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**Influences of organic loading, feed-to-inoculum ratio, and different pretreatment strategies on
the methane production performance of eggplant stalk**

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Authors' contributions

ZZ and SZ performed experiment and calculation, wrote the original draft and revised the manuscript; CS, LW and FC helped revise the manuscript; CC and GL provided ideas and resources for the experiment, supervised the experiment process and helped revise the manuscript. All authors read and approved the final manuscript.

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Abstract

A large amount of eggplant straw (ES) is incinerated after harvesting of eggplant every year, which aggravates environmental pollution and waste of resources. Converting ES into methane through anaerobic digestion (AD) technology may be a potential treatment method, considering the low environmental impact and high energy recovery. Firstly, this study explored the effects of organic loading (OL) and feed to inoculum ratio (F/I ratio) on the AD of ES by response surface methodology (RSM). In order to achieve higher AD efficiency, various pretreatments (acid, alkali, alkaline hydrogen peroxide (AHP), microwave and ultrasound) were introduced and comprehensively assessed with regard to methane production performance, organic matter destruction and kinetic parameters. Results showed that OL had a more significant impact on AD process compared to F/I ratio and methane production was enhanced remarkably when the OL and F/I ratio were 35.0 g VS/L and 3.0, respectively. XRD, FTIR, and SEM analyses of pretreated ES showed that alkali and AHP pretreatments performed better in delignification. Under optimal conditions, the ES pretreated with 1.5% AHP (adjusted by KOH) performed the maximum methane production of 262.2 mL/g VS with a B_d of 95.0%, which increased by 334.1% than untreated ES. This paper not only provides the theoretical data about methane production performance of ES but also gives practical guidance for efficient utilization of similar vegetable straw biowastes, which is also promising for large-scale industrial applications in the future.

Keywords: Eggplant stalk, Anaerobic digestion, Methane yield, Response surface methodology, Pretreatment

1. Introduction

Eggplant is one of the most popular vegetables mainly planted in Asia, with an production volume of approximately 48.28 million metric tons (MTS) in 2019 (Tridge 2019). As an inedible fraction, about 14.48 million MTS of eggplant straw (ES) is discarded after harvesting of eggplant (Ravi et al. 2018; Cai et al. 2019). Because of its organic content, poor management of ES not only causes waste of biomass resources but also poses serious environmental risks. Currently, a considerable amount of ES is combusted in the open field (Sharma et al. 2019), which causes serious air pollution, accelerates the earth's greenhouse effect and conflicts with the concept of green development. This calls for the development of an efficient way to utilize ES to reduce the environmental pollution and resource waste.

Anaerobic digestion (AD) technology is regarded as a highly economical-effective solution that can balance energy recovery and environmental impact, and already has a wide application in the stabilization of biomass wastes with abundant organic matters like carbohydrates (Zhang et al. 2020; Amin et al. 2021). As an organic waste with high carbon content, ES is likely to be a suitable feedstock for AD. Furthermore, the methane yield of lignocellulosic biowaste is deeply influenced by several parameters, in which OL and F/I ratio are recognized as important operational parameters (Mamun and Torii 2017; Sun et al. 2017). Enhancing OL can achieve higher volumetric methane productivity, but extremely high OL may exceed the processing capacity of anaerobic system, leading to the accumulation of volatile fatty acids (VFAs) and VFAs inhibition (Tanimu et al. 2014; Zhang et al. 2019). F/I is closely associated with biodegradability, and higher feedstock concentration decreases its biodegradability, resulting in lower AD efficiency (Kawai et al. 2014). It can be seen that the two parameters of OL and F/I ratio are crucial to obtain satisfactory methane production and stable AD performance throughout the AD process. However, no relative literatures have systematically reported the utilizing ES for AD up to now. Hence, it is necessary to explore the responses of AD of ES with different OL and F/I ratio.

