Supporting Information

The role of lipids in fermentative propionate production from the co-fermentation of lipid and food waste

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1. Supplemental materials and methods

1.1 Illumina Miseq sequencing

Illumina Miseq sequencing technology was used to analyze the microbial community in the fermentation system. Samples were collected on the 2nd, 6th, 12th, 15th and 21st day of the fermentation reaction in all reactors for DNA extraction and microbial analysis. Genomic DNA was extracted using the DR4011 kit (Bioteke, China) according to the manufacturer's instructions. The quality (A260/A280) and quantity (A260) of genomic DNA extracted were determined using a Nanodrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was amplified using polymerase chain reactions and the universal 16S rRNA primers F (CAAGC AGAAG ACGGC ATACG AGATG TGACT GGAG TTCA GACGT GT GCT CTTCC GATCT (barcode) ACTCC TACGG GAGGC AGCAG) and R (A ATGA TACGG CGACC ACCGA GATCT ACACT CTTTC CCTAC ACGAC GCTCT TCCGA TCT (barcode) GGACT ACHVG GGTWT CTAAT). DNA sequences were grouped by comparison and 97% of similarities were grouped into the same operational taxon (OTU). The sequence phylogeny was then classified and assigned to phylum and genus levels.

1.2 Calculations

Acidification degree was the parameter chosen to describe substrate conversion (in COD basis), based on the concentration of measured VFA and expressed as (Bevilacqua et al., 2021):

$$\text{Acidification degree}(\%) = \frac{\Sigma C_{VFA}}{C_{BSA}} * 100\%, \quad (1)$$

where C_{VFA} stands for the total concentration of the measured VFAs (in g COD-VFA/L) in the reactor effluent and C_{BSA} for the total protein concentration (in g COD/L) in the feeding of the reactor.

Ammonification was also used as a proxy to monitor protein conversion to VFA, as amino acid fermentation is always related to $N\text{-}NH_4^+$ release. It was expressed as follows (Bevilacqua et al., 2021):

$$\text{Ammonification } (\%) = \frac{C_{N\ effluent} - C_{N\ feeding}}{C_{N\ max}} * 100\%, \quad (2)$$

where $C_{N\ effluent}$ is the concentration of ammonium nitrogen (mg $N\text{-}NH_4^+$/L) measured in the reactor effluent and $C_{N\ feeding}$ is the concentration of ammonium nitrogen (mg $N\text{-}NH_4^+$/L) in the reactor feeding derived from the macronutrients supplementation and $C_{N\ max}$ is the maximum concentration of ammonium nitrogen (mg $N\text{-}NH_4^+$/L) achieved if complete degradation of protein occurs. $C_{N\ max}$ was estimated based on Total Kjeldahl Nitrogen measurements (SM4500C) of protein, which yielded a mg $N\text{-}NH_4^+$/g protein ratio of 160 for BSA.

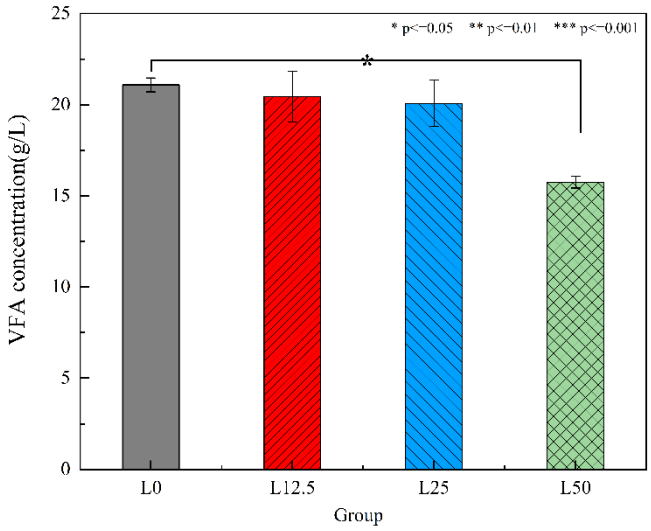

Fig. S1. Significance difference of maximum VFA production at different lipid contents during acidogenic fermentation.

