TALLINNA TEHNIKAÜLIKOOL
TALLINN UNIVERSITY OF TECHNOLOGY

School of Engineering

BIOGAS PRODUCTION POTENTIAL OF BIOMATERIAL FROM DIFFERENT STAGES OF BIOETHANOL PRODUCTION PROCESS AND ITS PURIFICATION

BIOGAASI TOOTMISE POTENTSIAAL BIOMATERJALIST ERINEVATES BIOETANOOLI TOOTMISE ETAPPIDES JA SELLE PUHASTAMINE

MASTER THESIS

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AUTHOR'S DECLARATION

Hereby I declare, that I have written this thesis independently.
No academic degree has been applied for based on this material. All works, major viewpoints and data of the other authors used in this thesis have been referenced.

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THESIS TASK

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Thesis topic: "Biogas production potential from biomaterial at different stages of bioethanol production process and its purification" (in English); "Biogaasi tootmise potentsiaal biomaterjalist erinevates etanooli tootmis etappides ja selle puhastamises" (in Estonian).

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1. Analyze the biogas production of samples from all stages of bioethanol production;
2. Find the optimum stage for biogas production, and study how these stages influence biogas yields;
3. Review and suggest the best stage of the production process for biogas purification.

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PREFACE

This topic was initiated by the supervisor Prof. Timo Kikas, together with the co-supervisor PhD student Lisandra Meneses. The major work of the thesis as well the experiments were carried out in the Institute of Technology (Laboratory of Biofuels) and Institute of Agricultural and Environmental Sciences (Laboratory of Bio- and Environmental Chemistry) of Estonian University of Life Sciences. Main assistance in the data collection and analysis was done by the co-supervisor.

I kindly express my gratitude to the Estonian University of Life Sciences for the support and all the facilities that were provided during the experiments. On my way to graduation I got very huge inspiration and support from my co-supervisor PhD student Lisandra Meneses.

This thesis aims to analyse the most effective ways for bio-gasification after bioethanol production and focus on possibilities of using the production-waste that is left over after the separation of bioethanol in the distillation process. This production-waste still has high energetic value and can be further utilized to increase the energy output from the biomass. In this work, all the possibilities for bio-gasification were considered, using biomass samples from all the stages of bioethanol production process. The results were compared to each other and with untreated biomass samples.

The results suggest that bioethanol production-waste is highly valuable, and it is reasonable to use it for further bio-gasification. Also, a review of purification techniques is presented, and the best possibilities for biogas purification are reported. Gaseous fuels such as biogas or biomethane have great potential for the sustainable energy supply, and are promising solutions for the transport sector, as well as for the natural gas substitution.

Key words: anaerobic digestion, bioethanol, biofuel, biogas purification, production-waste, zero-waste.

LIST OF ABBREVIATIONS

AD	Anaerobic digestion
AP	Acidification process
BG	Biomass gasification
BMP	Biomethane potential
CNG	Compressed natural gas
EP	Eutrophication process
EPA	Environmental Protection Agency
ERoEI	Energy return on energy invested
GHG	Greenhouse gas
GWP	Global warming potential
Mtoe	Million Tonnes of Oil Equivalent
NED	N_2 explosive decompression
NEG	Net energy gain
ODP	Ozone depletion potential
POCP	Photochemical oxidant creation potential
TS	Total solids
VS	Volatile solids
WO	Wet oxidized

LIST OF SYMBOLS

$CH_{4\,F}$	Molar quantity of final methane gas in the test bottle, mol
$CH_{4\,I}$	Molar quantity of initial methane gas in the test bottle, mol
B_{max}	Maximum biogas yield, mol /100g of raw biomass
$CH_{4\,C}$	Cumulative methane produced, mol/100g
k	Kinetic rate constant, $mol \cdot L^{-1}$
MF	Methane fraction, %
P_F	Final partial pressure in the headspace, Pa
P_I	Initial partial pressure in the headspace, Pa
R	Ideal gas constant, $Jmol^{-1}K^{-1}$
R^2	Correlation coefficient
T	Temperature, ºC
t	Time interval, days
V_{HS}	Volume of the headspace, m^3

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INTRODUCTION

Since the European Commission has included energy policy in its agenda, great attention has been paid to climate change, energy security, and to solutions that will help to move to a more sustainable energy path, from 2020 to 2030. Biofuels are expected to become one of the most important sources of energy in the future. They can help with the reduction of CO_2-emissions and provide energy security [1].

Biofuels produced from biological sources are particularly interesting for the transportation sector and will play an important role in sectors where electrification and other renewable sources of energy are not feasible. Advanced biofuels are expected to be a partial substitution of fossil fuels, which are currently being used as a main source of energy for the transportation sector. According to a recent report by General Directorate for Research and Innovation (European Commission) [2], it is expected that advanced biofuels will replace 0,8 Mtoe of fossil-based fuels by 2020, and cover almost 50% of the EU transport sector's energy needs by 2050.

The first biofuels produced from conventional energy crops have been highly criticized because the cultivation had taken place on croplands that were previously used for agricultural production. Utilization of these areas brought along environmental impacts, as indirect land use change and increment of atmospheric emissions (because the area for absorbing CO_2 decreased). Thus, the quantity of biofuels produced from food crops grown decreased, and great attention has been paid to agricultural biomass residues. For instance, unlike first-generation biofuels, second-generation releases less GHG emissions, making them better candidates for biofuel production. Besides, second-generation feedstock in the form of lignocellulosic biomass is one of the most abundant bioresources on Earth, it does not compete with food production, and has low cost [1, 2].

Lignocellulosic biomass consists of three main components: cellulose (40-60%), hemicellulose (20-40%) and lignin (10-25%) [3]. While cellulose does not contain chemical bonds with lignin and hemicellulose, hemicellulose contains chemical bonds with lignin, what makes the structure of the biomass highly rigid and impermeable. Lignin plays an important role in the protection of plant against microbial attacks and oxidative stress [4]. Due to the fact that lignocellulosic biomass has a complex structure, converting it into liquid or gaseous biofuels requires four sequential steps pretreatment, hydrolysis, fermentation and distillation. Each one of these steps contributes to the quantity of the ethanol produced and influence the overall production cost [5].

At the same time, in the biogas conversion process, it is only possible to convert 20-30% of the substrate into fuel, while 70-80% remains undigested, and can be further utilized for example as a fertilizer [6]. Other handling options currently available for bioethanol production-waste include anaerobic digestion. It has been reported as an attractive solution due to its low environmental impact, high potential for energy generation, and as a solution to increase the energy output from the biomass [7]. Additionally, as it uses production-waste that has already been pretreated at the beginning of the process, it may significantly reduce the production cost of the anaerobic digestion, since the process will start more rapidly.

This study aims to investigate the potential of bioethanol production-waste for biogas recovery, using the biomethane potential (BMP) assay. Barley straw (*Hordeum vulgare*) was used as a biomass crop in all the experiments. The biogas potential from bioethanol production-waste was further analyzed and compared with biogas potential of samples from different stages of bioethanol production process (pretreatment, hydrolysis and fermentation) and with biogas potential of raw barley straw. Also, this works reviews and discusses the main technologies currently used for biogas purification.

1. LITERATURE REVIEW

1.1. Classification of biofuels

There are many types of fuels produced from biomass such as ethanol, methanol, biodiesel, and hydrogen. All these fuels can be classified as primary and secondary biofuels. The primary biofuels use biomass in its unprocessed form like fuelwood, wood chips and pellets, and can be directly utilized for heating, electricity generation and cooking. The secondary biofuels which are processed into liquid or gaseous form such as ethanol, biodiesel or methane are mainly consumed by transport and industry sectors. They are further divided into first, second, third and fourth generation, based on the type of organic material and technology used for their production [8].

Biofuels are also classified according to their source of origin. Biomass can be obtained from forest or agricultural residues, fishery products, municipal waste and may also include by-products of food industry and services [9]. The overall classification of biofuels is displayed in **Figure 1.**

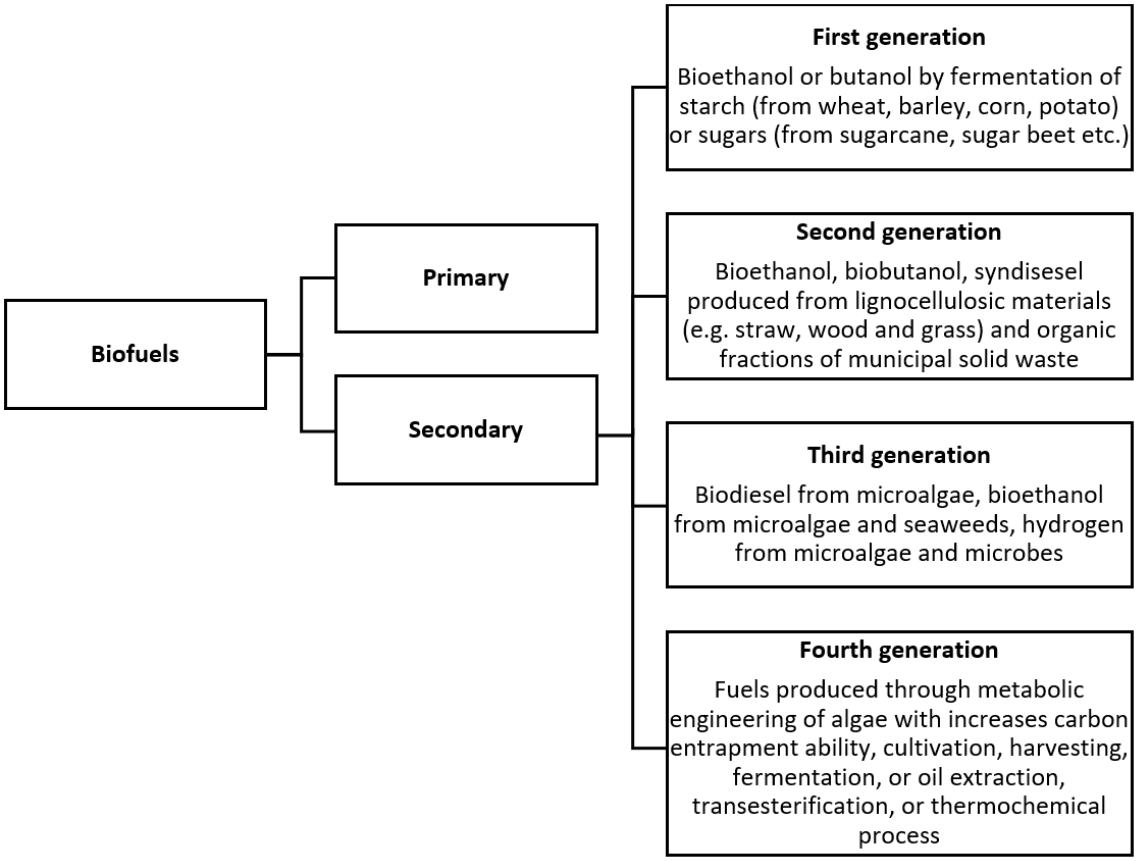

Figure 1. Classification of biofuels

First-generation

First-generation of biofuels are made of vegetable oils or sugars which can be found in eatable crops, and easily extracted by using conventional technologies. The most popular feedstocks for fuels such as biodiesel, bioethanol or biobutanaol are sugars, grains or seeds as corn, wheat, barley and sugarcane. The main processing technologies used in this generation are esterification and transesterification of oils, fermentation of sugars and thermochemical process. Currently, the largest volume of biofuels is produced in the form of ethanol. As a result, about 80% of first-generation bioethanol in the world comes from corn and sugarcane. Although first-generation biofuels may result in fuel-food competition due to the land requirement for growing crops, a reduction in carbon dioxide and monoxide, particulate matter and smoke emissions in exhaust are experienced [5].

