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Sequential Analysis of Phosphorus Compounds Contained in the Substrates and the Digestate

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Abstract: The aim of this study was to determine the properties of the components used for fermentation and digestate. The content of phosphorus and its fraction in the substrate mixture undergoing fermentation in the real agricultural biogas plant and in the digestate was determined. The research was carried out based on substrate and digestate samples from an agricultural biogas plant with a production capacity of up to 1 MW (Mega Watt). The biogas plant operates in a continuous system. To ensure optimal operating conditions of the installation, it is necessary to provide substrates with appropriate quality and composition throughout the year. The substrate consists mainly of maize silage, poultry manure and potato pulp. In the study, samples of individual substrates and digestate were taken in four terms. The time of collection depended on the fraction of individual substrates in the charge composition. In the first term, the fraction of three substrates in the orchard was as follows: 79%—maize silage, 15%—poultry manure, and 6%—potato pulp. In the second term, it reached 82%, 10% and 8%, respectively, in the third 83%, 8% and 9%, and the fourth 80%, 6% and 14%. Eight samples of the substrates and digestate were collected every time. The samples were determined considering the content of dry matter, organic matter and the total content of phosphorus, magnesium, calcium, potassium, nitrogen, iron, and the pH value. After drying the samples, the fractions were determined by the phosphorus method with the Sequential Chang–Jackson extraction with subsequent modifications by Petersen and Corey. Measurements were made using the ICP-AES method. The following fractions were isolated: P-lab. (labile) labile phosphorus, P-Al phosphorus in aluminium phosphates, P-Fe phosphorus in gel phosphates, P-red.(reduced), P-ok. (occluded) the fraction of occluded phosphates absorbed on the surface of mineral particles, P-Ca phosphorus in calcium phosphates. It was found that the physicochemical composition of the feed and digestate was determined by the content of about 80% of maize silage in fermented substrates. The addition of substrates in the form of poultry manure and potato pulp influenced the content of total phosphorus and slightly modified the content of individual fractions in it. The fermentation process, to some extent, decreased the share of fraction I (mobile) and fraction II (combined with aluminium), increased the share of fraction VI (combination with calcium) and had no significant effect on the others. Digestate is a material rich in macronutrients and should be used as a fertilizer. The dosage of digestate used for fertilisation should be preceded by a knowledge of the soils and the requirements of the cultivated plants in order to prevent mobile phosphorus fractions from entering surface waters and increasing the eutrophication process.

Keywords: substrate and digestate composition; phosphorus; phosphorus fractions

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1. Introduction

Technological changes initiated in the nineteenth century influenced the global evolution of civilization, which has been developing until now. Civilization and technological progress essentially regulate the level of its consumption. Over the last century, the accelerated global nature of economic and technological development, aimed at improving living conditions, has led to increased threats to environmental protection of a global nature [1]. Rapid demographic growth, intensive farming, urbanization and industrialization have contributed to a steadily increasing demand for energy. It is assumed that by 2035 the global demand for energy will increase by 35%, even with the implementation of all solutions aimed at increasing energy efficiency [2]. Meeting these needs with the use of only conventional solutions would lead to significant environmental degradation, intense pollution of the atmosphere, and depletion of energy resources and finally dramatically cause the rising energy costs [3]. For many years, the intensive consumption of natural resources without respecting the laws of nature have led to an imbalance in nature and many ecological disasters. Constant fluctuations in economic activity, rising prices of raw materials, and increasing climate changes aroused the interest of the world market in new energy sources and modern energy technologies in the 1960s [4]. Along with the deteriorating condition of the environment, the emphasis was placed on expanding new development concepts. One of the concept is sustainable development, which is based on integrating economic, social and political activities while maintaining environmental balance [5]. Increasing use of renewable energy sources allow the country to be more independent from imported energy supplies. Moreover, the rising use of renewable energy sources impacts diversification of source supplies and creates conditions to develop distributed energy based on locally available raw materials [6]. One of the sources of renewable energy is biomass. Biomass consists of animal or plant biodegradable substances [7]. Biomass, which is obtained from the residues and waste of forest and agricultural production, the agri-food industry, and municipal management, can be used to produce electricity, heat, and biogas. The following types of biomass are used for energy purposes:

