energies

MDPI

Article

Enzymatic Activity in the Anaerobic Co-Digestion of Cavitated Coffee Waste and Sewage Sludge

Elżbieta Wołejko [1,*], Urszula Wydro [1], Aleksandra Szaja [2], Agnieszka Montusiewicz [2] and Magdalena Lebiocka [2]

[1] Department of Chemistry, Biology and Biotechnology, Bialystok University of Technology, Wiejska 45A Street, 15-351 Bialystok, Poland; u.wydro@pb.edu.pl
[2] Faculty of Environmental Engineering, Lublin University of Technology, Nadbystrzycka 40B, 20-618 Lublin, Poland; a.szaja@pollub.pl (A.S.); a.montusiewicz@pollub.pl (A.M.); m.lebiocka@pollub.pl (M.L.)
* Correspondence: e.wolejko@pb.edu.pl

Abstract: Hydrodynamic cavitation (HDC) as a pre-treatment method is innovative and has potential for wide-scale industrial applications. The novelty of this research involves evaluating the enzymatic activity in the anaerobic co-digestion (AcD) of hydrodynamically cavitated coffee waste (CW) and municipal sewage sludge (SS) as well as its influence on the AcD performance. The effectiveness of AcD was assessed on the basis of changes in the physico-chemical composition of the feedstock and digestate as well as the biogas/methane yield, and attention was paid to the effect of coffee waste on enzyme activity, including that of β-Glucosidases (β-Glu), protease (PR), urease (URE), phosphomonoesterases acid (ACP) and alkaline (ALP). Moreover, the changes in the heavy metal content after the AcD of CW and SS were investigated. Comparing the enzymatic activity of the feedstock and digestate, we observed that the URE, ACP and ALP activities were 4.5 to 11 times higher for the feedstock than the enzyme activities in the digestate. Moreover, when using CW cavitated for 30 min, the highest enzymatic activity in both the feedstock and digestate occurred. The results indicated that the relationship between the β-Glu activity and biogas yield showed the strongest positive correlation (r = 0.98 at $p \leq 0.05$). At the same time, a positive correlation between the PAC, PAL, URE and PR activity and methane yield and methane content at $p \leq 0.05$ was observed. The obtained results allow us to conclude that, in the future, such a digestate could be used as a bio-fertilizer to improve degraded soil to activate microbial populations.

Keywords: spent coffee grounds; anaerobic co-digestion; ALP and ACP; PR; URE; hydrodynamic cavitation; caffeine; municipal sewage sludge; methane production; macro- and micro-elements

Academic Editors: Anna Grobelak and Aneta Kowalska

Received: 9 December 2024
Revised: 30 December 2024
Accepted: 2 January 2025
Published: 4 January 2025

Citation: Wołejko, E.; Wydro, U.; Szaja, A.; Montusiewicz, A.; Lebiocka, M. Enzymatic Activity in the Anaerobic Co-Digestion of Cavitated Coffee Waste and Sewage Sludge. *Energies* **2025**, *18*, 187. https://doi.org/10.3390/en18010187

1. Introduction

Coffee is one of the most common beverages consumed in the world. It is estimated that the preparation of this beverage produces approximately 60 million kg of coffee waste (CW) per day, which is incinerated or deposited in landfills [1,2]. Such management of these wastes may have a negative impact on the environment and contribute to global warming [3]. Stylianou et al. [3] showed that a matrix of CW consisting of various compounds such as carbohydrates—45.3%; proteins—13.6%; lipids from 7 to 20%; and polyphenolic compounds, which—when deposited in landfills—can be readily converted into toxic substances [4].

Therefore, coffee grounds are increasingly used commercially in the pharmaceutical, food, cosmetic, and/or spirits industries [4]. They also have an additional application which is the use of this type of bio-waste for bioremediation or absorbent production [2,5]. In addition, data in the literature report a growing interest in the use of CW in anaerobic digestion (AnD), which might be an alternative for involving this bio-waste with energy and nutrient recovery [6]. However, CW indicated a very complex matrix with potential AnD inhibitors, such as caffeine, tannins, terpenes, polyphenols, and terpenoids [7,8]. As noted by Atelge et al. [9] the use of coffee grounds in mono-digestion may result in low process efficiency. Therefore, pre-processing this waste may reduce the negative effects associated with the presence of inhibitors as well as destroy the complex lignocellulosic structure of CW, consequently resulting in increased methane production [6]. Zheng et al. [10] reports that among pre-treatment methods, hydrodynamic cavitation (HDC) has several advantages, such as low cost, easy scale-up and high efficiency. Furthermore, research conducted using HDC indicates that this method is effective in fragmenting down lignocellulosic fibers, thus ensuring an increase in available surface area, the occurrence of subsequent hydrolysis, and the formation of a low quantity of AnD inhibitors [11].

Despite several studies on HDC, there is still a need to conduct detailed research for each new waste type individually, mainly due to the possibility of generating the toxic by-products within HDC that might adversely influence the subsequent anaerobic digestion process [12].

AnD is known to be one of the complex biological processes that involves the degradation, by different biological reactions of various groups of microorganisms, of a complex organic matrix [13]. One of the effects of microbial activity is the production of enzymes involved in the transformation of organic compounds and release into the circulation of components, such as carbon, nitrogen and phosphorus, which are also food for the microflora in the bioreactor. The properly performed anaerobic decomposition of organic matter determines the high efficiency of biogas production [14]. Frequently, to support the AcD process, different groups of enzymes are added to the pre-treatment of waste that promote hydrolysis by decomposing hemicellulose, lignins, polysaccharides and protein [15]. Taha et al. [16] noted the interference of anaerobic fermentation processes of waste was dependent on the amount of hemicellulose and lignin in the cell walls, which form barriers and limit enzyme contact with cellulose and inhibit the hydrolysis of polysaccharide polymers into sugars. Therefore, the removal of lignocellulosic biomass by the addition of enzymes to AcD improves the decomposition of anaerobic digestion conditions and thus enhances the yield of methane [17]. However, as reported by Koppram et al. [18], enzymatic hydrolysis requires the use of expensive enzymes to decompose polysaccharide polymers into sugars and the results may not necessarily be satisfactory and cover the costs incurred.

The most important enzymes responsible for the transformation of organic matter are considered to be β-glucosidase (decomposes cellulose into glucose), phosphatases (decompose organic forms of phosphorus), urease (decomposes urea), or protease (decomposes protein). As reported in the literature, an appropriate microorganism population is important in the AnD process, and its activity depends on the parameters of the environment, in which microbes occur [14,17].

In the first stage of AnD, compounds with a high molecular weight, such as proteins, carbohydrates and lipids, are broken down and the microorganisms involved (strict and facultative anaerobes) release various groups of enzymes [13]. These support the decomposition of extracellular polymeric substances and cell walls release readily available organic material necessary for subsequent processes [19]. Moreover, during AnD, the bacterial flora is constantly reconstituted, decomposing the introduced biomass and transforming it into

a mixture of gases consisting mainly of carbon dioxide and methane, as well as hydrogen, ammonia, hydrogen sulfide, and other compounds [14]. Depending on the composition of the feedstock, different microorganisms may be involved in transformations and significant changes in their enzymatic activity may also occur. Thus, for anaerobic co-digestion (AcD), which processes different types of substrates, each type requires an analysis in this area. The novelty of this research involves evaluating the enzymatic activity in the anaerobic co-digestion of hydrodynamically cavitated CW and municipal sewage sludge (SS) as well as its influence on AcD performance. Moreover, the changes in the heavy metal content after AcD of CW and SS was investigated. Thus far, such a multi-aspect study has not been performed. It should also be noted that such an evaluation has not been conducted for these substrates. Additionally, there is limited knowledge indicating the metabolic pathways involving functional enzymes that play important roles in the biotransformation of macromolecules and methane production in AD.

Importantly, the obtained research results will allow for the presentation of changes occurring during the anaerobic decomposition of selected substrates. Moreover, the conducted analysis provides an indication of the mechanisms of changes occurring during the anaerobic decomposition of these waste types. Therefore, the obtained results will be of great significance to scientists.

2. Materials and Methods

2.1. Applied Materials

In this study, SS was chosen as a main component, while CW was used as a co-substrate. The first material was taken from a municipal wastewater treatment plant (WTTP) located in Lublin (Poland). It was a mixture of thickened primary and waste sludge (60:40 v/v). the inoculum for AcD experiment was obtained from the same WWTP. This sample was originated from the effluent of a mesophilic digester. It was characterized by TS of 22.48 $\pm$ 0.16, VS of 12.89 $\pm$ 0.22 g/kg and pH of 7.31 $\pm$ 0.04. CW was obtained from the coffee machines located on the campus of Lublin University of Technology. Both non-cavitated and cavitated CW was used to enrich the SS feedstock composition. The physico-chemical characteristics of all materials are presented in Table 1.

Table 1. Physico-chemical characteristics of materials used in the experiments.

