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The Effect of Detoxification of Lignocellulosic Biomass for Enhanced Methane Production

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Abstract: The aim of this research is to examine the effect of lignocellulosic biomass detoxification on the efficiency of the methane fermentation process. Both for corn straw and rye straw, the methane yield was expressed per volume of fermentation medium and per mass of volatile solids (VS) added. Lignocellulosic biomass was subjected of thermo-chemical and enzymatic sequential pretreatments. It was found that methane yield was higher by 22% when using the detoxification process. In these variants, CH_4 yield was 18.86 L/L for corn straw and 17.69 L/L for rye straw; while methane yield expressed per mass of VS added was 0.31 m^3/kg VS for corn straw and 0.29 m^3/kg VS for rye straw. The inclusion of a detoxification step in pretreatments of biomass lignocellulosic increases the degree of organic substance decomposition and enhances methane yield. The results show that a two-step pretreatment, alkaline/enzymatic with a detoxification process, is necessary for the effective generation of high methane concentration biogas.

Keywords: lignocellulosic biomass; biofuels; enzymatic hydrolysis; simultaneous fermentation

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1. Introduction

The rapid depletion of fossil fuels and their negative impact on the environment (greenhouse effect, pollution of air with sulfur compounds, ammonia, smog formation) increases interest in the contribution of renewable raw materials in the energy market.

Renewable energy sources can provide energy security, help to protect the environment, and can stimulate the economy [1]. Therefore, there is a growing interest in the use of lignocellulosic material in biorefining processes, in which lignocellulose can be processed both to second-generation fuels (ethanol and methane) and to other organic chemical compounds [2–5].

For the production of second-generation biofuels, both biomass and any organic by-products or waste products should be used, because these substrates are readily available in large quantities and do not affect food shortage issues [6]. According to the literature, lignocellulosic biomass contains a large energy potential that has not yet been fully used for the production of biomethane. Consider that the worldwide annual production of biomass containing the lignocellulosic structure is approximately 200×10^9 ton per year, which is equal to 2.2×10^{21} Joules. This is 300 times more than the global energy demand [7]. Lignocellulosic biomass is not only a renewable energy source, but its conversion is also a way to reduce the excessive accumulation of waste products in agriculture (straw, leaves, haulms) [5].

Thanks to the use of non-food cellulosic materials in energy production, the biofuel sector will not be competitive to food production and will not contribute to higher food prices. Agricultural by-products are more available and much cheaper than, for example, cereal grain [8].

The latest European strategy for the use of biomass in transport clearly focuses on by-products and waste. The EU Renewable Energy Directive (2018/2001) "RED II" assumes

that the share of renewables in the total EU energy consumption by 2030 will be up to 32%, through increasing the role of second-generation biofuels. For transport, a specific sub-target is to have a 14% share of renewables. RED II incentivizes to a gradual phasing-out of conventional raw materials, up to complete phasing-out in 2030. Additionally, the Directive implements a "double counting" of the volumes for specific raw materials (including lignocellulosic biomass) towards the RED II target [9].

Biogas obtained in the anaerobic digestion process can be treated as a source of renewable energy, the main advantage of which is the wide variety of energy use—such as the production of electricity, heat, and conversion to liquid fuels [10,11]. In recent years, anaerobic degradation of biomass—the stage of preliminary processing of the raw material: chemical and enzymatic hydrolysis—has been introduced to technology. Polysaccharides such as cellulose and hemicellulose are not being directly used in the bioconversion process to soluble sugars. This makes the process of raw material pretreatment an essential stage in biogas production [12,13]. The objective of the different pretreatment methods is to change the morphological structure of lignocellulosic biomass by changing the amount and proportion of lignin to polysaccharides. Pretreatment causes degradation of the bonds connecting lignin to the other polymers, which leads to the complex's partial liquefaction and a removal of a portion of lignin (which microorganisms are not able to use). Usually after the pretreatment, the surface area and porosity of the material are increased, which facilitates the contact of the enzymes with cellulose [14]. With the lack of appropriate pretreatment, the decomposition of lignocellulosic biomass in the hydrolytic phase of methane fermentation is slow and incomplete [15].

The differences in a chemical composition among the raw materials have a large impact on the formation of inhibitors during pretreatment, which inhibit further biochemical processes. These are groups of volatile compounds that include the degradation products of pentose and hexose sugars, e.g., 5-hydroxymethylfurfural, as well as carboxylic acids and phenolic compounds formed mainly as a result of lignin degradation [14,16,17]. The coexistence of these various products may negatively affect methane bacteria, and results in lower biogas efficiency during methane fermentation [18,19].

The concentration of these compounds depends on the type of raw material subject to decomposition, as well as the pretreatment method used [20]. Detoxification or conditioning of lignocellulose hydrolysates is one of the most effective methods to remove compounds that inhibit microorganisms [21,22]. In order to remove them, processes involving the addition of various chemicals, physical methods such as heating and evaporation, as well as biological treatment were tested [21,23,24]. The effectiveness of the applied detoxification method depends both on the type of lignocellulosic biomass pretreatment and on the species of microorganisms. This process affects their activity and the ability to conduct methane fermentation [25]. One of the methods of detoxification is adsorption on activated carbon (AC). Activated carbon is characterized by a large surface area (500–3000 $m^2\ g^{-1}$), high microporosity, and adsorption capacity, which allow it to be used as an effective adsorbent for purification and/or separation of toxic compounds [26]. A significant advantage of activated carbon is that its use does not significantly change the amount of fermenting sugars obtained during the pretreatment [27]. Ma et al. [28] investigated the possibility of using modified activated carbon to limit the amount of carboxylic acids and phenolic compounds. Zhang et al. [29] evaluated the effect of the use of adsorption on activated carbon on the effective purification of the hydrolyzate from toxic compounds. An important criterion for the effectiveness of the detoxification process is to determine the effect of the use of activated carbon on the efficiency of methane fermentation process and the final amount of product obtained (methane). The research was aimed at determining the impact of lignocellulosic biomass detoxification on the efficiency of methane fermentation process. The amount and composition of biogas obtained was verified.

