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Nitrogen-rich and lignocellulosic biomass for biogas production: methane yield potentials, process stability and nutrient management

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Nitrogen-rich and lignocellulosic biomass for biogas production: methane yield potentials, process stability and nutrient management

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Poem “Methane Tank”

from Ukrainian “Метантенк”

(My Ukrainian poem from 4 June 2011, written during my bachelor’s project, lyrically describes the engineering period of a methane reactor for its construction and successful operation in Ukraine)

„Надіюсь,
Сподіваюсь,
Вірю,
Що якось я змонтую,
Втілю у життя
Це диво, що я зараз конструюю…
Дорога ця аж зовсім нелегка,
Та каяття мене вже не турбує.
Забула сльози, що були пролиті,
Безсонні ночі, темряву і біль.
Усі проблеми врешті пережиті,
Я бачу свою мрію – це не тінь.
Я не жалію себе ані трохи,
Все серце покладаю я у це.
Творю, збираю, конструюю, вірю.
Ця віра мене зігріває, береже.“

Contents

Abbreviations

AD	anaerobic digestion
AMY	areal methane yield
BBCH-code	code of phenological development stage of plants. "BBCH" derives from German "Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie", translated as "Federal Biological Research Centre, Federal Plant Variety Office and Chemical Industry"
Ca	calcium
$CaCl_2$	calcium chloride
CH_4	methane
CO_2	carbon dioxide
C/N ratio	carbon-to-nitrogen ratio
CSTR	continuously stirred tank reactor
d	day
DAP	diammonium phosphate
DM	dry matter
DMY	dry matter yield
EU	European Union
FAO	food and agriculture organization
FAN	free ammonia nitrogen
FM	fresh matter
FR	feeding regime
g	gram
h	hour
H_i	harvesting time
ha	hectare
HAc	acetic acid

HBT	Hohenheim biogas yield test
HRT	hydraulic retention time
HTC	hydrothermal carbonization
Inhibition	inhibition in specific methane yields
J	joule
K	potassium
L or l	liter
m	minute
m^2	square meter
m^3	cubic meter
Mg	magnesium
$MgCl_2$	magnesium chloride
N	nitrogen
NH_3	ammonia
NH_4^+	ammonium
NLR	nitrogen loading rate
oDM	organic dry matter
OLR	organic loading rate
p	p-value, probability value
P	phosphorus
PO_4^{3-}	phosphate
R^2	coefficient of determination
SMY	specific methane yield
STP	standard temperature and pressure
TAN	total ammonia nitrogen
TE	trace elements

TRL	technology readiness level
VFA	volatile fatty acid
VS	volatile solids
W	watt

1. Introduction

1.1 Sustainable biobased economy

A biobased economy, or simply bioeconomy, is a lever for the common good of our society that requires the continued growth and development of this sector. Bioeconomy can be defined as the sustainable and viable production, conversion and utilization of renewable biological resources (Iris Lewandowski, 2018; Nagothu, 2020). A sustainable bioeconomy involves, at least in part, the recycling of waste and the simultaneous environmentally friendly use of the remaining waste (Nagothu, 2020). A sustainable biobased production or economy should not compromise food security; at the same time, the cultivation and use of agricultural or forestry crops are suitable for the bioeconomy to largely replace fossil-based products or fossil energy sources (Viaggi, 2018). In addition, the necessary measures must be taken to achieve the goal of climate neutrality with zero net emissions of greenhouse gasses by 2050, as set out in the 2009 European Renewable Energy Directive (Dabbert et al., 2017). Therefore, prerequisites for the development of the bioeconomy include scientific know-how in biomass conversion with a relatively high technology readiness level (TRL), policy incentives, consumer acceptance, and market availability (Viaggi, 2018).

1.2 Anaerobic digestion

A key component of many bioeconomic approaches and biorefineries is the anaerobic, microbial conversion of biomass to produce biogas. Anaerobic digestion (AD) is a process for converting organic-rich substrates, used as a feedstock, into biogas (mainly methane (CH_4) and carbon dioxide (CO_2)) and degraded material – digestate. The anaerobic digestion process involves four stages of conversion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Different microorganisms are involved in each of the four stages of conversion (Bischofsberger et al., 2004).

The amount of biogas obtained at AD is related to the theoretical biogas (methane) potential of a feeding substrate (substrates) and microbiological efficiency (Bischofsberger et al., 2004; Chernicharo, Carlos Augusto de Lemos, 2007; Raposo et al., 2012). The theoretical biogas (methane) potential is related to the gross energy content of the substrates and their anaerobic degradability. It is described as specific methane yield (SMY), which must be determined experimentally. Theoretically, up to 85% of the energy contained in biomass can be converted to methane (Mittweg et al., 2012). Microbial efficiency is directly related to physical and chemical operating conditions. Operating conditions include organic loading rate, retention time (hydraulic retention time or solid retention time for wet or dry fermentation process, respectively), temperature, pH, carbon-to-nitrogen ratio in a feeding regime (FR), availability of inhibitors and sufficient amount of trace elements in the substrates, and mixing conditions (Bischofsberger et al., 2004; Pandey et al.). In contrast to the full-scale application, an inoculum is used for each experiment to start the biogas system at the laboratory scale. This inoculum has a significant impact on process efficiency and is either cultivated by the operator or taken in the form of digestate (effluent) from the operating biogas plant (Hülsemann et al., 2020; Mittweg et al., 2012).

To operate a biogas plant efficiently, the construction of anaerobic reactor(-s) has to be considered. There are biogas plants where all four stages of AD can take place in one reactor, a so-called one-stage anaerobic system. The two-stage anaerobic system refers to an installation with separate reactors for the hydrogenic stage (it includes hydrolysis, acidogenesis, and acetogenesis) and the methanogenic stage (Chernicharo, Carlos Augusto de Lemos, 2007). Agricultural biogas plants are usually the one-stage anaerobic systems (Bischofsberger et al., 2004).

1.3 Biogas feedstock and potential in Europe

Substrates suitable for biomethane production include municipal waste, animal waste, agricultural residues and energy crops, food waste, and organic wastes from the food processing industry (Pandey et al; Taherzadeh et al., 2019). They can be divided into readily degradable and poorly degradable substrates (e.g. substrates containing lignin, cellulose and hemicellulose). For AD based on poorly degradable substrates, additional pretreatment is recommended, e.g., mechanical comminution that increases the surface area of the substrates (Agbor et al., 2011; Lehtomäki, 2006; Schumacher, 2008). Co-fermentation of animal waste and energy crops or by-products of agricultural production (plant residues) is commonly used in practice (Scarlat et al., 2018). By utilizing animal wastes in AD, the carbon footprint can be minimized (Chernicharo, Carlos Augusto de Lemos, 2007). Maize silage is the most commonly used co-substrate for biogas production due to its high SMY, thus improving biogas (methane) yields (Amon et al., 2007; Herrmann et al., 2012; Mukengele, 2017; Schumacher, 2008). New maize varieties are constantly being investigated to further increase areal methane yields. The cultivation of perennial bioenergy crops as feedstock is an attempt to improve the energy balance of biogas production and minimize the nutrient requirements for cultivation (Mast et al., 2014). Sustainable crop cultivation includes reduced use of pesticides and mineral fertilizers (Lehtomäki, 2006; Ruppert et al., 2013; Ruppert and Ibendorf, 2017). For sustainable crop production, nutrients should be recovered from digestate and applied to the field at times of crop demand (Deublein and Steinhauser, 2008; Ruppert and Ibendorf, 2017).

According to (International Energy Agency, 2020), the total biogas production in Europe in 2020 was 18 Mtoe (mega ton of oil equivalent), while the production potential for biogas in Europe is 114 Mtoe. This 114 Mtoe is based on the following feedstocks: ~23% crop residues (26 Mtoe), ~28% animal manure (32 Mtoe), ~17% municipal solid waste (19 Mtoe), ~2% municipal wastewater (2 Mtoe), and ~30% woody biomass (35 Mtoe). The total biogas production in Germany in 2022 equaled to 7.83 Mtoe which corresponds to 91.27 TWh (German Biogas Association, Status 2023). In Germany in 2018, energy crops accounted for 47% of feedstocks for biogas production, excrement for 48%, municipal biowaste for 2%, and residues from industry, manufacturing and agriculture for 3% (Statista, 2020).

According to statistical data, the total primary energy supply in Ukraine in 2020 equaled 86.36 Mtoe (State Statistics Service of Ukraine. Energy Balance of Ukraine 2020; State Statistics Service of Ukraine. Express release on Ukraine's energy balance for 2020). Energy of biofuels and wastes contributed in Ukraine in 2020 to 4.90% of this value or 4.24 Mtoe. Biogas production accounted for Ukraine in 2020 for 3862 TJ or 1.07 TWh (State Statistics Service of Ukraine. 2020 Ukraine Energy Balance (By Products)). According to Geletukha, respectively Geletukha et al. (Geletukha, 2022; Geletukha et al., September/2022), the potential share of biomethane in Ukraine amounts to 2.36 Mtoe or 27.45 TWh, mainly due to the considerable biomass potential of bioenergy crops and agricultural residues.

The prerequisites for biogas production in Ukraine are the following (Babych, 2018; Geletukha, 2022; Geletukha et al., September/2022): Ukraine has a relatively low population density of around 69.5 inhabitants·km^{-2}. At the same time, Ukraine has one of the largest specific arable land areas in the world equal to 0.74 ha·person^{-1}. As of the outbreak of hostilities in Ukraine in February 2022, there were around 3 million hectares of underutilized agricultural lands. These lands could be used for growing bioenergy crops for biogas and other biofuels without harming Ukraine’s food and feed supply. About 1 million hectares were occupied by degraded and marginal lands, where bioenergy crops could be cultivated. On October 21, 2021, the Amendments to the Law of Ukraine “On Alternative Fuels” on the development of biomethane production” №5464 were adopted (Verkhovna Rada of Ukraine. Legislation of Ukraine, 2021).

The potential annual biomethane production in Ukraine (before the outbreak of hostilities, starting from 24 February 2022) was calculated based on feedstock availability. The share of maize silage, which could be grown on underutilized and marginal lands amounted to about 30%. Here the potential of Ukrainian bioenergy crops, other than maize silage, was not considered due to a knowledge gap on this topic (Geletukha et al., September/2022).

1.4 Legal framework

The main legal document regulating biomass biofuel production at the EU level is the Renewable Energy Directive (The European Parliament and the Council of the European Union, 2018). Incentives for biogas production vary at the national level. In Germany, for example, the legally guaranteed feed-in tariffs for the electricity produced depend, among other things, on the year in which the biogas plant is commissioned, the substrates used, and the proportion of heat used. However, the remuneration ceases after 20 years of operation of the biogas plant (German Combined Heat and Power Act 2020; Germany's Renewable Energy Act 2021).

For the designing and operation of a biogas plant under economic conditions, the constant availability of feedstock is required. A high diversity of crops is recommended for the use of plants and plant residues as feedstock. Sustainable biogas production requires not only the selection of suitable substrates, but also a technology adapted to the specific applications (Bischofsberger et al., 2004; Chernicharo, Carlos Augusto de Lemos, 2007). Covering manure storage tanks and covering digestate storage are among the measures to improve the greenhouse gas balance of a biogas plant.

Especially in rural areas, biogas plants can be efficiently integrated into the energy supply structure (Dittmer et al., 2021). The biogas produced can be used in a combined heat and power unit to meet local demand. The biogas upgraded to “biomethane” by removing CO_2 can be used as fuel or fed into the gas grid. Additional profit can be made by recovering nutrients from digestate and using it as fertilizers, thereby reducing the cost of feedstock (Pandey et al.).

Many bioenergy villages in Germany prove that a sustainable energy supply is already possible today. In the course of this, regional nutrient cycles are closed, greenhouse gas emissions are efficiently reduced and jobs are created in rural areas. Furthermore, by forming a consortium, the initial investment required by each partner (or proactive local citizen) to establish and maintain a bioenergy village can be minimized (Nagothu, 2020; Ruppert and Ibendorf, 2017).

1.5 Objectives of the study

A sustainable energy supply is also crucial for the future economic development of many countries in Eastern Europe. While in Germany more than 40% of gross electricity generation is already based on renewable energy (Statista, 2021), this share is only 7.3% in Ukraine (Ukrainian National Commission for State Regulation of Energy and Utilities, 2020). Due to the very large agricultural potential, bioenergy villages, also based on biogas plants, appear to be an interesting option for the future sustainable development of this country. While in Germany the cultivation and use of biomass for energy and bioeconomic purposes is already very well studied, such data for Ukraine is still completely lacking.

The aim of this thesis is to investigate the suitability of various agricultural crops grown in Ukraine for use in biogas plants. In addition to the biogas yield potential of the plants depending on the time of harvest, questions regarding process stability and nutrient recovery from the resulting digestates are to be investigated, so that a "cradle-to-cradle approach" is pursued.

This cradle-to-cradle approach combines three research objectives of the study, which are as follows:

Objective 1. Biogas yield potential for different crops. Experimental investigation on the influence of plant varieties, and harvest times on the methane yield potentials of miscanthus and other lignocellulosic bioenergy crops grown in Ukraine.

Objective 2. N-rich substrates: Process stability and reactor performance. Experimental investigation on the process stability during anaerobic digestion of N-rich biomass.

Objective 3: Separation of digestate: A study on nutrient management technologies.

1.6 Thesis at a glance

A cradle-to-cradle approach is a long-term approach to the production of goods in terms of ecological health, ecological abundance, and accompanying economic growth (Braungart et al., 2007). Under the cradle-to-cradle approach, materials are used in the most efficient way in closed-loop systems that allow products to be fully recycled without generating waste (Ceschin and Gaziulusoy, 2020; Iris Lewandowski, 2018). In this dissertation, a cradle-to-cradle approach is offered, as shown in the figure below. This approach contributes to a sustainable biobased economy and is recommended for building new bioenergy villages.

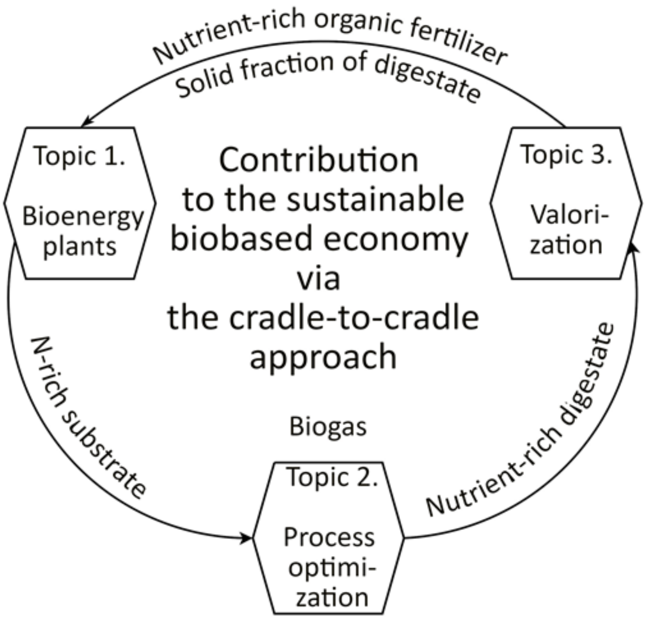

Figure: The cradle-to-cradle approach applied in the study

While there should be a steady supply of feedstock for biogas production, bioenergy crops can be used as feedstock in areas with poorly developed livestock and poultry farming (Topic 1). The use of organic fertilizers is recommended for sustainable cultivation of bioenergy crops. Crops with the highest biogas (biomethane) yields per hectare and the lowest fertilizer requirement per 1 m^3 of methane produced are preferred for use as feedstock (Lehtomäki, 2006). Bioenergy crops and other proteinaceous substrates are generally rich in nitrogen. When degraded in conventional AD, nitrogen forms ammonia, which in high concentrations causes microbial process disturbances and leads to losses in biogas production or, in the worst case, process failure (Rajagopal et al., 2013). Therefore, the technology of biogas production with nitrogen-rich substrates needs to be optimized, which is investigated in Topic 2. The nutrients recovered from the digestate after biogas production can serve as organic fertilizer for the cultivation of bioenergy crops. Nutrients recovery from the digestate, mainly into the solid fraction, after its pretreatment and mechanical separation, is studied in Topic 3. The proposed cradle-to-cradle approach for bioenergy villages consists of land, biogas production plant and nutrient recovery capacity.

Based on the figure, an overview of the thesis is given below.

Objective 1. Biogas yield potential for different crops. Experimental investigation on the influence of plant varieties, and harvest times on the methane yield potentials of miscanthus and other lignocellulosic bioenergy crops grown in Ukraine. The results of this research are published in **Paper 1**: "Assessment of Areal Methane Yields from Energy Crops in Ukraine, Best Practices".

Objective 2. N-rich substrates: Process stability and reactor performance. Experimental investigation on the process stability during anaerobic digestion of N-rich biomass. The main results are described in **Paper 2**: "Effects of Increasing Nitrogen Content on Process Stability and Reactor Performance in Anaerobic Digestion".

Objective 3. Separation of digestate: A study on nutrient management technologies. The results of this research are described in **Paper 3**: "Nutrient Recovery from Digestate of Agricultural Biogas Plants: A Comparative Study of Innovative Biocoal-Based Additives in Laboratory and Full-Scale Experiments".

2. Publication 1: Assessment of Areal Methane Yields from Energy Crops in Ukraine, Best Practices

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Article

Assessment of Areal Methane Yields from Energy Crops in Ukraine, Best Practices

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Abstract: Growing and utilizing bioenergy crops as feeding substrates in biogas plants may aid the development of the biogas sector in Ukraine. Therefore, research was done on potential methane yields from 22 high-yield varieties of 7 different crops grown in Ukraine for their biogas production suitability. Annual crops (maize, soybean, sweet sorghum and sorghum hybrids) and perennials (miscanthus, paulownia and switchgrass) harvested at three different harvesting times (H1, H2 and H3) related to specific stages of phenological development were investigated. The perennial crops studied were from different vegetation years. The samples were analysed in Ukraine on their dry matter- and volatile solids contents, dry matter yield (DMY) and crop nitrogen (N) uptake. The 55 °C-dried samples were delivered to Germany for their analysis with the Hohenheim Biogas Yield Test (HBT) on their specific methane yield (SMY). Based on DMY and SMY, the areal methane yields (AMY) were calculated. The highest SMY and AMY were found for maize, sweet sorghum and miscanthus. The highest average SMY of 0.35 ± 0.03 m^3_{CH4} $kgVS^{-1}$ was found for maize samples harvested at H2. Miscanthus "Giganteus" from the 8th vegetation year harvested at H1 has shown the highest AMY of 7404.50 ± 199.00 m^3_{CH4} ha^{-1}.

Keywords: anaerobic digestion; biogas; maize; soybean; sorghum; miscanthus; switchgrass; paulownia; BBCH-code; nitrogen use efficiency

1. Introduction

In 2018, the natural gas import in Ukraine amounted to 35.7% of the total gas consumption [1]. The topical issue is to reduce the natural gas import, which can be done through the production of biogas. According to Scarlat et al. [2], the biogas production in Ukraine in 2015 sums up to 600 TJ or 117 mil m^3, while the biogas share in the natural gas use was only 0.1%. In 2018 this share increased to 0.2% [1]. End of 2019, the installed power capacity of biogas plants in Ukraine amounted to 70 MW power capacity, where 47 MW of these units are based on agricultural wastes and the remaining run on landfill gas [3].

In the development of Ukraine's biogas sector, the availability of feedstock plays one of the key roles. As of February 2020, the existing agricultural biogas plants in Ukraine utilize the following substrates: pig and cattle manure, poultry litter, sugar-beet pulp, sugar sorghum silage and maize silage. It is noteworthy that in Ukraine there is a limited amount of manure due to a constant decreasing trend in livestock breeding except for poultry [1]. Therefore, alternative biogas substrates other than manure or poultry litter should be investigated.

To provide continuous availability of substrates supplies in biogas plants, different bioenergy crops cultivated in Ukraine specifically for biogas production can be utilized. Currently, Ukraine does not have a deficiency in the food crops availability which is in correspondence to its relatively low population density of 69.49 inhabitants per km^2 (in Germany the population density is 233, in China—146); moreover, Ukraine is one of the leading grain exporting nations in the world [1,4]. As the area of agricultural lands in Ukraine as of 01.01.2016 amounted to 42,726.4 thousand hectares or 70.8% of the total area of Ukraine [5], a part of bioenergy crops can be grown on underutilized agricultural lands or in sustainable rotations with other crops. Furthermore, experts assess the area of marginal lands in Ukraine to be ~4 million hectares for the inland territory [6,7], where the bioenergy crops can be additionally cultivated. Therefore, due to the land's availability, bioenergy crops are a potentially attractive substrate for biogas production in Ukraine. Additionally, since December 2019 the State Agency on Energy Efficiency and Energy Saving of Ukraine has been requesting the approval of the amendments to the Ukrainian legislation that should provide a state support program for the cultivation of bioenergy crops [8].

The biogas production from bioenergy crops is directly related to the value of crop's areal methane yield (AMY), measured in $m^3_{CH4} \cdot ha^{-1}$ [9]. For bioenergy crops, the AMY depends on many parameters: crop species [9–11], crop variety [12–14], soil-climatic conditions [11], average temperature and precipitation during the cultivation period [15–18], dosage of fertilizer applied [16,19,20], harvesting time related to specific stages of crop phenological development [9,11–15,19,21–24], pretreatment, especially for lignocellulosic biomass [23,25–28] among others.

No literature was found on AMY of bioenergy crops grown in Ukraine. Since the soils and climatic conditions in Ukraine differ significantly from those of Western European countries, the experience gained in energy crops cultivation in these countries cannot be directly transferred to Ukraine. For this reason, the study on the assessment of AMY of high-yield bioenergy crops grown in Ukraine has to be conducted.

In this study, the effects of harvesting time and vegetation year (for perennials) on AMY of potential Ukrainian energy crops are studied. Additionally, the effects of dry matter yield (DMY) and crop N uptake on AMY are determined.

2. Materials and Methods

2.1. Experiment Overview

Ukraine has a temperate climate except on the southern coast of Crimea, which has a subtropical climate. Ukrainian climate conditions are favourable for growing the following bioenergy crops further discussed in this paper: soybean, maize, sweet sorghum and sorghum hybrids (e.g., rice sorghum, known in Ukraine as soriz) [29], switchgrass, miscanthus and paulownia (see Table 1). For this research, high-yield varieties of the above-mentioned crops suitable for dissemination in Ukraine according to the State Register were selected [30], thus leading to a total of 22 varieties of 7 crops, each harvested at three different harvesting times. For perennials, additionally, the effect of the age of plantation (known also as crop vegetation year) on AMY was investigated.

Table 1. The basic characteristics of the bioenergy crops.

Name of Plant	Climate Preference	Carbon Fixation	Annual/Perennial	Class	Reference
Soybean	Cool season	C3	Annual	Legume	[11,16,31–35]
Maize	Warm season	C4	Annual	Grass	
Sweet sorghum	Warm season	C4	Annual	Thick-stemmed grass	
Sorghum oryzoidum or rice sorghum (soriz)	Warm season	C4	Annual	Thick-stemmed grass	
Switchgrass	Warm season	C4	Perennial	Thin-stemmed grass	
Miscanthus	Cool season	C4	Perennial	Thick-stemmed grass	
Paulownia	Warm season	C3	Perennial	Fast growth coppice	

2.2. *Field Trials and Plant Material*

2.2.1. Field Trials

The analysed crops were cultivated in 2017 at the fields of the Agrarian Academy of Sciences of Ukraine. The location of the fields: Kiev and Kiev region (Vasylkiv district, urban-type settlement Grebinky; the research enterprise and research household "Salyvinkivske"; latitude 49.6° N, longitude 30.1° E, altitude 178 m). 18 varieties were cultivated in the Kiev region; 4 varieties were grown on the research fields of the Institute of Bioenergy Crops and Sugar Beet in Kiev.

The fields belong to an area with a mean annual temperature of 10 °C. The crops were grown in a zone of unstable humidity with a mean annual precipitation of 341.1 mm.

The crops were cultivated on the typical medium-loamy black soils with loessial loam and a humus range of 2.68% ± 0.35%. The soils contained low-medium nitrogen (N) contents: 181.67 ± 78.72 mg N per g air-dry soil. The pH of the soils was 6.64 ± 0.09.

During the crop cultivation period in 2017 at the field locations, there was a higher average monthly temperature and a lower average monthly precipitation in comparison to those values in the years 1985–2016 (see Figure 1). Due to the unfavourable weather conditions, the losses in DMY and thus in AMY could take place.

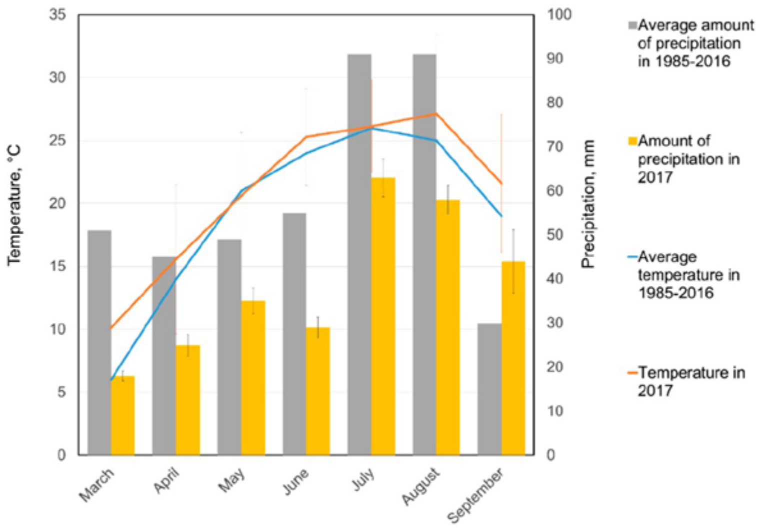

Figure 1. The comparison of weather conditions, such as monthly temperature and monthly precipitation, at the field locations during the cultivation period of the crop samples in 2017 with those values in years 1985–2016. Error bars indicate the monthly variability of temperature and precipitation in 2017.

