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RESEARCH ARTICLE

ALKALINE PRETREATMENT AND AIR MIXING FOR IMPROVEMENT OF METHANE PRODUCTION FROM ANAEROBIC CO-DIGESTION OF POULTRY LITTER WITH WHEAT STRAW

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KEYWORDS

sodium hydroxide, air injection, cumulative methane yield, kinetic modeling analysis, digestate

HIGHLIGHTS

- Integration of alkaline pretreatment and air mixing for co-digestion was validated.
- Alkaline pretreatment enhanced hydrolysis of poultry litter and wheat straw.
- Cumulative methane yield was improved by 46.7% compared to the control.
- The cone model best fitted the methane yield kinetics with $R^2 \geqslant 0.9979$.
- Total volatile solids removal was improved by 2.3 times in the digestate.

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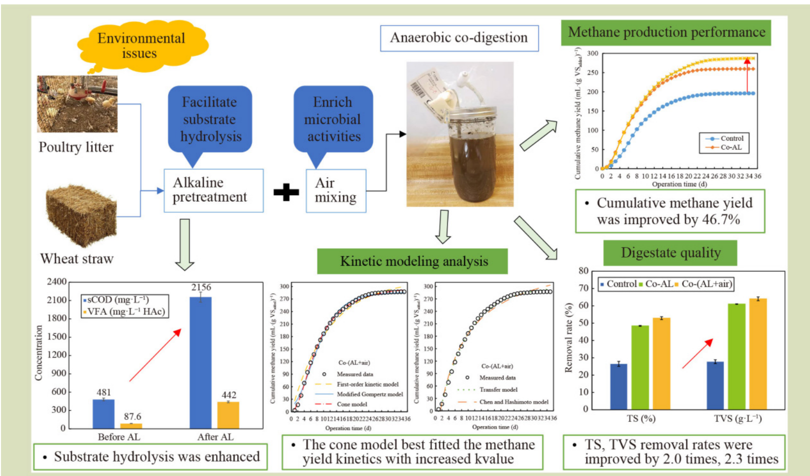
GRAPHICAL ABSTRACT



ABSTRACT

Alkaline pretreatment (AL) and air mixing (air) both have the potential to improve anaerobic co-digestion (Co-AD) of poultry litter with wheat straw for methane production. In this study, the effects of the combination of AL (pH 12 for 12 h) and air mixing (12 mL·d^{-1}) on the Co-AD process were investigated. The substrate hydrolysis was enhanced by AL, with soluble chemical oxygen demand increased by 4.59 times and volatile fatty acids increased by 5.04 times. The cumulative methane yield in the group of Co-AD by AL integrated with air (Co-(AL + air)), being 287 mL·(g VS$_{added}$)$^{-1}$, was improved by 46.7% compared to the control. The cone model was found the best in simulating the methane yield kinetics with $R^2 \geqslant 0.9979$ and root mean square prediction error (rMSPE) $\leqslant$ 3.50. Co-(AL + air) had a larger hydrolysis constant k (0.14 d^{-1}) and a shorter lag phase λ (0.99 d) than the control (k = 0.12 d^{-1}, λ = 2.06 d). The digestate improved the removal of total solids and total volatile solids by 2.0 and 2.3 times, respectively. AL facilitated substrate degradation, while air can enrich the microbial activity, together enhancing the methane generation. The results show that AL + air can be applied as an effective method to improve methane production from the Co-AD process.

1 INTRODUCTION

The state of Arkansas ranked third in broiler production in 2021 in the USA as reported by the United States Department of Agriculture[1], which resulted in a large amount of poultry litter (PL) left in the chicken houses. Worldwide, the increase of poultry farms with the increasing meat demand has also led to serious environmental problems due to the potential pollution caused by PL[2,3]. Anaerobic digestion (AD) has been widely acknowledged as a sustainable management technology for animal waste treatment and simultaneously renewable biogas production[4,5]. However, the utilization of PL as the single substrate for AD might encounter ammonia inhibition problems[6]. Agricultural straw, such as corn stover, rice straw and wheat straw (WS), has been commonly added as co-substrate to balance the carbon to nitrogen ratio for anaerobic co-digestion (Co-AD) with PL thanks to its high carbon content and low nitrogen content[7]. However, the lignin in the WS is not readily degraded to the soluble materials, leading to a low conversion efficiency to methane production by direct AD process[8]. The accumulation of undegraded straw particles might also cause an inhibition effect on the Co-AD process. In addition, PL comprises chicken feces mixed with bedding materials such as wood shavings, rice hulls, and straw[9,10], which means that there is also lignocellulosic biomass in PL that does not readily degrade. Thus, a proper pretreatment process for the substrate is needed before the Co-AD of PL and WS.

Based on the mechanism used to enhance substrate hydrolysis efficiency[11], the different methods of pretreatment reported in the previous studies included mechanical, thermal, chemical and biological processes, as well as their combinations. Chemical pretreatment includes acid pretreatment and alkaline pretreatment, which are usually completed by adding acids, such as sulfuric acid, hydrochloric acid and acetic acid, or alkalines, such as sodium hydroxide, potassium hydroxide and lime, to the substrate suspension[8] to enhance the biodegradation of complex materials[12]. Also, the amount of acid or alkaline needed for addition is usually decided by the desired pH. Alkaline pretreatment could improve the digestibility of the biomass in the substrate by the destruction of the lignin structure, thus improving methane yield of the AD process[13]. In addition, alkaline pretreatment has been reported to have a significant effect on improving the methane production from AD of wastewater sludge due to the accelerated hydrolysis of the sludge[14], and simultaneously enhance the degradation of exogenous pollutants in the wastewater sludge, which not only had potential environmental benefits but also might cause severe inhibitory effect on the AD process[15]. As a result, alkaline pretreatment has the potential to significantly improve both the efficiency and sustainability of the AD process for wastewater treatment.

Air mixing is also a newly emerged and attractive pretreatment that could enhance the AD process efficiency by enhancing the microbial community[16,17]. Air mixing is usually conducted by injecting a controlled small amount of oxygen or air, or by aeration with a flow rate controlled by the sensed oxidation-reduction potential value[18]. It has been reported that air mixing facilitates, processing efficiency and system stability by accelerating hydrolysis, scavenging hydrogen sulfide, augmenting the activity and diversity of the microbial consortia that promoted syntrophic interactions among different microbial groups[18,19].

Since chemical pretreatment and air mixing are conducted at different stages of the whole Co-AD process with the former in the stage of substrate pretreatment and the latter during the fermentation process, it is hypothesized that the combination of chemical and air mixing might work with a higher efficiency in improving the methane yield performance. However, there are currently no studies that have specifically validated the combination of alkaline pretreatment and air mixing for improving the AD of agricultural wastes. This study was the first to investigate the effects of alkaline pretreatment integrated with air mixing on the Co-AD of PL and WS. The substrate solubilization by alkaline pretreatment, the methane yield with kinetic modeling analysis and the digestate quality of the Co-AD process with alkaline pretreatment combined with air mixing was compared with the control. The aim was to validate the feasibility and efficiency of the combination of alkaline pretreatment and air mixing (AL + air) for improving methane production from the Co-AD process.

