

Components and their assessment in different biogas slurries for enhanced waste management

Ayten Namlı, Hanife Akça, Muhittin Onur Akça *

Ankara University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Ankara, Türkiye

Abstract

In this study, liquid fermented wastes from 15 licensed biogas plants located within different regions of Türkiye were determined and the parameters that are important to waste management evaluations were revealed. Accordingly, some physical, chemical, and biological analyses include moisture, dry matter (DM), organic matter (OM), pH, EC, total N, P, K, *Salmonella*, *Escherichia coli*, *E.coli* O157:H7, and *Enterobacteria* were conducted. Spearman's correlation analysis was conducted to determine the relationship among the results, and a regression analysis was conducted to reveal the effect of the results on each other. In the wastes, DM values were between 0.53-9.71%, OM values were between 0.53-7.76%, N contents were between 0.10-0.74%, P contents were between 0.04-0.22%, K contents were between 0.15-0.56%, EC values were between 1.50-6.51 dSm⁻¹, B contents were between 16.96-34.63 mg kg⁻¹, and Na contents were between 0.11-0.40%. A correlation analysis was conducted to reveal the relationship of OM and DM to other parameters. OM content had a significant correlation with N (73.9%), P (80.4%), Fe (71.4%), Mn (75.7%), EC (53.2%), K (60.7%), and Mg at 72.1%. The DM contents had a significant correlation with N (68.2%), P (95.4%), Cu (60.0%), Fe (88.2%), Mn (94.3%), Zn (67.5%), EC (76.1%), K (81.4%), and Mg at 83.9%. A significant regression model and the variances of DM and OM variables were 37.8 and 24.8% for N (%), 61.7 and 31.5% for P (%), 53.9 and 22.4% for K (%), 46.6 and 23.8% for Ca (%), and 70.0 and 45.7% for Mg (%), respectively. Finally, these observations should be used to demonstrate the usability of liquid fermented wastes for agricultural purposes.

Keywords: Biogas, slurry, dry matter, organic matter, correlation, regression.

© 2023 Federation of Eurasian Soil Science Societies. All rights reserved

Article Info

Received : 25.01.2022

Accepted : 07.09.2022

Available online : 20.09.2022

Author(s)

A.Namlı



H.Akça



M.O.Akça *



* Corresponding author

Introduction

Interest in the anaerobic digestion of organic wastes is gaining more and more attention for helping to reduce greenhouse gas emissions and facilitating the sustainable development of biogas production and energy supplies. It has been observed that as a result of the daily increases in agricultural activities based on the increase in the population and the investments made in agriculture, large amounts of agricultural biomass wastes are being released.

Biogas power plants are one of the most suitable alternatives for eliminating the negative effects of these wastes on soil and surface waters by processing manure and other organic wastes from livestock and creating a waste management plan. Nearly 19,000 biogas plants operating in Europe and investments in these plants have increased in recent years. The biogas industry has the potential to reduce greenhouse gas emissions worldwide by 10-13% and create jobs for thousands of people by 2050 by increasing the volume of renewable energy sources (Anonymous, 2021).

Biogas power plants produce clean energy from waste and are indispensable disposal facilities for modern, sustainable, environmental policies that not only generate electricity from natural resources, such as renewable energy facilities but are also taken under record. Biogas facilities are important in terms of finding

solutions to the energy deficit of Turkey, preventing environmental pollution, and serving the liberation of the major agricultural areas of the country that are about to experience desertification; however, the industry is already facing the issue of what to do with the liquid fermented wastes. Biogas facilities for the use of liquid fermented products in agricultural areas are attempting to resolve the issues within their means and knowledge within their regions. The Ministry of Environment and Urbanization and the Ministry of Agriculture and Forestry, who are the investigators of these issues, evaluate them from their perspective and have different legislation for implementation. In addition, the high cost of pasteurization of the wastes from biogas enterprises and the lack of standard quality and the continuity of security for the raw material source are the main problems of the biogas sector in Turkey. The most important of these problems is how to evaluate the liquid fermented waste. Under current conditions, the current use and storage methods of liquid waste in agriculture cause significant environmental problems. This rich byproduct has the potential to seriously pollute the environment if not appropriately handled (Yan et al., 2019). Many environmental problems are created from the irresponsible use of these wastes (Nasir et al., 2012).

Biogas slurry/waste is defined as an easily absorbable byproduct of plants, rich in macro- and micronutrients, such as N, P, and K, also known as anaerobic digestion products (Wang et al., 2018). The nutritional content of this waste may change depending on the raw material and anaerobic process. It has been stated that the composition of these slurries from biogas power plants generally comprises 93-99% water and 1-7% dry matter (DM), most of which are organic but some of which inorganic (Stinner et al., 2008; Fouda et al., 2013; Wentzel and Joergensen, 2016).

The current system in biogas facilities is continuously fed. The products released are separated into solid and liquid phases. The solid waste is fermented biogas fertilizer, while the liquid waste is $\sim 200 \text{ T d}^{-1}$ for every 1 MWh. Considering the power of biogas plants operating in Turkey at 150 MWh, $\sim 10.950.000 \text{ m}^3$ of waste is generated annually. With the foresight that this amount of waste will continue to increase each day, the resulting wastes must be well identified and characterized to create a sustainable management plan. If not, the damage to the environment will be considerably greater.

One example of the negative effects of fermented wastes has to do with the negative effects on the soil of applying higher than the recommended doses of fertilizer, depending on the content of the waste, the creation of diseases as a result of this application without pathogen removal, and the effect of high doses on climate change by increasing N_2O and releasing ammonia (Warnars and Oppenoorth, 2014). Some studies conducted on biogas wastes have considered such criteria as nutritional value, compliance with hygiene legislation, application time, application method, effects on climate change, and economic value when evaluating the products of fermentation from biogas plants (Al Seadi et al., 2013; Dahlin et al., 2017; Herbes et al., 2020). Without determining these properties, no wastes should be delivered directly to the soil or the environment.

