Supporting information

Industrial-scale biorefinery for *n*-caproate production from food waste

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Content:

Table S1 Gibbs free energy analysis for the possible reactions in CCE reactors.

	Glucose	Acetate	Ethanol	Lactate	Propionate	Butyrate	Valerate	Caproate	Heptylate	H_2O	CO_2	CH_4	H_2	H^+	$\Delta_r G_m^\Theta$
$\Delta_f G_m^\Theta$	-919.8	-369.5	-181.8	-517.1	-361.1	-352.6	-344.3	-336.0	-327.7	-237.2	-394.4	-50.7	0	0	kJ/mol
Homolactic fermentation	-1			2										2	-114.4
Ethanol fermentation	-1		2								2				-232.5
Butyric fermentation	-1					1					2		2	1	-221.5
Acetic fermentation	-1	2								-2	2		4	2	-133.4
Lactate oxidised to acetate		1		-1						-1	1		2		-9.5
Lactate oxidised to propionate				-3	2					-1	3		4	-1	-116.8
Acetate reduced to butyrate by lactate		-1		-1		1				1	1			-1	-97.6
Butyrate reduced to caproate by lactate				-1		-1		1		1	1			-1	-97.9
Acetate reduced to butyrate by ethanol		-1	-1			1				1					-38.5
Butyrate reduced to caproate by ethanol			-1			-1		1		1					-38.8
Propionate reduced to butyrate by ethanol			-1		-1		1			1					-38.6
Valerate reduced to Heptanoate by ethanol			-1				-1		1	1					-38.8
Aceticlastic methanogenesis		-1									1	1		-1	-75.6
Syntrophic acetate oxidation		-1								-2	2		4	-1	55.1
Hydrogenotrophic methanogenesis										2	-1	1	-4		-130.8

Estimation of the relationship between ammonification rate of DON in mixed MCCAs and probable increased value of effluent TN in WWTP

After the addition of the carbon source, COD and TN concentrations is increased in equations (S1-S2) in the conditioned wastewater.

$$\mathrm{COD_{mix}} = \frac{\mathrm{COD_{ww}} + \alpha \cdot \mathrm{COD_c}}{1 + \alpha} \tag{S1}$$

$$\mathrm{TN_{mix}} = \frac{\mathrm{TN_{ww}} + \alpha \cdot \mathrm{TN_c}}{1 + \alpha} \tag{S2}$$

The ratio of $\mathrm{COD_{mix}}$ to $\mathrm{TN_{mix}}$ is f_{dn}, then

$$f_{\mathrm{dn}} = \frac{\mathrm{COD_{ww}} + \alpha \cdot \mathrm{COD_c}}{\mathrm{TN_{ww}} + \alpha \cdot \mathrm{TN_c}} \tag{S3}$$

α is the dosage rate of mixed MCCAs in wastewater. α can be calculated by the deformed equation (S3).

$$\alpha = \frac{f_{\mathrm{dn}} \cdot \mathrm{TN_{ww}} - \mathrm{COD_{ww}}}{\mathrm{COD_c} - f_{\mathrm{dn}} \cdot \mathrm{TN_c}} \tag{S4}$$

The increased value of conditioned wastewater can be further identified.

$$\Delta\mathrm{TN_{mix}} = \mathrm{TN_{mix}} - \mathrm{TN_{ww}} \tag{S5}$$

Plug equation (S2) into (S5), then

$$\Delta\mathrm{TN_{mix}} = \frac{\alpha \cdot (\mathrm{TN_c} - \mathrm{TN_{ww}})}{1 + \alpha} \tag{S6}$$

Plug equation (S4) into (S6), then

$$\Delta\mathrm{TN_{mix}} = \frac{(f_{\mathrm{dn}} \cdot \mathrm{TN_{ww}} - \mathrm{COD_{ww}}) \cdot (\mathrm{TN_c} - \mathrm{TN_{ww}})}{\mathrm{COD_c} - \mathrm{COD_{ww}} - f_{\mathrm{dn}} \cdot (\mathrm{TN_c} - \mathrm{TN_{ww}})} \tag{S7}$$

DON in MCCAs typically cannot be conversed to N_2 in the activated sludge system for both nitrification-denitrification and nitritation and denitritation processes.