In addition, as a lignocellulosic substrate mainly consisting of cellulose, hemicellulose and lignin, ES also faces challenges in methane production. The protective barrier of lignin for the lignocellulose restricts hydrolysis rate of cellulose and hemicellulose, thus limiting the AD efficiency of vegetable straw biomass (Monlau et al. 2013; Phitsuwan et al. 2013; Gu et al. 2020; Song et al. 2021). In recent years, pretreatment (including chemical, physical and biological methods) has achieved increasing great attention in AD process

because it can break down complex and compact structure of lignocellulose and increase the methane production potential of lignocellulosic biomass (Paudel et al. 2017; Wang et al. 2021). Among all the pretreatment strategies, chemical and physical pretreatments are the most common and intensive methods due to their simplicity and effectiveness, which mainly includes acid (H₂SO₄, HCl, H₃PO₄, etc.), alkali (e.g. NaOH, KOH, Ca(OH)₂, etc.), alkaline hydrogen peroxide (AHP), microwave, ultrasound strategies and so on. Considering the recalcitrant nature of vegetable straw biomass, proper pretreatment strategies might be helpful to further improve the utilization efficiency and methane yield of ES. Up to now, no relative researches have been performed to explore the effects of various pretreatments on ES for AD, and the evaluation of different pretreatments is important for the large-scale utilization of ES for methane production in future.

This research set up to (1) explore the methane production performance of ES, (2) identify the effects of different OL and F/I on the AD of ES by RSM, and (3) investigate an effective pretreatment strategy to maximize AD efficiency of ES.

2. Materials and methods

2.1. Materials

ES was obtained from a farm in Anhui, China and milled to a particle size of 10 mm by a high-speed miller (Xingshilihe, Beijing, China), and then carefully reserved at 25° C for subsequent use. The inoculum was kindly acquired from a methane plant in Beijing and remove the background methane at 25° C for 14 days.

2.2. Theoretical maximum methane yield and biodegradability

The theoretical maximum methane potential (MMP) was computed by Eqs. (1) and (2) (Ji et al. 2017; Allison and Simmons 2018). The biodegradability (*B_d*) was calculated from Eq. (3), where EMY is the experimental methane yield.

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_4 \quad (1)$$

$$MMP(mL / g \text{ VS}) = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8} \right)}{12n + a + 16b + 14c} \times (1 - \text{lignin}\%) \quad (2)$$

$$B_d = \frac{EMY}{MMP} \times 100\% \quad (3)$$

2.3. RSM parameters and analysis

RSM is recognized as a mathematical and statistical method for determining optimal operating parameters and analyzing the influence of an independent factor on the response variable (Feng et al. 2017; Arhin et al. 2018). It already has a wide application in experiment design and process optimization of AD (Song et al. 2012). In this research, the RSM was conducted by central composite design (CCD) and each factor was set at five levels: 0, ± 1 , and $\pm \alpha$. The α was defined by Eq. (4) (Ahmad et al. 2009) :

$$\alpha = 2^{\frac{k}{4}} \quad (4)$$

where k represents the number of variables, OL and F/I ratio are separately set in the ranges of 3.0-35.0 g VS/L and 0.3-3.0 in terms of previous study (Zhang et al. 2018a). The values of these variables were listed in Table S1 (Supplementary materials) and the values of variables are shown in Table 2. A second-order polynomial function is employed as:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB \quad (5)$$

where Y , A , and B represent EMY, F/I ratio, and OL; β_0 represents a constant; β_1 , β_2 , β_{11} , β_{12} , and β_{22} denote the coefficients of the corresponding terms of the polynomial.

