Bioenergy from agricultural residues in Ghana

Thomsen, Sune Tjalfe; Østergård, Hanne; Kádár, Zsófia

Publication date:
2014

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Thomsen, S. T., Østergård, H., & Kádár, Z. (2014). Bioenergy from agricultural residues in Ghana. Technical University of Denmark (DTU).

Bioenergy from agricultural residues in Ghana

DTU Chemical Engineering
Department of Chemical and Biochemical Engineering

PhD thesis

Submitted January 2014
Supervisors: Hanne Østergård and Zsofia Kádár
Center for Bioprocess Engineering (BIOENG)
Department of Chemical and Biochemical Engineering
Technical University of Denmark
DTU Risø Campus

Abstract

There are strong incentives for increased bioenergy production in Ghana, since it may bring energy self-sufficiency for farmers and communities, cleaner fuels, and the possibility for closing the nutrient-cycle. Therefore, this PhD thesis is investigating production of residue-based ethanol and biomethane under Ghanaian conditions.

Detailed characterisations of thirteen of the most common agricultural residues in Ghana are presented, enabling estimations of theoretical bioenergy potentials and identifying specific residues for future biorefinery applications. When aiming at residue-based ethanol production, it is advised to utilise starchy residue at first (peelings of yam, cassava and plantain), since these hold the largest potentials per unit of mass while they have simpler conversion routes. Furthermore, only residues concentrated at processing facilities are applicable for ethanol production due to poor rural infrastructure, spatial distribution of the residues and the lack of tradition in collecting residues.

Pretreatment methods aimed at low-tech production of cellulosic ethanol from West African biomasses are assessed. Evaluated on the overall ethanol yield, the low-tech pretreatment methods (boiling-, soaking in aqueous ammonia-, and white rot fungi pretreatments) are viable alternatives to the high-tech method hydrothermal treatment. However, these methods are not as versatile as hydrothermal treatment as they each have satisfactory effect on only a few of the biomasses. Silage pretreatment is also assessed but is not adequate as stand-alone pretreatment of dry lignified biomass. However, combined with hydrothermal treatment, silage treatment decreases the optimal pretreatment temperature significantly, thereby reducing the energy inputs for hydrothermal pretreatments.

It is recommended to pursue increased implementation of anaerobic digestion in Ghana, as the first bioenergy option, since anaerobic digestion is more flexible than ethanol production with regard to both feedstock and scale of production. If possible, the available manure and municipal liquid waste should be utilised first as these are verified substrates for low-tech systems. Beside manure, the most recommendable agricultural feedstock for anaerobic digestion are processing residues with high biomethane potentials (BMP) such as starchy peelings, cocoa husks, maize husks and maize cobs.

Biomethane based on agricultural processing residues, manure and municipal liquid waste can theoretically replace approximately 20% of current utilisation of heat energy in households. However, a need is revealed for resilient small-scale anaerobic digestion solutions, designed for utilising agricultural residues under manure- and water shortage.

A novel model for estimating BMP from compositional data of lignocellulosic biomasses is derived. The model is based on a statistical method not previously used in this area of research and the best prediction of BMP is: $BMP = 347\, x_{C+H+R} - 438\, x_L + 63\, D_A$, where x_{C+H+R} is the combined content of cellulose, hemicellulose and residuals, x_L is the content of lignin while D_A is describing the applied analysis method, $D_A \begin{cases} 0, & \text{Forage analysis method} \\ 1, & \text{Fibre analysis method} \end{cases}$.

Potential bioenergy solutions should be a part of the fragile equilibria in the Ghanaian agricultural system. Additionally, current data on available crop residues should be used with caution to predict a future situation since 1) the current utilisation of residues is not clear, 2) changes in infrastructure and farming practices are expected, and 3) a growing bioenergy sector will bring self-inflicting changes to its surroundings.

Dansk resumé

Der er mange gode grunde til at ekspandere produktionen af bioenergi i Ghana, da dette kan føre til energiuafhængighed for både landbrug og lokalsamfund, renere brændsler, samt muligheden for at recirkulere næringsstoffer. Derfor undersøger denne Ph.d. afhandling produktion af ethanol og biogas fra reststrømme i den Ghanesiske landbrugssektor.

På basis af detaljerede karakteriseringer af 13 af de mest almindelige Ghanesiske afgrøderester estimeres teoretiske bioenergipotentialer, og interessante råmaterialer for fremtidige bioraffinaderier udpeges. I forbindelse med afgrøderestbaseret ethanolproduktion er det fordelsagtigt at udnytte de stivelsesholdige afgrøderester først (skræller fra yams, kassava og madbananer), da disse afgrøderester er simple at konvertere samtidigt med at de har høje ethanoludbytter. Derudover er det kun realistisk at udnytte afgrøderester der er opkoncentreret i forbindelse med processering da den Ghanesiske infrastruktur er mangelfuld, da afgrøderesterne er meget spredt, og da der mangler en tradition for at indsamle disse afgrøderester.

Forbehandlingsmetoder designet til lavteknisk produktion af cellulosebaseret ethanol fra Ghanesiske afgrøderester vurderes. Set ud fra det samlede ethanoludbytte, er de lavteknologiske forbehandlingsmetoder (simpel kogning, forbehandling med ammoniakopløsning og forbehandling med hvidrådsvampe) brugbare alternativer til den til hydrotermisk forbehandling. Imidlertid er disse metoder ikke så alsidige som hydrotermisk forbehandling, da de hver især kun har en tilfredsstillende effekt på få af afgrøderesterne. Ensileringsforbehandling har ikke tilstrækkelig effekt som separat forbehandlingsmetode af tørt lignocellulosisk biomasse, men kombineret med hydrotermisk forbehandling, nedsætter ensilering den optimale forbehandlings-temperatur, og dermed også energiforbruget under hydrotermisk forbehandling.

Det anbefales at øge implementering af bioforgasning i Ghana som den første mulighed inden for bioenergi, da bioforgasning er mere fleksibel end ethanol produktion med hensyn til både mulige råvare og skalering af anlæg. Hvis det er muligt, bør det tilgængelige husdyrgødning og spillevand udnyttes først, da disse er verificeret substrater for lavteknologiske systemer. De mest anbefalelsesværdige Ghanesiske afgrøderester til

bioforgasning er afgrøderester fra processering med højt metanpotentiale såsom de stivelsesholdige skræller, kakaofrøskaller, majsavner og majskolber. Biometan baseret på afgrøderester fra processering, husdyrgødning og spildevand kan erstatte omkring 20 % af det nuværende forbrug af brændsel i husholdninger. Der er dog et behov for småskala biogasanlæg designet til at udnytte afgrøderester på trods af mangel på husdyrgødning og vand.

En ny model er udledt til at estimere biogaspotentialer (BMP) ud fra kompositionen af lignocellulosiske biomasser. Modellen er baseret på en statistisk metode, der ikke tidligere anvendt inden for dette forskningsområde. Den bedste estimering af BMP er: $pBMP = 347x_{C+H+R} - 438x_L + 63D_A$, hvor x_{C+H+R} udgør koncentrationen af cellulose, hemicellulose og residualer lagt sammen, x_L udgør koncentrationen af lignin, imens D_A beskriver den anvendte analysemetode, $D_A \begin{cases} 0, & \text{Foderanalysemetoden} \\ 1, & \text{Fiberanalysemetoden} \end{cases}$.

Potentielle bioenergiløsninger bør in gå i de skrøbelige balancer i det ghanesiske landbrugssystem. Derudover bør de nuværende data om tilgængelige afgrøderester anvendes med forsigtighed til at forudsige en fremtidig situation pga. 1) da den nuværende udnyttelse af restprodukterne er næsten ukendt, 2) da konsekvenser af ændringer i infrastruktur og landbrugspraksis er svære at forudsige, og 3) da en kommende bioenergi sektor i sig selv vil have konsekvenser for det omgivende samfund.

Acknowledgments

Foremost I want to acknowledge Danida for funding this project, as well as all the project partners for a fruitful and enlightening collaboration.

I wish to express gratitude to main supervisor Hanne Østergård and co-supervisor Zsófia Kádár for inspiring collaboration and for guiding me safely into still waters. In addition, I have been blessed with inputs from several skilled supervisors in the course of my studies. Jens Ejbye Schmidt, Pablo Kroff and Henrik Bangsø Nielsen are sincerely thanked on these grounds.

My fellow authors, Jorge E. G. Londoño, Anne S. Meyer, Henrik Spliid, Francis Kemausuor, Edem C. Bensah and Andreas Kamp, as well as the brilliant technical team with Tomas Fernqvist, Ingelis Larsen, Ulla Lilholt and Annette E. Jensen, are recognised for their huge efforts.

Sincere thanks are also due to my truly inspiring colleagues; among the previously unmentioned are Stefan, Mads, Christina, Guotao, Ming, Tobias, Henrik, Esben, Thalia, Nadja, Piotr, Anne Belinda, and Zsuzsa, as well as my scientific partner in crime Morten Ambye-Jensen.

Finally, I wish to thank my family and friends for eternal support and for coping with my lack of presence. Especially, my wonderful fiancée Julie and our daughter Elin, for encouragement and understanding, and for making everything meaningful.

Preface

The PhD thesis you are about to read consists of two parts. Firstly, a treatise in four sections, and secondly the five papers that were produced as part of the project. In the treatise, I have strived to describe the context in which my research has taken place. Furthermore, state-of-the-art within the relevant fields of research are established, which includes the papers produced as a part of this PhD thesis. Therefore, the treatise will not include a systematic and chronological assessment of my own papers, but rather a story in its own right where the relevance of the enclosed papers will be illuminated. Furthermore, the methods I have applied are presented in the respective papers and will not be described in the treatise. When I refer to one of the papers that are a part of the PhD thesis, then the citation will be followed by a paper number in reference to the list of papers on page x, such as (Thomsen et al., 2014, Paper V). Throughout the treatise, drawings of common Ghanaian crops will be distributed without being further referred (IITA, 2012; Archives Larousse, 2013). I hope my way of designing the treatise will result in an interesting read.

The PhD project was carried out in the Center for Bioprocess Engineering (BIOENG), Department of Chemical and Biochemical Engineering, Technical University of Denmark at the DTU Risø Campus near Roskilde Denmark. It was carried out from December 1st 2010 to January 25th 2014, only interfered by two months of paternity leave in the summer of 2011. The PhD project was supported with a grant from Danida Fellowship Centre (DFC) of the Danish Ministry of Foreign Affairs, as a part of the project “Biofuel production from lignocellulosic materials – 2GBIONRG”, DFC journal no. 10-018RISØ. I have been supervised by main supervisors Hanne Østergård (1/9/2012 – present) and Jens Ejbye Schmidt (1/12/2010 – 1/9/2012), as well as co-supervisors Zsófia Kádár (1/9/2012 – present), Pablo Kroff (1/9/2012 – 1/10/2013) and Henrik Bangsø Nielsen (1/12/2010 – 1/5/2011).

Yours sincerely

Sune Tjalfe Thomsen

List of publications

The PhD thesis is based on work reported in the following peer reviewed articles:

Paper I: Kemausuor F., Kamp A., **Thomsen S.T.**; Bensah E.C., Østergård H., Assessment of biomass residue availability and sustainable bioenergy yields in Ghana, *submitted after review to Resources, Conservation and Recycling (2014),* first submitted on July 3 2013

Paper II: Thomsen S.T., Kádár Z., Schmidt J.E., Compositional analysis and theoretical biofuel potentials from various West African agricultural residues, *submitted after review to Biomass & Bioenergy (2014)*, first submitted on January 7 2013

Paper III: Ambye-Jensen M., **Thomsen S.T.**, Kádár Z., Meyer A.S., Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment, *Biotechnology for Biofuels* (2013) 6:116

Paper IV: Thomsen S.T., Londoño J.E.G., Schmidt J.E., Kádár Z., Screening of pretreatments of common West African lignocellulosic biomass residues for ethanol production, *submitted to Renewable Energy (2014)*, submitted on November 20 2013

Paper V: Thomsen S.T., Spliid H., Østergård H., Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass, *Bioresource Technology (2014)* 154, 80-6

Papers written, revised or contributed to, which are not part of the PhD thesis:

Thomsen S.T., Jensen M., Schmidt J.E., Production of 2nd generation bioethanol from lucerne – optimization of hydrothermal pretreatment, *BioResources* (2012) 7(2), 1582-93

Thomsen T.P., Ahrenfeldt J., **Thomsen S.T.**, Assessment of a novel alder biorefinery concept to meet demands of economic feasibility, energy production and long term environmental sustainability, *Biomass & Bioenergy* (2013) 53, 81-94

Schultz-Jensen N., Thygesen A., Leipold F., **Thomsen S.T.**, Roslander C., Lilholt H., Bjerre A.B., Effect of pretreatment on bioethanol production from macroalgae with emphasis on Chaetomorpha linum, *Bioresource Technology* (2013) 140, 36–42

Carter M.S., Hauggaard-Nielsen H., Heiske S., Jensen M., **Thomsen S.T.**, Schmidt J.E., Johansen A., Ambus P., Consequences of field N_2O emissions for the environmental sustainability of plant-based biofuels produced within an organic farming system, *GCB Bioenergy* (2012) 4, 435–52

Table of Contents

List of abbreviations

pBMP	Biomethane potential
BP	Boiling pretreatment
COD	Chemical oxygen demand
d	Day
DM	Dry matter
EFB	Empty fruit bunches
EWS	Ensiled wheat straw
g	Gram
Ha	Hectare
HPLC	High-performance liquid chromatography
HTT	Hydrothermal treatment
J	Joule
L and l	Litre
m	Metre
pBMP	Predicted biomethane potential
RPR	Residue to product ratio
RWS	Raw wheat straw
SAA	Soaking in aqueous ammonia pretreatment
SFF	simultaneous saccharification and fermentation
t	Ton (10^6 g)
TS	Total solids
VS	Volatile solids
w	Weight
W	Watt
Wh	Watt hour
WRF	White rot fungi pretreatment
WS	Wheat straw
yr	Year

Metric prefixes used:

P	T	G	M	k	m	µ
10^{15}	10^{12}	10^{9}	10^{6}	10^{3}	10^{-3}	10^{-6}
Peta	Tera	Giga	Mega	Kilo	Milli	Micro

1. Setting the scene

"Bioenergy systems, if properly harnessed and planned, provide a number of environmental and socio-economic opportunities for Africa" (Mangoyana, 2009).

The introducing quote of this PhD thesis is largely capturing a truth about bioenergy – it can be a part of a sustainable solution to some of the most eminent problems faced in Ghana as well as globally, but it does require knowledge and thoughtfulness in order to be beneficial. In the first part of the treatise, I will elaborate on the context that bioenergy in Ghana is situated in. Threats and possibilities are inevitable in all technical progress, and without seeking an understanding of the context, we are likely, unintentionally, to cause more harm than progress.

Biomass

The term biomass is originating from Greek with bio meaning life and maza meaning mass. It refers to non-fossilised and biodegradeable organic material originating from plants, animals and micro-organisms.

Biomass includes produces, by-products and waste residues from agriculture and forestry, as well as biodegradable organic fractions of industrial and municipal wastes.

The Republic of Ghana is located in West Africa bordered to the north by Burkina Faso, the south by the Atlantic Ocean, the west by Cote d'Ivoire and the east by Togo, and it covers 239,460 km^2. Ghana is known for an abundance of natural resources, such as gold, timber, diamonds, aluminium, manganese, fossil oil, fish, rubber, and hydropower (Mihyeon Jeon et al., 2006). Agricultural products accounts for 45% of the gross domestic product, where cocoa and timber alone accounts for 35% of the exports (Mihyeon Jeon et al., 2006). While Ghana in the past decades has become accustomed to growth in both economy, population and prosperity, the country now finds itself in the midst of several global crises, which will shape the future. One is the current energy crisis where irregularities in oil supplies and distributions have caused general uncertainty regarding global reliability on oil (Tverberg, 2012). A recent PhD thesis from the Department of Chemical and Biochemical Engineering at DTU illuminated how an inevitable peak and decline in the global extraction of fossil resources entails a reduction in societal complexity and that food and bioenergy systems will again need to provide net energy for the society (Markussen, 2013). Moreover, combustion of fossil fuels is the main contributor to global warming and the current climate crisis, which to some degree also can be counteracted by substituting fossil energy with renewable energy (Bernstein et al., 2007).

Two-thirds of the energy use in sub-Saharan Africa consists of highly inefficient combustion of traditional biomass, which often is utilised in very inefficient forms, predominantly as fuel-wood and charcoal for cooking and heating (Parker et al., 2010; Kemausuor et al., 2014). Therefore, the transition to a post-oil society might be more straightforward than in the developed world. However, the utilisation of the biomass resources should be made far more efficient, and balanced than presently to obtain long-term sustainability. The main challenge in the quest for a more sustainable future is to manage the available renewable resources in the best possible way, in a situation where the population has multiplied vastly compared to the pre-oil era. It has often been suggested that small-scale decentralised bioenergy systems have a potential responding to the described problems by providing energy independency, energy services in remote areas, and opportunities for economic development in marginalised areas, as well as providing means to mitigate climate change (Amigun et al., 2008; Mangoyana and Smith, 2011). However, since most of the bioenergy research and development have been focussed on large-scale technologies suited for the developed world, information on the breadth and depth of the small-scale technology is lacking (Ejigu, 2008). In the present work, focus is strictly on residue-based bioenergy, thus dedicated energy crops and potential food items are not assessed. This does not mean that all conflicts regarding land use can be eliminated, but focussing on the residue-based bioenergy pushes towards solutions that may become ecologically and socially sustainable – also in the long term.

1.1. Biomasses and current use in Ghana

In Fig. 1, the most common Ghanaian crops are presented with respect to cultivated area. Cocoa, which is associated with forest agriculture, is the most widespread crop, and furthermore, it is the most significant crop for export with 430,000 t exported per yr (FAOSTAT, 2013). The cropped area for most crops has increased over time (Fig. 1), and actually the share of agricultural land has increased with more than 30% since 1982 (FAOSTAT, 2013). In the same period, the annual yields of cassava, yam and plantain have approximately doubled, while other crop types have shown relatively stable yields. Presently the starchy stables - cassava, yam and plantain is the most high yielding crops with more than 10 t harvested per ha/yr, while other important crops like maize, groundnut and rice have annual yields of approximately 2 t/ha/yr (FAOSTAT, 2013). The most widespread crop in respect to area, cocoa, is among the crops with the lowest yield only of 0.4 t/ha/yr on average (FAOSTAT, 2013).

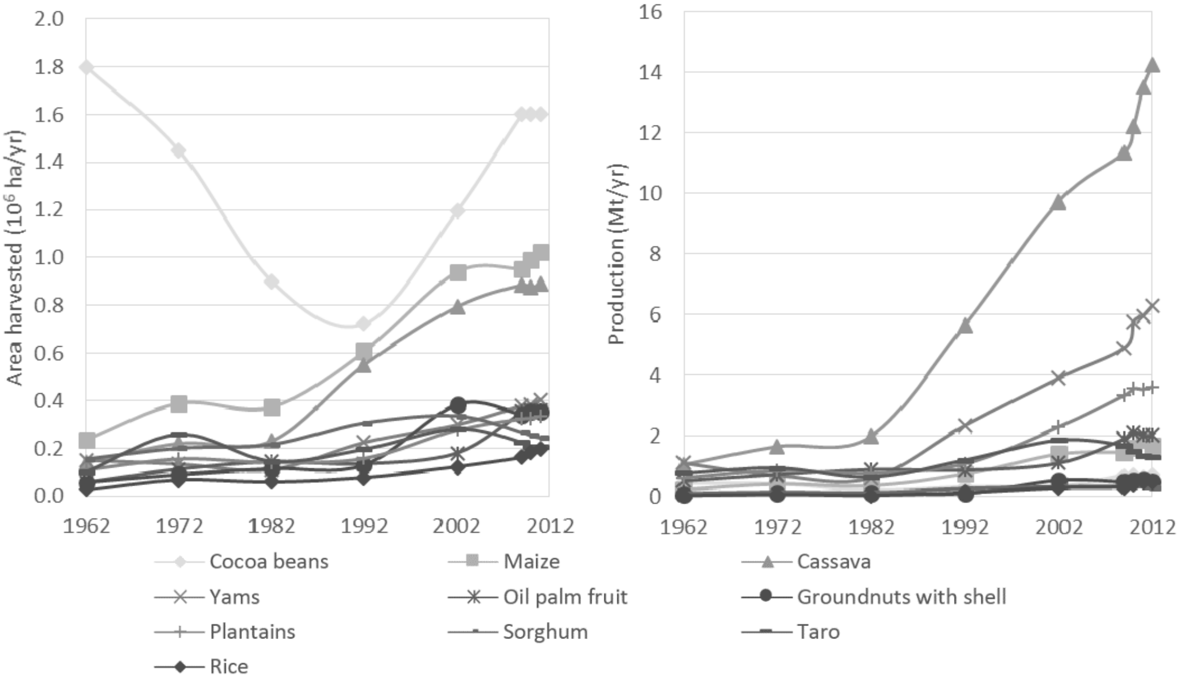

Fig. 1: The cultivated area and the total annual production of the 10 most common Ghanaian crops (FAOSTAT, 2013).

The annual production of the most common crops in Ghana is graphically presented in Fig. 1, and from these data, it is possible to estimate the total amounts of produced agricultural residues (Kemausuor et al., 2014, Paper I). The residues are what is left when the main parts of the produce have been utilized. Using cocoa as an example, there are two main residues from cocoa production. The cocoa pods, which are the outer leathery shell of the cocoa fruit that contain the cocoa beans. These pods are removed as the first cocoa residue. Hereafter, the beans are fermented to remove some of the pulp surrounding the beans and finally the beans are dried. After, the drying process the second cocoa residue can be separated, namely the cocoa husks.

When estimating amounts of residues produced, a product to residue ratio (RPR) is often introduced. The RPR is a measure of how much residue that is produced for each mass unit of product. Now using maize as an example, maize farming provides three different significant residues, stalks, husks and cobs, and these residues have different RPR's. The largest amount comes from stalks (1.60 g/g), while the husks and the cobs yield significantly less (0.20 and 0.29 g/g respectively) (Kemausuor et al., 2014, Paper I). These residues can be differentiated further by assessing where they are typically found. While a straw fraction most often is left on the field, the husks and cobs can be characterised as a processing residue, since they are typically separated from the main produce at a processing facility or alternatively in the household. Using this methodology, as well as the data presented in Fig. 1, a potential for available biomass residues in Ghana can be estimated (Table 1).

Table 1: Recoverability fraction (RF), residue to product ratio (RPR), technical potential, and residue type of most common agricultural residues in Ghana, (Kemausuor et al., 2014).

Crop	**Residue**	**RF** g/g	**RPR** g/g	**Biomass residues** Mt TS/yr	**Residue type**[*] F or P
Maize	Stalks	0.80	1.60	2.2	F
	Husks	1.00	0.20	0.34	P
	Cobs	1.00	0.29	0.49	P
Rice	Straw	0.25	1.70	0.19	F
	Husks	1.00	0.26	0.12	P
Millet	Stalks	0.80	1.80	0.27	F
Sorghum	Straw	0.80	2.00	0.46	F
Groundnut	Shells	1.00	0.37	0.18	P
	Straw	1.00	2.20	1.0	P
Cowpea	Shells	1.00	1.80	0.42	P
Cassava	Stalk	0.80	0.06	0.71	F
	Peelings	0.20	0.25	0.72	P
Plantain	Trunks and leaves	0.80	0.50	1.5	F
	Peelings[ƚ]	0.20	0.25	0.18	P
Soybean	Straw and pods	0.80	0.16	0.46	F
Yam	Straw	0.80	0.50	2.5	F
	Peelings[ƚ]	0.20	0.25	0.32	P
Taro (cocoyam)	Straw	0.80	0.50	0.54	F
	Peelings[ƚ]	0.20	0.25	0.067	P
Oil palm	EFB	1.00	0.17	0.33	P
	Kernel shells	1.00	0.07	0.13	P
	Fibre	1.00	0.14	0.28	P
Cocoa	Pods	0.80	0.93	0.67	F

*** F indicates a field-based residue while P indicates a processing residue. ƚ Not a part pf Paper I, added for the treatise, with RF and RPR assumed equal to cassava peelings.**

It is not easy to make general estimations of the current use of specific biomass residues in Ghana, and to the knowledge of the author, there is no inventory analysis in this field. Looking at nearby West African country Nigeria it seems that the main part of the residues is left on the fields, while only minor fractions are used for incineration or as livestock feed (Onwuka et al., 1997). The exceptions being cassava peelings, yam peelings and the cowpea husks, which are being used for livestock feed, in the given study it was 68, 44 and 43% of the residues respectively (Onwuka et al., 1997). Furthermore, approximately a third of the maize cobs as well as minor fractions of other biomass residues were used as cooking fuel (Onwuka et al., 1997). However, these estimations were made in another country almost two decades ago and much could be different in present day Ghana. Some residual biomasses are known to be used as low-grade fuels for cooking in Ghana, but this has been associated with high levels of particle pollution, and reduction in solid fuel use is essential for reducing this air pollution exposure and its health effects in Ghana (Zhou et al., 2011).

1.2. Ghanaian agricultural practices

Bioenergy is closely connected to agriculture, since the largest source of biodegradable residues is found in this sector (Kemausuor et al., 2014, Paper I). Therefore the production of bioenergy influences, and is influenced by, the nature and cultural practices of contemporary agriculture. In Ghana about half of the working force is engaged in agriculture, for the most part as smallholders with average holding sizes between 2 and 6 ha, and still many farmers are categorised as subsistence farmers who primarily produce food for their own family (Quaye et al., 2010). For that reason, if new bioenergy solutions influence the agriculture, it will have direct impacts on many Ghanaians. Furthermore, the agricultural sector is evolving continuously, thus affecting our possibilities to foresee and predict future bioenergy solutions based on agricultural residues.

Ghana consists of different agro-ecological zones that are affecting the type of agriculture being performed, ranging from regions with humid rainforest over deciduous forest to dry savannah. Correspondingly, the crop types differ from the more or less closed canopy farming with oil palms and cocoa, over cultivated crops, to herding of livestock in the arid northern parts. In large parts of Ghana the most predominant agricultural practice is shifting cultivation, which involves long cycles where land is cultivated for one or two years and then left fallow for 2-10 years (depending on agro-ecological zone) to restore its productive capacity (López, 1997; Quaye et al., 2010). In this system, the natural vegetation generated during the fallow period is used as green manure in the subsequent cultivation, having positive effects on both soil structure, soil organic carbon content, and erosion hazards (López, 1997). When the fallow period is brought to an end, the traditional Ghanaian method of preparing land for re-cultivation is by burning vegetation, which has a net negative impact on the soil organic carbon as well as on the greenhouse gas balance (Parker et al., 2010). Even though the fallow periods regenerate the soil, the burning practice is likely to result in rapid soil fertility decline (Parker et al., 2010). Nowadays, agriculture with fallow periods are declining throughout Ghana, and the use of more intensive farming systems through increased use of fossil fertilizer, machinery, intercropping with legumes etc. are more widespread (Fening et al., 2005; Codjoe, 2006). However, further intensification might prove difficult in a future of constrained global oil availability (Quaye et al., 2010).

With some crops, special circumstances prevail. In the case of cocoa, farmers usually start a growing cycle in virgin forest where they replace forest with cocoa trees. Cocoa production starts after 5 years, and after 25 years production has already been declining for a few years and farmers start a new cycle in a new forest (Jedwab and Moradi, 2011). Thus, as the tropical forest is decreasing, so will cocoa production, unless farmers switch to intensive production strategies.


Yam

It has been found that the steady increase in food production in Ghana over the past two decades is correlated with cropped area and the increase in population (Quaye et al., 2010). Thus, it seems that in order to sustain sufficient food production in a situation with increased population, the agricultural area will eventually increase. When pressure on the agricultural system intensifies, for one reason or another, shortening of fallow periods and more widespread land cultivation are probable, which is likely to alter these systems for a short-term benefit, even though this might have a negative effect on long-term productivity of the soils (Codjoe, 2006). This is because, less fallow areas, larger cropped area, and increased machinery use are all associated with increased turnover and depletion of organic material in the agricultural sector, which will cause enhanced soil erosion. Consequently, even though an increase in agricultural land has an output-increasing effect it will happen at the cost of reducing the natural capital or the long-term agricultural productivity (López, 1997).

Often, development of a crop-based biofuel production is associated with direct land use change (Hellmann and Verburg, 2011). Also in Ghana interest has been drawn from several nations including Brazil, Norway, Israel, China, Germany, Netherlands and Italy to cultivate jatropha and other biofuel crops on large scale for biodiesel production (Kemausuor et al., 2013). As an example, the Norwegian company ScanFuel Ltd. has been given governmental approval to start a jatropha oil production on 400,000 ha (Duku et al., 2011). However, the latest eyewitness reports tell that the initiated plots of jatropha have been abandoned while no more than 750 ha of maize have been established by the company (Bolwig and Hauggaard-Nielsen, 2011). Thus, so far, no sign of large-scale direct land-use change is detected in Ghana because of crop-based biofuel production. Land use change is considered one of the major threats to the sustainability of any biofuel regime especially in the already poverty-stricken region of Africa. Here, selected feedstock for bioenergy purposes must not affect availability and access to food since it is likely to lead to food shortage or directly to expansion of farmland elsewhere, often in forest areas (Mangoyana, 2009).

Different types of livestock are naturally kept in connection with Ghanaian farms and households. These are of interest as well, since biomethane can be produced from the manure. Both cattle, sheep, goats, pigs and poultry are kept, but utilising the manure fractions are often difficult since the majority are kept free-range during the day and only some are housed at night. Furthermore, there is not an even distribution of the livestock since the majority of the cattle are found in the northern part of the country (Bensah et al., 2011). Only a minor part of the livestock are in industrialised intensive farming systems and here mostly chickens are raised.

1.3. Ghanaian infrastructure

Like the agricultural sector, the Ghanaian infrastructure will have defining consequences on possible production of residue-based bioenergy in Ghana. African countries are in general underprovided with transport infrastructure (Jedwab and Moradi, 2011). However, rural transport improvement reduces poverty in connected villages by integrating labour and goods markets, thus providing new economic opportunities to the rural population (Jacoby and Minten, 2009; Renkow et al., 2004). In a comparative study of transportation standards, it was found that Ghana has a poor infrastructure and that a significant share of agricultural exportable produce decays due to deplorable rural road conditions (Mihyeon Jeon et al., 2006). If improvement to the transport infrastructure are made in the future, it will influence the availability of the biomass residues and thereby the potentials for bioenergy. There is a historic tendency that improved transportation infrastructure has an effect on the type of agriculture performed in Ghana, where improved transportation has induced production of cash crops such as cocoa while simultaneously inducing demographic growth and urbanisation (Jedwab and Moradi, 2011). Recently, Ghana has signed a contract to redevelop the existing railroad network and build a new line to the northern border, which will be the largest rail investment in Africa in at least 50 years (Jedwab and Moradi, 2011). Therefore, caution should be used when using current data on available crop residues to predict a future situation where also the transportation has been improved since this will most likely change agricultural practices. A consequence of the poor transport infrastructure on the perspective for improved bioenergy solutions is that large-scale bioenergy facilities are at present unfeasible. In the large-scale bioenergy plants projected for the European or North American market, more than 500 tons of dry lignocellulosic biomass should be brought in per day to ensure feasibility (Larsen et al., 2012). This requires a very fine-tuned infrastructure to support collection, storage and transport of such vast amounts of biomass, which is unavailable in the current Ghanaian context. Therefore, the process of making cellulosic bioenergy has to be optimised, not according to European or North American conditions, but within constraints that dictate a smaller scale than that envisioned in most scientific studies. How much of the biomass residues that could potentially be collected, is very difficult to evaluate based on the available literature, also since it eventually is a question about costs versus profit. Nevertheless, transportation of biomass can reasonably be assumed to be one of the bottlenecks for increased bioenergy production in Ghana.

1.4. The energy situation

It has been found that the living standards in Sub-Saharan Africa are very closely dependent on energy security, while approximately 90% of households in sub-Saharan Africa relies on traditional fuels for cooking and heating (Brew-Hammond and Kemausuor, 2009). Likewise, the traditional fuels of wood and charcoal are the most common fuels in Ghana, where they contribute to approximately 63% of the total energy consumption (Kemausuor et al., 2013). This is in spite a relatively high degree of electrification where about 56% of the Ghanaian households have access to modern energy services, produced mainly by hydropower (Brew-Hammond and Kemausuor, 2009). However, extending the electrical grid to rural domains is in many cases economically unfeasible (Mohammed et al., 2013). Even though a sustainable utilisation of fuel-wood is possible, it is not the current situation in Ghana. A recent study found that unrestrained exploitation of the common forest resources is taken place, which could ultimately completely deplete the forest resource. This is put into perspective by the fact that while Africa's population grew by 2.5%, between 1990 and 2004, Africa's consumption of traditional fuels rose by 42% (Ejigu, 2008). In total, Ghana lost 26 per cent of its forest cover (~2 Mha) between 1990 and 2005 due to the demand for agricultural lands and fuel wood (Owusu et al., 2012).

Though Ghana is an emerging oil producer, current oil production wells in Ghana is estimated to run out by about 2040, notably with current consumption rates, while higher consumption would mean faster depletion (Edjekumhene et al., 2010; Markussen, 2013). Therefore, the plausible benefits of the produced oil such as income, energy self-sufficiency, and resilience towards higher oil prices, will be temporary. Due to this, as well as due to the global peak oil situation, it is sensible to develop other energy options for Ghana, such as those possible with bioenergy. In addition, it is prudent to start such a transition in due time, since the time needed for adapting

Plantain

to a post-peak oil situation is counted in decades rather than years (Odum and Odum, 2001; Hirsch et al., 2005).

The primary end-uses of the bioenergy carriers assessed in this PhD thesis are within household energy for cooking and lightning, as well as for local transport. Both biomethane and ethanol can be used for both purposes. The easiest end-use for biomethane are direct combustion in households, but it is also possible to clean and compress the gas and use it in modified diesel engines, even though this is a much more complex technology (Patterson et al., 2008). Ethanol is most often mentioned as transport fuel substituting gasoline, but already now, ethanol of fossil origin has a marked share in Ghana used for cooking and lightning in households. Both biomethane and ethanol burn clearly with significantly reduction in particle emissions, thereby improving health and wellbeing as compared to fuel-wood.

Previously, political incentives have been used in the agricultural sector in order to increase the output of certain products such as cocoa, oil palm or cassava starch (Codjoe, 2006). Over the years, policies have also been directed at increasing access to energy services, but in spite of the good intentions, it has not delivered effective results, especially in the rural areas (Kemausuor et al., 2013). Lately, such efforts have also been pointed toward utilisation of biomass for energy purposes. This is indicated by the development of key documents such as the draft Bioenergy Policy of 2011 and the Renewable Energy Law of 2011 where targets for biofuels in the energy mix has been set (Kemausuor et al., 2011).

1.5.Ghanain bioenergy potentials

It has recently been pointed out that there is a lack of knowledge within the field of residue-based biofuels in Ghana (Duku et al., 2011). However, a few studies have tried to estimate the potential of bioenergy in the country. Firstly, Duku et al. (2011) published a study entitled “A comprehensive review of biomass resources and biofuels potentials in Ghana”. In this study, they are presenting yields of selected biomasses, both of crop and of residue, but they do not estimate any related biofuel yields and they exclude some of the main crops in Ghana such as cassava and yam. A residue-based energy potential is presented, however this is based on the lower heating value without any conversion efficiencies or estimations of potential utilisation. Furthermore, these residue energy potentials are a factor 1,000 too small compared to work presented subsequently (Duku et al., 2011).

In a recent study, the potential for transportations fuels based on cassava or oil palm is examined (Afrane, 2012). In spite of apparent problems of land use changes, which is raised in the paper, the authors are advocating for the use of cassava starch as a mean to respond to a future of declining oil supply (Afrane, 2012). Likewise, another study assessed the potential feedstock for ethanol production in Ghana and found that using cassava tubers would be the best substrate for making ethanol as substitute for gasoline (Osei et al., 2013). However, this study does not consider the implications of using a so-called 1st generation feedstock with respect to sustainable agriculture, food security and energy balance (Osei et al., 2013). A third study find that using 2.5% of uncultivated arable land in Ghana dedicated to four traditional crops, namely maize, cassava, sweet sorghum and oil palm could potentially replace 9.3% of transportation fuels by 2020 (Kemausuor et al., 2013). However, these potentials were once again based on usage of the starchy crops and not the crop residues.

Maize

The most extensive assessment of bioenergy potentials based on biomass residues was made by Kemausuor et al. (2014, Paper I). The aim of this study was to establish the amount of Ghana's energy demand that can be satisfied by using the lignocellulosic residues, including the agricultural residues presented in Table 1. The study estimates Ghana's bioenergy potentials based on detailed computations using literature and scientifically based assumptions to estimate the conversion of the residues into ethanol or biomethane. We found that the technical potential of bioenergy from residues was 2,700 Mm^3 of biomethane (96 PJ) or 2,300 ML of cellulosic ethanol (52 PJ) (Kemausuor et al., 2014, Paper I). The predicted national biomethane production would be sufficient to replace more than a quarter of Ghana's present fuel-wood use while the estimated cellulosic ethanol could replace more than 70% of the transport fuel (compared on energy content) (Kemausuor et al., 2014, Paper I). In the paper, it is argued that energy production based on biomass is not necessarily sustainable, and it is calculated that to utilise the full bioenergy potentials would be a huge challenge in terms of infrastructure requirements. For instance, hundreds of thousands of small-scale biomethane plants should be built in order to utilise the agricultural residues that constitute the main part of the potential residues.

1.6. Research questions

The research questions for the treatise are intended to tie together the findings from existing literature and the papers enclosed as part of the PhD thesis. The enclosed papers all have their own individual hypotheses that will not be repeated here.

Main research question for the treatise:

> *What is the potential of bioenergy, i.e. ethanol and biomethane, from agricultural residues that can be produced in Ghana? – Now and in a foreseeable future.*

With the sub-questions:

> *Is it possible to produce cellulosic ethanol as a part of a low-tech system?*
>
> > *How can the existing pretreatment methods be optimised for low-tech settings?*
> >
> > *What are the most significant prerequisites and primary constraints of increased cellulosic ethanol production in Ghana?*
> >
> > *Based on pretreatment technologies adaptable for Ghanaian conditions, what is the most realistic estimate for cellulosic ethanol production from agricultural residues?*
>
> *Is it possible to produce biomethane as a part of a low-tech system?*
>
> > *What are the most significant prerequisites and primary constraints of increased biogas production in Ghana?*
> >
> > *How can BMP be predicted precisely from biomass composition?*
> >
> > *Based on the derived model for determining biomethane potentials, what is the most realistic estimate for biogas production from agricultural residues in Ghana?*

To address these questions in the best possible way the next two sections will discuss the state-of-the-art within residue based ethanol and biomethane respectively. The findings will be put into a Ghanaian context and the bioenergy potentials presented in Paper I will be revised. Finally, the main conclusions and future perspectives will be presented.

2. Cellulosic ethanol and pretreatment

One of the bioenergy types assessed in this study is residue-based ethanol. Biomass-derived ethanol is one of our oldest inventions since beer- and wine-like beverages have been brewed for thousands of years. The simplest method of producing ethanol is from a biomass with a high free sugar content, such as fruits, where either added yeast or natural fermenting microorganisms present on the feedstock ferment the sugars into ethanol. Nearly as simple is the production of ethanol from starch, since starch is easily hydrolysed into free glucose by enzymes. These enzymes can be present in the feedstock, e.g. in the endosperm of a grain, or they can be added as it is often the case for large-scale starch-based ethanol production. Much more complicated is the production of ethanol from lignocellulosic feedstock.

Lignocellulose

Lignocellulose is made of three biochemical polymers: cellulose and hemicellulose two complex polymeric carbohydrate subunits of glucose (C6) and pentoses (C5) respectively, as well as a phenyl-propane macromolecule known as lignin.

The three components are closely connected with covalent cross-linkages making it very stable and thus difficult to degrade.

Cellulosic ethanol is produced by disrupting the rigid lignocellulosic matrix in a pretreatment step, and thereafter hydrolysing the cellulose into glucose with specialised enzymes. Hereafter, the sugars can be fermented into ethanol. The final process is a distillation, which is similar for all methods for producing ethanol from biomass. This is a well-known chemical unit operation, but since it is one of the most energy intensive processes, it lays constraints on some of the other steps such as the pretreatment and the choice of feedstock. It has been stated that at least 4 w/w % ethanol is needed in the fermentation liquor in order to make a cost- and energy competitive process, whereas higher concentrations obviously are advantageous (Larsen et al., 2008). Even though there could be interesting scientific achievements ahead within improved distillation in a low-tech small-scale setting, distillation will not be further addressed in this PhD thesis. Another bottleneck in production of ethanol from lignocellulose lies within fermentation of the C5 sugars originating from hydrolysis of the hemicellulose fraction, an ongoing field of research that has progressed for more than 20 years (Hahn-Hägerdal et al., 1991; Ahring et al., 1996). Even though this methodology is moving towards technical maturity (Biswas et al., 2013), it does still not exhibit the same stability as regular C6 sugar fermentation at large-scale and it is only briefly addressed in this PhD thesis (Sarkar et al., 2012). A third bottleneck, which by some are regarded as the main technical challenge, is the liberation of the sugars from the rigid lignocellulosic matrix. Succeeding in this requires an effective pretreatment of the biomass assisting an enzymatic attack on the cellulose fibres (Sarkar et al., 2012). Due to the advanced processes, cellulosic ethanol is more expensive to produce than for instance biogas and, even though it is compensated by a higher selling price, it not necessarily profitable to produce (Barta et al., 2013).

Pretreatment is regarded as the most technologically complicated process in production of cellulosic ethanol, as well as the most energy consuming activity, and the unit operation requiring the most expensive equipment (Hu and Ragauskas, 2012). Furthermore, since biomasses are different and quite complex, there is not a single pretreatment method that is “perfect” for all biomasses. The optimal pretreatment will always rely on the feedstock composition as well as the products aimed for – keeping in mind that most often ethanol is not the only product but one of several products in a biorefinery portfolio (Kurian et al., 2013). A thorough assessment pretreatment and the different modes of action employed within the different state-of-the-art pretreatment methods will be presented in section 2.2.

2.1. Composition

When choosing the strategy for ethanol production in a country such as Ghana, it is imperative to gain detailed knowledge of the substrates to be used for the production, since the composition has a determining impact on the key processes in the biorefinery. However, the main scientific attention has until now been on residues from industrialised countries or from the so-called BRIC countries (Brazil, Russia, India, China and South Africa) (Hellmann and Verburg, 2011; Pakarinen et al., 2011; Mabee and Mirck, 2011). Such thoroughly addressed agricultural residues contain maize stalks (Xu et al., 2010), wheat straw (Maas et al., 2008; Ambye-Jensen et al., 2013, Paper III), rye straw (Ingram et al., 2009), legumes (Petersson et al., 2007; Thomsen et al., 2012), sugar cane bagasse (Martin et al., 2007), and rice straw

Biorefinery

Many definitions exist, but being a field of rapid development, there is a tendency that these definitions do not withstand time.

However, a biorefinery is often thought of as a unit that converts biomass into several products, often energy carriers, materials, feed or chemicals, as a possible alternative to a fossil oil refinery.

(Chang et al., 2011). In contrast, agricultural residues originating from Ghana for the most parts have not been investigated. Of the Ghanaian residues listed in Table 1, residues common worldwide, e.g. from maize, soybean, sorghum and rice, have been thoroughly assessed, but not on residues originating from West Africa (Kim and Dale, 2004; Reddy and Yang, 2009; Van Eylen et al., 2011; Mahmood and Honermeier, 2012). Of the residues more unfamiliar outside of Africa, the peelings from yam, taro and cassava are partly used for animal feed, but only the cassava stalks have previously been evaluated for the composition (Martin et al., 2006). Millet has been evaluated for cellulosic ethanol purposes but only the whole-crop hay and, to the knowledge of the author not the residual straw after harvest (Chen et al., 2007). Cocoa and groundnut residues have mainly been considered for animal feed, additional information are therefore required to make bioenergy evaluations (Donkoh et al., 1991; Larbi et al., 1999; Aregheore, 2002). Oil palm residues have been studied by several authors, but large deviations in the published results exist in the case of oil palm empty fruit bunches (EFB) (Shibata et al., 2008; Abdullah et al., 2011). Plantain residues have not yet been assessed. A forthcoming paper will try to remedy the lack of knowledge in the field (Thomsen et al., 2014b, Paper II). In this work, a thorough compositional analysis was made in order to enable usage of the residues for ethanol or biorefinery purposes. In the study, we were able to obtain representative biomass samples of 13 of the most important biomass residues from our Ghanaian project partners.

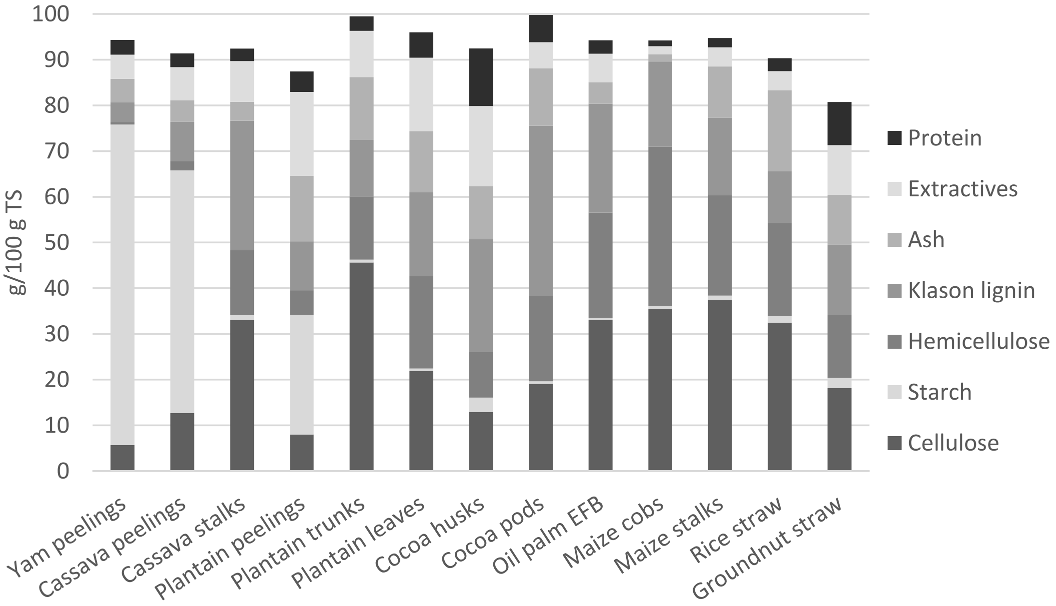

Fig. 2. Composition of common agricultural residues from Ghana (Thomsen et al., 2014b, Paper II).