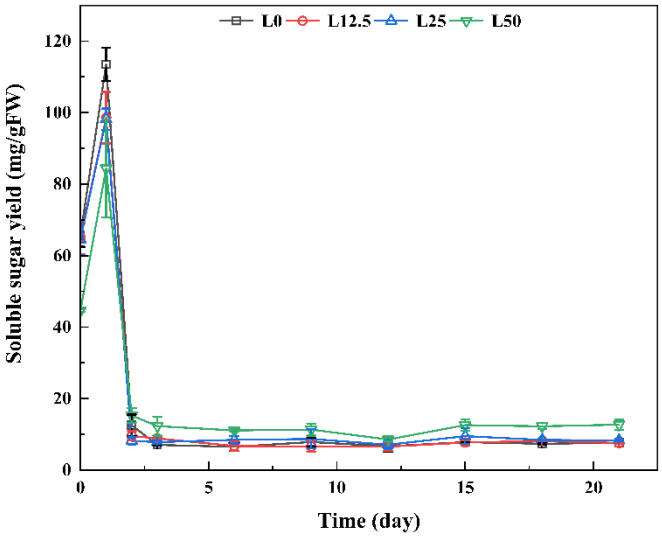

Fig. S2. Changes in concentrations of soluble sugar yield under different FW content at different lipid contents during acidogenic fermentation.

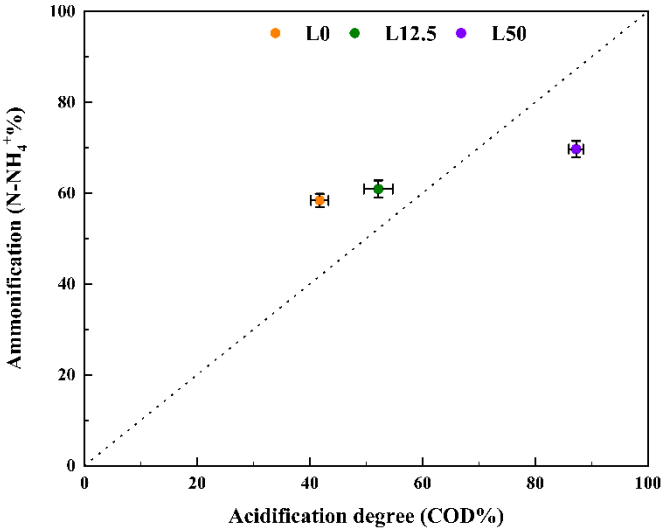

Fig. S3. Comparison between acidification degree (x-axis) and ammonification (y-axis) during continuous fermentation of BSA.

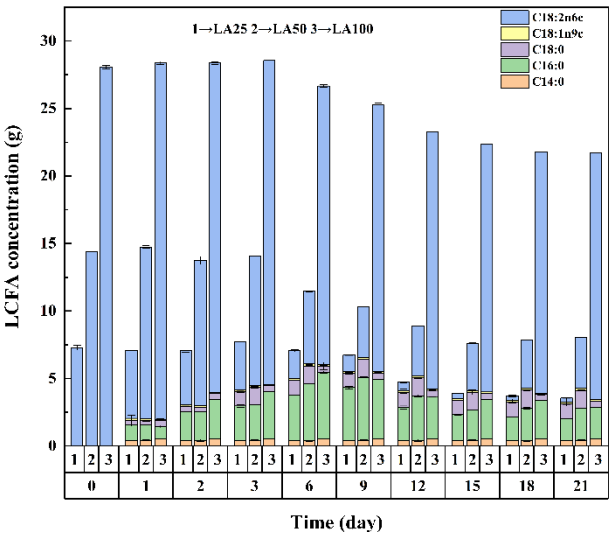

Fig. S4. Composition changes of LA in experimental group with different LA contents during acidogenic fermentation.