Second-generation

Second-generation of biofuels or, in other words, advanced biofuels are the fuels that can be produced from different types of non-food biomass. The word 'non-food biomass' is understood as lignocellulosic materials. The feedstocks commonly used may include by-products such as cereal straw, sugarcane, bagasse, forest residues, waste (such as organic components of municipal solid waste) and dedicated feedstock (in a form of purposely-grown vegetative grasses, short rotation forest and other energy crops). However, it is important to understand that energy crops as any type of first-generation source require land for its growth, so they may end up competing with food and fibre production. The processing technologies associated with second-generation biofuel production are physical, chemical, biological pretreatment of feedstock, fermentation and thermochemical process. The products that can be obtained from the processing are usually bioethanol, biobutanol, biodiesel, syngas, biooil and biochar [10].

Even though second-generation biofuel has a lot of advantages such as greenhouse gas (GHG) savings, utilization of food waste and usage of non-arable land for growing energy crops, it still demands costly pretreatment of lignocellulosic feedstock and highly advanced technology for the effective conversion of biomass into fuel [5].

Third-generation

Third-generation of biofuels uses algae and seaweeds as a substrate. This type of feedstocks does not compete with food and other crops and can be cultivated in places that are not appropriate for others vegetations, such as shadowed and closed ponds. Besides, biofuels made of algae can be produced during the whole year and the oil yield might even exceed that of the best oilseed crops. The most typical biofuels made of algae are biodiesel, bioethanol, biobutanaol, syngas, biohydrogen and biomethane. The processing technologies required for the production of the fuels are cultivation, harvesting, oil extraction, transesterification, or fermentation, or thermochemical process [11]. Despite the fact that algae do not compete with food production, they are easy to grow and they have higher growth rate [5].

Fourth-generation

Since algae have received a significant interest as an alternative biofuel feedstock, recent research activities have been focused on the search for an ideal combination of algal species with high lipid content and their optimum growth conditions. Meanwhile, genetic modification or metabolic engineering could be a possible alternative to increase the lipid content and biomass yield of algae. For the fourth-generation biofuels, the more typical processing technologies are metabolic engineering of algae with increases carbon entrapment ability, cultivation, harvesting, fermentation, or oil extraction, transesterification, or thermochemical process. The products are the same as for the third-generation biofuels and they are usually biodiesel, bioethanol, biobutanol, syngas, biohydrogen, methane [6].

Important to realize that initial investment into fourth-generation biofuels is high and current research is at its primary stage. But at the same time, the possibility of cultivating algae with high lipid-containing yield and heightened CO2 capture ability is a promising solution for overcoming the challenges previous generations of biofuels are facing now [5].

1.2. Characteristics and composition of lignocellulosic biomass

The most abundant raw material for biofuels production is plant matter, or in other words lignocellulosic biomass. This type of biomass exists in natural plants such as grass, bushes or trees. Moreover, it includes waste biomass from industrial sectors such as agriculture and forestry, or special energy crops, which are produced specifically with the purpose of further biofuels conversion [12].

Lignocellulosic biomass consists of three main polymers cellulose (40-50%), hemicellulose (30-40%) and lignin (10-25%), as well as proteins, sugars and inorganic components, from which celluloses and lignin make up approximately 75-85% of dry matter [13]. These proportions are the main factor for determination of the energy conversion [14]. Biochemical composition and plant cell structure are shown in **Figure 2.**

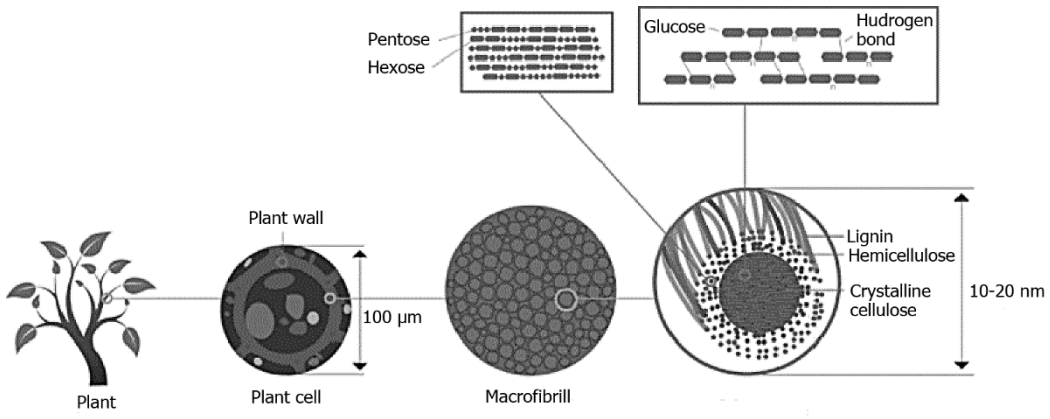

Figure 2. Biochemical composition of lignocellulosic biomass [15]

The most valuable component of biomass for biofuels production is cellulose $(C_6H_{10}O_5)_n$. It is an important structural organic compound of the primary cell wall of most plants. Lignocellulosic biomass has a cellulose content of 25–55%, depending on plant species. Usually, hardwood contains higher amounts of cellulose than softwood [6].

The second valuable component is hemicellulose $(C_5H_8O_4)_n$. It has a complex structure of carbohydrate and unlike cellulose, hemicellulose is composed of combinations of pentoses, hexoses and glucose. It is mostly found in plant cell wall together with cellulose and its content depending on plant species is about 20–40% [11].

The third main component of lignocellulosic biomass is lignin. It is so because lignin is a slowly decomposing component of plants, that prevents degradability of cellulose and hemicellulose in the plant structure. Lignin is a phenolic polymer, and it creates a physical barrier to enzymes when they need to act on the carbohydrate fraction of a lignocellulosic biomass. Its content is around 10-20% and in a plant, lignin provides structural strength of a plant [12].

1.3. Biomass conversion technologies

Production of bioethanol is a complex process composed of four sequential steps: pretreatment, hydrolysis, fermentation and distillation [16].

1.3.1. Pretreatment

In lignocellulosic materials, pretreatment is a stage of deformation of rigid components, which structured of lignin, cellulose and hemicellulose. Pretreatment is highly recommended due to the benefits it brings consequently. It increases the yield of fermentable sugars and prevents premature degradation of the yielded sugars. Additionally, it prevents the formation of inhibitors in the following steps as hydrolysis and fermentation, it lowers the processing costs and the demand for conventional energy in general. There are several pretreatment methods for bringing up the amorphous form of the celluloses, and more sugar monomers at the end of the process. One of the sufficient pretreatment is physical size reduction. It is usually done for easier access to hydrolysis. However, physical reduction alone will not be effective enough, even though it can be applied as the only one pretreatment method. Further chemical pretreatment, for instance, would bring out a better yield of reducing sugars at the end of hydrolysis [12].

Pretreatment methods can be divided into separate mechanical, thermal, chemical and biological as well as into any combinations of them. As an example of mechanical pretreatment, it can be chopping, milling, grinding or blending. Following pretreatment can be steam explosion of biomass and thermal hydrolysis. Acid, alkali or oxidizing agents are commonly used in the chemical pretreatment [17].

1.3.2. Hydrolysis

Hydrolysis is required for conversion of carbohydrates into its sugar components. It usually occurs with the addition of water molecule and it is catalyzed by enzyme or acid. Although enzymatic hydrolysis is considered impractical for commercial purposes due to its excessive cost, enzymes in comparison to

acids, work at a mild environment and consequently demand less for the equipment maintenance. The stage of hydrolysis has a huge importance for the further bioethanol production because the quality of hydrolysate will affect the subsequent fermentation [18].

1.3.3. Fermentation

The stage of direct ethanol production from the metabolic activity is called fermentation. It occurs with the presence of specific fermentation agents (yeast or bacteria) which consume sugars under supporting conditions in the absence of oxygen. Besides, additional factors that play important role in the fermentation process are the microbes growth rate and genetic stability, tolerance of inhibitors, osmosis and alcohol, productivity, and the yield of ethanol [19].

1.4. Anaerobic digestion

Conversion technology of anaerobic digestion (AD) is one of the low energy-consuming process among all bioenergy production technologies. One of the sources commonly used for AD is lignocellulosic biomass, that was discussed previously in this work. Although biogas production from lignocellulosic biomass has a big potential, its complex structure create recalcitrance in AD. In order to overcome these challenges, feedstock pretreatment and consequent co-digestion should be applied [20].

Anaerobic decomposition of organic matter is a complex process involving several microorganisms, with a wide variety of metabolic functions. It involves four key stages such as hydrolysis, acidogenesis, acetogenesis and methanogenesis. The overall process of AD can be described as a chemical reaction of organic materials digestion into carbon dioxide and methane by means of anaerobic microorganisms [21].

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$$

The first stage of hydrolysis breaks down the organic material into simple sugars, amino acids and fatty acids with the help of hydrolytic bacteria. After that, hydrolysis of acidogenic bacteria breaks down the components of the first step into volatile fatty acids, ammonia, carbon dioxide, hydrogen sulfide and other by-products within the second step of acidogenesis. Then, the step of acetogenesis is followed and the molecules created in the previous step are digested by acetogenic bacteria, in order to produce acetic acid, carbon dioxide and hydrogen. Finally, during the last step of methanogenesis, methanogenic bacteria convert the intermediate products into methane, carbon dioxide and water,

making up biogas output. This process is sensitive to pH levels, which must be between 6.5 and 8.0. The process itself can be run in batches or in a continuous flow system.

Temperature is a key component to the efficiency of anaerobic digesters. The more energy put into a reaction, the faster the reaction runs. Special organisms are responsible for the digestion process and it is vital to ensure that the process is kept within a certain temperature range in order to maximize reaction speed. The most widely used types of microorganisms are mesophiles and thermophiles. Moderate temperature range between 25-40°C allows mesophiles to be most efficient while higher temperature range between 45-80°C allows thermophiles to run most efficiently. These two types of temperature conditions named mesophilic or thermophilic regimes. If the temperature is monitored during digestion process and kept under specific range, the rate of reaction will be at its highest value producing more gas in the same amount of time [20].

The method utilized to measure anaerobic biodegradability of substrates is BMP test. It allows at the laboratory scale to determine the methane production from a specific substrate. Moreover, there are several advantages of the test such as easy setup and low-cost. However, the test require time and may take from 20 to 60 days, depending on the substrate [22].

Environmental aspects of AD

As far as organic waste has become an ecological problem, it also has been recognized as a valuable resource that can be converted into useful products, via microbial transformations. Thus, AD appears to be one of the most promising solutions for organic waste reduction and decreasing GHG emissions due to the fact, that those gases are not releasing into the atmosphere but captured for further use. Biogas can be used directly for water and space heating, or for electricity generation and internal combustion engines. While AD for biogas production is seen as a useful tool for green energy, remains of this process also can be sustainably used as a fertilizers what can increase economic profitability and environmental sustainability of AD plants [23].

Agriculture is a large contributor to global warming as it responsible for around 30% of the total global anthropogenic emissions of GHGs. The most significant emissions that result from agriculture are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), nitric oxides (NO_x) and ammonia (NH_3) [24].

Impact on environment from AD could be estimated according to the following parameters:

1. Acidification and eutrophication (AP and EP) - assessment according to the measurements of ammonia produced in a liquid digestate, and escape during its open-air storage.

2. Global warming potential (GWP) - usually carbon dioxide emissions from biogas combustion are not considered as they are biogenic in nature. However, having the level of this parameter in mind very important for the overall influence to the environment.

