Microbial ecological mechanism for long-term production of high concentrations of *n*-caproate via lactate-driven chain elongation

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Supplementary Material: 12 tables, 5 figures

TABLE S1. The microbial structural biodiversity

Days	Chao1	Observed OTUs	Shannon	Good Coverage (%)	Num. seqs.
0	223.538	146	4.432	99.73	25975
20	314.333	141	4.778	99.53	17030
40	210.588	120	4.082	99.69	9855
60	190.125	104	3.721	99.73	40068
80	326.167	155	4.713	99.68	-
100	314.870	174	5.063	99.69	-
120	332.250	186	5.191	99.65	-
140	283.241	177	5.205	99.73	-
160	387.222	185	4.581	99.61	-
180	421.118	164	5.064	99.53	-
202	256.875	177	4.621	99.52	60704
224	259.885	156	5.241	99.25	39664
246	241.316	145	4.879	99.40	68780
268	347.050	193	5.699	99.37	35065
290	266.621	176	5.010	99.25	27611
312	331.500	174	4.558	99.52	41395
334	382.091	226	5.983	99.16	28821
356	385.897	194	4.859	99.20	12751
378	342.097	210	5.358	99.13	31305
400	374.667	223	5.658	99.16	23409
425	399.276	211	4.902	98.87	18439
450	333.615	183	4.922	99.18	14159
475	357.677	198	4.786	99.34	15934
500	277.652	157	4.602	99.30	11974
525	345.750	163	4.230	99.03	19411
550	371.042	185	4.905	99.38	16300
575	365.156	201	5.069	99.53	53320
600	370.000	188	4.370	99.42	17730
625	205.000	142	3.759	99.42	16129
650	345.200	190	4.477	99.66	57283
661	269.526	135	4.176	99.73	42929
672	358.444	186	4.634	99.45	18019
683	434.550	216	5.895	99.69	47338
694	578.526	209	5.089	99.60	42698
705	300.476	150	3.752	99.34	19419
716	753.927	316	5.420	99.57	132524
727	694.560	324	5.467	98.04	28707
740	832.022	349	5.675	98.02	277976
752	702.889	349	5.700	97.42	188058

TABLE S2 Relative abundance at family and genus levels of microbial community of real wastewater

Organisms	Relative abundance (%)
Lactobacillaceae	99.8
Lactobacillus	
Methanobacteriaceae	0.004
Methanobacterium	
Lactobacillales;Other	0.004
Lactobacillales;Other;Other	
Geobacteraceae	0.004
Geobacter	
Others	0.008

TABLE S3 Reactor performances comparison during different phases

	HRT[a]		Acetate	*n*-Butyrate	*n*-Caproate	Propionate	*n*-Valerate
Concentration (g L^{-1})	**15**	Phase 1	1.97 ± 1.04	1.98 ± 1.94	23.87 ± 2.74	0.78 ± 0.82	1.26 ± 1.50
		Phase 2	3.94 ± 2.25	2.87 ± 2.23	19.35 ± 3.24	3.28 ± 1.83	6.59 ± 1.66
	8	Phase 3	1.65 ± 0.89	2.85 ± 1.25	16.08 ± 2.53	1.03 ± 0.89	5.05 ± 1.38
		Phase 4	2.16 ± 1.48	3.33 ± 1.22	27.01 ± 4.18	0.81 ± 0.92	4.65 ± 1.95
Specificity (mM C %)	**15**	Phase 1	4.43 ± 2.34	6.06 ± 5.94	83.23 ± 9.55	2.14 ± 2.24	4.16 ± 4.95
		Phase 2	7.65 ± 4.37	7.58 ± 5.89	58.24 ± 9.75	7.74 ± 4.32	18.79 ± 4.73
	8	Phase 3	4.21 ± 2.27	9.92 ± 4.35	63.71 ± 10.02	3.21 ± 2.77	18.95 ± 5.18
		Phase 4	3.8 4± 2.63	8.06 ± 2.95	74.24 ± 11.49	1.74 ± 1.98	12.12 ± 5.08

[a]: HRT is the abbreviation of hydraulic retention time

TABLE S4 Biogas content during the long-term operation period

Biogas	Content (%)			
	Phase 1	**Phase 2**	**Phase 3**	**Phase 4**
H_2	12.6 ± 3.09	9.67 ± 3.46	13.2 ± 1.63	18.3 ± 1.16
N_2	11.4 ± 2.02	11.6 ± 2.04	11.3 ± 1.62	8.07 ± 1.91
CO_2	62.9 ± 4.60	60.6 ± 5.80	64.5 ± 2.10	66.9 ± 2.86
CH_4	12.7 ± 5.53	16.9 ± 7.10	10.7 ± 1.55	7.00 ± 1.21

TABLE S5 Significant difference of microbiomes in reactor during different phases

