

1 ***Supplementary Material***

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3 **Fixed-point titration method for the determination of ammonium in anaerobic**

4 **systems**

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19 In the solution, the charge balance at pH₁ 8.75 can be obtained as follows:

$$\begin{aligned}
 20 \quad & [\text{NH}_4^+]_1 + [\text{H}^+]_1 + n[\text{M}^{n+}]_1 = [\text{VFA}^-]_1 + [\text{OH}^-]_1 + [\text{X}^-]_1 + [\text{H}_2\text{PO}_4^-]_1 + 2[\text{HPO}_4^{2-}]_1 \\
 21 \quad & \quad \quad \quad + 3[\text{PO}_4^{3-}]_1 + [\text{HCO}_3^-]_1 + 2[\text{CO}_3^{2-}]_1 + [\text{HS}^-]_1 + 2[\text{S}^{2-}]_1 \quad (1)
 \end{aligned}$$

22 where [] indicates the molarity, Mⁿ⁺ is the metal ion, and X⁻ is the halide ion.

23 At this endpoint, the volume of sample solution increased from initial V₀ to V₁,

24 Eq. (1) multiplied by V₁ became:

$$\begin{aligned}
 25 \quad & [\text{NH}_4^+]_1 V_1 + [\text{H}^+]_1 V_1 + n[\text{M}^{n+}]_1 V_1 = [\text{VFA}^-]_1 V_1 + [\text{OH}^-]_1 V_1 + [\text{X}^-]_1 V_1 + [\text{H}_2\text{PO}_4^-]_1 V_1 \\
 26 \quad & \quad \quad \quad + 2[\text{HPO}_4^{2-}]_1 V_1 + 3[\text{PO}_4^{3-}]_1 V_1 + [\text{HCO}_3^-]_1 V_1 \\
 27 \quad & \quad \quad \quad + 2[\text{CO}_3^{2-}]_1 V_1 + [\text{HS}^-]_1 V_1 + 2[\text{S}^{2-}]_1 V_1 \quad (2)
 \end{aligned}$$

28 The sodium hydroxide solution at a concentration of C_b continued to titrate until

29 pH₂ 9.75. The volume of sample solution became V₂ with added volume V_X. The

30 equation of charge balance can be reformulated:

$$\begin{aligned}
 31 \quad & [\text{NH}_4^+]_2 + [\text{H}^+]_2 + n[\text{M}^{n+}]_2 + [\text{Na}^+]_2 = [\text{VFA}^-]_2 + [\text{OH}^-]_2 + [\text{X}^-]_2 + [\text{H}_2\text{PO}_4^-]_2 \\
 32 \quad & \quad \quad \quad + 2[\text{HPO}_4^{2-}]_2 + 3[\text{PO}_4^{3-}]_2 + [\text{HCO}_3^-]_2 + 2[\text{CO}_3^{2-}]_2 \\
 33 \quad & \quad \quad \quad + [\text{HS}^-]_2 + 2[\text{S}^{2-}]_2 \quad (3)
 \end{aligned}$$

34 Similarly, Eq. (3) multiplies by V₂ became:

$$\begin{aligned}
 35 \quad & [\text{NH}_4^+]_2 V_2 + [\text{H}^+]_2 V_2 + n[\text{M}^{n+}]_2 V_2 + [\text{Na}^+]_2 V_2 = [\text{VFA}^-]_2 V_2 + [\text{OH}^-]_2 V_2 + [\text{X}^-]_2 V_2 \\
 36 \quad & \quad \quad \quad + [\text{H}_2\text{PO}_4^-]_2 V_2 + 2[\text{HPO}_4^{2-}]_2 V_2 + 3[\text{PO}_4^{3-}]_2 V_2 \\
 37 \quad & \quad \quad \quad + [\text{HCO}_3^-]_2 V_2 + 2[\text{CO}_3^{2-}]_2 V_2 + [\text{HS}^-]_2 V_2 \\
 38 \quad & \quad \quad \quad + 2[\text{S}^{2-}]_2 V_2 \quad (4)
 \end{aligned}$$

39 Eq. (4) subtracts Eq. (2) to obtain:

$$\begin{aligned}
 40 \quad & [\text{NH}_4^+]_2 V_2 + [\text{H}^+]_2 V_2 + n[\text{M}^{n+}]_2 V_2 + n[\text{B}^{n+}]_2 V_2 - \{[\text{NH}_4^+]_1 V_1 + [\text{H}^+]_1 V_1 + n[\text{M}^{n+}]_1 V_1\} = \\
 41 \quad & [\text{VFA}^-]_2 V_2 + [\text{OH}^-]_2 V_2 + [\text{X}^-]_2 V_2 + [\text{H}_2\text{PO}_4^-]_2 V_2 + 2[\text{HPO}_4^{2-}]_2 V_2 + 3[\text{PO}_4^{3-}]_2 V_2 + [\text{HCO}_3^-]_2 V_2 \\
 42 \quad & + 2[\text{CO}_3^{2-}]_2 V_2 + [\text{HS}^-]_2 V_2 + 2[\text{S}^{2-}]_2 V_2 - \{[\text{VFA}^-]_1 V_1 + [\text{OH}^-]_1 V_1 + [\text{X}^-]_1 V_1 + [\text{H}_2\text{PO}_4^-]_1 V_1 \\
 43 \quad & + 2[\text{HPO}_4^{2-}]_1 V_1 + 3[\text{PO}_4^{3-}]_1 V_1 + [\text{HCO}_3^-]_1 V_1 + 2[\text{CO}_3^{2-}]_1 V_1 + [\text{HS}^-]_1 V_1 + 2[\text{S}^{2-}]_1 V_1\} \quad (5)
 \end{aligned}$$