3. Human and eco-toxicity potentials - the use of fossil fuels as an energy source for manufacture of the plants is the major contributor to water toxicity. Short-term emissions of nickel and long-term emissions of beryllium, cobalt and vanadium must be measured and compared with the standard values.

4. Ozone depletion potential (ODP) - ozone depletion is caused by the release of halons such as bromotrifluoromethane during the combustion of the biogas.

5. Photochemical oxidant creation potential (POCP) - this impact is estimated according to the emissions of methane from the digestate storage.

Replacing a fossil-fuel system with heat and electricity generated in AD cogeneration system considered here could lead to significant reduction in most impacts. However, every particular project requires more exact evaluation of environmental impact depending on the specific details of it [23].

Energy return on energy invested and energy balances

One of the methods that can be applied to evaluate the energy balance is spatial and statistical analyses. While the spatial analysis was conducted in order to identify the location of the feedstock, their size, distance to both a biogas plant and the road network. Various statistical methods applied for assessing the potentially obtainable biomass yields as well as for identifying the factors that influence the achievable biomass yields from the agricultural fields. The spatial and statistical analyses and the corresponding results are applied to assess the yields of biomass that can be obtained and to estimate the energy balances of utilizing agricultural waste from the fields for biogas production [23].

For the energy balance estimation two main criteria should be taken into account:

1. The annual net energy gain (NEG) that shows the difference between output and input energy:

2. Energy returned on energy invested (ERoEI) that corresponds to the ratio of the amount of usable energy delivered from a particular energy to the amount of energy used to obtain that energy resource.

a. Energy outputs

For estimating the obtainable energy yields, the methane content in biomass materials should be assessed. However, the biomass quality and thereby the methane yield is highly influenced by the harvesting time [25].

b. Energy inputs

The processes used to estimate the energy consumption of the utilization of agricultural waste for biogas production can be divided into the following sub-steps:

- Harvest and collection
- Transport of harvest machinery
- Baling of the grass
- Loading to tractor
- Transport to road
- Offloading from tractor and loading to truck
- Transport to a biogas plant
- Offloading from truck
- Pre-treatment
- Feeding to digester
- Operation of the biogas plant and management of the digestate
- Fertilization with digestate

However, depending on particular grain production chain, some of the energy inputs might be equal to zero. For example, if we talk about agricultural straw, then energy input such as harvest and collection, transportation and bailing should not be added into the overall energy inputs.

On the whole, the estimated areas should be assessed according to this two parameters and compared for finding the better energy balance for the future AD production place [20].

The added value of anaerobic digestion

Depending on the fuel, different added value by-products could be obtained. For instance from various crop residues and by-products of sugarcane like bagasse, sugarcane tops, molasses and vinasse such by-products as bioethanol, biodiesel, biobutanol, 2, 3-butanediol, biohydrogen, bioelectricity, biopolymer, different enzymes, organic acids, amino acids, pigments, animal feed, composite, chelating agents, and alkaloids can be produced [26].

In case of anaerobic digestion, the added value to the organic waste products can be obtained in form of transforming the digestate into the useful products. An increase in economic sustainability of small/middle size biogas plants can be achieved due to treatment cost reduction of digestate as green fertilizers. The investment cost of anaerobic digestion is moderate and the potential of self-help is relatively high. The use of digestate as fertilized thus can create added value, making biogas production economically feasible. Besides, biogas plants could contribute to regional development and waste management at the same time [21].

1.5. Biogas purification

Biogas obtained from AD consists mainly of methane (CH_4, 40-75%), carbon dioxide (CO_2, 15-60%) and small amounts of water (H_2O, 5-10%), ammonia (NH_3 <1%), hydrogen sulphide (H_2S, 0.005-2%), oxygen (O_2, 0-1%), nitrogen (N_2, 0-2%) and carbon monoxide (CO < 0.6%). To get pure biomethane with a methane content >95vol% comparable with natural gas, impurities should be removed. For that reason, biogas upgrading technologies are required. Some of the specific technologies used are water scrubbing, pressure swing adsorption and chemical absorption [20].

Due to the fact that biogas can be produced at different places (sewage treatment plants, landfills or digestion plants for agricultural waste) and different operational conditions can be applied, the chemical composition of the biogas outputs can also vary. Depending on the biogas content and the

requirements for natural gas substitutions, different technologies should be chosen. For example, the biogas obtained from lignocellulosic biomass and biogas obtained from animal waste contain different amount of ammonia that should be removed. Thus, different approaches should be chosen for its purification. **Table 1** explains the impurities and their possible consequences for biogas usage as a fuel.

Table 1. Biogas impurities and its consequences [27]

Impurity	Possible impacts
Water	Corrosion in compressors, gas storage tanks and engines due to reaction with H_2S, NH_3 and CO_2 to form acids; Accumulation of water in pipes; Condensation and/or freezing due to high pressure
Dust	Clogging due to deposition in compressors, gas storage tanks
H_2S	Corrosion in compressors, gas storage tanks and engines; Toxic concentrations of H_2S (> 5 cm^3 m^{-3}) remain in the biogas; SO_2 and SO_3 are formed due to combustion, which is more toxic than H_2S and causes corrosion with water
CO_2	Reacts with to form acids
Siloxanes	Formation of SiO_2 and microcrystalline quartz due to combustion; Deposition at spark plugs, valves and cylinder heads abrading the surface
Hydrocarbons	Corrosion in engines due to combustion
NH_3	Corrosion when dissolved in water
O_2/air	Explosive mixtures due to high concentrations of O_2 in biogas
Cl^-	Corrosion in combustion engines
F^-	Corrosion in combustion engines

Generally, biogas treatment consists of two stages:

1. The cleaning process, where the trace components harmful for the convenient end-users are removed (ex. natural gas grid);

2. Upgrading process, where mainly CO_2 is removed in order to adjust the calorific value and relative density for meeting the specific standards.

After that transformation, the amount of CH_4 will increase up to 95-97% while CO_2 decrease to 1-3%. This composition ratio is an alternative for natural gas. However, end consumer sets its parameters for gas depending on the equipment requirements they use.

The techniques of biogas cleaning and upgrading processes are presented in the next subchapters.

A. Removal of water

For the different quality standards, biomethane requires different water content percentage. For example, in the gas pipeline water content should be about 100 mg m^{-3}, while for the compressed natural gas (CNG) vehicle fuels require a specific dewpoint. Usually the value of it is 10 °C below average winter temperature [26].

Depending on the temperature, the water content in the raw biogas varies. The lower the temperature, the lower the water content. For instance, the water content will be around 5% at a temperature of 35° C. In order to remove water from biogas (at the same time it can help to remove impurities, such as foam and dust) mainly two methods are usually applied: physical separation of condensed water or chemical drying.

A1. Physical drying methods

This method is based on removing water vapour through refrigeration. Physical drying methods prevent water contact with downstream equipment like compressors, pipes, activated carbon beds and others. This method is based on removing water vapour through refrigeration, but dewpoint can be lowered only to 0.5 °C, due to the problems with freezing on the surface of the heat exchanger. In order to achieve lower dewpoint, the gas has to be compressed before cooling and then later expanded to the desired pressure. The lower the dew point, the higher pressure needs to be applied [27].

A2. Chemical drying methods

The application of such techniques should be usually done at higher pressures. Only small amounts of water can be removed [27].

Methods based on gas drying include:

- adsorption of water vapour on silica, alumina or equal chemical components that can bind water molecules (adsorption dryer);
- absorption of water in tri-ethylene glycol;
- absorption of water with hygroscopic salts

The advantages and disadvantage of these techniques are presented in **Table 2**.

Table 2. Advantages and disadvantages of techniques for removal of water [26]

Method	Advantages	Disadvantages
Condensation methods: **Demister** **Cyclone** **Moister trap** **Water taps**	▪ Higher HC's dust and oil are removed ▪ Simple techniques ▪ Often used as a pretreatment before other techniques	▪ Atmospheric pressure: dew point minimum 1°C ▪ Gas at a higher pressure to reach lower dew point (minimal-18°C) but freezing can occur
Adsorption dryer: **Silica** **Aluminum**	▪ High removal: dew point from -10-20°C ▪ Low operational cost ▪ Regeneration possible	▪ More expensive investment: pressure 6-10 bar ▪ Dust and oil need to be removed in advance
Absorption with glycol	▪ High removal: dew point from - 5-15°C ▪ Higher HC's and dust are removed ▪ Not toxic or dangerous	▪ More expensive investment: high pressure and 200°C for regeneration ▪ Higher gas volumes (>500 m^3/h) to be economical
Absorption with hygroscopic salts	▪ High removal efficiency ▪ Not toxic or dangerous	▪ No regeneration done

A3. Removal of hydrogen sulphide

H_2S in raw biogas causes damage in the pipelines and motors, so it should be removed on an early stage of biogas upgrading process. Thus, cleaning technique of H_2S removal could be applied during the digestion process, as well as after. It means that hydrogen sulphide can be treated directly in the digester vessel, and also after we collect a certain amount of biogas in the gas holder. All advantages and disadvantages of the techniques for H_2S removal are mentioned in **Table 3.**

Table 3. Advantages and disadvantages of techniques for H_2S removal [26]

Method	Advantages	Disadvantages
Biological with oxygen/air (in filters, scrubbers, digester)	▪ Cheap investment and exploitation: low electricity and heat requirements, no extra chemicals or equipment required ▪ Simple operation and maintenance	▪ Concentration H_2S still high (100-300 cm^3 m^{-3}) ▪ Excess O_2/N_2 in biogas implies difficult upgrading or additional cleaning ▪ Overdosing air results in an explosive mixture
$FeCl_3/FeCl_2/FeSO_4$ (in digester)	▪ Cheap investment: storage tank and dosing pump ▪ Low electricity and heat requirements ▪ Simple operation and maintenance ▪ Compact technique ▪ H_2S not in biogas wire	▪ Low efficiency (100-150 cm^3 m^{-3}) ▪ Expensive operation (iron salt) ▪ Changes in pH/temp not beneficial for the digestion process ▪ Correct dosing is difficult

	▪ No air in biogas	
$Fe_2O3/Fe(OH)_3$-bed Rust steel wool impregnated wood chips or pellets	▪ High removal efficiency: > 99% ▪ Mercaptanes are also captured ▪ Cheap investments ▪ Simple	▪ Sensitive for water ▪ Expensive operation costs ▪Regeneration is exothermic: risk of chips ignition ▪ Reaction surface reduced each cycle ▪ Released dust can be toxic
Absorption in water	▪ H_2S <15 cm^3 m^{-3} ▪ Cheap when water is available (not regenerative) ▪ CO_2 is also removed	▪ Expensive operation: high pressure, low temperature ▪ Difficult technique ▪ Clogging of the absorption column possible
Chemical absorption NaOH $FeCl_3$	▪ Low electricity requirement ▪ Smaller volume, less pumping, smaller vessels (compared to absorption in H_2O) ▪ Low CH_4 losses	▪ Expensive investment & operation ▪ More difficult technique ▪ Not regenerative
Chemical absorption $Fe(OH)_3$ Fe-EDTA CooabTM	▪ High removal efficiency: 95-100% ▪ Cheap operation ▪ Small volume required Regenerative ▪ Low CH_4 losses	▪ Difficult technique; ▪ Regeneration through oxygenation $CO_2 \rightarrow H_2CO_3$ (using EDTA) leads to precipitation ▪ The buildup of thiosulfates from chelates + H_2S (using EDTA) ▪ Expensive
Membranes Biological	▪ Removal of > 98% is possible ▪ CO_2 is also removed	▪ Expensive operation and maintenance ▪ Complex
Biological filter	▪ High removal possible: > 97% ▪ Low operational cost	▪ Extra H_2S-treatment to reach pipeline quality ▪ O_2/N_2 in biogas implies difficult and additional upgrading steps
Adsorption on activated carbon (Impregnated with KI 1-5 %)	▪ High efficiency (H_2S <3 cm^{-3} m^3) ▪ High purification rate ▪ Low operation temperature ▪ Compact technique ▪ High loading capacity	▪ Expensive investment and operation ▪ CH_4 losses ▪ H_2O and O_2 needed to remove H_2S ▪ H_2O can occupy the binding places of H_2S ▪ Regeneration at 450°C Residue present till 850°C

A4. Removal of organic silicon-containing compounds (siloxanes)

Siloxanes are a group of components that contain a Si-O bound and organic radicals (methyl, ethyl and other organic groups) bound to the silicon atom. Siloxanes are used in cosmetics, pharmaceuticals and as anti-foam products. They share useful properties like high compressibility, low flammability, low surface tension and water repelling properties, high thermal stability, low toxicity (non-allergenic) and biodegradability. Both, linear and cyclic siloxanes can be present in biogas.