2.4. Pretreatments

In this study, H_2SO_4 and H_3PO_4 , NaOH, KOH and $Ca(OH)_2$, AHP (adjusted by KOH, NaOH and $Ca(OH)_2$) respectively microwave (210 W, 420 W and 630 W) and ultrasound (40kHz, 200 W) were introduced for pretreatments of raw ES. The mass concentration of H_2SO_4 , H_3PO_4 and AHP was set to 1.5%, 3.0%, 4.5% and 6.0%; while NaOH, KOH and $Ca(OH)_2$ was 1.0%, 2.0%, 3.0% and 4.0%, respectively. The pH of AHP pretreatment solutions were adjusted to 11.5 by NaOH, KOH and $Ca(OH)_2$, respectively. The power of microwave was set to 210 W, 420 W, and 630 W, respectively. The diagram of pretreatments setup

was presented in Table S2. The moisture content (MC) was adjusted by Eq. (6) until 90% in the pretreated vessel (Zhang et al. 2018b).

$$MC(\%) = \left(1 - \frac{\text{dry matter weight of ES}}{\text{weight of ES + water added}} \right) \times 100\% \quad (6)$$

All samples were mixed every 6 h under room temperature and pretreated for 24 h, and then were kept at 4°C.

2.5. Anaerobic digestion

The AD experiments were conducted in 500 mL serum vials with 250 mL of working volume at 37°C and each group had two replicates. To create an anaerobic environment, the headspace of reactors was flushed with nitrogen gas for about 2 min and then these reactors were sealed. The headspace pressure was measured before and after releasing methane to calculate cumulative methane yield (CMY), as shown by Eq. (7) (Rincón et al. 2012):

$$V_{biogas} = \frac{\Delta P \times V_{head} \times C}{R \times T} \quad (7)$$

where V_{biogas} means the daily methane volume (L), ΔP refers to the absolute pressure difference (KPa), V_{head} stands for the volume of the headspace (L), C denotes the molar volume (22.41 L/mol), R indicates the gas constant (83.14 L mbar/(K mol)), and T is the absolute temperature (K).

2.6. Analytical methods

Total solids (TS) and volatile solids (VS) of ES were determined as reported by Zimmerman (Zimmerman et al. 2003). The elemental compositions (C, H, N and S) were measured by an elemental detector (VarioEL cube, Germany) and then the oxygen content was estimated regarding to $C + H + O + N = 99.5\%$ on the basis of VS (Shen et al. 2018; Rincón et al. 2012).

The lignocellulosic contents in ES were measured by an A2000 fiber analyzer (ANKOM, USA), whose operation procedure was reported elsewhere (Shen et al. 2019). The functional groups and chemical bonds were examined by Fourier transform infrared spectroscopy (FTIR, Nicolet 6700, USA), the morphological characteristics were detected by surface electron microscopy (SEM, Hitachi S-4700, Japan), and the

crystallinity index (CrI) was determined by X-ray diffraction (XRD, Bruker D8-Advance, Germany).

2.7. Kinetic models

Three traditional mathematical models (first-order, Cone, and modified Gompertz) have been introduced to fit the CMY curves, as shown by Eqs. (8), (9), and (10), respectively (Siles et al. 2007; Li et al. 2012; Shen et al. 2018; Cai et al. 2019).

$$B = B_0 [1 - \exp(-kt)] \quad (8)$$

$$B = \frac{B_0}{1 + (kt)^{-n}} \quad (9)$$

$$B = B_0 \cdot \exp \left\{ -\exp \left[\frac{\mu_M e}{B_0} (\lambda - t) + 1 \right] \right\} \quad (10)$$

where B and B_0 denotes the simulated CMY (mL/g VS) and the simulated maximum CMY (mL/g VS), k is the rate constant (1/d), t means the AD time (d), μ_M stands for the maximum methane yield rate (mL/g VS/day), λ refers to the lag phase time (d), e is a constant ($e=2.718$), and n is a dimensionless factor.

2.8. Statistical analysis

The OriginPro 2021 Student Edition (Origin lab, Massachusetts, USA) was used for data calculation, modeling, and charting. The accuracy of the models is evaluated by analysis of variance in Excel statistic.

