rotation, soil conditions, etc. should be taken for the calculations to achieve more exact results. The entire pointed land area is only needed when the production is concentrated on just one of the products. In case of the production of both ethanol and biogas, the land area could be combined. In the production of ethanol, only part of the crop is used (grain), resulting in significantly higher land use when compared to the needs of biogas. If second generation technology was introduced, the efficiency of land use would be improved.

In the presented scenarios, the production of on-farm energy, in order to increase self-sufficiency of a 100 ha Danish organic farm, requires around 16.2% and 8.2% of the farm land and a very small livestock farm to produce either bioethanol or biogas respectively.

These initial results rise the question as to whether it could be economical feasible to establish such a small on-farm bioenergy production facility or whether it would be better to build a centralized biorefinery to join approximately 10 organic farms for the area of 1000 ha. The third scenario combines both processes and, at the same time, two farms. In this way, approximately 8% more biogas is produced (due to utilization of effluent from ethanol process). Moreover, the farmers would benefit from having diversified fuels, which they could use in the most efficient way. Bioethanol is stored more easily than biogas and could be applied when more energy is required. It is also a highly efficient car fuel. On the

other hand, the biogas process is more effective (smaller area of land is needed to produce the same amount of energy), and it is also very good fuel for CHP units to constantly produce heat and electricity. Additionally, having both processes would benefit in two ways - the excess heat from biogas running CHP unit would supply ethanol distillation with the necessary energy, and effluent from ethanol fermentation could be additionally streamed to the anaerobic digester.

The ethical issue is to which extend we should use raw materials such as rye (grains) or maize (the whole crop silage) for energy. In the future, on-farm bioenergy production should mainly focus on non-food crops e.g. clover grass and agricultural by-products such as animal manure or whey permeate.

Conclusions

We showed that there is definitely enough land and agricultural waste products to generate renewable energy directly on the farm (16% and 8% for ethanol or biogas production respectively). However, the total capital investments have not yet been estimated, which might likely be too high for a single farm biorefinery. Centralized system, the joining together of several of organic farms (for an area of approximately 1000 ha) should be reflected upon. The limitation of centralized bioenergy production is the broad distribution of organic farms in Denmark, which was also the most significant argument behind developing a single farm system.

In further development of the simulation model, more raw materials should be taken into consideration. The laboratory trials with different mixtures of feedstock will help to optimize the processes. Second generation ethanol production from lignocellulosic materials should also be investigated.

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Paper VII

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Techno-economic analysis of bioethanol and biogas production in organic farming

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Abstract
Bioethanol and biogas production are promising ways to provide renewable energy in organic farming. Five scenarios for bioenergy production at 1000 ha organic farm were simulated: Biogas I/II (10%/20% clover grass silage and cattle manure), Bioethanol I/II (10%/20% rye grains and whey) and combination of both. Combined scenario was characterized by the highest investment (3,330,000 USD) and the largest energy produced (29244 GJ/year). Biogas II was second best (26409 GJ/year energy produced) and it was characterized by lower investment cost (1,963,000 USD) compared to the Biogas I (19970 GJ/year energy produced; 2,016,000 USD). Bioethanol I and Bioethanol II presented the lowest investment costs (1,115,000 USD and 1,047,000 USD, respectively); they generated the least energy (4034 GJ/year and 5610 GJ/year, respectively). Bioethanol I indicated higher total capital investment compared to the scenario Bioethanol II. If the energy needs for 1000 ha organic farm are assumed for 1800 GJ, and the energy conversion efficiency of 30%, only the Biogas scenarios and the Combined one can meet the energy requirements. The remaining energy can be sold and add extra profit to the farm. In case of Ethanol scenarios, energy conversion efficiency of 45% (Ethanol I) or 32% (Ethanol II) is necessary to support farm with own produced energy.

Key words
Bioethanol, biogas, biorefinery, organic farming

INTRODUCTION

Bioethanol and biogas production are promising ways to provide renewable energy. If produced sustainable, both can significantly contribute to the reduced greenhouse gasses emission (Fleming et al., 2006; Jury et al., 2010). Bioethanol produced via yeast fermentation is a proven technology and the first generation (from starch) is already widely applied in a commercial scale (Zuurbier and van de Vooren, 2008). Ethanol has been verified to be an excellent vehicle fuel (Mandil eds., 2004). Biogas, on the other hand, is produced through anaerobic digestion process, which is already a mature technology (Mata-Alvarez et al., 2000) and which has been at a commercial scale for at least 30 years. Anaerobic digestion has been recognized as one of the most environmentally-friendly and energy efficient technology for bioenergy production form biomass (Wieland, 2010). The obtained gas can be both an efficient vehicle fuel (Borjesson and Mattiasson, 2007) and fuel for heat and electricity generation (Tippayawong et al., 2007). Each of those two fuels can be produced from variety of biomass available in agriculture, preferable from agricultural residuals (Jacques et al., 1999; Borjesson and Berglund, 2007; Thomsen and Haugaard-Nielsen, 2008) and both can be successfully applied in the organic agriculture.

In organic farming there is a trend that the whole production chain should use natural and renewable resources (IFOAM, 2010), including fuel, heat and electricity generation. The possible feedstock for

bioethanol and biogas potential in organic farming were evaluated and presented in (Oleskowicz-Popiel et. al., 2010), showing maize and rye silages being the most promising raw materials for biogas production whereas dried rye and fresh maize were characterized by the highest ethanol potentials among investigated crops.

The idea in self-sufficiency in fuel, heat and electricity production directly on the farm is gaining popularity among organic farmers. Several studies have been investigating different scenarios. An evaluation of energy balances and environmental impacts for farm systems supplied with rape methyl ester (RME), ethanol or biogas was described by Fredriksson et al. (2006). It was concluded that the global warming effects of all three examples could be reduced by 58-72% compared to farm using diesel. A follow up work was presented by Hansson et al. (2007), however this time the fuels were produced industrially instead of on-farm. The costs of using different biofuels were calculated, concluding that the ethanol scenario could provide a comparatively low cost fuel. However, in a case of rape methyl ester, the energy efficiency was in favor. The biogas scenario, fed with ley crops, was evaluated as the least economic valuable among all three due to high costs of transport and storage as well as due to assumed need for cleaning it from carbon dioxide and corrosive substances (Hansson et al., 2007). In most of the CPH units, biogas does not have to be upgraded and can be efficiently burnt with methane content of 50-60%. It reduces the costs of biogas production which makes it very suitable for organic farming.

