Journal of the Iranian Chemical Society (2024) 21:2459–2468
https://doi.org/10.1007/s13738-024-03083-x

ORIGINAL PAPER

Response surface method Box-Behnken design-based optimization of organic fertilizer enrichment using bat guano

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Received: 2 February 2024 / Accepted: 10 August 2024 / Published online: 23 August 2024
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Abstract
Agricultural environmental pollution needs to be reduced in our future. The biggest obstacles to preventing this situation are that agricultural inputs such as synthetic fertilizers increase product yield and scattered agricultural areas. This study involves the optimization of the production process of an easy-to-apply organic fertilizer with high nutritional value by subjecting bat guano, one of the valuable organic fertilizers, to anaerobic digestion together with cattle manure. Response surface method Box-Behnken design, a statistical model, was used for process optimization. The process input parameters were temperature, cattle manure, and bat manure, and the response value was considered as 2% total nitrogen. As a result of the optimization for a total nitrogen value of 2%, the optimum mixture ratio was determined as cattle manure 77.0448%, bat manure 12%, and temperature 40 °C. Analysis results showed that the addition of bat manure increased the N, P, K, and humic and fulvic acid values in the fertilizer. The study revealed that strong organic fertilizers can be used to increase the value of cattle manure organically. It also offers very promising results based on reducing agricultural pollution and ensuring agricultural sustainability.

Keywords Bat guano · Cattle manure · Response surface method · Fertilizer · Organic fertilizer

Introduction

The rapid increase in the human population necessitates the same increase in agricultural production for nutrition. Agricultural pesticides and fertilizers used extensively to meet demand cause air, soil, and water pollution. Nitrogen pollution, especially in groundwater and soil, reaches significant levels [1]. Crop yield in agriculture is largely due to the use of fertilizer [2]. It is known that the use of chemical fertilizers has increased greatly in the last 50 years and the majority of this use consists of agricultural countries and middle-income countries [1]. Increasing the area of fertilizer application and the amount of fertilizer use has brought about unprecedented growth in crop yield and overall crop production. This situation has brought to the agenda that global food security should be considered [3]. The International Fertilizer Association (IFA) has declared that there is a significant increase in synthetic fertilizer production every year [4].

Unfortunately, reducing fertilizer use causes significant losses in product yield and quality. Modern agricultural practices have triggered the process of agricultural pollution. This process can lead to degradation of ecosystems, soil, and the environment due to by-products of modern agriculture [5, 6]. Excessive use of synthetic fertilizers causes significant water pollution in surface water or groundwater [7]. Excessive use of chemical fertilizers and contaminated water for irrigation causes further damage to agriculture. For this reason, it has become impossible to advance the future scenario of the food and agriculture sector [5, 8].

The positive effects of nitrogen on plants were proven [9–16]. Nitrogen is the most important plant nutrient that is most lacking in plant production, but also most needed and increases productivity [11, 16, 17].

Humus is formed through chemical activity in the natural environment, including sediment, aquatic ecosystems, and soil [18–21]. Humic and fulvic acids play an important role in regulating acidity and alkalinity, as well as maintaining soil fertility, improving the properties of soil aggregates, and increasing soil buffer capacity [21–23]. Humic acids

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consist of minerals obtained from humic and fulvic acids, salts, and humus, which are formed by the combination of substances such as carbohydrates, amino acids, and phenols, which are collectively formed as a result of the decomposition of organic substances in the soil.

Phosphorus (P) is very important for plants because P is an important element of nucleic acids. Phosphorus was also important in plant developmental stages such as cell formation, tissue growth, synthesis of organic compounds, energy transfer and root development, flowering, seed and fruit formation. Therefore, it is an essential element for plant growth and has a significant impact on the quality and quantity of crop yield [24–27].

Nitrogen and phosphorus are the top two agricultural pollutants [28–30], and source of these two nutrients is synthetic inorganic fertilizers [30–32]. It is necessary to pay attention to nitrogen, which is generally mobile in soil and water. Soil ammonia is microbially converted to nitrates and can leach beneath the root zones of crops, contaminating groundwater or reaching streams [33, 34]. Nitrogen also reaches streams via runoff from fertilized crop fields and pastures, through drainage pavements or roadside ditches and drains [5]. Excessive application of nitrogenous and phosphorus fertilizers causes pollution in soil and water, and nitrogen oxide (NO, N_2O, NO_2) emissions cause air pollution [35, 36]. Fertilizer applications are widely applied worldwide to ensure high efficiency in agricultural production.