2. Materials and Methods

2.1. Substrate and Inoculum

The research material included corn straw and rye straw. Dried biomass was subjected to physical treatment. The method of mechanical comminution (grinding) of the raw material was used. In order to crush the raw material (20–25 mm particle size), special cutting shears were used. Then, the pre-crushed biomass was ground on a mechanical mill to obtain particles less than 1.0 mm in diameter. Yong et al. [30] found that optimum particle size was in the 0.30 mm to 1.0 mm range for better economics and energy management. The inoculum was a digestate from an anaerobic digestion plant, where stillages with plant biomass were used as raw material for methane fermentation at 36 °C. The inoculum had a pH value of 6.95. The main physicochemical characteristics of inoculum and both the corn and rye straw are reported in Table 1.

Table 1. Characteristics of the substrates and the inoculum.

Parameter	Corn Straw	Rye Straw	Inoculum
TS (%)	92.1 ± 1.8	91.9 ± 0.9	6.0 ± 0.1
VS (%)	93.8 ± 1.3	92.7 ± 1.8	72.5 ± 1.1
Ash (%)	6.2 ± 0.1	7.3 ± 0.1	27.5 ± 0.3
Crude fibre (%)	38.71 ± 1.51	40.41 ± 2.13	n.d.

Notes: The table shows mean values and standard deviations.

2.2. Chemical and Enzymatic Sequential Pretreatments

2.2.1. Alkaline Thermo-Chemical Pretreatment

The alkaline thermo-chemical pretreatment was carried out in an alkaline environment using a calcium hydroxide solution (prepared by dissolving 0.50 g/g $Ca(OH)_2$ in 130 mL of distilled water) at a temperature of 135 °C for 30 min. Then, the sample was cooled to 50 °C in a water bath. The experiments were conducted in duplicate.

2.2.2. Enzymatic Hydrolysis

The samples obtained from the thermo-chemical pretreatment was subjected to an enzymatic hydrolysis using cellulase and cellobiase. Cellulase (Celluclast 1.5 L, Novozymes Company, Bagsværd, Denmark) from the fungus *Trichoderma reesei* had an activity of 700 U/g of substrate. An enzyme from *Trichoderma reesei* degrades the cellulose into glucose, cellobiose, and higher glucose polymers. Cellobiase from the fungus *Aspergillus niger* used under the trade name Novozyme 188 (Novozymes Company, Bagsværd, Denmark) had an activity of 30 U/g of substrate. This enzyme supports the activity of the Celluclast 1.5 L preparation and is a biocatalyst for the breakdown of cellobiose into glucose. Celluclast 1.5 L and Novozyme 188 were added at 6% (*w*/*w*) g/g cellulose. The enzymatic hydrolysis process was conducted at pH of 4.8 in flasks incubated in a shaker with a shaking speed of 150 rpm. The mixture was incubated at 50 °C for 24 h. The experiments were conducted in duplicate.

2.2.3. Detoxification

In order to remove toxic compounds produced during the pretreatment of lignocellulosic biomass, a detoxification process was carried out. The optimum temperature, stirring rate, and process time were at 80 ± 2 °C, 150 rpm, and 2 h, respectively.

2.3. Anaerobic Digestion

The inoculum was added at a 10% (*v*/*v*) inoculation ratio to the laboratory bioreactor. The pH value of the mixture was adjusted to 7.5 ± 0.5 with the use of either NaOH or H_2SO_4. The pH value was determined during the highest activity of the methane-producing bacteria. Fermentation lasted for 12 days, until there was a significant decrease in biogas production. Lignocellulosic biomass was the only fermentation medium.

During methane fermentation, the samples were analyzed to determine the pH value, TS, VS, and VFAs component. The methane yields were related to the quantity of fermentation medium (L CH_4/L-waste) and volume of volatile solids (m^3 CH_4/kg-VS).

2.4. Reactor

The methane fermentation was performed at mesophilic conditions (37 °C) in a tank bioreactor with a working volume of 1.5 L with a stirrer. We studied the anaerobic digestion of lignocellulosic biomass in a one-stage digestion system, where all four sequential stages of AD including hydrolysis, acidogenesis, acetogenesis, and methanogenesis were carried out in one reactor vessel. During the process, the temperature of the fermentation medium was controlled by means of a thermostat connected to the water jacket. The volumes of the produced biogas and methane were measured by the water displacement method as shown in Figure 1. The gases produced by fermentation were directed to the pressure and balance tank.

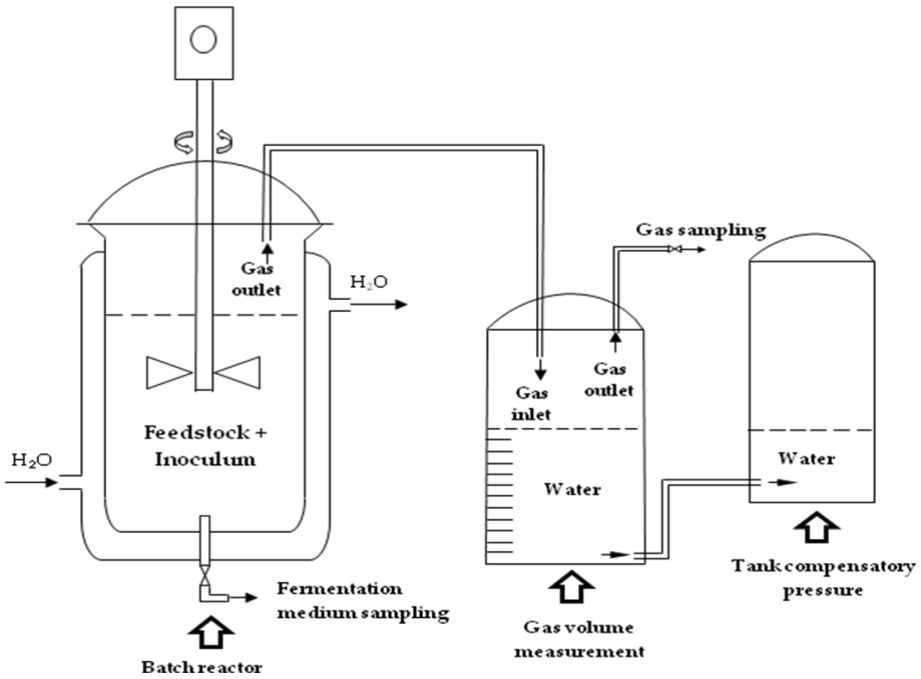


Figure 1. Schematic of the experimental set-up.