The minimal increased value of TN in the effluent of WWTP $\{\Delta TN_{eff}\}_{min}$ is derived in equation (S8).

$$\{\Delta TN_{eff}\}_{min} = \frac{f_{DON} \cdot \alpha \cdot TN_c}{1+\alpha} = \frac{f_{DON} \cdot TN_c \cdot (f_{dn} \cdot TN_{ww} - COD_{ww})}{COD_c - COD_{ww} - f_{dn} \cdot (TN_c - TN_{ww})} \quad (S8)$$

Table S2 Nomenclature list and information of the value range.

Parameters	Essence	Values
α	Dosage rate of mixed MCCAs in wastewater.	Calculated by Equation (S4)
COD_{ww}	Easily biodegradable COD concentrations of the wastewater with low COD/TN.	Depend on the type of wastewater
COD_c	Variable COD concentration of the mixed MCCAs.	Seeing **Fig. 4**
COD_{mix}	COD concentration of the conditioned wastewater.	Calculated by Equation (S1)
TN_{ww}	TN concentration of the wastewater with low COD/TN.	Depend on the type of wastewater
TN_c	Variable TN concentration of the mixed MCCAs.	Seeing **Fig. 4**
TN_{mix}	TN concentration of the conditioned wastewater.	Calculated by Equation (S2)
f_{dn}	Coefficient of TN and COD for nitrogen removal.	5 for nitrification-denitrification, and 3−3.5 for nitritation and denitritation process[1]
ΔTN_{mix}	Increased TN concentration of the wastewater after the addition of carbon source.	Calculated by Equation (S7)
f_{DON}	Ammonification rate of DON in the mixed MCCAs.	0−100%
$\{\Delta TN_{eff}\}_{min}$	Probable increased value of TN in the effluent of WWTP.	Calculated by Equation (S8)
$Q_{tap\ water}$	Consumed tap water during the pre-treatment process.	9−12 m^3/d
$\theta_{tap\ water}$	Unit price of tap water.	4.00 RMB/m^3
$C_{n\text{-caproate}}$	Concentration of caproate in broth after CCE reactor.	55−70 mM Average: 62 mM
Q_{FW}	Influent flow of this biorefinery plant.	131−150 m^3/d
$Q_{electric\text{-}CCE}$	Consumed electric power during the pre-treatment and CCE process.	3300−3800 kwh/d
$\theta_{electric}$	Unit price of electric power.	0.70 RMB/kwh

$C_{\text{p, water}}$	Constant-pressure specific heat capacity of water.	4.187 kJ/(kg·℃)
$C_{\text{v, water}}$	Enthalpy of evaporation of water.	2738 kJ/kg
ρ_{FW}	Density of the food waste.	Approximately 1000 kg/m^3
η	Excess ratio of steam.	15%
t_1	Primitive and heated temperature of food waste.	10−20 ℃
t_2	Heated temperature of food waste.	35 ℃
θ_{steam}	The unit price of steam.	180 RMB/tonne
R_{NaOH}	Dosage ratio of NaOH for pH condition in per tonne broth.	4−12 kg/tonne-FW[2-4]
θ_{NaOH}	The unit price of NaOH (30%).	2.00 RMB/kg
R_{ethanol}	Dosage ratio of ethanol as electron donor in per tonne broth.	3.6−6 kg/tonne-FW
θ_{ethanol}	The unit price of ethanol.	6.00 RMB/kg
R_{HCl}	Dosage ratio of HCl in the refinement and purification process.	3−8 kg/tonne-broth
θ_{HCl}	The unit price of HCl (31%).	0.30 RMB/kg
R_{sol}	Loss ratio of solvent in LL process.	0.5%−1% (Assumed)
Q_{sol}	Amount of solvent used in LL process.	100−135 m^3/d (Assumed)
θ_{sol}	The unit price of solvent, example of cyclohexane.	7.40 RMB/kg
Q_{MCCAs}	Amount of produced mixed MCCAs after CCE.	135 m^3/d
$C_{\text{v, sol}}$	Enthalpy of evaporation of solvent of LL, example of cyclohexane.	367 kJ/kg[5]
P_1	Costs of consumed tap water.	Calculated by Equation (S9)
P_2	Costs of consumed electric power in pre-treatment and CCE reactor.	Calculated by Equation (S10)
P_3	Saving costs of steam.	Calculated by Equation (S11)
P_4	Saving costs of NaOH.	Calculated by Equation (S12)
P_5	Saving costs of ethanol.	Calculated by Equation (S13)
P_6	Estimated costs of HCl for acidification.	Calculated by Equation (S14)
P_7	Estimated costs of wasted solvent.	Calculated by Equation (S15)

P_8	Estimated costs of energy of distillation.	Calculated by Equation (S16)
P_9	Estimated costs of consumed electric power during the ED-PS process.	Calculated by Equation (S17)

Note: 1 $=6.8 RMB (15 years average).

