

Measurement of Un-ionized Ammonia in Complex Mixtures

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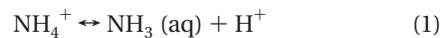
The toxicity of un-ionized ammonia, NH₃ (aq), in anaerobic digestion of high-nitrogen wastes has been researched extensively. Previous estimates of NH₃ (aq) concentration have relied on a simple speciation approach, based only on the acid dissociation constant and the sample pH and total ammonia concentration. The distinction between concentration and chemical activity has generally not been made, despite the potential for resulting errors in the calculation of NH₃ (aq) concentration, and the greater applicability of activity to toxicity work. The currently accepted approach for estimating NH₃ (aq) concentration is based on assumptions that are not valid in digested animal manure or other concentrated wastes. This work presents an approach for directly measuring NH₃ (aq) activity in complex mixtures using gaseous/aqueous equilibrium across microporous tubing. Application of this approach to anaerobic digester samples confirms that the currently accepted approach is not accurate; it overestimated NH₃ (aq) activity in unaltered samples by 45–200%. Previous work on the toxicity of ammonia to methanogenesis has probably overestimated the tolerance of consortia to NH₃ (aq), due to overestimation of concentrations. The method introduced here is expected to be useful in a range of research on ammonia toxicity and volatilization.

Introduction

Anaerobic digestion of high-nitrogen wastes such as animal manure may lead to the accumulation of high concentrations of total ammonia (i.e., several g NH₃/kg H₂O). Resulting ammonia toxicity can lead to poor performance and digester failure and has been a topic of considerable research (1–7). Un-ionized ammonia (NH₃ (aq)) is the form responsible for most toxic effects, although NH₄⁺ can be toxic at much higher concentrations (1, 2). Determining the aqueous activity of NH₃ in reactors is therefore essential to understanding and preventing ammonia toxicity. Previous work has relied on a simple speciation approach based on assumptions that are not appropriate for concentrated wastes such as digested manure. This work presents a method for directly measuring NH₃ (aq) activity in complex mixtures and demonstrates the inaccuracy of the currently accepted approach for anaerobic digester samples.

In very dilute aqueous solutions where ionic strength effects are negligible and generally the only relevant reaction

for ammonia is the acid–base reaction shown in reaction 1, NH₃ (aq) concentration can be estimated given the total ammonia concentration and pH. In this case, the fraction of total ammonia as NH₃ (aq) (α_1) is dependent only on the acid dissociation constant and the pH (8, 9):



$$\frac{m_{\text{NH}_3}}{m_{\text{total ammonia}}} = \alpha_1 = \frac{K_a}{K_a + m_{\text{H}^+}} \quad (2)$$

where m_i = the molal concentration of species i (mol/kg H₂O) and K_a = the acid dissociation constant of ammonium, which is 10^{-9.245} mol/kg H₂O at 25 °C (10). The activity and concentration of NH₃ (aq) in this case are approximately equal. The magnitude of K_a is dependent on temperature: $\text{p}K_a = 8.948$ and 8.408 at 35 and 55 °C, respectively (10). Using this approach, the fraction of total ammonia as NH₃ (aq) varies widely over the range of temperature and pH experienced by anaerobic digesters, for example, from 1% of total ammonia at 35 °C, pH 7, to 30% at 55 °C, pH 8. This underscores the need for accurate determination of NH₃ (aq) activity.

The use of total ammonia measurement and some form of eq 2 to estimate NH₃ (aq) concentration has been widespread in work on anaerobic digestion (1, 2, 4–6, 11–18). However, the presence of other solutes and high concentrations of dissolved and suspended organic matter in concentrated wastes make this approach questionable. Binding of NH₄⁺ to negatively charged functional groups on organic matter (e.g., carboxylic or phenolic), precipitation as struvite (MgNH₄PO₄·6H₂O) (19–21), and the formation of the carbamate ion (22, 23) are all potentially important in reducing the activity of NH₃ (aq). Even in the absence of these reactions, solute concentrations would be expected to lead to activity coefficients substantially different from unity. From the mass action expression for reaction 1, eq 3, it is clear that any reduction in the activity of NH₄⁺, $\gamma_{\text{NH}_4^+}m_{\text{NH}_4^+}$, leads to a proportional reduction in the aqueous concentration, m_{NH_3} , and activity, $\gamma_{\text{NH}_3}m_{\text{NH}_3}$, of NH₃ (aq), all else being constant:

