

Supplementary information for:

**Amending anaerobic bioreactors with pyrogenic carbonaceous materials:
the influence of material properties on methane generation**

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Supplementary methods

1. Soluble COD of particle amended deionized water (Table S1).

Deionized water (20 mL) was mixed with particles in 50 mL tubes, and the tubes were shaken by hand and then allowed to stand for 24 hours (25 °C). Supernatant was filtered through polyethersulfone syringe filters (pore size 0.2 µm; VWR International, Radnor, PA, USA) and soluble COD (SCOD) was measured using Hach Method 8000 (DR/890 Portable Colorimeter; Hach, Loveland, CO, USA). The highest particle-to-working volume ratio (3.4 g-particles per 100 mL) was used.

2. pH of particle-amended deionized water and swine wastewater (Table S2).

Deionized water (20 mL) or the swine wastewater mixture [seed:feed = 1:30 (v:v); seed and feed collected on May 17, 2016] was mixed with particles in 50 mL tubes, and the tubes were shaken by hand and then allowed to stand for 5 min (25 °C). Supernatant pH was measured using an Orion 3-Star benchtop pH meter equipped with an Orion ROSS Ultra Refillable pH/ATC Triode (Thermo Scientific, Waltham, MA, USA). The highest particle-to-working volume ratio (3.4 g-particles per 100 mL) was used.

3. Abiotic volatile fatty acid (VFA) adsorption in sterile swine wastewater amended with particles (Fig. S2).

The swine wastewater mixture [seed:feed = 1:30 (v:v); seed and feed collected on May 17, 2016] and amended particles were autoclaved before use. The sterile swine wastewater mixture (100 mL) was mixed with sterile particles in 125 mL serum bottles. The bottles were incubated on shakers at 30 °C for 19 days. Supernatant VFAs were determined using a Dionex ICS-5000+ Ion Chromatography system with a conductivity detector and Dionex IonPac AS11-HC column

(Thermo Scientific, Waltham, MA, USA). The lowest particle loading (2.2 g-particles per g-VS_{seed} before sterilization) was used.

4. Abiotic CH₄ adsorption in deionized water amended with particles (Fig. S3).

Gas containing either 100% CH₄ or 10% CH₄ was directly injected into 20 mL of deionized water in 25 mL serum bottles amended with particles (headspace pressure balanced with air during injection). The highest particle-to-working volume ratio (3.4 g-particles per 100 mL) was used. The bottles were incubated at 30 °C and the gas composition analyzed after 24 hours with a gas chromatograph (Model 8610C; SRI Instruments, Torrance, CA, USA) equipped with a thermal conductivity detector (TCD) and CTR I Column.

5. Abiotic total ammonia nitrogen (TAN) adsorption in NH₄Cl solutions amended with particles (Fig. S4).

NH₄Cl solutions (20 mL; initial TAN = 220 mg L⁻¹) were mixed with particles in 25 mL bottles and the bottles incubated on shakers at 30 °C for 19 days. The highest particle loading (3.4 g-particles per 100 mL) was used. The initial pH of the mixture was adjusted to 7.0 with 6 M HCl. The final TAN concentrations in the supernatant were measured using Hach Method 10031 (DR/890 Portable Colorimeter; Hach, Loveland, CO, USA).

57 **Table S1.** Soluble COD of particle-amended deionized water.

	Particle size	Graphite	Biochar	Activated carbon	Glass
SCOD (mg L ⁻¹)	G P	13 ± 7 21 ± 7	8 ± 2 16 ± 4	11 ± 5 23 ± 17	13 ± 1 17 ± 5

58 Average ± range ($n = 2$). G – granular; P – powdered. A loading of 3.4 g-particles per 100 mL was used.

59 **Table S2.** pH of particle-amended deionized water and swine wastewater.

