

Supplementary Information

for

Carbon-based Materials in Anaerobic Digestion for Methane Production: Unraveling the Multi-faceted Mechanisms and Shaping Future Perspectives

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Methanogen Genus / Group	Primary Metabolic Pathway	Typical generation time/incubation period	Typical Optimal pH Range	Typical Optimal Temperature Range	Representative Maximum Methane Yield Reported (Context)
<i>Methanosarcina</i>	Aceticlastic methanogenesis (cleavage of acetate to CH ₄ and CO ₂); some utilize methanol/methylamines	~12.6-36.5 h	~7.0	35-37°C	368 mL CH ₄ /g acetic acid (1); 8.1 mL CH ₄ (15.5 mg±0.5 mg methanol) (2)
<i>Methanotherix</i> (<i>Methanosaeta</i>)	Obligate aceticlastic methanogenesis (exclusive use of acetate)	>10 d (low acetate concentration, syntrophic growth)	7.0-7.5	37°C	373 mL CH ₄ /g acetic acid (3)
<i>Methanoculleus</i>	Hydrogenotrophic methanogenesis (H ₂ /CO ₂ as main substrates); some use formate/secondary alcohols	~1-2 d (inferred from acetate-assimilating activity)	6.7-8.0	37°C	37.6-41.6 mmol CH ₄ gVSS ⁻¹ d ⁻¹ (4)
<i>Methanobacterium</i>	Hydrogenotrophic methanogenesis (H ₂ /CO ₂ utilization)	~5.4-24 h	7.0-7.5	37°C	9.21 mL CH ₄ ·L ⁻¹ ·h ⁻¹ (5)
<i>Methanomassiliicoccus</i>	Methylotrophic methanogenesis (methyl compounds + H ₂)	~24-30 h	7.0-7.5	37°C	192 CH ₄ nmol min ⁻¹ mg ⁻¹ protein (6)
<i>Methanospirillum</i>	Hydrogenotrophic methanogenesis (H ₂ /CO ₂ , formate); some use 2-propanol	~23-35 h	7.0-7.5	35-45°C	1.1-1.8 μmol CH ₄ · h ⁻¹ mg ⁻¹ protein (7)
<i>Methanobrevibacter</i>	Hydrogenotrophic methanogenesis (H ₂ /CO ₂ , formate)	~24-48 h	7.0-8.0	36-42°C	71 mL CH ₄ (554 mg VFAs) (8)

Table S1. Core Metabolic Characteristics and Fermentation Parameters of Methanogen Genera

Table S2. Comparison Table of Methane Yields in Anaerobic Co-Digestion (AcoD) Systems with Different Carbon Sources

Category	Carbon Source Combination	Methane Yield (with units and key conditions specified)
Previous Research	Food Waste (FW) + Algae (AL) (9)	334 mL CH ₄ /g COD _(input) (optimal ratio AL:FW = 2:8)
	Durian Shell (DS) + Pig Manure (PM) (10)	224.8 mL/g VS (optimal ratio DS:PM = 1:1)
	Food Waste (FW) + Waste Activated Sludge (WAS) (11)	293.6 mL/g VS (bio-co-pretreatment group, OLR = 11.25 g VS/(L·d))
	Poultry Dung (PD) + Alkali-Pretreated Compressed Wheat Straw (BWS _(add) , 2% KOH) (12)	254.25 NL CH ₄ /kg VS (thermophilic condition, 53°C)
	Raw Sludge (S) + Food Waste Leachate (F) + Microalgae Biomass (A) (13)	176 mL CH ₄ /g VS (optimal ratio 1S:1F:1A)
	Buckwheat Hull (BH) + Slaughterhouse Waste (SW) (14)	485 NL kgVS ⁻¹ (optimal combination)
	Lipids + Food Waste (FW) (15)	Thermophilic: 288 mL CH ₄ /g COD; Mesophilic: 275 mL CH ₄ /g COD (Lipids/TS ≤ 50%)
	Corn Straw (CS) + Straw Depolymerization Wastewater (SDW) (16)	214.81 mL/g-VS (optimal substrate concentration 36.2 g/L, thermophilic 55±1°C)
Research on the Addition of Carbon-Based Materials	Organic Fraction of Municipal Solid Waste (OFMSW) + Steam-Exploded Giant Reed (GR) (17)	235.9 mL/g VS (optimal ratio OFMSW:GR = 75:25, mesophilic 37°C)
	Aloe Peel (AP) + Dairy Manure (DM) (18)	339.64 mL/g VS (0.36 wt.% aloe peel-derived carbon quantum dots AP-CQDs, mesophilic 36±1°C)
	Dairy Manure (DM) (19)	454.1 mL/g VS (UBC composite accelerant, containing activated carbon, mesophilic 36±1°C)
	Acorn Slag Waste (ASW) + Dairy Manure (DM) (20)	385.8 mL/g VS (2.16 g/L aloe peel-derived biochar AP-BC, optimal ratio ASW:DM = 1:3, mesophilic 36±1°C)
	Cow Manure (CM) + Aloe Peel Waste (APW) (21)	226.84 NmL/g VS (MEC 0.6V + 0.15 wt.% coconut shell-derived biochar CBC, mesophilic 36±1°C)

