

1           **The synthesis of *n*-caproate from lactate, a new efficient process for**  
2                                   **medium chain carboxylate production**

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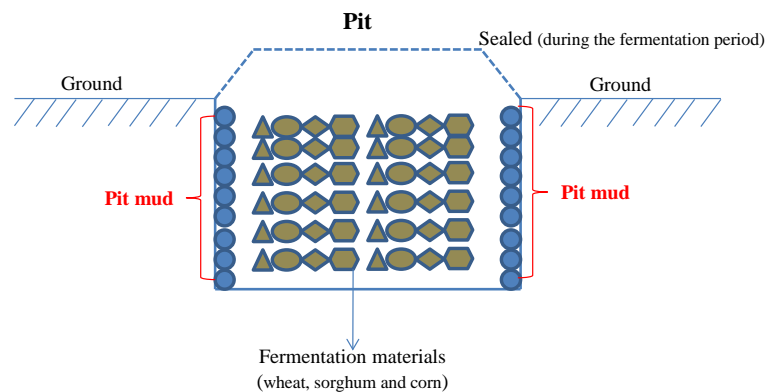
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18 **Materials:**

19 **Chinese strong-flavor liquor brewing process**

20 Chinese strong-flavored liquor (CSFL) is produced by traditional solid-state  
21 fermentation method, which has several thousand years of history in Chinese. In brief,  
22 fermentation materials (wheat, sorghum and corn) are put into a pit, in which the entire  
23 inner wall is covered with pit mud, which contains various flavor producing  
24 microbiomes. For every 2-3 months of airtight fermentation, fermentation diffusate  
25 (called “yellow water”) is pumped out, and then fermentation mash are taken out for  
26 wine distilling. CSFL is famous for its unique flavor. The main flavor component is  
27 ethyl caproate, which is produced from *n*-caproic acid. The *n*-caproic acid concentration  
28 controls the quality of CSFL.



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30 **Inoculum**

31 The pit mud acts as a source of inoculum and a habitat of microbes in the brewing  
32 fermentation pit for the CSFL production, in which microbes can produce various flavor  
33 components such as butyric acid, caproic acid and ethyl caproate. In this study, the  
34 initial inoculum was the pit mud of an old fermentation pit, which had been used to  
35 produce CSFL continuously for over 20 years. The pit is located in Mianzhu city,

36 Sichuan province, China.

37 **“Yellow water”**

38 A diffusate from fermentation mash, is brown and sticky muddy liquid produced  
39 from solid-fermentation of mixed materials including wheat, sorghum and corn.

40 Dominated compositions of yellow water in this study were (g/L), lactic acid,  $96.5 \pm 4.2$ ,  
41 ethanol,  $50.5 \pm 2.9$ , glucose,  $15.6 \pm 2.2$ .

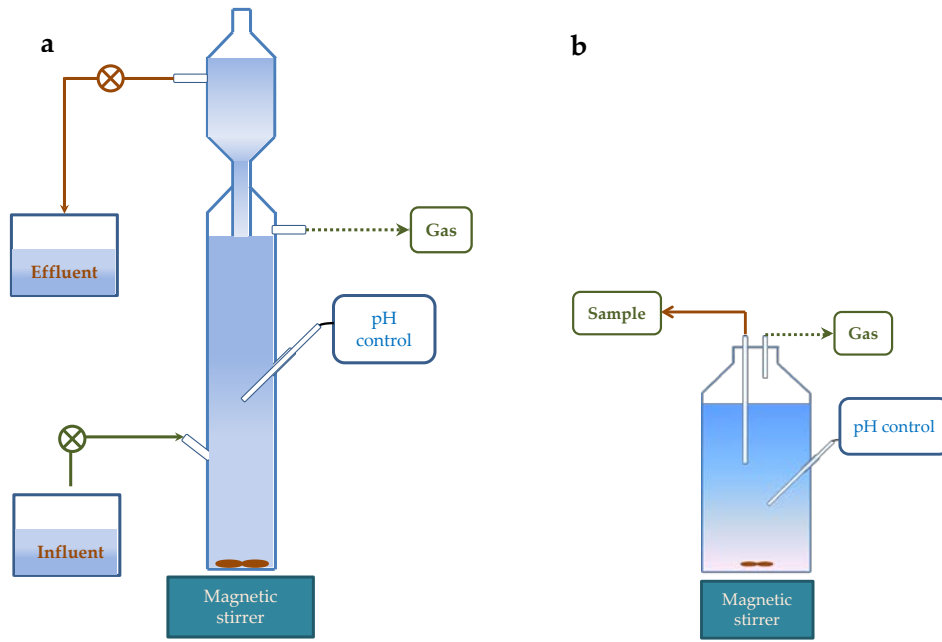
42 **Vitamin and trace elements solution**

