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# Impacts of Anaerobic Thermophilic Fermentation on Physicochemical Characteristics of Effluents Derived from Diverse Organic Feedstocks



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### ABSTRACT

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#### Keywords:

agricultural waste, anaerobic digestion, effluent, organic fertilizer, soil fertility, thermophilic fermentation

The study primarily investigates the chemical and physical properties of effluent produced via anaerobic thermophilic fermentation, aimed at addressing critical agricultural issues such as low soil fertility, diminished crop yields, and soil cover degradation. Raw materials, specifically cattle manure and vegetable food waste, were subjected to anaerobic thermophilic fermentation in a bioreactor. Potentiometric methods were employed to ascertain the acidity index, while the quantification of total nitrogen content was achieved through the wet oxidation of the test sample's organic substances, facilitated by heated sulfuric acid in the presence of catalysts. A widely accepted flotation method was utilized to detect helminth eggs, followed by a quantitative counting process. The study strived to determine the most effective ratios of organic waste components and other factors, thereby ensuring the production of an organic fertilizer with superior physicochemical and microbiological properties. During the thermophilic fermentation process, the total content of major nutritional elements and humus-forming substances remained stable. The outcomes revealed that the anaerobic thermophilic process significantly amplified the concentration of ammonium nitrogen in the fermented biomass (by 40-60%), while the total carbon content decreased (by 15-30%). Furthermore, a decline was observed in dry and organic matter content, and the C/N ratio reduced. An inverse relationship between the effluent's acidity and the content of ammonium nitrogen was noticed. Anaerobic treatment also enhanced the rheological properties of the fertilizers. Compared to pre-fermentation effluent, post-fermentation effluent exhibited a lower total content of suspended solids, fewer large particles, and reduced biomass density. The resulting product, an organic fertilizer, was characterized by high nitrogen and carbon content, absence of pathogenic microflora, and was deemed ready for use.

# 1. INTRODUCTION

#### **1.1 Problem statement**

The global challenge of organic waste management is pressing, as an alarming figure of roughly 35% of food produced by the food industry ends up in landfills [1]. Various methods have been explored to recycle this waste, one of the promising ones being the bioconversion of food waste through Hermetia illucens [2]. In the current technology landscape, diverse equipment types, such as aerated machines employing thermophilic conditions, are being designed to expedite the composting process and produce effective soil additives [3].

Animal waste processing techniques include enzymatic fermentation involving the addition of various substances, biological products, fungi, and microorganisms [4, 5], and anaerobic digestion under mesophilic conditions  $(35\pm1^{\circ}C)$  [6]. Thermophilic conditions are particularly favored due to their capability to eliminate pathogens, even in high concentrations, and achieve high purification efficiency [7]. This approach to

waste recycling not only has environmental benefits but also yields products that can be feasibly used in agriculture [8].

# 1.2 Contextual background

As reported by the Agency of the Republic of Kazakhstan, as of November 1, 2021, severe degradation has afflicted 26.6 million ha of the country's 187.9 million ha of pastures. Saline and solonetz land constitute a considerable 94.9 million ha [9]. The most recent land quality assessment (2021) revealed that out of the total area (21.7 mln ha, including 1.3 mln ha of irrigated land), the saline lands encompass 2.0 mln ha, inclusive of 0.47 thousand ha of irrigated land. Lands exposed to water and wind erosion comprise 1.59 mln ha, of which 65.0 thousand ha are irrigated [9].

In Kazakhstan, a 22% increase in the expanse of soil subjected to wind erosion has been observed since 1990, which equates to an additional 5 million ha. Concurrently, 5 million ha experience water erosion, including 1.0 million ha of arable land. Northern Kazakhstan is particularly impacted,

with soils exhibiting significant depletion. More than 1.4 million tons of humus have been lost over half a century of virgin land development, representing a third of the initial volume. Notably, average annual losses of humus in Kazakh agriculture range from 0.5 to 1.4 t/ha, with these losses being especially pronounced on eroded lands [10].

