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Anaerobic co-digestion of poultry litter and wheat straw affected by solids composition, free ammonia and carbon/nitrogen ratio

Jun Zhu\textsuperscript{a}, Sarah Wu\textsuperscript{b}, and Jiacheng Shen\textsuperscript{a}

\textsuperscript{a}Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, Arkansas, USA; \textsuperscript{b}Department of Biological Engineering, University of Idaho, Moscow, Idaho, USA

**ABSTRACT**

In this study, the effects of three total solids levels (2\%, 5\% and 10\% TS) of the mixtures of poultry litter and wheat straw at different percent volatile solids from wheat straw (0\%, 25\% and 50\% VSWS), free ammonia nitrogen (FAN) and C/N ratio on co-digesting poultry litter with wheat straw were studied in batch experiments operated at 37°C. The results showed that adjusting the substrate C/N ratio alone could not always result in high methane yields and biogas volumes. The maximum CH\textsubscript{4} yield of 201 mL g\textsuperscript{-1} initial VS was observed in the 5\% TS category with VSWS being 50\%. The highest specific biogas volume produced (318 mL g\textsuperscript{-1} initial VS) was also obtained in the same solid category. A polynomial regression between FAN and the methanogenic activity was obtained with the correlation coefficient being 0.9472. A FAN inhibitory threshold concentration of 253.9 mg L\textsuperscript{-1} was found, and a total loss of methane production occurred when the FAN concentration reached 1000 mg L\textsuperscript{-1}. An optimal TS content for anaerobic digestion of poultry litter with wheat straw was around 4.15\% to achieve the best biogas yield. Similarly, for digesting poultry litter without addition of wheat straw, the TS content should be kept at around 6.8\%.

**Introduction**

Poultry production is a major industry in Arkansas, which generates 1.4–1.7 million tons of manure litter every year.\textsuperscript{[1]} This litter has potential to be hazardous or beneficial to Arkansas’s economy and environment, depending on how it is managed. Unlike other animal manures, the composition of poultry litter is complex, consisting of not only bedding materials and birds’ dropping, but residual feed and feathers, leading to higher nitrogen content but lower carbon content.\textsuperscript{[2]} Traditionally, poultry litter has been land applied as a fertilizer for crops; however, this practice has proved to be not environmentally sustainable due to either insufficient land area available for disposal or the negative impacts on the environment in terms of soil phosphorus accumulation and ground/surface water contamination.\textsuperscript{[3]} In addition to land application, other treatment techniques of poultry litter were also experimented, such as combustion and pyrolysis, but these endeavors were later on proven to be problematic as well due to the emissions of ammonia and other particulates to the air and the failure to recover the nutrients (mainly nitrogen) from the litter.\textsuperscript{[4]}

Anaerobic digestion (AD) has long been considered a potential technology for poultry litter treatment, and was researched at great length in the past.\textsuperscript{[5]} There are advantages of using AD to treat poultry litter including bioenergy production to offset the operating expenses associated with the AD process and conservation of nutrients contained in the litter to soils.\textsuperscript{[5,6]} However, there are disadvantages of applying AD to poultry litter as well. One of the drawbacks in using the AD technology is related to the low carbon to nitrogen ratio in the litter (normally less than 10),\textsuperscript{[6]} as opposed to the established optimal range of between 15 and 30 for satisfactory digestion of any waste streams.\textsuperscript{[7]} To overcome this problem, past researchers evaluated co-digestion of poultry litter with other carbon rich substrates (preferably locally available agricultural production waste materials such as crop residues) to improve the digestion efficiency.\textsuperscript{[8–16]} Since winter wheat is a major crop grown in Arkansas, with 395,000 acres harvested in 2014,\textsuperscript{[17]} the volume of residues from the crop is huge and often causes a disposal dilemma. Therefore, it is worthwhile to study the potential use of the wheat straw produced from wheat cultivation as an added carbon source to improve the carbon to nitrogen ratio in AD of poultry litter to achieve better biogas production. A literature search has not led to similar information in the published domain as of this date.