2.5. Analytical Methods

2.5.1. Total Solids (TS), Volatile Solids (VS), Chemical Oxygen Demand (COD)

TS and VS were determined in accordance with Polish Standard Methods PN-92/P-50092. To determine TS, the samples were dried at a temperature of 105 °C to constant weight. After this, to quantitate VS, the materials were mineralized in an oven at 550 °C for three hours. COD analysis was performed by the photometric method, using the cuvette test (Spectroquant, Merck, Kenilworth, NJ, USA). Samples were analyzed in triplicate.

2.5.2. Chemical Composition

The chemical composition of the samples was determined by the method van Soest using a fiber analyzer Fibertec^{TM} 8000 (FOSS Analytical A/S, Hillerød, Denmark) equipped with a hot and cold extraction unit. The following fibre fractions of neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined. The fibre fractions allowed to determining the content of cellulose as ADF–ADL, and hemicellulose as NDF–ADF.

2.5.3. Composition of the Biogas Produced

The analysis of methane and carbon dioxide content in biogas was performed using the chromatographic method. A gas chromatograph (Laboratorní Přístroje, Praha, Czech Republic) equipped with a TCD detector was used. The oven temperature was set to 70 °C with a carrier gas (mobile phase) flow of 30 mL/min, whereas the temperature of the katharometer was 150 °C for 60 mA electric current. Chromatographic analysis was performed once daily.

3. Results and Discussion

During the alkaline pretreatment and enzymatic hydrolysis process, differences were noted in the content of polysaccharides (cellulose and hemicellulose), lignin, and simple sugars, which had the potential impact on improving the biomass biodegradability and methane yield. The influence of lignocellulosic biomass detoxification on the biogas productivity was analyzed.

3.1. Chemical Composition

The volatile solid (VS) content of the dry corn and rye straw was 93.8% and 92.7%, respectively, whereas, an inoculum as a digestate was characterized by low VS, i.e., 72.5%. Preparation of raw materials for research consisted of their milling to small particles. The aim of the mechanical size reduction was to have the plant biomass increase the accessible surface area of raw material and also decrease cellulose crystallinity. This allows for improvement in the accessibility of the biomass for enzymes and increases the substrate's sensitivity to chemical agents and susceptibility to microbials. The selection of mechanical pretreatment methods depends first of all on moisture content of the biomass [31]. Akhand and Blancas [32] confirmed that increasing the available surface of wheat straw to microorganisms through the process of grinding lignocellulosic biomass, results in increased methane efficiency. The mechanical pretreatment of six different lignocellulosic biomasses in two different treatment phases was performed by Dahunsi [33], who reported that mechanical pretreatment caused a reduction in the duration of AD and an increased methane yield up to 22%. Importantly, physical pretreatment does not generate any toxic compounds, which inhibit the AD process [34].

Alkaline thermal pretreatment enabled the delignification and caused more porosity, surface area, and reduction in the degree of polymerization of lignocellulosic biomass. The hydrolytic phase is the stage before anaerobic fermentation, thus providing easily decomposable products at this stage that affect the intensification of the entire fermentation process. In their research, Chandra et al. [35] obtained an increase in biogas yield by 88% and methane yield by 112%, using an alkaline pretreatment (NaOH). Khor et al. [36] showed that the highest increase in methane yield of 37% was obtained with 7.5% $Ca(OH)_2$ pretreatment.

After thermo-chemical pretreatment, the lignocellulosic biomass was subjected to the enzymatic hydrolysis with the use of cellulase and cellobiase. Two enzyme preparations were used for polysaccharide decomposition: Celluclast 1.5 L (cellulase) and Novozymes 188 (cellobiase). The simultaneous use of the enzymes allowed for an increase in efficiency of the hydrolysis of polysaccharides to fermentable sugars. The content of cellulose, hemicellulose, and lignin was calculated based on the loss of acid and neutral detergent fibers after two-stage pretreatment.

Table 2 shows the content of hemicellulose, cellulose, and lignin in the corn straw (45.40%, 11.80%, and 2.31%, respectively) and rye straw (20.72%, 43.45%, and 7.41%, respectively). The rest was ash and soluble matter (32.8%—corn straw; 28.42%—rye straw) or extractives, which include pectins, proteins, and fats, among others. The content of polysaccharides and lignin in various lignocellulosic substrates can be influenced by the variety and maturity of plants, growing conditions, and harvesting methods [37]. The pretreatment process is aimed at dissolving lignin, which limits the free access of alkalis and cellulolytic enzymes to cellulose microfibrils. This statement is confirmed by the results

obtained by Michalska et al. [38]. They reported that cellulose was enzymatically degraded with higher efficiency due to the delignification process carried out earlier.

Table 2. Chemical composition and yield of polysaccharides degradation before and after thermo-chemical and enzymatic sequential pretreatments.