	Organic Fraction of Municipal Solid Waste (OFMSW) + Waste Activated Sludge (WAS) (22)	0.193 NL/g VS (granular activated carbon 5 g/L); 0.201 NL/g VS (graphite 5 g/L); 0.211 NL/g VS (graphene oxide 0.1 g/L); 0.206 NL/g VS (carbon nanotubes 0.1 g/L) (mesophilic 35°C, HRT=21 d)
	Cow Manure (CM) + Aloe Peel Waste (APW) (23)	350.21 mL/g VS (0.20 wt.% N, P co-doped biochar, mesophilic 37±1°C)
	Cow Manure (CM) + Vegetable Residue (VR) (24)	526 mL/g VS (biochar 0.20 g/L, cumulative biogas yield, mesophilic 36±1°C)
	Cow Manure (CM) + Food Waste (Rice + Bread) (25)	78.3 mL/g VS (granular activated carbon/nano zero-valent iron 15 g/L, co-digestion, thermophilic 55°C, COD removal rate 78.29%)
Research on the Addition of Carbon-Based Materials	Cow Manure (CM) + Aloe Peel Waste (APW) (26)	348.9 mL/g VS (5 mT magnetic field + 0.36 wt.% aloe peel-derived carbon dots CQDs, mesophilic 36±1°C)
	Cow Manure (CM) + Food Waste (FW) + Rice Straw (RS) (27)	469.72 mL/g VS (coconut husk biochar 15 g/L, optimal ratio CM30:FW50:RS20, mesophilic 35-40°C)
	Cow Manure (CM) + Corn Straw (CS) (28)	309 mL/g VS (granular activated carbon 15.38 g/L, optimal OLR=3.0 g VS/(L·d), C/N=25, mesophilic, VS removal rate 62.41%)
	Hulless Barley Straw + Pig Manure (29)	362.89 mL/g VS (activated carbon, mesophilic, 40.47% higher than control)
	Cow Manure (CM) + Aloe Peel Waste (APW) (30)	396.09 mL/g VS (2.625 g/L Fe/Ni-N-C, bimetal-modified nitrogen-doped coconut shell biomass carbon, mesophilic 37°C)

Table S3. Summary of Dominant Microbial Community Succession in Anaerobic Digestion Systems with Different Carbon-based Materials (CBMs)