The scenario of supplying organic farm with second generation biofuels (Fisher-Tropsch diesel and dimethyl ether) was shown in (Ahlgren et al., 2008), where up to 82-95% of the global warming effects could be reduced compare to diesel, depending on a raw material use for production of bioenergy. Different view was presented by Svensson et al. (2005), where the focus was on a farm scale biogas process in order to supply an organic farm with energy. Different available systems were tested and discussed. The conclusion was drawn that the high-solids single-stage fed-batch technology would be the most suitable. Additionally, using agricultural by-products for biogas production, instead of ploughing them as green manure, would increase profit of such a farm. Ahlgren et al. (2009), on the other hand, evaluated three different scenarios of making organic farm self-sufficient in a tractor fuel. The scenarios included usage of wheat straw, salix or ley to produce hydrogen either through gasification or anaerobic digestion combined with reforming. The authors indicated that in those scenarios the global warming potential effect can be reduced as much as by 89-97% compared with diesel.

As indicated above, using renewable energy and/or producing it directly on-farm has been already environmentally justified, therefore this study focuses on techno-economic analysis of such a possibility. Several techno-economic studies evaluating diverse full-scale biofuels production has been published (Marchetti and Errazu, 2008; Aden and Foust, 2009; Eggeman and Elander, 2005; Klein-Marcuschamer et al., 2010). However, none of them deals with applying it in the organic farming environment.

This work focuses on a farm scale technology. The area of an organic farm is assumed to be 1000 ha, where 10% or 20% of it is dedicated for energy production. On the farm diverse grains, cash crops and the whole crops are cultivated, it also have livestock production. Five different scenarios for production biogas, bioethanol or both for an organic farm from clover grass silage, rye grains, cattle manure and whey permeate were evaluated. The obtained results were compared to previous studies and economic estimation performed.

METHODS

Principles

The basic principles of the process were described in (Oleskowicz-Popiel et al., 2009), however new scenarios and improved models were developed in this study. The simulation software applied was SuperPro Designer (v.8.0 academic version, Intelligen Inc.). The design of the flow sheets was partly based on a model presented by Klein-Marcuschamer et al. (2010), however due to different scale of the process some of the unit operation were simplified or skipped. The technical model consists of raw materials composition, unit operations representing different processes: feedstock handling, inoculum production, ethanol fermentation, anaerobic digestion process, ethanol distillation. The ethanol and biogas yields were validated against data published in Oleskowicz-Popiel et al. (2010). The schematic view of the simulated scenarios is presented on the figure 1.

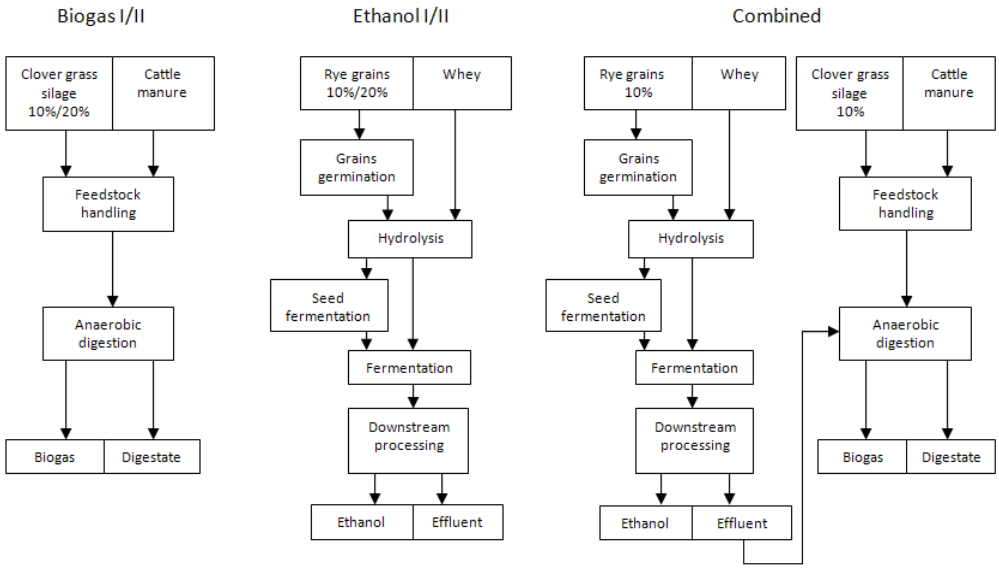

Figure 1. Scematic representation of the different scenarios

Process design overview

The concept for bioethanol and biogas production directly at the organic farm was developed. Three different scenarios were studied: separate biogas and bioethanol production and the combination of those two. The main purpose is to compare each of the technologies and their possible application for the organic farms and to discuss if any of those concepts can be suitable from technical and economic point of view. The processes are designed in continuous mode.

The area of the farm is 1000 ha; the percentages dedicated for diverse agriculture products are depicted in table 1. The description of the organic farm and its products distribution was presented in details in (Pugesgaard et al., 2010). The amount of livestock on the farm depending on the scenario is also shown. Whey for ethanol comes from dairy farm and manure for biogas comes directly from livestock.

Briefly describing the basics of the model: the raw materials are transported to the biorefinery and the first section is the feedstock handling. Ethanol is produced from rye grains and whey. The grains are washed from any impurities and germinated according to (Kadar et al., 2010): soaked in a room

temperature until moisture reaches 40-50%. Furthermore, they are germinated for 24 hours at 25°C; during this process the amylase enzymes are produced. The germinated grains are dried at 35°C. The dried grains (moisture content of approximately 20%) are then grinded and mixed with whey. The mixture is pumped to the prehydrolysis reactor, where amylase enzymes are activated. The process takes place at 50°c for 24 hours. The mixture is fermented by *Kluyveromyces marxianus* at 40°C, obtained yields are according to (Kadar et al., 2010 and Christensen et al., 2010).

Table 1. Characteristic of the organic farm, depending on the scenario

		Scenarios				
		Biogas I	Biogas II	Bioethanol I	Bioethanol II	Combined
Grain	[ha]	164.3	326.2	164.3	326.2	81.9
Cash crops	[ha]	28.9	57.5	28.9	57.5	14.4
Whole crops	[ha]	700.6	410.3	700.6	410.3	700.6
Set aside	[ha]	6.0	6.0	6.0	6.0	3.0
Energy crop	[ha]	100.0	200.0	100.0	200.0	200.0
Dairy cows	[heads]	496	291	496	291	496
Heifers	[heads]	536	314	536	314	536

The inoculum is produced in three seed fermentors; each one is 10% volume of the next one. The residence time is set up for 8 hours, the biomass productivity according to (Barba et al., 2001). The ethanol concentration in the effluent is approximately 36g/L, it is recovered in two step distillations, reaching the concentration of 88%, the final one (99.2%) is achieved through molecular sieve.

For anaerobic digestion process clover grass silage and cattle manure are the two substrates. Clover grass silage for biogas production is firstly washed to remove soil and dirt, shredded and then mixed with cattle manure before entering the reactor. Anaerobic digestion is performed in two stage process, each run in thermophilic conditions (55°C) with hydraulic retention time of 20 days based on (Oleskowicz-Popiel et al., 2010 and Nielsen et al., 2010). The amount of methane in biogas is approximately 60%, the rest of it consist of carbon dioxide. Obtained biogas can be directly used in nearby CHP (combined heat and power) unit or specially adjusted tractor.