Experimental section

The solid samples were manually sorted to remove bones, shells, and other inorganic debris, and then the residual part was smashed, homogenized, and stored at -20 ℃ until use. The liquid samples were centrifuged at 10000 rpm for 15 min, and the supernatant was filtered subsequently through a 0.45 μm syringe filter prior to soluble parameters measurement. Both the filtered and unfiltered liquid samples were stored at -20 ℃ until analysis. The biochemical properties of solid sample were analysed.

pH was acquired by a glassy probe (FiveEasy Plus, Mettler Toledo, Switzerland). Suspended solids (SS) were obtained by change of dry mass with 0.45 μm filter paper. Total solid (TS) and volatile solid (VS) were determined by the standard protocol No. 2540[6]. Chemical oxygen demand (COD) was detected by kits (COD/2000, Palintest, UK) after digestion, and ammonium-nitrogen (NH_4^+-N) by the kits (Ammonia/100N, Palintest, UK). Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were tested by a total organic carbon analyser (TOC-L CPN, Shimadzu, Japan). Dissolved Kjeldahl nitrogen (DKN) was conducted with a Kjeldahl apparatus (UDK 139, VELP, Italy). The compositions of carboxylates and alcohols were analysed on a gas chromatograph (Trace 1300, Thermo, USA) equipped with a flame ionization detector, and the lactate was measured by a high-performance liquid chromatograph (LC-2030C 3D Plus, Shimadzu, Japan) equipped with a diode array detector. Concentrations of nitrate-nitrogen (NO_3^--N), and nitrite-nitrogen (NO_2^--N) were quantified with an ion-chromatography (Dionex Aquion, Thermo Fisher, USA). The phosphate (PO_4^{3-}) concentrations were assayed by a flow injection analyser (AQ400, SEAL, USA). The

soluble phosphorus (SP) content quantification was executed with an inductively coupled plasma mass spectrometry (ICP-MS) (iCAP RQ, Thermo Fisher, USA). Dissolved organic nitrogen (DON) was calculated by DN minus inorganic nitrogen NH_4^+-N, NO_3^--N and NO_2^--N. Crude Protein in solid sample was determined by Kjeldahl method (UDK 139, VELP, Italy). Carbohydrates was tested by the phenol-sulfuric acid method. Crude fat was tested and extracted by anhydrous ether according to a Chinese standard GB 5009.6-2016. Cellulose, hemicellulose, and lignin was sequentially extracted with neutral and acid detergent solvent by using FiberCap (FOSS, Denmark).

The precipitate of slurry samples after centrifugation was used for total DNA extraction with the PowerSoil DNA isolation kit (Mo-Bio Laboratories Inc., CA). The archaeal and bacterial compositions were obtained by amplification of the V4 region of the 16S rDNA using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA) and 806R (5'GGACTACVSGGGTATCTAAT) (Caporaso et al., 2012). 16S rRNA gene was sequenced on a MiSeq platform (Illumina, San Diego, CA) according to the standard protocols. Amplification, library preparation, and sequencing were performed by Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China. Data processing was conducted on our homemade servers (Duan et al., 2021). Amplicon sequence variants (ASVs) were identified by using QIIME2 (https://docs.qiime2.org/2020.2/ tutorials/), and further classified according to SILVA database (version 138) (Quast et al., 2013).