$$K_a = \frac{\gamma_{\text{NH}_3}m_{\text{NH}_3}\gamma_{\text{H}^+}m_{\text{H}^+}}{\gamma_{\text{NH}_4^+}m_{\text{NH}_4^+}} \quad (3)$$

where γ_i = the activity coefficient of species i . Any of these effects invalidate eq 2, which is based on the assumptions that all ammonia is present as either NH₃ (aq) or NH₄⁺ and that activity coefficients are equal to unity. Additionally, m_{H^+} has been exclusively estimated as the antilog of the negative pH, although the accuracy of this is unclear except where the calibration buffer composition matches the sample composition (24).

Previous work on ammonia toxicity has generally ignored the distinction between solute activity and concentration. This approach is not reasonable for concentrated wastes. Besides the error this imparts to the calculation of NH₃ (aq) concentration, this distinction is important to recognize to select the best measure for a given application. In terms of evaluating toxicity, an estimate of NH₃ (aq) activity is more useful than concentration, because activity affects the gradient responsible for mass transfer. The current work focuses on measurement of activity. Because different solutes have strongly differing effects on γ_{NH_3} (10, 25), prediction of NH₃ (aq) activity from NH₃ (aq) concentration (or vice versa)

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may be difficult for complex mixtures. However, in many cases, γ_{NH_3} may be sufficiently close to unity that concentration can be assumed to equal activity (see parameters in refs 10, 25).

Some researchers have noted that, in animal manure, NH_3 (aq) activity is lower than predicted by eq 2 using literature values of K_a . Zhang (26) estimated the fraction of total ammonia as NH_3 (aq) in anaerobically stored swine manure liquid (<1% total solids (TS)) by comparing volatilization rates at pH 9.45 and pH 12.55 at 21 °C. This fraction was estimated as 0.20 at pH 9.45, while eq 2 predicts 0.55 using $\text{p}K_a = 9.369$ (10). Arogo et al. (27) applied a similar approach to swine waste liquid from an anaerobic lagoon (<0.5% TS) and estimated the fraction of total ammonia as NH_3 (aq) at pH 9 and 35 °C as 0.40, while eq 2 predicts 0.53. These researchers assumed that their results could be incorporated into eq 2 by reducing the magnitude of K_a . However, this approach has not been tested, and the potential interactions discussed above suggest that it may not be suitable.

Modeling approaches other than eq 2 may be suitable for predicting NH_3 (aq) activity in complex mixtures. Where ionic strength is low, an equilibrium approach that includes the Debye–Hückel equation or a similar expression for activity coefficients may be used. However, all significant ion associations must be included. At ionic strengths above approximately 0.5 (depending on the expression used and the solution composition), this approach is not reliable, but Pitzer's ion-interaction approach is (28–31). Currently, more research is needed to identify mechanisms controlling ammonia speciation in complex mixtures.

A method for direct measurement of NH_3 (aq) activity would be useful for toxicity, volatilization, and other research. Although NH_3 (aq) activity can be measured using an ammonia electrode, this method responds to volatile amines as well as NH_3 (aq) (32), and high partial pressures of CO_2 are a negative interference. The approach used by Zhang (26) and Arogo et al. (27) has the potential to provide estimates of relative NH_3 (aq) activity. However, it cannot provide absolute estimates, is not suitable for viscous or solid samples, and is very sample-intensive. There is currently no method available to measure NH_3 (aq) activity in anaerobic digester samples or similar complex mixtures. Therefore, our objective was to develop a simple method for measuring the activity of NH_3 (aq) in complex mixtures.