- primary energy resources (solid biofuels), including straw-cereal, legume and oilseeds, as well as hay, wood-waste from the wood industry and forestry, and used wooden packaging and production waste, yields from energy crops, dehydrated sewage sludge, pellets, briquettes, biocarbon and waste from the processing industry;
- secondary energy resources (gas biofuels), biogas produced, among others, from agricultural waste (slurry, manure, plant biomass), sewage sludge, waste in municipal landfills, agricultural and food industry waste: pulp, bagasse, molasses, wine, oil, cheese and dairy waste, as well as spoiled and expired vegetables and fruits;
- processed energy raw materials (liquid biofuels): biodiesel, vegetable oils, esters, biomethanol and bioethanol from agro-refineries and distilleries [8,9].

The use of biomass is beneficial when stable quality and low price of the produced energy or heat are ensured [10,11]. An adequately located biomass installation can reduce the use of conventional raw materials by limiting greenhouse gas emissions [7]. Biomass is an unsuitable raw material due to its susceptibility to microorganisms and the need to ensure adequate humidity. However, it is thoroughly prepared and has a high-energy potential [9]. Biogas plants make possible to use much troublesome waste on farmland, providing energy at the local level. The remainder of this process is a waste called digestate, which is not fully recognized in terms of its fertilizing values. The substrates subjected to the methane fermentation process are not entirely degraded. With this waste, a significant amount of carbon and other components, including phosphorus, can be introduced into the soil, which becomes a faulty component due to the limited resources of its raw materials [12–14]. Therefore, it is essential to use secondary sources of phosphorus, such as waste, and determine their potential use for fertilization purposes. Phosphorus mobility is related to the interaction of various macronutrients, such as calcium, magnesium, aluminium and iron. Therefore, it is worth knowing that in different fractions phosphorus is present. The analysis of phosphorus speciation can provide valuable information about the possibility of releasing and transforming this element from raw materials in the fermentation process, which gives recognition of its availability to crops. However, using hydrated waste for fertilisation, which contributes to the movement of constituents including phosphorus to water, can result in dangerous situations. Many pollutants penetrate beyond natural ecosystems and agroecosystems [15,16]. Environmental pollution by chemical compounds including phosphorus compounds has been increasing. Palanisamy and Parthasarathy report that an increase in chemical pollution in all elements of the environment, including phosphorus compounds, is observed [17]. Phosphorus is a biogen that comes from many sources in the environment, including the digestate. The digestate can be a source of nitrate emissions to groundwater, ammonia and nitrous oxide emissions to the atmosphere [18–20]. Phosphorus has also been included in the application of digestate for fertilization in accordance with the provisions of the Water Framework Directive [21].

It mainly influences the eutrophication process in surface waters. Eutrophication leads to reduced light penetration into the body of water and the death of plants and aquatic organisms that depend on them. The organic load and rotting of biomass falling to the bottom of the reservoir is observed, followed by the deoxygenation of the water and release of phosphorus to be reused in a new cycle, an increase in turbidity, hydrogen sulphide concentration and deterioration of taste and odour. This process leads to the degradation of reservoirs and rivers, which in turn contributes to the death of larger aquatic organisms such as fish. The limiting phosphorus concentration, above which intensive algal growth is observed, is 0.1 mg P-PO4/dm^3.

Most authors have carried out studies related to phosphorus speciation under laboratory conditions. The previous studies were related to real plants and the substrates used for fermentation in the proportions that are used in the plant. Therefore, the research aimed to determine the properties of the components used for fermentation and digestate, as well as the content of phosphorus and the share of its fraction in the mixture of substrates and the digestate.