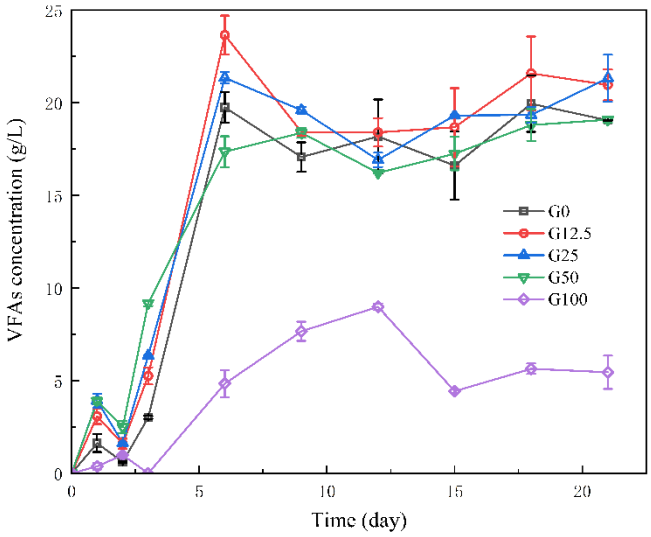

Fig. S5. Changes in concentrations of VFAs at different glycerol contents during acidogenic fermentation.

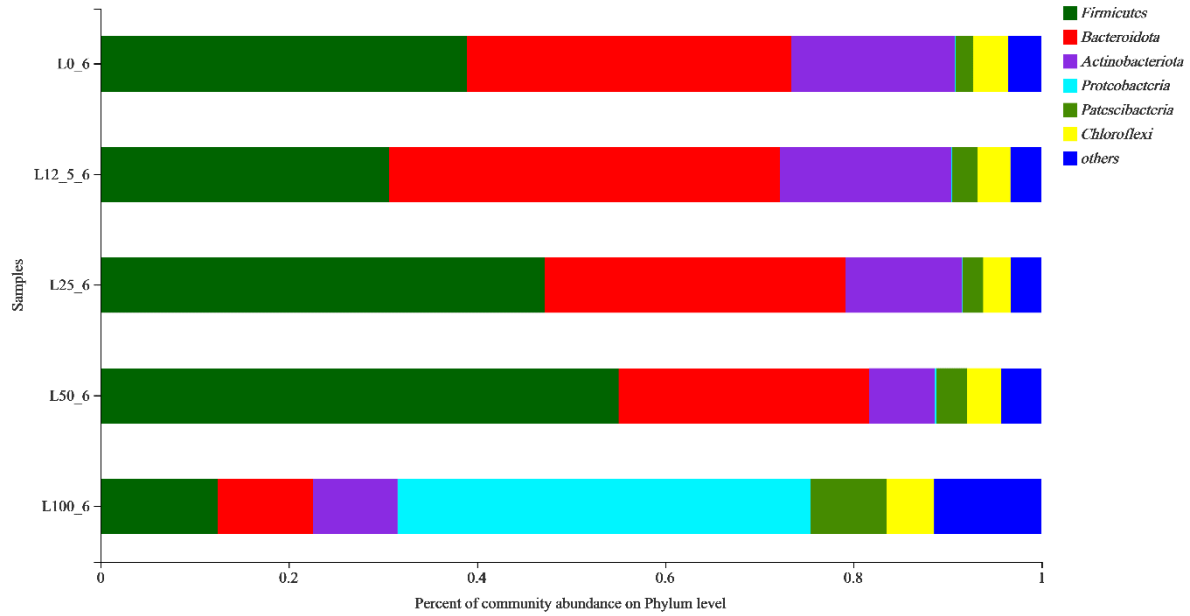

Fig. S6. Taxonomic classification of MiSeq sequencing of the bacterial community of inoculum sludge for samples from L0, L12.5, L25, L50, and L100 treatments at phylum on day 6. The relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample. Phyla making up less than 5% of the total composition in all treatments were classified as ‘‘others”.