Experimental Section

Principles and System for NH_3 (aq) Measurement. A simple system for measuring NH_3 (aq) activity based on the application of Henry's law was developed. According to Henry's law, given the partial pressure of ammonia gas that is in equilibrium with an aqueous solution, the activity of NH_3 (aq) can be calculated from eq 4 (25):

$$\gamma_{\text{NH}_3} m_{\text{NH}_3} = K_{\text{H}} P_{\text{NH}_3} \quad (4)$$

where K_{H} = Henry's constant (m/atm), and P_{NH_3} = partial pressure of gaseous ammonia (atm). The use of molal (or similar) units, which are independent of changes in density, provides an ideal approach for concentrated mixtures with variable density and substantial amounts of suspended solids at a range of temperatures. The system developed here (Figure 1) is similar to that of Dasgupta and Dong (33). Microporous poly(tetrafluoroethylene) (PTFE) tubing (6.35-mm outer diameter, 3.18-mm inner diameter; Markel Corp., Plymouth Meeting, PA) is used to equilibrate a measured volume of sampling gas with the mixture sample. This PTFE tubing has elongate pores with an approximate length of 10 μm at the outer surface. Assuming sufficient retention time, gas that moves through the length of the tubing reaches approximate

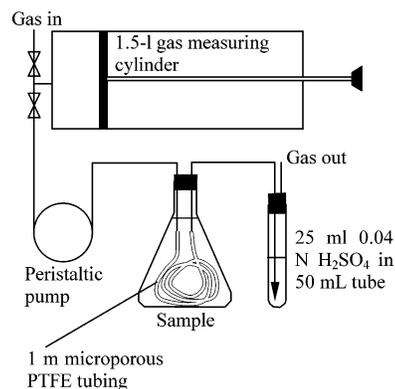


FIGURE 1. A diagram of the system developed for measurement of NH_3 (aq) activity. See text for a description of its use.

equilibrium with the sample ammonia. Sampling gas volume is controlled using a 1.5-L gas measuring cylinder, which is manually filled. After passing through the microporous tubing, sampling gas (now containing ammonia in equilibrium with the sample) is bubbled through a 0.04 N H_2SO_4 trap, using a 200 μL pipet tip (Fisher Scientific, Fair Lawn, NJ). Although this bubbler would be expected to have lower transfer efficiency than a fritted diffuser, it has the advantages of a low pressure differential (<0.015 atm) and minor carry-over between samples. The capture efficiency of this method was found to be high (>98%). The measured concentration of ammonia in the acid trap is used to determine the total mass of N transferred in the sampling gas, and the ideal gas law, eq 5, is used to calculate the partial pressure of ammonia in the sampling gas:

$$P_{\text{NH}_3} = \frac{n_{\text{N}} RT}{V_t} \quad (5)$$

where n_{N} = moles of N captured as NH_3 in the acid trap, R = the universal gas constant (0.082057 L atm/K·mol) (34), T = the temperature of the gas (K), and V_t = the volume of gas transferred (L). NH_3 (aq) activity is then calculated by eq 4.

Experimental Trials. The required tubing length and gas flow rate for approximate equilibrium was determined by measuring the response of P_{NH_3} to flow rate, from 85 to 770 mL/min, using two tubing lengths (0.5 and 2.0 m), and a 50 mg un-ionized NH_3 -N/kg H_2O standard solution. Standards were made by diluting concentrated NH_4OH in deionized distilled water and increasing the pH to 11.8–12 by adding NaOH. The NH_3 (aq) concentration and activity of these standards were estimated to be approximately equal to the total ammonia concentration. The standards were not otherwise buffered, to maintain a low ionic strength (0.01 m) to keep activity coefficients close to unity. Following the determination of sufficient tubing length and flow rate, the system was validated and calibrated at 25 °C using four standards of NH_4OH : 5, 50, 500, and 5000 mg NH_3 -N/kg H_2O . Partial pressure of NH_3 in the sampling gas was calculated using eq 5. Measured K_{H} was calculated using eq 4.

The measured K_{H} from this system should compare well with values of K_{H} reported in the literature. Additionally, according to Henry's law, P_{NH_3} should be directly proportional to NH_3 (aq) activity; that is, measured K_{H} should be independent of NH_3 (aq) activity. Documentation of constant K_{H} values over a range of NH_3 (aq) activities and comparison of calculated K_{H} to literature values was taken as validation of the system. NIST has compiled measured and calculated values of K_{H} for ammonia (35), and these were used for the first comparison. For more precise validation, the results of Dasgupta and Dong (33) were used. Dasgupta and Dong (33)

measured K_H (M/atm) at a range of temperatures and reported the following response:

$$K_H = e^{4092/T-9.70} \quad (6)$$

Predictions of K_H from eq 6 were adjusted to molal units (m/atm) assuming the concentration of water was equal to the density of pure water, using values given in ref 34.