Parameter	Units	SS	Non-Cavitated CW	CW Cavitated for 20 min	CW Cavitated for 30 min
COD	mg/L	47650 $\pm$ 235	9750 $\pm$ 190	6580 $\pm$ 164	6830 $\pm$ 125
TS	g/kg	45.3 $\pm$ 4.1	9.28 $\pm$ 2.7	8.21 $\pm$ 1.5	7.94 $\pm$ 0.8
VS	g/kg	33.35 $\pm$ 2.9	8.21 $\pm$ 0.5	7.43 $\pm$ 0.6	7.08 $\pm$ 0.5
phenols	mg/L	3.7 $\pm$ 0.06	16.30 $\pm$ 1.5	23.60 $\pm$ 2.7	26.10 $\pm$ 3.3
pH		5.97 $\pm$ 0.05	7.28 $\pm$ 0.1	7.82 $\pm$ 0.1	7.93 $\pm$ 0.1
caffeine	ppb	nd	nd	nd	505 $\pm$ 15

COD—chemical oxygen demand, TS—total solids, VS—volatile solids, CW—coffee waste, nd—non-detected, ppb—parts per billion.

2.2. HDC and AnD Experiments

HDC was selected as the pre-treatment technique for CW prior to its AcD with SS. These experiments were conducted using a laboratory device consisting of a cavitation reactor with replaceable cavitation inducers, circulation tank with an active volume of 30 L, pump and inverter. All mentioned elements were connected with pipelines and equipped with control valves. Moreover, this laboratory equipment had measurement devices, e.g., electromagnetic flow meter and three piezoelectric pressure gauges. In this laboratory device, the wastewater with suspended coffee circulates in a closed loop, repeatedly passing through the cavitation reactor. The operational parameters of HDC

experiments are presented in Table 2. The operational conditions to conduct HDC were chosen based on optimization studies, the results of which were presented in previous work [2]. The criteria taken into account were improved biodegradability and low energy consumption for pre-treatment. Therefore, two times were chosen differing in terms of caffeine presence; importantly, caffeine release was noted only at 30 min (Table 2).

Table 2. Operational parameters of HDC pre-treatment.

Parameter	Description
inlet pressure	5 bar
HDC inducer configuration	single hole plate with total area of hole 7.0686 mm^2
time	20 and 30 min
cavitation number	0.065

In the second stage of the experiment, batch anaerobic digestion was conducted. Therein, our series were provided with a differing feedstock composition; the detailed operational setup is presented in Figure 1. The cavitated CW was added at an amount of 1.5 g to 400 mL of SS (R2 and R3 for CW cavitated for 20 and 30 min, respectively). Furthermore, the mono-digestion of SS (R0) and the AcD series supplied with raw CW (R1) were performed as reference tests.

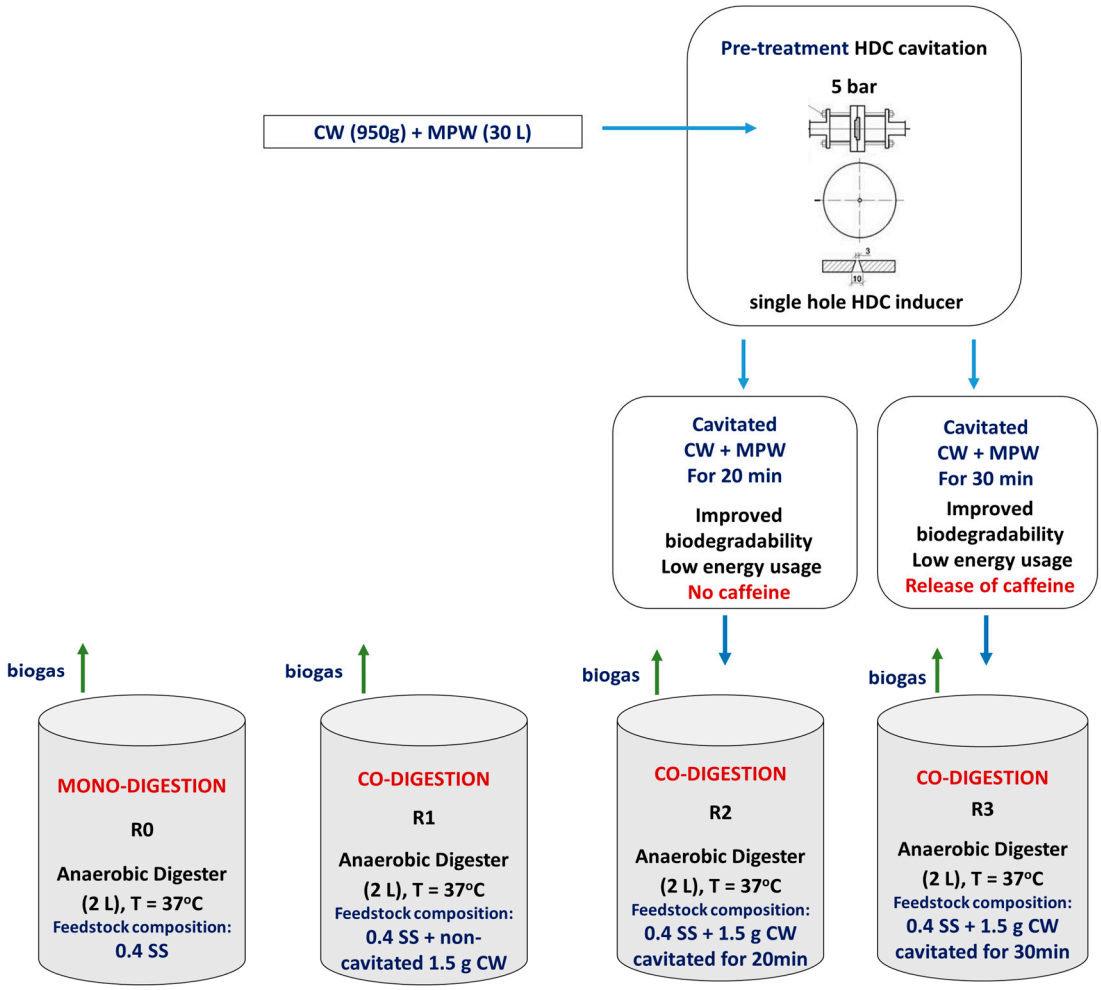

Figure 1. The main parameters of experiment (CW—coffee waste, MPW—mechanically pre-treated wastewater, HDC—hydrodynamic cavitation, SS—sewage sludge).

The specially created BioReactor Simulator (Bioprocess Control AB, Lund, Sweden) was used to carry out the batch tests. This equipment is equipped with six reactors, each with a 2.0 L working volume. Each reactor had a mechanical stirrer that ran for a cycle of five minutes for mixing and twenty-five minutes for resting. All reactors were kept in water baths to maintain the temperature under mesophilic conditions (37 °C). Using a wet gas flow-measuring apparatus that worked on the basis of buoyancy and liquid displacement, along with a system for continuous data collecting, the biogas output was automatically tracked and recorded. All reactors were flushed with inert gas (N2) to provide anaerobic conditions for the experiments.

2.3. Analyses of Physico-Chemical Parameters of the Applied Substrates

In the substrates, COD, soluble chemical oxygen demand (sCOD) and phenols were controlled using standard cuvette tests and the DR 3900 spectrophotometer (Hach Lange, Loveland, CO, USA). For TS and VS indication, the procedure provided by APHA was applied [20]. Meanwhile, the CP-411 pH-meter (Elmetron, Zabrze, Poland) was used to control the pH level in the analyzed materials.

The caffeine content was detected using an Agilent gas chromatograph mass spectrometer 8890/5977B (Waldbron, Deutschland). An HP-5 ms capillary column (30 m × 0.25 mm) was applied for the analyses. Helium with a flow rate of 1.0 mL/min was chosen as a carrier gas. The temperature program was as follows: 75 °C for 1 min, a linear ramp to 300 °C at 7.5 °C/min.

A ThermoTrace GC-Ultra gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) was used to control the composition of the biogas. For the analyses, the conductivity detector with divinylbenzene (DVB) packed columns (RTQ-Bond, Brisbane City, QLD, Australia) was used. The injector's temperature was 50 °C, while the detector's temperature was set at 100 °C. Helium at a flow rate of 1.5 cm^3/min was employed as the carrier gas. Chromatography was also used to determine the caffeine level. The Equity5MS Supelco (Sigma-aldrich, Missouri, USA) column was used, and the injector's temperature was kept at 270 °C.