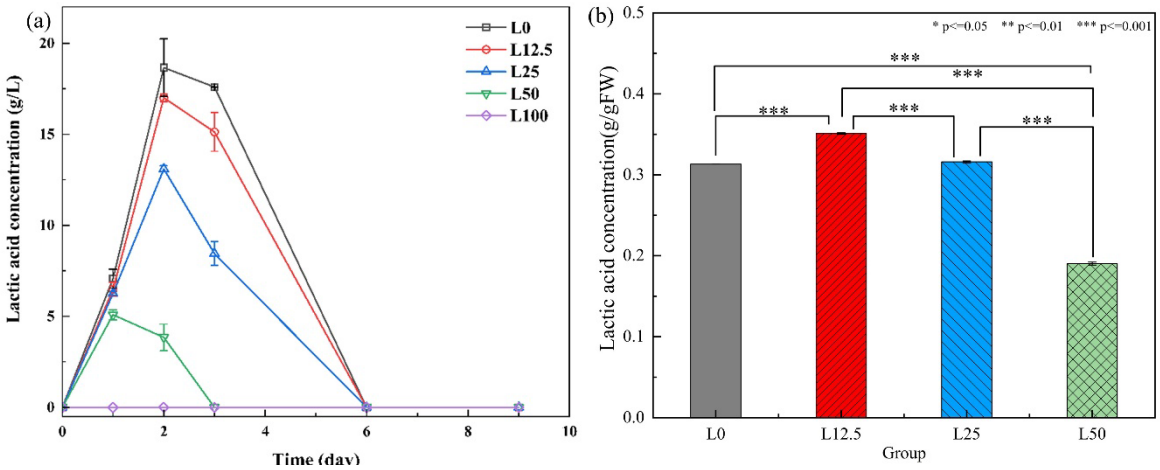

Fig. S7. Changes in lactic acid concentration at different lipid concentrations during acidogenic fermentation. Lactic acid production(a); theoretically the lactic acid produced per gram of FW at the highest Lactic acid production (b).