Table S3. Biochemical composition of the feedstock (% in total solid)

Crude Protein	Carbohydrates	Crude fat	Cellulose	Hemicellulose	Lignin
22.4±0.3	12.0±1.8	16.4±0.5	20.4±1.8	12.1±1.5	9.0±1.4

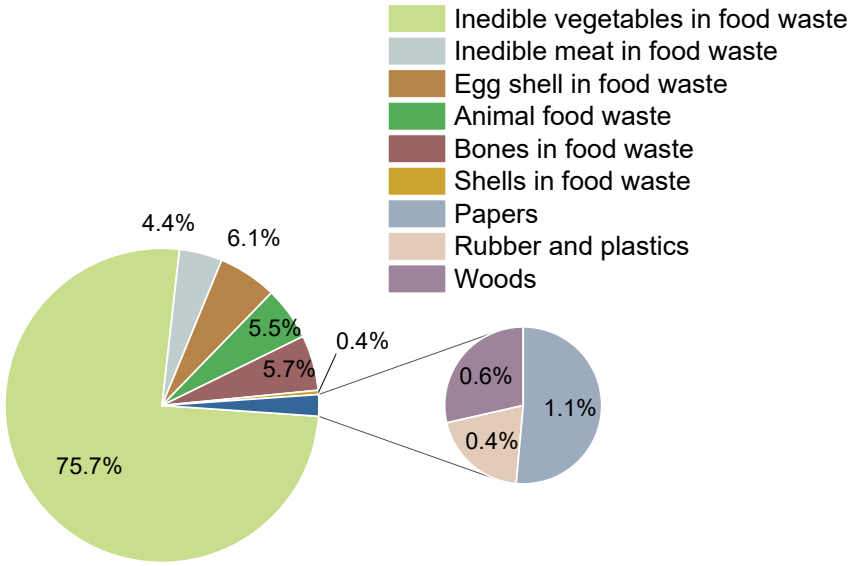

Fig. S1. Typical physical composition of raw food waste for production of *n*-caproate.

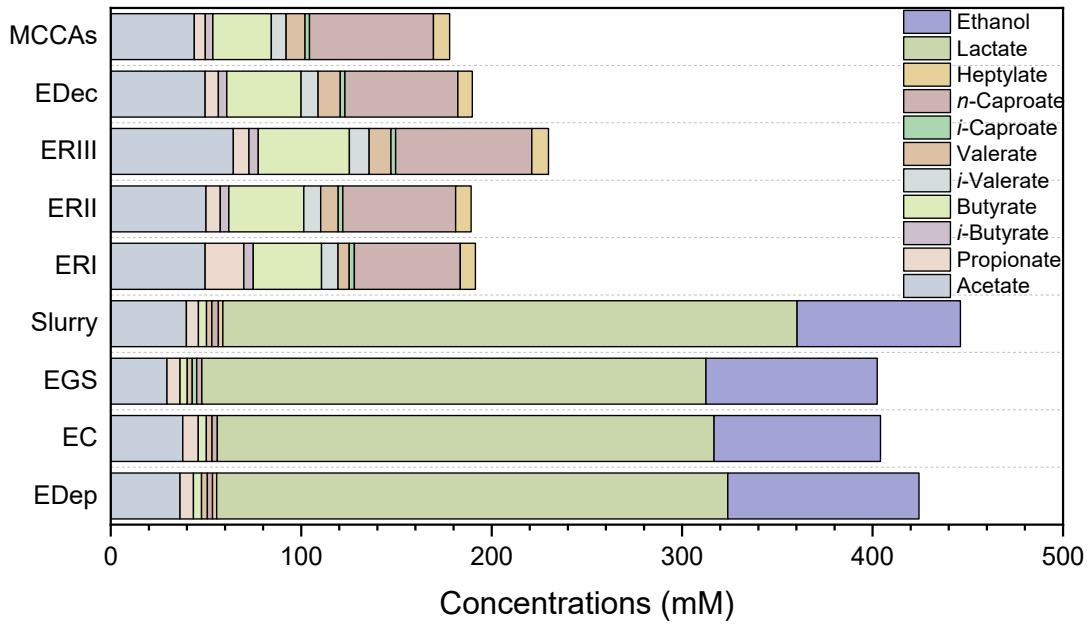

Fig. S2. The C_2–C_7 organic acids and ethanol concentrations during the pretreatment and CCE sections.