As the amount of rainfall on-site was considered to be sufficient for cultivation, the field plots were not artificially irrigated.

2.2.2. Plant Material

Plant material was provided by the Institute of Bioenergy Crops and Sugar Beet of the Agrarian Academy of Sciences of Ukraine. The high-yield varieties were selected for this investigation. The analysed amount of varieties per crop were as follows: 5 for soybean; 6 for maize; 4 for sweet sorghum; 3 for sorghum orysoidum; 1 for switchgrass harvested at the 2nd and the 8th vegetation years; 2 for miscanthus, where both varieties were harvested at the 3rd year of vegetation and one of them was also harvested at the 8th vegetation year; 1 variety of paulownia from the 1st vegetation year.

The fertilizers were applied to the fields in the dosage as recommended for each crop by the Institute of Bioenergy Crops and Sugar Beet taking into account the chemical content of the soil and the plants' demands (see Table 2). Herbicides and fungicides were conventionally applied at the individual locations.

Table 2. Fertilizer application at the fields where the analysed bioenergy crops were cultivated.

Name of Plant	Fertilizer	Dosage Applied	Application Time
Soybean	Ammonia nitrate (NH_4NO_3, consists 34.5% N)	100 kg ha^{-1}	Application during presowing cultivation
Maize	Nitroammophos (NH_4NO_3 + $NH_4H_2PO_4$, consists 21.0–25.0% N, 20.0–25.5% P_2O_5)	150 kg ha^{-1}	Application in autumn before plowing
	Ammonia nitrate (NH_4NO_3, consists 34.5% N)	300 kg ha^{-1}	Post-emergence fertilizing
Sweet sorghum, Sorghum oryzoidum or rice sorghum (soriz)	Superphosphate (consists 14.0–21.0% P_2O_5)	200 kg ha^{-1}	Post-emergence fertilizing
Switchgrass	Superphosphate (consists 14.0–21.0% P_2O_5)	200 kg ha^{-1}	Application during presowing cultivation
Miscanthus	Superphosphate (consists 14.0–21.0% P_2O_5)	200 kg ha^{-1}	Application during cultivation, before planting the rhizomes
Paulownia	Ash (consists ~4.6% P_2O_5, ~3.2% K_2O)	16,000 kg ha^{-1} (1 kg of ash per plant)	Application during hole planting of seedlings

The investigated crop varieties were harvested at three harvesting times, related to the different stages of crop phenological development which correspond to specific BBCH-codes [14,32,33,35,36]. The first harvesting time (H1) was between 01.08.17–03.08.17, the second (H2) between 19.08.17–21.08.17, and the third (H3) between 31.08.17–01.09.17, as recommended by the Institute of Bioenergy Crops and Sugar Beet.

For each sample, the above-ground part of the crops was cut. For each harvesting time and for each investigated crop variety, the fresh matter (FM) yields in kg·ha^{-1} were determined. These FM yields were determined based on the average weight of the plants, their germination rate, the planting density, and the plants' hectare population (amount of plants per hectare).

The dry matter (DM) content related to FM (DM_{FM}) in the collected samples was measured immediately after harvesting. Subsequently, the dried samples were analysed on volatile solids content related to DM (VS_{DM}). The DM_{FM} and VS_{DM} of the samples (in %) were determined by differential weighing before and after drying at 105 °C for 24 h and by subsequent ashing at 550 °C for 8 h, respectively by using standard methods [37,38].

DMY for the samples, in t·ha^{-1} were determined based on the FM yields and the DM_{FM} values. Total Kjeldahl nitrogen (TKN) is expressed as total nitrogen or N if not stated otherwise. The total nitrogen in the samples was determined by Kjeldahl analysis [37,38]. The crop N uptake for the collected samples, in kg_N·ha^{-1} was determined based on the crop N concentrations and their DMY.

For further analysis on the SMY with the Hohenheim Biogas Yield Test (HBT), collected crop samples were ground and dried at 55 °C to a constant weight and they were subsequently delivered to Germany.

2.3. *SMY and AMY*

2.3.1. SMY

The delivered dry crop samples were ground with a 1 mm grid size in the PULVERISETTE 19 cutting mill (Fritsch GmbH, Markt Einersheim, Germany). The samples were analysed in the HBT system as described in the literature [13,39]. The HBT experiment was conducted in 100 mL syringes, each filled with 400 mg of the grounded substrate (55 °C–dried crop sample) and 30 g of inoculum (30 mL, 4.66% DM, 2.82% VS) according to an inoculum to substrate ratio based on DM of 2:1 as recommended by VDI 4630 [40]. The standardized inoculum is cultivated in a 40 L laboratory reactor that was initially filled in with a biogas slurry and fed by a mixture of shredded wheat, soybean meal, rapeseed oil, maize silage and manure as described by Hülsemann et al. [41]. The experiment was conducted in accordance with standard methods [40] under mesophilic conditions of 37 ± 0.5 °C for a period of 35 days. In course of the fermentation process, the gas volume was manually recorded directly at the glass syringe in different time intervals (if at least 20 mL of gas was formed). The methane content was determined using an infrared-spectrometric methane sensor (Pronova Analysetechnik, Berlin, Germany). The experiment was performed in three repetitions. The results of the experiment are expressed in the determined specific methane yields for the samples in $m^3 \cdot kg^{-1}$VS. Gas yield was corrected at conditions of 273 K, 1.013 bar (STP - standard temperature and pressure).

2.3.2. AMY

The areal methane yield of the crop samples, in $m^3_{CH4} \cdot ha^{-1}$ was calculated as defined by the following equation:

$$AMY_{ij} = SMY_{ij} \cdot DMY_{ij} \cdot (VS_{DM})_{ij}$$

where i is related to an i-th crop variety, j represents the j-th harvesting time; SMY_{ij}, DMY_{ij} and $(VS_{DM})_{ij}$ are specific methane yield, dry matter yield and volatile solids content related to dry matter for i-th variety and j-th harvesting time.

2.4. *Statistical Analysis*

For data processing and visualization, Microsoft EXCEL 2016, R and RStudio (version 1.1.463) and SAS 9.4 were used. In the statistical analysis, the Tukey-test and the generalized linear model function were applied.

3. Results and Discussion

The maize, sweet sorghum and miscanthus have shown the highest values of SMY and AMY. Therefore, the results of these plants are first discussed separately. In the following section, there are combined the research results for soybean, soriz, switchgrass and paulownia. Finally, the effect of DMY on AMY and the specific nitrogen use efficiency of the plants were investigated.

3.1. *Maize*

Five varieties of maize (*Zea mays L.*) were analysed: "Varta MV" (FAO 280), "Shedevr MV" (FAO 320), "Slobozhans'ky MV" (FAO 290), "Svitanok MV" (FAO 250) and "Kardynal MV" (FAO 280). The selected varieties represented a wide ripeness spectrum (FAO 250–320). While "Varta MV" and "Shedevr MV" are especially recommended for steppe zones, the variety "Slobozhans'kyi MV" is preferably grown on humid sites and the varieties "Svitanok MV" and "Kardynal MV" grow best in the steppe, forest-steppe and marshlands covered with shrubs known as "Polesia".

According to literature, the highest methane yields for maize can be obtained when harvesting it in the vegetation stage of milk to wax ripeness (BBCH-codes 73–85) [12,23]. However, the timing of these vegetation stages depends on the crop variety and weather conditions. The values of DM_{FM}, VS_{DM}, DMY and crop N uptake are provided in Table 3; the SMY, AMY and BBCH-codes of the maize samples are given in Figure 2.

Table 3. Maize: DM and VS- content at harvest time, dry matter yield per hectare and N-uptake. Units are given in square brackets. Values are given as mean; standard deviation is given in round brackets.

Cultivar	Harvesting Time	DM_{FM} *, [%]	VS_{DM} **, [%]	DMY ***, [t_{DM} ha^{-1}]	Crop N Uptake, [kg_N ha^{-1}]
Kardynal MV	H1	24.4 (1.10)	93.9 (4.22)	7.30 (0.33)	55.5 (2.46)
Shedevr MV	H1	25.0 (1.13)	93.2 (4.19)	7.70 (0.35)	87.8 (3.95)
Slobozhans'kyi MV	H1	27.7 (1.25)	93.4 (4.20)	10.1 (0.46)	71.0 (3.19)
Svitanok MV	H1	24.3 (1.09)	93.7 (4.22)	9.75 (0.44)	116.9 (5.26)
Varta MV	H1	26.9 (1.21)	94.3 (4.25)	8.91 (0.40)	76.6 (3.49)
Kardinal MV	H2	25.8 (0.52)	96.5 (1.93)	7.71 (0.15)	58.6 (1.17)
Shedevr MV	H2	27.4 (0.55)	96.1 (1.92)	8.44 (0.17)	96.2 (1.92)
Slobozhans'kyi MV	H2	36.9 (0.74)	96.1 (1.92)	13.5 (0.27)	94.6 (1.89)
Svitanok MV	H2	25.6 (0.51)	81.3 (1.63)	10.3 (0.21)	123.2 (2.47)
Varta MV	H2	35.4 (0.71)	96.9 (1.94)	11.7 (0.23)	100.8 (2.02)
Kardinal MV	H3	39.4 (1.10)	95.4 (2.67)	11.8 (0.33)	89.5 (2.51)
Shedevr MV	H3	32.2 (0.90)	95.7 (2.68)	9.91 (0.28)	113.0 (3.17)
Slobozhans'kyi MV	H3	45.6 (1.28)	95.9 (2.69)	16.7 (0.47)	116.8 (3.27)
Svitanok MV	H3	44.0 (1.23)	97.3 (2.72)	17.7 (0.49)	211.8 (5.93)
Varta MV	H3	52.2 (1.46)	97.2 (2.72)	17.3 (0.48)	148.7 (4.16)

* The dry matter content related to fresh matter, ** Volatile solids content related to dry matter, *** Dry matter yield.

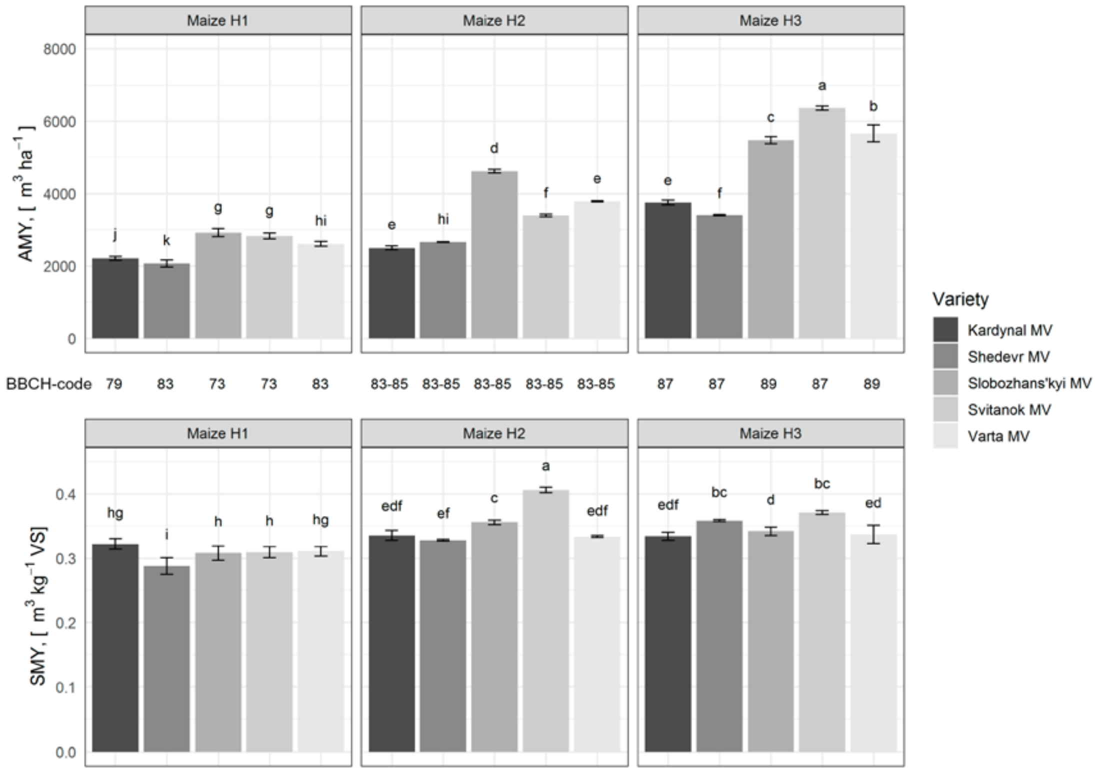

Figure 2. Specific methane yield (SMY) and areal methane yield (AMY) of the maize varieties harvested at H1, H2 and H3 harvesting times, which correspond to a certain BBCH-code related to a specific stage of crop phenological development. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences between all the maize samples according to the results of the Tukey test.

According to the research results for all the maize varieties, the samples harvested at H3 harvesting time (BBCH-codes 87–89) had the highest average values of DM_{FM} (42.68 ± 7.44%), VS_{DM}

(96.30 ± 0.86%), DMY (14.67 ± 3.56 t_{DM} ha^{-1}), crop N uptake (135.98 ± 47.34 kg_N ha^{-1}) and AMY (4929.99 ± 1285.53 m^3 ha^{-1}). However, the highest average values of SMY (0.35 ± 0.03 m^3 kg^{-1}VS) were measured for the maize samples harvested at H2 harvesting time (BBCH-codes 83–85).

As it is shown in Figure 2, the highest SMY of 0.41 ± 0.00 m^3 kg^{-1}VS was measured for the variety "Svitanok MV" from the harvesting time H2 (BBCH-codes 83–85), while the highest AMY value (6365.67 ± 55.49 m^3 ha^{-1}) was determined for the same variety, which was harvested at H3 (BBCH-code 87).

For Western European countries (Austria, Belgium, Germany) specific methane yields between 0.295 and 0.430 m^3 kg^{-1}VS are reported [10,12,14,22,42–44], for Southern European countries (Italy, Spain) between 0.203 and 0.419 m^3 kg^{-1}VS [25,45] and for Northern European countries (Sweden) between 0.280 and 0.370 m^3 kg^{-1}VS [20]. The investigations from the different countries show that areal methane yields of between 2900 and 12,390 m^3 ha^{-1} can be achieved with maize silage [12,22,43,44], with large fluctuations between the individual years. The results of these investigations on the specific methane yields are in the middle range of those values for the Western European countries. The data on areal methane yields should be verified in multi-year studies.

3.2. *Sweet Sorghum*

Four varieties of sweet sorghum (species *Sorghum saccharatum (L.) Moench*) were analysed: "Sylosne 42", "Favoryt", "Zubr", and "Mamont". The selected varieties have the following characteristics and recommended growing zones in Ukraine: "Sylosne 42" and "Favoryt" grow best in the Polesia zone with mid-ripening group of ripeness; "Zubr" grows best in the steppe and forest-steppe; "Mamont" grows best in the steppe zone with mid-ripening group of ripeness.

The values of DM_{FM}, VS_{DM}, DMY and crop N uptake are provided in Table 4; the SMY, AMY and BBCH-codes of the sweet sorghum samples are given in Figure 3.

Table 4. Sweet sorghum: DM and VS- content at harvest time, dry matter yield per hectare and N-uptake. Units are given in square brackets. Values are given as mean; standard deviation is given in round brackets.

Cultivar	Harvesting Time	DM_{FM} *, [%]	VS_{DM} **, [%]	DMY ***, [t_{DM} ha^{-1}]	Crop N Uptake, [kg_N ha^{-1}]
Favoryt	H1	19.7 (0.89)	92.6 (4.17)	14.2 (0.64)	136.8 (6.16)
Mamont	H1	24.0 (1.08)	92.8 (4.17)	17.8 (0.80)	284.6 (12.8)
Sylosne 42	H1	22.3 (1.00)	94.1 (4.24)	11.0 (0.49)	76.8 (3.46)
Zubr	H1	23.8 (1.07)	92.8 (4.18)	12.7 (0.57)	178.1 (8.01)
Favoryt	H2	20.9 (0.42)	95.3 (1.91)	15.1 (0.30)	145.1 (2.90)
Mamont	H2	23.6 (0.47)	94.5 (1.89)	17.5 (0.35)	279.8 (5.60)
Sylosne 42	H2	22.6 (0.45)	94.1 (1.88)	11.1 (0.22)	77.9 (1.56)
Zubr	H2	24.1 (0.48)	92.3 (1.85)	12.9 (0.26)	180.3 (3.61)
Favoryt	H3	24.0 (0.67)	95.9 (2.68)	17.4 (0.49)	166.7 (4.67)
Mamont	H3	23.4 (0.66)	95.1 (2.66)	17.3 (0.49)	277.5 (7.77)
Sylosne 42	H3	23.8 (0.67)	94.9 (2.66)	11.7 (0.33)	81.8 (2.29)
Zubr	H3	24.8 (0.69)	95.8 (2.68)	13.3 (0.37)	185.6 (5.20)

* The dry matter content related to fresh matter, ** Volatile solids content related to dry matter, *** Dry matter yield.

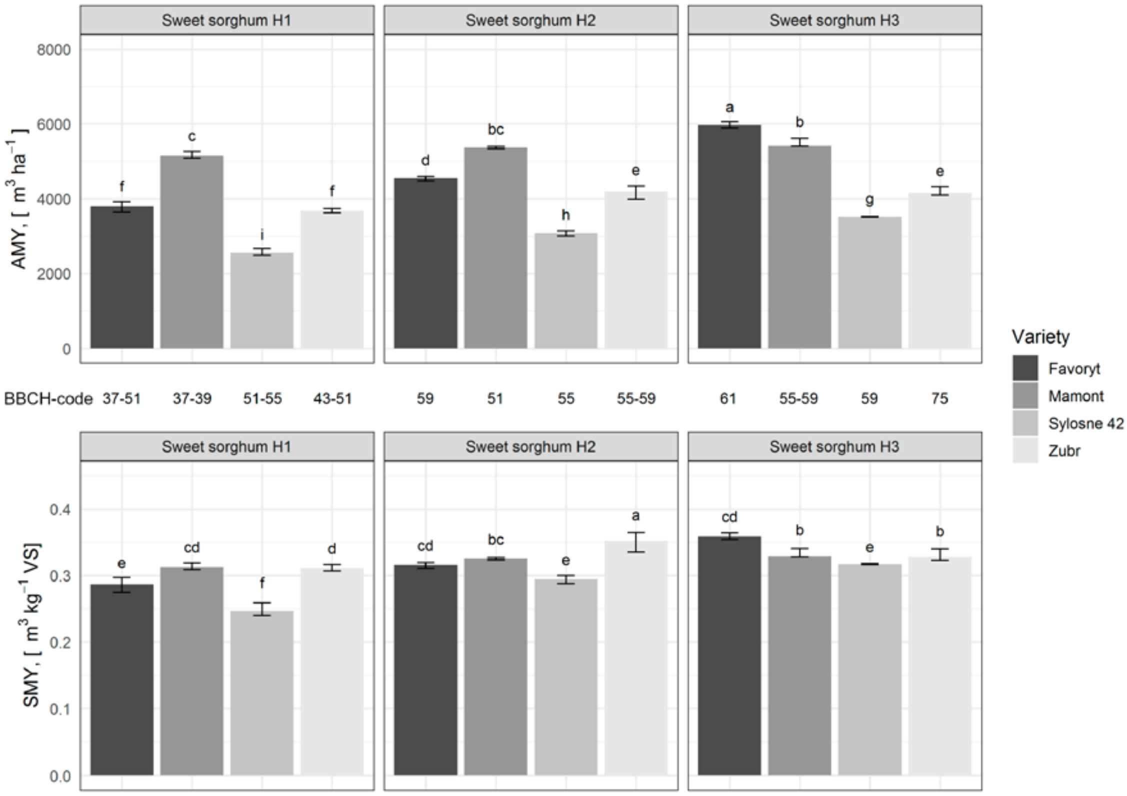

Figure 3. Specific methane yield (SMY) and areal methane yield (AMY) of the sweet sorghum varieties harvested at H1, H2 and H3 harvesting times, which correspond to a certain BBCH-code related to a specific stage of crop phenological development. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences between all the maize samples according to the results of the Tukey test.

The vegetation stages of the samples for the three analysed harvesting times varied between the stage of shooting/ the appearance of the last leaf (BBCH-code 37) and the mid-stage of milk ripeness (BBCH-code 75). The H3 harvesting time was related to the period between the mid-stage of inflorescence (earing) and the mid-stage of milk ripeness (BBCH-codes 55–75). The sweet sorghum samples harvested at H3 had the highest average values of DM_{FM} (23.99 ± 0.59%), VS_{DM} (95.43 ± 0.48%), DMY (14.91 ± 2.89 t_{DM} ha^{-1}), crop N uptake (177.88 ± 80.26 kg_N ha^{-1}), SMY (0.33 ± 0.02 m^3 kg^{-1}VS) and AMY (4767.17 ± 1125.41 m^3 ha^{-1}).

As shown in Figure 3, the highest SMY of 0.35 ± 0.02 m^3 kg^{-1}VS was measured for the variety "Zubr" from the harvesting time H2 (BBCH-codes 55–59), while the highest AMY value of 5968.90 ± 82.70 m^3 ha^{-1} was determined for the variety "Favoryt" harvested at H3 (BBCH-code 61).

For sorghum from Western European countries (Germany), specific methane yields between 0.263 and 0.328 m^3 kg^{-1}VS are reported [10,43]; for Southern European countries (Italy, Spain), the SMY varied between 0.240 and 0.386 m^3 kg^{-1}VS [25,45]. For German sorghum, areal methane yields of between 2900 and 3722 m^3 ha^{-1} can be achieved [43]. The results of these investigations on the specific methane yields are in the middle range of those values for the Southern European countries. The maximal results of the areal methane yields determined for Ukrainian sorghum were higher than those values for German sorghum. However, the data on areal methane yields should be verified in multi-year studies.

3.3. *Miscanthus*

For biogas production from perennial grasses, harvesting time after the ear-emergence stage is recommended [12]. According to Kiesel and Lewandowski (2017) [15], the SMY of miscanthus decreases with later harvesting times, and the AMY obtained from miscanthus is positively correlated with its DMY and SMY.

Two varieties of miscanthus bred and patented by the Institute of Bioenergy Crops and Sugar Beet of Ukraine for energy purposes were analysed in this study:

- Species Giant Chinese Silver Grass: *Miscanthus x giganteus J.M Greef & Deuter ex Hodkinson Renvoiz*, the variety name "Osinnii zoretsvit". We refer to the analysed variety simply as "Giganteus".
- Species Chinese Silver Grass: *Miscanthus sinensis Anderss.*, the variety name "Misiachnyi promin'". We refer to this variety simply as "Sinensis".

Both analysed miscanthus varieties are recommended for growing in the Polesia and the forest-steppe zones in Ukraine.

As it is stated in literature [11,16,46], the age of plantation and environmental factors, such as site, climate and weather conditions, have a direct impact on miscanthus yields, furthermore, mature or stabilized crop yields start from second to fourth year of vegetation and last for at least 15 years. The year of vegetation corresponds to the age of miscanthus rhizomes in soil. For this reason, both analysed miscanthus varieties were harvested from the 3rd vegetation year. For examining whether there is an effect of the age of miscanthus on its SMY and AMY, the miscanthus "Giganteus" (variety "Osinnii zoretsvit") from the 8th vegetation year was additionally analysed.

The values of DM_{FM}, VS_{DM}, DMY and crop N uptake are provided in Table 5; the SMY, AMY and BBCH-codes of the miscanthus samples are given in Figure 4.

Table 5. Miscanthus: DM and VS- content at harvest time, dry matter yield per hectare and N-uptake. Units are given in square brackets. Values are given as mean; standard deviation is given in round brackets.

Cultivar, Vegetation Year	Harvesting Time	DM_{FM} *, [%]	VS_{DM} **, [%]	DMY ***, [t_{DM} ha^{-1}]	Crop N Uptake, [kg_N ha^{-1}]
Giganteus, 3rd year	H1	44.4 (2.00)	93.8 (4.22)	20.5 (0.92)	115.0 (5.18)
Giganteus, 8th year	H1	45.6 (2.05)	92.9 (4.18)	27.5 (1.24)	110.1 (4.96)
Sinensis, 3rd year	H1	36.6 (1.65)	91.0 (4.10)	11.0 (0.50)	55.2 (2.48)
Giganteus, 3rd year	H2	45.6 (0.91)	97.5 (1.95)	21.1 (0.42)	118.0 (2.36)
Giganteus, 8th year	H2	46.9 (0.94)	96.8 (1.94)	28.3 (0.57)	113.3 (2.27)
Sinensis, 3rd year	H2	38.6 (0.77)	94.4 (1.89)	11.6 (0.23)	58.2 (1.16)
Giganteus, 3rd year	H3	48.9 (1.37)	95.5 (2.67)	22.6 (0.63)	126.7 (3.55)
Giganteus, 8th year	H3	50.4 (1.41)	96.8 (2.71)	30.4 (0.85)	121.7 (3.41)

* The dry matter content related to fresh matter, ** Volatile solids content related to dry matter, *** Dry matter yield.

