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Research paper

Energy recovery from poultry manure in the process of semi-continuous anaerobic co-digestion with sewage sludge

Anna Jasińska [a,*], Anna Grosser [a], Erik Meers [b], Ana Robles Aguilar [c]

[a] Faculty of Infrastructure and Environment, Department of Environmental Engineering and Biotechnology, Czestochowa University of Technology, Częstochowa, Poland
[b] Faculty of Bioscience Engineering, Department of Green Chemistry and Technology, Ghent University, Ghent, Belgium
[c] Technological Centre in Biodiversity, Ecology and Environmental and Food Technology, Central University of Catalonia, Catalonia, Spain

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ABSTRACT

In the face of significant legislative changes in renewable energy sources and conservation of natural resources, bio-waste must be managed efficiently. Developing wastewater treatment technologies generate a large amount of sewage sludge requiring appropriate use. A well-known and practiced process is the anaerobic digestion of sewage sludge, but due to its characteristics, the production of biogas is not sufficient enough to ensure the energy self-sufficiency of a wastewater treatment plant. Another waste whose management is a challenge is poultry manure, whose production in Poland is high due to intensive poultry farming. This study investigated the possibility of co-digestion of poultry manure and sewage sludge and its effect on increasing biogas production compared to processing sludge alone. The process was performed in two continuously stirred-tank glass reactors at mesophilic conditions, with 20 days of hydraulic retention time. In the first stage, the batch assay with sewage sludge was applied to promote the development of an anaerobic community. The second stage involved feeding the reactors on a semi-continuous regime with sewage sludge for the control sample and a mixture with the gradual addition of poultry manure from 2.5 % to 60 %. During the experiment, the most important parameters affecting the process and the quantity and quality of the obtained products in the form of biogas and digestate were monitored. The study's results indicated an advantage of the co-digestion process of poultry manure and sewage sludge over the digestion of sludge alone in terms of process efficiency. The highest biogas production was obtained with a co-substrate ratio of 42 % manure to 58 % sewage sludge (ratio based on volatile solids). Co-digestion had no significant effect on gas quality; in both cases, the methane content was more than 60 %. Moreover, a digestate with fertilizer potential was obtained for each sample.

1. Introduction

Due to the high nutritional value of meat, its consumption is increasing worldwide (Ghosh and Saha, 2020). In European countries, in particular, the poultry industry is growing rapidly. Fig. 1 shows the production of poultry meat in the EU based on data collected from 2011 to 2022 (Eurostat, 2023). Poland occupies a leading position in the depicted list, reaching a production of more than 2.7 million tons in 2022, nearly 190.000 tons more compared to 2021 and nearly twice as much as in 2011. It is worth mentioning that the total production of poultry, including broiler chickens, egg-laying hens and turkeys, is estimated there at an average of 4 million tons per year (Dróżdż et al., 2020).

The successive increase in the number of poultry farms results in the generation of more animal manure, one of the main by-products of farming. In 2017, the amount of poultry manure (PM) produced in Poland was estimated at 2121750 Mg per year (Dróżdż et al., 2020). According to the legal definition, manure, as a by-product of animal husbandry, is any excrement and/or urine of farmed animals other than farmed fish, even with bedding. This waste must undergo appropriate treatment based on technologies that are available, economical and as environmentally safe as possible. Improper manure management poses

Abbreviations: A, alkalinity; AcD, anaerobic co-digestion; AD, anaerobic digestion; CPI, co-digestion performance index; CSTR, completely stirred tank reactors; D-AcD, digestate from anaerobic co-digestion; D-Con., digestate from anaerobic digestion of sewage sludge; FA, free ammonia; HRT, hydraulic retention time; N-NH_4^+, ammonium nitrogen; OLR, organic loading rate; PM, poultry manure; SD, standard deviation; SS, sewage sludge; TC, total carbon; TKN, total Kjeldahl nitrogen; TS, total solids; VFAs, volatile fatty acids; VS, volatile solids; WWTP, wastewater treatment plant; Y_B, biogas yield; Y_M, methane yield.

* Corresponding author.
E-mail addresses: anna.jasinska@pcz.pl (A. Jasińska), anna.grosser@pcz.pl (A. Grosser), erik.meers@ugent.be (E. Meers), ana.robles@uvic.cat (A.R. Aguilar).

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the risk of undesirable consequences, such as the production of odours, attraction of rodents, insects and other pests, release of animal pathogens, contamination of soils and groundwater, surface water runoff and emission of greenhouse gases (Dróżdż et al., 2020; Böjti et al., 2017).

Poultry manure is characterized by a large amount of organic matter and contains in its composition many nutrients and trace elements, such as potassium, copper, zinc, cobalt, iron, selenium, molybdenum, boron and manganese. Unlike other types of animal manure, it has a higher content of nitrogen, phosphorus, calcium, magnesium and sulfur (Dróżdż et al., 2020; Kacprzak et al., 2023a). Due to its significant share of valuable nutrients, poultry manure can be used as a soil additive to improve soil properties and fertility. However, due to the excessive amount of this waste, Poland lacks sufficient agricultural land for such poultry manure management. This method is also fraught with the risk of environmental problems related to the physicochemical and hygienic-sanitary properties of the manure. Hence the need for a suitable strategy for its appropriate further processing, transportation or storage (Dróżdż et al., 2020; Bayrakdar et al., 2017; Abouelenien et al., 2014).

The promising strategy for managing poultry manure is anaerobic digestion (AD) while obtaining a valuable energy source in the form of biogas. This waste is an interesting substrate for the process due to its high organic matter content, 63–80 % of total solids (TS), and its buffering capacity (Dróżdż et al., 2020). Anaerobic digestion is a long known and used process, economically viable and socially acceptable due to its environmental friendliness (ACWilkie, 2005). Due to the biological character of this method, its efficiency depends primarily on the activity of microorganisms, which show varying tolerance to process conditions and the presence of toxic substances such as ammonia, hydrogen sulphide and heavy metals (Magrel, 2002a; Murto et al., 2004). In the case of poultry manure digestion, a particular problem is the high nitrogen content in the ammonium form, which transforms into an inappropriate carbon to nitrogen ratio in the substrate. Intense accumulation of ammonia released by the decomposition of uric acid and undigested proteins usually results in process inhibition. Hence, inadequate preparation of the feedstock in the form of manure is the reason for the failure of anaerobic decomposition (Kacprzak et al., 2023b).

There are many methods to reduce the negative effects of ammonia on the digestion process. The simplest of these is to dilute the feedstock with water, but then biogas production decreases, a large amount of secondary waste is generated, and technological water consumption increases (Jiang et al., 2019; Carlini et al., 2015; Ellersdorfer et al., 2020). Among the more economical techniques are the stripping process using air or steam, based on the principle of mass transfer, or the use of adsorbents such as biochar or zeolite (Limoli et al., 2016; Malińska, 2015). In recent years, researchers have focused on the intensification of anaerobic digestion of manure through the parallel use of other organic substrates in the process. Co-digestion (AcD) of several appropriately selected materials simultaneously makes it possible to increase the degradation rate of the processed materials, higher biogas yields, improve the nutrient balance, optimize the C/N ratio of the substrates and dilute toxic compounds. This strategy creates new opportunities for processing such organic wastes that are difficult to digest separately (Dróżdż et al., 2020; Borowski and Weatherley, 2013a).

Due to the high C/N ratio of agricultural materials such as corn silage, the agricultural industry is the most convenient source of co-substrates for processing poultry manure. However, the necessity to cope with seasonality and increase methane production efficiency has led to significant interest in other biodegradable waste (Mata-Alvarez et al., 2014). Undoubtedly, the aspect of using sewage sludge (SS) for co-digestion with poultry manure deserves special attention. Currently, no sludge-free wastewater treatment technology has been developed, nor is there a known solution to completely eliminate sludge from the environment. Although there is a progressive modernization and