Fig. 2 (page 16) is graphically presenting the compositional data obtained in the study and it is apparent that the different biomasses exhibit quite different compositional traits. The high content of starch in the yam and cassava peelings enables high ethanol potentials of 0.61 and 0.53 L ethanol/kg TS respectively, where 0.56 and 0.43 L ethanol/kg TS respectively are based on starch and free sugars only (Thomsen et al., 2014b, Paper II). Yam-, cassava- and possible also taro peelings are, on these grounds, the most obvious agricultural residuals for ethanol production, enforced by the relatively easier production route compared to lignocellulosic feedstock. However, it should be noted that current use and distribution is not clear. Lignocellulosic residues high in cellulose were found to have lower potentials. E.g. plantain trunks that, in spite of a very high cellulose content of 46 g/100 g, had a potential of only 0.21 L ethanol/kg TS based on the cellulose. If foreseeing a situation where both cellulose and hemicelluloses could be utilised, maize cobs were found to have high potentials of 0.32 L ethanol/kg TS.

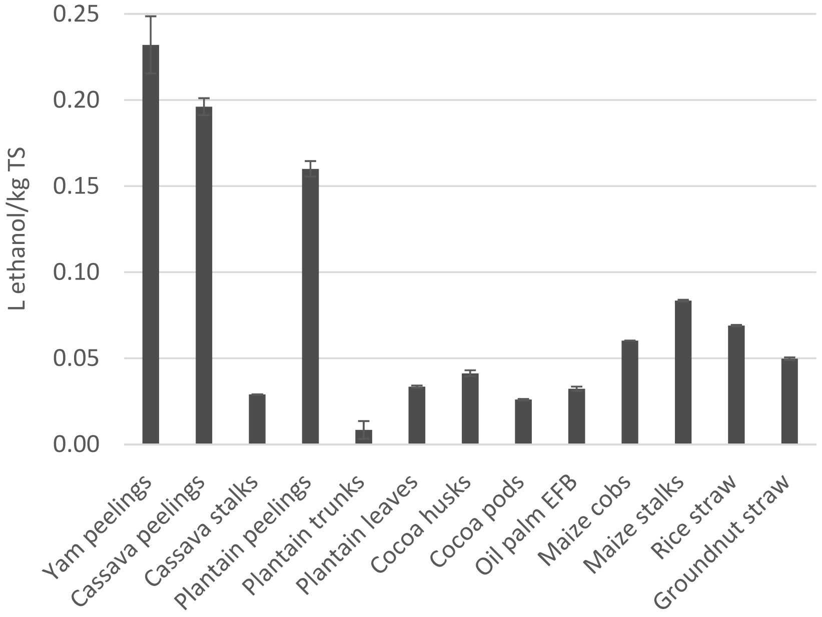

Fig. 3. SSF results on raw Ghanaian biomasses (results of plantain peelings, plantain trunks, maize cobs and maize stalks (Thomsen et al., 2014a, Paper IV), results of remaining biomasses are previously unpublished and not part of any current manuscripts but prepared as described in the materials and methods of Paper IV). Error bars describe standard deviations.

The 13 Ghanaian residues were in a previously unpublished study subjected to a simultaneous saccharification and fermentation (SSF) of the untreated biomasses (Figure 3). These fermentation results are significantly lower than the results projected from the

Cassava

composition (Thomsen et al., 2014b, Paper II). This tendency is most clear for plantain trunks where only 4% of the theoretical ethanol were produced in the actual fermentation of the raw materials.

None of the lignocellulosic residues had ethanol yields close to the theoretical potential of ethanol based on cellulose. This is due to the recalcitrance of these residues, which were not taken into account when determining the yields. As mentioned above, it is well known that lignocellulosic residues should be pretreated in order to achieve acceptable yields of ethanol.

The compositional data presented in Fig. 2 (page 16) do not only reveal the potentials for ethanol production. Biomethane potentials can be estimated, which will be discussed in section 3.2. Additionally, a whole range of products and intermediates has been suggested as a part of lignocellulosic biorefineries in the past years. Assessing the Ghanaian agricultural residues for other purposes than ethanol, makes other residues stand out. As an example, high-lignin residues such as the cocoa pods, which contain 37.2 g lignin/100 g TS, are attractive for incineration since lignin is more chemically reduced than the other biomass constituents are. Yet, other more sophisticated possibilities for utilising lignin exists, such as lignin-based polymers or aromatic chemicals (Laurichesse and Avérous, 2013; Olcese et al., 2013). Likewise, the presence of 5.6 g arabinan/100 g TS in the plantain leaves could indicate an arabinoxylan backbone in the hemicellulose fraction of the residue (Thomsen et al., 2014b, Paper II). Arabinoxylan has been considered for making bio-derived packaging films for the food industry (Sárossy et al., 2012). Likewise, numerous alternative uses for cellulose and hemicellulose have been proposed.

2.2. Pretreatment

Pretreatment is pivotal to make lignocellulosic biomass accessible for enzymatic attack and thus for decomposing the carbohydrates into free monomeric sugars. Many methods have been deployed on a scientific level, each of them building on different strategies with what can be described as different modes of action. Included in the modes of action of different pretreatments are heat, pressure, pressure release causing explosive decomposition, water and autohydrolysis, acid, alkali, enzymes, and effect of various ions (Carvalheiro et al., 2008; Hu and Ragauskas, 2012; Bensah and Mensah, 2013).

During the pretreatment, the effects on biomasses can be various. Most often the outer wax-layer is disrupted, some methods aim for hemicellulose removal, some for lignin relocation or removal, and others for cellulose swelling or decrystallisation. All pretreatment methods aim at a high subsequent utilisation of the glucan fraction, however, this most often comes at the expense of three disadvantages: 1) degradation of hemicellulose, 2) increased energy consumption, and 3) production of inhibitors. Inhibitors from pretreatments are somewhat ambiguous. On one side, if they exceed certain levels they will inhibit the subsequent enzymatic hydrolysis and/or fermentation (Thomsen et al., 2009), but on the other side, controlled levels of inhibitors can limit the level of contaminant growth in fermentations (Larsen et al., 2012). In addition, it is found more efficient to control the pretreatment settings in order to reduce inhibition, than to apply post treatment methods such as washing of fibres (Leu and Zhu, 2013). It has been found that hemicellulose removal is more important than lignin removal, however partial lignin removal is important in order to achieve high conversion of cellulose when the lignin content is high (Leu and Zhu, 2013). In Table 2 (page 20), the most employed pretreatment methods for lignocellulosic biomass are collected and their modes of action are described.

When optimising on pretreatment method such as the methods described in Table 2 (page 20), it is often the case that different methods and modes of action are combined in order to obtain beneficial synergies. A common example is the combination of mild acidic or alkali effect in HTT, liquid hot water or steam explosion pretreatments, thereby using the additive as a catalyst for a desired effect (Sorensen et al., 2008; Xu et al., 2010). Oxygenating additives, such as air, pure oxygen, or hydrogen peroxide are also applied, since these increase dissolving of hemicellulose (Martin et al., 2007).

The drawback of the various catalysts are often fossil origin and added costs. Recently a master study from the Department of Chemical and Biochemical Engineering at DTU investigated, if cheap alternative catalysts from agricultural or biorefinery waste could reduce optimal pretreatment temperature in HTT, however this will need further studies (Engelbrechtsen, 2012; Thomsen and Kádár, 2012, unpublished).

Table 2: The most common pretreatment methods and their respective mode of action and effect.

Method	Mode of action, effect and scale*	Reference
Hydrothermal treatment (HTT)	Autohydrolysis with water at 160-230°C, which causes cleavage of acetyl groups from the hemicellulose, thus pH decreases and the dissolved acids further catalyse hydrolysis of hemicellulose-lignin bonds and carbohydrates. Scale: Full-scale.	(Larsen et al., 2012; Ambye-Jensen et al., 2013, Paper III; Thomsen et al., 2014a, Paper IV)
Liquid hot water	Autohydrolysis. 160-230°C elevated pressure. Since the water to a higher extent is on liquid form, it has slightly different reaction patterns than HTT. Scale: Demonstration.	(Ingram et al., 2009)
Steam explosion	Autohydrolysis. Decrease in pressure makes moisture expand and breaks inter- and intra- molecular bonds in the lignocellulose (Shear forces). Scale: Demonstration.	(Martin et al., 2008; Di Risio et al., 2011)
Dilute acid	Dilute acids hydrolyse hemicellulose and expose cellulose to enzymes. Done at various temperatures. Scale: Demonstration.	(Van Eylen et al., 2011)
Concentrated acid	Hydrolyse hemicellulose and cellulose directly to monomeric sugars. Done at various temperatures. Scale: Demonstration.	(Liu et al., 2012)
Lime or NaOH	Alkali breaks ester bonds and glycosidic side chains thereby degrading lignin, solubilising hemicellulose and causing swelling and decrystallisation of cellulose. Scale: Pilot.	(Maas et al., 2008)
Ammonia fibre explosion/ expansion	Alkali. Decrease in pressure causes expansion of the lignocellulose and breaks inter- and intra- molecular bonds (Shear forces). Done at 60-100°C. Scale: Pilot.	(Krishnan et al., 2010)
Soaking in aqueous ammonia (SAA)	Alkali. Can be done with long retention times and ambient temperatures. Scale: Pilot.	(Kim and Lee, 2005; Thomsen et al., 2014a, Paper IV)
Organosolv	Primarily used for delignification, but can also hydrolyse hemicellulose (depending on choice of solvent). Solvents include ethanol, methanol, acetone, ethyl acetate, formic acid, etc. Scale*: Full-scale in paper and pulp industries.	(Pan et al., 2008)
Ionic liquids	Ionic liquids are salts that are liquid at, or close to, room temperature. Different combinations of various anions and mostly organic cations are used. Either partial lignin and hemicellulose solubilisation or complete solubilisation of the biomass. Scale: Lab-scale.	(Brandt et al., 2013)
Silage treatment	Combined storage and pretreatment. Long-term impregnation of the biomass with organic acids. Scale: Full-scale for forage and biogas purposes.	(Larsen and Petersen, 2012; Ambye-Jensen et al., 2013, Paper III)
White rot fungi pretreatment (WRF)	White rot fungi degrades lignin and carbohydrates through extracellular enzymes over an extended time.	(Isroi et al., 2011; Thomsen et al., 2014a, Paper IV)

***Represents the biggest current scale of the method to the knowledge of the author (lab-scale < pilot < demonstration < full-scale). However, up-scaling is not always published in scientific journals, thus larger scale might have been achieved.**

Another example of advances in pretreatment, is from a recent study by Ambye-Jensen et al. (2013, Paper III). This work started out as an investigation of the possibilities of using ensiling as a stand-alone pretreatment method of lignocellulosic biomasses, among others applicable for Ghanaian conditions. We were able to develop a novel method of ensiling dry lignocellulosic biomass, which in its natural state does not contain enough free sugars to obtain a successful ensiling. In this new methodology, xylose was added to the biomass while adjusting the water content of the material (Ambye-Jensen et al., 2013, Paper III). The added sugar was xylose for dual reasons, both since C5 sugars induce the production of acetic acid, and since xylose in a biorefinery perspective is often considered of less value than glucose. It was found that ensiling could not thoroughly pretreat the lignocellulosic biomass, which in this case was wheat straw used as a model biomass (Ambye-Jensen et al., 2013, Paper III). Instead, it was tested if ensiling could be combined with hydrothermal treatment (HTT). It was found that ensiling significantly reduced the required temperature of an optimised HTT, thus the energy consumption for the HTT can be reduced when ensiling is used as pre-step to HTT (Ambye-Jensen et al., 2013, Paper III). At the reduced HTT temperature of 170°C, the overall glucose yield after pretreatments of ensiled wheat straw was 1.8 times better than the corresponding yields for the non-ensiled straw. Since pretreatment is among the most energy intensive unit operations in the production chain of cellulosic ethanol, decreasing the temperature will also be making the entire process more cost competitive and thus more realistic for Ghana as well as other countries.

In a similar but previously unpublished study, ensiling was combined with WRF in a combination of biological pretreatments. The hypothesis of this study was that the ensiling procedure would decrease the recalcitrance of the biomass by decreasing the outer wax layer of the straw and by long-term impregnation with acids, consequently enabling a more efficient pretreatment with WRF. Wheat straw was again used as a model material, and the study has shown promising results when evaluated on glucan conversion after pretreatment (Fig. 4, page 22) (Thomsen and Londoño, 2013, unpublished and not part of any current manuscripts). The results indicate a clear effect of WRF on wheat straw and an even larger effect when the WRF is preceded by ensiling. However, these results should be verified in further studies.

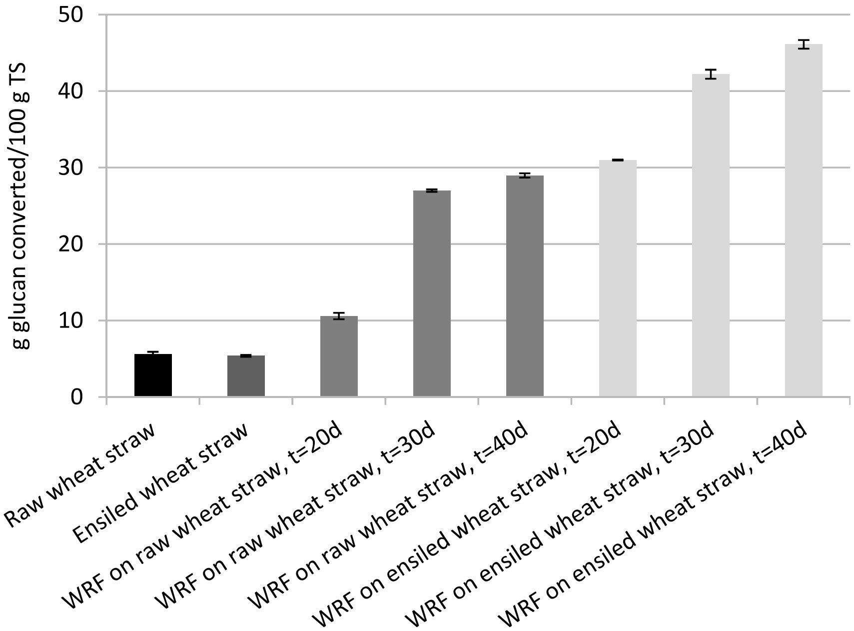

Fig. 4. Glucan converted by cellulase after, ensiling, WRF, or combined biological pretreatment at different times (Thomsen and Londoño, 2013, unpublished and not part of any current manuscripts). Silage prepared as described in the materials and methods of Paper III, WRF and enzymatic hydrolysis performed as the materials and methods of Paper IV. Error bars describe standard deviations.

If the results of Fig. 4 can be verified, the combination of biological pretreatments might be interesting in a Ghanaian context since high-tech equipment can be avoided. Meanwhile, the extended time and labour requirement might be outbalanced by the decreased equipment costs. In that case, the combination of biological pretreatments could be applied on Ghanaian lignocellulosic residues. Yet, it should be noted that WRF requires a sterilisation step, and that up-scaling of the method under Ghanaian conditions has not been investigated. It should also be noted that choosing wheat straw for the initial investigations of new pretreatment methods is not accidental. Wheat straw is a well-known residue that is used as model biomass since we, at the Technical University of Denmark, have worked with wheat straw for more than a decade (Bjerre et al., 1996; Klinke et al., 2002; Thomsen et al., 2008). Therefore, the focus on wheat straw should be seen as providing a proof of concept, which can be transferred to other biomasses in subsequent studies.

Pretreatment of the most common Ghanaian biomasses for cellulosic ethanol has until now focussed on the same high-tech methods as those applied to common residues from industrialised countries. For instance, the pretreatment of cocoa waste with ionic liquids has been investigated as well as wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and groundnut for ethanol production (Martin et al., 2007; Idi et al., 2012). Besides, the residues have mostly been addressed in optimisation studies on one or a few biomasses, and only with single pretreatment methods. In addition, no agricultural residues originating from Ghanaian soils have been investigated. On these grounds, a combined comparative study has recently been submitted for publication (Thomsen et al., 2014a, Paper IV). Here, 11 common lignocellulosic agricultural biomasses were subjected to pretreatment with three pretreatment methods that are believed to be favourable for low-tech, small-scale conditions in Ghana. The biomasses were cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches (Thomsen et al., 2014a, Paper IV). The pretreatment methods were boiling pretreatment (BP), soaking in aqueous ammonia (SAA) and fungal pretreatment (WRF), which were benchmarked against HTT. It was found that four of the biomasses, i.e. plantain peelings, plantain trunks, maize cobs and maize stalks, were the most promising for production of cellulosic ethanol with enzymatic conversion of glucan of more than 30 g glucan per 100 g TS (Thomsen et al., 2014a, Paper IV). HTT performed better, in both enzymatic convertibility and in fermentation, but assessed on the overall ethanol yield the low-tech pretreatment methods were viable alternatives with similar levels to the HTT (ranging from 13.4 to 15.2 g ethanol per 100 g TS raw material) due to a limited loss of glucan during pretreatments (Thomsen et al., 2014a, Paper IV). It was argued that the alternative methods of PB, SAA and WRF have more selective modes of action compared to HTT, and therefore they each performed well on a few of the biomasses only. Since the study suggested that less than half of the evaluated West African lignocellulosic biomasses were feasible for cellulosic ethanol production, this should be considered

Cocoa

when estimating cellulosic ethanol potentials of the region. Feedstock choices concerning farming systems and current use were not addressed, but this should be done in order to determine the overall sustainability of small-scale decentralised bioenergy systems in Ghana. Farming practices and current biomass use affect the choice of evaluated pretreatment technologies was made with respect to labour requirements, energy requirements and applicability in different contexts. Other chemical pretreatment methods could have been tested, but as a general sustainability requirement, the use of chemicals was avoided unless easy recoverability was mentioned in the literature (as the case for SAA).

It should be noted, that the threshold of 30 g glucan converted per 100 g TS applied in Paper IV was based on two constraints. Firstly, the necessity to reach a minimum ethanol concentration after fermentation of 4 w/w % (Larsen et al., 2008). Secondly, an assumption of 25% DM content in the enzymatic hydrolysis. DM contents in the enzymatic hydrolysis of 40% have already been reached in lab scale but the latest reports from large-scale test reports running at 20-30% DM (Jørgensen et al., 2007; Larsen et al., 2012).

2.3. Implementation of residue-based ethanol production in Ghana

It has been stated that the capital cost of investment on large-scale bioenergy plants is unaffordable in Ghana (Mohammed et al., 2013). Similarly, other authors have found that in an African context, it is unfeasible to set-up and implement technological schemes with high-performance technologies that are proven worldwide generally at the pilot plant level (Quintero et al., 2012). The same study showed that small-scale solutions are feasible to implement but they produce lower yields and have higher production costs per unit of output (Quintero et al., 2012). Labour is significantly cheaper in Africa than even in Asia or Eastern Europe and for that reason it might be conceivable to successfully employ methods that are more labour intensive than methods developed for the industrialised world (Osagie, 2008). Furthermore, the infrastructure necessary for a projected full-scale HTT-based cellulosic ethanol plant as planned for the European or North American market is far from reality in present days Ghana. In a country as Ghana where rural roads are mostly unpaved, transportation costs are inevitable higher, providing an incentive for reduced scale of production. Transporting the biomass is in an industrial scheme is estimated to constitute to a fifth of the total costs of producing cellulosic ethanol with decreasing costs for smaller scale due to smaller working range (Kurian et al., 2013). Furthermore, similar residues must be available year-round in order to keep the biorefinery running and pay back the capital investment. It is advisable to utilise the easily convertible and easily collectable residues first, such as starchy industrial residues. Thereafter, one may venture into the lignocellulosics. Likewise, production will be most realistic in an industrial setting where the processing residues are already concentrated.

Changing the energy paradigm is in development and a political imperative. Small-scale production and processing of bioenergy, with appropriate incentives and policy support, enables farmers to produce their own energy and achieve livelihood security (Ejigu, 2008). The raised points advocate for small-scale solutions contradicting the usual argument that high-tech equipment such as distillation columns and pressure vessels are relatively cheaper in large-scale. Therefore, further investigations should be done in the area of small-scale low-tech solutions, keeping in mind that a commercial opportunity should be available before the technology will be spread. In the meantime, a pool of expertise and skilled employees should be developed in Ghana, but the realism in this quest can be questioned. Whether or not there will be a net energy gain from the process should also be investigated, since the energy return on invested (EROI) for cellulosic ethanol do not always come out positive (Pimentel and Patzek, 2008; Hall et al., 2011).

Rice

2.4. Ghanaian ethanol potentials revisited

Based on the information presented in section 1. and 2. it is possible to revisit the ethanol potentials presented in Paper I (Kemausuor et al., 2014), aiming at a more realistic estimation. Thus, a set of new assumptions will be specified here.

As elaborated upon, starchy processing residues are the most realistic category of agricultural residues to initiate an ethanol production from in Ghana. In previous work, only one starchy residue, cassava peelings, was assessed (Kemausuor et al., 2014, Paper

I). However, the peelings from yam, plantain and taro, would also be appropriate to include in the assessment since these crops are also significant in a Ghanaian context as seen in Fig. 1 (page 3). Using the same recoverability fraction (0.20 g/g) and residue to product ratio (0.25 g/g) for the peelings of yam, plantain and taro as used for cassava peelings, will in this case be a fair assumption since these crops have similar end-uses (Kemausuor et al., 2014, Paper I). The relative low recoverability fraction of 0.20 g/g was assumed since the major fractions of the peelings will end up as household waste or as feed, while only a minor fraction is used for agro-industrial applications. Municipal household waste (MHW) is not assessed in this treatise since optimal utilisation of MHW in Ghana is currently investigated by a fellow PhD student in the 2GBIONRG project (Miezah, 2014, unpublished).

In Paper I, the ethanol potentials were calculated from compositional data found in literature. At this point, I will instead include the ethanol potentials presented in Fig. 3 (page 17), since these real data on residues found originating from Ghana. For taro peelings, which were not analysed in this study, an ethanol potential equal to the similar residue cassava peelings is assumed. When comparing the ethanol potential based on starchy processing residues with the total residue-based ethanol potential in Ghana as found in Paper I (Table 3), it is seen that ethanol from the starchy processing residues constitutes only 12%. However, this amount would be enough to substitute 8% of the transport fuel required in 2020.

Table 3: Up-dated ethanol potentials for agricultural residues in Ghana. The calculations were as described in Paper I (Kemausuor et al., 2014), with the exception presented above.

Residue category	ML ethanol / year	PJ /yr
Total agricultural residues (Kemausuor et al., 2014, Paper I)	2,000	53
Starchy processing residues	280	6.3
Processing residues, including lignocellulosic (according to presented assumptions)	860	20

A scenario where all lignocellulosic residues are utilised for ethanol is questionable in contemporary Ghana. Therefore, only processing residues are assessed in the following estimation of cellulosic ethanol in Ghana. Furthermore, it was found in Paper IV that only some Ghanaian lignocellulosic residues were promising for cellulosic ethanol production (maize straw, maize cobs, plantain peelings and plantain trunks) (Thomsen et al., 2014a, Paper IV). Furthermore, it is assumed that rice husks, sorghum stalks and sugarcane bagasse are promising due to successful evaluations in literature. At this point, I will again include real ethanol potentials, as presented in Paper IV, instead of the theoretically found potentials used in Paper I. For rice husks, sorghum stalks and sugarcane bagasse an overall ethanol yield of 14.8 g ethanol produced per 100 g TS raw material is assumed, since this was the average of the pretreated materials in Paper IV.

When adding the ethanol potential from the promising lignocellulosic residues to the potential from the starchy residues it sums up to a potential of 860 ML ethanol (Table 3). This could substitute 62% of the transport fuel required in 2020. Yet, there is no guaranty that ethanol produced in Ghana would be used locally since it might as well be exported to a market that subsidises bioenergy such as in the European Union. In addition, producing 860 ML ethanol would demand in the range of 10-25 full-scale plants as projected for the European or North American market with huge capital costs associated with the construction (Larsen et al., 2012). Furthermore, even though the waste is categorised as processing waste, it might still be too spatially distributed for large-scale implementation thereby requiring transportation under difficult conditions. Therefore, further research into the production regimes applicable for smaller scale is imperative. This should include focus on low-input pretreatment and distillation regimes. An area where solar powered units might prove interesting (Pablo Vargas-B. et al., 2013).

It can be speculated, that creating a market for residue-based ethanol might encourage farmers to produce dedicated energy crops for making ethanol. This would alter the land-use away from food and feed production and objectives of long-term sustainability would be harder to achieve. Likewise, it is important to manage the carbon and nutrient cycle and bring back remains after ethanol production to the agricultural system as not to exhaust the Ghanaian soils. Such remains would contain relatively recalcitrant lignin, silicates and important nutrients such as phosphorous.

3. Anaerobic digestion and prediction of BMP

3.1. Anaerobic digestion

Anaerobic digestion (AD) is the anaerobic biological degradation of organic matter to produce methane, CO_2 and traces of other gases, a process occurring in many anoxic natural habitats (Nallathambi Gunaseelan, 1997). This process can be utilised to convert diverse organic feedstocks to methane-rich biogas as a potentially resource-efficient and environmentally friendly method of producing energy. AD is more flexible concerning feedstock than ethanol fermentation, since most biological substances can be converted, including carbohydrates (all kinds), lipids, proteins, organic acids, alcohols, and more (Ahring, 2003). Consequently, a very large fraction of the waste produced in Ghana can be anaerobically digested including lignocellulosic residues, manure, municipal waste, many industrial wastes and wastewater. The most widespread biological substance that cannot be anaerobically digested is lignin. Besides, inert silicates are also present in most biomasses.

Unlike ethanol, methane is not produced by a single organism but by many different groups of microorganisms living in synergy, which each is responsible for only a part of the complete AD (Ahring, 2003). There can be found numerous microorganisms in an AD but the most important groups are: 1) The hydrolysing and fermenting bacteria that hydrolyse the carbon chains into monomers via extracellular enzymes and ferment them into acetate, volatile fatty acids, alcohols, hydrogen, and carbon dioxide. 2) The syntrophs, which are the obligate hydrogen-producing acetogenic bacteria that converts fatty acids into hydrogen, carbon dioxide or acetate. 3) The methanogenic *Archaea* that converts hydrogen, CO_2, and acetate into methane (Ahring, 2003). In addition to the main groups of microorganisms a broad variety of different species are present that might be either beneficial or inhibitory to the main process (Hattori, 2008; Deublein and Steinhauser, 2008). Methane production is efficient when the right microbial consortia

Oil palm

are present to perform all the different steps of complete anaerobic conversion of the substrates. Conveniently, manure generally contains microbial consortia, which are able to sustain some of the activity that took place in the digestion system of the animal source (Nallathambi Gunaseelan, 1997). Furthermore, manure also contains remaining organic substances. Thus, biomethane will be produced when manure is contained in anoxic environments, and therefore the most simple and withstanding AD set-ups are using manure as primary substrate (Bond and Templeton, 2011). The restrictions of using manure as substrate are a relatively low content of convertible carbon, and a high content of ammonia that can cause instabilities in the process (Nielsen and Angelidaki, 2008). This can be diminished by co-digesting other biomasses high in carbon together with the manure, e.g. lignocellulosic residues.

AD can be performed in very complex and high-tech set-ups, where the described microbial interactions are monitored and regulated by strict feeding regimes, novel reactor types, precise stirring and temperature control (Nielsen et al., 2004; Parawira et al., 2008). However, one of the advantages of AD is that the operator of an AD facility do not have to be aware of the rather complicated microbial interaction described above. If a smaller gas yield than achieved under optimal conditions is satisfactory, AD can be a relatively easy and robust technology. This is what can be exploited in a low-tech set-up. The performance in low-tech plants compared to Biomethane potentials (BMP) is very difficult to predict. It depends heavily on a number of factors, including hydraulic retention time, temperature, solid loadings, balances in the microbial community, and recalcitrance of the substrate (Surendra et al., 2013). Further, the daily managing of the system is very important. Therefore, choosing one factor describing low-tech performance in relation to BMP might be far-fetched. Nonetheless, 80% (biomethane yield compared to BMP) have been used (Kemausuor et al., 2014, Paper I), while 50% might be more realistic (Sasse, 1987).

An alternative to manure based AD and co-digestion regimes, is AD performed at high DM content solely on relatively dry biomasses such as grass, silages, or the organic fraction of municipal solid waste (Chanakya et al., 1995). The DM content of high-solid AD ranges from 10 to over 30%, compared to 2 to 8% DM used generally (Jha et al., 2011). Thus, high-solid AD offers an opportunity of reduced water use.

3.2. Prediction of biomethane potentials

Biomethane potential (BMP) tests are the most common way of estimating the maximum amounts of methane that can be produced from a given substrate. BMP is measured by quantifying the amount of produced methane in a complete AD of a known mass of substrate, which is a key parameter for assessing design, economic and managing issues for the full-scale implementation of anaerobic digestion processes. (Angelidaki et al., 2009). In addition, when estimating bioenergy potentials, correct BMPs are important for the accuracy of the estimations. There have been made numerous BMP measurements on various feedstock, but again West African feedstock has mostly been overlooked so far. In Figure 5, BMP are presented from the 13 biomasses previously described (section 2.1.), and differences among the feedstock are clear. Once again, the starchy peelings from yam, cassava and plantain performed well and gave rise to high BMP of with 393, 366 and 363 ml CH_4/g VS (Volatile Solid) respectively. Yet other biomasses are also in the high range of BMP, these are cocoa husks, maize cobs, maize stalks, and rise straw, which all had BMP over 300 ml CH_4/g VS. On the other hand, plantain leaves and oil palm EFB had BMP on the level of manure (~200 ml CH_4/g VS), while cassava stalks and cocoa pods performed poorest with approximately 100 ml CH_4/g VS (Figure 5). These results indicate that not all Ghanaian agricultural residues are fitted for AD.

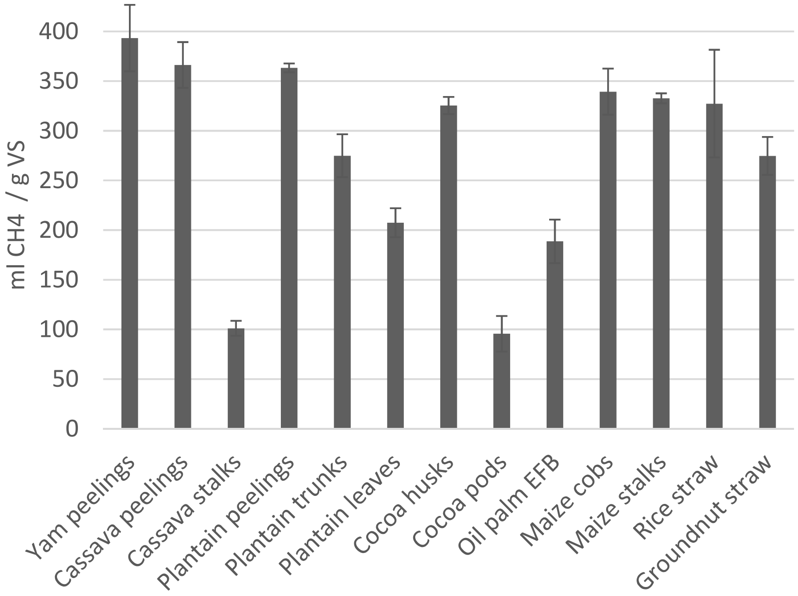

Fig. 5. Biomethane potentials of common Ghanaian biomasses (Thomsen et al., 2014c, Paper V), BMP for peelings of yam, cassava and plantain are previously unpublished but prepared as described in materials and methods for Paper V. Error bars describe standard deviations.

Measuring BMP is a quite time-consuming activity considering that up to 90 days are required as a standard incubation time (Hansen et al., 2004; Gerber et al., 2013). Consequently, it is desirable to use alternative methods to estimate BMP. This is particularly true, when making theoretical studies where real experiments are unfeasible or when time is an issue. During the studies on previously unanalysed biomasses from Ghana, it was found that the existing methods for predicting BMP from compositional data of the respective biomasses were insufficient. Either the methods did not take the recalcitrance of the materials into account or they were counter-intuitive (Thomsen et al., 2014c, Paper V). Recalcitrance of lignocellulosic biomass is, as mentioned, dependent on composition and are determined by the interaction in the lignocellulosic matrix (Albersheim et al., 2011). For instance, the cassava stalks seem to be quite recalcitrant by giving a low BMP despite a high carbohydrate content (Fig. 5, page 30 and Fig. 2, page 16).

Therefore, the amounts of biomass constituents such as lignin, hemicellulose, cellulose, waxes, pectin etc. are believed to have an impact (Thomsen et al., 2014c, Paper V). In addition, the specific biomass constituents can vary in between plant species. Of the methods used for predicting BMP that is not considering recalcitrance, three should be mentioned. 1) BMP estimated from the volatile solid (VS) content, 2) BMP estimated from the chemical oxygen demand (COD) level of the biomass, and 3), BMP determined with Buswell's formula that expresses the maximum output of methane gas in a complete AD of organic matter (Symons and Buswell, 1933). Most often Buswell's formula are used on chemical sum formula of the organic material, but it can also be used on of the biomass constituents individually.

The methods that we found to be counterintuitive were all regression models, which found that the lignin content is the only important biomass constituent for the outcome of BMP (Triolo et al., 2011; Monlau et al., 2012). This is not in line with AD theory since lignin does not contribute to the formation of methane in AD, but is merely tying the lignocellulosic matrix together while making a physical barrier around the carbohydrates (Albersheim et al., 2011). Thus, the content of otherwise degradable cellulose and hemicellulose is not accounted for in the models. Furthermore, Triolo et al. (2011) found that when including both lignin and cellulose as regression variables, cellulose contributed negatively to the model. This is also contrary to AD theory, since carbohydrates are degradable in AD and should result in a positive regression coefficient. Another weakness with the existing regression models was, that they were made on a small dataset ($n \leq 10$), thus they were not applicable for predicting BMP from lignocellulose in general (Triolo et al., 2011; Monlau et al., 2012).

Due to the presented motives, we were intrigued to find a better model for predicting BMP (Thomsen et al., 2014c, Paper V). In the first part of this study we collected a large dataset ($n = 64$) from peer-reviewed sources, in which BMP and the composition of a non-pretreated lignocellulosic biomasses were given. From this dataset, statistical methods was used to derive a new prediction model to find predicted BMP (pBMP). In the study, two different type of models was investigated both a canonical linear mixture model, as well as a canonical quadratic mixture model (Thomsen et al., 2014c, Paper V). Mixture models are models known from describing effects of chemical mixtures that take into account that the variables are proportionate nonnegative amounts of different constituents, $0 \leq x_i \leq 1,\ i = 1, 2, \ldots, q$ which sum to one, $\sum_{i=1}^{q} x_i = 1$ (Cornell, 2011). Since the aim was to determine pBMP from the composition of lignocellulosic biomass, the variables were the content of the most important biomass constituents of lignocellulosic biomass: Cellulose (x_C), Hemicellulose (x_H), and Lignin (x_L) (Thomsen et al., 2014c, Paper V). Biomass composition is most often given in relative numbers, in units such as % or w/w, and for that reason the variables can be expressed in a unit so that the variables sum up to one. To be in line with the mixture model theory an additional variable (x_R) called 'residuals' was included in the model. Thereby, everything that is not carbohydrates or lignin is characterised as residuals, $x_R = 1 - (x_C + x_H + x_L)$. x_R has not been considered in previous models, which is problematic since x_R might contain biomass constituents that are degradable in AD, such as lipids, fatty acids, pectin, and proteins. In the following, betas (β_i) will be regression coefficients, while x's will constitute as the variables. The tested canonical linear mixture model resembles a standard linear regression, however, without the intercept, $pBMP = \sum_{i=C,H,L,R} \beta_i x_i$ (where index *i* refer to the biomass constituents Cellulose, Hemicellulose, Lignin and Residuals). While the canonical quadratic mixture model also included interaction terms describing interactions between the variables, $pBMP = \sum_i \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j$ (where indices *i* and *j* refer to the biomass constituents).

Even though interactions between the variables are known to have an effect on recalcitrance, e.g. the bonds between hemicellulose and lignin, we were not able find a significant effect of any of the tested interaction in the canonical quadratic mixture model, and this model was rejected (Thomsen et al., 2014c, Paper V).

Cowpea

On the other hand, all components of the lignocellulosic biomass, x_C, x_H, x_L, and x_R, were statistically highly significant in the full canonical linear mixture model ($BMP \sim x_C \beta_C + x_H \beta_H + x_L \beta_L + x_R \beta_R + \varepsilon$). This resulted in the following prediction of BMP: $pBMP = 378\ x_C + 354\ x_H - 194\ x_L + 313\ x_R$ (Thomsen et al., 2014c, Paper V). In addition, the model ensured normal distribution of the data and homoscedasticity, as well as satisfactory regression diagnostics (Thomsen et al., 2014c, Paper V).

Since the regression coefficients (β_i) of cellulose, hemicellulose and residuals in the full linear model were in the same order of magnitude, it was tested if the full model could be reduced. We found, that we could reduce model in two steps without losing validity of the model ($BMP \sim x_{C+H+R}\ \beta_{C+H+R} + x_L\ \beta_L + \varepsilon$). Furthermore, we were able to prove a difference in the outcome of the two most common methods for determining biomass composition, the forage method (Van Soest et al., 1991), or the fibre method (Sluiter et al., 2010).When including this effect in the model, described with a dummy variable (D_A), the model was improved (Thomsen et al., 2014c, Paper V). Thus, the best prediction of BMP was found to be $pBMP = 347x_{C+H+R} - 438x_L + 63D_A$, $D_A \begin{cases} 0, & \text{Forage analysis method} \\ 1, & \text{Fibre analysis method} \end{cases}$.

The presented results are interesting for several reasons. Firstly, the model is in line with AD theory since the regression coefficients for carbohydrates are positive while the regression coefficient for lignin is negative. Secondly, the concept of mixture models is introduced to area of predicting BMP, which we believe will be used in future studies since it describes the relative nature of biomass constituent concentrations satisfactory. Further, the derived model are applicable to predict BMP of lignocellulosic biomasses based on compositional data in general, due to a large and diverse dataset. Lastly, we are now able to make estimations of BMP of those Ghanaian agricultural residues that were not tested in real AD determinations, which are more precise.

3.3. Anaerobic digestion in Ghana

Anaerobic digestion (AD) is not new to Ghana. In the range of 250 plants have been built within the past 3 decades and at least 5 companies are presently dedicated to building and maintaining biomethane plants in Ghana (Bensah et al., 2011; Mohammed et al., 2013). The majority of the digesters are of the fixed-dome type, with combined volumes of both reactor and gasholder of 10 to 100 m^3 (Bensah et al., 2011). The only industrial plant consist of an 800 m^3 upflow anaerobic sludge blanket (USAB) reactor running on brewery waste. Yet, the biomethane sector in Ghana cannot be regarded mature. Firstly, only approximately half of the existing plants are functioning, and secondly some developing countries of comparable population have considerably larger sectors, e.g. Tanzania and Nepal who have more than 6,000 and 260,000 plant installed respectively (Surendra et al., 2013). The limited distribution of the technology is in spite of numerous studies, which have found that use of biogas reduces the wood consumption and workload of families in the developing world significantly, while potentially providing energy independency and economic development opportunities in remote and marginalised areas (Mangoyana and Smith, 2011; Gurung and Oh, 2013). The possibility to bring back nutrients and lignin as recalcitrant carbon, as part of the digested slurry, is yet another opportunity that can potentially reduce the need for fossil derived fertilisers or even create a local market for bio-fertiliser. The appliance of digested material to Ghanaian soils is currently investigated by a fellow PhD student in the 2GBIONRG project (Adjei-Gyapong, 2014, unpublished).

In addition, Ghanaian women generally cook on simple, inefficient stoves or open fires in poorly ventilated kitchens, where inefficient burning of poor fuels leads to very high concentrations of indoor air pollutants with associated health impacts, which can be mitigated by the use of biomethane (Pennise et al., 2009; Gurung and Oh, 2013). The reasons for the limited distribution of the technology despite the advantages, lies within high costs of biogas digesters, poor image of biogas as a modern energy source, and socio-cultural hindrances on the use of 'faecal gas' for cooking and 'faecal fertilizer' in agriculture (Atakora, 2000; Bensah et al., 2011). Furthermore, low commitment from government, lack of political intensives, pour support system and difficult financing options, are mentioned as restrictions (Bensah and Brew-Hammond, 2010). Moreover, there is a lack of evidence regarding the many claims of positive social impacts of biofuels at local levels, especially in Ghana, where better reliability and performance of AD technologies are important (Hodbod and Tomei, 2013).

Groundnut

Other obstacles for further distribution of AD technology are limited access of two main aspects; namely manure supply and water supply. Regarding manure, as mentioned in section 1.2. the manure is not evenly distributed over the country, and the major part of the manure is not collectable since the livestock are kept free-range. Therefore, it is difficult to assess in which cases there are enough manure available to run a well-functioning AD. In some cases, municipal liquid waste (night soil / faeces) could be a valid alternative, but then issues with sterility and public acceptance should be addressed. Regarding water, even though it has been found that AD in Ghana would be unlikely to suffer from water scarcity (Bensah et al., 2011), then the need for collecting considerable amounts of water for a biogas plant might be a constraint, especially in those parts of the country where water is collected manually. Furthermore, draughts and water scarcity might be increased due to global climate change. In the light of these two limiting factors, a strategy for a technology specially adapted for the Ghanaian circumstances is under development among project partners. The hypothesis being that a high-solid regime specially adapted for low-tech and small-scale, could limit the need for water and manure, while enabling utilisation of agricultural residues to a higher degree (Heiske, 2013, unpublished). However, research need to address difficulties in deviating gas production, process- and reactor design applicable for small-scale low-tech solutions. Furthermore, only processing residues are likely to be used, since the extra labour needed for collecting the field-based residues has been found to exceed the current time spend on collecting fuel-wood (Kamp and Østergård, 2014). However, this might change if fuel-wood is further depleted in the future.

Pretreatment of biomass for AD, additional to size reduction, can be necessary to achieve higher methane yields and related research have been initiated in recent years (Zhong et al., 2011; Barta et al., 2013; Jurado et al., 2013). Introducing pretreatment for biogas in the developing world might be a welcoming opportunity for enhancing the methane yield in new as well as existing plants. However, related costs will have to be kept at an absolute minimum, as the produced methane is a low-value product that has to be used locally. In the current project, pretreatment for AD have been considered, and it could especially be interesting for the lignified materials. Therefore, appliance of the biological methods of ensiling and WRT will be tested in a future setup, for ethanol as well as biomethane. In addition, a mobile low-tech AD demonstration unit, for testing and demonstrating AD technologies on various feedstock on-site in Ghana, was developed as part of the project Fig. 6. It was tested on grass silage at low TS, however further work are needed prior to on-site implementation (Thomsen and Heiske, 2012, unpublished and not part of any current manuscripts). Furthermore, similar demo-scale versions of high-solid AD reactors should be developed for demonstrating the technology on-site in Ghana.

Figure 6: Mobile low-tech AD demonstration unit and feeding tube, designed as part of the study (Thomsen and Heiske, 2012, unpublished and not part of any current manuscripts).

There is a quite large potential for increased AD in Ghana, according to several authors, more than 200,000 potential small-scale plants are reported (Bensah and Brew-Hammond, 2010; Arthur et al., 2011; Kemausuor et al., 2014, Paper I). One study is also assessing the marked potential and estimated a potential of 16,000 plants (Bensah and

Brew-Hammond, 2010). The huge effort it would take to construct thousands of plants are not discussed but it represent a huge effort. However, most studies agree that the biggest hindrance to a large role-out of AD technology in Ghana is lack of political initiatives, while high constructing costs also are mentioned. (Atakora, 2000; Bensah and Brew-Hammond, 2010; Antwi et al., 2010; Arthur et al., 2011; Bensah et al., 2011). As a lesson from other developing initiatives, the importance of a strong initiating framework is important, where allocation of responsibilities, benefits and ownership seem crucial for long-term success of technology. Additionally, a well-functioning system of service providers has had catalytic in other developing countries such as Nepal (Surendra et al., 2013).

3.4. Ghanaian biomethane potentials revisited

As described with the ethanol potentials in Section 2.4. it is possible to revisit the biomethane potentials presented in Paper I (Kemausuor et al., 2014), now based on the information presented in in section 1. and 3. Thus, assumptions deviating from the ones presented in Paper I will be described here.

First of all, the BMP used for the agricultural residues in Paper I will be substituted. When available with real BMP data presented in Fig. 5 (page 30), and for the remaining lignocellulosic residues, pBMP values have been determined by using the prediction model derived in Paper V (Thomsen et al., 2014c) and presented in Section 3.2. (page 30). The compositional data used as variables are enclosed as appendix of Paper I (Kemausuor et al., 2014). In addition, a factor describing low-tech performance (biomethane yield compared to BMP) is now assumed 50%, which might be more realistic than the 80% assumed in Paper I.

Furthermore, based on the discussion presented in Section 1., the assumption is made that only processing residues are available for biomethane production from agricultural residues. Finally, only the processing residues with BMP or pBMP higher than 200 ml CH_4/g VS is considered, since to low-yielding biomasses will assumable not be rentable. BMP's are presented at Fig. 5 (page 30), while processing residues with pBMP > 200 ml CH_4/g VS are maize husks, rice husks and sugarcane bagasse, with pBMP equal to 297, 215 and 265 ml CH_4/g VS respectively.