Genus	Phase 1	Phase 2	Phase 3	Phase 4	p-Value
	Average relative abundance (%)	Average relative abundance (%)	Average relative abundance (%)	Average relative abundance (%)	
Caproiciproducens	47.77^{ab}	39.74^{a}	56.98^{ab}	64.47^{b}	0.010
Lactobacillus	8.84^{ab}	14.34^{a}	14.74^{a}	3.81^{b}	0.013
Methanobacterium	15.92	11.01	2.68	4.47	0.045
Lachnospiraceae uncultured	5.49^{a}	0.95^{b}	0.98^{b}	3.77^{ab}	0.003
Clostridium sensu stricto 12	3.60	3.25	1.75	1.41	0.676
Enterococcus	0.31^{a}	4.60^{b}	3.39^{ab}	1.60^{ab}	0.019
Cutibacterium	0.35^{a}	6.08^{b}	1.11^{a}	0.48^{a}	0.002
Garciella	0.31	6.30	0.05	0.69	0.360
Clostridiaceae 1 Other	2.31	0.19	4.16	1.27	0.217
Pseudomonas	5.09	0.30	0.02	0.07	0.444
Clostridiaceae 1 uncultured	3.30^{a}	0.00^{b}	0.00^{b}	0.53^{b}	0.000
Gordonibacter	0.00^{a}	0.82^{ab}	3.51^{b}	0.68^{ab}	0.026
Sporanaerobacter	0.04^{a}	0.63^{a}	0.23^{a}	3.60^{b}	0.003
Corynebacterium 1	0.00	1.73	1.61	0.35	0.088
Caldicoprobacter	1.69^{a}	0.10^{b}	0.11^{b}	0.88^{ab}	0.000
Anaerocolumna	0.24	0.40	0.71	0.27	0.111
Anaerosporobacter	0.20	0.72	0.60	0.17	0.473
Rummeliibacillus	0.05	0.36	0.35	0.44	0.165
Family XI uncultured	0.32	0.28	0.28	0.38	0.970
Kurthia	0.00^{a}	0.16^{ab}	0.08^{a}	0.91^{b}	0.011
Tyzzerella	0.00	0.64	0.21	0.08	0.078
Georgenia	0.32	0.05	0.44	0.31	0.133
Arcanobacterium	0.32	0.16	0.27	0.31	0.522
Oscillibacter	0.01	0.41	0.23	0.40	0.142
Olsenella	0.05	0.35	0.45	0.11	0.144
Clostridium sensu stricto 13	0.23	0.14	0.46	0.05	0.158
Clostridium sensu stricto 15	0.34	0.43	0.06	0.04	0.324
Family XI Sedimentibacter	0.00^{a}	0.71^{b}	0.08^{a}	0.01^{a}	0.004
Bacillaceae Bacillus	0.21	0.13	0.15	0.30	0.646
Ruminiclostridium 5	0.02^{a}	0.04^{a}	0.06^{a}	0.75^{b}	0.000
[Eubacterium] fissicatena group	0.20	0.26	0.13	0.13	0.311
Ruminococcaceae uncultured	0.01^{a}	0.06^{a}	0.06^{a}	0.57^{b}	0.000
Proteiniphilum	0.00	0.53	0.08	0.01	0.232
[Eubacterium] nodatum group	0.40	0.01	0.08	0.11	0.053

Ruminococcaceae Other	0.05^{a}	0.04^{a}	0.13^{a}	0.38^{b}	0.000
Tissierella	0.13	0.17	0.10	0.15	0.887
Haloimpatiens	0.11	0.19	0.09	0.11	0.813
Family XIII S5-A14a	0.37	0.08	0.21	0.01	0.326
Lachnospiraceae Other	0.09	0.14	0.09	0.16	0.399
Lachnospiraceae Blautia	0.04	0.12	0.22	0.09	0.361
Anaerotruncus	0.02^{a}	0.26^{b}	0.07^{ab}	0.07^{ab}	0.040

Different superscripts are shown for values that differ significantly from each other ($P < 0.05$, Tukey test).

TABLE S6 Taxonomic compositions of microbiomes in reactor

Genus	Phase 1	Phase 2		Phase 3	Phase 4	
	Average relative abundance (%)	Average relative abundance (%)	Adjusted *p*-Value[a]	Average relative abundance (%)	Average relative abundance (%)	Adjusted p-Value[b]
Caproiciproducens	47.77 ± 15.61	39.74 ± 16.62	0.2106	56.98 ± 8.74	64.47 ± 19.6	0.3237
Lactobacillus	8.84 ± 4.55	14.34 ± 8.81	0.0883	14.74 ± 8.82	3.81 ± 2.59	0.0079
Methanobacterium	15.92 ± 11.28	11.01 ± 14.98	0.5092	2.68 ± 3.90	4.47 ± 3.85	0.3700
Lachnospiraceae uncultured	5.49 ± 4.71	0.95 ± 0.60	0.0366	0.98 ± 0.74	3.77 ± 2.89	0.0266
Clostridium sensu stricto 12	3.60 ± 7.40	3.25 ± 4.41	0.9044	1.75 ± 1.49	1.41 ± 2.06	0.7109
Enterococcus	0.31 ± 0.41	4.60 ± 4.38	0.0166	3.39 ± 3.35	1.60 ± 1.46	0.1926
Cutibacterium	0.35 ± 0.42	6.08 ± 6.08	0.0219	1.11 ± 1.81	0.48 ± 1.36	0.4438
Garciella	0.31 ± 0.60	6.30 ± 16.62	0.2972	0.05 ± 0.05	0.69 ± 1.05	0.1195
Clostridiaceae 1 Other	2.31 ± 3.33	0.19 ± 0.23	0.1305	4.16 ± 6.21	1.27 ± 2.32	0.2447
Pseudomonas	5.09 ± 15.28	0.30 ± 0.60	0.1748	0.02 ± 0.03	0.07 ± 0.19	0.4407
Clostridiaceae 1 uncultured	3.30 ± 2.89	0.00 ± 0.00	0.0079	0.00 ± 0.00	0.53 ± 0.45	0.0111
Gordonibacter	0.00 ± 0.00	0.82 ± 0.12	0.0719	3.51 ± 4.14	0.68 ± 1.10	0.0934
Sporanaerobacter	0.04 ± 0.07	0.63 ± 1.39	0.2284	0.23 ± 0.30	3.60 ± 3.94	0.0418
Corynebacterium 1	0.00 ± 0.04	1.73 ± 1.56	0.0097	1.61 ± 3.20	0.35 ± 0.35	0.2999
Caldicoprobacter	1.69 ± 1.37	0.10 ± 0.17	0.0146	0.11 ± 0.11	0.88 ± 0.86	0.0356
Anaerocolumna	0.24 ± 0.25	0.40 ± 0.45	0.3706	0.71 ± 0.89	0.27 ± 0.18	0.2048
Anaerosporobacter	0.20 ± 0.24	0.72 ± 1.53	0.2900	0.60 ± 0.81	0.17 ± 0.18	0.1720
Rummeliibacillus	0.05 ± 0.07	0.36 ± 0.51	0.0997	0.35 ± 0.19	0.44 ± 0.52	0.6415
Family XI uncultured	0.32 ± 0.31	0.28 ± 0.70	0.8759	0.28 ± 0.40	0.38 ± 0.55	0.6768
Kurthia	0.00 ± 0.00	0.16 ± 0.31	0.1551	0.08 ± 0.17	0.91 ± 1.15	0.0780
Tyzzerella	0.00 ± 0.00	0.64 ± 0.88	0.0575	0.21 ± 0.52	0.08 ± 0.07	0.5165
Georgenia	0.32 ± 0.39	0.05 ± 0.06	0.1359	0.44 ± 0.47	0.31 ± 0.27	0.5073
Arcanobacterium	0.32 ± 0.34	0.16 ± 0.13	0.2206	0.27 ± 0.36	0.31 ± 0.12	0.7561
Oscillibacter	0.01 ± 0.02	0.41 ± 0.61	0.0805	0.23 ± 0.43	0.40 ± 0.33	0.3850
Olsenella	0.05 ± 0.10	0.35 ± 0.39	0.0525	0.45 ± 0.61	0.11 ± 0.13	0.1639
Clostridium sensu stricto 13	0.23 ± 0.25	0.14 ± 0.12	0.2330	0.46 ± 0.64	0.05 ± 0.11	0.1113
Clostridium sensu stricto 15	0.34 ± 0.54	0.43 ± 0.86	0.7251	0.06 ± 0.07	0.04 ± 0.06	0.5702
Family XI Sedimentibacter	0.00 ± 0.00	0.71 ± 0.84	0.0319	0.08 ± 0.10	0.01 ± 0.03	0.0919
Bacillaceae Bacillus	0.21 ± 0.36	0.13 ± 0.16	0.5112	0.15 ± 0.11	0.30 ± 0.35	0.3003
Ruminiclostridium 5	0.02 ± 0.03	0.04 ± 0.07	0.3446	0.06 ± 0.07	0.75 ± 0.69	0.0220
[Eubacterium] fissicatena group	0.20 ± 0.15	0.26 ± 0.16	0.3374	0.13 ± 0.18	0.13 ± 0.14	0.9894
Ruminococcaceae uncultured	0.01 ± 0.02	0.06 ± 0.07	0.0576	0.06 ± 0.06	0.57 ± 0.45	0.0133

