Process optimization of the anaerobic co-digestion of Alternanthera philoxeroides and corn straw enhanced by biochar and Fe3O4

Qiongbo Fan
Northwest Agriculture and Forestry University College of Mechanical and Electronic Engineering
Zhijiang Shao
Northwest Agriculture and Forestry University College of Mechanical and Electronic Engineering
Xiaohui Guo
Northwest Agriculture and Forestry University College of Mechanical and Electronic Engineering
Qinqin Zhou
Northwest Agriculture and Forestry University College of Mechanical and Electronic Engineering
Ling Qiu
xbgzzh@163.com

Northwest A&F University: Northwest Agriculture and Forestry University https://orcid.org/0000-0001-8002-2717

Research Article

Keywords: Alternanthera philoxeroides , anaerobic co-digestion , biochar , Fe3O4 , process optimization

Posted Date: November 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1998624/v2

Abstract

Alternanthera philoxeroides (AP), a highly invasive plant species, poses a serious threat to agriculture, ecological environment. In this study, the methane production properties of the anaerobic co-digestion (coAD) of AP and corn straw (CS) were studied. The interaction effects of total solids content (TS%), biochar, and Fe_3O_4 on methane yield were evaluated by regression model and response surface method (RSM). The regression model was extremely significant ($p < 0.0001$). The results show that the order of the effect of three factors on the methane yield of coAD was: TS% > Fe_3O_4 > biochar. With the aid of RSM, the optimum values of TS%, Fe_3O_4 and biochar were: 9.67 %, 8.41 g/kg and 5.51 %, respectively. The effects of three factors on the performance of coAD for methane yield were as follows: the content lower than the predicted optimal value was positively correlated with the methane yield, and the content higher than the predicted optimal value was negatively correlated with the methane yield. To improve the methane quality of AP, coAD could be carried out at a lower TS%, biochar and Fe_3O_4 were added to enhance the methane yield performance. This paper optimized the operational parameters of the coAD of AP and CS, which also provides new strategies for the treatment and utilization of AP.

1. introduction

Alternanthera philoxeroides (AP) is an amphibious, highly adaptable, widely distributed, and rapidly spreading weed (Wang et al., 2019). Due to the serious threat to agriculture, fisheries, ecological environment, water transportation, etc., AP has been announced by the State Environmental Protection Administration of China as one of the first invasive alien species (Huang et al., 2013). AP contains a lot of hemicellulose and cellulose and is a potential biomass energy (Bhattacharjee and Biswas, 2018). Thus, treating AP via anaerobic digestion (AD) is considered to be a feasible way due to its economy, high efficiency and energy recovery (Kunatsa and Xia, 2022).

According to previous studies, the accumulation of volatile fatty acids (VFAs), ammonia nitrogen, humic acid and other inhibitors might cause the failure of AD (Cai et al., 2021; Mozhiarasi et al., 2021). The addition of biochar, ferric oxide, activated carbon and other materials has been considered to effectively alleviate the acid inhibition of AD (Kumar et al., 2021; Zhang et al., 2020b; Shao et al., 2021). With high porosity, large surface area, and multifunctional groups, biochar can promote organic biodegradation, reduce the accumulation of VFAs, and improve methane yield (Qiu et al., 2019; Zhang et al., 2021). Cimon, C et al.(2020) explored the effects of biochar and wood ash amendment on biochemical methane yield of wastewater sludge during thermophilic AD, and found that biochar addition significantly increased methane yield by 192%-461% compared with the control. Trace elements (such as iron, cobalt and nickel) are essential constituents of cofactors and enzymes. Its addition to AD has been shown to stimulate and stabilize the performance of biogas processes (Abdelsalam et al., 2017). Compared with the Fe-free digester, Zhang et al.(2020) found that the accumulative methane production of the Fe_3O_4-added significantly increased by 50.7%. This might be attributed to iron ions promoting electron transfer between fermentative bacteria and methanogenic archaea. Feng et al.(2014) showed that the methane content increased gradually from 58.5% to 68.9% with the increase of iron ion dosage from 0 to 20 g/L. These suggest that the appropriate additives in AD can effectively improve biogas production. Anaerobic co-digestion (coAD) is more conducive to microbial growth due to its nutrient balance, metal and trace element supplementation, and enhanced buffering capacity (Hagos et al., 2017). Studies have shown that AP is a nitrogen-rich substrate (Zhang et al., 2016). Therefore, selecting carbon-rich corn straw (CS) to adjust the ratio of carbon to nitrogen for coAD is an efficient way to improve methane yield.

Response surface method (RSM) is an experimental design tool that combines mathematical and statistical techniques to evaluate the interactions of different factors on response variables, and to determine the optimal combination of process parameters(Mohtar et al., 2016). Cai et al.(2021) explored the effects of total ammonia nitrogen, pH and temperature on anaerobic digestion of chicken manure with the help of RSM, which provided a theoretical basis to develop the way of relieving ammonia inhibition. Deng et al.(2019) used quadratic regression model and RSM to evaluate the interactive effects of TS%, C/N ratio and inoculum volume percentage (I%) on total biogas production and methane content. With assistance of RSM, the best combination of TS%, C/N ratio and I% has been acquired. RSM can reduce the number of necessary experiments and minimize the use of test materials while performing parameter optimization(Mitraka et al., 2022).

The interaction between total solids content (TS%), biochar and Fe_3O_4 during the coAD of AP and CS was investigated in this study with the help of RSM. The specific objectives are: (a) modeling and analyzing the interaction effects of three factors (TS%, biochar and Fe_3O_4) on methane yield of coAD; (b) evaluating the effects of three independent factors on the methane yield; (c) optimizing the process parameters of coAD, and improving the resource utilization rate of AP.

2. Materials and methods

2.1 Experimental materials

AP was collected from Benxiang Agricultural family farm, Hanzhong City, Shaanxi Province. Fresh raw materials were cut into 2 ~ 3 mm pieces. CS was dried and crushed into powder (sifted through 20 mesh). The inoculum was taken from a lab-scale anaerobic reactor operated at 37 °C. The biochar was prepared from apple branches by pyrolysis at 550 °C for 2 hours and screened through 40 mesh. Fe_3O_4 (powder, 100~300 nm) was purchased from Macklin Biochemical Technology Co., Ltd., Shanghai, China. The C/N ratio of mixed material was adjusted to 25:1. Table 1 shows the physicochemical characteristics of the materials.

Table 1 Physical and chemical characteristics of materials			
Materials	TS%	VS%	C/N
AP	15.07±0.17 [a]	12.87±0.12 [a]	10.63±0.41
CS	88.97±0.59 [b]	79.38±0.94 [b]	61.56±0.38
Inoculum	5.38±0.11 [a]	3.76±0.12 [a]	ND[c]

Values are presented as mean ± standard deviation (n = 3)

[a] Based on wet matters. [b] Based on dry matters. [c] Not determined.

2.2 Experimental design

According to the Central Composite Design (CCD), TS%, biochar and Fe_3O_4 were used as independent variables of coAD of AP and CS, and methane yield was used as response variable. The five coding levels of -β, -1, 0, +1 and +β are presented in Table 2, and the β value was 1.68 (Zhu et al., 2021). A total of 23 treatment was carried out including 14 non-central points and 9 central points. Combined with previous studies, the effective test range of the three factors in this experiment was determined (Laid et al., 2021; Abid et al., 2021; Qi et al., 2021b; Li et al., 2021). To explore the influence of wet-dry fermentation on the biogas yield performance of AP, the TS% range was selected from 3.6 % to 20.4 %, and the central point value was 12 %. The center point values of biochar and Fe_3O_4 were 5 % (a mass percentage compared to the total solid of AP and CS) and 12.5 g/kg (mass ratio of Fe_3O_4 to the total mass of the filling), respectively. The CCD experimental design and results were shown in Table 3.