Under the given assumptions, the potential yield of biomethane based on agricultural processing residues is 390 Mm^3 CH_4/yr, which is only half the potential found in Paper I (Table 4, Page 38). The potentials found in Paper I for the collectable part of manure and municipal liquid waste were 120 Mm^3 CH_4/yr, but since these residues from an AD technology perspective are the simplest to digest, this potential should also be considered.

When revising the biomethane potentials and aggregating the agricultural processing residues, manure and municipal liquid waste, an estimation of 500 Mm^3 CH_4/yr is found realistic in contemporary Ghana. However, this will still demand large investments and dedication from various stakeholders, keeping in mind that this will demand a five-digit number of small-scale plants.

Table 4: Up-dated potential for biomethane production in Ghana. The calculations were made as described in Paper I (Kemausuor et al., 2014), with the exceptions presented above.

Residue category	Mm^3 CH_4/yr	PJ/yr
Total (Kemausuor et al., 2014, Paper I)	2,700	97
Total agricultural processing residues (Kemausuor et al., 2014, Paper I)	750	27
Agricultural processing residues with BMP > 200 (according to presented assumptions)	390	14
Agricultural processing residues, manure and municipal liquid waste (according to presented assumptions)	500	18

In a situation where agricultural processing residues, manure and municipal liquid waste are utilised 18 PJ/yr of heat energy will be available, which should be compared with the approximate current fuel-wood consumption of 360 PJ/yr. However, an uncritical comparison would be unfair, since use of a biogas cooking stove instead of fuel-wood fired stoves, increases the thermal efficiency from 10-20% to 40-65% (Bhattacharya and Abdul Salam, 2002; Gurung and Oh, 2013). Thus, biogas can replace approximately 20% (10-33%) of current consumption of heat energy in households. In addition, this potential would hypothetically be fulfilled in rural regions where energy scarcity is most profound. If only manure is utilised only 4% of current consumption of heat energy in households could be replaced, therefore is will significant to establish low-tech solutions for AD under manure-scarcity for the relatively larger amounts of agricultural residues.

4. Conclusion

The Ghanaian agricultural sector is still shaped by subsistence farming, while recent intensification has enhanced the vulnerability of the system due to decreased fallow periods, increased use of pesticides and inorganic fertiliser, increased deforestation and consequent soil erosion. Therefore, potential bioenergy solutions should pay attention not to disrupt fragile equilibria. Furthermore, rural transportation is inefficient, which is affecting future bioenergy systems rendering large-scale production plants unfeasible. Moreover, the utilisation of residues is made difficult by the spatial distribution of biomass and the lack of tradition in collecting the residues. Furthermore, the costs of bioenergy are currently outbalancing the advantages and stakeholders are eager for political incentives and for creation of a well-functioning support system. On the other hand, strong incentives exist for bioenergy systems, since they may bring energy self-sufficiency for farmers and communities, cleaner fuels in households, possibilities for closing the nutrient-cycle, and sound waste management. These constraints and opportunities for bioenergy in Ghana are defining a need for resilient, small-scale bioenergy solutions designed specifically for the Ghanaian context.

An assessment of the most abundant residues found that residues from yam, maize, plantain, cassava, oil palm and cocoa are produced in large amounts. However, the current utilisation is not clear and furthermore, current data on available crop residues should be used with caution when predicting a future situation, due to expectable changes in infrastructure, farming practices and the self-inflicting changes of a growing bioenergy sector.

Thirteen of the most common agricultural residues in Ghana were compositionally characterised. This enabled estimations of theoretic bioenergy potentials, but the possibility to pinpoint specific residues for future biorefinery applications might be more significant on longer terms. Pretreatment methods aimed at low-tech production of cellulosic ethanol for West African biomasses were tested. BP, SAA and WRF were generally not as efficient as high-tech HTT based on enzymatic convertibility and fermentation. However, when evaluated on the overall ethanol yield the low-tech pretreatment methods were viable alternatives to HTT (13.4-15.2 g produced ethanol per 100 g TS raw material).

Extended work was done in the field of biological pretreatment methods of silage pretreatment and WRF, since these are believed to be applicable for the Ghanaian context. Silage pretreatment was tested on wheat straw, but was not adequate as stand-alone pretreatment, but combining ensiling of dry lignified biomass with HTT proved that the optimal pretreatment temperature of HTT could be decreased significantly, thereby reducing the energy input to hydrothermal pretreatment. The high temperature, is seen as the main drawback of using HTT both in industrialised countries as well as in the developing world.

It is advisable to initiate Ghanaian bioenergy with the low-hanging fruits, i.e. processing residues. In the case of ethanol, the starchy residues concentrated at processing facilities should be utilised first (peelings of yam, cassava and plantain). According to presented estimations, ethanol of starchy residues may substitute 8% of the transport fuel required in Ghana by 2020.

Anaerobic digestion is arguably more flexible than ethanol production with regard to both feedstock and scale of production, while the associated costs are significantly diminished. Therefore, it is recommendable to pursue increased implementation of AD in Ghana, as the first bioenergy option. If possible, the available manure and municipal liquid waste should be utilised first, since these are proven substrates for low-tech systems. However, most manure is difficult to collect and a shift in farming practices might be necessary to enable collection. Beside manure, the most recommendable agricultural feedstock for AD are processing residues with high BMP such as the starchy peelings, cocoa husks and maize cobs. Biomethane based on agricultural processing residues, manure and municipal liquid waste can replace approximately 20% of current utilisation of heat energy in households. However, it should be noted that the residues


Sorghum

can be used only once, and the estimated potentials of ethanol and biomethane are based partly on the same feedstock.

During the study, is was found that existing methods of predicting BMP from compositional data of lignocellulosic biomasses were either not taking biomass recalcitrance into account or they were not in accordance to AD theory. Therefore, a novel method of estimating biomethane potentials (BMP) from compositional data of lignocellulosic biomasses was derived. This method was based on a statistical model not previously used in this area of research. The best prediction of BMP was found to be: $pBMP = 347x_{C+H+R} - 438x_L + 63D_A$. Where x_{C+H+R} is the combined content of cellulose, hemicellulose and residuals, x_L is the content of lignin while D_A is describing the applied analysis method, $D_A \begin{cases} 0, & \text{Forage analysis method} \\ 1, & \text{Fibre analysis method} \end{cases}$. This model was applied when estimating BMP of those Ghanaian biomasses that where not assessed prior in the studies.

4.1. Future perspectives

The future breakthroughs within bioenergy in Ghana will lie in the interphase between science and implementation. There is a need of specially adapted scientific solutions, but also for feedback from on-site test of technologies.

Moreover, a thorough analysis of current use of residues in Ghana is needed, both for estimating correct potentials and to enable sustainable utilisation of residues. In that regard, locations where residues are concentrated should be identified in order to initiate conversion technologies on the most promising feedstock. In addition, a mapping of the spatial distribution of water- and manure shortage together with the biomass availability could clarify in which areas of Ghana an introduction of an AD or ethanol regime would have the largest chances of success.

Biomass types interesting for future work are municipal solid waste and natural vegetation collected from fallow lands. Mixed feedstock of e.g. elephant grass and siam grass could be used for bioenergy purposes and thereby give renewed value to fallow periods in agricultural systems.

Biological pretreatment methods such as white rot fungi and silage deserves sustained scientific attention, since these methods might hold the key to lowering energy use in pretreatment. Likewise, up-scaling of these methods under Ghanaian conditions should be investigated. However, the biggest impact of bioenergy in Ghana will most likely be in the field of AD. Especially design of low-tech processes applicable for low manure- and water accessibility could have big impacts. However, research needs to address difficulties in fluctuating gas production, and novel process- and reactor design. In addition, assessing low-tech pretreatment methods such as WRF for AD can hold potential. Likewise, low-energy distillation methods could alter the energy balance of residue-based ethanol.

I believe that upcoming work on estimating BMP from biomass composition will be based on mixture models as proposed in this thesis. Our model can also be improved by eliminating errors in a large homogeneous dataset and estimate new regression coefficients. Moreover, the method can be extended to other types of biomass such as silage, municipal solid waste or manure.

Furthermore, future ground-breaking advances in pretreatment, enzyme technology, C5 fermentation, consolidation bioprocessing, etc. might change the perspectives on what is realistic under Ghanaian conditions.

References

Abdullah, N., Sulaiman, F., Gerhauser, H., 2011. *Characterisation of oil palm empty fruit bunches for fuel application,* Journal of Physical Science. 22(1), 1-24.

Afrane, G., 2012. *Examining the potential for liquid biofuels production and usage in Ghana,* Energy Policy. 40(1), 444-51.

Ahring, B.K., Jensen, K., Nielsen, P., Bjerre, A.B., Schmidt, A.S., 1996. *Pretreatment of wheat straw and conversion of xylose and xylan to ethanol by thermophilic anaerobic bacteria,* Bioresource Technology. 58(2), 107-13.

Ahring, B., 2003. *Perspectives for Anaerobic Digestion,* Advantages in Biochemical Engineering. 81, 1-30.

Albersheim, P., Darvillis, A., Robertsis, K., Sederoffis, R., Staehelinis, A., 2011. *Plant Cell Walls: From Chemistry to Biology*. Taylor & Francis Group.

Ambye-Jensen, M., Thomsen, S.T., Kádár, Z., Meyer, A.S., 2013 (Paper III). *Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment,* Biotechnology for Biofuels. 6(1).

Amigun, B., Sigamoney, R., von Blottnitz, H., 2008. *Commercialisation of biofuel industry in Africa: A review,* Renewable and Sustainable Energy Reviews. 12(3), 690-711.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. *Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays,* Water Science and Technology. 59(5), 927-34.

Antwi, E., Bensah, E.C., Quansah, D.A., Arthur, R., Ahiekpor, J., 2010. *Ghana's biofuels policy: challenges and the way forward,* International Journal of Energy and Environment. 1(5), 805-14.

Archives Larousse, *Archives Larousse,* (Accessed 2013), http://www.larousse.fr/encyclopedie/.

Aregheore, E.M., 2002. *Chemical Evaluation and Digestibility of Cocoa (Theobroma cacao) Byproducts Fed to Goats,* Tropical animal health and production. 34(4), 339-48.

Arthur, R., Baidoo, M.F., Antwi, E., 2011. *Biogas as a potential renewable energy source: A Ghanaian case study,* Renewable Energy. 36(5), 1510-6.

Atakora, S. 2000. *Biomass technologies in Ghana,* The ninth biennial bioenergy conference.

Barta, Z., Kreuger, E., Björnsson, L., 2013. *Effects of steam pretreatment and co-production with ethanol on the energy efficiency and process economics of combined biogas, heat and electricity production from industrial hemp,* Biotechnology for Biofuels. 6(1).

Bensah, E.C., Brew-Hammond, A., 2010. *Biogas technology dissemination in Ghana: history, current status, future prospects, and policy significance,* International Journal of Energy and Environment. 1(2), 277-94.

Bensah, E.C., Mensah, M., 2013. *Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations,* Int J Chem Eng. 2013, 21.

Bensah, E.C., Mensah, M., Antwi, E., 2011. *Status and prospects for household biogas plants in Ghana – lessons, barriers, potential, and way forward,* International Journal of Energy and Environment. 2(5), 887-98.

Bernstein, L., Bosch, P., Canziani, O., Chen, Z., Christ, R., Davidson, O., et al., 2007. *Climate Change 2007: Synthesis Report - An Assessment of the Intergovernmental Panel on Climate Change (IPCC),* IPCC.

Biswas, R., Uellendahl, H., Ahring, B.K., 2013. *Conversion of C_6 and C_5 sugars in undetoxified wet exploded bagasse hydrolysates using scheffersomyces (pichia) stipitis cbs6054,* AMB Express. 3(1), 1-7.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploger, A., Schmidt, A.S., 1996. *Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose,* Biotechnology and bioengineering. 49(5), 568-77.

Bolwig, S., Hauggaard-Nielsen, H., 2011. *Travel Report - 2GBIONRG Project,* Unpublished material*.*

Bond, T., Templeton, M.R., 2011. *History and future of domestic biogas plants in the developing world,* Energy for Sustainable Development. 15(4), 347-54.

Brandt, A., Gräsvik, J., Hallett, J.P., Welton, T., 2013. *Deconstruction of lignocellulosic biomass with ionic liquids,* Green Chemistry. 15(3), 550-83.

Brew-Hammond, A., Kemausuor, F., 2009. *Energy for all in Africa - to be or not to be?!* Current Opinion in Environmental Sustainability. 1(1), 83-8.

Carvalheiro, Duarte, Girio, 2008. *Hemicellulose biorefineries: a review on biomass pretreatments,* Journal of Scientific and Industrial Research. 67(11), 849-64.

Chanakya, H.N., Ganguli, N.K., Anand, V., Jagadish, K.S., 1995. *Performance characteristics of a solid-phase biogas fermentor,* Energy for Sustainable Development. 1(6), 43-6.

Chang, K., Thitikorn-amorn, J., Hsieh, J., Ou, B., Chen, S., Ratanakhanokchai, K., Huang, P., Chen, S., 2011. *Enhanced enzymatic conversion with freeze pretreatment of rice straw,* Biomass & Bioenergy. 35(1), 90-5.

Chen, Y., Sharma-Shivappa, R.R., Keshwani, D., Chen, C., 2007. *Potential of agricultural residues and hay for bioethanol production,* Applied Biochemistry and Biotechnology. 142(3), 276-90.

Codjoe, S.N.A., 2006. *Population growth and agricultural land use in two agro-ecological zones of Ghana, 1960-2010,* International Journal of Environmental Studies. 63(5), 645-61.

Cornell, J.A., 2011. *A retrospective view of mixture experiments,* Quality Engineering. 23(4), 315-31.

Deublein, D., Steinhauser, A., 2008. *Biology,* Anonymous Biogas from waste and renewable resources. Wiley-VCH, pp. 121.

Di Risio, S., Hu, C.S., Saville, B.A., Liao, D., Lortie, J., 2011. *Large-scale, high-solids enzymatic hydrolysis of steam-exploded poplar,* Biofuels, Bioproducts and Biorefining. 5(6), 609-20.

Donkoh, A., Atuahene, C.C., Wilson, B.N., Adomako, D., 1991. *Chemical composition of cocoa pod husk and its effect on growth and food efficiency in broiler chicks,* Animal Feed Science and Technology. 35(1-2), 161-70.

Duku, M.H., Gu, S., Hagan, E.B., 2011. *A comprehensive review of biomass resources and biofuels potential in Ghana,* Renewable and Sustainable Energy Reviews. 15(1), 404-15.

Edjekumhene, I., Agyemang, P.O., Edze, P., 2010. *Ghana's emerging oil industry: What Stakeholders need to know,* Kumasi Institute of Technology, Energy and Environment. Kumasi Institute of Technology, Energy and Environment.

Ejigu, M., 2008. *Toward energy and livelihoods security in Africa: Smallholder production and processing of bioenergy as a strategy,* Natural Resources Forum. 32(2), 152-62.

Engelbrechtsen, M., 2012. *Evaluation of Bio-derived catalysts for pretreatment of lignocellulosic biomass,* MSc, Technical University of Denmark, Department of Chemical and Biochemical Engineering, Roskilde, Denmark, Supervised by Thomsen, S.T. and Schmidt, J.E.

FAOSTAT, *The Statistics Division of the Food and Agriculture Organization of the United Nations,* (Accessed 2013), http://faostat3.fao.org/faostat-gateway/go/to/home/E.

Fening, J.O., Adjei Gyapong, T., Ababio, F., Gaisie, E., 2005. *Intercropping maize with planted fallows on smallholder farms in Ghana,* Tropical Science. 45(4), 155-8.

Gerber, M., Schneider, N., Kowalczyk, A., Schwede, S., Rehman, Z., Span, R. 2013. *The influence of pre-incubation, storage and homogenization of inoculum for batch tests on biogas production,* Proceedings of 13th World Congress on Anaerobic Digestion.

Gurung, A., Oh, S.E., 2013. *Conversion of traditional biomass into modern bioenergy systems: A review in context to improve the energy situation in Nepal,* Renewable Energy. 50, 206-13.

Hahn-Hägerdal, B., Lindén, T., Senac, T., Skoog, K., 1991. *Ethanolic fermentation of pentoses in lignocellulose hydrolysates,* Applied Biochemistry and Biotechnology. 28-29(1), 131-44.

Hall, C.A.S., Dale, B.E., Pimentel, D., 2011. *Seeking to understand the reasons for different energy return on investment (EROI) estimates for biofuels,* Sustainability. 3(12), 2413-32.

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J.l.C., Mosbæk, H., Christensen, T.H., 2004. *Method for determination of methane potentials of solid organic waste,* Waste Management. 24(4), 393-400.

Hattori, S., 2008. *Syntrophic acetate-oxidizing microbes in methanogenic environments,* Microbes and Environments. 23(2), 118-27.

Hellmann, F., Verburg, P.H., 2011. *Spatially explicit modelling of biofuel crops in Europe,* Biomass & Bioenergy. 35(6), 2411-24.

Hirsch, R.L., Bezdec, R., Wendling, R., 2005. *Peaking of world oil production: impacts, mitigation, & risk management,* Science Application International Corporation, at the United States Department of Energy, National Energy Technology Laboratory.

Hodbod, J., Tomei, J., 2013. *Demystifying the social impacts of biofuels at local levels: Where is the evidence?* Geography Compass. 7(7), 478-88.

Hu, F., Ragauskas, A., 2012. *Pretreatment and Lignocellulosic Chemistry,* BioEnergy Research. 5(4), 1043-66.

Idi, A., Salleh, M.M., Ibrahim, Z., Mohamad, S.E., 2012. *Pretreatment of cocoa waste for bioethanol production using ionic liquid,* Jurnal Teknologi (Sciences and Engineering). 59(SUPPL.1), 49-56.

IITA, *International Institute of Tropical Agriculture,* (Accessed 2012), http://old.iita.org/cms/details/who_details.aspx?articleid=75&zoneid=48.

Ingram, T., Rogalinski, T., Bockemühl, V., Antranikian, G., Brunner, G., 2009. *Semi-continuous liquid hot water pretreatment of rye straw,* The Journal of Supercritical Fluids. 48(3), 238-46.

Isroi, R.M., Niklasson, C., Cahyanto, M.N., Syamsiah, S., Lundquist, K., Taherzadeh, M.J., 2011. *Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review,* BioResources. 6(4), 5224-59.

Jacoby, H.G., Minten, B., 2009. *On measuring the benefits of lower transport costs,* Journal of Development Economics. 89(1), 28-38.

Jedwab, R., Moradi, A., 2011. *Transportation Infrastructure and Development in Ghana,* Paris Scool of Economics Working Paper N° 2011 – 24.

Jha, A.K., Li, J., Nies, L., Zhang, L., 2011. *Research advances in dry anaerobic digestion process of solid organic wastes,* African Journal of Biotechnology. 10(65), 14242-53.

Jørgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C., 2007. *Liquefaction of lignocellulose at high-solids concentrations,* Biotechnology and bioengineering. 96(5), 862-70.

Jurado, E., Gavala, H.N., Skiadas, I.V., 2013. *Enhancement of methane yield from wheat straw, miscanthus and willow using aqueous ammonia soaking,* Environmental Technology (United Kingdom). 34(13-14), 2069-75.

Kamp, A., Østergård, H. 2014. *Food and biogas production in a Ghanaian village - Results and modelling issues, 8th Biennial Emergy Research Conference, 2014.* 8th Biennial Emergy Research Conference.

Kemausuor, F., Akowuah, J.O., Ofori, E., 2013. *Assessment of Feedstock Options for Biofuels Production in Ghana,* Journal of Sustainable Bioenergy Systems. 3(2), 119-28.

Kemausuor, F., Kamp, A., Thomsen, S.T., Bensah, E.C., Østergård, H., 2014 (Paper I). *Assessment of biomass residue availability and sustainable bioenergy yields in Ghana,* Submitted Resources, Conservation & Recycling.

Kemausuor, F., Obeng, G.Y., Brew-Hammond, A., Duker, A., 2011. *A review of trends, policies and plans for increasing energy access in Ghana,* Renewable and Sustainable Energy Reviews. 15(9), 5143-54.

Kim, S., Dale, B.E., 2004. *Global potential bioethanol production from wasted crops and crop residues,* Biomass and Bioenergy. 26(4), 361-75.

Kim, T.H., Lee, Y.Y., 2005. *Pretreatment of corn stover by soaking in aqueous ammonia,* Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology. 124(1-3), 1119-31.

Klinke, H.B., Ahring, B.K., Schmidt, A.S., Thomsen, A.B., 2002. *Characterization of degradation products from alkaline wet oxidation of wheat straw,* Bioresource Technology. 82(1), 15-26.

Krishnan, C., Sousa, L.d.C., Jin, M., Chang, L., Dale, B.E., Balan, V., 2010. *Alkali-Based AFEX Pretreatment for the Conversion of Sugarcane Bagasse and Cane Leaf residues to Ethanol,* Biotechnology and bioengineering. 107(3), 441-50.

Kurian, J.K., Nair, G.R., Hussain, A., Vijaya, G.S.R., 2013. *Feedstocks, logistics and pre-treatment processes for sustainable lignocellulosic biorefineries: A comprehensive review,* Renewable and Sustainable Energy Reviews. 25, 205-19.

Larbi, A., Dung, D.D., Olorunju, P.E., Smith, J.W., Tanko, R.J., Muhammad, I.R., Adekunle, I.O., 1999. *Groundnut (Arachis hypogaea) for food and fodder in crop-livestock systems: forage and seed yields, chemical composition and rumen degradation of leaf and stem fractions of 38 cultivars,* Animal Feed Science and Technology. 77(1-2), 33-47.

Larsen, J., Petersen, M.Ø, Thirup, L., Li, H.W., Iversen, F.K., 2008. *The IBUS Process - Lignocellulosic Bioethanol Close to a Commercial Reality,* Chemical Engineering & Technology. 31(5), 765-72.

Larsen, J., Haven, M.Ø, Thirup, L., 2012. *Inbicon makes lignocellulosic ethanol a commercial reality,* Biomass and Bioenergy. 46, 36-45.

Larsen, J., Petersen, M.O., 2012. *Methods of processing ensiled biomass,* Official Gazette of the United States Patent and Trademark Office Patents.

Laurichesse, S., Avérous, L., 2013. *Chemical modification of lignins: Towards biobased polymers,* Progress in Polymer Science.

Leu, S.-., Zhu, J.Y., 2013. *Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding,* Bioenergy Research. 6(2), 405-15.

Liu, Z.S., Wu, X.L., Kida, K., Tang, Y.Q., 2012. *Corn stover saccharification with concentrated sulfuric acid: Effects of saccharification conditions on sugar recovery and by-product generation,* Bioresource technology. 119, 224-33.

López, R., 1997. *Environmental externalities in traditional agriculture and the impact of trade liberalization: The case of Ghana,* Journal of Development Economics. 53(1), 17-39.

Maas, R.H.W., Bakker, R.R., de Jong, E., Weusthuis, R.A., Boersma, A.R., Pels, J.R., Reith, H., Bisschops, I., Maas, R.H.W., de Jong, E., 2008. *Pilot-scale conversion of lime-treated wheat straw* *into bioethanol: quality assessment of bioethanol and valorization of side streams by anaerobic digestion and combustion,* Biotechnology for Biofuels. 1, 1-13.

Mabee, W.E., Mirck, J., 2011. *A Regional Evaluation of Potential Bioenergy Production Pathways in Eastern Ontario, Canada,* Annals of the Association of American Geographers. 101(4), 897-906.

Mahmood, A., Honermeier, B., 2012. *Chemical composition and methane yield of sorghum cultivars with contrasting row spacing,* Field Crops Research. 128, 27-33.

Mangoyana, R.B., Smith, T.F., 2011. *Decentralised bioenergy systems: A review of opportunities and threats,* Energy Policy. 39(3), 1286-95.

Mangoyana, R.B., 2009. *Bioenergy for sustainable development: An African context,* Physics and Chemistry of the Earth. 34(1-2), 59-64.

Markussen, M.V., 2013. *Sustainability assessment of food and bioenergy systems in a societal context – In a time of crisis,* PhD, Technical University of Denmark, Department of Chemical and Biochemical Engineering, Roskilde, Denmark.

Martin, C., Lopez, Y., Plasencia, Y., Hernandez, E., 2006. *Characterisation of agricultural and agro-industrial residues as raw materials for ethanol production,* Chemical and Biochemical Engineering Quarterly. 20(4), 443-7.

Martin, C., Klinke, H.B., Thomsen, A.B. 2007. *Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse,* Enzyme and Microbial Technology; International Conference on Enzyme Technology, 426-32.

Martin, C., Marcet, M., Thomsen, A.B., 2008. *Comparison between Wet Oxidation and Steam Explosion as Pretreatment Methods for Enzymatic Hydrolysis of Sugarcane Bagasse,* Bioresources. 3(3), 670-83.

Mihyeon Jeon, C., Amekudzi, A.A., Vanegas, J., 2006. *Transportation system sustainability issues in high-, middle-, and low-income economies: Case studies from Georgia (U.S.), South Korea, Colombia, and Ghana,* Journal of Urban Planning and Development. 132(3), 172-86.

Mohammed, Y.S., Mokhtar, A.S., Bashir, N., Saidur, R., 2013. *An overview of agricultural biomass for decentralized rural energy in Ghana,* Renewable and Sustainable Energy Reviews. 20, 15-22.

Monlau, F., Sambusiti, C., Barakat, A., Guo, X.M., Latrille, E., Trably, E., Steyer, J., Carrere, H., 2012. *Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials,* Environmental Science and Technology. 46(21), 12217-25.

Nallathambi Gunaseelan, V., 1997. *Anaerobic digestion of biomass for methane production: A review,* Biomass and Bioenergy. 13(1-2), 83-114.

Nielsen, H.B., Angelidaki, I., 2008. *Strategies for optimizing recovery of the biogas process following ammonia inhibition,* Bioresource technology. 99(17), 7995-8001.

Nielsen, H.B., Mladenovska, Z., Westermann, P., Ahring, B.K., 2004. *Comparison of two-stage thermophilic (68 degrees C/55 degrees C) anaerobic digestion with one-stage thermophilic (55 degrees C) digestion of cattle manure,* Biotechnology and bioengineering. 86(3), 291-300.

Odum, H.T., Odum, E.C., 2001. *A Prosperous Way Down: Principles and Policies*. University Press of Colorado.

Olcese, R.N., Lardier, G., Bettahar, M., Ghanbaja, J., Fontana, S., Carré, V., Aubriet, F., Petitjean, D., Dufour, A., 2013. *Aromatic chemicals by iron-catalyzed hydrotreatment of lignin pyrolysis vapor,* ChemSusChem. 6(8), 1490-9.

Onwuka, C.F.I., Adetiloye, P.O., Afolami, C.A., 1997. *Use of household wastes and crop residues in small ruminant feeding in Nigeria,* Small Ruminant Research. 24(3), 233-7.

Osagie, S., 2008. *African promise,* Engineering and Technology. 3(7), 68.

Osei, G., Arthur, R., Afrane, G., Agyemang, E.O., 2013. *Potential feedstocks for bioethanol production as a substitute for gasoline in Ghana,* Renewable Energy. 55, 12-7.

Owusu, V., Fosu, K.Y., Burger, K., 2012. *Intersectoral labor mobility and deforestation in Ghana,* Environment and Development Economics. 17(6), 741-62.

Pablo Vargas-B., J., Pérez-García, S.L., García-Cuéllar, A.J., Rivera-Solorio, C.I., 2013. *Thermal analysis of a solar distillation system for ethanol-water solutions,* Journal of Renewable and Sustainable Energy. 5(4).

Pakarinen, A., Maijala, P., Stoddard, F.L., Santanen, A., Tuomainen, P., Kymalainen, M., Viikari, L., 2011. *Evaluation of annual bioenergy crops in the boreal zone for biogas and ethanol production,* Biomass & Bioenergy. 35(7), 3071-8.

Pan, X., Xie, D., Yu, R.W., Saddler, J.N., 2008. *The bioconversion of mountain pine beetle-killed lodgepole pine to fuel ethanol using the organosolv process,* Biotechnology and bioengineering. 101(1), 39-48.

Parawira, W., Read, J.S., Mattiasson, B., Björnsson, L., 2008. *Energy production from agricultural residues: High methane yields in pilot-scale two-stage anaerobic digestion,* Biomass and Bioenergy. 32(1), 44-50.

Parker, B.Q., Osei, B.A., Armah, F.A., Yawson, D.O., 2010. *Impact of biomass burning on soil organic carbon and the release of carbon dioxide into the atmosphere in the coastal savanna ecosystem of Ghana,* Journal of Renewable and Sustainable Energy. 2(3).

Patterson, T., Dinsdale, R., Esteves, S., 2008. *Review of energy balances and emissions associated with biomass-based transport fuels relevant to the United Kingdom context,* Energy & Fuels. 22(5), 3506-12.

Pennise, D., Brant, S., Agbeve, S.M., Quaye, W., Mengesha, F., Tadele, W., Wofchuck, T., 2009. *Indoor air quality impacts of an improved wood stove in Ghana and an ethanol stove in Ethiopia,* Energy for Sustainable Development. 13(2), 71-6.

Petersson, A., Thomsen, M.H., Hauggaard-Nielsen, H., Thomsen, A.B., 2007. *Potential bioetanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean,* Biomass & Bioenergy. 31(11-12), 812-9.

Pimentel, D., Patzek, T., 2008. *Ethanol production using corn, switchgrass and wood; Biodiesel production using soybean,* Biofuels, Solar and Wind as Renewable Energy Systems: Benefits and Risks., 373-94.

Quaye, A.K., Hall, C.A.S., Luzadis, V.A., 2010. *Agricultural land use efficiency and food crop production in Ghana,* Environment, Development and Sustainability. 12(6), 967-83.

Quintero, J.A., Cardona, C.A., Felix, E., Moncada, J., Sánchez, ÓJ., Gutiérrez, L.F., 2012. *Techno-economic analysis of bioethanol production in Africa: Tanzania case,* Energy. 48(1), 442-54.

Reddy, N., Yang, Y., 2009. *Natural cellulose fibers from soybean straw,* Bioresource technology. 100(14), 3593-8.

Renkow, M., Hallstrom, D.G., Karanja, D.D., 2004. *Rural infrastructure, transactions costs and market participation in Kenya,* Journal of Development Economics. 73(1), 349-67.

Sarkar, N., Ghosh, S.K., Bannerjee, S., Aikat, K., 2012. *Bioethanol production from agricultural wastes: An overview,* Renewable Energy. 37(1), 19-27.

Sárossy, Z., Blomfeldt, T.O.J., Hedenqvist, M.S., Koch, C.B., Ray, S.S., Plackett, D., 2012. *Composite films of arabinoxylan and fibrous sepiolite: Morphological, mechanical, and barrier properties,* ACS Applied Materials and Interfaces. 4(7), 3378-86.

Sasse, L., 1987. *Die Biogasanlage: Entwurf und Details einfacher Anlagen*. Vieweg.

Shibata, M., Varman, M., Tono, Y., Miyafuji, H., Saka, S., 2008. *Characterization in chemical composition of the oil palm (elaeis guineensis),* Nihon Enerugi Gakkaishi/Journal of the Japan Institute of Energy. 87(5), 383-8.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. *Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods,* Journal of Agricultural and Food Chemistry. 58(16), 9043-53.

Sorensen, A., Teller, P.J., Hilstrom, T., Ahring, B.K., 2008. *Hydrolysis of Miscanthus for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic treatment,* Bioresource technology. 99(14), 6602-7.

Surendra, K.C., Takara, D., Jasinski, J., Kumar Khanal, S., 2013. *Household anaerobic digester for bioenergy production in developing countries: Opportunities and challenges,* Environmental Technology (United Kingdom). 34(13-14), 1671-89.

Symons, G.E., Buswell, A.M., 1933. *The methane fermentation of carbohydrates,* Journal of the American Chemical Society. 55(5), 2028-36.

Thomsen, S.T., Londoño, J.E.G., Schmidt, J.E., Kádár, Z., 2014a (paper IV). *Screening of pretreatments of common West African lignocellulosic biomass residues for ethanol production,* submitted to Renewable Energy.

Thomsen, M.H., Thygesen, A., Thomsen, A.B., 2008. *Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicellulose recovery, high cellulose digestibility and low lignin hydrolysis,* Bioresource Technology. 99(10), 4221-8.

Thomsen, M.H., Thygesen, A., Thomsen, A.B., 2009. *Identification and characterization of fermentation inhibitors formed during hydrothermal treatment and following SSF of wheat straw,* Applied Microbiology and Biotechnology. 83(3), 447.

Thomsen, S.T., Jensen, M., Schmidt, J.E., 2012. *Production of 2nd generation bioethanol from lucerne - Optimization of hydrothermal pretreatment,* BioResources. 7(2), 1582-93.

Thomsen, S.T., Kádár, Z., Schmidt, J.E., 2014b (paper II). *Compositional analysis and projected biofuel potentials from common West African agricultural residues,* submitted to Biomass & Bioenergy.

Thomsen, S.T., Spliid, H., Østergård, H., 2014c (paper V). *Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass,* Bioresource technology. 154(1), 80-6.

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. *A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential,* Bioresource technology. 102(20), 9395-402.

Tverberg, G.E., 2012. *Oil supply limits and the continuing financial crisis,* Energy. 37(1), 27-34.

Van Eylen, D., van Dongen, F., Kabel, M., de Bont, J., 2011. *Corn fiber, cobs and stover: Enzyme-aided saccharification and co-fermentation after dilute acid pretreatment,* Bioresource technology. 102(10), 5995-6004.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.* Journal of dairy science. 74(10), 3583-97.

Xu, J., Thomsen, M.H., Thomsen, A.B., 2010. *Investigation of acetic acid-catalyzed hydrothermal pretreatment on corn stover,* Applied Microbiology and Biotechnology. 86(2), 509-16.

Zhong, W., Zhang, Z., Qiao, W., Fu, P., Liu, M., 2011. *Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion,* Renewable Energy. 36(6), 1875-9.

Zhou, Z., Dionisio, K.L., Arku, R.E., Quaye, A., Hughes, A.F., Vallarino, J., Spengler, J.D., Hill, A., Agyei-Mensah, S., Ezzati, M., 2011. *Household and community poverty, biomass use, and air pollution in Accra, Ghana,* Proceedings of the National Academy of Sciences of the United States of America. 108(27), 11028-33.

Paper I

In-text citation:

(Kemausuor et al., 2014, Paper I)

Authors:

Kemausuor F., Kamp A., **Thomsen S.T.**, Bensah E.C., Østergård H.

Title:

Assessment of biomass residue availability and sustainable bioenergy yields in Ghana

Publication status:

First submitted on July 3 2013 to *Resources, Conservation and Recycling,* now submitted after review

Note:

Made as a collaboration between four of the PhD students in the 2GBIONRG project

Assessment of biomass residue availability and bioenergy yields in Ghana

Francis Kemausuor[a1]; Andreas Kamp[b]; Sune Tjalfe Thomsen[b]; Edem Cudjoe Bensah[c,d]; Hanne Østergård[b]

[a]Department of Agricultural Engineering, KNUST, Kumasi, Ghana

[b]Department of Chemical and Biochemical Engineering, Technical University of Denmark, DTU, DK-2800 Kgs. Lyngby, Denmark

[c]Department of Chemical Engineering, Kumasi Polytechnic, Kumasi, Ghana

[d]Department of Chemical Engineering, KNUST, Kumasi, Ghana

Abstract

Biomass is an important renewable energy source that holds large potential as feedstock for production of different energy carriers in a context of sustainable development, peak oil and climate change. In developing countries, biomass already supplies the bulk of energy services and future use is expected to increase with more efficient applications, such as the production of biogas and liquid biofuels for cooking, transportation and the generation of power. The aim of this study is to establish the amount of Ghana's energy demand that can be satisfied by using the country's crop residues, animal manure, logging residues and municipal waste. The study finds that the technical potential of bioenergy from these sources is 96 PJ in 2,700 Mm^3 of biogas or 52 PJ in 2,300 ML of cellulosic ethanol. The biogas potential is sufficient to replace more than a quarter of Ghana's present woodfuel use. If instead converted to cellulosic ethanol, the estimated potential is seven times the estimated 336 ML of biofuels needed to achieve the projected 10% biofuels blends at the national level in 2020. It is argued that energy production based on biomass is not necessarily sustainable and a range of recommendations for sustainability assessments of bioenergy projects is given. Utilizing the calculated potentials involves a large challenge in terms of infrastructure requirements, quantified to hundreds of thousands of small-scale plants.

Keywords: Biomass, bioenergy, biogas, cellulosic ethanol, Ghana

1 Introduction

Biomass is a renewable energy resource derived from living or recently living organisms (Fernandes and Costa, 2010). Biomass is today a very important energy source and forecasts for energy consumption suggest that it has a pivotal role to play as it can drastically reduce greenhouse gas[2] emissions compared to fossil fuels if produced sustainably (IEA Bioenergy, 2008; IEA, 2012). In developing countries, biomass already supplies the bulk of energy services albeit in very inefficient forms, particularly as firewood and charcoal for cooking and heating. Future use of biomass is expected to entail more efficient applications, such as the production of biogas and liquid fuels for cooking, transportation and the generation of power.

---

[1] Corresponding author – Email: Kemausuor@gmail.com; fkemausuor.soe@knust.edu.gh. Tel: +233207457532

[2] Greenhouse gases are gases that trap heat in the atmosphere. These gases include carbon dioxide and methane.

Many of the available studies on utilizing biomass for energy have targeted global and/or regional level assessments (e.g. Dasappa, 2011; Smeets et al., 2007) while others have targeted specific countries (see Cai et al., 2008; Kludze et al., 2010). The use of biomass residues, especially, for the production of non-food based biofuels is seen as a positive way to mitigate the effects of climate change (Gustavsson, 2007).

In the last few years, several countries with high agricultural potentials have sought to use their agricultural resources for the production of biofuels to limit the local use of fossil fuels and/or for export. Notable economic success stories are from Brazil, United States (US), Malaysia and Indonesia (Lamers et al., 2011). However, there have been criticisms of the use of agricultural land for the production of energy crops because of consequent direct or indirect effects of deforestation and increases in food prices (IEA, 2010). This has encouraged research towards biofuels that are based on lignocellulose in nonedible plant materials, typically in agricultural residues, and on residues and waste from other economic sectors. The bulk of residue-based biofuels (used throughout to refer to biofuels based on agricultural and forestry residues, manure and municipal waste) is expected to be produced from agricultural residues, reducing the negative effects of using cropland to produce biofuels instead of food. Political targets promote this development, particularly in the US and EU, through incentives for domestic production- and consumption targets that encourage production (US Government, 2007; EU, 2009). The EU Commission has published a proposal to limit the use of food-based biofuels to meet its 10% 'renewable energy for transport' target to only 5% with the rest expected to come from non-food based sources (EU, 2012).

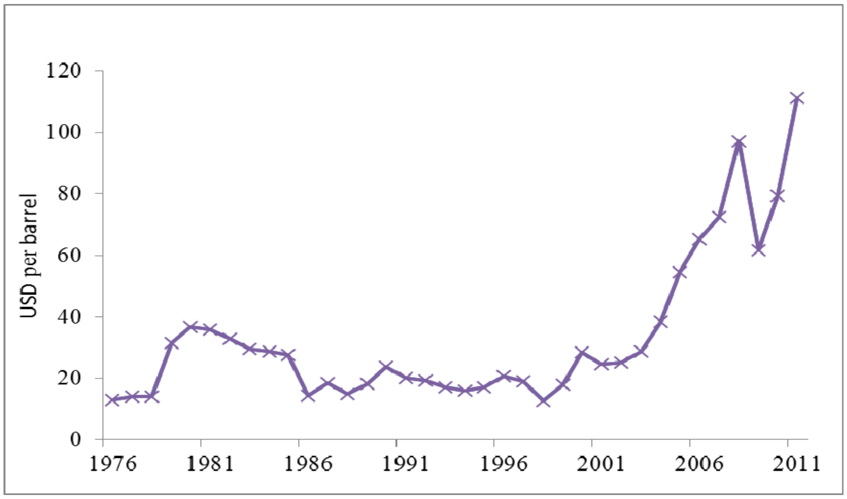

Figure 1: Historical Brent crude oil prices, nominal prices. Source: Data from BP (2012)

Though Ghana is an emerging oil producer, oil reserves will be depleted in the foreseeable future and it is prudent that alternative and more renewable energy sources are identified and made available. According to early estimates, current oil production wells in Ghana could run out by about 2040 (Edjekumhene et al, 2010) but additional discoveries have since been made which could extend this date. Globally, oil reserves are dwindling due to the combination of population growth, urbanization, and increasing per capita energy consumption. As a result, crude oil prices are rising (Figure 1) with implications for economies that are highly dependent on it. The irregularities in oil supplies and distributions, the challenges of accessing and procuring unconventional oil, and occasional political instabilities in oil producing nations in the developing world have caused general uncertainty regarding global reliability on oil, and have spurred renewed interest in renewable energy (IEA, 2012). Moreover, combustion of fossil fuels contributes to global warming (IPCC, 2007).

In view of the above, there is renewed interest in Ghana to further develop capacity in alternatives to fossil energy sources and thus 1) reduce the national carbon footprint, 2) reduce the country's dependence on oil, and 3) pursue political and economic goals through utilizing presently under-utilized and domestically available resources. Present political momentum is indicated by the development of key documents such as the Strategic National Energy Plan (SNEP) of 2006 (Energy Commission, 2006), the draft Bioenergy Policy of 2011 (Energy Commission, 2011a), and the Renewable Energy Law of 2011 (Ministry of Energy, 2012). Based on these documents, bioenergy is expected to contribute significantly to renewable energy supply from Ghana's energy sector. In order to pursue a strategy of increased bioenergy production while avoiding some of the problems associated with direct or indirect effects on food availability and deforestation rates, it makes sense for Ghana to explore its potentials for residue-based biofuels. This could make it possible for residue-based biofuels to contribute toward the proposed targets for all types of biofuels. Energy potentials from agricultural residues, logging residues, agro-industrial residues, municipal solid waste, food industry waste, industrial wastewater and animal waste could be explored for present and future energy needs.

Production of biofuels based on the mentioned residues avoids the problem of competition over land with food production. Residue-based biofuels, however, are not automatically environmentally benign nor do they ensure the development of a sustainable energy supply. To mention a couple of issues, the sustainability of feedstock supply is influenced by biomass production methods, and the transport and conversion of biomass into bioenergy requires additional inputs. Biomass production, transport and conversion are all likely to depend on non-renewable resources. Furthermore, the use of any biomass for fuel production entails a net loss of nutrients from the biomass production site that may cause a systematic deterioration of soil quality. These and similar issues require thorough assessment to avoid the substitution of one set of problems with another set of problems (Gopalakrishnan et al, 2009).

Bioenergy production in Ghana could be more than a means to reach present and short-term energy demands and politically established production targets; bioenergy could be a cornerstone of sustainable development. Therefore, the ability of specific bioenergy projects to sustain themselves over time and through changes in the surrounding environmental, social and economic context should be evaluated before decisions on which projects to develop further are made.

This paper assesses the potentials for bioenergy (biogas and cellulosic ethanol) to meet energy requirements in Ghana using residues and waste as feedstock. Important questions addressed in this paper include: 1) Which resources are available and how much is retrievable? 2) What amount of Ghana's projected energy demand can be satisfied by utilizing the country's crop residues, animal manure, logging residues and municipal solid waste? 3) How can we investigate the extent to which bioenergy production supports sustainable development, provides a net energy output, creates employment, and affects ecosystem functionality?

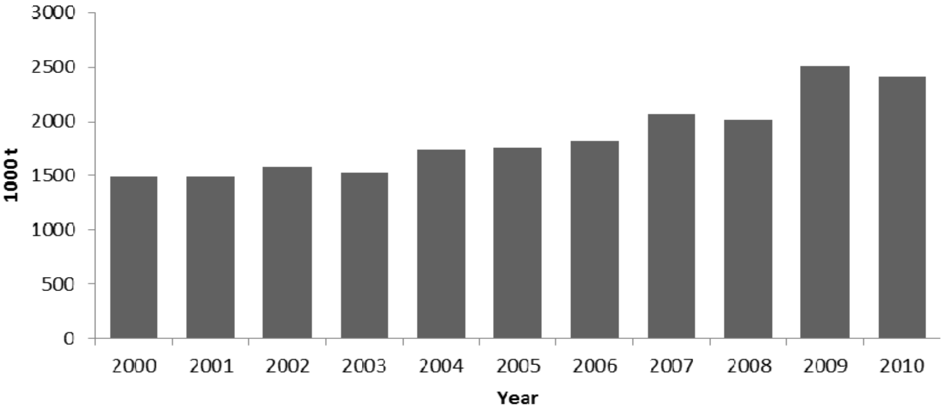

Figure 2: Petroleum Products Consumption (Energy Commission, 2011b)

2 Present and projected energy consumption and production

At the end of 2010, the energy consumption of Ghana was: 2.4 Mt of petroleum products[3] (equivalent to 111 PJ liquid fuel energy), 6,860 GWh of electricity (equivalent to 25 PJ electrical energy) and about 18 Mt of woodfuel (equivalent to 360 PJ of thermal energy, assuming 20 GJ/t of wood) (Energy Commission, 2011b). Even though animal manure is used to some extent in the northern parts of the country, its use is minimal (Arthur et al, 2011). There is currently no commercial production of liquid biofuels in the country. About 200 household and institutional biogas plants were estimated to have been installed at the end of 2009, out of which less than half were functioning (Bensah and Brew-Hammond, 2010).

Petroleum products dominate commercial fuels in Ghana and consumption has been growing over time. In the last decade alone, petroleum consumption increased 60% from 1.5 Mt (69 PJ) in 2000 to 2.4 Mt (111 PJ) in 2010 (Figure 2). Even though Ghana is producing oil and is likely to benefit from some of the effects of high oil prices, this luxury may be short lived as oil drilling activities in the country are not expected to go beyond the year 2040, even at a low production peak of 250,000 barrels per day (approximately 558 PJ/year) unless new reserves are discovered (Edjekumhene et al, 2010).

Table 1: Historical and projected use of woodfuel, petroleum products and electricity in Ghana.