Enhancing soil fertility and crop yield, and improving crop quality, are all interconnected with the rational application of organic fertilizers. This issue is of particular relevance in Northern Kazakhstan, where consistent high crop yields necessitate the systematic use of large quantities of organic fertilizer (15-20 tons per hectare of arable land). With the advent of intensive agriculture, the role of organic fertilizers in maintaining a balanced nutrient and humus profile in the soil, along with favorable physical properties, has become increasingly critical. Research conducted by the U.U. Uspanov Kazakh Research Institute of Soil Science and Agrochemistry suggests that an average application of approximately 10 tons of organic fertilizers per hectare of arable land annually is required to achieve a balanced humus profile in Kazakh soils, with the actual amount varying depending on the soil type [11].

Cattle manure and bird droppings are the primary potential sources of organic fertilizer in Kazakhstan. These materials accumulate in substantial quantities on farms. However, for them to be transformed into fertilizer, an extended period (6-7 months) is required. This duration leads to a considerable loss of nutrients, thereby highlighting the need for efficient processing [12].

#### 1.3 Organic waste management

The magnitude of household food waste is escalating on an annual basis. Urban refuse predominantly comprises organic matter, primarily attributed to kitchen waste, which makes up approximately 35% of the total waste. Other components include paper (34%), glass and porcelain (6%), iron (3%), and plastic (1%). Analysis reveals that 10 tons of urban waste can contain between 900 and 1,900 kg of organic matter, a figure comparable to the 1,800 kg found in 10 tons of manure. In terms of nutrient content, urban waste averages 0.6-0.7% nitrogen, 0.5-0.6% P<sub>2</sub>O<sub>5</sub>, and 0.8% K<sub>2</sub>O per dry substance, amounting to 8-10 kg of nitrogen, 10 kg of phosphorus, and 30-40 kg of potassium in 10 tons of waste [13].

However, the potential benefits of this organic content for agricultural applications are offset by the presence of harmful constituents. Household food waste may harbor significant quantities of heavy metals, phenols, cyanides, and detergents, presenting potential sources of infectious and parasitic diseases for both humans and animals. Consequently, the utilization of this waste necessitates the implementation of specialized processing technologies designed to avert negative soil impacts. Despite the inherent challenges, these waste types remain largely untapped as sources of organic fertilizer, representing an immense repository of organic substances critical for Kazakhstan's soils [14].

Identifying an optimal method for the production of organic fertilizer is thus paramount. This method should ensure eradication of weed seeds, facilitate the transformation of difficult-to-digest nutrients into bioavailable forms, and enhance the sanitary, hygienic, and physico-mechanical properties of fertilizers. Thermophilic fermentation in a bioreactor, a process that involves the preparation of fertilizers from manure or bird droppings, food, and other waste, emerges as a promising solution. Given the acute scarcity of organic fertilizers, the adoption of this technology could potentially revolutionize collective and private farming practices in Kazakhstan [15].

## 1.4 Significance of the Study

The scarcity of waste processing facilities presents a significant challenge in Kazakhstan [16]. This circumstance can potentially be attributed to a lack of comprehensive understanding of the subject matter. Czech scientists pioneered the use of thermophilic fermentation for household food waste treatment, drawing attention to a possible solution [17]. Thermophilic fermentation offers substantial disinfection benefits against both aerobic and anaerobic species. Current theories suggest that anaerobic fermentation ensures complete deworming, while mesophilic fermentation only eliminates 50-80% of helminth eggs. The fermentation process enhances the sediment's structure, transforming it into a uniform, non-disintegrating mass, thereby ensuring its sanitary safety and effective utilization [18].

The rising volumes of food waste, coupled with the diminishing soil fertility and loss of humus, underscore the urgent need for solutions. The lack of organic fertilizer production and their associated high costs, the diminishing crop productivity, and the limited understanding of anaerobic thermophilic fermentation technology for manure and household plant waste conversion into fertilizer, collectively highlight the importance of research in this domain.

The present study focuses on anaerobic fermentation under thermophilic conditions as a viable waste processing method. This method's advantage lies in its flexibility to utilize diverse raw materials, including food waste and animal husbandry waste.

## 1.5 Study objectives

This study is dedicated to investigating the chemical and physical characteristics of the effluent fertilizer produced from anaerobic thermophilic fermentation of a mixture of plant food residues and manure. The main scientific challenge of this work is to develop optimal strategies for processing organic food waste under anaerobic digestion in a bioreactor to yield high-quality organic fertilizers. In achieving this, the study will identify factors that ensure the production of organic fertilizer with optimal physicochemical and microbiological properties that meet sanitary requirements.

