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UTILIZATION OF LIGNOCELLULOSIC FIELD RESIDUES FROM AGRICULTURE FOR ENERGY GENERATION – IMPROVEMENT OF BIOLOGICAL CONVERSION THROUGH STEAM EXPLOSION PRETREATMENT

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Kurzfassung

Die Bereitstellung von Energie durch regenerative Technologien ist ein Schlüssel um den globalen Temperaturanstieg einzuschränken. Biomassebasierte Technologien werden dabei als Möglichkeit gesehen, fluktuierende Stromerzeugung aus Photovoltaik und Windkraftanlagen zu kompensieren und sind zudem geeignet, flüssige und gasförmige Treibstoffe bereitzustellen.

Landwirtschaftliche Reststoffe können als Ressource für die biologische Konversion zu Energieträgern dienen, die nicht in Konkurrenz zur Lebens- und Futtermittelerzeugung stehen. Die Umwandlung dieser Reststoffe in Biogas und Bioethanol hat den Vorteil, dass diese Energieträger bereits vielfach genutzt werden und daher in existierende Infrastruktur leicht integriert werden können.

Eine große Herausforderung stellt die Entwicklung eines effizienten Konversionspfades von der Biomasse zum Energieträger dar. Die Vorbehandlung der Biomasse gilt als Schlüsseltechnologie, um den Enzymen und Mikroorganismen für die Konversion zu Methan und Ethanol die organischen Komponenten nutzbar zu machen. Die Steam Explosion Technologie wird als Möglichkeit gesehen, ausreichend vorbehandeltes Material in hohen Durchsatzleistungen zur Verfügung zu stellen.

Der Fokus dieser Arbeit liegt in der Optimierung der Steam Explosion Vorbehandlung für die Anwendung an verschiedenen Biomassetypen. Der Effekt verschiedener Vorbehandlungstemperaturen sowie –zeiten auf die Zusammensetzung der Biomasse sowie die resultierenden Methan- und Ethanolpotentiale wurden untersucht. Abhängig von der Intensität der Lignifizierung konnten verschiedene Vorbehandlungstemperaturen als Optimum identifiziert werden, wobei höhere Temperaturen vor allem für stark lignifizierte Biomasse, wie zum Beispiel Miscanthus, vorzuziehen sind.

Es konnte beobachtet werden, dass es bei der Steam Explosion Vorbehandlung von weniger lignifizierter Biomasse zu einem Verlust organischer Trockenmasse kommt, da flüchtige organische Komponenten bei der plötzlichen Druckreduktion gasförmig abgeführt werden. Bei ungünstigen Vorbehandlungsparametern konnte bei der Steam Explosion von Heu ein Masseverlust von 35.2 % festgestellt werden. Bei der Umsetzung von Anlagen zur Steam Explosion Vorbehandlung von Biomasse sollte daher unbedingt ein Fokus auf die Rückgewinnung dieser Bestandteile gelegt werden, um eine möglichst hohe Ausbeute bei der Konversion zu biogenen Energieträgern zu gewährleisten.

Steigende Vorbehandlungstemperaturen führen zu einer kompletten Auflösung der Hemicellulose bei gleichzeitig steigendem Ligninanteil, welcher zum Teil auf die Bildung von Verbindungen zurückgeführt werden kann, die aus den Abbauprodukten der Hemicellulose entstehen. Es konnte jedoch gezeigt werden, dass diese Fraktion beim anaeroben Abbau ebenfalls abgebaut wird.

Der Cellulosegehalt von Weizenstroh und Miscanthus wurde durch die Steam Explosion Vorbehandlung, ungeachtet der gewählten Prozessparameter, nur gering beeinflusst. Bei der Steam Explosion Vorbehandlung von Heu kam es hingegen zu einer Reduktion des Cellulosegehalts um 8.2 %. Dieses Ergebnis unterstreicht die unterschiedlichen Effekte, die bei der Steam Explosion Vorbehandlung von stark und schwach lignifizierter Biomasse zu beobachten sind. Die Kombination der Steam Explosion mit einer biologischen Vorbehandlung durch Inokulation mit einer spezialisierten Hefe hatte keinen Effekt auf den potentiellen Energieoutput. Die kombinierte Produktion von Bioethanol und Biogas aus den Fermentationsresten zeigte vielversprechende Ergebnisse hinsichtlich des Gesamtenergieertrags.

Schlüsselwörter: Biogas, Bioethanol, Steam Explosion, Lignocellulose, Reststoffe

Abstract

The energy generation from renewable resources is a key factor for limiting the global temperature increase. Biomass driven technologies are seen as possibility to complement fluctuating energy generation from photovoltaic and wind power plants and, additionally, provide liquid and gaseous fuels.

Agricultural residues can serve as resource for the biological conversion to biogenic energy carriers, which do not compete with food and feed production. The conversion of those residual materials to biogas and bioethanol are of interest, as those energy carriers can be easily integrated in the present energy infrastructure.

The development of efficient conversion pathways from biomass to energy carriers is still a major challenge. The pretreatment of the biomass in order to enable the conversion to methane and ethanol through enzymes and microorganisms is a key factor. The steam explosion technology is regarded as a possibility to sufficiently pre-treat material at a high throughput.

The focus of this thesis is the optimisation of the steam explosion pretreatment for application to different types of biomass. The effect of pretreatment temperature as well as duration on biomass composition and potentials for methane and ethanol generation have been investigated. Depending on the intensity of lignification, different pretreatment temperatures have been identified as optimum, whereat higher temperatures are preferably used for strongly lignified biomass, e.g. miscanthus.

It could be observed that during steam explosion pretreatment of less lignified biomass, organic matter is lost as volatile organics are vaporized during the pressure drop and are subsequently discharged. Regarding the least favourable pretreatment conditions of hay from grassland, a loss of 35.2% of organic matter could be detected. Therefore, when constructing steam explosion equipment, a focus should be the recycling of steam after the pretreatment, in order to achieve a high efficiency of the process.

High pretreatment temperatures result in a complete degradation of hemicellulose at a simultaneous increase of lignin. This can be explained by the formation of compounds from hemicellulose degradation products. It could be shown that this fraction is degraded during the anaerobic digestion process.

The cellulose content of wheat straw and miscanthus was, regardless of the applied pretreatment temperature, only slightly altered. Regarding the steam explosion pretreatment of hay, a reduction of 8.2% of cellulose content could be detected. This result underlines the differing effects, which can be observed after pretreatment of strong and less lignified biomass. The combination of the steam explosion with a biological pretreatment using a specialised yeast has shown no effect on the potential energy output. The combined production of bioethanol and biogas showed promising results regarding the overall energy yield.

Keywords: biogas, bioethanol, steam explosion, lignocellulose, residues

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Content

1 Introduction 1

2 Structure 2

3 State of the art 3

3.1 Agricultural residues - overview 3

3.2 Structural components of agricultural residues 3

3.3 Pretreatment technologies 4

3.4 Steam explosion 5

4 Objectives 11

5 Materials and methods 12

5.1 Steam explosion setup 12

5.2 Dry matter, volatile solids and water content 12

5.3 Structural compounds 12

5.4 Biological potential for generation of biogenic energy carriers 13

5.4.1 Biological methane potential 13

5.4.2 Biological ethanol potential 13

6 Summary of publications 14

6.1 Biogas production from Steam-Exploded Miscanthus and Utilization of Biogas Energy and CO_2 in Greenhouses 14

6.2 Steam explosion pretreatment for enhancing biogas production of late harvested hay 15

6.3 Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion 16

6.4 The effect of combined biological and thermo-mechanical pretreatment of wheat straw on energy yields in coupled ethanol and methane generation 17

7 Results and discussion 18

7.1 Dry matter, volatile solids and mass loss 18

7.2 Structural compounds 18

7.3 Methane and ethanol yields 20

7.4 Inhibitors 21

8 Conclusions and further research 22

References 23

ANNEX 26

1 Introduction

The limitation of the utilization of fossil fuels as well as its negative impact on ecological systems and its contribution to climate change are a widely accepted fact by now [1]. Since the turn of the millennium, efforts have been made to substitute mainly coal and crude oil with renewable, eco-friendly alternatives [2].

The legislative base for actions concerning the endorsement of renewable energy in Europe is the renewable energy directive (RED) [3]. The RED defines, for the first time, obligatory goals concerning energy from renewable resources for the member states of the European Union. Until 2020, the share of renewable energy in the European energy sector should be 20 %. Additionally, energy efficiency should increase by 20 %. Those goals should, together with other measures, lead to a 20 % decrease of greenhouse gas emissions compared to 1990. A sub-goal is to reach a 10 % renewable share in the mobility sector.

As a continuation of the RED, the European Union concluded the Clean Energy Package (CEP), which contains further goals to be reached in 2030. The share of renewable energy should increase to 32 % by then [4].

In order to achieve the aspired goals, a wide variety of technologies are available. Among those, the utilization of biomass plays a major role, as it offers valuable advantages. Biomass can be found in nearly every region worldwide and can therefore reduce dependency, as compared to crude oil, for instance. Most technologies for the energetic utilization of biomass are well developed and tested. Moreover, biomass can be stored and therefore provides some flexibility, offering the possibility of energy generation when it is needed. This makes biomass technologies a viable complement to energy generation from wind or solar resources. Energy carriers derived from biomass, e.g., biodiesel or bioethanol, offer a high energy density and can be used in existing combustion engines [2].

In the European regulatory frameworks, the utilization of biomass plays a major role. Energy generation from biomass should fulfill certain sustainability criteria in order to guarantee a positive effect on climate and nature. Biomass used for the production of bioenergy and biofuels is not allowed to be harvested from areas with a high biodiversity or areas with a high carbon stock as wetlands. Furthermore, in order to be considered as sustainable, a certain amount of greenhouse gas has to be saved [5].

In order to establish a more sustainable feedstock for bioenergy production, the development of 2nd and 3rd generation biofuels is endorsed. In contrast to 1st generation biofuels, those types are not produced from biomass grown on agricultural land, but are either residues (2nd) or alternative biomass such as algae (3rd).

The utilization of residual material from agricultural land is considered as sustainable, as it does not need to be cultivated specifically, but can be obtained as a by-product of agricultural production. Examples for those by-products are cereal straw, husks and grassland, if obtained from areas which cannot be used for the production of animal feed. The utilization of miscanthus can be regarded as a special case, as it strongly depends on which areas it is cultivated.

A challenge still to be solved is the pretreatment of residual material rich in lignocellulose. In order to make the usable substances – cellulose and hemicellulose – available, the rigid structure of the lignocellulose has to be broken up. The efficiency and effectiveness of the biomass pretreatment is a key step in the production process of biomass-derived energy carriers in order to make them competitive to conventional fuels [6].

2 Structure

The first part of the thesis provides an overview concerning types and potentials of residues from agriculture, as well as a characterization of the types of residues chosen for the studies. The technological challenge of utilizing strongly lignified biomass and potential strategies to overcome this issue are explained, while an overview of possible pretreatment technologies is provided. Steam explosion technology is discussed and the relevance of pretreatment temperature and duration are especially addressed. In the materials and methods section, the applied steam explosion technologies are described. A central part of the thesis is the characterization of the pretreated material; therefore, the methods used for analyzing different compounds like cellulose, hemicellulose and lignin, as well as for determining the energy potential are described.

Subsequently, the publications are summarized and results are discussed. The thesis finishes with a conclusion of the key findings and addresses the further need for research and development.

3 State of the art

3.1 Agricultural residues - overview

On a global perspective, only a few crops are responsible for the majority of residue potential. Barley, maize, rice, soybean, sugar cane and wheat are cultivated on more than 50 % of the arable land worldwide and provide about three-quarters of the global residue potential. Locally, other crops such as canola, sorghum or sugar beet can also be of interest. The estimation of biomass and, subsequently, energy potentials is a challenge, as usually only crop yields are recorded. Therefore, methods have to be applied in order to calculate residue potentials. The residue-to-product ratio (RPR) is a factor describing the relation between crop yield and residue potential. Due to breeding efforts, the RPR has changed in the last decades towards a higher crop share of total biomass production. According to Bentsen et al. [7], wheat straw is one of the three most available agricultural residues worldwide, together with maize and rice. The estimated residue potential of the six most important crops is about 3.7 Pg yr^{-1}, of which 800 Tg yr-1 result from wheat. The most important regions are North and South America, as well as Southern Asia and Eastern Europe. The estimated potential of wheat straw in Eastern Europe is between 116 and 162 Tg yr^{-1}. Concerning the potential of agricultural residues, different factors like soil fertility, likeliness of soil erosion or weather conditions limit the amount of residue biomass which can be harvested [8].

Considering permanent grassland, local circumstances play a role for availability as a resource for energy production. An intensification of livestock production leads to a higher demand for feed like maize or soybean, while the importance of grass as feedstock is declining. Additionally, grassland in alpine areas, which is hard to cultivate, is abandoned. Prochnow et al. [9] estimates a grassland surplus of 14.9 x 10^6 ha for 2020 in the European Union.

The possibility of using perennial grasses as energy crops has frequently been discussed in recent years. Different properties are seen as advantages considering the cultivation as energy crops, e.g., low tillage and plant protection requirements. Therefore, they are considered as low-input crops while still promising high yields under favorable conditions [10]. Especially the sterile hybrid *Miscanthus x giganteus* is considered to be a good option for cultivation in Central and Southern Europe, as the nutrient and water use efficiency as well as the potential yield are very high [11]. There also is a high variety of soils on which miscanthus can be cultivated.

3.2 Structural components of agricultural residues

The main component of cereal straw, biomass from grassland, as well as perennial grasses is lignocellulose. This lignocellulose is composed of the polymers cellulose, hemicellulose and lignin. Depending on, e.g., the type, species and growth stadium of the plant, the amount as well as the linkage between those components varies. Wood biomass has a higher content of cellulose and lignin, while agricultural residues like wheat straw have a higher content of hemicellulose [12]. Cellulose is a polymer which consists of up to 15,000 glucose molecules. The main purpose of cellulose is to provide structural stability. Therefore, cellulose consists of linear molecules grouped together to form microfibrils and subsequently cellulose fibers. In order to provide fermentable sugars, the beta-(1,4)-glycosidic bondings between the glucose have to be broken down, e.g., by enzymes or acids [13].

In contrast to cellulose, hemicellulose consists of different C5 and C6 sugars, and is not a linear polymer, but a branched one. In agricultural biomass, hemicellulose consists mainly of xylan, while in softwoods glucomannan dominates.

Hemicellulose coats the cellulose fibers and should therefore be removed in order to increase the accessibility of cellulose for enzymes. Hemicellulose can be hydrolyzed easier than cellulose and is thermo-chemically sensitive [14] [15]; therefore it can be easily removed using thermochemical pretreatment technologies. When choosing pretreatment conditions, it has to be considered that high temperatures and long pretreatment durations can lead to the formation of inhibitors; therefore, a balance between biomass digestibility and probability of inhibitor formation has to be found [16].

Lignin is a very complex and large polymer structure which consists mainly of coniferyl, coumarly and sinapyl alcohol. It serves as "glue" between cellulose and hemicellulose and is responsible for protection against microbial attack. Besides being a physical barrier to degrading microorganisms, lignin adsorbs hydrolytic enzymes and interferes with cellulose degrading enzymes. Lignin derivatives which can form during pretreatment can be toxic for microorganisms [17]. During pretreatment, lignin is not being solved in a liquid, but melts and forms colloids, which can also show altered properties [18].

3.3 Pretreatment technologies

Pretreatment is used to modify biomass in order to fit the requirements of different biorefinery processes. Modifications include the reduction of particle size, and the alteration of the molecular structure and the chemical composition. The objective is to release monomers, usually sugars that can be fermented into different products. Key parameters for the development of pretreatment technologies are a low energy input, a minimal sugar degradation and avoiding the formation of compounds that inhibiting biological processes.

Different pretreatment technologies can be used for this purpose. In general, it can be distinguished between biological, chemical and physical pretreatment, whereas some combinations exist.

Biological pretreatment has been receiving more attention recently, as the required energy input is very small and it can be carried out without using chemicals. Different organisms are capable of degrading cellulose, hemicellulose or lignin. A oft-used organism for biological pretreatment is the white-rot fungi, which directly degrades lignin [19]. Nevertheless, pretreatment efficiency, the potential loss of sugars due to metabolic processes and the required time present a strong barrier to practical application [20].

Different chemicals can be used for chemical pretreatment. Strong acids such as H_2SO_4 dissolve hemicellulose, while alkalis like NaOH reduce the lignin content [21]. Various organic solvents like ethanol or ozone, as well as ionic liquids, are also effective chemicals for dissolving compounds of the lignocellulose complex [22]. Actual barriers for the utilization of most chemicals are corrosive effects, difficult recycling and high costs.

The easiest way to perform physical pretreatment is to reduce the particle size by using mills and comparable equipment. However, the high consumption of electrical energy and the moderate pretreatment effect prevent the practical application in most cases [14]. Ultrasound and microwave pretreatment have already shown good results regarding the digestibility of the pre-treated material [23]; the energy intensity as well as high investment costs are drawbacks for those technologies.

The utilization of thermal energy for pretreatment is common in the pulp and paper industry and has drawn huge interest in the pretreatment of different residual materials for energy production. Thermal energy can be provided more easily than electrical energy, e.g., waste heat from industrial processes or CHPs. The pretreatment times range from minutes to hours; therefore, high throughputs can be realized. Thermal pretreatment technologies can combine physical as well as chemical effects, as, e.g., hemicellulose degradation products form organic acids, which can contribute to the breakdown of the bonding between other structural compounds. Challenges include the formation of inhibitors under certain pretreatment parameters and poor lignin degradation compared to chemical methods [20].

3.4 Steam explosion

Steam explosion pretreatment is a commonly used technology for altering the properties of lignocellulose biomass in order to integrate it in some kind of biorefinery concept [24]. Typically, the biomass is subjected to saturated steam at a certain temperature for a defined duration. After that, the pressure is released abruptly, causing the liquid water in the biomass to evaporate very quickly, which leads to a disintegration of the biomass [25].

In the past decades, a wide variety of different biomass types was used to investigate the effects of a steam explosion pretreatment concerning parameters like biological degradability or the extraction of chemical substances. Commonly, some pretreatment parameters are varied in order to find a pretreatment setup that leads to optimal conditions for the desired application. Cellulose hydrolysis, e.g., is a problem, if the desired application is the utilization of the fibers, while it does not affect the process of bioethanol production.

Depending on the composition of the biomass (e.g., structural compounds), as well as on the severity of the steam explosion pretreatment, different processes occur during the pretreatment. Among those, hemicellulose solubilization, alteration of cellulose crystallinity and lignin deformation are already known [26].

In Table 1, an overview of investigated parameters and achieved results of different steam explosion settings and lignocellulosic biomass are presented. The presented publications all investigated some kind of agricultural lignocellulose-rich biomass which is either produced as a co-product of conventional agricultural production (e.g., grain) or as a fast-growing energy crop which can be cultivated on agricultural land (e.g., miscanthus). The most common biomass investigated is wheat straw [25, 27-32], due to its global importance and available quantity. Other agricultural co-products are oat [33], barley [34], triticale [35], and rapeseed straw [36, 37], as well as corn stover [38, 39]. Sugarcane straw [24] is of special interest in equatorial countries, e.g., Brazil. Reed [40] is an interesting biomass option, as it usually is not cultivated, but extracted from natural areas in order to prevent silting. Fast-growing perennial crops like miscanthus [41, 42], salix [43] and switchgrass [44] show comparably high biomass yields and usually require a minor production input (e.g., fertilizer) during cultivation.

The applied temperature range for steam explosion pretreatment is between 120 and 220°C for all presented studies. The duration of the pretreatment varies between 2 and 60 minutes, whereas most authors chose a maximum pretreatment time of 20 minutes. There were no chemicals used to catalyze the pretreatment process. The results in the table represent the values found for the steam explosion pretreatment conditions that resulted in the highest energy yields (methane, ethanol or total energy) or cellulose hydrolysis, respectively.

Common investigated parameters are the content of cellulose, hemicellulose and lignin, the efficiency of cellulose hydrolysis, the biological biogas and methane potential, the biological ethanol potential, as well as the formation of furfural and HMF.

Table 1: Overview of literature focusing on biomass pretreatment with steam explosion

author		Jorgensen [32]	Chen [27]	Bauer [28]	Horn [25]	Paschos [29]	Monschein [30]	Rajput [31]
publication year		2006	2008	2009	2011	2015	2016	2018
biomass type		wheat straw	wheat straw	wheat straw	wheat straw	wheat straw	wheat straw	wheat straw
steam explosion temperature range	[°C]	195-205	198	180-200	170-220	190	165	120-180
steam explosion time range	[min]	6-12	10	10-20	5-15	12	10	60
optimal pretreatment temperature	[°C]	195	198[1]	180	210	190[1]	-	180
optimal pretreatment time	[min]	12	10[2]	15	10	12[2]	-	60[2]
steam explosion pressure	[MPa]	-	1.5	-	-	-	-	-
relative change of cellulose content	[%)	+ 70	+ 23	-	+ 20	-	+ 5	+ 35
relative change of hemicellulose content	[%)	- 67	- 59	-	- 78	-	- 9	- 23
relative change of lignin content	[%)	+ 40	+ 105	-	+ 44	-	+ 13	slight reduction
cellulose hydrolysis	[%)	87.5	55	-	88	-	-	-
biological biogas potential (BBP)		-	-	+ 22 %[3]	-	-	-	+ 52 %[3]
biological methane potential (BMP)		-	-	+ 20 %[3]	-	-	-	-
ethanol		48 g kg VS^{-1}	30.1 g L^{-1}		210 g kg VS^{-1}	58 g L^{-1}	-	-
furfural		-	-		-	-	-	-
HMF		-	-		-	-	-	-
remarks			ethanol yield 82.4 % of theoretical maximum	Theoretical ethanol potential 200 g kg VS^{-1} (200°C, 10 min)				

[1] only one pretreatment time applied, [2] only one pretreatment duration applied, [3] compared to untreated sample

author		Dererie [33]	Iroba [34]	Agudelo [35]	Chang [38]	Lizasoain [39]	Vivekanand [37]	Lopez-Linares [36]
publication year		2011	2014	2016	2012	2017	2012	2015
biomass type		oat straw	barley straw	triticale straw	corn stover	corn stover	rapeseed straw	rapeseed straw
steam explosion temperature range	[°C]	190	140-180	180-200	-	140-220	190-230	185-215
steam explosion time range	[min]	10	5-10	5-15	3.3	2-15	5-15	2.5-7.5
optimal pretreatment temperature	[°C]	190[1]	-	200	-	160	-	215
optimal pretreatment time	[min]	10[2]	-	10	3.3[2]	2	-	7.5
steam explosion pressure	[MPa]	-	0.5-1.1	-	2.5	-	-	-
relative change of cellulose content	[%)	-	- 77	+ 41	- 8	+ 1	-	+ 50
relative change of hemicellulose content	[%)	-	- 90	- 82	- 55	+ 1	-	- 90
relative change of lignin content	[%)	-	+ 91	+ 127	- 37	+ 2	-	+ 169
cellulose hydrolysis	[%)	-	-	92	-	-	-	95
biological biogas potential (BBP)		-	-	-	-	+ 25 %[3]	-	-
biological methane potential (BMP)		230 L_N kg VS^{-1}	-	-	-	+ 22 %[3]	+ 17 %[3]	-
ethanol		150 g kg VS^{-1}	-	-	-	-	-	124 g kg VS^{-1}
furfural		-	-	46 mg g^{-1}	0.35 mg g^{-1}	-	-	23 mg g^{-1}
HMF		-	-	14 mg g^{-1}	ND	-	-	9.0mg g^{-1}
remarks		combined energy yield 9.5 MJ kg VS^{-1}	no energy potential investigated, therefore; no optimum				similar results for all pretreatment combinations	

[1] only one pretreatment time applied, [2] only one pretreatment duration applied, [3] compared to untreated sample

author		De Paoli [45]	Oliveira [24]	Horn [43]	Lizasoain [40]	Yeh [42]	Li [41]	Capecchi [44]
publication year		2011	2013	2011	2016	2016	2016	2016
biomass type		sugarcane straw	sugarcane straw	salix	reed	miscanthus	miscanthus	switchgrass
steam explosion temperature range	[°C]	180-200	180-200	170-230	160-220	175	153-198	195
steam explosion time range	[min]	15	15	10-15	5-20	40	3-10	5-15
optimal pretreatment temperature	[°C]	190	200	210	200	175[1]	198	195
optimal pretreatment time	[min]	15[2]	15	15	15	40[2]	10	15
steam explosion pressure	[MPa]	-	-	-	-	-	0.5-1.5	-
relative change of cellulose content	[%)	-	+ 22	+ 27	+ 1	+ 47	+ 17	-
relative change of hemicellulose content	[%)	-	- 87	- 46	-100	- 41	-36	-
relative change of lignin content	[%)	+ 91	+ 86	+ 26	+ 4	+ 15	+ 37	- 11.5
cellulose hydrolysis	[%)	-	80	-	-	-	-	88
biological biogas potential (BBP)		+ 267[3]	-	+ 69[3]	+ 123[3]	-	-	-
biological methane potential (BMP)		+ 190[3]	-	+ 71 %[3]	+ 89 %[3]	-	+ 50 %[3]	137.5 L_N kg VS^{-1}
ethanol		-	-	-	-	69 g kg VS^{-1}	-	29.8 g L^{-1}
furfural		-	-	0.09 g L^{-1}	-	-	-	1 g L^{-1}
HMF		-	-	0.05 g L^{-1}	-	-	-	0.25 g L^{-1}
remarks								combined energy yield 7 MJ kg VS^{-1}

[1] only one pretreatment time applied, [2] only one pretreatment duration applied, [3] compared to untreated sample

Considering the composition of the structural compounds, it can be seen that most authors found a decrease of hemicellulose, as well as an increase of cellulose and lignin content. Iroba [31] found an untypical 77 % decrease of cellulose after steam explosion of barley straw at a pretreatment time of 180°C and a duration of 10 minutes. The hemicellulose content decreases between 9 and 100 % in most studies, while the lignin content increases between 2 and 169 %. Lizasoain [39] finds almost no change in the structural composition (cellulose +1 %, hemicellulose +1 %, lignin +2 %) for the optimal pretreatment conditions. Bauer [28], Paschos [29], Dererie [33] and Vivekanand [37] provided no information considering the structural composition of the pretreated samples.