2. Materials and Methods

2.1. Description of the Research Object

The research was carried out based on substrate and digestate samples from an agricultural biogas plant called Adler Biogaz, with a production capacity of up to 1 MW (Mega Watt). The biogas plant is located near the city of Bialystok and the geographical coordinates of the centre of this location are: 52.9333333 latitude degrees and 23.2666667 longitude degrees. The biogas plant operates in a continuous system. To provide optimal operating conditions of installation, it is necessary to ensure substrates with appropriate quality and composition throughout the year. The substrate consists mainly of maize silage, poultry manure and potato pulp. The substrate is brought by a screw feeder into the shredded substrate digesters. Approximately 30–35 m^3 of substrate is transferred daily from the first tank to the second one and from the second tank to the digester. Silage and potato pulp are stored in first tank and manure in second one. It is assumed that the daily demand of the system is approximately 42–44 tons of substrate. The composition of the substrate can be modified every day; however, the swapped substrates must be suitably energetic. The substrates undergo anaerobic fermentation in the digesters at temperature of 38–43 °C, which result in biogas. The entire process of agricultural biogas plant operation is controlled by the German automatic control system created in cooperation with BiogazTech and S.-S.B. Automatisierung. In addition to the gas converted into energy, the products of the reactions in the biogas plant are unfermented organic compounds and minerals; the so-called digestate.

2.2. Sampling and Testing Methodology

In the study, samples of individual substrates and digestate were taken four times. Substrates were sampled prior to their introduction into the tanks, from which they were directed to the fermentation chamber. The digestate was sampled directly after the fermentation process. The term of collection depended on the share of individual substrates in the charge composition. In the first term, the content of three substrates in the orchard was as follows: 79% of maize silage, 15%—poultry manure, and 6%—potato pulp. In the second term, it was 82%, 10% and 8%, respectively, in the third 83%, 8% and 9%, and in the fourth 80%, 6% and 14%. Eight samples of substrate and digestate were taken in each term. The samples were then mixed and taken for mean analysis. Meanwhile, analyses of each sample were performed three times. The results in the paper are given as means.

The samples were determined considering the content of dry matter, organic matter and the total content of phosphorus, magnesium, calcium, potassium, nitrogen, iron, and the pH value. The dry mass was determined as the residue after drying the samples at 105 °C to constant mass following the recommendations of PN-78/C-04541. The determination of the total organic compound consisted in burning the dried and homogenized sediment samples at a temperature of 600 °C following the recommendations of PN-EN 12879:2004. Total nitrogen was determined using the Kjeldahl method, PN-EN 13342:2002. The content of calcium, magnesium, phosphorus, potassium, and iron was determined after dry mineralization of the samples and dissolving the ash in nitric acid using the ICP-AES method. After drying the samples, the fractions were determined by the phosphorus method with the Sequential Chang–Jackson extraction with subsequent modifications by Petersen and Corey [22]. Measurements were made using the ICP-AES method (inductively coupled plasma atomic emission spectroscopy). In order to obtain correct and reliable results, each sample was analysed in triplicate. Before analysis, optimization of apparatus conditions was performed with a mixture of Ce, Co, Li, Tl, Y, Mg standards (Inorganic Ventures) with a concentration of 1 ppb in 2% HNO_3 solution. During the analysis, the addition of an internal standard (mixtures of Ge, Hf, Mo, Nb, Sb, Si, Sn, Ta, Ti, W, Zr) of 100 ppb was used to control the stability of the analytical process. Certified reference materials BCR-145R sewage sludge (Institute for Reference Materials and Measurements) and NCS DC 73,023 soil (National Analysis Center for Iron & Steel) were used during the analytical procedure. Recoveries of the tested analytes in the range of 91–103% were obtained.

The following fractions were distinguished:

- P-lab. (labile): labile phosphorus, the fraction of easily soluble phosphorus compounds, extracted with 0.1 M NH_4Cl solution;
- P-Al: phosphorus in aluminium phosphates, extracted with 0.5 M NH_4F solution,
- P-Fe: phosphorus in iron phosphates, extracted with 0.1 M NaOH solution;
- P-red. (reduced): extracted with 0.3 M sodium citrate solution and sodium dithionite;
- P-okl. (occluded): fraction of occluded phosphates, absorbed on the surface of mineral particles, extracted with 0.1 M NaOH solution;
- P-Ca: phosphorus in calcium phosphates, extracted with 0.25 M H_2SO_4.

