

Article

Precipitation of Struvite from Supernatants Separated from Enzymatically Disintegrated Digested Sewage Sludge

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Abstract: This paper presents the results of a study on the precipitation of struvite from filtrate liquids obtained from fermented sewage sludge subjected to prior disintegration with the enzyme papain. The methane fermentation process was carried out for sewage sludge with the addition of four different doses of papain: 0, 1, 2, and 3% (*w/w*) with respect to the dry weight of sludge sent for fermentation. After 20 days of methane digestion, struvite precipitation was carried out from the obtained supernatants using different pH values (9, 10, and 11) and a $\text{PO}_4^{3-}:\text{NH}_4^+:\text{Mg}^{2+}$ molar ratio of 1:1:1. The results showed that the efficiency of removing phosphorus, ammonium nitrogen, and organic compounds from the supernatant after the enzymatic disintegration of sludge was high under all analyzed conditions. The highest efficiency of the precipitation of phosphorus (93.1%), ammonium nitrogen (59.8%), and organic compounds (35.0%) in the form of struvite was obtained for filtrate supernatants derived from sludge disintegrated with the addition of 3% (*w/w*) enzyme at pH = 11. The purity of precipitated struvite was determined by thermal analysis methods, and an appropriate theoretical model was used to describe the crystallization process in batch reactors.

Keywords: struvite; precipitation; phosphorus; ammonium nitrogen; supernatants; sewage sludge; disintegration; papain; fermentation; DSC/TG; struvite crystallization kinetics



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1. Introduction

Phosphorus recovery from wastewater can be carried out using a number of technologies. Among the most important are the technologies of biological phosphate removal from wastewater and chemical precipitation. The biological methods of phosphorus removal from the wastewater use processes of increased orthophosphate accumulation in the cells of selected microorganisms under anaerobic and anaerobic conditions include, for example, EBPR (enhanced biological phosphorous removal process) [1]. The chemical precipitation of orthophosphate (V) involves adding precipitating agents to wastewater and converting them into insoluble compounds, followed by removal through sedimentation. Precipitation can be carried out using salts of aluminum, iron, or calcium hydroxide [2–4]. The recovery of this element from wastewater and sewage sludge is of both ecological and economic importance. It is predicted that the world's reserves of phosphate deposits could run out within 50 to 300 years [5,6]. Most of the phosphorus extracted is processed into fertilizers, detergents, animal feed, and other products. Chemical and physical methods are also available, such as the MAP (magnesium–ammonium–phosphate) process and ammonia

stripping. The consequence of removing nutrient compounds from wastewater is an increase in phosphorus and nitrogen in excess sludge and the formation of chemical sludge. There is also uncontrolled precipitation and accumulation of compounds containing these elements at various points in the process line, such as from supernatants containing high concentrations of nitrogen and phosphorus.

The accumulation of struvite in sewage and sludge systems can result in the need for the frequent cleaning or replacement of infrastructure components, which generate additional operating and maintenance costs. In extreme cases, serious failures can occur, requiring costly repairs and the temporary halting of treatment plant operations. In addition, struvite can alter the hydrodynamic conditions of wastewater flow, which affects the efficiency of biological and chemical treatment processes. This is especially true of the spontaneous precipitation of hydrated ammonium magnesium phosphate ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, MAP—magnesium–ammonium–phosphate) [7–10].

The precipitation of phosphorus and ammonium nitrogen in the form of struvite has a high recovery efficiency of up to 90%. The biogenic elements recovered in this way are reusable and provide a valuable multi-nutrient fertilizer. There are many processes in the literature for recovering struvite from wastewater. The costs of these processes cannot be compared well due to differences in process conditions. The most effective strategies are a combination of biological methods for nitrogen removal, phosphorus recovery by struvite precipitation, and the use of sludge in agriculture or its thermal conversion. Combining these methods ensures high efficiency, cost minimization, and long-term sustainability [11,12].

The controlled precipitation and crystallization of difficult-soluble phosphate salts is a complex issue, both theoretically and practically. Decisive influences on the course and outcome of the process are pH, temperature, induction time, and the presence of impurities. Impurities can inhibit or catalyze the course of chemical precipitation reactions closely integrated with nucleation and crystal growth, significantly affecting crystal shapes and the chemical purity of the product [13–15].

Phosphorus can be recovered in the form of struvite using fluidized beds, fixed-fill beds, ion exchange, and fermentation processes, among others [16–19].

The disintegration of sludge prior to the anaerobic stabilization process is used to break strong chemical bonds, hindering the biodegradation of sludge, leading to the acceleration of the hydrolysis process. The disruption in the biological membrane and lysis of microorganism cells occurs, and as a result of this, the organic components of the cell become available to heterotrophic biomass, and bound intracellular water is released. One method of disintegrating sewage sludge is biochemical disintegration, which is based on the use of enzymes to biologically break down the organic parts of the sludge. Various enzymes can be used to disintegrate sewage sludge, such as cellulase, hemicellulose [20], papain, and lipase [21]. Papain is a proteolytic enzyme belonging to the cysteine protease group, which catalyzes the hydrolysis of peptide bonds in proteins. Its mechanism of action is based on the presence of a cysteine residue in the active center, which plays a key role in breaking peptide bonds. Cellulases degrade polysaccharides by hydrolyzing β -1,4-glycosidic bonds. Lipases, on the other hand, are enzymes that catalyze the hydrolysis of fats (triacylglycerols) to glycerol and free fatty acids, while amylases break down macromolecular carbohydrates by catalyzing the hydrolysis of glycosidic bonds. Proteolytic enzymes are distinguished from other enzymes by their broad specificity and ability to operate over a wide range of pH and temperature, making them an effective tool in sludge processing. Depending on the composition of the sludge, papain can be used alone or in combination with other enzymes (cellulase, amylase, and lipase) to comprehensively disintegrate the sludge [20–28].

The aim of this study was to precipitate magnesium–phosphate–ammonium salts from supernatants separated from digested sludge disintegrated with papain enzyme.