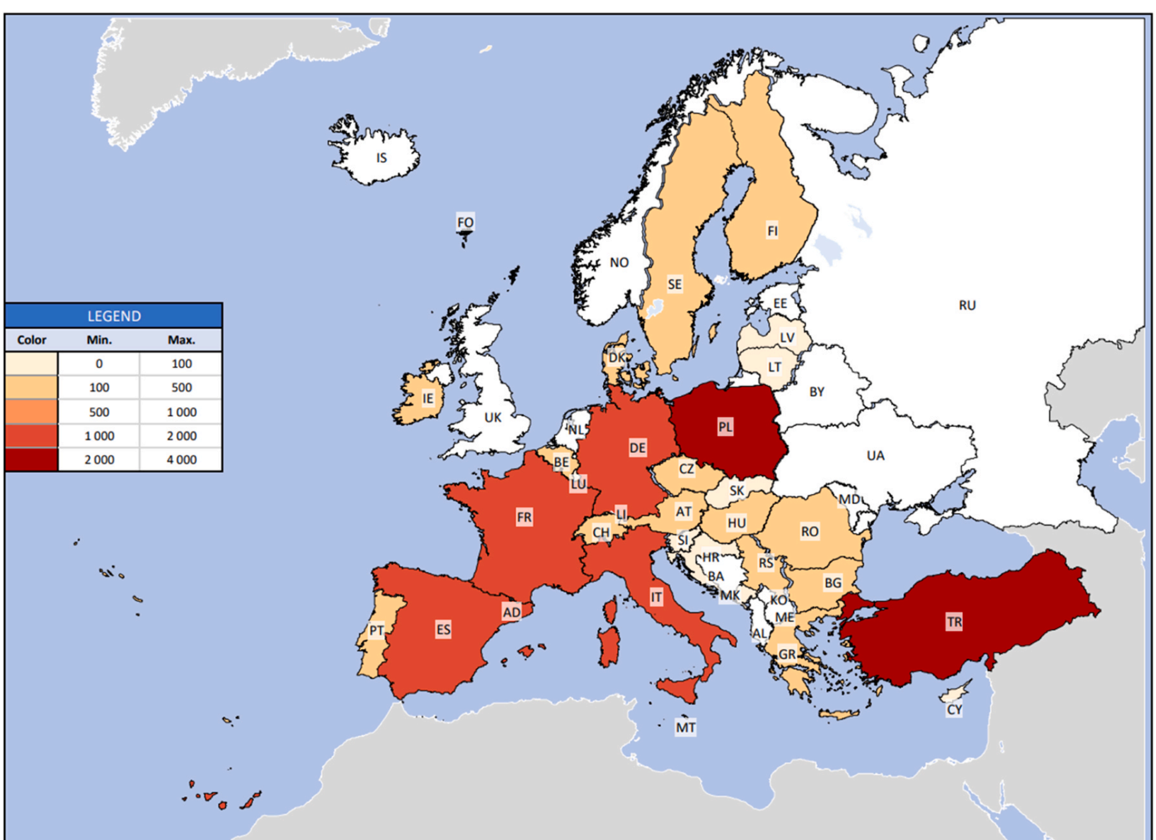

Fig. 1. Poultry meat production in Europe in 2022 (mln Mg) ($author1$ et al., 2023).

expansion of wastewater collection and treatment infrastructure, most digesters are oversized, so the organic load in them is low (Ghosh and Saha, 2020; Jasińska, 2018). Therefore, co-digestion of sewage sludge with other wastes appears to be a favourable strategy due to the possibility of increasing methane yield, as well as fully utilizing the available facilities at wastewater treatment plants (Xu et al., 2020; Yang et al., 2019).

This paper discusses the possibility of co-digestion of poultry manure and sewage sludge, with a particular focus on biogas production. To the best of the authors' knowledge, to date no one has carried out the process with such a high content of manure (up to 60 % based on volatile solids) in the co-digestion mixture and focused on the quality of the digestate, which is the novelty of the study. Only a few papers have been written on the co-digestion of these two materials, so the issue presented here provides a basis for a broader understanding of the possibilities associated with the aforementioned method of their management. Animal manure is most commonly processed by co-digestion in: (i) centralized plants that co-digest manure collected from several farms along with organic residues from industry and cities, and (ii) decentralized plants that co-digest manure with other agricultural wastes and increasingly with energy crops (Kadam et al., 2024). In our research, we decided to go against the grain of typical trends. Taking into account recent legislative changes (revision of the Wastewater Treatment Directive) ($author1$ et al., 2022), which talk about energy self-sufficiency of wastewater treatment plants, we decided to determine the effect of poultry manure on anaerobic digestion of sewage sludge. The identification of new potential co-substrate, so far not particularly considered, seems to be a very good solution, especially since changes in the law will result in stiffer competition in the raw material market. An additional argument in favour of this approach was the fact that this waste in Poland (which is one of the largest poultry producers in Europe) is found in large quantities, and its management is becoming increasingly problematic. Given these facts, co-digestion of sewage sludge with this waste stream seemed an attractive and sustainable approach. In addition, our research tested the methanogenic potential of locally generated waste, which could prompt its generators and stakeholders to manage such materials sustainably and economically. The scope of the work included performing mesophilic co-digestion of poultry manure and sewage sludge in a semi-continuous operation regime. In addition, a physicochemical analysis was made of the studied substrates, mixtures constituting the feedstock, and products of the process in the form of biogas and digestate. Potential greenhouse gas emissions were also estimated for various methods of poultry manure management.

2. Materials and methods

2.1. Substrates and inoculum

Poultry manure was obtained from a laying poultry farm in the Silesia region (Poland). The manure was produced by animals of adult age. During the experiment, poultry manure was collected twice, homogenized using an automatic mixer and stored at −20℃.

Sewage sludge, precisely a mixture of waste activated sludge and primary sludge was collected at the municipal wastewater treatment plant located in the Silesia region (Poland). The WWTP produces annually approx. 3200 Mg dry mass of sewage sludge and treats about 90 000 m^3/d of wastewater. Sludge was collected every two weeks and stored at 4℃ prior to use.

As inoculum, digested sludge was used and collected from the mentioned plant. The addition of inoculum in the feedstock during the start-up of the digester was equal to 10 % (v/v).

The co-digestion mixtures consisting of poultry manure and sewage sludge were prepared every nine days and stored at 4℃ prior to use.

Characteristics of the raw materials and prepared feedstock used in this study are presented in Table 1.

2.2. Experimental procedure

The process was performed in a semi-continuous mode under mesophilic conditions (37℃). The hydraulic retention time (HRT) was 20 days, which is a typical value on WWTP (Bolzonella et al., 2005).

The anaerobic digestion process was carried out in two continuously stirred glass reactors (CSTR) with a working volume=15 l. The reactors were equipped with thermostatic water jackets, mechanical stirrers with variable speed control (170 rpm), and sensors: temperature, redox. The test stands consisted of measuring cylinders (capacity 15 l) filled with saturated sodium chloride solution and equalization tanks (capacity 20 l).

At start-up, batch anaerobic digestion was carried out (Stage 1). Once steady state operation was reached, both reactors were fed once per day with sewage sludge. In the next stage, a co-digestion feedstock containing poultry manure and sewage sludge was added to the reactors, starting with a 2.5 % on volatile solids (VS) basis proportion of manure in the mixture. For the control trial, one of the reactors was fed with sewage sludge for the entire duration of the experiment. The proportion of poultry manure in each co-digestion mixture was systematically increased up to 60 % VS_{added} (Fig. 2). The gradual increase in the co-substrate content in the mixture was intended to provide the microorganisms with the opportunity to adapt to new environmental conditions (Grosser and Neczaj, 2018).

2.3. Sample analysis

The following parameters were measured during the experiment: total solids (TS), volatile solids (VS), pH value, volatile fatty acids (VFAs), alkalinity (A), ammonium nitrogen (N-NH_{4+}), total carbon (TC) and total Kjeldahl nitrogen (TKN). The volatile fatty acids (VFAs), pH levels, alkalinity, ammonium nitrogen concentration were determined in the supernatants after centrifugation at 12100 relative centrifugal force (rcf) for a time span of 15 minutes, and then filtration through filter papers (3w). All analytical evaluations were carried out in triplicate to assure precision and reliability. All mentioned measurements were performed according to the standard method (APHA, 1999). Additionally, free ammonia (NH_3 or FA) was calculated based on pH value, temperature and ammonium nitrogen, using the formula as described by Ref (Yang et al., 2019; Grosser, 2017).]. Concentrations of

Table 1
Characteristics of substrates used in this study.