2.4. Analyses of Macro- and Micro-Elements

Elemental analyses were conducted using an Agilent 8900 ICP-MS Triple Quad inductively coupled plasma mass spectrometer (Agilent, Santa Clara, CA, USA). The following heavy metals were examined in feedstock (F) and digestate (D) samples: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), potassium (P), iron (Fe), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), manganese (Mn) and mercury (Hg). The mentioned elements were analyzed in the He mode (gas flow rate of 5.5 mL/min). The nebulizer gas flow was 1.07 L/min, the auxiliary gas flow was 0.9 L/min, and the plasma gas flow was 15 L/min while operating in general purpose mode with 1.550 kW RF power. Depending on the anticipated elemental concentration, the acquisition time ranged from 0.1 to 2 s. The analyses were conducted using the internal standard ISTD (Sc, Y, Lu) at a concentration of 0.5 ppm. The recoveries achieved fell between 80 and 120 percent. All samples for analyses were mineralized; for this purpose, 1 g of the sample was digested with 5 mL of HNO_3 (69–70% Romil Super Purity Acid) and 2 mL of HCI (POCH 30% Ultranal). The microwave-assisted acid digestions were performed in Tefon reactors using a Topex microwave mineralizer (Preekem, Shanghai, China). The heating program included 4 steps, in which the initial temperature was maintained at 120 °C for 3 min and with maximum temperature up to 190 °C for 20 min. The obtained digests were transferred to and completed in a 25 mL volumetric flask with MilliQ water (Milllipore, Burlington, MA, USA).

2.5. Analyses of Enzyme Activity

Acid phosphomonoesterase (PAC) pH 5.0 and alkaline (PAl) pH 10.0 were determined in samples according to Schinner et al. [21]. Volumes of 1 mL of substrate solution (disodium p-nitrophenyl phosphate hexahydrate) and 4 mL of buffer solution were added to 1 g of samples. The samples were incubated for 1 h at 37 °C in a water bath. After incubating, 4 mL of 0.5 M NaOH and 1 mL of 0.5 M $CaCl_2$ were added, shaken vigorously and filtered. The released p-nitrophenol (pNP) was measured with a Vis Spectrophotometer (BioSens V5600, Wilmington, DE, USA) at 400 nm.

The protease activity (PR) measurement protocol was developed by Ladd and Butler [22]. In this procedure, 1 g of sample was weighed and 5 mL of substrate solution 2% casein as well as 5 mL of Tris buffer pH 8.1 were added. The samples were incubated for 2 h at 50 °C on a rotatory shaker. Next, 5 mL of TCA was added, shaken vigorously and filtered. For analyses, 5 mL of filtrate, 7.5 mL of mix alkali reagent (sodium carbonate + copper(II)sulfate pentahydrate + sodium potassium tartate) and 5 mL of Folin–Ciocalteu's were taken, allowed to stand for 90 min at room temperature and the extinction was measured at 700 nm.

β-Glucosidase (β-Glu) was determined according to Hayano [23]. Volumes of 1 mL of substrate solution pH 6.0 (4-Nitrophenyl β-D-glucopyranoside) and 4 mL of buffer solution pH 6.0 were added to 1 g of sample. The samples were incubated for 1 h at 37 °C. Next, 4 mL of 1 M NaOH and 1 mL of 0.5 M $CaCl_2$ were added. The extinction was measured with a Vis Spectrophotometer (BioSens V5600) at 400 nm.

Urease activity (URE) was determined in samples according to Kandeler and Gerber [24]. The process involved 5 g samples to which 2.5 mL of urea solution was added and incubated for 2 h at 37 °C. After the incubation, 1 mL filtrate was taken and reagent A (mix: 0.3 M NaOH + 1.06 M sodium salicylate + distilled water) as well as 39.1 mM dichlorisocyanurate were added. The extinction was measured at 690 nm.

2.6. Statistical Analyses

The results were analyzed statistically by using basic descriptive statistics. Results are presented as mean ± SD (standard deviation) or mean ± SE (standard error). The difference between the means for the tested parameters was verified using analyses of variance ANOVA, while the means were compared using Tukey's test at $p < 0.05$. The relationships between variables were evaluated using Pearson's correlation analyses at $p < 0.05$. Analyses were performed in R studio (ver. 2024.09.0 + 375).

3. Results

3.1. AnD and AcD Efficiency

The efficiency of AnD and AcD was evaluated in reference to organic compound removal as well as biogas and methane production. Figure 2 shows the results of TS, VS and COD removal, depending on the experiment variants. The application of pre-treated CW resulted in a decrease in the VS and TS removal, particularly in R3 (when using an HDC time of 30 min) by approx. 8% compared to R0. Importantly, in the presence of CW cavitated for 20 min (R2), comparable results to reference trials were achieved. However, a slight improvement in the COD removal was noticed in this case. A minor effect was noted for the mono-digestion of SS and for the AcD of non-cavitated CW with SS.

In other experiment variants, the VS content was at the same level at approx. 55.8%. In turn, the content of TS in R0 (with SS) and R1 (non-cavitated and with CW) was at a similar level of approx. 66.4% and decreased a little in the experiments with cavitated CW. This is not surprising, as cavitation leads to the decomposition of organic compounds, diminishing the TS and VS content, in particular with HCD duration (Table 1). The COD

content in the different experiment variants ranged from 61.94% (in R3) to 65.61% (in R2) (Figure 2).

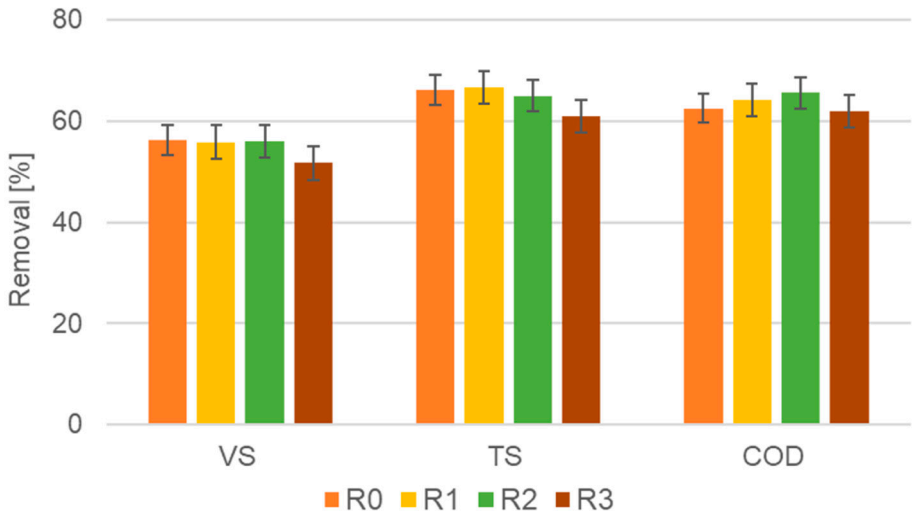

Figure 2. The organic compound removal in the investigated variants. The results are presented as mean ± SD. COD—chemical oxygen demand, TS—total solids, VS—volatile solids, R0—with SS, R1—non-cavitated and with CW, R2—CW cavitated for 20 min, R3—CW cavitated for 30 min.

Figure 3 presents the changes in the biogas/methane yield and methane content depending on the investigated variants. The application of pre-treated CW increased the methane yield by approx. 5% and 12% in both R2 and R3, respectively, compared to R1. It should be noticed that the highest biogas yield of 725.3 mL/gVS was found in R1 supplied by non-cavitated CW; however, in the presence of cavitated CW, a major enhancement of the methane content was observed. This fact corresponded to obtaining such beneficial results in terms of methane production (R2 and R3).

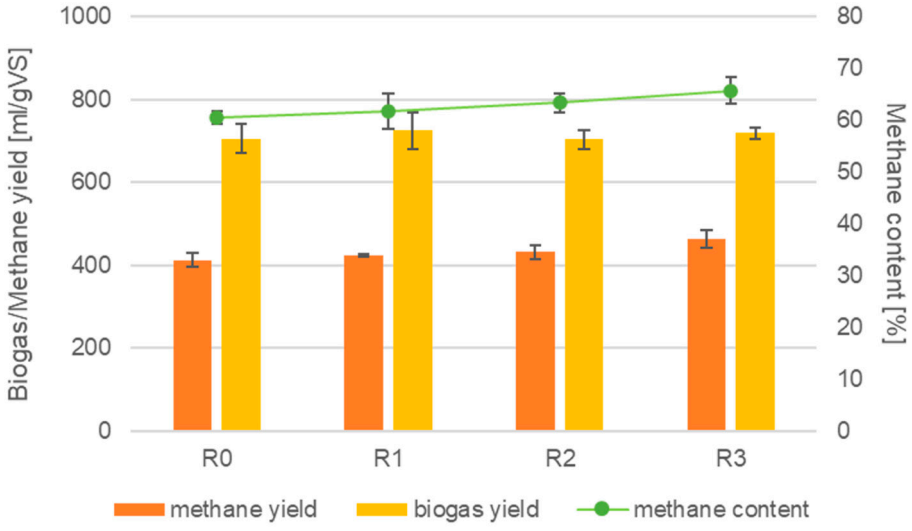

Figure 3. The changes in biogas/methane yield and methane content depending on the investigated variants. The results are presented as mean ± SD. R0—with SS, R1—non-cavitated and with CW, R2—CW cavitated for 20 min, R3—CW cavitated for 30 min.

3.2. Macro- and Micro-Element Content of Feedstock and Digestate

In the conducted study, higher macro- and micro-element concentrations were observed in the digestate, as compared to those in the feedstock. A significant increase in the

metal concentration in the digestate was due to the reduced TS during the fermentation process (Figures 4 and 5).