2. Results and Discussion

2.1. Characteristics of Supernatants

The characteristics of supernatants separated from digested sludge at four doses of papain enzyme at 0, 1, 2, and 3 (*w/w*) enzyme content to sludge dry weight content are shown in Table 1.

Table 1. Characteristics of supernatants after the methane fermentation process.

Indicator	Stage I 0%	Stage II 1%	Stage III 2%	Stage IV 3%
Phosphates, mg PO_4^{3-} /L	27.2	47.7	78.4	92.4
Phosphorus, mg P^- /L	8.9	15.6	25.6	30.2
Ammonium nitrogen, mg N-NH_4^+ /L	685.8	667.7	660.0	655.5
COD, mg O_2 /L	1800	1900	1900	2000

In the supernatants, after the methane fermentation process, the phosphate concentration was in the range of 27.2 to 92.4 mg PO_4^{3-} /L, and ammonia nitrogen was in the range of 655.5 to 685.8 mg N-NH_4^+ /L, and increased with the increase in the amount of the enzyme introduced into the reactor compared to the sludge introduced into the reactors. The content of organic compounds expressed by the COD index after the fermentation process was in the range of 1800 to 2000 mg O_2 /L. During fermentation, organic compounds containing nitrogen and phosphorus were hydrolyzed and decomposed, leading to the release of nutrients into the supernatants. An increase in the concentration of ammonium nitrogen resulted from ammonification. The introduction of the enzyme contributed to an increase in the release of nutrients compared to the control sample, probably due to the increased metabolic rate [29].

2.2. Precipitation of Struvite from Supernatants Separated from Non-Disintegrated Sewage Sludge

The phosphate concentration prior to the process was 27.2 mg PO_4^{3-} /L, while the concentration of ammonia nitrogen was 685.8 mg N-NH_4^+ /L. As the process time increased, the concentration of phosphate and ammonia nitrogen in the supernatants decreased. The efficiency of phosphate removal after 120 min, depending on pH, ranged from 78.3 to 80.5% (Figure 1), and of ammonia nitrogen, from 42.1 to 53.5% (Figure 2). The average phosphate removal rate after 0 min at pH = 9.0 was 62.9%, after 60 min, 70.2%, and after 120 min, 78.3%. In supernatants in which the pH was adjusted to 10.0, the average level of phosphate removal after 0, 60, and 120 min was 61.0, 74.0, and 79.0%, respectively. At pH = 11.0, the average level of phosphate removal was 65.4, 72.1, and 80.5% after 0, 60, and 120 min, respectively. A similar trend was observed for ammonium nitrogen. The removal of ammonium nitrogen after 0 min was 35.8, 36.4, and 42.4%, after 60 min, it was 40.7, 37.5, and 43.8%, and after 120 min, it was 42.1, 39.2, and 53.5% at pH = 9.0, 10.0, and 11.0, respectively. The removal efficiency of organic compounds was 11.1, 16.7, and 22.2% at pH = 9.0, 10.0, and 11.0, respectively.

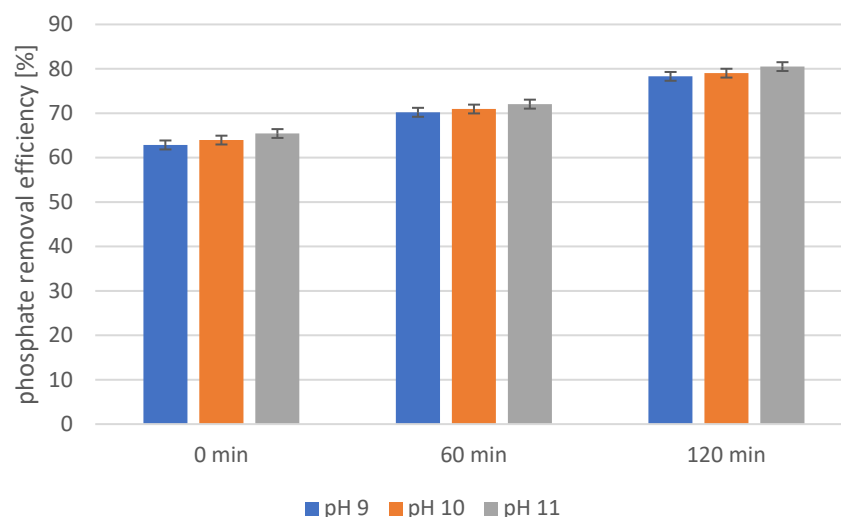


Figure 1. Efficiency of phosphate removal from supernatants separated from non-disintegrated sewage sludge.

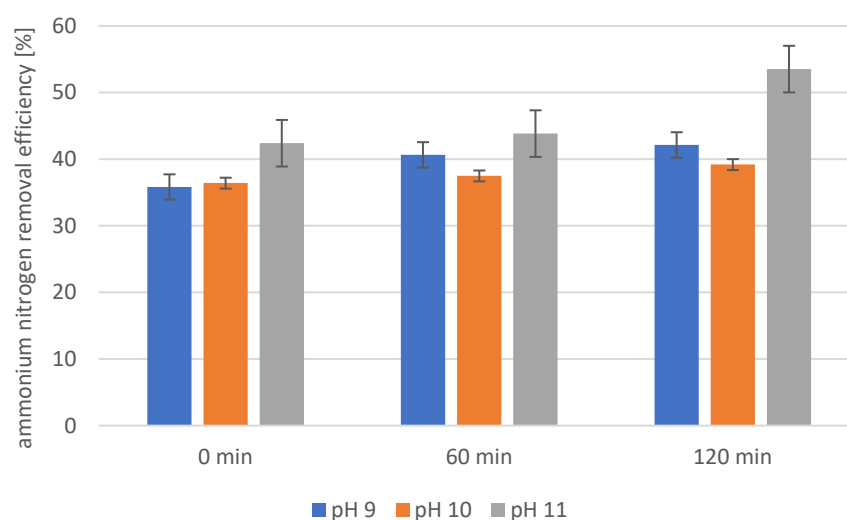


Figure 2. Efficiency of ammonium nitrogen removal from supernatants separated from non-integrated sewage sludge.