Siloxanes cause severe damage to engines. During incineration they are oxidized to silicon oxide and can consequently deposit as microcrystalline quartz in the combustion chamber, at spark plugs, valves or cylinder heads, abrading the inner surface of the motor. Engine manufacturers claim maximum limits of siloxanes in biogas, ranging from 0.03 mg m^{-3} to 28 mg m^{-3} [27].

Today there are several techniques for removing siloxanes available. A short summary presented in **Table 4**.

Table 4. Advantages and disadvantages of techniques for siloxanes removal [26]

Method	Advantages	Disadvantages
Absorption with organic solvents	▪ High removal efficiency (97%)	▪ Complete removal not possible
Absorption in strong acid	▪ High removal efficiency (<95%)	▪ Corrosion ▪ Environmental issues ▪ Hazardous chemicals
Absorption in strong base	*Not used due to CO_3^{2-} precipitation	▪ Corrosion; ▪ CO_3^{2-} precipitation ▪ Hazardous chemicals
Adsorption on silicagel	▪ High removal efficiency (<95%) ▪ Higher removal capacity *vs* activated carbon (50% extra) ▪ Regeneration possible (95% desorption at 250°C)	▪ High pressure needed ▪ Moisture decreases efficiency
Adsorption on activated carbon	▪ High removal efficiency (95%) ▪ Regeneration possible (desorption < desorption with silicagel at 250°C)	▪ High pressure needed (higher adsorption capacity) ▪ Moisture decreases removal efficiency
Cryogenic separation	▪ High removal efficiency (99.3% at -70°C); ▪ Removal of several impurities	▪ Expensive investment and operation (high pressure and low temperature)

A5. Removal of halogenated carbon hydrates

Higher and halogenated carbon hydrates are mainly found in landfill gas. They cause corrosion in engines and can be removed with activated carbon. Little molecules like CH_4, CO_2, N_2 and O_2 can migrate through the pores, while larger molecules are adsorbed. Generally, two tubes are used in parallel: one for treatment and one for regeneration. Regeneration is done by heating the activated carbon to 200 °C, thus evaporating the adsorbed components which are thereafter removed by an inert gas flow [27].

A6. Removal of ammonia

In industrial large-scale cleaning processes, NH_3 is often removed from the gas by a washing process with diluted nitric or sulfuric acid. The use of these acids demands installations made of stainless steel, that can be expensive for small-scale applications like biogas cleaning. NH_3 can also be removed with units filled with activated carbon and is also eliminated in some of the CO_2-removing units, like adsorption processes and absorption processes with water [26].

A7. Removal of carbon dioxide

Upgrading biogas to natural gas quality is a multiple step procedure. After removal of water (vapour), H_2S, siloxanes, carbon hydrates and NH_3, the removal of CO_2 is necessary in order to obtain the quality that meets the needed standards. As the CO_2 of the upgraded gas is removed, is the relative density decreased and the caloric value increased.

Depending on its intended use (pipeline or vehicle fuel), biomethane consists typically of 97-99% methane and 1-3% CO_2. Typical pipeline specifications require a CO_2 content of less than 3% whereas vehicle fuel specifications require a combined CO_2N_2 content of 1.5-4.5%. One of the following techniques can be used to remove CO_2 from the biogas: physical and chemical CO_2-absorption, Pressure Swing Adsorption (PSA) and Vacuum Swing Adsorption (VSA), membrane separation, cryogenic separation and biological methane enrichment. A short summary presented in **Table 5**.

Table 5. Advantages and disadvantages of techniques for carbon dioxide removal [26]

Method	Advantages	Disadvantages
Absorption with water	▪ High efficiency (>97% CH_4) ▪ Simultaneous removal of H_2S	▪ Expensive investment ▪ Expensive operation

	When H_2S < 300 cm^3 m^{-3} ▪ Easy in operation ▪ Capacity is adjustable by changing pressure or temperature ▪ Regeneration possible ▪ Low CH_4 losses (<2%) ▪ Tolerant for impurities	▪ Clogging due to bacterial growth ▪ Foaming possible ▪ Low flexibility toward variation of the input gas
Absorption with polyethylene glycol	▪ High efficiency (>97% CH_4) ▪Simultaneous removal of organic S components, H_2S, NH_3, HCN and H_2O ▪ Energetic more favourable than water ▪ Regenerative ▪ Low CH_4 losses	▪ Expensive investment ▪ Expensive operation ▪ Difficult in operation ▪ Incomplete regeneration when stripping/vacuum (boiling required) ▪ Reduced operation when dilution of glycol with water
Chemical absorption with amines	▪ High efficiency (>99% CH_4) ▪ Cheap operation ▪ Regenerative ▪ More CO_2 dissolved per unit of volume (compared to water) ▪ Very low CH_4 losses (<0.1%)	▪ Expensive investment ▪ Heat required for regeneration ▪ Corrosion ▪ Decomposition and poisoning of the amines by O_2 or other chemicals ▪ Precipitation of salts
PSA/VSA **Carbon molecular sieves** **Molecular sieves (zeolites)** **Alumina silicates**	▪ Highly efficient (95-98% CH_4) ▪ H_2S is removed ▪ Low energy use: high pressure, but regenerative ▪ Compact technique ▪Also for small capacities ▪ Tolerant to impurities	▪ Expensive investment ▪ Expensive operation ▪ Extensive process control needed ▪ CH_4 losses when malfunctioning of valves
Membrane technology **Gas/gas** **Gas/liquid**	▪ H_2S and H_2O are removed ▪ Simple construction ▪ Simple operation ▪ High reliability ▪ Small gas flows treated without a proportional increase of costs ▪ Gas/gas ▪ Removal efficiency: <92% CH_4 (1 step) or > 96% CH_4 ▪ H_2O is removed ▪ Gas/liquid ▪ Removal efficiency: > 96% CH_4 ▪ Cheap investment and operation ▪ Pure CO_2 can be obtained	▪ Low membrane selectivity: compromise between purity of CH_4 and amount of upgraded biogas ▪ Multiple steps required (modular system) to reach high purity ▪ CH_4 losses ▪ Little operational experience
Cryogenic separation	▪ 90-98% CH_4 can be reached ▪ CO_2 and CH_4 in high purity ▪ Low extra energy cost to reach liquid biomethane (LBM)	▪ Expensive investment and operation CO_2 can remain in the CH_4
Biological removal	▪ Removal of H_2S and CO_2 ▪ Enrichment of CH_4	▪ Addition of H_2

	▪ No unwanted end products	▪ Experiments not possible at large scale

2. AIMS AND OBJECTIVES OF THE STUDY

Gaseous fuels as biogas or biomethane have a huge potential as a sustainable source of energy. These fuels are a promising solution for the transport sector, as well as renewable solutions to diminish the share of fossil fuels in the energy mix.

The aim of this thesis is to analyze the potential for anaerobic digestion of biomass samples from bioethanol production-waste and compare its results with samples from all the stages of bioethanol production process (pretreatment, hydrolysis, fermentation and distillation). Also, the results are compared with samples of untreated biomass. Based on the results, it will be possible to find the best stage for biogas production and propose a possible and more reasonable way for biogas purification.

This research will focus on the challenges of bioethanol production and on the production-waste that is generated and left over after the distillation process. This production-waste still has high energetic value and can be further utilized to increase the energy output from the biomass. The objectives set up for the development of the thesis are:

1. Analyze the biogas production of samples from all stages of bioethanol production process;
2. Study how these stages may influence biogas yield of biomass samples from barley straw;
3. Find the best stage for biogas production;
4. Review and suggest possible purification technics for the produced biogas.

While first three objectives are based on experimental data, the fourth one is mainly theoretical due to the absence of the concrete requirement for further cleaned biogas usage. Since it is very difficult to define the best biogas purification technique, the criteria for this choose were suggested and explained.

Figure 3 illustrates the pathway proposed for the development of this research.

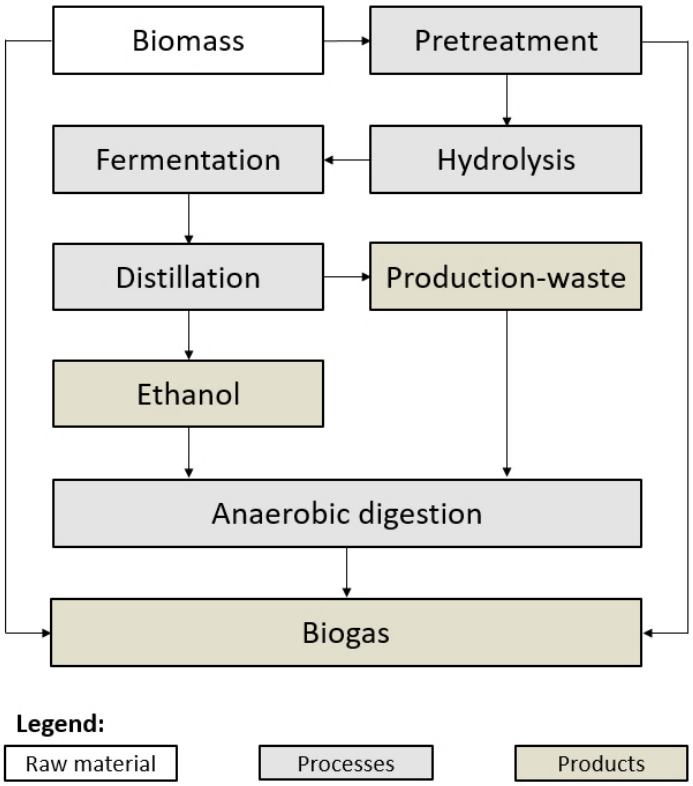

Figure 3. Pathway proposed for the utilization of bioethanol production-waste (adapted from [7]).

3. MATERIALS AND METHODS

Samples from all stages of bioethanol production process were analyzed in this work. This process includes four steps – pretreatment of biomass, hydrolysis of cellulose to sugars, fermentation of sugars into ethanol, and distillation to get high-quality ethanol [28].

3.1. Bioethanol production process

3.1.1. Biomass

Barley Straw (Hordeum vulgare) was the biomass used in all experiments. This feedstock was grown in Tartu area (Estonia). The samples were dried to a moisture content less than 10% or 100 g kg^{-1}, further milled for the particle size reduction (to 1-3mm or less), and sieved for the experimental procedure, using a Cutting Mill ZM 200 (Retsch GmbH) [25].

3.1.2. Pretreatment

Since the cellulose fibres in the biomass are densely packed with hemicellulose and lignin, the conversion of cellulose into ethanol is quite difficult. In order to break down this structure and to gain access to the sugars from cellulose and hemicellulose, the application of pretreatment is needed. The main goal of the pretreatment is to improve further conversion steps.

