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Supplementary material

2 **Enhancing protein pre-hydrolysis via effluent recirculation towards high-rate**

3 **household food waste anaerobic digestion using expanded granular sludge bed**

4 **reactor**

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23 Fig. S1. Changes in $\text{NH}_4^+\text{-N}$ concentration (mg/L) in the expanded granular sludge bed
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25 phases (Phase I to VI) corresponding to organic loading rates ranging from 2.0 to 9.0 g
26 COD/L/d, respectively.

27 Fig. S2. Bacterial community composition at the phylum level in the expanded granular
28 sludge bed reactor under different organic loading rates (seed sludge, OLR = 4.0 g
29 COD/L/d, and OLR = 9.0 g COD/L/d).

30 Fig. S3. Archaeal community composition at the genus level in the expanded granular
31 sludge bed reactor under different organic loading rates (seed sludge, OLR = 4.0 g
32 COD/L/d, and OLR = 9.0 g COD/L/d).

33 Fig. S4. Changes in (a) $\text{NH}_4^+\text{-N}$ concentration (mg/L), (b) alkalinity (mg $\text{CaCO}_3\text{/L}$), and
34 (c) free ammonia (FA) (mg/L) in the pre-hydrolysis reactors with and without expanded
35 granular sludge bed effluent recirculation over the operational period.

36 **Table S1 The composition of household food waste.**

Category	Material	Proportion (%)	Source / Brand
Food	Rice	10	School canteen
	Noodles	4	School canteen
	Steamed bun	4	School canteen
	Bread	4	Taoli Chunjiao Sliced Bread
Vegetables	Cabbage	8	Cabbage head
	Potato	8	Potato
	Carrot	8	Carrot
	Bok choy	8	Shanghai qing
Fruits	Banana	8	Tete Banana
	Apple	8	Red Fuji apple
	Pomelo	4	Yellow-hearted honey pomelo
	Orange	4	Dekopon
Meat, fish, eggs & tofu	Chicken	5	Fresh chicken breast
	Pork	5	Lean pork
	Fish fillet	4	Basa fillet
	Tofu	4	Soft tofu
	Eggshell	4	Yonghui Farm fresh eggs

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38 **Table S2 Physicochemical properties of household food waste.**

Parameters	Units	HFW
TS	g/L	193.4 ± 0.2
VS	g/L	10.7 ± 0.1
VS/TS	-	0.9
Moisture content	%	81.0
TSS	g/L	110.2 ± 3.0
VSS	g/L	109.0 ± 2.6
TCOD	g/L	162.9 ± 12.7
SCOD	g/L	64.8 ± 14.4
pH	-	4.7
NH ₄ ⁺ -N	mg/L	11.5 ± 0.1
Total Carbohydrate	g/L	69.0
Total Carbohydrate	%TS	35.7
Soluble carbohydrate	g/L	46.7 ± 5.2
Total protein	g/L	38.7
Total protein	%TS	20.0
Soluble protein	g/L	26.3 ± 0.4
Lipids	%	12.0
Lipids	g/L	23.2
Crude fibers	g/L	27.4
Crude fibers	%TS	14.1

39 TS: Total solids; VS: Volatile solids; TSS: Total suspended solids; VSS: Volatile suspended solids;

40 TCOD: Total chemical oxygen demand; SCOD: Soluble chemical oxygen demand.

41 **Table S3 Experimental design and operating conditions of treating household food**
42 **waste using EGSB.**

Phase	Day	HRT (d)	OLR (g COD/L/d)	T (°C)
1	1–26	9	2	35
2	27–71	9	3	35
3	72–100	9	4	35
4	101–117	9	5	35
5	118–131	9	7	35
6	132–164	9	9	35

43 OLR: Organic loading rate; T: Temperature; HRT: Hydraulic retention time.

44 **Table S4 Alpha diversity index statistics of bacteria.**

	Chao	Ace	Simpson	Shannon	Coverage
Seed sludge	535.600	538.5	0.088	3.703	0.999
OLR: 4 gCOD/L/d	296.395	298.087	0.032	4.108	1.000
OLR: 9 gCOD/L/d	235.000	235.000	0.056	3.663	1.000

45 OLR: Organic loading rate.

46 **Table S5 Alpha diversity index statistics of archaea.**

	Chao	Ace	Simpson	Shannon	Coverage
Seed sludge	69.2	69.4	0.306	1.939	0.999
OLR: 4 gCOD/L/d	50.000	50.000	0.230	1.723	1.000
OLR: 9 gCOD/L/d	46.000	46.000	0.321	1.480	1.000