2 MATERIALS AND METHODS

2.1 Substrate and inoculum sludge

Information on the sources and preparation methods of the substrates (PL and WS) has been presented in the previously published paper[20]. The properties of the PL and WS are summarized in Table 1. The inoculum sludge was sampled from an operating anaerobic sequencing batch reactor for methane fermentation in the laboratory[21] and was pre-incubated for 7 d and fully degassed in the incubator at 37 °C before being used for methane fermentation of the co-substrates in the batch reactors. The properties of the inoculum sludge are summarized in Table 1.

2.2 Alkaline pretreatment for substrate suspension

Alkaline pretreatment (AL) of the dry substrate was made after mechanical cutting. After the formulation of the substrate suspension in the glass container, the alkaline solution was added to increase the pH to 12 measured by a glass probe linked to a pH meter (XL 600, Fisher Scientific, Hampton, NH, USA). 1 mol·L^{-1} NaOH solution was used to increase the pH of the substrate suspension for alkaline pretreatment considering its high impact on alkalinity and reasonable price, as well as suggested in previous research[22,23]. After the pH adjustment, the glass container containing the mixture substrate was placed on a shaker at 150 r·min^{-1} in a water bath at 25 °C for 12 h to keep the substrate mixture uniform. Then the glass container was removed from the water bath and allowed to cool to room temperature and the pretreated substrate suspension was then conditioned with 1 mol·L^{-1} HCl solution to a pH of 8.0.

2.3 Air mixing for the co-digestion system

The air mixing was conducted during the Co-AD process after the inoculum incubation. The same volume of air (12 mL) was injected daily into the glass container from the tube that reached the container bottom using a syringe. The amount of air added to the glass reactor was decided according to previous research[24] as well as the substrate total solids levels.

2.4 Batch reactors for anaerobic co-digestion

Identical glass containers were used for the alkaline pretreatment and the Co-AD process. The glass container had a total volume of 648 mL, with a working volume of 500 mL. A gas tube was connected to an outlet for biogas collection which was closed by a rubber switch, and another tubing reached the bottom of the reactor in the sludge layer for nitrogen gas flushing (to empty air), air mixing and the sludge sampling if needed. The prepared dry PL and WS substrates were added to the glass container with a total weight of 9.2 g at a 1:1 ratio (4.6 g each). Tap water was then added to the reactor to formulate a mixture substrate suspension of 300 mL, which was then subject to AL treatment. When the substrate suspension was finally prepared, 200 mL of the inoculum sludge was added to reach the ratio of $TS_{inoculum}/TS_{substrate}$ of 0.5[25]. The reactor was flushed with gaseous nitrogen for 5 min to empty the inside air before it was fully sealed. The Co-AD process was immediately operated after the above procedures were completed. A Tedlar gas bag (Tedlar Bag, CEL Scientific Corp., Cerritos, CA, USA) was used to collect the biogas from the outlet on the top of the container. The reactors were then placed in a programmed incubator maintained at 37 °C for methane fermentation. The Co-AD process was operated in a batch mode and continued for 35 d until biogas production was negligible. During the Co-AD process, the gas bag was replaced daily with a new one for each reactor.

2.5 Experimental design

Three experimental groups were included to examine the

Table 1 Physiochemical properties of the substrate and the inoculum sludge

Properties	Poultry litter (%)	Wheat straw (%)	Inoculum sludge
TS	90.7 ± 0.0	96.7 ± 0.2	2.18% ± 0.01%
TVS	72.0 ± 0.3	92.6 ± 0.2	16.5 ± 0.5 g·L^{-1}
TC	25.4 ± 1.3	45.0 ± 3.3	–
TN	3.37 ± 0.65	0.76 ± 0.03	205 ± 23 mg·L^{-1}
pH	–	–	8.02
TAN	–	–	100 ± 5 mg·L^{-1}
FAN	–	–	2.04 mg·L^{-1}

Note: TS, total solids level; TVS, total volatile solids level; TC, total carbon content; TN, total nitrogen content; TAN, total ammonia nitrogen concentration; and FAN, free ammonia nitrogen concentration by calculation from Eq. (1). The values are presented as mean ± standard deviation (n = 3).

effects of AL and the combination of alkaline pretreatment and air mixing (AL + air) on the Co-AD process of PL and WS. Identical reactors and the same amount of mixture substrate suspension as well as inoculum were used for each experiment. The experiment group with no treatments was designated as the control. The same control was used in a previous study[20]. The group with AL of substrate was Co-AL, while the group of Co-(AL + air) meant that both AL and air mixing of substrate during Co-AD process were conducted.

2.6 Sample analysis

2.6.1 Physiochemical parameters

Total solids (TS) and total volatile solids (total VS, or TVS) were analyzed following the gravity method[26] using an oven (BINDER Inc., Bohemia, NY, USA) set at 105 °C and a muffle furnace (Cole-Parmer, Vernon Hills, IL, USA) set at 550 °C. Soluble chemical oxygen demand (sCOD, mg·L^{-1} O_2), volatile fatty acids (VFA, mg·L^{-1} acetic acid equivalent (HAc)), total ammonia nitrogen (TAN, mg·L^{-1} nitrogen (N)) and total alkalinity (TA, mg·L^{-1} $CaCO_3$) of the digestate samples were analyzed by standard methods[26]. All parameters were measured in triplicate and the mean values with standard deviations were obtained.

Free ammonia nitrogen (FAN) concentration was calculated according to the equilibrium equation:

$$\frac{\mathrm{FAN}}{\mathrm{TAN}} = \left(1 + \frac{10^{-\mathrm{pH}}}{10^{-\left(0.9018 + \frac{2729.92}{\mathrm{T(K)}}\right)}}\right)^{-1} \quad (1)$$

where, FAN and TAN is the concentrations (mg·L^{-1}) of free ammonia nitrogen and total ammonia nitrogen, respectively; the pH value and the temperature T (Kelvins (K)) were measured from the digestate.

2.6.2 Biogas analysis

The biogas volume was measured using a wet gas test meter (Model XMF-1, Shanghai Cixi Instrument Co., Ltd., China). The gas collected in gas bags was sampled and the composition was analyzed using a Shimadzu Gas Chromatograph (GC 2014, Shimadzu Scientific Instruments, Inc., Maryland, CO, USA) according to the operation details previously described[10].

2.7 Kinetic analysis

There are five kinetic models were used in this study to fit the methane yield from the different Co-AD batch experiments: first-order model, modified Gompertz model, cone model, transfer model, and the Chen and Hashimoto model. The first-order kinetic model (Eq. (2)) assumed that hydrolysis was the rate-limiting step that governed the overall process[27,28]. The modified Gompertz model (Eq. (3)) described the maximum methane production potential and lag phase[29]. The cone model (Eq. (4)) estimates the methane yield rate and the maximum cumulative methane yield[30,31]. The transfer model (Eq. (5)) and Chen and Hashimoto model (Eq. (6)) were also used to fit the methane production data from Co-AD process[32]. The equations of these kinetic models are given in Table 2.