The characteristics of the wastes may change at the end of the production processes based on the properties of the raw material used or the protocols of the biogas plant operations. For example, thermal pretreatment may be suitable for protein-rich waste but not for lipid-rich waste. The products released can be different depending on waste composition and quality. Certain features, such as high moisture content of the wastes, low energy density, storage requirements, and deterioration during storage, reveal the importance of determining the waste characteristics (Egieya et al., 2018). It is of great importance to determine waste quality for a sustainable evaluation.

In the present study, liquid fermented biogas wastes taken from 15 biogas plants licensed within different regions of Turkey were used. The aim of the present study was to (i) determine and characterize the liquid fermented biogas wastes, (ii) determine the waste pathogenicity, and (iii) obtain information about the nature of the waste by making certain inferences based on the data obtained.

Material and Methods

Collection and preparation of biogas wastes

Biogas liquid fermented wastes were taken from the lagoons of 15 licensed biogas plants located within different regions of Turkey and saved in clean polyethylene containers 40 cm high by 15 cm in diameter with the mouth of the container open and the container placed upside down at a distance of at least 1 m. After collecting the waste, we measured $\sim 5 \text{ L}$ after removing any air (e.g., it's the mouth was tightly closed and wrapped with aluminum foil). The sampled liquid fermented wastes were carefully transported to the Department of Soil Science and Plant Nutrition Laboratories, Ankara University and stored at $4 \text{ }^\circ\text{C}$ (liquid fermented wastes were kept until the analysis was completed) in the fridge.

Composition of biogas wastes

The composition of the liquid fermented wastes taken from the biogas plants comprised animal manure (13 samples) and urban wastes (2 samples). The samples were first filtered to remove the coarse particles in the

waste and then kept at 4°C before the analysis process. Some of the chemical properties of the liquid fermented wastes are shown in Table 1. In determining the wastes composition, moisture (%), dry matter (DM, %), organic matter (OM, %), pH (1:10), EC (1:10), total N (%), P (%), Ca (%), Mg (%), K (%), Na (%), Fe (mg kg⁻¹), Zn (mg kg⁻¹), Cu (mg kg⁻¹), Mn (mg kg⁻¹), B (mg kg⁻¹), Cd (mg kg⁻¹), Cr (mg kg⁻¹), Pb (mg kg⁻¹) and Ni (mg kg⁻¹) were analyzed.

Standard methods commonly used in liquid fermented wastes were used to determine the basic properties of the slurries examined in the study as follows: The moisture and dry matter (DM) content of the slurry was determined by oven drying at 70 °C. Organic matter content (OM) in the slurry was determined after ashing in an oven at 550°C and weighing (Bauer et al., 2009). pH and electrical conductivity (EC) in a 1:10 slurry/water mixture were determined as potentiometrically (Jackson, 1958); total nitrogen (N) by Kjeldahl method (Bremner, 1965); slurry samples digested with HNO₃-HClO₄ acid mixture (Kalra, 1997). Total P, Ca, Mg, K, Na, Fe, Zn, Cu, Mn, B, Cd, Cr, Pb, and Ni in the acid digest concentrations were determined by ICP-OES (Perkin Elmer Optima 2100 DV, Waltham, MA, USA).

Biogas liquid fermented wastes were analyzed for pathogens in their original state and after the heat treatment at 70°C for 1 h; *Salmonella*, *Escherichia coli* (*E. coli*), *E. coli* O157:H7, and *Enterobacteria* presence were analyzed.

Determination of *Salmonella*:

Salmonella was determined using a 25 g or 1 mL sample and the following methods (Mooijman et al., 2019):

- i. **Pre-enrichment:** 1 mL biogas sample was added to a nonselective liquid medium (buffered peptone water; BPW) and incubated at 37 ± 1°C for 18 ± 2 h.
- ii. **Growth on selective media:** After pre-enrichment, 100 µL sample taken from the culture medium was diluted with phosphate-buffered saline (PBS) up to 10⁻⁶, and 100 µL each dilution was transferred to xylose lysine deoxycholate (XLD) agar and inoculated using the smear plate method in bismuth sulfite agar medium. The agar plates were incubated at 37 ± 1°C for 24 ± 3 h.
- iii. **Verification:** Colonies with a black center and black precipitate zone with a metallic glow around bismuth sulfite agar and colonies with a black center on XLD agar medium were determined to be *Salmonella*.

Determination of *E. coli*:

Escherichia coli was determined according to the following methods for 25 g or 1 mL samples:

- i. **Pre-enrichment:** 1 mL biogas sample was added to BPW and incubated at 37 ± 1°C for 18 ± 2 h.
- ii. **Growth on selective medium:** After pre-enrichment, 100 µL sample taken from the culture medium was diluted with PBS up to 10⁻⁶, and 100 µL each dilution was transferred to eosin methylene-blue lactose sucrose (EMB) agar medium and smear inoculated. The agar plates were incubated at 37 ± 1°C for 24 ± 3 h.
- iii. **Verification:** After incubation, the colonies with a violet color and greenish metallic glow with reflected light on the EMB agar medium were determined to be *E. coli* (Leininger et al., 2001).

Determination of *E. coli* O157:H7:

Escherichia coli O157:H7 was determined according to the following methods for 25 g or 1 mL samples:

- i. **Pre-enrichment:** 1 mL biogas sample was taken onto tryptone soy liquid medium containing novobiocin and enriched at 41.5 ± 1°C for 6 h and then for 12-18 h.
- ii. **Isolation:** After pre-enrichment, cefixime telluride sorbitol was inoculated on MacConkey agar (CT-SMAC) selective medium from the culture medium. The agar plates were incubated at 37°C for 18-24 h.
- iii. **Verification:** MacConkey agar with sorbitol, cefixime, and tellurite inhibit the growth of most noncytotoxicogenic *E. coli* strains and other strains of *E. coli* that cannot ferment sorbitol. Other microorganisms that can be confused with *E. coli* O157:H7 on traditional MacConkey agar with sorbitol were inhibited by CT-SMAC (Zadik et al., 1993). Sorbitol-negative colorless colonies formed on the CT-SMAC agar surface were determined to be *E. coli* O157:H7.

