

Application of the Thermophilic Fermentation Method to Obtain Environmentally Friendly Organic Fertilizer

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ABSTRACT

In this study, the authors obtained samples of biological fertilizer by thermophilic fermentation of substrates of different compositions. Two types of effluent were studied in the experiment: food plant waste with the addition of cattle manure and liquid pig manure with litter cattle manure. To activate the process of obtaining fertilizer, the Agrarka biological preparation was added containing a complex of microorganisms and bacteria that accelerate the decomposition process. A detailed chemical analysis of the liquid and solid fractions of the raw materials and the obtained products was carried out for the content of total phosphorus (P), carbon (C), potassium (K), nitrogen (N), ammonium nitrogen (NH_4^+), organic matter, dry matter, cadmium (Cd), cobalt (Co), manganese (Mn), copper (Cu), nickel (Ni), mercury (Hg), lead (Pb), chromium (Cr), zinc (Zn), and arsenic (Ar). The indicators of acidity and ash content were determined and microbiological analysis was carried out. The obtained results showed that the thermophilic temperature regime of fermentation accelerated the decomposition process, positively affected the total content of the main nutrients in the studied substrates, and negatively affected the vital activity of microorganisms. In two types of effluents, there was a significant increase in the level of ammonium nitrogen by up to 60%, a decrease in the total carbon content by 15–30%, and dry and organic matter by 10–12% in both liquid and solid phases. However, in the effluent from food plant waste with the addition of cattle manure, the indicators increased by 13% in total carbon and by 8.2% in dry matter, and the ash content was 3 times lower than in the second effluent. Thus, thermophilic fermentation obtained two types of effluents with high-quality indicators corresponding to environmental and sanitary standards, since the content of microorganisms and heavy metals did not exceed the permissible limits.

Keywords: effluent, fertilizer, feedstock, total carbon, humus formation, digestate, centrate.

INTRODUCTION

Analyzing the current state of the issue of the use of organic fertilizers, it should be noted that over the past 10–12 years, their total number has decreased by 3–4 times [United Nations, n.d.; QazaqZerno, 2017; Eskov et al., 2018]. The shortage of organic fertilizers for the main consumers alone, primarily agricultural enterprises of various forms of ownership, is more than 65% [Zelonyye pobedy, 2022]. The consumer market has been significantly replenished by farms, mainly grain producers, and horticultural societies that do not

have and do not produce their organic fertilizers [Arthurson, 2009; Kosnikov et al., 2021]. In addition to a rather noticeable lack of organic fertilizers, problems of a different order arise when using them [Sanitary Control in the Food Industry, 2009; Al Seadi and Lukehurst, 2012]. Manure, as a rule, is used without appropriate preparation by direct application to the fields or, at best, accumulates and is kept in storage bunkers for some time, which is accompanied by a significant loss of organic matter and nitrogen [Sadchikov, 2017]. In Kazakhstan, manure is the most affordable organic fertilizer, but requires preliminary preparation,

without which the technological process can be disrupted. Depending on the method and duration of storage, organic waste loses 25–50% of organic matter and nutrients (primarily nitrogen N). Even greater losses are observed during freezing followed by thawing up to 70% [Kuszel and Lorenkowicz, 2015; Cavalli et al., 2016; Kurmanbayeva et al., 2022].

Manure treatment technology

Carbohydrates, fiber, and proteins have their optimal pH and retention time for decomposition [Cavalli et al., 2016]. This means that the decomposition of waste can be improved, as well as accelerated, by adapting environmental conditions to the degree of decomposition. Therefore, one way to solve the problem is anaerobic thermophilic fermentation of manure and manure effluents and their subsequent use as fertilizers [Bayazitova et al., 2022; Salkhozhayeva et al., 2022].

Table 1 [Bayazitova et al., 2022] shows the average losses of nitrogen and organic matter depending on the storage period. The use of fresh manure is associated with certain agrotechnical difficulties, which leads not only to the contamination of cultivated areas with weed seeds but also carries the risk of environmental pollution.

To eliminate the negative phenomena discussed above, a special technology of manure treatment is needed, which allows for increasing the concentration of nutrients, eliminating unpleasant odors, suppressing pathogenic microorganisms, and reducing the content of carcinogenic substances [Bayazitova et al., 2022]. Therefore, the study is aimed at obtaining high-value organic fertilizers using anaerobic thermophilic fermentation in a bioreactor to obtain natural biofertilizer, which contains a large amount of biologically active substances and trace elements. The main advantages of biofertilizers over traditional fertilizers (manure, litter, etc.), concerning nutrients, are their form, availability, ensuring a balanced ratio of nutrients for crops, and a high level of humification of organic matter [Kumar et al., 2015]. This type of fertilizer contains easily degradable

organic nutrients that are available for decomposition by microorganisms, improve soil fertility and structure, have a balanced composition, and maintain the stability of the ecosystem. They also contain a large amount of moisture, macro-, and microelements, which are gradually released and used by plants.

Effluent

Researchers studying the process of anaerobic digestion consider it exclusively as a process for the production of biogas and its processing [Zdeb, 2021; Shitophyta et al., 2022]. However, in the process of anaerobic digestion of organic matter, another valuable product is obtained, namely, the effluent which is a liquid material with solid particles in suspension, having such chemical-physical and agronomic characteristics that can be considered a good fertilizer [Alfa et al., 2014]. The process of anaerobic digestion leads to a decrease in organic matter but does not reduce the amount of nitrogen and phosphorus, while it mineralizes part of the organic nitrogen into ammonia nitrogen, making it easily accessible [Helias and Brockmann, 2014]. For biomass suitable for anaerobic digestion, agricultural waste, food plant waste, manure, etc. are included. The countries where the use of biogas technologies appeared among the first are Japan, Nepal, India, and China [Abanades et al., 2022]. There are practically no implemented biogas technologies in Kazakhstan. It should be noted that the waste of biogas plants (BGP) does not have a generally accepted name [Comparetti et al., 2013]. In the literature, they are designated by various terms: BGP waste, namely the solid and liquid fractions together (effluent), digestate (biogas sludge, biosludge), and centrate (liquid product of centrifugation, which is released during the dehydration of bulk material) [Eickenscheidt et al., 2014]. After the separation of the effluent, solid and liquid fractions are formed.