The objectives of this study were to investigate the mixing ratios of poultry litter and wheat straw based on different total solids (TS) and volatile solids added from wheat straw (VSWS) for biogas production via co-digestion and study the impacts of free ammonia concentration and VSWS on the methane producing process. Also, the optimal levels of TS (with or without VSWS) for the highest specific biogas volume produced (biogas yield) were revealed in AD, or co-digestion, of poultry litter.
Materials and methods

Co-digestion substrates

The poultry litter used in the experiments was collected from a broiler house owned by the University of Arkansas System Division of Agriculture. After collection, the litter was sieved through a 2.38-mm screen, and stored in a walk-in refrigerator prior to use. The wheat straw used was collected from a local farm, which, after collection, was also stored in a 4°C walk-in refrigerator if not used. The wheat straw was then processed using a Wiley mill to a size of 20 mesh (0.85 mm) before being used in the experiments. The characteristics of both the litter and straw were analyzed in our lab and the results are shown in Table 1.

Experimental apparatus and design

The digesters used for the co-digestion experiments consisted of 1000 mL graduated glass flasks, which were sealed using rubber stoppers at the openings of the flasks, through which gas bags were connected to the digesters (Tedlar Bag, CEL Scientific Corp., Cerritos, CA, USA). Valves were installed between the flasks and gas bags to prevent air from entering into the digesters when replacing the gas bags to reduce disturbance to the anaerobic condition established inside the flasks. The activated anaerobic sludge as inoculum was prepared 25 days before inoculation using 2% TS poultry litter mixed with municipal activated sludge collected from the Fayetteville Municipal Wastewater Treatment Plant (85% moisture content). The experimental substrates were prepared using poultry litter and wheat straw mixed with tap water to form three TS levels, i.e., 2%, 5% and 10%, and for each TS level, the percent VS from wheat straw was further specified at 0%, 25% and 50% (this means that either 0%, or 25%, or 50% of total VS in the mixed substrate was from wheat straw VS, and the rest was from the VS in poultry litter). The total working volume for each flask (digester) was 500 mL (the flask volume was 1000 mL). Based on this experimental design, a total of nine treatments were employed (3 TS levels × 3 VS% from wheat straw) to understand the effect of the VS content from wheat straw on the biogas production yield. Please note that the TS content in all experiments were calculated and adjusted according to the data from the materials dried at a constant temperature of 37°C for incubation. During the experiment, all the flasks were manually shaken twice a day for about 30 sec for mixing. Biogas sampling was conducted once a day in the first two days and then once every three days until the end of the biogas production process, which were 47, 59 and 47 days for 2%, 5% and 10% TS, respectively. Liquid samples taken at the beginning and end of the experiment were analyzed for physical and chemical properties such as TS, VS, chemical oxygen demand (COD), free ammonia-nitrogen, total nitrogen and volatile fatty acids (VFAs) following the Standard Methods. The control digesters, which were composed of 100 mL of activated sludge and 400 mL of tap water, were also sampled and analyzed to eliminate the effect of activated sludge on biogas production from the test digesters. All experiments were run in duplicate, and the averaged values were reported.

Sample analysis

The levels of TS and VS in the liquid samples were determined using the gravity method. Total nitrogen and free ammonia nitrogen (FAN) were analyzed using a DR 3900 Hach spectrophotometer coupled with a DRB 200 Hach digester according to the Hach Manuals and the Standard Methods. The volumes of biogas produced from the digesters were recorded using a wet gas meter (Model XMF-1, Shanghai Cixi Instrument Co., Ltd, Shanghai, China), and the biogas composition was analyzed using a Shimadzu Gas Chromatograph (GC 2014, Shimadzu Corp. in North America, Columbia, MD, USA) equipped with a thermal conductivity detector (TCD) and a ShinCarbon column (length 2 m × ID 2 mm, Restek Corp., US, Bellefonte, PA, USA). For biogas analysis, the gas sample of 0.2 mL was injected manually into GC using a syringe, and the column temperature started at 40°C for 2 min, then increased at a rate of 25°C per minute until reaching 150°C, and then stayed there for 1 min. The temperatures of the injector and TCD were set at 180°C and 185°C, respectively. Calibration gases were purchased from The Gas Co. (BuyCalGas.com) to calibrate the GC for methane, carbon dioxide and nitrogen. For liquid analysis, the VFAs in the digested samples were analyzed by the same GC equipped with a flame ionization detector (FID), an auto injector (Shimadzu, AOC-20i) and a Stabilwax®-DA column (Restek, length 30 m × ID 0.53 mm) using the same temperature operating profile as that described above. The temperatures for both the injector and FID were set at 250°C, and the volume of the injected liquid sample was 0.006 mL.

Table 1. The chemical composition of poultry litter and wheat straw.