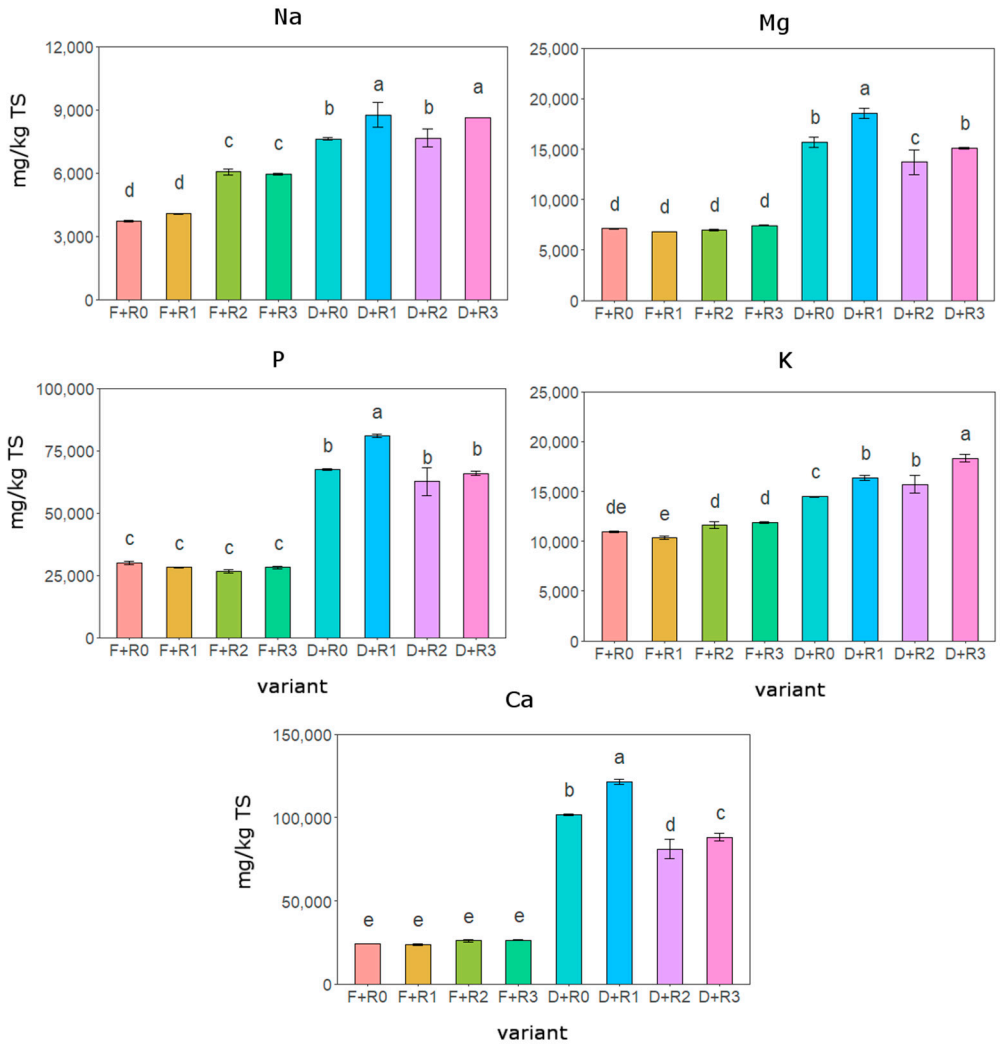

Figure 4. Effects of CW and HDC on changes in macro-element concentrations in feedstock and digestate. The results are presented as mean ± SD. Different letters above the error bars indicate statistically significant differences evaluated by the Tukey test at $p < 0.05$.

The macro-element concentrations of the feedstock and digestate are shown in Figure 4. The magnesium content, depending on the experimental variant, in the feedstock was from approx. 6832 mg/kg TS (F + R1) to 7442 mg/kg TS (F + R3) while in the digestate, it was from approx. 13,707 mg/kg TS (D + R2) to 18,567 mg/kg TS (D + R1). The use of the 20 min and 30 min HDC indicated a decrease in the Mg content of the digestate compared to D + R1.

The largest phosphorus content in the feedstock was observed for variant F + R0 while, for D + R1, this was in the digestate. An increase in the P concentration of about approx. 6% was observed for both feedstock and digestate after the 30 min HDC compared to the 20 min pre-treatment duration. The potassium content of the feedstock was highest after the use of HCD F + R3 (11 894 mg/kg TS), by approx. 8%, compared to F + R0. A similar dependence was observed in the digestate, where the highest K content was for D + R3, approx. 26%, as compared to D + R0.

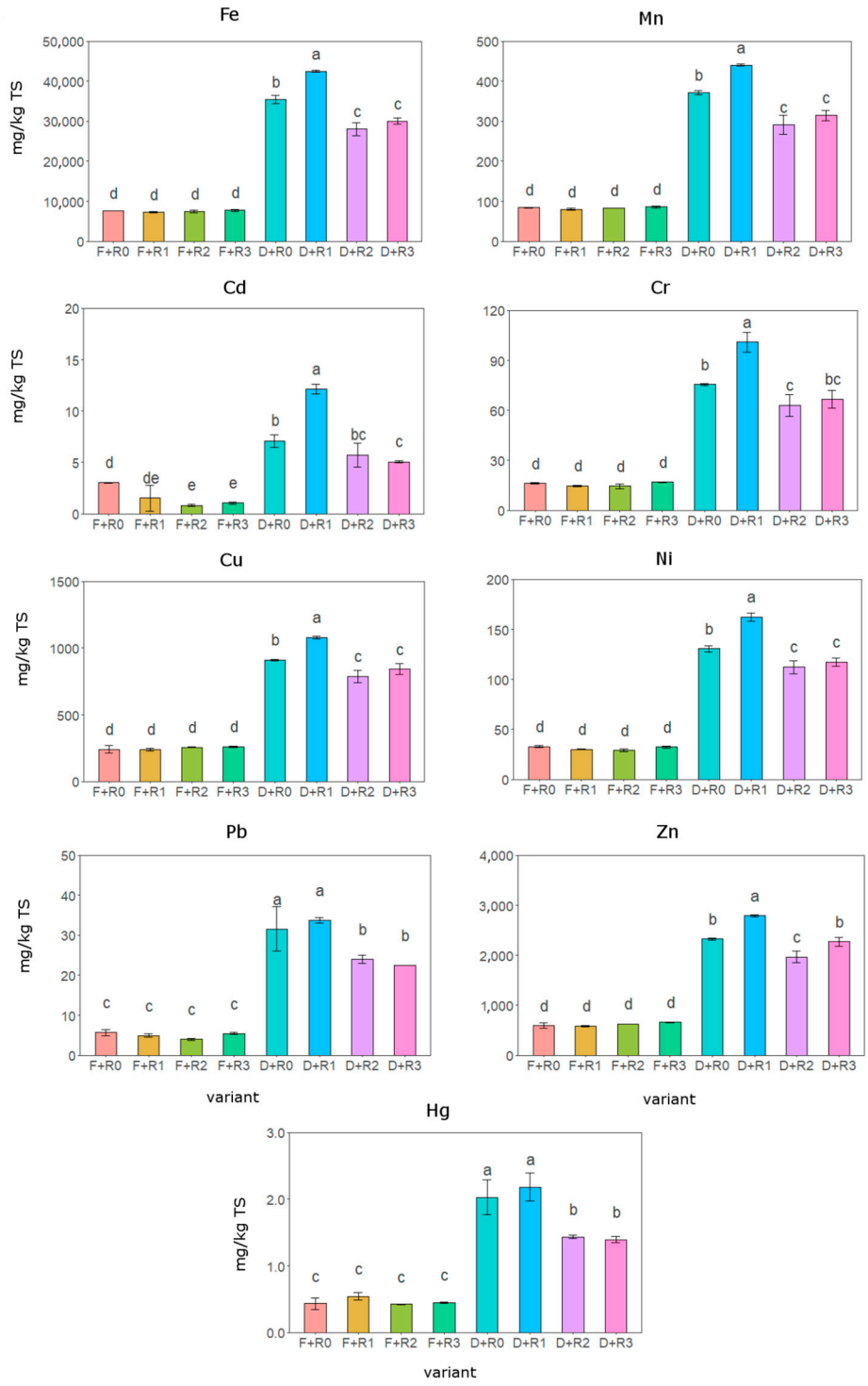

Figure 5. Effects of CW and HDC on changes in micro-element concentrations in feedstock and digestate. The results are presented as mean ± SD. Different letters above the error bars indicate statistically significant differences evaluated by the Tukey test at $p < 0.05$.

The content of calcium in the feedstock was at a similar level of approx. 25,000 mg/kg TS for all variants. In contrast, the significantly highest Ca content for the variant in the digestate was observed in D + R1. The concentration of Ca in the digestate after the use of HCD at 20 min and 30 min was at a similar level of approx. 84,707 mg/kg. In the samples with CW after HCD, a significant increase in sodium in the feedstock by approx. 60% was observed compared to F + R0. In this study, the content of sodium in the digestate ranged from 7620 mg in D + R0 to 8760 mg/kg TS in D + R1 (Figure 4).