Chemical Composition (TS%)	Corn Straw		Rye Straw	
	Raw	Treated	Raw	Treated
Crude fibre	$38.71^{c} \pm 1.51$	$12.33^{a} \pm 0.30$	$40.41^{c} \pm 2.13$	$15.87^{b} \pm 0.18$
NDF	$59.52^{b} \pm 5.10$	$18.44^{a} \pm 0.46$	$71.58^{c} \pm 1.42$	$25.09^{a} \pm 0.29$
ADF	$14.11^{b} \pm 0.92$	$8.67^{a} \pm 0.96$	$50.86^{d} \pm 0.32$	$18.57^{c} \pm 0.25$
ADL	$2.31^{b} \pm 0.08$	$1.30^{a} \pm 0.02$	$7.41^{d} \pm 0.08$	$3.15^{c} \pm 0.02$
Cellulose	$11.80^{b} \pm 0.86$	$7.37^{a} \pm 0.96$	$43.45^{d} \pm 0.40$	$15.42^{c} \pm 0.17$
Hemicellulose	$45.40^{c} \pm 4.87$	$9.77^{a} \pm 1.37$	$20.72^{b} \pm 2.05$	$6.52^{a} \pm 0.08$
Total lignin	$2.31^{b} \pm 0.08$	$1.30^{a} \pm 0.02$	$7.41^{d} \pm 0.08$	$3.15^{c} \pm 0.02$
Other (ash + extractives)	$40.49^{b} \pm 5.18$	$81.56^{c} \pm 4.25$	$28.42^{a} \pm 2.14$	$78.06^{c} \pm 5.07$
Y_D *	-	70.03 ± 2.51	-	65.81 ± 3.19

Notes: The table shows mean values and standard deviations. Mean values designated by different letters and placed in the same row differ statistically significantly at $p < 0.05$; $n = 3$. * Y_D—yield of polysaccharides degradation.

By implementing initial processing along with enzymatic hydrolysis, components of lignocellulosic biomass—hemicellulose, cellulose, and lignin—were decomposed. Their amount has been reduced by 78%, 38%, 44%, respectively (corn straw), and by 69%, 64%, and 57%, respectively (rye straw).

In order to finally determine the efficacy of alkaline hydrolysis and its influence on the total degradation of biomass, the decomposition yield of plant materials was calculated for the entire two-stage process. The results are shown in Table 2. The yield of polysaccharides degradation calculated as a sum of individual steps of pretreatment was 70.03% for corn straw and 65.81% for rye straw. The effectiveness of the two-step process was also determined by total sugars obtained in the hydrolysate. Without the step of chemical pretreatment, enzymatic degradation and obtaining the monosaccharides in hydrolysates would not be possible. Thus, when the polysaccharides (cellulose and hemicellulose) are not degraded by cellulosic enzymes, methane production is limited.

Table 3 shows the values of basic indicators (TS, COD, Tsu, VFA) of the hydrolysates after thermo-chemical pretreatments and enzymatic hydrolysis. The total concentration of sugars in the supernatants showed that the alkaline pretreatment dissolved the lignin and released the cellulose. This step is necessary for the effective degradation of lignocellulosic biomass as it increases the accessible surface area of polysaccharides for cellulolytic enzymes, resulting in an increase in the amount of monosaccharides. The reduced sugars yield found in the alkali and enzymatic hydrolysates was 33.5 g/L (corn straw) and 29.6 g/L (rye straw). Similar results were obtained in the hydrolysate of wheat straw [39], in which the content of total sugars (carbohydrates) was 30.5 g/L. The sugar composition in the hydrolysates presented by Tovar et al. [13] indicates that to achieve a high biogas yield, it is not necessary to completely degrade the polysaccharides to C-6 sugars. The microbial used for AD are characterized by a wide variety in the metabolism of both C-6 sugars and other sugars determined as total sugars [40].

After alkaline pretreatment and enzymatic hydrolysis for corn and rye straw, TS were about 102.9 g/L and 107.2 g/L, respectively. This value is optimal for methane fermentation and ensures that the fermentation medium can be mixed during the process. If the substrate was too diluted, then the solid particles could fall and settle in the fermentation chamber, which would hinder the flow of gas formed in the bottom of the reactor. This would lead to a reduction in biogas production. According to Mazumdar [41], fermentation medium humidity should be around 90%, because if the humidity is too low, acetic acid may accumulate, which inhibits the biogas production process.

Table 3. The parameters determined in the hydrolysates thermo-chemical and enzymatic treatments.

Raw Material	TS (g/L)	pH	COD (g O_2/L)	Tsu (g/L)	VFA (mg/L)
Corn Straw treated	102.9 ± 9.8	5.1 ± 0.1	59.21 ± 7.1	33.5 ± 5.8	327.3 ± 26.8
Rye Straw treated	107.2 ± 13.1	5.2 ± 0.0	63.64 ± 11.2	29.6 ± 5.8	338.9 ± 19.7

Notes: The table shows mean values and standard deviations. TSu, Total sugars.

The literature reports that the average increase in methane production as a result of enzymatic hydrolysis of lignocellulosic biomass is 25%. Gerhardt et al. [42] conducted research on the effect of enzymatic hydrolysis of different lignocellulosic biomass on biogas production. They found that using enzymatic pretreatment improves biogas yield by 4–35%. Very often in research, enzymatic hydrolysis is used as a biological pretreatment of lignocellulosic biomass due to the short duration and low loss of sugars during the reaction. The efficiacy of degradation polysaccharides can be improved by using a complex of various cellulolytic and hemicellulolytic enzymes [43].

3.2. Characteristics of AD

3.2.1. Methane Production

This study examined the influence of the detoxification process in the pretreatment of lignocellulosic biomass on the effectiveness of biogas and methane production. The study was conducted in a way where lignocellulosic biomass was subjected to two-stage preliminary hydrolysis: chemical and enzymatic in one variant and the same processes but with detoxification in the second variant. Such research has been carried out both for corn straw and rye straw. All research (under detoxification conditions and without) was performed with the same amount of biomass. The amount of corn and rye straw researched was approximately 50 g.

Figure 2 shows the daily biogas yield from corn and rye straw, considering pretreatments performed with detoxification and without the detoxification process. Both corn and rye straw showed higher biogas production when the detoxification process was used.