Energy carriers	Unit	2000	2015	2020
Woodfuel, mass	Mt	14	38-46	54-66
Woodfuel, thermal energy equivalents	Quads	0.27	0.72-0.87	1.02-1.25
Petroleum products, mass	Mt	1.6	3	4.5
Petroleum products, liquid energy equivalents	PJ	74	139	208
Electricity*	GWh	6,900	18,000	24,000

*Excludes transmission and distribution lossesSource: Modified from Energy Commission (2006).

---

[3] Petroleum products refer to refined petroleum fuels such as gasoline and diesel. It does not include crude oil used for electricity generation.

Comparing consumption levels up until 2020 (Table 1) with the suggested oil production peak, the need for replacing petroleum-based fuels with biofuels does not seem imminent. It must be understood, however, that the majority of oil being produced in Ghana is produced by foreign companies and leaves the country for export, never seeing the Ghanaian market. Moreover, in a longer perspective it seems prudent to develop the country's bioenergy sector. As noted by Hirsch et al. (2005), the time needed for preparing for and transition to a post-peak oil situation is counted in decades.

Traditionally, Ghana's electricity production has come from two hydropower dams (Akosombo and Kpong dams) which together contributed 69% to the over 10,000 GWh total electricity generated in 2010. From just 8% in 2000, electricity from thermal plants, driven by petroleum fuels, contributed 31% to electricity generation in 2010. With the increase in electricity demand and poor rainfall experienced in recent years, the share of electricity from thermal plants is expected to increase significantly (GRIDCO, 2010).

Various projections conducted by the Ghanaian Ministry of Energy and its agencies (Energy Commission, 2006) suggest that energy demand will increase for all sectors of the economy in line with future economic growth (Table 1). In view of the demand for petroleum resources and mindful of the environmental impacts of increased consumption of petroleum fuels, the Strategic National Energy Plan 2006-2020 and the draft bioenergy policy call for 10 and 20% substitution of national petroleum fuels consumption with biofuels by 2020 and 2030, respectively (Energy Commission, 2006; Energy Commission, 2011a). According to Antwi et al. (2010), Ghana would have to produce roughly 336 ML of biofuels to substitute 10 per cent of expected transport fuels in 2020. Besides transportation, there is also the potential to replace portions of cooking fuel use (firewood and especially charcoal) with biogas that is much more efficient at the point of use. Demand for woodfuel is projected to increase steadily to more than 50 Mt in 2020, creating a worrying situation because of the resulting net increase in forest degradation, averaging about 115,000 ha/yr during the period 2000-2005 (FAO, 2010). In order to reduce woodfuel usage and hence reduce the rate of forest degradation, biogas for cooking should be encouraged and implemented with locally produced biogas. In the following, we will estimate the potential for replacing petroleum-based transport fuel and woodfuel use with cellulosic ethanol and biogas, respectively. The possibility of converting biogas into electricity remains, albeit with considerable conversion loss. The production of electricity has not been elaborated on in this paper.

3 Methodology

The estimation of available biomass residues in Ghana and bioenergy potentials was based on detailed computations using published literature when possible. In the cases where no scientifically solid data were available for the computations, assumptions have been made which will appear in the specific cases. Figure 3 illustrates how the residue- and energy potentials were obtained. Different productions (p), yields (y), concentrations (c), and efficiencies (η) are indicated. Table 2 explains the indices used in Figure 3. In the following sections the calculations will be elaborated on.

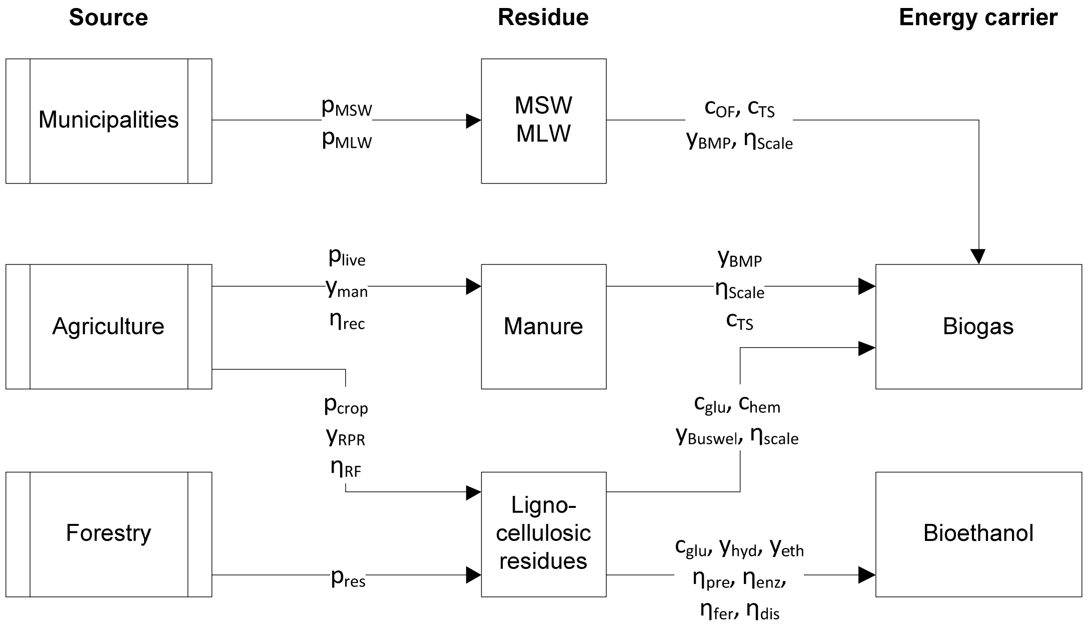

Figure 3: Computations of biogas and ethanol yields from Ghanaian biomass residues. Productions (p), yields (y), concentrations (c), and efficiencies (η) are indicated. The various indices are presented in Table 2.

Table 2: Indices of productions (p), yields (y), concentrations (c), and efficiencies (η) used in the determination of biogas and ethanol yields from Ghanaian biomass residues. TS is total solids.

Index	Explanation	Unit
c_{glu}	The concentration of glucan (cellulose or starch) in a specific residue	g/100g TS
c_{hem}	The concentration of hemicellulose in a specific residue	g/100g TS
c_{OF}	The concentration of the organic fraction in MSW	g/100g TS
c_{TS}	Total solids concentration	g TS/100g
p_{crop}	The production of specific crop residues annually	t TS/yr
p_{live}	Number of specific livestock populations	heads
p_{MLW}	Production of municipal liquid waste	t TS/yr
p_{MSW}	Production of municipal solid waste	t TS/yr
p_{res}	The annual production of forestry residuals	t TS/yr
y_{BMP}	Biomethane potentials - from literature	m^3 CH_4/kg TS
y_{Buswel}	Biomethane potentials – calculated with Buswel's formula	m^3 CH_4/kg TS
y_{eth}	Ethanol stoichiometric yield from glucose	g/g
y_{hyd}	Glucose yield when enzymatic hydrolysed from glucan	g/g
y_{man}	Manure produced of one specific livestock annually	kg/yr/head
y_{RPR}	Residue to product ratio	w/w
η_{enz}	Efficiency of enzymatic conversion (cellulose converted)	%
η_{pre}	Efficiency of pretreatment (glucose conserved)	%
η_{rec}	Recoverability of manure for specific livestock	w/w%
η_{scale}	The average efficiency of continuous biogas production compared with BMP (depending of reference of BMP)	%

3.1 Estimation of crop residue potentials

The estimation of residue potentials can be done in several ways, according to different types of potential. These include theoretical, technical, economic, implementation and sustainable biomass residue potentials (Biomass Energy Europe, 2008). The theoretical potential is the most straightforward to estimate since it takes into consideration all biomass that is available for collection and use and can be based on available crop production statistics. In practice, however, not all the biomass may be available for collection and use due to different inhibiting, economic, social and environmental factors. In this paper, we assess the theoretical and the technical potentials. The technical potential is the fraction of the theoretical potential that is technically recoverable. Due to the unavailability of data, we are unable to estimate beyond the technical potential. Thus, the economic, implementation and sustainable biomass potentials, which should be much lower, are not estimated in this paper. The estimated potential should be regarded as the maximum that could be obtained based on current technology.

The main agricultural crops in Ghana, in terms of area cultivated and production capacity, are cocoa, maize, cassava, yam, oil palm, groundnut, plantain, sorghum, cocoyam and rice (MOFA, 2011). Residues produced from these crops, relevant to bioenergy are the straw, stalk, husks, trunks and sometimes their peels after harvesting and/or processing. The theoretical potential of crop residues was estimated using the output of crops multiplied by the residue to product ratio (y_{RPR}). There are y_{RPR} values for crop types grown in Ghana but these are from different test fields and regions making accurate computation difficult (Duku et al., 2011; Jekayinfa and Scholz, 2009; Kartha and Larson, 2000; Koopmans and Koppejan, 1997). Some parameters that may vary from farm to farm include moisture content at time of measurement, yield of crops, and yield of biomass, which all depend on climatic conditions and the level of management. The computation for this assessment was done using the mean value from three different sources of y_{RPR} (Table 3).

Following the example of previous studies (Lemke et al., 2010; Zheng et al., 2010) we use a recoverability fraction (y_{RF}) to estimate the technical potential (Table 3). The y_{RF} is the ratio between the residues that realistically can be collected and the total production of residues (the theoretical potential) (Smeets et al., 2007). The y_{RF} differs according to crop type, soil type, typical weather conditions, and the tillage system used. To our knowledge, no study has established the y_{RF} for agricultural crops in Ghana. Doing so would demand a detailed procedure as described by Kludze et al. (2010), which is beyond the scope of this paper. The y_{RF} used in other assessments varies from one study to the other. In a global assessment of bioenergy potentials, Smeets et al. (2004) used an y_{RF} of 25% for rice straw, 80% for stalks, 100% for processing residue and 50% for wood process residues. In an earlier assessment of maize residues for energy production in the Eastern region of Ghana, the Kumasi Institute for Technology, Energy and Environment (KITE, 2009) used 80% y_{RF} taking into consideration the fact that farming in Ghana is largely no-tillage and with no existing regulation for residue management. In this paper, we assume recoverability fractions based on previous studies to estimate the technical biomass potential (Table 3).

Table 3: Indices for specific agricultural and forestry residues. ^{F} indicates a field based residue while ^{P} indicates a processing residue.

Crop	Residue	p_{crop} (p_{res})	(y_{RF})	y_{RPR}*	y_{Buswel}
		Mt TS/yr	g/g	g/g	m^3 CH_4/kg TS
Maize	Stalks^F	1.7	0.80	1.59[abc]	0.268
	Husks^P	1.7	1.00	0.20[b]	0.238
	Cobs^P	1.7	1.00	0.29[bc]	0.348
Rice	Straw^F	0.47	0.25	1.66[abc]	0.264
	Husks^P	0.47	1.00	0.26[bc]	0.232
Millet	Stalks^F	0.18	0.80	1.83[abc]	0.181
Sorghum	Straw^F	0.29	0.80	1.99[bc]	0.285
Groundnut	Shells^P	0.48	1.00	0.37[bc]	0.227
	Straw^P	0.48	1.00	2.15[bc]	0.154
Cowpea	Shells^P	0.24	1.00	1.75[b]	0.65
Cassava	Stalk^F	14	0.80	0.06[b]	0.192
	Peelings^P	14	0.20	0.25[b]	0.323
Plantain	Trunks and leaves^F	3.7	0.80	0.50[c]	0.213
Soybean	Straw and pods^F	0.16	0.80	3.50[b]	0.225
Yam	Straw^F	5.9	0.80	0.50[c]	0.167
Cocoyam	Straw^F	1.3	0.80	0.50[c]	0.167
Sweet potato	Straw^F	0.044	0.80	0.50[b]	0.144
Oil palm	EFB^P	2.0	1.00	0.17[ab]	0.253
	Kernel shells^P	2.0	1.00	0.07[b]	0.280
	Fibre^P	2.0	1.00	0.14[b]	0.146
Coconut	Husks^P	0.30	1.00	0.42[b]	0.141
	Shells^P	0.30	1.00	0.25[abc]	0.289
Sugarcane	Leaves^F	0.15	0.80	0.11[abc]	0.204
	Bagasse^P	0.15	1.00	0.18[bc]	0.221
Cotton	Stalks^F	0.027	0.80	2.88[bc]	0.225
Cocoa	Pods^F	0.90	0.80	0.93[ab]	0.80
Forestry	Slabs^P	(0,019)	1.00	-	0.276
	Wane^P	(0,0071)	1.00	-	0.276
	Bark^P	(0,0034)	1.00	-	0.159
	Sawdust^P	(0,0076)	1.00	-	0.218

^{F} indicates a field based residue while ^{P} indicates a processing residue. *: Average based on literature sources; [a] indicates Duku et al, 2011, [b] Jekayinfa and Scholz, 2009, and [c] Kartha and Larson, 2000

3.2 Estimation of animal manure, wood residues and Municipal Solid and Liquid Waste (MSW and MLW)

The potential quantities of animal manure resources are estimated using number of animals, average annual manure production per animal, coefficient of manure collection and dry manure fraction (Cai et al., 2008). Amount of manure per head per day depends on factors such as body size, kind of feed, physiological state (lactating, growing, etc.), and level of nutrition (Junfeng et al., 2005). The manure theoretically available was estimated by multiplying the number of animals (p_{live}) by the estimated manure produced per day (y_{man}). Data on animal production was obtained from the Ghanaian Ministry of Food and Agriculture (MOFA, 2010).

The recoverability fraction used in the estimation of technically available animal manure is based on a study by KITE (2008).

Wood residue results as a co-product of logging and timber processing. Wood residue can be collected and used from in-forest cutover, log landing or wood processing sites. In Ghana, the landing and processing sites are often the same since the tree-length materials are transported straight from the forest to the processing sites. Data on wood production was obtained from the FAO (2012). The data obtained is categorized into slabs, wane, bark and sawdust.

With regard to MSW, data was collected only for the regional capitals and other major cities. These cities were considered because of relatively higher population and therefore higher generation of solid and liquid waste and better waste collection systems as compared to other locations in the country. Besides, there is poor data on waste management activities outside the major cities. The possibility of collecting and using MSW from a number of smaller towns is not being ruled out but the possible contribution from this has not been considered in this paper.

3.3 Estimation of energy potentials

Eq. 1 shows the formula for the computation of annual potential biogas production from MSW (in m^3 methane per yr). Here the yield of biogas (y_{biogas}(MSW)) is equal to the production of municipal solid waste (p_{MSW}) multiplied by the concentration of the organic fraction in MSW (c_{OF} =64%) (Asase et al, 2009), the total solids concentration in the organic fraction of MSW (c_{TS}) (Unpublished results from Accra, Ghana), and the biomethane potentials of organic fraction of MSW (y_{BMP}=0.32 m^3 CH_4/kg TS) based on an average of continuous mesophilic biogas plants from Gunaseelan (1997).

Eq. 1: $$y_{biogas}\,(MSW) = p_{MSW} \cdot c_{OF} \cdot c_{TS} \cdot y_{BMP}$$

Similarly, Eq. 2 describes the computations of the biogas potentials from municipal liquid waste (MLW). In Eq. 2 the total solids concentration in the organic fraction of MLW (c_{TS}) equals 8.9 g TS/100g, and the biomethane potential (y_{BMP}) of MLW is assumed to be 0.34 m^3 CH_4/kg TS (Arthur and Brew-Hammond, 2010). Eq. 3 describes the computation of biogas potentials from livestock manure. The indices are presented in Table 4.

Table 4: Indices of livestock populations (p_{live}), manure produced (y_{man}), the recoverability of the manure (η_{rec}) (KITE, 2008), the total solid concentration (c_{TS}), and the biomethane potential (y_{BMP}).

Type of livestock	p_{live}[a]	y_{man}[b]	η_{rec}[c]	c_{TS}[d]	y_{BMP}[e]
	1000 heads	**kg manure /head/day**	**kg/kg**	**g TS /100g**	**m^3/ kg TS**
Cattle	1,454	12	0.2	12	0.22
Sheep	3,759	1.2	0.2	25	0.22
Goats	4,855	1.5	0.2	25	0.22
Pigs	536	3.6	0.5	11	0.22
Poultry	47,752	0.02	0.5	25	0.22

[a](MOFA, 2010), [b](Kartha and Larson, 2000; Milbrandt, 2009), [c](KITE, 2008), [d](Randall et al., 2006), [e]Estimated from Angelidaki and Ellegaard, (2003) and Randall et al. (2006).

Eq. 2: $$y_{biogas}\,(MLW) = p_{MLW} \cdot c_{TS} \cdot y_{BMP}$$

Eq. 3: $$y_{biogas}\,(manure) = p_{live} \cdot y_{man} \cdot \eta_{rec} \cdot c_{TS} \cdot y_{BMP}$$

The computations of the potential biogas produced from lignocellulosic residues from agriculture or forestry is described in Eq. 4. The y_{Buswel} is calculated from compositional data from literature which is compiled in the Appendix. Note that in the case of the forestry residues, composition and dry matter content of hardwood is assumed to be the same as the common Ghanaian specie Awiemfosamina (*Albizzialucida*) based on Kataki and Konwer (2001) and sawdust is assumed to be 50% trunk wood and 50% bark. The average efficiency of continuous biogas production compared with BMP, η_{scale}, for the forestry residues are assumed to be 50% due to the recalcitrant nature of the biomass. For agricultural residues η_{scale} are assumed to be 80%.

Eq. 4: $$y_{biogas}(lignocellulose) = p_{crop} \cdot y_{RPR} \cdot (y_{Buswel,glu} \cdot c_{glu} + y_{Buswel,hem} \cdot c_{hem}) \cdot \eta_{Scale}$$

Eq. 5 and Eq. 6 express how the yields of ethanol from lignocellulosic agricultural residue and forestry residues, respectively, were calculated. The crop specific indices have been shown in Table 3 while the general indices assumed for ethanol production are: a 90% conservation of glucan in the pretreatment ($\eta_{pre} = 90\%$) and 80% enzymatic conversion of glucan ($\eta_{enz} = 80\%$). The stoichiometric yields of hydrolysing glucan to glucose ($y_{hyd} = 1.11$ g/g) and fermenting glucose into ethanol ($y_{eth} = 0.51$) are theoretically determined. Ethanol from C5 sugars are not considered and distillation losses are assumed to be negligible.

Eq. 5: $$y_{bioethanol}(agriculture) = p_{crop} \cdot y_{RPR} \cdot c_{glu} \cdot y_{hyd} \cdot y_{eth} \cdot \eta_{pre} \cdot \eta_{enz}$$

Eq. 6: $$y_{bioethanol}(forestry) = p_{res} \cdot c_{glu} \cdot y_{hyd} \cdot y_{eth} \cdot \eta_{pre} \cdot \eta_{enz}$$

Energy potentials have been converted from Mm^3 of methane and ML of ethanol to PJ of heat energy and PJ of liquid energy, respectively, using the factors 0.0360 GJ/m^3 methane and 22.8 MJ/L ethanol.

4. Results and discussions

4.1 Energy potential from crop and wood residues

The production data for crops in Ghana and the potential residue generated from these crops in 2011 as well as the potential energy from the residues are shown in Table 5. The residue available is dominated by residues from cassava, yam, maize, plantain and groundnut. Together, residue from these five crops constitutes more than 72% of the total residue available. In 2011, the total crop residue generated was approximately 20 Mt. The assumption here is that all processing residues can be available for energy generation as processing involves already concentrated material. An exception to this is cassava peels, of which only 80% are considered available because cassava peels are sometimes fed to livestock or dumped in MSW, depending on the point of usage and the quantities available. For other crops, it is estimated that between 25 and 80% of field based residues could be available for collection in Ghana, depending on the crop. The amount of residues recoverable for energy generation thereby becomes equal to the total agricultural residue generated minus the amount used for

Table 5: Energy potential from crop residues in 2011. ^{F} indicates a field based residue while ^{P} indicates a processing residue.

Crop	Residue	Theoretical potential of residue	Technical potential of residue	Biogas		Cellulosic ethanol	
		Mt/yr	Mt/yr	Mm^3 CH$_4$/yr	PJ/yr	ML/yr	PJ/yr
Maize	Stalks^{F}	2.7	2.2	470	17	410	9.4
	Husks^{P}	0.34	0.34	65	2.3	62	1.4
	Cobs^{P}	0.49	0.49	140	4.9	86	1.9
Rice	Straw^{F}	0.77	0.19	41	1.5	38	0.9
	Husks^{P}	0.12	0.12	22	0.8	19	0.4
Millet	Stalks^{F}	0.34	0.27	39	1.4	38	0.9
Sorghum	Straw^{F}	0.57	0.46	100	3.8	98	2.2
Groundnut	Shells^{P}	0.18	0.18	32	1.2	33	0.8
	Straw^{P}	1.0	1.0	130	4.6	200	4.5
Cowpea	Shells^{P}	0.42	0.42	22	0.8	17	0.4
Cassava	Stalk^{F}	0.89	0.71	190	6.7	210	4.7
	Peelings^{P}	3.6	0.72	250	9.0	260	5.9
Plantain	Trunks and leaves^{F}	1.8	1.5	80	3.0	90	2.0
Soybean	Straw and pods^{F}	0.58	0.46	110	3.9	120	2.8
Yam	Straw^{F}	3.2	2.5	340	12	330	7.4
Cocoyam	Straw^{F}	0.67	0.54	72	2.6	70	1.6
Sweet Potato	Straw^{F}	0.022	0.017	2.0	0.1	1.8	0.0
Oil palm	EFB^{P}	0.33	0.33	67	2.4	52	1.2
	Kernel shells^{P}	0.13	0.13	29	1.0	31	0.7
	Fibre^{P}	0.28	0.28	33	1.2	23	0.5
Coconut	Husks^{P}	0.12	0.12	14	0.5	14	0.3
	Shells^{P}	0.075	0.075	17	0.6	7.8	0.2
Sugarcane	Leaves^{F}	0.016	0.013	2.2	0.1	2.2	0.0
	Bagasse^{P}	0.025	0.025	4.5	0.2	4.7	0.1
Cotton	Stalks^{F}	0.076	0.061	11	0.4	13	0.3
Cocoa beans	Pods^{F}	0.84	0.67	43	1.5	37	0.8
	Total field based residues			1,600	57	1,500	35
	Total processing residues			750	27	750	17
	Total			**2,300**	**84**	**2,300**	**52**

other purposes or un-recovered. The potential can be exploited for either biogas or ethanol, or a mixture of the two. The total biogas potential from agricultural residues is approximately 2,300 Mm^3 of methane containing 83 PJ of heat energy. The total ethanol potential from the residues is estimated to approximately 2,300 ML or 51 PJ of liquid fuel energy. Field based residues account for about two thirds of the potential in both the biogas and ethanol case. As the energy content figures show, the conversion to biogas is more efficient than conversion to ethanol. It should be kept in mind, however, that the different energy carriers have different energy qualities, that they can be used for different purposes, and that they therefore are not directly comparable. Also noteworthy is that fact that the estimated ethanol and biogas potentials are based on the technical potential of biomass, but actual potentials could be much lower in practice.

The energy potentials from wood residues in Ghana have been calculated (Table 6). Wood residues are usually available at centralized sites, making it relatively easier to recover significant volumes, which can then be used as feedstock. Currently, wood residues in Ghana are mainly used as firewood and for the production of charcoal. The actual production of residues generated from the manufacture of wood products differs from one plant to another and depends on several factors: from the properties of the wood to the type, operation and maintenance of the processing plant (FAO, 1990). In 2010, about 1.3 Mm^3 of industrial roundwood was produced in Ghana (FAO, 2012). Based on estimations deployed by Ghana's Energy Commission (2010), the total amount of slabs, wane, bark and sawdust from wood residues amounted to about 0.35 Mt. Biogas potential from these residues amounts to 19 Mm^3 of methane or 0.67 PJ. Ethanol potential is 48 ML corresponding to 1.1 PJ.

Table 6: Energy potential from processing wood residues

Forestry residue	**Production**	**Biogas**		**Cellulosic ethanol**	
	Mt/yr	**Mm^3 CH_4/yr**	**PJ/year**	**10^6 L/yr**	**PJ/year**
Slabs	0.16	10	0.37	28	0.64
Wane	0.061	3.9	0.14	11	0.24
Bark	0.047	1.1	0.039	1.9	0.043
Sawdust	0.081	3.3	0.12	7.9	0.18
Total	**0.35**	**19**	**0.67**	**48**	**1.1**

4.2 Energy from animal manure

In terms of quantity, the most important livestock raised in Ghana are cattle, sheep, goats, pigs and poultry (mainly chicken). Populations of livestock in Ghana, the estimated manure, and corresponding energy potentials are shown in Table 7. The majority of cattle are kept free-range during the day and some of them are housed at night. It is therefore assumed that for half the day, manure produced from most cattle is not recoverable. It has also been established that cattle breeds reared in Ghana and many other West African countries are small and undernourished, with less manure production as compared to better fed cattle breeds (KITE, 2008). Sheep and goats are also mostly kept on free range and allowed to stable around farmer residences during the night, which means that only manure produced by night can be collected from these animals. Commercially produced chicken and pigs are largely kept in intensive farming systems. Manure produced from these types of livestock is more easily recoverable as compared to the cattle, sheep and goats. About 100 Mm^3 of methane can be produced from the recoverable fraction; this corresponds to 3.6 PJ (Table 7).

Table 7: Energy potential from animal manure

Type of Livestock	Population	Estimated amount of manure	Recoverability Fraction	Manure available	Potential biogas	Energy potential
	10^6 heads	kg/head/day	g/g	Mt/yr	Mm^3 CH_4/yr	PJ/year
Cattle	1.5	12	0.2	1,300	34	1.2
Sheep	3.8	1.2	0.2	330	18	0.66
Goats	4.9	2	0.2	710	30	1.1
Pigs	0.54	3.6	0.5	350	8.6	0.31
Poultry	47	0.02	0.5	170	10	0.35
Total					**100**	**3.6**

Source: Livestock population data from MOFA (2010); Recoverable fraction estimated from KITE (2008); Manure estimation is from Milbrandt (2009); Kartha and Larson (2000)

4.3 Energy from Municipal Solid and Liquid Waste

The potential for energy generation from municipal waste in Ghana has been considered for a while because of its potential dual ability to abate environmental pollution problems (Fobil et al., 2005). Table 8 shows estimates of solid and liquid waste production in the major cities in Ghana. Together, these 11 cities produce about 50% of the solid waste generated in the country due to their large populations. The other half of the waste generated is scattered across 150 municipalities and districts in the country where collection is not as effective compared to the major cities considered in this paper. Around 2.1 Mt of solid waste and about 0.56 Mt of liquid waste are produced annually in the major cities. About 230 Mm^3 methane can be produced from solid waste and about 17 Mm^3 of methane from liquid waste (sewage sludge) amounting to a total of 9.0 PJ.

Table 8: Energy potential from municipal solid and liquid waste in major cities

City	Solid Waste	Potential biogas	Energy Potential	Liquid Waste	Potential biogas	Energy Potential
	Mt/yr	Mm^3 CH$_4$/yr	PJ/year	Mt/yr	Mm^3 CH$_4$/yr	PJ/year
Accra	0.72	80	2.9	0.29	8.7	0.31
Tema	0.5	56	2.0	0.039	1.2	0.043
Kumasi	0.54	60	2.2	0.078	2.4	0.085
Other 8 Regional Capitals	0.34	37	1.3	0.15	4.6	0.16
TOTAL	**2.1**	**230**	**8.4**	**0.56**	**17**	**0.61**

Source: Municipal waste data from Energy Commission (2010)

4.4 Discussion

The maximum technical potential of bioenergy from crop residues, wood residues, animal manure, municipal solid wastes and municipal liquid waste in 2011 is 2,700 Mm^3 of biogas, equivalent to 97 PJ of heat energy, or 2,300 ML cellulosic ethanol, equivalent to 52 PJ of liquid fuel energy (Table 9). It is important to note, however, that because of technical constraints cellulosic ethanol production is considered only from crop and wood residues whereas biogas production is considered from all residue types discussed in this paper. This selection is done since manure, MSW, and MLW are characterized as being high in water content, low in cellulose content, and with a quite inconsistent composition which significantly complicates industrial implementation of the substrates.

Table 9: Summary of energy potential from selected residues in Ghana.

Residue category		Biogas		Cellulosic ethanol	
		Mm^3 CH$_4$/yr	PJ/year	ML ethanol/yr	PJ/year
Crop residue	Field based residue	1,600	56	1,500	34
	Processing residue	750	27	750	17
Forestry	Processing residue	19	0.67	48	1.1
Manure	Recoverable	100	3.6		
Municipal waste	Solid (MSW)	230	8.4		
	Liquid (MLW)	17	0.61		
Total		**2,700**	**97**	**2,300**	**53**

The biogas potential is equivalent to 27% of the heat energy in woodfuel consumed in 2010. In essence, biogas from lignocellulosic materials has the potential to replace approximately 5 Mt of woodfuel. The substitutable share may arguably be higher since combusting biogas is likely to transfer heat more efficiently than in the combustion of wood or charcoal, i.e. 96 PJ in biogas may cook more meals than 96 PJ in woodfuel. The result shows a large potential for biogas production in Ghana. With respect to transport fuels, the total potential of 2,300ML is about seven times the estimated 336 ML of biofuels needed in 2020 to achieve a 10% biofuels blends at the national level.

As emphasized in the methodology, the aforementioned potentials are the maximum bioenergy potentials attainable based on the technical potential of biomass. In practice however, several factors will limit the actual amount of bioenergy than can be produced. Factors such as capital cost of plants, infrastructural development and the skill level of available labor would determine how much biofuel (ethanol or biogas) potential could realistically be implemented. Ethanol potential would be affected by 1) biomass recalcitrance, which varies with maturity, time and method of harvest, extent of drying, and mode of storage, and 2) the cellulose content of biomass after pretreatment, which is dependent on the pretreatment method and process severity, as well as the biomass composition. This is also likely to influence the level of ethanol in the fermentation broth. Also for biogas, the extent to which inoculum is made available to ensure a stable digestion of lignocellulosic waste is a challenge which is linked to the spatial distribution of livestock. These and other factors will affect final bioenergy availability but are not addressed in this paper.

The bioenergy potentials estimated in this study are higher by a factor of one thousand, compared to previous studies on biomass energy in Ghana. Duku et al. (2011) estimated 75.2 TJ heat energy (theoretical potential) from residues of nine major agricultural crops in Ghana using crop production data for 2008. Their study did not provide energy potentials for forestry residues, urban and other wastes considered in this paper. Mohammed et al. (2013) focused on agricultural residue based resources for decentralized energy production. Using methods and data similar to those used by Duku et al. (2011), Mohammed et al. (2013) found that the theoretical energy potential from residues of ten fruits and eight cash crops is 14.6 TJ and 86.6 TJ, respectively. Additionally, 47.6 TJ was calculated as theoretically available from livestock manure. The very large differences in calculated potentials between this study and the ones reported above may be explained by a factor 1000 error in computation in Duku et al. (2011) and Mohammed et al. (2013). Assuming that their TJ should be PJ, the findings are comparable.

Many factors affect the decision to choose between using the biomass for biogas or cellulosic ethanol. Even though it is in principle possible to produce both cellulosic ethanol and biogas from most of the residues available, biogas from residues is a much more mature technology as opposed to cellulosic ethanol, which still requires more research. Another characteristic of cellulosic ethanol is that it is much more expensive to produce due to the cost of pretreatment (in terms of energy, solvents, etc.) and the use of enzymes in both saccharification and fermentation. Biogas is more versatile with respect to feedstock as it can be produced from all the residues considered in this paper. Further, biogas conversion more easily allows for local nutrient recycling which could reduce dependence on inorganic fertilizer. On the other hand, ethanol can be used directly as blends in transport fuels without further processing whereas biogas would need further processing to be used as a transport fuel, if there is a desire to do so.

Unlike dung and human feces, lignocellulose is recalcitrant and thus needs some form of pretreatment to disrupt its complex structure and thereby facilitate biological breakdown of cellulose and hemicellulose into simple forms under anaerobic conditions. It should be noted that simple manure-based biodigesters that are mostly promoted in Ghana would need to undergo design changes to handle plant materials that show high propensity to form surface scum, which occludes methane escape, and inhibit the biological process. If all residues considered are used for biogas generation, then the annual biomass potential is capable of supporting biogas production in about 6.1 million household digesters (1.2 m^3/day), in 25,000 institutional digesters (300 m^3/day), or in 3,600 large-scale plants (2000 m^3/day). The estimated number of domestic plants is more than the number of agricultural households in the country (2.5 million in 2010). Though the possibilities in Ghana's biomass resources appear obvious, moving from estimated potential to implementation and actual output presents huge challenges pertaining to technology and infrastructure.

Compared to biogas there is less experience in the design and installation of hardware required in the cellulosic ethanol production chain: pretreatment, enzymatic hydrolysis, and fermentation. Further, cellulosic ethanol has so far received little attention in the form of funding for research in Africa and thus only a small portion of potential biomass unique to Africa has been studied in detail. From the residues from crops and forest sources, about 180,000 small-scale (12,500 L/y) or 1800 demonstration units (1.3 ML/y) or 18 commercial-scale plants (120 ML/y) can be technically installed. Apart from the challenge of establishing infrastructure, advances in cellulosic ethanol production will require much effort in research and development along the entire chain – from raw material collection and transportation to ethanol purification – even in small farm-based systems. In the end, the country must decide how to best allocate feedstock between the production of biogas and cellulosic ethanol, based on its long-term energy strategies.

Although it has been shown that Ghana has technical potential for residue based bioenergy based on feedstock availability, technical feasibility is one thing; the desirability of realizing bioenergy projects in order to reach bioenergy production on such a scale is another thing. A transition to biofuels on the scale presently proposed should not be done without a thorough discussion of likely socio-economic and environmental consequences. Based on the identification of key issues related to the sustainability of bioenergy production, some general recommendations to guide sustainability assessments are presented.

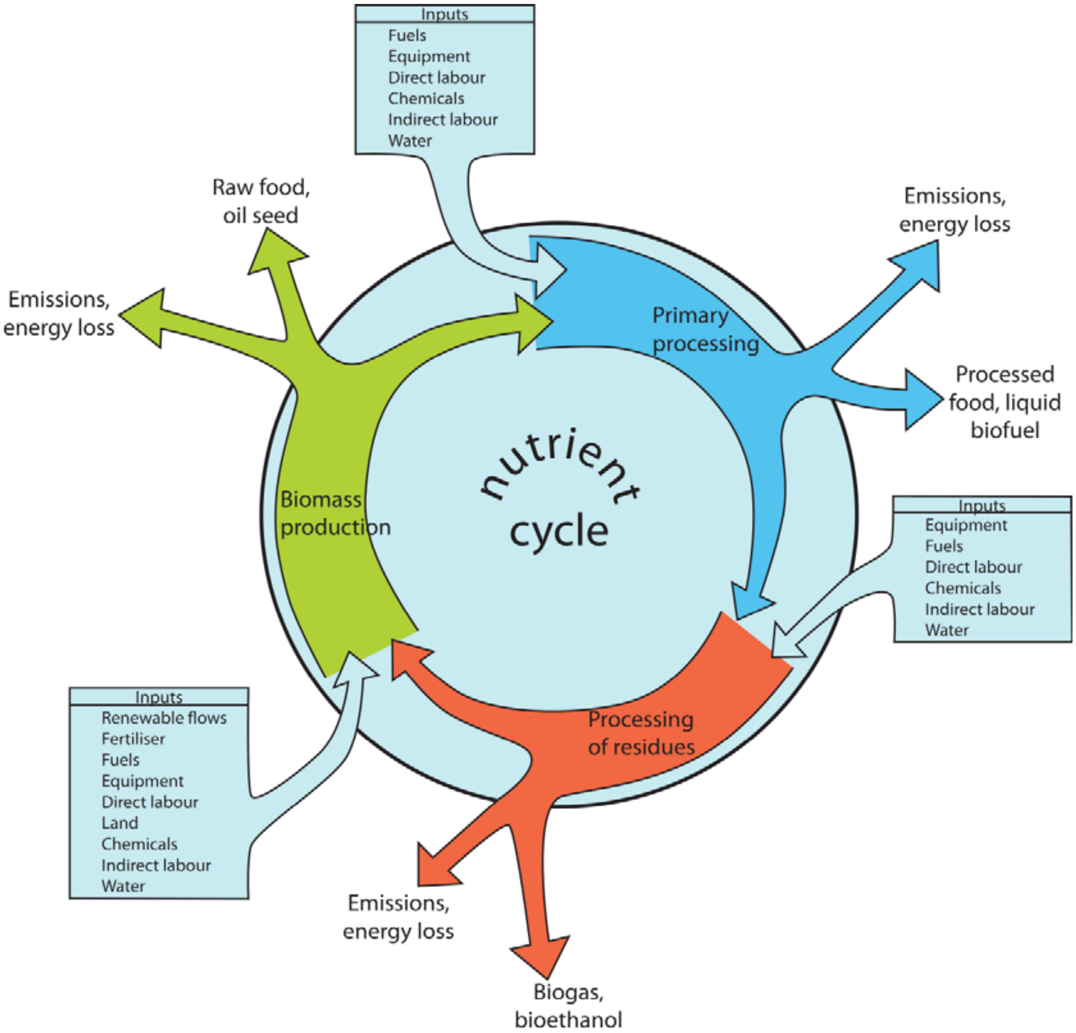

Figure 4: Conceptual overview of life-cycle approach to combined food and bioenergy production emphasizing nutrient re-cycling and the existence of multiple system outputs.

The sustainability of bioenergy production is influenced by several interrelating factors as indicated in Figure 4. These include:

- The continued flow of feedstock to the energy conversion stage which, in turn, is dependent on the reliable supply of inputs in the biomass production, transport and/or processing stage(s);
- The emissions to the environment related to production and transport of feedstock as well as from the conversion stage;
- The resulting outputs' ability to replace fossil-based energy carriers and thereby realize actual reductions in non-renewable resource use and emissions;
- The ability of the applied technology and practices to re-cycle nutrients in order to avoid soil degradation and reduce the use of non-renewable inorganic fertilizer;

- The ability of projects to make use of local resources including labor to facilitate societal support and improve resilience to changes in external support;
- The use of practices that do not undermine the social, environmental and economic foundations that the projects are based on.

The ability of bioenergy production to contribute to energy supply should be assessed through the calculation of net energy output or energy return on energy invested (Murphy et al., 2011, Giampietro and Mayumi, 2009). Energy inputs should be considered in a life-cycle perspective and ideally, be categorized according to origin (fossil or renewable) to highlight how dependent bioenergy production is on non-renewable energy resources. Moreover, assessing the degree to which energy inputs are from local and/or domestic sources can indicate the project's dependence on imports.

Bioenergy projects should be evaluated on their ability to create employment, generate income for local society and in general improve the livelihood of people involved in and affected by the development (EPFL, 2011). General socio-economic indicators include: expanded access to modern energy services, contribution to local economy, job creation, change in the food basket price, change in income, land use changes and effects for users, effect in changes in traditional uses of residues, and smallholder integration, to name a few.

Altering agricultural practices, e.g. through the removal of residue biomass may affect agro-ecosystem functioning and could possibly increase the susceptibility of agro-ecosystems to diseases and pests, especially in large-scale plantations. Since this may undermine the sustainability of ecosystem function, bioenergy projects should be assessed to indicate the impacts on biodiversity. Assessing biodiversity is complex and no straightforward method with easily calculated indicators is available at present. Attempts to establish a common framework are made however, e.g. by the Roundtable on Sustainable Biofuels (EPFL, 2011) who provides extensive guidance on conservation measures that also encompass the maintenance of biodiversity. By carrying out sustainability assessments, the impacts of bioenergy production can be modeled and estimated, both quantitatively and qualitatively.

In order for assessments of Ghanaian bioenergy production projects to be comparable and thereby ease decision-making, a reasonable degree of consistency in methods is recommended. Choosing among the approaches and indicators, however, can be overwhelming because of the amount of options and it is too much to hope for that an agreement can be made among sustainability assessors of Ghanaian bioenergy projects. As a starting point in the development of a framework for carrying out systematic and compatible assessments of environmental and socio-economic consequences of bioenergy production, a compilation of guiding principles is provided. For a more detailed study, see the Roundtable on Sustainable Biofuels guidelines (EPFL, 2011), Markandya and Halsnaes' (2002) discussion of sustainable development assessment in Clean Development Mechanism (CDM) projects, and Giampietro and Ulgiati's (2005) discussion on integrated assessment of biofuel production. Sustainability assessments of (Ghanaian) bioenergy projects should:

- Be considered in a life-cycle perspective (European Commission, 2006) and preferably with a consequential approach that takes alternative land use and likely ability of bioenergy carriers to substitute for alternatives into account;
- Focus on quantitative indicators to facilitate comparison;

- Include up- and downstream indicators from several categories to reflect the range of effects and the amount of stakeholders involved;
- Contain sensitivity analyses that emphasize the (highly) unstable economic environment of Ghana's economy and its dependence on oil, inorganic fertilizer and other non-renewable inputs; and
- Be transparent with respect to assumptions made.

Apart from addressing environmental impacts on the global scale, e.g. greenhouse gas emissions, the choice of indicators should reflect the conditions in the region where a specific bioenergy project is carried out. Site-specific problems of e.g. drought, deforestation, soil erosion, water pollution and/or potable water scarcity should be addressed with appropriate environmental sustainability indicators where those problems are present.

5. Conclusions

This paper assessed the potential contribution of bioenergy (biogas and cellulosic ethanol) to meet portions of energy demand in Ghana using residue and waste based biofuels. The types of feedstock considered were crop residues, forestry residue, animal manure and municipal waste. The most important feedstock in terms of energy potential is crop residues. The results show that it is technically possible to obtain Ghanaian biomass for an estimated 2,700 Mm^3/yr of biogas or 2,300 ML/yr of cellulosic ethanol. The biogas potential is sufficient to replace approximately a quarter of Ghana's present woodfuel use. If all the biomass is instead converted to cellulosic ethanol, the estimated potential is seven times the estimated 336 ML of biofuels needed in 2020 to achieve a 10% biofuels blends at the national level.

Two critical issues have been raised: sustainability and implementation. Projects that aim to produce biogas or cellulosic ethanol in Ghana must ensure sustainability from the feedstock production stage through transport and conversion to final use in order to be viable in the long term. Therefore, the ability of individual bioenergy projects to sustain themselves over time and through changes in the surrounding environmental, social and economic context should be evaluated before decisions are made to develop those projects. Utilizing the bioenergy potential involves an immense challenge of establishing the necessary infrastructure, with small-scale plants counted in hundreds of thousands for cellulosic ethanol and counted in millions for household-scale biogas digesters. The production of cellulosic bioethanol requires more additional research and development to be implemented compared to the more mature biogas technology.

Acknowledgement

This work was supported with a grant from Danida Fellowship Centre (DFC) of the Danish Ministry of Foreign Affairs, as a part of the project "Biofuel production from lignocellulosic materials – 2GBIONRG", DFC journal no. 10-018RISØ. For additional information, see http://2gbionrg.dk.

References

Aderemi FA, Nworgu FC. Nutritional Status of Cassava Peels and Root Sieviate Biodegraded with Aspergillusniger, American-Eurasian J. Agric. & Environ. Sci., 2007; 2(3); 308-11.

Amaducci S, Amaducci MT, Benati R, Venturi G. Crop yield and quality parameters of four annual fibre crops (hemp, kenaf, maize and sorghum) in the North of Italy. Ind. Crop. Prod. 2000;11(2/3); 179-86.

Angelidaki I, Ellegaard L. Status and Future Trends in Centralized Biogas Plants Codigestion of Manure and Organic Wastes, Applied Biochemistry and Biotechnology 2003;109(1-3); 95-105.

Antwi E, Bensah EC, Quansah DA, Arthur R, Ahiekpor J. Ghana's biofuels policy: challenges and the way forward, International Journal of Energy and Environment 2010; 1(5); 805-814

Arthur R, Baidoo MF, Antwi E. Biogas as a potential renewable energy source: A Ghanaian case study. Renew. Energy 2011; 36(5); 1510-6.

Arthur R, Brew-Hammond A. Potential biogas production from sewage sludge: A case study of the sewage treatment plant at Kwame Nkrumah University of science and technology, Ghana, International Journal of Energy and Environment 2010; 1(6); 1009-6.

Asase M, Yanful EK, Mensah M, Stanford J, Amponsah S. Comparison of municipal solid waste management systems in Canada and Ghana: A case study of the cities of London, Ontario, and Kumasi, Ghana, Waste Management 2009; 29(10); 2779-86.

Ayeni AO, Banerjee S, Omoleye JA, Hymore FK, Giri BS, Deshmukh SC et al. Biomass and Bioenergy 2013; 48; 130-138.

Ayeni AO, Hymore FK, Mudliar SN, Deshmukh SC, Satpute DB, Omoleye JA et al. Fuel 2013; 106; 187-194.

Benjamin Y, Cheng H, Görgens JF. The influence of variety type on dilute acid pretreatment-hydrolysis responses of sugarcane bagasse, Centre for Renewable and Sustainable Energy Studies, Stellenbosch University, 2011. Available online from http://www.crses.sun.ac.za/files/services/conferences/annual-student-symposium-2011/11-18nov_benjamin.pdf. Accessed May 8 2013.

Bensah EC, Brew-Hammond A. Biogas technology dissemination in Ghana: history, current status, future prospects, and policy significance. International Journal of Energy and Environment 2010;1(2); 277-94.

Bilba K, Arsene M, Ouensanga A. Study of banana and coconut fibers, Bioresource Technology 2007; 98(1); 58-68.

Biomass Energy Europe (2010). Status of Biomass Resource Assessments Version 3. Available online from http://www.eu-bee.info/GetItem.asp?item=digistorefile;247973;837&params=open;gallery. Accessed May 8 2013.

BP (British Petroleum) 2012. Excel workbook with historical data. Available from http://www.bp.com/extendedsectiongenericarticle.do?categoryId=9041229&contentId=7075080. Accessed March 14 2013.

Cai J, Liu R, Deng C. An assessment of biomass resources availability in Shanghai: 2005 analysis. Renewable and Sustainable Energy Reviews 2008; 12(7); 1997–2004.

Chen Y, Sharma-Shivappa RR, Keshwani D, Chen C. Potential of Agricultural Residues and Hay for Bioethanol Production, Appl Biochem Biotechnol 2007; 142(1); 276–90.