2.3. Crystallization Kinetics of Struvite from Supernatants Separated from Non-Disintegrated Sewage Sludge

In the case of a periodic-type crystallizer, in which the processes of nucleation, crystal growth, and disappearance, as well as aggregation and decay occur, the population balance equation can be expressed in the following form Equation (1) [30]:

$$\frac{\partial n(v, t)}{\partial t} + \frac{\partial [G(v)n(v, t)]}{\partial v} = b(v) - d(v) \quad (1)$$

where $n(v)$ is the density of particle population in the crystallizer, which is a function of particle volume v , $G(v)$ is the particle volume-dependent rate of crystal volume growth, $b(v)$ is the particle volume-dependent rate of particle formation (the “birth” rate), and $d(v)$ is the particle volume-dependent rate of particle disappearance (the “death” rate).

The initial condition for particle formation is Equation (2)

$$n(v, t = 0) = n_0(v) \quad (2)$$

and the rate of particle formation and disappearance due to aggregation can be described by the following Equation (3) [31]:

$$b_a(v) - d_a(v) = \int_0^{1/v} \beta(v-u, u)n(v-u)n(u)du - n(v) \int_0^\infty \beta(v, u)n(u)du \quad (3)$$

where $\beta(v, u)$ denotes the aggregation core (measure of the rate at which particles of volume $v-u$ collide with particles of volume u and the production of particles of volume v).

In the case of aggregate decay, the rate of formation $b_b(v)$ and the disappearance of particles $d_b(v)$ with volume v can be expressed by the following relation Equation (4) [32]:

$$b_b(v) - d_b(v) = \int_v^\infty S(w)\rho(v, w)n(w)dw - S(v)n(v) \quad (4)$$

where $S(v)$ denotes the aggregate decay rate, which is a function of particle size v , $\rho(v, w)$ denotes the particle size distribution function, defined as the probability that a particle fragment of dimension w will have dimension v .

The particle density distribution function can be described in the particle size variable x , instead of its volume variable v , then the population balance equation in the relation $n(x)$ instead of $n(v)$ is expressed, and the population balance (Equation (1)) takes the following form Equation (5):

$$\frac{\partial n(x, t)}{\partial t} + \frac{\partial [G(x)n(x, t)]}{\partial x} = b(x) - d(x) \quad (5)$$

This equation can be simplified, assuming that there is no formation and disappearance of particles due to aggregation (member (b) described by Equation (3) disappears) and no decay of aggregates (member (d) described by Equation (4) disappears) according to Equation (6):

$$\frac{\partial n(x, t)}{\partial t} + \frac{\partial [G(x)n(x, t)]}{\partial x} = 0 \quad (6)$$

If it is further assumed that the crystals are geometrically similar to each other and can be characterized by only one characteristic dimension (L), there is no dispersion of crystal growth (for the same L : $G(L) = \text{const.}$), and McCabe's principle is satisfied (the growth rate of crystals does not depend on their size); Equation (6) can be written in the following form Equation (7):

$$\frac{n(L)}{t} + G \frac{dn(L)}{dL} = 0 \quad (7)$$

and from the population balance, the population density distribution of $n(L)$ crystals can be determined according to Equation (8):

$$n(L) = n_0 \exp\left(-\frac{L}{Gt}\right) \quad (8)$$

where

n_0 —germ population density.

The germ population density n_0 is related to the nucleation rate B and the crystal growth rate G Equation (9):

$$B = n G_0 \quad (9)$$

Hence Equation (10),

$$n(L) = \frac{B}{G} \exp\left(-\frac{L}{Gt}\right) \quad (10)$$

The total number of crystals (N_k) formed at time t can also be determined Equation (11):

$$N_k = n_o G t = B t \quad (11)$$

and the total mass of crystals (M_k) in 1 m³ of the suspension Equation (12):

$$M_k = N_k \rho_k 6 k_v (G t)^3 = 6 k_v \rho_k B t (G t)^3 \quad (12)$$

where ρ_k —density of crystals.

The model presented above was used to calculate struvite crystallization from supernatants separated from non-disintegrated sewage sludge. The mass of crystals calculated from the model (M_k —Equation (12)) was compared with experimental values (M_k^{exp}) obtained for three different pH values (9.0, 10.0, and 11.0) after one hour (3600 s) and two hours (7200 s) of crystallization.

The experimental values of precipitated crystals' masses were determined based on the data of percentage mass loss of PO_4^{3-} and NH_4^+ (expressed in mg/L), for each solution, which were summed together with the mass of Mg^{2+} (mg/L), calculated from its proportional percentage in struvite ($[\text{PO}_4^{3-}]:[\text{Mg}^{2+}]:[\text{NH}_4^+] = 1:1:1$), which are shown (M_k^{exp}) in Table 2.

Table 2. Model parameters: G—crystal growth rates and B—nucleation rate.

pH	t [s]	G [m/s]	B [1/(s·m ³)]
9.0	900	2.07×10^{-8}	3.7×10^7
9.0	3600	7.68×10^{-9}	4.3×10^6
10.0	900	1.67×10^{-8}	5.3×10^7

To carry out the model calculations of precipitated struvite crystal mass (Equation (12)), it is necessary to know the crystal growth rate (G) as a function of its characteristic parameter (L) and nucleation rate (B). Both of these quantities depend on both pH and crystallization time. The literature lacks these data for our process conditions. Therefore, the calculations (Table 2) were based on the literature data [33] given for a pH equal to 9.0 and 10.0 and crystallization times of 900 s and 3600 s.

