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Research Article

Anaerobic treatment of N-(phosphonometyl) glycine using mixed culture in batch reactor

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ABSTRACT

Pesticides are chemicals and preparations used in agricultural control practice and research. They are used to prevent and control pests. Pesticides are toxic and biocidal substances. The unconscious and uncontrolled use of pesticides in order to provide high yields in agricultural areas is an important problem for human health and the environment. For this reason, biodegredation of pesticides was gained importance in recent years. N-(phosphonomethyl) glycine formulated as isopropylamine salt, is a broad spectrum herbicide with high activity and effective destruction. In this study, the optimization of anaerobic treatment of N-(phosphonomethyl) glycine was investigated by applying a statistical-based experimental design. Full factorial experiments with different initial pesticide concentrations and cosubstrate types were established and 9 different experimental setup were established. The experiments carried out in 2 replicates. The experiments were carried out in Oxitop C flasks in a working volume of 200 mL with stirring. The pH was adjusted to 7±0.2. The experiments carried out at 35 ^{o}C for 30 days. At the end of the process, the removal of inlet and outlet COD and pesticide values were analyzed. As a result, the most efficient COD removal was obtained with 99% at a pesticide concentration of 5 mg L^{-1} and glucose as cosubstrate. The highest pesticide removal was found to be 75% at a pesticide concentration of 25 mg L^{-1} and glucose as cosubstrate. 5 mg. L^{-1} pesticide containing inlet concentration had toxic effect over 19% of the Vibrio fischeria before treatment, while no toxic effect was observed after treatment. This shows that the toxic value of wastewater containing pesticides decreased.

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INTRODUCTION

Agriculture is the most important source of nutrition for the whole world and the need for nutrition is increasing with the increasing population, so it is aimed to obtain more agricultural products. For this purpose, all kinds of harmful weeds, plants and insects that will damage the agricultural product and affect the productivity of the product are tried to be prevented from damaging the product. Agricultural drugs developed to combat these weeds, plants, and insects are generally called pesticides. Pesticides are grouped as insecticides, herbicides, fungicides, acaricides, rodenticides, nematicides, avicides, tree protectants and defoliants according to their intended use [1]. Pesticides are further defined as aniline derivatives, carbamates, chlorophenoxy compounds, organochlorinated compounds, organophosphorus compounds, pyridine and pyridine derivatives, triazines, urea-containing compounds and unclassified compounds according to their active ingredients [2].

Agricultural production is one of the most important factors in Turkish economy. For this reason, a significant amount of pesticide are produced and consumed in our country. However, increasing urbanization has increased the use of pesticides for other purposes in addition to agricultural purposes. Türkiye is an agricultural country and it is known that agricultural areas cover about 50% of the country's surface area and the amount of pesticides used per hectare is proportional to the surface area of agricultural areas. However, pesticide consumption per hectare in countries such as the USA, Germany, the Netherlands and Italy, which have around 50% agricultural land, is much higher than in Türkiye [3]. The main problem for Türkiye is that the amount of pesticide use is high in some regions and excess pesticides are detected in agricultural products in these regions. For example, in Antalya province where fruit and vegetable production is high and these products are exported, the amount of pesticides used per hectare in arable agriculture areas is 26.85 kg. ha^{-1}, while this value is 10.9 kg. ha^{-1} in the Netherlands, the country with the highest pesticides use in Europe [3].

Pesticide contaminated wastewaters cause environmental pollution problems because they contain toxic substances, have high chemical oxygen demand (6000–10000 mg. L^{-1}), high biological oxygen demand (2000–5000 mg. L^{-1}), and high total dissolved solids (12000–13000 mg. L^{-1}) concentrations and have basic properties [4].

Pesticides applied to agricultural areas, are transferred and transformed in air, water and soil, and from there to other organisms living in these environments. Glyphosate (N-(phosphonomethyl)glycine) was developed in the early 1970s and at present is used as a herbicide to kill broadleaf weeds and grass. Glyphosate [N-phosphonomethyl glycine] is a broad-spectrum herbicide formulated as an isopropylamine salt with high activity and effective knockdown. It is an organophosphate and non-selective herbicide applied to the leaves of plants to kill both broad-leaved plants and grasses. The widely occurring degradation product aminomethylphosphonic acid (AMPA) is a result of glyphosate and amino-polyphosphonate degradation [5, 6].

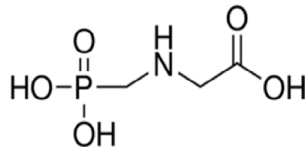

Figure 1. N- (phosphonomethyl) glycine.