The Combined scenario include both biogas and bioethanol production. It puts together those two processes in a way that effluent stream from ethanol fermentation is directed to anaerobic digestion resulting in additional biogas production.

Economic assumptions

The prices of the equipment were estimated based on (Aden et al., 2002), (Klein-Marcuschamer et al., 2010), and SuperPro Designer database. All the prices are shown in 2008 USD.

RESULTS

Scenarios

The scenarios showed in table 2 were modeled in the process simulation software. Five cases were designed to meet possible potentials of an organic farm: production of biogas from clover grass silage and cattle manure (two scenarios), production of bioethanol from rye grains and whey (two scenarios), and combination of those two to produce on-farm biogas and bioethanol.

Table 2 shows available biomass in tons dry matter per year and the input to the model in kg of wet biomass per hour. Furthermore, the theoretical ethanol yields (based on sugar content) and practical methane yield (based on batch experiments), measured and described in (Oleskowicz-Popiel et al., 2010), are shown (table 4). The annual operating time of the plant was 7920 hours. The simulated methane and ethanol are about 85-90% of the maximum theoretical ones.

Table 2. Five scenarios for production of biogas and/or bioethanol for an organic farm

		Scenarios				
		Biogas I	Biogas II	Bioethanol I	Bioethanol II	Combined
Clover grass silage	[tDM/year]	822.8	1645.6			822.8
Cattle manure	[tDM/year]	1071.5	627.9			1071.5
Rye grains	[tDM/year]			365.5	731.0	365.5
Whey	[t/year]			3557.4	2084.6	3557.4
		Input for SuperPro				
Clover grass silage	[kg wet/h]	597	1194	-	-	597
Cattle manure	[kg wet/h]	1853	1086	-	-	1853
Rye grains	[kg wet/h]	-	-	53	107	53
Whey	[kg wet/h]	-	-	449	263	449

Scenarios Biogas I and Biogas II described possible biogas production when 10% and 20% of the organic farm was dedicated for bioenergy. In those two scenarios it was assumed that clover grass silage would be main feedstock for the anaerobic digestion process (823 tons dry matter per years for the first one and 1646 tons dry matter per year for the second one). Cattle manure served as water and nutrients supply. When more clover grass was dedicated for biogas production, less manure would be available for this process (1072 tons dry matter/year compared to 628 tons dry matter/year for the second scenario).

Scenarios Bioethanol I and Bioethanol II presented option for an on-farm ethanol production from rye grains and whey permeate. Similar to biogas cases, it was assumed that rye production could occupy 10% or 20% of the farm cropping area. In Bioethanol I, 366 tons dry matter of rye grains was available for bioenergy, whereas in Bioethanol II scenario, the amount increased till 731 tons dry matter per year. The amount of whey permeate available to produce ethanol was higher in the first scenario (3557 tons/year) compared to the second option (2085 tons/year).

The last scenario called Combined, merged scenario Biogas I and Bioethanol I and resulted in co-production of both biogas and bioethanol. Effluent from ethanol production is rich in several chemical compounds such as proteins, remaining sugars (only C6 sugars were converted into ethanol), cell biomass, acetic acid. All of those can be converted into methane during the anaerobic digestion process. In the Combined scenario the whole effluent stream from the ethanol fermentation was directed to the anaerobic digestion process.

Table 3. Theoretical ethanol, practical biogas yields (Oleskowicz-Popiel et al., 2010) and results for simulated scenarios

		Scenario				
		Biogas I	Biogas II	Bioethanol I	Bioethanol II	Combined
		Theoretical methane				
Clover grass silage	[m3/h]	41.6	83.1	-	-	41.6
Cattle manure	[m3/h]	40.7	23.9	-	-	40.7
Sum	[m3/h]	82.3	107.0	-	-	82.3
		Theoretical ethanol				
Rye grains	[L/h]	-	-	16.2	32.5	16.2
Whey	[L/h]	-	-	10.7	6.3	10.7
Sum	[L/h]	-	-	27.0	38.8	27.0
Simulation methane	[m3/h]	70.4	93.1	-	-	88.9
Simulation ethanol	[L/h]	-	-	24.3	33.7	24.3

Economics

In table 4, prices of the most important equipments are indicated. For the anaerobic digestion process, the digestors were the most crucial pieces of equipment and at the same the most expensive ones. For the ethanol scenarios, cost of the pre-hydrolysis reactor, seed and main fermentors, distillation columns and molecular sieve are indicated.

Table 4. Main equipment price list for described scenarios in 2008 USD

	Biogas I	Biogas II	Bioethanol I	Bioethanol II	Combined
Prehydrolysis reactor	-	-	195.000	158.000	195.000
Seed fermentor 1	-	-	5.000	4.000	5.000
Seed fermentor 2	-	-	17.000	14.000	17.000
Seed fermentor 3	-	-	40.000	33.000	40.000
Main fermentor	-	-	263.000	213.000	263.000
Distillation column I	-	-	37.000	37.000	37.000
Distillation column II	-	-	43.000	45.000	43.000
Molecular sieve	-	-	34.000	42.000	34.000
Anaerobic digestor	910.000	867.000	-	-	997.000
Anaerobic digestor	901.000	853.000	-	-	985.000

The outcome of the simulation for all five scenarios is summarized in table 5. Substrates costs were significantly higher for the Bioethanol scenarios as well as the utilities costs compared to the Biogas scenarios. On the other hand, the equipment purchase costs were much lower for the Ethanol scenario, which resulted in double total capital investment for the Biogas scenarios compared to the Ethanol ones. The retention time in anaerobic digestion is longer compared to ethanol fermentation (20 days compared to 40 hours, respectively). Long retention time means much larger fermentors capacity, more power for stirring and for heating (biogas process run at 55°C whereas yeast fermentation at 40°C). Thus, much higher cost for anaerobic digestion installation. Typical retention time for thermophilic anaerobic digestion process is 15 days; in our case it was prolonged due to an energy crop input. All the costs were the highest for the Combined scenario.