Substrate	PM (% basedon VS)	TS (%)	VS (%)	VS/TS	pH	VFAs (mg CH3COOH/l)	VFAs/A (-)	N-NH4 (mg/l)	TC/TKN
Inoculum	-	2.52–2.56	1.44–1.46	0.57	7.80–7.82	480–514	0.21	537–543	7.51–7.57
SS	-	3.08–4.71	2.22–3.21	0.66–0.74	5.54–5.97	2131.42–2582.31	1.18–3.22	138.34–299.47	10.20–12.12
PM	-	27.44 ± 0.24	20.19 ± 0.81	0.74	5.41 ± 0.01	Nm	Nm	7000 ± 12.3	7.38 ± 0.51
Mixture 1	10	4.86 ± 0.01	2.81 ± 0.05	0.58	5.81 ± 0.01	2840 ± 9.9	2.48	294.93 ± 3.23	10.64 ± 0.04
Mixture 2	20	4.74 ± 0.05	3.13 ± 0.44	0.66	5.89 ± 0.01	3204 ± 36	3.01	396.06 ± 3.23	11.90 ± 1.41
Mixture 3	30	4.66 ± 0.02	3.37 ± 0.09	0.72	5.72 ± 0.01	2457.94 ± 36.69	2.21	461.61 ±5.6	8.22 ± 0.77
Mixture 4	40	5.4 ± 0.04	3.92 ± 0.29	0.73	5.73 ± 0.01	2488.52 ± 22.23	2.18	512.40 ± 3.96	8.20 ± 1.00
Mixture 5	50	5.05 ± 0.05	3.85 ± 0.49	0.76	5.75 ± 0.01	2589.31 ± 31.35	2.33	579.13 ± 4.7	7.94 ± 1.30
Mixture 6	60	6.21 ± 0.11	4.66 ± 0.65	0.75	5.69 ± 0.01	2742 ± 57,68	2.17	625.48 ± 9.54	7.89 ± 0.27

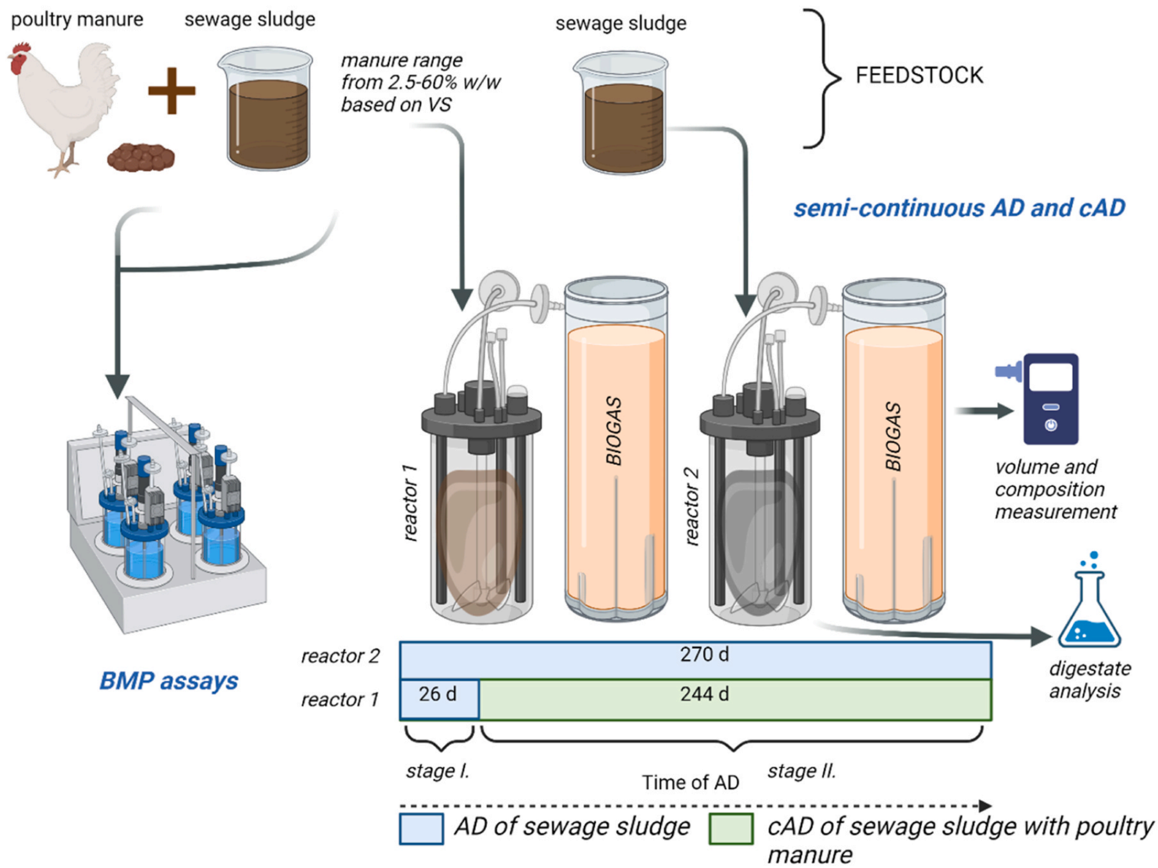

Fig. 2. Scheme of an experiment performed in this study.

heavy metals and nutrients were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES). Prior to analysis, samples were extracted in reverse aqua regia, sonicated for one hour and digested in a digestion chamber (Milestone ultraWAVE). Daily biogas production was measured using the water displacement method, while biogas composition was determined using a portable gas analyser (NANOSENS DP-27 BIO+). The biogas volume and composition were monitored daily, while digested sludge and substrate were analysed once every seven days. The volume of biogas was converted to standard conditions (temperature = 273 K, pressure = 1 atm).

In addition, to determine whether synergistic effects occurred between the co-substrates, the co-digestion performance index (CPI) was estimated according to the formula presented by Ebner et al (Ebner et al., 2016a). In the numerator, the methane production obtained during the study was considered. In the denominator, on the other hand, the calculation considered the methane production coefficients estimated for each component using the automatic methane potential test system (AMPTS II) (own research not presented in the manuscript). For sewage sludge, the methane production coefficient was 0.310 l/g VS_{added} (In the manuscript, the average production for methane yield was 0.303 l/g VS_{added}), and for chicken manure, it was 0.28 l/g VS_{added}. The tests were conducted in mesophilic conditions with an I/S ratio of 1.

2.4. Calculation of emissions

Based on the data on the characteristics of the tested poultry manure, the potential for greenhouse gases (CH_4, CO_2, N_2O) emissions was estimated. Calculations were made based on equations proposed by Kreidenweis et al (Kreidenweis et al., 2021). for four scenarios: storage (1) and composting (2) of fresh manure and its anaerobic co-digestion with sewage sludge at PM = 60 % in the feedstock, taking into account storage of the digestate in a closed (3) and open storage tank (4). The emission results obtained are presented in kg/t of poultry manure or co-digestion mixture.

2.5. Statistical analysis

The effect of poultry manure addition on the effectivity of anaerobic digestion was studied using a one-factor ANOVA analysis. The homogeneity of variances was checked using Levene's test. Data which failed the ANOVA assumptions were analyzed via the Kruskal-Wallis test. ANOVA was done with at least three replications for each combination of the nominal variables. The statistical analysis was carried out using STATISTICA software (STATISTICA 12 PL StatSoft, Inc.)

3. Results and discussion

3.1. Characterisation of raw materials and feedstock mixtures

Poultry manure had a relatively high TS content of about 27 %, but lower than the literature data indicate (for solid manure, the TS range is 33–79.4 %) (Ghirardini et al., 2020). It is worth noting that the dry matter content of manure depends on the form of storage and the presence of bedding material. From the point of view of the co-digestion process together with sewage sludge, whose hydration is high, the TS value of manure for wet digestion is satisfactory. However, both SS and PM had a high VS/TS ratio (<0.7), indicating their high biodegradability. As expected, with increasing the addition of PM in the feedstock mixture, the total solids and volatile solids contents increased.

A significant difference can also be seen in the C/N ratio of poultry manure was equal to 7.38, compared to references, where for manure the range of values for this parameter is 15–18 (Sadecka and Suchowska-Kisielewicz, 2016). The ammonium nitrogen content of the manure was high at 7000 mg/L. Mixing sewage sludge with poultry manure led to an increase in C/N and a significant reduction in N-NH_4 concentration, which offset the risk of inhibition associated with ammonia production during the co-digestion process.

Both poultry manure and sewage sludge were characterized by low pH, while a high content of volatile fatty acids was reported for sewage sludge. The wide range of results obtained for the second mentioned material was due to the variability of the wastewater composition, which consequently translates into a lack of homogeneity in the chemical composition of the sludge.

3.2. Process stability

The stability of the process in both reactors was evaluated mainly by measuring the value of the VFAs/A ratio. During start-up, this ratio averaged for R1 =0.17 and R2=0.46, respectively. The difference in value between the two reactors may have been due to the heterogeneous nature of the sludge. The stabilization process of the reactors was short, already on the 11. day the value of VFAs/A for R1 and R2 was reported below 0.2, which indicates good adaptation of the inoculum to the conditions. As the proportion of poultry manure in the co-digestion mixture increased, the ratio remained at a similar level, averaging 0.16–0.18. The highest VFAs/A ratio was reported for R1 on day 161, at 48 % proportion of poultry manure in the mixture, and it was 0.33. The literature data indicates the critical upper value of VFAs/A for proper anaerobic digestion as 0.3–0.4 (Grosser, 2017; Dąbrowska, 2015). Thus, the results obtained in both cases testify to the stable operation of the reactors, and single spikes in values above 0.3 did not disrupt the process. Fig. 3 shows the fluctuations in the values of VFAs and alkalinity in the R1 and R2 digestate during the experiment, as well as the quotient of these parameters.