Table 1. Characteristics of anaerobic sludge (AS) used in batch studies

Parameter	AS content
pH	7.4
TS (g. L^{-1})	36.4
TSS (g. L^{-1})	30.8
VSS (g. L^{-1})	14.6

The EPA divided the toxicity of Glyphosate (N-phosphonomethyl) glycine into slight toxicity with concentrations ranging from 10 to 100 mg/L and almost nontoxicity with concentration higher than 100 mg/L to fish species with acute LC50 values from >10 to >1000 mg/L. Lethal concentrations are various for 24, 48, and 96 h ranging from 0.295 to 645 mg/L for fish species; from 6.5 to 115 mg/L for amphibian's species; and from 35 to 461.54 mg/L for invertebrate species [6].

Complete and rapid degradation of glyphosate occurs microbiologically in soil and/or water, not chemically occurs. This study aim was to investigate the removal of N-(phosphonomethyl)glycine pesticide under anaerobic conditions in a batch study regardinf the effect of various operating conditions, such as concentration of pesticide and co-substrate type. The full factorial (3^2) experimental design was adopted to determine the statistical significance of each parameter on treatment performance.

MATERIALS AND METHODS

Pesticide Solution

In this study, N- (phosphonomethyl) glycine ($C_3H_8NO_5P$) (Fig. 1), which is a type pesticide that is widely used in agricultural areas. Commercial pesticide solutions containing these active ingredients were obtained from Eskişehir Green Agricultural Products, Pesticides, and Tools Company in order to be both economical in pesticide removal experiments in the batch reactors and to test the form commonly used in agricultural activities in Eskişehir province. The pesticide was stored in a refrigerator at +4 °C

Anaerobic Sludge

Anaerobic sludge (AS) used in the batch reactor was obtained from Eskişehir Sugar Factory Anaerobic Treatment Unit. Before use, the sludge was homogenized by thorough mixing and filtered through a filter with a pore diameter of 1 mm. The important properties of AS in terms of treatment, such as pH, total suspended solids (TSS),total solids (TS) and volatile suspendedn solids were determined (Table 1) [7].

Figure 2. Batch reactor (Oxitop C bottles).

Basal Medium

The composition of the basal medium used in the experiments is as follows (Concentrations of the components are given as mg. L^{-1}): NH_4Cl (1200), $MgSO_4.7H_2O$ (400), KCl (400), $Na_2S.9H_2O$ (300), $CaCl_2.2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2.4H_2O$ (40), $CoCl_2.6H_2O$ (10), KI (10.0), $MnCl_2.4H_2O$ (0.5), $CuCl_2.2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_2O$ (0.5), $NaMoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_2O$ (0.5), $NaWO_4.2H_2O$ (0.5), Na_2SeO_3 (0.5), and cysteine (10.0). This basal medium contains all the micro and macronutrients necessary for an optimum anaerobic microbial growth [8–10].

Factorial Experimental Design

Factorial experimental designs are widely used in experiments involving several factors to investigate the common effects of factors on the outcome [11]. Factorial (3^2) experimental designs were used to minimize the number of experiments. In this factorial design, 9 different experimental setups were established by matching three factors and three levels. In this study, a 3^2 factorial experimental design was used to investigate the effects of pesticide containing wastewater and co-substrate on COD and pesticide removal. The experiments were conducted in two parallel runs. Statistical analysis and interpretations were performed using IBM SPSS Statistics 22 software program.

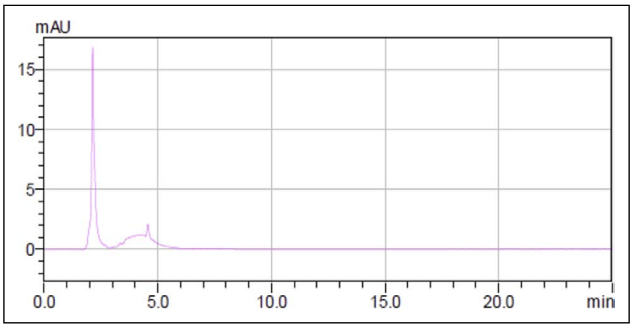

Figure 3. Chromatogram obtained for N phosphonomethyl glycine in HPLC.