#### 2. MATERIALS AND METHODS

#### 2.1 Period and place of study

From 1.03.2022 to 01.07.2022, at the laboratory of organic waste processing of the Kokshetau University named after Shokan Ualikhanov, studies were conducted to evaluate the effect of thermophilic fermentation regime on the chemical and physical characteristics of the effluent.

### 2.2 Stages of the study

Processing of organic waste presents a complex biotechnological task. The objects of the study were the kinds of waste that are formed in large quantities in public catering establishments and farms in Kokshetau and for which there is

Name of the Waste	Source of Origin	Date of Collection	Designation	Xenobiotics of Chemical Origin (Heavy Metals, Pesticides)
Food plant waste	University Canteen located at 194 Kuanyshev Street	15.03.2022	FPW (1)	not found
Food plant waste	University Canteen located at 76 Abaya Street	18.04.2022	FPW (2)	not found
Food plant waste	School No. 18 Canteen located at Kokshetau	25.05.2022	FPW (3)	not found
Cattle manure	Zhol Aman private farm at the village of Krasny Yar, 12 Symphelopolskaya Street	15.03.2022	Cattle manure (1)	not found
Cattle manure	Agrotrade-Kyzylzhar Limited Liability Partnership (LLP) farm, Krasny Yar village, 25 Zhidek Street	18.04.2022	Cattle manure (2)	not found
Cattle manure	Vita-30 LLP farm, Krasny Yar village, 24 Mira Street	25.05.2022	Cattle manure (3)	not found

 Table 1. Waste sources

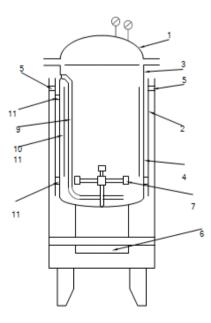
To obtain effluent biofertilizer by anaerobic thermophilic fermentation, the following stages of work were carried out:

1. Chemical and physical analysis of the raw materials represented by FPW and manure. The analysis was carried out according to the following parameters: acidity (pH), dry matter content, organic carbon content ( $C_{org}$ ), and total nitrogen content ( $N_{total}$ );

2. Production of fermentation mixtures of various compositions (three types of FPW (1, 2, 3) + cattle manure 1, 2, 3);

Chemical and physical analysis of fermentation mixtures;
 The process of anaerobic thermophilic fermentation in a bioreactor;

5. Chemical and physical analysis of the resulting product: the effluent biofertilizer.



# Figure 1. Diagram of the bioreactor BUG-R laboratory biogas plant

1: lid, hatch; 2: heating water with a HE; 3: vessel; 4: a temperature sensor; 5: mounting and turning unit of the bioreactor; 6: motor with a magnetic drive; 7: stirrer for mixing; 8: gas distribution bubbler; 9: opening for loading the substrate; 10: pressed partition (divider); 11: drainage opening for the substrate and for cleaning and flushing the internal space of the bioreactor

All experiments were carried out under thermophilic conditions (52-55°C) in a BUG-R Bioreactor (100 L volume) manufactured by the BMP Enterprises Association, Vologda, Russia. The appearance and general diagram of the laboratory bioreactor are presented in Figure 1. A bioreactor is a vertically positioned cylindrical container with a stirrer inside. The bioreactor was loaded once every 15 days. For this purpose, a mixture of cattle manure (25 L) and food plant waste (25 L) and 50 L of water (in a ratio of 0.25:0.25:0.5) were fed into the grinder and then into the bioreactor, where the homogenization and heating of the mixture to operating temperature took place for 30 minutes with constant stirring. In the lower part of the bioreactor, there is a water jacket, i. e. 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 (HE) (2) in automatic mode to a temperature of 52-55°C.

The fermentable mixture in the bioreactor was stirred once every day for 10 minutes. The resulting biogas was released into the open air through a gas distribution bubbler and outlet pipes. The retention time at all stages of the experiment was 15 days, which is considered optimal for the decomposition of organic waste in continuous processes of thermophilic fermentation and also prevents the leaching of thermophilic microorganisms.