43 Vitamin for medium: vitamin B12, 100.00 mg; p-aminobenzoic acid 80.00 mg;  
44 D(+)-biotin 20.00 mg; nicotinic acid 200.00 mg; calcium pantothenate 100.00 mg;  
45 pyridoxine hydrochloride 300.00 mg; thiamine-HCl  $\times 2$  H<sub>2</sub>O 200.00 mg; distilled water  
46 1000.00 ml.

47 Vitamin for artificial wastewater: biotin 2.0 mg; folic acid 2.0 mg; pyridoxine  
48 hydrochloride 10.0 mg; thiamine-HCl 5.0 mg; riboflavin 5.0 mg; nicotinic acid 5.0 mg;  
49 calcium D-(+)-pantothenate 5.0 mg; vitamin B12 0.1 mg; p-aminobenzoic acid 5.0 mg;  
50 thioctic acid 5.0 mg; distilled water 1000.00 ml.

51 Trace elements solution: nitrilotriacetic acid 2.0 g; MnSO<sub>4</sub>·H<sub>2</sub>O 1.0 g;  
52 Fe(SO<sub>4</sub>)<sub>2</sub>(NH<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O 0.8 g; CoCl<sub>2</sub> · 6H<sub>2</sub>O 0.2 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.2 mg; CuCl<sub>2</sub>·2H<sub>2</sub>O 20.0  
53 mg; NiCl<sub>2</sub>·6H<sub>2</sub>O 20.0 mg; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 20.0 mg; Na<sub>2</sub>SeO<sub>4</sub> 20.0 mg; Na<sub>2</sub>WO<sub>4</sub> 20.0 mg;  
54 distilled water 1000.00 ml.

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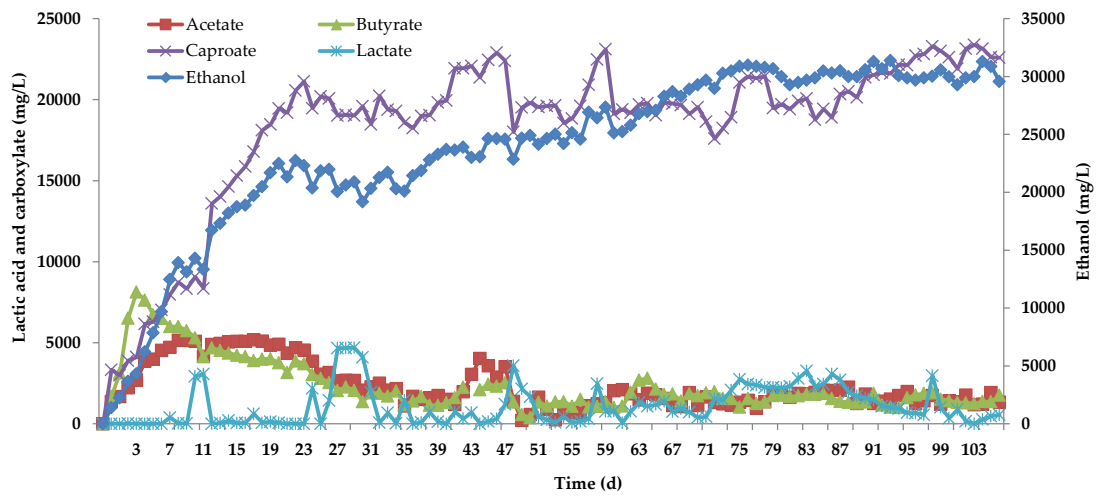
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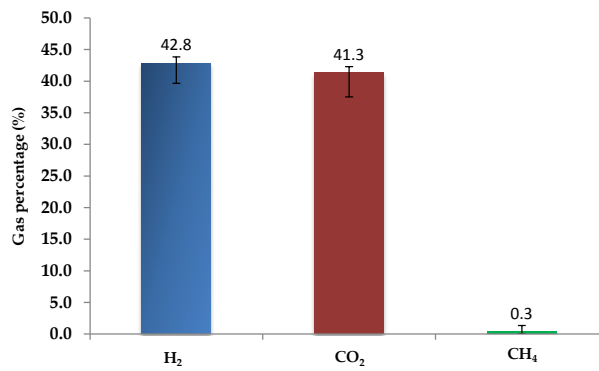
Figure S1 Schematic of the bioreactor setup: a, bioreactor fed with yellow water and operated semi-continuously(SCOR); b, bioreactor for substrates identification (batch reactor, BR).