A mandatory condition on which the quality of the effluent depends is the temperature regime of fermentation of biomass, pathogenic microorganisms are not neutralized at elevated

Table 1. Average nitrogen and organic matter losses depending on the storage period

Losses	Substrate	Storage period		
		2 months	4 months	6 months
Total nitrogen	Organic waste	15-20%	25-35%	40%
Organic matter		20-25%	35-35%	50%

temperatures, weed seeds die, etc. In this regard, it can be considered that thermophilic fermentation is the most optimal. Carbohydrates, fiber, and proteins have their optimal pH and retention time for decomposition [Sridhar and Arinola, 1991]. This means that the decomposition of food waste can be improved and also accelerated by adapting environmental conditions to the degree of decomposition [Denisov, 2006]. Therefore, anaerobic thermophilic fermentation of food waste and manure, as well as manure effluents, improves the quality and accelerate the formation of biofertilizers [Appels et al., 2008].

The liquid residue of the fermented mass is highly environmentally friendly, since during thermophilic anaerobic fermentation of organic raw materials, pathogenic microflora in the manure is destroyed, and the unpleasant odor is neutralized [Eickenscheidt et al., 2014].

Digestate

Digestate is a by-product of anaerobic digestion, which is rarely considered industrial waste (only when it does not comply with current regulations, for example, in terms of nitrogen or pollutants). For the most part, it is a raw material for the production of natural fertilizers [Lin et al., 2017].

The composition of the digestate also varies depending on the composition of the organic matter entering the anaerobic BGP. If it consists only of animal husbandry wastewater, nitrogen consists mainly of its ammonia form, while if it is formed by isolated crops or agri-food waste, nitrogen is predominantly organic [Mantovi et al., 2009]. Some studies emphasize how the pH of the culture strongly affects the solubility of phosphorus and trace elements. An increase in pH shifts the chemical balance towards the formation of phosphate ($\text{HPO}_4^{2-} \rightarrow \text{PO}_4^{3-}$), and then its precipitation in the form of calcium or magnesium phosphate (for example, $\text{Ca}_3(\text{PO}_4)_2$). Changes in phosphorus may also depend on the form of binding of other elements, such as iron. In addition, the mineralization of magnesium, phosphorus, and nitrogen in combination with an increased pH of the culture can lead to the formation and crystallization of ammonium magnesium phosphate (MgNH_4^+ phosphate hydrate) [Mantovi et al., 2009]. Sulfur, one of the most important nutrients for plants, is most available in oxidized form, that is, sulfate (SO_4^{2-}). The

reactions of sulfur are affected by the redox level: the organic fraction of sulfate reacts with protons in the absence, which leads to the formation of hydrogen sulfide (H_2S) and other molecules that lead to an increase in pH and a decrease in the concentration of sulfate.

The assessment of the quality of potential effluent fertilizers has been carried out in many studies [Sogn et al., 2018], but due to the wide variety of raw materials used during the production of biogas, the nitrogen content was significantly different. In the studies of G. Qi, Z. Pan, Y. Sugawa, et al. [2018], the properties of the digestate from cattle manure obtained under mesophilic and thermophilic fermentation conditions were compared. Special attention was paid to bacteria that stimulated plant growth. It has been established that the properties of the effluent depend on the feedstock and the conditions of anaerobic digestion. Anaerobic digestion for the production of biogas and effluent is considered a sustainable alternative to non-renewable energy sources and inorganic fertilizers, respectively. It has been proven that the potential use of anaerobic effluent is an alternative to manure as a fertilizer [Mukhuba et al., 2018]. Samples of the resulting effluent and manure were tested for the content of nutrients, the composition of bacteria that promote plant growth, and the amount of heavy metals (HMs). High concentrations of potassium, phosphorus, and HMs were contained in manure. Compared with cattle manure, the effluent of fermented biomass had a lower content of HMs, the presence of a large amount of nitrogen, and a significant number of bacteria that stimulated plant growth, which confirms the potential ability of the effluent to increase soil fertility. S.I. Tarasov, D.A. Kovalev, and Yu.V. Karaev [2018] conducted a study of the influence of thermophilic and mesophilic modes of operation of BGP for the use of substrates of various types (semi-liquid cattle manure, liquid pig manure, liquid poultry litter) on the agrochemical characteristics of the effluent. It was found that the main humus-forming substances and biogenic elements of plant nutrition were preserved, despite a decrease in the content of organic matter. We did not observe any influence of the temperature regime of anaerobic digestion on the change in the properties of the substrates. It was found that the higher the temperature and the longer the substrate stayed in the reactor, the better the disinfection effect would be.

Consequently, the death of pathogens and parasites begins already during the mesophilic fermentation regime (33–38 °C) and reaches a maximum during the thermophilic regime (53–55 °C) [Riva et al., 2016]. Therefore, BGP waste has a significant advantage as a raw material for obtaining organic fertilizer compared to compost, manure, and fresh litter. Determining the effect of anaerobic digestion on the change in the content of biogenic elements in the liquid and solid fractions of effluent from crop and livestock waste is an important issue in agricultural production with a view to its further application in agriculture.

The purpose of the study was to obtain samples of environmentally friendly organic fertilizer by thermophilic fermentation from substrates of different compositions with the determination of the content of biogenic elements and sanitary and environmental indicators. This study was aimed at obtaining two types of fertilizer from the organic waste of different compositions, with the addition of activator microorganisms and conducting their comparative analysis to identify changes in the content of nutrients, which was necessary to recommend their further use in agriculture and thereby reduce the anthropogenic load on the environment and preserve resources from depletion.