The number of experimental points in the literature [33] (only two or three points) did not allow for the precise determination of the functional dependence of both growth and nucleation rates on crystallization time and change in pH value. Therefore, the only possible solution was to assume a linear dependence on both of these values as a function of crystallization time and pH value changes. This assumption generates additional errors, but as long as there are no precise experimental data on the changes in G and N under process conditions (conducting precise experimental studies to verify our proposed model will be our next step), it provides the only possibility to check the correctness of the applied model.

For the model calculations of precipitated struvite crystal mass (Equation (12)) under the studied process conditions (pH = 9.0, 10.0, and 11.0 and $t = 3600$ s and 7200 s), the converted values of crystal growth rate (G) and nucleation rate (B) parameters presented in Table 1 were used, assuming their linear change both as a function of crystallization time and change in pH value. The results of calculations carried out in this way (M_k) are shown in Table 3.

Table 3. Comparison of the experimentally determined struvite precipitation mass (M_k^{exp}) with the crystal mass calculated from the model (M_k).

pH	t [s]	M_k^{exp} [mg/L]	M_k [mg/L]	RE [%]
9.0	3600	359.3	336.3	6.4
	7200	373.5	380.0	1.7
10	3600	336.1	339.6	1.0
	7200	356.5	418.0	17.2
11	3600	347.8	342.7	1.5
	7200	468.4	459.8	1.8

The results of model calculations were compared with experimentally obtained data by calculating the relative mass error of precipitated struvite crystals (RE—Table 3), according to Equation (13):

$$RE = \frac{|M_k^{exp} - M_k|}{M_k^{exp}} \quad (13)$$

where

M_k^{exp} —experimentally determined mass of precipitated struvite (mg/L);

M_k —mass of struvite crystals calculated from the model (mg/L).

The results obtained (Table 3) show very good agreement between the model's predictions and the experimental data. In four of the six cases tested, the relative prediction error was less than 2%. The worst result (error of 17.2%) was obtained for the prediction of crystal masses obtained after 2 h of crystallization at pH = 10. This error may be due to the too far extrapolation of parameters concerning the crystal growth rate (G) and nucleation rate (B). This is due to the fact that in this case, they were extrapolated (Table 2) from a crystallization time of 900 s, up to a time of 7200 s (in other cases, the extrapolation was for crystallization times from 3600 s to 7200 s). Another reason for the errors may be the assumption of linearity in the change in G and B parameters during crystallization and with the change in pH.

However, in general, it should be stated that the very simplified model used provides very close prediction results to the experimental data. It seems that in the studied cases of struvite crystallization, there is no significant agglomeration of particles, and then the disintegration of these agglomerates, as the lack of consideration of these mechanisms in the model (lack of members: $b(v)$ —rate of particle formation (rate of “birth”) and $d(v)$ —rate of particle disappearance (rate of “death”)) does not significantly affect the prediction result (RE—Table 2).

The obtained results also suggest that the assumption of the linearity of changes in the nucleation rate and crystal growth rate seems to be acceptable, provided that the extrapolation of the experimental data is not too far.

The primary goal of this work was experimental research on struvite precipitation, not a precise mathematical description of this process. However, we wanted to see what prediction results could be achieved by this very simplified model. The obtained results showed that such a model gives an acceptable prediction of the investigated process. The use of the full model (our next work), by eliminating errors caused by the assumptions about the linear dependence of G and B on the crystallization time and pH value, and by taking into account the agglomeration and disintegration of agglomerates during the crystallization of struvite, should result in a very good description of the studied process.

2.4. Precipitation of Struvite from Supernatants Separated from Disintegrated Sewage Sludge with 1% Papain Enzyme

The phosphate concentration prior to the process was 47.7 mg PO_4^{3-} /L. At pH = 9.0, the phosphate concentration was 24.6, 19.7, and 11.3 mg PO_4^{3-} /L, at pH = 10.0, it was 14.3, 13.5, and 10.8 mg PO_4^{3-} /L, and at pH = 11.0–13.7, 10.9, and 9.3 mg PO_4^{3-} /L after 0, 60, and 120 min, respectively. Phosphate removal ranged from 48.4 to 80.5% (Figure 3).

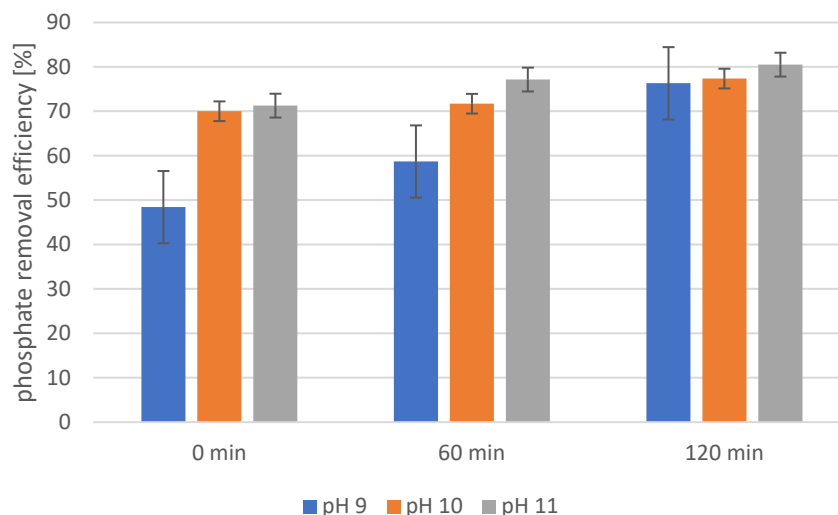


Figure 3. Efficiency of phosphate removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 1%.

The initial concentration of ammonium nitrogen was 667.7 mg N-NH_4^+ /L. At each pH value, the most ammonium nitrogen removed was at a process time of 120 min, and the concentrations were 53.1, 62.8, and 62.5 for pH = 9.0, 10.0, and 11.0, respectively (Figure 4).