The Type Of CBMs	Dominant Bacterial Community	Dominant Archaeal Community
Biochar (31)	<i>Geobacter sp.</i> (Detected in all sets); <i>Clostridium sp.</i> , <i>Bacillus sp.</i> , <i>Bacteroides sp.</i> , <i>Anaerobaculum sp.</i> (Present)	<i>Methanosarcina sp.</i> (215% increase); <i>Methanothermobacter sp.</i> , <i>Methanobrevibacter sp.</i> (Enriched)
Woody Biochar (32)	Lactobacillales (23.95%); Clostridiales (Predominant in later stage)	<i>Methanosarcina</i> (Dominant); <i>Methanoculleus</i> (Increased to 47.43% on day 2 vs control's 0.41%)
Carbon-Nanotube Hollow-Fiber (33)	<i>Pelobacter</i> (10.0% vs control's trace); <i>Geobacter</i> (6.9% vs control's 2.8%); <i>Aminiphilus</i> (10.0% vs control's 2.3%)	<i>Methanosaeta</i> (15.7% vs control's 4.4%)
Graphene (34)	<i>Pseudomonas</i> (11.73% vs control's 0%); <i>Clostridium</i> (Enriched)	<i>Methanosaeta</i> (47.1% vs control's 41.6%)
Biochar (35)	<i>Syntrophomonas</i> (19-21% increase); <i>Clostridium sensu stricto</i> (8-249% increase); <i>Petrimonas</i> (Enriched)	<i>Methanotherrix</i> (45.73-52.42% vs control's 45.73%); <i>Methanobacterium</i> (15.27% on day 3 vs control's 14.83%)
Carbon Quantum Dots (18)	<i>Proteiniclasticum</i> (39.70% vs 37.56%, +2.14%); <i>Sporosarcina</i> (5.41% vs 0.76%, +4.65%); <i>Petrimonas</i> (10.40% vs 6.27%, +4.13%)	<i>Methanosarcina</i> (54.20% vs 31.63%, +22.57%); <i>Methanobacterium</i> (30.01% vs 24.38%, +5.63%)
Carbon Cloth (36)	<i>Sporanaerobacter</i> (34% vs 2%, +32%); <i>Enterococcus</i> (10% vs <1%, +>9%)	<i>Methanosarcina</i> (68% vs control's dominant <i>Methanosaeta</i>)
NiFe ₂ O ₄ -MXene@Carbon Felt (37)	<i>Trichococcus</i> (23% vs <1%, +>22%); <i>Longilinea</i> (9% vs 4%, +5%); <i>Clostridium sensu stricto</i> 7 (7% vs 2%, +5%)	<i>Methanosaeta</i> (34% vs 3%, +31%)
Granular Activated Carbon (38)	<i>Acetoanaerobium</i> (34% vs 25%, +9%); <i>Proteiniborus</i> (5% vs 1%, +4%)	<i>Methanobacterium</i> (23% vs 16%, +7%); <i>Methanolinea</i> (11% vs 5%, +6%)

Graphene (39)	<i>Geobacter</i> (9.9% vs 8.4%, +1.5%); <i>Pseudomonas</i> (6.85% vs 1.91%, +4.94%)	<i>Methanobacterium</i> (34.87% vs 24.02%, +10.85%); <i>Methanospirillum</i> (7.76% vs 2.15%, +5.61%)
Reduced Graphene Oxide (40)	<i>Defluviitoga</i> (7-67%); <i>Syntrophomonas</i> (Enriched); <i>Clostridium</i> (Enriched); <i>Coprothermobacter</i> (Enriched)	<i>Methanothrix</i> (89-97%, higher than control); <i>Methanoculleus</i> (56% vs 49%, +7%)
N,P-Co-Doped Biochar (23)	<i>Proteiniclasticum</i> (39.70% vs 37.56%, +2.14%); <i>Sporosarcina</i> (5.41% vs 0.76%, +4.65%); <i>Petrimonas</i> (10.40% vs 6.27%, +4.13%)	<i>Methanosarcina</i> (54.20% vs 31.63%, +22.57%); <i>Methanobacterium</i> (30.01% vs 24.38%, +5.63%)
Carbon Quantum Dots (26)	<i>Acinetobacter</i> (44.90% vs control's 0%, +44.90%); <i>Clostridium</i> (13.71% vs 7.42%, +6.29%); <i>Petrimonas</i> (7.12% vs 5.35%, +1.77%)	<i>Methanosarcina</i> (Enriched); <i>Methanobacterium</i> (31.10% vs 4.38%, +26.72%); <i>Methanomassiliicoccus</i> (23.17% vs 5.47%, +17.70%)
Granular Activated Carbon (41)	<i>Smithella</i> (3.2% vs 2.6%, +0.6%; 14.4-82.9% increase in Phases I-III); <i>Mesotoga</i> (5.1% vs 3.3%, +53.3%)	<i>Methanothrix</i> (31.7% vs 34.2%, no increase); <i>Methanobacterium</i> (11.6-15.6%, stable vs control's 12.0-16.1%)

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