Additional validation was carried out by using the system to measure NH_3 (aq) activity in three simple solutions of NH_4Cl and NaOH , with 500 mg $\text{NH}_3\text{-N/kg H}_2\text{O}$, at pH 7.0, 7.5, and 8.0, at 35 °C. Measured activity was compared to results from an equilibrium model with the activity coefficient of NH_4^+ calculated using the extended Debye-Hückel equation (9). The ion size parameter was set to 2.5 (36). An expression for NH_3 (aq) activity in simple solutions can be found by substituting $m_{\text{total ammonia}} - m_{\text{NH}_3}$ for $m_{\text{NH}_4^+}$ in eq 3, and rearranging.

$$\gamma_{\text{NH}_3} m_{\text{NH}_3} = \frac{K_a \gamma_{\text{NH}_3} \gamma_{\text{NH}_4^+} m_{\text{total ammonia}}}{\gamma_{\text{NH}_3} \gamma_{\text{H}^+} m_{\text{H}^+} + \gamma_{\text{NH}_4^+} K_a} \quad (7)$$

Because activity coefficients are dependent on the concentrations of NH_4^+ and NH_3 (aq), eq 7 was solved iteratively. The activity of H^+ , $\gamma_{\text{H}^+} m_{\text{H}^+}$, was estimated as the antilog of the negative pH, and K_a values were calculated following ref 10. The value of γ_{NH_3} was calculated using parameters presented in refs 10 and 25, but was very close to unity for all solutions.

The simplifications made in this analysis potentially generate small biases, requiring calibration of the system under a given set of conditions. Determination of the measured K_H value at the temperature of interest was taken as a calibration of the system. One standard (50 mg N/kg H_2O) was used to calibrate the system at 35 and 55 °C, using both ambient and humidified air. The degree of humidification has the potential to influence calibration values of K_H by introducing errors in the measurement of gas volume (see Results and Discussion). Air was humidified by transfer through deionized distilled water in a gas-washing bottle with a fine fritted diffuser.

For all trials, at least four sequential volumes of gas were transferred through the tubing and acid trap, resulting in four replicate acid traps. The total ammonia concentration of the acid solutions was measured using the manual phenate method, following neutralization with 180 μL of 5 N NaOH . The manual phenate method followed ref 37, with the exception that the phenol solution was made by diluting 10 g of crystallized phenol to 100 mL with ethyl alcohol. Samples with total ammonia concentrations >0.5 mg N/L were diluted prior to analyzing. All 25 °C trials were carried out in a free-standing incubator, while the other trials used walk-in controlled-temperature chambers.

Prior to collecting the first acid trap sample in each set, 100 mL of sampling gas was passed through the tubing, to flush the system. However, the first acid trap sample in a sequence was occasionally higher or lower than the rest, probably due to carryover between samples. These results were excluded from analysis.

It is important that the sampling gas be in approximate equilibrium with major pH-influencing components of the sample, such as CO_2 for anaerobic digester samples. Otherwise a change in aqueous concentrations could change the sample pH and impact ammonia speciation. In the worst-case scenario, poor mixing in a viscous sample could lead to localized effects (i.e., immediately adjacent to the microporous tubing). Ambient and humidified laboratory air was successfully used as the sampling gas for the trials with standards. Biogas would be expected to be an ideal sampling

gas for anaerobic digester samples. Trials were carried out to compare the response of ammonia partial pressure and sample pH to biogas and air. In both cases, a mesophilic slurry reactor sample was used as described below. Biogas was used for all other reactor samples to eliminate the effect of CO_2 stripping on ammonia speciation. Because biogas is approximately saturated with water vapor, calibration for biogas trials was carried out using humidified air.