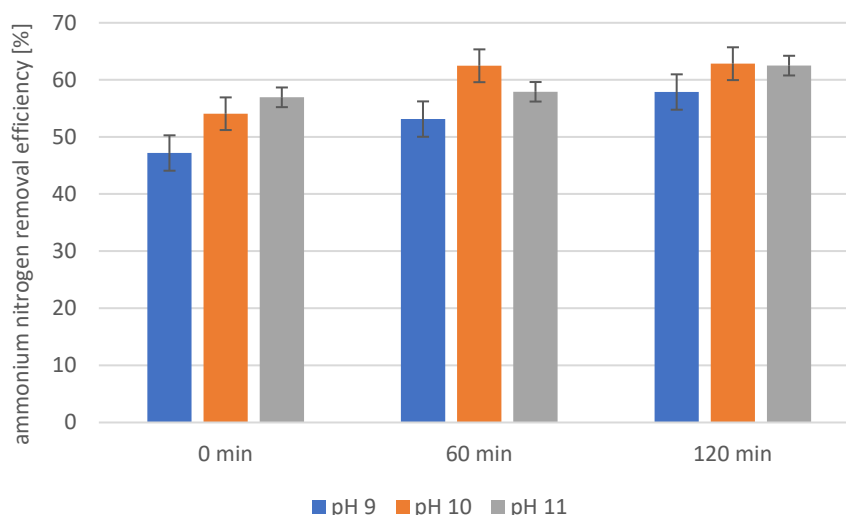


Figure 4. Efficiency of ammonium nitrogen removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 1%.

The removal efficiencies of organic compounds as expressed by the COD index were 10.5, 21.1, and 31.6% at pH = 9.0, 10.0, and 11.0, respectively.

2.5. Precipitation of Struvite from Supernatants Separated from Disintegrated Sewage Sludge with 2% Papain Enzyme

The efficiency of phosphate and ammonium nitrogen removal from supernatants separated from enzymatically disintegrated digested sludge with 2% papain enzyme is shown in Figures 5 and 6. The phosphate concentration prior to the process was $78.4 \text{ mg PO}_4^{3-}/\text{L}$. The efficiency of phosphate removal at pH = 9.0 was 77.3, 89.0, and 91.6%, at pH = 10.0, it was 85.8, 89.2, and 92.0%, and at pH = 11.0, it was 86.9, 89.9, and 92.3% after 0, 60, and 120 min, respectively.

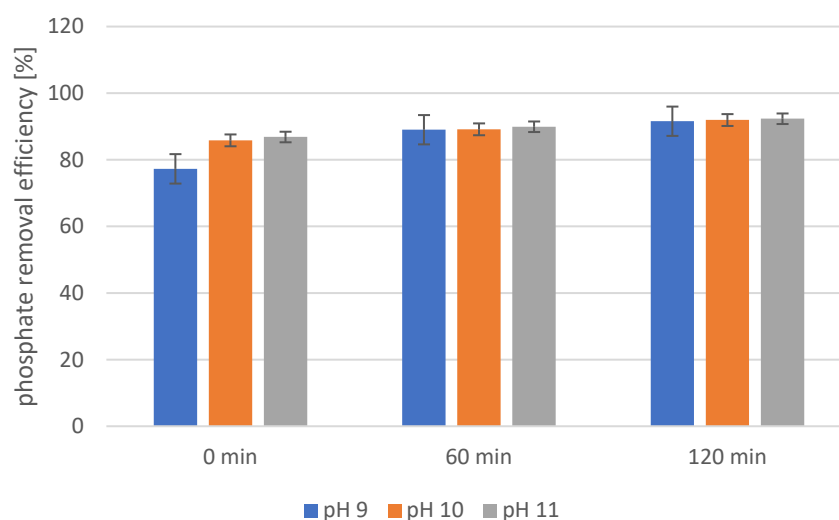


Figure 5. Efficiency of phosphate removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 2%.

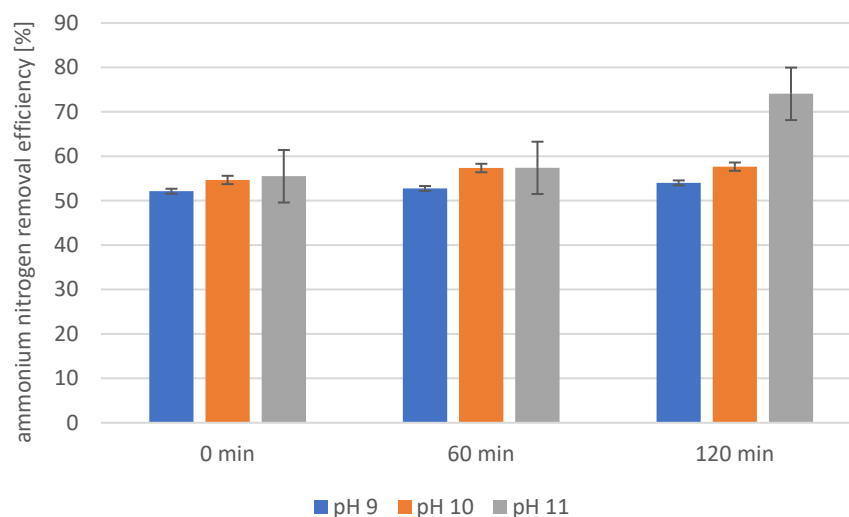


Figure 6. Efficiency of ammonium nitrogen removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 2%.

The initial concentration of ammonium nitrogen was $660.0 \text{ mg N-NH}_4^+/\text{L}$. The removal efficiencies of ammonium nitrogen at pH = 9.0 were 52.1, 52.8, and 54.0%, at pH = 10.0, they were 54.7, 57.4, and 57.7%, and at pH = 11.0, they were 55.5, 57.4, and 74.1% after 0, 60, and 120 min, respectively. COD removal efficiencies at pH = 9.0, 10.0, and 11.0 were 15.8, 21.1, and 31.6%, respectively.