Proteiniphilum	0.00 ± 0.00	0.53 ± 1.21	0.2200	0.08 ± 0.14	0.01 ± 0.03	0.2122
[Eubacterium] nodatum group	0.40 ± 0.58	0.01 ± 0.03	0.0668	0.08 ± 0.13	0.11 ± 0.08	0.5344
Ruminococcaceae Other	0.05 ± 0.05	0.04 ± 0.05	0.8793	0.13 ± 0.14	0.38% ± 0.23	0.0187
Tissierella	0.13 ± 0.10	0.17 ± 0.20	0.3583	0.10 ± 0.13	0.15 ± 0.28	0.6357
Haloimpatiens	0.11 ± 0.31	0.19 ± 0.19	0.5771	0.09 ± 0.09	0.11 ± 0.32	0.8541
Family XIII S5-A14a	0.37 ± 0.71	0.08 ± 0.13	0.5726	0.21 ± 0.43	0.01 ± 0.02	0.2272
Lachnospiraceae Other	0.09 ± 0.09	0.14 ± 0.14	0.3875	0.09 ± 0.08	0.16 ± 0.11	0.1849
Lachnospiraceae Blautia	0.04 ± 0.06	0.12 ± 0.24	0.3376	0.22 ± 0.26	0.09 ± 0.13	0.2159
Anaerotruncus	0.02 ± 0.06	0.26 ± 0.32	0.0516	0.07 ± 0.14	0.07 ± 0.07	0.9432

[a]: significant difference between phase 1 and phase 2

[b]: significant difference between phase 3 and phase 4

TABLE S7 Raw data quality control information

Sample	InsertSize (bp)	SeqStrategy	RawData (Mbp)	CleanData (Mbp)	Clean_Q30 (%)	Clean_GC (%)
A1	350	(150 : 150)	21,409.67	21,402.37	93.29	43.83
A2	350	(150 : 150)	21,380.62	21,374.82	93.54	45.05

TABLE S8 Metagenome assembly information

Statistical level	Original			Scaffold > 500bp		
	scaffold	contig	contig > (500)	scaffold	contig	contig > (500)
Total number (>)	69620 ± 4268	69620 ± 4268	69620 ± 4268	69620 ± 4268	69620 ± 4268	69620 ± 4268
Total length (bp)	148213513 ± 511039	148213513 ± 511039	148213513 ± 511039	148213513 ± 511039	148213513 ± 511039	148213513 ± 511039
Gap number (bp)	0	0	0	0	0	0
Average length (bp)	2137.93 ± 138.44	2137.93 ± 138.44	2137.93 ± 138.44	2137.93 ± 138.44	2137.93 ± 138.44	2137.93 ± 138.44
N50 Length (bp)	4253 ± 700	4253 ± 700	4253 ± 700	4253 ± 700	4253 ± 700	4253 ± 700
N90 Length (bp)	740.5 ± 14.5	740.5 ± 14.5	740.5 ± 14.5	740.5 ± 14.5	740.5 ± 14.5	740.5 ± 14.5
Maximum length (bp)	864770 ± 219749	864770 ± 219749	864770 ± 219749	864770 ± 219749	864770 ± 219749	864770 ± 219749
Minimum length (bp)	500 ± 0.00	500 ± 0.00	500 ±0.00	500 ±0.00	500 ±0.00	500 ±0.00
GC content (%)	45.65 ± 0.15	45.65 ± 0.15	45.65 ± 0.15	45.65 ± 0.15	45.65 ± 0.15	45.65 ± 0.15

TABLE S9 Gene catalogue information

ORFs NO.	182170 ± 400
integrity: end	31125 ± 1646
integrity: start	38336 ± 1581
integrity: all	102210.5 ± 3612.5
integrity: none	10498.5 ± 785.5
Total Len (Mbp)	131.255 ± 0.095
Average Len (bp)	720.51 ± 1.05
GC percent	45.845 ± 0.415

ORFs NO. means the numbers of genes in gene catalogue, integrity: end means the number of genes that contain only termination codons, integrity: start means the number of genes that contain only initial codons, integrity: all means the number of complete genes, integrity: none means the number of genes that have neither termination codon nor initial codon.