3. Results and discussion

3.1. Features of ES

The properties of feedstock and inoculum were listed in Table 1. It is apparent from this table that the TS of ES was 91.3% and the VS/TS ratio reached 93.3% to 93.8%, indicating the organic matter content of ES is high and suitable for methane production through AD. The cellulose, hemicellulose, and lignin contents of ES were 50.6%, 15.1%, and 19.9%, respectively. Moreover, the carbon-to-nitrogen (C/N) ratio is an important factor for nutrition balance in AD process, and the C/N ration of ES was 19.1, which is close to the preferred value (20.0-25.0) for AD (Shen et al. 2019). In summary, these characteristics suggested that ES could be utilizable as a feedstock for the AD process. According to its element compositions, the chemical formula of ES was determined to be $C_{22.7}H_{36.4}O_{21.3}N$, and its MMP is 275.9 mL/g VS.

3.2. Impacts of OL and F/I ratio on methane produce performance of ES

The 3D response surfaces and 2D contour plots simulated by EMY are shown in Figure 1. It could be observed that the EMY had a sharp increase with increasing OL from 3.0 g VS/L to 35.0 g VS/L at a constant F/I ratio, while it slowly changed with F/I ratio at a constant OL. Consequently, the OL perhaps was a major determinant on methane production during AD of ES and the highest EMY was achieved at the F/I ratio and OL of 3.0 and 35.0 g VS/L as shown in Figure 1a.

3.3. Determination of the suitable OL and F/I ratio for AD process of ES

The experiment obtained the second-order polynomial of ES and shown as follows:

$$Y = 40.11 - 22.88A + 3.19B + 0.62AB + 2.37A^2 - 0.06B^2 \quad (11)$$

where Y , A and B stand for EMY of ES, F/I ratio and OL, respectively. The results of the significance ANOVA of the RSM for ES are listed in Table S3. Over the whole RSM, the R^2 was 0.937, which exhibited a good fitting degree. Also, the equation had a high F-value of 20.75 and a low p-value of 0.001 (< 0.05), showing the significance of these parameters (Kleingesinds et al. 2018). The actual and predicted EMY were displayed in Table 2 and no obvious difference was observed, which also indicated the accuracy of RSM model. The predicted EMY of ES was 92.8 mL/g VS at an OL of 35 g VS/L and F/I ratio of 3.0 corresponding to the validated methane yield of 110.3 mL/g VS, further illustrating the credibility of the RSM.

3.4. Influences of various pretreatments on AD performance of ES

The CMY of the different pretreated ES is shown in Figure 2. Acid pretreatments showed little improvement of EMY of ES, the highest EMY of H_2SO_4 and H_3PO_4 pretreated ES were 70.5 mL/g VS and 86.2 mL/g VS, which were did not significantly differ from that (60.4 mL/g VS) of the untreated ES. The EMY of alkali and AHP pretreated ES improved significantly. It could be found that the highest EMY of alkaline pretreated ES were 202.8 mL/g VS in 4.0% NaOH, 159.0 mL/g VS in 4.0% KOH, and 151.7 mL/g VS in 2.0% $Ca(OH)_2$, respectively. For AHP pretreatments, the 6.0% AHP (adjusted by NaOH), 4.5% AHP (adjusted by KOH), and 1.5% AHP (adjusted by $Ca(OH)_2$) pretreatment groups had the maximum EMY of 261.6 mL/g VS, 251.9 mL/g VS, and 124.4 mL/g VS, respectively. The microwave and ultrasound pretreatments slightly increased methane yield of ES, and the highest EMY were 75.7 mL/g VS in microwave pretreatment (630 W, 10 min) and 80.2 mL/g VS in ultrasonic pretreatment (60 min), respectively. As shown

in Figure 2k, it is obvious that alkali and AHP pretreatments are better for enhancing methane production potential, and NaOH, KOH, and AHP (adjusted by NaOH and KOH) pretreated ES merited to be conducted AD experiment under optimal OL and F/I ratio conditions in order to further improve its methane yield.