Anaerobic Batch Reactor

The optimization of anaerobic treatment of synthetic wastewater containing pesticides was studied by applying statistical-based experimental design in batch studies. The batch studies were performed in 200 mLworking volume in 250 ml glass Oxitop C bottles (OxiTop® Control AN12, WTW, Weilheim, Germany) (Fig. 2). In the full factorial experimental design nine different experimental setups were investigated including the effects of different parameters such as different pesticide initial concentrations (5 mg. L^{-1}, 25 mg. L^{-1}, 45 mg. L^{-1}) and co-substrates (a-glucose (2000 mg L^{-1}, b-propionic acid (1000 mg L^{-1}), c-acetic- propionic-butyric acid mixture (ABP) (1500, 350, 350 mg L^{-1} respectively).

In all studies, a mineral medium containing substances necessary for the growth of anaerobic microorganisms was also used. pH stability was ensured by addition of $NaHCO_3$ and dissolved oxygen removal by $Na_2S.9H_2O$. pH was adjusted to 7±0.1. All experiments were carried out at 35 °C for 30 days. At the end of the period, COD and pesticide removal rates were determined [12].

The HPLC Analysis of N-Phosphonomethyl Glycine

Shimadzu UFLCXR model High-Pressure Liquid Chromatography System (HPLC) device was used for determining pesticide removal in a batch reactor. Chromatographic instructions of HPLC device with DAD detector for the determination of N- (phosphonomethyl) glycine: [13]

Column: C18 (inner diameter: 250 mm x 4.6 mm, particle size: 5 mm)

Mobile Phase: 0.05 M sodium phosphate buffer

Flow Rate: mL / min

Wavelength: 265 nm

The chromatogram obtained for the analysis of N phosphonomethyl glycine in HPLC is given in Figure 3. The calibration curves were created for all pesticides with the analysis made on the HPLC device. These curves include

Table 2. Batch reactor COD results obtained with full factorial (3^2) experimental design of synthetic wastewater containing N phosphonomethyl glycine and co-substrates (a-glucose (2000 mg L^{-1}, b-propionic acid (1000 mg L^{-1}), c-acetic- propionic-butyric acid mixture (ABP) (1500, 350, 350 mg L^{-1} respectively)

Experiment no	Pesticide concentration (A)		Co-substrate type (B)		Average COD removal (%)
	Real	Code	Real	Code	
1	**5 mg. L^{-1}**	**-1**	**Glucose**	**-1**	**99**
2	5 mg. L^{-1}	-1	Propionic acid	0	96
3	5 mg. L^{-1}	-1	ABP	1	98
4	25 mg. L^{-1}	0	Glucose	-1	96
5	25 mg. L^{-1}	0	Propionic acid	0	96
6	25 mg. L^{-1}	0	ABP	1	97
7	45 mg. L^{-1}	1	Glucose	-1	97
8	45 mg. L^{-1}	1	Propionic acid	0	95
9	45 mg. L^{-1}	1	ABP	1	97

ABP: C-acetic- propionic-butyric acid mixture.

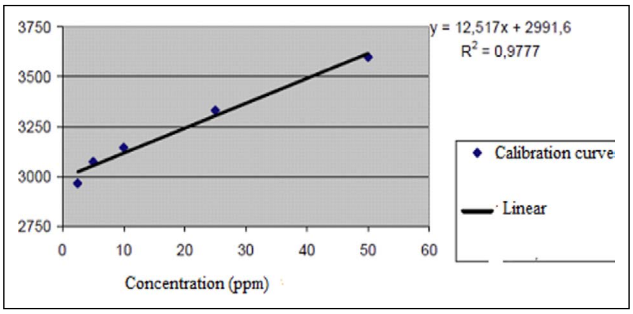

Figure 4. Calibration graph for N phosphonomethyl glycine in HPLC.

from 2.5 to 50 mg. L^{-1}. Standard solutions of N- (phosphonomethyl) glycine (Sigma) are used and the obtained calibration chart is given in Figure 4.

Toxicity Tests

Toxicity Test Using Vibrio Fischeri

The effect of wastewater containing pesticides on prokaryotic organisms before and after treatment was determined by the *Vibrio fischeri* toxicity test. The experiment was performed on SDI (United States) M500 Microtox® instrument. The determination of toxicity is based on the principle that the ability of the marine bacterium *Vibrio fischeri* culture decreases luminescence properties in the presence of toxic substances. The experiments were carried out in 2% NaCl solution at 15 °C. Luminescence was measured at 490 nm. The results were expressed as the concentration at which 50% of the luminescence was lost at 5 and 15 minutes (EC_{50}) [14].