The samples were harvested in the period between the stem elongation, booting and before the inflorescence emergence stage (BBCH-codes 36–47). All miscanthus samples had the highest average DM_{FM} (47.80 ± 3.29%) when harvested at H3 (BBCH-codes 41–47). The highest average VS_{DM} content (95.13 ± 1.90%) was measured for the samples harvested at H2 (BBCH-codes 39–43). All tested varieties showed the highest average DMY (22.12 ± 8.57 t_{DM} ha^{-1}) during the third harvesting period. However, the DMY values for "Giganteus" varied between the samples from different vegetation years. The maximum DMY (30.43 ± 0.85 t_{DM} ha^{-1}) was determined for "Giganteus" from the 8th vegetation year harvested at H3 (BBCH-code 41). The highest average crop N uptake (104.97 ± 33.38 kg_N ha^{-1}) of miscanthus is related to the H3 harvesting time. Nonetheless, N uptake varied between the varieties: at H3 the uptake for "Sinensis" was 66.50 ± 1.86 kg_N ha^{-1} (BBCH-code 47), while for two "Giganteus" samples from H3 the determined average crop N uptake was 124.19 ± 0.10 kg_N ha^{-1} (BBCH-code 41). In spite of that, the highest average SMY of 0.26 ± 0.04 m^3 kg^{-1}VS and the highest average AMY of 4805.44 ± 2357.94 m^3 ha^{-1} corresponded to the crop samples from H1 (BBCH-codes 36–39). There was also a big difference in SMY between the samples. The highest SMY (0.29 ± 0.02 m^3 kg^{-1}VS) and the highest AMY (7404.55 ± 199.00 m^3 ha^{-1}) were measured for "Giganteus" from the 8th vegetation year harvested at H1 (BBCH-code 36).

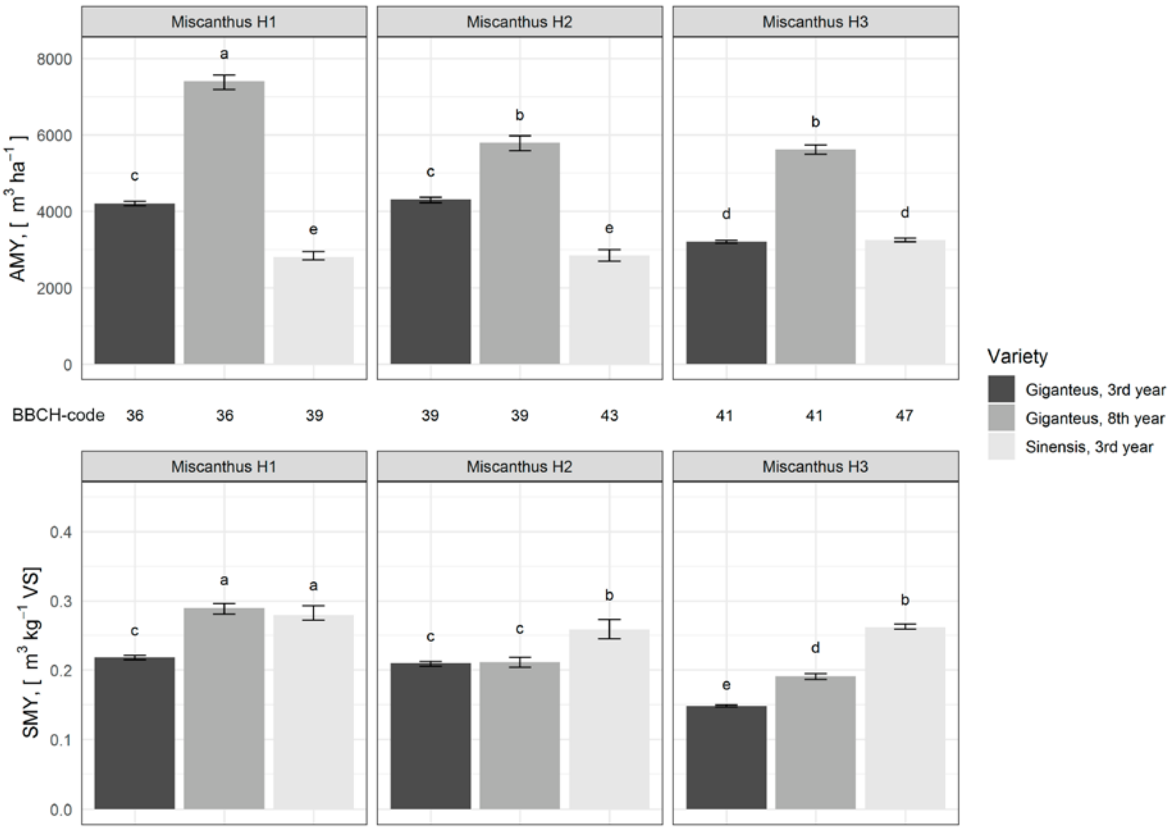

Figure 4. Specific methane yield (SMY) and areal methane yield (AMY) of the miscanthus varieties harvested at H1, H2 and H3 harvesting times, which correspond to a certain BBCH-code related to a specific stage of crop phenological development. The analysed varieties were: (1) Species Giant Chinese Silver Grass: *Miscanthus x giganteus J.M. Greef & Deuter ex Hodkinson Renvoiz*, the variety name "Osinnii zoretsvit", referred to as "Giganteus". (2) Species Chinese Silver Grass: *Miscanthus sinensis Anderss.*, the variety name "Misiachnyi promin'", referred to as "Sinensis". "Giganteus" from the 3rd and the 8th years of vegetation and "Sinensis" from the 3rd vegetation year were analysed. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences between the analysed miscanthus samples according to the results of the Tukey test.

For Germany, the specific methane yields for miscanthus between 0.179 and 0.280 $m^3 kg^{-1} VS$ are reported [10,15]. The areal methane yields for German miscanthus varied between 2300 and 6400 $m^3 ha^{-1}$ [15]. The results of these investigations are in the middle range of the values reported in the literature, except for miscanthus "Giganteus" from the 8th vegetation year with the higher SMY and AMY values.

3.4. Data Analysis for Maize, Sweet Sorghum and Miscanthus

A generalized linear model procedure was used for the analysis of data based on the results for maize, sweet sorghum and miscanthus. Based on the modelling results, the following conclusions can be drawn:

SMY was significantly affected by the BBCH-code ($p = 0.0009$) and by the variety ($p < 0.0001$). The highest SMY was determined for the analysed maize and sweet sorghum samples. The highest SMY values for the maize samples were related to the vegetation stage of wax to full ripeness (BBCH-codes 83–87). For sweet sorghum, the highest SMY corresponded to the period between the mid-stage of inflorescence (earing) and the flowering stage (BBCH-codes 55–61). For miscanthus, the highest SMY values were determined for the samples harvested at earlier harvesting times, which corresponded to the vegetation period between the stem elongation and booting (BBCH-codes 36–39).

AMY was significantly affected by the BBCH-code (p = 0.0024) and by the variety (p < 0.0001). The highest AMY among the three crops was found for miscanthus "Giganteus" from the 8th vegetation year harvested at H1 (7404.55 ± 199.00 m ha^{-1}, BBCH-code 36). For maize and sweet sorghum, the highest yields were found when collected at the third harvesting time. When harvested at H3, the mean AMY values for maize with the highest yields (4929.99 ± 1285.53 m^3 ha^{-1}, BBCH-codes 87–89) have slightly overperformed those values for sweet sorghum (4767.17 ± 1125.41 m^3 ha^{-1}, BBCH-codes 55–75). Thus, the AMY of miscanthus was about 50% higher than that of the traditional energy crops maize and sweet sorghum. Miscanthus has an additional advantage of being a perennial crop, which can be cultivated for more than 20 years; moreover, this crop can be grown on marginal and contaminated lands for soil phytoremediation [16,47]. Furthermore, the cultivation costs for miscanthus are lower, than those for maize and sweet sorghum. In further studies, SMY and AMY of miscanthus under other vegetation years up to the death of plantation have to be further investigated.

3.5. Other Analysed Crops

In addition to the "traditional" energy crops, also soybean, soriz, switchgrass and paulownia had been analysed according to their methane yield potential. These results are provided in Table 6. Soybean (*Glycine max (L.) Merrill*) can be successfully grown in rotation with a large variety of other plants [11], as well as in widely diverse climates and on varied soil types [48]. Soriz (*Sorghum oryzoidum*) is a hybrid of sorghum and rice, which was selected for this study for being nonexacting to soil with lodging resistance, as well as with resistance to smut diseases and lice [29]. Switchgrass (*Panicum virgatum L.*) is a perennial plant, which is valued for its soil stabilizing, phytoremediation and windbreaking capacities in crop fields [16,31]. Paulownia (*Paulownia Sieb. et Zucc.*, species *P. tomentosa x P. fortunei*) is a very fast-growing plant, which is extremely adaptive to a wide range of soils and climatic conditions and can also be grown on marginal lands [34,49].

Table 6. Soybean, soriz, switchgrass and paulownia: DM and VS- content at harvest time, dry matter yield per hectare and N-uptake. Units are given in square brackets. Values are given as mean; standard deviation is given in round brackets.

Crop	Analysed Cultivars	DM_{FM} *, [%]	VS_{DM} **, [%]	DMY ***, [t_{DM} ha^{-1}]	Crop N Uptake, [kg_N ha^{-1}]	SMY, [Nm^3 **** kg^{-1}VS]	AMY, [Nm^3 ha^{-1}]
Soybean	"Diona", "Muza", "Sharm", "Sprytna"	28.88 (4.74)	90.17 (2.76)	1.41 (0.71)	20.47 (9.72)	0.27 (0.025)	341.77 (183.31)
Soriz	"Kvarts", "Saliut", "Titan"	16.32 (3.07)	92.16 (1.89)	3.81 (1.21)	0.06 (0.02)	0.329 (0.006)	1164.05 (407.62)
Switchgrass	"Morozko" from the 2nd and the 8th vegetation years	38.21 (5.77)	95.21 (0.82)	2.99 (0.89)	24.78 (4.24)	0.258 (0.006)	732.77 (210.23)
Paulownia	"Shantong"	25.87 (0.01)	83.47 (5.58)	7.08 (0.36)	56.66 (2.85)	0.231 (0.055)	1363.95 (329.73)

* The dry matter content related to fresh matter, ** Volatile solids content related to dry matter, *** Dry matter yield, **** Nm^3 (273 K, 1.013 bar).

For switchgrass, specific methane yields between 0.191 and 0.309 m^3 kg^{-1}VS are reported for South European countries (Spain) [25]. Similar SMY were measured for switchgrass from Canada (between 0.210 and 0.365 m^3 kg^{-1}VS) with an AMY between 1500 and 3280 m^3 ha^{-1} [21]. The results of these investigations on the specific methane yields from Ukrainian switchgrass are in the middle range of those values reported in the literature. However, the maximal results of the areal methane yields determined for Canadian switchgrass were higher than those values achieved in our study.

3.6. Influence of DMY on AMY

For all the analysed samples, when comparing DMY and AMY, a high correlation ($R^2 = 0.97$) significant at the 0.001 probability level was found (see Figure 5). A rather small correlation was identified for only the samples from miscanthus "Giganteus". With the exception of paulownia and miscanthus "Giganteus", almost all varieties for all analysed crops have shown higher AMY values at higher DMY, harvested at the later stage of maturity.

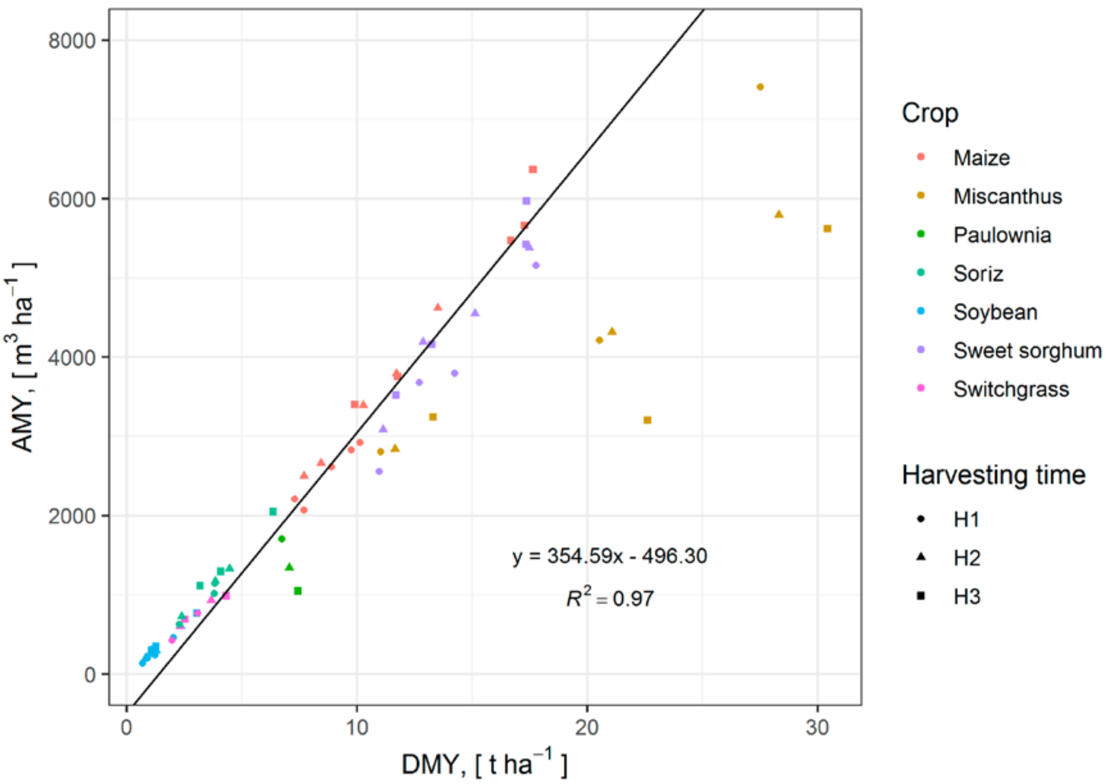

Figure 5. Correlation between dry matter yield (DMY) in t ha^{-1} and areal methane yield (AMY) in m^3 ha^{-1} for the total of 22 varieties from 7 crops harvested in three harvesting times (H1, H2 and H3) in 2017 (total amount of samples $n = 66$).

3.7. Influence of Crop N Uptake on AMY

Crop N uptake is related to crop N demand, the dosage of fertilizer supplied, and the N contents in soil [50,51]. The generalised mechanism of N uptake by plants related to N supply is described by Lawlor [51]. A small correlation ($R^2 = 0.29$, significant at the 0.001 probability level) was found between the crop N uptake and the AMY values for all the analysed samples (see Figure 6). The crop N uptake varied depending on plant, variety and harvesting time.

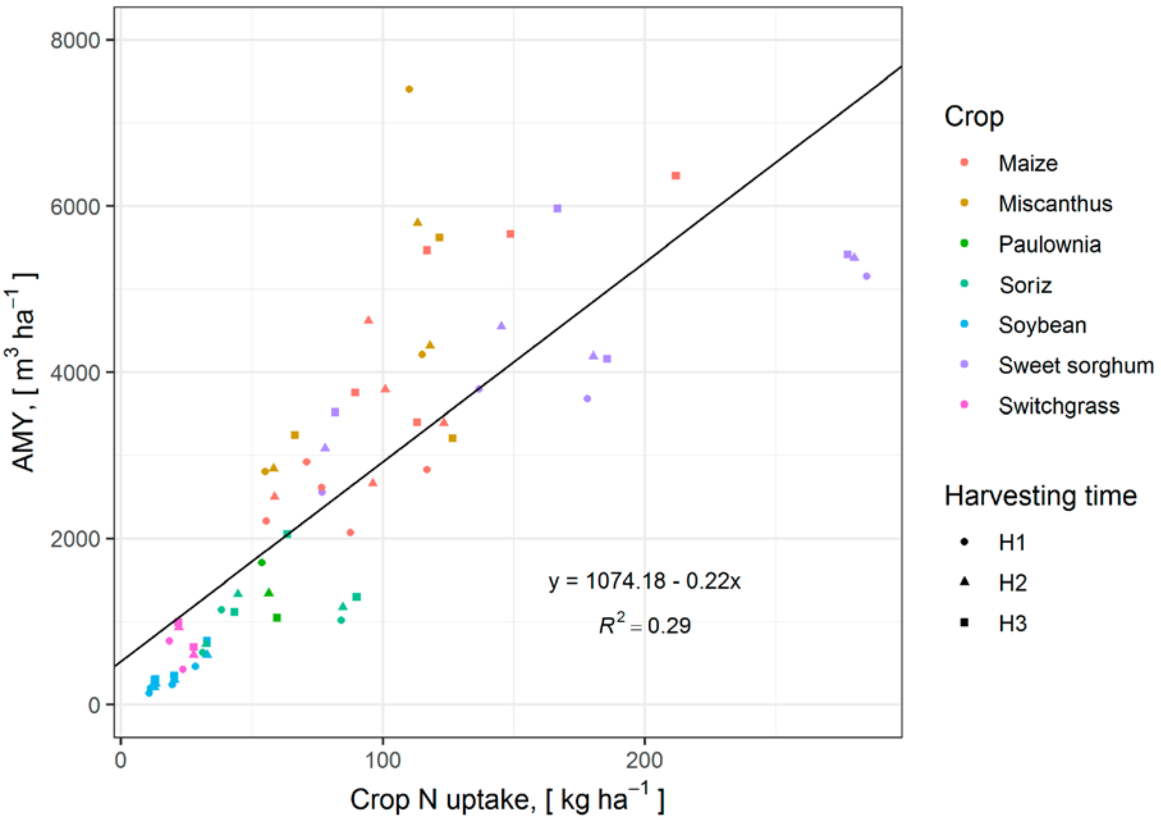

Figure 6. Correlation between crop nitrogen (N) uptake in kg ha^{-1} and areal methane yield (AMY) in m^3 ha^{-1} for the total of 22 varieties from 7 crops harvested in three harvesting times (H1, H2 and H3) in 2017 (total amount of samples n = 66).

Among all the analysed research crops, miscanthus has the lowest N-demand per 1 m^3 methane produced (23.41 ± 7.18 g_N m^{-3}). Maize has higher N-demand than miscanthus, but is more efficient in N-use compared to other analysed crops (29.58 ± 7.13 g_N m^{-3}). Switchgrass and sweet sorghum continue this list with their N-demand of 36.84 ± 13.87 g_N m^{-3} and 39.08 ± 11.35 g_N m^{-3}, respectively. Paulownia, soriz and soybean are the least efficient in N use for producing 1 m^3 methane among all the analysed plants.

4. Conclusions

In this study, Ukrainian energy crops were harvested at different harvesting times, related to the different stages of crop phenological development and analysed according to their dry matter content, volatile solids content, dry matter yield, crop nitrogen uptake, specific methane yield, and areal methane yield. Miscanthus, sweet sorghum and maize are, in that order, particularly well suited for use as energy crops in Ukraine. Whereas the AMY of maize and sweet sorghum are mainly influenced by DMY of the crops, the SMY of miscanthus has a great influence on its methane yield per hectare. In relation to the biogas formation potential, miscanthus and silage maize showed the highest nitrogen use efficiency. This means that they have the lowest N requirement relative to biogas formation. For the continental climate of Ukraine, miscanthus appears to be the most interesting energy crop under the aspects of cultivation costs, methane yield per area and nitrogen use efficiency.

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3. Publication 2: Effects of Increasing Nitrogen Content on Process Stability and Reactor Performance in Anaerobic Digestion

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Article

Effects of Increasing Nitrogen Content on Process Stability and Reactor Performance in Anaerobic Digestion

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Abstract: The aim of this study was to analyse the effect of different nitrogen increase rates in feedstock on the process stability and conversion efficiency in anaerobic digestion (AD). The research was conducted in continuously stirred tank reactors (CSTR), initially filled with two different inocula: inocula #1 with low and #2 with high nitrogen (N) concentrations. Three N feeding regimes were investigated: the "0-increase" feeding regime with a constant N amount in feeding and the regimes "0.25-increase" and "0.5-increase" where the N concentrations in feedstock were raised by 0.25 and 0.5 $g \cdot kg^{-1}$, respectively, related to fresh matter (FM) every second week. The N concentration inside the reactors increased according to the feeding regimes. The levels of inhibition (Inhibition) in specific methane yields (*SMY*), related to the conversion efficiency of the substrates, were quantified. At the N concentration in digestate of 10.82 ± 0.52 $g \cdot kg^{-1}$ FM measured in the reactors with inoculum #2 and "0.5-increase" feeding regime, the level of inhibition was equal to 38.99% ± 14.99%. The results show that high nitrogen increase rates in feeding regime are negatively related to the efficiency of the AD process, even if low volatile fatty acid (VFA) concentrations indicate a stable process.

Keywords: biogas; methane; ammonia; inhibition; acclimatization; trace elements

1. Introduction

Utilization of protein-rich substrates, such as kitchen waste, poultry manure, microalgae, green legumes, oilseeds, etc. may lead to high concentrations of nitrogen (N) in the reactor during anaerobic digestion (AD) [1–4]. High concentrations of N inside the reactor negatively affect process stability and efficiency due to ammonia formation. Total ammonia nitrogen (TAN), which is generally defined as the sum of free ammonia nitrogen (FAN, NH_3-N) and ammonium nitrogen (NH_4^+-N), is formed during the hydrolysis of proteins, urea and nucleic acids [5–8]. Ammonia freely passes through the cell membranes of methanogens and causes a proton imbalance [5,8,9]. Free ammonia changes the intracellular pH of methanogenic bacteria and inhibits specific enzymatic reactions [10]. Therefore, high concentrations of ammonia in anaerobic reactors lead to inhibition of methanogenesis and may cause complete failure of AD [6,11,12]. As reported by Chen et al. [13], temperature change has a direct impact on both microbial growth rates and free ammonia concentration: increased process temperature affects the metabolic rate of the microorganisms in a positive way; however, it also results in higher ammonia levels.

The chemical balance between NH_3 (free ammonia) and NH_4^+ (ammonium) is shown in Equation (1) [14,15].

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \quad (1)$$

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The shift of this equilibrium depends mainly on the process conditions, i.e., temperature and pH [7,9,14]. The concentration of free ammonia is positively correlated with temperature and pH [5,16].

Under high ammonia concentrations in the reactor, the acetoclastic methanogens (e.g., *Methanosarcina, Methanosaeta* spp.) are unable to degrade acetate, which results in its accumulation, depletion of buffer capacity and a subsequent drop in pH [16–19].

According to the literature [9,10,12,13,20], inhibition of the AD process by ammonia is indicated by the decrease in the specific methane yields along with the increase in volatile fatty acid (VFA) concentrations and a pH drop due to inhibition of bacterial growth. However, the limiting concentrations of TAN and FAN for maintaining AD without inhibition are subject to discussion (Table 1). In addition, there is a controversy whether TAN or FAN mainly inhibits methanogenesis [20].

Most authors in previous studies tend to agree that TAN ≥ 3.00 g·L^{-1} and FAN ≥ 0.20 g·L^{-1} have an inhibitory effect on AD (see Table 1). According to Table 1, very few studies have measured the level of inhibition in methane production when treating N-rich substrates.

For maintaining stable and efficient biogas production under high and/or increasing TAN and FAN concentrations, acclimatization strategies can be applied. A frequently used approach is to feed the reactor with a specific N- or ammonia-increase rate. However, no information on the maximum increase rates is available [1,4,12,21–24].

High nitrogen concentrations in the digestate are generally the result of a narrow carbon-to-nitrogen (C/N) ratio of the feedstock [9,12,25,26]. To reduce the concentrations of TAN and FAN in the digestate and thus to maximise biogas and methane yields, Shanmugam and Horan [26] recommend keeping the C/N ratio of the feedstock in the range of 15 to 20, while according to Kayhanian [9], this ratio should be between 27 and 32.

Currently, many operators of biogas plants suffer from AD inhibition and methane losses when utilizing N-rich substrates. The application of the acclimatization strategy with an optimal N-increase rate could stabilize AD and prevent or minimize methane losses. In this study, natural N-sources and microbial communities from full-scale biogas digesters were utilized. The research was conducted in continuously operated reactors under conditions similar to those in full-scale biogas plants. The aim of this investigation is to determine the effect of different nitrogen increase rates on anaerobic digestion in order to achieve an optimal process performance. The nitrogen increase in the feedstock was carried out every two weeks at rates of 0.25 and 0.5 g·kg^{-1} related to fresh matter. The N content of the digestate rose continuously in response to the two different increase rates in the feedstock. At the same time, the C/N ratio of the feedstock consistently decreased throughout the experiment. Thus, the influence of high nitrogen content on the stability of the fermentation process with regards to the C/N ratio could be investigated. By comparing the values of specific methane yield (*SMY*) obtained from the continuous experiment with those obtained from the batch experiment, the effect of increasing N content in the feedstock on the conversion efficiency of the substrates was studied.

Table 1. Limiting total ammonia nitrogen (TAN)- and free ammonia nitrogen (FAN)-concentrations (g·L^{-1}) for maintaining stable anaerobic digestion (AD) under different temperature conditions.