2.6. Precipitation of Struvite from Supernatants Separated from Disintegrated Sewage Sludge with 3% Papain Enzyme

The phosphate concentration prior to the process was 92.4 mg PO_4^{3-} /L. Phosphate concentration at pH = 9.0 was 9.3, 8.1, and 7.8 mg PO_4^{3-} /L, at pH = 10.0, it was 7.3, 6.6, and 6.3 mg PO_4^{3-} /L, and at pH = 11.0, it was 6.6, 6.5, and 6.4 mg PO_4^{3-} /L after 0, 60, and 120 min, respectively. The efficiency of phosphate removal is shown in Figure 7. The initial concentration of ammonium nitrogen was 655.5 mg N-NH_4^+ /L. The concentration of ammonium nitrogen at pH = 9.0 was 365.2, 340.4, and 309.4 mg N-NH_4^+ /L, at pH = 10.0, it was 297.6, 284.9, and 282.4 mg N-NH_4^+ /L, and at pH = 11.0, it was 296.9, 282.5, and 156.3 mg N-NH_4^+ /L after 0, 60, and 120 min, respectively. The removal efficiency of ammonium nitrogen is shown in Figure 8. The removal efficiencies of organic compounds expressed by the COD index were 15.0, 25.0, and 35.0% at pH = 9.0, 10.0, and 11.0, respectively.

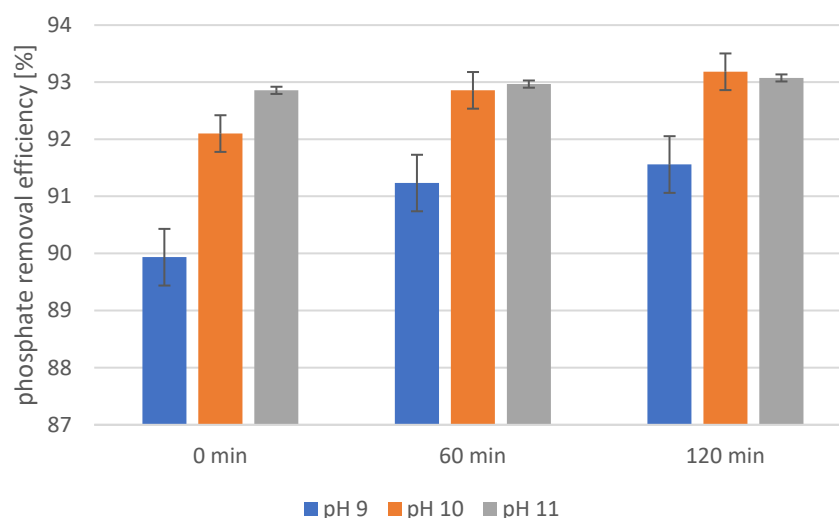


Figure 7. Efficiency of phosphate removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 3%.

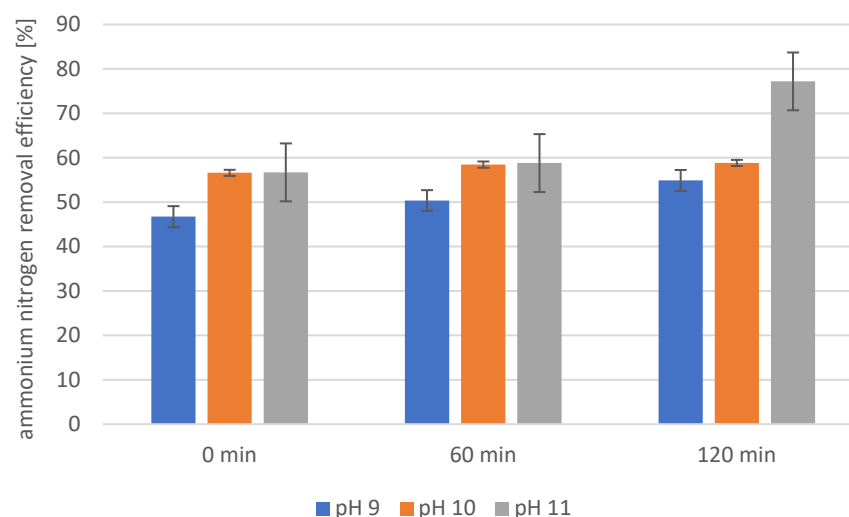


Figure 8. Efficiency of ammonium nitrogen removal from supernatant separated from disintegrated sewage sludge with the papain enzyme in the amount of 3%.

2.7. DSC/TGA Analysis

The amount of precipitated struvite ranged from 8.2 to 13.0 kg/m³ (Figure 9).

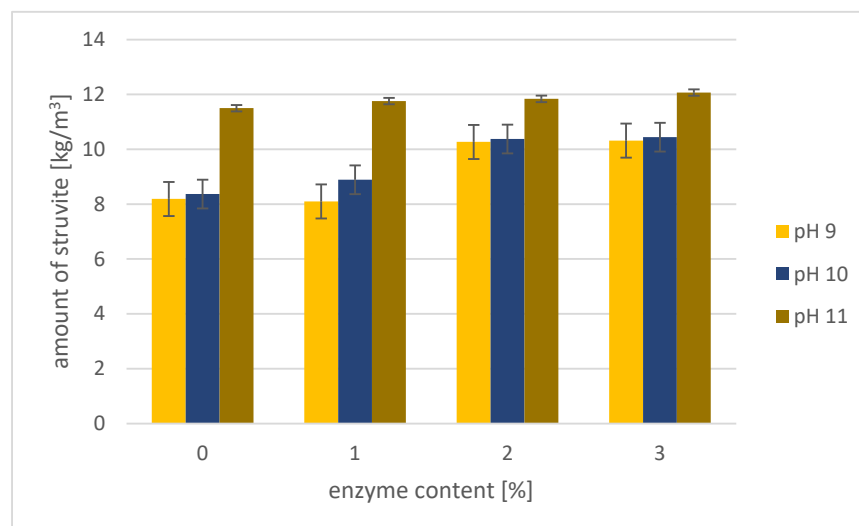


Figure 9. Amount of precipitated struvite.