Thus, after milling the biomass, it was pretreated with the N_2 explosive decompression method (NED). The simplified schematic of N_2 explosive decompression pretreatment system is shown in **Figure 4**.

For the second step of the pretreatment, 100 g of dry biomass was weighed, placed into a pressure vessel and mixed with distilled water until watery biomass paste was gained. The reactor was closed with customized pressure vessel cap and pressurized with N_2 gas to a pressure of 1-30 bars. The pressurized samples were then heated up in the reactor vessel from 25°C -150°C. When the intended temperature was reached, the reactor was cooled down to at least 80°C, and the pressure was released through the valve. After the pretreatment, the samples were cooled down to a temperature below 50°C and prepared for enzymatic hydrolysis [29].

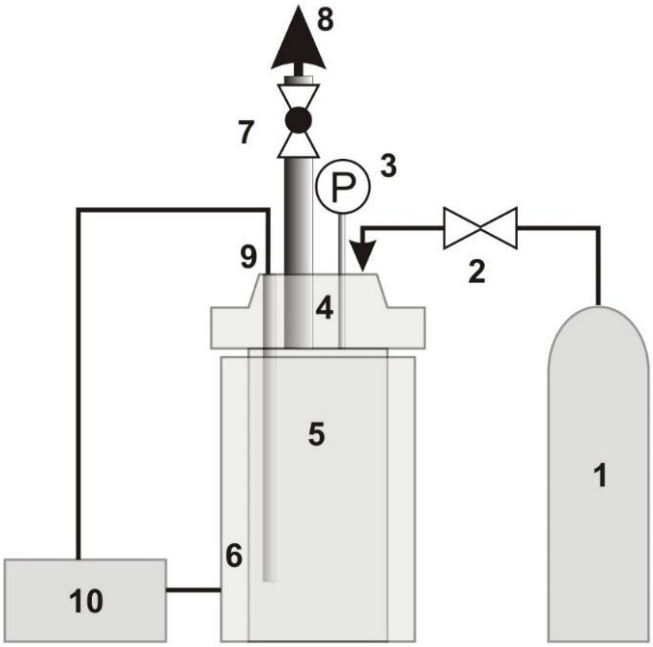

Figure 4. Diagram of N_2 explosive decompression pretreatment system.
1- N_2 tank, 2- pressure control valve; 3- manometer; 4- modified pressure vessel cap; 5 - pressure vessel; 6- ceramic contact heater; 7- pressure release valve; 8- ventilation system; 9- thermocouple; 10- temperature controller unit [30]

3.1.3. Hydrolysis

After the NED pretreatment was applied, the enzyme Accellerase 1500 was added to the samples, in a ratio of 0.3 mL per g of biomass, to perform the conversion of cellulose into glucose. Then, the incubation flask was filled with distilled water to a volume of 1000 mL, and the hydrolysis was carryout for 24h, at a temperature of 50°C, under constant stirring in the shaker Unimax 1010, Heidolph Instruments GmbH & Co.KG [31].

3.1.4. Fermentation

Afterwards, the dry yeast *Saccharomyces cerevisiae* was added to the hydrolyzed solution in the amount of 2.5 g per 100 g of biomass, to convert the glucose into ethanol. This process was carried out at room temperature, under low oxygen conditions in 1000 mL glass bottles sealed with a fermentation tube. The whole fermentation process lasted 7 days [32].

3.1.5. Distillation

In the next step, the distillation was processed to separate the ethanol from the liquid mixture. For this, the samples were kept in the oven at 95 °C, for one hour. The materials that are left after the distillation process are the production-waste, and are further used for AD.

3.2. Anaerobic digestion

For the anaerobic digestion, samples from all the stages of bioethanol production process were used (pretreatment, hydrolysis, fermentation and distillation). The biomethane potential test (BMP) was used to determine the methane yield of the organic substrates under specific conditions, and the biogas produced was estimated.

3.2.1. Inoculum

The inoculum was collected from the wastewater treatment plant in Tartu (Estonia), and incubated for 4 days at 36°C for degasification.

3.2.2. Experimental procedure

The BMP assay utilized in this research was based on a modified version of the guidelines described by Owen et al [33]. The experiments were carried out in triplicate, using 575 mL plasma bottles that were filled up with 150 mL of inoculum and 0.3 g TS of substrate, reaching a total volume of 200 mL. After, the bottles were flushed with nitrogen to remove the oxygen and to assure the anaerobic conditions. The bottles were incubated at the temperature of 36°C, during 42 - 45 days. The production of biogas was estimated by measuring the pressure increase in the test bottles. Thus, the pressure was measured before and after GC analysis, using a BMP-Testsystem WAL (WAL Mess- und Regelsysteme GmbH). The methane content was determined chromatographically in the gas chromatograph CP-4900 Micro-GC, Varian Inc. To study the methane production in each batch, a blank teste composed of just inoculum was prepared, and the methane production of the samples was subtracted to the methane production of the inoculum. All the results were expressed in mol of CH_4 per 100g of initial dry biomass. For each substrate, the duration of the BMP test was specifically determined. Biogas production and gas composition were determined periodically. The bottles were mixed manually once per day [22].

3.2.3. Analytical methods

The total solids (TS) and volatile solids (VS) content were analyzed according to method 1684 of U.S. Environmental Protection Agency (EPA) [34]. The TS content was determined after drying the samples overnight, under the temperature of 105°C. The VS content in the organic waste was found by subtracting the total solids content and the ash content after ignition, under the temperature of 550°C.

3.2.4. Calculations

The molar quantity of initial methane gas in the test bottle $[CH_{4\ I}]$ was determined with the help of equation 1:

$$[CH_{4\ I}] = MF \frac{P_I\, V_{HS}}{R\ (273{,}15+T)} \quad (1)$$

where P_I (Pa) is the initial headspace partial pressure, V_{HS} (m^3) is volume of the headspace, MF is the methane fraction measured by the gas chromatography in the current time interval, R is the ideal gas constant (8,314 $Jmol^{-1}K^{-1}$), and T the incubation temperature (ºC).

The molar quantity of final methane in the headspace of the bottle $[CH_{4\ F}]$ is given by equation 2:

$$[CH_{4\ F}] = MF \frac{P_F\, V_{HS}}{R\ (273{,}15+T)} \quad (2)$$

where P_F (Pa) is the final headspace partial pressure, measured after the GC analysis.

The cumulative methane $[CH_{4\ C}]_t$ produced during the current time interval was calculated with the help of equation 3:

$$[CH_{4\ C}]_t = ([CH_{4\ I}]_t - [CH_{4\ F}]_{t-1}) + [CH_{4\ C}]_{t-1} \quad (3)$$

where $[CH_{4\ I}]_t$ represents the initial concentration of methane in the headspace of the bottle during the current interval of time, $[CH_{4\ F}]_{t-1}$ characterizes the final methane concentration in the headspace of the bottle during the previous time interval, and $[CH_{4\ F}]_{t-1}$ is the cumulative methane produced in the previous time interval.

The methane production was modelled by the assistance of the software GraphPad Prism 7.0, using a non-linear regression model, that was fitted in a first-order exponential association equation (equation 4) [35, 36].

$$B = B_{max} (1 - e^{-kt}) \quad (4)$$

where B is the cumulative methane production during the time (t), B_{max} is the maximum methane yield and k is the rate constant.

The maximum methane yield B_{max}, corresponds to the cumulative methane yield during 45 days of incubation and it was calculated with the help of equation 4. The digestion time required to reach 85% and 95% of the methane yield, was calculated from the maximum methane yield.

3.3. Statistical analysis

The statistical analysis was performed with the assistance of the software GraphPad Prism 7. The TS and VS results were analyzed using descriptive statistics, and Kolmogorov-Smirnov's test for normality because the number of samples was small (n=3). The null hypothesis in this test is that both groups are samples with an identical distribution. To find the differences between the substrate groups Krustal-Wallis test was used. Moreover, the post hoc test Dunn's multiple comparison test was applied, to study which of the groups are different from each other.

The methane production was analyzed using descriptive statistics and the Shapiro-Wilk's test for normality, where the null hypothesis is that number is normally distributed. The Krustal-Wallis test was performed to determine the differences between the groups, and the post hoc test Dunn's multiple comparison test to analyze which groups differ from each other. The means are presented with their standard errors (±SE).

4. RESULTS AND DISCUSSION

In this chapter, the experimental results are compared and discussed. The research focuses on the challenges of bioethanol production, on the production-waste that is left over after the separation of bioethanol in the distillation process, and on the different possibilities for biogas purification.

4.1. Biomass analysis

Different types of feedstock can be characterized on the basis of its relative proportion of cellulose, hemicellulose, lignin, and ash. As it can be seen from **Table 6**, the barley straw used in these experiments as a sample biomass, contained 45.7% of cellulose, 32.6% of hemicellulose and 5.2% of lignin. Therefore, relatively high cellulose content in barley straw makes it a suitable biomass for bioethanol production [29].

Table 6. The results of biomass analysis determined using Ankom 2000 at Institute of Technology (Laboratory of Biofuels) of Estonian University of Life Sciences

Component Content	**%**
Cellulose	45.7 ± 0.2
Hemicellulose	32.6 ± 0.5
Lignin	5.2 ± 0.0
Ash	3.8 ± 0.1

4.2. Biogas composition

Figure 5 shows the chemical composition of the biogas generated from untreated biomass. The biogas production has an exponential growth from day 0 till day 14. The stationary phase starts from day 14 until the end of the whole experiments, which lasts in total 44 days. On the last day of the experiments, the amount of biogas produced was 1.91 mol^{-1} 100 g raw biomass. The highest fraction of methane was achieved on day 5 (62% of methane and 38% of other compounds). In the last day of the experiments, this fraction became 55% of methane and 45% of other compounds. The fraction of methane in the biogas is smaller in the beginning of the experiments and then it increases and starts to exceed the fraction of the other components (carbon dioxide, nitrogen, hydrogen and ammonia).

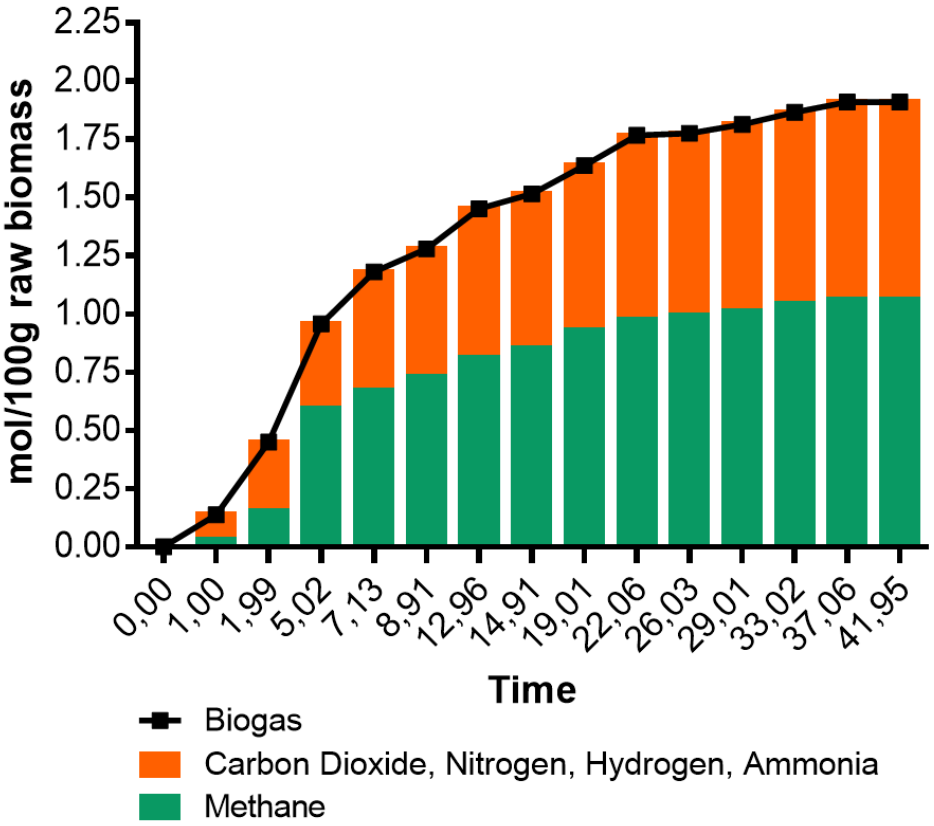

Figure 5. Biogas composition of the untreated biomass source

Figure 6 illustrates the biogas composition for pretreated biomass samples. The production of biogas has an exponential growth from day 0 until day 12. The stationary phase begins on day 12 and lasts until day 44 (end of the experiments). The amount of biogas produced during the last day of the experiments was 1,99 mol^{-1} 100 g raw biomass. The largest fraction of methane was found on day 1 (65% of methane and 35% of other compounds,) and by the last of the experiments this percentage ratio drops and becomes 56% of methane and 44% of other compounds. In fact, the fraction of methane in the biogas content in this experiment exceeds the fraction of other components (carbon dioxide, nitrogen, hydrogen and ammonia) during the experiments.