TABLE S10 The abundance of gene annotated to species at all levels information

Annotated on Unclassified:	4.78%
Annotated on Kingdom level:	95.2%
Annotated on Phylum level:	92.9%
Annotated on Class level:	85.1%
Annotated on Order level:	71.1%
Annotated on Family level:	55.9%
Annotated on Genus level:	54.1%
Annotated on Species level:	55.1%

TABLE S11 Relative abundance of functional genes at the metabolic level3

Metabolic pathway	Sequence assign (%)
Environmental Information Processing; Membrane transport; ABC transporters	4.7532 ± 0.2779
Genetic Information Processing; Translation; Ribosome	4.0897 ± 0.1952
Metabolism; Nucleotide metabolism; Purine metabolism	3.667 ± 0.054
Metabolism; Nucleotide metabolism; Pyrimidine metabolism	3.1975 ± 0.0773
Cellular Processes; Cellular community - prokaryotes; Quorum	2.4621 ± 0.0323

sensing	
Metabolism; Carbohydrate metabolism; Pyruvate metabolism	2.2288 ± 0.0241
Metabolism; Carbohydrate metabolism; Glycolysis / Gluconeogenesis	2.1734 ± 0.0212
Metabolism; Carbohydrate metabolism; Amino sugar and nucleotide sugar metabolism	2.1369 ± 0.0161
Environmental Information Processing; Signal transduction; Two-component system	1.9239 ± 5.44E-03
Genetic Information Processing; Translation; Aminoacyl-tRNA biosynthesis	1.8798 ± 0.0641
Metabolism; Energy metabolism; Carbon fixation pathways in prokaryotes	1.8233 ± 0.0797
Metabolism; Amino acid metabolism; Cysteine and methionine metabolism	1.8175 ± 0.0548
Metabolism; Amino acid metabolism; Alanine, aspartate and glutamate metabolism	1.6921 ± 0.0338
Metabolism; Energy metabolism; Oxidative phosphorylation	1.6614 ± 0.1605
Genetic Information Processing; Replication and repair; Homologous recombination	1.6463 ± 0.0335
Metabolism; Amino acid metabolism; Glycine, serine and threonine metabolism	1.6461 ± 0.0288
Metabolism; Carbohydrate metabolism; Starch and sucrose metabolism	1.5844 ± 0.05
Metabolism; Carbohydrate metabolism; Citrate cycle (TCA cycle)	1.5258 ± 0.1484
Metabolism; Carbohydrate metabolism; Pentose phosphate pathway	1.4561 ± 0.0285
Genetic Information Processing; Replication and repair; Mismatch repair	1.3871 ± 0.0336
Metabolism; Energy metabolism; Methane metabolism	1.3767 ± 0.0747
Metabolism; Carbohydrate metabolism; Glyoxylate and dicarboxylate metabolism	1.33 ± 0.0172
Metabolism; Carbohydrate metabolism; Propanoate metabolism	1.3214 ± 0.0313
Metabolism; Glycan biosynthesis and metabolism; Peptidoglycan biosynthesis	1.3206 ± 0.0338
Metabolism; Carbohydrate metabolism; Fructose and mannose metabolism	1.2007 ± 0.0103
Metabolism; Carbohydrate metabolism; Butanoate metabolism	1.1819 ± 0.0365
Genetic Information Processing; Replication and repair; DNA replication	1.1535 ± 7.21E-03
Metabolism; Energy metabolism; Carbon fixation in photosynthetic organisms	1.0546 ± 0.0113
Metabolism; Amino acid metabolism; Phenylalanine, tyrosine and tryptophan biosynthesis	1.053 ± 0.0355
Genetic Information Processing; Folding, sorting and degradation;	1.0184 ± 0.0183

RNA degradation	
Metabolism; Amino acid metabolism; Lysine biosynthesis	1.0116 ± 0.0323
Metabolism; Carbohydrate metabolism; Galactose metabolism	0.9926 ± 0.0496
Metabolism; Amino acid metabolism; Arginine biosynthesis	0.9788 ± 0.0143
Genetic Information Processing; Folding, sorting and degradation; Protein export	0.9056 ± 1.92E-03
Metabolism; Lipid metabolism; Glycerophospholipid metabolism	0.883 ± 0.0182
Metabolism; Metabolism of cofactors and vitamins; Porphyrin and chlorophyll metabolism	0.8828 ± 0.0168
Genetic Information Processing; Replication and repair; Nucleotide excision repair	0.8638 ± 0.0498
Metabolism; Lipid metabolism; Fatty acid biosynthesis	0.8346 ± 0.0614
Metabolism; Metabolism of cofactors and vitamins; Thiamine metabolism	0.8207 ± 0.0849
Metabolism; Metabolism of cofactors and vitamins; Pantothenate and CoA biosynthesis	0.8172 ± 0.0234
Environmental Information Processing; Membrane transport; Bacterial secretion system	0.7929 ± 3.63E-03
Cellular Processes; Cell growth and death; Cell cycle - Caulobacter	0.7904 ± 9.08E-03
Metabolism; Metabolism of cofactors and vitamins; Nicotinate and nicotinamide metabolism	0.7832 ± 0.0828
Metabolism; Metabolism of cofactors and vitamins; One carbon pool by folate	0.7336 ± 0.0379
Metabolism; Metabolism of terpenoids and polyketides; Terpenoid backbone biosynthesis	0.6989 ± 0.0244
Metabolism; Metabolism of other amino acids; Selenocompound metabolism	0.6944 ± 0.0766
Genetic Information Processing; Replication and repair; Base excision repair	0.6651 ± 0.022
Metabolism; Carbohydrate metabolism; Pentose and glucuronate interconversions	0.6644 ± 0.0138
Metabolism; Amino acid metabolism; Arginine and proline metabolism	0.6554 ± 0.0348
Metabolism; Metabolism of cofactors and vitamins; Folate biosynthesis	0.6447 ± 0.0945
Environmental Information Processing; Membrane transport; Phosphotransferase system (PTS)	0.6391 ± 0.1671
Human Diseases; Drug resistance: Antimicrobial; beta-Lactam resistance	0.6384 ± 7.15E-03
Metabolism; Energy metabolism; Nitrogen metabolism	0.6359 ± 0.058
Cellular Processes; Cell motility; Flagellar assembly	0.5963 ± 0.0754
Metabolism; Amino acid metabolism; Histidine metabolism	0.5739 ± 2.49E-03
Metabolism; Xenobiotics biodegradation and metabolism; Drug metabolism - other enzymes	0.5596 ± 3.31E-03