As shown in Figure 5, the concentration of trace elements in the feedstock and digestate depended on the experimental variants used. The Fe and Cu concentrations in the feedstock, regardless of the experimental variant, were at similar levels of approx. 7549 mg/kg TS and 250 mg/kg TS, respectively, while in the digestate, the highest value of Fe and Cu in D + R1 were observed. The use of HCD influenced a decrease in the Fe and Cu concentration in the digest by approx. 21% and 13%, respectively (after 20 min of HCD), while after 30 min of HCD, it decreased by approx. 15% and 8%, respectively, compared to D + R0. The Zn content of the feedstock ranged from approx. 582 mg/kg TS (F + R1) to 660 mg/kg TS (F + R3).

In the study, one observed that the use of HCD influenced an increase in the Zn in the feedstock by approx. 6% (20 min HCD) and 11% (30 min HCD). In turn, a decrease in the Zn content was also observed in the digestate after HCD. A lower Zn concentration was observed for D + S2 (by about 14%) and for D-R3 (about 2.5%) compared to D + R0. The chromium in the feedstock was at a similar level of approx. 15 mg/kg TS, irrespective of the experimental variants used. However, in the digestate, the highest value was observed for D + R1, at approx. 100 mg/kg TS and after the applied HCD, the Cr concentration in the medium was at a level of approx. 64 mg/kg TS. The results obtained indicate that the content of Cd, Pb and Hg decreased in both the feedstock and digestate after the HCD treatment (Figure 5).

3.3. Enzyme Activity of Feedstock and Digestate

Enzyme activity, such as that of β-Glu, PAC, PAL, PR and URE, in the feedstocks and digestate was observed depending on the experimental variants used. In the study, higher enzymatic activity of PAC, PAL, PR and URE was observed in the feedstock than in digestate, while the activity of β-Glu was at a higher level in the digestate (Figure 6).

The obtained results indicate that the activity of β-Glu was highest in D + R1 (3.34 mg pNP/g TS/h) and lowest in F + R2—2.08 mg pNP/g TS/h. Figure 6 shows the enzyme activity for the feedstock and digestate for the individual experiments. The activity of β-Glu was highest in D + R3 and lowest in F + R2. The addition of CW to SS resulted in a decrease in the activity of this enzyme of approx. 18% compared to F + R0. In turn, the use of 20 min HCD and 30 min HCD reduced the β-Glu activity by approx. 28% and 18%, respectively, compared to F + R0. Furthermore, increases in the β-Glu activity in D + R1, D + R2 and D + R3 by approx. 18%, 8% and 24%, respectively, were observed for the investigated variants with added CW.

The PAC activities of the feedstock ranged from 9.50 mg pNP/g TS/h (F + R0) to 12.24 mg pNP/g TS/h (F + R1), while in the digestate, these ranged from 0.787 mg pNP/g TS/h (D + R1) to 1.14 mg pNP/g TS/h (D + R3). The highest increase in this enzyme was observed in F + R1, which was higher by 29% compared to that in F + R0. Moreover, the use of HCD for 20 min and 30 min caused an increase in the activity of this enzyme by approx. 16% and 15%, respectively, compared to that in F + R0. In the digestate of D + R2 and D + R3, increased PAC activity by approx. 15% and 22%, respectively, was observed compared to D + R0. Alkaline phosphatase in the feedstock ranged from 7.9 mg pNP/g TS/h (F + R1) to 11.19 mg pNP/g TS/h (F + R3). In the digestate, the PAL activity significantly decreased on average by approx. 80% compared to the feedstock (Figure 6). Among the digestates, the lowest PAL activity was observed in D + R1 (1.56 mg pNP/g TS/h) while the highest was in D + S3 (2.38 mg pNP/g TS/h).

The PR activity in the digestate and feedstock ranged around a similar level (Figure 6). The average PR activity in the feedstock was approx. 68.30 mg tyr/g TS/2 h, while for the digestate, it was approx. 53.26 mg tyr/g TS/2 h. The applied HCD increased the PR activity in the feedstock by approx. 11.5% and 15%, respectively, compared to that in F + S0.

Moreover, the used HCD caused an increase in the activity of this enzyme by approx. 3% (for F + R2) and 4.5% (for F + R3) compared to F + R1.

In the samples from the feedstock, the URE activity was in the range from 8.24 mg N/g TS/2 h (F + R1) to approx. 14.45 (for F + R2 and F + R3). Compared to F + R0, a significant decrease in the URE activity of approx. 38% was observed only in F + R1. The activity of URE in the digestate was in the range from 1.47 mg N/g TS/2 h (D + R0) to 3.32 mg N/g TS/2 h (D + R3). Over 75% of the URE activity of the feedstock was increased in F + R2 and F + R3 after using HCD, compared to F + R1 (Figure 6).

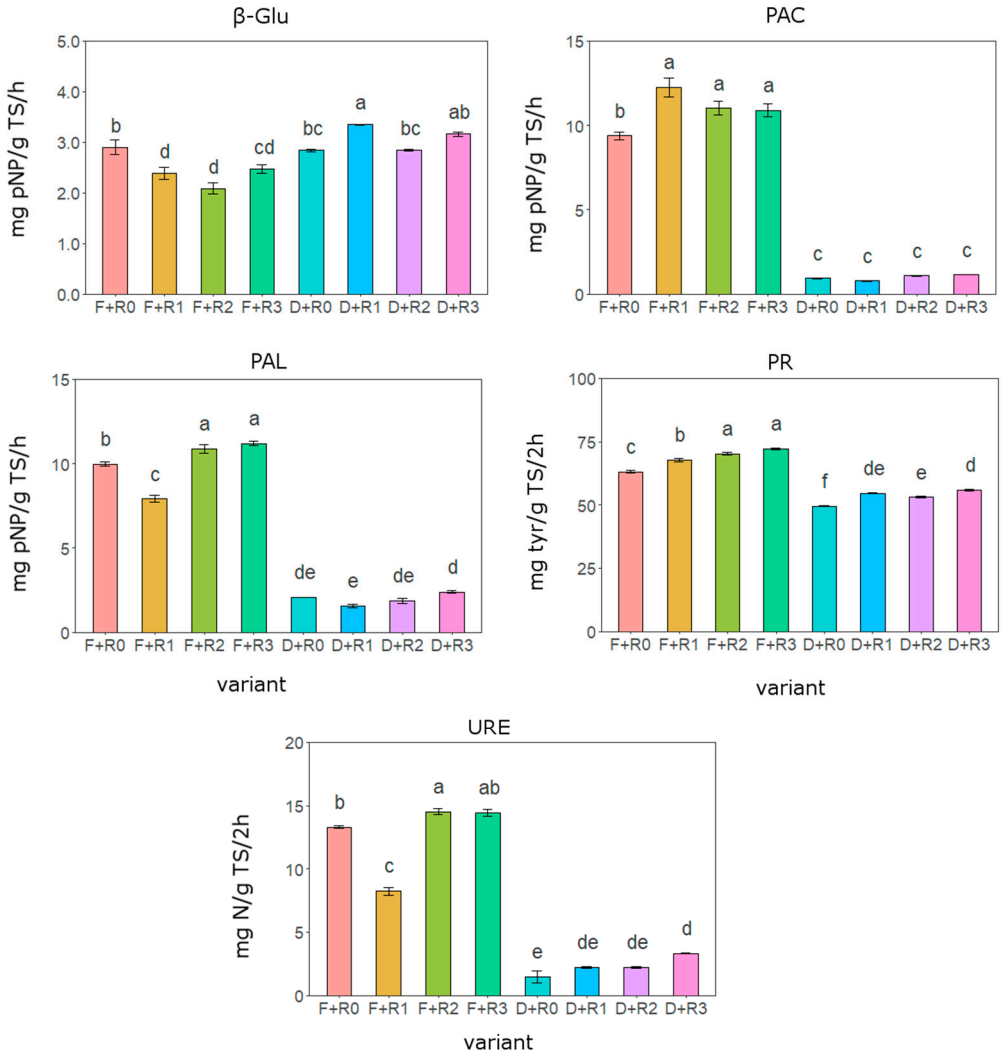

Figure 6. The influence of CW and HDC on the enzymatic activity in the feedstock and digestate. The results are presented as mean ± SE. Different letters above the error bars indicate statistically significant differences evaluated by Tukey's test at $p < 0.05$.

3.4. Coefficients of Correlations Between Parameters

Figure 7 shows the correlation analyses between the enzymatic activity, content of macro- and micro-elements and methane, biogas/methane yield and physico-chemical properties of the feedstock (A) and digestate (B) for each variant. The correlation analyses in the feedstock showed a positive relationship between the PR activity and the Na, K and Ca concentrations (r = 0.88, r = 0.61, r = 0.77, respectively), β-Glu activity and Ni, Cd and Pb concentrations (r = 0.57, r = 0.74, r = 0.75, respectively), and the PAL and URE activity and Na (r = 0.72, r = 0.63, respectively), Mg (r = 0.74, r = 0.69, respectively), K (r = 0.91, r = 0.88, respectively), Ca (r = 0.83, r = 0.77, respectively) and Mn concentrations (r = 0.75,

r = 0.77, respectively), according to $p \leq 0.05$. In comparison, a negative correlation was observed for the β-Glu activity and Na concentrations (r = −0.63), PAC activity and P, Fe, Mn and Ni concentrations (r = −0.52, r = −0.53, r = −0.59, r = −0.51, respectively), and the PR activity and the P and Cd concentrations (r = −0.72, r = 0.76, respectively).