Mesophilic conditions									
TAN	**Treated substrate**	**Operating temperature**	**Inhibition in CH_4 production**	**Reference**	**FAN**	**Treated substrate**	**Operating temperature**	**Inhibition in CH_4 production**	**Reference**
≤ 1.00	Mashed biowaste, residual food waste	Not indicated	Not indicated	[8]	≤ 0.03	Mashed biowaste, residual food waste, steers manure	Not indicated, 35 °C	Not indicated	[8,21]
≤ 2.00	Food waste	37 °C	Not indicated	[17]	≤ 0.49 (b)	Animal manure, food waste	37 ± 1 °C	Not indicated	[22]
≤ 2.40	Chicken manure, spent poppy straw	36 ± 1 °C	Not indicated	[27]	≤ 1.10	Thin stillage	38 °C	Not indicated	[28]
≤ 3.00	Municipal wastewater biosolids	36 ± 1 °C	Not indicated	[29]	≤ 1.20	Pig slurry, maize silage, other agricultural wastes	38.0 ± 0.5 °C	Not indicated	[30]
≤ 3.20 (b)	Municipal wastewater	~ 22 °C	Not indicated	[23]					
≤ 3.50	Municipal wastewater biosolids	37 ± 1 °C	Not indicated	[1]					
≤ 4.56 (b)	Jatropha press cake	37 °C	Not indicated	[31]					
≤ 5.00	Animal/ poultry manure, organic waste, municipal wastewater	30–38 °C	50% inhibition at TAN of 3.0 g l^{-1}	[11,32–34]					
≤ 6.00	Pig slurry, maize silage, other agricultural wastes	38.0 ± 0.5 °C	Not indicated	[30]					
≤ 7.00	Chicken manure, maize silage	37–41 °C	10 - 20% at TAN ≥ 7.0 g l^{-1}, FAN ~ 600 mg l^{-1}; 50% at TAN ≥ 8.8 g l^{-1}	[35]					

Table 1. *Cont.*

≤	10.00 (b)	Animal waste, food waste	37 ± 1 °C	Not indicated	[4]						
≤	11.80 (b)	Beet-sugar factory wastewater	30 ± 1 °C	Not indicated	[24]						
Thermophilic Conditions											
	TAN	**Treated substrate**	**Operating Temperature**	**Inhibition in CH4 Production**	**Reference**		**FAN**	**Treated substrate**	**Operating Temperature**	**Inhibition in CH4 Production**	**Reference**
≤	1.80–2.40	Dairy manure	55 °C	Not indicated	[36]	≤	0.20 (a)	Steer manure	55 °C	Not indicated	[21]
≤	4.32 (b)	Animal manure, food industrial organic waste	53 ± 1 °C	Not indicated	[22]	≤	0.39 (b)	Steer manure	55 °C	Not indicated	[21]
						≤	0.85	Municipal wastewater biosolids	55 °C	Not indicated	[1]
						≤	1.20	Cattle manure	53–55 °C	Not indicated	[37]
						≤	1.43 (b)	Animal manure, food industrial organic waste	53 ± 1 °C	Not indicated	[22]

if stated: (a) unacclimatised, (b) acclimatized.

2. Materials and Methods

2.1. *Reactor Design*

The experiment was conducted in 12 horizontal, stainless steel, continuously stirred tank reactors (CSTR) of 20 L total volume (working volume 17 L) each, as described in [38], in duplicate repetition according to the Guideline 4630 issued by the Association of German Engineers (VDI) [39]. Different N-increase rates in the CSTR were achieved by different feeding regimes described in Section 2.2. During the experimental period, the organic loading rate related to volatile solids (OLR_{VS}) was kept at 3 $kg \cdot m^{-3} \cdot d^{-1}$ with a hydraulic retention time (HRT) of 40 days. The temperature in each reactor was mesophilic at 37 ± 1 °C.

2.2. *Inocula and N-Increase in Feeding Regimes*

Each digester was filled with 17 L of inoculum at the beginning of the experiment. Inoculum #1 and inoculum #2 from two full-scale biogas plants were used in this trial. These inocula differed in total Kjeldahl nitrogen (TKN) concentrations, with inoculum #2 containing twice as much nitrogen as inoculum #1 (Table 2). Inoculum #1 was taken from a digester treating cattle manure (35–40%), maize silage (40%), grain whole plant silage (5%) and triticale (rest). Inoculum #2 was taken from a digester treating turkey manure (10%), cattle manure (8%), cereals (10%) and maize silage (62%).

Table 2. Characteristics of the substrates. Gas volumes are given under standard temperature and pressure conditions (0 °C, 101.325 kPa). Units are given in square brackets. Values are given as mean; the standard deviation is given in round brackets.

Parameter	Inoculum		Maize Silage	Soybean Meal
	#1	#2		
DM_{FM} [(a)] [$g \cdot kg^{-1}$]	59.80 (2.99)	103.75 (5.18)	377.61 (18.88)	887.99 (18.46)
VS_{DM} [(b)] [$g \cdot kg^{-1}$]	738.74 (36.93)	789.74 (39.48)	907.38 (45.37)	927.19 (4.06)
TKN_{FM} [(c)] [$g \cdot kg^{-1}$]	3.34 (0.70)	7.14 (0.36)	4.00 (0.20)	67.83 (3.39)
$NH_4{}^{+}{}_{FM}$ [(d)] [$g \cdot kg^{-1}$]	1.35 (0.07)	5.00 (0.25)	0.60 (0.03)	9.50 (0.48)
pH	7.44 (0.37)	8.42 (0.42)	NA	NA
SMY_{VS} [(e)] [$L \cdot kg^{-1}$]	25.78 (1.30)	88.43 (4.42)	330.66 (15.08)	423.16 (21.11)

[(a)] Dry matter (DM) related to fresh matter (FM), [(b)] volatile solids (VS) related to DM, [(c)] total Kjeldahl nitrogen (TKN) related to FM, [(d)] ammonium related to FM, [(e)] specific methane yield (SMY) related to VS.

The substrates were fed into the reactor daily as described in [38]. The daily feedstock consisted of fresh inoculum, maize silage (low nitrogen content) and soybean meal (N-rich substrate). Tap water was added in order to keep the HRT and OLR_{VS} constant, thus resulting in 425 g of fresh matter daily feedstock. Characteristics of the substrates are described in Table 2. The values of the specific methane yield for the feeding substrates were determined by the Hohenheim biogas yield test [40,41].

For each inoculum, the different feeding regimes were separately analysed, as shown in Figure 1. These feeding regimes represent the rate of N increase in the feeding ratio. The investigated feeding regimes were "0-increase", "0.25-increase" and "0.5-increase". Under the "0-increase" feeding regime, the nitrogen content did not change over the whole course of the experiment. For the other two regimes, there was an increase in nitrogen content in the feedstock (see Figure 1a). The increase in nitrogen concentration was achieved by adding soybean meal and simultaneously decreasing the share of maize silage. In the feeding regimes "0.25-increase" and "0.5-increase", the share of soybean meal was increased stepwise, thus leading to N-increase rates of 0.25 and 0.5 $g \cdot kg^{-1}$ FM every two weeks, respectively. By contrast, the C/N ratio in feedstock was decreasing as shown in Figure 1b.

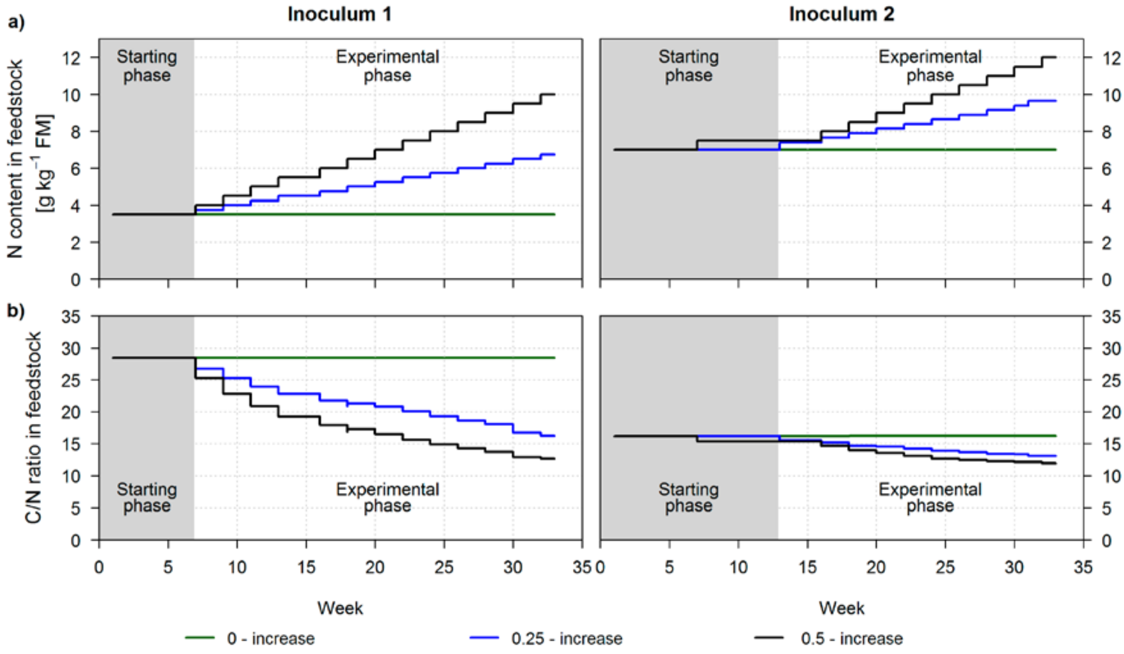

Figure 1. Experimental procedure: (**a**) N content in feedstock; (**b**) C/N ratio in feedstock. The results are given separately for inoculum #1 and inoculum #2. Different line colours in the graphs and in the legend correspond to the N-increase rates "0-increase", "0.25-increase" and "0.5-increase" in the feeding regimes. Grey and white backgrounds in the graphs are related to the starting phase and the experimental phase, respectively.

2.3. *Trace Elements Supplementation*

For AD process stability, the importance of micronutrients, i.e., iron, nickel, molybdenum, cobalt and selenium is described in literature [27,42–45]. After observing process instability for the reactors with inoculum #1 at the beginning of week 17, micronutrient levels were tested. In response to the identified deficiency in trace elements (TE) in the reactors with inoculum #1 and for keeping the TE in the range as recommended by Vintiloiu et al. [42], 1.23 g of BC.Pro Akut® was added to all the reactors (with #1 and #2) weekly, starting from the end of week 17 up to the end of the experimental trials. BC.Pro Akut® is a mixture of TE and other components comprising the following active substances in the ionic form: aluminium, boron, calcium, iron, cobalt, copper, magnesium, manganese, molybdenum, sodium, nickel, selenium, tungsten and zinc.

2.4. *Analytical Methods*

The produced biogas was collected in gas bags as described by Haag et al. [38]. A gas measuring unit automatically analysed the gas quantity (Hoentzsch FA MS40, Waiblingen, Germany), as well as the content of CH_4 and CO_2 (AGM 10, Sensors Europe, Erkrath, Germany). The measurements were carried out once per day before feeding.

Samples were taken from the reactors weekly. The dry matter content related to fresh matter (DM_{FM}) and volatile solids content related to dry matter (VS_{DM}) of the collected samples were determined by differential weighing before and after drying at 105 °C for 24 h and by subsequent ashing at 550 °C for 8 h, respectively. The pH was measured in each reactor three times per week with a WTW 323, using a SenTix 41 pH-electrode (WTW, Weilheim, Germany). Concentrations of VFA in the samples were determined by gas chromatography. The gas chromatograph Shimadzu GC-2010plus (Tokyo, Japan) was equipped with a FFAP 50 m × 0.32 mm column with a chemically bonded polyethylene glycol CP-Wax 58 FFAP CB 1.2 μm film, a flame ionization detector and helium as a carrier gas. Total ammonium concentrations in the digestate were determined by the automatic distillation system Gerhardt Vapodest 50s (Koenigswinter, Germany). Total Kjeldahl nitrogen (TKN) is expressed as total nitrogen or N if not stated otherwise. The total nitrogen in the samples was determined by Kjeldahl analysis. The potassium determination was done by means of flame atomic absorption spectroscopy (AAS, Eppendorf, ELEX 6361, Wesseling-Berzdorf, Germany), operated with an acetylene gas. For the determination of phosphorus, a cuvette test [46] and a spectrophotometer UV–VIS 1240 (Shimadzu, Tokyo, Japan) were used. All the analyses were carried out according to standard methods [46]. The analysis on trace element content in the samples was done by an external laboratory in accordance with standard methods [47–49].

2.5. Calculation of FAN and TAN

The NH_3 (free ammonia) concentration was calculated by using the equation described in [14].

$$NH_3 = K_{NH_4} \cdot \frac{NH_4^+}{H^+} \quad (2)$$

where NH_4^+ is the ammonium concentration in g·kg^{-1} related to FM; K_{NH_4} is the ionization constant of ammonium (for 37 °C, K_{NH_4} = 1.14·10^{-9} [21]); H^+ is the hydrogen ion concentration ($H^+ = 10^{-pH}$ [14]). NH_3 was recalculated to NH_3-N (FAN), and NH_4^+ was recalculated to NH_4^+-N (ammonium nitrogen) according to their molar masses. The concentration of TAN was calculated as the sum of FAN (NH_3-N) and NH_4^+-N.

2.6. Statistical Analysis

For data processing and visualization, Microsoft EXCEL 2016, SAS 9.4, R and RStudio (version 1.1.463) were used.

2.6.1. Inhibition in SMY

The inhibition in specific methane yields (Inhibition) for different N-increase rates in feeding regimes is defined by Equation (3):

$$\text{Inhibition} = \frac{1}{n}\sum_{i=1}^{n} \frac{SMY_t - SMY_m}{SMY_t} \cdot 100\% \quad (3)$$

where n is the number of observations over the experimental period taken for the analysis. The theoretical methane yields (SMY_t) were calculated based on the amounts of VS_{DM} added to the reactors and the SMY_{VS} of the substrates obtained by the Hohenheim biogas yield test (Table 2). The measured SMY (SMY_m) was based on the measured value of methane yield divided by the amount of VS added to the reactor. This inhibition can also be described as the conversion efficiency between the theoretical SMY_t values obtained from the batch experiment and the measured SMY_m values obtained from the continuous experiment.

The one-sided Tukey test was applied to identify whether the difference between the SMY_t and SMY_m was statistically significant. The analysis was done in Excel and Rstudio.

2.6.2. Analysis of the effect of TAN and FAN on inhibition

Based on the experimental data for the three investigated feeding regimes along with inocula #1 and #2, the effects of TAN and FAN concentrations in the reactor on the level of inhibition were analysed. For this purpose, mixed modelling for repeated measurements was applied [50]. This model was selected for serial correlation among observations on the same experimental unit. The experimental unit, in our case, was the reactor [50]. Analyses were based on the experimental data starting from week 17 of the trials after the TE supplementation was started. The applied data were checked by using the normality test on the studentized residuals. For meeting the requirements of the mixed model, the square-root transformation of the data on inhibition in *SMY* (sqrt_Inhibition) was used. Several types of models (independent, compound symmetry, autoregressive, unstructured) were checked; on the grounds of the normally distributed residual plots and the lowest Akaike information criterion (AIC) value, the compound symmetry type was selected as the best-fitting model.

The applied model is given in Equation (4):

$$y_{itk} = \mu + a_i + r_t + b_{tk} + e_{itk} \quad (4)$$

where y_{itk} is the dependent variable; i is the i-th observation, t is the weekly measurement and k is related to the interaction between the fixed factor and the point in time (t); μ describes the general effect of the model; a_i is the i-th observation of the fixed factor; r_t is the replicate of a weekly measurement; b_{tk} is the random effect of a week and the interaction between week and the fixed factor; e_{itk} is the random deviation associated with y_{itk}.

The sqrt_Inhibition was used as the dependent variable; the TAN and FAN were separately analysed as the fixed factor. The influence of time and interaction between time ("WEEK", in our case) and a fixed factor was analysed on a random effect in the model. The "MIXED" procedure of SAS was used to fit the model.

3. Results and Discussion

The reactors were continuously monitored over the whole period of the trials. The measured values for N, TAN, FAN, acetic acid (HAc), pH, SMY_m and inhibition are shown in Figure 2. The trial period included a starting phase and an experimental phase.

3.1. The Starting Phase

During the starting phase, the OLR_{VS} was increased until the aimed values were achieved.

The starting phase was needed for the microorganisms to adapt to the operating conditions. During this phase, all reactors were fed with a constant N feeding ratio equivalent to the "0-increase" variant (Figure 2a) to establish stable conditions. The stable operation was determined by monitored VFA concentrations (Figure 2d) and specific methane production (Figure 2f). In week four to six, the TE concentrations were additionally tested, which showed sufficient nutrient levels according to Vintiloiu et al. [42] (see Table 3). For inoculum #1, the starting phase lasted for 48 days. For inoculum #2, the starting phase took 90 days.

The values provided in Figure 2 for the starting phase can be relevant for farmers and biogas operators when utilizing protein-rich substrates in biogas plants. However, these values are excluded from the statistical analysis described in Section 3.3.

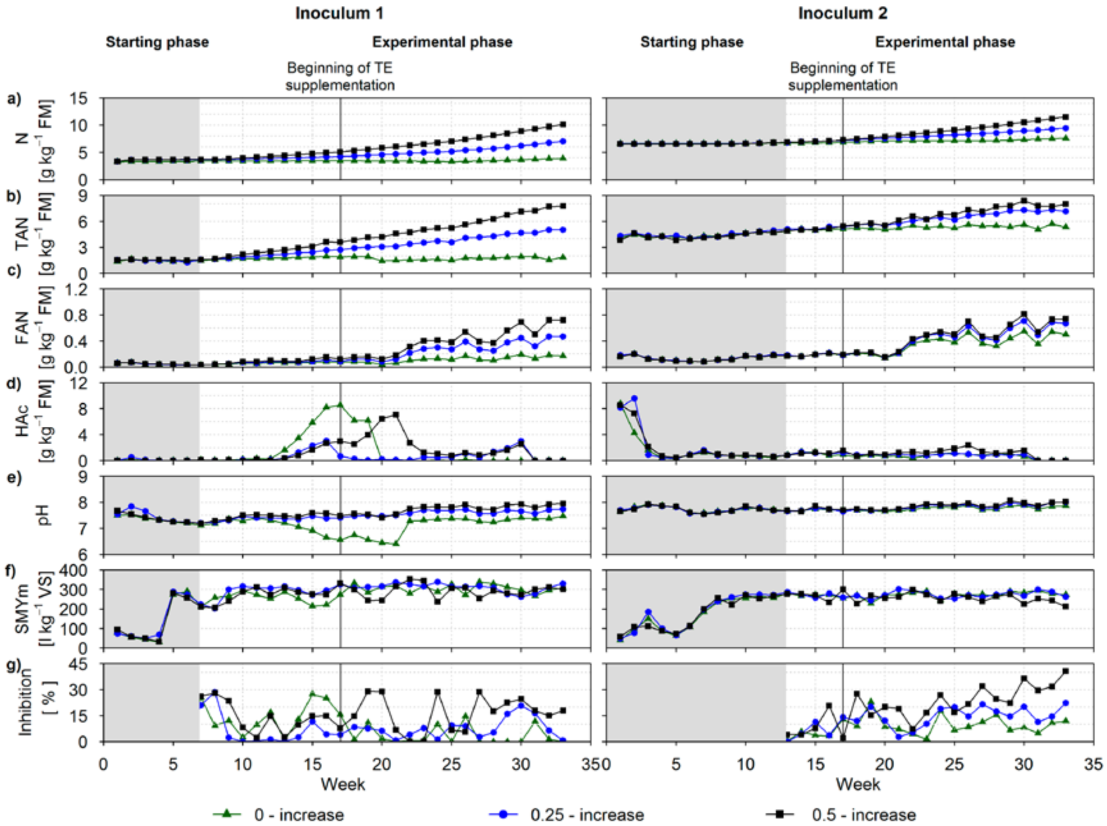

Figure 2. Measured values of the following parameters in the continuously stirred tank reactors (CSTR) under different N-increase rates in feeding regimes: (**a**) total nitrogen (N); (**b**) total ammonia nitrogen (TAN); (**c**) free ammonia nitrogen (FAN); (**d**) acetic acid (HAc); (**e**) pH; (**f**) the measured values of specific methane yield (SMY_m); (**g**) inhibition in specific methane yield (Inhibition). The results are given separately for inoculum #1 and inoculum #2. Different line colours along with different marks in the graphs and in the legend correspond to the N-increase rates "0-increase", "0.25-increase" and "0.5-increase" in the feeding regimes. Grey and white backgrounds in the graphs are related to the starting phase and the experimental phase, respectively. The vertical line in the graphs corresponds to the beginning of regular weekly trace elements (TE) supplementation.

Table 3. Concentrations of trace elements in the reactors over the trial period, in mg·kg^{-1} related to dry matter. Feeding regime expresses the N-increase rate in a feeding ratio. Values are given as mean; standard deviation is given in brackets.

Inoculum	Feeding Regime	Week 4	5	6	17	24
		Fe				
1	0-increase	1827.94 (91.40)	1625.38 (81.27)	1463.40 (73.17)	936.00 (77.78)	2105.00 (106.07)
1	0.25-increase	1515.01 (75.75)	1512.09 (75.60)	1449.05 (72.45)	1120.00 (367.70)	1810.00 (84.85)
1	0.5-increase	1625.33 (81.27)	1368.86 (68.44)	1402.16 (70.11)	837.50 (74.25)	1835.00 (134.35)
2	0-increase	3055.97 (102.47)	2986.89 (246.08)	2779.68 (138.98)	2835.00 (473.76)	3795.00 (700.04)
2	0.25-increase	NA	NA	NA	2840.00 (339.41)	3540.00 (650.54)
2	0.5-increase	3116.76 (155.84)	2846.76 (142.34)	2705.63 (135.28)	2910.00 (14.14)	3380.00 (183.85)
		Ni				
1	0-increase	12.06 (0.60)	11.70 (0.59)	10.99 (0.55)	5.33 (2.26)	21.25 (0.21)
1	0.25-increase	6.81 (0.34)	6.92 (0.35)	6.57 (0.33)	15.79 (14.02)	21.15 (8.41)
1	0.5-increase	7.72 (0.39)	6.29 (0.31)	6.76 (0.34)	8.17 (3.16)	21.20 (1.27)
2	0-increase	8.14 (0.29)	8.62 (0.06)	8.45 (0.42)	9.22 (1.05)	21.10 (0.28)
2	0.25-increase	NA	NA	NA	9.97 (0.18)	20.80 (0.85)
2	0.5-increase	13.82 (0.69)	13.81 (0.69)	13.10 (0.66)	12.85 (0.49)	20.45 (3.18)
		Mo				
1	0-increase	4.91 (0.25)	4.83 (0.24)	4.83 (0.24)	2.85 (0.08)	5.02 (0.15)
1	0.25-increase	4.32 (0.22)	4.66 (0.23)	4.62 (0.23)	4.39 (0.92)	6.49 (0.59)
1	0.5-increase	4.93 (0.25)	4.39 (0.22)	4.84 (0.24)	4.06 (0.33)	7.15 (0.49)
2	0-increase	5.18 (0.39)	5.57 (0.34)	5.59 (0.28)	5.22 (0.66)	7.32 (1.01)
2	0.25-increase	NA	NA	NA	5.29 (0.25)	7.61 (0.95)
2	0.5-increase	5.70 (0.28)	5.77 (0.29)	5.78 (0.29)	5.67 (0.40)	7.56 (0.35)
		Co				
1	0-increase	1.14 (0.06)	1.17 (0.06)	1.12 (0.06)	0.51 (0.02)	1.90 (0.08)
1	0.25-increase	0.98 (0.05)	1.07 (0.05)	1.03 (0.05)	0.76 (0.31)	1.71 (0.15)
1	0.5-increase	1.05 (0.05)	0.95 (0.05)	1.04 (0.05)	0.65 (0.09)	1.83 (0.15)
2	0-increase	1.23 (0.10)	1.28 (0.01)	1.25 (0.06)	0.91 (0.11)	2.11 (0.25)
2	0.25-increase	NA	NA	NA	0.94 (0.02)	1.90 (0.28)
2	0.5-increase	1.36 (0.07)	1.38 (0.07)	1.35 (0.07)	0.99 (0.01)	1.79 (0.06)

Table 3. *Cont.*

Inoculum	Feeding Regime	Week 4	5	6	17	24
		Se				
1	0-increase	0.51 (0.03)	0.49 (0.02)	0.73 (0.04)	0.34 (0.04)	1.60 (0.14)
1	0.25-increase	0.62 (0.03)	0.42 (0.02)	0.64 (0.03)	0.41 (0.05)	1.85 (0.07)
1	0.5-increase	0.61 (0.03)	0.50 (0.03)	0.68 (0.03)	0.40 (0.03)	2.00 (0.28)
2	0-increase	1.19 (0.18)	1.22 (0.07)	1.12 (0.06)	0.85 (0.13)	2.20 (0.42)
2	0.25-increase	NA	NA	NA	0.85 (0.05)	2.10 (0.28)
2	0.5-increase	1.25 (0.06)	1.12 (0.06)	1.18 (0.06)	0.88 (0.02)	1.90 (0.00)

3.2. The Experimental Period

After the starting phase, the reactors were continuously operated and monitored for 26 and 20 weeks for inoculum #1 and #2, respectively.

The lack of TE in the reactors with inoculum #1, which resulted in the accumulation of acetic acid up to 8.53 g·kg^{-1} FM, along with a drop in pH up to 6.40 (as described in [12,42]), was identified at the beginning of week 17 of the trials (see Figure 2d,e and Table 3). The weekly supplementation of the CSTR with TE was established thereafter in order to compensate for the deficiency in TE in the reactors with inoculum #1 and to ensure a sufficient TE supply for the remainder of the experiment. The vertical line shown at week 17 in Figure 2 marks the beginning of weekly TE supplementation. The positive effect of TE to AD process stability can be seen in Figure 2d,e in the stabilization of pH and HAc in the weeks following supplementation. The analysis of TE measured in week 24 showed that the amounts of these nutrients in the reactors were well-balanced (see Table 3).

Additionally, the total phosphorus and potassium concentrations inside the reactors were analysed. The availability of these nutrients may be of great interest when using digestate as a fertilizer. The concentrations of these macro elements within the research period were the following: for inoculum #1, P = 0.62 ± 0.13 g·kg^{-1} FM, K = 3.20 ± 0.35 g·kg^{-1} FM; for inoculum #2, P = 0.99 ± 0.09 g·kg^{-1} FM, K = 3.73 ± 1.11 g·kg^{-1} FM.

Over the experimental period, the concentration of N in the digestate was accumulating, as shown in Figure 2a. The accumulation of N in the reactors was related to the analysed N-increase rates. The average N-increase rate in the daily feedstock under the "0.5-increase" feeding regime was 35.7 mg·kg^{-1}·d^{-1} related to the fresh matter of the input substrates. At the end of the experiment, the highest values of total nitrogen in the digestate were 10.09 ± 0.08 g·kg^{-1} FM and 11.49 ± 0.01 g·kg^{-1} FM for the reactors with inoculum #1 and #2, respectively. Accordingly, a maximum "nitrogen loading rate" (NLR) can be given; the NLR was equal to 0.25 g·L^{-1}·d^{-1} for the reactors with inoculum #1 and 0.30 g·L^{-1}·d^{-1} for those with inoculum #2.