Toxicity Test Using Lepidium Sativum

The toxic effect of pesticide-containing wastewater on eukaryotic plant cells before and after treatment was evaluated by its effect on the germination of *Lepidium sativum* seed. The experiment was carried out in 10 cm petri dishes on 2 Whatman No: 1 papers sterilised paper and 10 seeds were placed in each petri dish. The petri dishes were with 5 ml each of the pre and post-treatment samples to wet the entire Whatman No: 1 paper. Afterwards, the seeds were placed with equal distance between them. After 7 days of incubation, measurements were made. The number of germination and root elongation for each concentration were used to determine the toxic effect [15]. Experiments were carried out in 2 replicates.

RESULTS AND DISCUSSION

Determine the Properties of Anaerobic Sludge

Some characteristics of anaerobic sludge used in batch reactor studies are given in Table 1.

Batch Reactor Studies of Synthetic Wastewater Containing N-(phosphonomethyl) glycine

In this work, a statistical approach was chosen based on a factorial experimental design that would allow us to infer about the effect of the variables with a relatively small number of experiments. The independent variables of the experimental design are presented in Table 2. Each one of the three variables received three values, a high value (indicated by the plus sign), a medium value (indicated by the zero sign) and a low value (indicated by the minus sign) [8].

COD Removal Results

Batch reactor experimental setups were prepared using different concentrations of N phosphonomethyl glycine and different types of cosubstrate and the average COD removal rates obtained from each experiment are given in Table 2.

In line with these results, variance analyses, single and pairwise interaction analyses of factors were performed. Experimental design results were calculated with IBM SPSS Statistics 22 software. The analysis of variance obtained in terms of COD removal as a result of the statistically based study is given in Table 3.

Table 3. Variance analysis table for COD removal

Source	Sum of squares	df	Average of squares	F	Sig.
Corrected model	19.240[a]	8	2.405	12.013	0.001
Interrupter	168428.211	1	168428.211	841253.064	0.000
Concentration	6.642	2	3.321	16.587	0.001
Co-substrate	5.828	2	2.914	14.555	0.002
Concentration * Co-substrate	6.770	4	1.693	8.454	0.004
Error	1.802	9	0.200		
Grand Total	168449.253	18			
Adjusted Total	21.042	17			

a R^2=0.914 (Adjusted R^2=0.838) df: Degree of freedom; F: Frequency; Sig: Significance level.

When Table 3 is analysed, it is seen that the significance value calculated for the model is less than 0.05 and pesticide concentration and 3 different levels of cosubstrate have a statistically significant effect on COD removal ($p<0.05$). It is seen that the single interactions and pairwise interactions of the factors in the experiments are also statistically significant at 0.05 significance level. In addition, it was determined that the established model explained 83.8% of the COD removal.

The relationship between pesticide concentration difference and COD removal is given in the homogeneous subset in Table 4.

Table 4. Relationship between pesticide concentration and COD removal

Concentration	N	Subset	
		1	2
45 mg L^{-1}	6	96	
25 mg L^{-1}	6	97	
5 mg L^{-1}	6		97
Sig.		0.060	1.000

Sig: Significance level.

When Table 4 was examined, it is seen that the best COD removal is at 5 mg L^{-1} and 25 mg L^{-1} pesticide concentration. The significance value calculated for the model is greater than 0.05 and there is no statistically significant effect between pesticide concentration and COD removal ($p>0.05$).

According to the statistical data, the comparison of pesticide concentrations in COD removal is given in Table 5.

According to Table 5, COD removal was enhanced by 1.49%, on average, when the pesticide concentration decreased from 45 mg L^{-1} (high level) to 5 mg L^{-1} (low level). When 25 mg L^{-1} pesticide concentration was used instead of 5 mg.L^{-1} pesticide concentration, COD removal decreased by 0.79%. When 25 mg L^{-1} pesticide concentration was used instead of 45 mg.L^{-1} pesticide concentration, COD removal increased by 0.69%. As a result of the significance test made regarding whether this difference is statistically significant, sig. the value was obtained as 0.06. It was found that the difference in COD removal between the use of 25 mg. L^{-1} and 45 mg L^{-1} concentrations were not statistically significant.

The relationship between co-substrate and COD removal was given in Table 6. When Table 6 was examined, it is seen that the best COD removal is in glucose use. In batch reactor studies, using propionic acid instead of ABP as a cosubstrate type increases COD removal by 1.13%, while the use of glucose increases COD removal by 1.27%. There is no statistically significant difference in the comparison of propionic acid and glucose use as co-substrate.