44 In the range of pH₁ 8.75 to pH₂ 9.75, a certain amount of ammonium would
 45 become NH₃ molecule. Nevertheless, the solubility of ammonia in the water is as high
 46 as 1:700 (v/v), the total ammonia nitrogen can be considered as constant. Thus, the
 47 ammonium concentration is expressed according to the ionization equilibrium:

$$48 \quad [NH_4^+]V = T_{AN} / (1 + K_{NH_4^+} / (H^+)) = T_{AN} / f_{NH_4^+} (H^+) \quad (6)$$

49 where T_{AN} is the mole number of ammonium in the sample, and K_{NH₄⁺} is the
 50 dissociation constant of ammonium (i.e., 9.25 at 25 °C), (H⁺) denotes the activity of
 51 H⁺.

52 Substituting Eq. (6) into Eq. (5) and eliminating the carbonate, bicarbonate,
 53 VFAs, sulfide and phosphate, the ammonium concentration can be expressed:

$$54 \quad C_{AN} = \frac{T_{AN}}{V_0} = \frac{(10^{-pH_2} - 10^{pH_2-14}) \times V_2 + (10^{pH_1-14} - 10^{-pH_1}) \times V_1 + (V_2 - V_1) \times C_b}{\left[1 / (1 + K_{NH_4^+} / 10^{-pH_1}) - 1 / (1 + K_{NH_4^+} / 10^{-pH_2}) \right] \times V_0} \quad (7)$$

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57 **Table S1.** Concentrations of chemical reagents used in the experiments.

Reagent	Concentration
NH_4Cl	From 15 to 230 mmol/L
KH_2PO_4	0, 2, 4, 6, 8, 10 mmol/L
CH_3COOH	0, 6, 12, 18, 24, 30 mmol/L
NaCl	0, 0.1, 0.2, 0.3, 0.4 mol/L
NaHCO_3	20 mmol/L
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	10 mmol/L
NaOH	0.3 mol/L
HCl	12 mol/L
$\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$	1.77 mol/L

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59 **Table S2.** Components and their concentrations in the synthetic reactor effluents

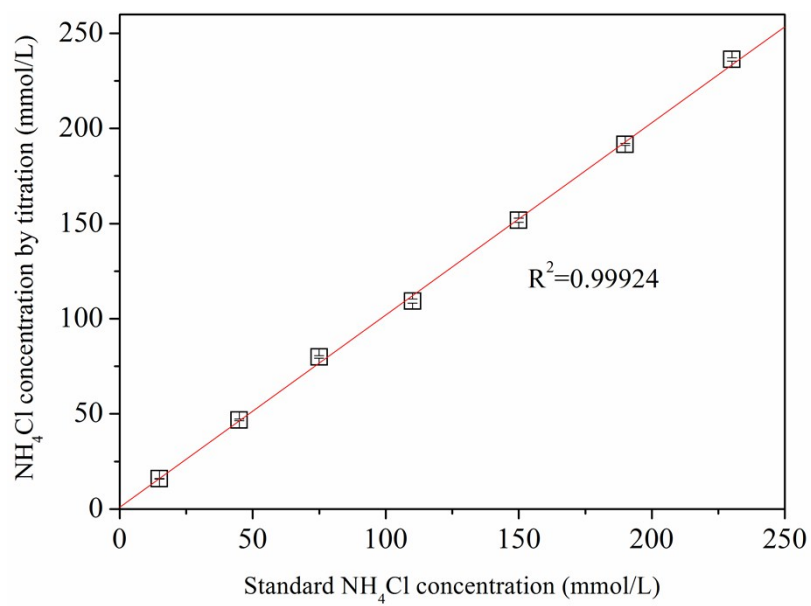
Component	Concentration
NH ₄ Cl	5, 25, 75, 125 mmol/L
KH ₂ PO ₄	0, 2, 4, 6, 8, 10 mmol/L
CH ₃ COOH	0, 6, 12, 18, 24, 30 mmol/L
NaHCO ₃	20 mmol/L
NaCl	0.2 mol/L

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62 **Table S3.** Comparison of titrated and standard ammonium concentrations.

Standard concentration (mmol/L)	Titrated concentration (mmol/L)	Relative standard deviation (%)
14.92	15.33 ± 0.02	1.05
44.94	46.83 ± 0.99	0.85
74.99	79.88 ± 1.70	0.85
110.08	109.29 ± 2.81	1.04
149.93	151.76 ± 2.68	0.71
189.98	191.60 ± 1.20	0.25
230.08	236.25 ± 2.25	0.38

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66 **Figure S1.** Comparison of ammonium concentration measured by titration and
67 standard ammonium concentration.