Table 2

Experiment factor level and code

Factors	-β	-1	0	1	β
TS (%)	3.60	7.00	12.00	17.00	20.40
Biochar (%)	1.64	3.00	5.00	7.00	8.36
Fe_3O_4 (g/kg)	4.10	7.50	12.50	17.50	20.90

Table 3

CCD experimental design and results

Run	TS (%)	Biochar (%)	Fe_3O_4 (g/kg)	Methane yield (mL/gVS)
1	1	1	1	189.81
2	1	1	-1	282.32
3	1	-1	1	189.92
4	1	-1	-1	221.00
5	-1	1	1	235.89
6	-1	1	-1	229.59
7	-1	-1	1	206.94
8	-1	-1	-1	203.94
9	β	0	0	1.02
10	-β	0	0	165.22
11	0	β	0	206.95
12	0	-β	0	185.63
13	0	0	β	198.35
14	0	0	-β	223.61
15	0	0	0	230.84
16	0	0	0	232.27
17	0	0	0	227.41
18	0	0	0	225.75
19	0	0	0	224.69
20	0	0	0	239.28
21	0	0	0	234.92
22	0	0	0	222.62
23	0	0	0	236.18

2.3 Experimental setup

Batch AD was performed in 1 L serum bottle. Each digestor contained a 400 g mixture of AP, CS, inoculum, additives (biochar and Fe_3O_4), and a certain amount of deionized water. The content of the inoculum was 40% of the filling mass (Deng et al., 2019). The top space of the bottle was purged N_2 for 3 minutes to create an anaerobic environment. AD was carried out in 37 °C constant temperature incubator (Mao et al., 2015)(HWS, Ningbo southeast Instrument Co., LTD). The biogas was collected by gasbag, and its yield and composition were determined daily.

2.4 Chemical analysis

The TS was determined by drying samples at 105°C to constant weight. The volatile solid content (VS) was determined by ashing the materials at 550 °C for 6 hours in a muffle furnace (Indren et al., 2020). The pH was analyzed by a pH probe (DZS-706China). The biogas was determined by a gas chromatograph (GC2014CJapan). The injector temperature was set to 110 °C and the column box temperature was 90°C with argon as the carrier gas, the flow rate was 25 mL/min (Deng et al., 2019). The C/N ratio was measured by an Elemental analyzer (Vario MACRO cube, Germany).

2.5 Statistical analysis

Statistical analysis of variance (ANOVA) and response surface analysis were implemented in Design-expert 10.0 (StatEase, America). $p < 0.05$ was indicated statistically significant. Figures were drawn using the Origin 2021 (OriginLab, America).

3. Results And Discussion

3.1 Establishment and analysis of methane yield regression model

In this experiment, methane yield (y) was taken as the response variable, TS% (x_1), biochar (x_2), and Fe_3O_4 (x_3) were taken as factors to build a regression model. Design-expert 10.0 was used to conduct multivariate regression analysis on 23 groups of experimental data, and the multivariate regression Eq. (1) was obtained as follows:

$$\mathrm{y} = 230.44 - 48.82x_1 + 6.34x_2 - 11.48x_3 + 0.86x_1x_2 - 16.61x_1x_3 - 7.24x_2x_3 - 52.09x_1^2 \\ -12.07x_2^2 - 6.88x_3^2 - 8.07x_1x_2x_3 + 8.17x_1^2x_2 + 49.65x_1x_2^2 + 60.53x_1^2x_2^2$$

1

Table 4
Analysis results of regression model

Source Prob > F	Sum of Squares	df	Mean Square	F-Value	P-Value	Signature
Model	57461.93	13	4420.15	96.52	< 0.0001	significant
x_1-TS%	13481.15	1	13481.15	294.37	< 0.0001	
x_2-Biochar	227.27	1	227.27	4.96	0.0529	
x_3-Fe3O4	1799.42	1	1799.42	39.29	0.0001	
x_1x_2	5.85	1	5.85	0.13	0.7291	
x_1x_3	2207.14	1	2207.14	48.19	< 0.0001	
x_2x_3	419.34	1	419.34	9.16	0.0143	
x_1^2	35514.78	1	35514.78	775.48	< 0.0001	
x_2^2	1908.36	1	1908.36	41.67	0.0001	
x_3^2	619.68	1	619.68	13.53	0.0051	
$x_1x_2x_3$	520.35	1	520.35	11.36	0.0082	
$x_1^2x^2$	221	1	221	4.83	0.0556	
$x_1x_2^2$	8169.54	1	8169.54	178.39	< 0.0001	
$x_1^2x_2^2$	11707.1	1	11707.1	255.63	< 0.0001	
Residual	412.17	9	45.8			
Lack of Fit	152.11	1	152.11	4.68	0.0625	Not significant
Pure Error	260.06	8	32.51			
Cor Total	57874.1	22				

The statistical analysis results of the multivariate regression model of the CCD experiment were shown in Table 4. It can be seen that the model was extremely significant ($P < 0.0001$) (Khodaei et al., 2016), and it greatly represents the relationship between predicted and observed value (Cheng et al., 2022). The lack of fit P-Value of the model was 0.0625 ($P > 0.05$), indicating that the regression model had a high degree of fit and could be used to predict the response value (Zhu et al., 2021). The P-Values of different factors were less than 0.05 except x_2, x_1x_2, $x_1^2x_2$, showing that they performed a significant impact on methane yield. This experiment adopted dimensionless linear code, thus there was no correlation among all regression coefficients. The influence of various factors on methane yield can be ranked directly according to the absolute value of the first item regression coefficient (Mohtar et al, 2016; Deng et al., 2019), and the order was TS% > Fe_3O_4 > Biochar (48.82 > 11.48 > 6.34). The regression coefficient was independent, thus the simplified regression equation (2) can be obtained by directly deleting the insignificant items from the above model.

$$\mathrm{y} = 230.44 - 48.82x_1 - 11.48x_3 - 16.61x_1x_3 - 7.24x_2x_3 - 52.09x_1^2 \\ -12.07x_2^2 - 6.88x_3^2 - 8.07x_1x_2x_3 + 49.65x_1x_2^2 + 60.53x_1^2x_2^2$$