The mass concentration of NaOH, KOH, and AHP (adjusted by NaOH and KOH) pretreatments was set to 1.5%, 3.0%, 4.5% and 6.0%, and experimental pretreatments setup is presented in Table S4. Then pretreated ES by above four methods were subjected to AD experiments under optimal digestion conditions and the CMY of pretreated ES during AD process were analyzed to determine the best pretreatment method. The methane performance of ES under each pretreatment is illustrated in Table 3 and Figure 3. As shown, the trends of the CMY in the AD process of ES were similar: they increased sharply at first and then slowed down gradually. As for the NaOH pretreatment, the maximum cumulative methane production was attained at 3% dose, followed by the ES with a concentration of 1.5% NaOH pretreatment. Furthermore, it could be seen that the improvement in CMY of 4.5% and 6% NaOH pretreated ES were relatively lower compared to 3% and 1.5% NaOH pretreatments ($P < 0.05$). There were two possible explanations for this result, much organics was lost in 4.5% and 6.0% KOH solutions, or the high concentration of NaOH caused excessive hydrolysis rate and the accumulation of VFAs, resulting in VFAs inhibition. For the KOH pretreatment, the highest cumulative methane production of ES gained at 6% dose, which was significantly higher than the results at other concentrations of KOH pretreatment ($P < 0.05$). As shown in Figure 3c, all pretreatments showed a significantly higher CMY than untreated ES and no significant difference can be found in terms of the maximum cumulative methane production of pretreated samples. Considering the economic factor and methane produce performance, 1.5% was determined to be the optimum concentration in AHP (adjusted by NaOH) pretreatment strategy. As shown in Figure 3d, the highest CMY of 1.5% and 3.0% AHP pretreated ES were significantly higher than the CMY of 4.5% and 6.0% AHP pretreated ES ($P < 0.05$). While the comparison between 1.5% AHP and 3.0% AHP pretreated ES reveal that no significant difference can be found in the highest methane yield. In order to reduce the cost, 1.5% was selected as the best AHP (adjusted by NaOH) dose. Based on the results above, the highest CMY of NaOH, KOH, and AHP (adjusted by NaOH or KOH) pretreatment groups occurred at the concentration of 3.0%, 6.0%, 1.5%, and 1.5%, respectively, which were 228.1 mL/g VS, 260.1 mL/g VS, 251.0 mL/g VS and 262.2 mL/g VS with a respective increase of 277.6%, 330.6%, 315.6% and 334.1% than untreated. Therefore, the optimal strategy for AD of ES was

1.5% AHP pretreatment (adjusted by KOH) under optimal digestion conditions.

3.5. Kinetic analysis

The first-order, Cone, and modified Gompertz models were applied for simulating the CMY of NaOH, KOH, and AHP (adjusted by NaOH or KOH) pretreated ES, respectively. All kinetic parameters were estimated and the corresponding results were exhibited in the Table 3. It is apparent from this table that the coefficients of correlation (R^2) in the first-order, Cone, and modified Gompertz models ranged from 0.955 to 0.987, 0.987 to 0.994, and 0.969 to 0.989, which suggested these models were well fitted to the CMY of all chosen samples. The all B_0 values were close to the EMY of all samples, which further confirmed the reliability of parameters analysis using the three models.

k is an important parameter for the first-order and Cone models, reflecting the hydrolysis rate of ES. In the two models, the k of all pretreated ES is lower than untreated ES, which means a lower hydrolysis rate. However, a low k doesn't indicate a low EMY because the hydrolysis rate alone can't fully determine the methane performance. Further analysis showed that the B_0 values from the two models were higher than the B_0 of untreated ES obviously, which suggested that all the pretreatment methods are useful for ES to improve its AD efficiency.