Dasappa S. Potential of biomass energy for electricity generation in sub-Saharan Africa, Energy for Sustainable Development 2011; 15; 203-13.

Duku MH, Gu S, Hagan EB. A comprehensive review of biomass resources and biofuels potential in Ghana. Renewable and Sustainable Energy Reviews 2011; 15(1); 404–15.

Dung NNX, Manh LH, Uden P. Tropical fibre sources for pigs -digestibility, digesta retention and estimation of fibre digestibility in vitro 1996. Animal Feed Science and Technology 2002; 102(1-4); 109-24.

Edjekumhene I, Agyemang PO, Edze P. Ghana's emerging oil industry: What Stakeholders need to know. Kumasi Institute of Technology, Energy and Environment 2010; Available online from http://kiteonline.net/images/downloads/emerog.pdf. Accessed February 8 2013

Energy Commission. Draft Bioenergy Policy of Ghana. Energy Commission of Ghana 2011a.

Energy Commission. National Energy Statistics – 2000-2010. Energy Commission of Ghana 2011b.

Energy Commission. Renewable energy policy framework for climate change mitigation in Ghana: data for the development of GIS maps, alternative business development scenarios, and toolkit for renewable energy planning and policy. Energy Commission of Ghana 2010.

Energy Commission. Strategic National Energy Plan 2006-2020: Main Report. Energy Commission of Ghana 2006. Available online from http://www.energycom.gov.gh/old/pgs/reports.php. Accessed December 15 2012.

EPFL.École polytechnique fédérale de Lausanne. RSB Impact Assessment Guidelines. 2011. Available online from http://rsb.org/sustainability/rsb-tools-guidelines/. Accessed April 19 2013.

EU Directive on the promotion of the use of energy from renewable sources. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009. Official Journal of the European Union 2009.

EU. Proposal for a Directive of the European Parliament and of the Council amending Directive 98/70/EC relating to the quality of petrol and diesel fuels and amending Directive 2009/28/EC on the promotion of the use of energy from renewable sources, 2012. Available online from http://ec.europa.eu/energy/renewables/biofuels/doc/biofuels/com_2012_0595_en.pdf. Accessed April 19 2013.

European Commission - Joint Research Centre - Institute for Environment and Sustainability. International Reference Life Cycle Data System (ILCD) Handbook - General guide for Life Cycle Assessment - Detailed guidance. First edition March 2010. EUR 24708 EN. Publications Office of the European Union, Luxembourg 2010.

FAO. Energy conservation in the mechanical forest industries. Food and Agriculture Organisation of the United Nations, FAO Forestry Paper 1990; 93.

FAO. Global Forest Resources Assessment. Food and Agriculture Organisation of the United Nations. FAO Forestry Paper 163, 2010.

FAO. Ghana Timber Production 2012. Available online from http://faostat3.fao.org/home/index.html#DOWNLOAD. Accessed June 5 2012.

Fernandes U, Costa M. Potential of biomass residues for energy production and utilization in a region of Portugal, Biomass and Bioenergy 2010;34;661-666

Figueiró GG, Graciolli LA. Influência da composiçãoquímica do substrato no cultivo de Pleurotusflorida, Ciência e Agrotecnologia, 2011; 35(5); 924-30.

Fobil JN, Carboo D, Armah NA. Evaluation of municipal solid wastes (MSW) for utilisation in energy production in developing countries, Int. J. Environmental Technology and Management, 2005;5(1); 76-86.

Garlock RJ, Chundawat SPS, Balan V, Dale BE. Optimizing harvest of corn stover fractions based on overall sugar yields following ammonia fiber expansion pretreatment and enzymatic hydrolysis, Biotechnology for Biofuels 2009;2(2); 29.

Giampietro, M., Mayumi, K. The Biofuels Delusion: The Fallacy of Large-Scale Agro-Biofuels Production. Earthscan. London, UK. 2009.

Giampietro, M. Ulgiati, S. Integrated Assessment of Large-Scale Biofuel Production, Critical Reviews in Plant Sciences 2005;24(5); 365-84.

Gopalakrishnan, G., Negri, M. C., Wang, M., Wu, M., Snyder, S. W., Lafreniere, L. Biofuels, land, and water: A systems approach to sustainability. Environmental Science & Technology, 2009;43(15), 6094-6100.

GRIDCO (Ghana Grid Company Limited). Ghana Transmissions and Distribution Master Plan. Ghana Grid Company Study, 2011

Gunaseelan VN. Anaerobic digestion of biomass for methane production: a review, Biomass and Bioenergy 1997;13(1); 83-114.

Gustavsson L, Holmberg J, Dornburg V, Sathre R, Eggers T, Mahapatra K, Marland G. Using biomass for climate change mitigation and oil use reduction Energy Policy 2007; 35(11); 5671-91.

Hirsch, RL, Bezdek, R; Wendling, R. Peaking Of World Oil Production: Impacts, Mitigation, & Risk Management, Science Applications International Corporation/U.S.Department of Energy, National Energy Technology Laboratory 2005.

IPCC (Intergovernmental Panel on Climate Change). IPCC Working Groups contributions to the Fourth Assessment Report (AR4) 2007. Available online from http://www.ipcc.ch. Accessed 19 March 2013.

IEA (International Energy Agency). Sustainable production of second-generation biofuels: potential and perspectives in major economies and developing countries. Information paper, Paris, France, 2010.

IEA (International Energy Agency). World Energy Outlook 2012. Available online from http://www.iea.org/. Accessed March 14 2013.

IEA Bioenergy. From 1st- to 2nd- generation biofuel technologies: an overview of current industry and R&D activities. IEA Bioenergy, Paris, France, 2008.

James R, Eastridge ML, Brown LC, Elder KH, Foster SS, Hoorman JJ, et al. Ohio Livestock Manure Management Guide, 2006.

Jekayinfa SO, Scholz V. Potential Availability of Energetically Usable Crop Residues in Nigeria, Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 2009; 31(8); 687-97.

Junfeng L, Runqing H,Yanqin S, Jingli S, Bhattacharya SC, Abdul Salam P. Assessment of sustainable energy potential of non-plantation biomass resources in China. Biomass and Bioenergy 2005; 29; 167–77.

Jung SH, Kang BS, Kim JS. Production of bio-oil from rice straw and bamboo sawdust. J. Anal. Appl. Pyrolysis 2008; 82; 240-7.

Kartha S, Larson ED. Bioenergy Primer: Modernised Biomass Energy for Sustainable Development. UNDP 2000. Available online from http://www.undp.org/content/dam/aplaws/publication/en/publications/environment-energy/www-ee-library/sustainable-energy/bioenergy-primer-modernised-biomass-energy-for-sustainable-development/Bioenergy%20Primer_2000.pdf. Accessed March 19 2013.

Kataki R, Konwer D. Fuelwood characteristics of some indigenous woody species of north-east India; Biomass & Bioenergy 2001; 20(2); 17-23.

KITE (Kumasi Institute of Technology, Energy and Environment). Feasibility Study Report on Domestic Biogas in Ghana – Report Submitted by Kumasi Institute of Technology, Energy and Environment (KITE) to the Shell Foundation 2008.

KITE (Kumasi Institute of Technology, Energy and Environment) Biomass Energy for Rural Development: An Assessment of the Feasibility of Using Agricultural Residues and Municipal Wastes to Produce Modern Energy Services in Selected Communities in the Eastern Region of Ghana. Final Report Submitted to American Physical Society 2009.

Kludze H, Deen B, Weersink A, Van Acker R, Janovicek K, De Laporte A. Assessment of the availability of agricultural biomass for heat and energy production in Ontario. A Report for the Ontario Ministry of Agriculture, Food and Rural Affairs 2010. Available online from http://www.uoguelph.ca/plant/research/agronomy/publications/pdf/Ontario_Biomass_Availability_Project_Final_Report_2011.01.27.pdf. Accessed March 19 2013.

Koopmans A, Koppejan J. Agricultural and forest residues - generation, utilization and availability. Paper presented at the Regional Consultation on Modern Applications of Biomass Energy, Kuala Lumpur 1997.

Leemhuis RJ, de Jong RM. Biomassa: biochemischesamenstelling en conversiemethoden (confidential report, in Dutch), Petten, ECN, ECN 7.2072-GR 21997; 16.

Lemke RL, Van den Bygaart AJ, Campbell CA, Lafond GP, Grant B. Crop residue removal and fertilizer N: effects on soil organic carbon in a long-term crop rotation experiment on a Udic Boroll. AgrEcosyst Environ 2010; 135; 42–51.

Madhukara K, Srilatha HR, Srinath K, Bharathi K, KrishnaN. Production of methane from green pea shells in floating dome digesters, Process Biochemistry 1997; 32(6); 509-13.

Markandya, A., Halsnaes, K. (Eds.). Climate Change & Sustainable Development. Earthscan. London, UK. 2002.

Martín C, Lopez Y, Plasencia Y, Hernandez E. Characterisation of Agricultural and Agro-Industrial Residues, Chem. Biochem. Eng. Q. 2006; 20(4); 443–7.

Milbrandt A. Assessment of Biomass. Resources in Liberia. Prepared for the U.S. Agency for International Development (USAID) under the Liberia Energy Assistance Program (LEAP).Technical Report NREL/TP-6A2-44808 2009.

Ministry of Energy. Renewable energy policy and regulatory framework and renewable energy law for Ghana – electricity generation. Volume 1, Ministry of Energy, Accra, Ghana, 2012.

MOFA. Agriculture in Ghana: Facts and Figures 2010. Ministry of Food and Agriculture, Government of Ghana. 2011.

Mohammed YS, Mokhtar AS, Bashir N, Saidur R. An overview of agricultural biomass for decentralized rural energy in Ghana. Renewable and Sustainable Energy Reviews 2013 ;20; 15-22

Mtui GYS. Oxalic acid pretreatment, fungal enzymatic saccharification and fermentation of maize residues to ethanol. African Journal of Biotechnology 2012; 11(4); 843-51.

Mtui G, Nakamura Y. Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam, Tanzania. Biodegradation, 2005; 16(6); 493-9

Murphy DJ, Hall CAS, Dale M, Cleveland C. Order from Chaos: A Preliminary Protocol for Determining the EROI of Fuels. Sustainability 2011; 3(10); 1888–1907.

Lamers P, Hamelinck C, Junginger M, Faaij A. International bioenergy trade—A review of past developments in the liquid biofuel market. Renewable and Sustainable Energy Reviews 2011; 15; 2655–2676.

Razvigorova M, Goranova M, Minkova V,Cerny J. On the composition of volatiles evolved during the production of carbon adsorbents from vegetable wastes. Fuel 1993; 73(11); 1718-22.

Richard T, Trautmann N. Substrate composition table 2006, Cornell University Ithaca, NY. Available online from http://compost.css.cornell.edu/calc/lignin.noframes.html. Accessed November 27 2012.

Samahadthai P, Vityakon P, Saenjan P. Effects of different quality plant residues on soil carbon accumulation and aggregate formation in a tropical sandy soil in northeast Thailand as revealed by a 10-year field experiment, Land Degrad. Develop 2010;21(5); 463–73.

Smeets E, Faaij A. Lewandowski I. A quickscan of global bioenergy potentials to 2050. An analysis of the regional availability of biomass resources for export in relation to the underlying factors. Report NWS-E-2004-109, Utrecht University, Netherlands 2004. Available online from http://www.riaed.net/IMG/pdf/A_quickscan_of_global_bioenergy_potentials_to_2050.pdf. Accessed March 19 2013

Smeets E, Faaij A, Lewandowski I, Turkenburg W. A bottom-up assessment and review of global bio-energy potentials to 2050, Prog. Energ. Combust. 2007; 33; 56–106

Shu-Lai M, Antal MJ. Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water. Ind. Eng. Chem. Res. 1992; 31(4); 1157-61.

Thomsen ST, Schmidt JE. 2nd Generation Biofuel Production from Selected Agricultural Waste Resources in Ghana, Poster presented at the Conference of Advanced Biofuels in a Biorefinery Approach, Copenhagen, Denmark, February 28 - March 1, 2012.

UNCTAD. Biofuel production technologies: status, prospects and implications for trade and development. UNCTAD/DITC/TED/2007/10, 2008.

US Government. Energy Independence and Security Act of 2007. Public Law 2007; 110–40.

Vadiveloo J, Fadel JG. Compositional analyses and rumen degradability of selected tropical feeds. Animal Feed Science and Technology 1992; 37(3-4); 265-79.

Zheng YH, Li ZF, Feng SF, Lucas M, Wu GL, Li Y. Biomass energy utilization in rural areas may contribute to alleviating energy crisis and global warming: A case study in a typical agro-village of Shandong, China. Renew Sust Energ Rev 2010; 14(9); 3132–9.

Appendix

Compositional data used for calculating y_{Buswel} and the references for the data. ^{F} indicates a field based residue while ^{P} indicates a processing residue.

Crop	**Residue**	**c_{glu}**	**c_{hem}**	**Source**
		g/100g TS (starch)	**g/100g TS**	
Maize	Stalks^F	36.8	27.4	Amaducci et al., 2000
	Husks^P	35.3	21.8	Garlock et al., 2009
	Cobs^P	34.0	49.1	Figueiró et al., 2011
Rice	Straw^F	37.8	25.3	Jung et al., 2008
	Husks^P	31.3	24.3	Leemhuis and de Jong, 1997
Millet	Stalks^F	26.9	16.5	Chen et al., 2007
Sorghum	Straw^F	41.6	26.7	Amaducci et al., 2000
Groundnut	Shells^P	35.7	18.7	Leemhuis and de Jong, 1997
	Straw^P	37.2	n.a.	Samahadthai et al., 2010
Cowpea	Shells^P	7.6	7.8	Madhukara et al., 1997
Cassava	Stalk^F	33.0	13.0	Martín et al., 2006
	Peelings^P	55.5 (50.0)	22.0	Aderemi and Nworgu, 2007
Plantain	Trunks and leaves^F	34.0	17.0	Thomsen and Schmidt, 2012
Soybean	Straw and pods^F	38.0	16.0	Richard and Trautmann, 1996
Yam	Straw^F	25.0	15.0	Estimate
Cocoyam	Straw^F	25.0	15.0	Estimate
Sweet potato	Straw^F	19.8	14.6	Dung et al., 2002
Oil palm	EFB^P	30.5	29.9	Shamsudin et al., 2010
	Kernel shells^P	45.4	21.7	Zhuang et al., 2009
	Fibre^P	15.7	19.1	Vadiveloo and Fadel, 1992
Coconut	Husks^P	21.2	12.7	Bilba et al., 2007
	Shells^P	20.0	48.8	Razvigorova et al., 1993
Sugarcane	Leaves^F	32.0	17.0	Mellows et al., 1993
	Bagasse^P	36.0	17.0	Shu Lai and Antal, 1992
Cotton	Stalks^F	42.0	12.0	Leemhuis and de Jong, 1997
Cocoa	Pods^F	10.7	8.5	Vadiveloo and Fadel, 1992
Forestry	Slabs	58	8	Energy Commission, 2010
	Wane	58	8	Energy Commission, 2010
	Bark	21	17	Energy Commission, 2010
	Sawdust	40	12	Energy Commission, 2010

Paper II

In-text citation:
(Thomsen et al., 2014b, Paper II)

Authors:
Thomsen S.T., Kádár Z., Schmidt J.E.

Title:
Compositional analysis and theoretical biofuel potentials from various West African agricultural residues

Publication status:
First submitted on January 7 2013 to *Biomass & Bioenergy*, now submitted after review

Compositional analysis and projected biofuel potentials from common West African agricultural residues

Sune Tjalfe Thomsen*, Zsófia Kádár, Jens Ejbye Schmidt†

Department of Chemical and Biochemical Engineering, Technical University of Denmark, DTU, DK-2800 Kgs. Lyngby, Denmark

Keywords: West Africa, biomass, residue, composition, bioethanol, biogas

Highlights:

- A complete biomass balance for 13 West African agricultural residues were completed
- Several of the residues were analysed for the first time
- Theoretical bioethanol potentials based on starch, C6- and C5 sugars were estimated
- Theoretical maximum biogas potentials were estimated
- Recommendations of optimal utilisation of West African biomasses were presented

Abstract

In recent years the focus on sustainable biofuel production from agricultural residues has increased considerably. However, the scientific work within this field has predominantly been concentrated upon bioresources from industrialised and newly industrialised countries, while analyses of the residues from most developing countries remain sparse. In this study the theoretical bioenergy potentials (bioethanol and biogas) of a spectrum of West African agricultural residues was estimated based on their compositions. We analysed 13 of the most common residues: yam peelings, cassava peelings, cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches. The yam peelings showed the highest methane and bioethanol potentials, with 439 L methane (kg Total Solids)$^{-1}$ and 0.61 L bioethanol (kg TS)$^{-1}$ based on starch and cellulose alone due to their high starch content and low content of un-biodegradable lignin and ash. A complete biomass balance was done for each of the 13 residues, providing a basis for further research into the production of biofuels or biorefining from West African agricultural residues.

---

* Corresponding author, Tel.: +45 2132 5181; Fax +45 4677 4109
Email addresses: sunt@kt.dtu.dk (S. Thomsen), zska@kt.dtu.dk (Z. Kádár), jschmidt@masdar.ac.ae (J. E. Schmidt)
† Present address: Institute Center for Energy – iEnergy, Masdar Institute for Science and Technology, PO Box 54224, Abu Dhabi, United Arab Emirates

1 Introduction

Following the 2007 report from the Intergovernmental Panel on Climate Change (IPCC) [1], the role of the greenhouse gas (GHG) effect on the climate has been widely accepted in the scientific community [2, 3]. At the same time, increasing fuel prices have massively increased the focus on energy security and energy independence globally, regionally, and locally [4-6]. One of the responses to the above-mentioned issues is increased focus on sustainable biofuels, since biofuel production can potentially reduce GHG emissions and yield energy.

The biofuels policies of many countries, currently fail to satisfy ethical principles of, e.g. environmental sustainability and equitable distribution of costs and benefits among stakeholders [7]. In that sense, these policies are still counter-constructive in terms of the most obvious environmental goals. However, there are other incentives for encouraging biofuels production besides its potential as a source of GHG neutral energy. Ejigu (2008) [8] found that in an African context modern bioenergy offers tremendous opportunities to meet growing household energy demands, increase income, reduce poverty, and mitigate environmental degradation. Mangoyana way of achieving sustainable development that is less land intensive, has positive net energy gains and environmental benefits, and provides local socio-economic benefits. However, despite the wide interest in biomass for energy production, scaling up experimental projects to commercial operations is far from easy, to a certain extent due to lack of investment capital but also due to lack of interdisciplinary approaches that account for the specific dynamics and interrelationships between environmental and socio-economic systems of the West African context [10]. A thorough knowledge of agricultural residues that can be used for biofuel estimations is a necessary starting point, in order to reveal such dynamics and interrelationships.

1.1 Residues from West Africa

Dealing with bioenergy from a scientific approach, the main focus has until now been on residues from industrialised countries or from newly developed countries e.g. China [11-13]. Such well-investigated residues include: maize stalks [14], wheat straw [15], rye straw [16], grasses [17, 18], legumes [19, 20], sugar cane bagasse [21], and rice straw [22]. On the other hand, the agricultural residues most common to developing countries of West Africa have not yet been prioritised.

According to the Food and Agriculture Organisation of the United Nations [23] residues from archetypical West African crop include yam peelings, cassava peelings, cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches (EFB). Of those 13 West African residues, the peelings from yam and cassava are partly used for animal feed, but only the cassava stalks have previously been evaluated for their composition by Martin et al. (2006) [24]. Plantain residues have not yet been assessed. A few studies have addressed a closely related banana residue, but they did not compare it with other residues [25]. Cocoa residues, as well as groundnut straw, have mainly been considered for animal feed and therefore some information required for bioenergy evaluations is missing [26-28]. Oil palm EFB has been studied by several authors, but with large deviations in the published results which justifies improved investigation [29, 30]. Internationally common residues such as maize cobs, maize straw and rice straw have been thoroughly investigated [31, 32], but not on residues originating from West Africa.

1.2 Biomass characterisation and bioenergy potentials

Since the beginning of the last century, great effort have invested in investigating the composition of residues [33, 34]. However, within optimal utilisation of the residues for bioenergy purposes, some features have not yet been scientifically addressed.

Ethanol fermentation is facilitated by pure cultures of microorganisms which metabolises only sugars, and calculating a theoretical bioethanol potential from the composition of the materials depends both on the biomass constituents and on the kind of fermenting microorganism applied. The traditional substrate for ethanol fermentation is free C6 sugars often originating from hydrolysed starch. Since some waste residues are containing free sugars and/or starch this traditional and simple way is investigated. However, present day's bioethanol can be produced as a sustainable fuel based on complex lignocellulosic residues with a wide range of different sugar monomers. Wild-type *Saccharomyces cerevisiae* strains readily ferment glucose, mannose and fructose, galactose as well as the disaccharides sucrose and maltose (C6 sugars), and therefore this has been the organism of choice for centuries [35, 36]. On the other hand, other of the most abundant sugar monomers from biomass D-xylose, L-arabinose, galacturonic acid and L-rhamnose (C5 sugars) requires either extensive metabolic engineering of *S. cerevisiae* [35] or other fermentative organisms such as *Kluyveromyces marxianus* [37], *Zymomonas mobilis* [38], *Pichia stipitis* [37], or the thermophilic anaerobic bacterium *Thermoanaerobacter* [39]. However, the challenge in C5 sugar fermentation is now to successfully transfer strains and concepts from the laboratory to industrial conditions, which opposes multiple challenges [35].

Whereas ethanol fermentation is facilitated by pure cultures of microorganisms, biogas is produced by a mixed natural consortium of microorganisms. Therefore, the anaerobic biological degradation of organic matter to produce biogas is a process found in many anaerobic habitats such as sediments, rice paddies, open manure silos, landfills, waterlogged soils, and in the mammalian gut [40, 41]. The same processes that take place in these habitats can be controlled and utilised to convert various biomass constituent, such as carbohydrates, proteins, lipids, sugar alcohols, fatty acids and more, into methane-rich biogas as a very resource efficient and environmentally friendly way of producing energy [42]. The theoretical biogas yield can be estimated from the biomass constituents of a residue with Buswell's formula [43, 44], or it can be computed from the chemical oxygen demand (COD) of the residues [44].

In this study, we thoroughly analysed 13 archetypical West African agricultural residues and we are presenting complete chemical composition of these residues. In addition, we estimate three different ethanol potentials: (1) based on starch and free sugars, (2) based on all C6 sugars fermentable by *S. cerevisiae*, and (3) a potential if all available sugars are utilised. Furthermore, we estimate theoretical maximum biogas potentials both based on compositional data calculated with Buswell's formula, and based on COD content.

2 Materials and methods

2.1 Raw materials

The agricultural residues were obtained from the test facilities of the Ghana Crops Research Institute of the Council for Scientific and Industrial Research, in Kumasi, Ghana. The crops were harvested during the first months of 2011 after which the residues were air dried and transported to Denmark.

2.2 Chemical analysis

The residues were knife milled (Retsch SM 2000, Haan, Germany) to pass a 2 mm sieve. The total solids (TS), volatile solids (VS), and ash content were measured using a standard method [46].

Lipophilic extraction was carried out by Soxhlet extraction in a reflux condenser for six hours with 99% ethanol. The total amount of extractives, including volatiles, was defined as the mass of material lost during extraction.

Strong acid hydrolysis was used to determine the carbohydrate content of lipophilic extracted residues: first, the 0.16 g dry biomass was subjected to 1.5 mL strong sulphuric acid (72 w/w%) at 30 °C for one hour; afterwards the remaining polymers were hydrolysed by diluting the samples to 4 w/w% sulphuric acid concentration and autoclaving in at 121 °C for ten minutes. The hydrolysates were then filtered, and the Klason lignin was measured as the dry weight of the filter cake taking the ash content into account. Derived sugars in the hydrolysates were analysed by High Pressure Liquid Chromatography (HPLC) as described in section 2.4.

Starch determination was performed according to the method of Sluiter and Sluiter [47] to classify the different kinds of glucan as either cellulose or starch. Following this method starch was hydrolysed in two phases: in the first phase, the starch was partially hydrolysed and totally solubilised by α-amylase. In the second phase, the starch dextrins were quantitatively hydrolysed to D-glucose by amyloglucosidase. Derived glucose was quantified by HPLC (see section 2.4). The cellulose content of the residues was defined as the glucan content as found by strong acid hydrolysis, subtracted the starch content.

To distinguish between structural carbohydrates and free sugar a water extraction was performed on the raw biomass samples using hot milli-Q water in a Soxhlet extraction with a reflux condenser for 6 hours. The derived sugars were evaluated on HPLC (see section 2.4).

Biomass samples were prepared for protein determination by wet milling in a Mannesmann wet mill (Remscheid, Germany) in a 1 g TS L^{-1} H_2O solution to a particle size of 50 μm. Thereafter, the content of nitrogen bound in protein (protein-N) was estimated by determining the content of total nitrogen in the samples and withdrawing the content of nitrogen bound as ammonia or nitrate. The nitrite content was assumed to be insignificant. The total nitrogen, nitrogen bound as ammonia, and nitrogen bound as nitrate were all measured using different commercially available kits from Hach Lange GmbH (Germany); Total Nitrogen LCK 138, Ammonium-Nitrogen LCK 303, and Nitrate LCK 339 respectively. The protein content was calculated by multiplying the protein-N content with the “nitrogen-to-protein conversion factor” of 5.6 determined by Mariotti et al. [48].

2.3 Chemical Oxygen Demand (COD) determination

Biomass samples were prepared for COD determination by wet milling as described in section 2.2. The COD of the wet-milled biomass samples was determined using commercial available COD kit, LCK 514 from Hach Lange GmbH (Germany).

2.4 High pressure liquid chromatography (HPLC)

Monomeric sugars were analysed on HPLC. Both with a Biorad Aminex HPX-87H column (Hercules, CA; USA) with sugar standard of D-glucose, D-xylose, L-arabinose, L-rhamnose using 4 mM H_2SO_4 as eluent (flow rate 0.6 ml $(min)^{-1}$ and temperature 63°C), and on a Biorad Aminex HPX-87P column (Hercules, CA; USA) using sugar standards of D-galactose, D-mannose and D-fructose, with H_2O as eluent (flow rate 0.5 ml $(min)^{-1}$ and temperature 80°C).

3 Calculations

Estimation of ethanol potentials was based on the carbohydrates content in the residues. Two stoichiometric factors were taken into account; firstly, a hydrolysis factor that describes the hydrolysis of carbohydrate polymers into free sugars, and secondly an ethanol factor that describes the mass fraction of sugar monomer converted to ethanol, cf. Table 1. Using the stoichiometric factors for each of the biomass constituents of a residue, as well as the density of ethanol ($\rho = 789$ g L^{-1}), the bioethanol potentials were calculated.

Table 1: The hydrolysis factor and ethanol factor of the different carbohydrates and the biogas yield of specific biomass constituent as calculated with Buswell’s formula, equation (1) and (2).

Biomass constituent	Sum formula	Hydrolysis factor *g monomer (g polymer)$^{-1}$*	Ethanol factor *g EtOH (g monomer)$^{-1}$*	Biogas yield *L CH_4 (g VS)$^{-1}$*
Cellulose	$C_6H_{12}O_6$	1.111	0.511	0.414
Starch	$C_6H_{12}O_6$	1.111	0.511	0.414
Xylan	$C_5H_{10}O_5$	1.136	0.511	0.423
Arabinan	$C_5H_{10}O_5$	1.136	0.511	0.423
Rhamnan	$C_6H_{12}O_5$	1.123	0.608	0.498
Galactan	$C_6H_{12}O_6$	1.111	0.511	0.414
Mannan	$C_6H_{12}O_6$	1.111	0.511	0.414
Fructose	$C_6H_{12}O_6$	-	0.511	0.373
Lipid	$C_{50}H_{90}O_6$	-	-	1.011
Protein	$C_5H_7O_2N$	-	-	0.495

Even though Buswell's formula was designed for estimating the ultimate methane yield from a biomass based on the sum formula, it can also be used on each of the biomass constituent containing only carbon, hydrogen and oxygen, see Equation (1) [43].

$$C_nH_aO_b + (n - a/4 - b/2)\ H_2O \rightarrow (n/2 + a/8 - b/4)\ CH_4 + (n/2 - a/8 + b/4)\ CO_2 \qquad (1)$$

Since proteins contain nitrogen, the biogas potentials of these cannot be determined by Buswell's formula. Thus, the biogas potentials of the proteins were determined by equation (2) [44].

$$C_5H_7O_2N + 4H_2O + H^+ \rightarrow 2.5CO_2 + 2.5CH_4 + NH_4^+ \qquad (2)$$

Using equation (1) and (2) the biogas potentials of every biomass constituent was calculated, as presented in Table 1. The biogas potentials were estimated by adding the contributions of each biomass constituent, as calculated with Buswell's formula, according to the composition of the agricultural residues. Since lignin cannot be converted by anaerobic digestion the contribution of lignin is not included in the biogas potentials obtained based on Buswell's formula.

Another way of estimating the biogas yield is based on the chemical oxygen demand (COD) content of the material. Since COD is a measure of organic matter in the residues the biogas yield can be stoichiometrically estimated from the COD measurement, where 1 g of COD has maximum methane potential of 0.35 L of CH_4 at standard conditions [44].

Theoretical methods, such as Buswell's formula and the COD method, often over-estimate methane yields since microbial maintenance and substrate biodegradability is not accounted [45]. However, since biodegradability depends highly on varying process parameters, this work will present the theoretical maximum biogas potentials.

4 Results

According to our analysis of West African agricultural residues, a broad compositional diversity was observed, cf. Table 2. Some of the residues such as yam peelings and cassava peelings have high starch contents of 70.1 and 53.1 g (100 g)$^{-1}$ respectively, while other are high in cellulose, e.g. plantain trunks, maize stalks or maize cobs, with 45.6, 37.5, and 35.4 g (100 g)$^{-1}$ respectively. Looking at the total content of hemicellulose carbohydrates (xylan, arabinan, rhamnan, and galactan), maize cobs have the highest content (31.3 g (100 g)$^{-1}$). Lignin content of the examined biomasses differs in a wide range between 4.1-37.2 g (100 g)$^{-1}$. Whereas, the three peelings (yam, cassava and plantain) with high starch content have low lignin content, cassava stalks, cocoa pods, cocoa husks and oil palm EFB all have lignin contents of more than 20 g (100 g)$^{-1}$ on dry basis. Biomasses with high content of total extractives – which is soluble material including waxes, lipids, chlorophyll, and other minor components [49] – are plantain peelings, plantain leaves and cocoa husks, with 18.3, 16.2 and 17.5 g (100 g)$^{-1}$ respectively. Cocoa husk are also high in protein (12.6 g (100 g)$^{-1}$) and so is groundnut straw (9.4 g (100 g)$^{-1}$).

The residuals, which is a measure of how well the mass closure was reached, should preferable be as close to 0 as possible. However plantain peelings and groundnut straw residuals had high residuals of 12.3 and 19.3 g (100 g)$^{-1}$ respectively, which indicates that these biomasses contain biomass constituents that are not detected for in this study such as pectin.

Table 2: Chemical composition of 13 West African agricultural residues.

g (100 g)$^{-1}$	Starch	Cellulose	Xylan	Arabinan	Rhamnan	Galactan	Fructose	Lignin	Ash	Extractives	Protein	Residual
Yam peelings	70.1	5.7	n.d.	0.6	n.d.	n.d.	4.7	4.1	5.1	5.3	3.2	1.2
Cassava peelings	53.1	12.7	n.d.	1.3	0.8	n.d.	3.4	8.2	4.8	7.2	3.0	5.6
Cassava stalks	1.1	33.1	13.7	0.5	n.d.	n.d.	2.8	28.3	4.1	8.9	2.7	4.8
Plantain peelings	26.2	8.0	n.d.	2.6	n.d.	2.8	1.0	10.0	14.3	18.3	4.5	12.3
Plantain trunks	0.6	45.6	9.6	2.6	n.d.	1.6	n.d.	12.4	13.7	10.1	3.2	0.5
Plantain leaves	0.6	21.9	9.0	5.6	1.6	4.1	n.d.	18.3	13.4	16.1	5.6	4.0
Cocoa husks	3.2	12.9	n.d.	1.5	1.7	6.7	n.d.	24.3	11.6	17.5	12.6	8.0
Cocoa pods	0.6	19.1	8.7	1.8	1.7	6.5	n.d.	37.2	12.6	5.7	5.9	0.3
Oil palm EFB	0.5	33.0	22.1	0.6	n.d.	0.3	n.d.	23.8	4.8	6.2	2.9	5.8
Maize cobs	0.7	35.4	31.3	3.5	n.d.	n.d.	n.d.	18.0	1.6	1.7	1.3	6.4
Maize stalks	1.0	37.5	18.8	2.7	n.d.	0.5	n.d.	17.0	11.2	4.2	2.0	5.3
Rice straw	1.4	32.5	17.3	2.5	n.d.	0.6	n.d.	11.3	17.8	4.2	2.8	9.7
Groundnut straw	2.2	18.1	7.7	2.6	1.7	1.7	n.d.	15.4	10.9	10.9	9.4	19.3

All standard deviations were below 5%. Not detected = n.d.

Bioethanol potentials from each of the West African residues are graphically shown on Figure 1. Since the utilisation of the different kind of carbohydrates for bioethanol are on different technological development stages, bioethanol potentials based on different carbohydrates were calculated. First, the potential bioethanol produced from starch and free fructose sugar is shown, since this is the most traditional and simple production method, and here yam peelings and cassava peelings have the highest potentials with 0.56 and 0.43 L ethanol (kg TS)$^{-1}$ respectively. Secondly, the potential ethanol from cellulose and galactan is added since these consist of sugars originating from lignocellulose, which can be metabolised by *S. cerevisiae* in a cellulosic bioethanol production facility. The highest cellulose and galactan based potential was from plantain trunks (0.34 L ethanol (kg TS)$^{-1}$). Last, the potential ethanol from the remaining detected carbohydrates is added to give the maximum theoretical bioethanol yield including a 2nd generation bioethanol production. The highest potentials only using this setup would be from maize cobs with 0.26 L ethanol (kg TS)$^{-1}$. The highest total potentials with all available sugars summarised are from yam peelings, cassava peelings and maize cobs with 0.61, 0.53 and 0.52 L ethanol (kg TS)$^{-1}$ respectively.

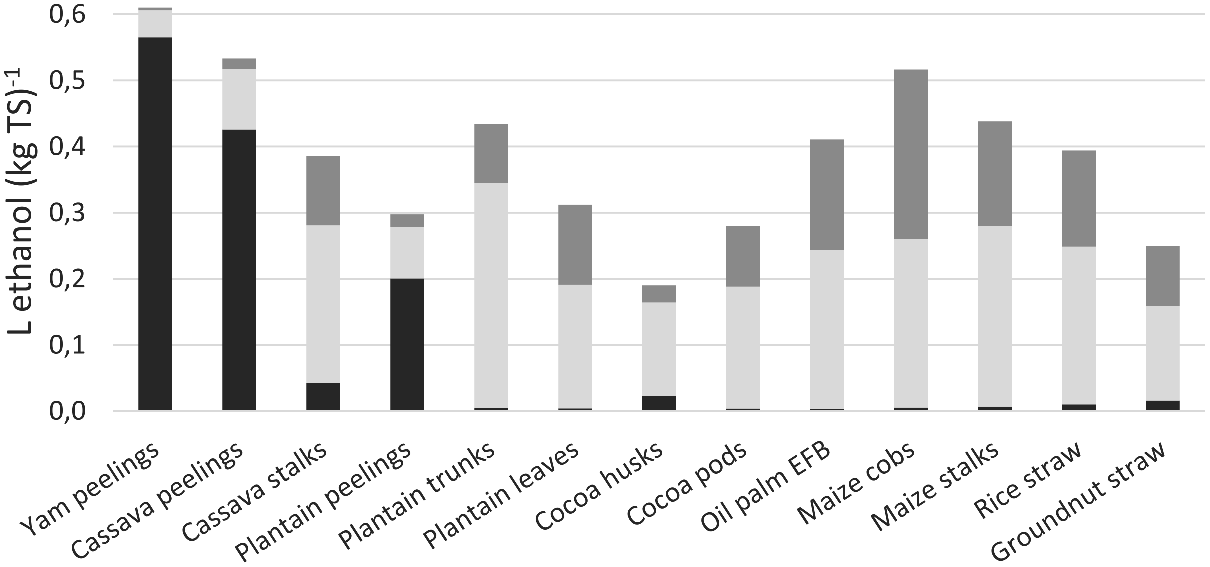

Figure 1: Theoretical bioethanol potential of 13 West African agricultural residues based on; only starch and free sugar content (black), the combined C6 sugar content fermentable by S. cerevisiae (light grey), or all potential sugars including C5 sugars (dark grey).

Table 3: Volatile solids (VS) and the chemical oxygen demand (COD) as a fraction of TS of West African agricultural residues.

	VS g VS (g TS)$^{-1}$	COD g COD (g TS)$^{-1}$
Yam peelings	0.948	1.159
Cassava peelings	0.957	1.149
Cassava stalks	0.949	1.303
Plantain peelings	0.852	1.141
Plantain trunks	0.792	0.976
Plantain leaves	0.881	1.046
Cocoa husks	0.908	1.290
Cocoa pods	0.880	1.217
Oil palm EFB	0.939	1.267
Maize cobs	0.985	1.234
Maize stalks	0.852	1.097
Rice straw	0.790	1.014
Groundnut straw	0.887	1.147

All standard deviations were below 5%.

As an alternative way of utilising the residues for energy, biogas was also investigated and potentials were calculated. For that purpose both volatile solids (VS) and COD were determined since there are both indicators of how much organic material is available. In Table 3 the VS and COD of the West African agricultural residues are presented. The biomass with the highest COD was cassava stalks with 1.303 g COD (g TS)$^{-1}$.

From the results presented in Table 2 and Table 3 the maximum biogas potentials of the West African agricultural residues have been calculated, cf. Figure 2, according to two different methods based on either Buswell's formula or COD, as described detailed at section 3. According to results obtained with Buswell's formula, Yam peelings and cassava peelings have the highest biogas potentials with 403 and 382 L CH_4 (kg TS)$^{-1}$ respectively. However, by means of the COD based method the highest biogas potentials are cassava stalks and coca husks with 427 and 426 L CH_4 (kg TS)$^{-1}$ respectively. A common trait is that the biogas potentials which are based on COD are higher than the ones calculated with the Buswell formula; this is especially the case when the residues are high in lignin, as e.g. cocoa pods where the COD based biogas potential is 58% higher than calculated with Buswell's formula.

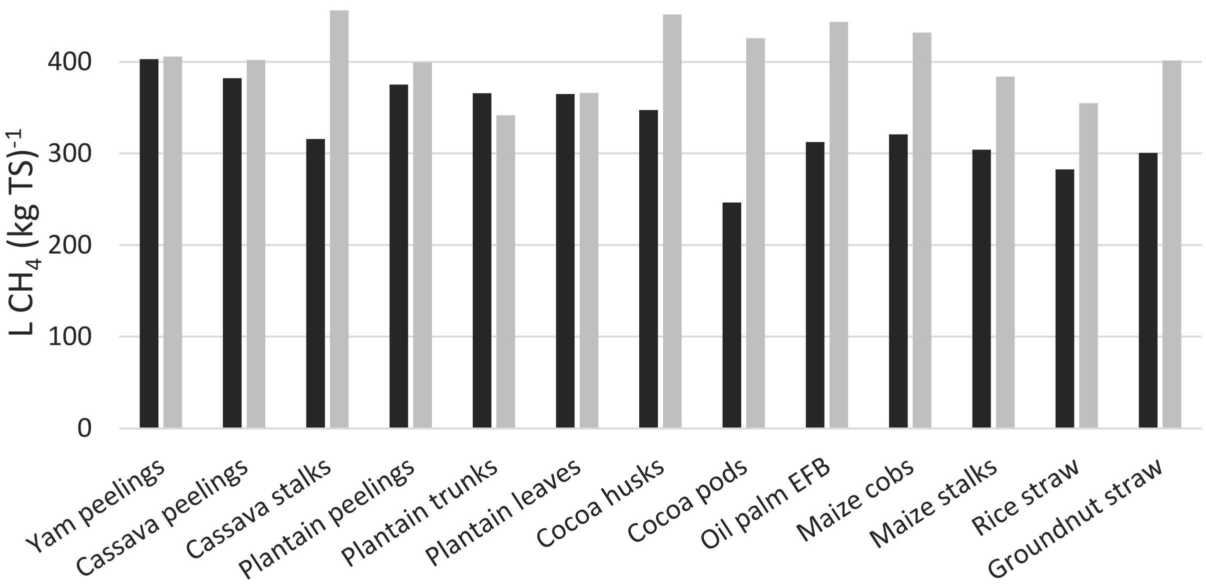

Figure 2: The theoretical biogas potentials of the West African agricultural residues calculated with Buswell's formula (black columns) or from COD (grey columns) respectively.

5 Discussion

13 archetypical West African agricultural residues were analysed and in all cases a comprehensive picture of the biomass constituents was achieved. Yam peelings, cassava peelings, plantain residues, cocoa residues and groundnut straw, were analysed in the context of

bioenergy for the first time, and this study show that especially the peelings have high potentials for both bioethanol and biogas production.

When comparing composition of residues with previously published work, our results are in line with these results. E.g. cassava stalks has been analysed earlier by Martin et al. (2006) and their results of 33 g (100 g)$^{-1}$ cellulose, 13 g (100 g)$^{-1}$ hemicellulose and 25 g (100 g)$^{-1}$ lignin are similar with our findings of 33.1 g cellulose (100 g TS)$^{-1}$, 14.3 g hemicellulose (100 g TS)$^{-1}$ and 28.3 g lignin (100 g TS)$^{-1}$. Also the composition of internationally common residues such as maize cobs, maize straw and rice straw are similar to previously published results, where small differences can be explained by factors like: differences in crop varieties, meteorological differences, seasonal changes, harvest maturity and agricultural practises unique to a West African context. It should be noted that the analysis of West African agricultural residues addressed in this work was made on only one biomass sample for each residue; thereby the above mentioned factors are not addressed in this work. As stated in section 1.1 large differences in published compositional data on oil palm EFB exists. The presented results reveals 33.1 g (100 g)$^{-1}$ cellulose, which is comparable to the 37.9 g (100 g)$^{-1}$ presented by Shibata et al. (2008) [30], but not with the relatively high 59.7 g (100 g)$^{-1}$ reported by Abdullah et al. (2011) [29].

The compositional data have laid basis for projecting theoretical potentials of bioethanol and biogas from the residues, and as expected the residues are different, which is reflected in the bioenergy potentials as well. Especially for potential bioethanol the high starch content of the yam and cassava peelings is advantageous, and gives rise to high bioethanol potentials of 0.61 and 0.53 L ethanol (kg TS)$^{-1}$ respectively, where 0.56 and 0.43 L ethanol (kg TS)$^{-1}$ respectively are based on starch and free sugars only (1stgeneration bioethanol). The composition of yam and cassava peeling resembles the composition of well-known residue from tempered regions; potato peelings, which also have a high starch content (52.1 g starch (100 g TS)$^{-1}$) with ethanol potential similar to cassava peelings [50]. Potato peelings have been among one of the first agricultural residues to be utilised for bioenergy purposes in industrialised countries, yam and cassava peelings seem to hold similar potentials among West African agricultural residues.

Also other residues, such as maize cobs have high potentials if all the lignocellulosic carbohydrates (cellulose and hemicelluloses) are utilised. The estimated bioethanol potentials are theoretical maxima, which in many cases will be difficult to achieve in reality, especially at more lignified residues, due to the recalcitrance of these residues. In order to make this material more accessible for enzymatic attacks a pretreatment step is necessary to open up the structure. Pretreatment is an essential step in the lignocelluloses based ethanol production process, and methods like hydrothermal pretreatment, wet oxidation, ammonia explosion and acid hydrolysis are the most common [18-20, 51]. Even though the recalcitrance will have an impact on the actual potentials, the presented results are still giving insights into the differences between the residues, thus providing a basis for choosing the most promising bioethanol candidates. How to pretreat the different biomasses are related to the composition and should be evaluated in future studies.

The maximum biogas potentials calculated with Buswell's formula or from COD measurements differ a lot. The biggest differences between the two methods are in the residues with high lignin content. This can be explained, since the COD method is measuring all organic carbon – including the lignin. Lignin is a chemically reduced compound, thus giving rise to a relatively high COD content per mass of biomass constituent. This is a drawback of the COD method since lignin cannot be converted in anaerobic digestion. Therefore, biomasses with high lignin content, such as cassava stalks, cocoa husks, cocoa pods, oil palm EFB, and rice straw, the COD based method are expected to overestimate the maximum biogas potential. The method based on Buswell's formula only includes biomass constituents which are degradable in anaerobic degradation, thus lignin contents are not contributing to the biogas potential. However, also with anaerobic digestion recalcitrance of the residues, which is correlated with lignin content, is influencing the yield. This is not accounted for in the presented results. Since there is such a big deviation between biogas potentials calculated with either Buswell's formula or from COD, and since recalcitrance is not taken into account, it is advisable to make practical biogas potential tests of specific residues in future studies.

Cocoa pods have an unusual high lignin content of 37.2 g (100 g)$^{-1}$ which courses quite low potentials of bioethanol, and low biogas potentials estimated with the method based on Buswell's formula. Thus it might be more attractive to incinerate or thermally gasify cocoa pods, even though some the valuable nutrients would be lost, which is not the case with bioethanol or biogas production where nitrogen, phosphor and other remaining nutrients are left in the residual after the bioenergy production [52].

The presented results do not only have relevance in the field of biofuels. Also in other fields of research the compositional data can provide new insights, e.g. production of building block chemicals such as lactic acid, or when searching for residues with high content of a specific biomass constituent for biorefining, e.g. galactan which is high in plantain leaves.

