

# A Simple Method for Measurement of Inorganic Carbon Concentration and Carbonate System Alkalinity in Anaerobic Digesters

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## ABSTRACT

This paper presents a simple approach for measurement of inorganic carbon (IC) concentrations and carbonate system alkalinity in anaerobic digester samples. The method described here, based on CO<sub>2</sub> volatilization from acidified samples, is a simplification of a previously described method and is well-suited for application to manure digesters. Simplifying assumptions result in a total systematic error smaller than 10% when applied to typical manure digester samples. Application to standards of NaHCO<sub>3</sub> showed accurate and precise results (for 5 g samples, accurate to within 3%, coefficient of variation <4%). Application to samples from mesophilic and thermophilic laboratory-scale anaerobic digesters fed dairy cattle manure showed good results (for 5 g samples, coefficient of variation < 10%).

**Keywords:** Anaerobic digestion, manure, inorganic carbon, carbonate, bicarbonate, alkalinity, carbon dioxide

## 1. INTRODUCTION

Anaerobic digestion of animal manure is an important technology for energy production and odor control (e.g. Ahring, 2003; Goodrich, *et al.*, 2005). The carbonate system is a major component of alkalinity in anaerobic digesters and is often used to monitor anaerobic digester stability (Speece, 1996). Carbonate system alkalinity declines in anaerobic digesters in proportion to increases in concentrations of volatile fatty acids (VFAs) as the latter drive pH downward. Therefore, carbonate system alkalinity can be used to detect an imbalance between acid production and methanogenesis, which is a sign of digester instability and may lead to digester failure in the absence of corrective measures. Carbonate system alkalinity can be estimated as the difference between total alkalinity (measured by titration) and the estimated contribution of volatile fatty acids (McCarty, 1964; Speece, 1996), but other weak acid/base systems are a positive interference. More complicated procedures can be used to differentiate between different weak acid/base systems (Moosbrugger, *et al.*, 1992; Lahav and Loewenthal, 2000), but these approaches require measurement of other solutes, and do not address interferences from uncharacterized organic material. Rozzi and Brunetti (1981) proposed that carbonate system alkalinity (assumed to be equivalent to bicarbonate concentration) could be

determined in digester samples by measuring CO<sub>2</sub> volume volatilized after equilibrating a sample with 1.0 atm CO<sub>2</sub>, followed by acidification to pH 4. Additional information on this method is found in Rozzi and Labellarte (1984) and Rozzi *et al.* (1985). A review of methods for alkalinity measurement for anaerobic digesters noted that this approach suffers from complexity and a need for specialized equipment (Lahav and Morgan, 2004).

Measurement of inorganic carbon (IC) concentrations is useful for constructing anaerobic digestion mass balances, measuring gaseous/solution distribution of IC, and understanding chemical speciation of IC and ammonia. Inorganic carbon species can influence the speciation of other chemical systems. For example, high concentrations of bicarbonate, HCO<sub>3</sub><sup>-</sup>, can reduce the toxic un-ionized ammonia concentration by effectively reducing the activity coefficient of ammonium (Hafner, 2007). This paper presents a simple method for measurement of IC concentration and carbonate system alkalinity in anaerobic digesters. The method described here is a simplification of the method of Rozzi and Brunetti (1981) that requires no specialized equipment and is well suited to manure digesters.

## 2. METHOD DEVELOPMENT

In this work it is proposed that a simplified gas volatilization approach can be used to easily measure IC concentrations and carbonate system alkalinity with a systematic error smaller than 10%. In this method samples are added to serum bottles and sealed, followed by acidification using HCl or another strong acid. Acidification causes all IC in equilibrium with CO<sub>2</sub> (aq) to be converted to CO<sub>2</sub> (aq). Subsequent volatilization of CO<sub>2</sub> from the acidified sample causes an increase in headspace pressure. Using a syringe, headspace gas is intermittently removed to reestablish a gauge pressure of zero. Control bottles, with water in place of a sample, are used to correct for the effect of water evaporation and slight temperature changes. Sample IC concentration can be estimated from the total volume of gas removed and carbonate system alkalinity can be estimated by assuming that each mole of IC contributes 1.0 equivalent of alkalinity. In this section the rationale for this simple approach is given and potential errors are discussed.