<table>
<thead>
<tr>
<th>Component</th>
<th>Poultry litter</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hernicellulose (%)a</td>
<td>19.85</td>
<td></td>
</tr>
<tr>
<td>Lignin (%)a</td>
<td>4.61</td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)a</td>
<td>28.44</td>
<td></td>
</tr>
<tr>
<td>Crude fat (%)a</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Starch (%)a</td>
<td>1.49</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>TS (%)</td>
<td>74.9</td>
<td>91.4</td>
</tr>
<tr>
<td>Volatile solids (VS)</td>
<td>(VS%)</td>
<td>70.9</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>(wet basis)</td>
<td>72</td>
</tr>
</tbody>
</table>

Based on dry matter.


Results and discussion

**CH₄ yields**

The cumulative specific methane yields (mL g⁻¹ initial VS), specific biogas volumes and ammonia and total nitrogen concentrations at three TS levels (2%, 5% and 10%), with each further containing three levels of VS from wheat straw (0%, 25%, 50% VSWS) are presented in Table 2.

The results showed profound influences of TS and VSWS on CH₄ yield and specific biogas volumes produced. For CH₄ yield, the maximum value (201 mL g⁻¹ initial VS) was observed in the 5% TS category with VSWS at 50%, which appeared to suggest that the VS from wheat straw might be an important factor governing CH₄ production. The value of specific CH₄ yield found in this study was consistent with the findings reported by past researchers. In one study, it was found that the specific CH₄ yield was between 140 and 220 mL g⁻¹ initial VS when poultry litter was co-digested with sawdust under mesophilic temperature.¹¹ While in another study, it was reported that the specific CH₄ yield was around 145 mL g⁻¹ initial VS when poultry litter was co-digested with a microbial species after thermal chemical pretreatment. However, the TS level in the second study was only 1%, which was much lower than the level used in this study. Compared to additional studies where chicken manure was co-digested with corn stover,¹⁹ the values of CH₄ yield obtained from this study were slightly lower (298–351 mL g⁻¹ initial VS vs. 201 mL g⁻¹ initial VS). In terms of the specific biogas volume produced, the highest value (318 mL g⁻¹ initial VS) also went to the TS category of 5% with 50% VSWS. However, the second highest specific CH₄ yield (163 mL g⁻¹ initial VS) and biogas volume produced (283 mL g⁻¹ initial VS) were associated with 2% TS at 50% VSWS instead. The data also showed that the specific biogas volumes in 50% VSWS in 2% and 5% TS categories were higher than those in the 0% and 25% VSWS within the same TS groups, largely as a result of the appropriate C/N ratios achieved in the 50% VSWS groups (Table 2). An interesting observation was that for TS categories of 2% and 5%, the specific biogas volume increased as the VSWS increased from 0% to 50% (in tandem with the C/N ratio increase). Nonetheless, this trend was not observed for the 10% TS category because the two highest values (CH₄ yield and biogas volume) were obtained at 0% VSWS, and the changing trend actually reversed, i.e., as the VSWS increased, the two values decreased. This finding appeared to negate the early impression that the VS from wheat straw could play a significant role for good methane production (later on in discussions, it was found that there was an optimal TS as well). It also ran against the common agreement on the importance of C/N ratio in AD because the C/N ratios of 25% and 50% VSWS in the 10% TS category were generally considered in the normal range while that of 0% VSWS was not (Table 2). Therefore, it seemed that there must be other factors in addition to the C/N ratio in determining the performance of AD. In this case, the above observation might be explained from the perspective of ammonia inhibition. When the TS was entirely composed of poultry litter, the concentrations of protein and amino acids in the 0% VSWS could be much higher than in the 25% and 50% VSWS substrates in the 10% TS category. These nitrogenous compounds were more readily available for bacteria to use than the lignocellulosic substances contained in wheat straw, and the fast decomposition of these compounds could lead to enhanced production of ammonia, as shown in Table 2 (1581, 1128 and 751 mg L⁻¹), thus inhibiting the methanogenic activities and sabotaging methane production. In comparison, in the 2% and 5% TS categories, all the free ammonia concentrations, except the one in the 0% VSWS of 5% TS, were lower than 700 mg L⁻¹, which indicated that methanogenesis was not affected substantially by ammonia²⁰ and could continue to proceed to produce methane from the substrates in these two TS categories. When the ammonia level went above 700 mg L⁻¹ (911 mg L⁻¹ in 0% VSWS under 5% TS), the specific CH₄ yield and biogas volume were both observed to be reduced (Table 2), which were similar to the findings from studies conducted by Angelidaki and Ahring,²⁰ in which ammonia inhibition to the methanogenic activity with acetate as substrate occurred at the free ammonia concentration of 150 mg L⁻¹, and the methanogenic process was completely stopped at 720 mg L⁻¹.