2

3.2 Effects of independent factor on coAD of AP and CS

3.2.1 Effect of TS% on methane yield

The effect of TS% on the coAD of AP and CS is shown in Fig. 1. Run 10 reached the maximum daily methane yield (DMY) (31.74 mL/gVS) on the 2nd day and was significantly higher than those of Run18 and Run9 (12.42 mL/gVS on the 21th day and 0.78 mL/gVS on 1st day) (Fig. 1a). This is attributed to the fact that a smaller TS% determines a larger IS ratio (ratio of inoculum to substrate) when the amount of inoculum was constant. During the start-up period, excessive organic loading rates lead to severe acid inhibition and even the cessation of AD (Deng et al., 2019). The biogas production of Run 18 was delayed until the 11th day, while Run 9 was terminated on the 3rd day. The reason for the delay or stop of biogas production might be that the AD process was inhibited due to the VFAs accumulation. The inhibition effect was more significant in Run 9, which caused the failure of the AD system (Abid et al., 2021). During AD, pH parameters can be used to estimate the degree of acidification in the reactor (Kunatsa and Xia, 2022), thus optimizing operating conditions. As shown in Fig. 1d, the pH value in the high TS% reactor dropped sharply in the initial stage. Run 9 was finally stabilized at around 5.0, which was far below the suitable pH (6.8 to 7.5) for the survival of methanogens (Mao et al., 2015). The acid accumulation severely inhibited the activity of methanogens and eventually led to the failure of the whole AD. In terms of cumulative methane yield (CMY), Run 18 and Run 10 obtained the highest yield of 209.03 mL/gVS and 165.22 mL/gVS, respectively, while Run 9 was only 1.02 mL/gVS under the influence of inhibition (Fig. 1b), suggesting that the CMY increased with the increase of the TS% within a certain range of TS%. However, a sharp decrease in CMY has occurred at excessively high TS%, which might be attributed to the higher organic loading, resulting in severe disruption of methanogenic pathways (Deng et al., 2019). The variation trend of methane content in each treatment was similar, ie., rose rapidly in the initial stage and remained in a stable state with the progress of anaerobic digestion (Fig. 1c). Run 10 obtained the highest percentage of 70.2%, indicating that the digester with a lower TS% can obtain a higher methane content.

3.2.2 Effect of biochar on methane yield

The effect of biochar on the coAD characteristics of AP and CS is shown in Fig. 2. As shown in Fig. 2a, the change trends of DMY in the three runs were similar. Run 18 obtained the highest CMY of 225.75 mL /gVS, followed by Run 11 of 206.95 mL /gVS, and Run 12 of 185.63ml /gVS (Fig. 2b). These results indicate that the appropriate addition of biochar can significantly promote methanogenesis, but high dose biochar would negatively affect methane yield. According to previous reports, the alkaline functional groups on the surface of biochar can improve the buffering capacity and stability of AD process (Qi et al., 2021b), effectively alleviate acid accumulation and provide a stable pH environment for methanogenic microorganisms. However, it is worth noting that AD requires a neutral environment, excessive biochar will cause the pH value of the digesting slurry increase, which will also inhibit the activity of anaerobic microorganisms (Cui al., 2021). Interestingly, the methane yield of Run 12 started 3 and 5 days earlier than those of Run 11 and Run 18, respectively. In terms of methane content (Fig. 2c), Run 12 was higher than these of Run 11 and Run 18. In fact, compared to Run 11 and Run 18, the pH of Run12 increased quickly after slight acidification. This provided a suitable environment forthe rapid growth of methanogenic microorganisms in Run12 and further improved methane content (Qi et al., 2021a).

3.2.3 Effect of Fe_3O_4 on methane yield

The effect of Fe_3O_4 on the AD characteristics of AP and CS is shown in Fig. 3. As shown in Fig. 3a, the maximum DMY of Run 14 was 16.46 mL /gVS on the 19th day, followed by 12.39 ml /gVS for Run 18 and 11.13 mL/gVS for Run 13. The CMY of Run 14 (223.61 mL/gVS) and Run 18 (225.75 mL/gVS) were close (Fig. 3b), both much larger than that of Run 13 (198.35 mL/gVS). With the Fe_3O_4 increased, the methane yield first increased and then decreased. Feng et al.(2014) and Zhao et al.(2018) reported that the addition of Fe_3O_4 could enhance the activities of major enzymes related to hydrolysis and acidification, and promote the possible direct interspecific electron transfer between bacteria and methanogenic archaea. However, the acceleration effect was at the low level, and high dose addition reduced methane yield, due to toxic effects on bacteria and methanogenic microorganisms (Kunatsa and Xia, 2022). The variation trend of methane content in these three runs was similar (Fig. 3c). Different from the CMY, The maximum methane content of 67.39% was obtained in Run 13 on the 25th day and obviously higher than those in Run 18 and 14. This indicates that a higher dose of Fe_3O_4 performed a a positive effect on methane content (Feng et al., 2014). Figure 3c shows that the variation trend of pH in these 3 Runs tended to be semblable, indicating that Fe_3O_4 had no significant effect on the pH of AD ($p > 0.05$).

3.3 Interaction effects of TS, biochar and Fe_3O_4 on AD characteristics of AP and CS

Interactions between different factors would affect methane yield, so regression models were employed to determine the interaction of different factors on methane yield (Hagos et al., 2017). Figure 4 shows the response surface and contour plot of the interaction effects of different factors on methane production. When the code value of Fe_3O_4 was 0 (12.5 g/kg), the response surface Eq. (3) was as follows:

$$y = 230.44 - 48.82x_1 - 52.09x_1^2 - 12.07x_2^2 + 49.65x_1x_2^2 + 60.53x_1^2x_2^2$$

3

Figure 4a and Fig. 4b show the response surface and contour of TS% and biochar. The response surface changed greatly, and the contour was irregularly circular, indicating that there was a significant effect between TS and biochar (Zhu et al., 2021). This might be due to the significant effect of high-order items in the regression model ($p < 0.05$). When the factors changed, high-order terms had a greater impact on the response value (methane yield). In the lower TS% range (< 10%), the methane yield first increased and then decreased with the addition of biochar. When large amounts of acidic intermediates accumulated, the alkaline properties of biochar began to neutralize acids, ensuring a suitable living environment for microorganisms (Zhao et al., 2020). However, the alkaline properties of biochar should to be rationally utilized. Too high addition might cause acid neutralization imbalance and form an alkaline environment, which is also not conducive to the survival of microorganisms. When biochar was maintained at a constant content, methane yield increased first and then decreases with the increase of TS%. Obviously, excessive organic loading rate led to serious acidification and inhibited the activity of methanogens (Indren et al., 2020). It can be seen from Fig. 4a that at a high level of TS%, methane yield dropped sharply at the larger code, and even stopped biogas production when the code is β, indicating that biochar could not completely alleviate acid accumulation when severe acid inhibition occurred in the AD system.

The increased biochar addition might decrease the mass transfer rate, and further lead to a decrease in methane yield, which was consistent with the results reported by Indren et al.(2020). It should be noted that in the regression model analysis in Table 4, the interaction between TS and biochar was not significant ($p > 0.05$), and the results obtained by the above two methods were contradictory, which might be because the P-value only indicates whether the experimental data is statistically significant or not, and cannot fully represent the significance in practical applications. The P-value needs to be considered in combination with practical issues (Yang et al., 2022).

Figure 4c and Fig. 4d show the response surface and contour plot of the interaction effects of TS% and Fe_3O_4. When the code value of biochar was 0 (5%), the response surface Eq. (4) was as follows:

$$y = 230.44 - 48.82x_1 - 11.48x_3 - 16.61x_1x_3 - 52.09x_1^2 - 6.88x_3^2$$

4

The contour in Fig. 4d was arc-shaped, indicating that there was a very significant interaction between TS% and Fe_3O_4 ($P < 0.001$). Figure 4c shows that when TS% was constant, the methane yield increased first and then decreased with the increase of Fe_3O_4. This might be because trace metals (such as iron, cobalt, and nickel) are cofactors and basic components of enzymes. Adding trace metals to the AD could stimulate and stabilize the performance of the biogas process, but the high content of trace elements could inhibit the biodegradation process (Abdelsalam et al., 2017). When Fe_3O_4 was fixed, the methane yield first increased and then decreased with the increase of TS%, showing a greater change range compared to that in the case of varying Fe_3O_4 with constant TS. According to the response equation, both the two factors had a primary term and a quadratic term, and the coefficient of TS% was larger than Fe_3O_4, so the change of methane yield with TS% was greater than Fe_3O_4.