For the modified Gompertz model, λ represents the lag-phase time and a lower λ suggests a shorter time to start anaerobic ferment. The λ of 3.0% NaOH, 6.0% KOH and 1.5% AHP (adjusted by NaOH) treated ES reduced compared to the untreated ES. However, the λ of 1.5% AHP (adjusted by KOH) treated ES was longer than the untreated ES, which is consistent with the results in Figure 3. In addition, the μ_M corresponds to the EMY rate and the μ_M of all pretreated samples is higher than untreated ES. Especially the μ_M of 1.5% AHP (adjusted by KOH) pretreated ES improved from 2.53 to 10.91, which suggested that the 1.5% AHP (adjusted by KOH) pretreatment had the most favorable digestion efficiency.

3.6. Composition changes of ES after different pretreatments

The characteristics (VS/TS, cellulose, hemicellulose, and lignin) of pretreated ES were measured and shown in Table 4. After pretreatments of NaOH, KOH, AHP (adjusted by NaOH and KOH), the VS/TS ratios corresponded to 59.7%, 68.8%, 64.9%, and 67.8% (reduced by 26.4%-36.1% than untreated ES), suggesting that some organic matters were dissolved and more contributable to the degradation of ES. Moreover, it was observed that a decrease in hemicellulose content from 15.1% to 7.8-10.5% after the above-mentioned

pretreatments. This might be related to that alkali and AHP pretreatments probably broke the ester bond between hemicellulose and lignin, which resulted to the degradation of hemicelluloses (Kaur and Phutela 2016). Furthermore, AHP (adjusted by NaOH and KOH) pretreatments were more effective in delignification, which decreased lignin contents from 19.9% to 15.6% and 16.2%. It might be inferred that AHP pretreatments ruin the bond between lignin and partially dissolved hemicellulose by cleavages of hydrogen bond, and thus decreased lignin content (Perendeci et al. 2018). However, the lignocellulose after acid, microwave, and ultrasound pretreatments hardly changed, indicating the limited impact of these pretreatment strategies structure of ES.

3.7. Structural changes of pretreated ES

3.7.1. FTIR analysis

The structural arrangement of the functional groups existed in untreated and pretreated ES was analyzed by FTIR (Figure S1). The absorbance at 3400 cm^{-1} , 2900 cm^{-1} , 1439 cm^{-1} and 1050 cm^{-1} were responsible for O-H, C-H, C-O, C-C bonds in cellulose. There were no significant differences between them, indicating that the cellulose content of ES almost unchanged. The absorbance at 1734 cm^{-1} is responsible for C=O bonds in acetyl and carboxylic acids and 1716 cm^{-1} is C=O or C=C bonds in aromatic ring, which were correlated with the lignin structure had flattened or disappeared completely with alkali and AHP pretreatments due to the action of strong oxidation (Yao et al. 2018). At the same time, the reduction of 1720 cm^{-1} denoting carboxylic acid and C=O bond in hemicellulose illustrated the effective hemicellulose removal (Kang et al. 2018). These absorbance bands were greatly reduced, indicating that alkali and AHP pretreatments had a good effect on changing the internal structure of ES.

3.7.2. SEM analysis

Figure S2 shows the SEM images of ES after different pretreatments. It is apparent from this figure that the surface of raw ES had a smooth and orderly fiber structure with regular and dense distribution. And the surface of NaOH, KOH, AHP (adjusted by NaOH and KOH) pretreated ES became deconstructed, shaggy, and irregular (Fig. S2D, Fig. S2E, Fig. S2G, and Fig. S2H, respectively). These structural changes can improve the exposure of lignin and cellulose, hence, the negative effect of lignin on AD hydrolysis process was effectively weakened. These results suggested that NaOH, KOH, and AHP (adjusted by NaOH and KOH) pretreatments were more useful to break down the structure of ES and may be helpful in enhancing AD

efficiency.