However, differences in the amount, type application time of fertilizers, and the lack of knowledge in this field have led to serious health and environmental problems. Since agricultural production is a component of the atmospheric carbon and nitrogen dynamics, three greenhouse gases, nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2), are the main consequences of agricultural activities [37–40]. Agricultural food efficiency projects should be implemented to prevent this pollution [29].

One way to reduce the pollution load in the atmosphere is controlled anaerobic digestion. Anaerobic digesters break down organic waste using CH4-producing bacteria (methanogenic bacteria). In addition, these digesters reduce the odor and pathogens in animal waste and enable the use of degraded manure as agricultural fertilizer. Anaerobic digestion is the most important and effective method of reducing all greenhouse gas emissions resulting from manure storage and use of manure on land, without any increase in NH_3 emissions [17]. Leaving animal manure in the open not only causes greenhouse gas emissions but also invites various diseases. Animal manure left to dry in the open is seen as the cause of various diseases [41].

Bat guano is natural fertilizer obtained naturally from feces left over from caves where bats live [42]. Bat manure is rich in nitrogen due to the fact that bats feed on living organisms [43, 44]. Bat guano provides high levels of nutritional support to plants as a natural fertilizer with its nitrogen (N), phosphorus (P), and potassium (K) content. Bat manure is used as fertilizer in agriculture because it contains macro- and microelements. Bat guano has a much higher microorganism content and efficiency than other organic fertilizers [42, 45, 46]. It was shown very positive effects on plants when used in addition to natural fertilizers [46, 47]. Bat manure, which is an excellent organic fertilizer with its mineral content, is generally applied to flowers and various ornamental plants in homes and workplaces [48]. However, bat guano also has drawbacks for home and workplace use due to odor, ectoparasites, and some health risks. Although it is a highly valuable organic fertilizer, its obtaining procedures such as initial extraction and processing are challenging [49–51]. Contact with humans is very dangerous because bats consume living organisms and manure contains various harmful bacteria and viruses. Fertilizers containing bacteria and viruses are subjected to heat treatment to eliminate these factors. The heat treatment process consists of either direct application of heat or processing the guano in high-temperature furnaces and then dissolving it in water; both require high energy consumption [52]. Fertilizers derived from bat manure cannot be applied directly to plants due to their very high nitrogen and phosphorus content, but can only be used after diluting with a method such as drip irrigation, which is stated to be difficult to apply. Cattle manure, another source of organic fertilizer, is a weak fertilizer and has negative environmental effects when used without anaerobic digestion [53, 54]. There are clear limitations on the use of raw manure in organic farming. These limitations are detailed in the National Organic Production (NOP) Regulation [55, 56].

Greenhouse gas emissions from uncontrolled organic fertilizer digestion are higher than transportation. It was also reported that significant amounts of greenhouse gases are produced during storage for digestive purposes. To estimate greenhouse gas emissions from animal waste, emission factors (EF) are required for accurate evaluation and comparison of existing composting scenarios [17, 57, 58]. Emission factors are one of the most useful metrics for assessing the environmental impacts of fertilizers and achieving accurate environmental and life cycle assessments (LCAs) [59, 60]. EF is used as an important limiting factor to assess the validity of analyses [61–63].

It is known that cattle manure used randomly other than natural gas production harms the environment and human health and increases greenhouse gas emissions [41]. In addition, cattle manure, which is an important source of natural gas, is not a rich organic fertilizer and needs to be enriched [64]. Bat guano, which is rich in nitrogen, cannot be used without pre-treatment because it is harmful to human and environmental health [49–51]. Recent studies emphasize that fertilizers with low nutritional value should be enriched with additional substances [65, 66]. This study involves the

production of an easy-to-use nitrogen-rich organic fertilizer as a result of anaerobic digestion of cattle manure, which is a nitrogen-poor organic fertilizer, together with bat manure, which is rich in nitrogen, and optimization of the process.