Determination of *Enterobacteriaceae*:

Enterobacteriaceae was determined by calculating the number in a 1 g or 1 mL sample. Inoculum suspensions were prepared using 10-fold dilutions of the sample taken from the test sample (ISO 21528).

- i. **Inoculation using selective media:** 100 µL each of the prepared suspensions was taken and smeared on violet red bile glucose (VRBG) agar medium. The agar plates were incubated at 37°C for 24 ± 2 h.
- ii. **Verification and calculation of colony-forming units:** After incubation, the red colonies surrounded by a reddish precipitate zone of 1-2 mm in diameter were counted as members of the *Enterobacteriaceae* family. Counted colonies were calculated according to the following formula:

$$\text{cfu / g (mL)} = \text{average of two parallel plates} \times \text{dilution factor}$$

Statistical analyses

SPSS ver. 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. While evaluating the obtained data, in addition to the descriptive statistical results (average, standard deviation, median, frequency, ratio, minimum, maximum), a Spearman's correlation analysis was conducted to determine the relationship among the measurements. A regression analysis was conducted to analyze the effects of the measurements on each other. Significance was determined at $p < 0.01$ and $p < 0.05$ levels.

Results and Discussion

The components and pathogen content values of the liquid fermented wastes are provided in Tables 1 and 2. Some of the physical properties were cloudy, clear, scented, odorless, and dense. We determined that the physical properties of the liquid wastes were considerably different based on the analysis results.

Table 1. Some chemical properties of liquid fermented wastes

No	Waste Type	Moisture	Dry matter	Organic matter	pH	EC	N	P	K	Ca	Mg	Na	Fe	Zn	Cu	Mn	B	Cd	Cr	Pb	Ni
		(%)			(1:10)	(1:10)	(%)						(mg kg ⁻¹)								
1	Manure	94.68	5.32	4.63	8.51	1.74	0.24	0.09	0.33	0.28	0.09	0.16	404	16.29	6.21	19.31	17.59	3.19	5.43	3.45	3.62
2	Manure	98.16	1.84	1.83	8.93	2.44	0.43	0.09	0.25	0.20	0.01	0.11	134	22.59	3.29	11.96	16.96	2.34	4.11	2.59	2.72
3	Manure	99.47	0.53	0.53	8.59	1.63	0.14	0.04	0.15	0.20	0.02	0.14	73.41	6.88	2.68	5.43	21.45	2.68	4.64	2.97	2.97
4	Urban	94.25	5.75	3.31	8.88	5.10	0.74	0.22	0.36	0.54	0.09	0.27	376	48.15	10.19	32.13	34.63	3.52	6.85	6.57	4.72
5	Manure	96.50	3.50	2.87	8.52	2.27	0.29	0.09	0.38	0.67	0.07	0.24	151	11.42	4.15	14.06	25.09	3.49	6.04	4.06	3.96
6	Manure	93.21	6.79	2.38	8.86	4.00	0.36	0.19	0.47	0.60	0.19	0.40	1638	32.25	10.78	37.16	27.55	3.53	6.57	4.02	4.80
7	Urban	98.64	1.36	1.36	8.67	1.50	0.25	0.05	0.21	0.15	0.02	0.31	78.89	6.67	1.94	3.89	33.43	3.43	5.83	3.89	3.98
8	Manure	97.96	2.04	1.19	8.86	1.82	0.10	0.09	0.31	0.20	0.02	0.18	199	23.80	4.10	6.40	29.20	3.70	6.40	4.10	4.20
9	Manure	94.53	5.47	2.93	8.89	3.31	0.62	0.11	0.56	0.40	0.03	0.22	298	46.83	9.13	27.46	22.86	2.94	4.92	3.10	3.73
10	Manure	96.84	3.16	1.20	8.75	2.19	0.12	0.06	0.43	0.27	0.04	0.32	322	104	33.17	22.31	27.40	8.27	11.73	8.75	9.04
11	Manure	98.88	1.12	0.47	5.62	1.84	0.11	0.06	0.16	0.23	0.02	0.25	60.00	18.80	9.10	9.40	26.90	8.60	12.20	8.90	8.70
12	Manure	98.51	1.49	0.82	7.87	2.05	0.10	0.06	0.17	0.22	0.04	0.24	86.50	17.60	7.50	8.30	25.20	8.50	11.70	9.00	8.60
13	Manure	90.29	9.71	7.76	8.08	3.12	0.48	0.16	0.42	0.48	0.16	0.33	381	35.60	8.00	37.30	28.38	6.80	5.10	5.60	5.20
14	Manure	93.35	6.65	4.65	8.56	3.27	0.40	0.14	0.38	0.55	0.09	0.26	457	34.5	14	35.9	21.30	n.d.	8.40	4.70	4.30
15	Manure	94.54	5.46	2.81	8.73	6.51	0.48	0.10	0.40	0.53	0.07	0.26	753	26.00	26.60	27.90	25.00	n.d.	1.40	0.60	2.10