MATERIALS AND METHODS

All experiments were carried out under anaerobic thermophilic conditions (52–55 °C) in a BUG-R bioreactor manufactured by the Association of BMP Enterprises, Vologda, Russia (Figure 1) with periodic stirring (1 time per day) for 15 days. A bioreactor is a vertically positioned cylindrical container with a stirrer inside it. In the lower part of the bioreactor, there is a cylinder jacket, a cavity filled with water through which the container with the substrate is heated. Water heating is carried out by one 5 kW heating element in automatic mode to a temperature of 52–55 °C.

The study was conducted in March–June 2022, at the laboratory of organic waste processing of the Shokan Ualikhanov Kokshetau University.

The Agrarka biological preparation was added to the effluents. Agrarka is a biological destructor for composting organic waste. It consists of living beneficial soil microorganisms, metabolites, and biologically active substances.

Agrarka was developed by scientists of the Saken Seifullin Kazakh Agrotechnical University (Kazakhstan) as a result of strain screening according to agroecological properties: soil nitrogen fixers; plant growth stimulators; destructors of complex compounds; inhibitors of growth



Figure 1. The BUG-R bioreactor, 1 – the heating element for water heating, 2 – the hatch, 3 – the valve for removing the resulting gas, 4 – the hole for draining the substrate and for cleaning and flushing the internal tank of the bioreactor, 5 – the shaft handle, 6 – the hole for loading the substrate

and reproduction of soil pathogens). Agrarka was added in a ratio of 1:1: 0.1 to the food plant waste (effluent No. 1) and animal husbandry waste (liquid pig manure and litter cattle manure, in a ratio of 1:4) (effluent No. 2).

Samples of organic raw materials and the obtained products were subjected to chemical analysis in the laboratory in a 3-time repetition.

The method for determining the mass fraction of moisture and the dry residue is based on determining the mass loss of an organic fertilizer sample when dried to a constant mass.

The samples prepared for analysis were taken after thorough mixing from at least five points. Samples weighing 15–20 g were taken to determine the mass fraction of moisture, and samples weighing 150–200 g were taken to determine the mass fraction of dry residue. The evaporation bowls were pre-dried in a drying cabinet at a temperature of 105–110 °C to a constant mass and weighed with an error of no more than 0.1 g.

To determine the mass fraction of moisture and dry residue, the samples of raw materials and fertilizers were put in a porcelain bowl and placed in a drying cabinet, preheated to a temperature of 105–110 °C, and dried for 5 hours. Then the bowl with the attachment was removed from the drying cabinet, air-cooled for 30 minutes, and weighed. Each subsequent weighing was carried out after drying for 30 minutes and air-cooling the bowl with the sample for 30 minutes. The analysis was considered complete if the difference between the results of the two subsequent weighings did not exceed 0.1 g [USSR Gosstandart, 1985b].

The method is based on the production of ash, the residue of mineral substances formed as a result of the complete combustion of the organic part of the product sample, and subsequent weight determination of the mass fraction of ash [USSR Gosstandart, 1977].

From an analytical sample of organic fertilizer, a weight of 3 to 5 g was taken into a glass beaker with an error of no more than 0.001 g. The weight of the sample was determined by the difference in the mass of the glass with and without the sample. The sample was wrapped in an ashless filter and placed in a porcelain crucible pre-calcined to a constant mass. When testing concentrates with significant moisture content, they were previously evaporated to a dry residue in a water bath and dried for 20 minutes in a drying cabinet at a temperature of 100–105 °C. The sample was then carefully charred on a small

flame of a gas burner. During the charring process, ignition is prevented, as it would lead to the loss of the sample. After charring the sample, the crucible was placed in a muffle furnace heated to a temperature of 500–600 °C (red-hot). The ashing was carried out until the black particles completely disappeared and the ash color turned white or slightly grayish. The crucible with calcined ash was transferred to the desiccator, cooled for 35–40 minutes, and weighed with an error of no more than 0.001 g.

The method of determining the pH is based on the preparation of an extract from a fertilizer followed by the determination of pH [USSR Gosstandart, 1985a].

The extract was prepared at a ratio of 1:5 (digestate: distilled water). After adding water, the sample was shaken for 3 minutes manually. Then part of the suspension was poured into a glass, into which the electrodes were then immersed. The readings were taken no earlier than 1.5 minutes after the drift stopped. The actual acidity was expressed in pH units.

Determination of the total nitrogen content in organic fertilizers is based on the mineralization of organic fertilizer when heated with concentrated sulfuric acid in the presence of hydrogen peroxide, a mixed catalyst, or in a solution of phenol in sulfuric acid.

To determine the total nitrogen, the dry residue of the organic fertilizer sample was used after determining the moisture content. The bulk of dry fertilizer was placed in a Kjeldahl flask, and 20 cm³ of concentrated sulfuric acid and 3 cm³ of hydrogen peroxide solution with a mass fraction of 30% were added. The contents of the flask were thoroughly mixed with light circular movements, ensuring complete wetting of the sample, and left for 12–15 hours. Then another 3–5 cm³ of hydrogen peroxide was added to the flask and placed in a hood on a flask heater with a gas burner. The Kjeldahl flask should incline 35° to the vertical. A special glass teardrop-shaped leaky stopper was placed in the opening of the flask and carefully heated, avoiding rapid boiling [Gosstandart of Russia, 1996].