Equilibrating a sample with CO<sub>2</sub> (g) before acidifying (Rozzi and Brunetti, 1981) prevents original CO<sub>2</sub> (aq) from contributing to CO<sub>2</sub> volatilization and eliminates uncertainty in residual IC. Without this step, as in the proposed method, the quantity of IC remaining in solution as CO<sub>2</sub> (aq) will be dependent on the partial pressure of CO<sub>2</sub> (g) in the vessel headspace, assuming equilibrium, and will not contribute to the volume of gas volatilized. This incomplete volatilization contributes a negative bias when this method is used to measure total IC. However, this source of error is small (generally < 5%) and counteracts other, positive, biases. The magnitude of this error, as well as the error due to water evaporation, discussed below, can be estimated using the model given in the Appendix. Conversely, this model can be used to correct measured values for these biases.

Allowing a wet sample to equilibrate with a gas phase leads to evaporation of H<sub>2</sub>O and a resulting increase in headspace pressure. In the proposed method gas volatilization from control bottles with water and acid only is subtracted from gas volatilization from sample bottles. However, these control bottles do not provide perfect estimates of water evaporation in sample bottles since the magnitude of water evaporation is dependent on the volume of gas removed.

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This difference contributes a small positive bias to measurements of IC. The magnitude of this error is small (generally  $\sim 2\%$ ) and can be addressed using the model given in the Appendix.

If a sample contains sulfide, acidification will lead to volatilization of  $\text{H}_2\text{S}$  (g), which will cause overestimation of  $\text{CO}_2$  volatilization (Rozzi and Brunetti, 1981) and therefore incorporate a positive bias in the measurement of IC. However, based on typical concentrations of total S in manure, sulfide is not a significant interference for typical samples. For example, assuming a total S concentration of 0.425% of TS in dairy manure (ASAE 1998), and a TS concentration of 10% in the digester feed, the total S concentration in a digester sample would be  $< 15$  mmol/kg (note that all concentrations in this paper used the molal scale). Assuming sulfide makes up 50% of total S the sulfide concentration in a typical manure digester sample would be  $< 8$  mmol/kg. Resulting error would be  $< 3\%$  for samples with IC concentrations of  $\sim 300$  mmol/kg as in the mesophilic digester samples shown below.

Volatilization of un-ionized volatile fatty acids (VFAs) present in anaerobic digesters could also lead to overestimation of  $\text{CO}_2$  volatilization (Rozzi and Brunetti, 1981). However, Henry's constants for important VFAs are so high (Sander, 2003) that VFAs cannot be a major source of error. As an extreme case, consider a digester sample with an acetic acid concentration of 80 mmol/kg (5 g/kg). With a low estimate of Henry's constant of 4000 mol/kg-atm (Sander, 2003), this corresponds to a partial pressure of  $2 \times 10^{-5}$  atm or an error less than 0.1% for the bottle and sample sizes used in this work.

The remaining errors discussed below do not cause biased estimates of total IC, but can contribute bias if results are interpreted as dissolved IC or carbonate system alkalinity. Where measurement of total IC alone is preferred or sufficient, these errors are not relevant. In samples that contain particulate IC, acidification will dissolve carbonate minerals and measurement of IC will include a contribution of particulate IC. Dolomite precipitation appears to be extremely rate-limited in the absence of high temperatures (Land, 1998; Arvidson and Mackenzie, 1999), leaving Ca and Mg carbonates as the two groups of minerals likely to be present in anaerobic digesters (Musvoto, *et al.*, 2000). Given typical concentrations of 1.3% and 0.59% (TS basis) for Ca and Mg in dairy cattle manure (ASAE, 1998) and a TS concentration in digester feed of 10%, reactor concentrations would be approximately 40 and 30 mmol/kg, respectively. Swine manure shows higher concentrations of Ca (3.0%) (ASAE, 1998), but is digested at much lower TS concentrations. Magnesite,  $\text{MgCO}_3$ , is probably not important in manure digesters, since struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ) formation is much faster (Musvoto, *et al.*, 2000). Additionally, both  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  bind to natural organic matter (Benedetti, 1995; Gustafsson, 2003) which explains greater solubility and reduced formation rates of  $\text{CaCO}_3$  minerals in the presence of dissolved organic matter (Musvoto, *et al.*, 2000). Lastly, calcium phosphates compete for  $\text{Ca}^{2+}$  and precipitate more quickly than  $\text{CaCO}_3$  minerals (Ferguson and McCarty, 1971; Musvoto, *et al.*, 2000). Given these interactions, it is unlikely that carbonate minerals are a major pool of IC in typical manure digesters, although further validation of this conclusion would be useful.