47 OLR: Organic loading rate.

48 **Table S6 Classification of typical methanogens and metabolic substrates in EGSB**
 49 **reactor.**

Archaea	Metabolic substrates
<i>Methanosaeta</i>	Acetic acid
<i>Methanosarcina</i>	H ₂ /CO ₂ , Acetic acid
<i>Methanobacterium</i>	H ₂ /CO ₂ , Formic acid, 2-Propano, 2-Butanol
<i>Candidatus_Methanofastidiosum</i>	H ₂ /CO ₂
<i>Methanospirillum</i>	H ₂ /CO ₂ , Formic acid
<i>Methanocorpusculum</i>	H ₂ /CO ₂ , Formic acid
<i>Methanomassiiicoccus</i>	H ₂ /CO ₂ , Formic acid
<i>Methanomethylovorans</i>	Trimethylamine, Dimethylamine, Methylamine, Methanol, Dimethyl sulfide, Methyl mercaptan
<i>Methanoculleus</i>	H ₂ /CO ₂ , Formic acid

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51 **Table S7 Characteristics of effluent from EGSB reactor.**

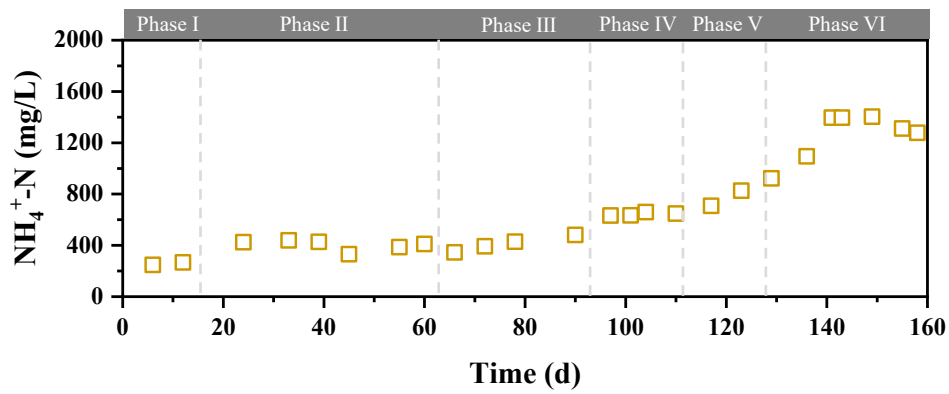
Indicators	units	value
pH		7.2 ± 0.1
TCOD	g/L	0.9 ± 0.2
SCOD	g/L	0.2 ± 0.1
Proteins	g/L	0.3 ± 0.1
Carbohydrates	g/L	0.1
NH ₄ ⁺ -N	mg/L	132.2 ± 4.9
Alkalinity	mg CaCO ₃ /L	953.1 ± 110.5
TSS	g/L	0.1
VSS	g/L	0.1

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53 **Table S8 Alpha diversity index statistics of bacteria.**

	Chao	Ace	Simpson	Shannon	Coverage
Seed sludge	286.941	289.585	0.059	3.585	1.000
No-recirculation	195.556	197.139	0.202	2.500	1.000
Recirculation	240.000	240.000	0.112	3.165	1.000