Figure 4e and Fig. 4f show the response surface and contour plot of the interaction effects of biochar and Fe_3O_4. When the code value of TS% was 0 (12%), the response surface Eq. (5) was as follows:

$$\mathrm{y} = 230.44 - 11.48x_3 - 7.24x_2x_3 - 12.07x_2^2 - 6.88x_3^2$$

5

The contour in Fig. 4f was oval, showing that there was a significant interaction between biochar and Fe_3O_4 ($P < 0.05$). As can be seen from Fig. 4e, a great change was presented in methane yield. It might be attributed to the Fe_3O_4addition slowly dissolved into $Fe^{2+}$and Fe^{3+} in the AD system, which could enhance the activity of methanogen microorganisms and promote direct interspecific electron transfer (Zhang et al., 2020a). When a small amount of Fe_3O_4 (< 12.5 g/kg) was added, the methane yield first increased and then decreased with the increase of biochar, indicating that the role of biochar in buffering acidification was effective, and the accumulation of acid was controlled within a certain range. Interestingly, the increase in methane yield still occurred in the case of the highest biochar and lower Fe_3O_4 additions, indicating that the negative effect of a high content of biochar was less than the positive stimulating effect of Fe_3O_4 on microorganisms. Judging from the regression equation, there were multiple x_3 in the model instead of x_2, which further demonstrated that Fe_3O_4(x_3)had a greater impact on methane production than biochar (x_2).

3.4 Optimisation of operational parameters

The methane yield can directly reflect the AD performance of raw materials (Cui al., 2021). Based on the experimental results and statistical analysis, the operation parameters were numerically optimized and the optimal level corresponding to the expected value was established.

Figure 5 presents the perturbation analysis of different parameters to the AD. x_1, x_2 and x_3 represents TS%, biochar and Fe_3O_4, respectively, and the ordinate is methane yield. The gradient of the parabola could be used to determine the impact of these three factors on methane yield (Deng et al., 2019). As can be seen from Fig. 5a, the gradient of TS% was the largest, indicating that TS% had the most significant effect on the response value of methane yield ($p < 0.0001$). This was consistent with the results in Section 3.1 that the TS% was the most significant factor among the three factors. Figure 5 (b) (c) (d) are the univariate perturbation curves of TS%, biochar, and Fe_3O_4 on methane yield analyzed by Design expert 10.0. In Fig. 5b, the optimal TS % was 9.67% at the code value of -0.47. The methane yield first rose and then decreased with the increase of TS%. When TS% was greater than 9.67%, the methane yield decreased rapidly because of a higher organic loading rate, which inhibited the overall methanogenesis process. In Fig. 5c, the optimal value of biochar was 5.51% at the code value of 0.26.

The methane yield also first increased and then decreased with the increase of biochar. When biochar was greater than 5.51%, the methane yield began to decrease, which may be caused by the alkaline properties of biochar. In Fig. 5d, the optimal value of Fe_3O_4 was 8.41 g at the coding value of -0.82. Methane yield increased slowly with the increase of Fe_3O_4, but when the amount of Fe_3O_4 was more than 8.41 g/kg, the methane yield decreased rapidly, and the high dose of Fe_3O_4 had negative effects on biogas production, showing the phenomenon of "low promotion but high inhibition" (Wang et al., 2021). Therefore, the optimal values of TS%, Fe_3O_4 and biochar were 9.67%, 8.41 g/kg and 5.51%, respectively.

4. Conclusions

In this study, the regression equation of the coAD of AP and CS was obtained through the CCD experiment, and the regression model was extremely significant ($p < 0.0001$). TS%, biochar, and Fe_3O_4 all had significant effects on methane yield, among which TS% and Fe_3O_4 had more significant effects on methane yield than biochar. The addition of biochar and Fe_3O_4 enhanced the methane yield of coAD. The optimal values of TS%, Fe_3O_4, and biochar were optimized to be 9.67%, 8.41 g/kg, and 5.51% by the response surface methodology, respectively. In the low TS% (< 12%), the addition of Fe_3O_4 could alleviate the negative effect of high content of biochar (> 7%) on methanogenesis performance. Therefore, the dosage of the additions for AD needs to be reasonably selected.

Declarations

Funding

This study was supported by the National Natural Science Foundation of China (31900105), and Agricultural Science and technology innovation drive project of Shaanxi Province, China (NYKJ-2022-YL(XN)17).

Competing Interest

The authors declare that they have no conflict of interest or competing interest that could have appeared to influence the work reported in this paper.

Author Contributions

Qiongbo Fan and **Zhijiang Shao**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Xiaohui Guo**: Formal analysis, Supervision, Funding acquisi-tion, Writing - review & editing. **Qinqin Zhou**: Data curation, Methodology, Resources, Visualization. **Ling Qiu**: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Data Availability

The authors declare all data generated or analyzed during this study included in this manuscript.

References

1. Abdelsalam E., Samer M, Attia Y. A., Abdel-Hadi M. A., Hassan H. E., Badr Y. (2016). Influence of zero valent iron nanoparticles and magnetic iron oxide nanoparticles on biogas and methane production from anaerobic digestion of manure. *Energy*, 120, 842-853. https://doi.org/10.1016/j.energy.2016.11.137
2. Abid M., Wu J., Seyedsalehi M., Hu Y. Y., Tian G. (2021). Novel insights of impacts of solid content on high solid anaerobic digestion of cow manure: Kinetics and microbial community dynamics. *Bioresource technology*, 333, 125205. https://doi.org/10.1016/j.biortech.2021.125205
3. Bhattacharjee N., Biswas A. B. (2017). Pyrolysis of Alternanthera philoxeroides (alligator weed): Effect of pyrolysis parameter on product yield and characterization of liquid product and bio char. *Journal of the Energy Institute*, 91 (4), 605-618. https://doi.org/10.1016/j.joei.2017.02.011
4. Cimon C., Kadota P., Eskicioglu C. (2020). Effect of biochar and wood ash amendment on biochemical methane production of wastewater sludge from a temperature phase anaerobic digestion process. *Bioresource technology*, 297, 122440. https://doi.org/10.1016/j.biortech.2019.122440
5. Cai Y., Gallegos D., Zheng Z., Stinner W., Wang X., Proter J., Schafer F. (2021). Exploring the combined effect of total ammonia nitrogen, pH and temperature on anaerobic digestion of chicken manure using response surface methodology and two kinetic models. *Bioresource technology*, 337, 125328. https://doi.org/10.1016/j.biortech.2021.125328
6. Cui Y., Mao F., Zhang J., He Y., Tong Y. W., Peng Y. (2021). Biochar enhanced high-solid mesophilic anaerobic digestion of food waste: Cell viability and methanogenic pathways. *Chemosphere*, 272, 129863. https://doi.org/10.1016/j.chemosphere.2021.129863
7. Cheng Y., Huang M., Shen X., Jiang C. (2021). Enhanced cornstalk decomposition by a psychrotrophic bacterial consortium comprising cellulose, hemicellulose, and lignin degraders with biochar as a carrier for carbonneutrality. *Bioresource technology*, 344 (Pt B), 126259. https://doi.org/10.1016/j.biortech.2021.126259
8. Deng Y., Qiu L., Shao Y., Yao Y. (2019). Process Modeling and Optimization of Anaerobic Co-Digestion of Peanut Hulls and Swine Manure Using Response Surface Methodology. *Energy & Fuels*, 33 (11), 11021-11033. https://doi.org/10.1021/acs.energyfuels.9b02381
9. Feng Y., Zhang Y., Quan X., Chen S. (2014). Enhanced anaerobic digestion of waste activated sludge digestion by the addition of zero valent iron. *Water Reserch*, 52, 242-250. https://doi.org/10.1016/j.watres.2013.10.072
10. Huang H., Deng Y. F., Cheng Z. P., Xu N., Xu J. M. (2013). Characteristics of Mixed Fermentation by Alternanthera Philoxeroides and Corn Stalk for Biogas Production. *Advanced Materials Research*, 805-806, 208-214. https://doi.org/10.4028/www.scientific.net/AMR.805-806.208