While determining the above fractions, some modifications were made: the pH value of the ammonium fluoride solution used to extract aluminium phosphates was raised from pH 7.0 to 8.5 because at a lower pH value in the extract, apart from the expected P-Al fraction, there were also significant amounts of phosphorus that were iron related; increasing the pH value to 8.5 allowed for selective determination of the mentioned forms [23].

2.3. Statistical Analysis of the Study Results

Statistical analysis was performed using the computer programme STATISTICA, version 12. An analysis of variance: one-way classification ANOVA was used to compare several populations. It is a technique for examining outcomes (dependent or explanatory variables) that depends on a single acting factor (clustering or classification factor). It was tested as to whether the analysed factors had an effect on the observed results. The aim was to test the significance of differences in population means. Using analysis of variance, the significance of differences between means from multiple groups (populations) was tested. If the means were significantly different from each other, it could be concluded that the analysed factor influenced the dependent variable.

Means belonging to two different groups differed significantly, and those belonging to one group were univariate (homogeneous) from a statistical point of view. A significance level of $p = 0.05$ was adopted ($p > 0.05$—non-significant differences, $p < 0.05$—statistically significant differences). Multiple comparison procedures were used; post-hoc tests, which are based on comparing differences between pairs of sample means with the size of the least significant difference (NIR).

The Tukey HSD (Honestly Significant Difference Test) was chosen, which is recommended for comparing pairs of means. The results of Fisher's NIR, Bonferroni test and

Scheffe test were also calculated and the same conclusions were obtained as from the Tukey HSD test.

3. Results and Discussion

3.1. Physico-Chemical Properties of Substrates and Digestate

Each of these substrates determines the final composition of the feed used for biogas production and impacts the composition of the digestate. The results of the properties of the substrates used in fermentation are presented in Table 1.

Table 1. The physicochemical composition of substrates used in fermentation.

Component/Substrate	DM	OC	N	P	K	Ca	Mg	Fe	pH
	g/kg	g/kg DM							
Maize silage	300	850	20.5	4.2	10.4	42	21	13	4.8
Poultry manure	400	760	17.2	21.8	20.0	40.0	7.2	1.5	7.3
Potato pulp	106	810	2.5	1.5	15.2	2.5	0.16	0.002	5.2

The highest amount of dry matter was determined in poultry manure, while potato pulp was the most hydrated. Maize silage was the richest in organic matter, which dominated among the substrates sent to biogas plants. Potato pulp was the poorest in macronutrients, with the highest addition to the feedstock at the level of 14%. The substrates' pH level varied, but in the case of the silage, it was the lowest, and for the manure, the highest. Significant differences were found for most components between the substrates. Potato pulp included significantly less calcium, while maize silage had significantly more iron compared to the other two substrates. The pH value of the substrates in maize silage and potato pulp was similar, and yet significantly higher in poultry manure.

After the fermentation of the substrates, the composition of the digestate changed. The dry matter content decreased and depended on the share of the input components (see Table 2). The content of macronutrients and organic matter in the digestate determined the types of raw materials used. At the same time, it was observed that the fermentation process contributed to the transformation of biogenic compounds and changed the physical and chemical properties of the digestate, comparing the raw materials used. In comparison to the batch substrates, the digestate was characterized by a lower organic matter and nitrogen content but a higher pH value. During the methane fermentation processes (hydrolysis, acidification, acetogenesis and methanogenesis processes), easily degradable organic compounds decomposed and passed into the gas phase, resulting in reduction in dry matter content and organic matter in the digestate compared to raw materials. Lignin and cellulose (hemicellulose) by Möller remained in the digestate (as the main components of organic matter) [24]. Their content was the highest in the digestate plant, where the content of poultry manure in the substrate reached 15%. It was found that the dry matter decreased with the lower proportion of poultry manure in the fermented substrate. There were no significant differences in dry matter content in the digestate taken in term II compared to term IV. In the other samples, dry matter content differed significantly. The organic matter content in most of the digestate samples was significantly differentiated. Sadecka et al. [25] report that the share of poultry manure was up to 20% and silage was up to 80%, which was fermented, resulting in the digestate containing the highest amount of dry matter and organic matter. Similar observations were made by Bachmann et al. [26], stating that the content of dry matter and organic matter in the digestate obtained from co-substrates with animal excrements was higher than in digestate without their participation. The composition of the feedstock influenced the composition of the digestate, as reported by Tuszyńska et al. [27]. The research team investigated the composition of animal manure, agricultural and food waste and sewage sludge and their digestate products.