In the upper part of the bioreactor, there is a hatch (1), in which there is a valve for removing the resulting gas. The hatch is hermetically sealed. On the side end side, there is a technological hermetically sealed opening (11) designed for complete drainage of the substrate and for cleaning and flushing the internal tank of the bioreactor.

Inside the bioreactor, there is a vertical shaft with blades (7) for mixing the substrate and destroying the surface film formed during fermentation. In the upper part of the container on the side, there is an opening (9) for loading the substrate. A filler pipe is installed to fill the water into the jacket, and a valve is provided for its discharge.

To start the process of thermophilic fermentation, fermentation mixtures were prepared from cattle manure (25 l) and FPW (25 l), and 50 l of water, in a ratio of 0.25: 0.25: 0.5.

As a result of anaerobic fermentation of the fermentation mixture with periodic stirring (1 time per day) for 15 days, we received a valuable product, an effluent, i. e. a liquid material with solid particles, a suspension. The chemical and physical and agronomic characteristics of the effluent correspond to the characteristics of a highly effective organic fertilizer.

The efficiency of anaerobic digestion of organic waste depends on many factors, such as the composition and amount of organic substances in the substrate, its loading rate and residence time in the reactor, the C/N ratio in the fermented mass, the activity of the inoculum, temperature, pH, humidity, the presence of inhibitors, design and type of operation of the bioreactor, etc.

Studying these factors in a controlled fermentation process in a bioreactor is important to allow the bacteria to work at maximum efficiency to produce biofertilizer.

To analyze the effectiveness of anaerobic digestion, we assessed the content of  $C_{org}$ ,  $N_{total}$ , pH, dry matter content, and infestation indicators of the initial mixtures and digestate at the beginning and end of the experiment.

Sampling for chemical analysis was taken after thorough mixing of the effluent from at least five points. The weight of the subsample for the determination of the moisture mass fraction was 15-20 g, and the weight of the subsample for the determination of the mass fraction of the dry residue was 150-200 g. The mass fraction of moisture and the dry residue was determined by the weight loss of the organic fertilizer sample (effluent) when dried to a constant mass. Samples of organic raw materials and the resulting products (effluent) were subjected to chemical analysis in the laboratory in a 3-time repetition.

An aqueous extract was prepared from the fertilizer suspension, in which the pH value was determined by the potentiometric method. Potentiometric determination of pH was carried out by measuring the potential difference between two suitable electrodes immersed in the test solution. One of the electrodes was sensitive to hydrogen ions (glass electrode), and the other was a reference electrode (silver chloride electrode). Together with a temperature sensor, they were combined into one compact electrode. The measuring device for measuring pH was a voltmeter with an input resistance 100 times greater than the resistance of the electrodes used.

Distilled water was used to prepare effluent water extracts (1:5), after which the mixture was stirred for 60 minutes, settled, and filtered. Before determining the pH, the suspension was stirred again and the measuring electrodes were lowered into it so that they were completely immersed in the liquid. The pH value of the studied sample was set after 0.5 to 1 min on the device display.

When determining the mass fraction of the dry residue, a subsample of organic fertilizer in a porcelain cup was placed in a water bath and evaporated dry with periodic stirring with a glass stick. Then the subsample was transferred to a preheated drying cabinet and dried at 105 to 110°C for five hours until it was brought to a constant weight. Weighing was carried out every hour, and each time before weighing, the bowl with the subsample was cooled in the air for 30 minutes. The analysis was considered complete if the difference between the results of two subsequent weighings (m<sup>2</sup>) did not exceed 0.1 g.

The N<sub>total</sub> content was determined by the Kjeldahl method. The essence of the method for determining the total amount of nitrogen involves the wet oxidation of organic substances of the test sample with sulfuric acid heated in the presence of catalysts. During the process of oxidation, organic substances in the test sample burn: carbon turns into  $CO_2$ , hydrogen to H<sub>2</sub>O, sulfur to SO<sub>2</sub>, nitrogen turns into NH<sub>3</sub>, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> is

formed by reaction with sulfuric acid. Part of the sulfuric acid that oxidizes organic substances is reduced to  $SO_2$ . Ammonia from ammonium sulfate is displaced by alkali and distilled into titrated acid. The ammonia distilled by steam was collected in a titration cell, into which a solution of boric acid had previously been poured. The concentration of the resulting ammonium borate solution was determined by titration by changing the color of the indicators. By the amount of acid bound to ammonia, the amount of nitrogen was determined.