Table 5. Plant capacity and main economic indicators for different scenarios in 2008 USD

	Biogas I	Biogas II	Bioethanol I	Bioethanol II	Combined
Raw material cost	30.000	46.000	192.000	213.000	222.000
Utilities cost	78.000	101.000	252.000	188.000	337.000
Equipment purchase cost	1.911.000	1.857.000	1.023.000	962.000	3.123.000
Total capital investment	2.016.000	1.963.000	1.115.000	1.047.000	3.330.000
Total operating cost	108.000	146.000	444.000	402.000	559.000

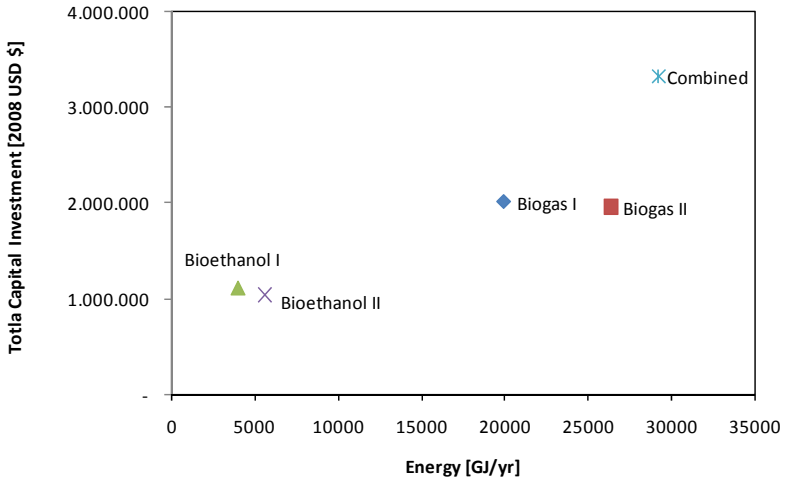

Figure 2. Relationship between total capital investment of each scenario (2008 USD) and produced energy (in GJ/yr)

The relationship between the amount of produced energy (GJ per year) and the total capital investment is shown on the figure 2. The costs were presented in 2008 USD. The Combined scenario was characterized by the highest investment but also by the largest energy produced (29244 GJ/year). Scenario Biogas II was second best in terms of amount of produced energy (26409 GJ/year) and it was characterized by slightly lower investment cost compared to the scenario Biogas I, which was also less effective in terms of energy (19970 GJ/year). Even though, Bioethanol I and Bioethanol II presented the lowest investment costs, they also generated the least energy (4034 GJ/year – Bioethanol I and 5610 GJ/year – Bioethanol II). Scenario Bioethanol I indicated slightly higher total capital investment compared to the scenario Bioethanol II.

DISCUSSION

There is growing interest for a small farm-scale production of renewable energy (Ahlgren et al., 2008). Especially organic farmers are interested and forced in improving their sustainability by using “green” energy and at the same time to make their farms self sufficient in terms of energy supply.

Five different scenarios for on-farm bioenergy production were evaluated. Scenarios Biogas II and Bioethanol II appeared to be more efficient in terms of produced energy and total capital investments compared to scenarios Biogas I and Bioethanol I, respectively. Higher costs of investment for scenarios “I” were caused by large amount of liquid raw materials available (cattle manure for anaerobic

digestion and whey for ethanol fermentation). Both, cattle manure and whey permeate are important feedstock for the fermentation processes, providing nutrients and water to the process. They also serve as carbon source; however, much less methane (cattle manure) or ethanol (whey permeate) can be produced from them compared to clover grass silage (methane) or rye grains (ethanol). Additionally, big volume of liquid substrate increased the volumes of the reactors, consequently the equipment costs. Increasing dry matter content in the process definitely would lead to cutting the overall costs. Svensson et al. (2005) discussed biogas production from crop residues on a farm-scale level, it was indicated that single stage high-solids digester was the most competitive.

Both Bioethanol scenarios were characterized by low energy generation and rather high cost of investment. Different configuration could be investigated in order to minimize those costs. Leaving out the seed fermentors (buying dry yeast, instead producing the inoculums directly on the farm) or reducing the downstream processing by moving out part of the distillation process to a bigger facility could be two possible solutions to consider. Ahlgren et al. (2008) considered on-farm biomass cultivation and then being transported to a large scale fuel production facility and the fuel returned and used on the farm. Ethanol is produced only from sugars, in our case only from C6 sugars. To increase the economic efficiency of the process, C5 sugars should also be converted in ethanol. Additional profit could be taken out of the effluent. It is rich in proteins stream and by simple separation/concentration could be sold as high valuable animal feed.

Labor demand was not accounted in the presented simulations. In general, biogas plant is simpler in operation, key elements are feeding and gas collection. In ethanol production, on the other hand, each step (grains germination, hydrolysis, seed and main fermentation, downstream processing) requires trained operators.

Advantage of combining biogas and bioethanol production process was noticed. The effluent stream from ethanol production provided an extra carbon source for anaerobic digestion. In the presented case (scenario Combined) it resulted in additional 10% of methane. Further investment cut could be done by integration the “feedstock handling” units. Integration of process gives also advantages in energy use. First one is that the ethanol effluent from distillation process could warm-up AD influent. Secondly, heat generated by burning methane in CPH unit, which in many cases is lost, could support distillation process.

The calculated cost of investment for biogas scenarios were similar to the one presented in a Danish report for biogas production in organic farming (Tersbøl and Jørgensen, 2009), indicating that the model could be applied as useful tool for designing such facilities. No report on a small scale organic ethanol plant was found. The obtained results for the ethanol scenarios were validated against the techno-economic models built for large scale facilities (Aden and Foust et al., 2009; Klein-Marcuschamer et al., 2010), Those authors showed total investment cost in a range of 220 MM USD (year 2007) or 315-370 MM (year 2008), respectively.

If the energy requirements for 1000 ha organic farm are 1800 GJ (personal communication), and if we account energy conversion efficiency of 30%, only the Biogas scenarios and the Combined one can meet the requirements. The remaining energy can be sold and add extra profit to the farm. In case of Ethanol scenarios, energy conversion efficiency of 45% (Ethanol I) or 32% (Ethanol II) is necessary to support farm with own produced energy. Even though, there is less energy produced (GJ) in the Ethanol scenarios, liquid fuel has several advantages over gaseous one. It is easier to store it and most

of the nowadays machinery run on liquid fuel. Combined scenario provides large energy amount (terms of GJ) divided in two different energy carries: biogas - commonly used for cogeneration of heat and electricity; and ethanol - a very suitable liquid fuel for agriculture machinery. Such an integration of the processes gives farmers not only an extra energy but also the diversity of products.

CONCLUSIONS

Possibility of bioenergy production directly at the organic farm was evaluated. Five different scenarios were simulated: two with biogas production, two with ethanol production and one with combination of both: biogas and bioethanol processes. Both fuels could be generated directly at the organic farm; moreover, there are enough raw materials to supply it with own-produced energy. Presented models are strong platforms for further development and adjusting them to the specific needs. Presented work aimed to help bring closer to reality the idea of the energy self-sufficient organic farm.

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Paper VIII

Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W.: Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries. *Submitted*

Techno-economic analysis of biofuels: a wiki-based platform for lignocellulosic biorefineries

Summary: An open-access, community-updatable, and transparent tool for evaluating the economic and environmental performance of a lignocellulosic ethanol biorefinery.

