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

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Abstract
A large amount of eggplant stalk (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, 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 biodegradability of 95.0%, which increased by 334.1% compared to 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 stalk biowastes, which is also promising for large-scale industrial applications in the future.

Keywords Chemical pretreatment · Physical treatment · Anaerobic digestion · Methane yield · Response surface methodology · Structural change · Kinetic analysis

Introduction
Eggplant is one of the most popular vegetables mainly planted in Asia, with a 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 stalk (ES) is discarded after harvesting of eggplant (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 organic loading (OL) and feed to inoculum ratio (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 further suppression of methane production (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, hemicelluloses, 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 limits the AD efficiency of vegetable stalk biomass (Gu et al. 2020; Song et al. 2021). In recent years, pretreatment has achieved increasing great attention in AD process, especially physical and chemical methods due to their simplicity and effectiveness (Preethi et al. 2022). For example, Abraham et al. applied microwave physical pretreatment for wheat straw, and found that the surface area of straw was disrupted to a certain degree and the methane yield of pretreated straw was significantly increased by 30% than untreated (Abraham et al. 2020). Chemical pretreatment such as acid (H2SO4, HCl, H3PO4, etc.), alkali (NaOH, KOH, Ca(OH)2, etc.), and oxidative primarily functions by removing the lignin present in the biomass. Chandra et al. used NaOH pretreatment to treat wheat straw, and the results showed that the lignin composition was removed and the methane production of pretreated straw significantly enhanced by 112% related to untreated one. Considering the recalcitrant nature of vegetable stalk biomass, proper pretreatment strategies might be helpful to further improve the utilization efficiency and methane yield of ES. However, it was well known that pretreatment effects varied with feedstocks, which induced that previous pretreatment experience could not be directly applied to ES. Up to now, little concern has been addressed on the effects of varies pretreatments on ES for AD, and the evaluation of different pretreatments on ES still has necessity for the large-scale utilization of ES for efficient methane production in future.

Therefore, this research aims 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 response surface methodology (RSM), and (3) investigate an effective pretreatment strategy to maximize AD efficiency of ES.

### Materials and methods

#### Materials

ES was obtained from a farm in Anhui, China, and milled to a particle size of 10 mm by a high-speed muller (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 removes the background methane at 25 °C for 14 days. And the reference and the provider of each chemical reagents are listed in Table S1 (Supplementary materials).

#### Theoretical maximum methane yield and biodegradability

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

\[
C_{6}H_{12}O_{6} + (a - \frac{n}{2} + \frac{b}{4} + \frac{c}{8})H_{2}O \rightarrow (\frac{n}{2} + \frac{a}{8} - \frac{b}{4} + \frac{c}{8})CH_{4} + (\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{c}{8})CO_{2} + cNH_{4}
\]

(1)

\[
MMP (mL/gVS) = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{c}{8}\right)}{12n + a + 16b + 14c} \times (1 - \text{lignin}\%)
\]

(2)

\[
B_d = \frac{EMY}{MMP} \times 100\%
\]

(3)

#### RSM parameters and analysis

RSM is recognized as a mathematical and statistical method for analyzing the influence of an independent factor on the response variable, as well as determining optimal operating parameters through predictive model (Arhin et al. 2018). It already has a wide application in experiment design and process parameters optimization of AD. In this research, the RSM was conducted by central composite design (CCD) and the two process parameters (OL and F/I ratio) were considered, where 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). Each factor was set at five levels: 0, ± 1, and ± 2. The α was defined by Eq. (4) (Ahmad et al. 2009):

\[
\alpha = 2^k
\]

(4)

where k represents the number of factors (k = 2). The total number of experiments for the two factors is 13 (= 2^k + 2 k + 5), where 5 is centered replicates (Sathish and Vivekanandan 2016). The coded and real values of these variables are listed in Table S2, and the values of variables
are shown in Table 1. A second-order polynomial function is employed as:

\[ Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB \]  

(5)

where \( Y, A, \) and \( B \) represent EMY, F/I ratio, and OL and \( \beta_0 \) represents a constant; \( \beta_1, \beta_2, \beta_{11}, \beta_{12}, \) and \( \beta_{22} \) denote the coefficients of the corresponding terms of the polynomial.

### Pretreatments

In this study, \( \text{H}_2\text{SO}_4 \) and \( \text{H}_3\text{PO}_4, \text{NaOH}, \text{KOH} \), and \( \text{Ca(OH)}_2, \text{AHP} \) (adjusted by \( \text{KOH}, \text{NaOH}, \) and \( \text{Ca(OH)}_2 \)), microwave and ultrasound were finally introduced for pretreatments of raw ES based on relevant literatures and pre-experiments (Gu et al. 2020; Kim et al. 2018). The mass concentrations of \( \text{H}_2\text{SO}_4, \text{H}_3\text{PO}_4, \) and AHP were set to 1.5%, 3.0%, 4.5%, and 6.0%, while \( \text{NaOH}, \text{KOH}, \) and \( \text{Ca(OH)}_2 \) were 1.0%, 2.0%, 3.0%, and 4.0%, respectively. The \( \text{pH} \) of AHP pretreatment solutions were adjusted to 11.5 by \( \text{NaOH}, \text{KOH}, \) and \( \text{Ca(OH)}_2 \), respectively. The power of microwave was set to 210 W, 420 W, and 630 W, respectively. The frequency and power of ultrasound were 40 kHz and 200 W. The diagram of pretreatments setup is presented in Table S3. The moisture content (MC) was adjusted by Eq. (6) until 90% in the pretreated vessel (Zhang et al. 2018b).