6 Conclusion

For the first time a wide array of West African agricultural residues have been thoroughly compositional analysed and bioenergy potentials have been presented. The 13 different Ghanaian residues have diverse compositions, thus different bioenergy potentials. The highest bioethanol potentials was from yam peelings due to a low content of lignin and ash and a high content of starch, here 0.61 L ethanol (kg TS)$^{-1}$ could be achieved, which is more than 3 times as much as the lowest potential found in cocoa husks. However, the results did not take potential need for pretreatment into account.

With respect to biogas, maximum theoretical potentials were presented and peelings of yam, cassava and plantain were most promising. However, it became apparent that theoretical potentials cannot stand alone, they need a correlation with practical biogas potential test since the present methods of calculating the theoretical biogas potentials – Buswell formula and the COD – are providing quite different results, and since these methods are not taking the biomass recalcitrance into account.

An evaluation of how to best utilise West African biomasses is outside the scope of this study, however, we will provide a few general recommendations: (1) For producing ethanol starchy residues such as yam and cassava peelings are preferable as it is easiest to process with current technologies, and since they have the highest potential yields. (2) When cellulose based ethanol is becoming a valid business opportunity plantain trunks, maize stalks, maize cobs, cassava stalks, oil palm EFB, and rice straw are the most interesting possibilities. (3) In longer terms, when all carbohydrates can be utilised for ethanol, maize cobs are advantageous compared to all other West African biomass residues except yam and cassava peelings. (4) With all residues it is possible to make biogas and for the non-starchy residues it will be the method of choice at least until the new bioethanol technologies are getting mature. If low-tech solutions are needed, biogas will be advantageous to ethanol; however, with very lignified biomasses such as cocoa pods combustion might be a better alternative. (5) Only utilise residues if they are not already utilised for food or feed purposes or for insuring sustained soil fertility.

8 Acknowledgements

This work was supported with a grant from Danida Fellowship Centre (DFC) of the Danish Ministry of Foreign Affairs, as a part of the project “Biofuel production from lignocellulosic materials – 2GBIONRG”, DFC journal no. 10-018RISØ. Furthermore the authors would like to thank Annette Eva Jensen for technical assistance and Dr Moses Mensah from the Kwame Nkrumah University of Science and Technology, Kumasi Ghana and Dr Edward Yeboah from the Council for Scientific and Industrial Research, Kumasi Ghana for assisting in providing residues.

9 References

[1] Bernstein L, Bosch P, Canziani O, Chen Z, Christ R, Davidson O, et al. Climate Change 2007: Synthesis Report - An Assessment of the Intergovernmental Panel on Climate Change (IPCC) 2007.

[2] Bjurström A, Polk M. Physical and economic bias in climate change research: a scientometric study of IPCC Third Assessment Report. Climatic Change 2011; 108: 1-22.

[3] Schneider SH. Science as a Contact Sport: Inside the Battle to Save Earth's Climate. 1st ed: National Geographic; 2009.

[4] International EA, IEA, OECD. World energy outlook 2010. Paris: International Energy Agency; 2010.

[5] Bazilian M, Hobbs BF, Blyth W, MacGill I, Howells M. Interactions between energy security and climate change: A focus on developing countries. Energy Policy 2011; 39: 3750-6.

[6] Antwi E, Bensah EC, Quansah DA, Arthur R, Ahiekpor J. Ghana's biofuels policy: challenges and the way forward. Int J Energy Environ (Iraq) 2010; 1: 805-14.

[7] Buyx A, Tait J. Ethical Framework for Biofuels. Science 2011; 332: 540-1.

[8] Ejigu M. Toward energy and livelihoods security in Africa: Smallholder production and processing of bioenergy as a strategy. Natural Resources Forum 2008; 32: 152-62.

[9] Mangoyana RB. Bioenergy for sustainable development: An African context. Phys Chem Earth 2009; 34: 59-64.

[10] Gilbert N. Local benefits: The seeds of an economy. Nature 2011; 474: S18-9.

[11] Hellmann F, Verburg PH. Spatially explicit modelling of biofuel crops in Europe. Biomass Bioenerg 2011; 35: 2411-24.

[12] Pakarinen A, Maijala P, Stoddard FL, Santanen A, Tuomainen P, Kymalainen M, et al. Evaluation of annual bioenergy crops in the boreal zone for biogas and ethanol production. Biomass Bioenerg 2011; 35: 3071-8.

[13] Mabee WE, Mirck J. A Regional Evaluation of Potential Bioenergy Production Pathways in Eastern Ontario, Canada. Ann Assoc Am Geogr 2011; 101: 897-906.

[14] Xu J, Thomsen MH, Thomsen AB. Investigation of acetic acid-catalyzed hydrothermal pretreatment on corn stover. Appl Microbiol Biotechnol 2010; 86: 509-16.

[15] Maas RHW, Bakker RR, de Jong E, Weusthuis RA, Boersma AR, Pels JR, et al. Pilot-scale conversion of lime-treated wheat straw into bioethanol: quality assessment of bioethanol and valorization of side streams by anaerobic digestion and combustion. Biotechnol Biofuels 2008; 1: 1-13.

[16] Ingram T, Rogalinski T, Bockemühl V, Antranikian G, Brunner G. Semi-continuous liquid hot water pretreatment of rye straw. The Journal of Supercritical Fluids 2009; 48: 238-46.

[17] Humphreys J, O'Kiely P. Effects of two mixtures of perennial ryegrass cultivars with contrasting heading dates, and differing in spring-grazing frequency and silage harvest date, on characteristics of silage from first-cut swards. Grass Forage Sci 2007; 62: 389-404.

[18] Redding AP, Wang Z, Keshwani DR, Cheng JJ. High temperature dilute acid pretreatment of coastal Bermuda grass for enzymatic hydrolysis. Bioresour Technol 2011; 102: 1415-24.

[19] Thomsen ST, Jensen M, Schmidt JE. Production of 2nd generation bioethanol from lucerne - Optimization of hydrothermal pretreatment. BioResour 2012; 7: 1582-93.

[20] Martin C, Thomsen MH, Hauggaard-Nielsen H, BelindaThomsen A. Wet oxidation pretreatment, enzymatic hydrolysis and simultaneous saccharification and fermentation of clover-ryegrass mixtures. Bioresource technology 2008; 99: 8777-82.

[21] Martin C, Klinke HB, Thomsen AB. Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse 2007; 40: 426-32.

[22] Chang K, Thitikorn-amorn J, Hsieh J, Ou B, Chen S, Ratanakhanokchai K, et al. Enhanced enzymatic conversion with freeze pretreatment of rice straw. Biomass Bioenerg 2011; 35: 90-5.

[23] FAOSTAT- The Statistics Division of Food and Agriculture Organization of the United Nations. Available online at: www.faostat3.fao.org. Assessed March 2, 2012.

[24] Martin C, Lopez Y, Plasencia Y, Hernandez E. Characterisation of agricultural and agro-industrial residues as raw materials for ethanol production. Chemical and Biochemical Engineering Quarterly 2006; 20: 443-7.

[25] Li K, Fu S, Zhan H, Zhan Y, Lucia LA, Lucia LA. Analysis of the chemical composition and morphological structure of banana pseudo-stem. BioResour 2010; 5: 576-85.

[26] Donkoh A, Atuahene CC, Wilson BN, Adomako D. Chemical composition of cocoa pod husk and its effect on growth and food efficiency in broiler chicks. Anim Feed Sci Technol 1991; 35: 161-70.

[27] Aregheore EM. Chemical Evaluation and Digestibility of Cocoa (Theobroma cacao) Byproducts Fed to Goats. Tropical Animal Health and Production 2002; 34: 339-48.

[28] Larbi A, Dung DD, Olorunju PE, Smith JW, Tanko RJ, Muhammad IR, et al. Groundnut (Arachis hypogaea) for food and fodder in crop-livestock systems: forage and seed yields, chemical composition and rumen degradation of leaf and stem fractions of 38 cultivars. Anim Feed Sci Technol 1999; 77: 33-47.

[29] Abdullah N, Sulaiman F, Gerhauser H. Characterisation of oil palm empty fruit bunches for fuel application. J Phys Sci 2011; 22: 1-24.

[30] Shibata M, Varman M, Tono Y, Miyafuji H, Saka S. Characterization in chemical composition of the oil palm (elaeis guineensis). Nihon Enerugi Gakkaishi/Journal of the Japan Institute of Energy 2008; 87: 383-8.

[31] Van Eylen D, van Dongen F, Kabel M, de Bont J. Corn fiber, cobs and stover: Enzyme-aided saccharification and co-fermentation after dilute acid pretreatment. Bioresour Technol 2011; 102: 5995-6004.

[32] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. Biomass & bioenergy 2004; 26: 361-75.

[33] Spaulding P. Studies on The Lignin and Cellulose of Wood . Missouri Botanical Garden Annual Report 1906; 1906: 41.

[34] Schwalbe CG. Cellulose . Journal of the Franklin Institute 1910; 170: 371-84.

[35] van Maris A, Abbott D, Bellissimi E, van den Brink J, Kuyper M, Luttik M, et al. Alcoholic fermentation of carbon sources in biomass hydrolysates by Saccharomyces cerevisiae: current status. Antonie van Leeuwenhoek 2006; 90: 418.

[36] Hahn-Hägerdal B, Lindén T, Senac T, Skoog K. Ethanolic fermentation of pentoses in lignocellulose hydrolysates. Appl Biochem Biotechnol 1991; 28-29: 131-44.

[37] Rouhollah H, Iraj N, Giti E, Sorah A. Mixed sugar fermentation by Pichia stipitis, Sacharomyces cerevisiaea, and an isolated xylose-fermenting Kluyveromyces marxianus and their cocultures. African Journal of Biotechnology 2007; 6: 1110-4.

[38] Pinilla L, Torres R, Ortiz C. Bioethanol production in batch mode by a native strain of Zymomonas mobilis. World J Microbiol Biotechnol World journal of microbiology & biotechnology 2011; 27: 2521-8.

[39] Georgieva TI, Ahring BK. Evaluation of continuous ethanol fermentation of dilute-acid corn stover hydrolysate using thermophilic anaerobic bacterium Thermoanaerobacter BG1L1. Appl Microbiol Biotechnol 2007; 77: 61-8.

[40] Brown RC. Biorenewable Resources Engineering New Products from Agriculture. 1st ed. Iowa: Blackwell Publishing; 2003.

[41] Ward AJ, Hobbs PJ, Holliman PJ, Jones DL. Optimisation of the anaerobic digestion of agricultural resources. Bioresour Technol 2008; 99: 7928-40.

[42] Lantz M, Svensson M, Björnsson L, Börjesson P. The prospects for an expansion of biogas systems in Sweden—Incentives, barriers and potentials. Energy Policy 2007; 35: 1830-43.

[43] Buswell AM, Neave SL. Laboratory studies of sludge digestion. State Water Survey, State Ill -- Bul 1930.

[44] Angelidaki I, Ellegaard L. Codigestion of manure and organic wastes in centralized biogas plants - Status and future trends. Appl Biochem Biotechnol 2003; 109: 95-105.

[45] Labatut RA, Angenent LT, Scott NR. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour Technol 2011; 102: 2255-64.

[46] Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater. Washington: American Public Health Association; 1998.

[47] Sluiter A, Sluiter J. Determination of Starch in Solid Biomass Samples by HPLC - Laboratory Analytical Procedure (LAP) - Issue Date: 07/17/2005. Technical Report National Renewable Energy Laboratory NREL/TP-510-42624 2008.

[48] Mariotti F, Tome D, Mirand PP. Converting nitrogen into protein - Beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr 2008; 48: 177-84.

[49] Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. J Agric Food Chem 2010; 58: 9043-53.

[50] Arapoglou D, Varzakas T, Vlyssides A, Israilides C. Ethanol production from potato peel waste (PPW). Waste Manage 2010; 30: 1898-902.

[51] Krishnan C, Sousa LdC, Jin M, Chang L, Dale BE, Balan V. Alkali-Based AFEX Pretreatment for the Conversion of Sugarcane Bagasse and Cane Leaf residues to Ethanol. Biotechnol Bioeng 2010; 107: 441-50.

[52] Thomsen MH, Hauggaard-Nielsen H. Sustainable bioethanol production combining biorefinery principles using combined raw materials from wheat undersown with clover-grass. J Ind Microbiol Biotechnol 2008; 35: 303-11.

Paper III

In-text citation:

(Ambye-Jensen et al., 2014, Paper III)

Authors:

Ambye-Jensen M., **Thomsen S.T.**, Kádár Z., Meyer A.S.

Title:

Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment

Publication status:

Published in *Biotechnology for Biofuels* (2013) 6:116

Note:

M. Ambye-Jensen and S. T. Thomsen contributed equally to this work.

Ambye-Jensen *et al. Biotechnology for Biofuels* 2013, **6**:116
http://www.biotechnologyforbiofuels.com/content/6/1/116

Biotechnology for Biofuels

RESEARCH **Open Access**

Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment

Morten Ambye-Jensen, Sune Tjalfe Thomsen, Zsófia Kádár* and Anne S Meyer

Abstract

Background: Ensiling is a well-known method for preserving green biomasses through anaerobic production of organic acids by lactic acid bacteria. In this study, wheat straw is subjected to ensiling in combination with hydrothermal treatment as a combined pretreatment method, taking advantage of the produced organic acids.

Results: Ensiling for 4 weeks was accomplished in a vacuum bag system after addition of an inoculum of *Lactobacillus buchneri* and 7% w/w xylose to wheat straw biomass at 35% final dry matter. Both glucan and xylan were preserved, and the DM loss after ensiling was less than 0.5%. When comparing hydrothermally treated wheat straw (170, 180 and 190°C) with hydrothermally treated ensiled wheat straw (same temperatures), several positive effects of ensiling were revealed. Glucan was up-concentrated in the solid fraction and the solubilisation of hemicellulose was significantly increased.
Subsequent enzymatic hydrolysis of the solid fractions showed that ensiling significantly improved the effect of pretreatment, especially at the lower temperatures of 170 and 180°C.
The overall glucose yields after pretreatments of ensiled wheat straw were higher than for non-ensiled wheat straw hydrothermally treated at 190°C, namely 74-81% of the theoretical maximum glucose in the raw material, which was ~1.8 times better than the corresponding yields for the non-ensiled straw pretreated at 170 or 180°C. The highest overall conversion of combined glucose and xylose was achieved for ensiled wheat straw hydrothermally treated at 180°C, with overall glucose yield of 78% and overall conversion yield of xylose of 87%.

Conclusions: Ensiling of wheat straw is shown to be an effective pre-step to hydrothermal treatment, and can give rise to a welcomed decrease of process temperature in hydrothermal treatments, thereby potentially having a positive effect on large scale pretreatment costs.

Keywords: Silage, Ensiling, Combined pretreatment, Hydrothermal treatment, Wheat straw, Enzymatic hydrolysis

Background

Lignocellulosic residues such as wheat straw (WS) are an attractive renewable resource for the production of fuel, feed and chemicals. Wheat is the most important crop in the EU with an annual average production of over 130 Mt grain [1] and around 200 Mt of straw residues (using a residue to product factor of 1.5 according to [2]). Replacement of conventional sugar or starch based feedstock with lignocellulosic agricultural residues, such as WS, for ethanol production is advantageous due to a more efficient use of the agricultural area. However, lignocellulosic residues require more advanced processing technologies. Lignocellulose consists of the polysaccharides cellulose and hemicellulose and the polyphenolic structure of lignin; together forming a rigid matrix structure in the secondary plant cell wall. This structure is naturally ‘engineered’ to resist degradation, thus creating great challenges in terms of biorefining. Physical and chemical pretreatments have been developed for lignocellulosic biomass in order to create accessibility for hydrolytic enzymes to hydrolyze the polysaccharides into readily fermentable sugars [3]. Bioethanol production from lignocellulosic residues has been the main driver for the technology development, and production is now on the verge of industrialization [4]. However the industry is facing huge difficulties in creating enough economic viability to engage in full scale production [5]. Pretreatment have been shown to

---

* Correspondence: zska@kt.dtu.dk
Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, DTU, Denmark

cover up to 33% of the processing costs [6-9]. The pretreatment step is most often based on hydrothermal principles of high temperatures (170-220°C) in aqueous solution, and is the most energy intensive and expensive process step in the lignocellulose to ethanol process, due to the need of high temperature, pressure, and/or chemicals as well as specialized equipment. Examples of pretreatment methods are hydrothermal treatment (HTT), dilute acid treatment (using H_2SO_4), and ammonia fiber explosion. HTT has been widely studied for pretreatment of WS and other cellulosic biomasses, where it facilitates high yields of enzymatic cellulose conversion (70-90%) and its simple approach without additives makes it advantageous to upscale [5,8,10,11] In the current Inbicon demonstration plant in Kalundborg, Denmark [5] the straw is hydrated to a dry matter (DM) mass fraction of 35% before it is continuously fed to a pressurized pretreatment reactor operating at 180-200°C for a retention time of 10-20 min [5]. Considering the low feed-in DM for lignocellulosic bioethanol, dry biomass storage processing is no longer an advantage as compared to traditional combustion. Furthermore drying of biomass increases the biomass recalcitrance towards biological degradation [12]. Alternatively wet storage (<40% DM) can be applied using ensiling.

Ensiling is the well-known preservation method for forages, based on anaerobic fermentation by lactic acid bacteria (LAB) that produce organic acids, reduce pH, and prevent growth of yeasts, fungi and competing bacteria. Lignocellulosic residues including WS, do not have sufficient available sugars to facilitate the necessary lactic acid fermentation required for preservation at low DM. Organic acids can be added directly instead of LAB fermentation [13], lignocellulytic enzymes can be applied to release fermentable carbohydrates from the lignocellulose [6], or sugars can be added as substrate for LAB fermentation [14]. This study applies the latter of the three strategies. The species of LAB are usually separated into homo- and heterofermentative LAB based on their type of hexose fermentation. The homofermentative utilizes the Empden-Meyerhof-Parnas pathway and produces only lactic acid, while the heterofermentative utilizes the phosphoketolase pathway and produce lactic- and acetic acid, ethanol and carbon dioxide [15]. However when pentoses are used as fermentation substrate, then both types of LAB may produce both lactic- and acetic acid, see Eq. 1, but variation do occur [16,17].

$$\overset{\text{Pentose}}{HOCH_2(CH(OH))_3CHO} \rightarrow \overset{\text{Lacti cacid}}{CH_3CH(OH)COOH} + \overset{\text{Aceti cacid}}{CH_3COOH} \qquad (1)$$

Ensiling has in the last 6 years gained increased focus as a method for combined storage and pretreatment in biorefinery applications [6,18-24]. Based on studies of grass ensiling for forage purposes [25], the effect of ensiling as pretreatment is known to be correlated to the produced organic acids that act primarily on hemicellulose.

Oleskowicz-Popiel *et al.* [26] combined ensiling with HTT (190°C, 10 min) on maize, clover grass, and whole crop rye, which all contain easily fermentable free sugars, however they were not able to prove a positive effect of the ensiling. Xu *et al.* [27] studied the effect of adding lactic- and/or acetic acid to the hydrothermal pretreatment of dry corn stover and found that addition of acetic acid performed better as a catalyst than lactic acid, and increased the ethanol yield in a subsequent simultaneous saccharification and fermentation from 78% to 87% of the theoretical yield [27].

The pretreatment factors of temperature, holding time and pH, are often combined to one factor expressing the severity of the pretreatment [28]. Reducing pH through ensiling will increase the severity factor of the pretreatment at same temperature and holding time, thus higher severity would result in higher sugar release. It has however been shown by Pedersen et al. [29] that the use of the one dimensional severity factor to predict sugar yields is not reliable, because lignocellulosic pretreatment is much too complex.

Based on the hypothesis that the acid produced during ensiling can assist pretreatment, the aim of this study is to investigate the effect of ensiling prior to HTT in order to decrease pretreatment temperature and thereby decrease energy consumption. The ensiling is facilitated by addition of xylose and a heterofermentative LAB inoculum, which will favor acetic acid production in the silage. The motivation for using xylose as silage fermentation substrate is the availability of cheap C5 sugars in internal biorefinery process streams such as C5 molasses condensed from a HTT liquid fraction.

Results and discussion

Ensiling wheat straw

Ensiling of WS successfully preserved the biomass, resulting in only 0.35% loss in total DM and produced both acetic and lactic acid which caused the pH to drop from 7.0 to 3.7 (Table 1). The addition of 7 (w/w)% xylose resulted in 2.8 (w/w)% acetic acid and 2.4 (w/w)% lactic acid weight base in relation to the initial WS DM before ensiling. Over 1% of the added xylose was recovered, thus preservation can be carried out with less addition of xylose. Following Eq. 1 and assuming xylose were the only substrate, it can be calculated that 6 (w/w)% of utilized xylose would yield 3.6 (w/w)% lactic acid and 2.4 (w/w)% acetic acid. This is presumably due to the inoculum of *Lactobacillus büchneri* which is capable of a secondary fermentation where lactic acid is converted to acetic acid, thus shifting the ratio between acetic- and

Table 1 Dry matter loss and pH after 4 weeks ensiling; the most significant organic compounds in water extraction after ensiling

DM loss (w/w)%	0.35
pH	3.69
Glucose	0.06 ± 0.00
Xylose	1.27 ± 0.02
Xylitol	0.17 ± 0.00
Lactic acid	2.46 ± 0.09
Acetic acid	2.79 ± 0.08
Propionic acid	0.36 ± 0.01
Total	7.06

Total includes the mentioned organic compounds.

lactic acid [30,31]. The motive to favor acetic acid to lactic acid is that it increases the effect of pretreatment [27].

Production of propionic acid and xylitol (Table 1) is due to minor secondary fermentations, which are still occurring during the stable phase of the ensiling. These secondary reactions can be carried out by a variety of acid tolerant microorganisms such as LAB, *Clostridium-*, *Bacillus-* or *Propioni* bacteria. It is well documented that secondary fermentation often utilizes other carbon sources than sugars including fatty acids, alcohols and amino acids derived from plant proteins [16]. This complicates the mass balance when products become substrates, for example parts of the produced lactic acid has most likely been further metabolized into propionic acid.

The ensiled wheat straw (EWS) was also analyzed for butyric acid, since butyric acidusually is due to presence of *Clostridium* bacteria and is a common indicator of insufficient preservation. The amounts detected were however below 0.01 (w/w)%, showing efficient preservation.

It was not possible in this experimental setup to distinguish between leftover xylose and the xylose released from hemicellulose. Preliminary experiments have shown xylose release during WS ensiling (unpublished observation, M. Ambye-Jensen and S. T. Thomsen), but in amounts less than 0.1 (w/w)%. It is therefore assumed that the released xylose only counts for a negligible fraction compared to leftover xylose. No arabinose was found in the water extractions and only insignificant amounts of released glucose were detected (Table 1).

The DM loss during ensiling was very limited and measured to below 0.5%. This was due to a fast and effective preservation facilitated by the efficient laboratory vacuum ensiling, however, losses cannot be expected to be as low in large scale.

Evaporation of fatty acids needs to be considered when determining DM content of silage, which can be done by using of volatilization coefficients to determine the acids lost during DM-determination [32]. In this work volatilization coefficients and the quantity of the total fatty acids in the EWS were used, to subtract the remaining fatty acids from the DM of the EWS as described at Material and Methods. Fatty acids originated from the added xylose were hereby not taken into account.

HTT pretreatment

Composition

The composition of the raw WS (RWS) and the solid fractions of hydrothermally pretreated WS (HTT WS) are compared with the EWS and the solid fractions of pretreated EWS (HTT EWS) (Table 2). The effects of increased temperature in the HTTs are up-concentration of cellulose and lignin in the solid fraction (Table 2).

Since xylan and arabinan levels in the solid fractions of HTTs are decreasing with increasing HTT temperature, and since levels are lower on EWS, the solubilisation of hemicellulose is concluded to be intensified when the WS is ensiled and the temperature of the HTT pretreatment is increased.

Comparing the glucan content of RWS with that of EWS confirmed that the ensiling effectively preserves the cellulose (Table 2). Likewise, the total amount of fatty acids produced during ensiling (Table 1) is corresponding to the amount of added xylose. Hence, there is no indication of loss of structural carbohydrates during the 4 weeks of ensiling.

Mass balance

The glucan content in the pretreated solid fraction plus the small amounts of solubilized glucan were compared to the amount of glucan in the RWS and a total recovery was calculated. The glucan in the EWS was preserved to the same extent as the RWS after HTT and all pretreatments had a recovery above 90% (data not shown).

The pretreatment effect of HTT lies in the mechanism of autohydrolysis, catalyzed by the high temperature steam; here water acts as a weak acid and initiates depolymerization of hemicellulose [28]. During this process acetic acid is released from the O-acetyl groups on the hemicellulose which further enhance the acid hydrolysis [3,29]. The solubilization of hemicellulose, simultaneously with a dislocation of lignin [33] is the reason for inlcreased accessibility to cellulose that facilitates enzymatic attack. Even though the hemicellulose solubilizition is attractive, the hemicellulose carbohydrates still holds potential value in a biorefinery context. The recovery of hemicellulose (xylan and arabinan) is therefore an important factor.

A clear trend was found that temperature increased solubilisation of hemicellulose (Figure 1). For all pretreatments, except HTT EWS 190°C, the hemicellulose was mainly recovered in the solid fraction, and the total recovery for these pretreatments was high (92-97%), while only 64% of the total hemicellulose was recovered

Table 2 Composition of raw wheat straw (RWS) hydrothermal treated wheat straw (HTT WS), ensiled wheat straw (EWS) and hydrothermal treated ensiled wheat straw (HTT EWS) in the solid fraction after HTT (if pretreated)

	Glucan	Xylan	Arabinan	Lignin	Ash	Extractives
			(w/w % of DM)			
RWS	**40.2 ± 0.2**	**22.3 ± 0.1**	**3.3 ± 0.0**	**18.6 ± 1.1**	**5.2 ± 0.2**	**6.3 ± 0.2***
HTT WS 170°C	40.3 ± 2.4	24.8 ± 0.8	2.3 ± 0.1	21.3 ± 0.1	4.8 ± 0.3	
HTT WS 180°C	45.1 ± 1.5	25.2 ± 0.2	2.0 ± 0.0	21.6 ± 0.3	4.0 ± 0.2	
HTT WS 190°C	50.5 ± 0.2	22.4 ± 0.4	1.5 ± 0.2	23.0 ± 0.2	5.0 ± 0.2	
EWS	**39.7 ± 0.0**	**24.1 ± 0.4**	**2.6 ± 0.0**	**17.5 ± 1.2**	**3.1 ± 1.1**	**6.9 ± 0.8**
HTT EWS 4w 170°C	40.2 ± 1.0	20.1 ± 1.3	1.3 ± 0.2	23.0 ± 0.4	4.2 ± 0.0	
HTT EWS 4w 180°C	43.2 ± 1.0	18.5 ± 1.2	1.6 ± 0.1	24.5 ± 0.4	4.2 ± 0.3	
HTT EWS 4w 190°C	54.3 ± 0.6	11.8 ± 0.6	0.4 ± 0.0	25.9 ± 0.6	4.0 ± 0.1	

*only ethanol extraction.

from HTT EWS 190°C (Figure 1). The solubilisation of hemicellulose was in general quite low compared to similar studies on hydrothermal pretreatments on WS (e.g. Petersen et al., (2009). [11]). This is most likely due to differences in biomass composition; e.g. Petersen et al. had significantly lower lignin and cellulose content compared to the WS used in this study.

It is clear from the results that ensiling significantly increased the solubilisation of hemicellulose, and the increase with pretreatment temperature was more pronounced (Figure 1). The relative high degradation of hemicellulose for EWS at 190°C indicates that severity of this pretreatment was too high.

It is well known that HTT at high temperature and acidic conditions cause degradation of xylose and forms furfural while degradation of glucose mainly forms hydroxymethyl furfural (HMF) and both are potential fermentation inhibitors [10,34]. Accordingly, the increase in hemicellulose degradation with temperature, enforced by the combination with ensiling, was recorded in the measurements of furfural in the hydrolysates (Figure 2). Although the furfural levels were significantly higher in the HTT EWS samples than the HTT WS samples, the maximum concentration did not exceed 0.53 g/L (HTT EWS 190°C), which is far below the critical inhibition levels of 2.0 g/L [35]. HMF concentrations were found not to exceed 0.03 g/L (data not shown) which is likewise much below inhibition levels [35].

For both WS and EWS the concentration of organic acid in the HTT liquid increased with temperature as expected (Figure 2) due to the higher biomass degradation at higher temperature. The HTT EWS liquids had significantly higher concentrations of total organic acids than HTT WS, which was due to both higher biomass degradation but also the organic acid content in the biomass before HTT. The levels on Figure 2 in (w/w)% of DM before HTT is

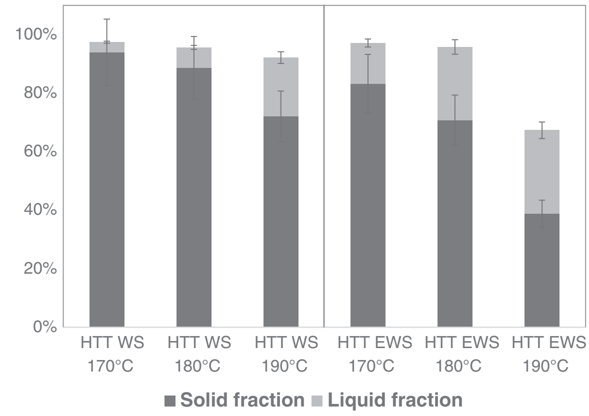

Figure 1 Recovery of hemicellulose. Recovery of hemicellulose (xylan and arabinan) in solid fraction (dark) and liquid fraction (light) on HTT treated wheat straw (HTT WS) and on HTT treated ensiled wheat straw (HTT EWS). HTT pretreatment was carried out at 170, 180 and 190°C.

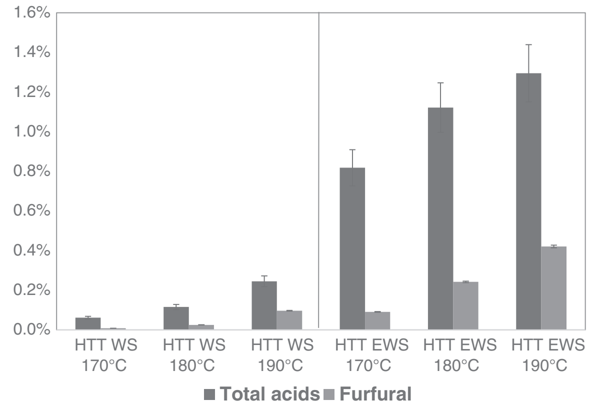

Figure 2 Organic acids and furfural in liquid fraction after HTT. Total organic acid (dark) and furfural (light) in (w/w)% of raw material DM. Analyzed in the liquid fractions after HTT treatment of wheat straw (WS) and ensiled wheat straw (EWS).

equivalent to between 1.5-1.9 g/L for HTT EWS and 0.1-0.4 g/L for HTT WS. The distribution of organic acids was also different for the WS HTT and EWS HTT. For HTT of WS it was mainly acetic acid and a bit of formic acid, a distribution of 82% and 15% respectively. For the HTT on EWS the distribution was 54% acetic-, 7% formic-, 34% lactic-, and 5% propionic acid (data not shown). The difference in organic acids in the pretreated liquids suggests that the mechanisms during pretreatment of the two different biomasses appear to be different, which is in line with the clear difference in hemicellulose solubilisation (Figure 1). Organic acids can have inhibitory effect in subsequent ethanol fermentation, but for that the concentrations should exceed 10 g/L [35]. On the other hand, it has been shown that inhibitors can serve as very efficient contamination control in large-scale lignocellulosic bioethanol production, preventing growth of especially *Lactobacillus* and thus avoid the need of expensive sterile fermentation equipment [5].

Enzymatic hydrolysis

The enzymatic hydrolysis on the pretreated fiber was effectively acting on both cellulose and hemicellulose due to the addition of both cellulase- and hemicellulase blends. The glucose conversion yields in the pretreated solid fraction of the HTT WS increased with temperature especially from 180°C to 190°C where the conversion yield jumped from 45.9 to 71.5% (Table 3). For the HTT EWS the glucose conversion yield ranged from 73.5-78.7% and did not differ significantly due to the standard deviations (Table 2). When addressing the actual release of glucose in (w/w)% of DM in the solid fraction after HTT it were apparent that HTT EWS 190°C gave the highest release of 43.9 (w/w)% (Table 3).

The glucose conversion yields after enzymatic hydrolysis were clearly improved by ensiling especially at the lower HTT temperature of 170°C and 180°C, which leads to a significant increase in the overall glucose conversion yields (Table 3). E.g. at the HTT at 180°C the overall glucose conversion yield increased from 44.4% to 78.5% of glucose in raw material when WS was ensiled.The data also showed that ensiling alone was not sufficient as pretreatment, since only 13% of the available glucose in the raw material could be enzymatically converted (Table 3). The low overall glucose conversion yield on WS at the two lower pretreatment temperatures shows that the pretreatment severities were insufficient.

The overall conversion yield of xylose (Table 4) showed the same trend as for glucose (Table 3). However for HTT EWS 190°C the released xylose was significantly lower compared to pretreatments at lower temperatures. This can be explained by the thermal degradation of hemicellulose at higher pretreatment severity. Furthermore, the xylose release of HTT EWS 170°C (17.2 (w/w)%) was similar to HTT WS 190°C (18.0 (w/w)%), corroborating that ensiling facilitated high xylose release at lower pretreatment temperature.

The positive effect of ensiling WS prior to HTT can be quantified by comparing the yields over the same pretreatment temperature. At 170°C and 180°C ensiling improves the total yield. Comparing the released glucose and xylose (Table 3 and Table 4) from HTT WS with HTT EWS it can be concluded that we gain substantial more released sugar than the 7% xylose spent facilitating the ensiling process. However, at 190°C this positive sugar balances is not observable due to xylose degradation.

The literature points at two main reasons for the improved sugar release of combining ensiling and HTT.

Table 3 Glucose conversion after enzymatic hydrolysis of raw wheat straw (RWS), hydrothermal treated wheat straw (HTT WS), ensiled wheat straw (EWS) and of hydrothermal treated ensiled wheat straw (HTT EWS)

	Released glucose	Glucose conversion yield	Overall glucose conversion yield		
			Liquid fraction	Solid fraction	Total
	In (w/w) % of DM in solid fraction	In % of glucose in solid fraction	In % of glucose in raw material	In % of glucose in raw material	In % of glucose in raw material
RWS				19.0 ± 2.6^{c}	19.0 ± 2.6^{c}
HTT WS 170°C	19.1 ± 0.5^{d}	43.0 ± 1.2^{b}	0.9 ± 0.0^{c}	38.3 ± 1.0^{b}	39.1 ± 1.0^{b}
HTT WS 180°C	22.8 ± 1.9^{d}	45.9 ± 3.9^{b}	1.4 ± 0.1^{b}	43.0 ± 3.6^{b}	44.4 ± 3.6^{b}
HTT WS 190°C	39.7 ± 2.9^{ab}	71.5 ± 5.1^{a}	1.8 ± 0.2^{a}	69.3 ± 5.0^{a}	71.1 ± 5.0^{a}
EWS				13.5 ± 0.8^{c}	13.5 ± 0.8^{c}
HTT EWS 170°C	33.5 ± 2.9^{c}	75.7 ± 6.7^{a}	0.8 ± 0.1^{c}	74.3 ± 6.4^{a}	75.1 ± 6.4^{a}
HTT EWS 180°C	37.4 ± 1.5^{b}	78.7 ± 3.0^{a}	1.3 ± 0.1^{b}	77.1 ± 3.4^{a}	78.5 ± 3.4^{a}
HTT EWS 190°C	43.9 ± 2.1^{a}	73.5 ± 3.5^{a}	1.6 ± 0.1^{a}	80.8 ± 3.8^{a}	82.3 ± 3.8^{a}

Released glucose is expressed as (w/w)% of DM in solid fraction after HTT. Glucose conversion yield is expressed as glucose release in % of glucose in the solid fraction after HTT. Overall glucose yield is the glucose release in the liquid fraction after HTT- and in the solid fraction after enzymatic hydrolysis in % of glucose in the raw wheat straw. The results in each row are grouped according to significance (p = 0.05%), where 'a' is significantly higher than 'b' and so forth.

Table 4 Xylose conversion after enzymatic hydrolysis of raw wheat straw (RWS), hydrothermal treated wheat straw (HTT WS), ensiled wheat straw (EWS) and of hydrothermal treated ensiled wheat straw (HTT EWS)

	Released xylose	Xylose conversion yield	Overall xylose conversion yield		
			Liquid fraction	Solid fraction	Total
	In (w/w) % of DM in solid fraction	In % of xylose in solid fraction	In % of xylose in raw material	In % of xylose in raw material	In % of xylose in raw material
RWS				14.8 ± 1.7^{e}	14.8 ± 1.7^{e}
HTT WS 170°C	11.1 ± 0.3^{c}	40.0 ± 1.0^{d}	3.1 ± 0.0^{f}	39.5 ± 0.9^{d}	42.6 ± 0.9^{d}
HTT WS 180°C	14.6 ± 0.7^{b}	51.6 ± 2.6^{c}	6.2 ± 0.3^{e}	48.6 ± 2.4^{c}	54.9 ± 2.4^{c}
HTT WS 190°C	18.0 ± 1.6^{a}	71.8 ± 6.2^{b}	21.1 ± 1.8^{c}	55.6 ± 4.8^{b}	76.7 ± 4.8^{b}
EWS				10.5 ± 0.4^{e}	10.5 ± 0.4^{e}
HTT EWS 170°C	17.2 ± 1.0^{a}	76.3 ± 4.6^{ab}	14.5 ± 0.0^{d}	67.5 ± 4.1^{a}	82.0 ± 4.1^{ab}
HTT EWS 180°C	16.7 ± 0.8^{a}	81.0 ± 5.0^{a}	26.7 ± 2.3^{b}	61.1 ± 3.1^{a}	87.8 ± 4.9^{a}
HTT EWS 190°C	11.7 ± 0.7^{c}	88.2 ± 5.5^{a}	30.6 ± 0.0^{a}	37.9 ± 2.3^{d}	68.5 ± 2.3^{d}

Released xylose is expressed as (w/w)% of DM in solid fraction after HTT. Xylose conversion yield is expressed as xylose release in % of xylose in the solid fraction after HTT. Overall xylose yield is the xylose release in the liquid fraction after HTT- and in the solid fraction after enzymatic hydrolysis in % of xylose in the raw wheat straw. The results in each row are grouped according to significance (p = 0.05%), where 'a' is significantly higher than 'b' and so forth.

First, the improved sugar release is connected to the natural long term impregnation of organic acids on the biomass where the lignocellulosic structure is loosened by weak acid hydrolysis accomplished by organic acids [6]. Due to the addition of xylose as substrate for ensiling, it could not be concluded to which extent hemicellulose was solubilized, but the combined results suggests very little solubilisation. Since this study did not look at the duration of the ensiling or included pretreatment of WS merely soaked in organic acids as a control, it cannot be unequivocally concluded that the improvement of HTT on EWS was directly due to the long term ensiling alone. Monavari *et al.* [36] did a study on impregnation with lactic acid on bagasse prior to steam explosion and found a significant difference between long term impregnation (4 weeks) and merely soaking, favoring the impregnation, proving that this is in fact a factor. Nonetheless, soaking of the dry wheat straw to a DM of 35%, do cause swelling of the cell wall, which is most likely improving the effect of pretreatment.

The second main effect of ensiling prior to HTT is the lowering of pH which causes higher severity, i.e. the action of the produced organic acids within the HTT pretreatment. Especially acetic acid, but also lactic acid has been shown to catalyze the autohydrolysis and improve the process as it was found by Xu *et al.* [27]. Recently it has been shown that addition of 0.04 g $(\text{g DM})^{-1}$ acetic acid to HTT of wheat straw increased glucose yield at both 190°C and 195°C, however not at 200°C, thus the effect of acetic acid was more significant at lower temperatures [37]. Results from the present study also determine that improvement by acid catalyzed autohydrolysis increases at decreasing pretreatment temperature. Furthermore, due to the large effect of ensiling at lower HTT temperatures i.e. 170-180°C, it would be interesting to test even lower HTT temperatures than 170°C in future studies.

Conclusion

Ensiling prior to hydrothermal treatment was shown to significantly increase the effect of the pretreatment, especially at 170°C, and 180°C. An effective ensiling of wheat straw was accomplished with the presented method in which both glucan and xylan was effectively preserved, and where the DM loss during ensiling was under 0.5%. Ensiled wheat straw hydrothermally treated at 180°C gave the highest overall conversion yield regarding both glucan and xylan, 73.6% and 83.5% respectively, but even pretreatment of ensiled wheat straw at 170°C provided satisfying results, 70.4% and 77.4% for glucan and xylan respectively. In both cases, more xylose was gained after the enzymatic hydrolysis than was used in the production of the wheat straw silage. The findings potentially enable a considerable decrease in the necessary process temperature in hydrothermal treatments of wheat straw, thereby having a positive effect on large scale pretreatment costs.

Materials and methods

Raw material

Wheat straw (*Triticum aestivum* L.) was supplied by DONG Energy (Skærbæk, Denmark). The straw was chopped to approximately 10 cm pieces and stored at ambient temperature. Dry matter content of the stored WS was 90%.

The process

Combined ensiling and HTT pretreatment was tested against conversion of glucose and xylose after subsequent enzymatic hydrolysis. The combined pretreatment

(HTT EWS) were compared to the conversion in raw wheat straw (RWS), ensiled wheat straw (EWS) and sole HTT pretreated wheat straw (HTT WS).

Ensiling

Ensiling was carried out on chopped WS (10 cm) adjusted to 35% final DM content. Due to the low free sugar content of WS, 7 g xylose per 100 g DM was added as determined to be optimal by Yang *et al.* [14]. Each batch of ensiling contained 1.5 kg DM WS. The ensiling was carried out using a vacuum based plastic bag system [38] and a Variovac EK10 vacuum packaging machine (Variovac Nordic A/S, DK-7100 Vejle, Denmark).

The commercially available inoculum LACTISIL CCM (Chr. Hansen, Hørsholm, Denmark) which consists of freeze dried pure heterofermentative *Lactobacillus buchneri* was applied. A suspension of 0.2 g L^{-1} water was prepared and added in the amount of 40 mL kg^{-1} WS to reach an initial inoculum size of 8 mg kg^{-1}.

The plastic bags were opened after 4 weeks. Weight loss was measured for calculation of DM loss. After ensiling, 1 kg DM of the ensiled WS was pretreated hydrothermally.

Hydrothermal pretreatment

Hydrothermal pretreatments (HTT) were carried out in the "Mini IBUS" equipment (Technical University of Denmark, Risø campus). 1 kg DM (corrected for volatile fatty acid) of the EWS was treated at different temperatures (170, 180 and 190°C) for 10 min. In order to verify the reproducibility of HTT, the EWS pretreated at 180°C were done in triplicate. After HTT the pretreatment reactor was cooled to below 70°C thereby avoiding evaporation of acids, and the material was separated by pressing. Each solid fiber fraction and each liquid fraction were analyzed separately. The solid fraction was kept in the freezer and used to evaluate the process efficiency by enzymatic hydrolysis.

Enzymatic hydrolysis

The enzymatic convertibility assay based on commercial CellicCTec2 (blend of cellulases) and CellicHTec2 (blend of hemicellulases) (Novozymes A/S, Denmark) was used to determine the efficiency of the pretreatment process. Enzymatic conversion of pretreated solids was performed at 5% DM content in a total volume of 25 mL using 50 mM citrate buffer (pH 5) and 0.25 mL sodium azide (2%) at 50°C shaken at 150 rpm for 72 h. Applied enzyme loadings were 15 FPU g^{-1} DM solids of CellicCTec2 supplemented with xylanase CellicHTec2 (90:10 ratio based on protein loading for all assays). The enzymatic hydrolysis was performed in triplicates and enzyme blanks were included. Samples were analyzed for carbohydrates on HPLC. Cellulose convertibility was calculated as the converted cellulose divided by the original cellulose content.

Chemical analysis

Raw wheat straw (RWS), ensiled wheat straw (EWS), hydrothermally pretreated wheat straw (HTT WS) and hydrothermally pretreated ensiled wheat straw (HTT EWS) were analyzed for chemical composition by methods based on standard laboratory analytical procedures developed by National Renewable Energy Laboratory (NREL), US [39]. Deviations from these standard procedures are stated in the following sections. The analysis of the solid fiber fraction included ash content determination, water extraction, ethanol extraction and strong acid hydrolysis for structural carbohydrates and lignin. The liquid fraction of the HTT was analyzed by weak acid hydrolysis.

DM determination

DM was determined using a standard method [39]. The contribution of fatty acids produced during ensiling was subtracted from the DM, since the acids originated from the added xylose, which likewise were not included in the original DM content of WS. Huida *et al.* [40] determined volatilization coefficients describing to which extent different fatty acids were evaporating during determination of DM at specific pH. These volatilization coefficients were used to determine how much of the different acids that were left after DM determination of EWS in order to correct for this amount. Fatty acids in RWS and solid fraction of HTTs EWS were negligible, thus no correction of DM were needed in these cases.

Analytical method

Concentrations of carbohydrates (D-glucose, D-xylose, L-arabinose), organic acids (lactic-, formic-, acetic-, propionic, and butyric acid) were quantified by HPLC using a Biorad HPX-87H column (Hercules, CA; USA), RI detector, 63°C and 4 mM H_2SO_4 as eluent, at flow rate of 0.6 ml min^{-1}.

Water extraction

0.3-0.4 g DM biomass from freshly disrupted silage bags was extracted in 10 ml MilliQ H_2O with 10 μl of the antibiotic ampicillin (10 mg/ml solution) to prevent microbial activity during extraction. The extraction samples were shaken for 2 hours at 25°C and 150 rpm. Extracts were analyzed for sugars, acids by HPLC as described above. Acids produced from additional xylose used for initiating ensiling process, were taken into account.

Weak acid hydrolysis of hydrolysates

The liquid fraction of HTT was further analyzed by weak acid hydrolysis to quantify the content of soluble oligomer carbohydrates. 10 ml HTT liquid fraction were autoclaved for 10 minutes at 121°C with 4 w/w % H_2SO_4. Derived sugars were analyzed by HPLC as described above.