Table 2. The physicochemical composition of the digestate.

Component/Term	DM	OC	N	P	K	Ca	Mg	Fe
	g/kg	g/kg DM						
I term	260	715	15.2	18.1	8.1	2.1	0.6	7.0
II term	230	720	13.2	17.4	7.3	1.5	0.8	7.3
III term	210	735	12.5	15.2	8.3	1.3	0.5	7.2
IV term	200	705	11.8	20.1	6.7	1.1	0.3	7.8

Notes: I term—79% of maize silage, 15%—poultry manure, and 6% potato pulp. II term—82% of maize silage, 10%—poultry manure, and 8% potato pulp. III term—83% of maize silage, 8%—poultry manure, and 9% potato pulp. IV term—80 % of maize silage, 6%—poultry manure, and 14% potato pulp. Phosphorus fractions I–VI.

The potassium content varied according to the composition of the input (term) and the differences were statistically proven. Calcium and iron were significantly less in the digestate samples with the highest proportion of ground pulp in the input. On the other hand, magnesium was significantly higher in the digestate with the highest proportion of poultry manure compared to the other samples. The content of nitrogen and phosphorus also depended on the proportion of poultry manure. The content of these components was significantly lowered by a higher proportion of potato pulp in the batch, which is evidenced by the results obtained in the digestate from the fourth term where the proportion of pulp achieved 14%. The composition of the feedstock had little influence on the value of the reaction of the digestate, which was close to neutral for all samples, but was significantly higher in the case of the digestate with the highest share of potato pulp.

3.2. Total Phosphorus Content of Substrates and Digestates and Their Sequential Analysis

The total phosphorus content in the digestate was higher than before fermentation (Tables 3 and 4). In both materials, the phosphorus content depended significantly on the poultry manure division and was higher in the samples with the highest proportion, which can be explained by the fact that easily soluble phosphates in the form of mineral additives are introduced into the poultry diet; hence, the high phosphorus content in the waste. The obtained results indicate that the fermentation process changed the share of phosphorus fraction in total phosphorus. The mobile fraction in the digestate decreased concerning the fermented substrates and was close to two percentage points on average. The highest amount of phosphorus was concentrated in the connections with aluminium in the substrates and the digestate. Tuszyńska et al. [27] found that phosphorus in the digestate occurred mainly in combination with aluminium and iron hydroxides.

Table 3. Phosphorus content in g/kg DM and the fraction share (%) in the substrates before fermentation.

Total Phosphorus/Fractions	I Term	II Term	III Term	IV Term	Mean
	7.2	6.8	6.2	5.1	6.3
I	13.5	14.6	18.8	19.2	16.5
II	62.7	62.4	57.0	59.9	60.5
III	12.2	15.5	12.0	12.0	12.9
IV	7.8	6.8	9.2	3.0	6.7
V	1.3	0.8	1.1	0.5	0.9
VI	2.4	1.0	3.0	2.8	2.3

Notes: I term—79% of maize silage, 15%—poultry manure, and 6% potato pulp. II term—82% of maize silage, 10%—poultry manure, and 8% potato pulp. III term—83% of maize silage, 8%—poultry manure, and 9% potato pulp. IV term—80% of maize silage, 6%—poultry manure, and 14% potato pulp. Phosphorus fractions I–VI.

Table 4. Phosphorus content in g/kg DM and the fraction share (%) in the digestate.