In this study, the 0% VSWS in the 2% TS category had achieved an ammonia concentration of 319 mg L⁻¹, coupled with the lowest specific CH₄ yield and biogas volume (5.45 mL g⁻¹ initial VS and 8.8 mL g⁻¹ initial VS) among all the TS categories. These results could be due to the low carbon to nitrogen ratio (C/N = 2.7, Table 2). Theoretically, a stoichiometric C/N ratio of 16 is sufficient for good glucose conversion to methane, but a practical C/N ratio should be higher than 16 due to the biodegradability of biomass containing lignin that prevents some carbon from being available for biological metabolisms. This situation could
overestimate the C/N ratio of substrate determined using an elemental analyzer. In addition, the 0% VSWS in 2%, 5% and 10% TS categories all had the lowest C/N ratio (2.7, 2.4 and 6.8) in the respective TS groups, likely explaining the reason for the low specific CH₄ yield and biogas volume obtained (less than 44 and 93 mL g⁻¹ initial VS) compared to the highest values of 201 and 318 mL g⁻¹ initial VS among all the measurements (Table 2). However, also from Table 2, although the 10% TS category with 50% VSWS had the highest C/N ratio (35.9), it generated the lowest specific CH₄ yield and biogas volume. Again, a high C/N ratio did not appear to guarantee a high specific CH₄ yield and biogas volume. This could be attributed to the high protein and amino acids content contained in the substrate as explained previously.

Effect of ammonia inhibition on the digestion process

Ammonium (NH₄⁺) and free ammonia (NH₃) were considered inhibitors to methanogens in AD, between which free ammonia concentration was considered a more potent factor due to its hydrophobic nature that enables it to migrate through cell membrane by the passive diffuse mechanism, leading to proton imbalance and potassium deficiency.[21] Ammonia inhibition to methanogens in AD happens at elevated concentrations and normally is characteristic of severe upset observed in AD performance as a result of substantial decrease of microbial activities.[22] This type of disturbance in AD can result from either free ammonia concentration or total ammoniac nitrogen (TAN), both of which are dependent upon the chemical equilibrium among ammonium, free ammonia and protons in the digestion liquid. By using the specific methane volume in each solid category and the maximum specific methane volume among all the solid categories, the methanogenic activity can be described in the following equation.

\[
M_a(\%) = \left(1 - \frac{y_{c_{max}} - y_c}{y_{c_{max}}} \right) \times 100(\%)
\]  

(1)

where \(M_a\) is the methanogenic activity (%); \(y_c\) and \(y_{c_{max}}\) are the specific and the maximum methane volumes in each solid category and among all categories (mL g⁻¹ initial VS).

The data from the above calculation are presented in Figure 1, which showed the impact of free ammonia concentration on the methanogenic activity at the end of the digestion experiments. It appeared that when the FAN concentration was below 200 mg L⁻¹, the methanogenic activity was not conspicuously impacted (all above 90%). However, when the FAN level went above 300 mg L⁻¹, a steep drop in the methane productivity to below 40% was observed, clearly indicating the inhibition of FAN to the activities of methane producing bacteria. As the trend of increasing FAN continued, so did the decrease in methanogenic activity. For instance, when the FAN level increased from 323 to 695 mg L⁻¹, the methane producing activity decreased from 38.4% to 15.2%, a 60% reduction. When the FAN level approached around 750 mg L⁻¹, the methane producing activity was almost nearing zero (only 1.6% left), signaling that the AD process largely ceased. Similar results were reported by early researchers in that when FAN concentration of 700 mg L⁻¹ in thermophilic digestion of cattle manure was reached, the methanogenic activity was drastically reduced to almost zero.[20]

Also according to Figure 1, the relationship between the FAN concentration and the methanogenic activity seemed to follow a polynomial curve with the correlation coefficient being 0.9472. When the FAN concentration hit around 1000 mg L⁻¹, the methane producing activity came close to a complete halt. However, this regression was not valid between FAN concentrations of around 1050 and 1400 mg L⁻¹ because the regression curve moved into a negative territory, which could not be the case in real situations. As such, it may be concluded that the polynomial regression equation obtained based on the experimental data in this study is only applicable for the FAN concentration range of up to 1050 mg L⁻¹.