3.7.3. XRD analysis

Furthermore, the XRD patterns of ES before and after pretreatment is shown in Figure S3. The three peaks at 16°, 22° and 35° of ES were similar, which represented the cellulose I and suggested the crystalline structure of cellulose was not changed. Further analysis shows that the CrI of raw ES was 39.25% and it increased to 48.19%, 47.86%, 49.13%, and 49.21% after NaOH, KOH, AHP (adjusted by NaOH and KOH) pretreatment. It could be that most of the non-crystalline components such as lignin and hemicellulose were effectively removed by above pretreatments.

Overall, NaOH, KOH, and AHP (adjusted by NaOH or KOH) pretreatments could efficiently minimize the fractions of hemicellulose and lignin that interfered in the hydrolysis process and at the same time increased the cellulosic fraction for AD.

4. Conclusion

This study firstly demonstrated that ES as a novel bioenergy feedstock is suitable for methane production via AD, and then assessed the influences of OL, F/I ratio, and different pretreatment strategies on the methane production performance of ES. The results of this study showed that the AD of ES performed best at OL and F/I ratio of 35 g VS/L and 3.0. Among all the pretreatment strategies, alkali and AHP pretreatments showed tremendous advantages in breaking down the complicated structure of lignocellulose. Under optimal operating conditions, 1.5% AHP (adjusted by KOH) pretreated ES achieved the maximum EMY of 262.2 mL/g VS and biodegradability (B_d) of 95.0%, with an improvement of 334.1% over the untreated ES. In conclusion, this study describes a suitable route to utilize ES and offers a promising pretreatment strategy to improve its methane production performance, which not only fills in the blank in scientific research but also provides a reference for the full utilization of other vegetable straw waste in an environment friendly way.

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456

457 **Tables captions**

458 **Table 1** Characterizations of the ES and inoculum

459 **Table 2** The values of the coded, actual, and response variables of RSM

460 **Table 3** The kinetic parameters of optimal pretreatments

461 **Table 4** The Characteristics a of pretreated ES

462 **Figures captions**

463 **Fig. 1** 3D response surfaces (a) and 2D contour plots (b) obtained by RSM.

464 **Fig. 2** The CMY of ES, (a) H_2SO_4 -pretreated ES; (b) H_3PO_4 -pretreated ES; (c) NaOH-pretreated ES; (d)
465 KOH-pretreated ES; (e) $\text{Ca}(\text{OH})_2$ -pretreated ES; (f) AHP-pretreated ES (adjusted by NaOH); (g) AHP-
466 pretreated ES (adjusted by KOH); (h) AHP-pretreated ES (adjusted by $\text{Ca}(\text{OH})_2$); (i) microwave-pretreated
467 ES; (j) ultrasonic-pretreated ES; (k) Highest cumulative methane yields of different pretreatments for ES.

468 **Fig. 3** The CMY during AD process of ES at suitable F/I and OL, (a) NaOH pretreatment; (b) KOH
469 pretreatment; (c) alkali hydrogen peroxide (AHP) pretreatment (adjusted by NaOH); (d) AHP pretreatment
470 (adjusted by KOH).

Figures

Figure 1

3D response surfaces (a) and 2D contour plots (b) obtained by RSM.

Figure 2

The CMY of ES, (a) H₂SO₄-pretreated ES; (b) H₃PO₄-pretreated ES; (c) NaOH-pretreated ES; (d) KOH-pretreated ES; (e) Ca(OH)₂-pretreated ES; (f) AHP-pretreated ES (adjusted by NaOH); (g) AHP-pretreated ES (adjusted by KOH); (h) AHP-pretreated ES (adjusted by Ca(OH)₂); (i) microwave-pretreated ES; (j) ultrasonic-pretreated ES; (k) Highest cumulative methane yields of different pretreatments for ES.

Figure 3

The CMY during AD process of ES at suitable F/I and OL, (a) NaOH pretreatment; (b) KOH pretreatment; (c) alkali hydrogen peroxide (AHP) pretreatment (adjusted by NaOH); (d) AHP pretreatment (adjusted by KOH).

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