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62 Figure S2 Dynamic changes of carboxylate, lactate, ethanol concentration during the acclimation  
63 period feeding with yellow water in reactor-SCOR.



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65 Figure S3 Average data of gas composition in the headspace of reactor (SCOR) during the acclimation  
66 period feeding with yellow water.

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68 Table S1 Comparison of product selectivities (based on electrons) by using different reducing  
 69 substrates

	products					
	formate <sup>a</sup>	acetate <sup>a</sup>	propionate <sup>a</sup>	butyrate <sup>a</sup>	valerate <sup>a</sup>	caproate <sup>a</sup>
ethanol <sup>d</sup>	n.d. <sup>c</sup>	- <sup>b</sup>	3.21	78.59	4.62	9.16
lactic acid	n.d. <sup>c</sup>	2.10	n.d. <sup>c</sup>	5.47	3.10	81.36
glucose	4.81	2.15	n.d. <sup>c</sup>	29.75	n.d. <sup>c</sup>	n.d. <sup>c</sup>

<sup>a</sup>: the unit was %.  
<sup>b</sup>: acetic acid was exhausted at the final day.  
<sup>c</sup>: concentration was always lower than the detecting line.  
<sup>d</sup>: fed with acetate at the same time

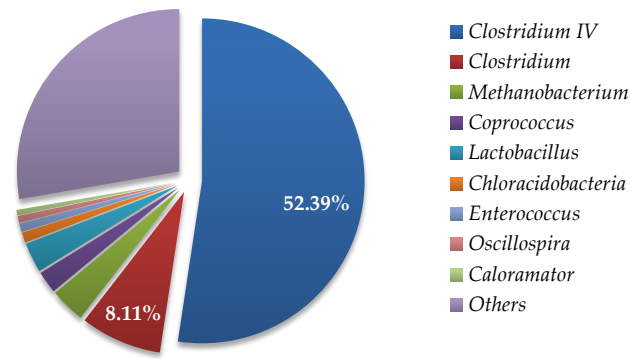
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72 Table S2 The relative abundance of prokaryotic community in the fermentation reactor SCOR #1 and  
 73 #2 on day 30 and 90 at genus levels.