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Abstract

We present a process model for a lignocellulosic ethanol biorefinery that is open to the biofuels academic community. Beyond providing plant design, operating conditions and economic performance, this wiki-based platform provides a transparent tool that can be revised by the academic and professional research community for analyzing, exploring, and communicating the impact of process advances and alternatives for biofuels production. The model is available for download (at http://econ.jbei.org) and will be updated based on feedback from the community of experts in biofuel-related fields. By making the assumptions and performance metrics of this model transparent, we anticipate this tool can help provide a consensus on the energy-related, environmental, and economic performance of lignocellulosic ethanol.

Introduction

Biofuels, particularly lignocellulosic ethanol, have attracted significant attention as one of the routes to address the world's concerns on energy and climate, though their potential as a sustainable solution remains somewhat controversial. Principally, substantial doubts remain regarding the economic and greenhouse gas (GHG)-abatement performance of biofuels (*1-4*). In the background of these discussions, researchers have focused on solving the challenges that have hitherto limited the commercialization and adoption of lignocellulosic biofuels. It is the outcome of these efforts that will assist in moving toward toward adoption of renewable transportation fuels, and thus the technological and economic progress brought about by biofuels research must be carefully and repeatedly evaluated.

Several technoeconomic studies based on process models have assessed the potential of biofuels and have provided invaluable guidance to research, investment, and policy endeavors (*5-8*). These studies usually rely on experimentally-derived or assumed parameters to estimate process performance values, including capital and operating costs, GHG emissions, and biofuel yield as a function of feedstock, among others. They have been also used to analyze how changes in the input parameters translate into changes in overall system performance. Naturally, but unfortunately, these studies can only study a limited set of scenarios, and are unable to address all possible parameter choices or scenarios that could be of interest to the biofuels community. Moreover, research in the field is multidisciplinary and dynamic and modeling advances brought about by one research group are rarely considered in models developed by others, primarily because there is, at present, no avenue for such an exchange to take place.

In response to these challenges, we have constructed a technoeconomic model of a lignocellulosic ethanol biorefinery that is accessible to the biofuels community at large. The model has been deposited online and is available for download and evaluation. Each unit operation in the process flowsheet has a dedicated discussion thread, making it possible for experts in different fields to collectively and publicly address issues associated with different sections of the biorefinery, contributing in their respective areas of expertise. To make the model interactive, collaborative, and to ensure its accuracy and robustness, the parameters and assumptions will be updated in response to feedback obtained from the community of users. This is an essential feature of the present contribution, as the results of any model are strongly dependent on the assumptions made, and the community as a whole will have the opportunity to monitor

all parameter values. The goal is to provide an open, community-based modeling tool that: (1) incorporates assumptions in a transparent manner, (2) allows its users to analyze the scenarios that are of most interest to them, (3) gathers meaningful parameters and other information from experts across disciplines in a centralized location and a unified model, (4) directs research efforts by communicating what parameters are in most need of experimental verification, and (5) disseminates findings across different, and many times unrelated, fields.

In order to show how different groups can benefit from the model, we highlight different targeted biorefinery scenarios. It must be noted that none of our cases has been optimized for a particular performance value, which is best left to the commercial sector (*1*); these cases are offered mainly as an illustration of how groups with different aims can explore parameter variations. Nonetheless, the model is intended to have immediate applicability within the biofuel community. All the parameters of the model were either taken from published studies or were chosen according to established industrial practice, and, regardless of whether the results provide optimistic or pessimistic outlooks, they are certainly representative of current established technology. A brief description of the base case is given in the supporting online text. Complementary to it, a list of the assumptions made and parameters used in each part of the model can be found online (http://econ.jbei.org).

Scenario 1: Reducing acetate content of the biomass feedstock

Acetyl functional groups are found in the hemicellulose and lignin constituents of biomass, and are liberated and solubilized during dilute acid pretreatment. Acetic acid is inhibitory to fermenting microorganisms, and especially to *Saccharomyces cerevisiae*, in addition to interfering with enzymatic hydrolysis during saccharification (*9, 10*). Plant biologists are targeting reducing the content of acetate in bioenergy crops. In this scenario, a reduction of 20% in the acetate content in biomass was modeled, relieving toxicity during fermentation. A reduction in saccharification time or enzyme loading was not modeled in this scenario, since we were not aware of published reports that quantified such an effect.

Scenario 2: Increasing cellulolytic enzyme activity

Cellulolytic enzymes are the second largest material costs after the feedstock material itself, even at the highly optimistic price of $2.70/kg of enzyme (corresponding to about $0.35/gal ethanol in our base case). Efforts in protein engineering have strived to reduce this contribution to the operating costs, for example, by increasing the kinetic activity of the enzymes during saccharification (*11*). Some have proposed enzymes that do not absorb as easily to lignin as native enzymes do (*12*), or that are engineered for stability (*13*). Consequently, a lower loading could be used in the process or the residence time of saccharification could be reduced. Here, we explored a 2-fold improvement in enzyme activity, which would halve the required enzyme loading.

Scenario 3: Reducing lignin content of the biomass feedstock

The effects of lignin have been described as some of the major hurdles that lie in the way of an effective process for lignocellulosic ethanol. Notably, lignin interferes with cellulose hydrolysis by limiting the accessibility of enzymes to the cellulose fibers during saccharification and by irreversibly adsorbing active enzyme (*12, 14*). The toxicity of lignin monomers to fermenting organisms has been reported (*15*). In this scenario, we modeled the use of biomass modified to have 20% lower lignin content with respect to the base case. This reduction was assumed to be compensated by an increase in cellulose, based on previously reported studies in transgenic aspen (*16*). While the saccharification residence time was left

unchanged, the sugar released during hydrolysis was increased by ~50% (*17*). Fermentation time was either left unchanged (scenario 3a), or increased so that the glucose was exhausted (scenario 3b), similar to the base case (see supporting online text). To partly compensate for the lost lignin and higher conversion in scenario 3b, purchased natural gas was added to the combustor for adequate steam and electricity production.

Scenario 4: Increasing the rate of xylose-fermentation by yeast

S. cerevisiae is the preferred industrial organism for the production of ethanol from cane or grain-derived sugars, because of its natural ability to rapidly ferment six-carbon sugars even in the presence of oxygen and its tolerance to the alcohol product (*18, 19*). Wild-type *S. cerevisiae*, however, cannot metabolize five-carbon sugars such as xylose, effectively reducing the overall yield of ethanol on biomass and increasing the cost of production. To overcome this limitation, several groups have focused on engineering strains of *S. cerevisiae* for uptake of five-carbon sugars (*20*). To model the effect of an increase in xylose metabolism, we doubled the growth rate on xylose in the anaerobic fermentation, while leaving residence time unchanged.