The mass fraction of total nitrogen (X%) in the analysis of a dry sample of fertilizer was calculated using the formula:

$$X\% = \frac{0.0014 \times (V_1 - V_0) \times 250 \times 100}{V_2 \times m} \quad (1)$$

where: 0.0014 – the mass of nitrogen corresponding to 1 cm³ of sulfuric acid solution with a molar concentration of 0.05 mol/dm³ consumed for titration of the analyzed solution, g;

V_1 – the volume of sulfuric acid solution with a molar concentration of 0.05 mol/dm³ spent on titration of the analyzed solution, cm³;

V_0 – the volume of sulfuric acid solution with a molar concentration of 0.05 mol/dm³ consumed for titration in the idle experiment, cm³;

250 – the volume of the initial solution, cm³;

V_2 – is the volume of the analyzed solution taken for distillation, cm³;

m – the weight of the sample, g.

The method for determining the content of ammonium nitrogen in organic fertilizers is based on the extraction of ammonium nitrogen from a sample of organic fertilizer with a solution of hydrochloric acid with a concentration of 0.05 mol/dm³ with the determination of the ammonium content. The fertilizer sample in the state of initial humidity was placed in a flask with a capacity of 500 and 200 cm³ of hydrochloric acid solution with a concentration of 0.05 mol/dm³ was added. The flask was placed on a rotator and shaken for 30 minutes. The resulting suspension was filtered through a dry folded filter. The contents on the filter were washed with 2–3 portions (30–50 cm³ each) of hydrochloric acid solution. The volume of the filtrate obtained in the flask was brought to the mark with the same acid. The resulting solution was used to determine ammonium nitrogen by distillation using the Kjeldahl method (the Kjeldahl method is a multi-stage analysis that allows quantifying the content of bound nitrogen in organic compounds) [Gosstandart of Russia, 1996].

When using the installation, 35–50 cm³ of mineralizate was placed in the distillation flask, 30–40 cm³ of boric acid solution with a mass fraction of 4% was placed in the receiver, and 3–5 drops of the mixed indicator were added. The receiver was placed under the refrigerator so that the bubbler of the refrigerator was completely immersed in the solution of boric acid. 25–30 cm³ of

sodium oxide hydrate solution with a mass fraction of 40% was carefully added to the distillation flask through a funnel. The funnel was rinsed with distilled water so that the volume of liquid in the reaction flask was 100–150 cm³, heating was started and the solution was brought to a boil. Distillation was carried out until 2/3 of the liquid volume was distilled. The completeness of distillation was controlled by the breakdown of condensate with Nessler reagent and by indicator paper (the pH of the distillate should be 6–7 units). The contents of the receiver were titrated with a solution of sulfuric acid with a molar concentration of 0.05 mol/dm³ until the green color turned crimson. An idle analysis was carried out according to the same scheme without a fertilizer sample.

The total phosphorus content was obtained after the mineralization of dry organic fertilizer when heated with concentrated sulfuric acid in the presence of hydrogen peroxide. The determination of phosphorus is based on the measurement of the optical density of a colored phosphorus-molybdenum complex reduced to molybdenum blue. For the analyses, we used a mineralizate prepared for the determination of total nitrogen [USSR Gosstandart, 1975].

Preparation of a sample solution. 1.916 g of monosubstituted potassium phosphoric acid (KH₂PO₄) pre-dried to a constant weight at 105–110 °C was dissolved in distilled water in a measuring flask at 1 dm³, after which the volume was brought to the mark. 1 cm³ of the resulting solution contains 1 mg P₂O₅ and 0.66 mg K₂O. The solution was used to prepare comparison solutions. To do this, the following volumes of sample solution were placed in measuring flasks with a capacity of 500 cm³ (Table 2):

Distilled water was added to each flask to half the volume, after which 15 cm³ of concentrated sulfuric acid was added. After cooling, the volume of the solution in the flasks was brought to the mark with distilled water and thoroughly mixed.

The experiment. In conical flasks with a capacity of 100 cm³, 2 cm³ of the analyzed mineralizate, control solution, and comparison solutions were taken, and 50 cm³ of reagent B was added, mixed, and left for 30 minutes for the full development of coloring. The optical density of the

Table 2. Volumes of sample solution placed in measuring flasks with a capacity of 500 cm³

Amount of sample solution, cm ³	0	2	4	6	8	10	12	16	20	25
Mass fraction of P ₂ O ₅ in a dry sample, %	0	0.1	0.2	0.3	0.4	0.5	0.6	0.8	1	1.25

solutions was measured on a photocolorimeter with a red light filter (710 nm) at a layer thickness of 10 mm [USSR Gosstandart, 1985c].

Processing of results. Using the calibration graph, according to the results of determining the optical density in the analyzed solutions, the mass fraction of phosphorus (P_2O_5) in the dry sample (X_2) was found. An adjustment was made for the contamination of reagents and water with phosphorus according to the formula:

$$X_2 = X_1 - X_0 \quad (2)$$

where: X_2 – the true mass fraction of P_2O_5 in the analyzed sample, % of the dry sample; X_1 – the mass fraction of P_2O_5 in the analyzed sample, found according to the calibration graph, % of the dry sample; X_0 – the mass fraction of P_2O_5 in the control experiment, found according to the calibration graph, %.

The total potassium content was determined after the mineralization of dry organic fertilizer heated with concentrated sulfuric acid in the presence of hydrogen peroxide. For the analysis, we used a mineralizate prepared for the determination of total nitrogen.

The potassium content in the mineralizate was determined with a flame photometric analyzer [Rosstandart, 2008]. Preparation for work. As an initial sample solution, we used a solution prepared in the same way as in the experiment for determining the phosphorus content, where 1 cm³ contained 0.66 mg K_2O . The following volumes of sample solution were placed in measuring flasks with a capacity of 500 cm³ (Table 3).

Each flask was topped up to half the volume with distilled water, after which 15 cm³ of concentrated sulfuric acid was added. After complete cooling of the contents of the flasks, the volumes of solutions were brought to the mark.