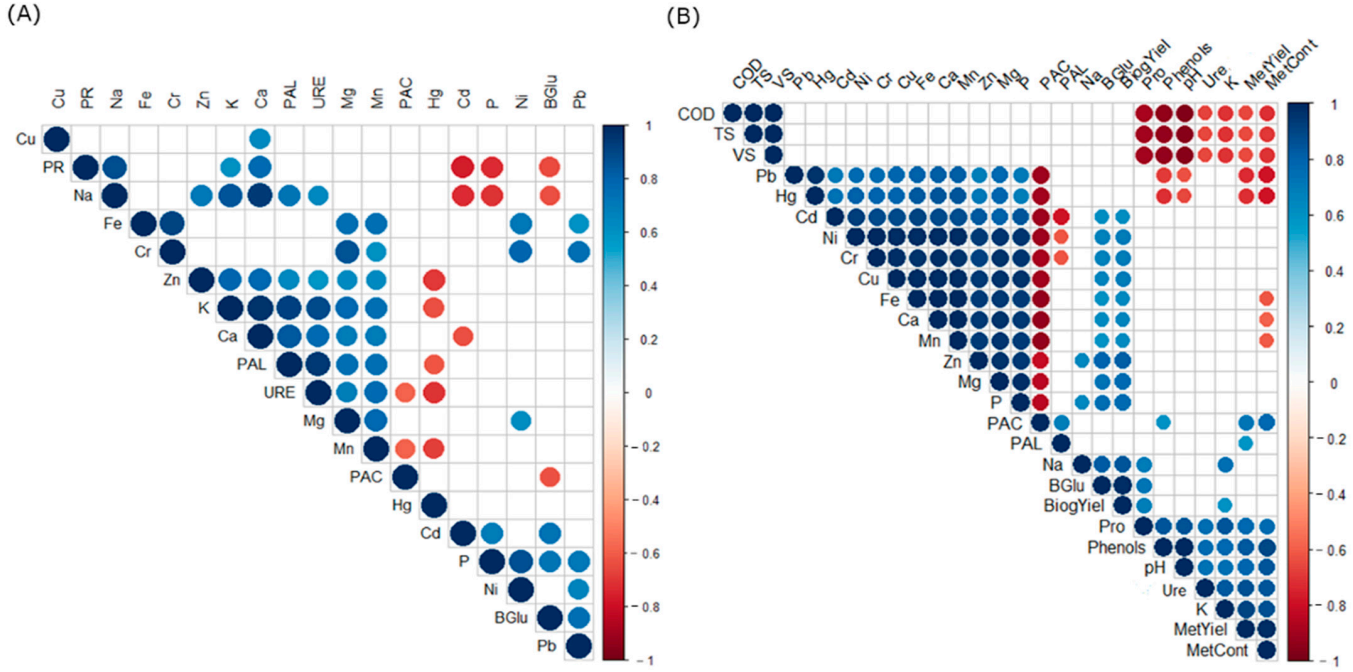

Figure 7. Correlation analyses between enzymatic activity, content of metals and methane, biogas/methane yield, and physico-chemical properties in the (**A**) feedstock and (**B**) digestate.

Moreover, digestate showed a positive correlation between the PR and URE activity and Na concentration (r = 0.69, r = 0.51, respectively) and K concentration (r = 0.85, r = 0.84, respectively), the β-Glu activity and the Na, P, K, Ca, Fe, Cu, Zn, Mn, Ni, Cd and Cr concentrations (r = 0.83, r = 0.74, r = 0.75, r = 0.58, r = 0.62, r = 0.60, r = 0.67, r = 0.79, r = 0.59, r = 0.67, r = 0.62, r = 0.67, respectively) at $p \leq 0.05$ (Figure 6). The analyses of the correlations in the digestate showed that the strongest negative correlation was between the PAC activity and the Mg, P, Ca, Fe, Cu, Zn, Mn, Ni, Cd, Cr, Pb and Hg concentrations (r = −0.84, r = −0.84, r = −0.92, r = −0.93, r = −0.90, r = −0.82, r = −0.92, r = −0.91, r = −0.90, r = −0.88, r = −0.90, r = −0.89, respectively) at $p \leq 0.05$. In addition, a negative correlation was observed between the PAL activity and Ca, Fe, Cu, Mn, Ni, Cd, Cr, Pb and Hg concentrations (r = −0.52, r = −0.55, r = −0.53, r = −0.52, r = −0.61, r = −0.78, r = −0.61, r = −0.54, r = −0.57, respectively), URE activity and Pb and Hg (r = −0.55, r = −0.54, respectively).

The results indicated that the relationship between the β-Glu activity and biogas yield showed the strongest positive correlation (r = 0.98 at $p \leq 0.05$). At the same time, a positive correlation was also observed between the PAC, PAL, URE and PR activity and methane yield (r = 0.72, r = 0.59, r = 0.86, r = 0.77, respectively) and methane content (r = 0.78, r = 0.55, r = 0.85, r = 0.76, respectively), according to $p \leq 0.05$ (Figure 7).

4. Discussion

In recent years, due to increased environmental awareness and stringent environmental regulations, the treatment and use of industrial and household waste has become an important area of research [25]. Gawande et al. [26] reported that waste or sewage sludge, depending on the region, may significantly differentiate by the industries which can form

specific mixtures. This necessitates the use of treatment techniques capable of degrading such complex matrices [25].

The AnD and AcD techniques are being increasingly used to convert various organic waste types to biogas that can be used as a renewable energy source to generate electricity or heat and digestate that can be applied as a fertilizer or improver for degraded soils [27,28].

SS originating from WWTPs is one of the most commonly applied substrates in AnD. However, due to its low organic content and unfavorable C/N ratio, its mono-digestion might be ineffective. Therefore, to overcome these difficulties, it should be co-digested with valuable additives. The obtained results indicated that the application of CW might lead to increased methane production. Nevertheless, the most favorable results were obtained using cavitated CW. Previous studies have indicated that the application of HDC to lignocellulose biomass, e.g., brewery spent grain, might allow for the effective destruction of complex compounds, contributing to enhanced methane production [29]. According to Gawande et al. [26], HDC generates intense pressure, heat and shock waves, causing the formation and sudden bubble collapse in the liquid. The rapid collapse of cavitated bubbles leads to the generation of highly reactive forms such as –OH (hydroxyl radicals) that degrade and oxidize organic contaminants. For instance, this type of degradation can effectively reduce the COD of sewage sludge, which can affect anaerobic digestion [26].

However, it should be noticed that the highest improvement was observed in trial R3, i.e., in the presence of caffeine, which might indicate a stimulatory effect of this compound. Importantly, in this case, the efficiency of removing organic compounds was reduced, as compared to other trails, due to the loss of organic matter occurring through HDC. In this research, relatively high biogas and methane yields were achieved compared to those reported in the study conducted by Neves [30], in which the methane yield varied between 240 and 280 $mLCH_4/gVS$. In turn, the AcD of defatted spent coffee grounds and spent tea waste mixed at a ratio of (50:50) resulted in a methane production rate of 318 ± 4 CH_4 mL/g VS [9].

According to Czatzkowska et al. [31], various heavy metals (HMs) (e.g., Fe, Cd, Pb, Cu, Cr, Zn, Ni, etc.) are introduced into digesters with sewage sludge, which can be one of the significant reasons for the interference and low efficiency of AnD. While introducing a co-substrate that has been chosen wisely regarding its macro- and micronutrient composition, it is possible to diminish the heavy metal content in the feedstock, thus minimizing the risk of their toxic effect.

Paulo et al. [32] reported that the toxicity of heavy metals is one of the main reasons for low productivity in the methane fermentation process. However, as the literature states, the toxicity of metals or the lack of this toxicity will depend on the pH of the solution, the redox potential and their concentration in the solution [31,33]. The use of HCD for CW in this study reduced the concentration of metals in the digestate, which indicates that HCD improves the conditions of the AcD process. In addition, due to the properties of the metals and the inability to pass into other forms, some metals may pass into a residual form, which is not susceptible to mineralization.

Moreover, as suggested by Chen et al. [34], the occurrence of heavy metals in larger quantities in the AnD process depends on the component that is fed to the bioreactors. Furthermore, foods added into bioreactors generally include various trace amounts of metals, so CW could also contain HMs in its composition. Guadalupe et al. [35] draw attention to the fact that the heavy metal content in coffee beans may depend on the location of cultivation, type of fertilization and atmospheric deposition. In this study, the application of CW to the feedstock did not significantly increase the HMs. Only after the AnD process, a significant increase was observed in the content of Fe, Cu, Zn, Mn, Ni, Cd, Cr, Pb and Hg in the digestate.