11. Khodaei B., Sobati M. A. & Shahhosseini S. (2016). Optimization of
12. ultrasound-assisted oxidative desulfurization of high sulfur kerosene using response surface methodology (RSM). *Clean Technologies and Environmental Policy*, 18(8), 2677-2689. https://doi.org/10.1007/s10098-016-1186-z
13. Hagos K., Zong J., Li D., Liu C., Lu X. (2016). Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and Sustainable Energy Reviews*, 76, 1485-1496. https://doi.org/10.1016/j.rser.2016.11.184
14. Indren M., Birzer C. H., Kidd S. P., Medwell P. R. (2020). Effect of total solids content on anaerobic digestion of poultry litter with biochar. *Journal of Environmental Management*, 255, 109744. https://doi.org/10.1016/j.jenvman.2019.109744
15. Kumar A. N., Dissanayake P. D., Masek O., Priya A., Ki Lin C. S., Ok Y. S., Kim S. H. (2021). Recent trends in biochar integration with anaerobic fermentation: Win-win strategies in a closed-loop. *Renewable and Sustainable Energy Reviews*, 149. https://doi.org/10.1016/j.rser.2021.111371
16. Kunatsa T., Xia X. (2022). A review on anaerobic digestion with focus on the role of biomass co-digestion, modelling and optimisation on biogas production and enhancement. *Bioresource technology*, 344 (Pt B), 126311. https://doi.org/10.1016/j.biortech.2021.126311
17. Laid T. M., Abdelhamid K., Eddine L. S., Abderrhmane B. (2020). Optimizing the biosynthesis parameters of iron oxide nanoparticles using central composite design. *Journal of Molecular Structure*, 129497. https://doi.org/10.1016/j.molstruc.2020.129497
18. Li Pengfei, Wang Qi, He Xiaoman,Yu Ran, He Chao, Shen Dekui, Jiao Youzhou. (2021). Investigation on the effect of different additives on anaerobic co-digestion of corn straw and sewage sludge: Comparison of biochar, Fe_3O_4, and magnetic biochar. *Bioresource technology*, 345. https://doi.org/10.1016/j.biortech.2021.126532
19. Mao C., Feng Y., Wang X., Ren G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews*, 45, 540-555. https://doi.org/10.1016/j.rser.2015.02.032
20. Mohtar S. S., Tengku Malim Busu T. N. Z., Md Noor A. M., Shaari N., Yusoff N. A., Che Yunus M. A. & Mat H. (2016). Optimization of coag-flocculation processes of a newly synthesized quaternized oil palm empty fruit bunch cellulose by response surface methodology toward drinking water treatment process application. *Clean Technologies and Environmental Policy*, 19(1), 191-204. https://doi.org/10.1007/s10098-016-1221-0
21. Mozhiarasi V., Balakumar R., Benish Rose P. M., Speier C. J., Weichgrebe D., Srinivasan S. V. & Suthanthararajan R. (2021). Enhancing methane production by anaerobic co-digestion of extruded organic wastes from slaughterhouse and vegetable market in batch and continuous processes. *Clean Technologies and Environmental Policy*, 23(9), 2727-2740 https://doi.org/10.1007/s10098-021-02198-6
22. Mitraka G. C., Kontogiannopoulos K. N., Tsivintzelis I., Zouboulis A. I., Kougias P. G. (2022). Optimization of supercritical carbon dioxide explosion for sewage sludge pre-treatment using

response surface methodology. *Chemosphere*, 297. https://doi.org/10.1016/j.chemosphere.2022.133989

23. Qiu L., Deng Y. F., Wang F., Davaritouchaee M., Yao Y. Q. (2019). A review on biochar-mediated anaerobic digestion with enhanced methane recovery. *Renewable and Sustainable Energy Reviews*, 115. https://doi.org/10.1016/j.rser.2019.109373
24. Qi Q., Sun C., Zhang J., He Y., Wah Tong. Y. (2021a). Internal enhancement mechanism of biochar with graphene structure in anaerobic digestion: The bioavailability of trace elements and potential direct interspecies electron transfer. *Chemical Engineering Journal*, 406. https://doi.org/10.1016/j.cej.2020.126833
25. Qi C., Wang R., Jia S., Chen J., Li Y., Zhang J., Li G., Luo W. (2021b). Biochar amendment to advance contaminant removal in anaerobic digestion of organic solid wastes: A review. *Bioresource technology*, 341, 125827. https://doi.org/10.1016/j.biortech.2021.125827
26. Shao Z., Guo X., Qu Q., Kang K., Su Q., Wang C., Qiu L. (2021). Effects of chlorine disinfectants on the microbial community structure and the performance of anaerobic digestion of swine manure. *Bioresource technology*, 339, 125576. https://doi.org/10.1016/j.biortech.2021.125576
27. Wang Y., Tian X., Zeng Z., Dai L., Zhang S., Jiang L., Wu Q., Yang X., Liu Y., Zhang B. et al. (2019). Catalytic co-pyrolysis of Alternanthera philoxeroides and peanut soapstock via a new continuous fast microwave pyrolysis system. *Waste Management*, 88, 102-109. https://doi.org/10.1016/j.wasman.2019.03.029
28. Wang Wenxin, Ding Weimin, Xiong Jiading, Muhammad Hassan, Sun Yuanhao. (2021). Effects of trace elemens Fe^{2+},Co^{2+},Ni^{2+} on ananrobic digestion of wheat straw and chicken manure. *Acta Energiae Solaris Sinica*, 42(06), 462-468. https://doi.org/10.19912/j.0254-0096.tynxb.2019-0228
29. Yang Lijuan, Wang Gang, Zhang Hang, Jiang Yufeng, Yang Kai. (2022). Response Surface Methodology to Optimize the Flocculation Condition of Cu(Ⅱ) by Heavy Metal Flocculant MAMPAM. *Research of Environmental Sciences*, 35(03), 789-795. https://doi.org/10.13198/j.issn.1001-6929.2021.11.21
30. Zhang Ya-ling, Yang Shu, Qin Xiang-dong, Lang Changyi, Zhang Chao. (2016). Anaerobic Fermentation Performance of Alternanthera Philoxeroides. *China Biogas*, 34(01), 29-31.
31. Zhao Z., Li Y., Quan X., Zhang Y. (2018). Improving the co-digestion performance of waste activated sludge and wheat straw through ratio optimization and ferroferric oxide supplementation. *Bioresource technology*, 267, 591-598. https://doi.org/10.1016/j.biortech.2018.07.052
32. Zhang Z., Guo L., Wang Y., Zhao Y., She Z., Gao M., Guo Y. (2020a). Application of iron oxide (Fe_3O_4) nanoparticles during the two-stage anaerobic digestion with waste sludge: Impact on the biogas production and the substrate metabolism. *Renewable Energy*, 146, 2724-2735. https://doi.org/10.1016/j.renene.2019.08.078
33. Zhang G., Shi Y., Zhao Z., Wang X., Dou M. (2020b). Enhanced two-phase anaerobic digestion of waste-activated sludge by combining magnetite and zero-valent iron. *Bioresource technology*, 306, 123122. https://doi.org/10.1016/j.biortech.2020.123122