	Particle size	Graphite	Biochar	Activated carbon	Glass	No-particle
Deionized water	G	5.2 ± 0.03	8.5 ± 0.02	9.3 ± 0.02	9.1 ± 0.02	
	P	5.3 ± 0.01	9.0 ± 0.01	9.4 ± 0.03	9.0 ± 0.02	
Swine wastewater	G	7.3 ± 0.01	7.7 ± 0.02	8.0 ± 0.02	7.8 ± 0.03	7.6 ± 0.04
	P	7.4 ± 0.02	7.8 ± 0.01	8.1 ± 0.03	7.7 ± 0.03	

60 Average ± range ($n = 2$). G – granular; P – powdered. A loading of 3.4 g-particles per 100 mL was used.

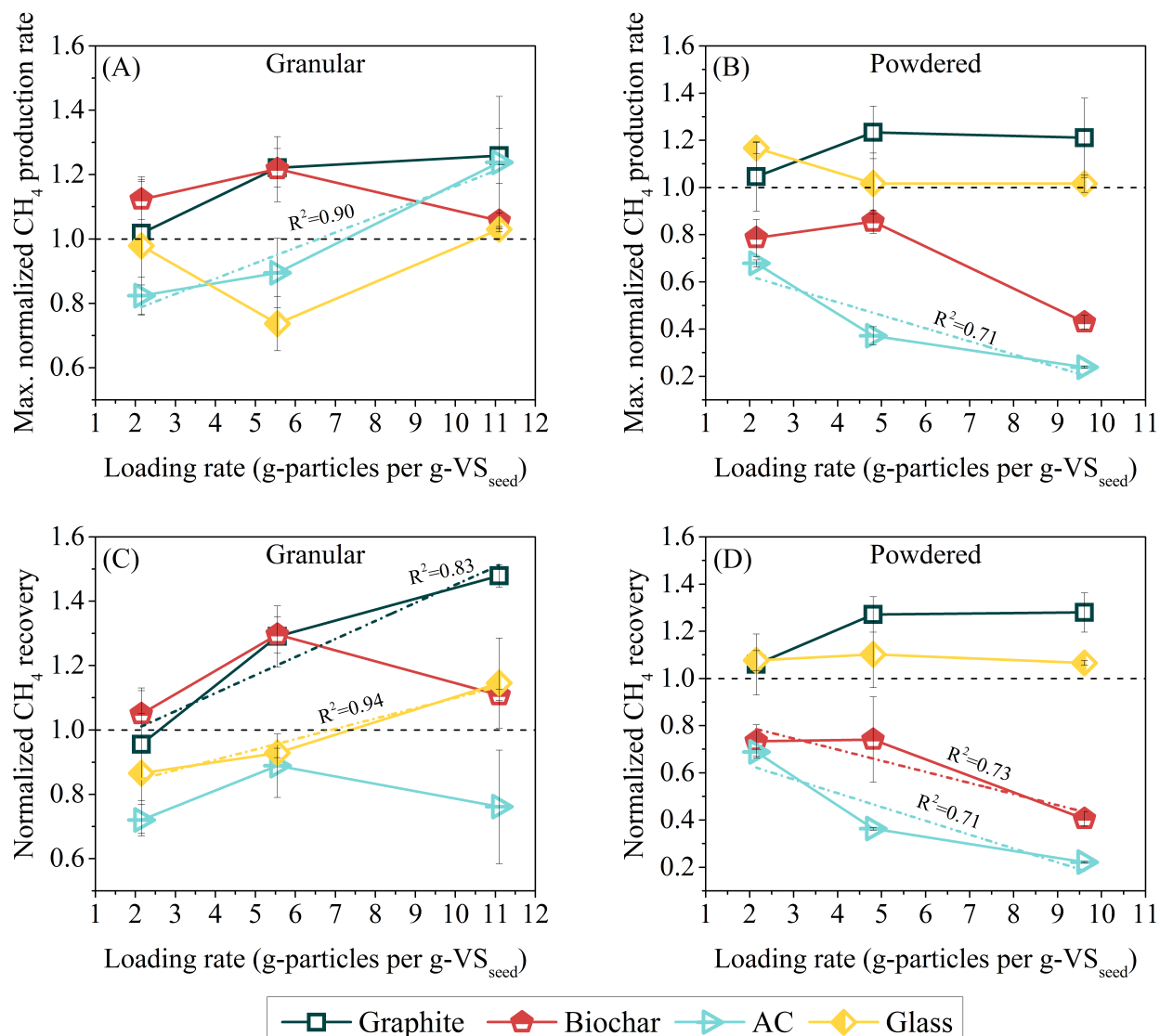


Fig. S1. Maximum CH₄ production rates as a function of particle loadings from (A) granule-amended reactors and (B) powder-amended reactors. CH₄ recoveries as a function of particle