n.d.: not determined

Table 2. Pathogen values of liquid fermented wastes

No	Waste Type	Before 70°C	After 70°C	Before 70°C	After 70°C	Before 70°C	After 70°C	Before 70°C	After 70°C
		<i>E.coli</i> (cfu mL ⁻¹)	<i>E.coli</i>	<i>E.coli</i> O157:H7	<i>E.coli</i> O157:H7	<i>Salmonella</i> (cfu mL ⁻¹)	<i>Salmonella</i>	<i>Enterobacteriaceae</i> (cfu mL ⁻¹)	<i>Enterobacteriaceae</i>
1	Manure	None	None	None	None	None	None	4.4 x 10 ⁴	None
2	Manure	Present (1.12 x 10 ⁴)	None	None	None	Present (4.55 x 10 ³)	None	1.45 x 10 ⁵	None
3	Manure	Present (4.8 x 10 ³)	None	None	None	None	None	8.4 x 10 ⁴	None
4	Urban	Present (6.55 x 10 ³)	None	None	None	Present (1.05 x 10 ³)	None	5.1 x 10 ⁴	None
5	Manure	Present (9.75 x 10 ³)	None	None	None	Present (4 x 10 ³)	None	2.42 x 10 ⁴	None
6	Manure	Present (4 x 10 ²)	None	None	None	Present (4 x 10 ²)	None	2.11 x 10 ⁴	None
7	Urban	Present (9 x 10 ²)	None	None	None	Present (4 x 10 ²)	None	5.4 x 10 ⁴	None
8	Manure	Present (3.7 x 10 ³)	None	None	None	Present (1.05 x 10 ³)	None	3 x 10 ⁴	None
9	Manure	Present (4.9 x 10 ³)	None	None	None	Present (5 x 10 ³)	None	7.4 x 10 ⁴	None
10	Manure	None	None	None	None	Present (2 x 10)	None	1.46 x 10 ⁵	None
11	Manure	Present (3.6 x 10 ²)	None	None	None	None	None	4.6 x 10 ⁴	None
12	Manure	Present (1.59 x 10 ⁵)	None	None	None	Present (1.15 x 10 ⁴)	None	4.7 x 10 ⁵	None
13	Manure	Present (7 x 10 ²)	None	None	None	None	None	4.75 x 10 ³	None
14	Manure	Present (1.8 x 10 ⁴)	None	None	None	Present (1.45 x 10 ³)	None	4.55 x 10 ⁵	None
15	Manure	Present (4.2 x 10 ³)	None	None	None	None	None	7.3 x 10 ³	None

E. coli: *Escherichia coli*; cfu: colony forming unit.

Because the substrate mixtures used in biogas production were very heterogeneous in terms of plant nutrients and OM composition, their chemical composition was also very diverse. The DM content of the liquid fermented wastes varied considerably, and the DM amounts varied between 0.53-9.71%. The OM content of the liquid wastes were between 0.53-7.76%. The pH of the liquid fermented wastes was slight to strongly alkaline. The EC values of the liquid fermented wastes were between 1.50-6.51 dS m⁻¹. The total N contents of the liquid wastes were between 0.10-0.74%, P contents were between 0.04-0.22%, and K contents were between 0.15-0.56%. The B contents of the liquid wastes were between 16.96-34.63 mg kg⁻¹, and the Na contents were between 0.11-0.40%. The Fe contents of the liquid wastes were between 60.00-1638 mg kg⁻¹ and the Mn contents were between 3.89-37.30 mg kg⁻¹. The Cd, Cu, Cr, Ni, Pb, and Zn contents of the liquid fermented wastes were below the limits determined by the "Regulation on Organic, Mineral and Microbial Originated Fertilizers Used in Agriculture" (Tables 1 and 3).

Correlation analyses

OM and DM contents of the fermented liquid biogas wastes are the main characteristics taken into consideration in the evaluation of wastes. In addition, these two parameters can be determined easily and quickly; therefore, correlation analyses were conducted to reveal the OM and DM relationships with other parameters.

Table 3. Distribution of statistical parameters

Parameters	Mean	Standard deviation	Maximm	Minimum	Median	Skewness	Kurtosis
Moisture (%)	96.0	2.7	99.5	90.3	96.5	-0.515	-0.451
Dry Matter (%)	4.0	2.7	9.7	0.5	3.5	0.515	-0.451
Organic Matter (%)	2.6	2.0	7.8	0.5	2.4	1.372	2.271
pH (1:10)	8.4	0.8	8.9	5.6	8.7	-3.095	9.460
EC (1:10)	2.9	1.4	6.5	1.5	2.3	1.509	1.979
N (%)	0.3	0.2	0.7	0.1	0.3	0.618	-0.401
P (%)	0.1	0.1	0.2	0.0	0.1	0.953	0.155
K (%)	0.3	0.1	0.6	0.1	0.4	-0.076	-0.725
Ca (%)	0.4	0.2	0.7	0.1	0.3	0.452	-1.480
Mg (%)	0.1	0.1	0.2	0.0	0.0	1.162	0.677
Na (%)	0.2	0.1	0.4	0.1	0.2	0.130	0.014
Fe (mg kg ⁻¹)	360.9	401.1	1638.2	60.0	297.7	2.588	7.773
Zn (mg kg ⁻¹)	30.1	24.0	103.7	6.7	23.8	2.198	6.189
Cu (mg kg ⁻¹)	10.1	8.8	33.2	1.9	8.0	1.828	3.015
Mn (mg kg ⁻¹)	19.9	12.3	37.3	3.9	19.3	0.181	-1.603
B (mg kg ⁻¹)	25.5	5.0	34.6	17.0	25.2	0.030	-0.171
Cd (mg kg ⁻¹)	0.5	0.2	0.9	0.2	0.4	0.951	-0.989
Cr (mg kg ⁻¹)	6.8	3.1	12.2	1.4	6.0	0.616	0.003
Pb (mg kg ⁻¹)	4.8	2.5	9.0	0.6	4.1	0.562	-0.351
Ni (mg kg ⁻¹)	4.8	2.2	9.0	2.1	4.2	1.116	0.161