Concurrently, the TAN and FAN concentrations in the digestate increased, as shown in Figure 2b,c. At the end of the experiment, the highest values of TAN were 7.72 ± 0.33 g·kg^{-1} FM (for the reactors with inoculum #1) and 7.95 ± 1.08 g·kg^{-1} FM (for the reactors with inoculum #2). The highest FAN concentration in the final samples was 0.72 ± 0.03 g·kg^{-1} FM and 0.74 ± 0.12 g·kg^{-1} FM for the reactors with inoculum #1 and #2, respectively.

The concentration of HAc in the reactors over the period of the trials is shown in Figure 2d. The average concentrations of acetic and propionic acids in the CSTR during the experimental period were 0.88 ± 0.46 g·kg^{-1} FM and 0.17 ± 0.32 g·kg^{-1} FM, respectively, independent of the inoculum. In the reactors with #1, acetate accumulation caused by TE deficiency decreased to a minimum after the start of TE supplementation, with no acetate found in weeks 31–33. In the reactors with inoculum #2, acetate remained at a stable low concentration over the entire experimental phase with zero-values at the end of the trials. The concentrations of other VFA, i.e., iso-butyric, n-butyric, iso-valeric, n-valeric and caproic acids were low over the research period; the concentration of these acids was 0.04 ± 0.16 g·kg^{-1} FM for both inocula.

The pH-values during the experimental phase were slightly higher than those in the starting phase. Over the entire experimental period, the pH levels in the CSTR were stable, except for the reactors with inoculum #1 under the TE deficiency with a drop in pH up to 6.40 (see Figure 2e). The average pH was 7.45 ± 0.21 for the experiments based on inoculum #1 and 7.77 ± 0.11 for those based on inoculum #2.

The values of SMY_m during the experimental phase are given in Figure 2f. The mean SMY_m was 289.93 ± 35.13 L·kg^{-1} VS and 267.20 ± 19.86 L·kg^{-1} VS for the reactors with #1 and #2, respectively.

The values of inhibition during the experimental phase are given in Figure 2g. At the end of the experiment, the values of inhibition for inoculum #1 were 0.57% ± 1.22% (in weeks 32–33), 18.02% ± 22.64% (in weeks 32–33) and 26.96% ± 22.88% (in weeks 29–33) for the "0-increase", "0.25-increase" and "0.5-increase" variants, respectively. At the final phase of the experiment (in weeks 29–33) the values of inhibition for inoculum #2 were 10.91% ± 4.58%, 19.38% ± 8.93%, 38.99% ± 14.99% for the "0-increase", "0.25-increase" and "0.5-increase" variants, respectively. The 38.99% ± 14.99% inhibition determined in the reactors with #2 and "0.5-increase" feeding regime was related to N, TAN and FAN concentrations of 10.82 ± 0.52 g·kg^{-1} FM, 7.92 ± 0.27 g·kg^{-1} FM and 0.69 ± 0.10 g·kg^{-1} FM, respectively. As seen in Figure 2g, inhibition levels in the reactors with both inocula appear to have reached higher levels at the higher N increase rate.

3.3. *Results of Statistical Analysis*

3.3.1. Results of analysis on inhibition in *SMY*

The results of the analysis on inhibition in *SMY* over the experimental phase are given in Table 4 and are shown in Figure 3. According to the results of the Tukey test, for all the analysed feeding regimes the difference between the SMY_t and SMY_m was statistically significant. The large variation in the SMY_m for the reactors with inoculum #1 and the "0-increase" variant can be explained by the instability of the AD process under the TE deficiency. The highest inhibition was determined in the reactors with inoculum #2 and the "0.5-increase" variant.

Table 4. The results of analysis on inhibition in specific methane yield (*SMY*). Feeding regime expresses the N-increase rate in a feeding ratio. Degrees of freedom (DF). SMY_t and SMY_m are the theoretical and measured values of specific methane yield, respectively. Gas volumes are given under standard temperature and pressure conditions (0 °C, 101.325 kPa). Units are given in square brackets. Values of SMY_t and SMY_m are given as mean; the standard deviation is given in round brackets.

Inoculum	Feeding Regime	DF	SMY_t (L·kg$_{VS}^{-1}$)	SMY_m (L·kg$_{VS}^{-1}$)	t-Value	*p*-Value
1	0-increase	148	304.65 (11.80)	298.68 (4.44)	1.70	0.05
1	0.25-increase	182	323.71 (18.17)	302.41 (51.70)	5.42	0.00 *
1	0.5-increase	182	333.36 (21.78)	289.81 (55.09)	9.53	0.00 *
2	0-increase	141	296.65 (10.57)	264.50 (55.87)	6.55	0.00 *
2	0.25-increase	141	313.72 (16.48)	269.99 (46.99)	10.29	0.00 *
2	0.5-increase	141	325.72 (22.40)	257.87 (60.36)	11.62	0.00 *

* Significant at *p*-value = 0.0001.

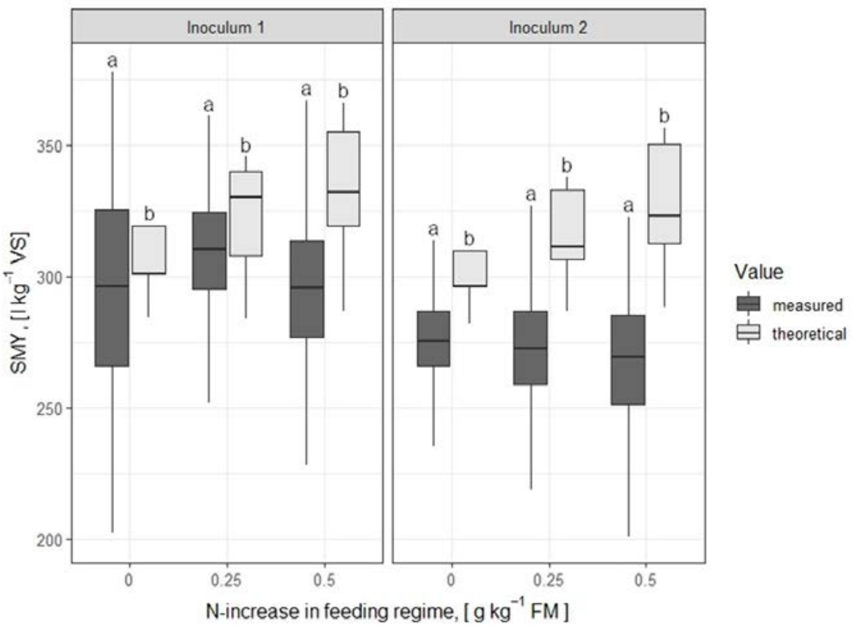

Figure 3. The results of analysis on inhibition in specific methane yield (*SMY*). The results are given separately for inoculum #1 and inoculum #2. Tick marks "0", "0.25" and "0.5" on the x-axis correspond to the N-increase variants of "0-increase", "0.25-increase" and "0.5-increase" in feeding regimes. The "measured" value is the measured *SMY* (SMY_m); the "theoretical" value is the theoretical *SMY* (SMY_t). Letters "a" and "b" denote the significant differences between the SMY_m and SMY_t for the same variant of N-increase according to the results of the one-sided Tukey test.

According to the results of the analysis, the N-increase rate in feeding regime had a negative effect on the AD process efficiency.

3.3.2. Results of analysis of the effect of TAN and FAN on inhibition

The results of the fitted model were the following: The increase in TAN levels resulted in an increase of inhibition in *SMY*, *p*-value = 0.0001 (Table 5 and Figure 4). The increase in FAN concentration in the AD reactor resulted in an increase of the inhibition level, *p*-value = 0.0012 (Table 5 and Figure 5). The observed noise in Figures 4 and 5 can be associated with the fact that the inhibition does not derive only from TAN or FAN concentrations inside the reactors; this inhibition can be also affected by other parameters.

Table 5. The effect of total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) on the inhibition in specific methane yield: the results of the fitted model. Degrees of freedom (DF). The square-root transformed values of inhibition in specific methane yield (sqrt_Inhibition); the transformation was done for meeting the requirements of the model.

Dependent Variable	Effect	Numerator DF	Denominator DF	F-Value	R^2	*p*-Value
sqrt_Inhibition	TAN	1	30.7	19.08	0.20	0.0001
sqrt_Inhibition	FAN	1	16.5	15.11	0.15	0.0012

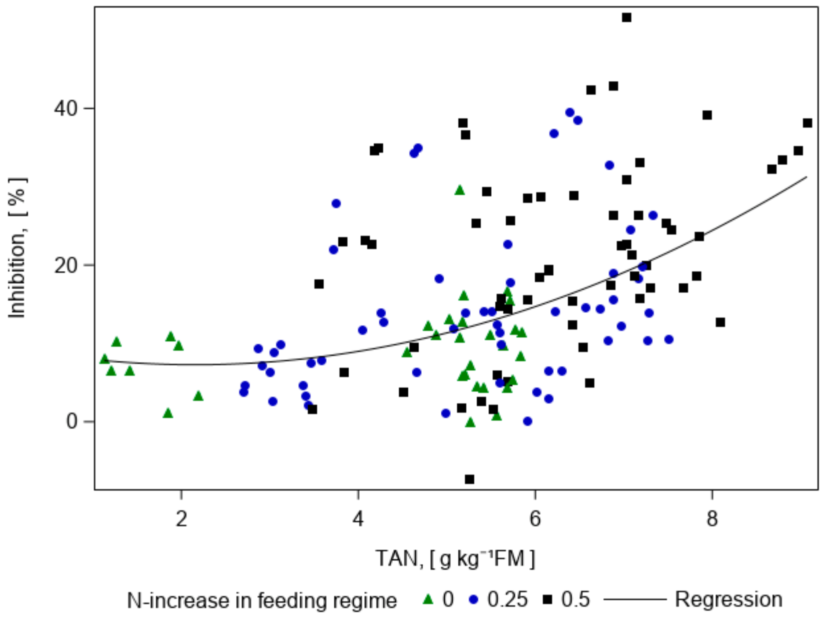

Figure 4. The correlation between the total ammonia nitrogen (TAN) and the inhibition in specific methane yield (Inhibition). The "0", "0.25" and "0.5" marks in the legend correspond to the N-increase variants of "0-increase", "0.25-increase" and "0.5-increase" in the feeding regimes. The regression line was built based on the results obtained from the model.

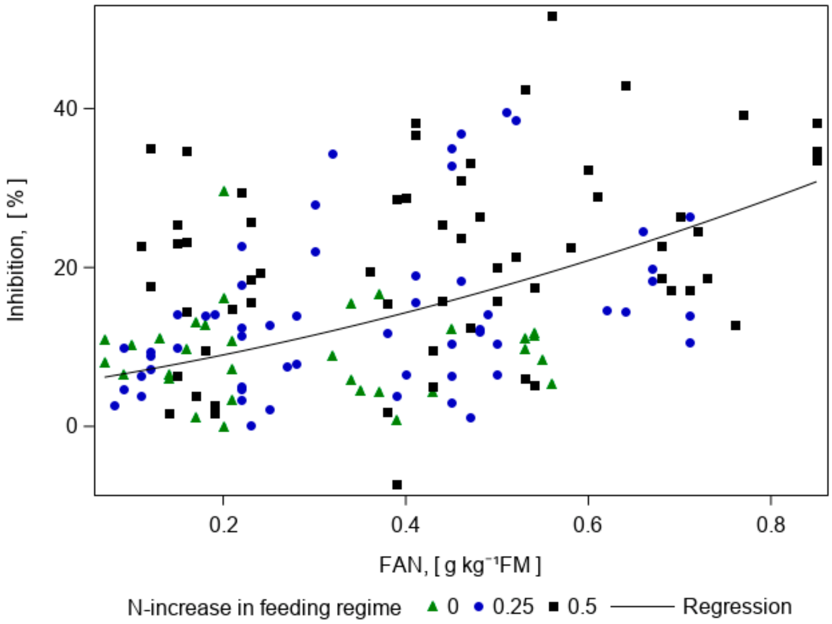

Figure 5. The correlation between the free ammonia nitrogen (FAN) and the inhibition in specific methane yield (Inhibition). The "0", "0.25" and "0.5" marks in the legend correspond to the N-increase variants of "0-increase", "0.25-increase" and "0.5-increase" in the feeding regimes. The regression line was built based on the results obtained from the model.

The results of the data analysis show that the analysed N-increase rates can be recommended for a stable AD process. However, the level of inhibition in *SMY* depends on the concentration of TAN and FAN inside the reactors and the N-increase rate in the feeding regimes (see Figures 4 and 5).

3.4. Discussion

The inhibitory effect of urea, NH_4Cl, TAN, FAN and high N concentration in feeding, as well as the effect of elevated ammonium (NH_4^+), elevated ammonium nitrogen (NH_4^+-N) and elevated TAN on biogas and methane yields, have been previously studied [1,4,15,17,22,32,51–53]. However, no results on the effects of N concentration in inoculum and N-increase rate in feedstock on the level of inhibition in specific methane yield were found.

Contrary to the research results reported by Siegrist et al. [18], Chen et al. [17], Meng et al. [19] and Theuerl et al. [16], the stable AD-process was found in this study as indicated by stable pH values and a minimal accumulation of acetate (except for the reactors with #1 under the TE deficiency) (see Figure 2e,d). During the experimental phase, the specific methane yields were kept stable in all the reactors, and their values were in a normal range (see Figure 2f). Base on the results obtained it can be assumed that the analysed feeding regimes enabled the microorganisms to adapt to changing N-conditions, which is indicated by a stable AD process. The regular supplementation of reactors with TE positively contributed to the process stability. The proposed increase rates did not have any negative effect on the process stability. Hereby the N-increase variants of "0.25-increase", "0.5-increase" and the NLR up to 0.30 $g{\cdot}L^{-1}{\cdot}d^{-1}$ can be recommended for maintaining a biogas plant in a stable way.

In contrast, the efficiency of AD, which in this study corresponded to the inhibition in specific methane yield (Inhibition), was affected by N-increase rate and the level of TAN and FAN inside the reactor. The conversion process in the reactors, which in this study is described as inhibition, became more and more inefficient due to the closer C/N ratio in feedstock (see Figure 1b). Chen et al. [17] has stated that the methane production was intensely inhibited when TAN increased to 5 $g{\cdot}L^{-1}$ and they recommended to maintain the ammonium concentration below 2 $g{\cdot}L^{-1}$ in the reactors for preventing the ammonium shock to the AD process. According to the review made by Chen et al. [15], in different studies there is controversial information on the level of inhibition in methane production depending on the TAN and FAN concentrations in the AD reactor: 50% of methane inhibition was observed at TAN of 1.44 $g \cdot L^{-1}$, 2.48 $g \cdot L^{-1}$ and 5.60 $g \cdot L^{-1}$ and FAN of 0.03 $g \cdot L^{-1}$ and 0.64 $g \cdot L^{-1}$; 100% of methane inhibition was identified at TAN values above 5.20 $g \cdot L^{-1}$ and FAN of 0.20 $g \cdot L^{-1}$ and 0.62 $g \cdot L^{-1}$. Fotidis et al. [32,53] specify that at the NH_4^+-N in the range of 3–5 $g \cdot L^{-1}$, an ammonia induced inhibited-steady state in the AD reactors was observed with inhibition in methane production of 30–40%, and the authors recommend a bioaugmentation strategy for overcoming an ammonia inhibiting effect. However, in our research, under NH_4^+-N of 5.03 ± 0.06 $g \cdot kg^{-1}$ FM, our TAN and FAN concentrations in the reactors were 5.34 ± 0.15 $g \cdot kg^{-1}$ FM and 0.31 ± 0.14 $g \cdot kg^{-1}$ FM, respectively, and the value of inhibition was equal to 9.46% ± 5.60%. According to the results of the data analysis, both TAN and FAN had a significant effect on the level of inhibition. As FAN levels are mostly affected by temperature and pH fluctuations [7,9,14,21], the effect of FAN was less significant than TAN in our research, since the reactors were operated under mesophilic conditions at stable temperature and pH (except for the pH values in the reactors with inoculum #1 under the TE deficiency). As the OLR_{VS}, HRT, temperature and pH in the reactors were kept stable, the results show that the N-increase rate in the feeding regime was negatively related to the efficiency of the AD process even if low VFA concentrations indicated a stable process. In further studies, the influence of the increasing N concentrations in the digestate on the microbial population should be investigated.

The results of this study can be applied by biogas operators running their systems at high nitrogen concentrations up to 11.5 $g \cdot kg^{-1}$ FM or utilizing substrates with varying nitrogen contents.

4. Conclusions

In this study, we analysed the effect of different inocula and different N-increase rates in feeding regimes on AD process stability and efficiency. The stepwise acclimatisation strategy used for microorganisms to adapt to a new nitrogen concentration according to the feeding regime prevented failure of the AD process under high and elevated ammonia levels. The research approach applied in this study enabled us to run the CSTR in a stable way under the elevated nitrogen loading rates up to 0.30 $g \cdot L^{-1} \cdot d^{-1}$. The highest N, TAN and FAN in the digestate at the end of the experiment were equal to 11.50 $g \cdot kg^{-1}$ FM, 9.07 $g \cdot kg^{-1}$ FM and 0.85 $g \cdot kg^{-1}$ FM. However, the study indicates that the N-increase rate was negatively related to the AD process efficiency. The level of inhibition in specific methane yield was positively correlated to the TAN and FAN concentrations in the digestate.

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4. Publication 3: Nutrient Recovery from Digestate of Agricultural Biogas Plants: A Comparative Study of Innovative Biocoal-Based Additives in Laboratory and Full-Scale Experiments

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Article

Nutrient Recovery from Digestate of Agricultural Biogas Plants: A Comparative Study of Innovative Biocoal-Based Additives in Laboratory and Full-Scale Experiments

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Abstract: Nutrients can be recovered from the digestate of an agricultural biogas plant in the form of solid fraction and serve as crop fertilizers. Removal of suspended solids with screw press separators is the most commonly used technique for treating digestate from biogas plants. To increase separation efficiency and nutrient transfer to the solid phase during separation, eight biocoal-based additives were investigated, which were based on beech wood and produced by pyrolysis at temperatures of 350 °C and 600 °C. Four of the biocoals were impregnated with $CaCl_2$ or $MgCl_2$ before pyrolysis. The reaction time between the additives and the digestate varied from 5 min to 2 weeks. The application of $MgCl_2$-impregnated biocoal synthesized at 600 °C for 20 h increased the nutrient removal efficiency by 76.33% for ammonium and 47.15% for phosphorus, compared to the control (the untreated digestate).

Keywords: digestate valorization; biocoal (biochar); phosphorus; ammonium; potassium; screw press; anaerobic digestion; separation; additives

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1. Introduction

Phosphate fertilizers such as diammonium phosphate (DAP), are essential for plant growth and are made from phosphate rock. Phosphate rock and DAP prices are expected to increase 1.6-fold and 1.4-fold, respectively, by 2035. The main reason for this is the increasing demand for phosphate and the depleting phosphate rock reserves [1–3]. On the other hand, there is a major problem of ammonia leaching, ammonia emissions, and eutrophication due to excess ammonia in regions with high livestock density, where meat processing factories are often located [4,5]. Both issues could be solved by sustainable nutrient recovery from slurry, either by direct treatment or by processing the digestate of biogas plants after anaerobic digestion of the slurry.

Various mechanical and non-mechanical techniques for nutrient recovery from digestate have been studied: sedimentation, centrifugation, drainage, pressure filtration and chemical pretreatment (by precipitation, coagulation and flocculation), use of specific additives (sorbents), among others [6–10]. In full-scale biogas plants, pressure filtration with a screw press is commonly used [11]. This method is costly and energy consuming, and its efficiency needs to be improved [10]. In this study, the efficiency of nutrient binding of biocoal-based additives was investigated when applied before a mechanical separation step (pressure filtration) to improve nutrient recovery in the solid fraction of the digestate. Although the liquid fraction of digestate is a valuable nutrient source [12–16], the focus on the solid fraction in this study can be explained by lower transportation and storage costs for its application as fertilizer for crops cultivation in times of their nutrient demand.

For cultivating crops, an improved nutrients retention and their slow release was confirmed on charcoal-rich soils (i.e. Terra Preta) due to the high cation exchange capacity and high porosity of charcoal [17]. In the same way, the application of biochar to the soils proved to improve plant yields by 15–17%: for arbuscular mycorrhizal fungi, it stimulated root development and increased root colonization; for rice, biomass yields and soil pH were increased; for maize, the stimulated plant growth with the improved root system was observed [17].

Literature sources use different terms, i.e. hydrochar or hydrocoal, biochar or biocoal depending on the origin and production technology [18–22]. The terms "biochar" and "hydrochar" are commonly used when the products are used as fertilizers and made of biomass, agricultural residues, or wastes [19–21]. In this study, nutrient recovery from digestate is investigated based on the sorption characteristics of these additives. Therefore, the terms "hydrocoal" and "biocoal" (referring to "hydrochar" and "biochar") will be used in the following. The main difference between biocoal and hydrocoal lies in their production process: biocoal is a product of a thermochemical process, such as pyrolysis or torrefaction (so-called mild pyrolysis), while hydrocoal is obtained by hydrothermal carbonization (HTC) [18–20,22]. The quality and properties of biocoal or hydrocoal are directly related to the process temperature and heating time (residence time of the material in the reactor); the heating rate (for slow pyrolysis and torrefaction), pressure, and moisture content of the material have an additional influence [18,21]. The pyrolysis process is carried out by heating the material in the temperature range between 300 °C and 650 °C in the absence of oxygen [18]; most commonly, biocoal is synthesized under N_2 flow [23–27]. Torrefaction is achieved by limiting the process temperature to 200–300 °C and the heating time from 30 min to several hours with heating rates of less than 50 °C min^{-1} [18,19]. HTC or the so-called wet torrefaction is carried out in the temperature range of 180–260 °C. In HTC, raw material is immersed in water and heated in a closed system under pressure (2–6 MPa) for 5–240 min [18,22].

The recovery of nutrients from municipal or industrial wastewater using biocoal has already been investigated in several studies [23–31]. Efficient phosphate recovery from piggery digestate when using biogas residue biocoal was described by Luo and co-authors [32]; however, this method is resource-consuming (due to chemicals application), and the recovered phosphate is available as a magnesium ammonium phosphate mixture. Although the aforementioned studies showed the positive effect of the biocoals they used for nutrient recovery, research into new types of biocoals for commercial application is needed. According to Harikishore and co-authors, the main obstacle for nutrient adsorption by using biocoal is the negatively charged surface of biocoal and the anionic nature of nitrate and phosphate molecules [33].

Impregnation of the feedstock with $MgCl_2$ or $CaCl_2$ prior to biocoal production has improved the sorption capacity of biocoal [9,10,24,25,34]. The usefulness of $MgCl_2$ and $CaCl_2$ can be described due to formation of porous crystals of magnesium oxide and calcium oxide during biocoal production, which are valuable adsorbents [34,35]. $MgCl_2$-salt is added to enhance phosphorus recovery via magnesium ammonium phosphate (MAP, sturvite) precipitation from wastewater or digestate [9]. Biocoal from Mg-enriched tomato leaves synthesized at 600 °C was studied by Yao and co-authors [24]. Corncob based biocoal enriched in Ca and Mg and pyrolyzed at 300 °C, 450 °C, and 600 °C was analyzed by Fang and co-authors [25]. Additionally, the temperature of the biocoal synthesis also seems to have an effect on nutrient recovery. Fang and co-authors revealed that higher phosphate recovery was achieved when biocoal synthesized at higher process temperatures was used [25]. Mg-modified sugarcane bagasse biocoal pyrolysed at 700 °C was efficient for P-absorption from acid-extract of incinerated sewage sludge ash [34].

In this study, the nutrient binding into the solid fraction after the pretreatment of biogas digestate with the biocoal-based additives and subsequent solid-liquid separation was investigated. For this purpose, digestate treated with the additives and untreated digestate were separated by pressure filtration. Biocoal-based additives of different origins synthesized at different process temperatures and both impregnated and not impregnated with $CaCl_2$ or $MgCl_2$ were investigated. Additionally, the effect of different reaction times, i.e., the period of digestate pretreatment with the additives, was analyzed. The research results were compared with the control variant, corresponding to the untreated digestate.

2. Materials and Methods

2.1. Experiment Overview

In this study, the experiments were conducted both in full-scale and under laboratory conditions. First, the separation experiments were conducted in the full-scale research biogas plant "Unterer Lindenhof" described in some published papers [36–38]. In the separation experiments, pressure filtration (400 mbar inlet pressure) was performed using a screw press. The aim of the full-scale separation experiments was to identify benchmark values for a comparable control in the laboratory. These benchmark values were used to adjust the pressure filtration in the laboratory to allow comparable results. During the full-scale separation, different screw press settings were used to evaluate minimum and maximum solid-liquid and nutrient separation efficiency. The laboratory-scale experiments comprised both the separation of untreated digestate and the separation experiments with the digestate pretreated with additives. First, the separation experiments with untreated digestate were performed and the optimum separation settings were determined. The optimum was selected based on the research results, which correspond to the mean benchmark values obtained in full-scale. The optimum separation settings were then applied to separate the digestate pretreated with additives. To evaluate the effect of the additives on nutrient removal efficiency, they were exposed to the digestate prior to separation at a defined ratio and for a specific reaction time.

2.1.1. Mechanical Separation Step in the Full-Scale Application

Full-scale mechanical separation experiments were conducted at the research biogas plant of University Hohenheim "Unterer Lindenhof" located at "Eningen unter Achalm", Baden-Württemberg, Germany. A filter screw press FSP-A 20150518 (UTS Products GmbH, Dorfen, Germany) was used for digestate separation (see Figure 1).

Figure 1. A filter screw press used for the digestate separation at the full-scale research biogas plant of University Hohenheim "Unterer Lindenhof".