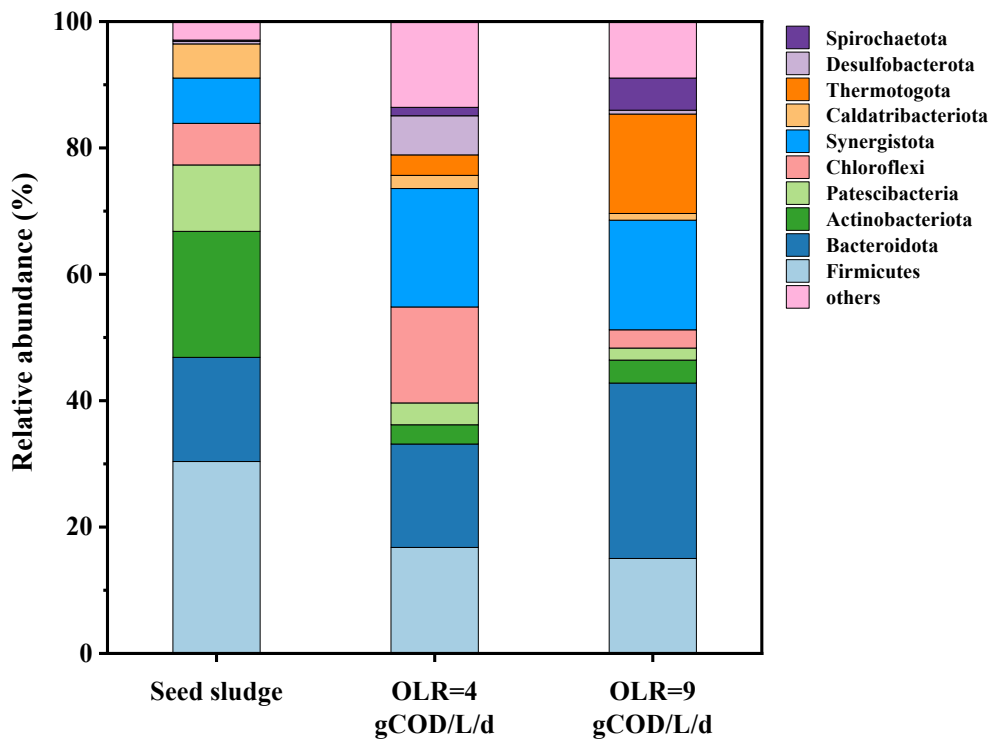
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56 **Fig. S1. Changes in NH₄⁺-N concentration (mg/L) in the expanded granular sludge**
 57 **bed reactor during the treatment of household food waste. The operation was**
 58 **divided into six phases (Phase I to VI) corresponding to organic loading rates**
 59 **ranging from 2.0 to 9.0 g COD/L/d, respectively.**

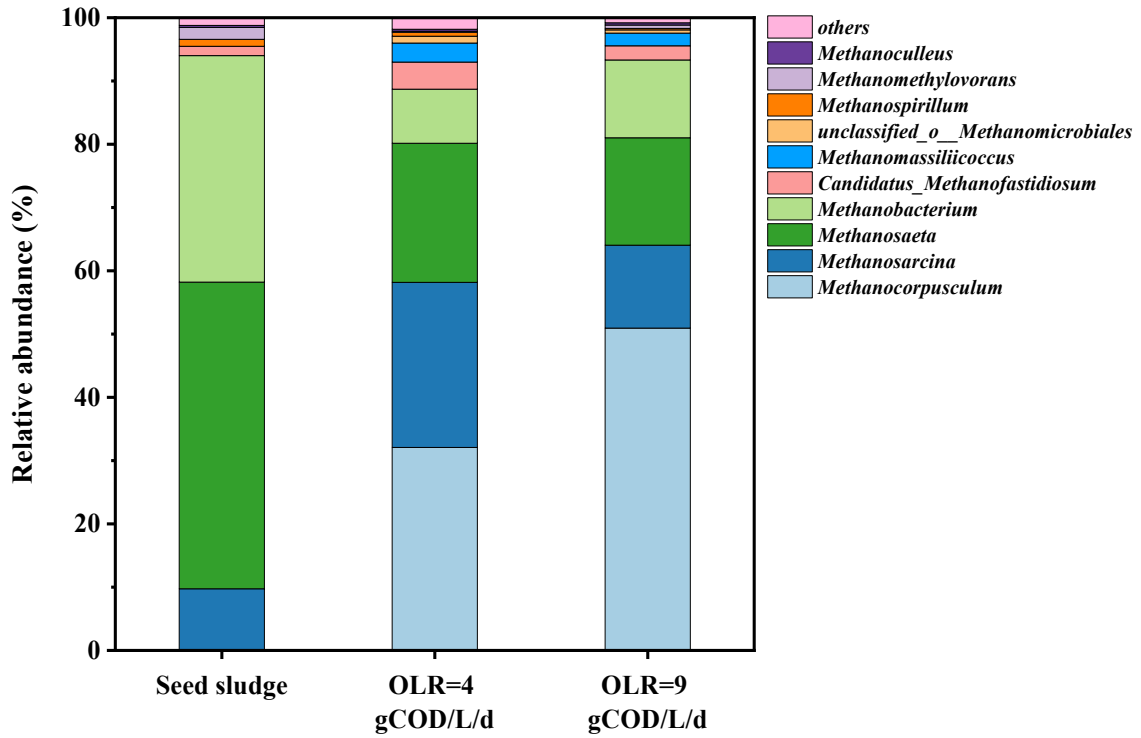
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62 **Fig. S2. Bacterial community composition at the phylum level in the expanded**
 63 **granular sludge bed reactor under different organic loading rates (seed sludge, OLR**
 64 **= 4.0 g COD/L/d, and OLR = 9.0 g COD/L/d).**