Metabolism; Amino acid metabolism; Valine, leucine and isoleucine degradation	0.5118 ± 0.0293
Metabolism; Amino acid metabolism; Valine, leucine and isoleucine biosynthesis	0.509 ± 0.0534
Metabolism; Lipid metabolism; Glycerolipid metabolism	0.5074 ± 0.0386
Cellular Processes; Cell motility; Bacterial chemotaxis	0.5007 ± 1.91E-03

TABLE S12 The detailed genomic characteristics of all the bins

Bin	Completeness (%)	Contamination (%)	GC (%)	Lineage	N50 (bp)	Size (bp)
			A1			
1	94.11	2.797	30.6%	o__Clostridiales	15678	2303007
9	76.01	1.785	47.6%	k__Bacteria	33937	1515065
10	79.95	3.705	65.2%	k__Actinobacteria	4680	2107664
11	95.70	0.0	51.6%	p__Euryarchaeota	56651	1141960
12	100.0	0.0	69.0%	o__Actinomycetales	66535	3333348
13	78.92	3.605	35.5%	o__Clostridiales	3745	1933843
14	89.81	0.125	33.8%	p__Bacteroidales	34176	2457727
17	93.71	0.0	46.0%	o__Clostridiales	87273	5683546
19	77.55	1.037	37.1%	p__Bacteroidales	3934	1563338
20	86.31	0.0	48.3%	o__Clostridiales	11703	3780825
22	89.71	3.021	35.2%	o__Clostridiales	43479	2329384
23	76.56	1.582	62.2%	o__Actinomycetales	4920	2160114
26	90.64	0.894	40.7%	o__Clostridiales	10758	1712818
28	84.56	2.439	34.8%	o__Clostridiales	11421	1713900
29	88.72	0.0	43.3%	k__Bacteria	14169	2673769
30	82.15	1.901	61.2%	o__Clostridiales	34491	1717832
31	98.73	2.887	42.5%	o__Clostridiales	48880	4068098
32	81.87	3.131	44.3%	o__Clostridiales	8486	1704623
34	87.83	1.308	38.8%	o_Lactobacillales	7779	1694662
36	92.42	0.0	39.0%	o__Clostridiales	33257	2777365
38	96.87	1.206	36.1%	c__Bacilli	25203	2676825
39	85.22	2.016	36.1%	c__Clostridia	5710	1760298
40	87.27	0.503	33.6%	o__Clostridiales	9603	2527697
43	98.8	0.8	38.3%	p__Euryarchaeota	27127	2054514
44	81.31	1.364	43.4%	f__Lachnospiraceae	7324	2241762
45	91.41	0.632	45.6%	o__Clostridiales	9039	4034241
46	97.68	1.348	71.5%	o__Actinomycetales	18560	1922448
47	78.48	0.0	53.9%	o__Clostridiales	82503	2040129
			A2			
11	71.55	2.586	34.8%	k__Bacteria	4401	3340318
12	92.82	0.0	43.7%	o__Clostridiales	59094	2268218
14	97.97	0.0	71.6%	o__Actinomycetales	85266	1926238

15	91.33	1.699	35.8%	c__Bacilli	21261	2627343
16	74.36	1.582	43.5%	o__Clostridiales	16772	2026195
17	97.14	0.317	50.2%	k__Bacteria	42964	2315694
18	80.54	0.0	43.3%	k__Bacteria	9065	2515969
22	80.73	1.420	33.6%	c__Clostridia	6910	1792635
23	82.73	0.0	51.4%	o__Clostridiales	13281	4232469
26	98.46	1.666	48.0%	p__Firmicutes	171709	2449457
29	80.28	1.273	46.5%	o__Clostridiales	7008	3927156
33	96.22	1.388	67.0%	o__Actinomycetales	28756	2978660
40	81.90	3.191	31.2%	o__Clostridiales	4800	1866415
43	70.45	1.724	61.9%	k__Bacteria	6110	1528936
45	89.13	1.308	39.4%	o__Lactobacillales	7064	2060655

TABLE S13 More information about enzymes in MCCA-production

Metabolic pathway	Abbreviation	Gene	Definition	KO_ID	EC No.
Lactate oxidation	LDH	*ldh*	lactate dehydrogenase	K00016	1.1.1.27
	PFOR	*por*	Pyruvate ferredoxin oxidoreductase	K03737	1.2.7.1
Fatty acid biosynthesis pathway	ACC	*accA*	acetyl-CoA carboxylase carboxyl transferase subunit alpha	K01962	6.4.1.2
		accB	acetyl-CoA carboxylase biotin carboxyl carrier protein	K02160	-
		accC	acetyl-CoA carboxylase, biotin carboxylase subunit	K01961	6.4.1.2
		accD	acetyl-CoA carboxylase carboxyl transferase subunit beta	K01963	6.4.1.2
	MAT	*fas*	fatty acid synthase	K11533	2.3.1.-
		fabD	[acyl-carrier-protein] S-malonyltransferase	K00645	2.3.1.39
	KAS	*fas*	fatty acid synthase	K11533	2.3.1.-
		fabB	3-oxoacyl-[acyl-carrier-protein] synthase I	K00647	2.3.1.41
		fabF	3-oxoacyl-[acyl-carrier-protein] synthase II	K09458	2.3.1.179
		fabH	3-oxoacyl-[acyl-carrier-protein] synthase III	K00648	2.3.1.180