Where an estimate of carbonate system alkalinity is desired, it is reasonable to assume that measured IC contributes 1.0 equivalent of alkalinity per mole. This approximation follows the typical assumption that carbonate system alkalinity is approximately equivalent to  $\text{HCO}_3^-$  alkalinity in digesters, since  $\text{CO}_3^{2-}$  is insignificant in the range of digester pH values (McCarty, 1964; Rozzi, *et al.*, 1985; Speece, 1996; Metcalf & Eddy Inc., 2003). In digester samples with high concentrations of ammonia and dissolved  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  carbonate system alkalinity also

includes the complexes  $\text{CaCO}_3$  and  $\text{MgCO}_3$ , and the carbamate ion,  $\text{NH}_2\text{COO}^-$  (Hafner, 2007). Quantification of the error associated with the approximation that IC contributes 1.0 eq/mol requires the use of speciation modeling. Speciation of the dissolved  $\text{CO}_2$  system is primarily a function of  $\text{CO}_2$  partial pressure and  $\text{H}^+$  activity, but other solutes influence speciation. Simulation of chemical speciation in manure digesters requires a modeling approach suitable for concentrated solutions, such as Pitzer's ion-interaction approach (Clegg and Whitfield, 1991; Pitzer, 1991; Millero and Pierrot, 1998). The following speciation results were generated using the Pitzer approach with the program PHREEQC v. 2.13.04 (United States Geological Survey, 2007). Given a solution in equilibrium with  $\text{CO}_2$  (g),  $\text{HCO}_3^-$  is the dominant IC species within the pH range of typical manure digesters (Fig. 1). Estimating carbonate system alkalinity from IC gives values within 5% of actual alkalinity from pH 7.5 to pH 8.5 (Fig. 1). In the presence of  $\text{NH}_3$ ,  $\text{NH}_2\text{COO}^-$  reaches significant concentrations at higher pH values (Fig. 2). However, since  $\text{NH}_2\text{COO}^-$  also contributes 1.0 eq/mol, carbonate system alkalinity estimated from IC is within 5% of actual alkalinity between pH 7.5 and pH 8.5 (Fig. 2).