Regarding the optimal steam explosion conditions for methane yields, it can be seen that a pretreatment temperature of around 180°C has been identified as optimum for agricultural residues by most authors. Comparing the results of Bauer [28] and Rajput [31], it can be said that, in addition, a longer pretreatment time further improves the biogas yield. Higher pretreatment temperatures do not further improve methane yields for agricultural residues. Regarding perennial grasses like miscanthus [41] and reed [40], higher temperatures (starting at 200°C) result in optimal methane yields.

If the ethanol yield is used as a parameter to optimize the steam explosion pretreatment, it can be seen that higher pretreatment temperatures are more favorable. Horn [25] and Lopez-Linares [36] find 210 and 215°C, respectively, as optimal pretreatment temperatures for achieving the highest ethanol yield from wheat and rapeseed straw, respectively.

Inhibitors that are frequently discussed are furfural and hydroxy-methyl-furfural (HMF). Furfural is formed by dehydration of C5 sugars like xylose and arabinose, which are major components of hemicellulose [46]. HMF in contrast is formed from hexose sugars like glucose, mannose and galactose, which are also components of hemicellulose but in a lower amount than pentose sugars [47]. Both formation reactions are accelerated by higher temperatures and the presence of acids to catalyze the process. Other reaction products of hemicellulose sugars after hydrolyzation are organic acids (e.g. acetic acid) [14]. Therefore, it can be concluded, that there are potentially favorable conditions for furfural and HMF generation during the steam explosion process. The actual formation of these inhibitors depends on the hemicellulose composition, pH (presence of acids from hemicellulose degradation) and temperature. The actual effect of inhibitors on microbiological processes is determined by the toxicity and the amount of the inhibitor present in the pretreated material. Furfural and HMF can be tolerated to some extent while inhibitors derived from lignin (e.g. phenolic compounds) are already problematic at low concentrations [16]. Agudelo [35] and Lopez-Linares [36] found the highest rates of cellulose hydrolysis (>90%) despite the presence of furfural and HMF (furfural 46 $g\ L^{-1}$ and 23 $g\ L^{-1}$; HMF 14 $g\ L^{-1}$ and 9 $g\ L^{-1}$).

Perennial energy crops, e.g., miscanthus, show a considerably higher content of structural compounds than agricultural residues, especially lignin. Therefore, it can be assumed that harsher pretreatment conditions may further improve the biological conversion of this type of biomass.

Another interesting feedstock for bioenergy generation is biomass from grasslands. In Austria, e.g., nearly half of the cultivated agricultural land consists of grassland [48]. Depending on the cultivation techniques applied, this type of biomass also shows a high lignocelluloses content. Therefore, a pretreatment using steam explosion may be beneficial for the efficiency of biological conversion. Nevertheless, the effect of steam explosion pretreatment on possible energy yields using, e.g., biogas technology has not been investigated yet.

Although the biological potential of biogas and methane production from different steam explosion pretreated biomass types have been investigated, the degradation kinetics have not been a major part of research yet. A better understanding of the behavior of compounds like cellulose, hemicellulose and lignin during anaerobic digestion may contribute to an improvement of steam explosion pretreatment, as it may help understand the biological processes during degradation.

In order to ensure an efficient utilization of the biomass, it may be beneficial to combine pretreatments to use synergies and maximize the output. While 2nd generation bioethanol production relies on the availability of glucose, the biogas process can handle a variety of organic compounds (C5 sugars, lipids, proteins). Therefore, it makes sense to combine those two processes in order to achieve a maximum energy output. For the realization of this concept, it is crucial to choose pretreatment conditions which allow an optimal conversion for both yeasts and the microbial consortium during anaerobic digestion. Dererie [33] has investigated this concept for one combination of steam explosion pretreatment temperature and time, but there is still a lack of knowledge as to whether this is the optimal combination. It is known that *Saccharomyces cerevesiae* is vulnerable to inhibitors [49]; therefore, it is desirable to reduce inhibitor concentration in the pretreated material. A possible approach for solving this issue could be, e.g., the combination of steam explosion pretreatment with a less harsh pretreatment option in order to reduce the required pretreatment temperatures, which leads to lower inhibitor formation.

4 Objectives

This project aims to determine a practical potential of selected lignocellulosic residues from agriculture for the utilization as a resource for bioenergy production, particularly for the production of biogas and bioethanol. A key issue for the utilization of residue biomass for this purpose is the processability of the organic fraction for microorganisms in anaerobic processes, particularly the biogas and ethanol processes. It is commonly accepted that lignocellulosic biomass has to be pretreated in order to be used in fermentation processes. In this study, the steam explosion technology was investigated to this end.

The key research questions were as follows:

- How does steam explosion pretreatment affect the structural composition of the investigated biomass?
- How does steam explosion pretreatment affect the biological potential for biomethane and/or bioethanol production?

Further research topics included a possible correlation of the structural composition of the biomass after pretreatment with observed biomethane and bioethanol production and the effect of the pretreatment on degradation kinetics of the biomass in the biogas process.

In all presented papers, different kinds of agricultural residues (miscanthus, hay, wheat straw) were pretreated using steam explosion. A substantial interest of paper I and paper II was to find out which effect the pretreatment has on the structural composition of the biomass, particularly the content of cellulose, hemicellulose and lignin. Furthermore, the biological methane potential of the steam exploded biomass was analyzed, in order to find optimal conditions for maximizing the energy output. In paper III those objectives are complemented by the investigation of the effect of steam explosion on the digestibility of structural compounds of the pretreated biomass (wheat straw). In paper IV, a biological pretreatment was combined with steam explosion. As in papers I-III the effect of the pretreatment on the structural compounds as well as on the biological methane potential were investigated. In addition, the biological potential for ethanol generation was analyzed. Finally, the concept of a combined ethanol and methane production was investigated and pretreatment parameters for an optimal overall energy output were identified.

5 Materials and methods

5.1 Steam explosion setup

For the steam explosion pretreatment of the chosen biomass types, two different technologies were applied.

In papers I, II and IV, the material was pretreated with a laboratory-size steam explosion unit by Cambi (Asker, Norway). The unit consisted of a reaction vessel (20 L), a removable bucket and a steam generation unit. After preheating the reaction vessel, the material was fed into the vessel without the addition of water or any catalyst. Steam was subsequently injected until the desired pretreatment conditions were attained, whereupon an automatic valve closed the reaction vessel. After the desired pretreatment duration was achieved, another automatic vessel opened, causing an abrupt pressure drop. Afterwards, the pretreated material was transferred into the removable bucket and filled into plastic bags, which were then vacuumed and stored at 4°C until further use.

In paper III, the material was pretreated using a demonstration-sized steam explosion unit by Biogas Systems GmbH (Parndorf, Austria). After cutting the straw to a size smaller than 5 cm, the material was mixed with water in order to obtain a dry matter content of 30 %. The material was then transferred into the reaction vessel, where it was preheated until a steam saturated atmosphere was obtained in the vessel. After that, the vessel was closed and further heated until the desired reaction conditions were reached. In contrast to the Cambi system, the heating was realized using a heating jacket wrapped around the reaction vessel. After the desired pretreatment duration had expired, the pressure was abruptly reduced to atmospheric level, which caused the water to vaporize immediately. The material was transferred to a flash tank, cooled to room temperature and was then collected, sealed in airtight containers and stored at 4°C until further use.

5.2 Dry matter, volatile solids and water content

The dry matter and volatile solids were analyzed according to DIN 12880 [50] and DIN 12879 [51] respectively. Briefly, the material was dried at 105°C until a constant weight was reached. The difference between the initial weight and the dry weight was considered as dry matter. To determine the volatile solids content, the dry matter was further dry oxidized in a muffle furnace at 550°C. The weight of the remaining ash was subtracted from the dry matter to determine the volatile solids. The determination of the water content was carried out using a Karl Fischer titrator (Mettler Toledo V20) with Hydranal Composite 5 and Hydranal Methanol dry from Sigma Aldrich.

5.3 Structural compounds

Two different methods were used to determine the structural compounds. The determination of cellulose, hemicellulose and lignin according to the method of van Soest and Wine [52], which was adapted by Naumann and Bassler [53], is a wet-chemical method originating from feed analysis. The material is briefly treated with a neutral detergent solution, which dissolves non-structural compounds of the biomass; the remains are called neutral detergent fiber (NDF). Subsequently, the material is treated with an acid detergent solution, with the acid detergent fiber (ADF) remaining. The difference between NDF and ADF is determined as the hemicellulose fraction. The ADF is then treated with 72 % sulfuric acid to determine the lignin (ADL) fraction. The difference between ADF and ADL is determined as the cellulose fraction.

For the determination of the structural compounds using the acid hydrolysis method [54], 150 mg of the milled sample material was mixed with 1.5 ml of 72 % sulfuric acid and incubated at 30°C for 60 minutes and then autoclaved at 121°C after diluting the acid to 4 %. The content of the sample containers was filtered, washed and dried at 105°C. The organic fraction of the solid remains corresponds to the lignin content. The liquid was captured and analyzed for the dissolved sugars (HPLC Dionex Ultimate 3000, 7.8 x 100 mm Rezex RFQ-Fast Fruit H+ column, 82°C, mobile phase 5mM sulfuric acid at a flow rate of 1.0 ml/min). The glucose content corresponds to the cellulose content of the sample. Xylose, arabinose and galactose content correspond to the hemicellulose content.

5.4 Biological potential for generation of biogenic energy carriers

For investigation of the effect of steam explosion pretreatment on the biological potential for the generation of biogenic energy carriers, two different parameters of the native material as well as the pre-treated material were analyzed. In order to estimate the energy potential of the investigated variations, the biological methane potential (I-III) and the biological ethanol potential (IV) were analyzed.

5.4.1 Biological methane potential

The biological methane potential was determined using the VDI 4630 standard batch method [55]. Briefly, 0.5 L digesters were filled with sample material and inoculation material from a biogas plant (volatile solids ratio 1:3). The digesters were placed in a water bath (37.5°C) and equipped with eudiometers to determine the volume of the produced biogas. The biogas composition was measured periodically with an X-am 7000 (Dräger, Germany) gas analyzer. The produced biogas and methane volumes are given in norm liters per kilogram of volatile solids (lN kg VS-1).

5.4.2 Biological ethanol potential

The biological ethanol potential was determined using a simultaneous saccharification and fermentation (SSF) method, described by Passoth et al. [56]. Briefly, the material was filled into sterile bottles and deionized sterile water was added until a volume of 100 ml was reached. 100 ml citrate buffer (pH 5, 0.2 M) was added before adjusting the content of the bottle to a pH of 5 using 10 M sodium hydroxide or 25 % citric acid. Subsequently, the bottles were closed with aluminum foil and boiled in a water bath in order to sanitize the biomass. Yeast cells (S. cerecisiae J672) were pre-cultured in YPD and then incubated in a shake flask (30°C for 20 h). Afterwards, the cells were harvested, centrifuged, washed and suspended in a saline solution before inoculation. The liquid containing the enzymes for saccharification (Accelerase 1500, Genecor, Palo Alto, CA, USA) was centrifuged and sterile-filtered (0.2 µm syringe filter) prior to suspending it to the bottles. Samples were withdrawn prior to incubation and then after 24, 48, 72 and 96 h. The ethanol content was determined using HPLC (high performance liquid chromatography) with a HC-75 column (305 by 7.8 mm, Hamilton, Nevada, USA) and an RI detector (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany).

6 Summary of publications

6.1 Biogas production from Steam-Exploded Miscanthus and Utilization of Biogas Energy and CO_2 in Greenhouses

Simona Menardo, Alexander Bauer, Franz Theuretzbacher, Gerhard Piringer, Paal Jahre Nilsen, Paolo Balsari, Oksana Pavliska, Thomas Amon

Bioenergy Research (2013) 6:620-630 doi: 10.1007/s12155-012-9280-5

The utilization of miscanthus as a resource for energy generation can provide several advantages. It can adapt to different soil and climate conditions easily, therefore making it a crop that can be cultivated in a wide range of locations. Especially the growth of miscanthus on sites not suited for food crop production is an interesting option. Moreover, miscanthus shows a high efficiency in CO_2 and water utilization, which result in harvestable yields of 5-55 t DM ha-1 per year.

The focus of the paper is the investigation of the effect of a steam explosion pretreatment on the structural composition and the biological methane potential of miscanthus. Additionally, the possibility of using miscanthus as an energy resource for both heat and CO_2 supply of greenhouses was investigated.

The miscanthus used for the study was composed mainly of structural components, with cellulose being the highest share (50.7 % DM), followed by hemicellulose (27.5 % DM) and lignin (15.6 % DM). In order to improve digestibility, the material was pre-treated using steam explosion with direct steam injection (no liquid was added in advance). The temperature range for the pretreatment varied from 180 to 220°C with a duration between 5 and 20 minutes, resulting in a severity factor between 3.1 and 4.7.

Results show that the structural composition of the miscanthus is already altered at low pretreatment temperature and duration. The hemicellulose content decreases 15 % at the lowest pretreatment severity (SF Log(R0) = 3.1) compared to the untreated sample. At a pretreatment severity of SF Log(R0) = 4, more than 97 % of hemicellulose is dissolved. In addition, a decrease of lignin with increasing pretreatment could be observed, being at a maximum of 59 % at SF Log(R0) = 4.5 compared to the untreated sample. The cellulose content stayed relatively stable between 50.2 and 55.2 % DM (untreated 50.7 % DM).

Regarding the biological methane potential, the highest yield was observed with a pretreatment severity of SF Log(R0) = 4.5 at a pretreatment temperature of 220°C and a duration of 10 minutes. The yield was 374 l_N kg VS^{-1}, which is a quadrupling compared to the untreated sample (84 l_N kg VS^{-1}). Compared to the theoretical maximum of carbon conversion to methane, the best result shows a conversion efficiency of 83 %.

The outcome of this paper is that steam explosion pretreatment is a viable option for improving the digestibility of miscanthus, resulting in a tremendous increase of the biological methane potential. For a practical implementation, it can be concluded that high pretreatment temperatures and a long duration have to be favored in order to achieve optimal results.

6.2 Steam explosion pretreatment for enhancing biogas production of late harvested hay

Alexander Bauer, Javier Lizasoain, Franz Theuretzbacher, Jane W. Agger, María Rincon, Simona Menardo, Molly K. Saylor, Ramón Enguidanos, Paar J. Nielsen, Antje Potthast, Thomas Zweckmair, Andreas Gronauer, Svein H. Horn

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Grassland areas are an important part of European landscapes. In recent years, the utilization of those areas for agricultural purposes decreased continuously, leading to a disappearance of about 15 % of the grassland in the last 20 years. This development can be explained by the economic considerations of farmers, who need to optimize their operations in order to be competitive on global markets. Still, there are several reasons for maintaining grasslands, among those ecological (biodiversity) and economical (tourism) ones. Therefore, biomass obtained from those areas can be considered as a potential residual material for energy generation. In order to preserve a maximum of biodiversity and at the same time reduce harvesting costs, an extensive management with one cut per year is considered as preferable. This also means a higher lignification of the obtained biomass and therefore leads to the need for pretreatment in order to improve digestibility.

The objective of the study is the investigation of the effect of a steam explosion pretreatment on the biological methane potential of late harvested hay. In addition, the samples were analyzed for the content of different carbohydrate fractions, protein, furfural and hydroxyl-methyl-furfural.

Concerning the composition of the samples, increasing pretreatment severity resulted in (a) a nearly complete dissolution of hemicellulose; (b) an increase of the lignin fraction; (c) a loss of organic compounds; and (d) an increase of furfural and HMF. The dissolution of hemicellulose is commonly observed in similar studies and was therefore expected. A possible increase of the lignin is well-documented in several publications; nevertheless, the observed increase from 5.8 % DM to 24 % DM can be considered as very high compared to other findings. This could have its reason in the composition of the used hay, which differs from other types of commonly used types of biomass, e.g., wheat straw. Another reason for this finding could be the relative increase of poorly soluble organic compounds, as those are likely to remain in the biomass while others like, e.g., organic acids are lost during the pretreatment. This effect could also be observed in this study, with a maximum loss of 39.2 % of organic compounds at the highest pretreatment severity (220°C and 15 minutes). Another finding was the increase of furfural and HMF with increasing pretreatment severity up to 2884 mg kg^{-1} VS and 2995 mg kg^{-1} VS respectively. Notably, the highest concentrations were not found at the highest pretreatment severity, but at 205°C and 190°C respectively. This can be explained by the high volatility of those compounds, which makes it likely that some fraction is lost in the process.

Considering the biological methane potential, the highest improvement could be observed at a relatively low pretreatment severity (175°C and 10 minutes), resulting in a methane yield of 281 l_N kg VS^{-1}. This is an improvement of 16 % compared to the untreated sample and is about 64 % of the theoretical potential based on the chemical composition of the untreated sample. Higher pretreatment severities resulted partly in a lower methane yield than the untreated sample. Explanations for this are the loss of organic compounds, an increase of the lignin fraction, as well as the formation of furfural and HMF.

The outcome of this paper is that steam explosion pretreatment has to be adapted for biomass with a lower grade of lignification. Lower pretreatment temperatures have to be preferred in order to increase the methane yield. The comparison with the theoretical methane potential still shows a wide range for improvement of the process.

6.3 Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion

Franz Theuretzbacher, Javier Lizasoain, Christopher Lefever, Molly K. Saylor, Ramón Enguidanos, Nikolaus Weran, Andreas Gronauer, Alexander Bauer

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Wheat straw is an abundant resource for bioenergy generation and can be obtained in large quantity worldwide. Therefore, it is a widely used material for studies with the objective of developing and improving pretreatment methods. Parameters usually investigated are the specific energy potential or the rate of enzymatic hydrolysis. A comparison of the biological degradation kinetics of native and pretreated material has not been investigated yet.

The objective of this study was to investigate the degradation kinetics of the structural compounds cellulose, hemicellulose and lignin during the anaerobic digestion of untreated and steam-exploded wheat straw. The steam explosion pretreatment was performed at temperatures from 140 to 178°C, with a duration between 30 and 120 minutes. This corresponds to a severity factor SF Log(R0) between 2.7 and 4.4. Subsequently, the samples were analyzed for their structural carbohydrate and protein content. In order to analyze the degradation kinetics, a modified method from feed analysis was used. Briefly, for each pretreatment combination several nylon bags were filled with sample material and transferred to a 20 L container filled with inoculum. After 1, 2, 5, 10, 15, 20, 30 and 45 days at a time, a sample bag was removed and analyzed. In parallel, the biological methane potential was analyzed using batch tests.

The dry matter content of the samples increased with increasing pretreatment severity, as more steam is needed to reach the desired pretreatment temperature. Hemicellulose started to hydrolyze at 140°C and was below detection limit at pretreatment severities of SF Log(R0) = 4.1 and 4.4. Lignin content increased with pretreatment severity, which can be attributed to the formation of compounds with similar properties as lignin. This fraction is formed from hemicellulose degradation products during the steam explosion process. The pretreatment temperature had a greater effect on the composition of the biomass than the duration of the pretreatment.

The specific methane potential of the pretreated samples did not exceed the yield of the untreated sample. This finding does not correspond with results from similar studies, which found increases of the methane yield after pretreatment. The reason for this could be the different steam explosion setup with lower pretreatment temperatures and considerably longer duration. In addition, the methane yield of the untreated wheat straw was unexpectedly high, which could be addressed to the milling of the material as well as the used inoculum, which was adjusted to lignocellulose-rich feedstock.

Concerning the methane formation rate, it could be observed that methane generation was faster for all pretreated samples. This effect could be observed until the 20th day of the experiment; afterwards no significant difference could be detected.

Regarding the degradation kinetics of the structural compounds, it could be observed that the hemicellulose fraction of the pretreated samples degraded faster than in the untreated sample. Considering the degradation speed of cellulose, no difference could be observed. As expected, the lignin fraction of the untreated sample could not be digested. In contrast to that, the fraction that formed from hemicellulose degradation products declined during the experiment until it reached the level of the lignin in the untreated sample. This result suggests that those compounds can be degraded during anaerobic digestion despite their properties similar to lignin.

6.4 The effect of combined biological and thermo-mechanical pretreatment of wheat straw on energy yields in coupled ethanol and methane generation

Franz Theuretzbacher, Johanna Blomqvist, Javier Lizasoain, Lena Klietz, Antje Potthast, Svein Jarle Horn, Paal J. Nilsen, Andreas Gronauer, Volkmar Passoth, Alexander Bauer

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Considering the research goals of this study, there are two approaches: in order to provide wheat straw as a resource for biological conversion into bioethanol and biogas, it has to be available for a whole year. In order to store wheat straw properly, a water content of 12 % should not be exceeded. Moreover, wheat straw has to be pre-treated prior to biological conversion in order to break the linkage between cellulose, hemicellulose and lignin. A possible approach for targeting both challenges is the utilization of microorganisms, which A) can prevent the growth of, e.g., moulds, and B) can improve the digestibility of the biomass.

In this study, wheat straw with a water content of 30 % was inoculated with *Scheffersomycesstipitis*. After a storage time of 42 days, the material was pre-treated using steam explosion with pretreatment temperatures of 180, 200 and 220°C and a duration of 15 minutes. The material was analyzed for water, hemicellulose, cellulose, lignin, furfural and hydroxyl-methyl-furfural content. After pretreatment, the material served as a substrate for A) a combined ethanol and biogas production, and B) sole biogas production. The ethanol potential was analyzed using a simultaneous saccharification and fermentation method, in which the hydrolysis of the cellulose fraction and the fermentation of the resulting sugars into ethanol are carried out in the same vessel.

The results show that the inoculation with *Scheffersomycesstipitis* leads to a decrease of cellulose, while other structural compounds are unaffected. With increasing steam explosion pretreatment severity, water content as well as the content of volatile compounds increase. In accordance to the findings of the other studies, lignin increased with higher pretreatment temperatures, while hemicellulose decreased. After steam explosion pretreatment, no difference between the inoculated and the native material could be observed.

Regarding the biomethane potential, the inoculated material showed an increase of 15 % compared to the native (ground) sample. The highest methane yield obtained was 20 % higher compared to the native sample. No difference could be observed between inoculated and non-inoculated material. Considering the ethanol potential, a strong influence of the pretreatment temperature could be observed. The highest yields were achieved using a pretreatment temperature of 220°C. Compared to the theoretical maximum, 70 % of the possible ethanol yield was obtained. The highest overall energy yield (combined ethanol and biomethane production) was obtained with inoculated wheat straw and steam explosion pretreatment at 200°C, whereas nearly 50 % results from ethanol.

Furfural and hydroxy-methyl-furfural were formed at pretreatment temperatures of 200 and 220°C, whereas the concentration of furfural was quite constant at 7 mg VS^{-1}, while the concentration of hydroxy-methyl-furfural increased from 5 mg VS-1 to 23 mg VS^{-1} (without inoculation) and 16 mg VS^{-1} (with inoculation) respectively.

It can be concluded that the inoculation of the wheat straw with *Scheffersomycesstipitis* did not have an effect on ethanol and biomethane generation. In contrast, the pretreatment temperature used for steam explosion had a strong effect on ethanol potentials. A key finding is that the combined production of ethanol and biomethane resulted in the highest overall energy yield.

7 Results and discussion

7.1 Dry matter, volatile solids and mass loss

The dry matter content of the pretreated material depends on the technology used for steam explosion, as well as on the pretreatment intensity. Biomass pretreated using steam explosion pretreatment with direct steam injection (Cambi process, paper I, II and IV) showed lower dry matter content with increasing pretreatment temperature and duration. This can be explained by the larger amount of steam needed for reaching the desired pretreatment conditions, as well as the need for additional steam to maintain those conditions for longer durations. In contrast, applying the steam explosion pretreatment using a heating jacket (paper III), the dry matter content mainly depends on the water added prior to feeding it into the reaction vessel. Nevertheless, high pretreatment intensity also leads here to a lower dry matter content. A possible explanation for this effect is the release of water through chemical reactions during the pretreatment. All samples showed a considerable decrease of dry matter content. In paper IV, the dry matter content of wheat straw dropped from 92.5 % to 15.2 % after steam explosion at 220°C and 15 minutes. The steam explosion pretreatment of miscanthus (paper I) showed less decline considering the dry matter content (from 88.4 to 27.0 %). In general, those results are in line with the findings of other studies. Bauer et al. [28], e.g., found a decrease of dry matter content of wheat straw from 92.4 to 18.5 % at a pretreatment temperature of 200°C and a pretreatment duration of 10 minutes.

The content of volatile solids of pretreated biomass can differ from the untreated biomass, as volatile compounds are formed during the pretreatment. When the pressure is reduced to atmospheric level, those volatile compounds can get lost. In paper II, the volatile solids content of hay decreased from 94.1 to 90.9 %. This corresponds to a mass loss of 35.2 %. In paper I, III and IV this effect could not be observed. A possible explanation for this effect is that the composition of hay differs from those of miscanthus and wheat straw. For example, the cellulose, hemicellulose and lignin content is lower, while the protein content is considerably higher. Also the hemicellulose composition differs between the investigated types of biomass. This could lead to an enhanced formation of volatile components which are lost during the pretreatment process. Lizasoain et al. [39] found a decrease of volatile solids after steam explosion of corn stover from 94.3 to 90.8 % at 220°C and 25 minutes. In general, biomass with a lower grade of lignification seems to be more vulnerable to mass loss through the loss of volatile compounds than biomass with a more rigid lignocellulose structure as, e.g., wheat straw or miscanthus.

7.2 Structural compounds

As described previously, the steam explosion pretreatment results in an alteration of the structural compounds of the biomass. This is a desired effect, as the lignocellulose complex is broken up, providing microorganisms and enzymes the possibility to degrade the polysaccharide compounds of the biomass.

Regarding the cellulose content, it can be seen that the steam explosion pretreatment only has a minor effect if wheat straw and miscanthus are used as substrate. In paper I the cellulose content varies in a range from 48.1 to 55.2 % (native 50.0 %), in paper III from 39.8 to 43.0 % (native 40.6 %), and in paper IV between 48.1 (native) and 46.7 %. Paper II is an exception, as the cellulose content decreases from 34.9 to 26.7 %. This is of particular interest, as also in paper II mass loss occurred due to the removal of volatile solids, which means that compared to the native biomass, the loss of cellulose is even higher.