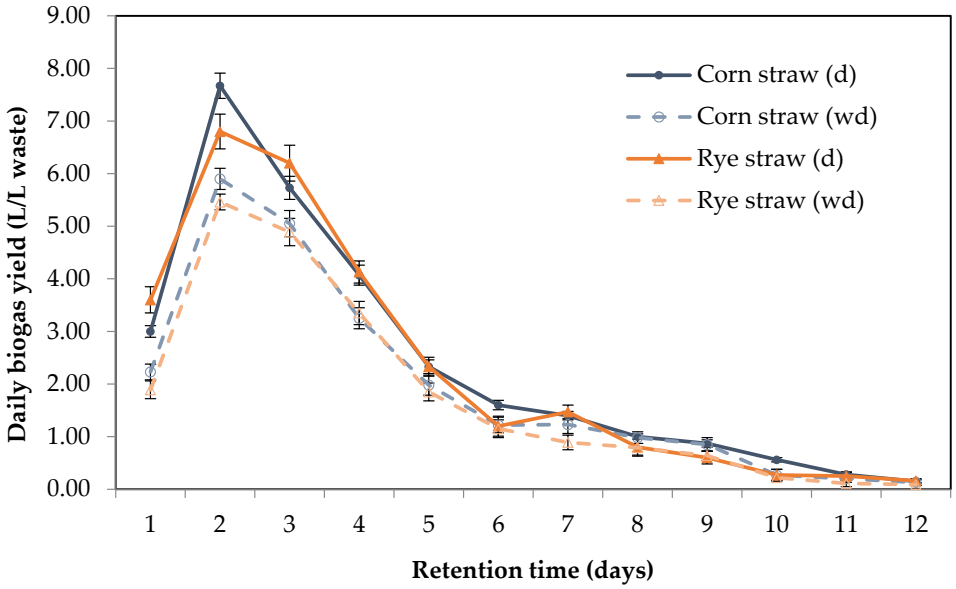


Figure 2. Daily biogas yield (d—with detoxification; wd—without detoxification).

It was found that the production of methane from lignocellulosic biomass subjected to two-stage preliminary hydrolysis—chemical and enzymatic—is very effective. Lignin

was partially degraded, making the polysaccharides (cellulose and hemicellulose) more accessible to cellulolytic enzymes and methane bacteria. Lignocellulose saccharification allowed for obtaining soluble fermentable sugars that were used in the methane production.

Due to the pretreatment of the raw material, the biomass subjected to AD fermentation was easily converted by methane bacteria. Based on the pH value and VFAs component results, the rapid course of the first two stages of AD—hydrolysis and acidogenesis—was found. The hydraulic retention time (HRT), which depends on the biodegradability of the substrate, was 12 days. After this time, the process slowed down and methane production decreased. As shown in Figure 2, the daily biogas production for both biomass from corn and rye straw reached 7.67 L/L and 6.80 L/L, respectively (using the detoxification process), during day 2 of digestion. Thereafter, the daily biogas yield decreased rapidly to 1.60 L/L and 1.25 L/L on day 6 and finally decreased to 0.2 L/L on day 12. For pretreated corn and rye straw without the detoxification process, daily biogas yields reached a value, respectively: 5.90 L/L and 5.46 L/L, on day 2, which decreased slowly to 12 days. The presence of readily biodegradable compounds in the medium resulted in the rapid beginning of methane production. At that time, the most turbulent period of methane fermentation took place, during which organic substances were transformed into volatile fatty acids, alcohols, aldehydes, and CO_2 and H_2 gas products. Carrying out the detoxification process resulted in obtaining more biogas during each day of the process. About 71% of biogas was obtained from biomass from corn straw (d) and 75% from rye straw (d) at the end of day 4 (from the total amount of biogas produced). Similar results were presented by Kacprzak et al. [44]. The highest biogas yield from energy crops was obtained in the first days, while from the third day, it fell and fluctuated.

The higher cumulative biogas yields (28.66 L/L for corn straw and 27.67 L/L for rye straw) for 12-day digestion were obtained with pretreated biomass with the detoxification process, which were 18.7% and 23.3% higher than that of the samples without detoxification. In this case, the cumulative biogas yield reached 23.29 L/L and 21.33 L/L for corn and rye straw, respectively. These results indicated that using the detoxification process on the pretreatments was effective in improving the biogas production. The residue of activated carbon in the fermentation medium after the detoxification process also had an impact on the activity of methanogenic bacteria and on the increase the cumulative biogas yield.

According to Yadvika et al. [45], the addition of such substances as bentonite, phosphorite, zeolite, as well as charcoal and others, can improve the efficiency, speed, and stability of methane fermentation, e.g., the addition of charcoal to a stable working fermenter (on a laboratory scale) resulted in an increase in production biogas by 17–35%. The mechanism of "catalysis" is that the charcoal added to the fermenter becomes the basis or a binder, leads to easier aggregation and immobilization of methane bacteria, and thus increases their population in the fermenter.

The methane content of biogas increased rapidly in the beginning of the fermentation process, exceeded 75% on the second day, and then stabilized to about 70%, Figure 3. The methane contents ranged between 70% and 80% for variants with detoxification process and 65–76% for variants without detoxification process. The methane content in biogas proves its energy value. Daily biogas flammability tests confirmed the obtained results. In the anaerobic digestion process, apart from methane, the presence of carbon dioxide and trace amounts of hydrogen sulphide and oxygen were also found.

Furan compounds and other degradation products of hemicellulose and polysaccharides have a negative effect on the methane production process, due to the low tolerance of microorganisms to inhibit compounds like furfural and HMF. With this fact, we performed methane fermentation of treated lignocellulosic biomass with and without the detoxification process. The results showed that the use of the detoxification process causes an increase in methane efficiency, whereas, both for corn and rye straw, the methane yield expressed per volume of fermentation medium was higher by 22%, when using the detoxification process, Table 4.