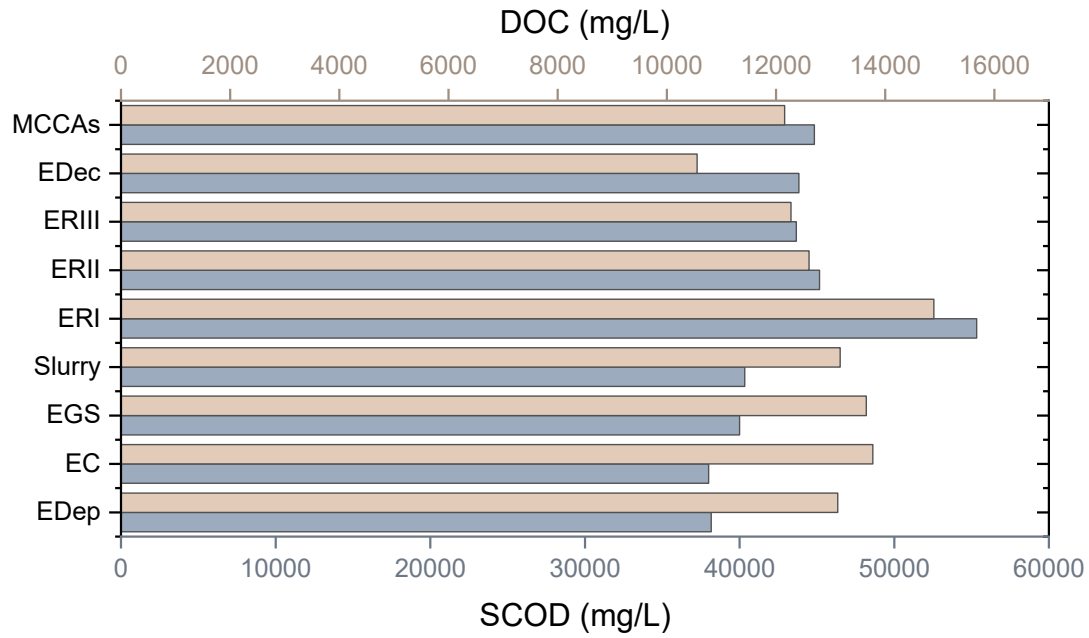

Fig. S3. The SCOD and DOC concentrations during the pretreatment and CCE sections.

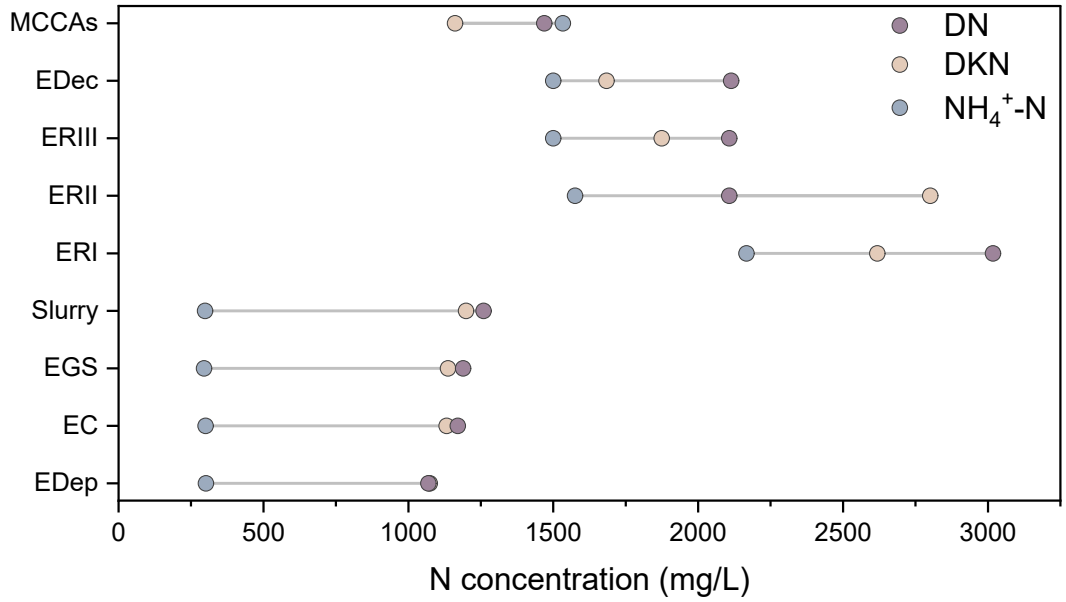

Fig. S4. N concentrations during the pretreatment and CCE sections.