While the moderate change of cellulose content is in line with some of the literature [30, 38-40], the decrease of cellulose in paper II is an exception, with only one author [34] obtaining a similar result using barley straw. A series of authors also found an increase of cellulose [24, 25, 27, 31, 32, 35, 36, 42]. A possible explanation for this effect is that amorphous cellulose is more vulnerable to hydrolysis than crystalline cellulose [57]. Therefore, biomass with a high content of amorphous cellulose could show a declining cellulose content, while biomass with a higher share of crystalline cellulose would show no effect or even an increase (if mass losses occur). In paper II hay was the investigated biomass. As discussed previously, hay shows differences regarding the share of cellulose, hemicellulose and lignin as well as the composition of those compounds compared to other investigated types of biomass like wheat straw and miscanthus.

The hemicellulose content of all types of biomass used in the presented studies decreased with increasing steam explosion pretreatment intensity. Hemicellulose hydrolysis was increased by higher pretreatment temperatures as well as longer pretreatment duration. In paper III the hemicellulose fraction of wheat straw was completely degraded at a pretreatment temperature of 178°C, whereas the pretreatment duration of 60 minutes was comparably long. In contrast, in paper IV the hemicellulose fraction of the used wheat straw was completely degraded at 200°C with a pretreatment duration of 15 minutes. The hemicellulose fraction of both miscanthus (paper I) and hay (paper II) was degraded at a pretreatment temperature of 190°C and a pretreatment duration of 10 minutes.

Other authors also find a major decrease of hemicellulose, although to a different extent. Iroba [34], Lopez-Linares [36] and Lizasoain [40] observed a 90 % decrease at pretreatment temperatures around 200°C, while Monschein [30] only found a 9 % decrease. An exception is Lizasoain [39], who found a 1 % increase of hemicellulose after steam explosion pretreatment of corn stover.

The analysis of the degradation kinetics, which was carried out in paper III, showed that the remaining hemicellulose degraded faster than without steam explosion pretreatment. The improved accessibility of the hemicellulose after partial disintegration could be a possible explanation for this effect.

The results considering the lignin content of the pretreated biomass differ considerably. The steam explosion pretreatment of miscanthus (paper I) showed a decrease of the lignin fraction from 15.6 % to 7.9 % at a steam explosion temperature of 220°C and a pretreatment duration of 15 minutes. In contrast, the lignin fraction of steam-exploded hay (paper II) increased from 5.8 % to 24.0 % at the same pretreatment conditions. Steam-exploded wheat straw also showed an increase of the lignin fraction from 7.5 % to 15.9 % at a pretreatment temperature of 178°C and a pretreatment duration of 120 minutes using the Biogassystems process (paper III), while no effect could be observed using the Cambi process (paper IV).

The obtained results considering the lignin content are similar to data obtained from literature. While Rajput [31] found no significant change in the lignin content of steam-exploded wheat straw, Chang [38] found a 37 % decrease of steam-exploded corn stover and Agudelo [35] found an increase of 127 % of steam-exploded triticale straw. In general, it can be said that most of the authors observed an increase of the lignin content of steam-exploded lignocellulose material.

A possible explanation is that components resulting from hemicellulose degradation form compounds which are similar in their properties to lignin. Nevertheless, this fraction does not share all of those properties. On the one hand, both applied methods for the measurement of structural compounds resulted in increased lignin contents. On the other hand, the analysis of the degradation kinetics carried out in paper III showed that this fraction is degraded until the original lignin content was reached.

7.3 Methane and ethanol yields

In papers I to III the intention was to improve the biogas and methane yields respectively, using steam explosion as a pretreatment technology. In paper IV the approach was different, as the optimization of the combined production of methane and ethanol was in the focus of the research. The steam-exploded material used in paper I to III was vacuumed and refrigerated immediately, and then used for the determination of the biological biogas and methane potential as described in the methods section. In paper IV the material was first used for the determination of the biological ethanol potential. After this, the ethanol was removed by evaporation, using a rotavapor in order to prevent additional alteration of the material by heat. The residue after ethanol removal was subsequently used for the determination of the biogas and methane potential.

In paper I every combination of pretreatment temperature and duration led to a significant improvement of the methane yield, with an optimum achieved at 220°C and 15 minutes. The methane yield could be improved from 84 l_N kg VS^{-1} to 347l_N kg VS^{-1} (+313 %). Increasing steam explosion temperature as well as increasing duration both led to higher yields. Compared to the literature, this improvement is considerably higher. Li [41] found an improvement of 50 % after steam explosion pretreatment of miscanthus at 198°C and 10 minutes. Nevertheless, the absolute yield he obtained was 274 l_N kg VS^{-1}, which is in the range of the obtained yield in paper I at the same pretreatment conditions (311 l_N kg VS^{-1}).

The steam explosion pretreatment of hay that was carried out in paper II showed a different picture. An increased methane yield could only be obtained at comparably moderate pretreatment conditions with a maximum of 281 l_N kg VS^{-1}(+16 %) at a steam explosion temperature of 175°C and a duration of 10 minutes. Increasing the severity of the pretreatment led to declining yields, even below the level of the native hay sample. A possible explanation for this result could be that the fraction analyzed as lignin showed a rapid increase after steam explosion pretreatment. Assuming this fraction is not – or at least hard – to digest and maybe even prevents degradation of cellulose and other usable fractions, this would lead to declining methane yields.

In paper III no relevant improvement of the methane yield of the used wheat straw could be achieved through steam explosion pretreatment. The highest methane yield was achieved using a steam explosion temperature of 140°C and a pretreatment duration of 120 minutes. Nevertheless, it has to be stated that the methane yield of the native wheat straw was very high, at a value of 276 l_N kg VS^{-1}. Literature values for the methane yield of native wheat straw vary between 210 l_N kg VS^{-1} [25] and 275.6 l_N kg VS^{-1} [28]. In paper IV the methane yield of the untreated sample was 210 l_N kg VS^{-1}. This means that both studies, which were focused on the improvement of energy yields of wheat straw through steam explosion pretreatment, delivered methane yields for untreated samples that are in accordance with literature, although representing the lowest and the highest margins of the spectrum. Nevertheless, the investigation of the degradation kinetics showed that the remaining hemicellulose and cellulose fractions degraded faster compared to the untreated wheat straw sample. This means that the speed of methane generation also increases, which can be an advantage for the operation of biogas plants.

The approach in paper IV was to investigate whether a combined ethanol and methane generation of steam-exploded wheat straw would result in higher energy yields than solely methane generation. In addition, the steam explosion pretreatment was combined with a biological pretreatment (integrated storage and pretreatment ISP) in order see if a further improvement of the pretreatment could be achieved. The results show that this combination did not have a significant effect on the composition of the material and on observed energy yields. The approach of combining ethanol and methane generation showed the highest overall energy yields (10.86 MJ kg VS^{-1}) at a steam explosion temperature of 200°C, which means a 44 % increase to the untreated sample. Without ethanol production, the maximum energy yield would have been 9.03 MJ kg VS^{-1}.

The ethanol yield for both variants (with and without ISP) was 160 mg g VS^{-1}. This value is a similar range as found by Dererie [33] after steam exploding oat straw at a pretreatment temperature of 190°C (ethanol yield 150 mg g VS^{-1}). Lopez-Linares [36] found a yield of 124 mg g VS^{-1} after steam exploding rapeseed straw at 215°C, while Horn [25] found an ethanol yield of 210 mg g VS^{-1} after steam exploding wheat straw at 210°C.

7.4 Inhibitors

The generation of inhibitors is often seen as a drawback concerning pretreatment technologies which rely on high temperatures. Therefore, in paper IV the concentration of the inhibitors furfural and HMF was analyzed. At a steam explosion temperature of 180°C, both furfural and HMF concentrations are below or a maximum 1 mg g VS^{-1}. After increasing the pretreatment temperature to 200°C it can be seen that the concentration of furfural increases to 7 (with ISP) and 8 mg g VS^{-1} respectively. The concentration of HMF is still moderate, with around 4 mg g VS^{-1} at the same pretreatment temperature. After steam explosion pretreatment at 220°C, the concentration of furfural decreases to 7 and 6 mg g VS^{-1}(with ISP) respectively, while the HMF concentration increases to 23 and 16 mg g VS^{-1} (with ISP) respectively. Agudelo [35] found a furfural concentration of 46 mg g VS^{-1} and a HMF concentration of 14 mg g VS^{-1} after steam exploding triticale straw at 200°C for 10 minutes. Lopez-Linares [36] found a furfural concentration of 23 mg g VS^{-1} and a HMF concentration of 9.0 mg g VS^{-1} after steam exploding rapeseed straw at 215°C for 7.5 minutes. It can be seen that, when comparing similar pretreatment conditions, the values obtained from the literature are higher than those found in this study. Nevertheless, the ration between furfural and HMF are similar.

As the highest ethanol potentials were found at a pretreatment temperature of 220°C, the results of this study indicate that HMF does not have a negative impact on yeast performance at this concentration level. Furthermore, furfural does not seem to have a negative impact on ethanol yields. The effect of furfural and HMF on the methane yield is unclear. The results of paper IV would indicate that the higher HMF concentration after steam exploding the wheat straw at 220°C has a negative effect on the methane yield. As described previously, the increasing lignin content (paper II and paper III) as well as mass losses (paper II) also have a possible negative effect on the methane yield of steam-exploded biomass. It is unclear if – and to which extent – those effects are relevant for the achievable biological methane yield.

8 Conclusions and further research

The objective of this study was to investigate whether steam explosion pretreatment improves the energy yields of lignocellulose material when used as substrate for methane or ethanol generation. In addition, the effect of the steam explosion pretreatment on the structural compounds cellulose, hemicellulose and lignin should be shown.

The severity of steam explosion pretreatment is defined by two factors: the steam explosion temperature and the duration of the pretreatment. The obtained results show that both factors have an effect on the energy yields, as well as on the composition regarding the structural compounds, both in a positive and negative direction.

Considering the link between the alteration of the structural compounds to achievable energy yields, it was shown that the analyzed amount of lignin plays a role in the methane yields. The best results were achieved when the lignin content declined. Nevertheless, for increasing lignin content as well, an improvement of methane yields could be observed in some cases. In general, it can be said that if the steam explosion severity was increased too much, the analyzed lignin fraction increased rapidly, while methane yields dropped. The only exception was miscanthus, which showed improved yields and declining lignin content also at the highest pretreatment severity.

There are several questions concerning the role of lignin in the course of the steam explosion pretreatment which still remain. The results of the investigation of the degradation kinetics showed that the share of the lignin fraction resulting from the pretreatment is degraded during anaerobic digestion. Therefore, it can be assumed that the properties of those compounds are considerably different to those of native lignin. If and how those compounds affect the biological degradability of the biomass as a whole is still unclear and needs further investigation.

Steam explosion pretreatment needs a considerable amount of thermal energy, which has to pay for itself in terms of improved energy yields, considering possible methane as well as ethanol potentials. For biogas plants that realize the biogas utilization by power generation in combined heat and power plants, it makes sense to use the produced waste heat to run the steam explosion process. The results presented in this thesis show that the revenue in terms of improved energy yields can vary considerably. In the case of using miscanthus, steam explosion pretreatment showed an extraordinary effect, leading to a methane yield that is comparable to silages of energy crops like maize. Nevertheless, also here the ratio between energy input through the pretreatment and energy output has to be taken into account for the evaluation of this concept.

The results considering the steam explosion pretreatment of hay and wheat straw are more differentiated. The methane yields are improved only to a minor extent, leading to the question whether they justify the energy input in the steam explosion pretreatment. On the other hand, the methane yields are in the range of other currently used substrates, e.g., grass silage. Moreover, material like wheat straw could not be used in biogas plants without pretreatment, due to its physical properties.

The approach of combining ethanol and methane generation of steam-exploded wheat straw showed a higher overall energy yield than solely producing methane. The advantage of this combination would be that ethanol is a more valuable energy carrier compared to biogas, which could improve the economic feasibility. On the other hand, the required technology for producing fuel-grade ethanol is very expensive; therefore, only large-scale applications are present today.

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Annex

Paper I	Biogas Production from Steam-Exploded Miscanthus and Utilization of Biogas Energy and CO2 in Greenhouses
Paper II	Steam explosion pretreatment for enhancing biogas production of late harvested hay
Paper III	Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion
Paper IV	The effect of a combined biological and thermos-mechanical pretreatment of wheat straw on energy yields in coupled ethanol and methane generation

I

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Biogas Production from Steam-Exploded Miscanthus and Utilization of Biogas Energy and CO_2 in Greenhouses

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Abstract The costs of producing protected vegetables comprise up to 78 % of the total operating costs in greenhouses. These expenses mainly result from energy consumption. Increasing energy efficiency and expanding the use of renewable energy sources are essential for global competitiveness. The aim of this study is to optimize methane production from miscanthus and to evaluate the potential use of miscanthus as a source of electrical energy, heat, and CO_2 in vegetable greenhouses. To optimize methane yield, miscanthus was pretreated by steam explosion using different time/temperature combinations. Pretreatment resulted in a more than threefold increase of methane yield from anaerobic digestion (374 $l_N kgVS^{-1}$) compared with untreated miscanthus. Based on technical parameters from two greenhouses (in Northern and Southern Europe), four different energy balances were established. The balances showed that using methane produced by pretreated miscanthus in vegetable greenhouses can enhance the entire process and therefore make it more sustainable.

Keywords Biogas · Greenhouses · Miscanthus · Steam explosion · Energy balance

Introduction

Worldwide, an area of more than 900,000 ha is covered by protected cultivations [1]. In Europe, 400,000 ha of crops are grown under protected cultivation (including high tunnel greenhouses), which are mainly concentrated in the Mediterranean Basin, specifically in Spain, Italy, France, and Greece [1]. Europe is the biggest supplier of greenhouse products in the world. Excluding high tunnel greenhouses, their distribution across Europe in the year 2000 was as follows:42,500 ha in Spain, 25,000 ha in Italy, 10,125 ha in The Netherlands, 8,500 ha in France, 5,000 ha in Greece, 4,600 ha in Germany, 2,250 ha in Belgium, and 1,000 ha in the UK [2].

The energy demand for greenhouse production is 220–320 MJ/m^2 of covered surface area per year for South European countries (Italy, Greece, Southern France) [3, 4]. In contrast, annual energy demand for countries in Central and Northern Europe (Poland, The Netherlands, Germany) can be up to 3,600 MJ/m^2 [5]. Artificial lighting and heating of greenhouses used for growing vegetables require a substantial amount of energy, mainly in the cooler regions of Europe. Such high energy demand has a considerable effect on production costs in greenhouses and can contribute up to 78 % of the total costs of the entire production chain [6]. The energy used in greenhouses is usually obtained from fossil fuels, and it is reasonable to evaluate the potential of substituting them with renewable energy sources. Combining biogas production with vegetable production in greenhouses could become a model for sustainable food production.

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Electrical and thermal energy is produced when biogas is used in a co-generation (CHP) unit. This energy could contribute substantially to the high energy demands of a greenhouse. It is also important to note that the thermal energy produced by a CHP unit is only partially used to heat biogas plants. Most of it dissipates into the atmosphere. Moreover, biogas production results in CO_2 output, which can be used as air fertilizer (after being purified), and the digestate can be used as soil amender. Whether biogas energy and its by-products can be harnessed effectively depends entirely on the proximity of the biogas plant to the consumer. The heat and CO_2 can be used only if consumers are located close to the biogas plant. Similarly, the utilization of fermentation residues is limited by the high transport volume.

While crops such as maize, sorghum, and sunflowers are frequently used for biogas production [7], miscanthus can also be used as a biogas substrate. Miscanthus is a perennial grass which originates from East Asia [8]. Its adaptability to different soils and climates makes it a suitable crop for the climatic conditions in Europe and North America [9]. Even at low temperatures, miscanthus remains productive and exhibits high efficiency for CO_2 assimilation [10]. In addition, miscanthus uses water efficiently, typically requiring 100–300 l of water to produce 1 kg of biomass [9]. For comparison, typical values for maize or sorghum are closer to the top of this range, around 300 lkg^{-1} [11–13]. Overall, studies show that harvestable miscanthus yields range from 5 to 55 tha^{-1}. In France, about 1,300 ha of surface area are cultivated with miscanthus in Austria, it is about 400 ha, in Germany about 325 ha, and in Italy just 50 ha [14]. The share of arable land cultivated with miscanthus is 0.13 % in Austria, 0.04 % in France, 0.02 % in Germany, and only 0.0037 % in Italy [15].

Recent studies analyzed the use of miscanthus to produce biogas by anaerobic digestion [16, 17]. However, the complex structure of its lignocellulosic biomass poses problems regarding its utilization as an energy source. Pretreatment is clearly needed to destroy the lignocellulosic structure. Steam explosion (SE) is generally considered one of the most cost-effective methods for pretreating lignocellulosic biomass because it does not require the use of an external catalyst [18]. Compared with some alternative pretreatment methods, SE has several advantages, including a significantly lower environmental impact, lower capital investment, and less hazardous process chemicals [19].

Varying the temperature and duration of SE pretreatment has been shown to be effective at optimizing the methane yield [20]. Therefore, one objective of this work was to evaluate how different temperature/time combinations for SE pretreatment can increase the degradability of miscanthus in order to increase its methane yield and make it a more suitable crop for biogas production. A second objective was to evaluate the feasibility of employing biogas from SE-pretreated miscanthus to meet the energy and CO_2 demands of two model greenhouses (one in Southern and one in Northern Europe).

Materials and Methods

Substrate and Pretreatment

The miscanthus used in the experiments was harvested in Groß-Enzersdorf in eastern Austria in December 2010. The harvested miscanthus was stored in a sheltered area until further use for 9 months. The moisture content of the untreated miscanthus was 11.6 %.

After cutting the whole plant miscanthus, 500 g units of miscanthus were pretreated with a steam explosion unit (CAMBI, Asker, Norway) at the University of Life Sciences (UMB) in Ås, Norway [21]. The samples were not mixed with water prior to the pretreatment. Fifteen samples of 500 g were pre-treated in a 20-l pressure vessel. The steam was injected directly into the container to facilitate heating. Temperature and pressure inside the reactor were monitored and controlled during the pretreatment procedure. After the reaction time elapsed, the biomass and steam were flashed into a tank. The pretreated samples were stored under vacuum at 4 °C for 2 weeks until further use.

The steam explosion pretreatments were assessed at different temperatures and durations (Table 1). For each temperature/duration combination, the severity factor (SF) was calculated as $Log(R_0)$, where $R_0=t*\exp((T\text{-}100)/14,75)$, T is the treatment temperature in degrees Centigrade and t is the reaction time in minutes. The severity factor is widely used to compare the effect of steam explosion on the chemical composition of samples and on the methane yields.

Chemical Analysis

The chemical composition of untreated miscanthus was determined by analyzing for the following parameters: total solids (DM), raw ash (XA), cellulose (CEL), hemicellulose (H-CEL), and lignin (ADL).

Table 1 Severity factor SF of the different pretreatment conditions of steam-explosion experiments

Temperature (°C) Time (min)	180	190	200	210	220
5	3.1		3.6		4.2
10	3.4	3.7	3.9	4.2	4.5
15	3.5	3.8	4.1	4.4	4.7
20		4.0		4.5	

To analyze the dry matter content, it was dried to constant weight in a chamber at 105 °C. The dried material was burned in a muffle furnace at 550 °C, and the residue was recorded as the raw ash content. The volatile solids were calculated by subtracting the raw ash content from the total solids [22]. Cellulose, hemicellulose, and lignin were determined by using standard procedures based on Van Soest and Wine [23]. The neutral detergent fiber (NDF) is the percentage of fiber, which is not soluble in a neutral detergent solution. NDF measures most of the structural components in plant cells (i.e., lignin, hemicellulose, and cellulose) excluding pectin. The acid detergent fiber (ADF) is the percentage of fiber which is insoluble in a weak acid detergent solution [23]. Hemicellulose can be calculated by determining the difference between NDF and ADF; cellulose can be calculated by determining the difference between ADF and acid detergent lignin (ADL). The ash content was subtracted from both the cellulose and hemicellulose content, according to Naumann and Bassler [22]. A Perkin–Elmer Elemental EA 1108 CHNS-O apparatus (Carlo Erba Instruments) was used to perform elemental analyses of all samples according to standard procedures. Analyses for cellulose the elemental analyses were performed in the Microanalytical Laboratory of the University of Vienna.

Specific Methane Yield

Anaerobic digestion batch trials were carried out according to VDI [24], employing eudiometer batch digesters of 0.25 l capacity. The temperature was set to 37.5 °C, and the trial lasted 45 days. The analyzed variants were mixed with an inoculum at a 1:3 ratio (based on volatile solids content). The inoculum was digestate obtained from a full-scale commercial biogas plant in Utzenaich, Austria, that uses manure and energy crops as feedstock. This was to ensure that experimental results can be adopted for the commercial plant. The biogas and methane yields were monitored on a daily basis. The portable gas analyzer Dräger, X-AM 7000 was used to determine the CH_4 and CO_2 concentrations in the biogas. Biogas and methane production were calculated at a temperature and pressure of 273 K and 1,013 hPa, respectively, and expressed as norm cubic meters per kilogram of volatile solids ($m^3{}_N kg VS^{-1}$) [24]. All gas volumes in this study are given at these conditions, unless noted otherwise. Biogas and methane from the inoculum were substracted from the yields. All experiments were conducted in triplicate.

The results from elemental analysis of untreated miscanthus can be utilized to estimate the carbon dioxide and methane yields as well as yields of trace gases ammonia and hydrogen sulfide (Eq. 1, [25]).

$$\begin{aligned} C_aH_bO_cN_dS_e + (a - b/4 - c/2 + 3\cdot d/4 + e/2)\cdot H_2O \rightarrow (a/2 + b/8 - c/4 - 3\cdot d/8 - e/4) \\ \cdot CH_4 + (a/2 - b/8 + c/4 + 3\cdot d/8 + e/4)\cdot CO_2 + dNH_3 + eH_2S \end{aligned} \tag{1}$$

Where *a* is the number of carbon atoms; *b*, the number of hydrogen atoms; *c*, the number of oxygen atoms; *d*, number of nitrogen atoms; and *e*, the number of sulfur atoms.

Energy Balance

A simple energy and CO_2 balance calculation was conducted for two vegetable greenhouse models, each representing the climate and technical conditions in a different part of Europe: Southern Europe (Italy (IT); Spain (ES)) and Northern Europe (The Netherlands (NL)). The methane produced via anaerobic digestion of miscanthus could be employed to feed a CHP unit. The thermal and electrical energy obtained from the CHP unit could be used initially for the steam explosion pretreatment of miscanthus and reused later for the greenhouse heating system and electrical system. Additionally, the CO_2 production balance was determined for both greenhouse models.

The average heat consumption of greenhouses in IT and ES lies at 200 kWh_{th} per hectare and hour [26]. In NL, the average heat requirement is estimated at 500 kWh_{th} per hectare and hour [27, 28]. In these studies, the heat produced by grow light bulbs was also considered; 1,000 lux raises the temperature by about 0.7 °C. The electrical energy consumption was assumed to be 100 kWh per hectare and hour [29]. In the NL, an additional 1,000 kWh of electricity per hectare and hour was assumed for the grow lights. The value was calculated based on the annual light demand for growing tomatoes, about 10,000 lux for 3,500 h [24]. The CO_2 fixed by the plants grown is estimated at an additional 300 kg CO_2 per hectare per hour [30] for both vegetable greenhouse models. These data were used to calculate the annual energy and CO_2 demand per hectare of the greenhouse models (assuming continuous operation at 8,760 h per year).

Considering the higher heating value of CH_4, which is 39.79 MJ/m^3 [31], the thermal output of a CH_4-fuelled CHP is 5.5 kWh_{th}/m^3 (50 % thermal efficiency η_{th}) and the electrical output is 4.4 kWh_{el}/m^3 CH_4 (40 % electrical efficiency η_{el}) [32].

An energy balance was calculated for untreated miscanthus (M_0) and for miscanthus pretreated at 220 °C per 10 min (M_1), which yielded the highest amount of methane.

For both M_0 and M_1, the thermal and electrical energy produced per tonne of dry matter was calculated. The following assumptions were made:

a. Heat and electricity produced by the CHP are used first to cover the demands of the biogas plant (about 30 % of produced thermal energy [33] and about 8 % of produced electrical energy).
b. M_0=70 % of the produced heat (at 80 °C) and 92 % of the produced electricity are used for the greenhouse [33].
c. M_1=65 % of the produced heat (at 80 °C) and 87 % of the produced electricity are used for the greenhouse; in comparison to M_0, additional 5 % of the overall heat and power are used for the SE pretreatment.

The solid yields of the steam-exploded samples were assumed to be 100 % based on measured solid losses below 5 % under the most severe conditions.

Considering that 1 m^3 of biogas produces 1.96 kg of CO_2 (44.61 mol of carbon per m^3 biogas; CO_2 from combustion is assumed negligible), the quantity of CO_2 produced per tonne of dry matter of M_0 and M_1 was calculated (Eq. 2).

$$\mathrm{CO_2produced(kg\ CO_2/t\ DM)} = \mathrm{Biogas\left(m^3/t\ DM\right)} * 1.96\ \left(\mathrm{kgCO_2/m^3biogas}\right) \quad (2)$$

The determination of the annual energy demand made it possible to calculate how many tonnes of M_0 and M_1 were necessary to supply the required thermal and electrical energy and CO_2 for greenhouse models NL and IT/ES. The quantity of miscanthus plantation was expressed in hectares (Eqs. 3 and 4), and the yields per hectare were 18 t DM/ha in NL and 36 tDM/ha in IT and ES. The dry matter content of the untreated miscanthus (M_0) was 88.4 %.

$$\text{Miscanthus area required to meet energy demand(ha)} = \text{Energy demand (kWh)/energy produced(kWh/t DM)/miscanthus yield(t DM/ha)}. \quad (3)$$

$$\text{Miscanthus area required to meet CO}_2\text{demand (ha)} = \text{CO}_2\text{demand (t)/CO}_2\text{produced (t/t DM)/miscanthus yield (t DM/ha)}. \quad (4)$$

These equations express the energy and CO_2 demand as areas of miscanthus required. They were compared with the biogas outputs of thermal energy, electricity, and CO_2 in order to identify which of them represents a limiting factor for the greenhouse needs.

Statistical Analysis

SPSS version 17 was used to conduct a statistical analysis. Data were analyzed using one-way ANOVA, assuming homogeneity of the variances, followed by Tukey's range test ($\alpha<0.05$). The Pearson correlation (two-tailed) was used to highlight statistically significant correlations between the specific methane yield and chemical parameters.