The mass fraction of  $N_{total}$  (X%) in the analysis of a dry sample of fertilizer was calculated by the formula:

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

where,

0.0014 is the mass of nitrogen corresponding to 1 cm<sup>3</sup> of sulfuric acid solution with a molar concentration of 0.05 mol/dm<sup>3</sup> consumed for titration of the analyzed solution, g;

 $V_1$  is the volume of sulfuric acid solution with a molar concentration of 0.05 mol/dm<sup>3</sup> spent on titration of the analyzed solution, cm<sup>3</sup>;

 $V_0$  is the volume of sulfuric acid solution with a molar concentration of 0.05 mol/dm<sup>3</sup> consumed for titration in the idle experiment, cm<sup>3</sup>;

250 is the volume of the initial solution, cm<sup>3</sup>;

 $V_2$  is the volume of the analyzed solution taken for distillation, cm<sup>3</sup>;

m is the weight of the subsample, g.

The study of the effect of the fermentation process on the indicators of infestation in manure waste and FPW was carried out before and after the fermentation process under thermophilic conditions. After sampling, containers with cattle manure and FPW waste were loaded into a bioreactor.

To detect helminth eggs, the most common flotation method was used (the Fulleborn technique). To carry out the flotation method, a saline solution was prepared (400 g of NaCl) per 1 l of boiled water and was constantly stirred until completely dissolved.

5 g of organic mass sample was diluted into <sup>3</sup>/<sub>4</sub> cup with flotation solution and thoroughly stirred. The resulting uniform suspension after removal of the surfaced large particles was filtered through gauze into glasses of smaller capacity, which were filled to the top and left alone for 40-60 minutes. During this time, the eggs float up.

For quantitative counting of helminth eggs, the Krasilnikov-Volkova method was used. When examined by this method, 1 g of the test sample was mixed in a glass test tube with a 1-1.5% detergent solution in a ratio of 1:10. The suspension was thoroughly shaken, transferred to a slide, and the number of eggs in the entire preparation was counted. To calculate the number of eggs in 1 g of the test sample, the resulting number was multiplied by 100. The data obtained were used for comparative accounting of the infestation of animals before and after deworming.

The intensity of infestation of the resulting fertilizer was determined by the number of helminth eggs and larvae in one g of the analyzed sample (Table 2).

Statistical processing of the results was carried out using the Microsoft Office Excel 2010 software. The reliability of the differences in the mean values was assessed using the Student's coefficient (P<0.05). All tabular and graphical data contain average values and standard errors.

Helminth Class	Intensity of Infestation Depending on the Number of Detected Helminth Eggs and Larvae, Specimens/1 g of Analyzed Sample				
	Low	Average	High	Very high	
Helminth class Nematodes, cestodes	0-50	51-100	101-200	201-500	

# 3. RESULTS AND DISCUSSION

#### 3.1 Technology efficiency

One of the main methods of processing organic waste is the method of thermophilic fermentation. The relative costeffectiveness, as well as the combination of sanitary safety and environmental cleanliness, makes the thermophilic fermentation technology very effective [19, 20]. Under anaerobic conditions, the rate of decomposition of organic matter of waste is significantly higher than with aerobic fermentation. The obtained organic fertilizers after anaerobic biofermentation of waste differ favorably in terms of sanitary and hygienic indicators from the products of biomass conversion under aerobic conditions [21]. Compared with mesophilic traditional fermentation, thermophilic fermentation can inhibit the growth of polluting bacteria, thereby increasing the efficiency of fermentation of food waste [22-24].

The results obtained by us 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 [25].

During anaerobic thermophilic fermentation of waste, there is practically no odor, which is created by harmful gases ammonia and hydrogen sulfide, which reduces the negative impact of waste on the environment. It should be noted that the ability to filter waste biomass during anaerobic digestion is higher than during aerobic fermentation. However, energy costs for providing anaerobic thermophilic fermentation processes are higher than for aerobic fermentation [26, 27]. That said, this technology is poorly studied and, as a result, has not found wide application in practice in the national economy.