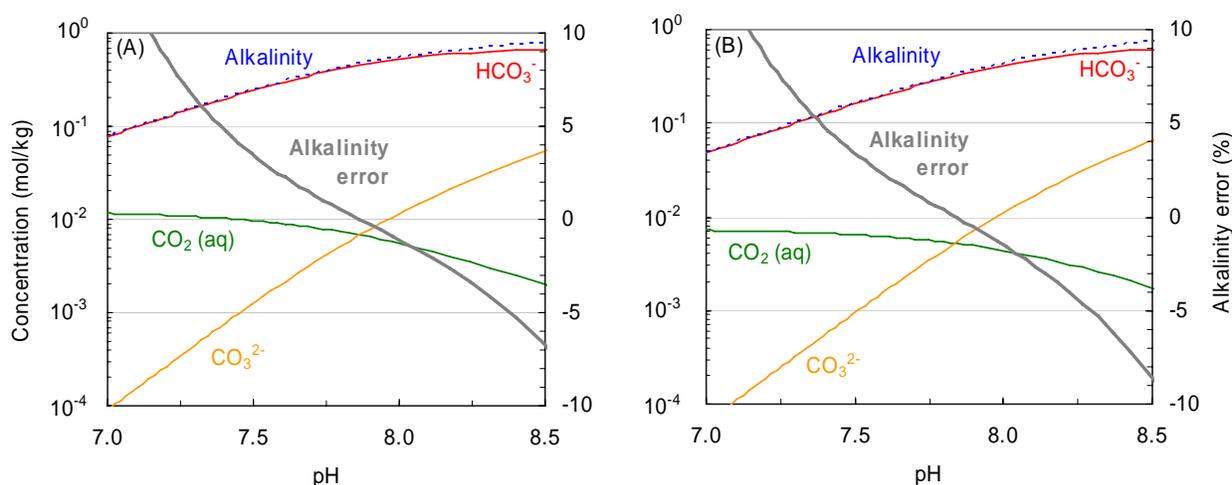


Figure 1. Simulated inorganic carbon speciation in simple solutions at 35°C (A) and 55°C (B). Concentrations are expressed using the molal scale, and axes are identical in parts A and B. The thick gray line labeled “Alkalinity error” shows the error in the estimate of carbonate system alkalinity made by assuming that alkalinity = 1.0 eq/mol  $\times$   $m_{\text{IC}}$  (where  $m_{\text{IC}}$  = the total inorganic carbon concentration). These simulations were produced using the PHREEQC database *pitzer.dat*, the derivation of which is described in Plummer *et al.* (1988). Both graphs show equilibration of 0.8 mol/kg each  $\text{CO}_2$  and  $\text{CH}_4$  between a gas phase and a solution, and use NaOH as the titrant.

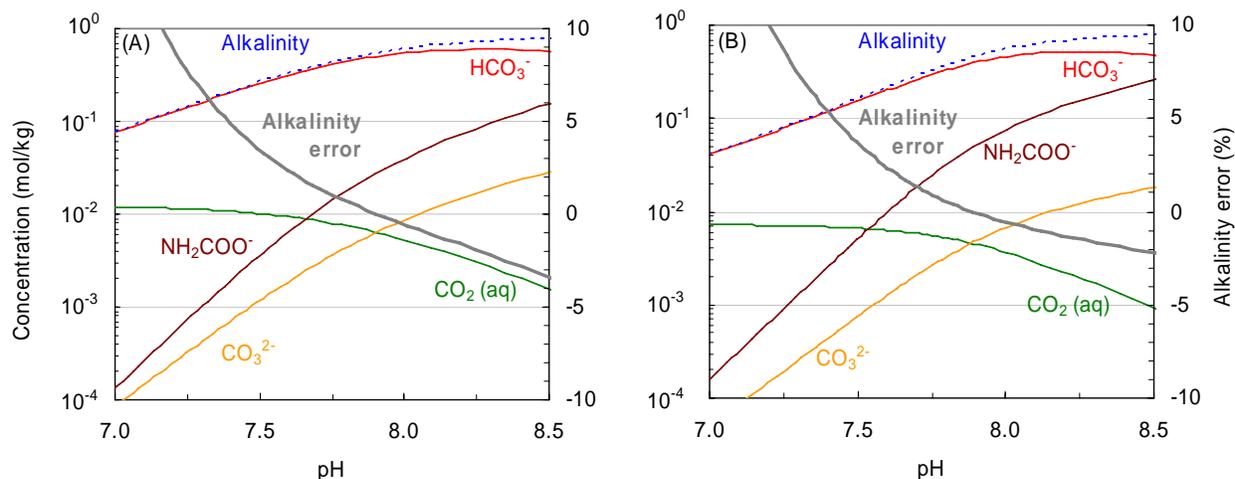


Figure 2. Simulated inorganic carbon speciation in simple solutions at 35°C (A) and 55°C (B). Concentrations are expressed using the molal scale, and axes are identical in parts A and B. The thick gray line labeled “Alkalinity error” shows the error in the estimate of carbonate system alkalinity made by assuming that alkalinity = 1.0 eq/mol  $\times$   $m_{IC}$  (where  $m_{IC}$  = the total inorganic carbon concentration). These simulations were produced using the database described in Hafner (2007). Both graphs show equilibration of 0.8 mol/kg each  $\text{CO}_2$  and  $\text{CH}_4$  between a gas phase and a solution, and use  $\text{NH}_3$  as the titrant.

### 3. METHODS

In the proposed method, an appropriately sized sample is weighed and washed into a serum bottle with a minimum volume of deionized distilled water, or added directly with no rinse water. Given a bottle size, an appropriate sample size can be estimated using the model given in the Appendix to select the largest possible sample size (to maximize precision) with an acceptable level of accuracy. After sealing, sufficient HCl (2.0 mol/L) is added using a syringe and needle to acidify samples to  $\text{pH} < 4$ . After mixing, bottles are held at a constant temperature, e.g. room temperature. Accumulated gas is intermittently removed and measured using a syringe, to reestablish a gauge pressure of zero, measured using a manometer. It is important that gas volume be measured at a gauge pressure of zero. Gas removal is stopped when pressure production has ceased or decreased to the level of the control bottles.