	KAR	*fas*	fatty acid synthase	K11533	2.3.1.-
		fabG	3-oxoacyl-[acyl-carrier protein] reductase	K00059	1.1.1.100
	HAD	*fas*	fatty acid synthase	K11533	2.3.1.-
		fabZ	3-hydroxyacyl-[acyl-carrier-protein] dehydratase	K02372	4.2.1.59
	EAR	*fas*	fatty acid synthase	K11533	2.3.1.-
		fabI	enoyl-[acyl-carrier protein] reductase I	K00208	1.3.1.9 1.3.1.10
		fabK	enoyl-[acyl-carrier protein] reductase II	K02371	1.3.1.9
		fabL	enoyl-[acyl-carrier protein] reductase III	K10780	1.3.1.104
	ACT	*paaI*	acyl-CoA thioesterase	K02614	3.1.2.-
Reverse β-oxidation pathway	ACAT	*fadA*	acetyl-CoA acyltransferase	K00632	2.3.1.16
		atoB	acetyl-CoA C-acetyltransferase	K00626	2.3.1.9
	HCD	*paaH/hbd/fadB*	3-hydroxybutyryl-CoA dehydrogenase	K00074	1.1.1.157
		fadJ		K01782	1.1.1.35
		fadN		K07516	1.1.1.35
	ECH	*crt*	enoyl-CoA hydratase	K01715	4.2.1.17
		echA/paaF		K01692	4.2.1.17
	ACD	*mmgC*	acyl-CoA dehydrogenase	K18244	1.3.99.-
		acd	acyl-CoA dehydrogenase	K00249	1.3.8.7
	EtfA	*etfA*	electron transfer flavoprotein alpha subunit	K03522	-
	EtfB	*etfB*	electron transfer flavoprotein beta subunit	K03521	-
	CoAT	*cat2*	4-hydroxybutyrate CoA-transferase	K18122	2.8.3.-
	PTA	*pta*	phosphate acetyltransferase	K00625	2.3.1.8
	ACK	*ackA*	acetate kinase	K00925	2.7.2.1

Energy conservation	RNF Complex	*RnfA*	electron transport complex protein RnfA	K03617	7.2.1.2
		RnfB	electron transport complex protein RnfB	K03616	7.2.1.2
		RnfC	electron transport complex protein RnfC	K03615	7.1.1.-
		RnfD	electron transport complex protein RnfD	K03614	7.2.1.2
		RnfE	electron transport complex protein RnfE	K03613	7.2.1.2
		RnfG	electron transport complex protein RnfG	K03612	7.2.1.2
Wood-Werkman cycle	MDH	*mdh*	malate dehydrogenase	K00024	1.1.1.37
	FUM	*fum*	fumarate hydratase	K01679	4.2.1.2
	SDH	*sdh*	succinate dehydrogenase	K00239 K00240	1.3.5.1 1.3.5.4
	MUT	*mut*	methylmalonyl-CoA mutase	K01847	5.4.99.2
Hydrogeno-trophic Methane production pathway	FWD	*fwdA*	formylmethanofuran dehydrogenase subunit A	K00200	1.2.7.12
		fwdB	formylmethanofuran dehydrogenase subunit B	K00201	1.2.7.12
		fwdC	formylmethanofuran dehydrogenase subunit C	K00202	1.2.7.12
		fwdD	formylmethanofuran dehydrogenase subunit D	K00203	1.2.7.12
		fwdE	formylmethanofuran dehydrogenase subunit E	K11261	1.2.7.12
	FTR	*ftr*	formylmethanofuran--tetrahydromethanopterin N-formyltransferase	K00672	2.3.1.101
	MCH	*mch*	methenyltetrahydromethanopterin cyclohydrolase	K01499	3.5.4.27
	MTD	*mtd*	methylenetetrahydromethanopterin dehydrogenase	K00319	1.5.98.1
	MER	*mer*	5,10-methylenetetrahydromethanopterin reductase	K00320	1.5.98.2

	MTR	*mtrA*	tetrahydromethanopterin S-methyltransferase subunit A	K00577	2.1.1.86
		mtrB	tetrahydromethanopterin S-methyltransferase subunit B	K00578	2.1.1.86
		mtrC	tetrahydromethanopterin S-methyltransferase subunit C	K00579	2.1.1.86
		mtrD	tetrahydromethanopterin S-methyltransferase subunit D	K00580	2.1.1.86
		mtrE	tetrahydromethanopterin S-methyltransferase subunit E	K00581	2.1.1.86
	MCR	*mcrA*	methyl-coenzyme M reductase alpha subunit	K00399	2.8.4.1
		mcrB	methyl-coenzyme M reductase beta subunit	K00401	2.8.4.1
		mcrC	methyl-coenzyme M reductase subunit C	K03421	-
		mcrD	methyl-coenzyme M reductase subunit D	K03422	-
		mcrG	methyl-coenzyme M reductase gamma subunit	K00402	2.8.4.1
Aceto-trophic Methane production pathway	CDH	*cdhD*	acetyl-CoA decarbonylase/synthase complex	K00194	2.1.1.245
	MTR	*mtrA*	tetrahydromethanopterin S-methyltransferase subunit A	K00577	2.1.1.86
		mtrB	tetrahydromethanopterin S-methyltransferase subunit B	K00578	2.1.1.86
		mtrC	tetrahydromethanopterin S-methyltransferase subunit C	K00579	2.1.1.86
		mtrD	tetrahydromethanopterin S-methyltransferase subunit D	K00580	2.1.1.86
		mtrE	tetrahydromethanopterin S-methyltransferase subunit E	K00581	2.1.1.86
	MCR	*mcrA*	methyl-coenzyme M reductase alpha subunit	K00399	2.8.4.1
		mcrB	methyl-coenzyme M reductase beta subunit	K00401	2.8.4.1
		mcrC	methyl-coenzyme M reductase subunit C	K03421	-
		mcrD	methyl-coenzyme M reductase subunit D	K03422	-