A comparison of different co-substrates in COD removal according to statistical data is given in Table 7. According

Table 5. Effect of pesticide concentrations on COD removal

(I) Concentration	(J) Concentration	Average difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower limit	Upper limit
5 mg. L-1	25 mg. L^{-1}	0.7967*	0.25834	0.032	0.0754	1.5179
	45 mg. L^{-1}	1.4867*	0.25834	0.001	0.7654	2.2079
25 mg. L-1	5 mg. L^{-1}	-0.7967*	0.25834	0.032	-1.5179	-0.0754
	45 mg. L^{-1}	0.6900	0.25834	0.060	-0.0313	1.4113
45 mg. L-1	5 mg. L^{-1}	-1.4867*	0.25834	0.001	-2.2079	-0.7654
	25 mg. L^{-1}	-0.6900	0.25834	0.060	-1.4113	0.0313

Sig: Significance level.

Table 6. Relationship between co-substrate and COD removal

Co-substrate	N	Subset	
		1	2
ABP	6	96	
Propionic acid	6		97
Glucose	6		97
Sig.		1.000	0.850

ABP: C-acetic- propionic-butyric acid mixture; Sig: Significance level.

to Table 7, the use of glucose as a co-substrate increased the COD removal rate by 1.27% compared to the use of propionic acid. When using ABP instead of glucose, the COD removal rate increased by 0.14%. As a result of the significance test made regarding whether this difference is statistically significant, sig. the value was obtained as 0.850. Since this value is higher than the significance level of 0.05, it was determined that the difference in COD removal between glucose and ABP use was not statistically significant. The use of propionic acid as a co-substrate reduces COD removal by 1.27% compared to glucose use and by 1.13% compared to ABP use.

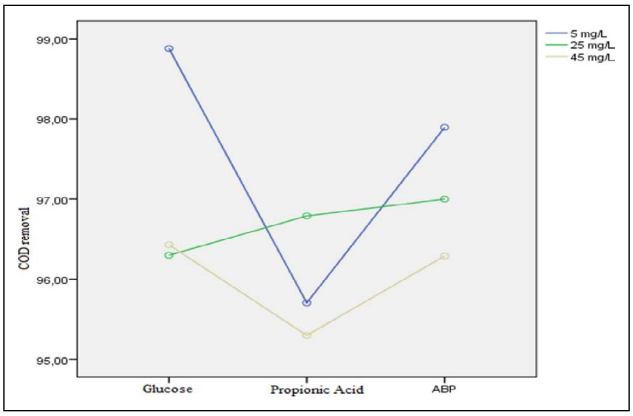

Figure 5. Profile graph of concentration and cosubstrate interaction for COD removal.

The profile graph of the pesticide concentration and cosubstrate interaction is given in Figure 5. When Figure 5 is analyzed, 5 mg L^{-1} pesticide concentration and glucose as cosubstrate, maximum COD removal was obtained.

Pesticide Removal Results

The N-(phosphonomethyl) glycine removal with different times and different co-substrate test setups and the average pesticide removal rates are given in Table 8.

Table 7. Effect of co-substrate on COD removal

(I) Co-substrate	(J) Co-substrate	Average difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower limit	Upper limit
Glucose	Propionic acid	1.2717*	0.25834	0.002	0.5504	1.9929
	ABP	0.1417	0.25834	0.850	-0.5796	0.8629
Propionic acid	Glucose	-1.2717*	0.25834	0.002	-1.9929	-0.5504
	ABP	-1.1300*	0.25834	0.005	-1.8513	-0.4087
ABP	Glucose	-0.1417	0.25834	0.850	-0.8629	0.5796
	Propionic acid	1.1300*	0.25834	0.005	0.4087	1.8513

ABP: C-acetic- propionic-butyric acid mixture; Sig: Significance level.

Table 8. Pesticide removal results by batch reactor obtained with full factorial design of synthetic wastewater containing N phosphonomethyl glycine

Experiment no	Pesticide concentration (A)		Co-substrate type (B)		Average pesticide removal (%)
	Real	Code	Real	Code	
1	5 mg. L^{-1}	-1	Glucose	-1	73
2	5 mg. L^{-1}	-1	Propionic acid	0	52
3	5 mg. L^{-1}	-1	ABP	1	71
4	**25 mg. L^{-1}**	**0**	**Glucose**	**-1**	**75**
5	25 mg. L^{-1}	0	Propionic acid	0	49
6	25 mg. L^{-1}	0	ABP	1	51
7	45 mg. L^{-1}	1	Glucose	-1	74
8	45 mg. L^{-1}	1	Propionic acid	0	69
9	45 mg. L^{-1}	1	ABP	1	54

ABP: C-acetic- propionic-butyric acid mixture.