Control bottles with water plus acid are included to correct for changes in headspace pressure due to both evaporation of water and small temperature changes throughout the incubation period. Assuming the difference in gas removal between the sample and control bottles is due only to  $\text{CO}_2$  (g) volatilization, the moles of  $\text{CO}_2$  volatilized can be estimated by Eq. (1) using a molar volume at standard temperature and pressure of 22.26 L/mol (Lemmon, *et al.*, 2005).

$$mol_{CO_2} = \frac{(V_s - V_c) 273.15}{V_m (273.15 + T)} \quad (1)$$

In Eq. (1),  $V_s$  = volume of gas removed from a sample bottle (L),  $V_c$  = volume of gas removed from a control bottle (L),  $V_m$  = molar volume of  $CO_2$  at standard temperature and pressure (L/mol), and  $T$  = temperature ( $^{\circ}C$ ). Given the moles of  $CO_2$  volatilized, the sample mass, and TS content, the concentration of IC can be calculated by dividing the moles of  $CO_2$  evolved by the mass of water in the sample. Carbonate system alkalinity can be estimated by multiplying this concentration by 1.0 eq/mol. Alkalinity can be converted to  $CaCO_3$  equivalent by multiplying by 50 g/eq. Conversion to volume-based units can be made given the density of the sample.

This method was applied to standards of  $NaHCO_3$  (300 mmol/kg) and effluent samples from laboratory-scale anaerobic digesters using 160 mL serum bottles. Acid addition caused bubbling of  $CO_2$  from the samples so the bottles were inverted when withdrawing the needle to prevent gas loss. Bottles were mixed by manually shaking and were allowed to equilibrate at room temperature for  $\geq 10$  min before gas was removed. Accumulated headspace pressure was reduced intermittently with syringes until the difference between volume removal from samples and volume removal from controls was small ( $<0.2$  mL). For the samples used here, this required four to six gas removals, using an interval of 10 minutes or more. A mechanical micromanometer (Meriam model 34 FB2TM, Cleveland, OH) was used to determine when gauge pressure in the bottles reached zero (stated precision  $2 \times 10^{-6}$  atm).

Standard solutions of  $NaHCO_3$  were made using certified  $NaHCO_3$ . Anaerobic digesters were 8.0 kg units (reacting mass) and were fed raw dairy cattle manure (TS = 10%, VS = 85% of TS, retention time  $\approx 30$  d) and incubated at  $35^{\circ}C$  (mesophilic) or  $55^{\circ}C$  (thermophilic). To explore the error inherent in this method due to  $CO_2$  (aq) remaining in solution, the concentration of  $CO_2$  in the bottle headspaces was measured at the end of the analysis using a Gow-Mac Series 580 gas chromatograph with a thermal conductivity detector and He as a carrier gas.

Measurement of TS for digester samples was made by drying at  $105^{\circ}C$ , following Standard Methods (Eaton, *et al.*, 1995). Digester pH was measured directly in the digesters just before sampling using an Orion ROSS electrode and an Orion 720A meter. Calibration was carried out at  $35$  or  $55^{\circ}C$  using pH 7.00 and pH 9.00 buffers (reported values at  $25^{\circ}C$ ). Methane and  $CO_2$  concentrations in digester headspace were measured by gas chromatography as described above.

Measured concentrations of IC were calculated using Equation (1). To estimate standard error an estimate of standard deviation was made for each set of samples from the standard deviation of the sample bottles and the control bottles. Standard deviation was corrected for a small sampling size following Gurland & Tripathi (1971). Standard deviation values calculated in this manner were used to calculate 95% confidence intervals for all samples (Zar, 1999). Measured IC concentrations in mesophilic and thermophilic samples were compared to measured total alkalinity. Total alkalinity was measured following Standard Methods (Eaton, *et al.*, 1995) to an endpoint of pH 4.5.

#### 4. RESULTS AND DISCUSSION

Measurements made using the method described above were close to total IC concentrations for the 5 g NaHCO<sub>3</sub> solution samples (Table 1). Small samples showed coefficients of variation (CVs) of 3% to 15%, due in part to the relatively greater importance of variability in the water-only controls. Larger samples showed CVs of 0.9% to 3%. It is clear that this method provides reasonable estimates of IC in simple solutions. Measurement of IC concentrations in anaerobic digester samples showed slightly higher variability than did the NaHCO<sub>3</sub> solutions, with CVs ranging from 3% to 26% (Table 2). Smaller samples showed greater variability, but both sample sizes gave similar mean results for digester samples. As expected, total alkalinity, as measured by titration, was greater than carbonate system alkalinity measured by the proposed method in two digester samples (Table 3).