Results and Discussion

Chemical Composition

The chemical composition of untreated and of steam-exploded miscanthus samples is shown in Table 2.

Untreated miscanthus had a DM content of 88.4 %. During pretreatment, most of the steam condensed on the miscanthus and was absorbed by the sample. As a consequence, the moisture content increased substantially, and the share of dry matter content expressed as DM decreased substantially in all treated samples. Compared with the untreated sample, the sample obtained through the weakest pretreatment (180 °C for 5 min) absorbed slightly more than its dry matter mass in condensate. This reduced the DM percentage by approximately half (to 41.9 %), and the other pretreated samples showed even further reductions in the share of dry matter. The lowest DM percentage was achieved during pretreatment at 210 °C for 20 min. At temperatures higher than 190 °C, the DM content appeared lower in samples treated for a longer duration.

Besides a higher sample moisture and resulting lower DM, SE pretreatment also lead to a relatively minor DM reduction due to losses of volatile solids. The untreated miscanthus sample had a VS content of 97.9 % DM. This value slightly decreased in all pretreated samples, and it ranged from 97.8 % DM to 97.3 % DM.

In order to study the combined effects of temperature and time on cellulose, hemicellulose, and lignin, their concentrations were determined for untreated and pretreated miscanthus samples (Table 2). Untreated miscanthus has a cellulose content of 50.7 % DM. In pretreated miscanthus samples, the percentage of cellulose ranged from 48.1 % to 55.2 % DM.

In contrast, hemicellulose was strongly affected by steam explosion (Fig. 1a). According to Garrote et al. [34], hemicellulose begins to solubilize at 150 °C. The correlation between SF and H-CEL degradation shows

Table 2 Chemical composition of untreated and pretreated miscanthus samples

Samples	SF Log(R_0)	DM	VS	XA	CEL	H-CEL	ADL
Unit of measurement		[% sample]	[% DM]	[% DM]	[% DM]	[% DM]	[% DM]
Miscanthus untreated	–	88.4	97.9	2.1	50.7	27.5	15.6
180 °C, 5 min	3.1	41.9	97.6	2.4	50.2	23.3	15.4
180 °C, 10 min	3.4	31.9	97.4	2.6	52.0	19.0	13.4
180 °C, 15 min	3.5	41.7	97.7	2.3	52.8	12.4	14.1
190 °C, 10 min	3.7	32.4	97.8	2.2	50.5	8.1	13.9
190 °C, 15 min	3.8	36.0	97.7	2.3	52.0	4.5	10.4
190 °C, 20 min	4.0	32.9	97.8	2.2	52.7	0.7	12.4
200 °C, 5 min	3.6	33.4	97.7	2.3	50.0	9.7	11.8
200 °C, 10 min	3.9	33.6	97.6	2.4	52.9	0.3	9.3
200 °C, 15 min	4.1	30.1	97.7	2.3	55.2	0.4	8.8
210 °C, 10 min	4.2	28.0	97.4	2.6	50.2	2.8	9.1
210 °C, 15 min	4.4	24.9	97.3	2.7	53.1	0.1	9.0
210 °C, 20 min	4.5	24.0	97.6	2.4	53.5	1.2	9.2
220 °C, 5 min	4.2	31.3	97.3	2.7	50.3	1.2	8.6
220 °C, 10 min	4.5	30.2	97.3	2.7	51.3	0.2	6.4
220 °C, 15 min	4.7	27.0	97.3	2.7	48.1	0.1	7.9

SF severity factor, *DM* dry matter, *VS* volatile solids, *XA* ashes, *CEL* cellulose, *H-CEL* hemicellulose, *ADL* lignin

that the intensity of pretreatment strongly affects H-CEL degradation. Untreated miscanthus samples had an H-CEL content of 27.5 % DM. Pretreatment at 180 °C for 5 min (SF 3.1) reduced the H-CEL by about 15 % compared to the untreated sample. When the temperature was kept steady at 180 °C but the pretreatment duration was increased to 10 and 15 min (that is, SF 3.4 and 3.5, respectively), H-CEL degradation increased by 19 % and 55 %, respectively, relative to the untreated sample.

At a pretreatment temperature over 200 °C (SF greater than 3.8–4.0), the average H-CEL content was below 2 % DM. Further increasing the temperature above 200 °C and extending the pretreatment duration did not have a strong effect on H-CEL degradation.

ADL was also affected by steam-explosion pretreatment. The ADL content in untreated miscanthus was 15.6 % DM, and this percentage decreased with pretreatment intensity (Fig. 1b).

The weakest pretreatment (SF 3.1) did not significantly affect the amount of ADL, but pretreatment at 180 °C for 10 min reduced ADL content to about 14 % compared with the untreated sample. The strongest pretreatments, 220 °C for 10 and 15 min (SF 4.5 and 4.7, respectively) reduced ADL content by more than 50 %. These results show that the ADL content of miscanthus can be substantially reduced by increasing the severity factor of steam explosion pretreatment. Lignin degradation and modification during steam-explosion pretreatments were reported by Martin-Sampedro et al. [35, 36]. The lignin degradation after steam explosion at 183 °C for 13 min was higher than 47 % [36]. These results are in accordance with those reported by Martín-Sampedro et al. [37], confirming that lignin starts to dissolve into water at the temperature level around 180 °C under neutral conditions.

The untreated miscanthus sample was composed of the following elements 47.4 % carbon, 6.23 % hydrogen, 0.30 % nitrogen, 0.02 % sulfur, and 46.08 % oxygen (percent of organic dry mater). The elemental composition of miscanthus biomass samples corresponded to values reported by Lewandowski [38]. According to the authors, carbon and hydrogen content (percent of DM) of miscanthus ranged from 47.8 to 49.7 for carbon and from 5.64 to 5.92 for hydrogen.

Specific Biogas and Methane Yields

Results obtained from batch experiments are presented in Table 3. The specific biogas yield of untreated miscanthus was 130 $l_N kgVS^{-1}$ and the specific methane yield, 84 $l_N kgVS^{-1}$. Klimiuk et al. [16] reported that the specific methane yield for miscanthus was 100 $l_N kgVS^{-1}$. This result was obtained in an experiment with continuously operated reactors, employing miscanthus silage with a lower content of cellulose (about 28 % of DM) and lignin (about 8 % of DM), compared with the miscanthus sample used in the present study. Higher methane yield for miscanthus (200 $l_N kgVS^{-1}$) can be achieved using miscanthus harvested in autumn [16]. The dry matter content of miscanthus harvested in autumn is about 35 % lower than miscanthus harvested in winter–spring [42, 43].

Each of the steam-explosion pretreatments resulted in a significant increase of the biogas and methane yield for

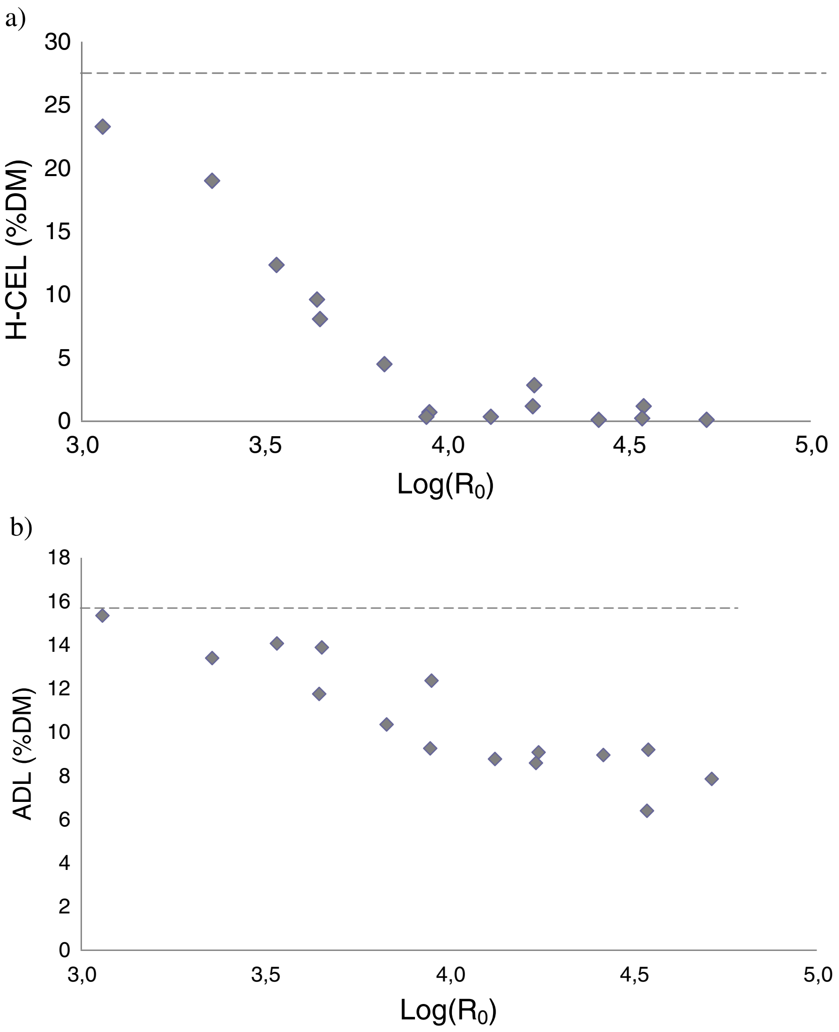


Fig. 1 H-CEL (**a**) and ADL (**b**) degradation as a function of the severity factor (SF) of the steam explosion pretreatment. The *dashed line* indicates the H-CEL/ADL percentage of untreated miscanthus

Table 3 Biogas and methane production of untreated and steam-exploded miscanthus

Samples	Log(R_0)	Biogas	SD	CH_4	SD	CH_4
Unit of measurement		$[l_N \cdot kgVS^{-1}]$	$[l_N \cdot kgVS^{-1}]$	$[l_N \cdot kgVS^{-1}]$	$[l_N \cdot kgVS^{-1}]$	[%]
Miscanthus untreated	–	$130^{(i)}$	15.7	$84^{(h)}$	10.4	65
180 °C, 5 min	3.1	$248^{(h)}$	7.7	$149^{(g)}$	25.8	60
180 °C, 10 min	3.4	$360^{(g)}$	8.2	$242^{(f)}$	4.6	67
180 °C, 15 min	3.5	$360^{(g)}$	9.8	$244^{(f)}$	11.1	68
190 °C, 10 min	3.7	$363^{(g)}$	19.6	$248^{(f)}$	18.3	68
190 °C, 15 min	3.8	$448^{(e)}$	29.0	$279^{(de)}$	18.1	62
190 °C, 20 min	4.0	$466^{(de)}$	11.4	$308^{(cd)}$	11.1	66
200 °C, 5 min	3.6	$409^{(f)}$	8.2	$260^{(ef)}$	7.7	63
200 °C, 10 min	3.9	$489^{(cd)}$	7.8	$311^{(c)}$	1.6	64
200 °C, 15 min	4.1	$481^{(cde)}$	11.9	$309^{(cd)}$	10.2	64
210 °C, 10 min	4.2	$541^{(b)}$	11.8	$345^{(ab)}$	9.2	64
210 °C, 15 min	4.4	$517^{(bc)}$	11.3	$333^{(bc)}$	8.2	64
210 °C, 20 min	4.5	$511^{(bc)}$	4.4	$331^{(bc)}$	4.6	65
220 °C, 5 min	4.2	$539^{(b)}$	3.0	$349^{(ab)}$	5.9	65
220 °C, 10 min	4.5	$600^{(a)}$	3.2	$374^{(a)}$	5.2	62
220 °C, 15 min	4.7	$525^{(b)}$	10.6	$347^{(ab)}$	5.3	66

The letters in parenthesis show the results of statistical analysis (Tukey's range test, α=0.05); different letters indicate significant differences among the samples miscanthus, in agreement with other studies dealing with the steam explosion pretreatment of biomass for the biogas production [39, 40]. The weakest pretreatment (SF 3.1) improved the biogas yield by about 90 %. The best outcome was obtained at 220 °C for 10 min (SF 4.5). This pretreatment resulted in a biogas production of 600 $\mathrm{l_N kgVS^{-1}}$.

The specific methane yield of steam-exploded miscanthus lies at 149 $\mathrm{l_N kgVS^{-1}}$ when pretreated at 180 °C for 5 min (SF 3.1) and at 374 $\mathrm{l_N kgVS^{-1}}$ when pretreated at 220 °C for 10 min (SF 4.5).

Based on the results fom the elementary analysis, the methane yield of untreated miscanthus calculated with the Boyle equation [25] is equal to 450.8 $\mathrm{l_N kgVS^{-1}}$, assuming that all carbon is converted to biogas (including lignin, which is considered non-digestible by anaerobic degradation). Up to 83 % (SF 4.5) of the calculated potential was converted to biogas/methane. This outcome is in line with the data supplied by Boyle [25], who reported that the maximum realistic rate for converting carbohydrates into biogas is about 85 %. Steam-explosion pretreatment resulted in an increased anaerobic degradation for volatile solids of miscanthus. Many studies have reported that the removal of a large part of hemicellulose and the break of lignocellulosic bonds cause an increase of cellulose fiber reactivity and ensures that cellulose is easily accessible for the enzymes [44–46].

The percentage of methane in biogas ranged from 60 % (180 °C for 5 min) to 68 % (180 °C for 15 min and 190 °C for 10 min), and the average was about 65 % (Table 3). Steam-explosion pretreatment did not have a detectable effect on the quality of the biogas.

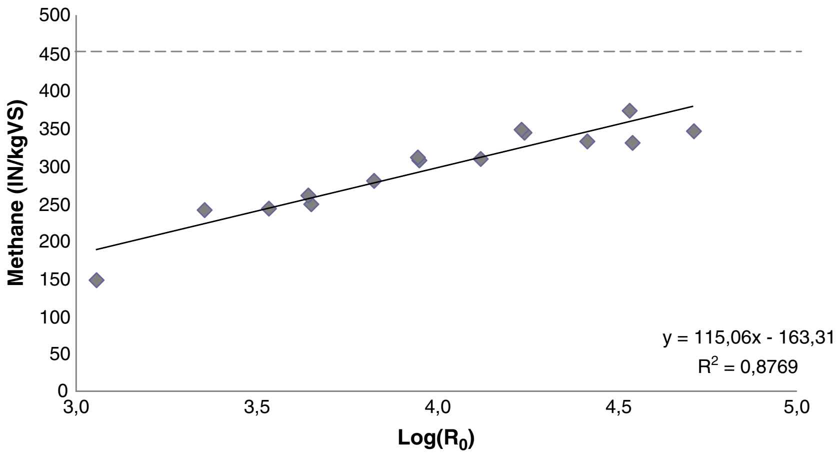
The present study shows a significant, negative correlation between specific methane yields and H-CEL (α=0.01; Pearson R^2=−0.942) and ADL (α=0.01; Pearson R^2=−0.902) contents. As expected, methane production of the pretreated samples increased with an increased degradation of hemicelluloses into simpler sugars, compounds that are more easily digested by anaerobic bacteria.

All pretreatments significantly improved the methane yield of untreated miscanthus (α=0.05), as a function of the SF (Fig. 2).

The temperature/time combination of 180 °C for 5 min (SF 3.1) resulted in the lowest increase of specific methane yield for miscanthus (77 %). The highest methane yield increase (344 %) was achieved at 220 °C for 10 min (SF 4.5). Compared with other studies [39, 41, 47], steam-explosion pretreatment on miscanthus was very effective. In terms of methane production, the pretreatments of 180 °C for 10 min and 15 min (SF 3.4 and 3.5, respectively) and of 190 °C for 10 min (SF 3.7) did not differ significantly from each other (α=0.05). When grouping the pretreatments by temperature, it became obvious that, at lower temperatures (180 °C, 190 °C, and 200 °C), only the shortest pretreatments (5, 10, and 15 min, respectively) are significantly different from each other. The same trend recurred also in other studies applying steam explosion to increase the biogas yield of lignocellulosic biomass [39, 41]. The samples pretreated for longer durations did not significantly differ from each other (α=0.05). At 210 °C and 220 °C, the pretreatment duration did not have any additional effect on the specific methane yield. The three pretreatments at 210 °C did not significantly differ from each other and neither did the pretreatments at 220 °C. Moreover, at the highest temperatures, a slightly negative effect on the specific methane yield occurred with respect to the pretreatment duration, as reported in the literature [39, 41]. This may be due to the formation of substances such as furfural and phenolics in high concentrations at harsher steam-explosion conditions [48–50], which may inhibit the microbial activity [51].

Methane production increased with temperature. For a 15 min pretreatment duration, the following methane production increase occurred 190 % at 180 °C, 232 % at 190 °C, 268 % at 200 °C, 296 % at 210 °C, and 313 % at 220 °C.

Fig. 2 Correlation between the specific methane yield and severity factor, SF. The *dashed line* indicates the calculated methane potential using the Boyle equation (Boyle, 1976)



The outcome shows that steam-explosion pretreatment is an effective way to considerably increase the methane yield of miscanthus. Furthermore, steam explosion induces a degradation of the lignin content of about 50 %. This causes an increase of the specific methane yield of pretreated miscanthus up to 3.5 times that of untreated miscanthus, so that pretreated miscanthus reached 83 % of its methane potential, as calculated with the formula of Boyle [25]. The specific methane yield increase exhibited a significant positive correlation with the SF and the temperature. Longer pretreatment times at the highest temperature resulted in a slightly inhibitory effect on methanogenic bacteria activity.

Energy Efficiency Evaluation of Biogas Utilization in Greenhouses

Greenhouse energy demand is strongly influenced by climate, temperature, and solar radiation. The latter is defined as the amount of incoming solar electromagnetic radiation per unit area. Two different balances were calculated, specifically adapted to the different climate conditions and the solar radiation in The Netherlands (NL) and in Italy/Spain (IT/ES). For the energy balance, 1 ha of greenhouse surface area was considered.

In NL, the annual temperature normally ranges from −1 to +23 °C, and the solar radiation is about 900 kWh/m^2. The thermal energy necessary to maintain an adequate temperature for growing vegetables in a greenhouse is about 500 kWh$_{th}$, corresponding to an annual demand of 4,380 MWh$_{th}$ per hectare (8,760 h) (Table 4) [52]. The annual electricity demand is also rather high (about 9,636 MWh$_{el}$) due mainly to the grow light bulbs, which supply about 10,000 lux per hour for 3,500 h per year (based on tomato growth parameters). In The Netherlands, the amount of CO$_2$ necessary for growing vegetables in a greenhouse is 2,628 t per year.

In the greenhouse model in Southern Europe (IT/ES), the thermal energy demand is much lower, only 1,752 MWh$_{th}$ per hectare. Temperatures in the Mediterranean basin are on average higher than in The Netherlands and range from 5 to 30 °C. Solar radiation is also stronger than in Northern Europe. It can reach 2,000 kW/m^2 per year [52]. The use of grow light bulbs is not necessary in Southern Europe, and consequently, the electricity demand is lower (only 876 MWh$_{el}$ per hectare). The assumed CO$_2$ amount for greenhouse vegetable production in IT/ES did not differ from the NL model.

The thermal energy produced by untreated miscanthus (M_0) can reach 0.32 MWh$_{th}$/tDM (excluding heat consumed for operating the biogas plant itself), while the electrical energy can reach 0.34 MWh$_{el}$/tDM. Steam-explosion pretreatment (200 °C for 10 min) resulted in a significant increase in methane yield and energy production. From the pretreated miscanthus (M_1), 1.31 MWh$_{th}$/tDM thermal and 1.40 MWh$_{el}$/tDM electrical energy can be produced respectively, considering deductions of operational energy for the biogas plant and for the steam explosion. Steam-explosion pretreatment yields a steady heat output of about 80 °C, which could be immediately reused in the greenhouse heating system without energy losses. This is possible because the initial temperature of the gases exiting the CHP is very high (over 200 °C). Although the pretreatment process

Table 4 Results of the energy and CO$_2$ balance for two greenhouse models (NL and IT/ES)

	Energy demand per ha greenhouse area per year					
Models	NL			ITA/ES		
Parameter	Thermal	Electricity	CO$_2$	Thermal	Electricity	CO$_2$
Unit of Measurement	MWh$_{th}$/ha	MWh$_{el}$/ha	t/ha	MWh$_{th}$/ha	MWh$_{el}$/ha	t/ha
	4,380	9,636	2,628	1,752	876	2,628
Sample	Produced Energy and CO$_2$					
Parameter	Thermal	Electricity	CO$_2$			
Unit of Measurement	MWh$_{th}$/t DM	MWh$_{el}$/t DM	t/t DM			
M$_0$	0.32	0.34	0.25			
M$_1$	1.31	1.40	1.15			
Sample	Surface area demand (hectares)					
Models	NL			ITA/ES		
Parameter	Thermal	Electricity	CO$_2$	Thermal	Electricity	CO$_2$
M$_0$	764	1598	585	153	73	292
M$_1$	186	383	127	37	17	64

absorbs a certain amount of energy, M_1 energy yields were substantially higher compared with M_0. This is due to the low energy consumption of steam explosion technology. The same trend was shown for CO_2 production. The CO_2 yield was more than four times higher for M_1 than for M_0 (1.15 t/t DM as compared with 0.25 t/t DM, see Table 4).

The volume of miscanthus required to supply sufficient greenhouse energy and to meet the CO_2 demand was calculated for untreated miscanthus (model M_0) and for miscanthus pretreated at 220 °C for 10 min (model M_1). The results were expressed in hectares of surface area cultivated with miscanthus.

The estimated energy consumption for the NL greenhouse per hectare is presented in Table 4. To meet the thermal energy demand, it would be necessary to cultivate 764 ha using M_0 (without pretreatment) or 186 ha using M_1 (with pretreatment). However, more than 1,598 ha of untreated miscanthus would be required to meet the electrical energy demand. This surface area could be reduced to 383 ha, if steam-exploded miscanthus was used to feed the biogas plant. Furthermore, the required area to meet the CO_2 demand for NL greenhouses was substantial.

The surface areas of miscanthus needed to meet the thermal and CO_2 demands were comparable. The results showed that the use of steam explosion led to a significant reduction in cultivated area requirements as compared with untreated miscanthus to obtain the same amount of energy. The balance showed that the use of this particular renewable energy source could be interesting for greenhouse use in Northern Europe, but only if another additional energy source is used. The required area for miscanthus cultivation (more than 150 ha for M_1 and more than 750 ha for M_0) is much larger than the utilized agricultural area (UUA) per farm in the European countries (UUA range between 1 and 125 ha, with an average of about 25 ha per farm [53]). The energy demand of a greenhouse in Northern Europe is too high to be met only by energy produced in a biogas plant fed with miscanthus.

The lower energy consumption estimate for a greenhouse in Southern Europe suggests that this location is more suitable for miscanthus use as a source of energy. To meet the thermal energy demand, 37 ha were required in the case of M_1 and 153 ha when using M_0. The electrical energy demand was substantially lower in Southern Europe due to the high solar radiation in this geographical area. A grow light system is not necessary, and electricity is used only for auxiliary services. To meet the electrical demand, only 17 ha of cultivated area were required with M_1; the area needed increased to 73 ha for M_0. This greenhouse model underlines the necessity of pretreating miscanthus to increase the output in the energy balance. In the IT/ES greenhouse model, CO_2 represented the limiting factor. To meet the CO_2 demand, it was necessary to cultivate 292 ha when using M_0 and 64 ha in the case of M_1. The advantage of using steam-exploded miscanthus is significant since the surface area need was reduced to less than one fourth compared with untreated miscanthus. All the energy produced to meet the CO_2 demand would be sufficient to also meet the thermal and electrical energy demand.

Conclusion

This study demonstrates that pretreating miscanthus with steam explosion increases its methane yield. The effect is well correlated with the severity factor. The best outcome is obtained for pretreatment at 220 °C for 10 min. At higher temperatures, further increasing the pretreatment length does not positively affect the methane yield. Moreover, when the methane produced in a CHP system is used, the CO_2 could be employed to meet the CO_2 demand of a vegetable greenhouse. The thermal and electrical energy produced would be sufficient to meet the low energy demand of greenhouses in Southern Europe but not the higher energy demand of Northern European greenhouses. From this perspective, it would make sense to plan a vegetable greenhouse in Southern Europe, where the energy is supplied by a biogas plant, since the agricultural land required is closer to the average European farm size. There, miscanthus could be used as an alternative to food or feed crops. Owing to its extreme adaptability, miscanthus can be cultivated also on marginal land and has low water and fertilizer needs. Miscanthus' very high lignocellulosic content makes it unsuitable for biogas production without pretreatment, but pretreatment increases its methane yield and, consequently, its energy potential. The use of steam explosion for pretreatment appears to be an interesting solution, in particular, employing temperatures over 200 °C and a treatment duration of at least 10 min.

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Steam explosion pretreatment for enhancing biogas production of late harvested hay

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HIGHLIGHTS

- Hay was pretreated with steam explosion from 160 °C to 220 °C for 5, 10 and 15 min.
- Increasing severity in the pretreatment induced degradation of hemicellulose.
- Enzymatic hydrolysis showed maximum glucose yields under the harshest conditions.
- Enzymatic hydrolysis showed maximum xylose values under mild conditions.
- Steam explosion increased 15.9% the methane yields.

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ABSTRACT

Grasslands are often abandoned due to lack of profitability. Extensively cultivating grassland for utilization in a biogas-based biorefinery concept could mend this problem. Efficient bioconversion of this lignocellulosic biomass requires a pretreatment step. In this study the effect of different steam explosion conditions on hay digestibility have been investigated. Increasing severity in the pretreatment induced degradation of the hemicellulose, which at the same time led to the production of inhibitors and formation of pseudo-lignin. Enzymatic hydrolysis showed that the maximum glucose yields were obtained under pretreatment at 220 °C for 15 min, while higher xylose yields were obtained at 175 °C for 10 min. Pretreatment of hay by steam explosion enhanced 15.9% the methane yield in comparison to the untreated hay. Results indicate that hay can be effectively converted to methane after steam explosion pretreatment.

1. Introduction

Among the largest habitat type in the world, grasslands are characterized by multiple functions and values; they provide forage for animals, they have a positive influence on the recharging of water tables and a protective effect on water quality. Grasslands have a big potential to sequester carbon in the soil, while safeguarding soil from erosion, as well as improving its fertility. In addition, grasslands support biodiversity and contribute significantly to rural economies (Peeters, 2009).