The experiment. The working sample solutions were used to calibrate the device and build a calibration graph. Then, the radiation intensity of mineralizate obtained from samples of organic fertilizers and a control solution was measured. Processing of results. Using the calibration graph, the mass fraction of K_2O in the dry sample (X_1) was found as a percentage based on the results

of measuring the intensity of the radiation of the mineralizate. An adjustment was made for the contamination of reagents and water with potassium according to the formula:

$$X_2 = X_1 - X_0 \quad (3)$$

where: X_2 – the true mass fraction of K_2O in the analyzed sample, % of the dry sample; X_1 – the mass fraction of K_2O in the analyzed sample, found according to the calibration graph, % of the dry sample; X_0 – the mass fraction of K_2O in the control experiment, found according to the calibration graph, %.

The experimentally established values of MPC (maximum permissible concentration) (hygienic norms (HN) 2.1.7.2041-06) were used as sanitary and hygienic standards for the content of chemicals. However, the establishment of MPC is not always possible (n/a). In such cases, for rationing, approximately permissible concentrations are used (APC). The APC of cadmium is 1–2 mg/kg. The MPC of the gross arsenic content in fertilizers is 2.0 mg/kg [Al Seadi and Lukehurst, 2012].

The study of the effect of the fermentation process on the indicators of invasiveness of manure waste and food plant waste was carried out before and after the fermentation process under thermophilic conditions. Previously, the organic mass was diluted with water in a ratio of 1:1. Then samples were taken to identify the number of eggs and larvae of helminths. After sampling, containers with cattle manure and plant food waste were loaded into a bioreactor.

The detection of helminth eggs was carried out by the Fulleborn method (flotation method). For quantitative counting of helminth eggs, the method of V.N. Trach (1981) was used, which is not inferior in accuracy to foreign methods of counting helminth eggs in one unit of fecal mass and can be used for comparative accounting of infestation in animals before and after deworming.

To determine the number of microorganisms in 1 g of the sample, the method of sequential decimal dilutions was used, followed by seeding the material on nutrient media. The microflora of the selection was tested by seeding samples on

Table 3. Volumes of sample solution placed in measuring flasks with a capacity of 500 cm³

Amount of sample solution, cm ³	0	1	5	10	15	20	25	30	35
Mass fraction of K_2O in a dry sample, %	0	0.03	0.16	0.33	0.5	0.66	0.82	0.99	1.16

selective media. The identification of bacteria was carried out according to Bergey's manual of determinative bacteriology.

To study the presence of salmonella, the sample was placed in a flask with peptone buffer water. They were incubated at a temperature of 37 °C for 16–20 hours, followed by seeding on media with cystine selenite and Rappaport Vasiliadis (RV) broth. After incubation, a bacteriological loop was seeded from the storage media onto dishes with solid media: diamond green violet red agar and bismuth sulfite agar, followed by incubation.

The HM content in the obtained fertilizer samples was determined by atomic spectrometry with atomization in an Analyst 300 Perkin Elmer graphite furnace, according to the Price method [Price, 1976]. The control of the convergence and reproducibility of the measurement results was carried out by comparison in two parallel measurements.

RESULTS

When studying the agrochemical composition of effluent No. 1 (food plant waste and cattle manure with the addition of Agrarka), as well as effluent No. 2 (liquid pig manure and litter cattle manure), before and after fermentation (Table 4) it was found that the content of N total had decreased by 1.02 times, and N-NH₄ ammonium had increased by 2.3 times.

The composition of total phosphorus and potassium remained virtually unchanged in both samples. The organic matter content decreased by 1.49 and 1.47 times, respectively. The hydrogen index (pH) of both effluents was close to neutral.

The resulting effluents are an organic mass with a humidity of 63.5–99% of the alkaline reaction and a slight odor. The water pH of effluent samples from various raw materials in the alkaline range: centrate pH 6.8 to 8.2 and digestate 7.2 to 9.1 (Table 5).

Table 4. Agrochemical composition of the effluent obtained before and after fermentation in a BGP

Indicators	Effluent No. 1	
	Before fermentation	After fermentation
Acidity, pH	6.50±0.06	6.71±0.3
Humidity, %	90±0.20	89.1±0.2
N total, %	1.41±0.11	1.38±0.13
N-NH ₄ , %	0.28±0.03	0.65±0.16
Total phosphorus, %	0.93±0.06	0.92±0.03
Total potassium, %	4.10±0.06	4.09±0.13
Organic matter, %	36.7±0.20	24.6±0.1
Indicators	Effluent No. 2	
	Before fermentation	After fermentation
Acidity, pH	6.80±0.04	6.71±0.3
Humidity, %	92±0.40	89.1±0.2
N total, %	1.34±0.15	1.31±0.11
N-NH ₄ , %	0.21±0.03	0.55±0.15
Total phosphorus, %	0.78±0.02	0.76±0.01
Total potassium, %	5.10±0.06	5.09±0.12
Organic matter, %	31.2±0.10	20.3±0.2

Table 5. Agrochemical composition of organic raw materials, solid and liquid residues after the anaerobic fermentation process

Raw material	Dry matter/ Dry residue, %	pH	Ash content, %	C _{total} , %
Food plant waste with cattle manure and the addition of Agrarka. Effluent No. 1:	32.5	6.1	9.2	43.2
Liquid fraction	4.2	6.8	14.5	7.1
Solid fraction	39.8	7.2	8.2	35.6
Liquid pig manure and litter cattle manure, in a ratio of 1:4. Effluent No. 2:	24.3	9.2	35.2	30.2
Liquid fraction	10.2	8.2	22.7	38.2
Solid fraction	52.3	8.5	14.3	76.3