34. Zhao L., Xiao D., Liu Y., Xu H., Nan H., Li D., Kan Y., Cao X. (2020). Biochar as simultaneous shelter, adsorbent, pH buffer, and substrate of Pseudomonas citronellolis to promote biodegradation of high contents of phenol in wastewater. *Water Reserch*, 172, 115494. https://doi.org/10.1016/j.watres.2020.115494
35. Zhu S. (2021). Optimization of Extraction of Polyphenol from Panax Quinquefolius L.Using Central Composite Design/Response Surface Methodology. *Starch - Stärke*, 73 (7-8). https://doi.org/10.1002/STAR.202100020
36. Zhang B., Wang L., Ghimire S., Li X., Todd M. S. & Shahbazi A. (2021). Enhanced biomethane production via thermophilic anaerobic digestion of cattail amended with potassium phosphate and magnesium-modified biochar. *Clean Technologies and Environmental Policy*, 23(8), 2399-2412. https://doi.org/10.1007/s10098-021-02154-4

Figures

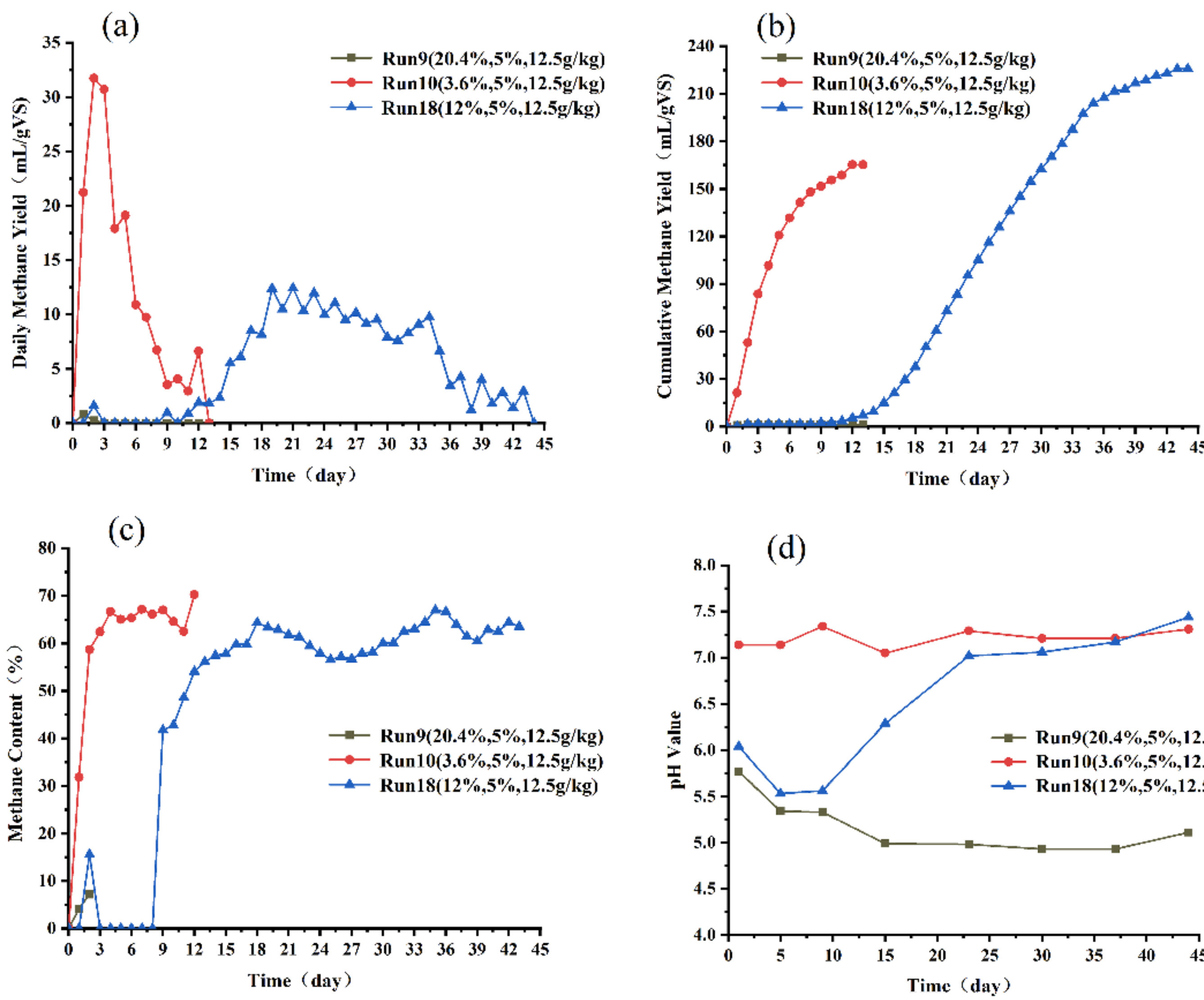

Figure 1

Univariate effect of TS% on daily methane yield (a), cumulative methane yield (b), methane content (c) and pH (d) of coAD