Scenarios 5 and 6: Increasing the tolerance of yeast to acetic acid and ethanol

Acetic acid has a pronounced toxic effect in yeast; the minimum inhibitory concentration (MIC) of the undissociated form of acetic acid can be as low as 7.5 g/L or less (*9, 21, 22*). Typical fermentation conditions for yeast have an initial pH of ~4.5 (pK_a = 4.75), so that even relatively low concentrations of the acid can have a detrimental effect on fermentation performance. Ethanol is also toxic, and overcoming its negative effects on fermentation has been the area of intense study. In order to account for toxic effects, the inhibition of both ethanol and acetic acid were included in the fermentation kinetic models. The decrease in growth rate with increasing concentrations of both compounds was assumed to be linear, based on previous studies (*21, 23, 24*). For scenario 5, the MIC of acetic acid (assuming a pH of 4.5) was increased by 50%. A similar case was studied with ethanol (scenario 6).

Results and Discussion

The results from the different scenarios are summarized in Table 1. The capital investment for all scenarios was comparable, at approximately $315 – $370MM, for a facility processing 2000 MT/day of wet biomass (moisture content ~15%). For the base case, about 40% of the production cost is derived from raw materials, and corn stover in particular, while about the same figure is facility-dependent (see supporting online text). Such strong dependence in feedstock implies that gains in yield have a large effect in the minimum ethanol selling price (MESP). In cases where conversion of biomass to ethanol increases with respect to the base case, a concomitant reduction in electricity production is observed, as less biomass is available for burning. This is particularly seen in scenario 3b, where natural gas is assumed to be purchased to supply the steam and electricity needed for plant operation. The CO_2 credit, computed from the displaced gasoline and fossil-derived electricity minus the CO_2 emitted from burning purchased gas, is highest for this scenario because producing more ethanol offsets methane-associated emissions (see supporting online text).

Even for our base case scenario, the performance values of our process – the MESP, operating cost, yield on biomass, etc. – contrast to others in the literature. While the capital expenditure is approximately the same as previous estimates for this configuration (*5*), the conversion of sugars is lower, which has a marked effect on yields and, thus, on operating cost per unit of output. The main reason for this is our

choice of fermentation and lignocellulose pretreatment technologies. The parameters and performance assumptions for these operations were derived from studies in the literature that were complete enough to be accurately represented in our simulations (these references are found in the wiki). This approach ensured that our choices were representative of current technology. Future experimental work will be needed to obtain the necessary details for alternative technologies to be modeled, and we invite the biofuels research community to contribute their results in these areas.

Although none of the cases has been optimized for MESP minimization, our "all-else-being-equal" analysis already suggests possible future directions for biofuels research. For example, biomass engineering strategies offer great potential for aiding commercialization of lignocellulosic ethanol, though their economic impact has not been properly studied. Strain and enzyme engineering also offer interesting prospects, although some of these are in areas different from those explored most extensively (e.g. acetate impairs yeast fermentation more than ethanol does). One main reason behind why these and other observations may escape experts is the lack of time and resources needed to develop a full technoeconomic model. Even if each research group were to create a model of their own, the likelihood of different studies agreeing in the assumptions and parameters is quite low, making the observations and conclusions non-comparable and diminishing their usefulness. Partial analyses are common and have continued to be a weakness in the field, fostering unrealistic expectations that cannot be fulfilled by any single technology. It may still be possible, however, to bring about economical and environmentally sustainable renewable liquid fuels, but only if new technologies are developed and evaluated in the context of other advances. The aim of this study was not to determine what technology is best, but rather to make available a dynamic modeling tool and a communication avenue for such exchanges to occur. Without a concerted effort, diverging arguments about the advantages and limitations of biofuels and different biofuel technologies might completely halt progress in the area.

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Table 1. Summary of the explored scenarios.

Case	Throughput (million gal/yr)	Yield (gal/ton)[a]	C6-conv.[b]	C5-conv.[c]	Electricity (GWh/yr)	CO_2 credit (thousand tons/yr)	TPI[d] (MM$)	AOC[e] (MM$/yr)	MESP ($/gal)	Δ MESP[f]
Base case	30.9	44.2	71%	50%	27.3	197	337.1	138.5	$ 4.58	$ -
Scenario 1	34.7	49.7	71%	68%	20.8	215	336.7	139.0	$ 4.11	$ 0.47
Scenario 2	30.9	44.1	71%	49%	27.5	196	335.3	133.2	$ 4.41	$ 0.17
Scenario 3a	35.1	50.2	76%	55%	12.8	213	315.5	135.4	$ 3.98	$ 0.60
Scenario 3b	43.9	62.8	93%	64%	0.16	245	367.7	148.5	$ 3.53	$ 1.05
Scenario 4	35.2	50.4	65%	81%	24.2	220	334.7	138.5	$ 4.03	$ 0.55
Scenario 5	37.0	52.9	71%	80%	20.7	228	338.1	139.7	$ 3.88	$ 0.70
Scenario 6	31.7	45.3	71%	53%	27.5	201	334.5	138.0	$ 4.45	$ 0.13

[a] Yield in gallons of ethanol per wet ton of biomass

[b,c] Conversion of C6- and C5- sugars to ethanol based on total available sugar in biomass feedstock