It is interesting to note that the mean of the two FAN concentrations before (184.6 mg L⁻¹) and after (323.1 mg L⁻¹) the significant fall of the methanogenic activity was 253.9 mg L⁻¹, and its corresponding methanogenic activity was around 65%, thanks to the polynomial equation in Figure 1. This calculation may suggest that the FAN concentration of 253.9 mg L⁻¹ could be considered the inhibitory threshold of FAN in this study. When the FAN concentration fell below 253.9 mg L⁻¹, a methane producing activity of higher than 65% could always be guaranteed. When the FAN concentration increased from 253.9 mg L⁻¹, the inhibitory threshold concentration, to 323.1 mg L⁻¹, a 40% reduction in methanogenic activity would result. This observation was somewhat consistent with the findings reported by previous workers. Gallert et al.[23] observed a 50% inhibition of methanogenic activity when FAN concentration exceeded about 251 mg L⁻¹ in thermophilic digestion of peptone. Generally, a FAN concentration of lower than 200 mg L⁻¹ was commonly recognized as having minimal inhibition to anaerobic bacteria[24]; however, this rule of thumb was considered too general without taking into account the digestion temperature. In other words, the inhibitory threshold of FAN concentration could be much lower in mesophilic than in thermophilic digestion. Gallert et al.[23] investigated AD of peptone under both mesophilic and
Effect of solids composition on digestion performance

As discussed above, ammonia inhibition to the digestion process may be attenuated by co-digesting two substrates. However, little information in the literature is available with respect to the impact of the mixing ratio of the two co-substrates on digestion performance. According to the data from this study, this improvement in ammonia tolerance of methanogens appeared to be dependent upon the quantities of each substrate involved. Using the specific biogas volume as an indicator of ammonia inhibition, the mixing ratio effect was revealed in Figure 2, which showed the changes in specific biogas volume produced for different mixing ratios of poultry litter and wheat straw based on the volatile solids content from wheat straw (VSWS) in TS and the FAN concentrations.

Carefully examining Figure 2 has led to several observations. First, all the FAN concentrations decreased, regardless of the TS content, as the VSWS increased, in nearly a linear manner with correlation coefficients of 0.9532, 0.9997 and 0.9986 for 2%, 5%, and 10% TS (not shown in Fig. 2). For instance, for TS content of 2%, 5% and 10%, the FAN concentrations declined from 319, 911 and 1581 mg L\(^{-1}\) to 177, 456 and 751 mg L\(^{-1}\), respectively, at VSWS of 0%, 25% and 50% of TS, and the reductions were 44.5%, 49.9% and 52.5%, respectively, with the largest reduction observed at the 50% VSWS level. This observation showed the benefit of adding wheat straw to poultry litter to reduce the FAN concentrations, and also appeared to suggest that further reduction in the FAN levels might be obtained if the VSWS level in TS continued to increase beyond 50%, which could not be determined based on the data from this study. However, with reference to the C/N ratios listed in Table 2 for 50% VSWS in all TS categories, this level of straw addition should be considered maximal because it was almost certain that the C/N ratios would go beyond the optimal range\(^{[7]}\) if more straws were added, which could actually impair the digestion process because the improvement in the AD performance resulting from co-digesting poultry litter with wheat straw could be largely attributed to the improved C/N ratio of the digestion substrate according to the information presented in Table 2. Second, for 2% and 5% TS, the specific biogas production was positively related to the VSWS content in TS (e.g. for 2% TS, the biogas yield increased from 8.8 to 283 mL g\(^{-1}\) initial VS, while that for 5% TS increased from 143 to 318 mL g\(^{-1}\) initial VS, when the VSWS level in the substrates increased from 0% to 50% TS), while for 10% TS, increasing the VSWS content in TS did not show the similar benefit for biogas production. This observation might suggest that there could exist an optimal TS in digesting poultry litter. Figure 3 shows the relationship between TS% and the biogas yield under three different VSWS levels in TS. All three observations representing different percent VSWS in TS were fitted with polynomial equations, with which the optimal TS content under each VSWS level could be determined to achieve the best biogas yields. By setting the derivatives of the three regression equations in Figure 3 to zero, the best TS content for 0%, 25% and 50% VSWS was 6.77%, 4.15% and 4.14%, respectively. When the TS

thermophilic conditions and found that the FAN threshold concentrations were 92 mg L\(^{-1}\) for the former and 274 mg L\(^{-1}\) for the latter, respectively, so the tolerance of FAN by methanogens could be greatly improved under elevated digestion temperatures. In this study, all experiments were run under mesophilic conditions, but the FAN threshold concentration identified appeared to be much higher than that reported by Gallert et al.\(^{[23]}\) (92 mg L\(^{-1}\)). The reason for this discrepancy was unclear, and could be related to the different substrates used in respective investigations. Nonetheless, considering the key inhibitory effect of FAN on AD, controlling the FAN concentration below 200 mg L\(^{-1}\), as indicated by previous researchers,\(^{[24]}\) should be deemed appropriate in practical applications to safely guard the AD process against ammonia inhibition.