The least amount of struvite in each case was precipitated at pH = 9.0, and the most at pH = 11.0. In the first step, struvite standard (Thermoscientific ammonium magnesium phosphate hexahydrate, 98%) and pure struvite precipitated from water were tested according to the adopted method. A single endothermic peak was observed in the DSC curves at ~100–110 °C (Figures 10 and 11 for the standard and struvite precipitated from water, respectively).

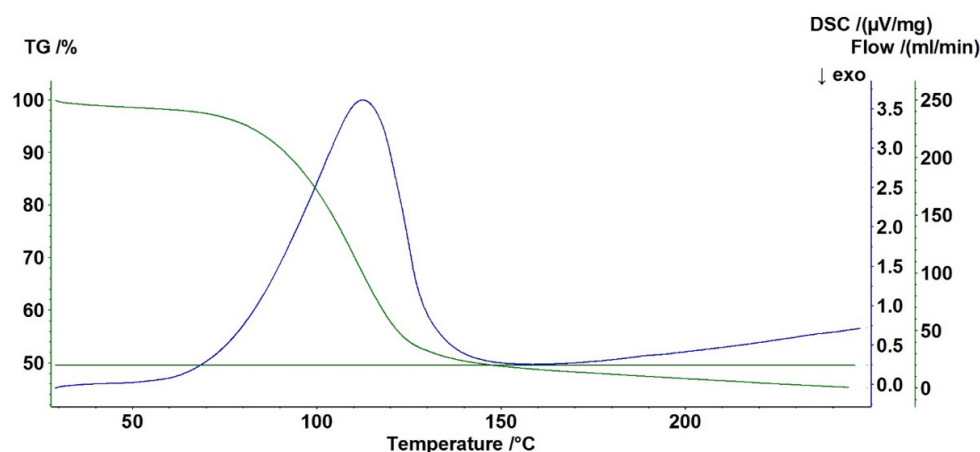


Figure 10. TGA/DSC curves of Thermoscientific ammonium magnesium phosphate hexahydrate, 98% for a heating rate of 5 °C/min from 30 to 250 °C.

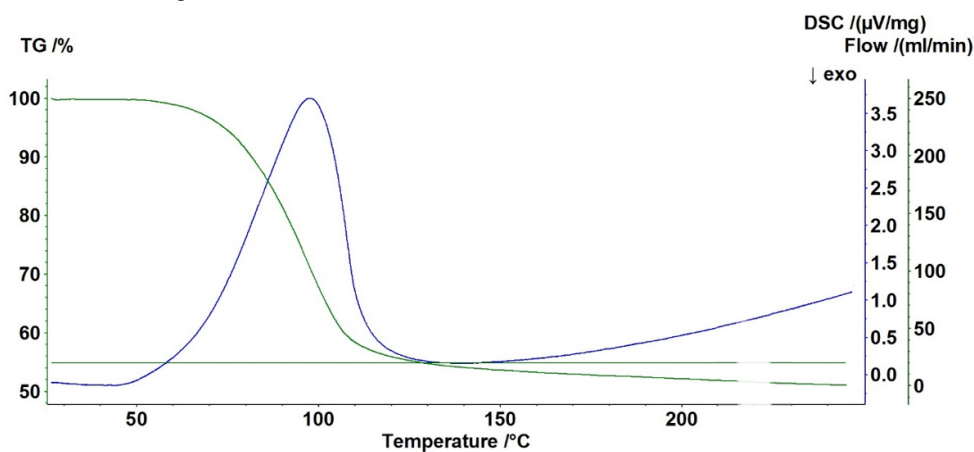


Figure 11. TGA/DSC curves of pure struvite for a heating rate of 5 °C/min from 30 to 250 °C.

The mass loss observed in the TGA curves (Table 4), in the temperature range from 30 to 150 °C (over a mass change range of 45–49%), corresponds to the theoretical mass loss associated with the complete dehydration of struvite (loss of one molecule of NH_3 and six molecules of H_2O -51%) [34].

Table 4. Weight loss of the struvite standard (Thermoscientific ammonium magnesium phosphate hexahydrate, 98%) and pure struvite precipitated from water in accordance with the method adopted in this work.

Temperature Range	Loss of Weight [%]	
	Standard	Pure Struvite
30 °C do 150 °C	49.22	46.89

For struvite precipitated from supernatants separated from non-disintegrated sewage sludge, a single endothermic peak at ~110 °C was also observed in the DSC curves in each case. From the DSC-TGA curves, it was found that the higher the pH, the purer the struvite was. Changes in struvite mass were observed in the range of 30 to 49% (Table 5). Very high agreement with the standard was obtained for struvite precipitated from enzymatically disintegrated liquids at 3% papain content. The presence of other ions such as magnesium, calcium, or sulfate ions affects the crystallization process of struvite, leading to the formation of competing precipitates and reducing the purity of struvite. The proper control of process parameters, particularly pH, can reduce competitive reactions and promote the precipitation of struvite formation. In addition, the use of selective precipitating reagents can be an effective method to improve recovery efficiency and final product quality.

Table 5. Mass loss of struvite precipitate from supernatant separated from non-disintegrated sewage sludge.

Temperature Range	Amount of Papain [%]	Loss of Weight [%]		
		pH = 9	pH = 10	pH = 11
30 °C do 150 °C	0	~30	~30	~35
30 °C do 150 °C	1	~30	~30	~35
30 °C do 150 °C	2	~40	~40	49.65
30 °C do 150 °C	3	48.10	48.70	49.14

3. Materials and Methods

3.1. Materials

Sewage sludge collected from the municipal wastewater treatment plant was used for the study. The municipal wastewater treatment plant carries out the biological treatment of wastewater, where the oxidation of organic compounds, defosfation, denitrification, and nitrification take place. Preliminary sludge is thickened by gravity, and excess sludge is thickened mechanically. The mixture of thickened sludge is subjected to methane fermentation. Stabilized sludge is mechanically dewatered. Supernatants from thickening and dewatering are returned to the main line of the treatment plant.