The correlation coefficients (R^2), root mean square prediction error (rMSPE), Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) were used to compare the models. The model with a higher R^2 and a lower rMSPE value provided a better fit[28]. The lower values of AIC and BIC indicate that the model is more likely to be suitable[35,36]. They

Table 2 Summary of the kinetic models used to fit the cumulative methane yield from different reactors for the Co-AD of poultry litter and wheat straw

Model	Equation	Equation number	Reference
First order	$P(t) = Y_m \times \left(1 - e^{-kt}\right)$	(2)	[33]
Modified Gompertz	$P(t) = Y_m \times \exp\left\{-\exp\left[\frac{R_m \times e}{Y_m}(\lambda - t) + 1\right]\right\}$	(3)	[34]
Cone	$P(t) = \frac{Y_m}{1 + (-kt)^{-n}}$	(4)	[31]
Transfer	$P(t) = Y_m \times \left\{1 - \exp\left[\frac{-R_m}{Y_m}(t - \lambda)\right]\right\}$	(5)	[32]
Chen and Hashimoto	$P(t) = Y_m \times \left(1 - \frac{k_{CH}}{HRT \times \mu_m + k_{CH} - 1}\right)$	(6)	[32]

Note: $P(t)$, the cumulative methane yield (mL·(g VS_{added})$^{-1}$) at the AD process operation time (d); Y_m, the maximum methane yield potential (mL·(g VS_{added})$^{-1}$); e, 2.718; k, the hydrolytic rate constant (d^{-1}); t, the digestion time (d); R_m, the maximum methane production rate (mL·(g VS_{added})$^{-1}$·d^{-1}); λ, the lag phase (d); n, the shape constant, which reveals whether there is a lag phase in the reactor; k_{CH}, Chen and Hashimoto constant (dimensionless); μ_m, Maximum specific growth rate of microorganisms (d^{-1}); HRT, digestion time or hydraulic retention time (d).

were calculated as the following equations, Eq. (7)[10], Eq. (8), Eq. (9), and Eq. (10), respectively.

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} = 1 - \frac{\sum_{i=1}^{m}(P_i - M_i)^2}{\sum_{i=1}^{m}\left(M_i - \overline{M}\right)^2} \quad (7)$$

$$\mathrm{rMSPE} = \sqrt{\sum_{i=1}^{m}\frac{(P_i - M_i)^2}{m}} \quad (8)$$

$$\mathrm{AIC} = m \times \ln\left(\frac{SS_{res}}{m}\right) + 2(N+1) + \frac{2(N+1)(N+2)}{m-N-2} \quad (9)$$

$$\mathrm{BIC} = m \times \ln\left(\frac{SS_{res}}{m}\right) + N \times \ln(m) \quad (10)$$

where, SS_{res} is the sums of squares of residuals, SS_{tot} is the total sum of squares of deviations, P_i is the predicted value of point i by the model, M_i is the measured value of point i, $\overline{M}$ is the mean of the measured value, m is the number of measurements, and N is the number of model parameters.

The difference Dif (%) between the model-predicted value of maximum methane yield potential (mL·(g VS_{added})$^{-1}$) and the measured value of the final cumulative methane yield (mL·(g VS_{added})$^{-1}$) was calculated as:

$$\mathrm{Dif} = \frac{Y_m - \mathrm{CMY}_m}{\mathrm{CMY}_m} \times 100\% \quad (11)$$

where, Y_m is the maximum methane yield potential (mL·(g VS_{added})$^{-1}$) predicted by the model, and CMY_m is the cumulative methane yield measured (mL·(g VS_{added})$^{-1}$).

2.8 Data analysis

The data in this study were recorded and processed using Excel 2016 (Microsoft, Corporation, Redmond, WA, USA). JMP software (JMP Pro Version 12.0, SAS Institute Inc., NC, USA) was used to determine the statistical significance by analysis of variance using a P-value of 0.05. Tukey's honest significant difference test was used to compare differences between the three groups.

3 RESULTS AND DISCUSSION

3.1 Effect of alkaline pretreatment

The mixture substrate suspension of PL and WS exhibited huge changes in sCOD concentration (mg·L^{-1} O_2) and VFA concentration (mg·L^{-1} HAc) after AL as shown in Fig. 1. sCOD increased 4.59 times and VFA increased 5.04 times after AL, indicating a significantly enhanced hydrolysis of the substrate by AL. Various previous studies that employed alkaline pretreatment have also reported enhanced substrate degradation[13,37]. According to Badiei et al.[13], the mechanism of AL lies in that it involves the addition of bases to biomass, leading to an increase of internal surface by swelling, a decrease of polymerization degree and crystallinity, destruction of links between lignin and other polymers, and lignin breakdown.

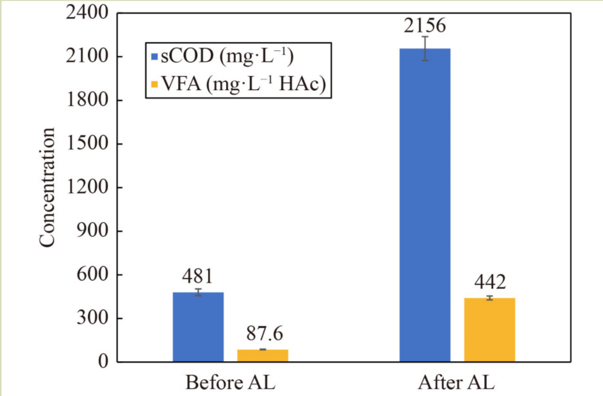


Fig. 1 Changes in soluble chemical oxygen demand (sCOD, mg·L^{-1}), volatile fatty acids (VFA, mg·L^{-1} HAc) in the substrate suspension of poultry litter and wheat straw by alkaline pretreatment (AL).

3.2 Methane production performance

The Co-AD batch experiments, including the groups of control, Co-AL (that with AL) and Co-(AL + air), were performed with the variation of daily methane production (DMP, mL) and methane content (%) is shown in Fig. 2. The cumulative methane yields (CMY, mL·(g VS_{added})$^{-1}$) of the different Co-AD groups were calculated from the daily methane production and the co-substrate VS addition and are shown in Fig. 2(c). AL obviously enhanced methane production efficiency in the Co-AD process. Compared with Co, Co-AL reached a higher maximum DMP (212 vs 158 mL) with a shorter lag phase time (4 vs 7 d). Also, the final measured CMY of Co-AL (260 mL·(g VS_{added})$^{-1}$) was improved by 32.5%, compared with the CMY of the control (196 mL·(g VS_{added})$^{-1}$). The results indicated that alkaline pretreatment was an efficient method to elevate methane production efficiency in the Co-AD of PL and WS.