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; DM, dry matter; FM, fresh matter; H-CEL, hemicellulose; HMF, hydroxymethylfurfural; KF, Karl Fischer; l_N, standard liter (dry gas volume at 0 °C and 1013 mbar); NDF, neutral detergent fiber; NREL, National Renewable Energy Laboratory (United States); ODW, oven dried weight; SE, steam explosion; SRS, sugar recovery standards; VS, volatile solids; XA, raw ash; XP, crude protein.

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The grassland area in Europe is estimated at 59 million hectares, which is 34% of the total agricultural area (FAOSTAT, 2013). In the last twenty years, the grassland area in Western Europe declined by about 15%, incurring negative consequences for ecology, economy and society. Moreover, the situation of Alpine agriculture is further complicated, where recent warming has been roughly three times the global average (OECD, 2007). It is therefore of vital importance that alternative land use concepts are developed to help maintain Alpine agriculture. One possible strategy is the biogas-based biorefinery concept, which entails using grassland biomass to produce energy and chemical components. Among the different ways for producing energy out of grassland biomass, biogas production is currently the most common practice in Europe. Since the orographic features in Alpine areas compromise the sustainability of this type of use, extensive agricultural techniques (e.g. only one cut per year) will help mitigate this handicap. Thus, grass would be reaped slightly later than its optimum quality stage and will contain a higher lignification level than the standard feedstock.

Hay is the oldest, and still the most important, conserved fodder. Haymaking turns green, perishable, grass into a product that can be safely stored and easily transported by reducing its moisture content from approximately 70–90% to 15–20%. It is especially appropriate for small-scale producers since, it can be made with little equipment or costs. Natural drying of mown grass by sun and wind is still the most common way of conserving this type of biomass. For efficient methane production from grass, a pretreatment of the biomass is necessary and can lead to an important economic gain (Thamsiriroj and Murphy, 2010). In order to break the lignocellulosic bonds in the biomass, autohydrolytic steam explosion has generally been accepted as one of the most effective pretreatment methods, since no addition of external catalysts is necessary.

Currently, steam explosion is one of the most intensive investigated pretreatment technology of lignocellulosic material for both ethanol and biogas production. The factors that most affect steam explosion pretreatment are temperature, residence time, particle size, and moisture content (Cara et al., 2006). The treatment is applied for a few minutes and then the pressure is abruptly reduced, which make the material suffer an explosive decompression. This produces the hydrolysis of the hemicellulose into water-soluble oligomers or to individual sugars, and also generates a good substrate for enzymatic hydrolysis by cellulases. The rupture of the union lignin–carbohydrates, together with the hemicellulose solubilization is responsible of the big increase of cellulose to enzymatic hydrolysis (Fernández-Bolaños et al., 2001). Besides, the rapid thermal expansion opens up the biomass particle structure leading to a reduction of the particle size and an increase of the pore volume.

Steam explosion is a well-documented pretreatment, tested by several researches and widely utilized nowadays since it is one of only a very limited number of cost-effective pretreatment technologies. It has been demonstrated to be an efficient pretreatment method for both ethanol and biogas production from such a different substrates as wood (Horn et al., 2011a), grasses (Prochnow et al., 2009), agricultural residues (Ballesteros et al., 2002; Bauer et al., 2009a), by-products (De Paoli et al., 2011) or municipal waste (Li et al., 2007). Moreover, commercial steam-explosion equipment is available.

Compared to alternative techniques, the advantages of steam explosion (SE) include a remarkably low environmental impact, lower capital investment and less hazardous process chemicals (Li et al., 2001). Like other physio-chemical pretreatment methods, it may create degradation products that have an inhibitory and toxic effect on the anaerobic digestion. Nelson et al. (1988) demonstrated the formation of aromatic compounds, such as furans, pyrroles, phenols, enols and carboxylic acids from carbohydrates and amino acids in slightly acidic aqueous solution under reflux or hydrothermolytic conditions. Since analogous conditions are set up in the steam explosion treatment, similar chemical substances may be produced and potentially have an inhibitory effect on the enzymatic hydrolysis and fermentation processes (Palmqvist and Hahn-Hägerdal, 2000). The composition and concentration of the inhibitors vary with the severity of the pretreatment, the raw material used, and the type and content of chemical catalyst (García-Aparicio et al., 2006).

While biogas production from permanent grassland has been widely investigated (Prochnow et al., 2009), the use of steam exploded hay has hardly been investigated. Thus, the objective of this work was to evaluate the effect of temperature and time of steam explosion pretreatment on the biogas production and enzymatic hydrolysis of Alpine hay. Steam explosion was tested for a range of different temperature and time combinations – the two most important parameters for optimizing methane production from biomass (Ballesteros et al., 2002). For every pretreatment, detailed chemical analyses of the substrate were carried out for a better understanding of the effect of the pretreatment severity on the degradation process.

2. Methods

2.1. Raw material and steam explosion pretreatment

The hay used in the experiments was harvested in Purgstall an der Erlauf (Lower Austria) in June 2010. The biomass was dried on the field and the samples were stored in a sheltered area for nine months until the start of the tests. The water content of the untreated hay determined by Karl Fischer (KF) titrator was 12.8% fresh matter (FM).

The harvested biomass was pretreated with a steam explosion unit at the University of Life Sciences (UMB) in Ås (Norway) (see Horn et al., 2011b). For this process, 300 g of hay was filled into the 20 L pre-heated reactor and the steam explosion pretreatments were performed at temperatures ranging from 160 °C to 220 °C, using intervals of 15 °C. Each temperature was maintained for 5, 10 or 15 min. The steam-exploded material was vacuum stored at 4 °C for one to two weeks until the biogas trials and laboratory analysis commenced.

2.2. Chemical analysis

The composition of native and steam exploded hay was determined by analyzing the following parameters: oven dry weight (ODW), water content, raw ash (XA), volatile solids (VS), crude protein (XP), cellulose, hemicellulose and acid detergent lignin (ADL). The ODW was analyzed by drying the biomass in an oven at 105 °C until constant weight was reached. The water content was determined with the KF titrator Mettler Toledo V20 (Columbus, Ohio, USA) using Hydranal Composite 5 and Hydranal Methanol dry from Sigma Aldrich (St. Louis, Missouri, USA). The XA was analyzed by determining the residue left after dry oxidation of the oven dried material in a muffle furnace at 550 °C (Sluiter et al., 2004). The volatile solids (VS) were calculated by subtracting the raw ash content from the total solids (Naumann and Bassler, 1993). Nitrogen composition was determined by combustion using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA) and the total crude protein was calculated by multiplying the amount of nitrogen by the factor 6.25.

Cellulose and hemicellulose were determined by using two different standard procedures, the Van Soest method (Van Soest and Wine, 1967) and the sulfuric acid hydrolysis procedure provided by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2011). This second method is able to provide additional information about the composition of the different hemicelluloses, which is not included in the Van Soest procedures. The ADL content was determined by using the Van Soest method.

According to Van Soest, samples were previously dried and grinded to pass through a 1 mm sieve. For determining the content of neutral detergent fiber (NDF), a neutral detergent solution, decahydronaphthalene and sodium sulfite was added to the sample material and boiled for 60 min. Afterwards, the remains were filtered using sintered glass crucibles and the filtrate was washed with deionized water and acetone. Filters with their respective filtrates were dried at 105 °C to constant weight. NDF measures most of the structural components in plant cells, including cellulose, hemicellulose and lignin. The acid detergent fiber (ADF) was determined by adding a mixture of an acid detergent solution and 0.5 M sulfuric acid to the samples, followed by decahydronaphthalene. The samples were boiled for 60 min, filtered, washed with deionized water and acetone and dried at 105 °C to constant weight. ADF is mainly composed of cellulose and lignin. Therefore, hemicellulose was calculated as the difference between NDF and ADF. The acid detergent lignin (ADL) was determined from the filtrates remaining in the glass filters after ADF determination. For this, 72% sulfuric acid was added to the remains and they were periodically stirred for 3 h and washed with deionized water until a pH of 7 was reached. Filters with their respective filtrates were dried at 105 °C to constant weight and placed afterwards in a muffle furnace at 500 °C for 3 h. Cellulose was calculated by determining the difference between ADF and ADL.

In the acid hydrolysis method, the structural carbohydrates analyses were carried out for native and pretreated samples in triplicate, following the Laboratory Analytical Procedure presented in the technical Report of NREL no. TP-510-42618 (Sluiter et al., 2011). The dried biomass was milled until the entire sample passes through the 1 mm screen. First, 150 mg ± 5 mg of the samples were weighted into pressure tubes and then incubated at 30 °C for 60 min after the addition of 1.5 ml 72% sulfuric acid. In a second stage, the samples were incubated for one hour at 121 °C in an autoclave after a dilution of the sulfuric acid to 4% using 42 ml of deionized water. In addition to the samples, a set of sugar recovery standards (SRS) was prepared, which are taken through the remaining hydrolysis with 4% sulfuric acid in order to correct losses due to destruction of sugars during the dilute acid hydrolysis. SRS included D-(+) glucose, D-(+)xylose, D-(+)galactose, -L(+)arabinose. The structural carbohydrates were analyzed in the hydrolysis liquor. After the dilute acid step was filtered with glass filters, the remaining insoluble residue was washed and dried at 105 °C overnight and weighed. Glucose concentrations correspond with the cellulose content in the hay sample. Xylose, arabinose and galactose are the major components of hemicelluloses.

The content of soluble sugars (glucose, xylose, galactose, arabinose) as well as inhibitors (furfural and HMF) were analyzed by isocratic HPLC run on a Dionex UltiMate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) set up with a 7.8 × 100 mm Rezex RFQ-Fast Fruit H+ column (Phenomonex) heated to 82 °C. The mobile phase consisted of 5 mM sulfuric acid and the flow rate used was 1.0 ml/min. The HPLC samples were prepared by diluting samples from the reactions 5-fold with the mobile phase followed by centrifugation and filtration (0.2 μm Sarstedt Filtropur S). Eluted glucose, xylose, arabinose and galactose were monitored by recording refractive index. Furfural and HMF concentrations were monitored using an UV detector at 280 nm. Analytes were identified and quantified by running standards. Chromatograms were recorded, integrated and analyzed using the Chromeleon 6.8 chromatography software (Dionex).

The Microanalytical Laboratory of the University of Vienna performed the elemental analysis of the untreated hay sample. For the analysis, an Element-Analyzer of Perkin Elmer (EA 1108 CHNS-O, Carlo Erba) was used, according to standard procedures (Theiner, 2008).

2.3. Enzymatic hydrolysis (EH)

Enzymatic hydrolysis was performed on both untreated and steam exploded hay samples using Cellic CTec2, consisting on a blend of cellulases, β-glucosidases and hemicellulases (from Novozymes, Bagsvaerd, Denmark), dosed at 20 FPU g^{-1} DM of substrate. Hydrolysis was carried out in triplicate using 30 ml reaction volumes in 50 ml screw-capped centrifuge tubes, which were preheated at 50 °C before the enzymes were added. The tubes were horizontally shaken at 130 rpm and maintained at 50 °C. The pH in the hydrolysis reactions was adjusted by adding succinate buffer, pH 5.0, to a final concentration of 100 mM. The substrate concentration in the tubes was 50 g VS l^{-1} and reactions were started by adding 200 μl of the enzyme preparation.

2.4. Specific methane yield according to VDI 4630

Anaerobic digestion batch trials were carried out in triplicate in accordance with VDI 4630 (VDI, 2006), employing eudiometer batch digesters of 0.25 l capacity. The substrates and the inoculum were weighed out in a ratio of 1:3 (based on volatile solids content). The inoculum utilized was taken from a biogas plant in Utzenaich, Upper Austria (detailed information about the biogas plant is published in (Bauer et al., 2009b). The digesters, incubated at 37.5 °C, were continuously stirred and the biogas yields were monitored on a daily basis during the whole digestion process. Biogas and methane production were measured in norm liters (273 K and 1013 mbar) per kg of volatile solids (l_N kg^{-1} VS). The portable gas analyzer Dräger X-AM 7000 was used to determine the biogas composition (CH_4 and CO_2). The results from the elemental analyses of untreated hay (Section 3.3) can be used to calculate the theoretical biogas and methane yields as well as the concentrations of the trace gases ammonia and hydrogen sulfide (Boyle, 1976). Hence, the theoretical potential can be calculated and fixed as the maximum achievable yields by the native and pretreated samples. Measured and potential yields can be compared in order to estimate the remaining capacity for process improvement.

2.5. Calculations and statistical analysis

2.5.1. Mass loss

During the steam explosion pretreatment some of the volatile solids of the biomass are lost. The amount of loss can be estimated through the ash content of both steam exploded and untreated biomass. This mass has been calculated in relation to the total mass as stated in the formula (1).

$$[\text{Mass loss}/M\ \text{total}] = \frac{XA_{SE} - XA_{untreated}}{XA_{SE}} \quad (1)$$

Mass loss is the loss of volatile solids, M total the total mass of the untreated biomass, XA_{SE} the ash content of the steam exploded biomass, $XA_{untreated}$ is the ash content of the untreated biomass.

2.6. Statistical analysis

Data provided in tables and figures present means and standard deviations of performed experiments. Statistical analysis was carried out using SPSS Version 18. Data was analyzed by one-way ANOVA, followed by Dunet-T3 test for post hoc comparison and *t*-test for paired samples. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Chemical composition

The chemical composition of untreated and pretreated samples is shown in Table 1. The native biomass had an ODW content (% FM) of 87.1%. This value decreased strongly in all treated samples since the steam used in the pretreatment added water to the samples. The ODW content of the pretreated samples ranged between 22.8% (220 °C for 15 min) and 40.6% (175 °C for 5 min). In general terms, the ODW content appeared to be lower in samples treated for longer durations, since the biomass was exposed to the steam for a longer time. Moreover, higher temperatures also resulted in lower ODW values. The DM (% FM) determined by KF titration followed a similar trend to that formed by the ODW content. While the native biomass had a DM (KF) content of 87.2%, the values for the pretreated samples ranged from 26.3% (220 °C for 15 min) to 40.3% (175 °C for 5 min). When comparing the values obtained by the two methods for pretreated samples, lower values were obtained by the oven drying than the KF titrator. These results are in accordance with those obtained by Agger et al. (2013). The untreated hay sample had a VS content (% ODW) of 94.1%. This value decreased in pretreated samples, ranging from 94.1% to 90.6%, the latter corresponding to the sample with the second strongest pretreatment (220 °C for 10 min). Noteworthy is also a clear drop in the pH produced by the pretreatment, decreasing from 5.75 in the native sample up to 3.65 (205 °C for 5 min). This phenomenon is probably caused by acetate release from the xylan.

The calculated mass losses are shown in Table 1. The highest mass loss was 39.2% ODW, which corresponded to the most intense pretreatment (220 °C and 15 min). Therefore, an increment of first the temperatures and secondly the pretreatment time lead to a significant decrease in biogas yield caused by the loss of biomass. No exhaust gas, which contains different volatile components, was collected during the pretreatment step. However, it is known that the water dissolved in the process biomass mainly contains acetic acid, levulinic acid, formic acid, furfural and HMF (Parawira and Tekere, 2011) and microorganisms can convert these components into biogas. According to Boyle (1976), the theoretical specific methane yields of the compounds cited above are 373, 531, 122, 583 and 533 $l_N\,kg^{-1}$ VS, respectively. Therefore, in a full-scale biogas plant all the steam has to be condensed using adapted technologies.

The original content of protein raised progressively as the intensity of treatment increased, ranging from 7.5% up to 10.6%. Table 1 also shows the results of ADL, cellulose and hemicellulose concentrations, based on the Van Soest method. The ADL content was strongly affected by the steam-explosion pretreatment. Untreated hay had an ADL concentration of 5.8% ODW, rising to 24% ODW under the pretreatment (220 °C for 15 min), which represents an increase of up to four-fold the original value. The cellulose content in the untreated hay sample was 34.9% ODW and its content after steam-explosion pretreatment ranged from 26.7 to 36.9% ODW. Its content did not follow any trend according to the pretreatment severity. Moreover, steam explosion led to a reduction in the content of hemicellulose as the pretreatment intensity increased, since it starts to solubilize at a temperature of 150 °C (Garrote et al., 1999). The untreated hay sample had a hemicellulose (H-CEL) content of 18.9% ODW. The pretreatment at 220 °C for 15 min reduced this content by about 99% compared to the untreated sample. It is also apparent that not only the temperature, but also the pretreatment duration plays an important role. At pretreatment durations of 5 or 10 min, the content of hemicellulose for each temperature level was markedly higher than at pretreatment durations of 15 min.

Some researchers have reported ADL increases and hemicellulose reductions for different steam-exploded biomasses (Ballesteros et al., 2004; Vivekanand et al., 2013). However, in the present study, variations of the cited components are exceptionally higher. These changes increase as a function of pretreatment intensity and are caused by different reactions occurring during this process. The ADL increase could be related to the degradation of hemicellulose sugars since their monosaccharides, under the acid conditions of steam explosion, lead to the formation of different degradation products (Nelson et al., 1988), which may be transformed to acidic insoluble pseudo-lignin (Sannigrahi et al., 2011) and then measured in the Van Soest analysis as ADL. Ballesteros et al. (2004) reported slight increases in the acid insoluble lignin content of herbaceous biomass caused by condensation and repolymerization reactions.

Table 1
Chemical composition of untreated and steam exploded hay using the Van Soest method.

Sample	ODW	Water	DM (KF)	XA	VS	Mass loss	pH	XP	CEL	H-CEL	ADL	Organic rest[a]
	[% FM]			[% ODW]								
Untreated	87.1	12.8	87.2	5.9	94.1	–	5.75	7.5	34.9	18.9	5.8	27.0
160 °C, 5 min	25.4	72.7	27.3	6.3	93.7	6.3	4.91	7.5	34.8	22.7	13.5	15.2
160 °C, 10 min	25.5	73.9	26.1	5.9	94.1	<5.0	4.60	8.1	36.9	22.3	12.9	13.9
160 °C, 15 min	34.5	67.3	32.7	7.4	92.6	20.3	4.34	7.5	28.9	15.6	15.6	25.0
175 °C, 5 min	40.6	59.7	40.3	6.5	93.5	9.2	4.32	7.5	31.9	15.6	13.5	25.0
175 °C, 10 min	35.1	64.9	35.1	7.1	92.9	16.9	4.24	8.1	31.6	8.6	15.1	29.5
175 °C, 15 min	27.4	71.5	28.5	7.7	92.3	23.4	3.91	8.8	32.0	2.2	16.9	32.4
190 °C, 5 min	32.6	65.7	34.3	7.5	92.5	21.3	3.95	8.1	30.3	2.1	15.4	36.6
190 °C, 10 min	30.8	66.4	33.6	7.8	92.2	19.2	3.72	9.4	33.5	0.1	14.5	34.7
190 °C, 15 min	26.4	69.8	30.2	8.2	91.8	25.3	3.70	10.6	30.2	0.1	18.2	32.7
205 °C, 5 min	31.7	64.4	35.6	8.2	91.8	28.0	3.65	9.4	34.4	0.1	14.4	33.5
205 °C, 10 min	30.4	67.4	32.6	9.1	90.9	35.2	3.76	10.0	32.4	0.2	19.3	29.0
205 °C, 15 min	28.5	70.5	29.5	9.0	91.0	34.4	3.71	10.0	30.4	0.1	20.5	30.0
220 °C, 5 min	30.3	68.3	31.7	9.3	90.7	33.7	3.76	10.6	26.7	0.1	21.9	31.4
220 °C, 10 min	24.1	73.2	26.8	9.4	90.6	31.4	3.86	10.6	30.4	2.9	23.2	23.5
220 °C, 15 min	22.8	73.7	26.3	8.6	91.4	39.2	3.78	10.6	32.6	0.1	24.0	24.1

ODW: oven dry weight; FM: fresh matter (mass of material in its original state with its natural water content); DM (KF): dry matter (Karl Fischer); VS: volatile solids; XP: crude protein; CEL: cellulose; H-CEL: hemicellulose; ADL: Acid detergent lignin.

[a] Organic rest was calculated by subtracting XA, XP, CEL, H-CEL and ADL values from 100%, mainly composed by lipids, organic acids and different carbohydrates such as starch, soluble sugars (e.g. monosaccharides) and pectin.

Table 2
Results of the carbohydrate analysis of untreated and steam exploded hay using the acid hydrolysis method.

Sample	Glucan		Xylan		Arabinan		Galactan	
	% ODW	SD	% ODW	SD	% ODW	SD	% ODW	SD
Untreated	36.9	4.50	17.3	0.48	3.9	0.13	1.8	0.09
160 °C, 5 min	35.1	4.07	20.1	1.43	4.3	0.27	1.8	0.08
160 °C, 10 min	29.7	1.42	17.8	0.81	3.6	0.18	1.7	0.07
160 °C, 15 min	32.7	1.65	18.3	0.68	2.9	0.08	1.7	0.07
175 °C, 5 min	33.0	0.28	17.5	0.25	2.9	0.06	1.5	0.06
175 °C, 10 min	32.9	0.07	15.9	1.26	2.2	0.21	1.4	0.13
175 °C, 15 min	31.9	–	11.6	2.09	1.1	0.19	1.1	0.18
190 °C, 5 min	31.7	5.46	14.0	2.54	1.4	0.33	1.5	0.09
190 °C, 10 min	35.9	0.75	9.6	0.26	0.6	0.00	1.0	0.07
190 °C, 15 min	40.3	0.17	6.6	0.18	0.4	0.00	0.7	0.00
205 °C, 5 min	37.0	0.26	7.1	0.11	0.5	0.00	0.8	0.01
205 °C, 10 min	33.0	0.30	2.2	0.06	–	–	0.3	0.07
205 °C, 15 min	37.2	1.14	1.9	0.08	0.1	0.00	0.2	0.00
220 °C, 5 min	33.4	0.67	1.5	0.02	0.1	0.00	0.2	0.00
220 °C, 10 min	34.8	1.01	0.6	0.00	–	–	–	–
220 °C, 15 min	32.1	1.28	0.8	0.07	–	–	–	–

As showed in Table 1, there is a relation between the increase in the percentage of ADL and the mass loss. This relation was expected as no loss of lignin is assumed in the pretreatment and the loss of volatile compounds reduces the quantity of remaining sample, thereby increasing its percentage in ADL. If the increase in ADL content were only due to mass loss, the 39% mass loss reported in the pretreatment at 220 °C for 15 min would have increased the ADL content from 5.8% to 9.5%. However, the observed ADL content is 24.0%, clearly showing the contribution of pseudo-lignin formation to the total amount of analyzed ADL. Similar observations have been done for steam exploded birch (Vivekanand et al., 2013).

The combined effects of temperature and time on cellulose and hemicellulose concentrations were also determined by the sulfuric acid hydrolysis procedure according to NREL (Sluiter et al., 2011) (Table 2). The sugar composition data for the pretreated samples showed similar trends when compared to the results obtained with the Van Soest method. No correlation was observed between the severity of the pretreatment and the glucan content, which ranged between 29.7% and 40.3% ODW. Hemicellulose sugars were extensively solubilized during steam explosion; xylose content decreased nearly 95% compared to the raw material, arabinose and galactose decreased from 3.9% and 1.8% respectively to non-detectable concentrations. As stated in the former method, the remaining compounds constituting the biomass are ash, protein, lignin, lipids, organic acids and other carbohydrates.

Comparing the results obtained by the acid hydrolysis and the Van Soest method, no differences could be detected regarding the cellulose composition. However, some differences were observed regarding the hemicellulose contents; when increasing the first the temperature and secondly the time of the pretreatment, the decrease in the hemicellulose content is more pronounced under the Van Soest method. This lower content is probably caused by a higher hemicellulose removed during the washing caused by the acid detergent solution and the neutral detergent solution used. Therefore, it is expected that a small part of hemicellulose is quantified as the organic rest.

Furfural and hydroxymethylfurfural (HMF) are furan compounds generated from pentoses and hexoses, respectively. Their concentration was determined from the 50 g VS l^{-1} hydrolysates after 4, 24 and 48 h hydrolysis (data not shown). Analyses revealed that there were no significant differences in the concentration of inhibitors after 4 and 24 h of hydrolysis. However, significant decreases in their concentration were detected after 48 h of hydrolysis. Therefore, 4 h of hydrolysis was chosen for the graphical representation of the concentration of HMF and furfural in the pretreated hay (see Fig. 1). The HMF content increased as a function of the pretreatment severity, starting at 211 mg kg^{-1} ODW (160 °C for 5 min) and raising up to 2884 mg kg^{-1} ODW (205 °C for 10 min). A temperature increase beyond this intensity reversed the trend and resulted in a reduction of the HMF content to 926 mg kg^{-1} ODW (220 °C for 10 min). A similar progression can be seen for furfural, which started to be detected under the pretreatment at 160 °C for 10 min, with a concentration of 23.3 mg kg^{-1} ODW, peaked at 2995 mg kg^{-1} ODW (190 °C for 15 min) and decreased up to 1348 mg kg^{-1} ODW (220 °C for 10 min). This decrease in the contents of HMF and furfural under severe steam explosion conditions can be explained by their volatility, degradation to other substances and formation of pseudo-lignin (Bösch et al., 2010). When compared to other test results from steam exploded *Salix* (Horn et al., 2011a) or wheat straw (Horn et al., 2011b), the HMF and furfural contents reached very high concentrations of inhibitors at relatively low temperatures. This is probably caused because hay is a premature agricultural product containing easily digestible components, which furthers the enzymatic susceptibility of their cellulose and hemicellulose components (Chen et al., 2007), leading to the formation of inhibitors.

3.2. Enzymatic hydrolysis

The hay samples were enzymatically hydrolyzed in order to evaluate the effect of the different thermal pretreatments on the glucose and xylose release. Fig. 2 shows the glucose yields after 4, 24 and 48 h of enzymatic hydrolysis in a solution with 50 g VS l^{-1}. The results indicate that the release of glucose is strongly affected by the severity of the pretreatment. After 4 h of enzymatic hydrolysis, the glucose yields reached their maximum value at 7 g l^{-1} for the samples pretreated at 175 °C for 15 min). The 24-h hydrolysis increased the glucose yields up to 16.6 g l^{-1} (pretreatment at 205 °C for 15 min), which corresponds to a yield of 332 g kg^{-1} VS. A comparison of the glucose yield with the glucan content of the untreated sample (368.7 g kg^{-1} VS) shows that 90% of the maximum potential is converted. The glucose content after 24 h is not markedly different to the content obtained after 48 h, except for the pretreatment at 220 °C for 15 min.