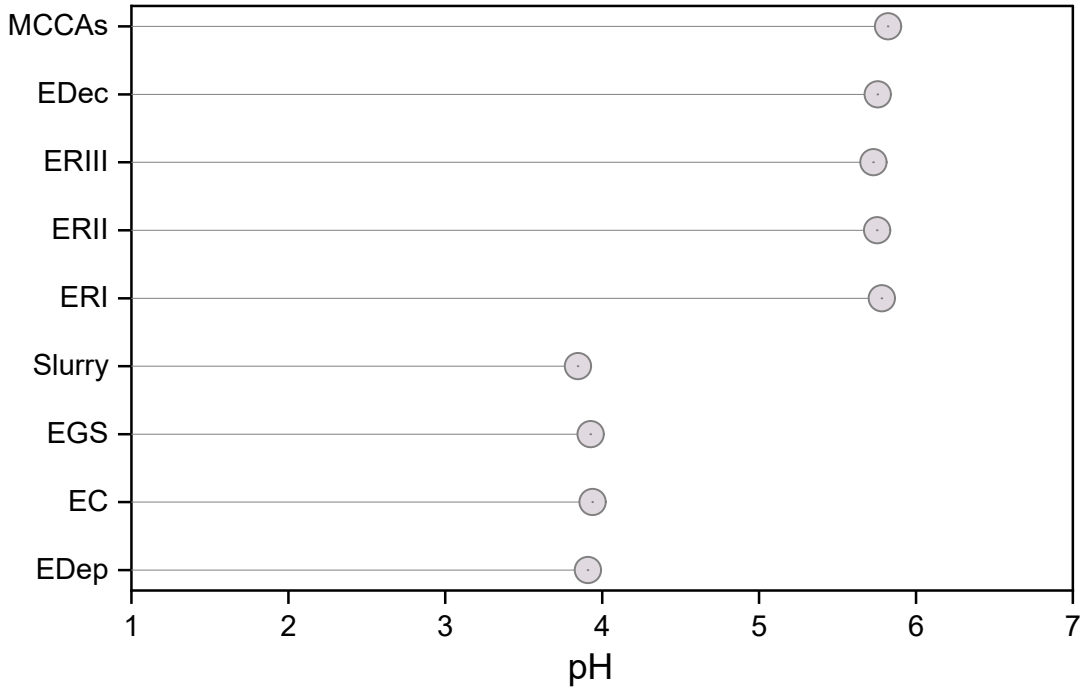

Fig. S5. pH transformation during the pretreatment and CCE sections.