#### 3.2 Physicochemical parameters of the raw materials

In the first stage of the work, a chemical and physical analysis of the raw materials represented by food plant waste and manure was carried out. The selected parameters for the chemical and physical analysis are limiting in the biological processes of transformation of organic matter [19, 20]. The data obtained are presented in Table 3.

Table 3. Physical and chemical parameters of the raw materials

Waste	Dry Matter, %	РН	Ntotal, %	Corg, %	C/N
FPW (1)	24.9±15.2	5.1±0.4	2.5±0.3	41.6±12.0	18
FPW (2)	38.8±4.5	5.5±0.3	2.3±0.2	45.6±3.6	20
FPW (3)	42.3±3.9	6.5±0.2	2.6±0.4	$54.6\pm5.9$	21
Cattle manure (1)	$16.8 \pm 2.5$	8.1±0.3	0.63±0.9	21.1±1.3	20
Cattle manure (2)	20.1±1.3	7.5±0.2	0.54±0.5	20.6±1.6	22
Cattle manure (3)	12.6±2.3	7.2±0.2	0.74±0.4	19.5±1.5	19

An important characteristic of the initial waste is its acidity. According to the pH value in the raw materials, the acidity had a neutral and slightly alkaline pH value (5.1-8.1). The waste of the same type, selected at different enterprises, had different characteristics: thus, FPW (1), from the University Canteen at 194 Kuanyshev Street had a more acidic pH value than FPW (3) selected in the School No. 18 Canteen (pH 6.5). Similarly, different characteristics were obtained from selected samples on cattle manure farms. The obtained data on the acidity of the waste correspond to the data in the literature. Thus, according to the literature, food waste is usually characterized by a low pH value from 4 to 5 [28-30]. Due to the high content of ammonia compounds, cattle manure is classified as alkaline waste, the pH of which varies in the range of 6.3-8.2 [31].

Analysis of waste moisture by dry matter content showed that the values of the indicators met the criteria of wet waste (with a dry matter content of 60-10%). The results obtained are similar to the data presented in the literature. Thus, the dry matter content in food waste varies in the range from 18.9 to 83.4% [28] and in cattle manure in the range of 10-80% [32].

The principal characteristics of waste that determine its availability to microorganisms are the carbon and nitrogen content, as well as the ratio of their values. In all waste, the  $C_{org}$  content ranged from 19-54% and the N<sub>total</sub> content from

0.54 to 2.6%. Concerning waste, the results obtained are consistent with the literature data. Thus, the content of  $C_{org}$  in the organic fraction in food waste ranged from 41-53% and  $N_{total}$  from 4-3.4%, respectively [20, 29, 33]. In the cattle manure,  $C_{org}$  and  $N_{total}$  had values of 18.0-38.8 and 2.7% [34].

# **3.3** Characteristics of fermentation of FPW and cattle manure

In the second stage of the study, fermentation mixtures were prepared from FPW and cattle manure selected from different sources and at different times. The same characteristics were studied in the obtained fermentation mixtures: pH, dry matter content, organic carbon content ( $C_{org}$ ), and total nitrogen content ( $N_{total}$ ) (Table 4).

To assess the possibility of anaerobic thermophilic fermentation, fermentation mixtures of waste were analyzed. The use of waste mixtures in the fermentation process makes it possible to optimize the pH of fermentation mixtures, the C/N ratio, and the dry matter content [21, 22]. An important criterion for anaerobic digestion is the acidity of the medium. According to literature data, food waste is usually characterized by a low pH value from 4 to 5 [25]. Due to the

high content of ammonia compounds, cattle manure is an alkaline waste where the pH varies in the range of 6.3-8.2 [26].

In the resulting fermentation mixtures (cattle manure and FPW), the pH varied in neutral values.