Following calibration and validation, the system was tested using samples from laboratory-scale dairy manure anaerobic digesters. All digesters were 8.0-kg units (reacting mass) and were fed weekly with raw undiluted manure, for an organic matter loading rate of 3–4 g/(kg reacting mass)-d. Raw manure contained 11.4% TS, of which 84.3% was organic matter. For measurement of NH_3 (aq) activity in slurry digester samples, approximately 450 g of reacting mass was added to a 600-mL flask containing a 1-m piece of microporous PTFE tubing. The reacting material in the high solids digester was maintained at a solids content of approximately 25% by recycling dried (50% TS) effluent back into the digester with the feed. For the high-solids trial, a wide-mouth jar was used to hold a 750-g sample during NH_3 (aq) measurement. Samples were incubated at 35 or 55 °C for at least 1 day prior to measurement. During this time, sample containers were sealed except for a water trap to allow biogas to escape. Sample containers were sealed during measurement. For the slurry digesters, the sample was mixed intermittently during measurement by shaking. For measurement of NH_3 (aq), at least 5 sequential volumes of biogas, collected previously from the corresponding 8.0-kg digester, were transferred. After collection of the acid trap samples, the sample pH was determined and total ammonia was measured by distillation followed by the manual phenate method (37). Sample pH was determined using an Orion AquaPro low maintenance combination electrode with an Orion 720A meter. Calibration of the meter was carried out at the temperature of the sample using two buffers (pH 7.00, $\text{KH}_2\text{PO}_4\text{-NaOH}$ buffer, 0.05 M; pH 10.00, $\text{K}_2\text{CO}_3\text{-K}_2\text{B}_4\text{O}_7\text{-KOH}$, 0.05 M; Fisher Scientific, Fair Lawn, NJ). The pH of one sample was increased to >12 using 6 N NaOH prior to measurement of NH_3 (aq) activity, to determine the possibility of disequilibrium that could cause reduced NH_3 (aq) activity, and to qualitatively assess the value of γ_{NH_3} . Additionally, the flow rate was varied for two samples to further test for the possibility of disequilibrium in the system. Measurements of NH_3 (aq) activity were compared to concentrations calculated using the accepted speciation approach, eq 2. The value of m_{H^+} was estimated as the antilog of the negative pH, and K_a values were calculated following ref 10.

Results and Discussion

Development, Validation, and Calibration. Using a 50 mg $\text{NH}_3\text{-N/kg}$ standard with a 2-m length of tubing, P_{NH_3} showed no response to flow rate over the range used, but a 0.5-m length showed a decrease in P_{NH_3} as flow rate increased, suggesting disequilibrium at higher flow rates. With 0.5 m of tubing, equilibrium was attained up to approximately 170 mL/min, or at a retention time ≥ 1.4 s. Based on these results, the flow rate was maintained at 170 mL/min, and the tubing length was increased to 1.0 m to incorporate a safety factor for all subsequent trials. This resulted in a retention time of 2.8 s. Equilibrium between a viscous or solid sample, where mixing is low, and gas within the PTFE tubing could possibly require a greater retention time than that suggested from trials with solutions. However, two trials using 1.0 m of tubing showed no response of P_{NH_3} to flow rate using samples from both mesophilic digesters.

Measurement of ammonia partial pressure in four standards at 25 °C yielded a highly linear response (Figure 2), with a relatively constant K_H value. Measured K_H values at

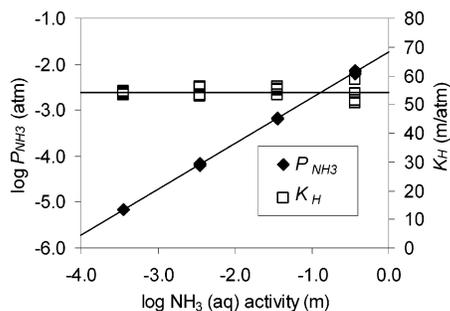


FIGURE 2. Measured partial pressure of NH_3 at 25°C as a function of NH_3 (aq) activity, and resulting measured K_H values. Note the linear response and the relatively constant measured K_H values.

TABLE 1. Measured Values of Henry's Constant As Compared to Ref 33

tempera- ture ($^\circ\text{C}$)	standards (mg $\text{NH}_3\text{-N}/$ kg H_2O) ^a	mean measured ^b K_H , ambient air (std. dev.)	mean measured K_H , humidified air (std. dev.)	refer- ence K_H ^c
25	5, 50, 500, 5000	54.3 (1.9) ^d	ND ^e	56.1
35	50	34.9 (1.5)	35.5 (1.4) ^f	36.1
55	50	14.8 (0.91)	16.5 (0.73) ^g	16.2