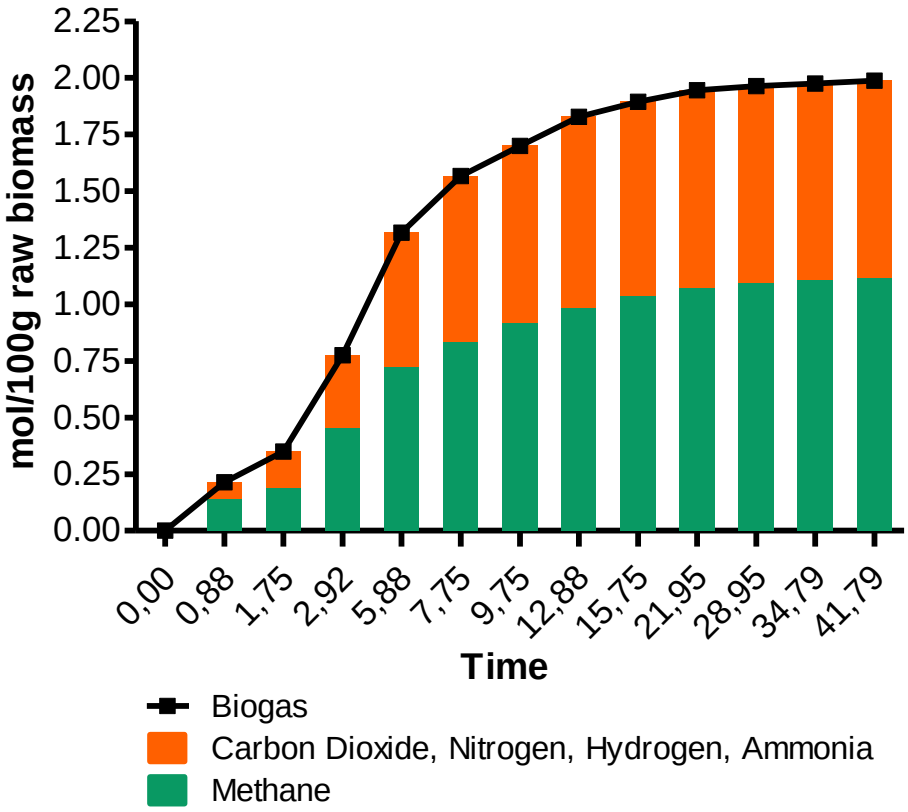

Figure 6. Biogas composition of the pretreated biomass source

Figure 7 corresponds to the chemical composition of the biogas generated from hydrolyzed biomass samples. The exponential growth begins on day 0 and continues until day 10. The stationary phase starts on day 10 and lasts until the end of the experiments. The amount of biogas produced during the last day of experiments is 2,11 mol^{-1} 100 g raw biomass. The largest fraction of methane was achieved on day 1 (59% of methane and 41% of other compounds), and by the last day, this percentage drops to 55% of methane and 45% of other compounds. Similarly to pretreated samples, for hydrolyzed material, the fraction of methane in the biogas exceeds the fraction of other components.

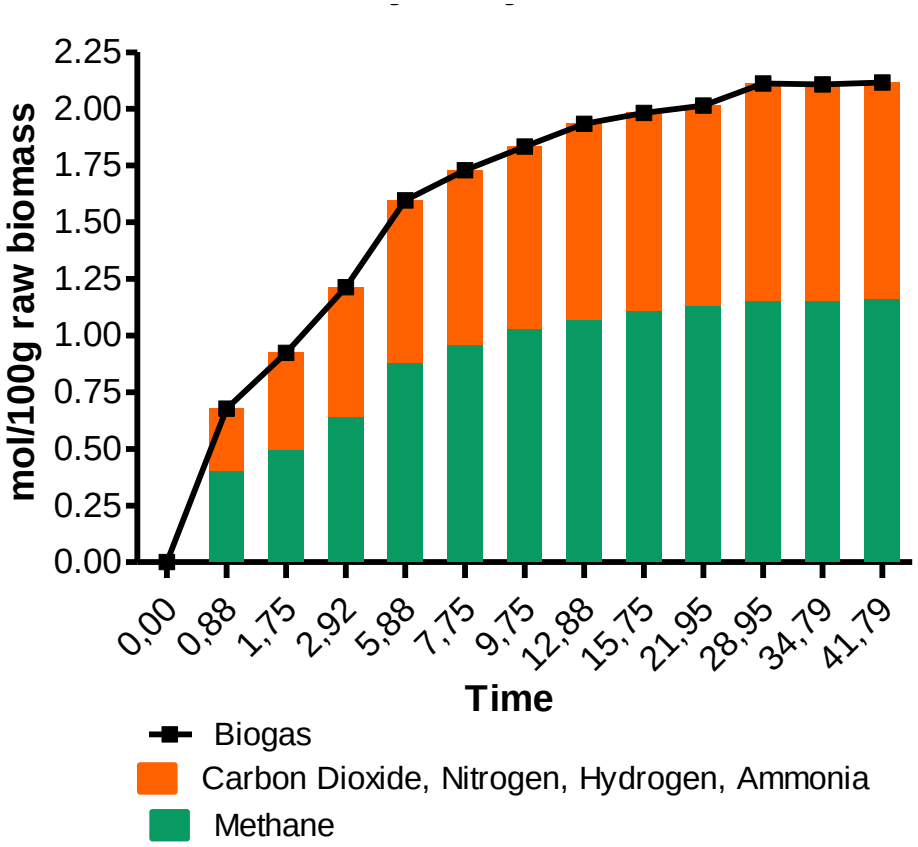

Figure 7. Biogas composition of the hydrolyzed biomass source

On **Figure 8** the chemical composition of the biogas generated from fermented biomass sample can be observed. The biogas production has an exponential growth from day 0 until day 14. The stationary phase starts from day 14 until the end of the experiments, which lasted in total 44 days. During the last day of the experiments, the amount of biogas produced was 2.06 mol^{-1} 100 g raw biomass. The highest fraction of methane was achieved on day 1 (67% of methane and 33% of other compounds), and in the last day, it became 59% of methane and 41% of other compounds. Also, the fraction of methane exceeds the fraction of other components (carbon dioxide, nitrogen, hydrogen and ammonia), especially in the beginning of the experiments.

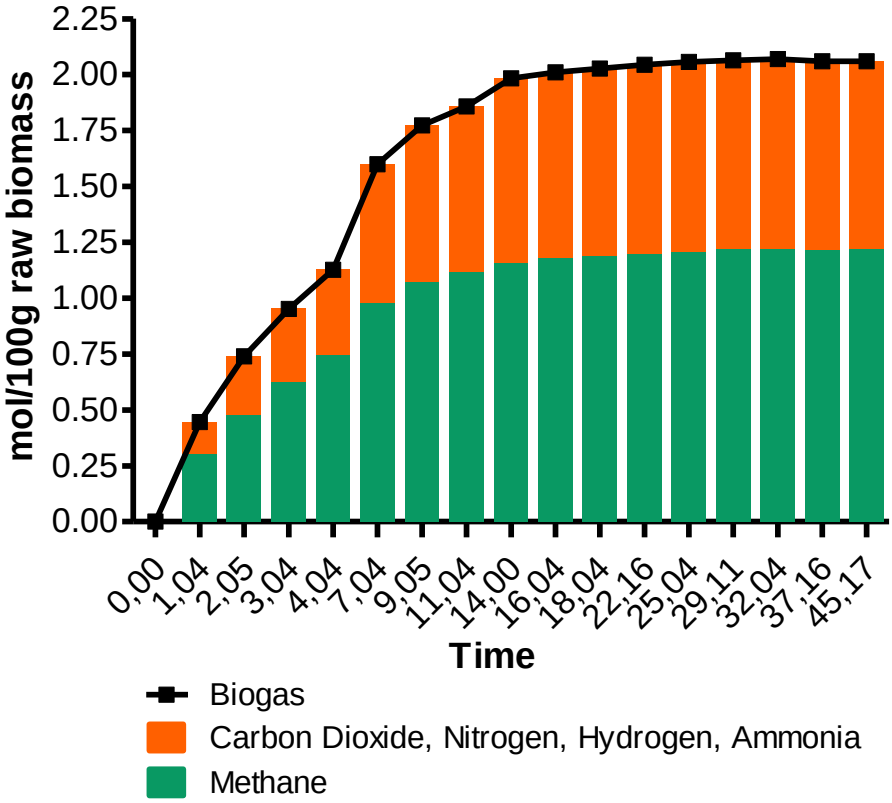

Figure 8. Biogas composition of the fermented biomass source

Figure 9 shows chemical composition of the biogas generated from bioethanol production-waste. The exponential growth begins on day 0 and continues until day 14. The stationary phase starts on day 14 and lasts until the end of the experiments. The amount of biogas produced during the last day is 2,00 mol/100 g raw biomass. The largest fraction of methane was achieved on day 1 (67% of methane and 33% of other compounds), and by the last day of experiments, these ratios drop to 59% of methane and 41% of other compounds.