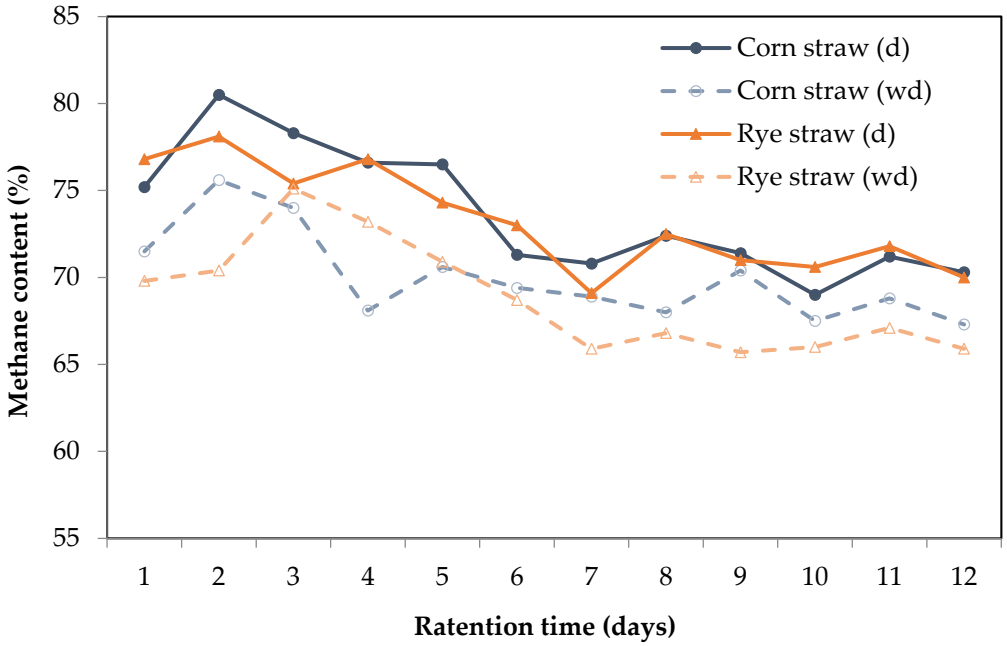


Figure 3. Methane content of biogas (d—with detoxification; wd—without detoxification).

Table 4. Parameters obtained from anaerobic digestion.

Variants	CH_4 Yield (L/L)	CH_4 Yield (m^3/kg VS)	DOSD (%)
Corn Straw (d)	18.86 ± 0.91	0.31 ± 0.03	62.59 ± 3.72
Corn Straw (wd)	14.80 ± 1.47	0.24 ± 0.02	59.73 ± 2.64
Rye Straw (d)	17.69 ± 1.07	0.29 ± 0.01	62.09 ± 2.88
Rye Straw (wd)	13.68 ± 0.31	0.25 ± 0.03	58.40 ± 2.17

Notes: The table shows mean values and standard deviations. DOSD: degree of organic substance. decomposition (d—with detoxification; wd—without detoxification).

In these variants, CH_4 yield was 18.86 L/L (corn straw) and 17.69 L/L (rye straw), while methane yield expressed per mass of VS added was 0.31 m^3/kg VS for corn straw (d) and 0.29 m^3/kg VS for rye straw (d). For variants without detoxification, the amount of methane after fermentation was 0.24 m^3/kg VS for corn straw (wd) and 0.25 m^3/kg VS for rye straw (wd). According to the literature, the yield of biogas from corn straw ranges from 0.20 m^3/kg to 0.60 m^3/kg VS [35].

Based on a parameter such as dry organic matter, the degree of organic substance decomposition (DOSD) was calculated. DOSD was calculated according to Equation (1):

$$\text{DOSD} = \frac{S_D - S_O}{S_D} \cdot 100\ (\%), \quad (1)$$

where:

S_D—the VS of the biomass, before process (kg/m^3), S_O—the VS of the biomass, after processing (kg/m^3).

For corn straw, the degree of organic substance decomposition (DOSD) was 62.59% (d) and 59.73% (wd), whereas for rye straw subjected to the detoxification DOSD, it was 62.09%, and without the detoxification process, it was 58.40%. The use of the detoxification process in the pretreatment of lignocellulosic biomass has therefore allowed an increase the DOSD during AD.

3.2.2. Variation of VFA and pH

Volatile fatty acids (VFAs) including acetic, propionic, and iso-butyric acid, produced during the acidogenic stage, are key intermediates in the biomethanation process that

is capable of inhibiting methanogensis at high concentration [46]. The degradation of propionate and butyrate by syntrophic acetogenic bacteria produces acetic acid that is subsequently degraded into methane and CO_2 by acetoclastic methanogens [47]. Figure 4 shows the content of volatile VFAs and pH in subsequent days of AD.