Table 4. Distribution of statistical parameters of animal manure and urban waste

Type of waste	Parameters	Mean	Standard Deviation	Maximum	Minimum	Median
Manure waste	Moisture (%)	95.9	2.7	99.5	90.3	96.5
	Dry Matter (%)	4.1	2.7	9.7	0.5	3.5
	Organic Matter (%)	2.6	2.1	7.8	0.5	2.4
	pH (1:10)	8.4	0.9	8.9	5.6	8.6
	EC (1:10)	2.8	1.3	6.5	1.6	2.3
	N (%)	0.3	0.2	0.6	0.1	0.3
	P (%)	0.1	0.0	0.2	0.0	0.1
	K (%)	0.3	0.1	0.6	0.1	0.4
	Ca (%)	0.4	0.2	0.7	0.2	0.3
	Mg (%)	0.1	0.1	0.2	0.0	0.0
	Na (%)	0.2	0.1	0.4	0.1	0.2
	Fe (mg kg ⁻¹)	381.4	424.9	1638.2	60.0	297.7
	Zn (mg kg ⁻¹)	30.5	24.5	103.7	6.9	23.8
	Cu (mg kg ⁻¹)	10.7	9.2	33.2	2.7	8.0
	Mn (mg kg ⁻¹)	20.2	12.0	37.3	5.4	19.3
	B (mg kg ⁻¹)	24.2	4.0	29.2	17.0	25.1
	Cd (mg kg ⁻¹)	0.5	0.3	0.9	0.2	0.4
Cr (mg kg ⁻¹)	6.8	3.3	12.2	1.4	6.0	
Pb (mg kg ⁻¹)	4.8	2.6	9.0	0.6	4.1	
Ni (mg kg ⁻¹)	4.9	2.4	9.0	2.1	4.2	
Urban waste	Moisture (%)	96.4	3.1	98.6	94.3	96.4
	Dry Matter (%)	3.6	3.1	5.7	1.4	3.6
	Organic Matter (%)	2.3	1.4	3.3	1.4	2.3
	pH (1:10)	8.8	0.2	8.9	8.7	8.8
	EC (1:10)	3.3	2.6	5	1.5	3.3
	N (%)	0.5	0.3	0.7	0.3	0.5
	P (%)	0.1	0.1	0.2	0.1	0.1
	K (%)	0.3	0.1	0.4	0.2	0.3
	Ca (%)	0.3	0.3	0.5	0.1	0.3
	Mg (%)	0.1	0.0	0.1	0.0	0.1
	Na (%)	0.3	0.0	0.3	0.3	0.3
	Fe (mg kg ⁻¹)	227.6	210.4	376.4	78.9	227.6
	Zn (mg kg ⁻¹)	27.4	29.3	48.2	6.7	27.4
	Cu (mg kg ⁻¹)	6.1	5.8	10.2	1.9	6.1
	Mn (mg kg ⁻¹)	18.0	20.0	32.1	3.9	18.0
	B (mg kg ⁻¹)	34.0	0.9	34.6	33.4	34.0
	Cd (mg kg ⁻¹)	0.3	0.0	0.4	0.3	0.3
Cr (mg kg ⁻¹)	6.3	0.7	6.8	5.8	6.3	
Pb (mg kg ⁻¹)	5.2	1.9	6.6	3.9	5.2	
Ni (mg kg ⁻¹)	4.3	0.5	4.7	4.0	4.3	

The OM content, in a positive direction, had a significant correlation with N at 73.9%, P at 80.4%, Fe at 71.4%, Mn at 75.7%, EC at 53.2%, K at 60.7%, and Mg at 72.1% (Figure 1). The numbers obtained show the correlation coefficient between the organic matter and the determined parameters. This order was determined as P > Mn > N > Mg > Fe > K > EC. There was no statistically significant relationship between OM and Cd, Cr, Cu, Ni, Pb, and Zn, or pH parameters ($p > 0.05$). This order given is based on the highest positive correlation coefficient between the organic matter and the determined parameters.