At the initial stage of the experiment - anaerobic mono-digestion of sewage sludge, the content of volatile fatty acids in the digestate averaged for R1 and R2, 662 and 818 mg CH3COOH/l, respectively. With the introduction of co-digestion in R1, increasing the proportion of poultry manure in the mixture resulted in an increase in the concentration of VFAs, and the maximum value was 1823 mg CH_3COOH/l at 60 % PM. An increase in the concentration of VFAs is observed with increasing FA content as methanogen activity decreases (Shi et al., 2017). Hence, the addition of manure, which is a substrate with high nitrogenous organic matters, resulted in higher production of VFAs. In the case of R2, the concentration of volatile fatty acids tended to decrease with the course of sludge digestion. As stated in the literature, the AD process is stable at values of 100–500 mg CH3COOH/l, with alkalinity of not less than 500 mg $CaCO_3$/l (Magrel, 2002b), and inhibition can occur at concentrations of VFAs of 2500–4000 mg CH_3COOH/l (Grosser, 2017). Co-digestion of sewage sludge and poultry manure significantly increased alkalinity, from an average of 3740 mg $CaCO_3$/l when co-substrate was introduced to 11080 mg $CaCO_3$/l at 60 % PM. During the sewage sludge mono-digestion process in R2, a decrease in $CaCO_3$ concentration to 2260 mg $CaCO_3$/l at day 260 was observed with the course of the experiment. Despite the relatively high values of VFAs reported during AcD, there was no interference with the process. Poultry manure has a high buffer capacity and high nitrogen content, thus showing the potential to raise the pH in the mixture. Many authors report the influence of a slightly acidic pH, close to 6, on improving the working conditions of hydrolytic-acid-forming bacteria and limiting the growth of methanogens (Ponsá et al., 2008). The stable pH of the AcD process, oscillating in the range of 7.70–8.30, prevented the transition of VFAs into dissociated forms detrimental to the process. In this case, the alkalinity of the manure is a neutralizing factor for the generated VFAs to offset the pH changes. Thus, the preference for VFA/A monitoring over pH is due to the fact that AD rarely returns to normal when pH drops dramatically. This makes pH less relevant, as Issah et al. (2021a) also point out in their study on co-digestion of palm nut paste

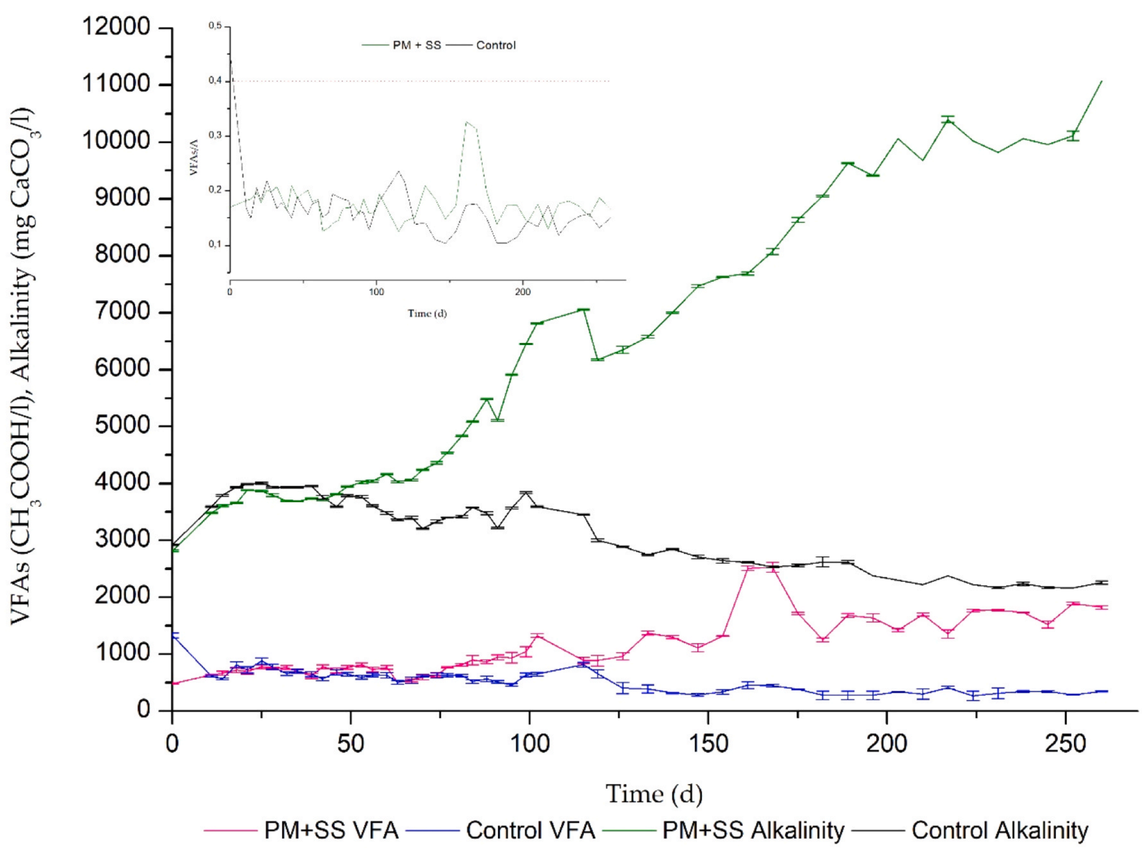

Fig. 3. Variations of volatile fatty acids, alkalinity and VFAs/A ratios during the experiment.

waste and anaerobic digested rumen waste (Issah and Kabera, 2021b). These researchers report that balancing high VFA production during anaerobic digestion requires maintaining alkalinity above 1.5 g $CaCO_3$/l.

The introduction of PM co-substrate into R1 also resulted in a significant increase in N-NH_4 concentration. While the analysis of digestate from R2 showed a decreasing trend in ammonium ions content with the course of the experiment, in R1, an accumulation of N-NH_4 was observed from 917 mg/l with 2.5 % PM (based on VS), to nearly 2750 mg/l with 60 % manure (based on VS). Borowski et al (Borowski and Weatherley, 2013b)., in their study on the co-digestion of sewage sludge with poultry manure in a ratio of 30:70 % (w/w VS), reported ammonium ions concentrations of 2094 mg/l at HRT=20 days, with no process interference. The mechanism of ammonia inhibition during AD is still not sufficiently defined, hence a wide range of reported concentrations, from 1500 to 7000 mg/l (Jasińska et al., 2023a), can be found in the literature. However, it is known that the main factor of process interference is the presence of FAN (free ammonium nitrogen), the concentration of which depends on the process parameters - pH and temperature, as well as the adaptability of microorganisms. Cook et al (Cook et al., 2017). indicate the instability of AD at FAN concentrations above 200 mg/L. Altinbas et al (Altinbas and Cicek, 2019)., in their study of co-digestion of cattle and poultry manure, found the maximum concentration of N-NH_3 tolerated by methanogens at 332 mg/l, but for a value of 253 mg/l, they already observed a 51 % reduction in methane production compared to the initial stage of the reactor operation only with cattle manure AD. The researchers also pointed out a trend of increasing free ammonia content with increasing the proportion of chicken manure in the co-digestion mixture. It is well known that this kind of manure contains more nitrogen than other manure because hens belong to monogastric animals (Borowski and Weatherley, 2013b).

The source of N-NH_4 in these wastes is nitrogen found in uric acid, excreted by the organisms along with urine. Fig. 4 illustrates the changes in pH and N-NH_4, and FAN content for the digestate in both reactors. However, the relatively high total concentration of ammonium ions and FAN in R1 did not lead to inhibition of the process, which means the good adaptation of the microorganisms to the process conditions, achieved, among other things, by the gradual introduction of the co-substrate in the form of a PM. In comparison to the studies of Sillero et al (Sillero et al., 2023a)., slightly larger pH fluctuations were noted (7.70–8.30 - current studies vs 7.5–7.9). However, lower indicator fluctuations result from the fact that the process was conducted in a two-stage digestion system, and in addition to sewage sludge and poultry manure, a third co-substrate was dosed to the anaerobic chambers.