Table 4. Characteristics of the fermentation mixture of cattle manure and food waste before the process of anaerobic
thermophilic fermentation

No.	Waste	Dry Matter, %	PH	Ntotal, %	Corg, %	C/N
1	FPW $(1)$ + cattle manure $(1)$	$20.8 \pm 8.2$	6.7±0.3	1.6±0.3	31.3±11.0	19
2	FPW $(2)$ + cattle manure $(2)$	29.4±3.5	$6.6\pm0.4$	$1.4\pm0.2$	33.1±3.1	21
3	FPW $(3)$ + cattle manure $(3)$	27.4±2.5	$7.0{\pm}0.2$	$1.7\pm0.4$	37.5±5.1	20

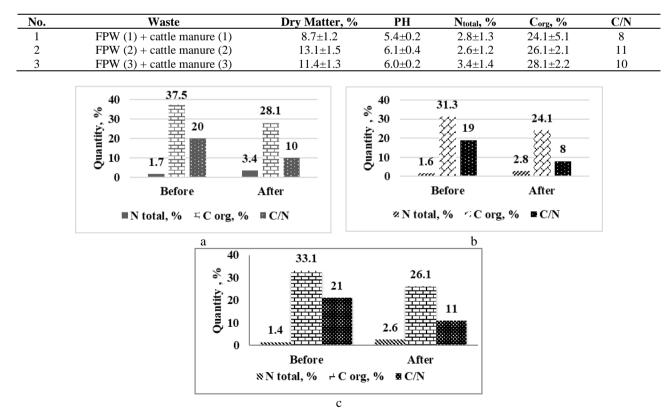
The process of anaerobic thermophilic fermentation in a bioreactor is carried out in mixtures with a humidity of 70-75%. The waste selected for fermentation is wet (where the dry matter content is 10-60%), therefore, water was added to the resulting fermentation mixture until a value of 70-75% humidity was obtained.

During the decomposition of organic matter, microorganisms consume 25-30 times more carbon than nitrogen. Therefore, the C/N ratio of 20:1-30:1 is preferable for microorganisms [32]. Waste with a high content of  $C_{org}$  can be combined with waste with a high nitrogen content to achieve a ratio of 30:1 [28]. Therefore, in some cases, joint fermentation of substrates is advisable. Thus, the content of  $C_{org}$  in food waste varied from 41.6 to 54-55%, and  $N_{total}$  from 2.5 to 2.6% [34]. In the cattle manure,  $C_{org}$  and  $N_{total}$  had values of 19.5-21.1 and 0.54-0.74%. In addition to the optimal C/N ratio, the combined fermentation of waste allows for optimizing the concentrations of macro- and microelements and the moisture content of the mixture, reducing the influence of inhibitory factors or toxic components, controlling the output of biogas and the stability of the digestate [29, 35].

After anaerobic thermophilic fermentation, an organic effluent mass was obtained from the fermentation mixture, with a humidity of 63.5-72% a slightly acidic reaction (5.4-6.1 pH) and a slight odor. In the resulting effluent, a solid mass (digestate) was studied, where we determined the same physicochemical parameters as in fermentation mixtures and initial waste separately (pH, dry matter content, C<sub>org</sub>, N<sub>total</sub>) (Table 5).

From the data in Table 5, it can be seen that after anaerobic thermophilic fermentation of waste mixtures, their acidification occurred compared to the fermentation mixture before fermentation (pH 6.7-7.0). The decrease in pH after fermentation is associated with sugars decomposed during acidogenesis in food plant waste increasing the concentration of volatile fatty acids. In all variants of effluents, the pH value was lower, in the range of 5.4-6.0. The efficiency of the anaerobic digestion process depends significantly on the pH value since most microorganisms prefer neutral pH concentrations between 6.4 and 7.0. but for some hydrolyzing and acidogenic microorganisms, the optimal pH value is in the range of 5.5-6.5.

 Table 5. Characteristics of effluent digestates from cattle manure and food waste after the process of anaerobic thermophilic fermentation



**Figure 2.** The content of nutrients in the fermentation mixture (before) and digestates of effluents (after). a: FPW (1) + cattle manure (1); b: FPW (2) + cattle manure (2); c: FPW (3) + cattle manure (3)

The highest dry matter content was observed in the mixture (FPW (2) + Cattle manure (2)) (29%). The minimum dry matter content of the mixture (FPW (1) + cattle manure (1)), may be due to the variety of FPW with a high moisture content (20%). The dry matter content after fermentation in the mixtures decreased by 40-60%, which is associated with the transformation of organic matter during anaerobic processing to volatile compounds (CH<sub>4</sub>, CO<sub>2</sub>) and water. The content of  $C_{org}$  in all variants of the mixtures varied in the range of (31.3-37.5%), and after anaerobic thermophilic fermentation, we observed a decrease in all variants (24.1-28.1%), which corresponds to a decrease in  $C_{org}$  by 25-30%.