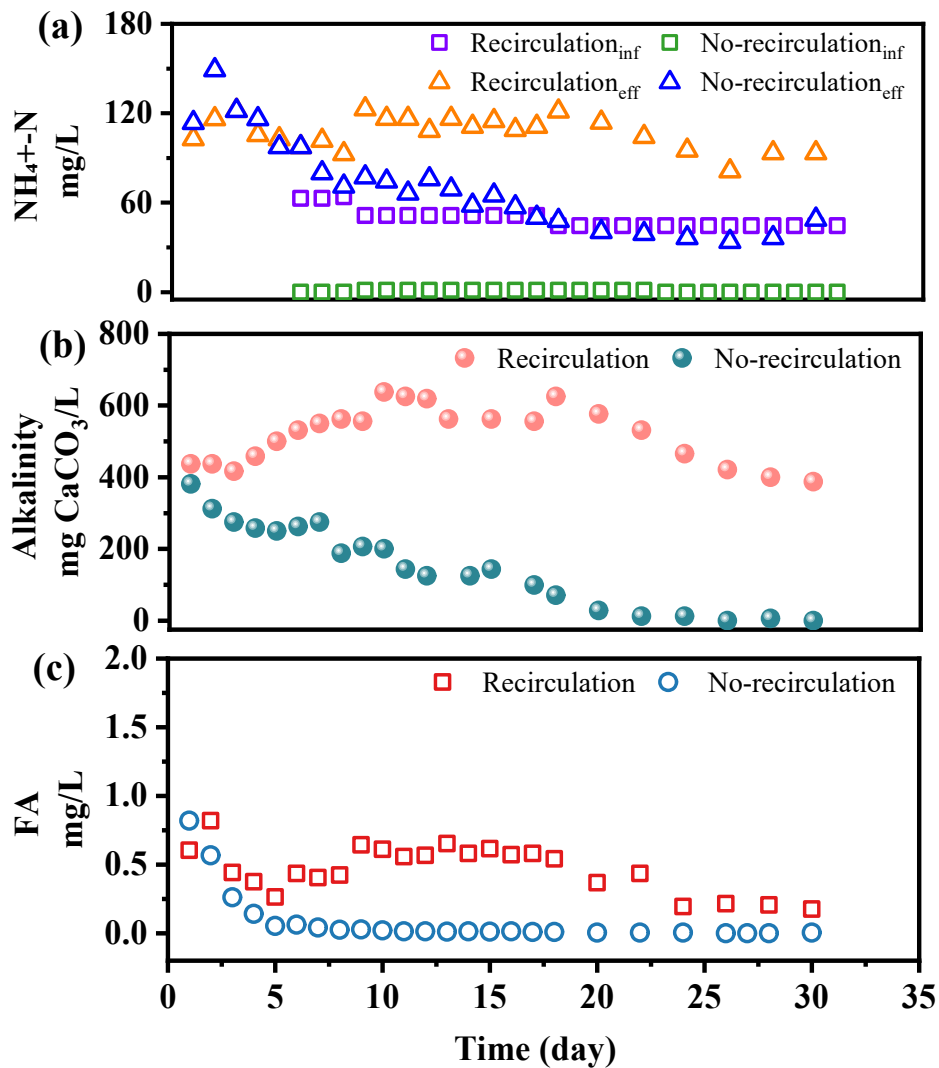
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67 **Fig. S3. Archaeal community composition at the genus level in the expanded**
 68 **granular sludge bed reactor under different organic loading rates (seed sludge, OLR**
 69 **= 4.0 g COD/L/d, and OLR = 9.0 g COD/L/d).**

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72 **Fig. S4. Changes in (a) $\text{NH}_4^+\text{-N}$ concentration (mg/L), (b) alkalinity (mg CaCO_3/L),**
 73 **and (c) free ammonia (FA) (mg/L) in the pre-hydrolysis reactors with and without**
 74 **expanded granular sludge bed effluent recirculation over the operational period.**