One other point that has not been paid much attention by previous researchers but probably deserves some discussion herein is the effect of co-substrates digestion on the tolerance of FAN by the methanogens. Nakakubo et al.\(^{[25]}\) conducted a study on ammonia inhibition using pig manure co-digested with the solid fractions separated from pig manure at a thermophilic temperature of 51\(^{\circ}\)C. Their results showed that only 50% decrease in methanogenic activity was observed at a FAN concentration of 1450 mg L\(^{-1}\) (much higher tolerance than reported by other researchers in literature). Therefore, they suggested that co-digestion of pig manure with its separated solid fraction could actually alleviate the ammonia inhibition effect, which, based on their understanding, was attributed to the environment created by the co-substrates that enabled methanogens to acclimate themselves to the higher FAN concentration without losing the methanogenic activity. Since co-digestion of two substrates was also experimented in this study, the findings by Nakakubo et al.\(^{[25]}\) may explain why the FAN tolerance threshold in our study (253.9 mg L\(^{-1}\)) was much higher than that reported by Gallert et al.\(^{[23]}\) (92 mg L\(^{-1}\)) under mesophilic digestion of a single substrate. As such, it may be inferred that the FAN tolerance of methanogens can be improved not only by increasing the digestion temperature but also by co-digestion of two different substrates. Further research in this area is thus warranted.

Figure 2. The changes in ammonia concentration with the VSWS content in the digested liquid.
content deviated from these optimal values, co-digesting poultry litter with wheat straw to improve digestion performance could no longer be guaranteed. This finding has thus confirmed the conjecture early that there existed a value in the TS content when using co-substrates for AD of poultry litter. For digesting poultry litter alone without straw addition, the optimal TS content was found to be 6.77% to achieve the best biogas yield in this study. Therefore, it may be advised that to guarantee good performance of co-digesting poultry litter with wheat straw, the TS content of the substrate should be controlled around 4.15%. If poultry litter is used as the sole substrate, the TS content should be maintained at around 6.8% to achieve the highest biogas yield.

Finally, the specific biogas volume produced appeared to vary with the VSWS content in the TS (Fig. 2). For the two lower TS categories, i.e. 2% and 5%, the specific biogas volume produced increased almost linearly as the VSWS level increased. In both cases, the experiment with no straw added produced the least biogas. This observation has verified the point made early that co-digesting poultry litter with wheat straw could improve the AD performance in these two TS levels, but not in the 10% TS category, due possibly to deviating from the optimal TS content discussed above.

**Conclusion**

Co-digestion of poultry litter and wheat straw at different TS and VS contents was investigated in this study. The results showed that maintaining an appropriate C/N ratio alone did not appear to guarantee a high specific methane yield and biogas volume, and easily available nitrogen compounds such as protein and amino acids could impact the digestion performance irrespective of the C/N ratio. A polynomial regression between FAN concentration and the methanogenic activity was obtained with the correlation coefficient being 0.9472. A FAN inhibitory threshold concentration was found to be 253.9 mg L⁻¹ in this study, above which a 40% reduction in methanogenic activity was observed. A total loss of methane producing capacity could result when FAN concentration reached 1000 mg L⁻¹. The findings from this study also implied that there could exist a limiting TS value for AD of poultry litter, either alone or with other substrates, because although it was found that co-digesting poultry litter with wheat straw could improve the FAN tolerance of methanogens, the TS content in the digestion substrate should be controlled at around 4.15% to achieve the best biogas yield. This finding is significant because such information is critical for design engineers to better understand the limitations of AD in poultry litter treatment. Similarly, for digesting poultry litter without addition of wheat straw, the TS content should be kept at around 6.8%.

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**ORCID**

Sarah Wu [http://orcid.org/0000-0002-2340-5225](http://orcid.org/0000-0002-2340-5225)

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