Ethanol extraction

Lipophilic extraction was carried out by Soxhlet extraction in a reflux condenser for six hours with 99 w/w% ethanol on water extracted samples of EWS. The amount of ethanol extractives, including volatiles, was defined as the mass of material lost through extraction.

Determination of structural carbohydrates and lignin

Strong acid hydrolysis was used to measure the carbohydrate and lignin content of the extracted bio residue, based on the NREL standard laboratory analytical procedure [32].

Statistical evaluation

One-way analyses of variances (one-way ANOVA): 95% confidence intervals were compared as Tukey–Kramer intervals calculated from pooled standard deviations (Minitab Statistical Software, Addison-Wesley, Reading, MA).

Abbreviations

HTT: Hydrothermal treatment; WS: Wheat straw; EWS: Ensiled wheat straw; RWS: Raw wheat straw; DM: Dry matter; HPLC: High-performance liquid chromatography.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MA participated in the experimental design, carried out the ensiling treatment, contributed to acquisition of data and drafted the manuscript. STT participated in the planning and executing the laboratory work, contributed to acquisition of data and reviewing the manuscript. ZK participated in the experimental design, contributed to acquisition of data and review the manuscript. AM contributed to acquisition of data, performed statistical analyzis and review the manuscript. MA and STT contributed equally to this work. All authors read and approved the final manuscript.

Acknowledgements

Tomas Fernqvist and Ingelis Larsen are acknowledged for their indispensable effort in the laboratory.
The work was financially supported by the European Commission's Seventh Framework Programme (PROETHANOL2G, Project no. 251,151) as well as by the EUDP project 'Silage pretreatment of green crops for 2nd generation bioethanol production' (Jr. no. 64,010–0005) funded by the Danish Energy Agency.

Received: 5 May 2013 Accepted: 22 July 2013
Published: 14 August 2013

References

1. *Eurostat.* http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/.
2. Sun R-C, Tong-Qi Y: *Cereal straw as a resource for sustainable biomaterials and biofuels: chemistry, extractives, lignins, hemicelluloses and cellulose.* 1st edition. Amsterdam, The Netherlands: Elsevier; 2010.
3. Hu F, Ragauskas A: Pretreatment and lignocellulosic chemistry. *Bioenergy Research* 2012, **5**(4):1–24.
4. Brown TR, Brown RC: A review of cellulosic biofuel commercial-scale projects in the United States. *Biofuels Bioprod and Biorefin* 2013, **7**(3):235–245.
5. Larsen J, Haven MT, Thirup L: Inbicon makes lignocellulosic ethanol a commercial reality. *Biomass Bioenergy* 2012, **46**:36–45.
6. Chen Y, Sharma-Shivappa RR, Chen C: Ensiling agricultural residues for bioethanol production. *Appl Biochem Biotechnol* 2007, **143**(1):80–92.
7. Bals B, Wedding C, Balan V, Sendich E, Dale B: Evaluating the impact of ammonia fiber expansion (AFEX) pretreatment conditions on the cost of ethanol production. *Bioresour Technol* 2011, **102**(2):1277–1283.
8. Nitsos CK, Matis KA, Triantafyllidis KS: Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process. *ChemSusChem* 2013, **6**(1):110–122.
9. Yang B, Wyman CE: Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels Bioprod Biorefin* 2008, **2**(1):26–40.
10. Thomsen MH, Thygesen A, Thomsen AB: Identification and characterization of fermentation inhibitors formed during hydrothermal treatment and following SSF of wheat straw. *Appl Microbiol Biotechnol* 2009, **83**(3):447–455.
11. Petersen MØ, Larsen J, Thomsen MH: Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. *Biomass Bioenergy* 2009, **33**(5):834–840.
12. Luo X, Zhu JY: Effects of drying-induced fiber hornification on enzymatic saccharification of lignocelluloses. *Enzyme Microb Technol* 2011, **48**(1):92–99.
13. Kung LJ, Stokes MR, Lin CJ: Silage additives. In *Silage science and technology.* Edited by Buxton DR, Muck RE, Harrison JH, Madison I, Wisconsin I. USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2003:305–360.
14. Yang HY, Wang XF, Liu JB, Gao LJ, Ishii M, Igarashi Y, Cui ZJ: Effects of water-soluble carbohydrate content on silage fermentation of wheat straw. *J Biosci Bioeng* 2006, **101**(3):232–237.
15. Rooke JA, Hatfield RD: Biochemistry of ensiling. In *Silage science and technology.* Edited by Buxton DR, Muck RE, Harrison JH, Madison, Wisconsin. USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2003:95–140.
16. Pahlow G, Muck RE, Driehuis F, Elferink SJWHO, Spoelstra SF: Microbiology of ensiling. In *Silage science and technology.* Edited by Buxton DR, Muck RE, Harrison JH, Madison, Wisconsin. USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2003:31–94.
17. Thomsen MH: Lactic acid fermentation of brown Juice in the Green Crop Drying Plant. In *PhD Thesis.* Denmark: University of Southern Denmark; 2004.
18. Shinozaki Y, Kitamoto HK: Ethanol production from ensiled rice straw and whole-crop silage by the simultaneous enzymatic saccharification and fermentation process. *J Biosci Bioeng* 2011, **111**(3):320–325.
19. Passoth V, Tabassum MR, Nair HAS, Olstorpe M, Tiukova I, Ståhlberg J: Enhanced ethanol production from wheat straw by integrated storage and pre-treatment (ISP). *Enzyme Microb Technol* 2013, **52**(2):105–110.
20. Thomsen M, Holm-Nielsen J, Oleskowicz-Popiel P, Thomsen A: Pretreatment of Whole-Crop Harvested, Ensiled Maize for Ethanol Production. *Appl Biochem Biotechnol* 2008, **148**(1):23–33.
21. Pakarinen A, Maijala P, Jaakkola S, Stoddard F, Kymäläinen M, Viikari L: Evaluation of preservation methods for improving biogas production and enzymatic conversion yields of annual crops. *Biotechnology for Biofuels* 2011, **4**(20). doi:10.1186/1754-6834-4-20.
22. Zheng Y, Yu C, VanderGheynst JS, Jenkins B, Zhang R, Cheng Y: Effects of ensilage on storage and enzymatic degradability of sugar beet pulp. *Bioresour Technol* 2011, **102**(2):1489–1495.
23. Sipos B, Kreuger E, Zacchi G, Björnsson L, Réczey K, Svensson S: Steam pretreatment of dry and ensiled industrial hemp for ethanol production. *Biomass Bioenergy* 2010, **34**(12):1721–1731.
24. Digman MF, Shinners KJ, Casler MD, Dien BS, Hatfield RD, Jung H-G, Muck RE, Weimer PJ: Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. *Bioresour Technol* 2010, **101**(14):5305–5314.
25. Dewar WA, McDonald P, Whittenbury R: The hydrolysis of grass hemicelluloses during ensilage. *J Sci Food Agric* 1963, **14**(6):411–417.
26. Oleskowicz-Popiel P, Thomsen AB, Schmidt JE: Ensiling - Wet-storage method for lignocellulosic biomass for bioethanol production. *Biomass Bioenergy* 2011.
27. Xu J, Thomsen MH, Thomsen AB: Enzymatic hydrolysis and fermentability of corn stover pretreated by lactic acid and/or acetic acid. *J Biotechnol* 2009, **139**(4):300–305.
28. Garrote G, Domínguez H, Parajó JC: Interpretation of deacetylation and hemicellulose hydrolysis during hydrothermal treatments on the basis of the severity factor. *Process Biochem* 2002, **37**(10):1067–1073.
29. Pedersen M, Meyer AS: Lignocellulose pretreatment severity - relating pH to biomatrix opening. *New Biotechnology* 2010, **27**(6):739–750.

30. Filya I: The effect of Lactobacillus buchneri, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. *J Appl Microbiol* 2003, **95**(5):1080–1086.
31. Holzer M, Mayrhuber E, Danner H, Braun R: The role of Lactobacillus buchneri in forage preservation. *Trends Biotechnol* 2003, **21**(6):282–287.
32. Kreuger E, Nges I, Björnsson L: Ensiling of crops for biogas production: Effects on methane yield and total solids determination. *Biotechnology for Biofuels* 2011, **4**(44).
33. Hansen MAT, Kristensen JB, Felby C, Jørgensen H: Pretreatment and enzymatic hydrolysis of wheat straw (Triticum aestivum L.) - The impact of lignin relocation and plant tissues on enzymatic accessibility. *Bioresour Technol* 2011, **102**(3):2804–2811.
34. Klinke HB, Olsson L, Thomsen AB, Ahring BK: Potential inhibitors from wet oxidation of wheat straw and their effect on ethanol production of Saccharomyces cerevisiae: wet oxidation and fermentation by yeast. *Biotechnol Bioeng* 2003, **81**(6):738–747.
35. Palmqvist E, Grage H, Meinander NQ, Hahn-Hägerdal B: Main and interaction effects of acetic acid, furfural, and p- hydroxybenzoic acid on growth and ethanol productivity of yeasts. *Biotechnol Bioeng* 1999, **63**(1):46–55.
36. Monavari S, Galbe M, Zacchi G: Influence of impregnation with lactic acid on sugar yields from steam pretreatment of sugarcane bagasse and spruce, for bioethanol production. *Biomass Bioenergy* 2011, **35**(7):3115–3122.
37. Petrik S, Kádár Z, Márová I: Utilization of hydrothermally pretreated wheat straw for production of bioethanol and carotene-enriched biomass. *Bioresour Technol* 2013, **133**:370–377.
38. Johnson HE, Merry RJ, Davies DR, Kell DB, Theodorou MK, Griffith GW: Vacuum packing: A model system for laboratory-scale silage fermentations. *J Appl Microbiol* 2005, **98**(1):106–113.
39. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D: Determination of Structural Carbohydrates and lignin in Biomass. *NREL Technical Report* 2011, **510**:42618.
40. Huida L, Vaatainen H, Lampila L: Comparison of dry-matter contents in grass silages as determined by oven drying and gas-chromatographic water analysis. *Ann Agr Fen* 1986, **25**(3):215–230.

doi:10.1186/1754-6834-6-116
Cite this article as: Ambye-Jensen *et al.*: Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment. *Biotechnology for Biofuels* 2013 **6**:116.

Paper IV

In-text citation:
(Thomsen et al., 2014a, Paper IV)

Authors:
Thomsen S.T., Londoño J.E.G., Schmidt J.E., Kádár Z.

Title:
Screening of pretreatments of common West African lignocellulosic biomass residues for ethanol production

Publication status:
Submitted on November 20 2013 to *Renewable Energy*

Screening of pretreatments of common West African lignocellulosic biomass residues for ethanol production

Sune Tjalfe Thomsen[a], Jorge Enrique González Londoño[a], Jens Ejbye Schmidt[a,b], Zsófia Kádár[a,*]

[a]Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Kgs. Lyngby, DTU, Denmark

[b]Institute Center for Energy – iEnergy, Masdar Institute for Science and Technology, PO Box 54224, Abu Dhabi, United Arab Emirates

Abstract

Pretreating lignocellulosic biomass for cellulosic ethanol production in a West African setting requires smaller scale and less capital expenditure compared to current state of the art. In the current study, three low-tech methods applicable for West African conditions, namely Boiling Pretreatment (BP), Soaking in Aqueous Ammonia (SAA) and White Rot Fungi pretreatment (WRF), were compared to the high-tech solution of hydrothermal pretreatment (HTT). The pretreatment methods were tested on 11 West African biomasses, i.e. cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches. It was found that 4 biomass' (plantain peelings, plantain trunks, maize cobs and maize stalks were most promising for production of cellulosic ethanol with profitable enzymatic conversion of glucan (>30g glucan per 100 g total solids (TS)). HTT did show better results in both enzymatic convertibility and fermentation, but evaluated on the overall ethanol yield the low-tech pretreatment methods are viable alternatives with similar levels to the HTT (13.4-15.2 g ethanol per 100 g TS raw material).

Keywords

Cellulosic ethanol; lignocellulose; biomass; pretreatment; West Africa

---

[*] Corresponding author. Tel.: +45 21328345, E-mail address: zska@kt.dtu.dk, Postal address: DTU Risø Campus, Frederiksborgvej 399, P.O. Box 49, Building 301, 4000 Roskilde, Denmark

1. Introduction

In a West African context, there are several drivers and constraints shaping the path toward increased utilisation of lignocellulosic biomass for ethanol production. Among the drivers, there is a vision of energy self-sufficiency both in local and regional scale. In addition, it has been found that bioenergy systems, if properly harnessed and planned, can provide a number of additional environmental and socio-economic opportunities for Africa [1, 2]. Moreover, several authors have investigated the possibilities for bioenergy production in the region showing the theoretical ethanol potentials, while some countries have recently made political goals for increased bioenergy production [3-6]. However, according to other studies, it is unfeasible to set-up and implement technological schemes with large-scale, high-performance technologies in a current African context [7, 8]. This is reinforced by the scarce biomass infrastructure of the region, which in most cases do not support collection, storage and transport of huge amounts of the biomass needed for a large-scale facility. This implies that the process of making cellulosic ethanol has to be optimised, not as proven for a European or North American setting, but within the constraints of a smaller scale than envisioned in most scientific studies. However, labour is significantly cheaper in Africa than in e.g. Asia or Europe, making it conceivable to employ methods that are more labour intensive than methods developed for the industrialised world [9].

The main technical challenge when producing ethanol from lignocellulosic feedstock is liberating the sugars from the rigid lignocellulosic matrix, which consist of cellulose, hemicellulose and lignin [10]. Succeeding in this exercise requires an effective pretreatment of the biomass enabling an enzymatic attack on the cellulose fibres, thereby liberating glucose sugar monomers, which are easily fermentable into ethanol. Pretreatment of the most common West African biomasses for cellulosic ethanol, have previously not been investigated in a combined comparative study. Moreover, only a few of the most common residues have been addressed in optimisation studies on single pretreatment methods, and these studies have not been taking the scale of production into account [11-13]. Different pretreatment methods exhibit different modes of action that are more or less effective on a given biomass. Among the factors affecting the mode of action of different pretreatments are heat, pressure, water, acid, alkali, enzymes, ions, and more [14-16]. In this study, three pretreatment methods are tested which are believed to be favourable for low-tech, small-scale conditions, namely boiling pretreatment (BP), soaking in aqueous ammonia (SAA) and fungal pretreatment (WRF). These will be compared to the more advanced and established pretreatment method of hydrothermal treatment (HTT).

HTT refers to the use of hot water in either the gaseous or the liquid phase to pretreat lignocellulosic biomass [15]. HTT is most often done at 160-220°C where autohydrolysis of the hemicellulose is taking place and where degradation of glucan is limited. Autohydrolysis occurs when water acts as a weak acid that causes depolymerisation of hemicellulose by hydrolysing glycosidic linkages in the hemicellulose side-chains [17]. Thus, the main effect of HTT is solubilisation of hemicellulose consequently increasing cellulose digestibility, while most of the cellulose and lignin of a biomass remain in the solid phase after HTT [15, 18, 19]. HTT is one of the pretreatment technologies closest to commercial reality since large demonstration plants have already been operated, however, since this method needs highly specialised equipment, due to high pressure, high temperature and corrosive environment, HTT seem only to be feasible in large scale [20]. A projected full-scale HTT based cellulosic ethanol plant will have a capacity of 500 to 1200 tons TS lignocellulosic biomass per day [20], which will require a very high degree of biomass infrastructure that is not realistic in the current West African context.

Boiling pretreatment (BP) is performed without pressurised equipment and unlike HTT it cannot exceed temperatures of 100°C. This substantially limits the effect on lignocellulosic materials since the autohydrolytic effect does not take place [16]. When BP has been applied as lignocellulose pretreatment method, it has been with a limited effect [21, 22]. However, BP can be performed in small-scale low-tech set-ups, and the method solubilises some non-structural components such as proteins, waxes, inorganic compounds and free sugars [23], while starch fractions swell and become exposed for enzymatic breakdown [24]. Therefore, some biomasses might be prone to boiling pretreatment as a low-tech solution.

Soaking in aqueous ammonia (SAA) takes advantage of a mild alkali effect on the biomass and the method has been used on a variety of lignocellulosic materials [25-27]. The major effects of SAA are swelling of the cellulose and delignification due to cleavage of ether bonds in lignin, as well as the ether and ester bonds coupling lignin to hemicellulose [28][26, 28, 29]. Since SAA can be performed at ambient temperatures and atmospheric pressure, the method is highly scalable and can be a part of a low-tech solution. However, in order to be cost-effective and to reduce chemical demands, a recovery system for the ammonia is needed. Due to the volatility of the ammonia, this has been claimed viable [27], but to the knowledge of the authors, this has not yet been as a part of a low-tech solution.

White rot fungi pretreatment (WRF) of biomass, takes advantage of the unique and extracellular oxidative enzymes produced by white rot fungi. These enzymes are efficient to degrade lignin and open the phenyl rings, thus increasing the accessibility to the carbohydrates in the biomass [30]. Fungal pretreatment is an aerobic process, initiated by inoculating fungal mycelia in moist biomass. The degradation process is preferential as it is interdependent on both the type of pretreated biomass and the fungal species used [31]. The success of the pretreatment therefore depends on optimizing the growth conditions of the fungi, which are usually more environmental friendly compared to traditional chemical of physiochemical pretreatments. An attractive feature of WRF is the ambient operation temperatures, while the mayor drawbacks that can make WRF unattractive are slow rates and potential carbohydrate loss. The carbohydrate loss can be controlled by the choice of fungi, e.g. the white-rot fungus *Ceriporiopsis subvermispora* lacks a complete cellulolytic enzyme complex that makes it selectively decay lignin and hemicellulose and not cellulose. *C. subvermispora* has shown effective ligninolytic activity in WRF of different types of biomass [32].

The chosen pretreatment methods will be applied on 11 common West African biomasses in a screening set-up. The biomasses assessed are cassava stalks, plantain peelings, plantain trunks, plantain leaves, cocoa husks, cocoa pods, maize cobs, maize stalks, rice straw, groundnut straw and oil palm empty fruit bunches (EFB). These biomasses have undergone a thorough compositional analysis in a previous study [6].

2. Materials and Methods

2.1. Raw materials

The agricultural residues were obtained from the test facilities of the Ghana Crops Research Institute of the Council for Scientific and Industrial Research, in Kumasi, Ghana. The crops were harvested during the first months of 2011, air dried and transported to Denmark. The residues were knife milled to pass a 2 mm sieve (Mill: MF 10 basic, IKA-Werke GmbH & Co. KG, Germany). The total solids (TS), volatile solids (VS), and ash content were measured using a standard method [33].

2.2. Pretreatments

2.2.1. Hydrothermal pretreatment (HTT)

Hydrothermal pretreatments of biomass residues were performed in a 2 L loop reactor (Technical University of Denmark, Risø Campus). The loop reactor was operated in a batch setup and was fed with 1 L tap water and 60 g TS of biomass, and was submitted to continuous circulation and stirring. The design of the loop reactor as well as heating and cooling intervals has been described previously [34, 35]. The pretreatments were performed at 195°C for 10 minutes for each biomass without any added chemicals. After the HTT, the liquid and solid fractions were separated by vacuum filtration with a 100 µm nylon filter. The solid fractions were washed with 2 L tap water of ambient temperature and dried at 20 to 40°C to a TS content of approximately 95%. The chosen HTT conditions are based on our previous studies on different biomasses where optimised conditions, with high cellulose convertibility and low hemicellulose degradation, most often have been in the region of 195°C for 10 minutes [18, 19, 19, 36]. The maize stalks could unfortunately not be pretreated with HTT due to clocking of the apparatus.

2.2.2. Boiling pretreatment (BP)

Boiling pretreatments were carried out in batch setup where 60 g TS of biomass residue was fed with 1 L tap water in a 1 L blue cap flask. The bottles were heated in an autoclave (Getinge VS 70, Switzerland) to 100°C with a holding time of 10 minutes (internal temperature sensor). After the boiling pretreatments, the liquid and solid fractions were handled as described for HTT (section 2.2.1.).

2.2.3. Soaking in aqueous ammonia (SAA)

40 g TS of biomass residues were soaked in 28 % aqueous ammonia in 1L polyethylene plastic flasks, at solid to liquid loadings of 1:4 (w/w). After soaking for 10 days at 30°C, the ammonia was evaporated from the biomass residues in a fume hood, and finally the samples were dried at 40°C. The ammonia concentration, time, temperature, and solid to liquid loadings were based on previous studies [29].

2.2.4. White rot fungi pretreatment (WRF)

The white rot fungi *C. subvermispora* (CBS 347.63) was used for fungal pretreatment. The pure culture was obtained from The Fungal Biodiversity Center (The Netherlands), and maintained on malt extract agar (Difco, France) at 28°C. Preparation of liquid preculture for inoculation was performed in 250 ml Erlenmeyer flasks, cultivating 6 x 1cm squares of the plate culture into 50 ml malt extract broth supplemented with 0.05 % v/v of Tween 80. The preculture was incubated for 10 days at 28°C. Afterwards the liquid inoculum was blended to obtain a homogenous mixture.

For the WRF pretreatment 5 g of biomass residues were weighted and corrected to 25% initial TS content with deionized water into 250ml Erlenmeyer flasks that served as bioreactors for fungal pretreatment. The samples were sterilised in autoclave at 121°C for 30 min, adequately covered with hydrophobic cotton plugs. Inoculation of sterile samples was performed with the liquid inoculum at 5:1 biomass to inoculum ratio. The pretreatment of the biomass residues was carried out in a growth chamber for 20 days at 28°C at 90% relative humidity. Triplicates were prepared for every sample. After the pretreatment period, 100 ml of deionized water was supplemented into each flask and incubated at 50°C for 3 hours under agitation at 120 rpm in order to separate the fungal mycelia from the biomass residues, which were filtered (Whatman filter paper #41), and the collected solids were freeze-dried. The conditions for WRF pretreatment was based on literature survey and incubation time were optimised in a pre-study (data not shown) [37, 38]. *C. subvermispora* was chosen since it preserves more glucan as compared to other white rot fungi proposed for biomass pretreatment [32].

2.3. Enzymatic hydrolysis

The enzymatic convertibility assay based on commercial Cellic CTec2 and Cellic HTec2 (Novozymes A/S, Denmark) was used to determine the efficiency of the pretreatment processes. Enzymatic conversion of pretreated solids was performed at 5% TS content in a total volume of 25 mL using 50 mM citrate buffer (pH 5) and 0.25 mL sodium azide (2%) at 50°C shaken at 150 rpm for 72 h. Applied enzyme loadings were 9.51 ml Cellic CTec2 supplemented with 1.05 ml xylanase Cellic HTec2 per 100 g TS. The enzymatic hydrolysis was performed in triplicates and were corrected with enzyme blanks. The carbohydrates from the samples were quantified using HPLC as described in section 2.6.

2.4. Ethanol fermentation

Fermentations of raw and solid fractions of pretreated biomass was done in 100 mL blue cap flasks containing 5 g TS and 50 ml liquids to a final TS content of 10% TS. Each of the biomasses selected for fermentation were tested in triplicates. The fermentations were performed as a simultaneous saccharification and fermentation (SSF), but with two short liquefaction steps. Since some of the biomasses contained starch, the first liquefaction steps was done by adding 45 mL 50 mM citric acid buffer at pH 5.0 as well as 2 µL Liquozyme® SC DS alpha-amylase (Novozymes, Denmark) to the biomass and incubating at 80°C for 90 min at 150 rpm. After the samples were cooled to below 50°C and 1 ml stock solution were added, which contained: 0.5 mg Tetracycline hydrochloride (Sigma-Aldrich, USA) dissolved 0.62 ml citric acid buffer mixed with 6 µL Spirizyme® Fuel glucoamylase (Novozymes, Denmark), 340 µL Cellic CTec 2® cellulase complex (Novozymes, Denmark) and 38 µL Cellic Htec 2® endoxylanase (Novozymes, Denmark). The samples were incubated for 2 hours at 50 °C (liquefaction) at 150 rmp. After the liquefaction steps, the samples were cooled to below 35°C, and 4 ml citric acid buffer containing 0.1 g TS of *Saccharomyces cerevisiae* yeast (Ethanol Red®, Fermentis) was added. The yeast was harvested from an over-night culture (in YPD media) and was washed twice to remove excess growth media. The flasks was flushed with nitrogen, closed with yeast locks, and incubated for 144hours at 35°C, 100 rpm.

2.5 Chemical analysis.

Raw and BP plantain peelings; Raw, HTT and WRF plantain trunks; Raw, HTT and SAA maize cobs; as well as raw and SAA maize stalks; were analysed for chemical composition by methods based on standard laboratory analytical procedures developed by National Renewable Energy Laboratory (NREL), US [23]. Deviations from these standard procedures are stated in the following sections. The analysis of the solid fibre fraction included ash content determination, lipophilic extraction and strong acid hydrolysis for structural carbohydrates (glucan, xylan, arabinan) and lignin.

2.5.1. Lipophilic extraction

Lipophilic extraction was carried out by Soxhlet extraction in a reflux condenser for six hours with 99% ethanol. The total amount of extractives, including volatiles, was defined as the mass of material lost during extraction.

2.5.2. Strong acid hydrolysis

Strong acid hydrolysis was used to determine the carbohydrate content of lipophilic extracted residues. 0.16 g TS biomass was subjected to 1.5 mL concentrated sulphuric acid (72 w/w%) at 30 °C for one hour. The remaining polymers were hydrolysed by diluting the samples to 4 w/w% sulphuric acid concentration and autoclaving at 121 °C for 10 minutes. The hydrolysates were filtered, and the Klason lignin was measured as the dry weight of the filter cake taking the ash content into account. Derived sugars in the hydrolysates were analysed by high-performance liquid chromatography (HPLC).

2.6. High-performance liquid chromatography (HPLC)

Monomeric sugars and ethanol were analysed on HPLC using a Biorad Aminex HPX-87H column (Hercules, CA; USA) with sugar standard of D-glucose, D-xylose, L-arabinose and ethanol using 4 mM H_2SO_4 as eluent (flow rate 0.6 ml $(\text{min})^{-1}$ and temperature 63°C).

2.7. Calculations:

Three measures were calculated in order to evaluate the efficiency of the pretreatments. These were the glucan recovery, the ethanol conversion efficiency, and the overall ethanol yield, as given below:

$$\text{Glucan recovery } \left(\frac{w}{w}\%\right) = \frac{c_{\text{glucan in pretreated sample}} \cdot (100\% - \%\text{ TS loss during pretreatment})}{c_{\text{glucan in raw sample}}}$$

$$\text{Ethanol conversion efficiency } \left(\frac{g\ ethanol}{100\ g\ potential\ eth.from\ pret.\ mat}\right) = \frac{c_{\text{ethanol after fermentation}}}{c_{\text{glucan in pretreated sample}} \cdot 1.11\frac{\text{g glucose}}{\text{g glucan}} \cdot 0.51\frac{\text{g ethanol}}{\text{g glucose}}}$$

$$\text{Overall ethanol yield } \left(\frac{g\ ethanol}{100\ g\ TS\ raw\ mat.}\right) = c_{\text{ethanol after fermentation}} \cdot (100\% - \%\text{ TS loss during pretreatment})$$

3. Results and Discussion

3.1. Enzymatic convertibility

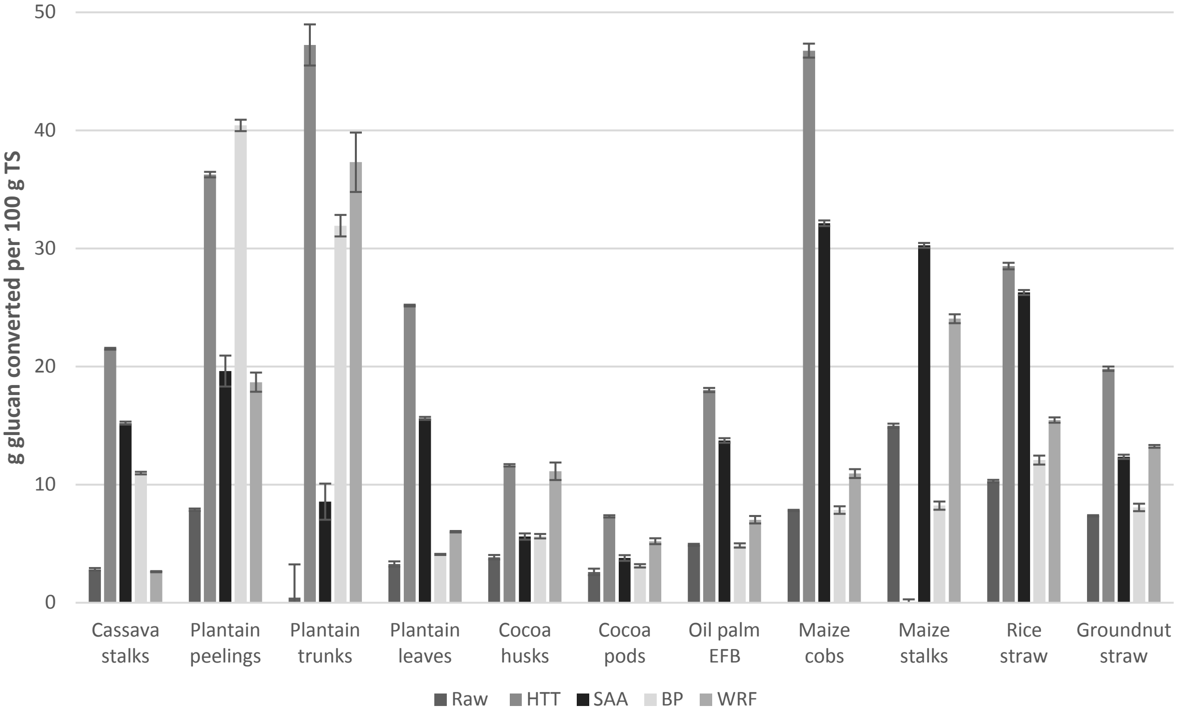

Fig. 1: Glucose yield after enzymatic conversion with cellulase of raw and pretreated West African biomasses.

The enzymatic convertibility expressed as the amounts of glucan (cellulose and starch) converted per gram TS of the raw and pretreated biomasses are presented in Fig. 1. It is apparent the biomasses are different and that the pretreatment methods have a substantial impact on the convertibility. For every biomass, HTT was the pretreatment that generated the highest convertibility among the pretreated biomasses, except for plantain peelings where BP was sufficient due to the relatively high starch content in this biomass [6]. Even though HTT gave the highest convertibilities, in most cases an alternative pretreatment method performs almost as well. This tendency is exemplified with plantain trunks where WRF is almost as effective as HTT (37.3 and 47.2 g glucan converted (100 g TS)$^{-1}$ respectively), or with rice straw where SAA is almost as effective as HTT (26.3 and 28.5 g glucan converted (100 g TS)$^{-1}$ respectively).

Where HTT have a profound effect on all biomasses, SAA, BP and WRF only affect some of the biomasses. SAA affects especially cassava stalks, oil palm EFB, maize cobs, maize stalks and rice straw, which is characterised by having high cellulose contents [6]. However, SAA had only limited effect on plantain trunks, even though that biomass contains 45 g cellulose (100g TS)$^{-1}$. BP significantly affected only cassava stalks, plantain peelings and plantain trunks, converting 11.0, 40.4, and 31.9 g cellulose (100g TS)$^{-1}$ respectively. WRF affects especially plantain trunks, maize stalks, rice straw and groundnut straw, converting 37.3, 24.0, 15.5 and 13.2 g cellulose (100g TS)$^{-1}$. There is no apparent correlation between the effect of BP or WRF and the content of any given biomass constituent. Thus, the expected correlation between mode of action and effect on the biomass cannot be confirmed in the current study.

High ethanol concentration in the fermentation liquor of an ethanol production is crucial, since this affects the distillation cost and energy balance. One of the most advanced cellulosic ethanol demonstration plants demand at least 4 w/w % ethanol in the fermentation liquor, whereas higher concentrations obviously are advantageous [39]. This places a constraint on the biomass as well as on the pretreatment method, since a high cellulose content, and a high enzymatic cellulose convertibility, after pretreatment will be required. Furthermore, the TS content in the feed for the enzymatic hydrolysis is seldom able to exceed 25 w/w %, which adds an additionally constraint [20]. The combination of at least 4 w/w % ethanol after fermentation, and maximum 25 % TS in prehydrolysis can be calculated into a required conversion of glucan of at least 30 g per 100 g of TS required in order to be cost effective. When evaluating the results presented in Fig. 1 in this context, only 4 of 11 biomasses tested had an enzymatic convertibility of more than 30g glucan converted per 100 g TS. Those were HTT and BP plantain peeling (36.3 and 40.4 g glucan/100 TS respectively), HTT, BP and WRF plantain trunks (47.2, 31.9 and 37.3 g glucan/100 TS respectively), HTT and SAA maize cobs (46.7 and 32.1 g glucan/100 TS respectively), and SAA maize stalks (30.3 g glucan/100 TS). Cassava stalks, plantain leaves and rice straw, were near the threshold and pretreatment of these could be subjected to optimisation studies in order to increase enzymatic convertibility. On the other hand, cocoa husks, cocoa pods, oil palm EFB, and groundnut straw, are not regarded as ideal for cellulosic ethanol production under the studied circumstances. This pinpoints that not all lignocellulosic biomasses serve as a potential feedstock for cellulosic ethanol, which often is not recognised in studies aiming to map bioenergy potentials of a specific region.

When addressing the enzymatic convertibility of xylan, all pretreatments have a positive effect but there is a tendency to highest conversion in the SAA treated material (data not shown). This is expected since a part of the xylan in the HTT material solubilises during the pretreatment, and the liquid fractions of HTT were not included in the study. Likewise, the WRF treatments were done with the fungus *C. subvermispora*, which preferably metabolises fractions of the hemicellulose components while degrading the lignin fraction, thus preserving cellulose.

Biomasses reaching the threshold of enzymatic glucan convertibilities over 30 g glucan $(100\text{ g TS})^{-1}$ (BP plantain peelings, HTT and WRF plantain trunks, HTT and SAA maize cobs, and SAA maize stalks) were evaluated further in fermentation studies, as well as in compositional analysis.

3.2. Compositional analysis

Table 1: Composition of selected raw and pretreated West African biomasses with enzymatic glucan convertibilities over 30g glucan (100 g TS)$^{-1}$.

g (100g TS)$^{-1}$		Glucan	Pentosan	Lignin	Ash	Extractives	Residual
Plantain peelings	**Raw[a]**	34.2	6.4	10.7	14.3	18.3	16.1
	BP	42.6	10.1	19.7	5.7	7.8	14.0
Plantain trunks	**Raw[a]**	46.3	13.8	12.4	13.7	10.1	3.7
	HTT	61.5	7.5	9.2	11.2	3.2	7.4
	WRF	51.3	12.6	11.7	12.7	4.6	7.1
Maize cobs	**Raw[a]**	36.1	34.8	18.7	1.6	1.7	7.1
	HTT	46.8	17.9	21.9	0.5	5.7	7.1
	SAA	29.2	34.1	14.9	1.7	9.8	10.3
Maize stalks	**Raw[a]**	38.4	22.0	17.0	11.2	4.2	7.3
	SAA	34.8	19.2	15.1	14.6	6.0	10.3

[a]raw materials were analysed in a previous study [6].

Compositional analysis of the biomasses were made in order to make mass balances in order to elaborate on the effects of pretreatment (Table 1).

In the case of plantain peelings, it appears that glucan, pentosan and lignin is increased during BP, this is however due to the relative nature of the compositional data. When ash components and extractives are reduced, due to the pretreatments, the relative share of the remaining components is enlarged. The same effect is seen for glucan for pretreated biomasses. The exception being SAA pretreated maize cobs and maize stalks. For the SAA no washing procedure was applied, thus degradation products from the pretreatment were left in the pretreated biomass thereby increasing the relative amounts of ash, extractives and residual. As indicated in the enzymatic convertibility and fermentation, the plantain trunks had the highest amount of glucan, especially the HTT treated and the WRF treated (61.5 and 51.3 g (100g TS)$^{-1}$ respectively). A high glucan content enables a high glucan concentration in the enzymatic prehydrolysis and thereby a high final ethanol concentration prior to distillation. The BP plantain peelings, the HTT and WRF plantain trunks, and the HTT and SAA maize cobs have not been addressed previously, to the knowledge of the authors. However, the maize stalks have been submitted to SAA in a previous study. Kim and Lee (2005) [29] found similar levels of glucan and xylan in SAA treated maize stalks as found in this study, however, they obtained a lower klason lignin content after the pretreatment than presented in Table 1 (7.5 compared to 15.1 g lignin (100 g TS)$^{-1}$ respectively).

3.3. Fermentation

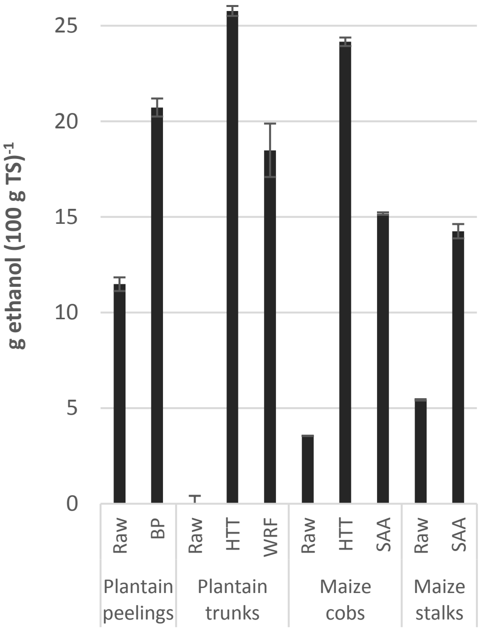

Figure 2: Ethanol yield from fermentation of raw and pretreated West African biomasses with enzymatic glucan convertibilities over 30g glucan (100 g TS)$^{-1}$.

The clear effect of pretreating experienced in the enzymatic convertibility is also apparent in the ethanol yields after fermentation, where all the pretreatments are significantly increasing the ethanol yields (Fig. 2). It is well-know that degradation products of certain pretreatments will act as a fermentation inhibitor if they exceed certain levels [40]. However, there is no sign of inhibition in any of the fermentations, which can be seen when comparing the ethanol yields in the fermentations, Fig. 2. with the enzymatic convertibility. Most of the fermentations have final ethanol concentrations corresponding to utilisation of 80 to 90 % of the glucose liberated in the enzymatic convertibility. The exception being plantain peelings, where both raw and BP is yielding more ethanol than expected from the enzymatic convertibility. This is due to addition of starch degrading enzymes in the prehydrolysis of the fermentation, which were not used in the enzymatic convertibility.

3.4. Mass balances

Table 2: Glucan recovery, ethanol conversion efficiency and overall ethanol yield.

		Glucan recovery *w/w %*	**Ethanol conversion efficiency** *g eth./100 g potential eth. from pretreated material*	**Overall ethanol yield** *g eth./100 g TS raw material*
Plantain peelings	**Raw**	100%	59.4	11.5
	BP	81%	85.9	13.4
Plantain trunks	**Raw**	100%	0.0	0.0
	HTT	77%	74.1	15.0
	WRF	89%	63.7	14.8
Maize cobs	**Raw**	100%	17.3	3.6
	HTT	81%	91.1	15.2
	SAA	81%	92.7	15.2
Maize stalks	**Raw**	100%	25.0	5.4
	SAA	90%	72.4	13.7

The glucan recovery after a pretreatment of biomass is indicating to which extent the potential of a biomass can be valorised. In Table 2 the glucan recovery of the most promising biomasses are given, and approximately 80 to 90 % of the glucan can be recovered for all the biomasses. The highest glucan recoveries found in the study were from SAA maize stalks and WRF plantain trunks (90 and 89% respectively), which indicates that these methods can be equal or superior compared to HTT on this parameter. Glucan recoveries of 90% or higher have been regarded satisfactory in previous studies [19, 41]

It is clear when evaluating the ethanol conversion efficiency, that the glucan of the HTT and SAA maize cobs are most readily converted to ethanol, since more than 90 % of the theoretical potential ethanol was produced from pretreated material (Table 2). In addition, it is seen that even though pretreated plantain trunks have very high glucan concentrations of 61.5 and 51.3 g $(100g\ TS)^{-1}$ respectively, the glucan is not fully accessible (Table 2). Moreover, untreated plantain could not be converted to ethanol under the conditions of the current study.

Assessing the overall ethanol yield of the combined processes displays that all the pretreated biomasses assessed in Table 2 are in the same range of between 13.4 and 15.2 g ethanol produced per gram of raw material. There is no significantly difference between HTT and WRF applied on plantain trunks when taking deviations in the assessment into account. Likewise, SAA has similar overall ethanol yield as HTT when applying the methods on maize cobs. Thus, it indicates that HTT can be substituted with methods like SAA or WRF without affecting the overall ethanol yield. Furthermore, it is once again emphasised that pretreatment is critical when utilising lignocellulosic biomasses for ethanol. However, higher overall ethanol yield might be reached if the pretreatment methods are optimised for the specific biomasses, though it cannot be concluded which method that will be most profitable in each case, since this will be highly dependent on local conditions such as the available biomass, infrastructure, and investment costs. The presented study is based solely on laboratory experiments and certain practices are not mimicking full-scale solutions perfectly, and therefore further laboratory and pilot studies are required. E.g., effect of regional differences and harvest time on the biomasses were not taken into consideration, and therefore further work should be done in this respect. Finally, feedstock selection for bioenergy purposes should be assessed further as not to affect availability to food or feed, especially in the already poverty-stricken region of West Africa.

4. Conclusion

In the presented study, it was shown that alternative pretreatment methods applicable for ethanol production in West Africa, such as BP, SAA and WRF, are comparable to HTT with respect to overall ethanol yield, even though HTT generally is the pretreatment method which give the highest enzymatic convertibilities. However, the alternative methods of PB, SAA and WRF have more selective modes of action than HTT, thus they each only performed well on a few of the biomasses.

The results of this study suggest that 4 out of the 11 addressed West African lignocellulosic biomasses were found to be feasible for cellulosic ethanol production, which should be taken into account when estimating cellulosic ethanol potentials of the region. Feedstock choices concerning farming systems and current use have not been addressed, although these, together with technology, play key roles in determining the overall sustainability of small scale decentralised bioenergy systems.

Acknowledgements

This work was supported with a grant from Danida Fellowship Centre (DFC) of the Danish Ministry of Foreign Affairs, as a part of the project “Biofuel production from lignocellulosic materials – 2GBIONRG”, DFC journal no. 10-018RISØ (http://2gbionrg.dk). Anne Eva Jensen, Tomas Fernqvist and Ingelis Larsen are acknowledged for their vital and professional assistance in the laboratory. Dr Moses Mensah and Dr Edward Yeboah are recognised for providing the residues. Professor Hanne Østergård is sincerely thanked for her help with planning and motivation.

References

[1] Mangoyana RB. Bioenergy for sustainable development: An African context. Phys Chem Earth 2009; 34: 59-64.

[2] Ejigu M. Toward energy and livelihoods security in Africa: Smallholder production and processing of bioenergy as a strategy. Nat Resour Forum 2008; 32: 152-62.

[3] Brew-Hammond A, Kemausuor F. Energy for all in Africa - to be or not to be?!. Curr Opin Environ Sustain 2009; 1: 83-8.

[4] Duku MH, Gu S, Hagan EB. A comprehensive review of biomass resources and biofuels potential in Ghana. Renew sust energ rev 2011; 15: 404-15.

[5] Kemausuor F, Kamp A, Thomsen ST, Bensah EC, Østgård H. Assessment of biomass residue availability and sustainable bioenergy yields in Ghana. Resour Conserv Recy 2013; in press.

[6] Thomsen ST, Kádár Z, Schmidt JE. Compositional analysis and projected biofuel potentials from common West African agricultural residues. Biomass Bioenerg 2013; in press.

[7] Amigun B, Sigamoney R, von Blottnitz H. Commercialisation of biofuel industry in Africa: A review. Renew sust energ rev 2008; 12: 690-711.

[8] Quintero JA, Cardona CA, Felix E, Moncada J, Sánchez ÓJ, Gutiérrez LF. Techno-economic analysis of bioethanol production in Africa: Tanzania case. Energy 2012; 48: 442-54.

[9] Osagie S. African promise. Eng Technol 2008; 3: 68.

[10] Sarkar N, Ghosh SK, Bannerjee S, Aikat K. Bioethanol production from agricultural wastes: An overview. Renew Energ 2012; 37: 19-27.

[11] Martin C, Thomsen AB. Wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and peanuts for ethanol production. Journal of Chemical Technology and Biotechnology 2007; 82: 174-81.

[12] Idi A, Salleh MM, Ibrahim Z, Mohamad SE. Pretreatment of cocoa waste for bioethanol production using ionic liquid. Jurnal Teknologi (Sciences and Engineering) 2012; 59: 49-56.

[13] Zhang H, Zhou Y, Li J, Dai L, Liu D, Zhang J, et al. Pretreatment of oil palm residues by dilute alkali for cellulosic ethanol production. Chin J Biotechnol 2013; 29: 490-500.

[14] Bensah EC, Mensah M. Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations. Int J Chem Eng 2013; 2013: 21.

[15] Hu F, Ragauskas A. Pretreatment and Lignocellulosic Chemistry. Bioenerg Res 2012; 5: 1043-66.

[16] Carvalheiro, Duarte, Girio. Hemicellulose biorefineries: a review on biomass pretreatments. J Sci Ind Res (India) 2008; 67: 849-64.

[17] Vegas R, Kabel M, Schols HA, Alonso JL, Parajó JC. Hydrothermal processing of rice husks: Effects of severity on product distribution. J ChemTechnol Biot 2008; 83: 965-72.

[18] Thomsen ST, Jensen M, Schmidt JE. Production of 2nd generation bioethanol from lucerne - Optimization of hydrothermal pretreatment. BioResour 2012; 7: 1582-93.

[19] Ambye-Jensen M, Thomsen ST, Kádár Z, Meyer AS. Ensiling of wheat straw decreases the required temperature in hydrothermal pretreatment. Biotechnol Biofuels 2013; 6.

[20] Larsen J, Haven MØ, Thirup L. Inbicon makes lignocellulosic ethanol a commercial reality. Biomass Bioenerg 2012; 46: 36-45.

[21] Harun MY, Dayang Radiah AB, Zainal Abidin Z, Yunus R. Effect of physical pretreatment on dilute acid hydrolysis of water hyacinth (Eichhornia crassipes). Bioresour Technol 2011; 102: 5193-9.