3.3. Process performance

The key parameters describing the efficiency of the anaerobic digestion process are the biogas/methane production ratio and the VS removal, the value of which largely depends on the ORL of the feedstock (Grosser et al., 2013). During the experiment, data on biogas production and volatile solids removal rate were reported for R1 and R2, as shown in Figs. 5, 6, 7 and 8. In the initial phase of anaerobic mono-digestion of sludge, irregular gas production was observed until the 11. day, which is related to the stabilization process of the reactors. From the 12. to the 25. day prior to the introduction of co-digestion, daily biogas and methane production averaged 7.7 and 5.2 l for R1 and 7.1 and 5.0 l for R2, respectively. For R2, where sludge mono-digestion was carried out, an average daily biogas production of 8.9 l and methane production of 5.64 l was reported from 26 to 260 days. In this case, the biogas and methane production yield, in this case, averaged 0.4 and 0.3 l/kg VS_{added} for R1 and R2, respectively. In the case of co-digestion, when the proportion of poultry manure in the mixture ranged from 10 % to 30 % (based on VS), there was no significant effect on increasing the efficiency of the process with respect to the control sample. In contrast, a

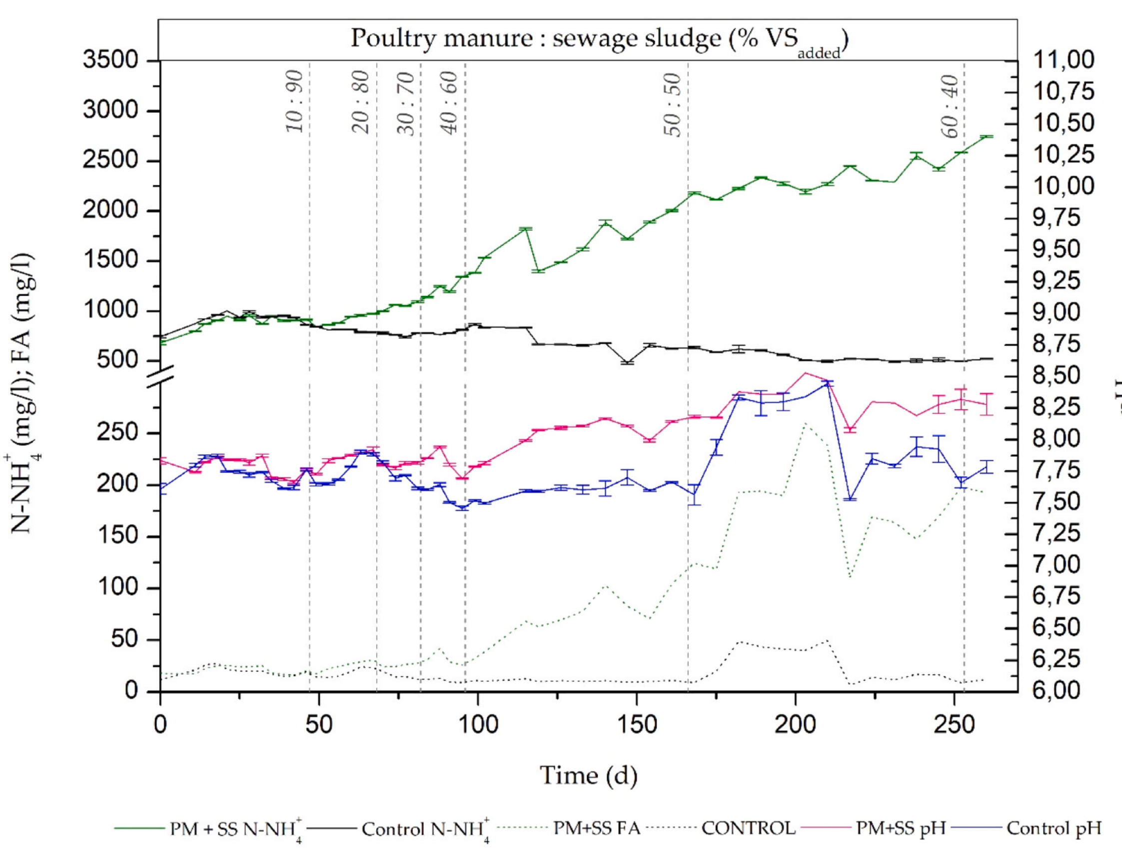

Fig. 4. Ammonium nitrogen, free ammonia concentrations and pH values for R1 and R2.

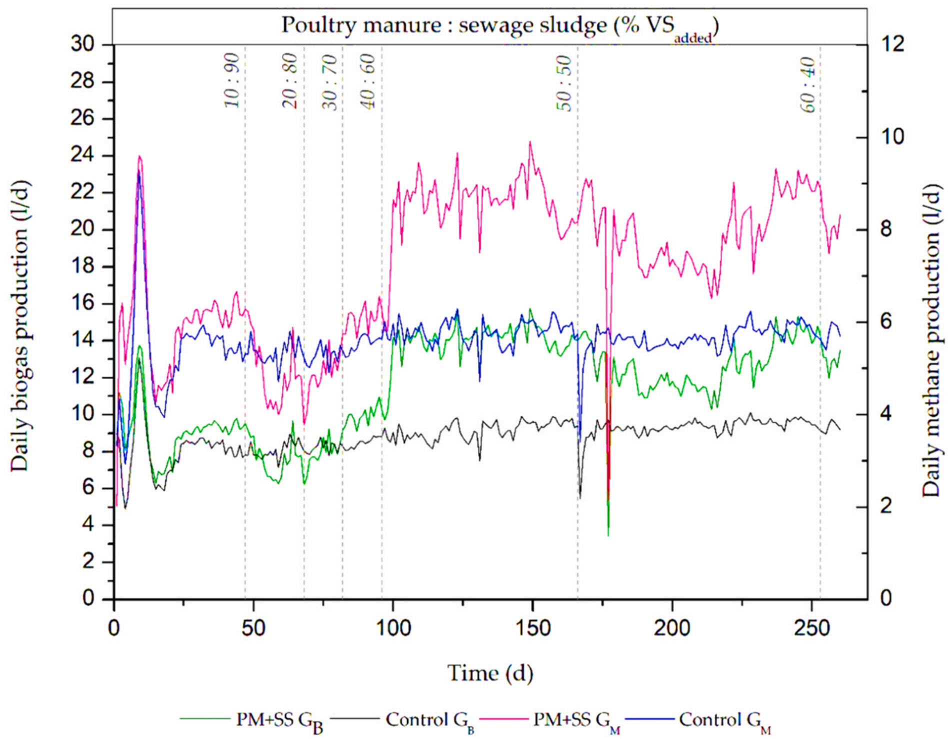

Fig. 5. Daily biogas and methane production reported during the experiment.

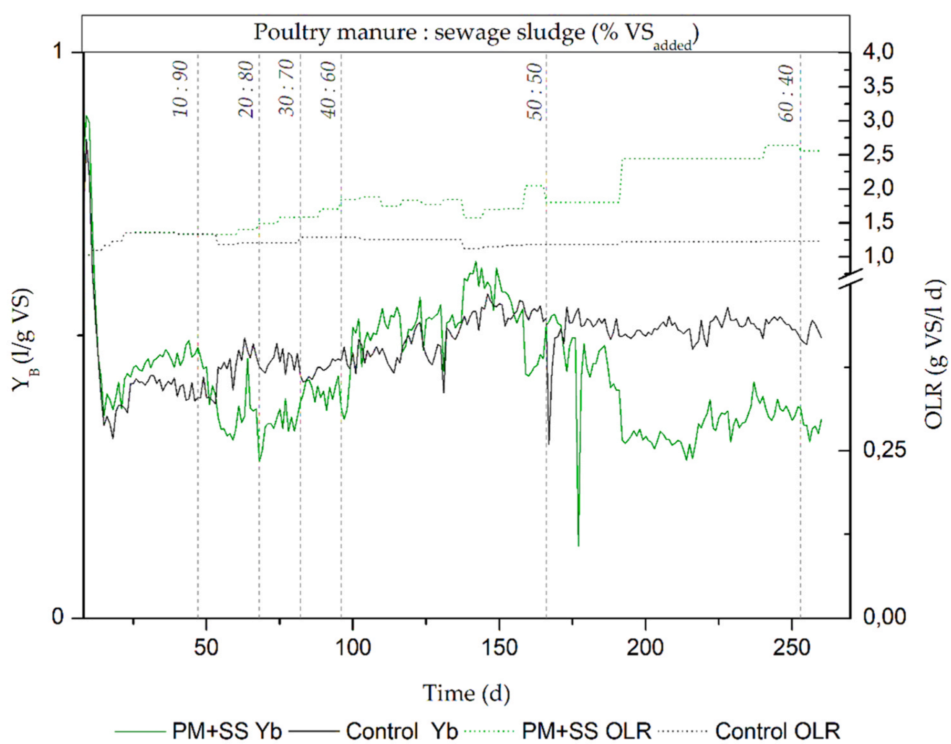

Fig. 6. Biogas production yield and organic loading rate of the anaerobic digestion processes.

significant increase in biogas production, by 35 % compared to the AD of sewage sludge, was obtained when the share of PM:SS co-substrates was 40:60 % w/w VS, respectively. Further increasing the proportion of PM in the mixture, up to 60 %, also resulted in a higher daily biogas gain ranging from 23 % to nearly 38 % with a PM:SS ratio of 42:58 % w/w VS.