^a Concentration units refer to mass of N present in the ammonia form per kg of water. ^b Measured values of Henry's constant are calibration values, incorporate small biases of the system, and should not be taken as true values of K_H for, for example, modeling work. ^c Values for K_H were calculated using eq 6, adjusted to molal units (all K_H values are in units of m/atm). ^d Standard deviation at 25°C is the mean of the standard deviation for each standard ($n = 4$ for each standard). ^e Not determined. ^f Ambient and humidified values are not significantly different ($P = 0.49$) as shown by a two-tailed t -test ($n = 10$ and 5, respectively). ^g Ambient and humidified values are significantly different ($P = 0.008$) as shown by a two-tailed t -test ($n = 5$ for each).

all temperatures were similar to, although, at 25 and 35°C , slightly lower than, the majority of the values compiled by NIST (35), and close to values calculated using eq 6 (Table 1). The coefficients of variation for the trials presented in Table 1 range from 3.5% to 6.1%. Measurement of NH_3 (aq) activity in simple solutions gave results very similar to those predicted using the equilibrium approach described above. Measured values for 500 mg $\text{NH}_3\text{-N}/\text{kg}$ at pH 7.0, 7.5, and 8.0 at 35°C were 4.76, 15.3, and 44.5 mg N/kg (SD = 0.68, 0.63, and 1.0 mg N/kg), while modeled values were 4.89, 15.1, and 44.5 mg N/kg, respectively.

These results show that the method introduced in this study is a reasonable approach for measuring NH_3 (aq) activity. The system appears to produce accurate results, with acceptable precision, using simple solutions. As long as the analytical method used for total ammonia in the acid solutions does not respond to amines, volatile amines will not interfere with the accuracy of this approach.

Application to Anaerobic Digester Samples. Measurement of sample pH and sampling gas P_{NH_3} confirmed that biogas is an appropriate sampling gas for anaerobic digester samples. Sample pH increased significantly with air as the sampling gas (0.07 pH units per L transferred), while biogas resulted in only a slight increase in pH before it stabilized 0.02 units above the initial value after transferring 4 L. Similarly, P_{NH_3} was greater with air than with biogas: 4.63×10^{-4} atm (SD = 3.2×10^{-5} atm) for air versus 2.27×10^{-4} atm (SD = 3.2×10^{-5}) for biogas ($n = 5$ for each). These results are most likely due to the stripping of CO_2 from anaerobic digester samples. Although biogas composition was not determined for each trial, the CO_2 content of biogas produced by these four digesters was 30–40%. This partial pressure (0.3–0.4 atm) has the potential to have a major effect on solution pH. The small (and insignificant) increase in sample pH when using biogas as the sampling gas is probably the result of a slightly higher CO_2 activity in the sample than in the sampling gas.

The system was applied to five anaerobic digester samples (Table 2). Mesophilic slurry samples showed NH_3 (aq) activity of approximately 90 mg $\text{NH}_3\text{-N}/\text{kg}$, which was $2/3$ of the value calculated using the currently accepted approach. A thermophilic slurry sample showed NH_3 (aq) activity of 250 mg $\text{NH}_3\text{-N}/\text{kg}$, which was just $>1/2$ of the calculated value. A high-solids mesophilic sample with a higher concentration of total ammonia showed an even greater discrepancy: the measured value was $1/3$ of the calculated value. These differences correspond to errors in the currently accepted approach of 45–200%. Increasing the pH of a mesophilic slurry digester sample to 12.2 led to a large increase in measured NH_3 (aq) activity. Measured NH_3 (aq) activity was 20% greater than the total ammonia concentration in this sample, showing that the differences observed in the other samples were not due to an artifact of the method. The precision of this method was lower using digester samples than when used with standards; coefficients of variation ranged from 2.1% to 16%.

The method presented in this work can easily be applied to anaerobic digesters to obtain in situ estimates of NH_3 (aq) activity without removing a liquid or solid sample. Variations of this method, for example, using a different flow rate or tubing with different porosity or dimensions, could be applied on larger or smaller scales.