Choong et al. [36] and Tian et al. [37] demonstrated that an increase in HMs during AnD, particularly regarding the Ni concentration, influences the growth of methanogenic bacteria, contributes to the synthesis of cofactor F430 for urease, and impacts the activity of cellulase during various steps of the AnD process. The correlation analyses showed a positive and negative correlation between the Ni concentration in the digestate and β-Glu and phosphomonoesterases activity, respectively. As shown by Romero-Güiza et al. [38], glucose metabolism correlates with the Cr concentration, while the concentration of Zn plays an important role as a metallic enzyme activator and cofactor of various enzymes and stimulates microbial growth in AnD. According to Chen et al. [39], the increase in trace metals in the feedstock can have a positive effect on the growth of methanogens as well as contribute to the growth of various enzymatic reactions and participate in the formation of methane. Enzymes are involved in the decomposition of organic matter in their catalytic centers, which include transition metals, which participate as cofactors in electron transport [14]. In addition, Romero-Güiza et al. [38] noted that when a bioreactor's trace element content is insufficient, this may cause improper AnD operation as well as increases in ammonia and volatile fatty acids.

Besides heavy metals, light metals, such as Ca, Mg, K and Na, are also present in bioreactors after feedstock addition and can have a significant impact on microbial growth [34]. In the present study, using HDC for CW in the feedstock influenced the increase in the concentration of Na and K as well as decreased the Ca content in the medium. In turn, the analysis of the digestate indicates a significant decrease in the concentration of Mg, P and Ca after using HDC. Probably, the cavitation removes absorbed compounds from the feedstock matrix, which can be utilized more easily by the microorganisms involved in the AcD process. As reported by Chen et al. [34], during the decomposition of organic matter, light metals are released into the solution, which can significantly affect the activity of microorganisms. However, when there are excessive amounts of these metals in solution, they can have an inhibitory effect on microbial growth by destabilizing cell membranes, with a consequent reduction in biogas production, which was not observed in this research. Enzymes are essential for the anaerobic digestion process; they play an important role in key biochemical responses, catalyzing reactions necessary for the proper functioning of microorganisms, the decomposition of organic pollutants and the formation of organic matter [40]. Many scientists have reported that there is still insufficient knowledge regarding the activities of enzymes and microorganisms participating in waste decomposition during the AnD process [38,41]. This is due to the fact that each AnD process is different because of the components introduced and it is impossible to replicate the same process 100% of the time. For example, a slight fluctuation in macro- or micro-elements can change the activity of the microbial population and thus the enzyme activity, influencing the AnD process and biogas production [13,38].

Various groups of microorganisms excrete or produce hydrolytic enzymes such as proteases, amylases or cellulases that participate in the AnD process [15]. As suggested by Parawira [42], AnD can be disrupted if the substrate contains solid particles. Therefore, to avoid this problem, HCD was used. This process affected the decomposition of particles and influenced the increase in the enzymatic activity of the feedstock, particularly regarding PR, URE and PAL. As shown by Bonilla et al. [15], glycosidases and proteases are important enzymes for pre-treatment in the AnD process. Thus, their activity can influence the proper functioning of the process. As reported by Liew et al. [13], proteins constitute the major part of the organic matter and total organic content, so during biodegradation, this can lead to a decrease in the total organic content and the release of easily available sugar required by methanogens in the AnD process. Teo and Wong [43] showed that protease in the pre-treatment stage can decrease the concentration of volatile suspended solids from

20% to 58% and total suspended solids by approx. 20%. In this study, a high positive correlation was obtained between the methane yield and PR activity as well as the biogas yield and β-Glu activity. β-glucosidase is responsible for the breakdown of cellulose, which is an important parameter for AnD. Therefore, it can be supposed that the activity of these enzymes may be an important indicator in determining the efficiency of biogas production.

5. Conclusions

The novelty of this research involves its evaluation of the enzymatic activity in the anaerobic co-digestion of cavitated CW and SS as well as its influence on AcD performance. The obtained results indicated that using the HDC as a pre-treatment method for CW might cause an increase in methane production. Using CW cavitated for 30 min as a co-substrate ensured the highest enzymatic activity of both the feedstock and digestate, which corresponded to improved methane production. Furthermore, in this case, lower metal concentrations in the digestate were achieved. It seems that such a digestate could, in the future, be used as a bio-fertilizer to improve degraded soil to activate microbial populations. Therefore, there are plans for future in situ studies to explain how such a digestate behaves in the environment. However, it should be remembered that one important limitation of the use of digestate as a potential bio-fertilizer is the relatively high concentration of heavy metals. Their introduction into the environment may constitute a potential risk for living organisms, including human health. Importantly, due to the limited number of scientific reports on this subject, the obtained results will be of great significance to researchers and technologists.

Author Contributions: Conceptualization, A.M. and E.W.; methodology, E.W., U.W., A.S. and A.M.; formal analysis, E.W., U.W., A.S. and A.M.; investigation, E.W., U.W. and A.S.; writing—original draft preparation, E.W., A.M., U.W., A.S. and M.L.; writing—review and editing, E.W., A.M., U.W., A.S. and M.L.; visualization, E.W., U.W. and A.S.; supervision, A.M.; funding acquisition E.W. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results has received funding from the commissioned task entitled "VIA CARPATIA Universities of Technology Network named after the President of the Republic of Poland Lech Kaczyński", contract No. MEiN/2022/DPI/2577 action entitled "In the neighborhood inter-university research internships and study visits".

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Fernandes, A.S.; Mello, F.V.C.; Thode Filho, S.; Carpes, R.M.; Honório, J.G.; Marques, M.R.C.; Felzenszwalb, I.; Ferraz, E.R.A. Impacts of Discarded Coffee Waste on Human and Environmental Health. *Ecotoxicol. Environ. Saf.* **2017**, *141*, 30–36. [CrossRef]
2. Szaja, A.; Montusiewicz, A.; Pasieczna-Patkowska, S.; Grządka, E.; Montusiewicz, J.; Lebiocka, M. Pre-Treatment of Spent Coffee Grounds Using Hydrodynamic Cavitation. *Energies* **2024**, *17*, 2229. [CrossRef]
3. Stylianou, M.; Agapiou, A.; Omirou, M.; Vyrides, I.; Ioannides, I.M.; Maratheftis, G.; Fasoula, D. Converting Environmental Risks to Benefits by Using Spent Coffee Grounds (SCG) as a Valuable Resource. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35776–35790. [CrossRef] [PubMed]
4. Zhao, N.; Liu, Z.; Yu, T.; Yan, F. Spent Coffee Grounds: Present and Future of Environmentally Friendly Applications on Industries-A Review. *Trends Food Sci. Technol.* **2024**, *143*, 104312. [CrossRef]
5. Qisse, N.; Fattah, G.; Elouardi, M.; Mabrouki, J.; El Azzouzi, L.; Ennouari, A.; El Belghiti, M.A.; El Azzouzi, M. Competitive Adsorption of Zn in Wastewater Effluents by NaOH-Activated Raw Coffee Grounds Derivative and Coffee Grounds. *Desalination Water Treat.* **2022**, *258*, 123–132. [CrossRef]
6. Kampioti, A.; Komilis, D. Anaerobic Co-Digestion of Coffee Waste with Other Organic Substrates: A Mixture Experimental Design. *Chemosphere* **2022**, *297*, 134124. [CrossRef]