The data on biogas and methane production yields, taking into account organic loading rates, were slightly different. During co-digestion at a share of PM up to 35 % in the mixture, fluctuations were observed in the values of biogas production yield in the range of 0.276–0.491 l/gVS_{added}, and methane production yield in the range of 0.168–0.334 l/gVS_{added}. When the share of PM was increased to 40 % in the input,

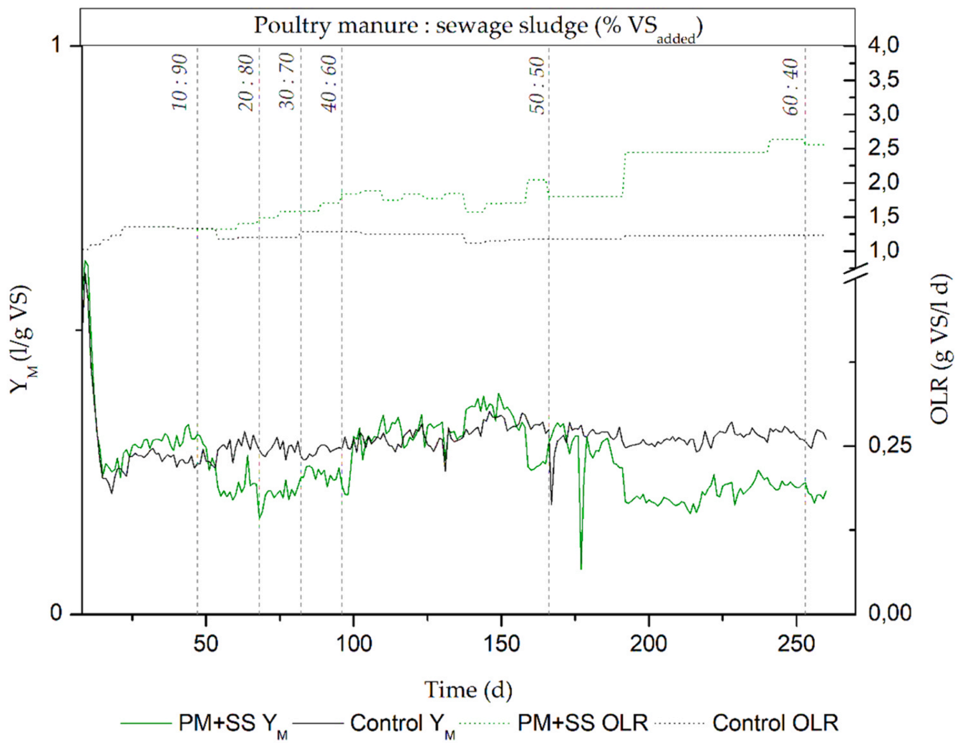

Fig. 7. Methane yield and organic loading rate of the anaerobic digestion processes.

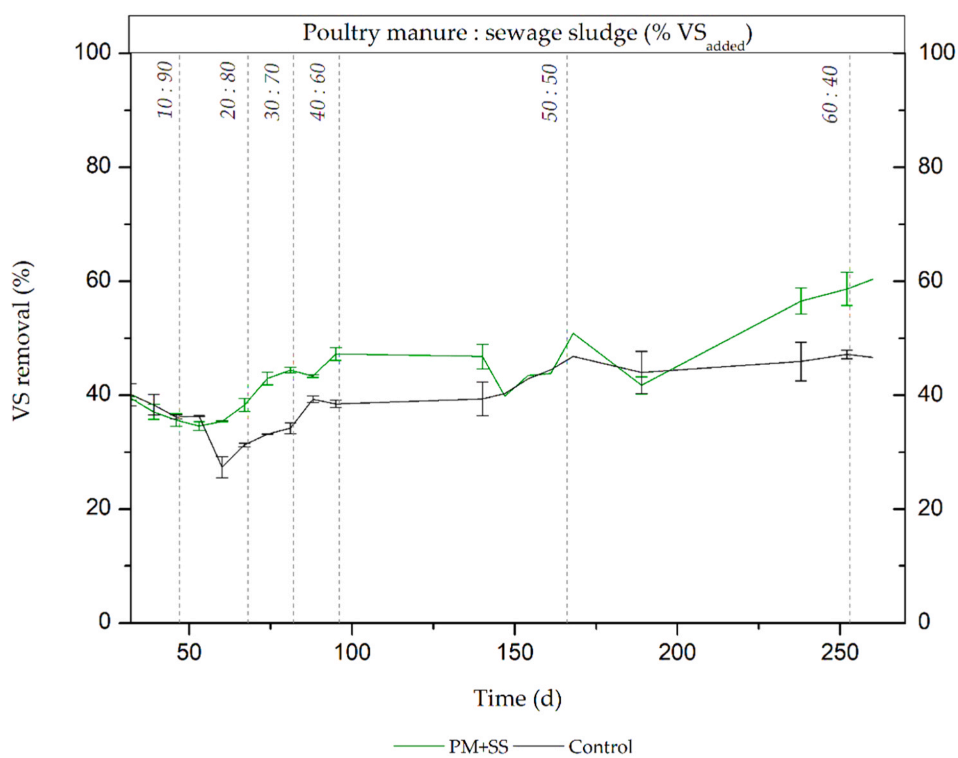

Fig. 8. Variations of volatile solids removal during the experiment.

higher values of these parameters were noted, averaging 0.503 l biogas/gVS$_{added}$ and 0.309 l CH4/gVS$_{added}$. The upward trend continued up to a PM share of 50 %, with the highest biogas production yield of 0.631 l/kgVS$_{added}$ at PM:SS = 48:52 % w/w VS and a methane production yield of 0.389 l/gVS$_{added}$ at PM: SS = 44:56 % w/w VS. Further increasing the proportion of PM in the co-digestion mixture resulted in a decrease in the values of both parameters to an average of 0.340 l biogas/g VS$_{added}$ and 0.213 l CH4/g VS$_{added}$.

In the case of R2, where sewage sludge mono-digestion was carried out, the values of the biogas production factor oscillated in the range:

Table 2
Average composition of the biogas during experiment.

Reactor (% addition of PM in the feedstock)	CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (p)	NH_3 (p)	CO (p)
R1 (2.5–10 %)	66.67 ± 0.86	27.00 ± 0.45	0.00	0.95 ± 0.22	0.00	14.86 ± 6.02
R1 (10–20 %)	64.24 ± 2.28	27.48 ± 1.57	0.00	0.71 ± 0.46	0.00	14.48 ± 6.95
R1 (20–30 %)	61.6 ± 1.24	29.73 ± 0.59	0.00	1.00	0.00	16.73 ± 8.61
R1 (30–40 %)	61.5 ± 1.45	30.57 ± 0.51	0.00	0.71 ± 0.47	0.07 ± 0.27	9.14 ± 5.29
R1 (40–50 %)	61.55 ± 1.29	28.5 ± 1.6	0.00	0.07 ± 0.26	0.00	5.71 ± 0.95
R1 (50–60 %)	62.78 ± 1.30	29.42 ± 1.16	0.00	0.02 ± 0.21	0.00	38.19 ± 18.03
R1 (60 %)	62.21 ± 0.37	30.04 ± 0.26	0.00	0.38 ± 0.74	0.00	76.38 ± 21.88
R2 (0 %)	63.14 ± 2.64	29.55 ± 2.25	0.00	5.89 ± 7.83	1.71 ± 5.51	18.29 ± 16.84

0.308–0.573 l/gVS$_{added}$, and the methane production yield in the range: 0.193–0.357 l/gVS$_{added}$. A similar discrepancy in the results for daily methane production and the production yield taking into account the amount of VS$_{added}$ was noted by Borowski et al (Borowski and Weatherley, 2013b). in their study on co-digestion of poultry manure and municipal sewage sludge at a ratio of 30:70 %VS$_{added}$. The lower gas yield per dry organic matter available in the reactor in the case of co-digestion may be due to the lower methanogenic potential of poultry manure relative to sewage sludge and the slight inhibition of the process due to ammonia release (Borowski and Weatherley, 2013b). Nevertheless, the author's research indicates an increase in the methane digestion efficiency of sewage sludge after introducing a 30 % addition of co-substrate in the form of poultry manure by about 50 %. There are few cases in the literature of joint digestion of the two wastes, but the available publications prove that co-digestion of sludge with poultry manure can increase the total biogas production volume and improve the sewage sludge treatment process (Borowski and Weatherley, 2013b; mahmoud et al., 2022; Mansour et al., 2023).

The observations were reflected in the estimated co-digestion performance index (CPI). Only for mixtures in which the proportion of poultry manure was between 40 % and 50 % based on VS, the value of the index was above 1, indicating a synergistic effect of co-digestion. In other co-digestion mixtures, CPI was lower than one, which indicates an antagonistic impact between substrates (Ebner et al., 2016b).The organic loading rate of the reactors for the R2 control trial ranged from 1.124 to 1.357 g/l·d. In the case of R1, where co-digestion was carried out, more considerable variations were observed in the value of this parameter, from 1.124 to 2.639 g/l·d, with a clear trend of increasing OLR with increasing the proportion of PM in the mixture. A similar relationship was observed in the case of the VS removal, where for the control sample, the average value was close to 40 %, while in the case of co-digestion, with the highest share of PM in the mixture, the value was 60 %.