Table 9. Variance analysis table for results of pesticide removal rates

Source	Sum of squares	df	Average of squares	F	Sig.
Corrected model	2007.987[a]	8	250.998	2086.630	0.000
Interrupter	71029.318	1	71029.318	590489.432	0.000
Concentration	191.264	2	95.632	795.021	0.000
Co-substrate	1097.180	2	548.590	4560.605	0.000
Concentration * Co-substrate	719.542	4	179.886	1495.446	0.000
Error	1.083	9	0.120		
Grand total	73038.387	18			
Adjusted total	2009.070	17			

a R2=0.999 (Adjusted R2=0.999) df: Degree of freedom; F: Frequency; Sig: Significance level.

In line with these results, analyses of variance, single and pairwise interaction analysis of the factors were performed. The results of the experimental design were calculated with IBM SPSS Statistics 22 software and the analysis of variance obtained in terms of pesticide removal as a result of the statistically based study is given in Table 9.

Table 9 shows that the significance value calculated for the model is less than 0.05. Then, the significance tests for the model coefficients were examined. It is seen that the single and pairwise interactions of the factors in the experiment are statistically significant at 0.05 significance level. In addition, it was determined that the established model explained 99.9% of the pesticide removal. As a result, 3 different levels of concentration and cosubstrate were considered and their effects were statistically significant.

The relationship between concentration difference and pesticide removal is given in the homogeneous subset table in Table 10.

When Table 10 was examined, it is seen that the best pesticide removal is at 5 mg L^{-1} and 45 mg L^{-1} pesticide concentration. The significance value calculated for the model is greater than 0.05 and there is no statistically significant effect between pesticide concentration and COD removal (p>0.05).

Comparison of the concentrations for pesticide removal according to statistical data is given in Table 11.

Table 10. Relationship between pesticide concentration and pesticide removal

Concentration	N	Subset	
		1	2
25 mg. L^{-1}	6	58	
5 mg. L^{-1}	6		65
45 mg. L^{-1}	6		65
Sig.		1.000	0.181

Sig: Significance level.

According to Table 11, when the pesticide removal rates obtained at 5 mg L^{-1} and 25 mg L^{-1} concentrations were analyzed, it was found that the pesticide concentration of 5 mg L^{-1} instead of 25 mg L^{-1} increased the pesticide removal by 6.71%. The removal rate decreased by 0.39% at 5 mg L^{-1} compared to 45 mg L^{-1}. As a result of the significance test for statistical significance of this difference, the sig. value was 0.181. Since this value is higher than 0.05 significance level, it is determined that the difference in pesticide removal between the use of 5 mg L^{-1} and 45 mg L^{-1} concentrations is not statistically significant.

The relationship between cosubstrate and pesticide removal is given in the homogeneous subset table in Table 12.

According to Table 12, it is seen that the best pesticide removal is in the use of glucose. In batch reactor studies, the

Table 11. Effect of pesticide concentration on pesticide removal

(I) Concentration	(J) Concentration	Average difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower limit	Upper limit
5 mg. L^{-1}	25 mg. L^{-1}	6.7117*	0.20024	0.000	6.1526	7.2707
	45 mg. L^{-1}	-0.3900	0.20024	0.181	-0.9491	0.1691
25 mg. L^{-1}	5 mg. L^{-1}	-6.7117*	0.20024	0.000	-7.2707	-6.1526
	45 mg. L^{-1}	-7.1017*	0.20024	0.000	-7.6607	-6.5426
45 mg. L^{-1}	5 mg. L^{-1}	0.3900	0.20024	0.181	-0.1691	0.9491
	25 mg. L^{-1}	7.1017*	0.20024	0.000	6.5426	7.6607

Std: Standart; Sig: Significance level.

Table 12. Relationship between cosubstrate and pesticide removal

Co-substrate	N	Subset		
		1	2	3
Propionic acid	6	56		
ABP	6		58	
Glucose	6			74
Sig.		1.000	1.000	1.000

ABP: C-acetic- propionic-butyric acid mixture; Sig: Significance level.

use of ABP instead of propionic acid as co-substrate increases pesticide removal by 1.67%, while the use of glucose increases pesticide removal by 17.33%. The use of glucose instead of ABP increases pesticide removal by 15.66%. These values are statistically significant. Recently, Feng et al. (2020) [16] reported that most of the organisms utilized glyphosate as a phosphorus source.

Comparison of different cosubstrates for pesticide removal according to statistical data is given in Table 13.