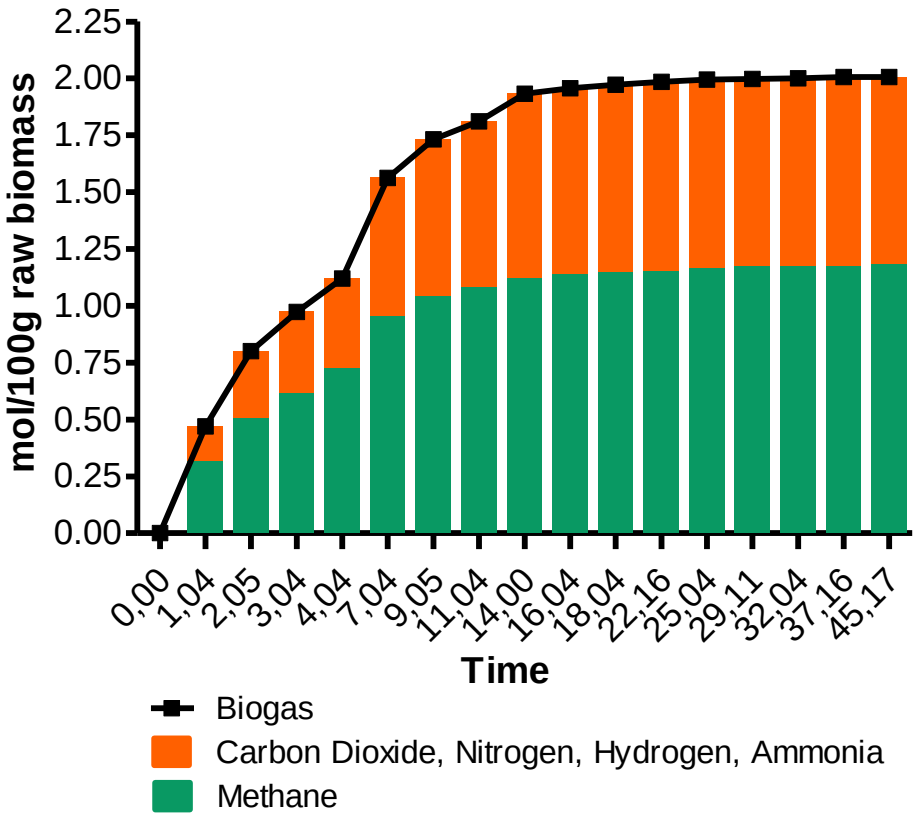

Figure 9. Biogas composition of the bioethanol waste-product biomass source

The highest ratio of methane in the overall biogas production was found in the samples of bioethanol production-waste and fermented substrates (59% of methane over 41% of other compounds). Then, it is followed by pretreated biomass material (56% of methane over 44% of other compounds) and the smallest ration was found from hydrolyzed and untreated biomass (55% of methane over 45% of other compounds).

4.3. Chemical composition of the substrates

In **Table 7**, the chemical composition (TS and VS) of samples from different steps of bioethanol production process is presented. The content of TS is higher for pretreated and hydrolyzed barley straw (94±7.6 g/kg) and lower for pretreated, hydrolyzed and fermented (79±0.93 g/kg). This differences between the groups (pretreated barley straw and production-waste) are statistically significant ($p \leq 0.001$). The reduction in the TS content after the pretreatment process shows that the dry matter

was partially decomposed, and because of this, it increased the amount of substrate available for anaerobic decomposition by the microorganisms. Moreover, the slight increase of TS after the hydrolysis may be because of the enzymes added during this step. The decrease in TS content just after the fermentation process may be due to sugars loss during the ethanol production [36]. Statistically significant differences have been found between the TS content of untreated barley straw *vs* bioethanol production-waste ($p \leq 0.001$); untreated *vs* fermented ($p \leq 0.05$) and hydrolysed *vs* waste products ($p \leq 0.05$).

The VS content varies between 896±12 g/kg TS for the raw substrate and 996±0.83 g/kg TS for fermented substrates. This parameter shows the potential of biogas production because it represents the fraction of solid material that will be converted into biogas. This differences between untreated and pretreated materials are statistically significant ($p \leq 0.001$).

Table 7. Total solids and volatile solids content for different steps of bioethanol production and bioethanol production-waste (n=3) (± represents the standard deviation)

	TS, g/kg	VS, g/kg TS
Raw	956+1.8	896+12
Pretreated	88±9.0	996±0.20
Pretreated and hydrolyzed	94±7.6	996±0.17
Pretreated, hydrolyzed and fermented	79±0.93	996±0.086
Waste products	84±2.8	996±0.83

4.4. Maximum biogas yield

The biogas production for all the steps of bioethanol production process was modelled, by fitting the experimental data with an exponential model (one-phase association equation). **Figure 10** represents the results obtained. It can be seen that the maximum biogas yield corresponds to the substrate that has been pretreated, hydrolyzed and fermented (2.07 ± 0.010 mol biogas per 100g of raw biomass), followed by pretreated and hydrolyzed substrates (2.04 ± 0.037 mol biogas per 100g of raw biomass), bioethanol production-waste (2.00 ± 0.014 mol biogas per 100g of raw biomass), pretreated samples (1,99 ± 0.030) and raw substrate (1.87 ± 0.29 mol biogas per 100 g of raw biomass). Statistically significant differences have been found between untreated *vs* pretreated/hydrolyzed/fermented/production-waste ($p \leq 0.0001$); pretreated *vs* hydrolyzed/fermented ($p \leq 0.0001$); and waste-products *vs* hydrolyzed/fermented ($p \leq 0.0001$).

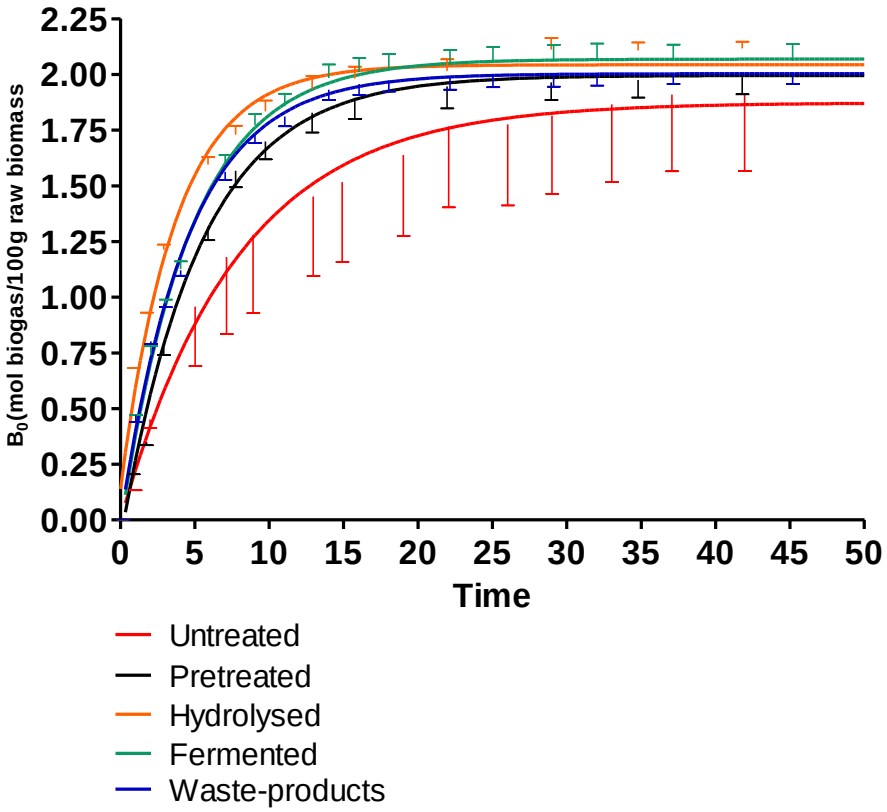

Figure 10. Amount of biogas produced based on experimental data and respective fitting curves for BMP tests of untreated, pretreated, hydrolyzed, fermented barley straw and bioethanol production-waste

4.5. Kinetic evaluation of biomass bioconversion

In order to characterize the conversion rate of the substrates, the maximum methane yield (B_{max}), kinetic constant (k) and correlation coefficient (R^2) were calculated for all substrates. **Table 8** illustrates the results obtained. The highest kinetic rate constant was found for pretreated and hydrolyzed substrates (0.27 ± 0.027), while the lowest was found for raw material (0.13 ± 0.0080). The kinetic rate of pretreated, pretreated hydrolyzed and fermented and bioethanol production-waste is 0.19 ± 0.011, 0.21 ± 0.0056 and 0.22±0.0083, respectively. Although the differences between the stages of bioethanol production and its chemical composition could explain the differences in the rates, no data about the kinetic rate for different steps of bioethanol production was found in the literature.

From Table 8 it can also be seen that the one-phase exponential association equation successfully expresses the variation of the data, between 98.54% (pretreated and hydrolyzed) and 99.85% (pretreated, hydrolyzed and fermented).

Table 8. Maximum biogas yield (B_{max}), kinetic rate constant (k) and correlation coefficient (R^2) of the one-phase exponential association equation for untreated, pretreated, hydrolyzed, fermented barley straw and bioethanol production-waste (n=2)

	B_{max} (mol biogas/100g)	k	R^2
Raw	1.87±0.29	0.13±0.0080	0.9935
Pretreated	1.99±0.030	0.19±0.011	0.9946
Pretreated and hydrolyzed	2.04±0.037	0.27±0.027	0.9854
Pretreated, hydrolyzed and fermented	2.07±0.010	0.21±0.0056	0.9985
Waste products	2.00±0.014	0.22±0.0083	0.9970

Table 9 shows the digestion time (85% B_{max} and 95% B_{max}) for substrates from different steps of bioethanol production process. The fastest bioconversion into biogas was found for pretreated and hydrolyzed substrate. It took 7 days to achieve 85% of the ultimate biogas yield, and 11 days to achieve 95% of the maximum biogas yield. To achieve 95% of the ultimate biogas yield, untreated straw took 23 days, pretreated samples 16 days, fermented material 14 days and bioethanol production-waste 13.5 days. It can be explained by the fact that pretreatment increased the amount of substrate available for anaerobic microorganisms. However, lately added enzymes after hydrolysis slowed the process of biogas generation due to increase of TS. After that, fermentation accelerated the process again due to the loss of sugars for bioethanol production. Thus, the fraction of the solid material that can be converted into methane influence actual potential for biogas production These obtained digestion periods can be explained by the amounts of TS which influence quantity of biogas that can be generated.

Table 9. Digestion time (85% B_{max} and 95% B_{max}) of the anaerobic digestion process for untreated, pretreated, hydrolyzed, fermented barley straw and bioethanol production-waste

Variable	85% B_{max}		95% B_{max}	
	mol CH_4/100g	Days	Mol CH_4/100g	Days
Raw	1.59	15	1.78	23
Pretreated	1.70	10	1.90	16
Pretreated and Hydrolyzed	1.74	7	1.94	11
Pretreated, hydrolyzed and fermented	1.76	9	1.97	14
Waste products	1.70	8.5	1.90	13.5

In order to discuss the results obtained, similar experiments carryout by Petersson *et al.* (2007) and Calicioglu & Brennan (2018) were analyzed [37, 38].

Petersson *et al.* (2007) [37] evaluate the potential for bioethanol and biogas production using winter rye straw, oilseed rape straw and faba bean straw. The crops were cultivated during summer (2005) in the experimental fields of Risø National Laboratory, Denmark. The three materials were pretreated by wet oxidation (195°C, 2 gl^{-1} Na_2CO_3, 12 bar O_2, 15 min). After the pretreatment, enzymatic hydrolysis took place at 50°C, pH 4.8, 2%DM content and an enzyme load of 30 FPU g^{-1} DM. The enzyme used was Cellubrix L. The experiments were carried out in triplicates for each solid pretreated fraction and for each raw material. Later, saccharification and fermentation were simultaneously performed on the raw materials, as well as on wet oxidized substrates. The ethanol yield from raw material was rather low (6-25% lower) than from pretreated materials. Bioethanol potential and methane yield were measured only for untreated raw material and wet oxidized (WO). Hydrolyzed, fermented and bioethanol waste-products were not analyzed in this research [37]. **Table 1**. Compares the results obtained in our study (using barley straw) with the results obtained by Petersson *et al.* (2007), using untreated and pretreated winter rye straw, oilseed rape straw and faba bean straw. Despite the results are presented for methane yields, it is expected a similar relationship for biogas production.