When the proportion of PM in R1 did not exceed 15 %, no significant effect of co-digestion on more effective VS removal was observed, while at 20 % PM addition, a clear increase in this parameter was noted. At a co-substrate ratio of PM: SS=40:60 %, a decrease in the degree of digestion was observed, which may have been related to the higher concentration of ammonium nitrogen and thus the need for acclimatization of microorganisms. More effective VS removal was also observed by Sillero et al (Sillero et al., 2022). when poultry manure was added at 10 g/l to a 50:50 % mixture of sewage sludge and vine vinasses. These authors studied mono-digestion of sewage sludge, co-digestion of sewage sludge and vine vinasses in a 1:1 ratio, and three-component co-digestion: sewage sludge, vine vineasse and poultry manure in a 49.5:49.5:1 % ratio. They obtained the most satisfactory results for the last variant, where at different HRTs (20−6), the VS removal was in the range of 40–57 %, and the highest methane yield was obtained at HRT=13, with 261 mL CH_4/g VS added. Borowski et al (Borowski and Weatherley, 2013b). also indicate that co-digestion of poultry manure with sludge resulted in a higher VS removal efficiency (43.16–49.35 %) compared to the digestion of sludge alone (33.85–36.33 %). As shown by the researches (Sillero et al., 2023a, 2023b, 2024), the increase in methane production can be achieved by introducing a third co-substrate and separating the acid phase from the methane phase in the processes of anaerobic co-digestion in the temperature phase (TPAD). However, the HRT for the individual phases should be selected experimentally. The mentioned authors, by reducing the length of the methane phase from 15 to 12 days, increased the methane yield from 320 (Sillero et al., 2023a) to 391 mLCH4/gVS$_{added}$ (Sillero et al., 2023b, 2024).

3.4. Quality of the anaerobic digestion products

Table 2. shows the approximate composition of biogas obtained during the various stages of the digestion process for the sludge alone and the mixture with poultry manure. Co-digestion did not significantly affect the quality of the gas, for both R1 and R2, the CH_4 content was more than 60 %, confirming the results obtained by Borowski et al (Borowski and Weatherley, 2013b). The highest percentage of methane was observed for PM addition at 2.5–10 % (VS$_{added}$), amounting to nearly 67 %. With sludge mono-digestion, relatively higher H_2S content was observed (5.89 ± 7.83 %) than co-digestion (0.02–1.00 %). Higher NH_3 concentrations were also observed for R2, at nearly 6 ppm, while for AcD, the value oscillated between 0.00 and 0.27 ppm. On the other hand, a marked increase in CO content in biogas was noted during co-digestion with PM addition above 50 % (VS$_{added}$), where its amount ranged from 38 to 76 ppm, while for AD of sewage sludge, it averaged 18 ppm.

It is well known that the anaerobic digestion process leads to the release of nutrients and trace elements from the substrates, causing them to accumulate more in the digestate (Borowski and Weatherley, 2013b; Czekała et al., 2020). Both poultry manure and sewage sludge belong to heterogeneous wastes, whose chemical composition varies depending on the diet and farming conditions in the case of manure and the source and technology of wastewater treatment. Agricultural management of these wastes, especially in the form of digestate, can positively affect crops and improve the physicochemical properties of soils, due to the presence of organic matter and nutrients, but it also involves the risk of secondary contamination with toxic substances (Jasińska et al., 2023b). Table 3. shows the elemental composition of the studied substrates and the digestate obtained from the anaerobic digestion and co-digestion processes. Among the processed wastes, poultry manure was characterized by a higher content of nutrients, such as Mg, Na, K and, in particular, calcium, which was determined at 82.014 mg/kg TS, which may be related to the presence of eggshell residues in the droppings of laying hens. On the other hand, sewage sludge was a better source of phosphorus and sulfur, which confirms literature reports that indicate high amounts of these elements in raw wastewater (Borowski and Weatherley, 2013b; Zerrouqi et al., 2020)]. Higher concentrations of metals such as Cr, Cu, Fe and Ni were also observed in the sewage sludge. Poultry manure contained more manganese and zinc, which is related to the high dietary requirements of animals for these elements, as they act as cofactors or activators for enzymes involved in the process of eggshell formation (Robert, 2004). For cadmium and lead, concentrations in both the raw waste and the digested sludge were below 1 mg/kg TS or below the detection levels. Cobalt was also not detected in the substrates, but in the digestate, its content was 13.31 mg/kg TS for R1 and 15.34 mg/kg TS for R2, which can be explained by the phenomenon

Table 3
Elemental content of the substrates and digestate (mg/kg TS).

Sample	Ca	Mg	Na	K	P	S	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
PM	82014 ±0.115	10018 ±0.149	3774 ±0.009	30094 ±0.071	22834 ±0.084	7541 ±0.018	1175 ±0.005	0.731 ±0.000	<dl	20.16 ±0.000	99.60 ±0.000	2437 ±0.011	566.6 ±0.002	23.00 ±0.000	<dl	584.2 ±0.001
SS	25158 ±0.097	3884 ±0.046	1936 ±0.044	3875 ±0.082	29091 ±0.216	10542 ±0.086	18442 ±0.332	0.947 ±0.000	<dl	351.4 ±0.005	216.0 ±0.002	23310 ±0.380	397.8 ±0.005	112.0 ±0.001	<dl	1897 ±0.021
D-AcD	59008 ±0.183	8213 ±0.327	15727 ±0.320	19899 ±0.413	36512 ±1.429	21236 ±0.898	8617 ±0.185	<dl	13.31 ±0.001	159.9 ±0.004	226.6 ±0.005	31827 ±1.097	786.9 ±0.033	117.7 ±0.003	<dl	1527 ±0.026
D-Con.	53429 ±0.460	10167 ±0.177	19214 ±0.451	14848 ±0.394	40163 ±1.090	21244 ±0.755	9773 ±0.301	<dl	15.34 ±0.000	204.4 ±0.006	236.6 ±0.007	39501 ±0.880	861.8 ±0.021	144.9 ±0.005	<dl	1672 ±0.053

PM – poultry manure; SS – sewage sludge; D-AcD – digestate from anaerobic co-digestion; D-Con. – digestate from anaerobic digestion of sewage sludge

of higher accumulation of metals in the AD product (Montusiewicz et al., 2020).

Undoubtedly, the products obtained from the anaerobic mono-digestion and co-digestion processes studied show potential for further use. The biogas obtained, both in the case of AD and AcD, was characterized by a high methane content, above 60 %, and a low percentage of pollutants. In practice, the treatment of digestion gas mainly focuses on the removal of H_2S (Angelidaki et al., 2018), which, in the case of the products obtained in this research, would only apply to biogas produced from sewage sludge mono-digestion. After eliminating H_2S, the biogas could be further upgraded to biomethane. The second product of the process, the digestate, could also find use in agriculture or as an additive for soil improvement due to the presence of organic matter and valuable elements. Therefore, this study carried out a physico-chemical study of the obtained digestate in terms of its fertiliser potential. In the case of poultry manure, the latest EU Fertilizer Regulation (Regulation, 2019) allows the use of this waste as a digestate, subject to subsequent thermal treatment in the case of mesophilic AD, such as through a pasteurization or composting process. However, the document excludes agricultural use of the product of AD of sludge, treating this material in the category of waste, the management of which is subject to other regulations.

In the European Union, the application of sewage sludge to soils is permitted, provided that the criteria contained in the Council Directive of June 12, 1986, on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (European Commission, 1986) are met. However, a new sewage directive is coming soon, which will include more extensive monitoring of the pollutants present in this waste. In Poland, however, the Regulation of the Minister of the Environment of February 6, 2015, on the use of municipal sewage sludge (Minister Środowiska, 2015) is in force. The main criterion determining the agricultural use of sewage sludge is its physicochemical and hygienic-sanitary properties. The resulting two types of digestate are characterized by trace element content below the upper limits of the value presented in the regulation, but the possibility of their use would require further testing for the presence of pathogens.

3.5. *Greenhouse gases emissions*

Management of bio-waste, including manure, is associated with the emission of gases responsible for the greenhouse effect. The most important of these include carbon dioxide, methane and nitrous oxide, with a global warming potential of 298 times higher for N_2O and 25 times for CH_4 compared to CO_2 (Pardo et al., 2015). Based on the results obtained from the substrate and methane production studies for anaerobic co-digestion, the emission potential of the above-mentioned gases was estimated for different poultry manure treatment scenarios. The calculations used equations proposed by Kreidenweis et al (Kreidenweis et al., 2021). in their study, who presented a new impact model that can assess the sensitivity of parameters and interdependencies between process emissions when comparing different broiler manure processing options. Fig. 8 shows the obtained results of CH_4, CO_2 and N_2O emissions calculations for poultry manure in the case of storage (1. scenario) and composting (2. scenario), and for anaerobic co-digestion of a mixture of poultry manure and sewage sludge, at a ratio of 60:40 % (VS_{added}), respectively, taking into account leakage when storing the digestate in an open (3. scenario) and closed (4. scenario) tank. First scenario assumes time-limited storage of manure in a closed concrete silo. Second scenario is a heap composting process on concrete slabs. For Scenarios 3. and 4., calculations were made for anaerobic co-digestion of a mixture of poultry manure and sewage sludge with the highest proportion of manure tested in the study, 60 %. This approach resulted from the limited practice of mono-digestion of manure, due to its properties and the associated risk of process inhibition (Kreidenweis et al., 2021). Fig. 9.