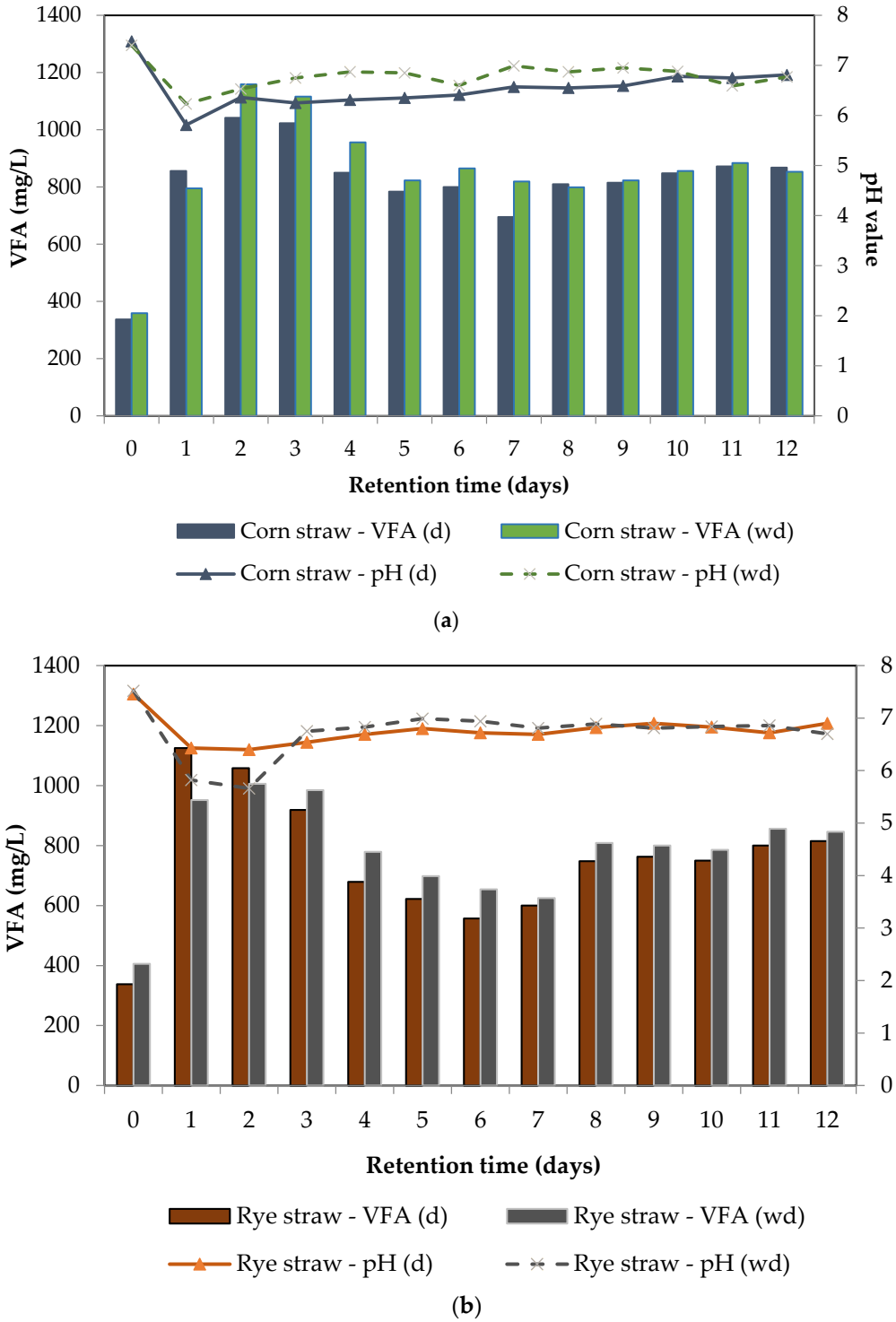


Figure 4. Variation of VFA and pH during AD, (**a**) corn straw, (**b**) rye straw (d—with detoxification; wd—without detoxification).

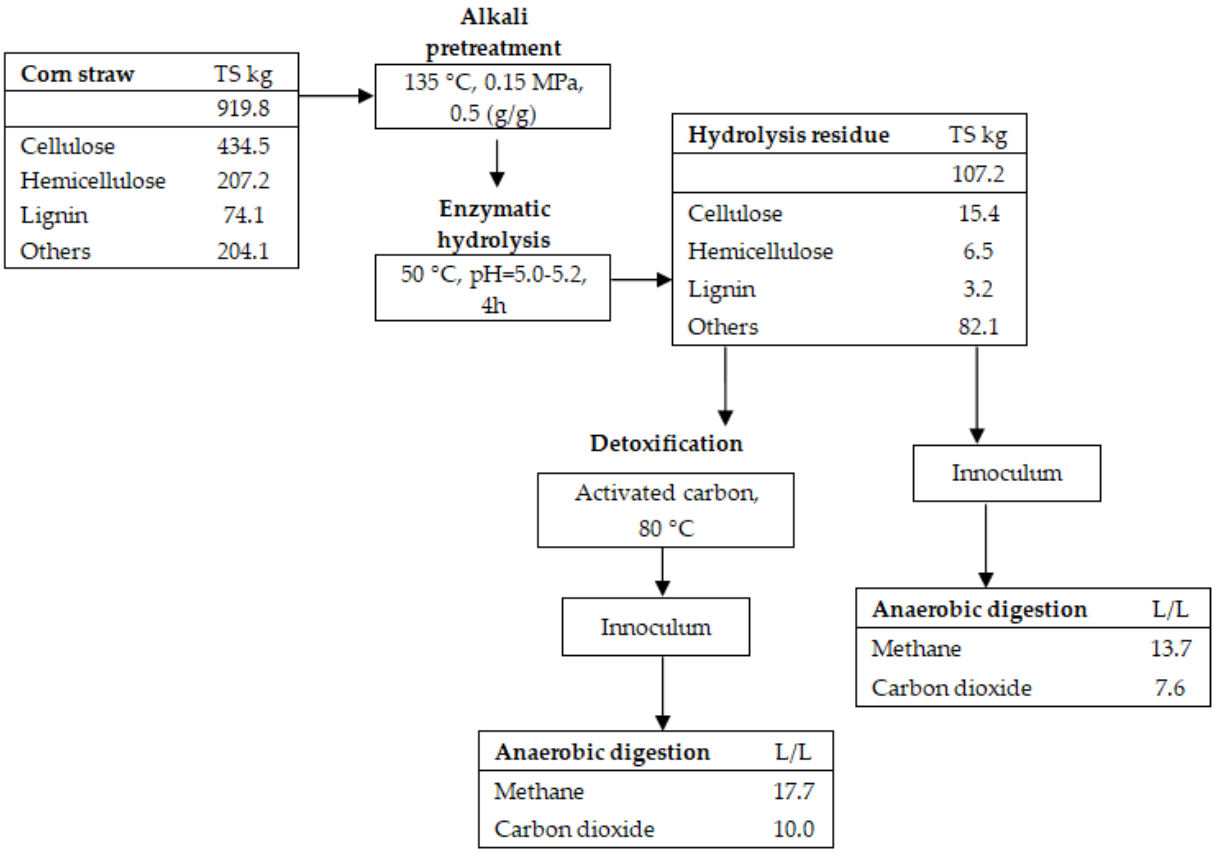
The high concentration of VFAs observed at the beginning of the process was characteristic for the initial phase of the process. In this phase, there was an intensive growth of acetic bacteria and the rapid course of fermentation, associated with the intensive consump-

tion of organic substrates and biogenic compounds. As the balance between individual groups of microorganisms was established, a systematic reduction of the VFAs values was observed. After methane fermentation, the average VFA concentration for both variants was 846 mg/L. There were no significant differences in the VFAs content. According to Chen et al. [48], the limited value of VFA is 2 g/L. Too high VFA concentration has an inhibitory effect on the activity of methanogens and strongly affects the pH value and alkalinity [49,50].