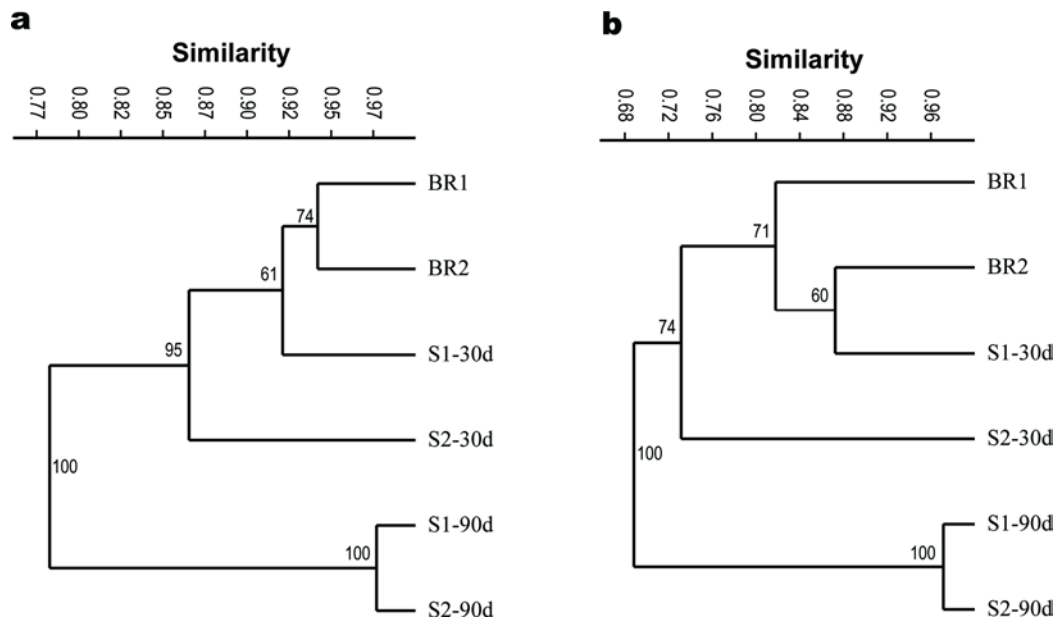
Family and genus	30 days			90 days		
	#1	#2	average	#1	#2	average
<i>Ruminococcaceae</i>	53.48	40.24	46.86 ± 9.36	77.91	80.55	79.23 ± 1.87
<i>Clostridium IV</i>	53.20	39.88	46.54 ± 9.42	77.74	80.41	79.07 ± 1.89
<i>Lactobacillaceae</i>	9.93	17.21	13.57 ± 5.15	4.24	3.55	3.90 ± 0.49
<i>Lactobacillus</i>	9.93	17.21	13.57 ± 5.15	4.21	3.52	3.87 ± 0.49
<i>Clostridiaceae</i>	5.91	18.76	12.34 ± 9.09	3.52	3.38	3.45 ± 0.10
<i>Clostridium</i>	3.19	16.93	10.06 ± 9.72	2.26	2.10	2.18 ± 0.11
<i>Caloramator</i>	2.07	0.09	1.08 ± 1.40	0.18	0.14	0.16 ± 0.03
<i>Lachnospiraceae</i>	2.16	2.30	2.23 ± 0.10	0.18	0.22	0.20 ± 0.03
<i>Coprococcus</i>	0.97	1.26	1.12 ± 0.21	0.14	0.13	0.14 ± 0.01
<i>Prevotellaceae</i>	0.04	0.02	0.03 ± 0.01	1.68	1.98	1.83 ± 0.21
<i>Prevotella</i>	0.04	0.02	0.03 ± 0.01	1.68	1.98	1.83 ± 0.21
<i>Tissierellaceae</i>	2.14	0.12	1.13 ± 1.43	1.36	1.28	1.32 ± 0.06
<i>Tissierella</i>	1.87	0.07	0.97 ± 1.27	0.14	0.11	0.13 ± 0.02
<i>Sedimentibacter</i>	0.05	0.02	0.04 ± 0.02	0.77	0.68	0.72 ± 0.06
<i>Methanobacteriaceae</i>	0.35	0.02	0.19 ± 0.23	0.36	0.29	0.33 ± 0.05
<i>Methanobrevibacter</i>	0.02	0.01	0.02 ± 0.01	0.21	0.15	0.18 ± 0.04
<i>Methanobacterium</i>	0.33	0.02	0.18 ± 0.22	0.14	0.14	0.14 ± 0.00

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76 Figure S4. The relative abundance of prokaryotic community in batch reactors (BR) at genus levels