Optimization of the process was done using response surface method Box-Behnken design RSM-BBD, central composite design, BBD, three-level factorial design, etc., value range. R^2, R^2 adj indicate the relationship between predicted and observed values including RSM approach. The BBD technique was chosen as the experimental design because it requires less time and fewer experiments than other approaches. The behavior of the system is described as a function of the quadratic polynomial equation Eq. (1) and independent variables involving quadratic interactions and square terms.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{i\,i}^2 + \sum_{ij=1}^{k} \sum_{j>1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

where Y is % total nitrogen compound (treatment response); k is the number of arguments; X_i and X_j are variables; β_0 is the estimated regression coefficient of the appropriate response at the center point of the design; β_i is the regression coefficient for linear effect terms; β_{ij} are interaction effects; and β_{ii} are quadratic effects [67].

Process variance analysis was performed with ANOVA based on the proposed model. ANOVA is used to check the validity of propose method and also the significance of coefficients in proposed model. One of the important parameters in this analysis is the model probability value p. This value determines the suitability of the model and should be within the recommended as well as the analysis of the residual. Model terms that are reliable are selected based on their suitability, while others are rejected.

It aims to utilize cattle manure, which is the waste of large amounts of cattle farming in eastern Turkey, reduce its impact on the environment, and direct it to biogas production facilities. It is a highly innovative study on the use of cattle manure as high-quality organic fertilizer when processed with bat manure and supports organic waste management.

Materials and methods

The bat manure used in the study was obtained from the caves in İspir district of Erzurum, and the cattle manure was obtained from the experimental animal farm of Kars Kafkas University Faculty of Veterinary Medicine. In order to purify the possible harmful microorganisms in the bat guano [49–52], it was subjected to heat treatment in an oven at 210 °C for 2 h. The heat-treated guano was diluted with water at a ratio of 1 (bat guano)/10 (water) by mass and added to wet cattle manure at different levels. The samples were prepared in the compositions given in the experimental design specified in Table 2. To prevent gas emission into the oven, an expanding balloon was connected to the gas outlets of the samples and closed.

Table 1 Process parameters and their levels

Parameters	Factor	Level -1 0 1		
Bad guano	A	5	12.5	20
Cattle manure	B	70	77.5	85
Temperature	C	20	30	40

Table 2 Planning matrix in coded and uncoded units

	Blocks	Bat guano	Cattle manure	Temperature
1	1	5	70	30
2	1	20	70	30
3	1	5	85	30
4	1	20	85	30
5	1	5	77.5	20
6	1	20	77.5	20
7	1	5	77.5	40
8	1	20	77.5	40
9	1	12.5	70	20
10	1	12.5	85	20
11	1	12.5	70	40
12	1	12.5	85	40
13	1	12.5	77.5	30
14	1	12.5	77.5	30
15	1	12.5	77.5	30

Used for analysis KOH, HNO_3, $Na_2H_2P_2O_7$, $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, NaOH, HCl and $KMnO_4$ were purchased from Sigma. $(NH_4)_2Ca_2O_4$, $Fe(NH_4)_2(SO_4)_2.6H_2O$, H_2SO_4, H_3BO_3, MgO, and quinoline, were purchased from Merk company. In the analyses, NÜVE EN400 oven, NCS Plasma 2000 ICP-OES, METTLER TOLEDO S220 pH meter, PROTHERM –PLF-120/6 muffle furnace, NÜKLEON Nitrogen Protein Determination Device Kjeldahl (NAP), STUART—SSL1—Orbital with Digital Display with 335 × 335 mm Plate Diameter Shaker – Labomar, THERMOMAC TMC815 Centrifuge devices were used.

The samples were left to anaerobically decompose in the oven at constant temperature for 40 days. The manure samples were then filtered, dried, and analyzed. All containers used at all stages of the experimental process were carefully cleaned to prevent possible mineral contamination. All samples were analyzed in triplicate laboratory replicates, and the resulting values were averaged across replicates. Total organic matter percentages were determined by the dry combustion method [68–70]. Total humic and fulvic acid composition is calculated according to ISO 5073:2013-TSE 5869.