The anaerobic digestion process at "Unterer Lindenhof" is organized in two steps. The main fermentation process takes place in the anaerobic reactors #1 and #2. In a secondary reactor the residual fermentation takes place. The digestate from the secondary reactor was selected for the separation experiments. The content of the reactor was mixed for five minutes prior to the separation step to achieve uniform distribution of total solids.

Three configurations of the screw press were tested. The digestate and collected separated fractions were frozen and stored at −20 °C before analysis in the laboratory.

2.1.2. Mechanical Separation Step in Laboratory Conditions

The separation experiments in the laboratory were conducted using a hydraulic tincture press (HAPA HPH 2.5 l, Achern, Germany), see Figure 2. The operation mechanism of the press is a combination of spindle and hydraulic system. The following operating conditions were tested in advance to find the optimal separation settings: (1) Different amounts of digestate in a range between 170 g and 1000 g. (2) Different pressure modes: atmospheric pressure, 25 bar, 50 bar, 75 bar, 80 bar, 100 bar and 125 bar. (3) Different duration of pressure application: 5 s, 60 s, 300 s. The settings resulting in the highest agreement with the benchmark values from the full-scale separation were selected for further experiments. In our case, the selected separation regime was 300 g sample pressed under 100 bar for 60 s.

Figure 2. A hydraulic tincture press used for the digestate separation experiments in the laboratory.

2.1.3. Pretreatment with Additives before the Mechanical Separation Step

Different types of biocoal-based additives were analyzed in this study. The biocoals produced within this research are specified in Section 2.2. In addition, a commercial biocoal was analyzed and tested either alone or in combination with $MgCl_2$-salt. The amounts of additives added to the digestate were calculated as described in Section 2.4. Different reaction conditions were analyzed, such as mixing of digestate with additives over different time intervals and storage with short mixing times (see Section 2.4). After pretreatment of the digestate with additives, it was immediately used for the mechanical separation step under the operating conditions assumed to be optimal as described in Section 2.1.2.

2.2. *Production of Biocoals*

Six biocoal variants were produced from beech wood by pyrolysis. Two biocoal variants were untreated beech wood synthesized at 350 °C (Biocoal V1) and 600 °C (Biocoal V4), respectively. Further, two biocoal variants were synthesized at 350 °C (Biocoal V2) and 600 °C (Biocoal V5), respectively, after impregnation with a $CaCl_2$-salt solution with a concentration of 5.69 mol/L. The last two biocoal variants were based on beech wood impregnated with $MgCl_2$-salt solution (5.69 mol/L) with the subsequent pyrolysis at either 350 °C (Biocoal V3) or 600 °C (Biocoal V6).

The wood was impregnated manually in twelve 30-l barrels for three weeks at the University of Hohenheim. Mixing was conducted by rolling each barrel for 30 min daily.

Excess liquid was removed by drying the impregnated wood at 60 °C for 17–18 days in a drying chamber (Robert Hildebrand Maschinenbau GmbH, Oberboihingen, Germany) to a constant weight.

Both impregnated and unimpregnated wood was delivered to the Clausthal Research Center for Environmental Technologies (CUTEC), where it was pyrolyzed in a rotary kiln [39,40]. For the synthesis of biocoal at either 350 °C or 600 °C, the walls of the pyrolyzer were heated accordingly for 8–12 h. The average retention time of the solid material in the pyrolyzer was 45–60 min. The pyrolyzer was filled to about 10–20% with wood. The synthesis of biocoal was carried out under N_2 flow, and it took about 18 h to produce a biocoal variant.

2.3. *Analysis of Biocoals*

The biocoals were analyzed for their specific water uptake (see Section 2.3.1), elemental composition, bulk density, and ash content (see Section 2.3.2).

2.3.1. Specific Water Uptake Analysis

For the analysis of specific water uptake, 250-mL bottles were filled with 125 mL biocoal and 125 mL deionized water at 20 °C. Specific water uptake for the biocoals was measured in triplicates after 1 h, 1 day, and 1 week. After the exposure period, the samples were filtered with a sieve (100 µm mesh size) to remove excess water. The biocoal was weighed before (m_{start}) and after (m_{end}) exposure, and specific water uptake was calculated according to Equation (1):

$$Specific\ water\ uptake = (m_{end} \cdot (m_{start})^{-1} - 1) \cdot 100\% \quad (1)$$

2.3.2. Analysis of Biocoals

The proportion of C, H, and N in the biocoals was measured using a Euro3000 EA CHNSO elemental analyzer (HEKAtech GmbH with Callidus software interface version 5.1, Wegberg, Germany). Analyses were conducted after spontaneous combustion at 1000 °C followed by chromatographic separation.

Ca and Mg contents of the biocoals were measured using inductively coupled plasma mass spectrometry (ICP-MS) with a NexION 2000 (PerkinElmer, Rodgau, Germany) after microwave digestion (Discover SP-D, CEM GmbH, Kamp-Lintfort, Germany).

For measuring bulk density, a bulk density cylinder was used. The dry matter (DM) content as DM relative to fresh matter ($g \cdot kg_{FM}^{-1}$) and the organic dry matter (oDM) content as oDM relative to DM of the samples (in $g \cdot kg_{DM}^{-1}$) were determined by differential weighing before and after drying at 105 °C for 24 h and after subsequent ashing at 550 °C for 8 h, respectively, using standard methods [41].

2.4. *Experimental Design*

The digestate was pretreated with additives in 2 L bottles. In total, seven variants of biocoal and six reaction times were tested. Reaction time refers to the period between the addition of biocoal to the digestate and solid-liquid separation. Among the six reaction times, four reaction times corresponded to periods of continuous mixing at 300 rpm on an orbital shaker (IKA KS260, Staufen, Germany) at 20 ± 2 °C for either 5 min, 1 h, 3 h or 20 h. Two further reaction times were established, when the digestate and the additives were first mixed for 5 min and then stored at 20 ± 2 °C for 1 or 2 weeks, respectively, with additional 5 min mixing intervals every second to third day.

An overview of the experimental design is shown in Table 1. The commercial biocoal was originally produced for the production of TerraPreta. It was added in the amount recommended by the manufacturer: 6 L of the biocoal per 1 m^3, which was equivalent to 8.33 g biocoal per kg digestate. The amounts of the $CaCl_2$-biocoals, $MgCl_2$-biocoals and $MgCl_2$-salt added to the digestate were calculated for the equimolar concentrations of Ca or Mg in the biocoals and of P in the digestate, allowing for the precipitation of magnesium-ammonium-phosphate possible. The amounts of the other biocoal variants were calculated under consideration of their individual bulk densities. For the pretreatment with the additives, 2 L bottles filled with 1.2 kg of the digestate were used, and thus, the presented amounts of additives applied corresponded to 1.2 kg of the digestate (see Table 1).

Table 1. Experimental design of the digestate pretreatment with additives. The check marks indicate the experiments performed. The added amounts corresponded to 1.2 kg of the digestate.

Variant	Additive, in g	Reaction Time					
		5 min	1 h	3 h	20 h	1 Week	2 Weeks
Biocoal V1, (350 °C)	6.05	✓	✓	✓	✓	✓	
Biocoal V2 (impregnated with $CaCl_2$, 350 °C)	8.35				✓	✓	✓
Biocoal V3 (impregnated with $MgCl_2$, 350 °C)	8.30	✓	✓	✓	✓	✓	✓
Biocoal V5 (impregnated with $CaCl_2$, 600 °C)	8.30				✓	✓	✓
Biocoal V6 (impregnated with $MgCl_2$, 600 °C)	5.79				✓	✓	✓
Commercial biocoal	9.99				✓	✓	✓
Commercial biocoal + $MgCl_2$	9.99 + 1.78				✓	✓	✓

2.5. *Analytical Methods*

The DM content of the collected samples was determined in the same way as for the biocoals, which is described in Section 2.3.2. NH_4^+ concentrations in the digestate and the separated fractions were determined using the Gerhardt Vapodest 50 s automatic distillation system (Germany). In the further text, NH_4 is written, which means the ammonium ion NH_4^+. Potassium was determined using flame atomic absorption spectroscopy (AAS, Eppendorf, ELEX 6361, Hamburg, Germany) operated with acetylene gas. For the determination of phosphorus, a cuvette test and a spectrophotometer UV-VIS 1240 (Shimadzu, Kyoto, Japan) were used. All analyses were carried out according to standard methods [41].

2.6. *Calculation of Removal Efficiency*

The influence of the different additives on the removal of nutrients during separation was evaluated on the basis of the parameter "removal efficiency", which describes the fraction of nutrients of the initial substrate that was contained in the solid phase after separation. The removal efficiency for different nutrients was calculated according to Equation (2):

$$Removal\ efficiency = Quantity\ in\ solid\ fraction \cdot Quantity\ in\ digestate^{-1} \cdot 100\% \quad (2)$$

2.7. *Calculation of Sorption Capacity*

The sorption capacity of P or PO_4^{3-} (q_e, $mg \cdot g_{biocoal}^{-1}$) was calculated as described by Li and co-authors [26] and defined by Equation (3):

$$q_e = M \cdot C_{s.f.} \cdot m^{-1} \quad (3)$$

where M is the quantity of digestate used for the pretreatment with the biocoals (g); $C_{s.f.}$ is the concentration of P or PO_4^{3-} in the solid fraction after separation ($mg \cdot g^{-1}$), m is the quantity of the biocoal supplied to M for its pretreatment under the specific reaction time according to the experimental design with the following separation step (g).

2.8. Statistical Analysis

Microsoft EXCEL 2016, R and RStudio (version 1.1.463) and SAS 9.4 were used for data processing and visualization. In the statistical analysis, the post hoc Tukey HSD test and the generalized linear model function were applied as described by some authors [42,43].

3. Results and Discussion

3.1. Results of the Mechanical Separation Step without Pretreatment: Comparison of Full-Scale and Laboratory Experiments

The digestate for the separation experiments was taken from the secondary reactor of the research biogas plant "Unterer Linderhof". During the experimental period, the reactors were fed with the following substrates: liquid manure, maize silage, grass silage, solid manure, horse dung, WPS (whole plant silage), sugar beet, cereals and water. The operating conditions during the experimental period and the properties of the digestate are shown in Table 2:

Table 2. Operating conditions of the research biogas plant "Unterer Lindenhof" and the properties of the digestate during the experimental period. OLR: organic loading rate; HRT: hydraulic retention time; FM: fresh matter; NA: not available.

Parameter	Reactor #1	Reactor #2	Secondary Reactor
OLR, in $kg_{oDM} \cdot (m^3 \cdot d)^{-1}$	3.42	3.26	2.24 *
			0.41 **
HRT, in d	61.50	58.00	32.40
Temperature (mean ± SD), in °C	44.00 ± 2.90	42.20 ± 3.20	52.50 ± 5.00
pH	NA		8.20 ± 0.41
DM, in %FM	NA		7.54 ± 0.84
oDM, $g \cdot kg_{DM}^{-1}$	NA		681.16 ± 15.23
NH_4, $g \cdot kg_{DM}^{-1}$	NA		61.58 ± 3.46
P, $g \cdot kg_{DM}^{-1}$	NA		13.86 ± 0.32
K, $g \cdot kg_{DM}^{-1}$	NA		83.37 ± 5.35
Ca, $g \cdot kg_{DM}^{-1}$	NA		26.78 ± 1.34
Mg, $g \cdot kg_{DM}^{-1}$	NA		7.76 ± 0.39

* Feeding plus digestate from primary digesters. ** Only feeding.

For the full-scale system, the FM removal efficiency in the solid fraction varied from 7.36% to 23.92%, depending on the different set-ups of the screw press. The screw press set-up with the highest FM removal efficiency yielded the highest removal efficiency for all parameters analyzed and was selected as the baseline setting that provided the benchmark results for the laboratory trials as shown in Figure 3.

The results of the laboratory separation experiments are comparable to those obtained in full-scale and no significant differences were found between both systems, except for P concentration. Although the absolute difference between P concentrations in laboratory and full-scale with $9.92 \pm 0.48 \ g \cdot kg_{DM}^{-1}$ and $11.67 \pm 0.23 \ g \cdot kg_{DM}^{-1}$, respectively, was very small, it was statistically significant due to the very small variance of the measured values. Mechanical separation in the laboratory for the untreated digestate is the control variant to evaluate the effect of the tested additives in further investigations.

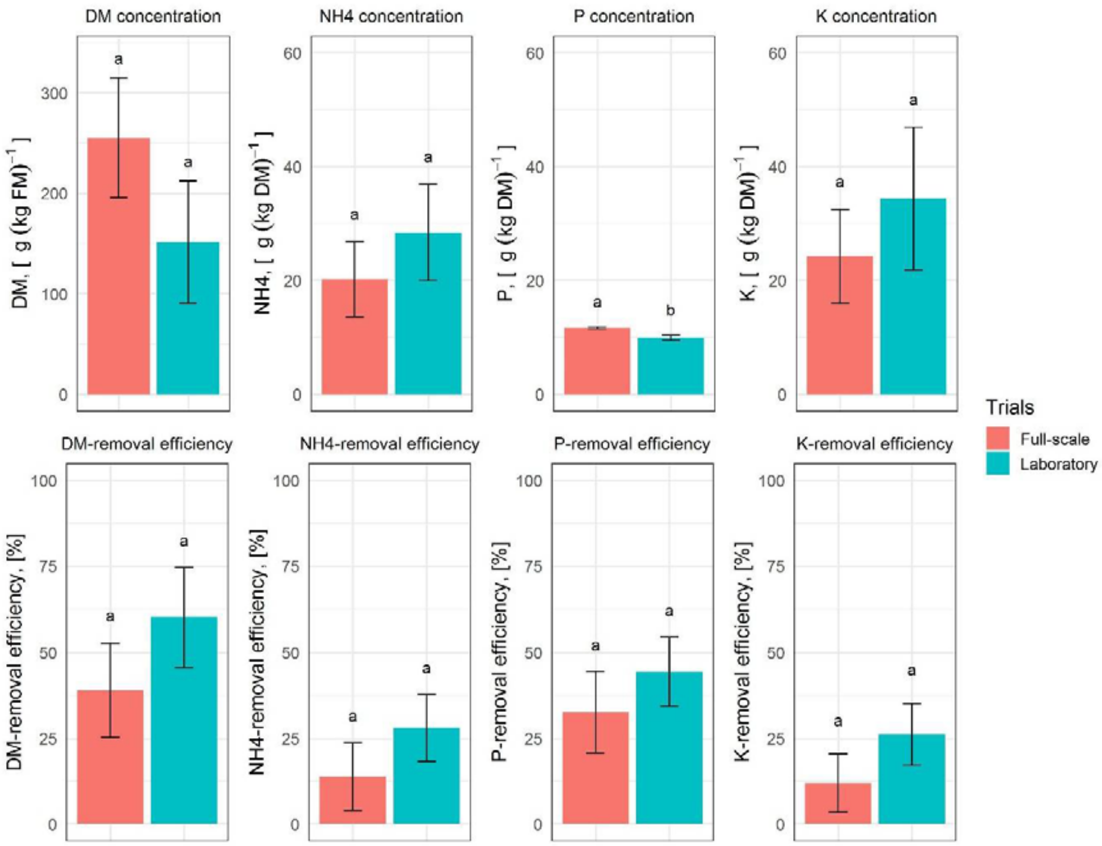

Figure 3. Results of the separation trials in the full-scale and laboratory systems. The upper diagrams show the concentration of nutrients in the full-scale and laboratory. The lower diagrams show the removal efficiency. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to the results of the Tukey test.

3.2. Results of the Laboratory Analysis of the Biocoals

The biocoals produced for this study were first analyzed for their chemical and physical parameters. The following table shows the results of elemental analysis of the biocoals without impregnation depending on pyrolysis temperature (Table 3).

Table 3. Elemental analysis of the biocoals in % of the dry weight.

Variant	C, in %	H, in %	N, in %
Biocoal V1 (350 °C)	64.32 ± 3.00	1.95 ± 0.49	0.26 ± 0.02
Biocoal V4 (600 °C)	77.56 ± 6.07	2.08 ± 0.48	0.25 ± 0.03

Higher process temperature during pyrolysis resulted in a higher carbonization, while H and N contents were in the same range for biocoal variants produced under both synthesis temperatures. The C contents of Biocoals V1 and Biocoal V4 were in the same range as the biocoal-based fertilizer described by Rasse and co-authors, with the C contents equal to 75 ± 15% [17].

The results of the supplementary analyses, such as bulk density, DM and oDM contents, Ca and Mg contents for all biocoals used are shown in Table 4.

Table 4. Laboratory analyses of the biocoals.

Variant	Bulk Density (Mean ± SD), in kg ·m^{-3}	Particle Size (Mean ± SD), in mm	DM, (Mean ± SD), in g ·kg$_{FM}^{-1}$	oDM, (Mean ± SD), in g ·kg$_{DM}^{-1}$	Ca, (Mean ± SD), in g ·kg$_{FM}^{-1}$	Mg, (Mean ± SD), in g ·kg$_{FM}^{-1}$
Biocoal V1, (350 °C)	176.55 ± 13.34	7.00 ± 3.00	1024.61 ± 2.72	969.97 ± 2.29	3.66 ± 0.58	1.53 ± 0.51
Biocoal V2 (impregnated with $CaCl_2$, 350 °C)	261.43 ± 1.53	10.00 ± 5.00	1047.02 ± 2.93	549.71 ± 3.59	165.79 ± 8.73	0.52 ± 0.04
Biocoal V3 (impregnated with $MgCl_2$, 350 °C)	243.55 ± 5.47	9.00 ± 3.00	1041.02 ± 0.98	811.11 ± 2.79	3.10 ± 0.19	101.16 ± 3.31
Biocoal V4, (600 °C)	158.19 ± 2.36	5.00 ± 2.00	1002.23 ± 0.92	1006.03 ± 98.63	7.95 ± 1.40	2.74 ± 0.79
Biocoal V5 (impregnated with $CaCl_2$, 600 °C)	284.48 ± 0.61	7.00 ± 3.00	1030.99 ± 0.57	482.36 ± 11.06	166.11 ± 22.29	0.56 ± 0.01
Biocoal V6 (impregnated with $MgCl_2$, 600 °C)	217.11 ± 1.44	7.00 ± 3.00	1033.45 ± 2.31	489.31 ± 3.41	3.67 ± 0.09	145.05 ± 4.68

Impregnation of wood with $CaCl_2$ or $MgCl_2$ prior to pyrolysis resulted in a higher density of the charcoal produced due to the increased mineral content. Higher synthesis temperatures resulted in the smaller biocoal particle size. Pretreatment with $CaCl_2$ increased the Ca content of the biocoal by a factor of 45 (Biocoal V2) and 21 (Biocoal V5), respectively, compared to the untreated biocoal variants at the same synthesis temperatures. Pretreatment with $MgCl_2$ increased the Mg concentration by a factor of 66 (Biocoal V3) and 53 (Biocoal V6), respectively, compared to the untreated biocoal variants at the same synthesis temperatures.

3.3. *Water Uptake of the Biocoals*

Specific water uptake is an important parameter for the application of biocoal as a soil conditioner. The results on specific water uptake for the six produced variants of biocoal under different exposure times (one hour, one day and one week) are shown in Figure 4.

With increasing exposure time, the specific water uptake of biocoals increased (see Figure 4). The highest specific water uptake of 250.49% was measured for Biocoal V3 after one week exposure. Pretreatment with $CaCl_2$ resulted in a lower specific water uptake compared to the other biocoal variants.

3.4. *Results on Nutrients Recovery after Pretreatment*

In some variants of the laboratory tests, no sufficient dewatering of the digestate could be achieved with the tincture press. Therefore, all variants with a fresh mass fraction of the solid phase after separation above 50% were excluded from further investigations. Thus, all experimental results obtained for the reaction time of one and two weeks and for Biocoal V2 were omitted. Digestate, solid- and liquid fractions were analyzed on their nutrient contents. The results on nutrient contents for the liquid fraction were considered for calculating mass balances and validating the nutrient recovery in the solid fraction; however, due to the main focus on the solid fraction, the results for the liquid fraction are not described in this study.

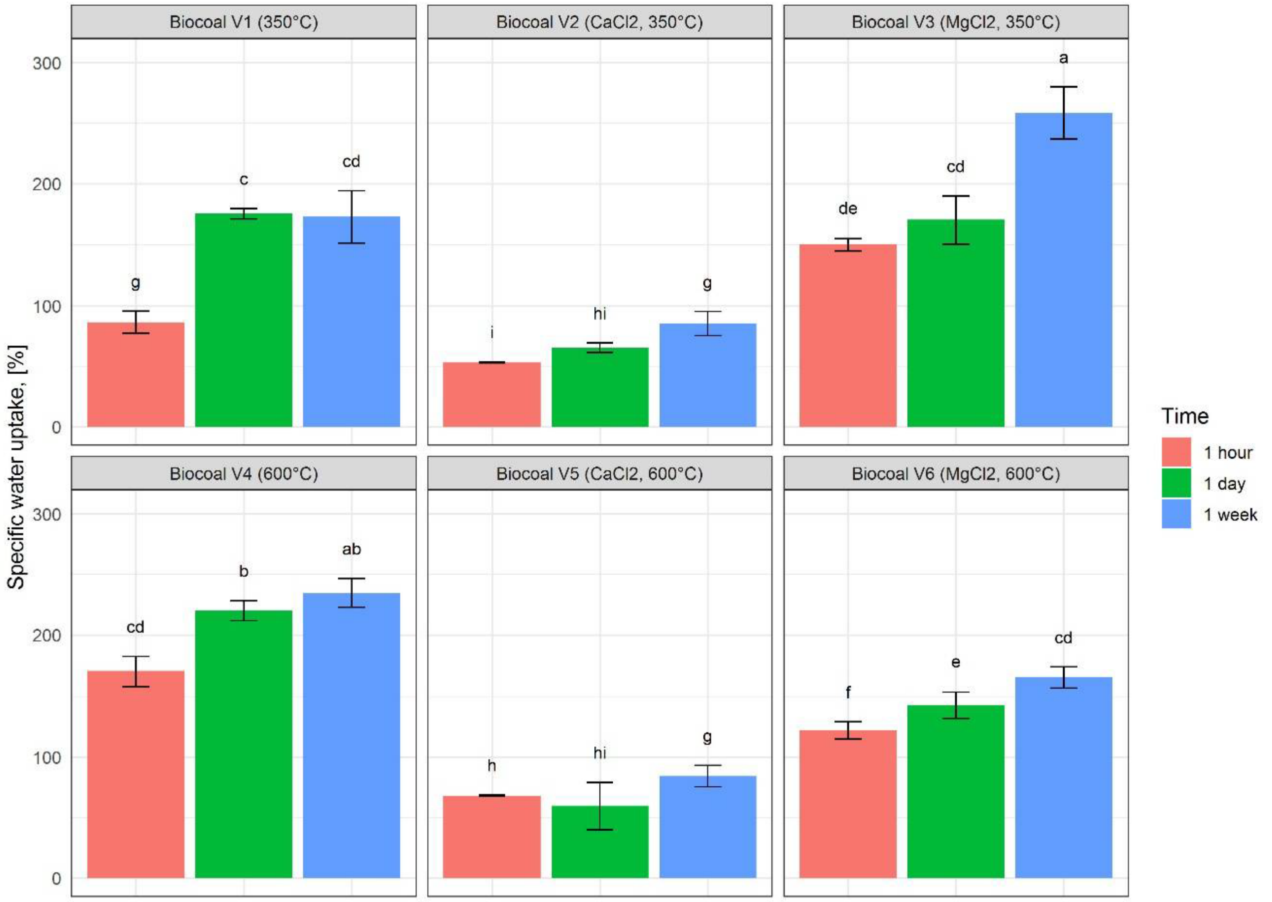

Figure 4. Specific water uptake for the six produced variants of biocoal under different exposure times (one hour, one day and one week). Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences between all the experiments according to Tukey test.

3.4.1. FM-Removal, DM Concentration and DM-Removal Efficiency

First, the effect of the additives on DM concentration of the solid phase after separation and on the degree of separation of the solids was investigated. The results on DM concentration and DM-removal efficiency for the ten tested variants are given in Figure 5.

The highest DM concentration in the solid phase for the four tested reaction times were measured for Biocoal V1 with 175.39 ± 21.20 $g{\cdot}kg_{FM}^{-1}$ (see Figure 5). The highest DM-removal efficiency was found for the reaction time of 20 h. However, the variant of biocoal also affected the FM and DM-removal efficiencies, with Biocoal V6 having the highest DM-removal efficiency of 78.06%.

3.4.2. NH_4 Concentration and NH_4-Removal Efficiency

Additives are used to increase the NH_4-concentration in the solid phase and thus, improve the removal efficiency. The highest NH_4 concentration of 34.63 $g{\cdot}kg_{DM}^{-1}$ was measured in the solid fraction after pretreatment with Biocoal V6 at the reaction time of 20 h (see Figure 6). The biocoal variants synthesized at higher temperatures had a higher NH_4-removal efficiency in the solid fraction. Among all tested variants, the highest NH_4-removal efficiency of 56.04% was measured for Biocoal V6 after pretreatment for 20 h.