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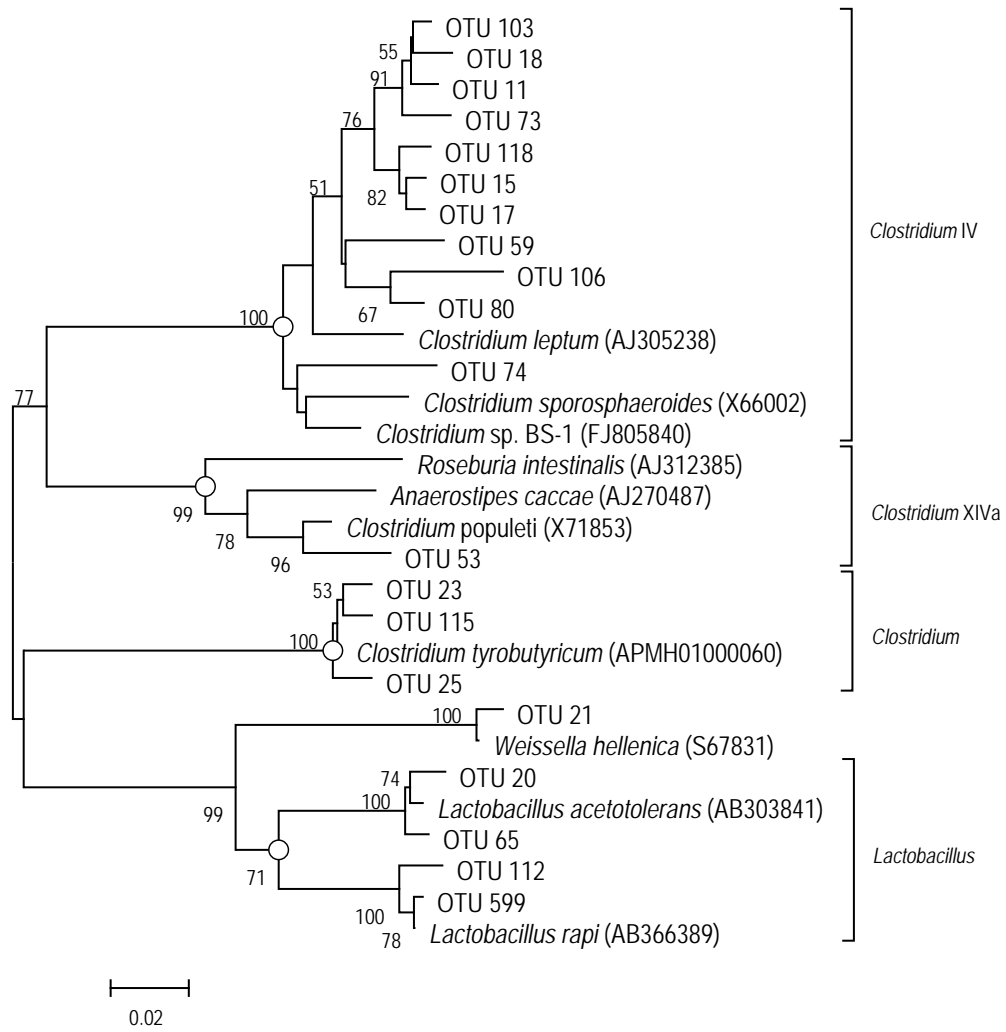
79 Figure S5. Cluster analysis (CA) of microbial communities in semi-continuously operated reactor (SCOR)

80 and batch reactor (BR) conducted using the Bray–Curtis distance in PAST based on at order level (a) and

81 genus level (b). The numbers at the nodes indicate the level of bootstrap of 1000 replications.

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84 Figure S6. Phylogenetic tree of the abundant OTUs of the microbiome and related species based on 16S  
 85 rRNA gene sequence similarity. GenBank accession numbers of the 16S rRNA gene sequences are  
 86 given in brackets. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are  
 87 shown at nodes. Bar, 0.02 substitutions per nucleotide position.

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