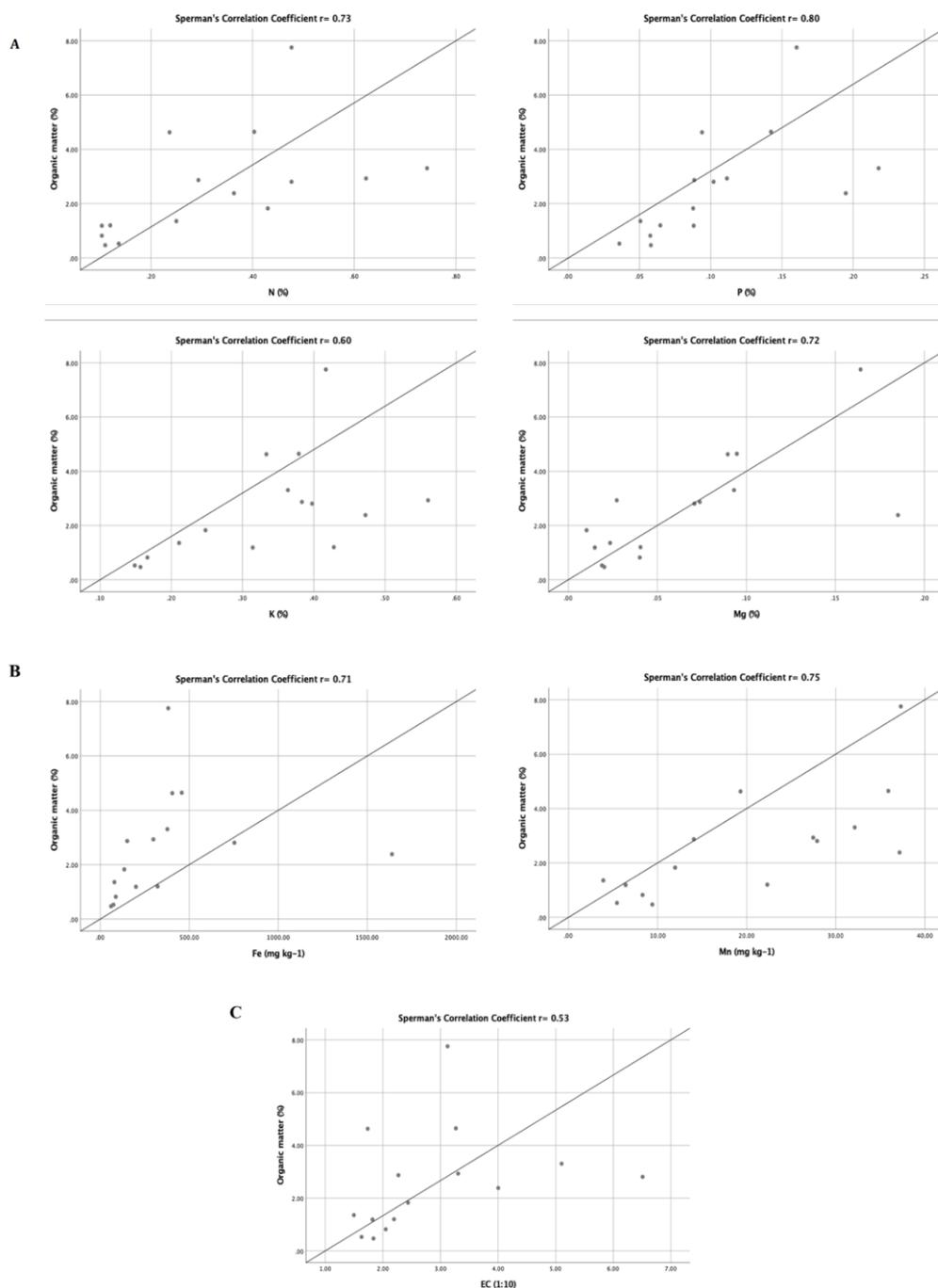


Figure 1. The relationship of organic matter with macro elements (A), micro elements (B) and EC (C)

Correlation analyses

OM and DM contents of the fermented liquid biogas wastes are the main characteristics taken into consideration in the evaluation of wastes. In addition, these two parameters can be determined easily and quickly; therefore, correlation analyses were conducted to reveal the OM and DM relationships with other parameters. The OM content, in a positive direction, had a significant correlation with N at 73.9%, P at 80.4%, Fe at 71.4%, Mn at 75.7%, EC at 53.2%, K at 60.7%, and Mg at 72.1% (Figure 1). The numbers obtained show the correlation coefficient between the organic matter and the determined parameters. This order was determined as P > Mn > N > Mg > Fe > K > EC. There was no statistically significant relationship between OM and Cd, Cr, Cu, Ni, Pb, and Zn, or pH parameters ($p > 0.05$). This order given is based on the highest positive

correlation coefficient between the organic matter and the determined parameters. The DM content, in a positive direction, had a significant correlation with N at 68.2%, P at 95.4%, Cu at 60.0%, Fe at 88.2%, Mn at 94.3%, Zn at 67.5%, EC at 76.1%, K at 81.4%, and Mg at 83.9% (Figure 2). This order was determined as P > Mn > Fe > Mg > K > EC > N > Zn > Cu. There was no statistically significant relationship between DM and Cd, Cr, Ni, Pb, and pH parameters ($p > 0.05$).