Analyzing the results, the highest methane emissions (0.4 kg/t of manure) were obtained for storage, and slightly lower levels of this gas

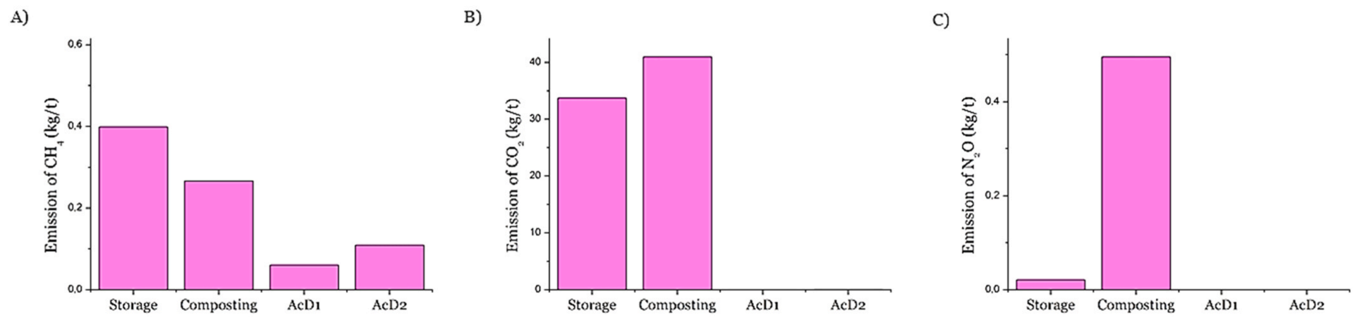

Fig. 9. Emissions of CH_4 (A), CO_2 (B) and N_2O (C) for four management options: storage of, composting of, anaerobic co-digestion of PM:SS = 60:40 % (VS_{added}) with closed digestate storage tank (AcD1), anaerobic co-digestion of PM:SS = 60:40 % (VS_{added}) with opened digestate storage tank (AcD2).

Table 4
Univariate Tests of Significance for VS removal.

Effect		F	p
Addition of	VS removal	36.92	0.00
	Methane yield	10.476	0.000000

were calculated for composting (0.27 kg/t of manure). In the case of co-digestion, the CH_4 emission values were lower, at 0.06 kg/t for AcD with a closed digestate storage tank and 0.11 kg/t for AcD with an open tank, with these emissions including potential leakage and not biogas production as the target product of digestion. For carbon dioxide, the highest emission value (40.99 kg/t) was calculated for the composting of manure and for storage the emission was slightly lower, amounted to 33.73 kg/t of manure. A significantly lower CO_2 level was calculated for anaerobic co-digestion, where for the AcD1 and AcD2 variants it was 0.04 and 0.07 kg/t of co-digestion mixture, respectively. Composting also produced the highest N_2O emissions, of 0.5 kg/t manure. For storage, the calculation result for this gas is 0.02 kg/t of manure, while no emissions were calculated for both AcD1 and AcD2.

The obtained results indicate the advantage of the process of anaerobic co-digestion of poultry manure over other methods of managing this waste in terms of GHG emissions, which confirms the conclusions of Kreidenweis et al (Kreidenweis et al., 2021). Nevertheless, in the case of calculations, it should be taken into account that for anaerobic co-digestion scenarios, the emission level refers to a ton of substrate in the form of a mixture of manure and sewage sludge, and not fresh manure under the assumptions for composting and storage. Therefore, these calculations are indicative, and a broader analysis should also take into account net GHG emissions from transport and further processing of

Table 6
Tukey HSD test; variable YM.

Addition of	YM	a	b	c	d	e
	Mean					
D20	0.201251	****				
D52	0.209673	****				
D60	0.211221	****				
D12.5	0.211621	****				
D25	0.216216	****	****			
D54	0.226471	****	****	****		
D15	0.234088	****	****	****		
D35	0.242527	****	****	****		
D30	0.250249	****	****	****		
D48	0.260553	****	****	****	****	
D0	0.281197	****	****	****	****	****
D10	0.287415	****	****	****	****	****
D50	0.287796	****	****	****	****	****
D2.5	0.299701		****	****	****	****
D5.0	0.305331			****	****	****
D40	0.309666			****	****	****
D7.5	0.312901			****	****	****
D46	0.341680				****	****
D44	0.365810					****
D42	0.389050					****

Table 5
Tukey HSD test; variable VS removal.

Addition of	VS removal	a	b	c	d	e	f	g	h
	Mean								
D10	34.58301	****							
D12.5	35.41606	****	****						
D7.5	35.63270	****	****						
D5.0	37.07645	****	****	****					
D15	38.27354	****	****	****	****				
D2.5	39.46969	****	****	****	****				
D0	39.58947	****	****	****	****				
D42	39.78799	****	****	****	****				
D50	41.75263		****	****	****	****			
D20	42.93402			****	****	****			
D30	43.35962			****	****	****			
D44	43.48969			****	****	****			
D46	43.80062				****	****			
D25	44.39653				****	****	****		
D40	46.81155					****	****		
D35	47.22913					****	****		
D48	50.89729						****	****	
D52	56.55506							****	****
D54	58.67072								****
D60	60.33229								****

the products of the analyzed manure management methods, such as compost management or CHP units in the case of the anaerobic digestion process.

3.6. Statistical analysis

Results achieved for one-way ANOVA (F-values for selected parameters), confirm the previous observation that the addition of PM has the highest positive impact on VS removal and methane yield (Table 4). However, the final effect depended on the addition in the feedstock. In the case of VS removal, an increase in the parameter, compared to the control sample, was recorded when the co-substrate addition was higher than 15 % (VS_{added}). On the other hand, for YM, the highest values were obtained when the addition was greater than 40 % (VS_{added} (Tables 5 and 6).

4. Conclusions

The following conclusions can be drawn from this study:

– The anaerobic co-digestion of poultry manure and sewage sludge has a beneficial effect on the process performance and the quantity of the obtained products in the form of biogas and digestate.
– At 40–60 % (VS_{added}) share of manure in the co-digestion mixture, more than an average 40 % (VS_{added}) increase in daily biogas production was observed compared to the digestion of sewage sludge alone. Moreover, better VS removal (34.6–60.3 %) was reported for co-digestion than for sludge mono-digestion (27.4–55.2 %).
– No ammonia inhibition or other process interference was observed when the proportion of poultry manure was increased, in the feedstock.
– Co-digestion did not significantly affect the composition of the obtained biogas, in both cases, the methane content was over 60 %, with the gas from the manure mixture being cleaner from H_2S and NH_3, while containing more CO on average.
– The digestate from the waste mixture contained more Ca, K and NH_4^+, while a higher amount of Mg, Na, P and Fe was found for the sludge digestate product. The concentrations of other elements were at similar levels for both samples, and the determined values met the criteria in the legal acts governing the possibility of agricultural use of the digestate.
– The GHG emission calculations results indicate the advantage of the process of anaerobic co-digestion of poultry manure over other methods of managing this waste.
– The treatment of multiple wastes, combined with an increase in the efficiency of anaerobic digestion (AD), is in line with the new EU policy supporting an increase in the share of renewable energy in the overall energy balance, as well as sustainable waste management and the rational use of natural resources.
– Nonetheless, implementing this solution within the wastewater treatment facility demands further investigations, including determining optimal anaerobic digestion parameters (e.g. HRT, OLR) and comprehensive solutions about wastewater treatment plant infrastructure (e.g., regarding pumping co-substrate into digestion chambers). In addition, in further research it is worth considering, among others: 1) the issue of the possibility of inhibiting the process not so much as a result of the accumulation of ammonia nitrogen but rather the impact on the efficiency of the process of antibiotics present in poultry manure (determination of inhibiting levels); 2) the evaluation of the ecological influence of the suggested resolution employing LCA instruments; 3) reduction of high concentrations of ammonia nitrogen in the supernatant, 4) changes in the population of microorganisms during the process.

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CRediT authorship contribution statement

Erik Meers: Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Ana Robles Aguilar:** Writing – review & editing, Methodology, Investigation. **Anna Jasińska:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Grosser:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests Anna Jasinska reports financial support was provided by Horizon 2020 (grant agreement No 773682). Anna Jasinska reports financial support was provided by Czestochowa University of Technology (Internal grant No BS_PB-400–301_24). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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