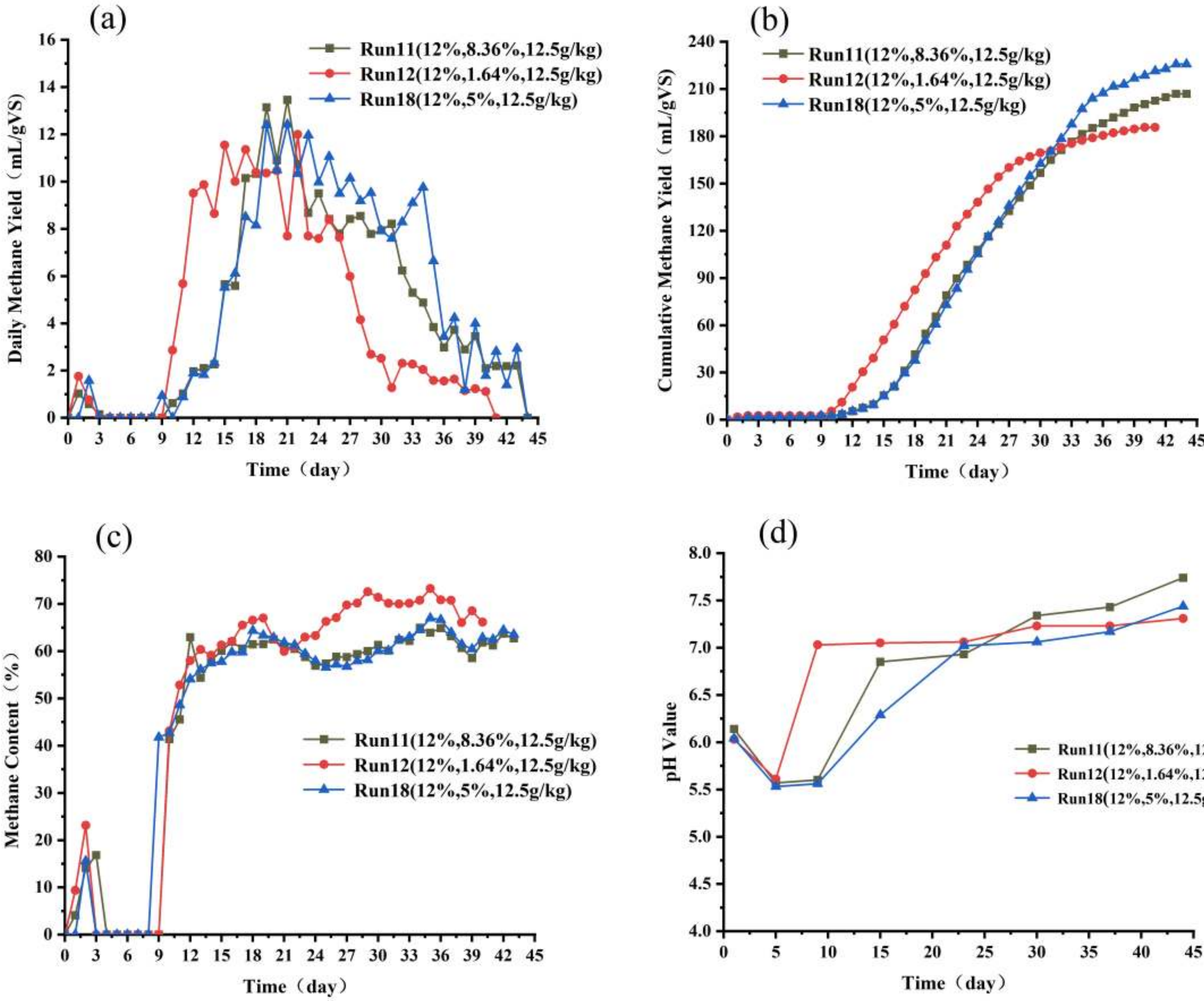

Figure 2

Univariate effect of biochar addition on daily methane yield (a), cumulative methane yield (b), methane content (c) and pH (d) of coAD

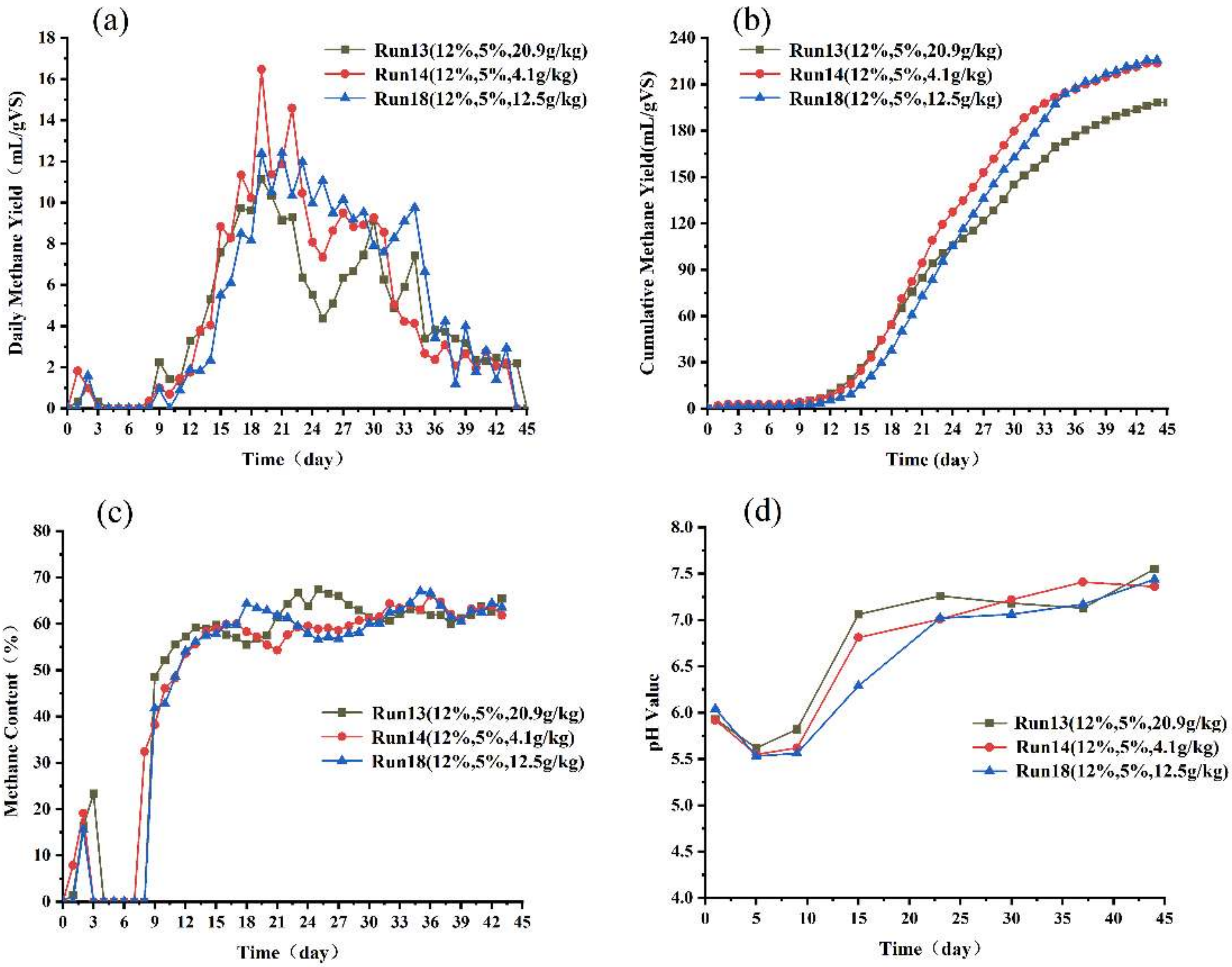

Figure 3

Univariate effect of Fe_3O_4 addition on daily methane yield (a), cumulative methane yield (b), methane content (c) and pH (d) of coAD