Zheng et al.[38] reported that the methane yield of NaOH-pretreated corn straw generated a methane yield of about 220 mL·g^{-1} VS, which was 73.4% higher than that of untreated corn straw. Similarly, it was reported that 2.5% KOH-treated corn straw resulted in a 95.6% improvement in maximum

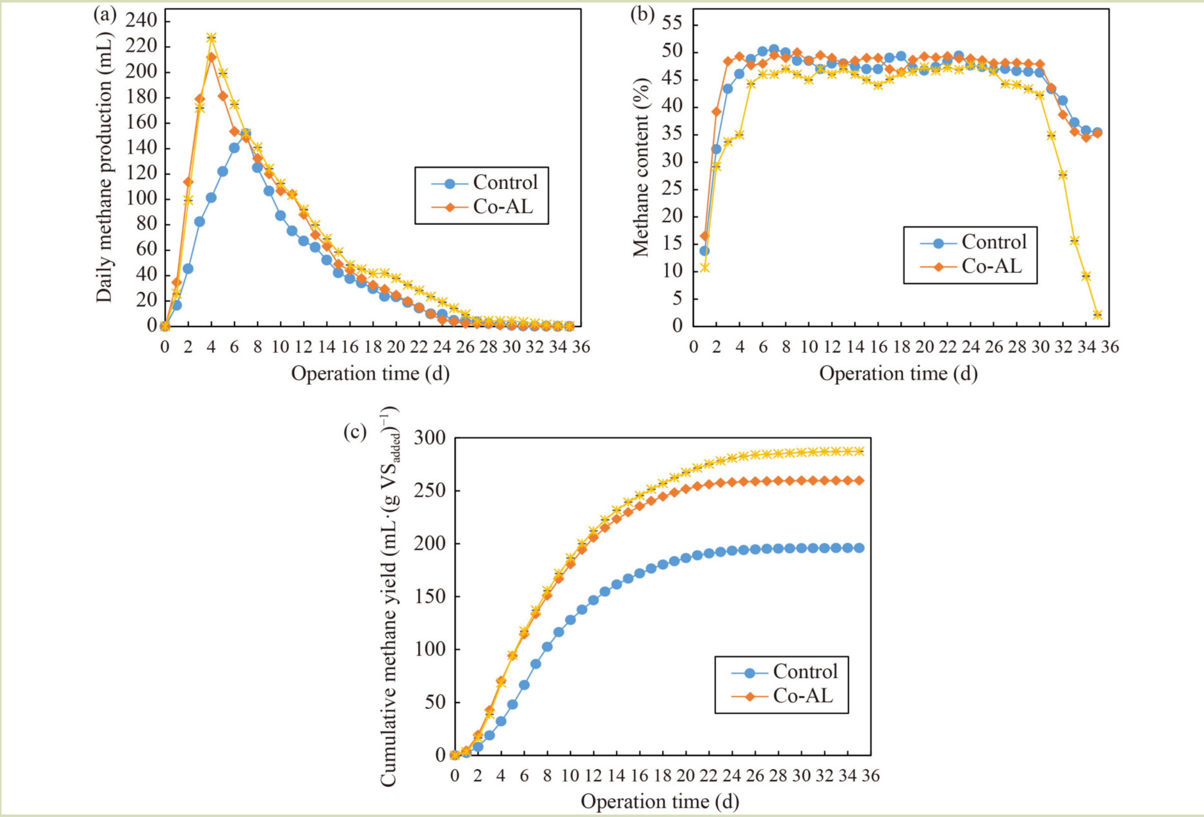


Fig. 2 The variation of daily methane production (mL) (a), methane content (%) (b), and cumulative methane yield (mL·(g $VS_{added})^{-1}$) (c) in the experimental groups with the Co-AD of poultry litter and wheat straw.

methane yield of 295 mL·g^{-1} VS compared to untreated corn straw[37].

In the case of the Co-AD with both alkaline pretreatment and air mixing employed (Co-(AL + air)), methane production efficiency was higher. Co-(AL + air) achieved the highest maximum DMP (227 mL) by day 4. Also, it reached the highest CMY (287 mL·(g $VS_{added})^{-1}$) of all the Co-AD batch groups, which was 46.7% and 10.7% higher than the control and Co-AL, respectively. These results indicate that air mixing can further improve methane production of Co-AD process with AL pretreatment of substrate. It has been reported that air mixing can improve methane production by maintaining low VFA concentration, or by promoting microbial activity[24] due to the small but continuous dosage of oxygen.

3.3 Kinetic analysis

3.3.1 Kinetic model evaluation

The five commonly used models listed in Table 2 were used to fit the measured CMY (mL·(g VS $_{added})^{-1}$ CH_4) of the different Co-AD batches, including the first-order kinetic model (FM), the modified Gompertz model (MGM), the cone model (CNM), the transfer model (TM) and the Chen and Hashimoto model (CHM). The measured data with fitting curves of the five models are shown in Fig. 3. Evaluation parameters for the model fitting, including R^2, rMSPE, AIC, BIC and Dif (%), are summarized in Table 3.

In general, all the five models provide good fits but CNM had a better goodness-of-fit than the other models with the highest R^2 and lowest rMSPE, AIC, BIC and Dif (%) according to the comparisons of model evaluation parameters shown in Table 3. It is also clear from the model curves in Fig. 3 that the CNM had the best fit to the measured data compared to the other models.

In different Co-AD groups, the R^2 values of the FM (0.9590, 0.9740 and 0.9792) and the CHM (0.9723, 0.9794 and 0.9874) were the lowest. The CNM had slightly higher R^2 values (0.9990, 0.9979 and 0.9994) than the MGM (0.9987, 0.9977 and