The change in pH values in effluents is associated with the transformation of organic compounds and is determined by the balance of organic acids, ammonia, and carbon dioxide. The liquid fraction (centrate) contains less than 10% dry matter and the main amount of potassium. Nitrogen in the centrate is contained mainly in ammonium form (up to 80% of the mass fraction of total nitrogen). The centrate from food plant waste contained 6 times more total nitrogen, 3.2 times more phosphorus, and 2.9 times more potassium compared to the feedstock (Figure 2). The utilization of organic waste by anaerobic digestion makes it possible to exclude wastewater

from livestock complexes from the category of hazardous waste and to obtain secondary raw materials in the form of liquid organic fertilizer. The centrate of pig manure and liquid cattle manure is characterized by a low content of total nitrogen, phosphorus, and potassium. Pig manure and litter cattle manure, compared with liquid manure, contain 3 times more nitrogen and phosphorus in their composition. The solid fraction after separation of the effluent contains about 30% moisture and is characterized by an increased pH (8.2–9.2) and low ash content (7.5–12.5%). The nitrogen content in digestate samples after processing of various raw materials is almost the same, at the

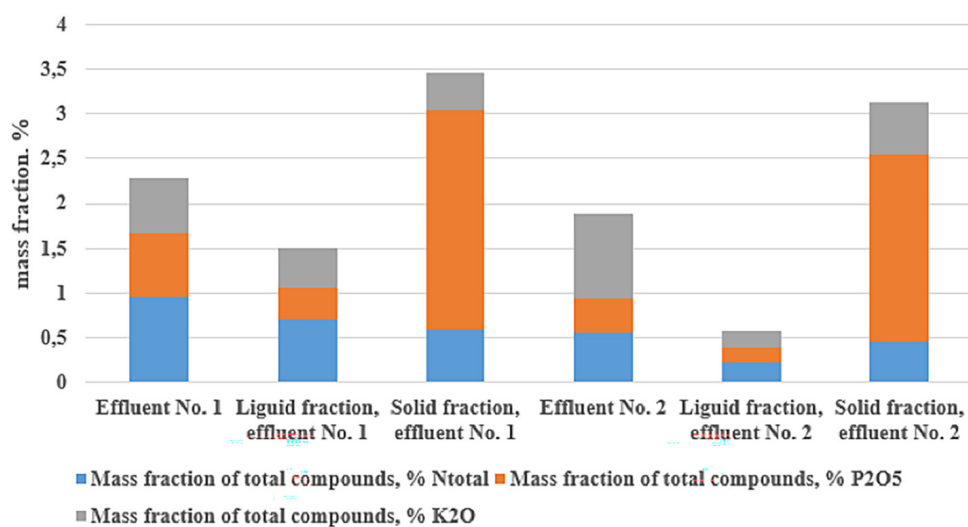


Figure 2. Mass fraction of total macronutrient compounds of organic matter after anaerobic thermophilic fermentation (raw matter)

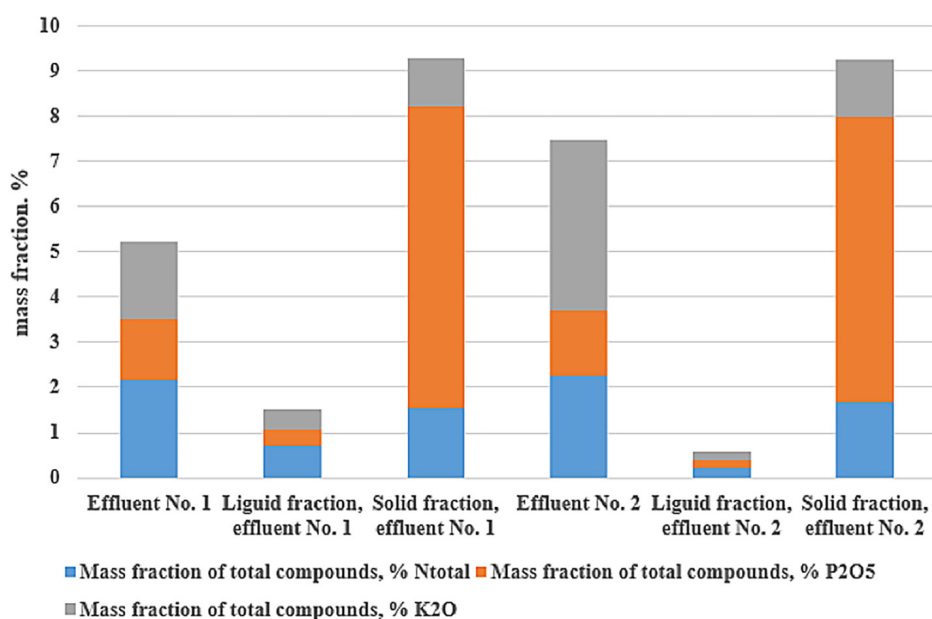


Figure 3. Mass fraction of total macronutrient compounds of organic matter after anaerobic thermophilic fermentation (dry matter)

level of 2% per dry matter, and the ammonium form is about half of the total nitrogen (Figure 3). The total phosphorus content varies from 6.68% per dry matter in the digestate after anaerobic digestion of food plant waste with cattle manure and the addition of Agrarka and up to 1.68% in the digestate from liquid pig manure and litter cattle manure. The phosphorus content in the digestate from food plant waste with cattle manure and the addition of Agrarka is 6.68% per dry matter. The solid fraction leaving the BGP is depleted in potassium compared to the liquid fraction.

Against the background of the low ash content of the digestate, a high total carbon content (more than 30%) was observed. The liquid fraction of the effluent after processing food plant waste with cattle manure and the addition of Agrarka contains C_{total} of 7.1% (Table 5). The liquid fraction of pig manure and cattle litter manure in its composition contains the largest amount of total carbon 38.2% (Table 5) from the studied raw materials. It was found that after anaerobic digestion, the liquid and solid fractions formed after separation are much more enriched with ammonium nitrogen (the most accessible form of nitrogen for plants) than the feedstock. The ammonium form of nitrogen is created as a result of the decomposition of nitrogenous organic compounds of the feedstock. Based on the generalization of study results on the agrochemical composition of liquid and solid effluent fractions, the possibility of their effective use in the production of organic fertilizers has been proved. 1 t of the centrate contains 0.6–7 kg of nitrogen, 0.4–2.3 kg of phosphorus, 1.2–4.2 kg of potassium, and 68–204 kg of carbon. From 1 t of the digestate, 11.8–15.3 kg of nitrogen, 5.4–66.5 kg of phosphorus, 4.9–12.2 kg of potassium, and 315–335 kg of carbon enter the soil.