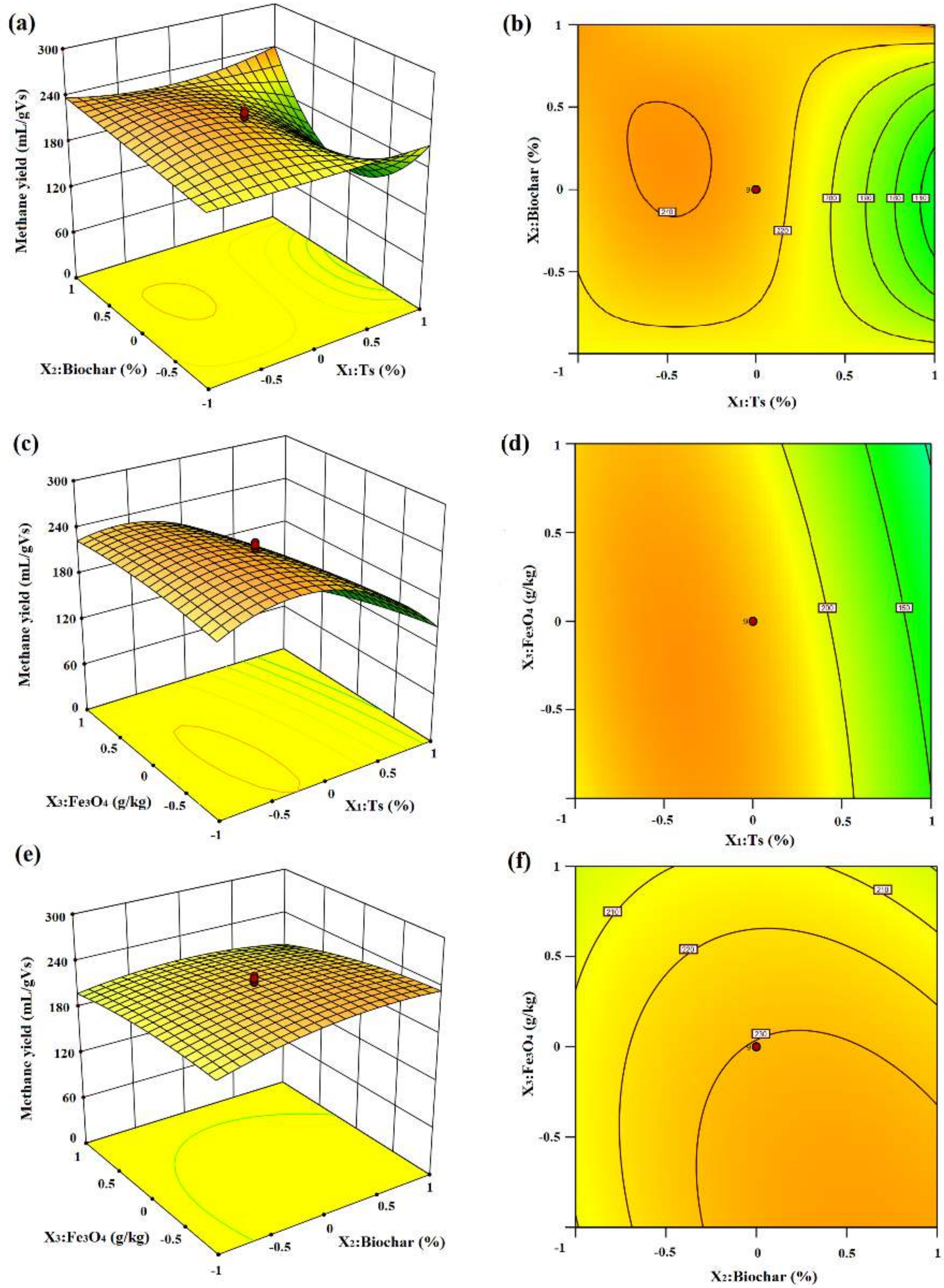

Figure 4

Response surface and contour plot of methane production: TS% and biochar(a-b)、TS% and Fe_3O_4(c-d)、Fe_3O_4 and biochar(e-f)

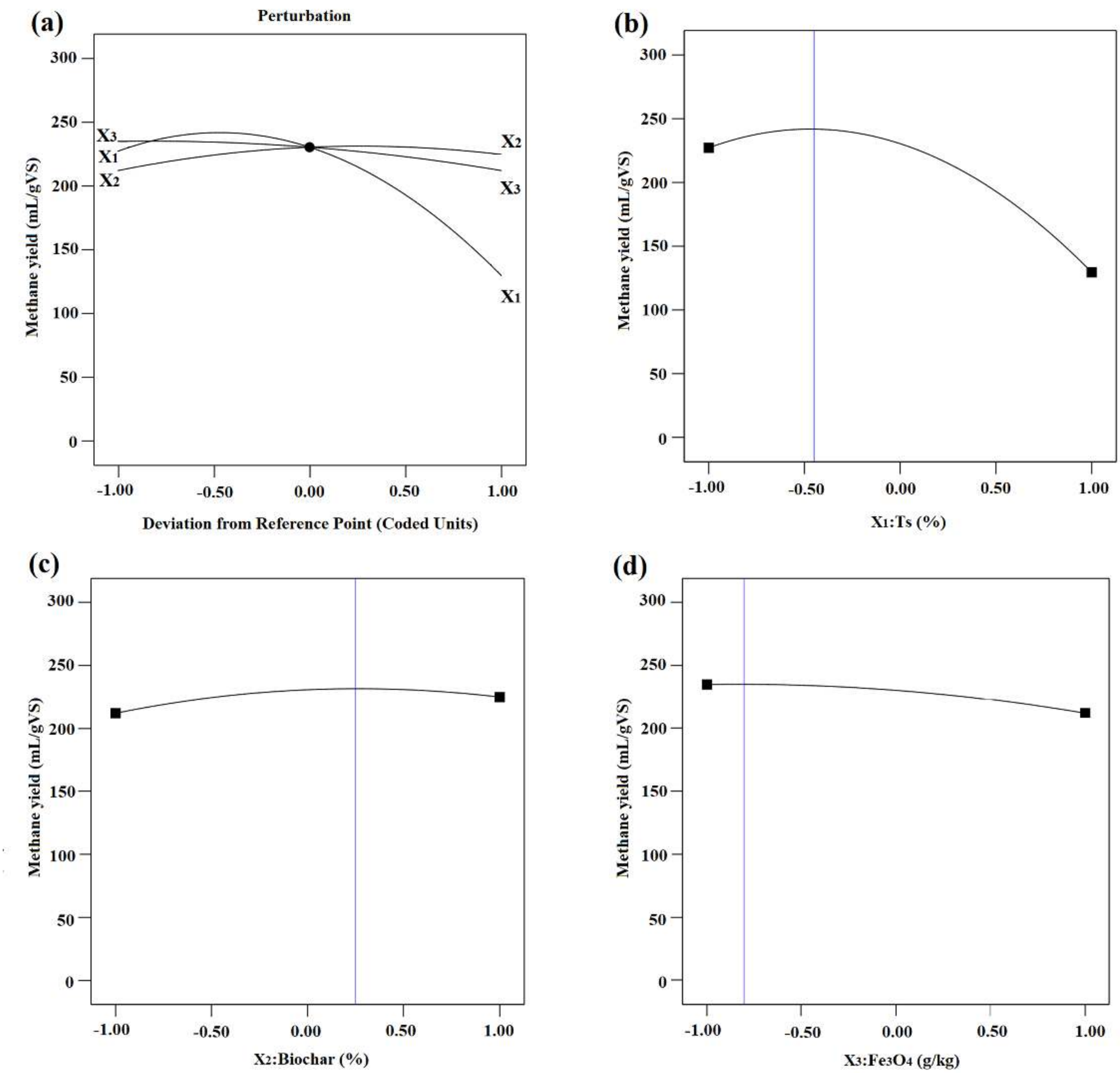

Figure 5

Perturbation analysis of anaerobic digestion with different parameters (a), TS% univariate perturbation (b), Biochar univariate perturbation(c), and Fe_3O_4 univariate perturbation(d)