[d] Total project investment

[e] Annual operating cost, including facility-dependent

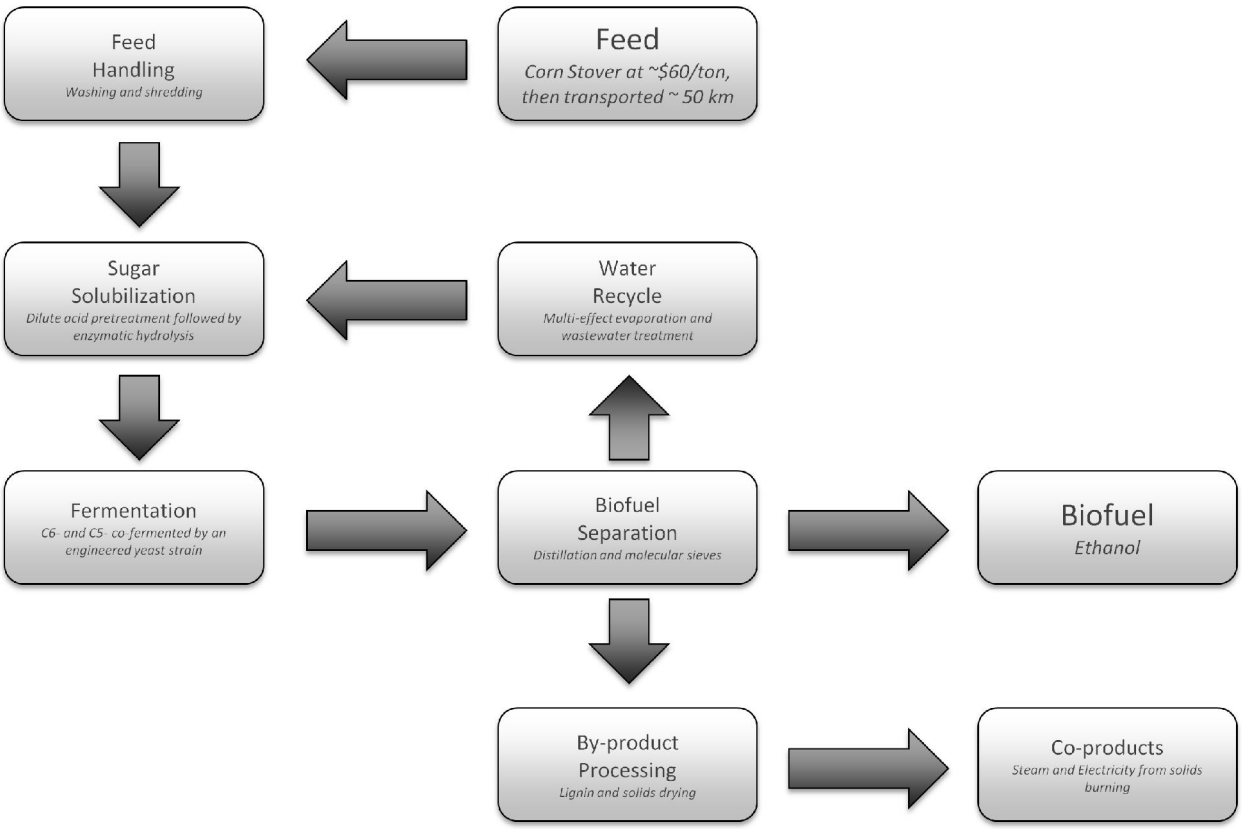
[f] Difference to Base Case

Supplementary Online Material

Description of the base case

A diagram illustrating the base case process is found in supplementary figure 1. Briefly, corn stover, priced at $60/ton (at the farmgate), is transported ~50km to the biorefinery, where it is unpacked from the bales, washed, and shredded. It enters the pretreatment reactor along with sulfuric acid, where high-pressure steam is used to heat the vessel to ~180 ^{o}C at a pressure of ~10-15 bar. The mixture is flash-cooled and the slurry is passed through a belt filter. The liquid filtrate is overlimed and neutralized to remove toxins before being slurried back with the solids, which contain most of the cellulose. This mixture enters the saccharification tank, cellulase enzymes are added to 20 mg/g cellulose, and the reaction is allowed to proceed for ~5 days. At this point, the saccharified slurry enters the fermentation section, where it supplies nutrients for yeast growth (in a train of seed fermentors) and ethanol fermentation. The fermentation proceeds until the C6 sugars are exhausted (see below), though some of the C5 sugars are also utilized. The exiting beer is passed through two distillation columns before entering

the molecular sieve columns, from which ethanol exits almost pure (~99.5%). The bottoms (i.e. the stillage) from the first distillation column, containing most of the lignin and other non-fermentable solids, is sent to a series of multi-effect evaporators for partial dewatering. Water is recycled back or treated in the wastewater treatment (WWT) section. The lignin and other solids, along with the biogas produced in the WWT digestors, are burned in a boiler, producing high pressure steam. This is used to run the turbogenerator for electricity production and for generation of lower pressure steam used in the pretreatment, product recovery, and water recovery sections.

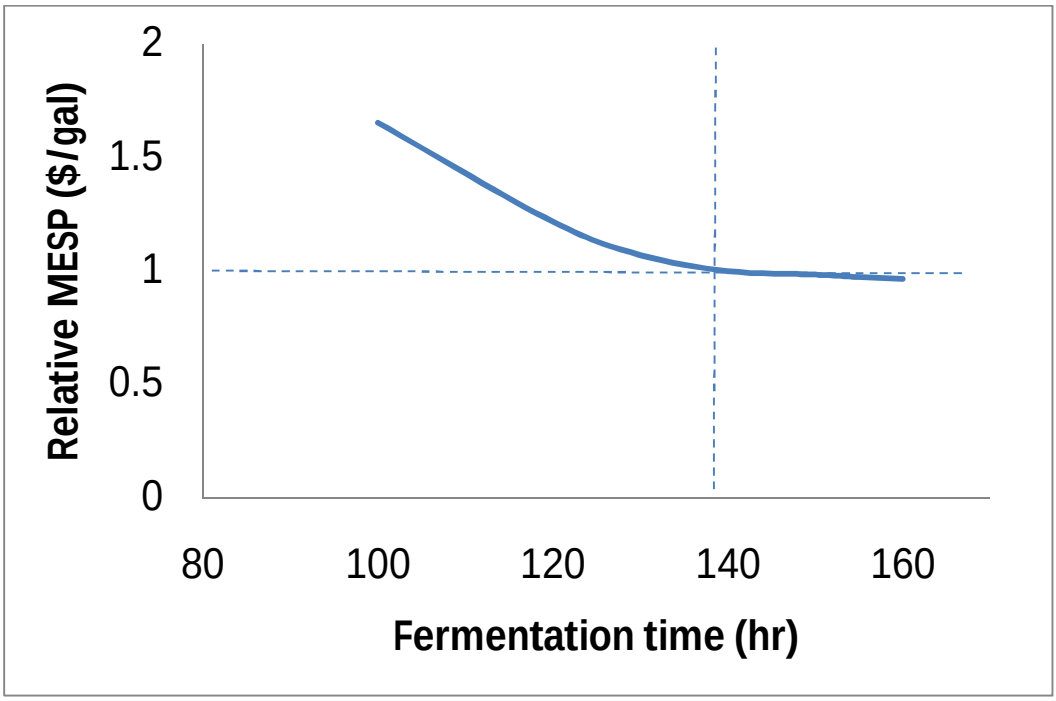


Supplementary Figure 1. General schematic of the lignocellulosic ethanol biorefinery

Price-dependence on fermentation residence time

As explained in the main text, the yield of ethanol on biomass has a clear effect in the minimum ethanol selling price (MESP). In turn, the fermentation residence time influences the yield, as a higher conversion of sugars can be achieved if the yeast is allowed to metabolize for longer periods of time. In general, longer times translate into larger or more fermentor units, and thus there is a tradeoff that arises from increasing the residence time of fermentation. To simplify the analysis, the fermentation residence time was chosen based on the dependence of MESP on this variable, as shown in supplementary figure 2. The relative MESP is defined such that the MESP at the time where all C6-sugar is consumed has the value of 1. For the base case, there is a clear and sharp decrease in MESP until ~140hr, the time at which the C6-sugars are depleted. After that, the decrease in MESP decelerates. Even though the cost of production continues to decrease, other performance values become less favorable, for example, electricity consumption increases quickly. To make the results comparable across all cases, the fermentation time was not changed, regardless of when C6-sugars were consumed, except for scenario 3b. For this case, for

which the C6-sugar content of the fermentor feed increases dramatically, the same rationale was used to select an appropriate residence time.