Table 10. Comparison of methane yields of different studies using winter rye, oilseed rape, faba bean and barley straw

Biomass feedstock	Pretreatment	Methane yield (g CH_4/ 100g)	Source
Winter rye	Untreated	18.2	[37]
	Pretreated	24.4	
Oilseed rape	Untreated	18.8	[37]
	Pretreated	20.4	
Faba bean	Untreated	18.9	[37]
	Pretreated	18.4	
Barley straw	Untreated	16.7	Our study
	Pretreated	17.8	

The differences in the values reported in Table 10 can be explained due to the chemical composition of these types of biomass, as well as the conditions the experiments were held. However, it is visible that the amount of methane produced from untreated biomass is smaller than for pretreated material. Although hydrolyzed, fermented and bioethanol production-waste were not assessed in this research, the aims of both studies were similar: to analyze the possibility of adding value to the bioethanol

production chain. The results are also expected to be comparable this is, when the pretreated material is further processed, the potential for methane conversion becomes higher.

In the study by Calicioglu & Brennan (2018) [38] the potential for biofuel production using duckweed was evaluated. In this research, dry biomass was directly added to anaerobic digestion, or sequential bioethanol and biogas production was carry out. The highest biomethane yield was 390 ± 0.1 ml CH_4 /gVS added, and it was achieved in a reactor containing fermented duckweed from the Living-Filter. Besides, this value was 51.2% higher than the biomethane yield of a replicate reactor with raw (non-fermented) duckweed. The combined bioethanol-biomethane process yielded 70.4% more bioenergy from duckweed than if anaerobic digestion would have be running alone. Bioethanol potential and methane yield were measured only for untreated raw material and fermented samples. Pretreated, hydrolyzed and bioethanol production-waste were not analyzed in this research. **Table 11** compares the results obtained in our study with the results obtained by Calicioglu & Brennan (2018).

Table 11. Comparison of methane yields for different studies using duckweed and barley straw

Biomass feedstock	Pretreatment	Methane yield (g CH_4/ 100g)	Source
Duckweed from Eco-Machine (EM)	Untreated	18.3	[38]
	Fermented	26.1	
Duckweed from Living-Filter (LF)	Untreated	19.2	[38]
	Fermented	28.9	
Barley straw	Untreated	16.7	Our study
	Fermented	17.8	

These differences between the values can also be explained by the chemical composition of the biomass as well as the conditions the experiments were held. However, it is visible that the amount of methane produced from untreated biomass is smaller (6-34%) than for fermented samples. Although pretreated, hydrolyzed and bioethanol production-waste were not assessed in this research, the aims of both research were similar. The biomethane yields from both studies are also in the same range.

4.6. Biogas purification potential

The calorific value is an important parameter to assess the efficiency of any fuel. The calorific value of biogas with 60% of methane is lower (21.5 MJ/m^3) than natural gas (35.8 MJ/m^3). This is due to the large volume of incombustible material present in the biogas (CO_2, H_2S, water vapour, nitrogen and oxygen), that reduces the heating value of the gas, and increases the costs for compression and

transportation. As a result, the economic feasibility of biogas production decreases. Moreover, such contaminants in biogas as H_2S and oxygen have a detrimental effect on the structure of the equipment where the biofuel will be burned. It leads to the corrosion of engines, boiler tubes and steel chimneys. Thus, great attention has been paid to the removal of impurities from biogas. This will allow the utilization of the biogas as a transport fuel, substitute natural gas, and contribute to the reduction of GHG emissions [38].

The ratio of methane obtained in this study varies between 55% (for untreated biomass) and 59% (for fermented and bioethanol production-waste). Usually, raw biogas consists of methane around 40-75% and carbon dioxide around 15-60% [25]. Actual results are in the middle of the range for biogas that can be obtained from different feedstock. The higher amount of methane the more reasonable and efficient transformation to biomethane will be. Therefore, further purification of obtained biogas is can be reasonable from the economic and technological perspective.

Unfortunately, it is not possible to propose the best and optimum solution for biogas purification, considering only the content of biogas that was obtained in this work. It can be explained by the not clear vision of how this purified biogas will be used later and is there a necessity to improve it and how. Nevertheless, in this chapter, the criteria for choosing possible biogas upgrading technologies are proposed, and different scenarios of further clean biogas application are discussed.

4.6.1. Economy and cost-efficiency of biogas upgrading technologies

Based on the literature review done in chapter 1, the cost shown to be one of the most crucial factors in determining the optimal solution for biogas upgrading technologies. However, the cheapest solution is not always the best. Such technologies like water scrubbing are cost-effective, but cryogenic and chemical absorption technologies provide higher efficiency. In terms of maintenance cost, in a large scale, cryogenic technology is economically feasible, but it requires high investments, in comparison with membrane separation, where investment is still high, but the operational costs are lower. This criteria for choosing a certain biogas upgrading technology is based on the amount of investment that can be spent and on the later operational costs [39]. **Table 12** illustrates the recent costs for comparison, advantages and disadvantages of six different biogas upgrading technologies.

Table 12. Comparative analysis of the different biogas upgrading technologies (adapted from [39])

Method	Cost (€/year) for 1000 m^3		Advantages	Disadvantages
	Investment	Maintenance		
Water scrubbing	10,000,000	15,000	▪ No pre-cleaning required ▪ Simple in operation	▪ Requires huge amount of fresh water
Physical absorption	10,000,000	39,000	▪ Economical operation ▪ High methane purity	▪ Use of chemicals
Chemical absorption	20,000,000	59,000	▪ Less methane loss	▪ Chances of biological contamination ▪ External heat required for regeneration
Pressure swing adsorption	17,500,000	56,000	▪ Dry process ▪ No chemical usage ▪ No water demand; ▪ No microbial contamination	▪ H_2S pretreatment required ▪ Complex setup ▪ High investment cost
Membrane	20,000,000	25,000	▪ Dry process ▪ No chemicals compact process ▪ Low mechanical wear	▪ Pre-treatment required ▪ High investment cost ▪ High energy demand ▪ Unstable over long term
Cryogenic separation	-	-	▪ Highest methane purity ▪ No chemicals required ▪ Upgraded biogas is at high pressure, thus no further compression is required for vehicular fuel	▪ High capital and operating cost ▪ A huge amount of energy required ▪ Pre-treatment required

4.6.2. Criteria for choosing a technology for biogas upgrading

Similar to the costs, the optimal technology may vary according to the requirements. For example, if the biogas is going to be used as a fuel for the transportation sector, it is important to ensure higher concentrations of methane in it. Thus, such technologies as chemical absorption and cryogenic separation are the most reliable in this sense. However, pressure swing absorption and membranes have a huge advantage, especially if biogas contains high amounts of O_2, N_2 and CO_2 because it removes these all components simultaneously. At the same time, cryogenic separation seems to be more effective because it produces high pressurized liquid fuel and ensures that no power will be consumed for the compression of the fuel. However, high capital and operating cost together with huge amount

of energy required for its operation of it cannot make this technology reasonable and available for the industry [39].

From the practical perspective, consumption of energy during the purification define both environmental sustainability and energy efficiency. Purification technologies as organic, water and amine scrubbing usually require more energy for its operation than pressure swing absorption or membranes, because achieve the steady state in a couple of seconds. Simultaneously, amine scrubbing and cryogenic separation are promising technologies due to its low methane slip, but the same results can be achieved with pressure swing absorption [39]. **Table 3** represents the energy demands, CH_4 purity, and methane loss for different technologies.

Upgrading costs depend mainly on the plant size. The purification price decreases with the increase of the capacity. Currently, it is still very expensive to clean biogas in a small scale (<200 Nm^3/h), due to high costs of investment and equipment. To make small-scale projects economically viable and sustainable, the operational costs should be reduced to €0.30-0.20 per Nm^3, and an approach to use locally produced biogas should be applied. Besides, cost reduction is also possible by diminishing the complexity of the control system and by keeping the methane content in upgraded biogas below 95% [39].

Table 13. Energy demand, purity of methane, and methane loss for different biogas upgrading technologies (adapted from [39])

Method	Energy consumption (kWh/Nm^3)	Purity of CH_4 (%)	Methane Loss (%)
Water scrubbing	0.2–0.5	95–98	<2, medium
Physical absorption	0.10–0.33	93–98	<4, high
Chemical absorption	0.05-0.18	>98	<0.5, low
Pressure swing adsorption	0.16-0.43	>96–98	<3, medium
Membrane	0.18-0.35	90–99	<5, high
Cryogenic separation	0.18-0.25	99	<0.1, the lowest

SUMMARY

Lignocellulosic biomass has become one of the most important feedstocks for biofuel production. Bioethanol and biogas are the most common biofuels in the transportation sector, together with other possibilities of its utilization. However, the production process of these fuels is still inefficient due to the enormous quantity of waste-products left over after the distillation process. In this work, analysis of biogas potential from bioethanol production-waste was performed, using barley straw as a biomass crop sample.

The results show that bioethanol production-waste has higher biogas yields (2.00 mol biogas/100g) than raw barley straw (1.87 mol biogas/100g). Moreover, bioethanol production-waste achieve 95% of the maximum biogas yield much faster (13.5 days) than untreated samples (23 days). Although production-waste samples have high results, experimental data showed that fermented samples have the highest biogas production (2.07 mol biogas per 100g of dry biomass). Hydrolyzed samples have a value of 2,04 mol biogas per 100g of dry biomass and pretreated samples only 1,99 mol biogas per 100g of dry biomass.

Barley straw was the biomass used in the experiments. The proportion of cellulose, hemicellulose and lignin were reported and taken into account. Higher cellulose content (45.7%) and lower contents of hemicellulose (32,6%) and lignin (5,2%) are appropriate conditions for bioethanol and biogas production. Moreover, the lower lignin content gives higher biogas yields due to the improvement cellulosebiodegradability.

The chemical composition (TS and VS) was also assessed. The TS content is higher in pretreated barley straw and lower in fermented samples. A reduction in the TS content after the pretreatment method indicates that the dry matter was partially decomposed during the pretreatment, increasing the amount of substrate available to anaerobic microorganisms. The VS content varies between 896 g/kg TS (untreated) and 996 g/kg TS (other steps). It represents the fraction of the solid material that can be converted into methane and this is actual potential for biogas production.

According to the results obtained in this study bioethanol production-waste have high energetic value and it is reasonable to use them to improve the overall efficiency of bioethanol production

chain. Thus, sequential fermentation and anaerobic digestion can increase the efficiency of the production chain.

Alongside with the possibility of biogas generation, the biogas content obtained in this research was studied for its further purification. Since the ratio of methane in the biogas content varies between 55% (untreated biomass) and 59% (fermented and bioethanol production-waste), it makes the purification of the biogas reasonable, from the economic and technological perspective. Even though it was not possible to propose the best and optimum solution for biogas purification based only on content of biogas, the criteria for choosing the particular cleaning technology was formulated. Since biogas can be used as a fuel for domestic stoves, boilers, internal engines, gas turbines, vehicles and fuel cells, or injected into natural gas grids to replace gaseous fuel, biomethane should have different requirements and characteristics depending on its application.

Definitely, by adding more data regarding how this purified biogas can be used, and which amount of biogas can be obtained from the lignocellulosic biomass available in a particular area, further research would give us possibility to estimate the efficieny of the conversion process, and its costs. Moreover, more accurate determination of biogas chemical composition would show the amount of balast components that should be removed. Based on these data way of proposed purification will be more reasonable and efficient as a result.

Although current market price of biofuels is higher compared to fossil natural gas, biomethane relies on numerous support schemes since under the current market conditions biomethane can not compete against natural gas in sales price. Currently in many countries production of biomethane relies on numerous support schemes such as feed-in tariffs for gas or electricity, biofuel quota or certificate systems, beneficial tax policy and investment aid. Moreover, biomethane projects have long development periods and therefore are reliant on long-term policies offering stable conditions and grandfathering. Keeping all this in mind, lignocellulosic biofuels as a part of renewable energy have huge potential for the research, cost-effective commercial production and environmental sustainability.

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