According to Table 13, the use of glucose increases the pesticide removal by 17.33% compared to the use of propionic acid and provides 15.66% more pesticide removal than the use of ABP. When propionic acid was used instead of glucose as a cosubstrate, pesticide removal decreased by 17.33%, while removal decreased by 1.67% when ABP was used. When ABP was preferred to glucose and propionic acid, pesticide removal decreased by 15.66% and increased by 1.67%, respectively. These values are statistically significant.

The profile plot of concentration and cosubstrate interaction is given in Figure 6. When Figure 6 is analysed, 25 mg. L^{-1} pesticide concentration and glucose as cosubstrate, maximum pesticide removal was obtained.

Most of the recent studies focused on the degradation of glyphosate pesticides in soil and water by aerobic microorganisms and reported successful degradation performances [16, 17]. However, there aren't any papers that show the degradation of this pesticide by anaerobic organisms. According to our present knowledge, this is the first paper that investigated the degradation of pesticides by anaerobic conditions.

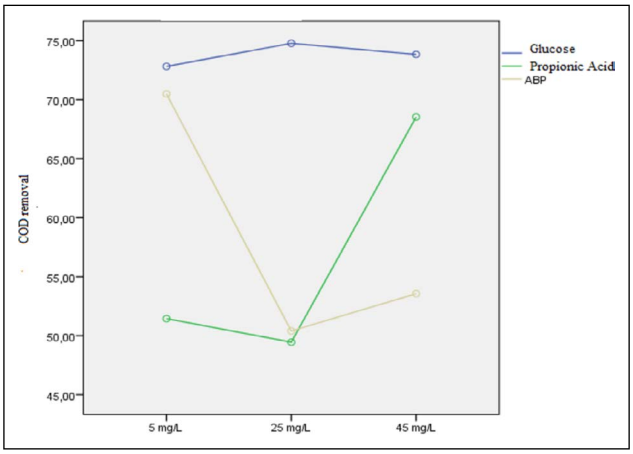

Figure 6. Profile graph of concentration and cosubstrate interaction for pesticide removal.

Ecotoxicological Studies

Toxicity Results with Prokaryotic Cells

In Tables 14 and 15, *Vibrio fischeri* toxicity test results of samples taken before and after the batch reactor using 5 mg L^{-1} as the concentration and glucose as co-substrate with the best efficiency in COD removal were given.

5 mg L^{-1}pesticide containing inlet concentration had toxic effect over 19% of the Vibrio fischeria before treatment, while no toxic effect was observed after treatment. This shows that the toxic value of wastewater containing pesticides decreased.

Toxicity Results with Eukaryotic Cells

Table 16 shows the results of Lepidium sativum toxicity test of the samples taken before and after the batch reactor with 5 mg L^{-1} pesticide concentration and glucose as cosubstrate.

The tested concentration 5 mg. L^{-1} pesticide showed toxic effect on root and stem growth *Lepidium sativum* before anaerobic treatment. After the anaerobic treatment in the batch reactor, root germination increased at a certain rate (15.2 mm) and stem germination decreased (18.4 mm). This indicates that the toxic effect of wastewater on root germination decreased after treatment. However, it is seen that the pressure on root and stem germination continues after treatment compared to control petri dishes.

Table 13. The effect of cosubstrate on pesticide removal

(I) Co-substrate	(J) Co-substrate	Average difference (I-J)	Std. error	Sig.	95% Confidence interval	
					Lower limit	Upper limit
Glucose	Propiyonic acid	17.3350*	0.20024	0.000	16.7759	17.8941
	ABP	15.6617*	0.20024	0.000	15.1026	16.2207
Propionic acid	Glucose	-17.3350*	0.20024	0.000	-17.8941	-16.7759
	ABP	-1.6733*	0.20024	0.000	-2.2324	-1.1143
ABP	Glucose	-15.6617*	0.20024	0.000	-16.2207	-15.1026
	Propiyonic acid	1.6733*	0.20024	0.000	1.1143	2.2324

ABP: C-acetic- propionic-butyric acid mixture; Sig: Significance level.