Studies of the microbial state have shown the presence in the digestate of bacteria of the *Escherichia coli* group (0.1), which indicates slightly polluted soil when in polluted soils the coli-titer is 0.001 and lower (Table 6).

The detection of *Cl. perfringens* in the soil also indicates its fecal contamination. The soil layer is enriched simultaneously with bacteria of the

E. coli group and *Cl. perfringens*. After 4–5 months, the death of *E. coli* is noted, and *Cl. perfringens* is still detected in the titer of 0.01. Therefore, *Cl. perfringens* has a sanitary-indicative value only if its titer is determined in combination with the coli-titer and other indicators. In our studies, the amount of *Cl. perfringens* in both samples (effluent No. 1 (solid fraction) and effluent No. 2 (solid fraction)) showed > 0.1 , which characterizes the soil as slightly polluted. The presence of thermophiles in the samples indicates its contamination with organic matters of animal origin, in our samples their ratio is 0.0001, which also characterizes them as slightly polluted soil.

The average cadmium content in both samples does not exceed the APC and corresponds to its background concentration (Tables 7 and 8). The MPC value of the gross lead content for all types of soils is 32.0 mg/kg, which corresponds to the APC, in the fertilizer samples effluent No. 1 (solid fraction) shows a value of 5.38 mg/kg, while in the second sample (effluent No. 2, solid fraction), it is 4.95 mg/kg.

Mercury was not detected in the studied fertilizer samples; the MPC of mercury in the soil is 2.1 mg/kg (Tables 7 and 8).

According to the results of the study, the average gross arsenic content in the samples of fertilizers equaled 1.270 mg/kg for effluent No. 1 (solid fraction). In the second sample of effluent No. 2 (solid fraction), it was 1.244 mg/kg and did not exceed the permissible concentrations of arsenic (Tables 7 and 8).

Manganese is a metal with high redox potential and can participate in biological oxidation reactions, so manganese plays the same role for plant cells as iron does for animals. Manganese enhances hydrolytic processes, as a result of which the number of amino acids increases, promoting the assimilates formed during photosynthesis from leaves to roots and other organs (Tables 7 and 8). As a result of the conducted studies, it was observed that the average content of manganese in the samples of fertilizers (effluent No. 1, solid fraction) was 1.258 mg/kg, while in the second sample (effluent No. 2, solid fraction), it was 1.246 mg/kg (Tables 7 and 8).

Table 6. Microbiological indicators

Name of the sample	Quantitative titer	Titer of anaerobes (<i>Cl.perfringens</i>)	Thermophiles
1st BGP (solid fraction)	0.1	> 0.1	0.0001
2nd BGP (solid fraction)	0.1	> 0.1	0.0001

Table 7. The HM content in organic fertilizer samples (food plant waste with cattle manure and the addition of Agrarka (effluent No. 1, solid fraction))

Name of indicators	Unit of measurement	Normal values according to the regulatory documents (RD)	Result of the study	RD for the test method
Lead (max)	mg/kg	32.0	5.38	National standard of the Republic of Kazakhstan (ST RK) 2.377-2015
Cadmium mg/dm ³ (max)	mg/kg	not specified	0.009	ST RK 2.377-2015
Zinc mg/dm ³ (max)	mg/kg	not specified	1.89	ST RK 2.377-2015
Copper mg/dm ³ (max)	mg/kg	not specified	4.91	ST RK 2.377-2015
Arsenic (max)	mg/kg	2.0	1.270	ST RK 2.377-2015
Cobalt (max)	mg/kg	5.0	0.031	ST RK 2.377-2015
Mercury (max)	mg/kg	2.1	0.000	Mineral Fertilizer (MU) 08-47/203
Nickel (max)	mg/kg	not specified	3.64	ST RK 2.377-2015
Chrome (6+) (max)	mg/kg	6.0	0.00	ST RK 2.377-2015
Manganese (max)	mg/kg	not specified	0.258	ST RK 2.377-2015

Table 8. The HM content in organic fertilizer samples (liquid pig manure and litter cattle manure, in the ratio of 1:4) (effluent No. 2, solid fraction)

Name of indicators	Unit of measurement	Normal values according to the RD	Result of the study	RD for the test method
Lead (max)	mg/kg	32.0	4.95	ST RK 2.377-2015
Cadmium mg/dm ³ (max)	mg/kg	1.0–2.0	0.007	ST RK 2.377-2015
Zinc mg/dm ³ (max)	mg/kg	not specified	1.77	ST RK 2.377-2015
Copper mg/dm ³ (max)	mg/kg	not specified	4.45	ST RK 2.377-2015
Arsenic (max)	mg/kg	2.0	1.244	ST RK 2.377-2015
Cobalt (max)	mg/kg	5.0	0.026	ST RK 2.377-2015
Mercury (max)	mg/kg	2.1	0.000	MU 08-47/203
Nickel (max)	mg/kg	not specified	3.60	ST RK 2.377-2015
Chrome (6+) (max)	mg/kg	6.0	0.000	ST RK 2.377-2015
Manganese (max)	mg/kg	not specified	0.246	ST RK 2.377-2015

DISCUSSION

According to our results, it can be concluded that manure deserves attention as an effective organic fertilizer, which also contains all the elements of mineral nutrition necessary for the plant, where thermophilic fermentation conditions accelerate the decomposition of each component of waste. Food waste and animal husbandry waste have a high energy content, and they are ideal for achieving the dual benefits of energy production and fertilizer production [Han and Shin, 2004]. Animals absorb only 25% of organic substances and energy contained in the feed, and 75% of it goes to waste [Sadchikov, 2017]. In particular, an average of 50–80% of nitrogen, 60–80% of phosphorus, 80–90% of potassium, up to 90% of calcium, and up to 60% of undigested substances and other components pass into manure waste.