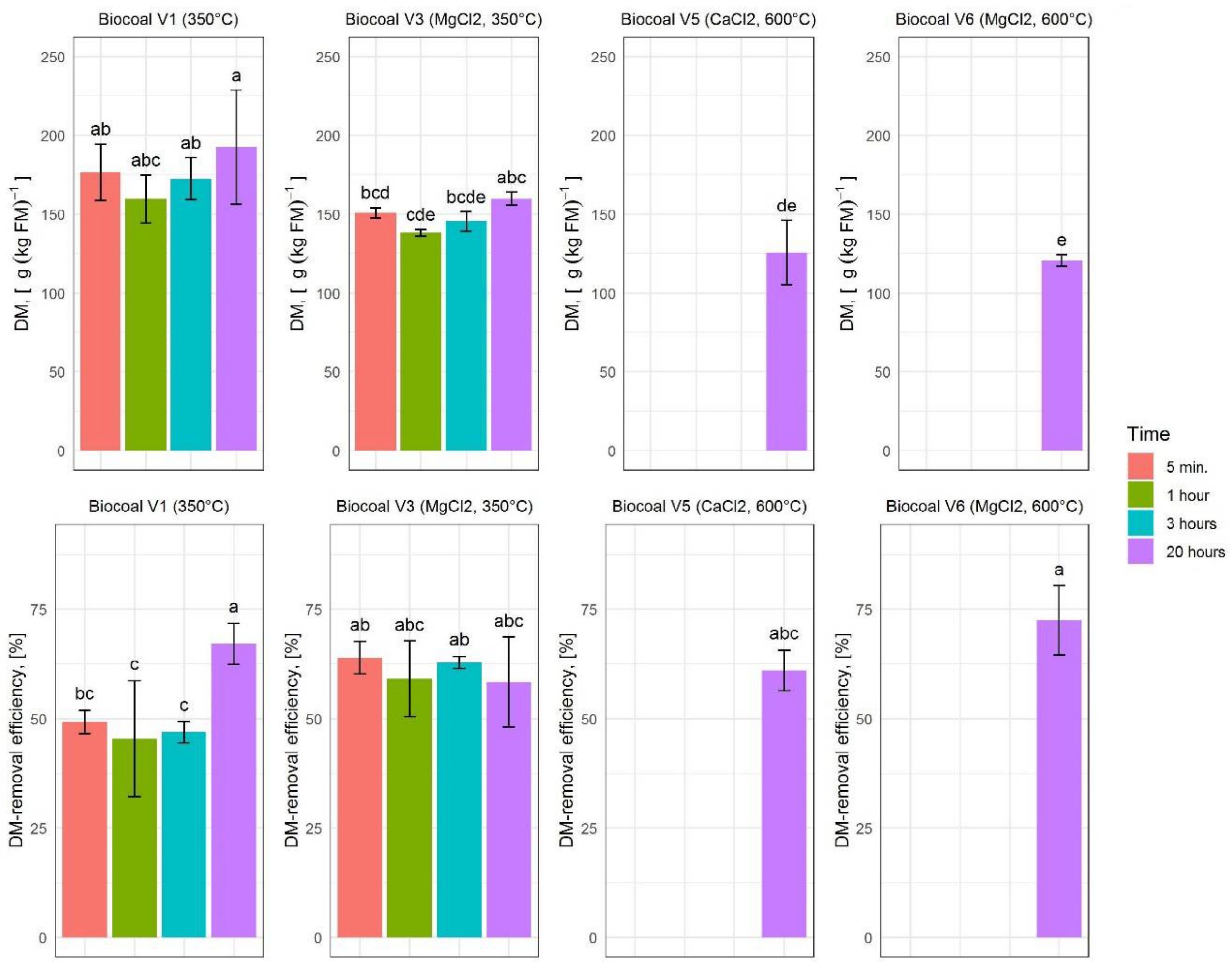

Figure 5. Dry matter (DM) concentration and DM-removal efficiency for the different variants of added biocoal. The upper diagrams show the DM concentration. The lower diagrams show the removal efficiency. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to Tukey test.

The NH_4-removal efficiency observed in this study is up to 1.8 times higher than reported by Kocatürk-Schumacher [28] and more than 3 times higher than reported by Takaya and co-authors [29]. Kocatürk-Schumacher applied biocoal from holm oak at a synthesis temperature of 650 °C and NH_4-removal efficiencies between 10% and 32% were observed [28]. Further, application of different types of hydrocoals and biocoals synthesized in the temperature range between 250 and 650 °C led to a removal efficiency of NH_4-N between 9% and 17% [29].

3.4.3. P Concentration and P-Removal Efficiency

According to the control experiments, the amount of P to be removed in the solid fraction is 38.41 ± 11.70%, based on the total amount of P in the digestate. The P-removal efficiency needs to be significantly improved for the commercial use of the P-rich solid fraction. Thus, by testing the additives, the P concentrations and P-removal efficiency were determined as shown in Figure 7.

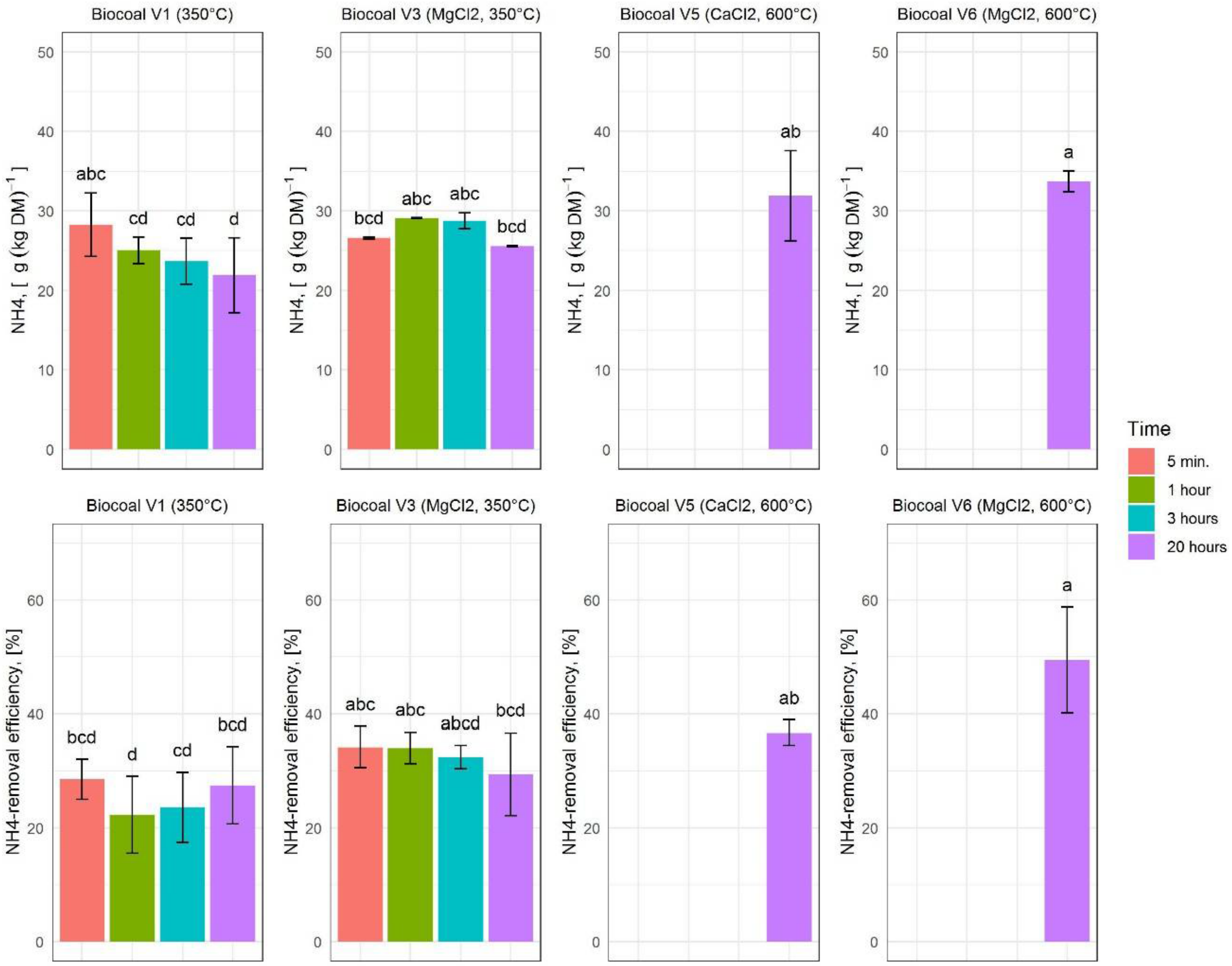

Figure 6. Ammonium (NH_4) concentration and NH_4-removal efficiency for the different variants of added biocoal. The upper diagrams show the NH_4 concentration. The lower diagrams show the removal efficiency. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to Tukey test.

The highest P concentrations were measured in the separated samples after the digestate pretreatment with the Mg-rich biocoals.

Application of biocoals loaded with $CaCl_2$ or $MgCl_2$ led to higher P-removal efficiencies than application of unloaded Biocoal V1. Mean P-removal efficiency for Biocoal V3 and Biocoal V5 was in the same range. The highest P-removal efficiency of 65.18 ± 1.48% was found after application of Biocoal V6 for 20 h.

To compare the results obtained in this study with those from literature, the sorption capacities for P and PO_4^{3-} were calculated. Neither the wood impregnation with $MgCl_2$ or $CaCl_2$ nor different synthesis temperatures improved the sorption capacities of P and PO_4^{3-} compared to Biocoal V1. Nevertheless, biocoals impregnated with $MgCl_2$ resulted in higher sorption capacities than biocoals impregnated with $CaCl_2$. The sorption capacities for all biocoal variants tested were much higher than those reported in literature [23,25–29,44].

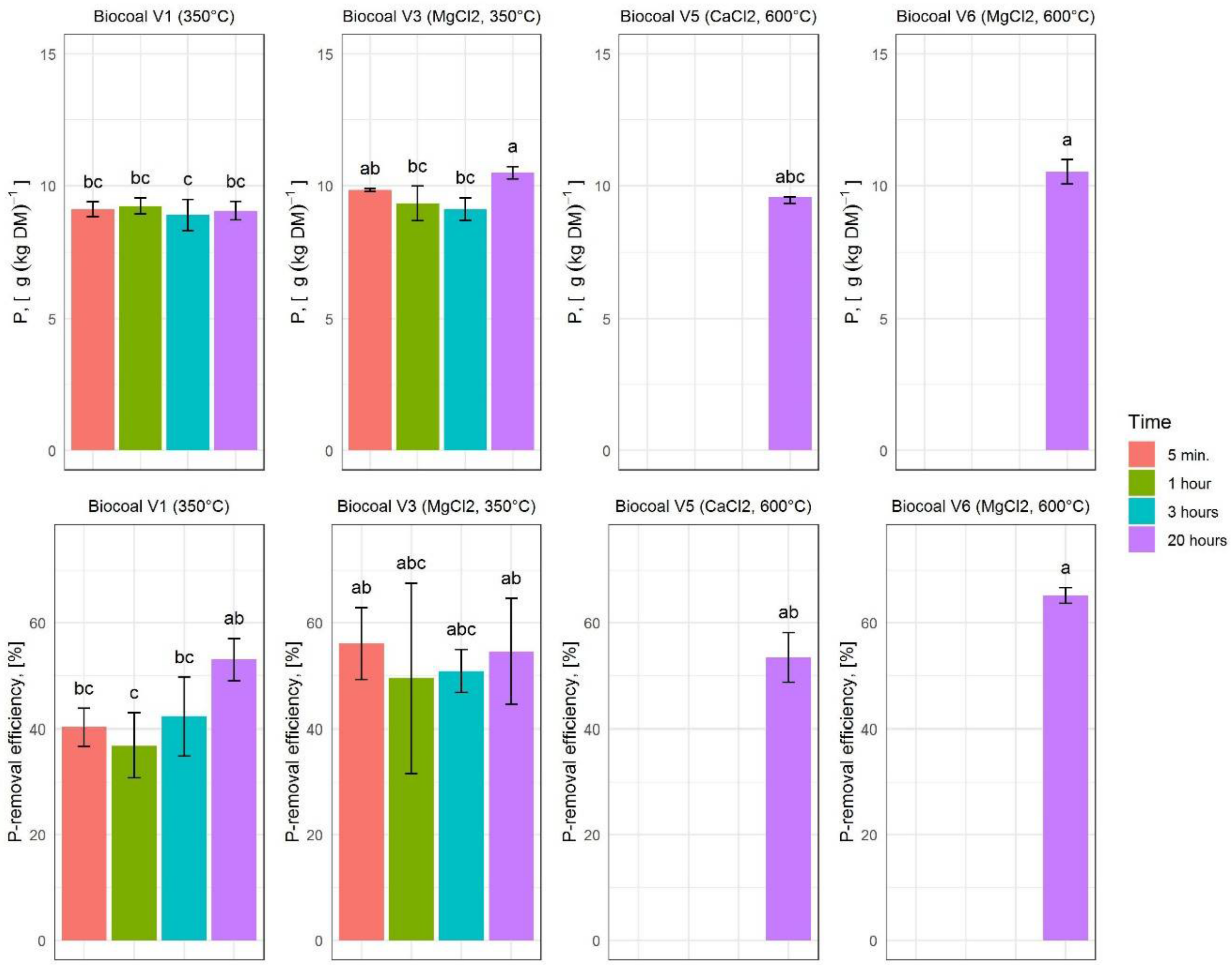

Figure 7. Phosphorus (P) concentration and P-removal efficiency for the different variants of added biocoal. The upper diagrams show the P concentration. The lower diagrams show the removal efficiency. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to Tukey test.

3.4.4. K-Concentration and K-Removal Efficiency

As digestate is rich in K, which is an essential crop nutrient, improved K-removal into the solid fraction is of relevance for plant nutrition. The K concentration and K-removal efficiency results for the analyzed variants are given in Figure 8 and described below.

3.5. *Comparison of the Control Variant with Those Pretreated with Additives with the Best Performance*

After evaluating the results in Section 3.4, it can be concluded that the application of Biocoal V3 and Biocoal V6 for the tested reaction time of 20 h resulted in the highest removal efficiency. The comparison of the results of these two variants with the control from the laboratory conditions is shown in Figure 9.

The results represented revealed that pretreatment of digestate with Biocoal V6 over the 20 h reaction time resulted in significantly higher mean removal efficiencies for NH_4, P, and K in the solid phase after separation compared to the control and the other variants studied. The application of Biocoal V6 resulted in a mean NH_4-removal efficiency, which was 76.33% higher than for the control under laboratory conditions and 67.81% higher than after pretreatment with Biocoal V3. The mean P-removal efficiency after Biocoal V6 application was 47.15%

of Biocoal V3. The K-removal efficiency in the solid fraction after the pretreatment with Biocoal V6 was 71.06% higher than the control and 78.67% higher than after application of Biocoal V3.

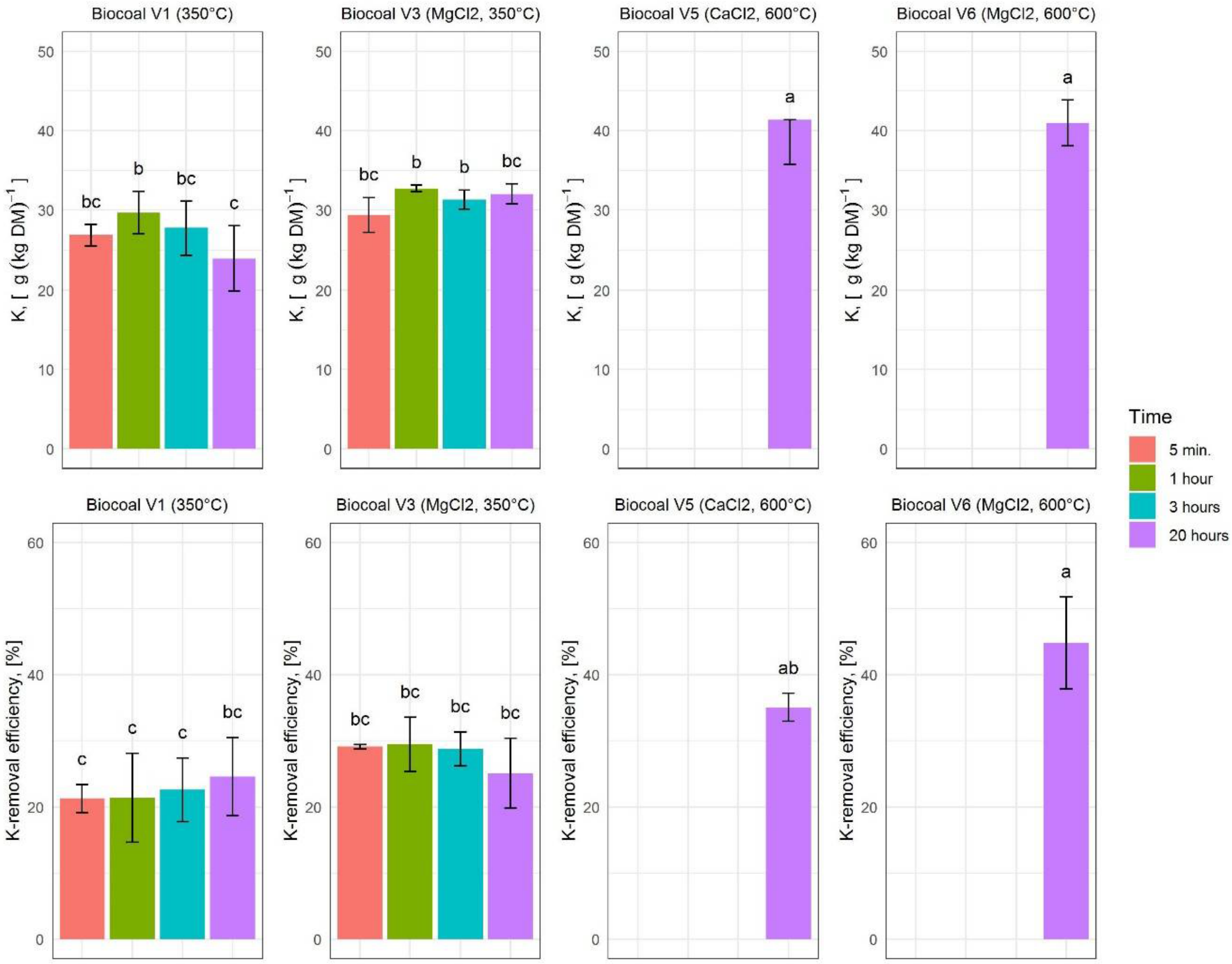

Figure 8. Potassium (K) concentration and K-removal efficiency for the different variants of added biocoal. The upper diagrams show the K concentration. The lower diagrams show the removal efficiency. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to Tukey test.

3.6. Summary of Results and Further Needed Research

In this study, the effect of various biocoal-based additives on nutrient removal during the separation of digestate was investigated at laboratory scale and at a full-scale biogas plant. A separation methodology was developed for the laboratory-scale, which led to similar results compared to the full-scale separation experiments. To improve the nutrient removal efficiency and increase the nutrient content in the solid phase after separation, the digestate was pretreated with different biocoal additives. First, the biocoals used in this research were tested on their specific water uptake and other characteristics. Then, the effects of pyrolysis temperature and pretreatment of the wood before pyrolysis on the sorption capacity of the biocoal was investigated. The digestate pretreatment with the biocoals impregnated with $MgCl_2$ for 20 h, synthesized at either 350 °C or 600 °C, resulted in higher nutrient recovery compared to the other tested variants. The application of Biocoal V6 for 20 h resulted in a significantly higher removal efficiency of NH_4, P and K compared to the untreated digestate and other tested variants. The enhanced adsorption effect of Mg-modified biocoal produced from corn stalk at 450 °C on ammonium nitrogen and phosphate was confirmed in the study [44]. In [44], the aforementioned biocoal has been investigated on its agronomic effect in pot analyses, proving the nutrient slow-release ability and the promoting of plant growth.

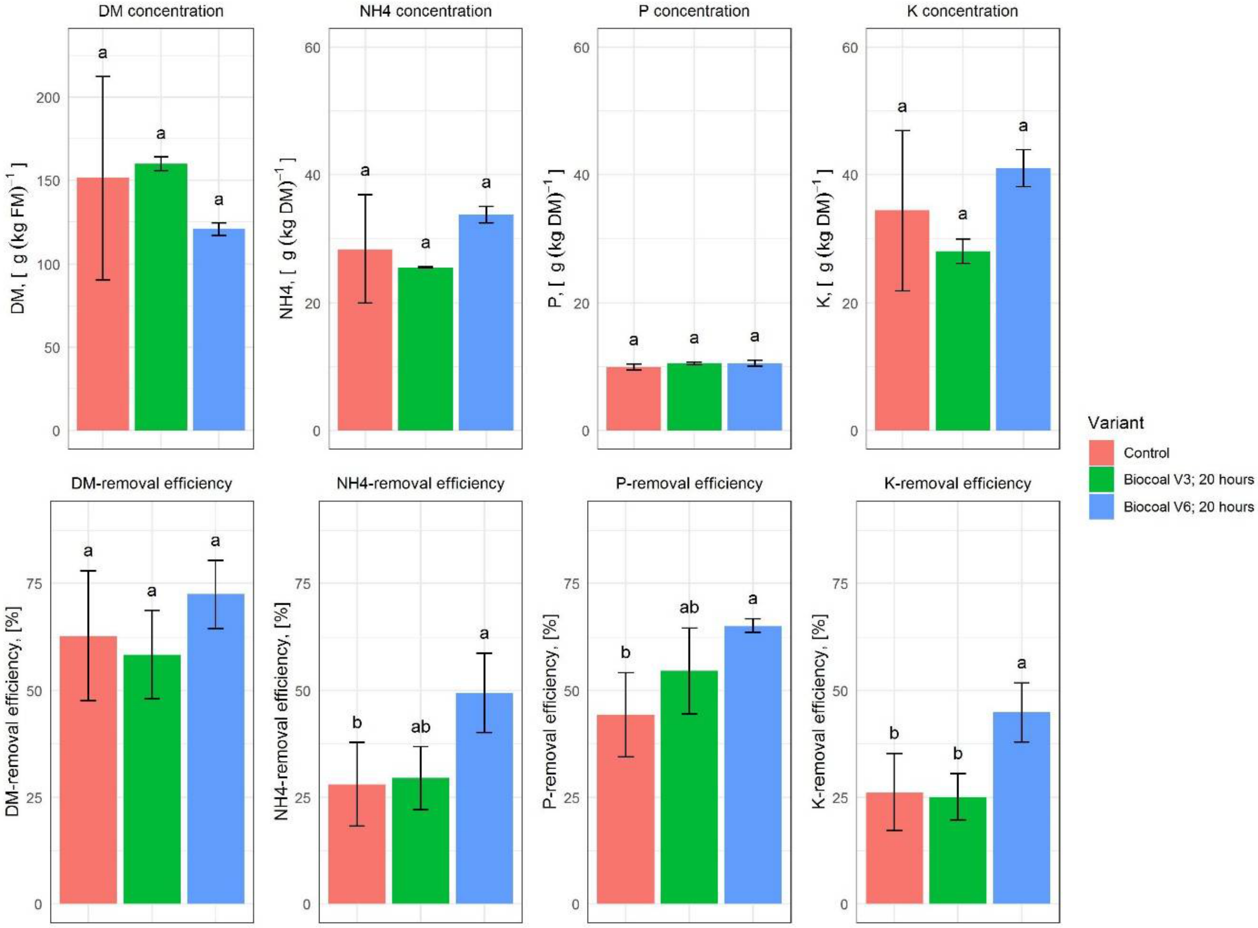

Figure 9. Comparison of the control variant (untreated digestate) with the two most promising additive variants and their best reaction times. Results were obtained in laboratory experiments. The upper diagrams show the concentrations. The lower diagrams show the removal efficiencies. Histograms are charted based on the mean values; error bars indicate the variability between the three replications. Lower case letters indicate significant differences according to the Tukey test.

Reducing the particle size of the biocoal by additional crushing in the mill could improve its sorption characteristics and lead to a higher nutrient removal efficiency. In addition to different reaction times, testing of higher temperatures (i.e., 40 °C) is also recommended and is currently being investigated in a follow-up research project.

4. Conclusions

A nutrient recovery in the solid fraction of the digestate has been investigated. A laboratory-scale digestate separation technology equivalent to full-scale pressure filtration has been developed. The nutrients recovery from the untreated digestate separated in the laboratory experiments, corresponded to the control variant. Pretreatment of the digestate with different additives before separation was tested in the laboratory conditions and the two most promising variants with the highest nutrient recovery in the solid fraction were identified: Biocoal from beech wood impregnated with $MgCl_2$, synthesized at either 350 °C or 600 °C and applied over a reaction time of 20 h. After the application of biocoal impregnated with $MgCl_2$ (600 °C, 20 h), the removal efficiency was increased by 76.33% for NH_4, 47.15% for P and 71.06% for K compared to the control variant at the laboratory-scale.

Author Contributions: Conceptualization, A.L. and I.M.; methodology, A.L. and I.M.; formal analysis, A.L. and I.M.; investigation, I.M.; resources, A.L. and I.M.; data curation, I.M.; writing — original draft preparation, I.M.; writing — review and editing, A.L. and I.M.; visualization, I.M.; supervision, A.L.; project administration, A.L. and I.M.; funding acquisition, A.L. and I.M. All authors have read and agreed to the published version of the manuscript.

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5. General discussion

5.1 Designing of biogas plant on the best performance bioenergy crops

When growing bioenergy crops as feedstock for biogas production, the soil-climatic characteristics and weather conditions affect areal biogas (methane) yields (Schumacher, 2008). For this reason, the dry matter yields may vary over the years. However, in this study, the crops were cultivated in Ukraine under extremely unfavourable weather conditions. Thus, the biomass yields obtained are likely below the long-term average. Therefore, based on this work, multi-year experiments on biomass and methane yields should be conducted with the most interesting crops. The novelty of this research lies in the analysis of high-yield Ukrainian species of different crops on their specific methane yields and areal methane yields. Both plant variety and harvesting time affected the yields. Miscanthus Giganteus (Species Giant Chinese Silver Grass: Miscanthus x giganteus J.M Greef & Deuter ex Hodkinson Renvoiz, the variety name “Osinnii zoretsvit”), harvested starting from the 3rd vegetation year, is the most promising crop for the establishment of biogas plants in Ukraine. The additional advantages of using miscanthus as a biogas substrate are the 15-year cultivation period of this crop, the lowest N-demand per unit of methane produced in this study, and the phytoremediation effect on the soil (Barbosa et al., 2015). Miscanthus is a perennial crop and can be harvested annually. Dry matter yields of miscanthus are low in the first two years of vegetation and increase with plantation age up to a certain point (which is not the subject of this study). The major challenge for miscanthus is timely fertilizer application and provision of pesticides, herbicides, and water as needed in the first and sometimes second year of cultivation (Zegada-Lizarazu et al., 2010). Additional costs are incurred by pretreatment of miscanthus due to its lignocellulosic composition (Agbor et al., 2011). Whether mechanical pretreatment, such as cross-flow grinding used to disintegrate horse manure (Mönch-Tegeder et al., 2014), is beneficial for miscanthus needs to be investigated in further trials.