Table 14. *Vibrio fischeri* toxicity test results of 5 mg L^{-1} concentration before anaerobic treatment

			5 Mins data			15 Mins data		
Sample	**Concentration (mg. L^{-1})**	**I_0**	**I_t**	**Gamma**	**% Effect**	**I_t**	**Gamma**	**% Effect**
Control	0.000	97.00	145.29	1.000#		178.39	1.000#	
1	0.019	89.40	150.25	0.0000#	-12.00%	178.42	-7.000*	-8.000%
2	0.039	90.93	139.58	-2.000*	-2.000%	176.42	-5.000*	-5.000%
3	0.078	83.28	136.31	-8.000*	-9.000%	173.30	0.0000*	-13.00%
4	0.156	90.15	147.85	-8.000*	-9.000%	178.42	-7.000*	-7.000%
5	0.312	96.37	148.60	-2.000*	-2.000%	178.42	-6.000*	0.0000%
6	0.625	92.10	149.11	-7.000*	-8.000%	177.32	-4.000*	-4.000%
7	1,250	89,91	133,87	5,000*	0,0000%	162,13	1,000*	1,000%
8	2,500	86,30	121,66	6,000#	5,000%	145,91	0,0000#	8,000%
9	5,000	81,47	102,60	0,0000*	15,00%	120,82	0,0000#	19,00%

Table 15. *Vibrio ficsheri* toxicity test results after anaerobic treatment of a concentration of 5 mg L^{-1}

			5 Mins data			15 Mins data		
Sample	**Concentration (mg. L^{-1})**		**I_t**	**Gamma**	**% Effect**	**I_t**	**Gamma**	**% Effect**
Control	0.000	95.44	112.49	1.000#		130.06	1.000#	
1	0.019	89.67	109.89	-3.000*	-3.000%	130.71	-6.000*	-6.000%
2	0.039	92.53	103.89	4.000*	4.000%	119.57	5.000	5.000%
3	0.078	87.46	105.84	-2.000*	-2.000%	125.80	-5.000*	-5.000%
4	0.156	90.66	111.53	-4.000*	-4.000%	130.62	-5.000*	-5.000%
5	0.312	92.11	121.95	0.0000*	-12.00%	144.54	0.0000*	-15.00%
6	0.625	90.72	122.56	0.0000*	-14.00%	150.42	0.0000*	-21.00%
7	1.250	81.86	103.00	-6.000#	-6.000%	118.84	-6.000*	-6.000%
8	2.500	75.90	122.88	0.0000*	-37.00%	133.19	0.0000*	-28.00%
9	5.000	74.79	122.28	0.0000*	-38.00%	152.05	0.0000*	-49.00%

Table 16. Lepidium sativum toxicity root and stem growths

Root (average)			Stem (average)		
Control	**Before batch reactor**	**After batch reactor**	**Control**	**Before batch reactor**	**After batch reactor**
21.85 mm	13.35 mm	15.2 mm	30.05 mm	22.95 mm	18.40 mm

Recently, de Castilhos Ghisi et al. (2020) [18] showed that glyphosate had toxic effects on living organisms. Similarly, in the results of ecotoxicology studies, the presence of this pesticide affected procaryotic and eucaryotic organisms, but the water treated with the anaerobic system contained less amount of pesticide and showed less toxicity to the test organisms.

CONCLUSION

Pesticides are plant protection drugs that are becoming more and more widespread day by day, although their damages to human and environmental health have been revealed by many scientific studies all over the world and in our country. The use of pesticides, which are used to protect plants against the negative effects of diseases and pests, has been shown by many scientific to be harmful to human and environmental health, ecological balance and agricultural products, especially cancer.

One of the most important sources of pesticide contamination is the discharge pesticide-containing domestic and industrial wastewater into receiving environments. In this context, the treatment process of pesticides is ecologically important. However, due to their complex chemical structure and synthetic origin, the treatment of pesticides is very difficult. Depending on the need, the structures of pesticides change and the removal process becomes more difficult.

In this study, the treatment potential of wastewater with different pesticide concentrations prepared with N- (phosphonomethyl) glycine in a laboratory environment was determined in a batch anaerobic reactor. When the analysis of variance obtained in terms of COD removal as a result of the statistically based study is examined, it is seen that the significance value calculated for the model is less than 0.05. This shows that the established model is statistically significant. Then, the significance tests for the model coefficients were examined. It is seen that the individual effects and the interaction effects of the factors in the experiment are statistically significant at the 0.05 significance level. In addition, it was determined that the established model explained 83.8% of the COD removal.

According to the results of the experimental design of anaerobic batch reactor studies, it was found that the best COD removal in the experimental set prepared with N-(phosphonomethyl) glycine was 99% when pesticide concentration was 5 mg L^{-1} and glucose was used as cosubstrate and the best pesticide removal was 75% when pesticide concentration was 25 mg L^{-1} and glucose was used as cosubstrate. In terms of binary interaction, it was determined that pesticide concentration and co-substrate type were not statistically significant on COD and pesticide removal.

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DATA AVAILABILITY STATEMENT

The author confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

USE OF AI FOR WRITING ASSISTANCE

Not declared.

ETHICS

There are no ethical issues with the publication of this manuscript.

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