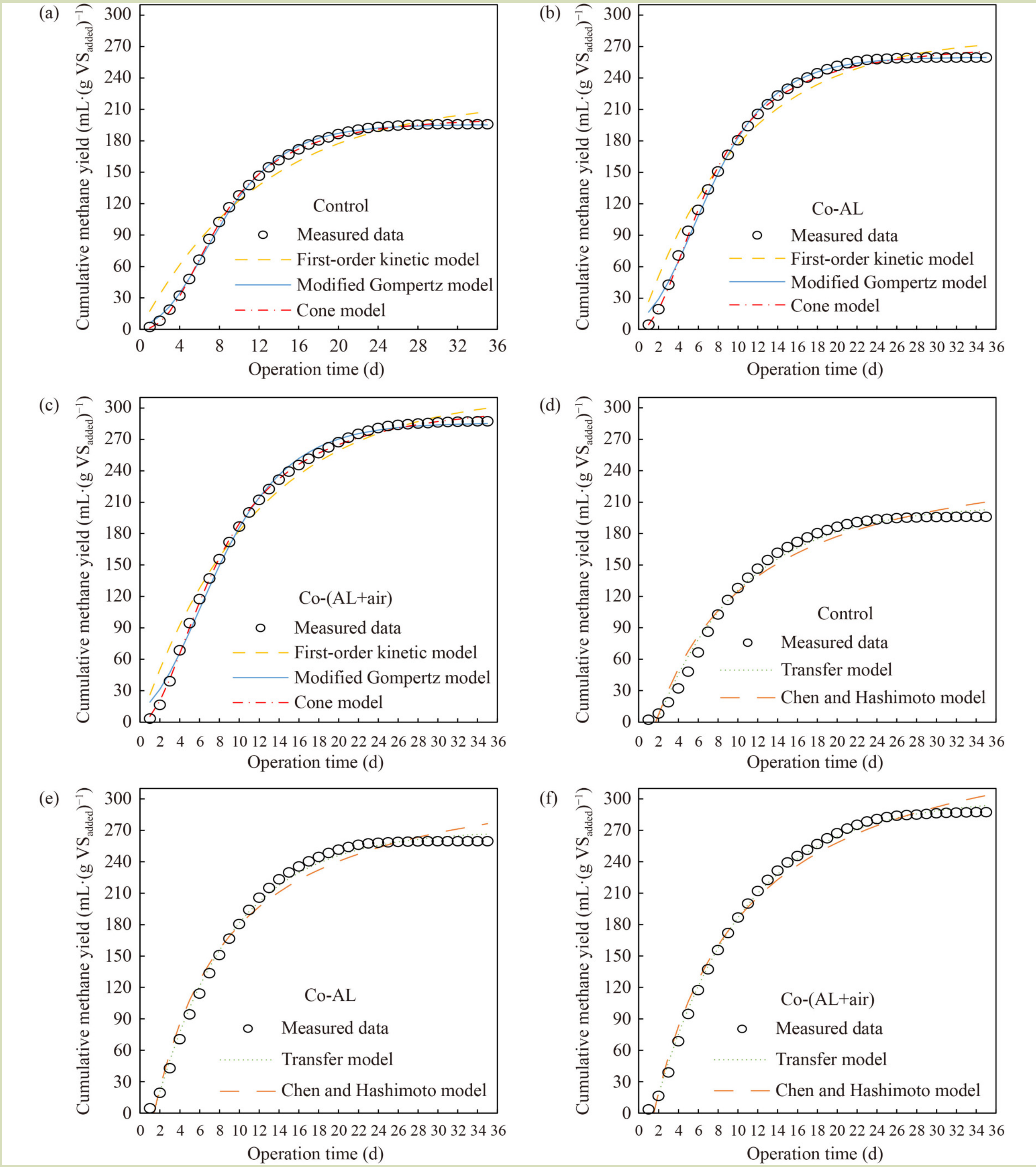


Fig. 3 Measured data of cumulative methane yield and the model fitting by the first kinetic model, the modified Gompertz model, and the cone model in experimental groups of control (a), Co-AL (b) and Co-(AL + air) (c); the transfer model, and the Chen and Hashimoto model for the experimental groups of control (d), Co-AL (e) and Co-(AL + air) (f) for Co-AD of poultry litter and wheat straw.

0.9956) and the TM (0.9873, 0.9941 and 0.9966), indicating that the CNM fitted better in the CMY of the Co-AD process in the three groups.

In addition, the CNM had lower rMSPE values (1.54, 3.50, 2.64) than the MGM (2.25, 3.65, 5.66) and the TM (6.95, 5.83 and 4.93). While rMSPE values were > 9.55 for the FM and the CHM. The comparisons suggested the best correlation between the predicted and the measured values by the CNM. Also, the lower values of AIC and BIC values of the CNM (39.5, 95.0, 77.3 for AIC and 40.8, 98.3, 78.7 for BIC), than the MGM (66.1, 100, 131 for AIC and 67.5, 101, 132 for BIC), the FM (both > 180), the CHM (both > 165), and the TM (both > 120) again demonstrated that the CNM was more suitable for fitting the experimental data from the Co-AD process. In addition, Co-AL gave higher AIC and BIC values than Co, but Co-(AL + air) had even higher values of AIC and BIC, for both the CNM and the MGM, indicating that alkaline pretreatment alone or with air mixing could have a negative impact on the model fitting to the experimental data of the Co-AD process. Finally, the overall Dif (%) was the smallest for the MGM, followed by the CNM, TM, FM, and CHM. The curves of the five models (Fig. 3) also illustrated that the CNM correlated with the measured data better than the MGM and much better than the

Table 3 Kinetic parameters of the first-order kinetic model, the modified Gompertz model, the cone model, the transfer model and the Chen and Hashimoto model for the Co-AD process in different experimental groups

Model	Kinetic parameters	Groups		
		Co	Co-AL	Co-(AL+air)
Measured CMY (mL·(g VS_{added})$^{-1}$)		196	260	287
First-order model	Y_m (mL·(g VS_{added})$^{-1}$)	220	280	315
	k (d^{-1})	0.082	0.10	0.09
	R^2	0.9590	0.9740	0.9792
	rMSPE	12.5	12.2	12.3
	AIC	183	182	182
	BIC	184	182	183
	Dif (%)	12.5	7.94	9.64
Modified Gompertz model	Y_m (mL·(g VS_{added})$^{-1}$)	196	260	286
	R_m (mL·(g VS_{added})$^{-1}$·d^{-1})	16.6	21.9	21.5
	λ (d)	2.06	1.05	0.99
	R^2	0.9987	0.9977	0.9956
	rMSPE	2.25	3.65	5.66
	AIC	66.1	100	131
	BIC	67.5	101	132
	Dif (%)	–0.13*	0.16	–0.46*
Cone model	Y_m (mL·(g VS_{added})$^{-1}$)	204	275	308
	k (d^{-1})	0.124	0.14	0.13
	n	2.44	2.08	1.95
	R^2	0.9994	0.9979	0.9990
	rMSPE	1.54	3.50	2.64
	AIC	39.5	97.0	77.3
	BIC	40.8	98.3	78.7
	Dif (%)	4.39	5.89	7.32
Transfer model	Y_m (mL·(g VS_{added})$^{-1}$)	207	270	300
	λ (d)	1.82	1.39	1.44
	R_m (mL·(g VS_{added})$^{-1}$·d^{-1})	23.7	35.1	34.1
	R^2	0.9873	0.9941	0.9966
	rMSPE	6.95	5.83	4.93
	AIC	145	133	121
	BIC	146	134	122
	Dif (%)	5.90	3.99	4.53

(*Continued*)

Model	Kinetic parameters	Groups		
		Co	Co-AL	Co-(AL+air)
Chen and Hashimoto model	Y_m (mL·(g VS_{added})$^{-1}$)	272	339	386
	k_{CH}	5.58	5.35	6.15
	μ_m (d^{-1})	0.57	0.70	0.67
	R^2	0.9723	0.9794	0.9874
	rMSPE	10.3	10.9	9.55
	AIC	172	177	167
	BIC	174	178	169
	Dif (%)	38.8	30.6	34.5

Note: Y_m, the maximum methane yield potential (mL·(g VS_{added})$^{-1}$ CH_4); k, the hydrolytic rate constant (d^{-1}); R_m, the maximum methane production rate (mL·(g VS_{added})$^{-1}$·d^{-1}); λ, the lag phase (d); n, the shape constant, which reveals whether there is a lag phase in the reactor; k_{CH}, Chen and Hashimoto constant (dimensionless); μ_m, maximum specific growth rate of microorganisms (d^{-1}); R^2, the correlation coefficients; rMSPE, root mean square prediction error; AIC, Akaike's information criterion; BIC, Bayesian information criterion; Dif (%), the difference between the model-predicted value of maximum methane yield potential, the negative values indicate that the predicted value is lower than the measured value.