Table 3 Analysis of variance

Source	DF	Adj SS	Adj MS	F Value	P Value
Model	9	1.86552	0.207279	21.10	0.002 significant
Linear	3	0.98647	0.328825	33.47	0.001
Bat guano	1	0.36125	0.361250	36.77	0.002
Cattle manure	1	0.00361	0.003612	0.37	0.571 not significant
Temperature	1	0.62161	0.621612	63.27	0.001
Square	3	0.74339	0.247797	25.22	0.002
Bat guano*Bat guano	1	0.54021	0.540208	54.98	0.001
Cattle manure*Cattle manure	1	0.24960	0.249600	25.40	0.004
Temperature*Temperature	1	0.02668	0.026677	2.72	0.160 not significant
Two-way interaction	3	0.13565	0.045217	4.60	0.067 not significant
Bat guano*Cattle manure	1	0.01103	0.011025	1.12	0.338 not significant
Bat guano*Temperature	1	0.04622	0.046225	4.70	0.082 not significant
Cattle manure*Temperature	1	0.07840	0.078400	7.98	0.037
Error	5	0.04912	0.009825		
Lack of Fit	3	0.04832	0.016108	40.27	0.024
Pure Error	2	0.00080	0.000400		
Total	14	1.91464			

R-sq (97.43%) R-sq (adj) (92.82%)

Total nitrogen N was calculated using the 1965 Bremner method [71, 72]. The percentage of NH_4 nitrogen and nitrate nitrogen was determined by the Kjeldahl method [72]. Percentage of organic nitrogen was determined using Mitchell, 1972 [73]. The total percentage of water-soluble P_2O_5 was determined gravimetrically, and the percentage of water-soluble K_2O was calculated according to ICP-OES [74].

Experimental design

RSM Box-Behnken design was used to optimize the process. Fixed input variables are bat manure rate, cattle manure, and temperature. The process response value is the percentage of total nitrogen compounds. Preliminary experiments determined the lower and upper limit values for Table 1 before the experiments. The values shown in Table 1 are the starting, ending, and midpoint values of the parameters in the experiments. Using these values, Table 2 for RSM-BBD was created in MINITAB software version 20. Table 1 presents these variables and their respective levels. RSM is a method used to design experiments and investigate the relationship between independent variables and system responses [75–77]. The planning matrix in the coded and uncoded units used in the experimental design is given in Table 2.

Results and discussion

As can be seen from Eq. 2, the selected model is quadratic, with an F value of 21.1 and a p value of 0.002, indicating that the model is suitable for analyzing and predicting the results. The correlation coefficient R^2 coefficient shows how much of the change in the dependent variable is due to the change in the independent variable. According to the results presented in Table 3, R^2 of 0.9743 or 0.95 indicates that the model is within the acceptable accuracy range. Additionally, estimated R^2 and adjusted R^2 were obtained as 0.9743 and 0.9292, respectively.

The closeness of these values to 1 and the small difference between the two indicate that the actual laboratory results are quite compatible with the results predicted by the model. The correlation of each variable with %total nitrogen compounds was determined by f and p values. p values < 0.05 indicate that model terms are significant. Parameters with p value < 0.05 are more significant in the regression model. As the p value increases, the effect of the parameters used in the model decreases. Parameters with a p value < 0.05 were considered significant and remained in the model [78]. Taking into account the health of the soil, the total nitrogen rate was determined as 2% and the mixture at this point was calculated. At 2% total nitrogen rate, the values of the process are 12.5% Bat manure, 7.0448 Cattle manure. The temperature is determined as 40 °C, and the optimization curves are presented in Fig. 3.

$$\%\ \text{Nitrogen} = -32{,}10 + 0{,}083\ B + 0{,}2056\ T - 0{,}0068\ B{*}B - 0{,}004622\ C{*}C - 0{,}001867\ C{*}T \quad (2)$$