		mcrG	methyl-coenzyme M reductase gamma subunit	K00402	2.8.4.1

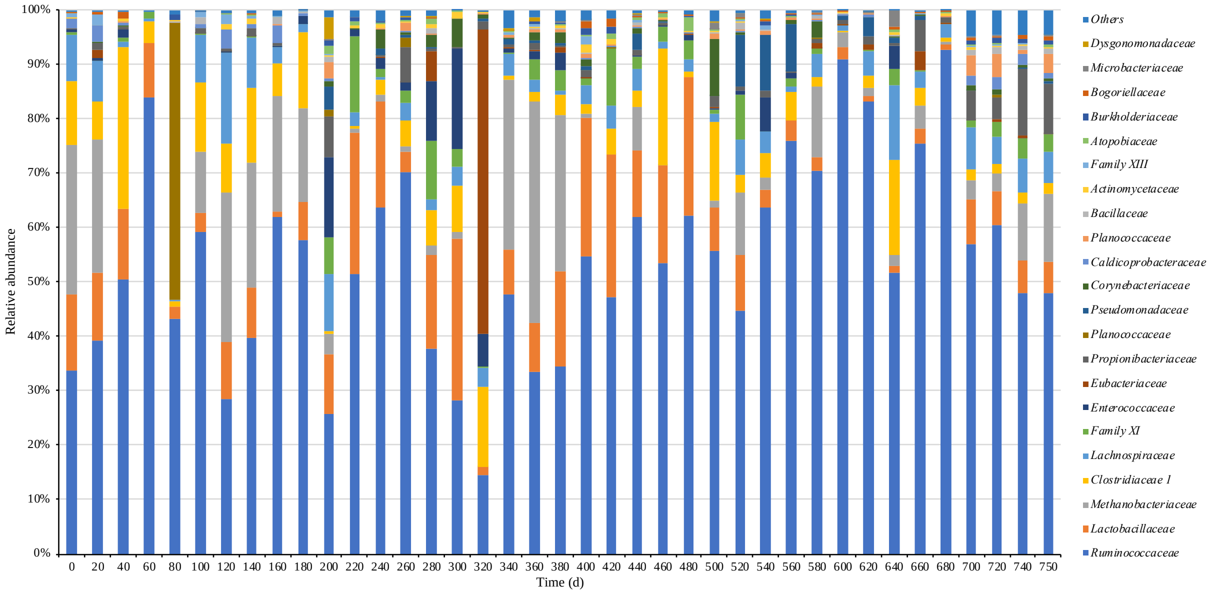

FIG. S1 Variations of taxonomic composition of reactor microbiome at family level. Thirty-nine samples were sequenced during the 780 days. 22 bacteria families were identified with a relative abundance of greater than 0.1% in samples.

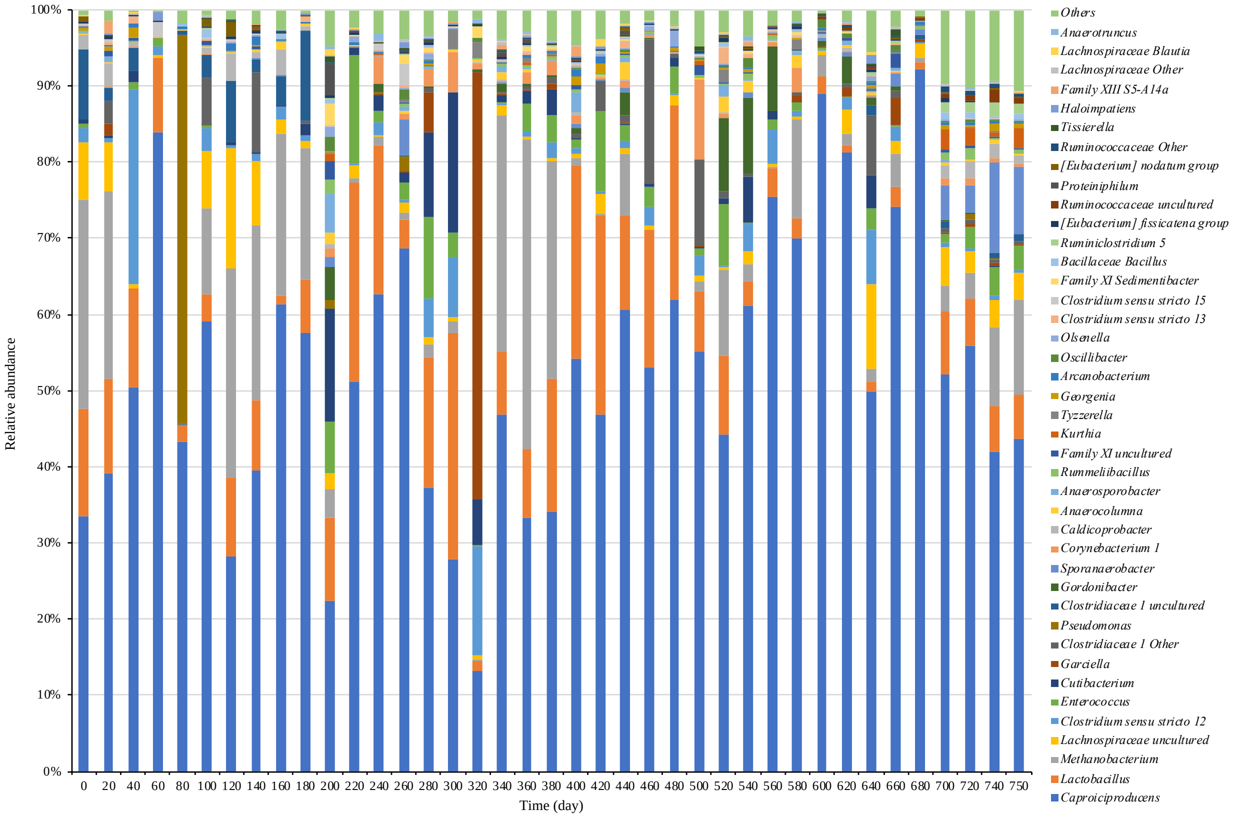

FIG. S2 Variations of taxonomic composition of reactor microbiome at genus level. 41 bacteria genera were identified with a relative abundance of greater than 0.1% in samples.