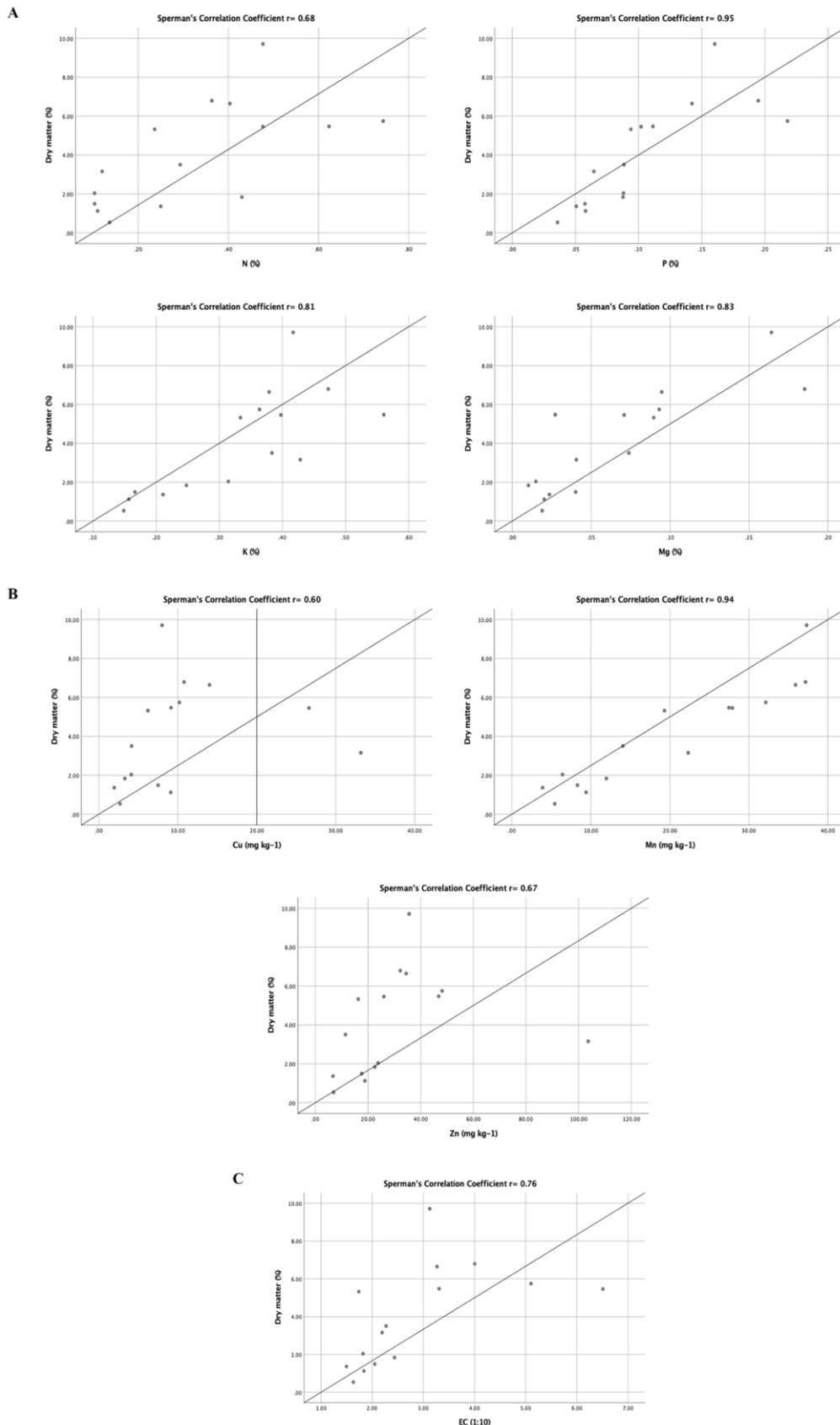


Figure 2. The relationship of dry matter with macro elements (A), micro elements (B) and EC (C)

Regression analysis

Regression analysis was conducted to predict how the independent variables affected the dependent variable in the parameters determined in the wastes. OM and DM contained in the fermented liquid biogas wastes as independent variables were examined for the reasons explained in section 3.1.

Table 5. Regression results of macro-elements in biogas waste according to dry matter and organic matter independent variables

N (%) **p < 0.01					
Independent variables	Univariate p-value	Odd's Ratio	95% C.I. for EXP(B)		R ² _{adjusted}
			Lower	Upper	
Dry Matter	0.009	0.049	0.015	0.83	0.378
Organic Matter	0.034	0.056	0.005	0.108	0.248
P (%) **p < 0.01					
Independent variables	Univariate p value	Odd's Ratio	95% C.I. for EXP(B)		R ² _{adjusted}
			Lower	Upper	
Dry Matter	0.001	0.016	0.009	0.023	0.617
Organic Matter	0.017	0.016	0.003	0.030	0.315
K (%) **p < 0.01					
Independent variables	Univariate p value	Odd's Ratio	95% C.I. for EXP(B)		R ² _{adjusted}
			Lower	Upper	
Dry Matter	0.001	0.035	0.017	0.053	0.539
Organic Matter	0.043	0.033	0.001	0.065	0.224
Ca (%) **p < 0.01					
Independent variables	Univariate p value	Odd's Ratio	95% C.I. for EXP(B)		R ² _{adjusted}
			Lower	Upper	
Dry Matter	0.003	0.047	0.019	0.075	0.466
Organic Matter	0.037	0.049	0.003	0.094	0.238
Mg (%) **p < 0.01					
Independent variables	Univariate p value	Odd's Ratio	95% C.I. for EXP(B)		R ² _{adjusted}
			Lower	Upper	
Dry Matter	0.001	0.017	0.011	0.024	0.700
Organic Matter	0.003	0.019	0.008	0.031	0.457

C.I.: confidence interval; EXP:Exponentiation

A regression analysis was conducted to estimate the values of N, P, K, Ca, and Mg using the results of DM and OM parameters found in the biogas waste. A significant regression model and variances of DM and OM variables were 37.8% ($R^2_{\text{adjusted}} = 0.37$) and 24.8% ($R^2_{\text{adjusted}} = 0.24$) for N (%), 61.7% ($R^2_{\text{adjusted}} = 0.61$) and 31.5% ($R^2_{\text{adjusted}} = 0.31$) for P (%), 53.9% ($R^2_{\text{adjusted}} = 0.53$) and 22.4% ($R^2_{\text{adjusted}} = 0.22$) for K (%), 46.6% ($R^2_{\text{adjusted}} = 0.46$) and 23.8% ($R^2_{\text{adjusted}} = 0.23$) for Ca (%), and 70.0% ($R^2_{\text{adjusted}} = 0.70$) and 45.7% ($R^2_{\text{adjusted}} = 0.45$) for Mg (%). According to these data, the DM and OM parameters and macro-elements can be predicted to be positive and significant (Table 5). Also according to these data, the DM result can be estimated to be sorted as Mg (70%) > P (61.7%) > K (53.9%) > Ca (46.6%) > N (37.8%). The OM result can be estimated to be sorted as Mg (45.7%) > P (31.5%) > N (24.8%) > Ca (23.8%) > K (22.4%). As a result of the regression analysis, the DM and OM parameters, micro element and the heavy metal analyses, no significant regression model was found for Fe, Zn, Cu, Mn, B, Cd, Cr, Pb or Ni parameters ($p > 0.05$).

Conclusion

The present study evaluated the liquid fermented wastes after production from 15 biogas plants (2 urban, 13 manure) being actively produced in Türkiye. The evaluation of the biogas liquid fermented wastes is one of the important factors in the construction of biogas plants. In the evaluation of these liquid wastes, it is possible that they can be considered as organic input and used in agricultural production; however, the properties of the liquid fermented waste is an important issue. In general, the type of waste used in the process of producing biogas the quality of that waste is based on the properties of the liquid fermented waste. The pathogen problem is important in liquid fermented wastes, especially those obtained from animals, and sound sanitation protocols are methods by which to overcome this problem. The fact that 15 plants contained important pathogens in the liquid fermented waste before sanitation and that no pathogenic microorganisms that may pose any risk were found after sanitizing at 70°C reveals the importance of sanitation. Because of some economic concerns, this process is not applied in biogas facilities; therefore, its release and application to the lands without pathogen removal should be strictly controlled. Another negative factor to using biogas liquid fermented wastes is their salt content. EC values in original liquid fermented waste samples, which give the total soluble salt content, can be high enough to limit agricultural use. The EC values of the 15 biogas liquid fermented wastes considered within the scope of the present study were within the range of 13-30 dS m⁻¹ in the original samples. This factor should not be ignored over long-term and overlapping applications of liquid fermented wastes to the soils. The most important properties in the application of liquid fermented wastes