FM, TM and CHM. Therefore, the CNM could be considered the best for simulating the methane yield kinetics of the Co-AD processes with alkaline pretreatment alone or with air mixing due to the higher R^2 and lower rMSPE, AIC, BIC and Dif (%). Previous research has reported that the CNM, as an empirical model, can estimate the methane yield rate and the maximum cumulative methane yield better than the other models when fitting data for methane yield from the Co-AD process[30,31].

3.3.2 Kinetic constants comparison

The kinetic constants from the kinetic models were used in comparison to assess the effect of alkaline pretreatment alone or with air mixing on the kinetic performance of AD processes. The results are summarized in Table 3 including the maximum methane yield potential (Y_m), maximum methane yield rate (R_m), hydrolysis rate constant (k), lag phase duration (λ) in different experiments.

Since the CNM gave a better fit for the measured CMY than the other models, the results of Y_m obtained by the cone model were used for comparison in different experimental groups. As given in Table 3, the difference of Y_m, which is a prediction of the potential methane production based on substrate usage[39], was consistent with the difference of the measured CMY as discussed in Section 3.2 for the three experimental groups, where Y_m increased in the Co-AL group by 34.4% and even further improved in the Co-(AL + air) by 50.8% compared to the control. The results demonstrated that both alkaline pretreatment w/o air mixing could enhance the methane yield potential in the Co-AD process.

The hydrolysis rate constant (k, d^{-1}) obtained by both the FM and the CNM could be used to characterize the degradation rate of the substrate, where a larger k value indicates a higher degradation rate[40]. Considering that the CNM fitted better than the FM, the k values of the three groups obtained by the CNM were used for comparison. Co-AL had a higher k value (0.13) than the control (0.12), indicating that the hydrolysis rate was enhanced after alkaline pretreatment. This could be explained by the degradation of complex substrate by alkaline pretreatment, which provided smaller molecules for easier hydrolysis during the Co-AD process. Also, Co-(AL + air) had an even larger k of 0.14, indicating that air mixing could improve hydrolysis during the Co-AD process with alkaline pretreatment. Previous studies have also shown that air mixing could enhance substrate hydrolysis during AD process[18].

Besides, the constants of R_m (mL·(g VS_{added})$^{-1}$·d^{-1} CH_4) and λ (d) obtained from the MGM and the TM, with the former provided a better fit of the measured data, and could be used to evaluate the variation of methane production activity during the AD process, where a higher value of R_m and a shorter λ indicated a higher maximum methane production activity and a shorter lag phase time, respectively. According to the MGM, the R_m values of the Co-AL (21.9 mL·(g VS_{added})$^{-1}$·d^{-1} CH_4) and Co-(AL + air) (21.5 mL·(g VS_{added})$^{-1}$·d^{-1} CH_4) were higher than that of the control (16.6 mL·(g VS_{added})$^{-1}$·d^{-1} CH_4). In addition, Co-AL and Co-(AL + air) also had a shorter λ (1.05 and 0.99 d, respectively) than the control (2.06 d). The results implied that the Co-AD process of PL and WS enhanced methane production activities with increased maximum methane yield rates and shortened lag phase time after alkaline pretreatment of substrate.

3.4 Digestate quality

The digestate from the batch Co-AD reactors of the three groups was analyzed for serval physiochemical parameters with the results summarized in Table 4. It could be inferred that the final pH of the digestate was significantly ($P < 0.05$) increased with AL and with further air mixing (AL + air). This could be explained by the changes of VFA and TA in the final digestate, where VFA was decreased ($P < 0.05$) but TA was increased ($P < 0.05$). This resulted in a decreased VFA/TA ratio in Co-AL and Co-(AL + air) compared to that in the control, which was commonly used as an indicator for evaluating AD system stability[41]. The results also indicated that AL not only increased the alkalinity in the final digestate but also improved substrate utilization. It was also observed that TS level, TVS and sCOD were all lower ($P < 0.05$) in Co-AL and Co-(AL + air) than that in the control, which also implied an enhanced substrate utilization due to AL in the Co-AD process.

The removal rates of TS and TVS in the digestate of different experimental groups compared to the feeding substrate are shown in Fig. 4, which showed that Co-AL and Co-(AL + air) both had higher ($P < 0.05$) values of removal rates of TS and TVS than the control. The removal of TS and TVS in the digestate were improved by 1.8 and 2.2, and 2.0 and 2.3 times in Co-AL and Co-(AL + air), respectively. In addition, air mixing further increased TA and decreased VFA, TS and TVS content in the digestate, resulting in a higher removal rate of both TS (from 48.4% to 52.9%) and TVS (from 61.0% to 64.1%) in the digestate as shown in Fig. 4. The results also indicated that air mixing might further improve the substrate utilization of the Co-AD process with AL. Previous studies have also reported improved methane yield and VS removal efficiency at an air injection rate of 12.5 $mL \cdot L_R^{-1} \cdot d^{-1}$ (L_R means litre of reactor volume) for a batch thermophilic AD process of corn straw[24].

In addition, the difference of TAN in the final digestate of the three groups was not significant ($P > 0.05$), while the FAN content was significantly ($P < 0.05$) higher in groups of Co-AL and Co-(AL + air) probably due to the increase of pH as mentioned above.

3.5 Mechanisms

Alkaline pretreatment and air mixing are both effective strategies to improve the methane production efficiency of AD process, and they have been reported to bring benefits in previous studies. AL can significantly facilitate substrate hydrolysis, improve methane production and remove exogenous pollutants as well as other benefits[14]. Air mixing was found to enhance hydrolysis, remove hydrogen sulfide and promote microbial diversity of AD process[18,19]. The results of the experiments presented here show that AL facilitated the degradation of the substrate, thus contributing to the enhancement of methane production. This was consistent with the results in the previously published studies that employed AL. AL is a chemical pretreatment method that could break down the bonds in macromolecules and transform non-biodegradable materials to biodegradable compounds[42], especially for lignocellulosic residues like wheat straw, which have the poor degradability and can barely be degraded by hydrolytic microorganisms under normal conditions.