The properties of the effluent depend on the feedstock and the conditions of anaerobic digestion. In the study [Qi et al., 2018], the properties of fertilizers from cow manure obtained by mesophilic and thermophilic fermentation were compared. As a result of the anaerobic thermophilic fermentation, the rheological properties of the effluent improved. In comparison with unfermented manure, homogeneity increased in the effluent, the number of large particles decreased, the total content of suspended solids decreased, and the density of biomass decreased. Thus, after the conducted studies, it was found that the effluent from plant food waste and cattle manure after the process of enzymatic fermentation had acquired the qualities of highly effective organic fertilizers, the use of which can increase soil fertility. They contain forms of mineral nutrition elements available for assimilation by plants. After fermentation, the organic mass acquired a darker

color, which may indicate the appearance of dark-colored humic compounds.

Mineralization in natural manure is 40%, and in fermented mass, it equals 60%, as a result of which, during anaerobic fermentation of manure, the content of N-NH_4 increases fourfold (20–30% of organic N passes into ammonium form), and the content of assimilated phosphorus doubles compared to the unfermented manure [Macadi et al., 2012]. According to the results of our study, the thermophilic temperature regime of fermentation has a positive effect on the total content of the main nutrients in the studied substrates. There is a significant increase in the level of ammonium nitrogen in the effluent by up to 60%, a decrease in the total carbon content by 15–30%, and in the dry and organic matter content by 10–12%. With an increase in the content of ammonium nitrogen in the fermented effluent, an increase in pH values has been noted. Despite the decrease in the organic matter content, the amount of the main humus-forming matter in the effluent has not changed.

The content of nutrients, as well as the number of bacteria that stimulate plant growth, were analyzed. The potential use of an anaerobic effluent as an alternative to manure as a fertilizer was carried out in the study [Mukhuba et al., 2018]. In the effluent samples after the thermophilic fermentation regime, a noticeable improvement in veterinary, sanitary, and hygienic characteristics was observed, and a decrease in microbial contamination and the number of viable helminth eggs was noted. The presence of more mineralized nitrogen, a lower content of HMs, as well as a significant number of bacteria that stimulate plant growth, confirms the potential ability of the effluent to increase soil fertility.

From an applied point of view, the most important representatives of the group of HMs are highly hazardous, namely, lead, cadmium, mercury, and arsenic [Eskov et al., 2018; Mineralnye udobreniya, 2022; Zelonyye pobedy, 2022]. Arsenic is not a metal, but for several properties in ecotoxicological studies, it is classified together with the HMs [Al Seadi and Lukehurst, 2012; Arthurson, 2009]. The toxic properties of HMs have been known for quite a long time, but attention has been paid to them only in the last few decades. This is due to the increased role of HMs in biological processes caused by an increase in the intake of these elements into the environment during human economic activity [Sadchikov, 2017].

The spent biomass from BGP is recommended to be used as an organic fertilizer by combining a centrate or digestate with organic components. Additional components can be clay minerals (bentonite), solid or liquid animal waste (manure, poultry litter), if necessary, moisture absorbing material (peat, straw), and the addition of microorganisms for a certain temperature regime (no more than 60 °C) to protect biologically active substances from thermal destruction. During the manufacture of organic fertilizers, it is necessary to observe the ratio of liquid/solid fractions of BGP waste at the level of 1–1.5. Organic fertilizers based on BGP waste have significant fertilizing potential and will be effective if applied on medium and slightly acidic soils (with a pH of 4.6–5.0 and 5.1–5.5) for winter wheat, buckwheat, soy, and vegetable crops (cabbage, onions, cucumbers, carrots, tomatoes).

CONCLUSIONS

The studied waste from BGPs operating on different raw materials (food plant waste with cattle manure and the addition of Agrarka, pig manure, and litter cattle manure), according to agrochemical indicators are not inferior to other raw materials and have significant fertilizing potential, especially on acidic soils, given their alkaline reaction. It was found that the nitrogen and potassium content in the centrate samples exceeded the corresponding digestate values, but the phosphorus content was higher in the solid fraction (almost by 3 times). According to the content of the main elements of plant nutrition, the effluent after anaerobic digestion had the best percentage of waste after processing food plant waste with cattle manure and the addition of Agrarka. Effluents are characterized by low ash content and high total carbon content (up to 35.2%), which confirms their value for potential humus formation in soils.

Based on the generalization of the study results on the agrochemical composition of liquid and solid fractions of BGP, the possibility of their effective use in the production of organic fertilizers has been proved. 1 t of the centrate contains 0.6–7 kg of nitrogen, 0.4–2.3 kg of phosphorus, 1.2–4.2 kg of potassium, and 68–204 kg of carbon. From 1 t of the digestate, 11.8–15.3 kg of nitrogen, 5.4–66.5 kg of phosphorus, 4.9–12.2 kg of potassium, and 315–335 kg of carbon enter the

soil. The spent biomass from BGP is recommended to be used as an organic fertilizer by combining a centrate or digestate with organic components.

As a result of anaerobic thermophilic fermentation, the level of readily available forms of nitrogen in effluents increased, fermentation allowed to obtain environmentally safe samples of organic fertilizer disinfected from viable eggs of helminth larvae and pathogens, with improved rheological properties, low ash content, and high total carbon content.

The process of complex processing of manure and food waste simultaneously has three advantages: obtaining biogas, improving the environmental situation around livestock farms, and obtaining environmentally safe and agronomically effective fertilizers.

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