Fig. 3 displays the xylose content during hydrolysis of the pretreated samples. The results differ greatly from those obtained for glucose: after 4 h of hydrolysis, the released xylose reached a maximum of 4.4 g l^{-1} for the samples pretreated at 175 °C for 15 min. As the pretreatment severity increases (pretreatment at 220 °C for 10 min), the xylose yields decrease down to 0.2 g l^{-1}. After the 24-h hydrolysis, the released xylose reached a maximum

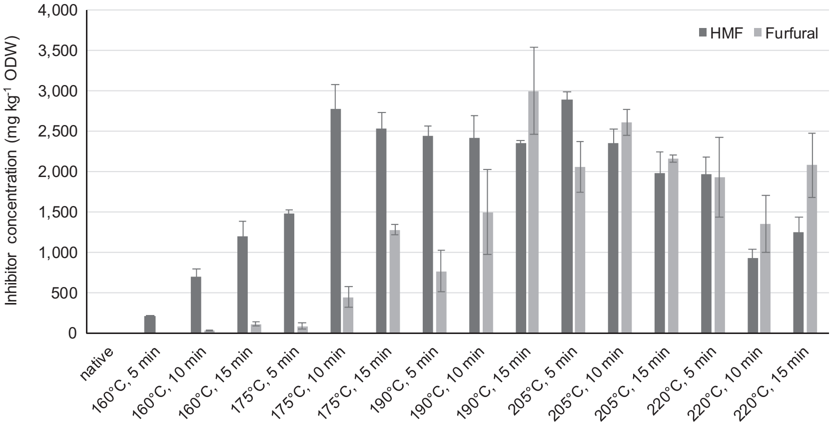

Fig. 1. Concentration of HMF and furfural after 4 h hydrolysis in the untreated and pretreated hay.

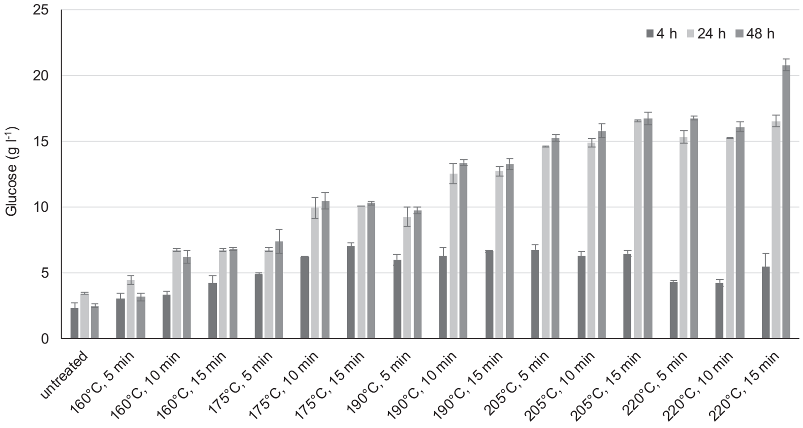

Fig. 2. Glucose released during enzymatic hydrolysis of pretreated hay after 0, 4 and 24 h.

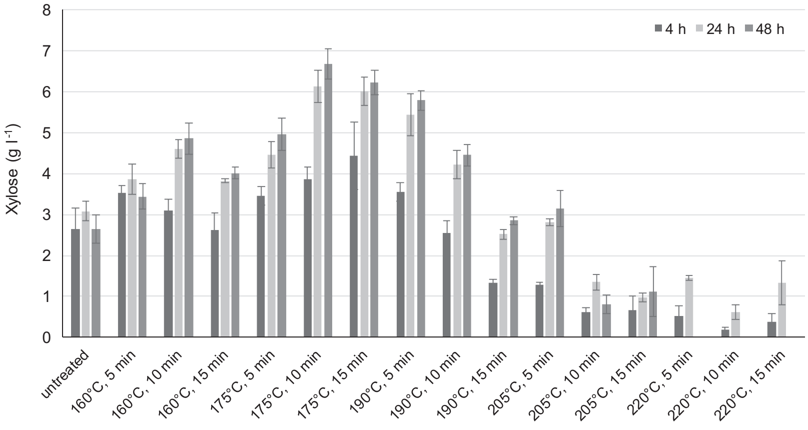

Fig. 3. Xylose released during enzymatic hydrolysis of pretreated hay after 0, 4 and 24 h.

of 6.1 g l^{-1} in samples pretreated at 175 °C for 10 min and decreased considerably down to 0.6 g, corresponding again to the pretreatment performed at 220 °C for 10 min. The released xylose after 48 h followed a similar pattern than that reported after 24 h, with the only difference of slightly higher maximum yields after 48 h.

Similar trends regarding the effect of the pretreatment severity on enzymatically released glucose and xylose have been reported for different biomasses (Horn et al., 2011a,b; Vivekanand et al., 2013). However, in the present study the maximum xylose yields are reached at lower temperatures. Since high concentrations of HMF and furfural were also detected in the lower temperature range, this is in accordance with the theory presented above stating that hay contains high quantities of easily degradable polysaccharides, resulting in the formation of inhibitors.

3.3. Specific biogas and methane yields

Fig. 4 shows measured specific biogas and methane yields for untreated and steam exploded hay. The specific biogas yield

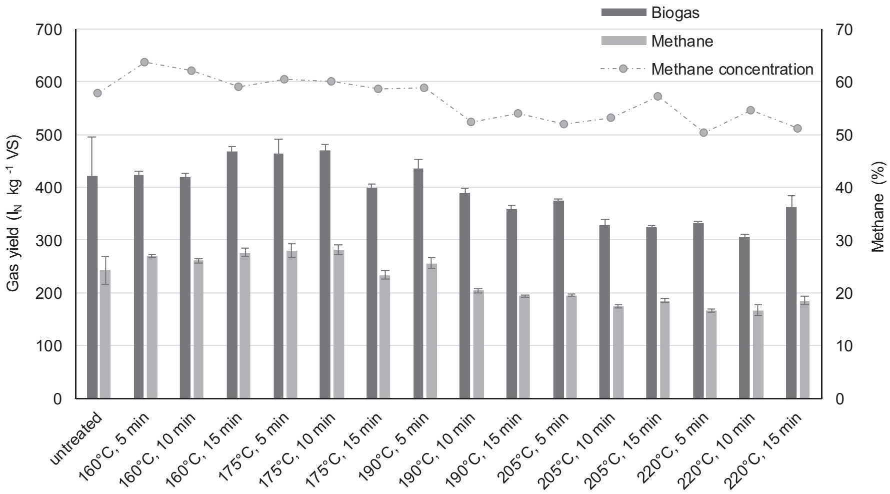

Fig. 4. Specific biogas and methane yields of untreated and steam exploded hay.

from untreated biomass was 420 l_N kg^{-1} VS. The highest yield was obtained from the material pretreated at 175 °C for 10 min, which increased the production up to 469 l_N kg^{-1} VS. Higher temperatures resulted in substantially lower specific biogas yields, ranging from 436 to 306 l_N kg^{-1} VS. The specific methane yield of untreated hay resulted in 243 l_N kg^{-1} VS. When the biomass was pretreated at 175 °C for 10 min, the methane yield increased 16%, up to 281 l_N kg^{-1} VS. The figure shows that an additional increase in the temperature markedly decreased the methane yield. The reason for this reduction under severe pretreatment conditions can mainly be attributed to the formation of substances inhibiting the microorganisms responsible for the anaerobic digestion process (e.g. phenolic compounds or furan derivatives) as well as to the loss of sugars due to pseudo-lignin formation. These results are in keeping with previous studies, which reported similar trends in the biogas yields after the steam explosion of other biomass such as wheat straw, sugarcane straw, *Salix*, miscanthus and rape straw. (Bauer et al., 2009a; De Paoli et al., 2011; Horn et al., 2011a; Menardo et al., 2012; Vivekanand et al., 2012). Nevertheless, the yield improvements recorded in the present study are lower than those obtained in the cited studies. Fig. 4 also shows the share of methane in the biogas, which varied from 50% to 64%, with the softer pretreatments being those that obtained the highest methane percentages.

The elemental analysis of the untreated hay showed that it is composed (% of volatile solids) of 46.15% carbon, 6.45% hydrogen, 1.55% nitrogen, 0.09% sulfur and 45.76% oxygen. The calculated methane potential of the untreated biomass according to Boyle (Boyle, 1976) was 440 l_N kg^{-1} VS. This is a theoretical value, which indicates the maximum methane potential that can be achieved with the native biomass, assuming that the organic matter is degraded and transformed completely into biogas. Hence, the highest methane yield obtained (281 l_N kg^{-1} VS) shows that 64% of the theoretical potential is achieved, which indicates that there is still scope for improvement in future investigations.

4. Conclusion

Results show a slight increase in the methane yield of hay after pretreatment with steam explosion. There is a remarkable decrease in the hemicellulose content and a raise in the ADL content, increasing the harshness of pretreatment. The degradation of the hemicellulose leads to the production of aromatic compounds. Compared to other biomasses, hay needs milder pretreatment conditions to achieve a high degradation, requiring less energy. The utilization of hay as a feedstock in biogas-based biorefinery concepts could help making the maintenance of grasslands in Alpine regions profitable. Further attempts to optimize the pretreatment conditions should be undertaken.

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III

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Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion

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HIGHLIGHTS

- Steam explosion treatment of straw from 140 °C to 178 °C for 30, 60, 120 min.
- Treatment caused a hydrolysis of hemicellulose and the formation of pseudo lignin.
- Investigation of the degradation process of biomass during anaerobic digestion.
- Increasing severity of pretreatment resulted in faster degradation of the biomass.
- Content of pseudo-lignin decreased during the anaerobic degradation experiment.

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ABSTRACT

Wheat straw can serve as a low-cost substrate for energy production without competing with food or feed production. This study investigated the effect of steam explosion pretreatment on the biological methane potential and the degradation kinetics of wheat straw during anaerobic digestion. It was observed that the biological methane potential of the non steam exploded, ground wheat straw (276 l_N kg VS^{-1}) did not significantly differ from the best steam explosion treated sample (286 l_N kg VS^{-1}) which was achieved at a pretreatment temperature of 140 °C and a retention time of 60 min. Nevertheless degradation speed was improved by the pretreatment. Furthermore it was observed that compounds resulting from chemical reactions during the pretreatment and classified as pseudo-lignin were also degraded during the anaerobic batch experiments. Based on the rumen simulation technique, a model was developed to characterise the degradation process.

1. Introduction

Producing energy from fossil fuels results in carbon dioxide emissions, which are largely responsible for the greenhouse gas effect. This, combined with finite and dwindling stocks of fossil fuels, has led to an increased interest in alternative energy sources. Policies have been enacted to increase the production of renewable energy. The European directive 2009/28/EC, states that by 2020 a share of 20% of the EU's overall energy consumption must come from renewable sources (Parliament, 2009). Alternative energy from solar radiation, wind and water faces technical challenges, such as uncertain availability and insufficient storage options. Energy production from biomass can overcome these problems as energy can be produced when it is actually needed. Biodiesel, bioethanol and biogas production have experienced a strong increase over the past several years. State-of-the-art technologies depend on sugar, starch and oil for conversion into energy carriers and therefore rely heavily on the availability of traditional energy crops such as maize, wheat or rapeseed (Borugadda and Goud, 2012). The use of energy crops can lead to a competitive situation between food (and respectively feed) and energy production (German National Academy of Sciences Leopoldina, 2012). Therefore, alternative biomass source are needed in order to ensure that

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renewable energy is being produced in an ecologically and socially sustainable fashion. The utilisation of lignocellulosic material is very promising, as it is the most abundant source of biomass worldwide and, depending on regional circumstances, is not in direct competition with food and feed production (Lin and Tanaka, 2006). The main components of lignocellulosic materials are cellulose, hemicellulose and lignin, which together form the lignocellulose complex. Depending on the type of plant and on the vegetation period in which it the biomass is harvested, the bond between lignin and the other components can be very resistant to digestion (Buruiana et al., 2013). In order to utilise this material for the production of energy carriers like ethanol or biogas, the material must be pretreated in order to make the cellulose and the hemicellulose available for the biological conversion to energy carriers. Due to its chemical structure, native lignin cannot be used for biological conversion into an energy carrier (Frei, 2013).

Depending on the type of biomass and on the desired biological conversion pathway, different strategies for lignocellulose pretreatment can be pursued. If lignocellulose is used as a feedstock for biogas production, reducing the particle size is a popular technique as it is easily implemented in biogas plants and is comparatively inexpensive. The disadvantage of this approach is that the process has a limited effect on feedstock properties but consumes large quantities of electrical energy in the process, leading to high operation costs (Kratky and Jirout, 2011; Taherzadeh and Karimi, 2008). The utilisation of thermophysical pretreatment technologies, such as steam explosion or liquid hot water pretreatment, is another promising strategy. Although these are more complicated to implement in a biogas plant, their significant effect on the lignocellulose feedstock promises high conversion rates in the biological degradation process (Hendriks and Zeeman, 2008). Steam explosion has proven to be a viable option for the pretreatment of several types of biomass (Bauer et al., 2014; Menardo et al., 2012). Pretreating biomass with steam explosion increase the specific biogas and methane yields significantly while also increasing the degradation speed of the biomass due to changes in the chemical composition. Chemical reactions take place during the pretreatment process due to high process temperatures (between 140 °C and 220 °C). Polysaccharide compounds such as hemicellulose are hydrolysed, leading to higher degradation speeds during the anaerobic process. These advantages allow the implementation of high performance fermenters with lower hydraulic retention times, reducing the energy required for fermenter homogenisation. Depending on the intensity of the pretreatment, inhibitors such as furfural and hydroxymethylfurfural and compounds classified as pseudolignin may also form (Bauer et al., 2014; Vivekanand et al., 2013).

The experimental setup for investigating biomass degradation in an anaerobic process is complex and cost intensive. Reliable models can provide an attractive alternative to the experimental approach, allowing the degradation process to be characterised and fundamental data to be calculated. The results of such models can be used to optimise the digestion process in existing biogas plants. Current models use the experimental determination of the methane yield at different points during fermentation to deduce the degradation of the biomass. In the field of feedstuff analysis there are models, which directly investigate the degradation of biomass based on its composition and a maximum degradation rate.

This study investigated the effect of steam explosion pretreatment on the biogas and methane yields of wheat straw. As the main fraction of wheat straw is lignocellulose, special focus was paid to the degradation kinetics of the structural compounds cellulose, hemicellulose and lignin, which were examined using a modified animal feed analysis method. Detailed knowledge of the digestibility and degradation speed of single compounds can serve as the basis for further improvements to the steam explosion pretreatment technology. The degradation behaviour of compounds resulting from reactions of sugars released by the hydrolysis of hemicellulose during steam explosion pretreatment is a yet unexplored field. Another objective of this study was to adapt a feedstuff analysis model for biomass degradation in an anaerobic process.

2. Methods

2.1. Steam explosion pretreatment

The biomass used for the experiments was wheat straw cultivated in eastern Austria in 2013. The steam explosion pretreatment was carried out at a testing facility at a biogas plant in Parndorf, Austria. The wheat straw was cut to a size smaller than 5 cm and then mixed with water to obtain a dry matter content of 30%. After transferring the material into the reaction vessel, it was preheated in order to obtain a steam-saturated atmosphere. The temperature was then increased until the final pretreatment temperature was reached. Pretreatment temperatures of 140 °C, 160 °C and 178 °C and pretreatment times of 0.5, 1 and 2 h were chosen for the experiment. After the retention time had elapsed, the pressure was reduced abruptly to atmospheric pressure, causing a sudden vaporisation of the water inside the vessel. The pretreated material was then transferred into a flash tank and, once cooled to room temperature, collected. The material was subsequently vacuum packed and stored at 4 °C for later analysis.

The severity factor combines both pretreatment temperature and time into one parameter (Overend and Chornet, 1987), allowing the results of the analysis to be displayed and analysed in a consistent manner. The severity factor is particularly useful when comparing the effects of pretreatment (see Eq. (1))

$$\mathrm{SF} = \log[t * \exp(T - 100/14,75)] \tag{1}$$

SF – severity factor,
t – pretreatment time,
T – pretreatment temperature.

2.2. Chemical analysis

In order to characterise the biomass, all samples were analysed for dry matter (DM) and volatile solids (VS) content as well as for cellulose (CEL), hemicellulose (H-CEL) and lignin (ADL) content.

The dry matter and the volatile solids content was determined according to the standard methods DIN 12880 (2001) and DIN 12879 (2001). The biomass was dried at 105 °C until a constant weight was reached. The dried material was then dry oxidised in a muffle furnace at 550 °C. After oxidation, the residue remaining was tagged as raw ash. The difference between dry matter and ash content represents the volatile solid content of the biomass.

Cellulose, hemicellulose and lignin content were determined according to the method of van Soest and Wine (1967) and the modification made by Naumann and Bassler (1976). After treatment with a neutral detergent solution, the insoluble residue is known as the neutral detergent fibre (NDF) content. After treatment with an acid detergent solution, the insoluble residue is known as the acid detergent fibre (ADF). The dried residue from the ADF determination was treated with 72% sulphuric acid to determine the lignin content (ADL). The hemicellulose content was calculated as the difference between NDF and ADF. Cellulose content was calculated as the difference between ADF and ADL.

After ADL determination, the dried residue was incinerated at 500 °C in order to determine the content of acid insoluble ashes.

Table 1
Chemical composition of the non steam exploded, ground sample and with steam explosion pretreated samples.

	SF –	DM (% FM)	VS (% DM)	CEL	H-CEL	ADL	XP
Non-steam-exploded, ground	–	89.6 ± 0.0	95.3 ± 0.1	40.6 ± 1.1	28.3 ± 0.9	7.5 ± 0.3	2.7
140 °C, 30 min	2.7	24.0 ± 0.1	95.8 ± 0.0	41.4 ± 0.5	22.3 ± 0.5	10.6 ± 0.1	3.8
140 °C, 60 min	3.0	24.6 ± 0.0	95.5 ± 0.1	42.2 ± 0.5	22.0 ± 0.5	10.7 ± 0.1	3.9
140 °C, 120 min	3.3	26.4 ± 0.1	95.4 ± 0.1	41.6 ± 0.3	19.1 ± 0.9	11.2 ± 0.1	4.0
160 °C, 30 min	3.2	22.1 ± 0.1	96.2 ± 0.1	40.0 ± 0.3	17.0 ± 0.8	9.4 ± 0.1	3.4
160 °C, 60 min	3.5	21.1 ± 0.3	96.7 ± 0.9	41.0 ± 0.6	13.8 ± 0.9	10.1 ± 0.3	4.8
160 °C, 120 min	3.9	23.1 ± 0.2	96.5 ± 0.3	40.5 ± 0.5	3.9 ± 0.4	12.1 ± 0.2	4.0
178 °C, 30 min	3.8	16.1 ± 0.0	96.6 ± 0.1	39.8 ± 0.9	3.8 ± 0.8	11.1 ± 0.4	3.8
178 °C, 60 min	4.1	14.6 ± 0.0	96.1 ± 0.1	40.8 ± 0.3	bdl	12.2 ± 0.1	4.2
178 °C, 120 min	4.4	12.5 ± 0.2	96.7 ± 0.3	43.0 ± 0.5	bdl	15.9 ± 0.3	4.5

SF: severity factor; DM: dry matter; VS: volatile solids; CEL: cellulose; H-CEL: hemicellulose; ADL: acid detergent lignin; XP: crude protein; bdl: below detection limit.

All chemicals used for the analysis were obtained from Ankom Technology (USA).

2.3. Biological methane potential

The biological methane potential (BMP) was determined using an anaerobic batch setup according to the VDI 4630 standard method (Association of German Engineers, 2006). Digesters with a volume of 0.5 L were filled with the variants to be analysed and inoculum (volatile solids ratio 1:3). The separated liquid fraction of fermentation residues taken from an agricultural biogas plant in eastern Austria (operated at 42 °C and fed with pig manure, maize and catch crop silage) was used as inoculum. Prior to the analysis, the inoculum was stored in a temperature controlled room to ensure the degradation of the remaining organic fraction. The final dry matter and volatile solids concentration of the inoculum was 3.4% and 2.5% respectively. The digesters were placed in a water bath at 37.5 °C and were connected to eudiometer systems in order to determine the volume of the biogas. The amount of biogas produced was monitored on a daily basis and its composition analysed whenever the produced volume exceeded 120 ml. An X-am 7000 (Dräger, Germany) gas analyser was used to analyse the composition of the biogas. The amount of biogas and methane produced is given in norm litres per kilogram of volatile solids ($l_N\ kg^{-1}$ VS). The analysis was carried out in triplicate and a standard (microcrystalline cellulose) was used to check for normal activity of the inoculum.

2.4. Degradation kinetics

In order to investigate the degradation kinetics of structural compounds in the biomass, a modification to the Rumen Simulation Technique (RUSITEC) developed by Czerkawski and Breckenridge (1977) was used. This method has already been widely tested in the field of feed analysis, such as the investigation of feed additives effects on methane production (Machmüller et al., 1998), or of the efficacy of digesting various low-quality feed types (Jayasuriya et al., 1987). There have been a few preliminary studies using this method in the field of biogas production. Stopp et al. (2009) and Bayané and Guiot (2011) explored the possibility of adapting effective animal digestion strategies for biogas production.

Table 2
Structural composition of the samples after washout of the water soluble fraction.

	CEL (% DM)	H-CEL	ADL	XP
Non-steam-exploded, ground	41.6	29.1	8.5	2.6
140 °C, 30 min	47.3	26.7	11.2	2.4
140 °C, 60 min	46.1	25.8	11.7	2.8
140 °C, 120 min	47.1	23.5	12.1	2.8
160 °C, 30 min	49.0	23.4	10.7	1.9
160 °C, 60 min	50.9	19.9	11.3	2.0
160 °C, 120 min	57.8	7.2	13.6	2.3
178 °C, 30 min	60.6	3.2	13.6	2.4
178 °C, 60 min	60.3	0.8	15.7	2.7
178 °C, 120 min	60.4	bdl	17.4	2.7

DM: dry matter; CEL: cellulose; H-CEL: hemicellulose; ADL: acid detergent lignin; XP: crude protein; bdl: below detection limit.

In the original method, perforated nylon filter bags containing feed were put into a digester filled with a solution of rumen microorganisms. Similarly, in this study, ash and nitrogen free nylon filter bags (type F57 with a porosity of 25 µm, Ankom Technology, United States) were filled with sample material, sealed (Welding impulse sealer Type AIE 200) and placed in a 20 L digester filled with inoculum as described in Chapter 2.3. The inoculum to sample DM ratio was set to 2:1 by adding water to the inoculum. The digesters were placed in a water bath tempered to 37.5 °C. The digesters were equipped with a water lock to allow the produced biogas to exit the digester. The headspace of the digesters was flushed with nitrogen at the start of the experiment and after the removal of each sample. For each variant analysis, eight nylon bags were placed in one digester. Samples were withdrawn at 1, 2, 5, 10, 15, 20, 36 and 45 days after the start of the experiment, washed with deionised water until the runoff was clear and then dried at 50 °C.

2.5. Calculations and statistics

2.5.1. Biomass degradation model

As the digestion of biomass in the rumen is analogous to the anaerobic degradation process in biogas plants, it has been suggested that degradation models from feedstuff analysis can be applied to the biogas process. Ørskov and McDonald (1979) described one of the most commonly used models (Eq. (2)):

$$P = a + b(1 - \exp^{-c*t}) \tag{2}$$

P – degradation rate,
a – degradable and water soluble organic fraction,
b – degradable and non-water soluble organic fraction,
c – degradation rate at time t,
t – time of degradation.

The parameters needed for modelling the biomass degradation curve include the percentage of the degradable water-soluble fraction (a) and the fraction solid (b), as well as the maximum degradation rate (c) and the duration of the degradation process (t). The organic water-soluble fraction was determined by weighing the samples before rinsing them until the runoff was clear. After washing, the samples were dried at 105 °C until a constant weight

Table 3
Methane yields and final methane concentration of the produced biogas of the non-steam-exploded, ground sample and with steam explosion pretreated samples.

	SF Log(R_0)	BMP (l_N kg VS^{-1})	SD (%)	Methane concentration
Non-steam-exploded, ground	–	276$^{(b)}$	5	45
140 °C, 30 min	2.7	275$^{(b)}$	3	50
140 °C, 60 min	3.0	286$^{(b)}$	16	49
140 °C, 120 min	3.3	273$^{(b)}$	1	49
160 °C, 30 min	3.2	261$^{(ab)}$	3	47
160 °C, 60 min	3.5	264$^{(ab)}$	9	48
160 °C, 120 min	3.9	260$^{(ab)}$	5	47
178 °C, 30 min	3.8	270$^{(ab)}$	7	48
178 °C, 60 min	4.1	245$^{(a)}$	0	47
178 °C, 120 min	4.4	272$^{(b)}$	9	47

SF: severity factor; BMP: biological methane potential; SD: standard deviation.

was attained. The difference in mass pre and post washing was recorded as the water-soluble organic fraction. The degradable solid fraction was determined by summing up the components cellulose, hemicellulose and crude protein. Lignin is considered to be nondegradable and is therefore not incorporated into the model. The maximum degradation rate was determined experimentally over the course of the degradation experiment. For practical application of the model, it would either be necessary to carry out a simplified form of the degradation experiment or to have a database with simple and applicable values available. The intention of introducing the model in this study is to develop a simplified method for investigating the degradation of biomass in the biogas process.

2.5.2. Statistical analysis

The tables and figures found throughout this paper present mean values and standard deviations of the performed experiments. Statistical analysis was carried out using SPSS Version 18. The obtained data was analysed using Scheffé's Test with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Characterisation of the variants

The changes in chemical composition of the non steam exploded and the steam exploded samples are shown in Table 1. The dry matter content of the non steam exploded ground sample was 89.6 (% FM) and decreased with the intensity of the pretreatment to 12.5 (% FM). This can be attributed to the water added prior to the steam explosion pretreatment as well as to the steam that was used to maintain the pretreatment temperature, which partly condensed in the flash tank. The volatile solids share of the dry matter remained constant for all investigated samples.