In addition, the results of the other experiment also demonstrated that AL integrated with air mixing could further improve the Co-AD process, with a further increased methane

Table 4 Parameters of the reactor digestate after the Co-AD process

Reactors digestate groups	Control	Co-AL	Co-(AL + air)
pH	8.01 ± 0.02b	8.27 ± 0.03a	8.32 ± 0.01a
TS (%)	1.71 ± 0.03a	1.20 ± 0.01b	1.10 ± 0.02c
TVS ($g \cdot L^{-1}$)	14.1 ± 0.4a	7.59 ± 0.11b	7.00 ± 0.14c
sCOD ($mg \cdot L^{-1}$)	623 ± 52a	520 ± 28b	483 ± 32b
VFA ($mg \cdot L^{-1}$ HAc)	83.3 ± 4.1a	63.0 ± 0.8b	55.1 ± 1.7c
TAN ($mg \cdot L^{-1}$)	311 ± 10a	334 ± 6a	323 ± 7a
FAN ($mg \cdot L^{-1}$)	6.1 ± 0.1b	11.9 ± 0.5a	12.8 ± 0.1a
TA ($mg \cdot L^{-1}$ $CaCO_3$)	375 ± 15c	1003 ± 42b	1106 ± 21a
VFA/TA (g HAc equivalent to g $CaCO_3$ equivalent)	0.22 ± 0.00a	0.06 ± 0.00b	0.05 ± 0.00b

Note: Data are shown as mean ± standard error (n = 3). Data in each row followed by the same letter are not significantly different ($P > 0.05$).

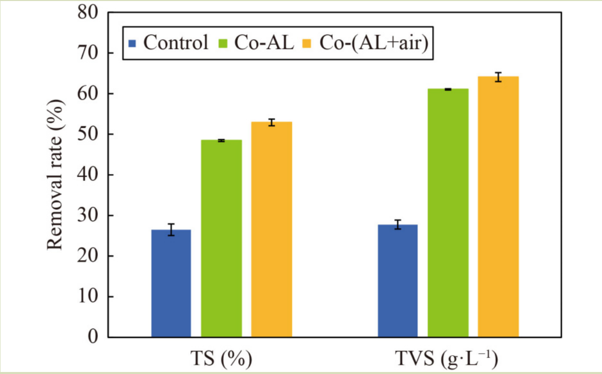


Fig. 4 Removal rates (%) of total solids (TS) and total volatile solids (TVS) in the digestate by the Co-AD process.

yield and an improved solids removal. Air mixing improves AD process by affecting the microbial activity in the AD system. The microbial mechanism of air mixing is that it could increase the growth and activities of hydrolytic bacteria as well as the hydrogenotrophic methanogens[43], thus contributing to a promoted process efficiency.

The results indicate that there was no inhibitory interaction between AL and air mixing, the two separate techniques could be combined as a new technique to achieve an even greater improvement in methane production from a Co-AD process. This might be related to that AL did not disturb the improving effect brought by air mixing. AL was applied to the substrates of PL and WS at the pretreatment stage, the degradation of substrates was facilitated by AL, resulting in an increased SCOD and VFA in the substrate suspension. When the microbial activity was enriched by air mixing, which was applied further during the Co-AD process using the pretreated substrate suspension, the process efficiency could be further enhanced due to the increased degradation of micromolecules that were more readily utilized and further degraded by the bacteria to methane[42]. This could explain the improved methane production and solids removal by the integration of AL and air mixing for the Co-AD process.

4 CONCLUSIONS

This study evaluated the combination of AL (pH 12 for 12 h) and air mixing (12 $mL·d^{-1}$) for the Co-AD process of PL with WS, intending to enhance methane production. The substrate hydrolysis was enhanced after AL with sCOD increased by 4.59 times and VFA increased by 5.04 times. The CMY was improved by 46.7% in Co-(AL + air) (287 $mL·(g\ VS_{added})^{-1}$ CH_4), compared to the control. The cone model was the best in simulating the methane yield kinetics in different Co-AD groups with $R^2 \geqslant 0.9979$ and rMSPE $\leqslant 3.50$. Co-(AL + air) also had a larger k (0.14 d^{-1}) and shorter λ (0.99 d) than the control (k = 0.12 d^{-1}, λ = 2.06 d). Co-(AL + air) improved the substrate utilization with the removal of TS and TVS in the digestate improved by 2.0 and 2.3 times, respectively. AL facilitated the substrate degradation, while air mixing enriched microbial activity, together enhancing methane generation. The results identified that the combination of AL and air mixing could be applied as an effective method to improve methane production from the Co-AD process.

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Compliance with ethics guidelines

Yuanhang Zhan, Jun Zhu, Yiting Xiao, and Leland C. Schrader declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

1. United States Department of Agriculture (USDA). Poultry—Production and Value 2021 Summary. *USDA*, 2022
2. Manogaran M D, Shamsuddin R, Mohd Yusoff M H, Lay M C, Siyal A A. A review on treatment processes of chicken manure. *Cleaner and Circular Bioeconomy*, 2022, **2**: 100013
3. Lee J, Choi D, Ok Y S, Lee S R, Kwon E E. Enhancement of energy recovery from chicken manure by pyrolysis in carbon dioxide. *Journal of Cleaner Production*, 2017, **164**: 146–152
4. Abdeshahian P, Lim J S, Ho W S, Hashim H, Lee C T. Potential of biogas production from farm animal waste in Malaysia.