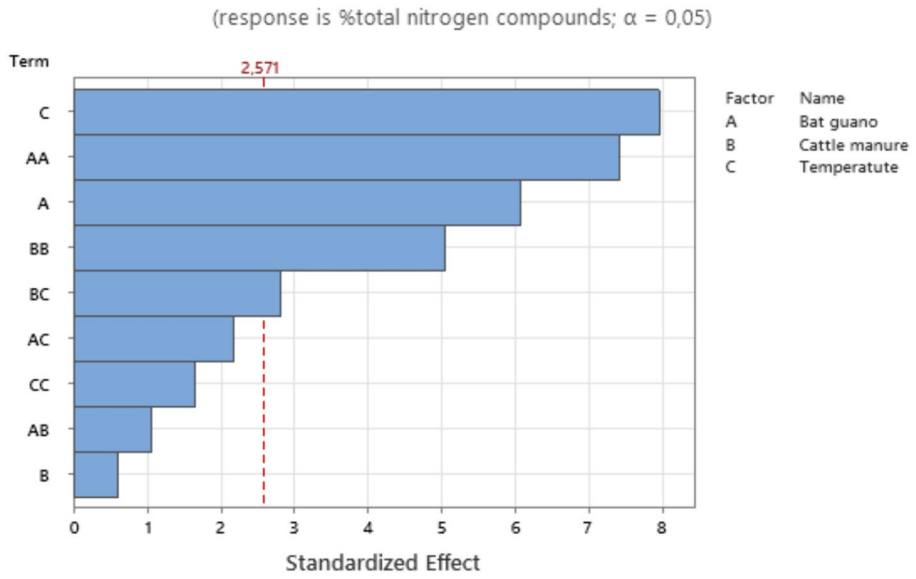


Fig. 1 Pareto card of the standardized effects

To obtain Eq. 2, values less than $p < 0.05$ in Table 3 were taken because they were significant, and values greater than 0.05 were removed. In the equation, T represents temperature, B represents bat guano, C represents cattle manure, and N represents nitrogen.

Here, the N value of 2% is a value chosen for general use. In real applications, optimization can be made for the required % N value by taking into account the needs of the soil and the plant to be grown. It is known that one of the most effective parameters in anaerobic digestion processes is temperature. It was clarified in many anaerobic digestion and biogas formation process studies [79–81]. When Fig. 1 and Fig. 2 Pareto and surface chart graphs are examined together, it is seen that the most effective parameter on the anaerobic digestion process is temperature. Since the calculations are made on a mass basis, the amount of organic component in the fertilizer will decrease as the methane gas formed as a result of the conversion of existing carbon into methane as a result of anaerobic digestion will increase [82–84]. When the graph of the change in total N ratio with bat guano and cattle manure is analyzed in Fig. 2, it is seen that the increase in bat guano affects the total nitrogen ratio more than cattle