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

(6)

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

### Anaerobic digestion

The AD experiments were conducted in 500 mL serum vials with 250 mL of working volume at 37 °C, and the initial \( \text{pH} \) of the reactors were adjusted to 6.5–7.0; 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 reactors of RSM experiment were operated for 52 days; the reactors of pretreatment experiments were operated for 55 days and 88 days, respectively. The headspace pressure was measured before and after releasing methane to calculate the biogas production, as shown by Eq. (7) (Rincón et al. 2012):

\[
V_{\text{biogas}} = \frac{\Delta P \times V_{\text{head}} \times C}{R \times T}
\]  

(7)

where \( V_{\text{biogas}} \) means the daily biogas volume (L), \( \Delta P \) refers to the absolute pressure difference (KPa), \( V_{\text{head}} \) 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). The methane production is calculated by biogas volume and biogas methane content, the latter of which be detected via gas chromatograph (Agilent 7890B, Santa Clara, CA, USA).

### Analytical methods

Total solids (TS) and volatile solids (VS) of ES were determined as reported by 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).
The lignocellulosic contents in ES were measured by an A2000 fiber analyzer (ANKOM, USA) by determining neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), whose operation procedure was reported elsewhere (Gu et al. 2020). The functional groups and chemical bonds were examined by Fourier transform infrared spectroscopy (FTIR, Nicolet 6700, USA). The morphological characteristics of eleven samples were detected by surface electron microscopy (SEM, Hitachi S-4700, Japan); samples were coated with a gold film to improve their conductivity and then observed with a Philips XL30 SEM at 20 kV. The crystallinity index (CrI) was determined by X-ray diffraction (XRD, Bruker D8-Advance, Germany).

**Kinetic models**

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

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

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

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

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), \( \mu_M \) stands for the maximum methane yield rate (mL/g VS/day), \( \lambda \) refers to the lag phase time (d), \( e \) is a constant (\( e = 2.718 \)), and \( n \) is a dimensionless factor.

**Statistical analysis**

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

**Results and discussion**

**Features of ES**

The properties of feedstock and inoculum are listed in Table 2. 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, which are similar to the biomass contents of ES measured by (Li and Chen 2014). 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.

**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 Fig. 1. It could be observed that the EMY had a sharp increase with increasing OL from 3.0 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 Fig. 1a.

**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:

![Springer](https://example.com/springer-logo.png)
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 S4. 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 are displayed in Table 1, 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.

**Influences of various pretreatments on AD performance of ES**

The CMY of the different pretreated ES is shown in Fig. 2. Acid pretreatments showed little improvement of EMY of ES; the highest EMY of H$_2$SO$_4$ and H$_3$PO$_4$ pretreated ES were 70.5 mL/g VS and 86.2 mL/g VS, which 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 Fig. 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 S5. 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 Fig. 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 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 Fig. 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 Fig. 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 (Table 3). On the other hand, the methane yield of pretreated ES was increased significantly compared with other lignocellulose biomass. For example, Zhang et al. applied 3% AHP for cotton stalk (CS) and found that the highest EMY of 192.4 mL/g VS was obtained with an increment of 254.3% compared to the untreated CS (Zhang et al. 2018b). Chandra et al. have used 4% NaOH pretreatment to wheat straw, and the results showed that the highest CMY was 165.9 mL/g VS and produced 111.6% increase compared with untreated one (Chandra et al. 2012). Therefore, the optimal strategy for AD of ES was 1.5% AHP pretreatment (adjusted by KOH) under optimal digestion conditions.
Kinetic analysis

The first-order, Cone, and modified Gompertz models were applied for simulating the highest CMY of NaOH, KOH, and AHP (adjusted by NaOH or KOH) pretreated ES, respectively. All kinetic parameters were estimated, and the corresponding results are 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 $k$ does not indicate a low EMY because the hydrolysis rate alone cannot 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, $\lambda$ represents the lag-phase time and a lower $\lambda$ suggests a shorter time to start anaerobic ferment. The $\lambda$ of 3.0% NaOH, 6.0% KOH, and 1.5% AHP (adjusted by NaOH) treated ES reduced compared to the untreated ES. However, the $\lambda$ of 1.5% AHP (adjusted by KOH) treated ES was longer than the untreated ES, which is consistent with the results in Fig. 3d. In addition, the $\mu_M$ corresponds to the EMY rate and the $\mu_M$ of all pretreated samples is higher than untreated ES. Especially the $\mu_M$ 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.

Composition changes of ES after different pretreatments

The characteristics (VS/TS, cellulose, hemicellulose, and lignin) of pretreated ES were measured and are shown in Table 4. After pretreatments of NaOH, KOH, and 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%, 9.1%, 7.9% and 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.
Structural changes of pretreated ES

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 C–H, O–H, C–O, and 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 and 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).

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, and 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.

XRD analysis

Furthermore, the XRD patterns of ES before and after pretreatment are 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 (Gu et al. 2020). For lignocellulosic biomass, the CrI implied the relative amount of crystal cellulose, which was strongly influenced by biomass composition. 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, and AHP (adjusted by NaOH and KOH) pretreatment, probably attributing to that most of the non-crystalline components such as lignin and hemicellulose were effectively removed by above pretreatments (Li et al. 2015).
Overall, the results of structural changes were highly consistent with the compositional variations, collectively suggesting that NaOH, KOH, and AHP (adjusted by NaOH and KOH) pretreatments could efficiently increase surface area, reduce cellulose crystallinity, minimize the fractions of lignin that interfered in the hydrolysis process; and therefore, increased the cellulosic fraction and improve the AD performance.

**Conclusion**

This research 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 $B_d$ of 95.0%, with an improvement CMY of 334.1% over the untreated ES. Furthermore, compared with other lignocellulose biomass (e.g., cotton stalk, wheat straw), pretreated ES also indicated significant superiority in methane production. 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 stalk waste in an environment friendly way.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-20940-5.
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