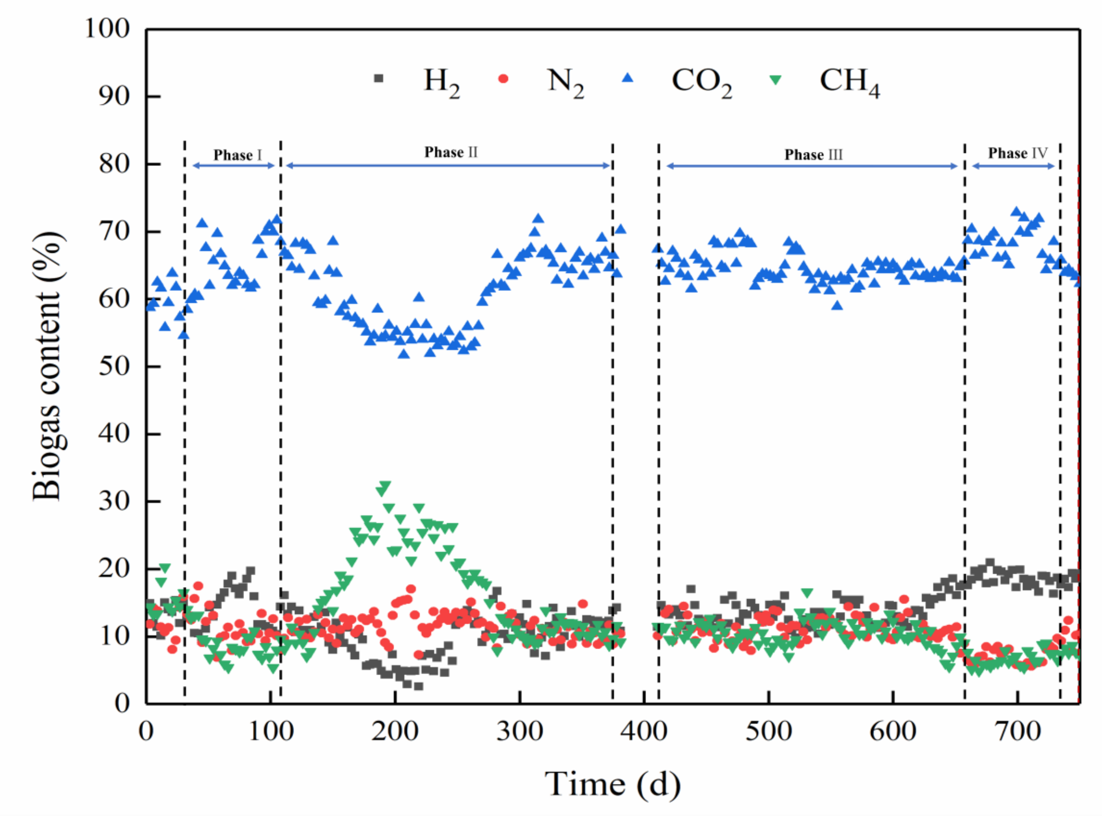

FIG. S3. Gas components of bioreactors treating with lactate artificial wastewater

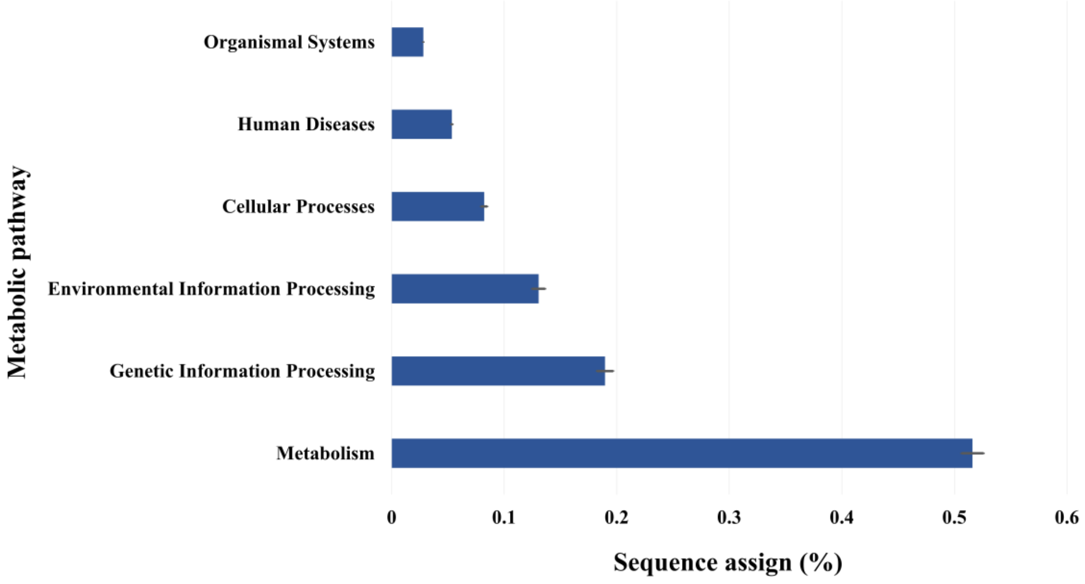

FIG. S4 Relative abundance of functional genes at the metabolic level 1

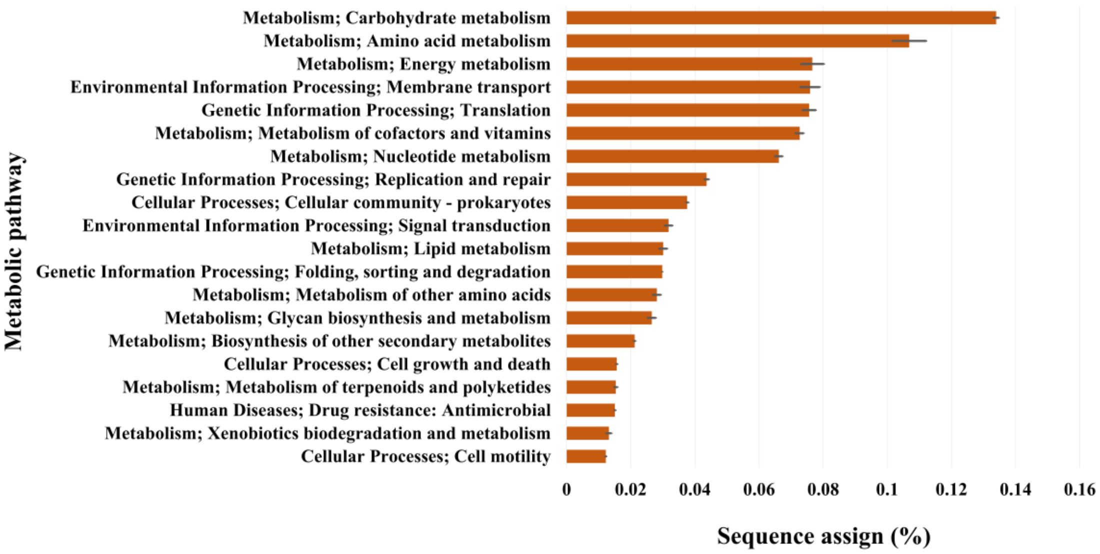

FIG. S5 Relative abundance of functional genes at the metabolic level 2