Table 1. Measured inorganic carbon concentrations in solutions of NaHCO<sub>3</sub> (300 mmol/kg), based on volatilized gas volume.

Mass (g)	Sample gas vol. (mL)	Control gas vol. (mL)	$m_{IC}$ (mmol/kg)	Recovery (%)
2.07	19.9	4.48	320 ± 79 (50)	107 ± 26
2.04	18.1	3.38	310 ± 19 (12)	103 ± 6.2
5.05	38.3	2.79	302 ± 15 (10)	101 ± 5.1
5.06	39.5	3.38	306 ± 4 (3)	102 ± 1.4

Notes: 95% confidence intervals are given with concentration estimates and recovery; values in parentheses are standard deviations. For all samples and controls,  $n = 4$ . Mass and volumes shown are mean values. Concentrations ( $m_{IC}$ ) are expressed using the molal scale.

Table 2. Measured inorganic carbon concentrations in anaerobic digester samples, based on volatilized gas volume.

Sample	Sample mass (g)	Sample gas vol. (mL)	Control gas vol. (mL)	$m_{IC}$ (mmol/kg) and carbonate sys. alk. (meq/kg)
M	2.04	19.9	4.48	340 ± 51 (32)
M	5.01	41.9	4.48	336 ± 24 (15)
T	2.16	10.4	4.48	126 ± 52 (33)
T	5.10	18.9	4.48	129 ± 20 (13)

Notes: M = mesophilic (TS = 6.95%), T = thermophilic (TS = 8.05%). Values in parentheses are standard deviations. 95% confidence intervals are given with concentration estimates. For all samples and controls,  $n = 4$ . Mass and volumes shown are mean values. Concentrations ( $m_{IC}$ ) are expressed using the molal scale.

Table 3. Measured inorganic carbon concentrations and measured total alkalinity for anaerobic digester samples, and digester characteristics.

Sample	TS (%)	T (°C)	pH	CH <sub>4</sub> (%)	CO <sub>2</sub> (%)	Total alkalinity (meq/kg)	$m_{IC}$ (mmol/kg) and carbonate sys. alk. (meq/kg)
M	7.96 (0.22)	35.8	7.67	55.8	38.9	401 ± 8	337 ± 15
T	8.02 (0.06)	55.8	7.62	53.5	32.9	370 ± 7	175 ± 9

Notes: M = mesophilic, T = thermophilic. Values in parentheses are standard deviation estimates (standard deviation for all gas concentration estimates is 0.4%). Gas concentrations are expressed as % of wet volume. 95% confidence intervals are given with measured concentration estimates. For TS and gas concentrations,  $n = 3$ ; for measured alkalinity and IC,  $n = 4$ . Concentrations ( $m_{IC}$ ) are expressed using the molal scale.

Serum bottle headspace samples showed CO<sub>2</sub> concentrations ranging from 8% to 20% of the wet volume depending on the sample size and sample IC concentration. Any CO<sub>2</sub> in the bottle headspace represents a negative systematic error for measurement of IC, since it corresponds to a quantity of CO<sub>2</sub> (aq) remaining in solution at equilibrium. Application of the model given in the Appendix can be used to evaluate the magnitude of the measurement error associated with a given IC concentration, sample size, and headspace volume, and can be used to select appropriate sample and headspace sizes. In general, this model predicts larger errors from samples with low IC concentrations and high masses of water. Application of this model to the samples shown in Table 1 gives predicted errors for measurement of IC of +0.2% for the 2 g samples, and -1.0% for the 5 g samples, using  $K_H = 0.039$  mol/kg-atm (Weiss, 1974). Predicted errors for the digester samples in Table 3, including the mass of water used for rinsing, were -3.4% for the mesophilic samples, and -5.5% for the thermophilic samples.