Among the annual crops, maize and sweet sorghum achieved the highest biomass yields in the cultivation trials in Ukraine. In particular, the maize variety “Svitanok MV” (FAO 250) and the sweet sorghum variety “Favoryt” can serve as biogas feedstock due to their high methane production. The results obtained in this study were compared with those for German maize varieties cultivated in Germany (Mukengele, 2017; Schumacher, 2008). Based on these two studies, the average specific methane yield (SMY, values related to STP) of the analyzed German varieties was 341.70 ± 13.88 $m^3 \cdot kg^{-1}$VS. These values are comparable to all Ukrainian maize varieties with SMY of 334.54 ± 29.72 $m^3 \cdot kg^{-1}$VS. The average SMY values of soryz and sweet sorghum were lower than those of maize: 325.38 ± 22.15 $m^3 \cdot kg^{-1}VS$ and 315.52 ± 30.10 $m^3 \cdot kg^{-1}VS$ for soryz and sweet sorghum, respectively. The SMY of miscanthus Gigantheus of the later vegetation year was relatively low: 230.56 ± 45.46 $m^3 \cdot kg^{-1}VS$. Comparing the areal methane yield (AMY, values related to STP) of maize grown in southern parts of Germany with the results of this study, the following conclusions can be drawn: The average AMY for the German maize (7373.71 ± 1796.81 $m^3 \cdot ha^{-1}$) was twice as high as this value for the Ukrainian maize (3597.35 ± 1295.73 $m^3 \cdot ha^{-1}$). The average AMY values of Ukrainian soryz and sweet sorghum were 1120.16 ± 368.41 $m^3 \cdot ha^{-1}$ and 4319.35 ± 1020.41 $m^3 \cdot ha^{-1}$, respectively. The average AMY value determined for miscanthus Gigantheus of the eight-year plantation was 6272.57 ± 865.84 $m^3 \cdot ha^{-1}$ and was comparable to the German maize. Nevertheless, the yield losses due to the unfavourable weather conditions during the cultivation of the crops must be taken into account.

Nitrogen is one of the essential plant nutrients. Due to the high energy required to produce N-containing fertilizers, energy crops with high N efficiency, in particular, have a good carbon footprint. The average nitrogen concentrations for Ukrainian crop varieties were as follows: 93.20 ± 22.57 $mg_N \cdot kg^{-1}$ for maize, 152.00 ± 61.58 $mg_N \cdot kg^{-1}$ for soryz, 116.50 ± 40.93 $mg_N \cdot kg^{-1}$ for sweet sorghum, 90.00 ± 42.43 $mg_N \cdot kg^{-1}$ for switchgrass, 146.50 ± 13.99 $mg_N \cdot kg^{-1}$ for soybean, 80.00 ± 4.00 $mg_N \cdot kg^{-1}$ for paulownia, and 48.00 ± 6.73 $mg_N \cdot kg^{-1}$ for miscanthus. The N contents of the most suitable crops and their respective harvesting times were the following: 40.00 ± 2.00 $mg_N \cdot kg^{-1}$ for miscanthus Gigantheus harvested at BBCH-code 36, 120.00 ± 6.00 $mg_N \cdot kg^{-1}$ for maize “Svitanok MV” harvested at BBCH-code 87, 96.00 ± 4.80 $mg_N \cdot kg^{-1}$ for sweet sorghum “Favoryt” harvested at BBCH-code 61.

This study is one of the first studies to systematically investigate the cultivation of energy crops in Ukraine and their methane formation potential. Due to the very large agricultural potentials, the cultivation of energy crops and the integration of biogas plants in bioenergy villages offer interesting opportunities for future sustainable energy supply in Ukraine. Plant breeders, biogas producers and policy makers can benefit from our results. The bioenergy crops technology readiness level can be assessed as TRL 4 in terms of experimental testing with field trials or validation experiments (United State Department of Agriculture. National Institute of Food and Agriculture. Institute of Food Production and Sustainability, 2018; Viaggi, 2018). The cultivation of the crops is associated with risk related to the political situation and weather conditions. In further studies, the economic and environmental analyses for the cultivation of the studied bioenergy crops need to be carried out.

5.2 Microbial adaptability to N-rich feedstock and increasing ammonia levels in an anaerobic reactor

Microorganisms in an anaerobic reactor can adapt to changes in the feeding regime, namely to changes in the N concentration in the feedstock, as confirmed in this study. Due to the chosen adaptation strategy, which allowed the microorganisms to adapt to higher N concentrations, stable fermentation processes were still achieved even at very high N concentrations of up to 12 g·kg^{-1}FM in the fermentation substrate. According to the experimental results, the system was able to adapt efficiently and with minimal inhibition within two weeks to the N increase rate in a feedstock of 0.25 g_N·kg^{-1}, N concentration of 5.92 g·kg^{-1}FM, TAN of 4.56 g·kg^{-1}FM and FAN of 0.38 g·kg^{-1}FM, respectively. This is in contrast to many previous studies that reported process disturbances at much lower N concentrations (Chen et al., 2008; Lauterböck et al., 2012; Li et al., 2017; Molaey et al., 2018). This seems to prove that not so much the absolute N concentration, but rather the rate of concentration change is crucial for stable fermentation processes.

Normally, low concentrations of volatile fatty acids in the digestate are indicative of a stable digestion process (Angelidaki and Ahring, 1994; Angenent et al., 2002; Fitamo et al., 2016; Hashimoto, 1986). However, this study showed that a stable AD process is not always an efficient process. In the research, the conversion efficiency of biomass to biogas was determined by measuring the methane yield potential of substrates in stable batch-tests (Hohenheim Biogas Yield Test – HBT) and comparing these results with continuously fed experiments. The difference in specific methane yields was defined as “inhibition”.

The continuous AD process organized at laboratory scale corresponded to the full-scale application (Steinbrenner et al., 2020). Different initial N levels in a feedstock and different feeding scenarios were investigated during the research trials. Inhibition levels under different N concentrations and N feeding scenarios were quantified. High N concentration in inoculum and feedstock resulted in high TAN and FAN concentrations in the digestate. There was a controversy in the literature about a method to measure TAN and FAN. When presenting results on ammonia, authors often refer to some other works, where the formulas were not clear and reproducible. At the same time, some authors did not differentiate between ammonium ion and TAN. In our work, we chose to use the established methodology for measuring TAN and FAN developed by (Hashimoto, 1986; Perry et al., 1961).

According to Shanmugam and Horan (Shanmugam and Horan, 2009), the C/N ratio in a feedstock should be kept between 15 and 20; Kayhanian (Kayhanian, 1999) recommends keeping the C/N ratio between 27 and 32. In this study, the stable and efficient process was determined for a C/N ratio in the feeding substrates between 19 and 28. Very high N contents in the digestate are causally triggered by a narrow C/N ratio of the feedstock. In the studies, the C/N ratio of the added substrates mixture decreased from 28.47 to 11.94. The studies show that at very high N concentrations in the digestate (or narrow C/N ratio of the feedstock), the conversion rate of the energy stored in the biomass to methane decreases significantly.

The research results are relevant for owners and operators of biogas plants using protein-rich feedstocks (bioenergy crops, biowaste, chicken manure etc.). For the full-scale trials, the levels of inhibition in SMY could be higher, which should be investigated. The TRL of the proposed AD process with high and increasing nitrogen concentrations in a feedstock can be estimated as TRL 7, corresponding to system prototype demonstration in operational environment (Viaggi, 2018).

5.3 Rational use of digestate as fertilizer for economic and environmental benefits

Digestate is the end product of feedstock decomposition and must be further utilized after the loss of volatile compounds that are converted to biogas (CH_4 and CO_2). The nutrient content of the digestate is related to the nutrient content of the feeding substrates. Due to the high water content of more than 80%, the transportability of the digestate is limited, so it can only be spread in a narrow radius around the biogas plant. The task was to produce a nutrient-rich fertilizer from digestate while reducing the water content in it. Separation tests were carried out for this purpose. The digestate is separated into a liquid and a solid, nutrient-rich fraction. The nutrient-rich solid fraction of the digestate can serve as an attractive organic fertilizer, which can be stored and applied to a field in times of high nutrient demand by the crop or sold to the market.

The aim of this study was to investigate new methods for pretreatment of digestate for nutrient recovery. The existing methods for nutrient recovery in the literature were mainly studied for waste water or nutrient-concentrated liquids (Chen et al., 2011; Fang et al., 2015; Kocatürk-Schumacher, 2016; Li et al., 2016; Takaya et al., 2016; Wang et al., 2015; Zhang et al., 2012). The results from the literature cannot be applied to digestate from agricultural biogas plants because it is generally a heterogenic liquid. It is important to properly mix the digestate to achieve a uniform distribution of solids in it before applying the pretreatment method and the solid-liquid separation process.

For the investigations, the digestate was taken from the full-scale biogas plant “Unterer Lindenhof”. In the laboratory, a methodology for digestate separation was developed that corresponds to the full-scale separation trials with a tincture press and an operating pressure of 100 bar. The pretreatment method studied was based on the use of innovative biocoal-based additives. It was fundamental research, including the production and testing of novel biocoals.

Nutrient recovery was affected by additive composition, additive synthesis temperature, and reaction conditions (mixing, storing conditions), as well as reaction time between additives and digestate. The results of the study are applicable to digestate from agricultural biogas plants.

The positive effect of biocoals with Mg on nutrients recovery has been described in the literature (Fang et al., 2015; Yao et al., 2013) and confirmed in this study. This could be due to the change of biocoal surface charge from negative (in conventional biocoal) to positive (in Mg-rich biocoal), which efficiently adsorbs nitrate and phosphate molecules of anionic nature. The application of biocoal from Mg-impregnated beech wood synthesized at 600 °C during the 20-hour reaction period with continuous mixing outperformed the other variants studied and the control variant.

Further research needs to test the biocoals with the smaller particle size and different reaction temperatures. For environmental benefits, pot trials are recommended to prove the accessibility of nutrients from the valorized solid fraction of the digestate to plants. The economics of the process need to be calculated. By testing the effects of the digestate pretreatment with the additives on the efficiency of nutrient removal from the digestate, the TRL 4 (corresponding to technology validated in laboratory) was achieved for this technology (Viaggi, 2018).

5.4 Perspectives and limitations of the investigated cradle-to-cradle concept

The perspective for the proposed cradle-to-cradle approach, i.e. from biomass cultivation to its utilization and return of digestate to the cultivated areas, is to verify the results in full-scale application at the farm level by building a new biogas plant or retrofitting the existing plant. Care should be taken to ensure that high-quality energy sources (electricity and heat) are also produced on an industrial scale without generating undesired by-products or waste. This is one of the major advantages of the sustainable approach described.

The scientific knowledge ("know-how"), resources, and legal basis can be seen as limitations of the concept. Governmental support and extension services for farmers, especially for small and medium farms, should be organized. Other factors influencing the practical implementation of the concept include the local situation (political situation, social acceptance, market demand i.a.), soil-water conditions, infrastructure, and market availability (for biomethane and nutrient-rich fertilizers).

5.5 Conclusion and Outlook

The technologies and methods developed in this study combine different disciplines: crop breeding (for bioenergy crops), physics (pretreatment of crops, production of biocoals), microbiology (batch and continuous experiments for AD), engineering (laboratory systems for AD processes and digestate separation; large-scale and laboratory separation experiments), biochemistry (pretreatment of fermentation residues with additives), chemistry (laboratory analyses) etc. This demonstrates the interdisciplinary, cross-disciplinary approach of the bioeconomic process described.

The first objective of the thesis was to experimentally investigate the influence of plant varieties and harvesting times on the methane yield potentials of miscanthus and other lignocellulosic bioenergy crops grown in Ukraine. Clear recommendations could be derived for this.

Miscanthus “Giganteus”, the Ukrainian variety “Osinnii zoretsvit” from the 8th year of vegetation, harvested at the stem elongation stage (BBCH-code 36) gave the highest AMY equal to 7404.55 ± 199.00 $m^3 \cdot ha^{-1}$ and the lowest N-demand per unit of methane produced up to 23.41 ± 7.18 $g_N \cdot m^{-3}$.

The highest SMY value of 0.41 ± 0.00 $m^3 \cdot kg^{-1}VS$ and the second highest AMY value of 6365.67 ± 55.49 $m^3 \cdot ha^{-1}$ were determined for “Svitanok MV” maize (FAO 250) harvested at the wax ripeness stage (BBCH-codes 83-85).

The Ukrainian sweet sorghum variety “Favoryt” with mid-ripening group of ripeness harvested at the beginning of the florescence stage (BBCH-code 61), resulted in the third-highest AMY value of 5968.90 ± 82.70 $m^3 \cdot ha^{-1}$ among the other analyzed varieties, after miscanthus “Giganteus” and maize “Svitanok MV”.

The second objective was to investigate the influence of N-rich biomass on process stability during anaerobic digestion. The investigated TKN contents in the reactors varied between 3.34 and 11.50 $g \cdot kg^{-1}$ over the experimental period; TAN values ranged between 1.35 and 7.97 $g \cdot kg^{-1}$; FAN values changed between 0.06 and 0.74 $g \cdot kg^{-1}$. High N concentrations in the inocula and the N-increase rates in the feeding regimes, which resulted in high TAN and FAN concentrations in the reactors, significantly affected inhibition of biogas (methane) production. The microorganisms in the reactor were able to adapt to high and increasing N concentrations in feedstock up to a maximum N loading rate of 0.30 $g_N \cdot L \cdot d^{-1}$. Thus, also with regard to the second objective, the knowledge about the relationships between C/N ratio of the substrates and the process efficiency could be significantly extended.

The third objective was to develop and investigate optimized separation processes to further improve the nutrient management of the digestate. Using $MgCl_2$-impregnated biocoal synthesized at 600 °C during the 20-hour reaction time as an additive prior to separation to improve nutrient recovery from the digestate, the highest removal efficiencies were obtained in the solid fraction compared to the control (unpretreated digestate) and other analyzed variants (application of other biocoal-based additives for the digestate pretreatment): For NH_4^+, P and K, the removal efficiency values were

56.04%, 66.66%, and 51.77% respectively. However, further investigations are necessary in this area. According to the results of our own investigations, the methods used in the laboratory for digestate separation can only be transferred to practice to a limited extent. Furthermore, some of the investigated variants showed a limited dewaterability of the digestate, which has not been described in this form in the literature so far. Further investigations are necessary before reliable recommendations for practice can be derived.

General conclusions:

The results of this study can be used for the establishment of new bioenergy villages to reduce dependence on fossil fuels, especially natural gas. The research concept was originally developed to promote the bioeconomy in Ukraine; however, the research results are transferable to other countries. The collected data and results form the basis for conducting environmental analysis (i.e. Life Cycle Assessment) and economic analysis of the production processes under study.

Summary

A sustainable energy supply and bio-based economic processes are of central importance for the future development of many Eastern European countries. Due to the large agricultural potentials of these countries, bioenergy systems can make a significant contribution to sustainable electricity and heat production if they are reasonably integrated into an energy supply structure based on various renewable energy sources. This requires the use of regenerative starting products and the complete utilisation of all by-products of the overall process. With such a cradle-to-cradle approach, biogas technology can be a central component of future energy systems.

The focus of this study is on Ukraine. In the future, bioenergy villages can make a decentralised contribution to a sustainable energy supply in this country. This study aims to determine the methane yield potential of various energy crops from Ukraine, investigate the process stability during fermentation in biogas plants and derive concepts for optimized digestate management.

Seven different crops with a total of 22 varieties were investigated for their specific biomass yields, methane yields and areal methane yields. The crops were cultivated in Ukraine. The biogas production potential of the collected crop samples was determined using the Hohenheim Biogas Test in Germany. The Ukrainian variety “Osinnii zoretsvit” of miscanthus, “Giganteus” species, from the 8th year of vegetation, harvested at the stem elongation stage, resulted in the highest areal methane yield of 7404.55 ± 199.00 $m^3 \cdot ha^{-1}$ and the lowest N requirement per unit methane produced (23.41 ± 7.18 $g_N \cdot m^{-3}$) among all the studied crops. The maize variety "Svitanok MV" (FAO 250) had the highest value of areal methane yield of 6365.67 ± 55.49 $m^3 \cdot ha^{-1}$ among the annual crops when harvested at the stage of wax maturity; remarkable was its unusually high specific methane yield of 0.41 ± 0.00 $m^3 \cdot kg^{-1}$VS. The Ukrainian sugar sorghum variety "Favoryt", harvested at the beginning of flowering, had an areal methane yield of 5968.90 ± 82.70 $m^3 \cdot ha^{-1}$, making it an attractive alternative energy crop for Ukraine.

In the second part of the work, experimental investigations were carried out to test how N-rich substrates influence the stability and efficiency of the biogas process. For this purpose, different variants with various N-increase rates of the input materials at two initial concentrations were evaluated in the laboratory. The continuous trials were conducted over a period of 33 weeks. The modelling procedure was applied to evaluate the effects of TAN (total ammonia nitrogen) and FAN (free ammonia nitrogen) on the degree of methane production inhibition for all scenarios studied. It was concluded that the higher the N-increase rate in the feeding regime, the more methane production is inhibited. The maximum nitrogen concentration in the digestate achieved during stable fermentation processes in this study was 11.5 $g \cdot kg^{-1}$FM, which corresponded to the values of TAN and FAN of

9.07 g·kg^{-1}FM and 0.85 g·kg^{-1}FM, respectively. These values are much higher than those reported up to now in the literature. At the same time, process efficiency decreased with increasing nitrogen concentrations.

As a final step, the technology for nutrients recovery from digestate was developed and tested in this work. First, the digestate separation with a screw press separator was carried out as a "benchmark" at the research biogas plant "Unterer Lindenhof" on a technical scale. Subsequently, a methodology for digestate separation at laboratory scale was developed based on a tincture press, which corresponds to the technology used in practice. The effect of pretreatment of digestate with various biocoal-based additives was studied. In this study, six variants of biocoals synthesized at either 350 °C or 600 °C and partially impregnated with Mg or Ca before pyrolysis were produced. Different reaction times and conditions between the biocoals and the digestate were tested. The results on nutrient removal showed that the biocoals impregnated with Mg prior to pyrolysis had a positive effect on nutrient removal efficiency. The Mg-impregnated biocoal synthesised at 600 °C showed removal efficiencies for NH_4^+, P and K of 56.04%, 66.66% and 51.77%, respectively. These values were much higher than those for the control variant and much higher than the values found up to now in the literature.

By using the nutrient-rich solid fraction of the digestate as fertiliser to cultivate bioenergy crops for further use in biogas production, the production cycle is closed, and the cradle-to-cradle approach is achieved. The technologies, products and methods developed, applied, investigated and validated in this dissertation form an initial basis for the expansion of the use of bioenergy in the countries of Eastern Europe. Due to weather-related yield fluctuations, in particular, the one-year cultivation trials must be validated in subsequent studies.

Zusammenfassung

Eine nachhaltige Energieversorgung sowie biobasierte Wirtschaftsprozesse sind für die zukünftige Entwicklung vieler osteuropäischer Länder von zentraler Bedeutung. Aufgrund der großen Agrarpotenziale dieser Länder können Bioenergiesysteme bei einer sinnvollen Integration in eine Energieversorgungstruktur auf Basis verschiedener erneuerbarer Energieträger einen wesentlichen Beitrag zur nachhaltigen Strom- und Wärmeproduktion liefern. Dies setzt die Nutzung regenerativer Ausgangprodukte sowie die vollständige Verwertung sämtlicher Nebenprodukte des Gesamtprozesses voraus. Mit einem solchen Cradle-to-Cradle-Ansatz kann die Biogastechnologie ein zentraler Bestandteil zukünftiger Energiesysteme sein.

In dieser Arbeit liegt der Untersuchungs-Schwerpunkt auf der Ukraine. Hier können zukünftig Bioenergiedörfer dezentral zu einer nachhaltigen Energieversorgung beitragen. Ziel der Arbeit ist es, die Methanertragspotenziale verschiedener Energiepflanzen aus der Ukraine zu ermitteln, die Prozessstabilität bei der Vergärung in Biogasanlagen zu untersuchen und Ansätze für eine optimiertes Gärrestmanagement abzuleiten.

Sieben verschiedene Kulturpflanzen mit insgesamt 22 Sorten wurden auf ihre spezifischen Biomasseerträge, Methanerträge und flächenbezogenen Methanerträge untersucht. Die Pflanzen wurden in der Ukraine angebaut. Das Biogasproduktionspotenzial der gesammelten Pflanzenproben wurde mit dem Hohenheimer Biogasertragstest in Deutschland ermittelt. Die ukrainische Miscanthus-Sorte "Osinnii zoretsvit", Art "Giganteus", aus dem 8. Vegetationsjahr, geerntet im Stadium der Internodienstreckung, ergab den höchsten flächenbezogenen Methanertrag von 7404,55 ± 199,00 $m^3 \cdot ha^{-1}$ und den niedrigsten N-Bedarf pro erzeugter Methaneinheit (23,41 ± 7,18 $g_N \cdot m^{-3}$) unter allen untersuchten Kulturen. Die Maissorte "Svitanok MV" (FAO 250) wies bei den einjährigen Pflanzen, wenn sie im Stadium der Wachsreife geerntet wurde, den höchsten Wert des flächenbezogenen Methanertrags von 6365,67 ± 55,49 $m^3 \cdot ha^{-1}$ auf; bemerkenswert war ihr ungewöhnlich hoher spezifischer Methanertrag von 0,41 ± 0,00 $m^3 \cdot kg^{-1}$VS. Die ukrainische Zuckersorghum-Sorte "Favoryt", die zu Beginn der Blüte geerntet wurde, wies einen flächenbezogenen Methanertrag von 5968,90 ± 82,70 $m^3 \cdot ha^{-1}$ auf und ist damit eine interessante alternative Energiepflanze für die Ukraine.

Im zweiten Teil der Arbeit wurde in experimentellen Untersuchungen geprüft, wie N-reiche Substrate die Stabilität und Effizienz des Biogasprozesses beeinflussen. Dazu wurden im Labor verschiedene Varianten mit unterschiedlichen N-Steigerungsraten der Inputstoffe bei zwei Ausgangskonzentrationen evaluiert. Die kontinuierlichen Versuche wurden über einen Zeitraum von 33 Wochen durchgeführt. Ein Modellierungsverfahren wurde angewandt, um die Auswirkungen von TAN

(Total Ammonia Nitrogen) und FAN (Free Ammonia Nitrogen) auf den Grad der Hemmung der Methanproduktion für alle untersuchten Szenarien zu bewerten. Es wurde festgestellt, dass die Methanproduktion umso stärker gehemmt wird, je höher die N-Anstiegs-Rate im Fütterungsregime ist. Die maximale Stickstoffkonzentration im Gärrest, die im Rahmen dieser Untersuchung bei stabilen Gärprozessen erreicht wurde, betrug 11,5 $g \cdot kg^{-1}$FM, was TAN- und FAN-Werten von 9,07 $g \cdot kg^{-1}$FM bzw. 0,85 $g \cdot kg^{-1}$FM entsprach. Diese Werte liegen deutlich über bisherigen Literaturangaben. Gleichzeitig nahm die Prozesseffizienz mit steigenden Stickstoffkonzentrationen ab.

Abschließend wurde in der Arbeit eine Technologie zur Nährstoffrückgewinnung aus Gärresten entwickelt und getestet. Zunächst wurde im technischen Maßstab an der Forschungsbiogasanlage „Unterer Lindenhof“ die Gärrestabtrennung mit einem Pressschnecken-Separator als „Benchmark“ durchgeführt. Anschließend wurde eine Methodik zur Gärrestabtrennung im Labormaßstab auf Basis einer Tinkturenpresse entwickelt, die der in der Praxis eingesetzten Technologie entspricht. Die Wirkung der Vorbehandlung von Gärresten mit verschiedenen biokohlen-basierten Additiven wurde untersucht. In dieser Studie wurden sechs Varianten von Biokohlen hergestellt, die entweder bei 350 °C oder 600 °C synthetisiert wurden und vor der Pyrolyse teilweise mit Mg oder Ca imprägniert wurden. Es wurden verschiedene Reaktionszeiten und -bedingungen zwischen den Biokohlen und dem Gärrest getestet. Die Ergebnisse zur Nährstoffabtrennung zeigten, dass Biokohlen, die vor der Pyrolyse mit Magnesium imprägniert wurden, einen positiven Effekt auf den Abscheidegrad der Nährstoffe hatten. Die Mg-imprägnierte Biokohle, die bei 600 °C synthetisiert wurde, zeigte eine Abtrenneffizienz für NH_4^+, P und K von 56,04%, 66,66% bzw. 51,77%. Diese Werte lagen deutlich über denen der Kontrollvariante bzw. deutlich über bisherigen Literaturwerten.

Durch die Nutzung der nährstoffreichen Feststofffraktion des Gärrestes als Düngemittel zum Anbau der Bioenergiepflanzen für die weitere Verwendung in der Biogasproduktion wird der Produktionszyklus geschlossen und der Cradle-to-Cradle-Ansatz erreicht. Die in dieser Dissertation entwickelten, angewandten, untersuchten und validierten Technologien, Produkte und Methoden bilden eine erste Grundlage zum Ausbau der Bioenergienutzung in den Ländern Osteuropas. Aufgrund witterungsbedingter Ertragsschwankungen müssen insbesondere die einjährigen Anbauversuche in folgenden Untersuchungen validiert werden.

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