Besides the VFAs concentration, another important parameter of AD is the pH value. It was found that along with the advanced course of methane fermentation, the VFAs concentration increases, while the pH value of fermentation medium decreases. In the first three days of methane fermentation, when the most turbulent period of digestion took place, the lowest pH value and the highest concentration of VFAs were observed. It was the period of the highest biogas productivity. In the following days of anaerobic digestion, the pH ranged from 6.3 to 6.9. The increase in pH value with a simultaneous decrease in the concentration of VFA indicated the conversion of VFA to methane.

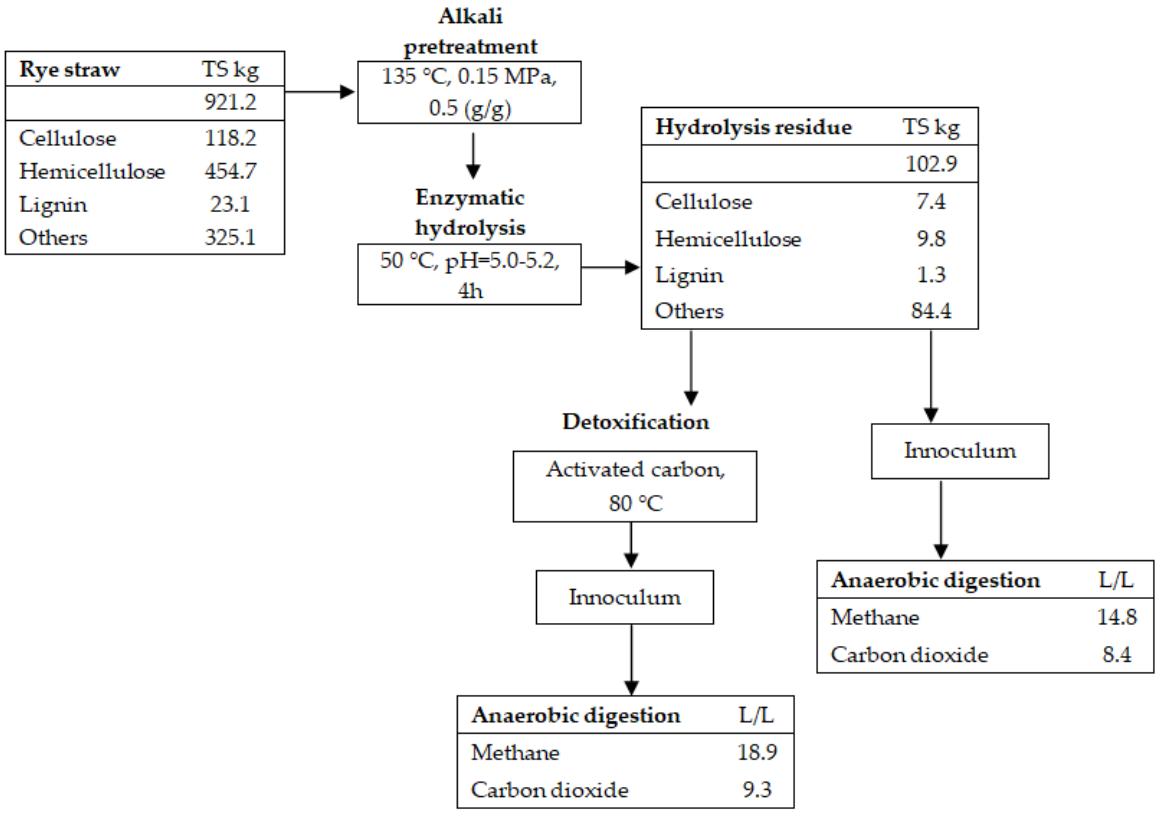
3.3. Mass Balance

The mass flows for the two substrates, corn straw (a) and rye straw (b), are presented in Figure 5 for both with and without the detoxification process. Mass balance was expressed in terms of total solids (TS) and the mass loss during chemical pretreatment of the biomass was considered in the calculation.



(**a**)

Figure 5. *Cont.*



Rye straw	TS kg
	921.2
Cellulose	118.2
Hemicellulose	454.7
Lignin	23.1
Others	325.1

Hydrolysis residue	TS kg
	102.9
Cellulose	7.4
Hemicellulose	9.8
Lignin	1.3
Others	84.4

Anaerobic digestion	L/L
Methane	14.8
Carbon dioxide	8.4

Anaerobic digestion	L/L
Methane	18.9
Carbon dioxide	9.3

Figure 5. Mass flow in the AD for pretreatment with and without the detoxification process: (**a**) corn straw; (**b**) rye straw.

For both corn and rye straw, the content of polysaccharides (cellulose, hemicellulose) of one tonne of raw material were 572.9 kg and 641.7 kg, respectively. After alkali pretreatment and enzymatic hydrolysis, TS was about 105 kg. The amount of the above-mentioned polysaccharides in hydrolysates were 17.2 kg for corn straw and 21.9 kg for rye straw.

4. Conclusions

Lignocellulosic substrates have a high potential for the biomethane production, but fibrous lignocellulosic composites limit their use in the anaerobic digestion process. Therefore, the pretreatment and the detoxification process are essential steps for the effective use of biomass in the biogas production. Our results showed that adsorption on activated carbon can significantly reduce the non-sugar compounds present in the hydrolysate, which directly influenced the biogas efficiency and the amount of methane.

Obtaining a high methane yield from organic biomass with lignocellulosic structure is a very important issue due to the increase of the efficiency of agricultural waste management. Utilization of waste materials in the anaerobic digestion process will be associated with an increase in the production of biofuels and the share of renewables in the transport sector. It will also be a response to EU directives aimed at limiting the use of raw materials that would compete with food or feed products. Additionally, the management of byproducts or waste products from agriculture in the anaerobic fermentation process brings with it further advantages, such as the reduction of pathogens and odors in the post-fermentation, which makes it a beneficial natural fertilizer after dehydration.

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supervision, K.K.; project administration, K.K. and W.D.; funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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