Renewable & Sustainable Energy Reviews, 2016, **60**: 714–723

5. Khalil M, Berawi M A, Heryanto R, Rizalie A. Waste to energy technology: the potential of sustainable biogas production from animal waste in Indonesia. *Renewable & Sustainable Energy Reviews*, 2019, **105**: 323–331
6. Nie H, Jacobi H F, Strach K, Xu C, Zhou H, Liebetrau J. Mono-fermentation of chicken manure: ammonia inhibition and recirculation of the digestate. *Bioresource Technology*, 2015, **178**: 238–246
7. Paranhos A G O, Adarme O F H, Barreto G F, Silva S Q, Aquino S F. Methane production by co-digestion of poultry manure and lignocellulosic biomass: kinetic and energy assessment. *Bioresource Technology*, 2020, **300**: 122588
8. Amin F R, Khalid H, Zhang H, Rahman S U, Zhang R, Liu G, Chen C. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express*, 2017, **7**(1): 72
9. Ogunwande G A, Osunade J A, Adekalu K O, Ogunjimi L A O. Nitrogen loss in chicken litter compost as affected by carbon to nitrogen ratio and turning frequency. *Bioresource Technology*, 2008, **99**(16): 7495–7503
10. Shen J, Zhu J. Methane production in an upflow anaerobic biofilm digester from leachates derived from poultry litter at different organic loading rates and hydraulic retention times. *Journal of Environmental Chemical Engineering*, 2017, **5**(5): 5124–5130
11. Wagner A O, Schwarzenauer T, Illmer P. Improvement of methane generation capacity by aerobic pre-treatment of organic waste with a cellulolytic *Trichoderma viride* culture. *Journal of Environmental Management*, 2013, **129**: 357–360
12. Zhou S, Zhang Y, Dong Y. Pretreatment for biogas production by anaerobic fermentation of mixed corn stover and cow dung. *Energy*, 2012, **46**(1): 644–648
13. Badiei M, Asim N, Jahim J M, Sopian K. Comparison of chemical pretreatment methods for cellulosic biomass. *APCBEE Procedia*, 2014, **9**: 170–174
14. Liu X, Fu Q, Liu Z, Zeng T, Du M, He D, Lu Q, Ni B J, Wang D. Alkaline pre-fermentation for anaerobic digestion of polyacrylamide flocculated sludge: simultaneously enhancing methane production and polyacrylamide degradation. *Chemical Engineering Journal*, 2021, **425**: 131407
15. Liu X, Du M, Lu Q, He D, Song K, Yang Q, Duan A, Wang D. How does chitosan affect methane production in anaerobic digestion. *Environmental Science & Technology*, 2021, **55**(23): 15843–15852
16. Xu H, Li Y, Hua D, Zhao Y, Chen L, Zhou L, Chen G. Effect of microaerobic microbial pretreatment on anaerobic digestion of a lignocellulosic substrate under controlled pH conditions. *Bioresource Technology*, 2021, **328**: 124852
17. Zhu R, Zhang Y, Zou H, Guo R B, Fu S F. The effects of micro-aeration on semi-continued anaerobic digestion of corn straw with increasing organic loading rates. *Renewable Energy*, 2022, **195**: 1194–1201
18. Nguyen D, Khanal S K. A little breath of fresh air into an anaerobic system: how microaeration facilitates anaerobic digestion process? *Biotechnology Advances*, 2018, **36**(7): 1971–1983
19. Chen Q, Wu W, Qi D, Ding Y, Zhao Z. Review on microaeration-based anaerobic digestion: state of the art, challenges, and prospectives. *Science of the Total Environment*, 2020, **710**: 136388
20. Zhan Y, Zhu J, Xiao Y, Schrader L C, Xiao Wu S, Aka Robinson N Jr, Wang Z. Employing micro-aeration in anaerobic digestion of poultry litter and wheat straw: batch kinetics and continuous performance. *Bioresource Technology*, 2023, **368**: 128351
21. Zhan Y, Cao X, Xiao Y, Wei X, Wu S, Zhu J. Start-up of co-digestion of poultry litter and wheat straw in anaerobic sequencing batch reactor by gradually increasing organic loading rate: methane production and microbial community analysis. *Bioresource Technology*, 2022, **354**: 127232
22. Liu X, Zicari S M, Liu G, Li Y, Zhang R. Improving the bioenergy production from wheat straw with alkaline pretreatment. *Biosystems Engineering*, 2015, **140**: 59–66
23. Solé-Bundó M, Eskicioglu C, Garfí M, Carrère H, Ferrer I. Anaerobic co-digestion of microalgal biomass and wheat straw with and without thermo-alkaline pretreatment. *Bioresource Technology*, 2017, **237**: 89–98
24. Fu S F, Wang F, Shi X S, Guo R B. Impacts of microaeration on the anaerobic digestion of corn straw and the microbial community structure. *Chemical Engineering Journal*, 2016, **287**: 523–528
25. Lim J W, Wang J Y. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. *Waste Management*, 2013, **33**(4): 813–819
26. Apha A. Standard Methods for the Examination of Water and Wastewater, 20th ed. Washington D.C.: *American Public Health Association, American Water Works Association, Water Environment Federation*, 1998
27. Li K, Liu R, Sun C. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. *Bioresource Technology*, 2015, **198**: 133–140
28. Zhang Y, Yang Z, Xu R, Xiang Y, Jia M, Hu J, Zheng Y, Xiong W, Cao J. Enhanced mesophilic anaerobic digestion of waste sludge with the iron nanoparticles addition and kinetic analysis. *Science of the Total Environment*, 2019, **683**: 124–133
29. Kafle G K, Chen L. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Management*, 2016, **48**: 492–502
30. Zahan Z, Othman M Z, Muster T H. Anaerobic digestion/co-digestion kinetic potentials of different agro-industrial wastes: a comparative batch study for C/N optimisation. *Waste Management*, 2018, **71**: 663–674
31. El-Mashad H M. Kinetics of methane production from the codigestion of switchgrass and *Spirulina platensis* algae. *Bioresource Technology*, 2013, **132**: 305–312

32. Karki R, Chuenchart W, Surendra K C, Sung S, Raskin L, Khanal S K. Anaerobic co-digestion of various organic wastes: kinetic modeling and synergistic impact evaluation. *Bioresource Technology*, 2022, **343**: 126063
33. Llabrés-Luengo P, Mata-Alvarez J. Kinetic study of the anaerobic digestion of straw-pig manure mixtures. *Biomass*, 1987, **14**(2): 129–142
34. Lay J J, Li Y Y, Noike T. Interaction between homoacetogens and methanogens in lake sediments. *Journal of Fermentation and Bioengineering*, 1998, **86**(5): 467–471
35. Motulsky H, Christopoulos A. Fitting models to biological data using linear and nonlinear regression: a practical guide to curve fitting. *Oxford University Press*, 2004
36. Nguyen D D, Jeon B H, Jeung J H, Rene E R, Banu J R, Ravindran B, Vu C M, Ngo H H, Guo W, Chang S W. Thermophilic anaerobic digestion of model organic wastes: evaluation of biomethane production and multiple kinetic models analysis. *Bioresource Technology*, 2019, **280**: 269–276
37. Li L, Chen C, Zhang R, He Y, Wang W, Liu G. Pretreatment of corn stover for methane production with the combination of potassium hydroxide and calcium hydroxide. *Energy & Fuels*, 2015, **29**(9): 5841–5846
38. Zheng M, Li X, Li L, Yang X, He Y. Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment. *Bioresource Technology*, 2009, **100**(21): 5140–5145
39. Shamurad B, Gray N, Petropoulos E, Tabraiz S, Membere E, Sallis P. Predicting the effects of integrating mineral wastes in anaerobic digestion of OFMSW using first-order and Gompertz models from biomethane potential assays. *Renewable Energy*, 2020, **152**: 308–319
40. Zhen G, Lu X, Kobayashi T, Li Y Y, Xu K, Zhao Y. Mesophilic anaerobic co-digestion of waste activated sludge and *Egeria densa*: performance assessment and kinetic analysis. *Applied Energy*, 2015, **148**: 78–86
41. Tang F, Tian J, Zhu N, Lin Y, Zheng H, Xu Z, Liu W. Dry anaerobic digestion of ammoniated straw: performance and microbial characteristics. *Bioresource Technology*, 2022, **351**: 126952
42. Neshat S A, Mohammadi M, Najafpour G D, Lahijani P. Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renewable & Sustainable Energy Reviews*, 2017, **79**: 308–322
43. Zhen F, Luo X, Xing T, Sun Y, Kong X, Li W. Performance evaluation and microbial community analysis of microaerobic pretreatment on thermophilic dry anaerobic digestion. *Biochemical Engineering Journal*, 2021, **167**: 107873