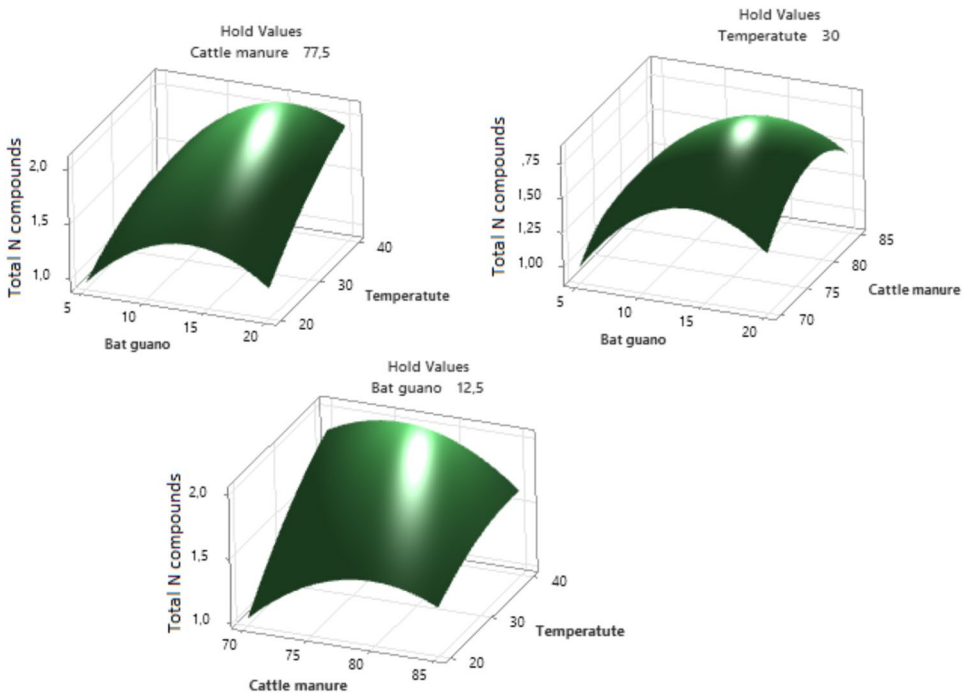


Fig. 2 Surface plot of %total nitrogen compounds related independent parameters

manure. If the change with temperature is taken as a basis, it is seen that the increase in total N content with the addition of bat guano is faster than the increase with cattle manure. This can be explained by the high amount of nitrogen contained in bat guano. The second most effective part of the process was bat manure. Bat guano is one of the organic fertilizers with high nitrogen content. Bat guano used in small amounts significantly affected the amount of nitrogen in the manure [85, 86]. Among these three parameters, the parameter that has the least impact on nitrogen value was cattle manure, which is poor in nitrogen.

Cattle manure is a fertilizer with low fertilizer value, high production amount, and a high potential to be used as a natural gas source [64, 87, 88]. However, it is insufficient to be used as fertilizer and various additives are required to enrich it. In this study, the fertilizer value was increased organically by combining it with bat manure. In the study, bat manure, which is a recommended fertilizer for organic agriculture due to its content, became easily applicable by adding it to cattle manure during biogas formation. Since bat manure is very rich in nitrogen, direct application to plants may cause burning and it means loading unnecessary nitrogen into the soil. This problem has been eliminated with the process [42, 45, 46].

This study also evaluated the effect of bat guano on the content of various nitrogen compounds in plant nutrition. The results revealed that nitrogen and its derivatives increased in the new manure parallel to the bat manure level (Table 4). While nitrogenous organic fertilizers increase plant productivity in agricultural environments, they also eliminate the negative environmental effects of synthetic fertilizers [89, 90]. The mixture ratios obtained in this study were calculated according to the existing fertilizer characterization. Since the fertilizer content changes each time, the mixture ratios must be re-determined in each study, taking into account the fertilizer characterizations used.

Considering the compositional differences between cattle and bat manure in Fig. 3, it was determined as a result of RSM-BBD optimization that the most suitable mixture ratio for 2% N rate in the study was 12.5 g bat, 70 g cattle manure and 40 °C operating temperature.

Some valuable water-soluble minerals and compounds, such as P and K, remained in the permeate water. This is one of the reasons for the low presence of water-soluble phosphorus and potassium components in Table 4. Phosphorus (P) is the second most important nutrient for plant growth after nitrogen. 30–40% of arable land worldwide has low P [91], and the soil needs P from outside for plant production. Table 2 shows that the total amount of K and P in cattle manure and the addition of bat manure slightly increase the amount of K and P. Since the phosphate value of the bat guano used was not very high, the change in P value could not be observed exactly.

Table 4 Analysis results of the samples produced in accordance with the experimental design

Properties	Unit	Method	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PH	-	1:10 w/v	6.23	6.58	6.33	8.32	7.5	8.11	6.33	8	7.65	6.35	7.84	8.43	8.02	8.03	8.2
Humidity	%		50.2	52.4	17.84	51.75	53.2	59.51	17.84	59.61	60.2	59.91	60.11	59.78	55.72	58.2	56.4
Organic Matter (550 °C)	%	Dry Burning Combustion Loss	76.5	75.3	81.86	78.08	74.2	79.17	81.86	78.26	76.3	79.85	79.68	79.56	75.5	74.3	76.2
Total Humic And Fulvic Acid	%	TSE 5869 ISO5073	28.3	29.2	27.39	32.08	26.8	28,.55	27.39	28.11	28.9	29.71	28.6	29.57	25.4	27.8	29.2
Total Nitrogen N	%	1965 Bremmer	1.02	1.2	1.01	1.4	0.9	1.25	1.2	1.98	1.02	1.29	1.9	1.61	1.8	1.78	1.82
Ammonium Nitrogen NH_4-N	%	KJELDAL	0.06	0.08	0.1	0.05	0.04	0.05	0.1	0.05	0.04	0.1	0.1	0.1	0.09	0.07	0.08
Nitrate Nitrogen -NO_3-N	%	KJELDAL	0.04	0.04	0.05	0.05	0.05	0.1	0.05	0.1	0.04	0.1	0.12	0.05	0.05	0.04	0.05
Organic Nitrogen	%	Mitchell,1972	1.23	1.3	0.86	1.39	1.29	1.21	0.86	1.89	1.5	1.09	1.68	1.46	1.3	1.32	1.31
Water-Soluble P_2O_5	%	Gravimetric	0.2	0.12	0.03	0.12	0.02	0.02	0.13	0.08	0.08	0.07	0.08	0.08	0.09	0.07	0.08
Water-Soluble K_2O	%	ICP-OES	1.02	1.03	1.07	1.25	1.05	0.73	1.07	1.16	1.08	1.04	0.94	0.85	0.86	0.9	0.92