[22] Haque MA, Barman DN, Kang TH, Kim MK, Kim J, Kim H, et al. Effect of dilute alkali on structural features and enzymatic hydrolysis of barley straw (Hordeum vulgare) at boiling temperature with low residence time. J Microbiol Biotechn 2012; 22: 1681-91.

[23] Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. J Agric Food Chem 2010; 58: 9043-53.

[24] Brown RC. Biorenewable Resources Engineering New Products from Agriculture. 1st ed. Iowa: Blackwell Publishing; 2003.

[25] Kim TH, Gupta R, Lee YY. Pretreatment of biomass by aqueous ammonia for bioethanol production. Methods Mol Biol 2009; 581: 79-91.

[26] Isci A, Himmelsbach JN, Pometto III AL, Raman DR, Anex RP. Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation. Appl Biochem Biotechnol 2008; 144: 69-77.

[27] Jurado E, Skiadas IV, Gavala HN. Enhanced methane productivity from manure fibers by aqueous ammonia soaking pretreatment. Appl Energy 2013; 109: 104-11.

[28] Kim M, Aita G, Day DF. Compositional changes in sugarcane bagasse on low temperature, long-term diluted ammonia treatment. Appl Biochem Biotechnol 2010; 161: 34-40.

[29] Kim TH, Lee YY. Pretreatment of corn stover by soaking in aqueous ammonia. Appl Biochem Biotechnol 2005; 124: 1119-31.

[30] Sánchez C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnol Adv 2009; 27: 185-94.

[31] Isroi, Isroi, Niklasson C, Millati R, Cahyanto MN, Syamsiah S, et al. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. BioResour 2011; 6: 5224-59.

[32] Wan C, Li Y. Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv; 30: 1447-57.

[33] Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater. Washington: American Public Health Association; 1998.

[34] Bjerre AB, Sørensen E. Thermal and oxidative decomposition of lower fatty acids with special attention to formic acid. Risø National Laboratorium 1990.

[35] Bjerre AB, Olesen AB, Fernqvist T, Ploger A, Schmidt AS. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnol Bioeng 1996; 49: 568-77.

[36] Schultz-Jensen N, Thygesen A, Leipold F, Thomsen ST, Roslander C, Lilholt H, et al. Pretreatment of the macroalgae Chaetomorpha linum for the production of bioethanol - Comparison of five pretreatment technologies. Bioresour Technol 2013; 140: 36-42.

[37] Salvachua D, Prieto A, Lopez-Abelairas M, Lu-Chau T, Martinez AT, Martinez MJ. Fungal pretreatment: An alternative in second-generation ethanol from wheat straw. Bioresour Technol 2011.

[38] Wan C, Li Y. Effectiveness of microbial pretreatment by Ceriporiopsis subvermispora on different biomass feedstocks. Bioresour Technol 2011; 102: 7507-12.

[39] Larsen J, Petersen MØ, Thirup L, Li HW, Iversen FK. The IBUS Process - Lignocellulosic Bioethanol Close to a Commercial Reality. Chem Eng Technol 2008; 31: 765-72.

[40] Thomsen MH, Thygesen A, Thomsen AB. Identification and characterization of fermentation inhibitors formed during hydrothermal treatment and following SSF of wheat straw. Appl Microbiol Biot 2009; 83: 447.

[41] Xu J, Thomsen MH, Thomsen AB. Investigation of acetic acid-catalyzed hydrothermal pretreatment on corn stover. Appl Microbiol Biotechnol 2010; 86: 509-16.

Highlights

- Screening of low-tech small-scale pretreatment methods for cellulosic ethanol
- Pretreatment performed on 11 West African biomasses, of which 4 were promising
- White rot fungi pretreatment and soaking in aqueous ammonia are viable alternatives
- Alternative methods have similar overall ethanol yields as hydrothermal treatment

(Thomsen et al., 2014a, Paper IV)

Paper V

In-text citation:
(Thomsen et al., 2014c, Paper V)

Authors:
Thomsen S.T., Spliid H., Østergård H.

Title:
Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass

Publication status:
Published in *Bioresource Technology* (2014) 154, 80-6

Bioresource Technology 154 (2014) 80–86

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass

Sune Tjalfe Thomsen[a], Henrik Spliid[b], Hanne Østergård[a,*]

[a] Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, Technical University of Denmark DTU, DK-2800 Kgs. Lyngby, Denmark
[b] Section of Statistics and Data Analysis, Department of Applied Mathematics and Computer Science, Technical University of Denmark DTU, DK-2800 Kgs. Lyngby, Denmark

HIGHLIGHTS

- A statistical model for predicting *BMP* from lignocellulosic material is developed.
- The true effect of lignin and carbohydrates on *BMP* is described.
- The best prediction is proposed using a canonical linear mixture model.
- An expression for prediction founded on the largest dataset to date, is presented.

ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form 3 December 2013
Accepted 7 December 2013
Available online 13 December 2013

Keywords:
Biomethane potential (*BMP*)
Mixture model
Lignocellulose
Biogas
Anaerobic digestion (AD)

ABSTRACT

Mixture models are introduced as a new and stronger methodology for statistical prediction of biomethane potentials (BPM) from lignocellulosic biomass compared to the linear regression models previously used. A large dataset from literature combined with our own data were analysed using canonical linear and quadratic mixture models. The full model to predict *BMP* ($R^2 > 0.96$), including the four biomass components cellulose (x_C), hemicellulose (x_H), lignin (x_L) and residuals ($x_R = 1 - x_C - x_H - x_L$) had highly significant regression coefficients. It was possible to reduce the model without substantially affecting the quality of the prediction, as the regression coefficients for x_C, x_H and x_R were not significantly different based on the dataset. The model was extended with an effect of different methods of analysing the biomass constituents content (D_A) which had a significant impact. In conclusion, the best prediction of *BMP* is $pBMP = 347x_{C+H+R} - 438x_L + 63D_A$.

1. Introduction

Biomethane potential (*BMP*) measurements are very time-consuming, as up to 90 days are required as a standard incubation time (Hansen et al., 2004; Gerber et al., 2013; Angelidaki et al., 2009). Therefore, it is attractive to use faster methods when estimating how much methane gas it is possible to produce from a given biomass. This is especially the case when making theoretical studies without access to laboratory facilities, or when a fast prediction of *BMP* from a new biomass is required.

Theoretical methods of predicting *BMP* (*pBMP*) have been available since 1933 when Symons and Buswell made their theoretical and laboratory studies of anaerobic digestion of carbohydrates where they presented what later would be known as Buswell's formula (Symons and Buswell, 1933). This formula expresses the maximum output of methane gas in a complete anaerobic digestion of organic matter, and is calculated from the chemical sum formula of the organic material:

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2$$

Even though Buswell's formula were designed for estimating the ultimate *BMP* from a biomass based on the sum formula, it can also be used on each of the biomass constituents. This means, that the formula can determine the theoretical *BMP* on cellulose (x_C), hemicellulose (x_H), protein, lipids, etc. of biomass, if compositional data are available. In these cases, it is also possible to exclude a contribution from non-convertible biomass constituents such as lignin (x_L) and ash.

Even though *BMP* can be predicted with Buswell's formula, one important factor is not taken into account, namely the recalcitrance of the biomass in question. When dealing with pure substrates, such as sugars or lipids recalcitrance is not important to include. However, when dealing with e.g., lignocellulosic substrates, the shielding effect of the lignocellulosic matrix will decrease the *BMP* (Azhar and Stuckey, 1994). The extent to which

---

* Corresponding author. Address: DTU Risø Campus, Frederiksborgvej 399, P.O. Box 49, Building 301, 4000 Roskilde, Denmark. Tel.: +45 21326955.
E-mail address: haqs@kt.dtu.dk (H. Østergård).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.12.029

such an effect takes place tends to be correlated with the compositions of the biomass (Labatut et al., 2011).

Often chemical oxygen demand (COD) is used to estimate *BMP*, however this method suffers from some of the same inconsistencies as Buswell's formula. When measuring COD a total oxidation of organic material is made, and therefore neither biomass recalcitrance nor the contribution of non-convertible lignin is taken into account, forcing the COD method to over-estimate the *pBMP*.

Determining *pBMPs* through regression models is a relatively new methodology initiated within the last decade (Monlau et al., 2012; Triolo et al., 2011; Gunaseelan, 2007, 2009). The current study focuses only on determining *pBMP* from lignocellulosic biomasses. This has only been addressed in a few previous studies which are presented in Table 1.

As seen in Table 1, the previously proposed regression models assume that the lignin content is the single most import biomass constituent, when predicting *BMP* (Triolo et al., 2011; Monlau et al., 2012). This is paradoxical since lignin does not contribute to the formation of methane in the anaerobic digestion (AD) process, but rather is acting as the glue that ties the lignocellulosic matrix together while making a physical barrier around the carbohydrates (Albersheim et al., 2011). In that way, the regression models proposed so far have been contradictory to AD theory, since the content of degradable biomass constituents such as cellulose and hemicellulose is not accounted for in the models. This implies that a biomass with low content of lignin will give rise to a high *pBMP* regardless of the carbohydrate content. Furthermore, Triolo et al. (2011) found that when including both x_L and x_C as regression variables, x_C contributed negatively to the model (Table 1, row 3). This is also contrary to AD theory, since carbohydrates are the main substrates in the AD and, therefore, should imply a positive regression coefficient.

The possible misinterpretations in the previous prediction models may reflect the relative nature of the compositional data. Biomass composition is most often presented as % of total solids (TS), % of volatile solids (VS) or w/w%. This results in a constraint on the data, since the components add up to 100%. Normally, regression coefficients are interpreted as the change in the dependent variable due to a unit change in the independent variable while keeping everything else constant, but with compositional data it is not possible to change one proportion while keeping the others constant. Due to this constraint, the space in which each component can be varied is obviously strongly restricted, which previously has not been addressed in relation to *BMP*. Similar issues have been taken into account elsewhere, especially for chemical mixtures where compositional data also are predominant. Here, a wide range of regression models, known as mixture models, have been developed (Cornell, 2011; Prakasham et al., 2009; Scheffe, 1963). It might be advantageous to view the compositional data as a chemical mixture, thus investigating the effect of the different biomass constituents on *pBMP* in a mixture model.

In mixture models, the variables are proportionate nonnegative amounts of different constituents, $0 \leqslant x_i \leqslant 1$, $i = 1,2,\ldots,q$ where $\sum_{i=1}^{q} x_i = 1$. In our case, the variables are the main biomass constituents of lignocellulosic biomass: Cellulose (x_C), hemicellulose (x_H), and lignin (x_L). Since the variables sum up to one, an additional variable (x_R) which is often called 'residuals' in relation to biomass composition, is included in the model. In this way everything which is not carbohydrates or lignin is characterised as residuals, $x_R = 1 - (x_C + x_H + x_L)$. Introducing residuals is not new to the area of determining biomass composition (Sluiter et al., 2010; Thomsen et al., 2012). However, x_R has not been considered in previous models as a regression variable (Table 1), which might be problematic, since x_R might contain methane yielding biomass constituents such as lipids, fatty acids, pectin, proteins and tannins.

In the present study, both a canonical linear mixture model, $pBMP = \sum_{i=C,H,L,R} \beta_i x_i$, as well as a canonical quadratic mixture model, $pBMP = \sum_i \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j$ (where indices *i* and *j* refer to the components *C*, *H*, *L* and *R*) will be investigated. In this way, models for predicting *BMP*, which are in accordance to AD theory, will be developed. The regression coefficients will be estimated from a large dataset from literature combined with data prepared for this study.

2. Methods

2.1. BMP test performed for this study

Biomasses tested for *BMP* for this study were cassava stalks, cocoa pods, groundnut straw, lucerne cake, maize cobs, maize stalks, oil palm empty fruit bunches (oil palm EFB), plantain leaves, plantain trunks, rice straw, vetch hay and rye straw mixed, rye straw and vetch hay. For the determination of biogas potentials prepared for this study, triplicate-samples of all biomasses were distributed in 1 l serum flasks (effective volume 1125 ml) in amounts of 1 g volatile solids (VS) per 100 ml active volume. The samples were inoculated with 150 ml of effluent from a lab-scale biogas reactor treating cattle manure and water was added to a total active volume of 300 ml. For subtraction of biogas produced by the inoculum, flasks containing only inoculum and water were also prepared. The flasks were sealed with rubber septum and metal screw plugs and the samples were incubated at 55 °C for a period of 50 days, hereafter, no more gas production was observed. The CH_4 production in the flasks was measured by collecting 0.5 ml of headspace gas using a gas tight syringe and analysing the CH_4 concentration in the sample by gas chromatography (HP 6890; Agilent). Measurements were carried out in increasing intervals ranging from 2 days in the beginning to 8 days in the end of the digestion trials. Biomass composition has been assessed previously (Thomsen et al., 2012; Carter et al., 2012).

2.2. Literature search and selection

In order to find relevant data for determining the best possible regression model, we aimed to construct as large a dataset as possible. The literature search was done with a systematic approach where all combinations of two lists of search criteria (Table 2) were applied. The search engine Scopus was used until April 5 2013 and

Table 1
Previously presented regression models for determining *pBMP* from composition of lignocellulosic biomass.

Regression model	Prediction model[a]	Reference	Biomass used (number of samples used generating the equation)	R^2
$\alpha_0 + \alpha_L x_L$	$pBMP = 461 - 258\ x_L$	Triolo et al. (2011)	Energy crops ($n = 10$)	0.76
$\alpha_0 + \alpha_L x_L$	$pBMP = 380 - 65\ x_L$	Monlau et al. (2012)	Raw and pretreated sunflower stalks ($n = 8$)	0.92
$\alpha_0 + \alpha_L x_L + \alpha_C x_C$	$pBMP = 447 - 277\ x_L - 7\ x_C$	Triolo et al. (2011)	Energy crops ($n = 10$)	0.77
Buswell on carbohydrates	$pBMP = 414\ x_C + 423\ x_H$	Symons and Buswell (1933)	Theoretical model	–

[a] The regression coefficients have been transformed to the unit of the variables used in this study which is w/w instead of w/w% used in the references. x_L is the lignin content, x_C is the cellulose content and x_H is hemicellulose.

Table 2
Search criteria used in the data search.

1st search criteria	2nd search criteria
Anaerobic digestion	Acid detergent fib[a] (ADF)
Batch test	Acid detergent lignin (ADL)
Biochemical methane potential (*BMP*)	Cellulose/Glucan
Biogas	Composition[a]
Biogas potential[a]	Hemicellulose/Pentosan
Biomethane potential[a] (*BMP*)	Lignin
Ultimate methane yield	Lignocellulose[a]
	Natural detergent fib[a] (NDF/aNDF)

[a] Indicates a wild-card operator, it was used to search for alternative endings of words, e.g. fib* searches for both fibre and fiber simultaneous.

the search was made in the fields of article title, abstract and keywords. Furthermore, data was only sought in peer-reviewed articles, and only English language sources were assessed. The search generated 2692 potential articles, which were assessed for relevance individually. In order for a published dataset to be included in this study, *BMP* as well as biomass composition should be available. Further requirements were:

- Biomass composition was either determined with the fibre analysis methods and presented as cellulose, hemicellulose and lignin, or determined with forage analysis methods and presented as ADL (acid detergent lignin), ADF (acid detergent fibre) and NDF (natural detergent fibre).
- The incubation time when measuring the *BMP* was more than 60 days or to a state of no further gas production.
  Low substrate loading was applied to counter inhibition of various kinds (<2 g VS/100 ml active volume).
- The feedstock is lignocellulosic, thus substrates containing more than 0.1 g/g TS of starch, lipids, proteins, free sugars, pectin, or fatty acids, was disregarded due to disproportionate large expected influence on *BMP*.
- The amount of lignocellulosic components ($x_C + x_H + x_L$), was more than 0.5 g/g TS, and each component should be present in at least 0.05 g/g TS (x_C, x_H, $x_L > 0.05$ w/w).
- The material has not been pretreated since this would have disrupted the lignocellulosic matrix. The only exception is milling to pass a sieve of minimum one mm, which is often used for homogenisation purposes in laboratory tests.
- Incubation temperature should be either mesophilic (32–37 °C) or thermophilic (52–55 °C).

2.3. Statistical analysis

2.3.1. Software

The open source software 'R' was used for statistical computing and some graphics. Add-on packages used were: boot, car, DAAG, datasets, lattice, leaps, MASS, methods, nnet, randomForest, rpart, splines, stats, survival, and utils.

2.3.2. Regression models to predict BMP

A canonical full linear mixture model was investigated and used for prediction of *BMP*:

$$BMP \sim \beta_C x_C + \beta_H x_H + \beta_L x_L + \beta_R x_R + \varepsilon$$

where x_C, x_H, x_L, and x_R are the observed composition of cellulose, hemicellulose, lignin and residuals, respectively, and the error term ε is representing uncertainty. The following assumptions for the mixture models were made:

Normal distribution of the data assessed graphically with Q–Q plots.

Homoscedasticity, i.e. the standard deviation of the error term is assumed constant and independent of the *x*-values.

Independence of the estimated errors assessed by plotting them against the fitted values. In this case the errors should be randomly distributed around 0.

A number of hypotheses were tested to reduce the model in the specific dataset:

$$H_1 : \beta_C = \beta_H = \beta_{C+H}$$

Given this hypothesis, the prediction would be

$$pBMP = \beta_{C+H}x_{C+H} + \beta_L x_L + \beta_R x_R, \quad \text{where } (x_{C+H} = x_C + x_H)$$

The validity of the reduced model was assessed by analysis of variance (ANOVA), and by assessing diagnostics plots. Further reduction of the model was assessed in a similar way:

$$H_2 : \beta_{C+H} = \beta_R = \beta_{C+H+R}$$

The final model was expanded to include the effect of whether the forage or the fibre analysis method was applied when determining content of biomass constituents. The prediction model was, thus, expanded with a dummy variable:

$$pBMP = \beta_{C+H+R}x_{C+H+R} + \beta_L x_L + \gamma_A D_A, \quad D_A = \begin{cases} 0, & \text{Forage analysis method} \\ 1, & \text{Fibre analysis method} \end{cases}$$

where $x_{C+H+R} = x_C + x_H + x_R$.

Other differences among the studies were not tested since this was not conceivable with the available dataset where homogeneity was aimed at in the selection of studies as described in Section 2.2. As an example, with respect to incubation temperature the number of thermophilic studies was too small for a statistical test.

When comparing the ability of the models to predict *pBMP*, R^2 and relative root mean square error (*rRMSE*) was used:

$$R^2 = \frac{\sum_i (pBMP_i - \overline{BMP})^2}{\sum_i (pBMP_i - \overline{BMP})^2 + \sum_i (BMP_i - pBMP_i)^2}$$

$$rRMSE = \frac{\sqrt{\frac{\sum (BMP - pBMP)^2}{n}}}{\overline{BMP}}$$

Here *n* is the number of data points and is the mean of the measured *BMP*'s. *rRMSE* is a measure of all the associated uncertainties of the model, unavoidable measure inaccuracies, differences between laboratories, interactions otherwise unaccounted for, and other limitations to the model. Furthermore, *rRMSE* is relative to the level of the values as opposed to R^2.

3. Results and discussion

3.1. The combined dataset

Using the search criteria (Table 2), as well as the criteria for selecting the data, it was possible to retrieve a dataset of 48 samples found in literature. Furthermore, 16 samples were analysed specifically for this study adding up to 64 samples (Table 3), which is a much larger sample size than used in predictive models presented previously (Table 1). The dataset contains a broad spectrum of lignocellulosic biomasses, originating from various plant genera, ecological niches and growing conditions, which results in diversity in both biomass constituent composition and in the measured *BMP*'s. This is an advantage to the study since it enables high significance of the regression coefficients, whereby the validity of the model increases. On the other hand, the presented results are retrieved from 12 studies, which may add to the uncertainties of

Table 3
Combined dataset. Names in quotation marks specify the samples in the references.

Biomass	Cellulose x_C w/w	Hemicellulose x_H w/w	Lignin x_L w/w	Residual x_R w/w	Biomethane potential *BMP* l CH_4/kg VS	Reference
Barley straw	0.468	0.300	0.096	0.136	229	Dinuccio et al. (2010)[e]
Bulrush DWC[a]	0.361	0.210	0.150	0.279	165	Wang et al. (2010)[e]
Cassava stalks	0.341	0.170	0.283	0.206	101	This study[f]
Clover DWC	0.263	0.183	0.142	0.412	269	Oleskowicz-Popiel et al. (2011)[f]
Cocoa pods	0.196	0.186	0.372	0.245	96	This study[f]
Energy grass 'Szarvasi' DWC	0.379	0.273	0.097	0.252	322	Alaru et al. (2011)[e]
Foxtail millet DWC	0.330	0.316	0.053	0.300	349	Alaru et al. (2011)[e]
Giant reed leaves	0.331	0.185	0.245	0.239	228	Monlau et al. (2012)[f]
Giant reed stalks	0.209	0.177	0.254	0.360	178	Monlau et al. (2012) [f]
Grape stalks	0.235	0.159	0.233	0.373	98	Dinuccio et al. (2010)[e]
Groundnut straw	0.204	0.060	0.154	0.582	275	This study[f]
Hedge cuttings	0.253	0.102	0.139	0.506	200	Triolo et al. (2011)[e]
Hemp 'fibre variety' 1	0.550	0.108	0.072	0.270	326	Alaru et al. (2011)[e]
Hemp 'fibre variety' 2	0.539	0.106	0.088	0.268	310	Alaru et al. (2011)[e]
Lucerne hay	0.183	0.121	0.119	0.576	276	This study[f]
Maize cobs 1	0.361	0.348	0.180	0.110	339	This study[f]
Maize cobs 2	0.298	0.346	0.192	0.164	228	Monlau et al. (2012)[f]
Maize DWC 1	0.396	0.195	0.079	0.330	363	This study[f]
Maize DWC 2[b]	0.224	0.269	0.061	0.446	311	Oslaj et al. (2010)[e]
Maize DWC 3[c]	0.266	0.287	0.075	0.372	319	Oslaj et al. (2010)[e]
Maize DWC 4[d]	0.318	0.284	0.071	0.327	288	Oslaj et al. (2010)[e]
Maize leaves	0.309	0.286	0.204	0.201	257	Monlau et al. (2012)[f]
Maize stalks 1	0.384	0.219	0.170	0.227	333	This study[f]
Maize stalks 2	0.271	0.212	0.232	0.285	206	Monlau et al. (2012)[f]
Maize stalks 3	0.301	0.190	0.103	0.406	360	Tong et al. (1990)[f]
Miscanthus DWC	0.420	0.302	0.070	0.209	349	Alaru et al. (2011)[e]
Napier grass DWC	0.360	0.187	0.105	0.348	288	Tong et al. (1990)[f]
Oil palm EFB	0.335	0.230	0.238	0.197	189	This study[f]
Perennial grass DWC	0.291	0.260	0.058	0.392	271	Triolo et al. (2011)[e]
Plantain leaves	0.225	0.202	0.183	0.390	207	This study[f]
Plantain trunks	0.463	0.138	0.124	0.275	275	This study[f]
Rice straw 1	0.339	0.204	0.113	0.344	327	This study[f]
Rice straw 2	0.262	0.188	0.270	0.280	217	Monlau et al. (2012)[f]
Rice straw 3	0.334	0.282	0.074	0.310	360	He et al. (2008)[e]
Rye DWC	0.500	0.182	0.107	0.211	417	Oleskowicz-Popiel et al. (2011)[f]
Rye straw 1	0.365	0.240	0.177	0.218	220	This study[f]
Rye straw 2	0.408	0.246	0.193	0.153	297	This study[f]
Smooth cordgrass DWC	0.340	0.299	0.097	0.264	358	Triolo et al. (2011)[e]
Sorghum 'Akklimat'	0.309	0.232	0.055	0.404	232	Mahmood and Honermeier (2012)[e]
Sorghum 'Biomass variety'	0.222	0.194	0.214	0.370	250	Monlau et al. (2012)[f]
Sorghum 'Bovital' 1	0.296	0.199	0.045	0.460	302	Mahmood and Honermeier (2012)[e]
Sorghum 'Bovital' 2	0.292	0.223	0.045	0.440	282	Mahmood and Honermeier (2012)[e]
Sorghum 'Forage variety'	0.183	0.217	0.207	0.393	280	Monlau et al. (2012)[f]
Sorghum 'Goliath' 1	0.363	0.199	0.053	0.385	320	Mahmood and Honermeier (2012)[e]
Sorghum 'Goliath' 2	0.334	0.193	0.053	0.420	280	Mahmood and Honermeier (2012)[e]
Sorghum stalks 'Seed variety'	0.291	0.261	0.225	0.223	236	Monlau et al. (2012)[f]
Sorghum 'Sweet variety' 1	0.201	0.209	0.185	0.405	314	Monlau et al. (2012)[f]
Sorghum 'Sweet variety' 2	0.197	0.200	0.198	0.405	307	Monlau et al. (2012)[f]
Sorghum 'Sweet variety' 3	0.181	0.185	0.213	0.421	289	Monlau et al. (2012)[f]
Sunflower stalks 1	0.310	0.156	0.292	0.242	185	Monlau et al. (2012)[f]
Sunflower stalks 2	0.312	0.143	0.277	0.268	190	Monlau et al. (2012)[f]
Sunflower stalks 3	0.312	0.143	0.300	0.245	183	Monlau et al. (2012)[f]
Sunflower stalks bark	0.274	0.135	0.350	0.241	161	Monlau et al. (2012)[f]
Vetch hay	0.293	0.164	0.214	0.328	191	This study[f]
Vetch hay and rye straw 1	0.336	0.219	0.185	0.259	236	This study[f]
Vetch hay and rye straw 2	0.364	0.225	0.193	0.218	236	This study[f]
Wheat straw 1	0.480	0.284	0.064	0.172	290	Triolo et al. (2011)[e]
Wheat straw 2	0.490	0.340	0.065	0.105	208	Sambusiti et al. (2012)[f]
Wheat straw 3	0.337	0.186	0.174	0.303	302	Tong et al. (1990)[f]
Wheat straw 4	0.350	0.182	0.172	0.296	333	Tong et al. (1990)[f]
Wild grass DWC	0.360	0.229	0.060	0.351	306	Triolo et al. (2011)[e]
Wild plants	0.337	0.229	0.101	0.333	214	Triolo et al. (2012)[e]
Wood cuttings	0.224	0.120	0.225	0.431	172	Triolo et al. (2012)[e]
Wood grass	0.390	0.149	0.273	0.188	291	Tong et al. (1990)[f]

[a] DWC = dried whole crop.
[b] Average of different hybrids at maturity class FAO 500–600 (mature).
[c] Average of different hybrids at maturity class FAO 400–500 (less mature).
[d] Average of different hybrids at maturity class FAO 300–400 (young).
[e] Indicates for age method used for biomass composition analysis.
[f] Indicates fiber method used for biomass composition analysis.

the results, e.g. due to differences in the laboratory procedures applied.

3.2. Regression models to predict BMP

Regression coefficients in the full canonical linear mixture model ($BMP \sim \beta_C\, x_C + \beta_H\, x_H + \beta_L\, x_L + \beta_R\, x_R + \varepsilon$) were estimated from the dataset (Table 3) resulting in the following prediction of the *BMP*:

$$pBMP = 378x_C + 354x_H - 194x_L + 313x_R$$

All components of the lignocellulosic biomass, x_C, x_H and x_L, as well as the remaining biomass constituents described by x_R, were statistically highly significant (Table 4). Furthermore, the model showed *rRMSE* of 19.7% and a high R^2-value of 0.96.

A confirmation of the full canonical linear mixture model was made by evaluating the residuals of the model (Fig. 1). In Fig. 1a, a Q–Q plot graphically compares the probability distributions of *BMP* and *pBMP*, by plotting the quantiles of the observed distribution of the errors against the quantiles of theoretical normally distributed observations. Since the Q–Q plot forms a straight line, it is reasonable to apply the normality assumption. Furthermore, the error distribution does not exhibit unusual patterns (Fig. 1b). Normally distributed data and unsystematic distribution of the errors are required in order to confirm the model. The samples with the larges errors are 'Rye DWC', 'Grape stalks', and 'Wheat straw 2' (marked on the plots with 25, 43 and 44, respectively), but since these samples are in line with the other samples on the Q–Q plot, they are not regarded as outliers.

The regression coefficients of x_C and x_H are only slightly smaller than the theoretical values determined via Buswell's formula (378 compared to 414 for x_C, and 354 compared to 423 for x_H), which supports that the estimation of *pBMP* using mixture model is reflecting AD theory better than the previous regression models. Furthermore, only lignin is a negative parameter indicating the inhibitory effect on *BMP*, while the positive sign of the remaining coefficients are consistent with AD theory. Since all available data from literature, as well as the data prepared for this work, are used, the derived regression model is considered to be the best possible model to predict *BMP* from lignocellulosic biomass to date.

A linear model assumes that there are no interactions between the biomass constituents. On the other hand, it is well known that the biomass constituents are chemically linked and that the interactions between the different components of the lignocellulosic matrix, e.g. between hemicellulose and lignin, is of crucial importance to the recalcitrance of the biomass and thereby to *BMP* (Albersheim et al., 2011). When testing the canonical quadratic mixture model $pBMP = \sum_i \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j$ (indices *i* and *j* refers to the components *C*, *H*, *L* and *R*), where the interaction terms would describe such interactions, we cannot prove statistically significance of any of these blending terms. However, it might be possible to prove interaction in a more homogeneous set of data with fewer underlying errors caused by e.g. differences in laboratory practices. Therefore, other more advanced mixture models with interaction terms might be identifiable in future studies.

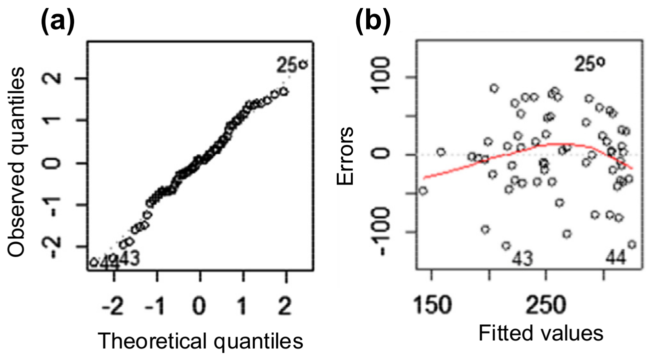

Fig. 1. (a) A normal Q–Q plot of the derived model ($pBMP = 378\,x_C + 354\,x_H - 194\,x_L + 313\,x_R$). (b) Errors vs. fitted values from the same model.

3.2.1. Reducing the model

Since the regression coefficients of cellulose, hemicellulose and residuals in the full linear model are of the same order of magnitude (Table 4), it was tested if the full model could be reduced by summing the two regression variables and testing H_1: $\beta_C = \beta_H = \beta_{C+H}$. The validation of the reduction of the model was done by ANOVA, and since the *p*-value of the reduction was large (0.87, data not shown) H_1 could not be rejected, and the reduced model was accepted. The model was validated by a normal Q–Q plot and by plotting errors vs. fitted values from the derived model (plots not shown). These plots did not differ substantially from the plots derived from the full model (Fig. 1), and the reduced model could be accepted. The regression diagnostics of the reduced model showed that the effect of x_{C+H} and x_L and x_R are highly significant. Furthermore, *rRMSE* was unchanged compared to the full model (Table 4).

The prediction model could be reduced even further by adding all energy yielding biomass constituents in one regression variable ($x_{C+H+R} = x_{C+H} + x_R$), and testing H_2: $\beta_{C+H} = \beta_R = \beta_{C+H+R}$. Since the *p*-value of the reduction again was relatively large (0.39, data not shown) and since the diagnostics plots once again showed normally distributed data and randomly distributed errors, the reduced model could be accepted.

The R^2-values were large and almost identical in the full model and the two reduced models (Table 4). Furthermore, the *rRMSE* of the models increased only slightly when the models were reduced, from 19.7% in the full model to 19.8% in the finally reduced model. This shows that the accuracy of the models are only slightly

Table 4
Regression diagnostics on the presented canonical linear mixture models.

	Model	Applied to data			R^2	*rRMSE* (%)
		Estimate	(St. dev.)	Significance		
Full canonical linear mixture model	$pBMP = \beta_C\, x_C + \beta_H\, x_H + \beta_L\, x_L + \beta_R\, x_R$	$\beta_C = 378$ $\beta_H = 354$ $\beta_L = -194$ $\beta_R = 313$	(62) (94) (69) (45)	*** *** ** ***	0.96	19.7
Reduced model H_1: $\beta_C = \beta_H = \beta_{C+H}$	$pBMP = \beta_{C+H}\, x_{C+H} + \beta_L\, x_L + \beta_R\, x_R$	$\beta_{C+H} = 369$ $\beta_L = -194$ $\beta_R = 312$	(28) (68) (45)	*** ** ***	0.96	19.7
Reduced model H_2: $\beta_{C+H} = \beta_R = \beta_{C+H+R}$	$pBMP = \beta_{C+H+R}\, x_{C+H+R} + \beta_L\, x_L$	$\beta_{C+H+R} = 349$ $\beta_L = -196$	(14) (68)	*** **	0.96	19.8

*** For $0.001 \geqslant p$ (statistically extremely significant).
** For $0.01 \geqslant p > 0.001$ (statistically highly significant).

reduced compared to the full model. Therefore, based on this set of data, the reduced model, H_2 seems adequate.

For design of future predictions models, it is important to start again from the full model and not from a reduced model since the reduction depends on estimated regression coefficients, and in a different dataset, the coefficients will have different numerical values reflecting the type of biomass. In addition, models based on biomasses with other characteristics, such as high content of lipids or proteins, should include regression coefficients reflecting this fact. For design of future models, it might also be appropriate to use more advanced mixture model as developed for the chemical and pharmaceutical fields, see e.g. (Focke et al., 2007; Mandlik Satish et al., 2012).

3.2.2. Comparison to previous models

The inherent mathematical relation between canonical linear mixture models and models with intercept as applied previously is exemplified by the following calculations:

$$\begin{aligned}
&\beta_C x_C + \beta_H x_H + \beta_L x_L + \beta_R x_R \\
&\quad = \beta_C x_C + \beta_H x_H + \beta_L x_L \\
&\quad + \beta_R(1 - x_C - x_H - x_L) \\
&\quad = (\beta_C - \beta_R)x_C + (\beta_H - \beta_R)x_H + (\beta_L - \beta_R)x_L + \beta_R \\
&\quad = \alpha_0 + \alpha_C x_C + \alpha_H x_H + \alpha_L x_L,
\end{aligned}$$

where

$$\alpha_0 = \beta_R, \alpha_C = \beta_C - \beta_R, \alpha_H = \beta_H - \beta_R, \text{ and } \alpha_L = \beta_L - \beta_R$$

Therefore, it is possible to mathematically transform regression coefficients from one to another manner of expressing the model. However, the regression coefficients would not necessarily make intuitive sense. For example, if β_C and β_R are both positive and of the same order of magnitude then $\alpha_C = \beta_C - \beta_R$ will be small, thus seemingly insignificant. If $\beta_C < \beta_R$, the sign will be negative even though this is contrary to AD theory. This was the case with the prediction model proposed by Triolo et al. (2011) (Table 1):

$$pBMP = 447 - 277x_L - 7x_C.$$

Further, since β_L is a negative regression coefficient, $\alpha_L = \beta_L - \beta_R$ may be even more negative and, thereby, highly significant. Thus, the role of lignin may previously have been exaggerated; not necessarily in the statistical calculations, but also in the way we understand and interpret previous results.

Predictions based on our model H_2, $pBMP = \beta_{C+H+R}\, x_{C+H+R} + \beta_L\, x_L$, can be recalculated similarly into $\alpha_0 + \alpha_L\, x_L$, where, $\alpha_0 = \beta_{C+H+R}$ and $\alpha_L = \beta_L - \beta_{C+H+R}$. In this notation, $pBMP = 348 - 544\, x_L$ is the same as the model previously proposed by both Triolo et al. (2011) and Monlau et al. (2012) (Table 1). Comparing our regression coefficients to those previously published (Table 1), our effect of lignin is more negative. It should be noted that the previously published regression coefficients are determined on homogeneous biomasses and on quite small sample-sizes, and apparently, they are not applicable for describing *pBMP* from lignocellulosic biomass in general.

3.2.3. Effect of biomass analysis method

Among the factors affecting the outcome of *BMP* measurements, using the forage or the fibre method for analysing biomass composition turned out to be of large importance especially for the lignin content (Table 3). We estimated a statistically highly significant effect of whether the compositional data was generated with either of the two methods (Table 5). This indicates that the two methods do not generate fully comparable results, and thus when determining *pBMP* it is important to know which of the methods had been used in the specific case. The dummy variable seems to be related to the lignin content as the β_L changes very much when the dummy variable is introduced while β_{C+H+R} stays unchanged. The effect that *pBMP* is 63 units larger, when the fibre method has been applied compared to when the forage method has been applied, could be explained by that the fibre method estimates a higher lignin content compared to the forage method. In this case, predictions made by means of the fibre method, which results in a larger negative contribution to *pBMP*, will be compensated by the dummy variable. R^2 is increased while *rRMSE* is decreased compared to the final reduced model. In addition, the diagnostics plots confirmed normal distribution of the data as well as independence of the residuals (data not shown).

3.3. Limitations of the study

Some factors expected to influence *BMP* were not taken into account in the statistical analysis and this may have affected the outcome of the study. Among these factors are:

Activity, activation and adaptation of the inoculum for *BMP* measurements. These factors have been shown to have a large impact on *BMP* (Gerber et al., 2013).

- End-point inaccuracy and various inhibitions of the *BMP* measurements.
- Deviating laboratory practices when determining *BMP*, such as biomass particle size, mixing, and incubation temperature.
- Additional differences in determination of biomass constituents other than fibre vs. forage method.

These factors most likely account for a large part of the variation in the dataset. This might have been avoided if more strict criteria for selecting data were applied. However, a large dataset with a broad variety of lignocellulosic biomasses were prioritised on the expense of presumable larger variation. In future studies, a prediction based on a large dataset from strictly standardised laboratory procedures, would presumably result in regression coefficients with lower standard deviation. In addition, it would be beneficial with a more thorough biomass composition analysis, where other biomass constituents also were accounted for.

An indication of deviating data in this study can be seen when comparing composition of rice straw compiled in this article. Rice straw 1 and Rice straw 2 were analysed with the fibre analysis method but their lignin content was very different (0.113 and 0.270 w/w, respectively). This might be a result of large natural variation, but it is likely also to be influenced by differences in

Table 5
Regression diagnostics on the alternative models.

	Model	Applied to data			R^2	*rRMSE*
		Estimate	(St. dev.)	Significance		
Including dummy-variable regressor for analysis method	$pBMP = \beta_{C+H+R}\, x_{C+H+R} + \beta_L\, x_L + \gamma_A\, D_A$	$\beta_{C+H+R} = 347$	(13)	***	0.97	17.7%
	$D_A = \begin{cases} 0 & \text{Forage method} \\ 1 & \text{Fiber method} \end{cases}$	$\beta_L = -438$	(87)	***		
		$\gamma_A = 63$	(16)	***		

*** For $0.001 \geqslant p$ (statistically extremely significant).

laboratory practices. Likewise, the data from Triolo et al. (2011) had unusually low lignin contents. These samples were analysed with the forage analysis, however, the low lignin values might also be a result of other specific practices used in that study. Even though a dummy variable was included in our model to describe the two different analysis methods, the difference between different research groups were not assessed.

Further, variation in the nature of the biomass constituents has not been taken into account. Hemicelluloses differ according to the biomass in question, in respect to both structural backbone and side chains, and likewise the exact structure of lignin can vary from one biomass to the other (Albersheim et al., 2011). Due to the availability of data, this has not been assessed in the current study.

Finally, it should be noticed that some biomasses commonly used for biogas production are omitted from the dataset due to the criteria of selecting the data. For instance, ensiled biomasses were excluded on beforehand due to a presumed large amount of fatty acids. Likewise, waste residues important for AD, such as manure, starchy materials, marine biomass, household waste, or industrial wastes, were omitted. In future studies, these biomasses may also be addressed, either individually or combined. The corresponding mixture model for predicting *BMP* should include the predominant biomass constituents as explanatory variables, in order to test the significance of the estimated regression coefficients.

4. Conclusion

Using canonical linear mixture models instead of standard linear regression models to predict *BMP* provides highly significant regression coefficients for the different biomass constituents in accordance with AD theory. Based on the large dataset ($n = 64$), the following equation to predict *BMP* was developed:

$$pBMP = 378x_C + 354x_H - 194x_L + 313x_R.$$

It was possible to reduce this model while including a dummy variable for the biomass composition analysis method without losing validity of the model:

$$pBMP = 347x_{C+H+R} - 438x_L + 63D_A,\ D_A\begin{cases}0, & \text{Forage analysis method}\\ 1, & \text{Fibre analysis method}\end{cases}$$

Furthermore, it is suggested that prediction of *BMP* in future studies with other types of biomasses should also be carried out using mixture models.

Acknowledgements

This work was supported with a grant from Danida Fellowship Centre (DFC) of the Danish Ministry of Foreign Affairs, as a part of the project "Biofuel production from lignocellulosic materials – 2GBIONRG", DFC journal no. 10-018RISØ. For additional information, see http://2gbionrg.dk. P. Kroff is recognized for his valuable initial inputs. H. Carrere is acknowledged for providing additional data to enable use of the reference by Monlau et al. (2012).

References

Alaru, M., Olt, J., Kukk, L., Luna-delRisco, M., Lauk, R., Noormets, M., 2011. Methane yield of different energy crops grown in Estonian conditions. J. Agric. Res. 9, 13–22.

Albersheim, P., Darvillis, A., Robertsis, K., Sederoffis, R., Staehelinis, A., 2011. Plant Cell Walls: From Chemistry to Biology. Taylor & Francis Group.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927–934.

Azhar, N.G., Stuckey, D.C., 1994. The influence of chemical structure on the anaerobic catabolism of refractory compounds: a case study of instant coffee wastes. Water Sci. Technol. 30, 223–232.

Carter, M.S., Hauggaard-Nielsen, H., Heiske, S., Jensen, M., Thomsen, S.T., Schmidt, J.E., Johansen, A., Ambus, P., 2012. Consequences of field N2O emissions for the environmental sustainability of plant-based biofuels produced within an organic farming system. GCB Bioenergy 4, 435–452.

Cornell, J.A., 2011. A retrospective view of mixture experiments. Qual. Eng. 23, 315–331.

Dinuccio, E., Balsari, P., Gioelli, F., Menardo, S., 2010. Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. Bioresour. Technol. 101, 3780–3783.

Focke, W.W., Sandrock, C., Kok, S., 2007. Weighted-power-mean mixture model: application to multicomponent liquid viscosity. Ind. Eng. Chem. Res. 46, 4660–4666.

Gerber, M., Schneider, N., Kowalczyk, A., Schwede, S., Rehman, Z., Span, R., 2013. The influence of pre-incubation, storage and homogenization of inoculum for batch tests on biogas production.

Gunaseelan, V.N., 2007. Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour. Technol. 98, 1270–1277.

Gunaseelan, V.N., 2009. Predicting ultimate methane yields of Jatropha curcus and Morus indica from their chemical composition. Bioresour. Technol. 100, 3426–3429.

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J.l.C., Mosbæk, H., Christensen, T.H., 2004. Method for determination of methane potentials of solid organic waste. Waste Manage. 24, 393–400.

He, Y., Pang, Y., Liu, Y., Li, X., Wang, K., 2008. Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. Energy Fuel. 22, 2775–2781.

Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255–2264.

Mahmood, A., Honermeier, B., 2012. Chemical composition and methane yield of sorghum cultivars with contrasting row spacing. Field Crop. Res. 128, 27–33.

Mandlik Satish, K., Saugat, A., Deshpande Ameya, A., 2012. Application of simplex lattice design in formulation and development of buoyant matrices of dipyridamole. J. Appl. Pharm. Sci. 2, 107–111.

Monlau, F., Sambusiti, C., Barakat, A., Guo, X.M., Latrille, E., Trably, E., Steyer, J.-P., Carrere, H., 2012. Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. Environ. Sci. Technol. 46, 12217–12225.

Oleskowicz-Popiel, P., Thomsen, A.B., Schmidt, J.E., 2011. Ensiling - Wet-storage method for lignocellulosic biomass for bioethanol production. Biomass Bioenerg. 35, 2087–2092.

Oslaj, M., Mursec, B., Vindis, P., 2010. Biogas production from maize hybrids. Biomass Bioenerg. 34, 1538–1545.

Prakasham, R.S., Sathish, T., Brahmaiah, P., Subba Rao, C., Sreenivas Rao, R., Hobbs, P.J., 2009. Biohydrogen production from renewable agri-waste blend: optimization using mixer design. Int. J. Hydrogen Energy 34, 6143–6148.

Sambusiti, C., Ficara, E., Rollini, M., Manzoni, M., Malpei, F., 2012. Sodium hydroxide pretreatment of ensiled sorghum forage and wheat straw to increase methane production. Water Sci. Technol. 66, 2447–2452.

Scheffe, H., 1963. The simplex-centroid design for experiments with mixtures. J. R. Stat. Soc. B Met. 25, 235–263.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J. Agric. Food Chem. 58, 9043–9053.

Symons, G.E., Buswell, A.M., 1933. The methane fermentation of carbohydrates. J. Am. Chem. Soc. 55, 2028–2036.

Thomsen, S.T., Jensen, M., Schmidt, J.E., 2012. Production of 2nd generation bioethanol from lucerne – optimization of hydrothermal pretreatment. BioResource 7, 1582–1593.

Tong, X., Smith, L.H., McCarty, P.L., 1990. Methane fermentation of selected lignocellulosic materials. Biomass 21, 239–255.

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. Bioresour. Technol. 102, 9395–9402.

Triolo, J.M., Pedersen, L., Qu, H., Sommer, S.G., 2012. Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. Bioresour. Technol. 125, 226–232.

Wang, J., Yue, Z., Chen, T., Peng, S., Yu, H., Chen, H., 2010. Anaerobic digestibility and fiber composition of bulrush in response to steam explosion. Bioresour. Technol. 101, 6610–6614.