Supplementary Figure 2. Dependence of the minimum ethanol selling price (MESP) on fermentation residence time

Calculation of carbon credits

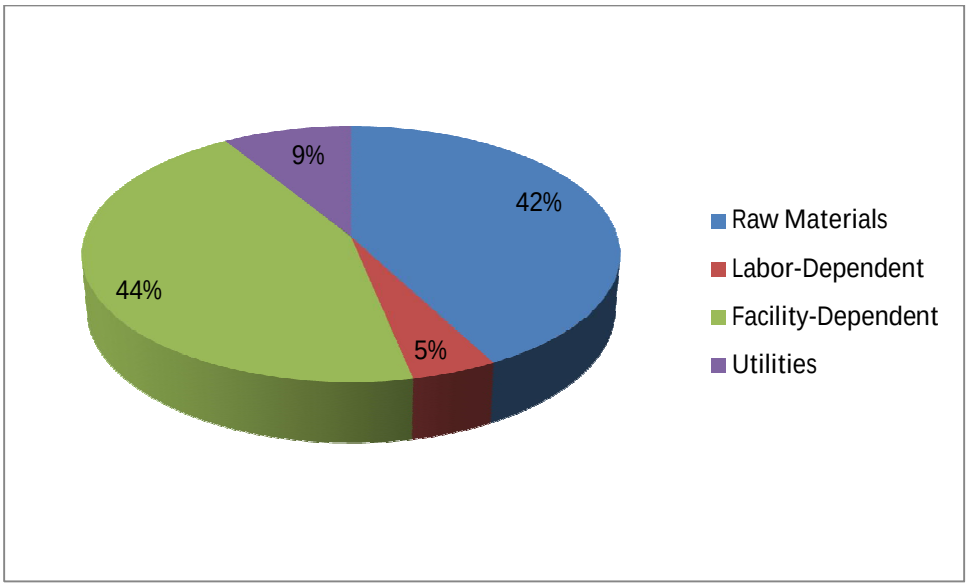
Without performing a full life-cycle analysis, one can analyze the greenhouse gas (GHG) emission reduction that stems from operating a biorefinery. Though the results of such analysis are by definition incomplete, they are useful for comparing different scenarios, i.e., when they are evaluated in relative terms. In essence, we performed the calculations expecting that the results could be used as part of the data needed in a full life cycle analysis. In addition, depending on the specific details of a cap-and-trade scheme, these numbers can also be used to calculate credits as the amount of GHGs that are prevented from entering the atmosphere due to the activity of the biorefinery.

In a simple case, a biorefinery can potentially diminish fossil fuel-generated CO_2 by two mechanisms: (1) the electricity displaced by that produced from burning biomass residues, and (2) the gasoline displaced by ethanol. To quantify the "credits" from such displacements, we calculated the CO_2 that would be "saved" because green alternatives were used instead of fossil fuels. For fossil fuel-based electricity, we assumed that emissions are those of the US, on average 0.606 MT CO_2/MWh (EIA, 2002). For ethanol, the value was calculated by assuming that gasoline produces 8.8 kg CO_2/gal (EPA, 2005), and adjusting for ethanol's lower energy content. In the cases where natural gas was used to supplement the energy contained in biomass residues, the emissions from completely burning the gas were subtracted from the CO_2 credit to give a lower number.

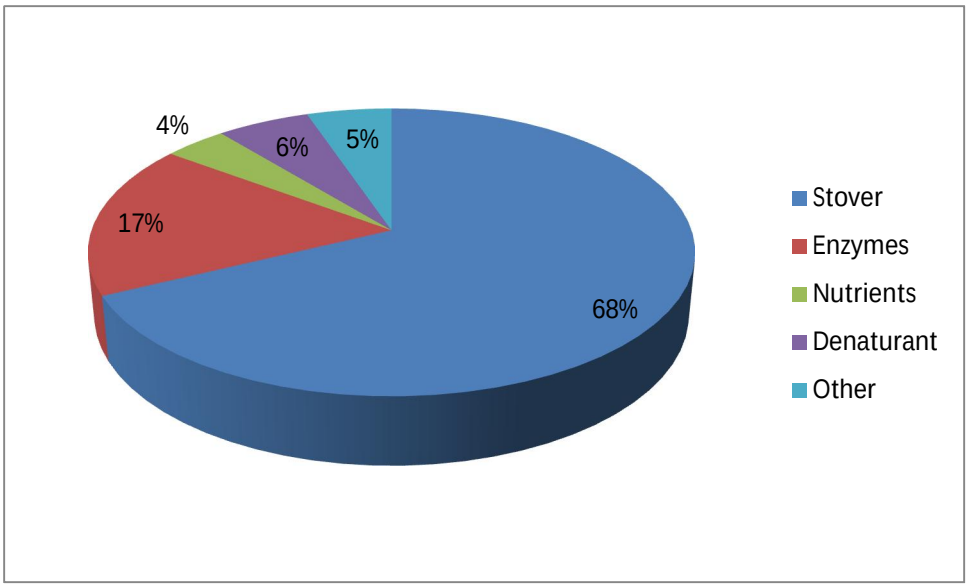
Distribution of annual operating and material costs

As part of our analysis, we plotted the contributions of materials, utilities, labor, and facility-dependent payments to the annual operating cost (AOC) for the base case (supplementary figure 3). In addition, we

plotted the distribution of different raw materials in the contribution of the material-associated costs (supplementary figure 4). As shown in the figures, the majority of the AOC is made up by facility-dependent and raw material costs. The stover is by far the largest contribution to the material costs, with the cost of enzymes being second. This trend may not hold had we not made such optimistic assumptions regarding the cost of enzymes.



Supplementary Figure 3. Annual operating cost (AOC) breakdown for the base case



Supplementary Figure 4. Contributions of different raw materials to total material cost

Accessing and updating the model

The model and supporting information, as well as all discussions associated with different assumptions, has been deposited at http://econ.jbei.org. The model is freely available to all academic users, which will have the opportunity to register, making their contributions easier to track and manage. After the affiliation information of the users is confirmed, the users will be granted access to the wiki site, and will have permission to discuss and contribute to any of the pages of the site. The model has been made possible thanks to the kind support of various governmental and industrial sponsors, which precludes us from distributing the model for commercial purposes. Non-academic users can inquire about licensing the model by writing to the authors.

The technoeconomic model is meant to be a community-updatable tool, and we encourage and welcome suggestions, corrections, and modifications to the assumptions and parameters used. In order to ensure that the model is updated in an orderly fashion and using relevant and accurate data, instructions on how to make contributions have been posted in the wiki. The model will be updated with data available in peer-reviewed publications or obtained directly from equipment vendors. Suggestions about how to improve the updating process are also welcome, and can be posted in the appropriate discussion thread in the wiki.

Risø DTU is the National Laboratory for Sustainable Energy. Our research focuses on development of energy technologies and systems with minimal effect on climate, and contributes to innovation, education and policy. Risø has large experimental facilities and interdisciplinary research environments, and includes the national centre for nuclear technologies.

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