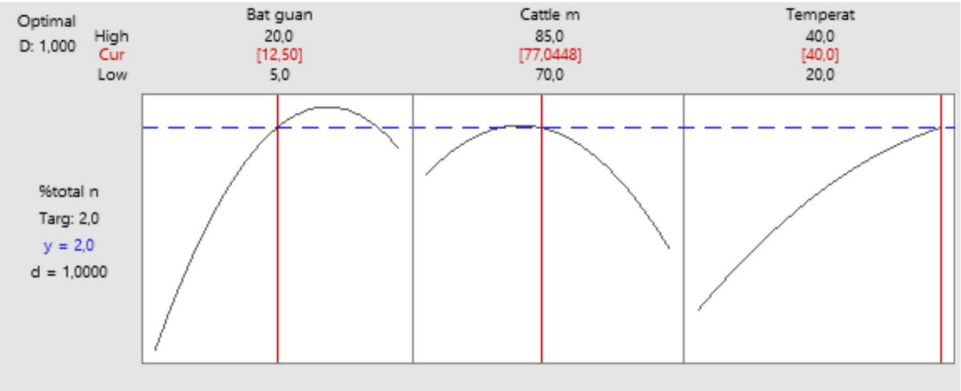


Fig. 3 Response optimization: % 2 total nitrogen compound

When Table 2 is examined, it is seen that the total organic matter amounts of all samples are between 74.3 and 81.86%. Additionally, this change may be caused by the anaerobic digestion composition of the manure. Enriching soil organic matter, increasing total N and alkali-N concentrations, and increasing the activity of soil enzymes play important roles in crop cultivation [92].

Other compounds that increase fertilizer quality are humic and fulvic acid content. Humic acid and fulvic acid are two important components of humus that are essential for soil improvement [18–21]. The changes in humic and fulvic acid values in the final product were observed depending on the guano addition (%). It was determined that the value of both increased as the content of bat manure increased (Table 4).

In order to maintain soil fertility, the nutrients absorbed by plants must be supplemented in the soil [93–95]. Therefore, fertilizers always maintain their importance.

Conclusions

This study was designed to combine animal feces to produce a fertilizer that is easy to apply and has high fertilizer value by biodegradation. An organic fertilizer with the desired N value was produced with the necessary optimization for recycling waste into a circular economy and a sustainable agricultural policy. The concept of sustainability generally refers to the capacity to maintain a situation or process for an indefinite period of time. Contemporary consumption-based economies have created a consumer society. This is important for our environment and our future. In the study, the optimum application temperature of the process for the optimum mixing ratio of fertilizers and 2% nitrogen rate was determined with the RSM Box-Behnken design. Conditions for this nitrogen value were determined as 15.15% bat manure and 84.85% cattle manure by mass at 40 °C. In addition, it was observed that the added bat manure increased the nitrogen in the final manure as well as the values such as humic–fulvic acid, K, P, total organic matter, and other nitrogen compounds. Minerals in the permeate water could not be evaluated in the study. When applied to a suitable biogas facility, the permeate can be recycled, so there will be less mineral loss in each recycling. In addition, if bat manure is added to a facility where electricity is generated from biogas, as in this study, the manure can be thermally treated with the waste heat generated during electricity production, providing extra economic gain. Contemporary consumption-based economies have created a consumer society. While meeting the needs of the consumer society, the production and use of fertilizer from waste materials without harming the environment should be supported.

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