Total Phosphorus/Fractions	I term	II term	III term	IV term	Mean
	9.3	8.1	7.5	6.8	7.9
I	10.2	11.6	15.8	21.8	14.9
II	52.7	48.4	47.0	46.9	48.8
III	12.2	11.5	14.0	12.0	12.4
IV	7.8	11.8	9.2	8.0	9.2
V	1.3	0.8	1.1	0.5	0.9
VI	12.4	11.0	9.2	8.8	10.4

Notes: I term—79% of maize silage, 15%—poultry manure, and 6% potato pulp. II term—82% of maize silage, 10%—poultry manure, and 8% potato pulp. III term −83% of maize silage, 8%—poultry manure, and 9% potato pulp. IV term—80% of maize silage, 6%—poultry manure, and 14% potato pulp. Phosphorus fractions I–VI.

There were slight differences in the proportion of individual fractions between the various ratios of substrates both before and after the fermentation process. The increasing share of poultry manure resulted in a significant reduction in the share of the soluble fraction in the substrates and in the digestate, and the increase in the share of the decoction in most samples increased it significantly. Similar relationships were noted in case of the second fraction. The proportions of the substrates did not cause such clear dependencies in the share of the third and fourth fractions in the mixture of substrates and in the digestate. The share of fraction III in the mixture collected in the second term differed significantly from the others and was higher than the others. In the fourth term, the share of fraction IV in the mixture was significantly lower. The digestate showed a slightly higher share of the fraction collected in the third term, and the IV fraction in the samples in the third term.

The results are different from those obtained by Bauer et al. [28]. Their research results results concerning the solid fraction of the digestate indicated that more than 52% of phosphorus was associated with calcium and magnesium ions contained in the crystalline forms hydroxyapatite and struvite. Kuo et al. [29] and Möller and Müller [30] confirmed that the fermentation contributed to the reduction of the content of readily soluble forms of phosphorus (compared to the total phosphorus) in the by-product of these processes, which is digestate, compared to the fractions contained in the input components. These researchers conducted research on digestate from catering waste, where phosphorus was mainly in the form of insoluble calcium salts ($3Ca_3(PO_4)_2 \cdot Ca(OH)_2$ and $Ca_8H_2(PO_4)_6 \cdot 5H_2O$), and in animal waste digestate, inorganic phosphorus fractions in combination with iron and aluminium were predominantly present. According to Pagliari [31], Pagliari and Laborski [32] mobile phosphates are found in animal faeces. Furthermore, phosphorus in digestate fractions occurred mainly as inorganic phosphorus and its content was determined by the input biomass. However, the proportion of phosphorus with high mobility potential (fractions I and II) was lower than the used raw materials. The results indicate similar relationships because the dominant fractions in the digestate are combinations of phosphorus with aluminium and iron. Bachmann et al. [26] report that the mobile forms of phosphorus in the digestate are about 70%, which is in accordance with the obtained results. The distribution of phosphorus in individual fractions depends on the composition of the feedstock and its susceptibility to degradation in the fermentation process. The obtained results differ slightly depending on the composition of the feedstock because the dominant substrate was maize silage, and the additives in the form of poultry manure and potato pulp slightly modified the content of individual fractions in total phosphorus, while affecting the total phosphorus content.

4. Conclusions

On the basis of conducted research, the following conclusions can be formulated:

1. The chemical composition of waste used in fertilisation, including the phosphorus content and its mobility, is crucial in terms of providing nutrients for plants, but also in terms of water protection and its susceptibility to eutrophication, which is determined by the availability of phosphorus.

2. The content of about 80% of maize silage in fermented substrates determined the physicochemical composition of the feed and digestate.
3. The addition of substrates in the form of poultry manure and potato pulp influenced the content of total phosphorus and slightly modified the content of individual fractions in it.
4. The fermentation process, to some extent, decreased the share of fraction I (mobile) and fraction II (combined with aluminium), increased the share of fraction VI (combination with calcium) and had no significant effect on the remaining ones. The share of phosphorus in the most mobile fractions decreased by 10% to 30% compared to the share of the raw materials used.
5. The share of bioavailable phosphorus in the fermentation was about 60% in relation to the total phosphorus content.
6. The digestate is a material rich in macronutrients and should be used as a fertilizer in appropriate doses for specific plants, so that nutrients, including phosphorus, do seep into surface waters.

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