In order to insure comparability between the biomass prior to anaerobic digestion with the samples withdrawn from the fermenters, it was necessary to calculate the relative share of the analysed components without the water-soluble fractions. Table 1 displays the biomass composition of non-steam-exploded, groundsamples, as well as the and steam-exploded samples. It can be seen that with increasing severity of the pretreatment the hemicellulose content declined while the lignin and protein contents increased. Garrote et al. (1999) showed that hemicellulose becomes soluble at 150 °C, therefore the reduction in hemicellulose content can be attributed to both pretreatment temperature and time.

The increase of the lignin fraction can be explained by the formation of cross-linked compounds, caused by reactions of released sugars from the hydrolysed hemicellulose fraction (Nelson et al., 1988). Vivekanand et al. (2013) designated these compounds as pseudo-lignin. As a dedicated analysis of these compounds was beyond the scope of this study, the increase in lignin was considered to be pseudo-lignin and will be addressed as such throughout the paper. The cellulose content differed slightly when comparing pretreatment intensities, however no clear tendency could be observed. In general, it was observed that the pretreatment temperature had a greater effect on the biomass composition than the duration of the pretreatment. This observation is in accordance with other studies investigating the effect of steam explosion on biomass composition (Bauer et al., 2014; Menardo et al., 2012; Vivekanand et al., 2013, 2012).

Table 2 shows the share of the structural components cellulose, hemicellulose, lignin and crude protein after the removal of the water-soluble fraction.

When removing the water-soluble substances from the biomass, a portion of the crude protein was removed. As the water-soluble fraction was removed during rinsing, the relative shares of cellulose, hemicellulose and lignin increased. Comparing the non-steam-exploded, ground sample with the steam explosion pretreated samples; it was observed that the cellulose content increased with the intensity of the pretreatment. This effect could not be observed for the samples, which included the water-soluble fraction. This has its reason in the method used for the analysis of the biomass. The reference base for the presentation of analysed compounds is the mass weighed for the analysis. For the original samples this means that water soluble compounds are included while for those washed prior to the analysis only the non-water soluble substance is left. This leads to an increase of all remaining compounds compared to the original samples. Compounds which have a higher share of the entire mass are influenced more by this effect than those with a lower share.

Table 4
Biological methane potential with standard deviation of the investigated samples after 5, 10, 20, 41 and 45 fermentation days. Level of significance applicable for same fermentation days (columns).

	FD_5 BMP (l_N kg VS^{-1})	SD_5	FD_{10}	SD_{10}	FD_{20}	SD_{20}	FD_{41}	SD_{41}	FD_{45}	SD_{45}
Non steam exploded, ground	46$^{(a)}$	7	98$^{(a)}$	8	216$^{(a)}$	7	268$^{(b)}$	5	276$^{(b)}$	5
140 °C, 30 min	104$^{(b)}$	1	168$^{(b)}$	0	240$^{(abc)}$	3	270$^{(b)}$	3	275$^{(b)}$	3
140 °C, 60 min	112$^{(bcd)}$	5	191$^{(bc)}$	13	262$^{(c)}$	14	291$^{(b)}$	16	296$^{(b)}$	16
140 °C, 120 min	107$^{(bc)}$	2	175$^{(bc)}$	2	244$^{(bc)}$	1	269$^{(b)}$	1	273$^{(b)}$	1
160 °C, 30 min	107$^{(bcd)}$	2	168$^{(b)}$	2	233$^{(abc)}$	4	257$^{(ab)}$	3	261$^{(ab)}$	3
160 °C, 60 min	114$^{(bcd)}$	1	175$^{(bc)}$	5	236$^{(abc)}$	7	260$^{(ab)}$	8	264$^{(ab)}$	9
160 °C, 120 min	117 $^{(cd)}$	3	185$^{(bc)}$	4	234$^{(abc)}$	5	255$^{(ab)}$	5	260$^{(ab)}$	5
178 °C, 30 min	119$^{(d)}$	1	194$^{(cd)}$	4	243$^{(bc)}$	8	265$^{(ab)}$	8	270$^{(ab)}$	7
178 °C, 60 min	107$^{(bc)}$	1	182$^{(bc)}$	1	221$^{(ab)}$	1	241$^{(a)}$	0	245$^{(a)}$	0
178 °C, 120 min	109$^{(bcd)}$	3	212$^{(d)}$	8	251$^{(c)}$	9	269$^{(b)}$	9	272$^{(b)}$	9

FD: fermentation day; SD: standard deviation; BMP: biological methane potential.

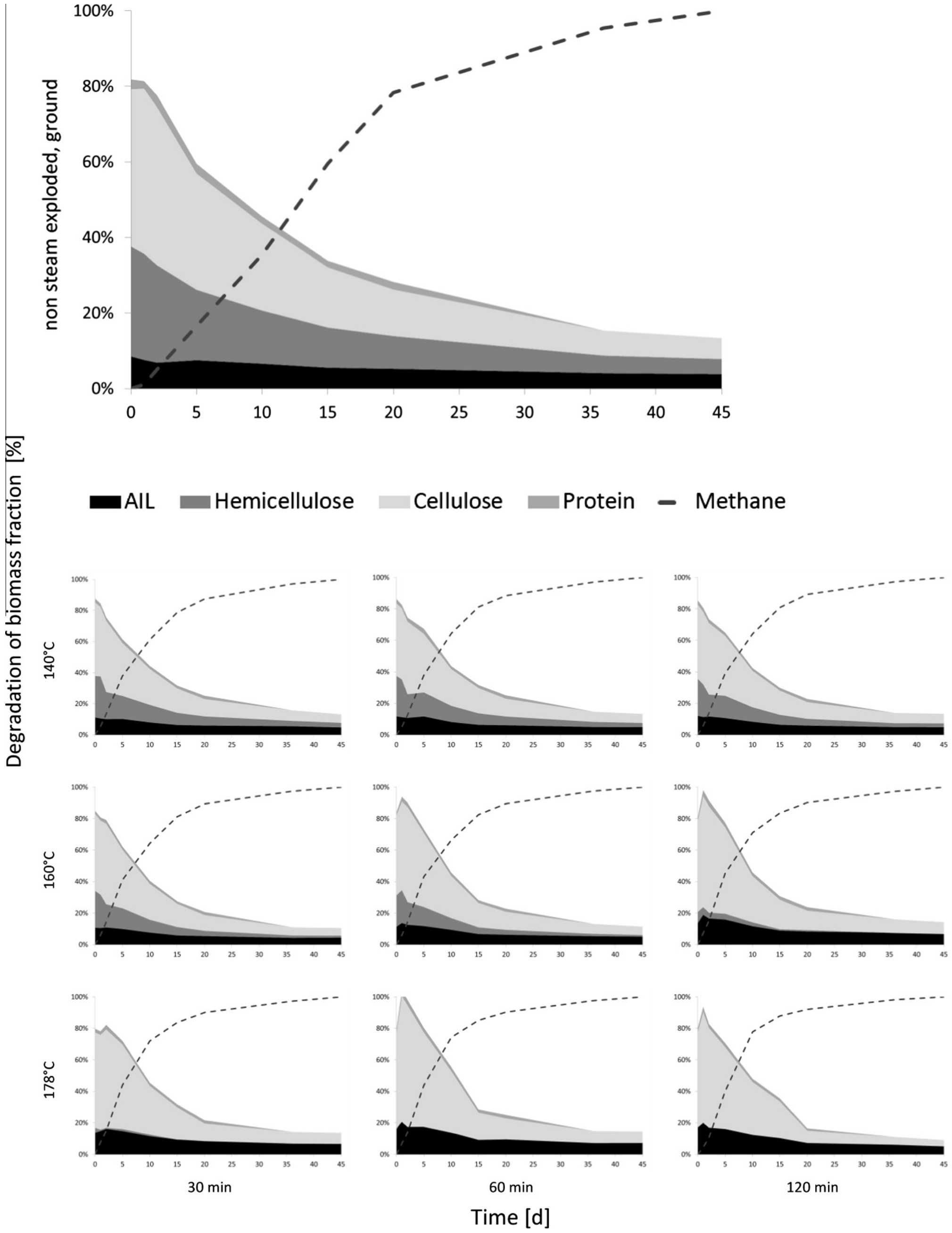

Fig. 1. Relative degradation of cellulose, hemicellulose, lignin and crude protein as well as relative methane formation of the analysed samples during the experiment.

3.2. Specific biogas and methane potential

The biological methane potential, determined according to VDI 4630, resulted in a specific methane yield of 276 l_N kg VS^{-1} for the non-steam-exploded, ground sample. Yields from the pretreated samples were similar or slightly lower. This is in contrast to other studies, which found significant increases in methane production using steam explosion pretreated wheat straw as compared to the non steam exploded, ground sample. Ferreira et al. (2014) found an increase of BMP of 27% after steam explosion at 200 °C for 5 min. Bauer et al. (2009) found a 20% increase of BMP after steam explosion at 180 °C and 15. This study's findings may differ for a variety of reasons, for example different experimental steam explosion setups with varying time until final pretreatment temperatures were reached in the reaction vessel were used. Different studies show that the pretreatment temperature in particular has a major effect on the methane yield of different types of biomass (Bauer et al., 2014; Risberg et al., 2013). For strongly lignified biomass like e.g. miscanthus a higher pretreatment temperature of 220 °C also leads to a higher methane yield of 374 l_N kg VS^{-1} (Menardo et al., 2012). This indicates that the pretreatment conditions provided by the experimental facility used in this study do not match the optimal conditions for maximising the methane yield of wheat straw.

Furthermore, for this experiment the non-steam-exploded wheat straw was ground to a particle size of <5 mm for the sake of homogenisation, which also has a positive effect on the digestibility of the biomass. The control sample was homogenised in order to make the results from non-steam-exploded sample more comparable to the steam-exploded sample. Another reason for the small difference between the untreated and the steam-exploded samples could also have been that the inoculum used

Table 5
Parameters used for model calculations and coefficient of determination.

	a (% DM)	*b*	*c* (h^{-1})	R^2
Non-steam-exploded, ground	1.8	71.6	0.0042	0.998
140 °C, 30 min	14.6	67.5	0.0041	0.992
140 °C, 60 min	14.4	68.1	0.0042	0.994
140 °C, 120 min	13.5	64.7	0.0049	0.994
160 °C, 30 min	22.8	60.4	0.0046	0.963
160 °C, 60 min	38.0	59.6	0.0027	0.971
160 °C, 120 min	41.7	48.4	0.0032	0.973
178 °C, 30 min	42.5	47.4	0.0032	0.915
178 °C, 60 min	52.2	44.7	0.0018	0.975
178 °C, 120 min	49.3	45.4	0.0026	0.995

a: water soluble fraction; *b*: non-water soluble degradable fraction; c: degradation rate; R^2: coefficient of determination of the model.

to the determine the BMP was more active. The biogas plant where the inoculum was withdrawn used high amounts of lignocellulose material. Therefore it can be assumed that the microbial consortia in the inoculum was well acclimatised to lignocellulose material leading to a improved degradation of the untreated wheat straw.

The highest BMP, $286\ l_N\ kg\ VS^{-1}$, was obtained with the sample pretreated at 140 °C for 60 min. As can be seen in Table 3, only a small difference in specific methane production was observed between the variants. The pretreatments at 160 °C and 178 °C resulted in a slightly lower methane yield (−11%) after a fermentation time of 45 days.

In contrast to the absolute yields, a significant difference could be seen in the methane formation rates. All steam explosion pretreated variants showed faster gas production rates in the first days than the non-steam-exploded, ground variant. With increasing intensity of the pretreatment, the rate of biogas and methane formation improved. This can be explained by the increase in area available to the degrading microorganisms and also by the partial hydrolysis of some components, particularly hemicellulose. This improvement was observed until the 20th day of fermentation. On fermentation days 41 and 45, methane yields of all variants reached a similar value (Table 4).

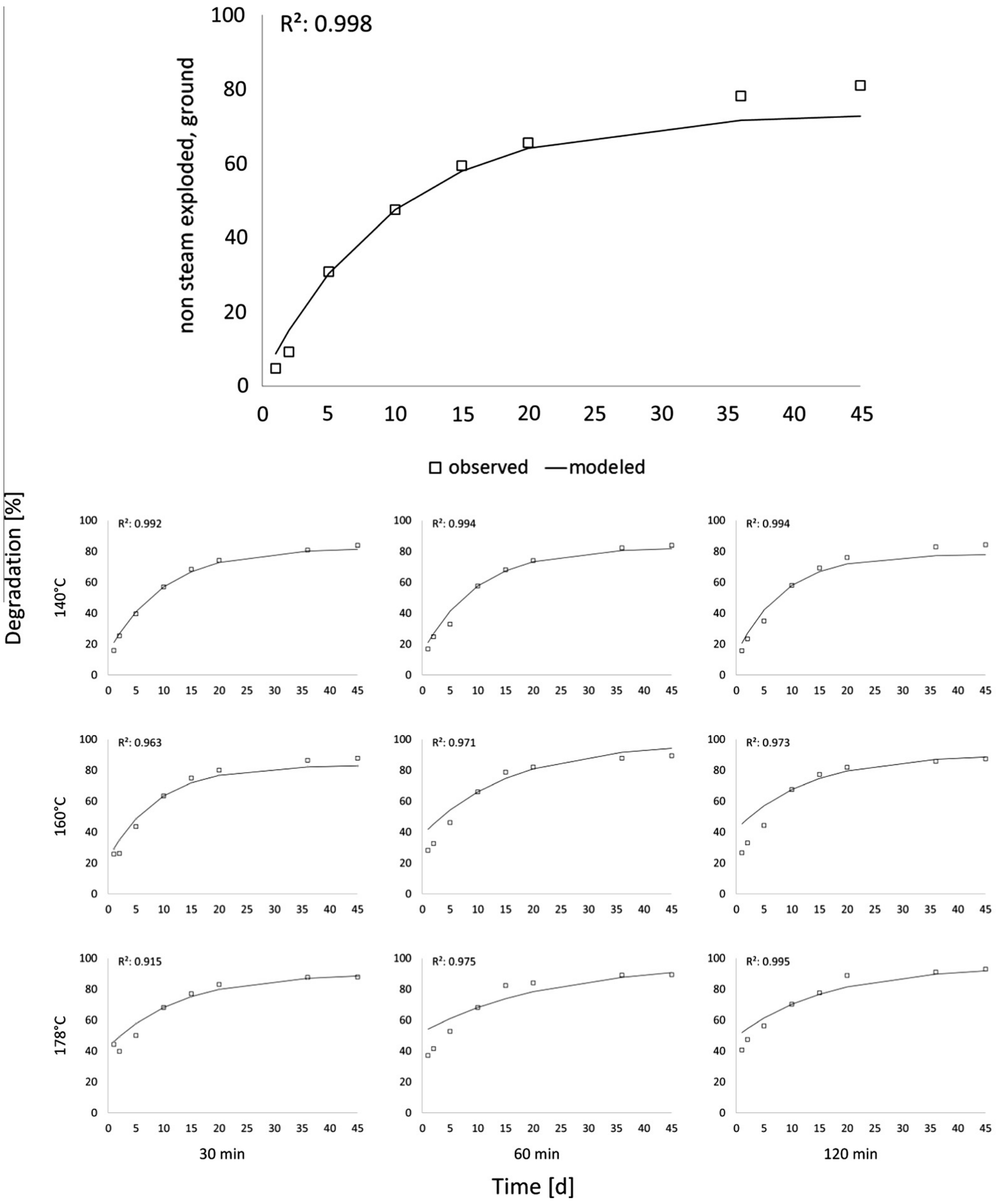

Fig. 2. Observed degradation of the analysed samples and comparison to the calculated model.

3.3. Biomass degradation

Fig. 1 shows the relative degradation and relative methane formation of the investigated samples. Relative degradation or methane formation indicates the level of degradation or formation at a specific point in time during the experiment relative to the final value. As previously mentioned, Fig. 1 shows that methane formation was accelerated with a higher pretreatment intensity. Consequently, biomass degradation in the pretreated samples was more rapid. In the case of the samples treated at 140 °C, the hemicellulose fraction remaining after steam explosion was more quickly hydrolysed than was the case with the non-steam-exploded, ground sample. In comparison to the non-steam-exploded sample, the cellulose fraction did not seem to be influenced in terms of degradation speed. It was also observed that the lignin fraction of the pretreated samples declined during the degradation process. At the end of the experiment the lignin content was at the same level as the non-steam-exploded, ground sample. Hence it can be concluded that the compounds classified as pseudo-lignin were degraded during anaerobic degradation.

For the samples treated at 160 °C for 30 and 60 min, as well as all samples treated at 178 °C, total solids increased at the beginning of the process instead of declining due to degradation as predicted. A possible explanation for this effect is that organic molecules from the inoculum formed bonds with the sample biomass inside the nylon bags due to the influence of the pretreatment on the biomass properties.

3.4. Degradation model

Table 5 displays the parameters used to calculate the model. The value for c, which resulted in the highest R^2 for the degradation model, occurred after 10 days. Values a and b were determined with standard laboratory methods. If applicable values for c are available, it is possible to determine the degradation curve with a high degree of certainty.

Fig. 2 displays both the observed and modelled biomass degradation according to Section 2.5.1 for the investigated samples. It can be seen that the model fits the observed data better for the non-steam-exploded, ground sample and for the steam-exploded samples pretreated at 140 °C, than for the samples, which underwent a more intense pretreatment. The calculated data an the measured values in the first 10 fermentation days differ strongly. Nevertheless, R^2 does not decrease to under a value of 0.915.

4. Conclusion

Depending on the pretreatment intensity, a substantial portion of the hemicellulose is hydrolysed to smaller organic compounds, which are more easily degraded. It was observed that the pseudo-lignin, which is assumed to be formed during the pretreatment, was degraded during anaerobic digestion. A new approach based on the Rumen Simulation Technique was developed to study the degradation of biomass in an anaerobic process. It can be concluded that the method is an adequate tool for investigating the evolution of certain biomass components during the process. The derived model is a viable option for describing the degradation process.

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IV

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The effect of a combined biological and thermo-mechanical pretreatment of wheat straw on energy yields in coupled ethanol and methane generation

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HIGHLIGHTS

- Integrated storage and pretreatment using *Schefferomyces stipitis* for inoculation.
- Steam explosion pretreatment of wheat straw at 180, 200 and 220 °C.
- Combined ethanol and methane utilisation scenario and balancing of overall energy yields.
- Increasing severity supporting conversion of cellulose to ethanol.
- Combined ethanol/methane production equal to exclusive AD considering energy yields.

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ABSTRACT

Ethanol and biogas are energy carriers that could contribute to a future energy system independent of fossil fuels. Straw is a favorable bioenergy substrate as it does not compete with food or feed production. As straw is very resistant to microbial degradation, it requires a pretreatment to insure efficient conversion to ethanol and/or methane. This study investigates the effect of combining biological pretreatment and steam explosion on ethanol and methane yields in order to improve the coupled generation process. Results show that the temperature of the steam explosion pretreatment has a particularly strong effect on possible ethanol yields, whereas combination with the biological pretreatment showed no difference in overall energy yield. The highest overall energy output was found to be 10.86 MJ kg VS^{-1} using a combined biological and steam explosion pretreatment at a temperature of 200 °C.

1. Introduction

The production of renewable energy from lignocellulosic feedstocks has recently garnered substantial interest as a replacement for fossil-derived energy. Not only could this source of renewable energy help in the transition away from fossil fuels, but it does not compete with food and feed production to the same extent as ethanol or biogas production from dedicated energy crops such as maize.

One of the most interesting feedstocks for lignocellulose-derived fuels is cereal straw (Chen et al., 2007). Straw is co-produced with cereal grain and has a broad field of application in agriculture. One of these applications is as a raw material for bioethanol production, as, for instance, demonstrated by the Inbicon-pilot plant in Denmark (Larsen et al., 2008). However, the moisture content of straw must be below 18% to prevent mould growth, regardless

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of its subsequent application. Field storage is commonly employed as a method of drying straw. If air humidity is too high, the low moisture content required for storage cannot be reached. For instance, the potential of straw production in Sweden is about one million tons, however, due to frequent high humidity, only a small proportion can be utilized (Nilsson, 2000).

Due to its lignocellulosic structure, the biomass is recalcitrant to biological conversion and hence requires a pretreatment prior to further processing (Taherzadeh and Karimi, 2008). Most pretreatment technologies presently in use are energy intensive or require chemicals, which are derived from fossil resources and may also require large amounts of energy for their production (Alvira et al., 2010). As such, the ratio between inputs necessary to process the feedstock and outputs (in terms of liquid or gaseous fuels) is crucial to economic and ecological viability. Decreasing inputs while increasing outputs has the potential to make the pretreatment process more competitive than conventional energy production.

Steam explosion is a commonly used pretreatment technology, typically carried out at temperatures of 160–260 °C and retention times of several minutes (Sun and Cheng, 2002). The feasibility of using steam explosion for the pretreatment of this feedstock has already been shown. Bauer et al. (2009) found an increase in methane yield from wheat straw pretreated with steam explosion ranging from 275 l_N kg VS^{-1} to 331 l_N kg VS^{-1} (+20%) at a temperature of 180 °C and a retention time of 15 min. Dererie et al. (2011) investigated the effect of steam explosion on the combined ethanol and methane yield of oat straw and found an overall energy output of 9.5 MJ kg DM^{-1} when using a 190 °C pretreatment temperature and a 10 min retention time. The amount of thermal energy that is required for the steam explosion pretreatment is very high; hence it is crucial to have a source of waste heat that is large enough to provide enough thermal energy for the pretreatment. Moreover, due to the harsh thermochemical pretreatment, compounds are formed that inhibit the subsequent fermentation processes. Acetic acid, furfural, hydroxy-methyl furfural (HMF) and phenolic compounds derived from hemicellulose and lignin can inhibit the yeasts from ethanol fermentation (Klinke et al., 2004; Larsson et al., 1999; Parawira and Tekere, 2011). Furfural and HMF inhibit the growth of yeast cells but are themselves degraded to furfuryl alcohol and furoic acid (Boyer et al., 1992; Taherzadeh et al., 2000). The inhibitory effect of those compounds on the consortium that is responsible for anaerobic digestion is not yet clear.

For the operation of a biomass-based fuel-generation plant to be economical, it is necessary to guarantee a good degree of capacity utilization. As wheat straw is harvested only once a year it is crucial to store it properly. As mentioned above, in some colder climatic regions it is a problem to remove water from the straw by drying it on the field. Microorganisms that are able prevent mould growth may offer one possibility to conveniently store the wheat straw. Moreover, Passoth et al. (2013) showed that the yeast *Scheffersomyces stipitis* is also capable of increasing ethanol yields of conserved wheat straw, likely due to its ability to partially degrade hemicellulose.

A combination of a low energy biological pretreatment combined with steam explosion could reduce the required thermal energy input while delivering the same energy output in ethanol and methane. Reducing the required temperature level could also lead to the reduced formation of furfural and HMF, which are formed during the degradation of hemicellulose (Larsson et al., 1999). Although separation (Qi et al., 2011) and biotransformation (Boopathy, 2009) are discussed as strategies to overcome inhibition caused by reaction products (resulting from thermophysical pretreatment), a reduction in their formation is considered to be the more economically efficient option.

The objective of this study was to investigate the effect of a combined biological (ISP) and thermochemical (steam explosion) pretreatment on ethanol and methane yields of wheat straw. As the concept of combined ethanol and methane production is regarded as a promising production system for fuels in the future, the residues from ethanol fermentation were also investigated in anaerobic batch experiments.

The goal of this study is to obtain the overall energy yield of combined ethanol and methane generation. This means that, after biomass is pretreated, ethanol is produced. The fermentation residues resulting from ethanol fermentation subsequently serve as a substrate for anaerobic digestion for the generation of methane.

2. Methods

This section explains the experimental setup for the biological and steam explosion pretreatments as well as the determination of methane and ethanol yields in detail. The experiments and analysis of sample material was performed strictly in order to determine the overall energy yield of coupled methane and ethanol generation.

2.1. Biomass

The biomass used in the trials was wheat straw that had been grown in 2012 in Lower Austria. Prior to the experiments it was ground to a particle size of <5 mm using a cutting mill (Retsch SM100). The dry matter content of the untreated sample was 92.52%. The content of volatile solids was 94.62%.

2.2. Strains and culture media

S. stipitis CBS 5774 was used for the ISP experiments and *Saccharomyces cerevisiae* J672 was used for simultaneous saccharification and fermentation. Both yeasts were from the strain collection at the Dept. of Microbiology, SLU, Uppsala. *S. cerevisiae* (Blomqvist et al., 2010) was subcultured on YPD-agar (yeast extract 10 g L^{-1}, bacteriological peptone 20 g L^{-1}, glucose 20 g L^{-1}, bacteriological agar 16 g L^{-1}) and *S. stipitis* on YM-agar (yeast extract 3 g L^{-1}, malt extract 3 g L^{-1}, bacteriological peptone 5 g L^{-1} and glucose 10 g L^{-1}, bacteriological agar 16 g L^{-1}). For the pre-cultures, *S. stipitis* was grown in YM (yeast extract 3 g L^{-1}, malt extract 3 g L^{-1}, bacteriological peptone 5 g L^{-1} and glucose 10 g L^{-1}) and *S. cerevisiae* in YPD (yeast extract 10 g L^{-1}, bacteriological peptone 20 g L^{-1}, glucose 20 g L^{-1}, bacteriological agar 16 g L^{-1}).Integrated storage and pretreatment

The inoculation of the wheat straw samples with *S. stipitis* (CBS 5774) was performed at the Department of Microbiology, SLU, Uppsala, Sweden. The experiment was carried out as described by Passoth et al. (2013). For the pre-culture, *S. stipitis* was grown in a 300 ml shake flask containing 100 ml YM-medium at a rotary table for 24 h at 25 °C. The optical density (OD_{600}) was measured and the volume of cell suspension needed to reach a starting OD of 0.1 was harvested. The cell suspension was subsequently centrifuged for 10 min at 4000*g* and washed with sterile saline (NaCl 9 g L^{-1}), centrifuged again, resuspended in 1 ml saline and inoculated in 300 m YM in a 1 L shake flask. The main culture was incubated at 25 °C for 20 h at a rotary table. Cells were counted in a Bürker chamber, washed and then resuspended in sterile deionized water. Before inoculation of the wheat straw with the yeast cells, water was added to reach a final water content of 30% in the wheat straw. Samples were subsequently inoculated with yeast cells to reach a ratio of about 10^5 cells per g DM of wheat straw. Wheat straw samples were then stored in plastic bags for 42 days at 4 °C until the steam explosion pretreatment.