are the OM and DM amounts. Detection of these two parameters is both easy and fast; therefore, OM and DM parameters were considered independent variables, and correlation and regression analyzes were conducted to estimate the distribution of macro-and micronutrients and heavy metals, which affect crop production and productivity, based on the presence of these two parameters. According to the results, DM and OM parameters, and macro-elements can be predicted significantly. The amount of macro-elements in the liquid fermented waste varied depending on the increase and decrease in OM and DM. On the other hand, a significant correlation and a regression model were not determined between OM and DM and their heavy metal contents. It is important to determine the pathogenicity, EC values, and OM and DM contents in studies to determine the usability of liquid fermented wastes for agricultural purposes.

Acknowledgements

We thank Berfu Arıkan, Ozge Gun, and Murat Aksit for assistance in the laboratory and data analysis. We are also grateful to “Biyotar Corp.” and “Renewable Energy and Environmental Technologies Cluster” for providing the necessary facilities for undertaking this research. This study was supported by the “Investigation of the Agricultural Usage Potential of Liquid Fermented Products From Biogas Power Plants” project.

References

- Al Seadi, T., Drosch, B., Fuchs, W., Rutz, D., Janssen, R., 2013. Biogas digestate quality and utilization. In: The biogas handbook. The Biogas Handbook : Science, Production and Applications. Wellinger, A., Murphy, J., Baxter, D. (Eds.). Woodhead Publishing, pp. 267-301.
- Anonymous, 2021. European Biogas Association: Number of Biogas Plants in Europe. Available at [Access date: 01.06.2021]. <https://www.europeanbiogas.eu/the-contribution-of-the-biogas-and-biomethane-industries-to-medium-term-greenhouse-gas-reduction-targets-and-climate-neutrality-by-2050/>
- Bauer, A., Mayr, H., Hopfner-Sixt, K., Amon, T., 2009. Detailed monitoring of two biogas plants and mechanical solid-liquid separation of fermentation residues. *Journal of Biotechnology* 142(1): 56-63.
- Bremner, J.M., 1965. Total nitrogen, In: Methods of soil analysis. Part 2. Chemical and microbiological properties. Black, C.A., Evans, D.D., White, J.L., Ensminger, L.E., Clark F.E. (Eds.), Soil Science Society of America. Madison, Wisconsin, USA. pp. 1149-1176.
- Dahlin, J., Nelles, M., Herbes, C., 2017. Biogas digestate management: Evaluating the attitudes and perceptions of German gardeners towards digestate-based soil amendments. *Resources, Conservation and Recycling* 118: 27-38.
- Egieya, J.M., Cucek, L., Zirngast, K., Isafiade, A.J., Pahor, B., Kravanja, Z., 2018. Biogas supply chain optimization considering different multi-period scenarios. *Chemical Engineering Transactions* 70: 985-990.
- Fouda, S., von Tucher, S., Lichti, F., Schmidhalter, U., 2013. Nitrogen availability of various biogas residues applied to ryegrass. *Journal of Plant Nutrition and Soil Science* 176(4): 572-584.
- Herbes, C., Roth, U., Wulf, S., Dahlin, J., 2020. Economic assessment of different biogas digestate processing technologies: A scenario-based analysis. *Journal of Cleaner Production* 255: 120282.
- ISO 21528-1, Microbiology of the food chain-Horizontal method for the detection and enumeration of *Enterobacteriaceae*-Part 1: Detection of *Enterobacteriaceae*.
- Jackson, M.L. 1958. Soil Chemical Analysis. Prentice Hall Inc., Englewood Cliffs, 498p.
- Kalra, Y. 1997. Handbook of reference methods for plant analysis. CRC press. 300p.
- Leininger, D.J., Roberson, J.R., Elvinger, F., 2001. Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. *Journal of Veterinary Diagnostic Investigation* 13(3): 273-275.
- Mooijman, K.A., Pielaat, A., Kuijpers, A.F.A., 2019. Validation of EN ISO 6579-1- Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1 detection of *Salmonella* spp. *International Journal of Food Microbiology* 288: 3-12.
- Nasir, I.M., Ghazi, T.I.M., Omar, R., 2012. Production of biogas from solid organic wastes through anaerobic digestion: a review. *Applied Microbiology and Biotechnology* 95(2): 321-329.
- Stinner, W., Moller, K., Leithold, G., 2008. Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming systems. *European Journal of Agronomy* 29(2-3): 125-134.
- Wang, H., Xu, J., Sheng, L., Liu, X., 2018. Effect of addition of biogas slurry for anaerobic fermentation of deer manure on biogas production. *Energy* 165: 411-418.
- Warnars, L., Oppenoorth, H., 2014. Bio slurry: A supreme fertilizer. A study on bio slurry results and uses. Hivos People Unlimited, FSC. Hague, Netherlands. 49p.
- Wentzel, S., Joergensen, R.G., 2016. Quantitative microbial indices in biogas and raw cattle slurries. *Engineering in Life Sciences* 16(3): 231-237.
- Yan, L., Liu, Q., Liu, C., Liu, Y., Zhang, M., Zhang, Y., Gu, W., 2019. Effect of swine biogas slurry application on soil dissolved organic matter (DOM) content and fluorescence characteristics. *Ecotoxicology and Environmental Safety* 184: 109616.
- Zadik, P.M., Chapman, P.A., Siddons, C.A., 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *Journal of Medical Microbiology* 39(2): 155-158.