3.2. Precipitation of Struvite

The test stand consisted of three magnetic stirrers and three 500 mL reactors into which reagents were dispensed. In the supernatants obtained from the centrifugation of digested sludge, the content of magnesium ions, phosphate, and ammonium nitrogen was

determined in order to identify the molar ratio of $\text{PO}_4^{3-}:\text{NH}_4^+:\text{Mg}^{2+}$. To obtain a molar ratio of 1:1:1, KH_2PO_4 and MgCl_2 were supplemented. MgCl_2 in amounts ranging from 168.7 to 176.8 g/L and KH_2PO_4 in amounts ranging from 3.7 to 8.7 g/L were dosed to the filtrate liquids [35].

3.3. Course of Study

Four mixtures were prepared for methane fermentation studies. One was municipal sludge (excess and digested sludge added as inoculum). To three mixtures (municipal sludge with composition as above), papain (Glentham Life Sciences, Corsham, UK, 6000 U/mg) was introduced in an amount representing 1, 2, and 3 (*w/w*) by weight with respect to the dry weight content of the sludge. Fermentation studies were conducted in 1 L glass reactors, with a single feed, no light, and with the ability to measure biogas pressure. The sludge was incubated in a thermostat for 20 days, at a constant temperature of 37 ± 2 °C. After the digestion process, the sewage sludges were centrifuged to obtain the supernatants [27,36].

Supernatants in a volume of 300 mL were introduced into glass reactors and set on a magnetic stirrer. The study was conducted in a batch reactor. The appropriate reactants were then dosed to obtain a molar ratio of $\text{PO}_4^{3-}:\text{NH}_4^+:\text{Mg}^{2+} = 1:1:1$. The study was conducted in four stages corresponding to the dose of papain enzyme—0 (control sample), 1, 2, and 3% (*w/w*) of enzyme content to the dry weight content of sludge headed for methane fermentation. Each stage was divided into three series differing in pH value. The pH was adjusted to pH = 9.0, pH = 10.0, and pH = 11.0 using NaOH. In each series, experiments were conducted in three parallel replicates. The samples were then stirred for 30 min at about 100 rpm. After this time, the stirrers were turned off and the samples were left over for sedimentation. After 0 (moment of stopping mixing), 60, and 120 min, phosphate concentration (by the molybdenum method using a DR6000 spectrophotometer (Hach, Loveland, CO, USA)) and ammonium nitrogen (Macherey-Nagel Nanocolor tube tests using a UV/Vis spectrophotometer (NANOCOLOR® UV/VISII, Macherey–Nagel, Dueren, Germany)) were determined, and chemical oxygen demand was determined prior and post-process (Macherey-Nagel Nanocolor tube tests using a UV/Vis spectrophotometer). After 120 min, the reactor contents were filtered through a previously dried and weighed tissue paper filter. The sieve and its contents were placed in the dryer for two hours, then weighed to determine the precipitate formed by weight. Struvite samples prepared in this way were subjected to thermal analysis. The simultaneous measurement of thermogravimetry (TG) and differential scanning calorimetry (DSC) was carried out using a STA 449 F3 Jupiter Netzsch (NETZSCH-Gerätebau GmbH) with a heating rate of 5 °C/min from 30 to 250 °C in a nitrogen atmosphere.

4. Conclusions

Based on this study, the following conclusions were made:

The chemical precipitation process of phosphate ions can be carried out with high efficiency at a pH of 9.0–11.0 and molar ratio $\text{PO}_4^{3-}:\text{NH}_4^+:\text{Mg}^{2+} = 1:1:1$.

Supernatants formed after the methane fermentation process are characterized by high concentrations of phosphorus and ammonium nitrogen of 27.2–30.2 mg P/L and 655.5–685.8 mg N- NH_4^+ /L, respectively, and a high content of organic compounds determined as the COD of 1800–2000 mg O_2 /L.

As the amount of added enzyme in the supernatants increased, the phosphorus and ammonium nitrogen concentrations and organic compound content were increasingly higher.

The efficiency of phosphorus removal from supernatants separated from non-disintegrated sludge was in the range of 62.9 to 80.5%, and for ammonium nitrogen, it was in the range of 35.8–53.5%.

The efficiency of phosphorus removal from supernatants separated from sludge disintegrated with papain at 1% (*w/w*) was in the range of 48.4 to 80.5%, and for ammonium nitrogen, it was in the range of 47.2–62.5%.

The efficiency of phosphorus removal from supernatants separated from sludge disintegrated with papain at 2% (*w/w*) was in the range of 77.3 to 92.3%, and for ammonium nitrogen, it was in the range of 52.1 to 74.1%.

The efficiency of phosphorus removal from supernatants separated from sludge disintegrated with papain at 3% (*w/w*) was in the range of 89.9 to 93.1%, and for ammonium nitrogen, it was in the range of 46.7 to 59.8%.

The removal efficiency of phosphorus, ammonium nitrogen, and organic compounds determined as COD depended on the pH and was highest at pH = 11.0.

The most struvite formed was from liquids separated from papain-disintegrated sludge at 3% (*w/w*) at pH = 11.

Thermal analysis methods used and the comparison with struvite standard allowed the determination of the optimal conditions for struvite precipitation: 3% papain content and pH = 9.0, 10.0, and 11.0.

The obtained results of struvite crystallization prediction allow us to recommend the used model for the description of the crystallization process in batch reactors.

The results highlight the importance of the enzymatic treatment of sludge as a method to increase the bioavailability of nutrients, which can help improve the efficiency of phosphorus recovery processes in wastewater treatment plants.

The enzymatic treatment of sludge as a method to increase nutrient bioavailability, which can help improve the efficiency of phosphorus recovery processes in wastewater treatment plants.

The phosphorus recovery method is of economic significance, fitting in with the concept of a closed-loop economy and the goals of sustainable development.

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