loading from (C) granule-amended reactors and (D) powder-amended reactors. Error bars represent the range of replicate experiments ($n = 2$). Correlation coefficients greater than 0.7 are shown.

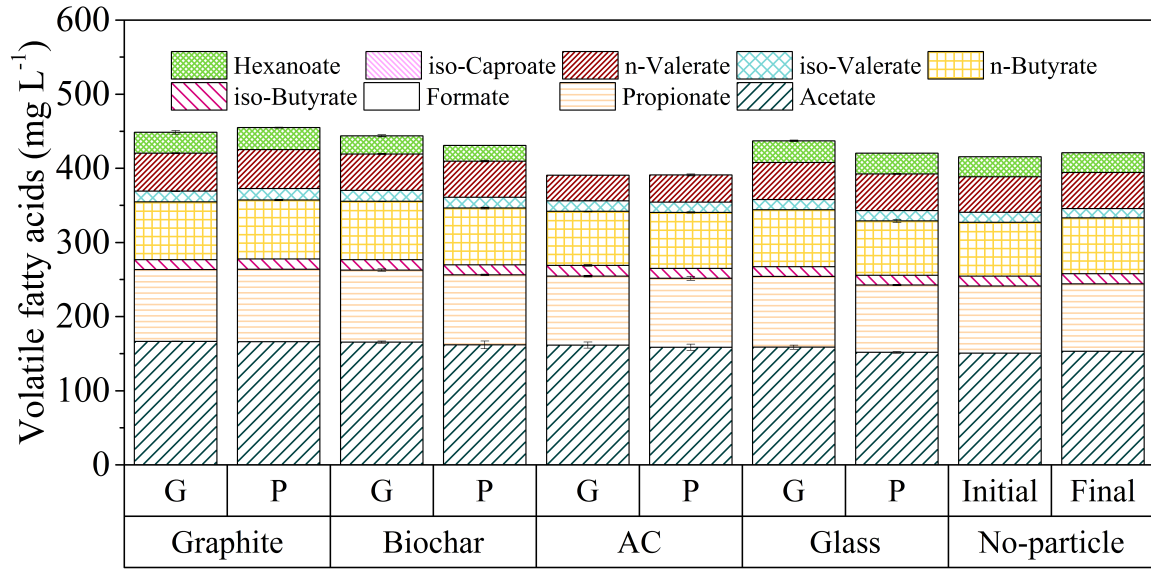


Fig. S2. Volatile fatty acid (VFA) adsorption in sterile swine wastewater amended with particles. The minimum detection limit was 20 mg L^{-1} . Concentrations below this limit are not shown. The lowest particle loading ($2.2 \text{ g-particles per g-VS}_{\text{seed}}$ before sterilization) was used. G – granular; P – powdered. Error bars represent the range of replicate experiments ($n = 2$).