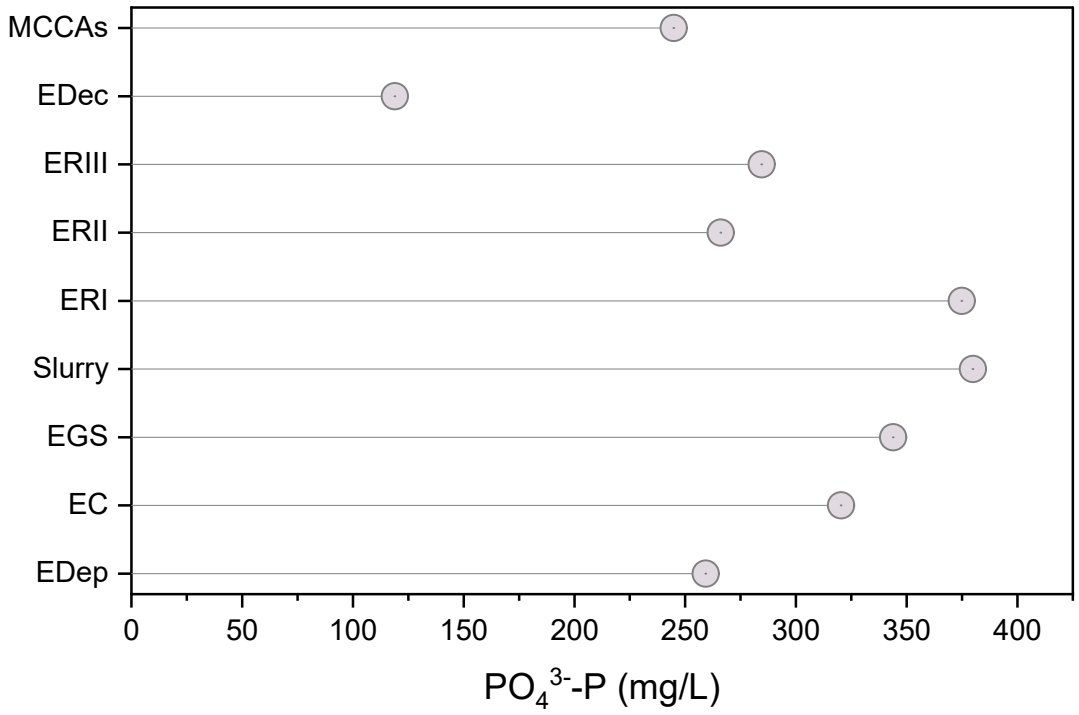

Fig. S6. Orthophosphate concentrations during the pretreatment and CCE sections.

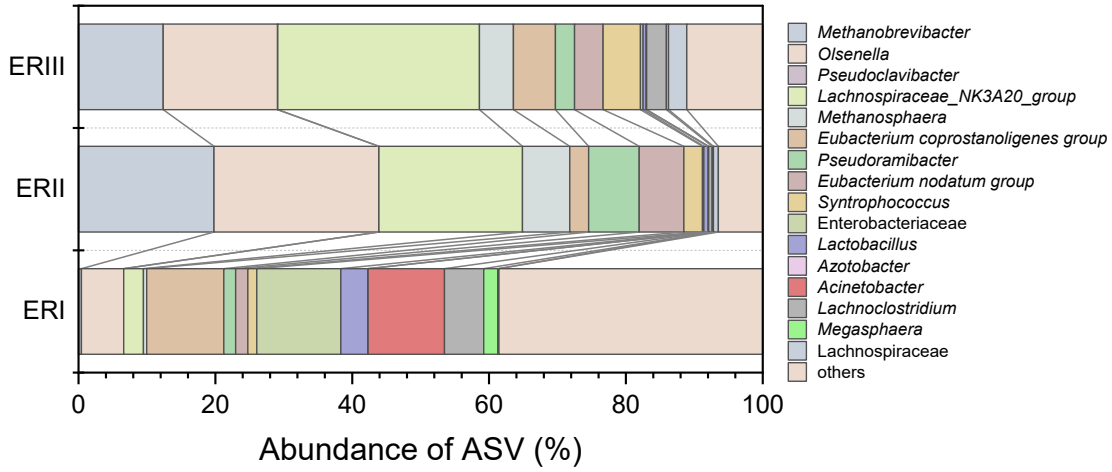

Fig. S7. Stacked bars of microbes in the CCE reactors at the temperature of 38−40°C.

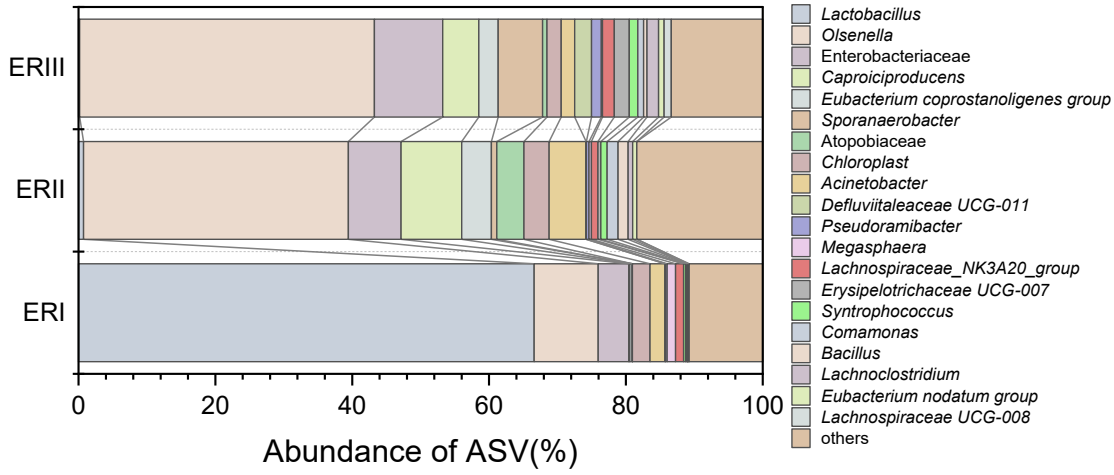

Fig. S8. Stacked bars of microbes in the CCE reactors at the temperature of 10−15°C.

Reference

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