7. Jomnonkhaow, U.; Plangklang, P.; Reungsang, A.; Peng, C.-Y.; Chu, C.-Y. Valorization of Spent Coffee Grounds through Integrated Bioprocess of Fermentable Sugars, Volatile Fatty Acids, Yeast-Based Single-Cell Protein and Biofuels Production. *Bioresour. Technol.* **2024**, *393*, 130107. [CrossRef]
8. Rabii, A.; Aldin, S.; Dahman, Y.; Elbeshbishy, E. A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration. *Energies* **2019**, *12*, 1106. [CrossRef]
9. Atelge, M.R.; Atabani, A.E.; Abut, S.; Kaya, M.; Eskicioglu, C.; Semaan, G.; Lee, C.; Yildiz, Y.Ş.; Unalan, S.; Mohanasundaram, R.; et al. Anaerobic Co-Digestion of Oil-Extracted Spent Coffee Grounds with Various Wastes: Experimental and Kinetic Modeling Studies. *Bioresour. Technol.* **2021**, *322*, 124470. [CrossRef]
10. Zheng, H.; Zheng, Y.; Zhu, J. Recent Developments in Hydrodynamic Cavitation Reactors: Cavitation Mechanism, Reactor Design, and Applications. *Engineering* **2022**, *19*, 180–198. [CrossRef]
11. Mitraka, G.-C.; Kontogiannopoulos, K.N.; Batsioula, M.; Banias, G.F.; Zouboulis, A.I.; Kougias, P.G. A Comprehensive Review on Pretreatment Methods for Enhanced Biogas Production from Sewage Sludge. *Energies* **2022**, *15*, 6536. [CrossRef]
12. Ge, M.; Sun, C.; Zhang, G.; Coutier-Delgosha, O.; Fan, D. Combined Suppression Effects on Hydrodynamic Cavitation Performance in Venturi-Type Reactor for Process Intensification. *Ultrason. Sonochem.* **2022**, *86*, 106035. [CrossRef]
13. Liew, Y.X.; Chan, Y.J.; Manickam, S.; Chong, M.F.; Chong, S.; Tiong, T.J.; Lim, J.W.; Pan, G.-T. Enzymatic Pretreatment to Enhance Anaerobic Bioconversion of High Strength Wastewater to Biogas: A Review. *Sci. Total Environ.* **2020**, *713*, 136373. [CrossRef] [PubMed]
14. Lim, J.W.; Park, T.; Tong, Y.W.; Yu, Z. The Microbiome Driving Anaerobic Digestion and Microbial Analysis. *Adv. Bioenergy* **2020**, *5*, 1. [CrossRef]
15. Bonilla, S.; Choolaei, Z.; Meyer, T.; Edwards, E.A.; Yakunin, A.F.; Allen, D.G. Evaluating the Effect of Enzymatic Pretreatment on the Anaerobic Digestibility of Pulp and Paper Biosludge. *Biotechnol. Rep.* **2018**, *17*, 77–85. [CrossRef]
16. Taha, M.; Foda, M.; Shahsavari, E.; Aburto-Medina, A.; Adetutu, E.; Ball, A. Commercial Feasibility of Lignocellulose Biodegradation: Possibilities and Challenges. *Curr. Opin. Biotechnol.* **2016**, *38*, 190–197. [CrossRef]
17. Uddin, M.M.; Wright, M.M. Anaerobic Digestion Fundamentals, Challenges, and Technological Advances. *Phys. Sci. Rev.* **2023**, *8*, 2819–2837. [CrossRef]
18. Koppram, R.; Tomás-Pejó, E.; Xiros, C.; Olsson, L. Lignocellulosic Ethanol Production at High-Gravity: Challenges and Perspectives. *Trends Biotechnol.* **2014**, *32*, 46–53. [CrossRef]
19. Lin, Y.; Wang, D.; Wu, S.; Wang, C. Alkali Pretreatment Enhances Biogas Production in the Anaerobic Digestion of Pulp and Paper Sludge. *J. Hazard. Mater.* **2009**, *170*, 366–373. [CrossRef]
20. 2720 Anaerobic Sludge Digester Gas Analysis. In *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2017; ISBN 978-0-87553-299-8.
21. Schinner, F.; Öhlinger, R.; Kandeler, E.; Margesin, R. (Eds.) Enzymes Involved in Carbon Metabolism. In *Methods in Soil Biology*; Springer: Berlin/Heidelberg, Germany, 1996; pp. 185–207. ISBN 978-3-642-60966-4.
22. Ladd, J.N.; Butler, J.H.A. Short-Term Assays of Soil Proteolytic Enzyme Activities Using Proteins and Dipeptide Derivatives as Substrates. *Soil Biol. Biochem.* **1972**, *4*, 19–30. [CrossRef]
23. Hayano, K. A Method for the Determination of β-Glucosidase Activity in Soil. *Soil Sci. Plant Nutr.* **1973**, *19*, 103–108. [CrossRef]
24. Kandeler, E.; Gerber, H. Short-Term Assay of Soil Urease Activity Using Colorimetric Determination of Ammonium. *Biol. Fertil. Soils* **1988**, *6*, 68–72. [CrossRef]
25. Bargole, S.; George, S.; Kumar Saharan, V. Improved Rate of Transesterification Reaction in Biodiesel Synthesis Using Hydrodynamic Cavitating Devices of High Throat Perimeter to Flow Area Ratios. *Chem. Eng. Process.-Process Intensif.* **2019**, *139*, 1–13. [CrossRef]
26. Gawande, G.; Mali, P.; Dhavane, P.; Nahata, Y. Hydrodynamic Cavitation—A Promising Technology for Water Treatment. *Mater. Today Proc.* 2024; in press. [CrossRef]
27. Kumar Khanal, S.; Lü, F.; Wong, J.W.C.; Wu, D.; Oechsner, H. Anaerobic Digestion beyond Biogas. *Bioresour. Technol.* **2021**, *337*, 125378. [CrossRef]
28. Montoneri, E.; Koutinas, M.; Padoan, E.; Negro, V.; Licignano, C.; Leone, S.; Photiou, P.; Kallis, M.; Vyrides, I.; Liendo, F.; et al. Integrated Chemical and Biochemical Technology to Produce Biogas with a Reduced Ammonia Content from Municipal Biowaste. Validating Lab-Scale Research in a Real Operational Environment. *Environ. Sci. Adv.* **2022**, *1*, 746–768. [CrossRef]
29. Szaja, A.; Montusiewicz, A.; Lebiocka, M.; Bis, M. A Combined Anaerobic Digestion System for Energetic Brewery Spent Grain Application in Co-Digestion with a Sewage Sludge. *Waste Manag.* **2021**, *135*, 448–456. [CrossRef]
30. Neves, L.; Oliveira, R.; Alves, M.M. Anaerobic Co-Digestion of Coffee Waste and Sewage Sludge. *Waste Manag.* **2006**, *26*, 176–181. [CrossRef]
31. Czatzkowska, M.; Harnisz, M.; Korzeniewska, E.; Koniuszewska, I. Inhibitors of the Methane Fermentation Process with Particular Emphasis on the Microbiological Aspect: A Review. *Energy Sci. Eng.* **2020**, *8*, 1880–1897. [CrossRef]

32. Paulo, L.M.; Stams, A.J.M.; Sousa, D.Z. Methanogens, Sulphate and Heavy Metals: A Complex System. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 537–553. [CrossRef]
33. Wang, X.; Huang, S.; Wang, S.; Chen, S.; Dong, S.; Zhu, Y. Effect of D-Limonene on Volatile Fatty Acids Production from Anaerobic Fermentation of Waste Activated Sludge under pH Regulation: Performance and Mechanisms. *J. Environ. Manag.* **2024**, *370*, 122828. [CrossRef]
34. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of Anaerobic Digestion Process: A Review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [CrossRef] [PubMed]
35. Guadalupe, G.A.; Chavez, S.G.; Arellanos, E.; Doménech, E. Probabilistic Risk Characterization of Heavy Metals in Peruvian Coffee: Implications of Variety, Region and Processing. *Foods* **2023**, *12*, 3254. [CrossRef] [PubMed]
36. Choong, Y.Y.; Norli, I.; Abdullah, A.Z.; Yhaya, M.F. Impacts of Trace Element Supplementation on the Performance of Anaerobic Digestion Process: A Critical Review. *Bioresour. Technol.* **2016**, *209*, 369–379. [CrossRef] [PubMed]
37. Tian, Y.; Zhang, H.; Chai, Y.; Wang, L.; Mi, X.; Zhang, L.; Ware, M.A. Biogas Properties and Enzymatic Analysis during Anaerobic Fermentation of Phragmites Australis Straw and Cow Dung: Influence of Nickel Chloride Supplement. *Biodegradation* **2017**, *28*, 15–25. [CrossRef] [PubMed]
38. Romero-Güiza, M.S.; Vila, J.; Mata-Alvarez, J.; Chimenos, J.M.; Astals, S. The Role of Additives on Anaerobic Digestion: A Review. *Renew. Sustain. Energy Rev.* **2016**, *58*, 1486–1499. [CrossRef]
39. Chen, J.; Yun, S.; Shi, J.; Wang, Z.; Abbas, Y.; Wang, K.; Han, F.; Jia, B.; Xu, H.; Xing, T.; et al. Role of Biomass-Derived Carbon-Based Composite Accelerants in Enhanced Anaerobic Digestion: Focusing on Biogas Yield, Fertilizer Utilization, and Density Functional Theory Calculations. *Bioresour. Technol.* **2020**, *307*, 123204. [CrossRef]
40. Yin, M.; Chen, H. Unveiling the Dual Faces of Chitosan in Anaerobic Digestion of Waste Activated Sludge. *Bioresour. Technol.* **2022**, *344*, 126182. [CrossRef]
41. Li, W.; Zhu, L.; Wu, B.; Liu, Y.; Li, J.; Xu, L.; Huangfu, X.; Shi, D.; Gu, L.; Chen, C. Improving Mesophilic Anaerobic Digestion of Food Waste by Side-Stream Thermophilic Reactor: Activation of Methanogenic, Key Enzymes and Metabolism. *Water Res.* **2023**, *241*, 120167. [CrossRef]
42. Parawira, W. Enzyme Research and Applications in Biotechnological Intensification of Biogas Production. *Crit. Rev. Biotechnol.* **2012**, *32*, 172–186. [CrossRef]
43. Teo, C.W.; Wong, P.C.Y. Enzyme Augmentation of an Anaerobic Membrane Bioreactor Treating Sewage Containing Organic Particulates. *Water Res.* **2014**, *48*, 335–344. [CrossRef]