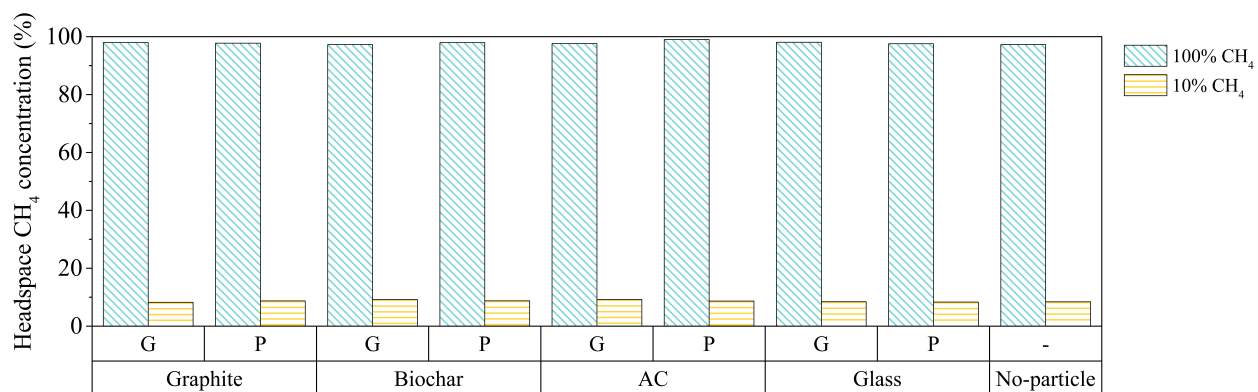


Fig. S3. Abiotic CH₄ adsorption in deionized water amended with particles. Blue diagonal lines represent the CH₄ concentrations after adsorption with 100% CH₄ initially injected. Yellow horizontal lines represent the CH₄ concentrations after adsorption with 10% CH₄ initially injected. The highest particle-to-working volume ratio (3.4 g-particles per 100 mL; $n = 1$) was used. A 24 hour incubation was used. G – granular; P – powdered.

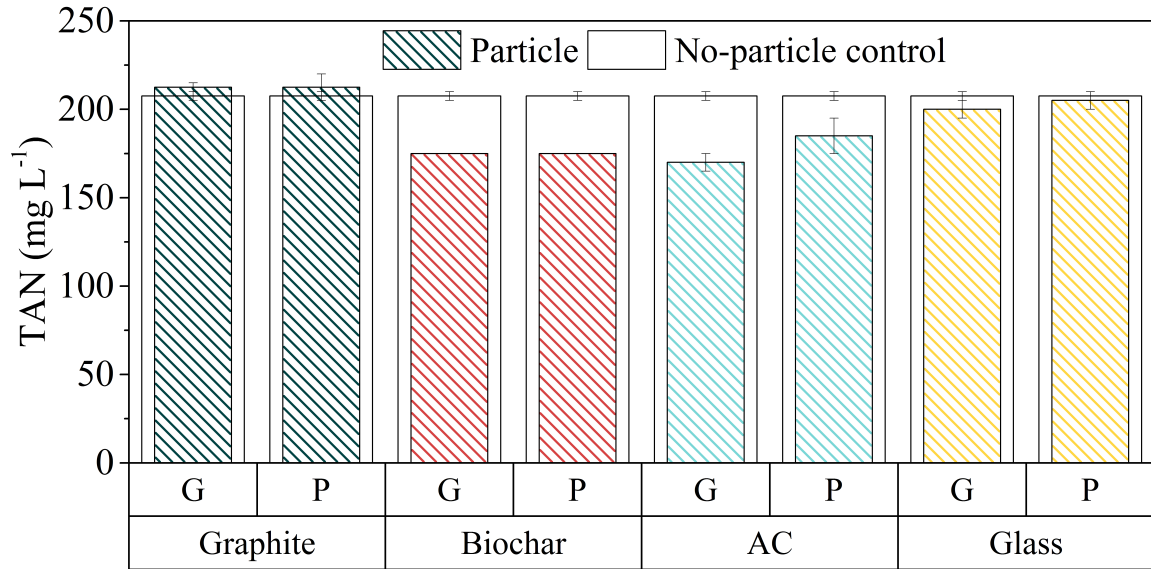


Fig. S4. Total ammonia nitrogen (TAN) adsorption in NH_4Cl solutions amended with particles under abiotic conditions. The highest particle-to-working volume ratio (3.4 g-particles per 100 mL) was used. A 19 day incubation was used. G – granular; P – powdered. Error bars represent the range of replicate experiments ($n = 2$).