Although these results do not constitute a large data set, they clearly confirm that the currently accepted approach for estimating NH_3 (aq) activity or concentration is not accurate in digested manure. Future work should recognize this limitation. Assuming an activity coefficient for NH_3 (aq) greater than unity, the currently accepted approach is less accurate for estimating NH_3 (aq) concentration than it is for estimating NH_3 (aq) activity. More research is needed to understand factors controlling NH_3 (aq) activity in complex

TABLE 2. Measured NH_3 (aq) Activity As Compared to that Calculated Using the Accepted Approach in Anaerobic Digester Samples

sample	total solids (%)	organic matter (%) of total solids)	total NH_3 concentration ^b	T ($^\circ\text{C}$)	pH	calculated NH_3 (aq) concentration/ activity ^c	measured NH_3 (aq) activity
mesophilic slurry	8.97 (0.084) ^a	80.3 (0.24)	3550 (65)	35	7.52	130	86.1 (5.5)
mesophilic slurry	8.82 (0.073)	78.8 (0.81)	3380 (54)	35	7.55	134	92.4 (6.4)
mesophilic slurry ^d	10.7 (0.028)	57.0 (0.68)	2980 (90)	35	12.20	2980	3590 (77)
thermophilic slurry	9.16 (0.049)	80.2 (0.19)	3720 (130)	55	7.57	470	249 (39)
mesophilic high solids	24.6 (0.93)	70.2 (0.86)	4700 (310)	35	7.77	292	98.0 (16)

^a Values in parentheses are standard deviations ($n = 2$ for total solids and organic matter, $n = 5$ or more for NH_3 (aq) activity, and $n = 3$ for total ammonia). ^b All ammonia concentrations and activities are in units of mg $\text{NH}_3\text{-N}/\text{kg}$ H_2O . ^c Calculated using eq 2. ^d Sample pH was adjusted using 6 N NaOH.

mixtures. Ammonia speciation in solutions with other inorganic solutes has been thoroughly studied and successfully modeled (10, 22, 23, 25, 38), and parameters from these studies will be useful for evaluating the significance of inorganic solutes.

Based on the inaccuracy of the currently accepted approach, it is likely that previous work on ammonia toxicity in anaerobic digesters has overestimated the limits of NH_3 (aq) tolerance by overestimating NH_3 (aq) concentrations. However, because the capacity of the matrix to reduce NH_3 (aq) activity in anaerobic digesters has generally not been considered, some limits to anaerobic digestion may be higher than previously assumed. The recognition that NH_3 (aq) activity is not solely a function of pH and total ammonia concentration, and the availability of an accurate method for measurement, should facilitate research on the control of ammonia toxicity. Future work that addresses NH_3 (aq) in complex mixtures should assess the accuracy of the currently accepted approach and consider direct measurement.

Calibration and Use of the System. The conditions under which the microporous tubing system is used can influence the measured value of K_H ; therefore, calibration should be carried out under conditions similar to those used for samples. The temperature of the system can influence the calibration value of K_H through both errors in gas volume measurement, and by influencing the true value of K_H . The change in gas volume with temperature is described by Charles' law and is a relatively small source of error in this system.

Temperature differences have the potential to cause condensation of water in the tubing exiting the sample container. Although this may not be a problem in some cases, the dissolution of NH_3 in water droplets could possibly lead to substantial underestimation of NH_3 (aq) activity, especially when the sampling gas includes a high partial pressure of CO_2 . A simple solution to this problem is to maintain the external environment at a slightly higher temperature than the sample (33).

Henry's constant itself is sensitive to temperature, as shown in eq 6. Near 35 °C, as little as a 1 °C difference between calibration and measurement temperatures can lead to a 4% error in results. Careful monitoring of the sample and standard temperatures is therefore important for accurate measurement.

As mentioned above, the composition of the sampling gas could influence the measurement of P_{NH_3} through changes in sample pH. This is potentially problematic for organic samples with microbial activity due to a high partial pressure of CO_2 in the sample that must be matched in the sampling gas. In samples with well-defined gas evolution, this is not a difficulty. In other settings, it may be necessary to determine and match the composition of gas in equilibrium with the sample.

The moisture content of the sampling gas is another variable that could influence the results, by introducing errors in the measurement of sampling gas volume. Measuring the volume of a dry gas before passing it through the microporous tubing (where it may approach saturation) results in an underestimation of volume transferred. The potential magnitude of this error increases with temperature and can be determined using values of water vapor pressure at saturation (39) and the ideal gas law. At 35 °C, the use of a dry gas for samples and a saturated gas for calibration, or vice versa, can lead to an error >5%. This difference increases to 16% at 55 °C. Assuming calibration takes place using gas of a humidity similar to that of the sampling gas, this effect will not reduce the accuracy of the method.

Acknowledgments

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