## 5. CONCLUSIONS

This work describes a simple method for measuring total inorganic carbon concentrations and carbonate system alkalinity in anaerobic digester samples. By making several simplifying assumptions this approach requires minimal equipment but still eliminates interferences inherent in simple titration approaches. The systematic error in this method is expected to be less than 10% based on characteristics of typical anaerobic digester samples. Application of this method to standard solutions and manure digester samples showed good results (accurate to within 3% for 5 g standard samples), with acceptable precision (coefficient of variation < 26% for all samples, < 10% for most samples).

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## 8. APPENDIX

Symbols used in the model are defined as follows:

$m_c$  = molal concentration of CO<sub>2</sub> (aq) in bottle solution (mol/kg)

$K_H$  = Henry's constant (mol/kg-atm)

$C_H$  = CO<sub>2</sub> in bottle headspace (mol)

$C_T$  = Total IC in bottle (mol)

$V_H$  = Bottle headspace (L)

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$R$  = Ideal gas constant = 0.08205 L-atm/mol-K (adjusted value for CO<sub>2</sub> near 20°C = 0.08150 L-atm /mol-K)

$M_w$  = Total mass of water in bottle, including acid solution and rinse water (kg)

$P$  = Total or partial headspace pressure (atm)

$V_A$  = volume of acid added (L)

Additional subscripts:

$E$ , indicates at equilibrium, just before gas removal

$I$ , indicates at the start of an iteration, before equilibrium is attained

Removal of headspace gas in the method described in this paper is an iterative process. In the following model, the iteration step is identified by the subscript  $i$ . As used in this model, the first iteration starts just after adding acid to the bottles and ends just after the first gas removal. Subsequent iterations start and end just after gas is removed. After equilibrium is achieved following acidification headspace CO<sub>2</sub> (g) and solution CO<sub>2</sub> (aq) are related by Eq. (2). Equation (2) is valid for any iteration at equilibrium.

$$P_{CO_2} = m_c / K_H \quad (2)$$

Note that Eq. (2) assumes that the activity coefficient for CO<sub>2</sub> (aq) is approximately unity. Based on the Pitzer model for inorganic interactions, this is a reasonable assumption. By mass balance:

$$C_H = C_T - m_c M_w \quad (3)$$

And by the ideal gas law:

$$C_H = \frac{P_{CO_2} V_H}{RT} \quad (4)$$

Equation (4) is valid at any time during the analysis. At 1.0 atm, CO<sub>2</sub> shows a deviation of about -0.7% in volume from the ideal gas law in the range of interest here. Considering this, the adjusted value of the ideal gas constant given in the list of symbols above can be used near 20°C if greater accuracy is desired. Setting Eqs. (3) and (4) equal, and solving for  $P_{CO_2}$  gives Eq. (5).

$$P_{CO_2} = \frac{RT}{V_H} (C_T - m_c M_w) \quad (5)$$

Equating Eq. (5) and Eq. (2) and solving for  $m_c$  gives:

$$m_c = \frac{C_T K_H RT}{(V_H + K_H M_w RT)} \quad (6)$$

Equation (6) can be used to find the equilibrium concentration of CO<sub>2</sub> (aq) in the sample solution after acidification, but before gas removal. Given this value, the partial pressure of CO<sub>2</sub> in the bottle headspace can be found from Eq. (2). The volume of gas removed for the first removal event is proportional to the total headspace pressure, given in Eq. (7).

$$P_T = 1.0 \text{ atm} + P_{CO_2} + P_{H_2O,E} - P_{H_2O,I} + V_A / V_H \quad (7)$$

This equation is also valid for control bottles, with no CO<sub>2</sub>. The partial pressure of water vapor can be calculated as the product of the relative humidity and the saturation partial pressure, which is 0.023 atm at 20°C (ASME, 2000).

The quantity of CO<sub>2</sub> in the bottle headspace at this point can be calculated from Eq. (4). Assuming the gas mixture in the bottle headspace behaves ideally, the volume that must be removed to bring the pressure to 1 atm can be calculated from Eq. (8), and the quantity of CO<sub>2</sub> removed in this gas can be calculated from Eq. (9):

$$V_R = \frac{P_T - 1.0 \text{ atm}}{1.0 \text{ atm}} V_H \quad (8)$$

$$C_R = \frac{C_H V_R}{P_T V_H} \quad (9)$$

Subtracting  $C_R$  from the original value of  $C_T$  gives the quantity of CO<sub>2</sub> remaining in the bottle (in solution and headspace). Subtracting  $C_R$  from  $C_H$  gives the moles of CO<sub>2</sub> remaining in the headspace at the start of the next iteration, before equilibrium is achieved. The partial pressure of CO<sub>2</sub> in the bottle headspace at the start of the iteration can then be calculated from Eq. (10).