The content of  $N_{total}$  in digestates of fermented mixtures (effluents) increased by 50-52% (equaling 2.6-3.4%) relative to the initial value (1.4-1.7%) (Figure 2). Thus, during anaerobic thermophilic processing, due to the transition of organic matter into methane and carbon dioxide, the waste mass decreases, and the percentage of nitrogen increases. Besides, at high temperatures, nitrogen turns into volatile

ammonium compounds. Due to the change in the nitrogen content in all effluent samples, the C/N ratio decreased by almost 50%.

# **3.4** Effect of thermophilic fermentation on bacterial contamination

The results of studies on the influence of thermophilic temperature regime on bacterial contamination and infestation of the resulting fertilizer are presented in Table 6. The intensity of infestation of the resulting fertilizer was determined by the number of helminth eggs and larvae in one g of the analyzed sample (Table 2). From the data in Table 6, it can be seen that on the 7th day of effluent fermentation under thermophilic temperature conditions, the number of eggs of the helminth Neoascaris vitulorum decreased by 3.2 times. Oesophagostomum radiatum by 2.8 times, and on the 14th day of fermentation of helminth no larvae of the suborder were detected.

Table 6. Effect of thermophilic fermentation on effluent deworming

	Numb	er of Helminth Eggs in 1 g of Effl	uent
Type of Helminth	Control	Active fermentation	End of fermentation
	(Before fermentation)	(Day 7)	(Day 14)
Neoascaris vitulorum	85.7±3.30	26.5±1.40	not found
Oesophagostomum radiatum	6.5±1.04	$2.3{\pm}0.30$	not found

The data in Table 6 indicate that cattle manure with a significant level of contamination by helminth eggs with Ascaris poses a danger to human health if it is introduced into the soil without prior disinfection. With an increase in the activity of fermentation, an increase in the efficiency of deworming is observed, but the number of viable helminth eggs is still high (up to 30% and higher, depending on the thermal tolerance of the type of helminth). It was found that the thermophilic temperature regime in the range of 50-55°C for 14 days ensured the complete death of invasive pathogens.

# 4. CONCLUSION

As a result of the conducted studies, based on the determination of the physicochemical characteristics of organic waste, it was found that the waste was characterized by a wide range of acidity values (5.1 to 8.1), dry matter (12.6 to 42.3%),  $C_{org}$  content (19.5 to 54.6%), and  $N_{total}$  content (0.54 to 2.6%). Differences in waste characteristics make it possible to create artificial systems optimal for the functioning of microbial communities and control processes by forming mixtures for anaerobic digestion without the use of additional reagents.

In the process of thermophilic fermentation, the total content of the main nutrition elements and humus-forming substances has not changed noticeably. As a result of the anaerobic thermophilic process, the concentration of ammonium nitrogen in the fermented biomass significantly increased (by 40-60%), the total carbon content decreased (by 15-30%), and the dry and organic matter content and the C/N ratio narrowed. With an increase in the content of ammonium nitrogen, the acidity of the effluent decreased. As a result of anaerobic processing, the rheological properties of fertilizers improved. In comparison with the effluent before fermentation,

it was noticed that after fermentation, the total content of suspended solids and the number of large particles in the effluent decreased, and the density of biomass decreased. The resulting product (organic fertilizer) is an effluent, with a high content of nitrogen and carbon, does not contain pathogenic microflora, and is completely ready for use.

Obtaining organic fertilizers using a bioreactor, by the method of anaerobic thermophilic fermentation from food waste will reduce the amount of organic waste and the volume of their accumulation, and, consequently, environmental pollution; increase soil fertility, increase crop yields without the use of mineral fertilizers, restore disturbed land; solve several sanitary and hygienic tasks, namely improve the epidemiological situation as a result of the death of pathogenic microflora contained in waste, reduce unpleasant odors, etc. In other words, the method of anaerobic thermophilic fermentation is the most radical, environmentally friendly, waste-free way of processing, recycling, and neutralization of various organic wastes of plant origin.

Further research can be aimed at studying the effect of organic fertilizers obtained from food waste on increasing crop yields and soil fertility.

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