2.3. Steam explosion pretreatment

The samples were pretreated with the CAMBI steam explosion process at a test facility at the University of Life Sciences (UMB) in Ås, Norway. 500 g of the biomass were filled into the 20 L reaction vessel. As soon as the desired steam temperature was reached in the steam generator, steam was let into the reactor by opening a valve. In order to maintain constant conditions, the steam inlet valve was regulated to maintain pretreatment temperature and pressure for the entire pretreatment time. After the retention time had elapsed, the steam valve was closed and biomass and steam were flashed into the flash tank. The biomass was collected in a product container. The temperatures used for the combined ISP and steam explosion pretreatment were 180, 200 and 220 °C; pretreatment time was 15 min.

2.4. Chemical analysis

The dry matter content of the samples was analysed by drying them at 105 °C in an oven until a constant weight was reached (DIN 12880, 2001). In order to determine the volatile solids, the dried samples were dry-oxidised in a muffle furnace at 550 °C for at least 5 h. Volatile solid content was calculated as the difference between dry matter and ash content (DIN 12879, 2001).

As volatile compounds, such as acetic acid, can be lost during drying, the water content was also measured with the volumetric Karl Fischer titrator Mettler Toledo V20 (Columbus, Ohio, USA) using Hydranal Composite 5 and Hydranal Methanol dry from Sigma Aldrich (St. Louis, Missouri, USA). For this, 1.25 g of the samples were mixed with 10 ml of methanol and placed on a rotator for 18 h. Subsequently, 200 μL of the liquid phase was injected into the titrator. The water content was calculated using Eq. (1) (Bauer et al., 2014).

$$wc = \frac{(weq * meq)}{m\ (sample)} \tag{1}$$

wc ... water content of the sample
weq ... result of Karl Fischer titration
meq ... mass of sample plus methanol
$m\ (sample)$ ... mass of the sample

Cellulose, hemicellulose and lignin content were determined according to the method of Van Soest and Wine (1967) and its modification by Naumann and Bassler (1976). The neutral detergent fibre (NDF) content is the insoluble residue after treatment with a neutral detergent solution. The acid detergent fibre (ADF) represents the insoluble residue after treatment with an acid detergent solution. The dried residue from the ADF determination was used to determine the lignin (ADL) by treating the residue with 72% sulphuric acid. The hemicellulose content is calculated as the difference between NDF and ADF. Cellulose content can be calculated as the difference between ADF and ADL. After ADL determination, the dried residue was dry-oxidised at 500 °C in order to determine the content of acid-insoluble ashes. All chemicals used for the analysis were obtained from Ankom Technology (USA).

Furfural and HMF concentrations were analysed as described by Bauer et al. (2014). The analysis was carried out using an isocratic HPLC (Dionex UlitMate 3000 HPLC, Sunnyvale, USA) set up with a 7.8 × 100 mm Rezex RFQ-Fast Fruit H + column (Phenomenex) heated to 82 °C. 5 mM sulphuric acid was used as the mobile phase at a flow rate of 1.0 ml/min. Samples were prepared by diluting them with the mobile phase followed by centrifugation and filtration. Concentrations of furfural and HMF were monitored using a UV detector at 280 nm.

In order to determine the pH value of the samples, 5 g of fresh material was mixed with 10 ml of tap water. The pH was measured using a Consort C933 pH meter.

2.5. Analysis of the biological methane potential (BMP)

The biological methane potential (BPM) of the samples was determined in anaerobic batch digestion tests according to the method of the Association of German Engineers (2006). The experiments were carried out in triplicate using 250 ml digesters at 37 °C. For inoculation, the separated liquid fermentation residue of a biogas plant with steam exploded lignocellulose feedstock (amongst others, maize straw) was used. Prior to the experiments the inoculum was stored for 3 weeks at 37 °C for degassing. During the experiment a blank series was carried out to determine the biogas production caused by the inoculum, which was then subtracted from the biogas production of the samples. The amount of the produced biogas was measured on a daily basis; the duration of the batch experiment was 48 days. The composition was analysed using a Dräger X-am 7000 multi-gas analyser calibrated at regular intervals with a standard gas. The produced biogas was analysed for CO_2, CH_4, NH_3, H_2S, H_2 and O_2 content. Biogas and methane yields are expressed in norm litres (273 K and 1013 mbar) per kg of volatile solids ($l_N\ kg^{-1}$ VS). The biological methane potential was determined for the samples directly after steam explosion pretreatment, as well as for the residues after ethanol fermentation. The percentage of citric acid added to the samples during ethanol fermentation was determined in order to correct the analysis of methane yields after ethanol fermentation.

2.6. Simultaneous saccharification and fermentation

The simultaneous saccharification and fermentation (SSF) experiments were carried out as described by Passoth et al. (2013) with some modifications. The bottles used for fermentation were prepared as follows: 11 g DM of the sample biomass was weighed into the bottles. Deionised, sterile water was added to reach a volume of 100 ml. Then, 100 ml of citrate buffer pH 5 and a concentration of 0.2 M was added. The pH of the bottle content was then adjusted to 5 using either 10 M sodium hydroxide or 25% citric acid. The bottles were then covered with aluminium foil and boiled in a water bath for 20 min to sanitise the biomass from possible bacterial contaminants.

Cells of *S. cerevisiae* J672, were inoculated in YPD and incubated in a shake flask at 30 °C for 20 h. Cells were harvested from the pre-culture to reach a start OD of 1 for SSF, centrifuged and washed as described above (Section 2.3). The pellet was suspended in sterile saline before inoculation.

The enzyme mixture used for saccharification was Accelerase 1500 (Genecor, Palo Alto, USA) with an endoglucanase activity of 2200–2800 CMC U/g and a beta-glucosidase activity of 450–775 pNPG U/g. The preparation of the enzymes was conducted as follows: the required amount of enzymes was put into a centrifuge tube and then centrifuged at 4000*g* for 10 min. The liquid was then sterile-filtered using a 0.2 μm syringe filter. The concentration of the enzyme solution in the bottle was 0.1 ml per g of DM.

After mixing, an initial sample of the liquid phase was drawn. Then, the bottles were closed with rubber stoppers, flushed with nitrogen for two minutes and incubated on a shaker at 37 °C. Samples of the liquid phase were withdrawn after 24, 48, 72 and 96 h using a syringe. After 48 h the bottles were flushed again with nitrogen. After the final sample was withdrawn, the bottles were frozen to prevent further activity of the yeast and enzymes. Before analysing the methane potential of the wheat straw silage, ethanol was removed by evaporation using a Rotavapor (Büchi, SUI).

Ethanol, as well as the glucose, xylose, acetic acid, and glycerol content of the samples (withdrawn during ethanol fermentation), were measured using HPLC (high performance liquid chromatography) as described in Blomqvist et al. (2010). For the analysis, a HC-75 column (305 by 7.8 mm; Hamilton, Reno, Nevada) and an RI detector (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany) were used. The column was eluted with 5 mM sulphuric acid at 60 °C and at a flow rate of 0.6 ml min^{-1}.

2.7. Energy output

Total energy output is given in MJ kg VS^{-1}. In order to calculate the direct methane production after pretreatment, the biological methane potential, given in l$_N$ kg VS^{-1} was multiplied with the lower heating value of methane (0.03589 MJ l$_N^{-1}$). In the case of combined production of ethanol and methane, the energy output resulting from ethanol was calculated by multiplying the ethanol yield given in mg g VS^{-1} with the lower heating value of ethanol (26.96 MJ kg^{-1}) and adding the energy output from the methane fermentation of the remaining bagasse (U.S. Department of Energy, 2011).

2.8. Statistical analysis

The tables and graphics shown in this paper represent mean values and standard deviation of the obtained measuring values. Statistical analysis was carried out using SPSS Version 22 (IBM, New York, USA). The obtained data was analysed using Tukey's Test with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Characterisation of wheat straw after steam explosion pretreatment

Table 1 shows the dry matter, volatile solids content, water content determined using a Karl Fischer titrator, and pH. The untreated (ground) wheat straw had a high dry matter content, typical for cereal straw harvested in Europe (Bauer et al., 2009; Dererie et al., 2011; Ferreira et al., 2014). For the ISP, the dry matter content of those samples was reduced to about 70% through the addition of distilled water. As it could not be guaranteed that autochthon microbes present on the wheat straw did not also have some pretreatment effect, one uninoculated, ground sample (water content 30%) was sealed and stored for the same period of time as the inoculated samples. For investigation of the ISP without steam explosion pretreatment, the uninoculated control was examined. Increasing the severity of the treatment reduced the dry matter content of the steam explosion pretreated samples. This effect occurs due to the addition of steam during the pretreatment process, which condenses when pressure is released from the reaction vessel. In addition to increasing the pretreatment severity, more steam is needed to maintain the pretreatment conditions, which also increases water content in the final samples. The volatile solids content was constant for all pretreatment variants. It was observed that the pH of the pretreated samples decreased with increasing pretreatment intensity. This can be explained by the formation of organic acids as by-products of the hemicellulose hydrolysis (Larsson et al., 1999). The formation of volatile compounds, such as organic acids for example, was confirmed by comparing the water content, determined by the Karl Fischer method, to the dry matter content (Table 1).

Table 1
Dry matter, volatile solids, water content and pH of the investigated samples.

	DM [% FM]	VS [% DM]	Water content	pH –
Ground	92.5 ± 0.0	94.6 ± 0.2	n.a.	n.a.
Ground (mc 30%)	66.3 ± 0.5	95.4 ± 0.1	32.5 ± 0.8	n.a.
Ground (no SE, with ISP)	68.1 ± 0.1	95.6 ± 0.0	31.8 ± 0.6	n.a.
180 °C, no ISP	21.5 ± 2.2	95.5 ± 0.1	80.1 ± 1.4	4.8 ± 0.1
200 °C, no ISP	16.9 ± 1.6	95.4 ± 1.4	85.2 ± 2.0	3.8 ± 0.1
220 °C, no ISP	15.2 ± 1.0	94.3 ± 0.2	83.7 ± 0.8	3.6 ± 0.0
180 °C, ISP	26.4 ± 1.0	95.5 ± 0.1	77.2 ± 0.3	5.3 ± 0.8
200 °C, ISP	17.6 ± 0.7	95.0 ± 0.1	82.5 ± 0.9	3.8 ± 0.0
220 °C, ISP	17.5 ± 1.4	94.2 ± 0.1	81.7 ± 0.8	3.5 ± 0.0

FM: fresh matter, DM: dry matter, VS: volatile solids, n.a.: not analysed, wc: water content, SE: steam explosion.

Table 2
Cellulose, hemicellulose and lignin content of the investigated samples.

	CEL [% DM]	H-CEL	ADL
Ground	48.1 ± 0.3	25.2 ± 0.6	12.5 ± 0.4
Ground (mc 30%)	45.8 ± 0.3	24.2 ± 0.0	11.2 ± 0.2
Ground (no SE, with ISP)	45.2 ± 0.5	26.8 ± 0.2	11.9 ± 0.0
180 °C, no ISP	47.4 ± 0.5	14.3 ± 1.1	13.1 ± 0.2
200 °C, no ISP	46.8 ± 0.9	b.d.l.	13.3 ± 0.6
220 °C, no ISP	46.5 ± 0.9	b.d.l.	15.2 ± 0.8
180 °C, ISP	46.3 ± 0.5	17.1 ± 1.0	13.6 ± 0.9
200 °C, ISP	45.8 ± 0.5	b.d.l.	12.5 ± 0.5
220 °C, ISP	46.7 ± 0.5	b.d.l.	15.1 ± 1.1

CEL: cellulose, H-CEL: hemicellulose, ADL: Lignin, wc: water content, SE: steam explosion, b.d.l: below detection limit.

Table 2 shows the composition of the samples, including the structural compounds cellulose, hemicellulose and lignin. Only small differences were observed in the cellulose content. Compared to the untreated sample, the cellulose content of the ISP sample and the uninoculated control decreased. This can be explained by microbial activity, for which cellulose, as well as hemicellulose and lignin, can be a substrate. The pretreatment using steam explosion does not have an explicit effect on the cellulose content. No effect of the ISP could be observed on the hemicellulose content. Contrary to the cellulose content, the steam explosion pretreatment had a strong effect on the hemicellulose content. With increasing pretreatment temperature, the hemicellulose content decreased. At pretreatment temperatures of 200–220 °C hemicellulose content was lower than 0.3%, which was below the detection limit of the method (Naumann and Bassler). The massive degradation of hemicellulose after steam explosion pretreatment is in accordance with previous findings (Weil et al., 1997). It was observed that the ISP as well as the uninoculated control did not show a significant difference in lignin content. Steam explosion pretreatment caused an increase in lignin content up to approximately 15% of dry matter at a pretreatment temperature of 220 °C. This can be explained by the formation of compounds resulting from hemicellulose hydrolysis, which are then detected as lignin (and hence referred to as pseudolignin). This effect has been reported in literature previously (Vivekanand et al., 2013). Pseudolignin is thought to inhibit cellulose hydrolysis and therefore can be problematic for ethanol fermentation (Hu et al., 2012). Recent R&D activities investigate possibilities for preventing the formation of pseudolignin during steam explosion pretreatment (Hu and Ragauskas, 2014) e.g. by addition of a water–dimethyl sulfoxide mixture to the reaction. It is unclear whether or not pseudolignin is degradable during anaerobic digestion (Theuretzbacher et al., 2015).

3.2. Effect of the combined biological and steam explosion pretreatment on methane yields

Fig. 1 displays the biological methane yields of the investigated pretreatment combinations. The first three columns represent samples without steam explosion pretreatment. The first column shows the methane potential after grinding the wheat straw (<1 mm), the second column the potential after the addition of water (no ISP) and the third column the potential with addition of water and ISP. Columns four through six show the methane potential of the ground wheat straw after steam explosion pretreatment. The last three columns show the methane potential after a combination of ISP and steam explosion pretreatments. The letters on the top of the columns represent the statistical difference of the groups.

The methane yield of the ground wheat straw was 210 l_N kg VS^{-1}, which is in accordance with literature values. Ferreira et al. (2014) and Bauer et al. (2009) found methane yields of 232 l_N kg VS^{-1} and 276 l_N kg VS^{-1} for ground (<1 mm) wheat straw. Kaparaju et al. (2009) found a higher methane yield of 297 l_N kg VS^{-1}. In general it should be mentioned, that results for the biological methane potential found in literature vary due to different experimental setups (e.g. different inoculum sources, fermentation temperatures and dry matter concentration in the fermenters). Nevertheless, if different biomass samples are investigated within one batch experiment, those effects can be neglected. The biological pretreatment using ISP already showed a significant increase up to 243 l_N kg VS^{-1} (+15%) in the methane yield. The highest methane yield, 254 l_N kg VS^{-1} (+21%), was obtained from a steam explosion treatment of the wheat straw at 180 °C without ISP. There were no significant differences between the methane yields of the samples combining ISP with steam explosion pretreatment at 180 °C (250 l_N kg VS^{-1}) and 200 °C (252 l_N kg VS^{-1}). Pretreatment with steam explosion at 220 °C did not result in a significant increase in methane yield compared to the ground sample.

3.3. Effect of the combined biological and steam explosion pretreatment on ethanol yields

Fig. 2 displays the ethanol yields of the investigated samples. SSF was only run on the steam exploded samples. It can be seen that the temperature of the steam explosion pretreatment has a very strong influence on the ethanol yield. The highest ethanol yields were achieved with a pretreatment temperature of 220 °C with 169 mg VS^{-1} (no ISP) and 167 mg VS^{-1} (ISP) respectively (corresponding to 180 mg DM^{-1} and 178 mg DM^{-1}). The theoretical ethanol yield of the untreated wheat straw is 244 mg g VS^{-1} (corresponding to 258 mg DM^{-1}) meaning that approximately 70% of the theoretical maximum ethanol yield was reached. Kaparaju et al. (2009) found a conversion efficiency of 80% after fermenting the solid fraction of hydrothermally pretreated wheat straw. A significant effect from combining steam explosion pretreatment with an ISP pretreatment could not be observed. For all samples, except for the steam explosion pretreatment at 180 °C and no combination with ISP, the highest ethanol yields were achieved after 96 h of fermentation.

3.4. Effect of the combined biological and steam explosion pretreatment on overall energy yields

The total energy output from the produced ethanol and methane potentials was calculated in order to compare sole production of methane to combined production of ethanol and methane from remaining bagasse. Fig. 3 displays the calculated energy yields in MJ kg VS^{-1}. With increasing pretreatment temperatures, the share of energy from ethanol increased. This can be explained by the fact, that cellulose, which has already been converted to ethanol, is no longer available for anaerobic digestion. In general, the combined ethanol and methane production resulted in the same or higher overall energy yields compared to methane production alone, however, this difference could not be proven to be statistically significant (Fig. 3). The highest overall energy yield, 10.86 MJ kg VS^{-1}, was the sample pretreated with a combination of ISP and steam explosion pretreatment at 200 °C, 44% higher compared to the untreated (ground) sample.

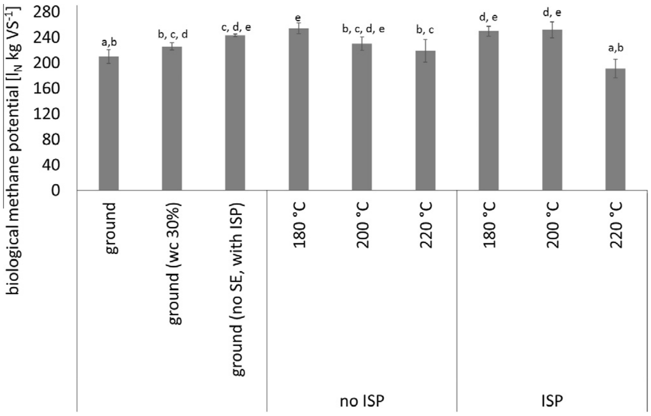

Fig. 1. Biological methane potential of the investigated samples.

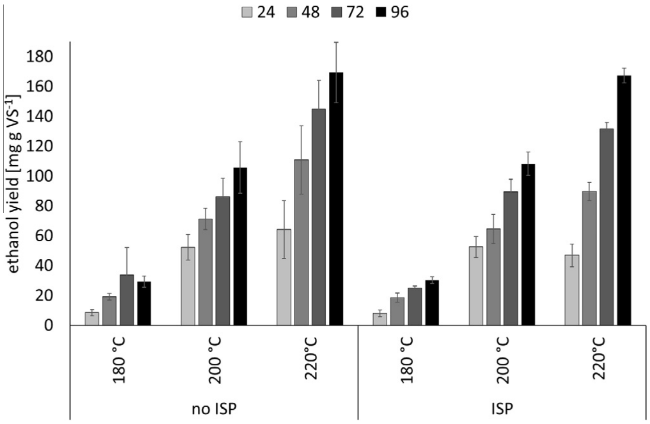

Fig. 2. Ethanol potential of samples pretreated by steam explosion (with and without combination with ISP).

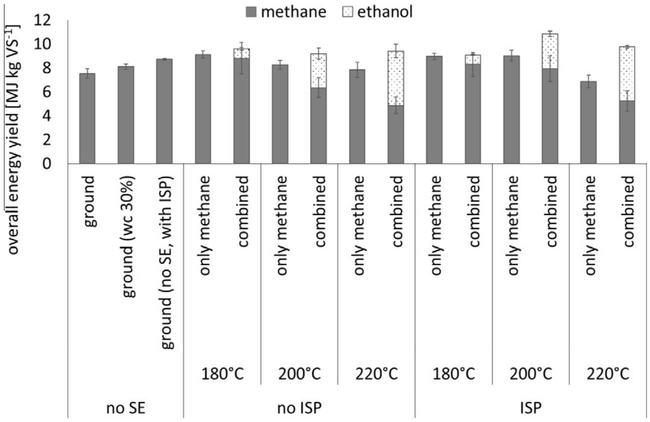

Fig. 3. Overall energy outputs from methane fermentation (only) as well as from a combined ethanol and methane fermentation of the analysed samples.

The share of ethanol from the overall energy yielded from samples steam exploded at 220 °C is nearly 50%. Kaparaju et al. (2009) found an energy yield of about 9.4 MJ kg VS^{-1} when using untreated wheat straw for methane production, which is about 20% higher than the energy output compared to the ground wheat straw sample in this study. The higher energy output of direct biogas fermentation of untreated and steam exploded wheat straw can be explained by the higher biological methane potential reported by Kaparaju (see Section 3.2). Kaparaju et al. (2009) obtained an energy yield of 9.1 MJ kg VS^{-1} for the combined production of ethanol and methane, which is in the same range as the energy yields found in the study by Dererie et al. (2011) and in this study. Also, the distribution between energy output resulting from biogas and bioethanol is within the same range. In a recent study on steam exploded bagasse Vivekanand et al. (2014) obtained a total energy yield of 7.6 MJ kg VS^{-1}, where 4.4 MJ kg VS^{-1} was in the form of ethanol.

Comparing all scenarios investigated in this study, the combined production of biogas and bioethanol seems to result in the highest overall energy outputs. The high standard deviations caused by the experimental setup and the analytical methods hinder a more distinct interpretation. This is in contrast to the findings of Kaparaju, where direct biogas fermentation of the pretreated material resulted in the highest energy output, which can also be attributed to the generally high methane potentials for the investigated biomass found in his study.

For the practical application of combined ethanol and methane generation it should be considered that the equipment needed for ethanol fermentation is more complex and expensive than for methane generation. Considering economy of scale aspects, the required size of an ethanol plant is several times larger than that of a biogas plant. As the retention time of the biomass during ethanol fermentation is shorter than during anaerobic digestion, the capacity of the biogas plant would have to be increased in order to handle the substrate flow. In summary, this means that besides optimal pretreatment conditions, other major factors have to be considered in order to develop an economically feasible lignocellulose driven ethanol/methane plant.

3.5. Concentration of inhibitors

The concentration of inhibitors like furfural and HMF can affect the conversion of sugars into ethanol (Palmqvist and Hahn-Hägerdal, 2000) as well as the conversion of organic compounds into methane (Horn et al., 2011). To determine the impact of pretreatment intensity, inhibitor concentrations were measured after steam explosion treatment. Fig. 4 displays the concentrations of furfural and HMF in steam explosion pretreated samples. By increasing the steam explosion temperature to 220 °C, the concentration of HMF increased to approximately 23 mg g VS^{-1} (without ISP) and 16 mg g VS^{-1} (with ISP) respectively. The concentration of furfural reached its maximum with 8 mg g VS^{-1} at a pretreatment temperature 200 °C (no ISP). The combination of steam explosion at 200 °C with ISP resulted in a slightly lower furfural concentration (7 mg g VS^{-1}). Di Girolamo et al. (2013) showed that higher temperatures and longer retention times result in increased concentrations of furfural and HMF.

Fig. 5 shows the concentration of acetate from the SSF experiment. It can be seen that the acetate concentration is higher for the samples steam exploded at 200 and 220 °C with a maximum of 31 mg g DM^{-1} for the sample pretreated with a combination of ISP and steam exploded at a temperature of 220 °C. No significant degradation of acetate was observed during ethanol production.

As the highest ethanol yields were found from samples steam exploded at 220 °C, it could not be proven that the measured concentrations of acetate, furfural and HMF had a negative effect on the conversion of cellulose to ethanol. This may be different if the organic loading rate during ethanol fermentation is increased.

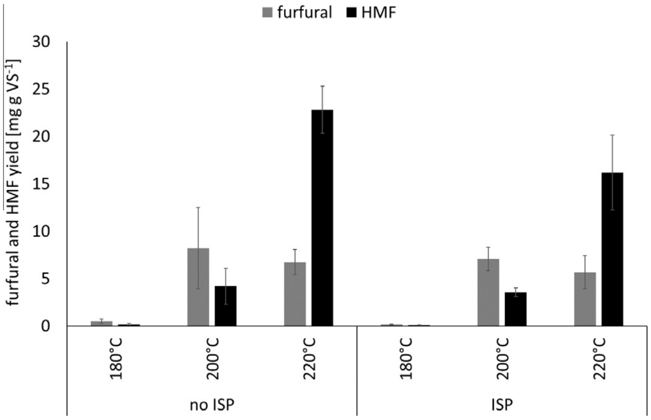

Fig. 4. Furfural and HMF concentrations of the steam explosion pretreated samples.

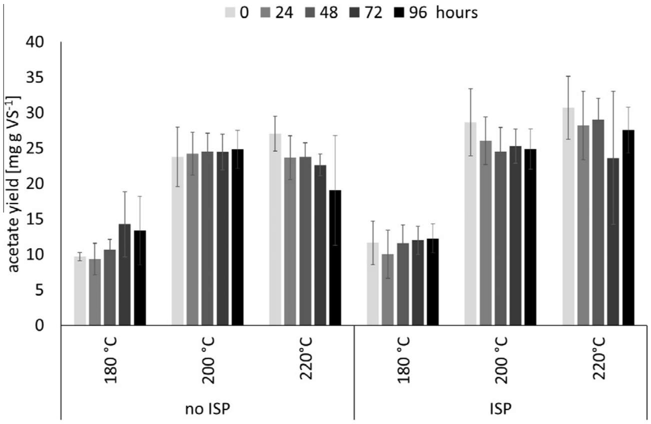

Fig. 5. Acetate concentration of the steam explosion pretreated samples during the ethanol fermentation experiment.

Considering the biological methane potential of the samples without ethanol fermentation, the high HMF and acetate concentrations could offer a possible explanation for the lower methane yields after steam explosion pretreatment at 220 °C.

Besides the partial transformation of cellulose to ethanol, another reason for the low methane yields of the samples steam exploded at 220 °C after ethanol fermentation could also have been the high concentration of HMF and acetate.

The combination of ISP with steam explosion pretreatment showed no difference in the formation of furfural and acetate. The measured HMF concentrations are lower for the combined ISP/steam explosion samples, although this difference could not be proven to be statistically significant due to high standard deviations. Still, the proposed pretreatment combination could be an option for reducing possible inhibitory substances.

4. Conclusions

The results of this study show that the temperature of the steam explosion pretreatment has a significant impact on ethanol yields. Further improvements using a combination of steam explosion and biological pretreatment could not be achieved in this first attempt. Combined ethanol and methane production after steam explosion at 200 °C showed the highest energy output and therefore is the most promising option for practical application. Low methane yields found with the samples showing the highest HMF concentrations indicate that further investigations concerning the possibilities to reduce HMF in the substrate may be important for additional optimization of the pretreatment process.

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Eidesstattliche Erklärung

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