$$P_{CO_2,I} = \frac{C_H RT}{V_H} \quad (10)$$

The new value of  $C_T$  is then used in Eq. (6) for a subsequent iteration, and  $P_{CO_2,E}$  is calculated as above using Eq. (2). Because there is some CO<sub>2</sub> in the bottle headspace at the start of all iterations  $i > 1$ , Eq. (7) is not valid for  $i > 1$ , and Eq. (11) must be used.

$$P_{T,i} = 1.0 \text{ atm} + P_{CO_2,i,F} - P_{CO_2,i,I} + \frac{P_{H_2O} V_{R,i-1}}{V_H} \quad (11)$$

Equations (8) & (9) are then applied as with the first iteration. This model identifies two sources of error in the simple method for measurement of total IC: CO<sub>2</sub> (aq) remaining in solution, which causes a negative bias, and H<sub>2</sub>O (g) that contributed to headspace pressure and, therefore, gas volume removed, which causes a positive bias. To estimate errors in the simple method presented in this paper, this model can be used to predict volume removals for a sample bottle with a defined sample, and a control bottle. Equation (1) can then be applied to predict total volumes removed and the error can be predicted by comparing IC calculated from Eq. (1) to the specified value.

Conversely, this model can be used to correct measurements for the error due to water evaporation and CO<sub>2</sub> (aq) remaining in solution. For the first headspace gas removal, control bottles fully compensate for water vapor effects, and no correction is needed. For subsequent bottles, water vapor can only increase headspace pressure in proportion to the volume of gas removed in the previous sampling event, as in Eq. (11). The effect of water vapor partial pressure can be easily eliminated, by application of Eq. (12) to sample and control bottles.

$$V'_{R,i} = V_{R,i} - P_{H_2O} V_{R,i-1} \quad (12)$$

The error caused by CO<sub>2</sub> (aq) remaining in solution can easily be estimated given an estimate of  $P_{CO_2}$  in the bottle headspace at the end of the analysis (this can be estimated based on the volume of gas removed). It is necessary to have an estimate of  $P_{CO_2}$  after equilibration but before removal of the current headspace sample. This is necessary because it is this value, and not the partial pressure after headspace volume removal, that determines CO<sub>2</sub> (aq) in solution during the time of the headspace gas removal. Where gas removal occurs sequentially until pressure accumulation is insignificant, this value will be essentially equal to the partial pressure after headspace gas removal. At the end of the first iteration, this pressure can be estimated using Eq. (13).

$$P_{CO_2,E,i} = \frac{V_{R,S,i} - V_{R,C,i}}{V_H} 1.0 \text{ atm} \quad (13)$$

At the start of the second iteration, before any further transfer of CO<sub>2</sub> takes place from the solution to the headspace,  $P_{CO_2}$  can be estimated from Eq. (14).

$$P_{CO_2,I,i} = \frac{V_{R,S,i-1} - V_{R,C,i-1}}{V_H + V_{R,S,i-1}} 1.0 \text{ atm} \quad (14)$$

To calculate the partial pressure of CO<sub>2</sub> beyond this Eqs. (15) & (16) must be used.

$$P_{CO_2,E,i} = \frac{P_{CO_2,I,i} V_H + 1.0 \text{ atm} V_{R,i} - P_{H_2O} V_{R,i-1}}{V_H} \quad (15)$$

$$P_{CO_2,I,i} = \frac{P_{CO_2,I,i-1} V_H + 1.0 \text{ atm} V_{R,i-1} - P_{H_2O} V_{R,i-2}}{V_H + V_{R,i-1}} \quad (16)$$

Given an estimate of  $P_{CO_2,E}$  before the final gas removal, CO<sub>2</sub> (aq) remaining in solution at this point can be calculated using an estimate of Henry's constant. A corrected estimate of IC in the sample can then be made, by using the sum of corrected volumes removed in Eq. (1), and adding the CO<sub>2</sub> (aq) remaining in solution. A spreadsheet that implements this model is available from the corresponding author. Application of this model requires an estimate of the relative humidity of the bottle headspace at apparent equilibrium.