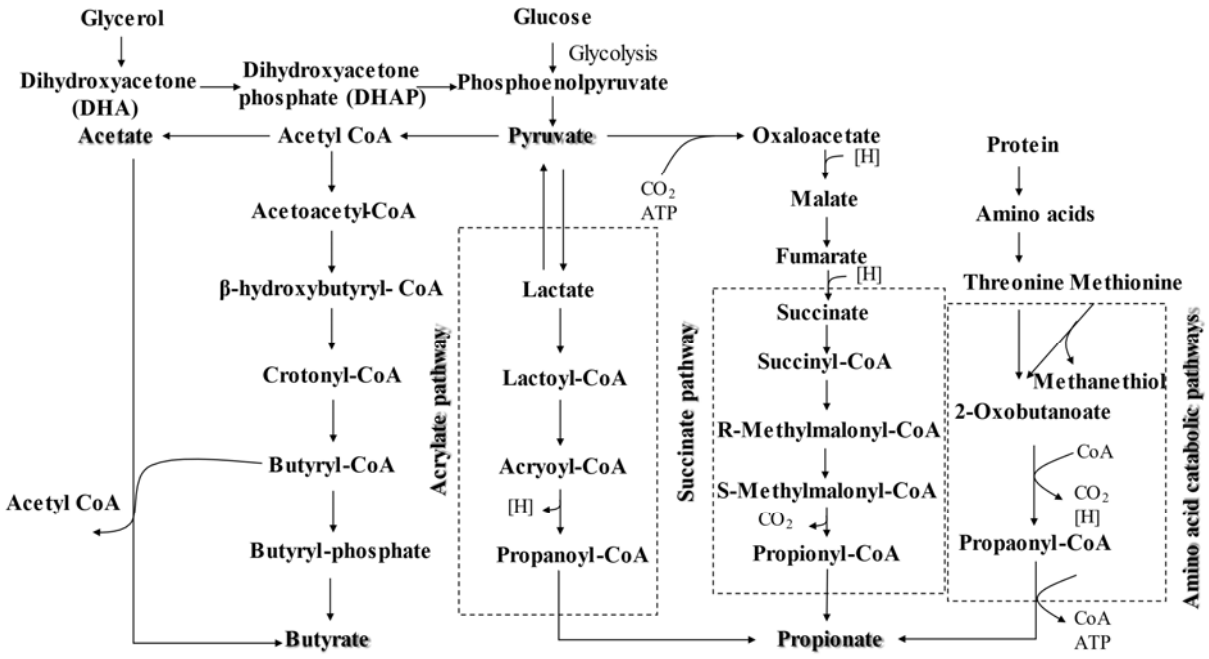

Fig. S8. Proposed pathway for metabolism of glucose, glycerol, lactate into PA in the co-fermentation. This pathway is extended and modified from previous models (Zhuge et al., 2013).

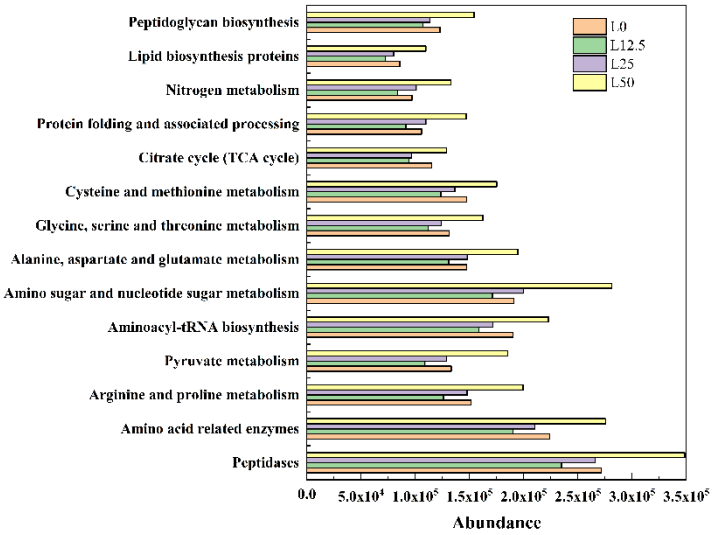

Fig. S9. Effects of lipid on the dynamics of metabolic functions of bacterial community in the co-fermentation based on KEGG database at level 3.

Table S1. Characteristics of the FW, anaerobic sludge used in the present study.

Parameter	FW	Anaerobic sludge
TS（%）	25.9	13.4
VS（%）	98.6	82.4
TCOD（%）	1.1	0.9
C/N	25.9	5.3
Cellulose and hemicellulose（%）	37.2	40.2
T-lipids (%)	0.27	-
T-sugar (%)	54.1	1.4

Table S2. Substrate total solid loading for acidogenic fermentation. Each condition was run in duplicate vessels containing 70 g/L of TS (35 g TS =28 g substrate +7 g sludge).

Lipid content	% Substrate loading	
	FW	Lipid
0% (L0) control	100	0
12.5% (L12.5)	87.5	12.5
25% (L25)	75	25
50% (L50)	50	50
100% (L100) control	0	100

Lipid content refers to the proportion of lipid in the fermentation substrates (FW and lipid), i.e. Lipid%=Lipid/(Lipid +FW).

Table S3 VFA yield and kinetic parameters estimated with the first-order rate equation and modified Gompertz model fitting.

	First order model		Modified Gompertz model			
(A)Test samples	Rate Constant (d^{-1})	R^2	Max. VFA yield (g TVFA/L)	Max. VFA production rate (g TVFA/L/d)	Lag Phase (d)	R^2
L0	0.29±0.08	0.87	18.90±0.81	2.93±0.14	0.92±0.05	0.94
L12.5	0.27±0.06	0.93	18.88±0.54	3.04±0.12	0.57±0.03	0.97
L25	0.34±0.09	0.88	17.16±0.76	3.04±0.13	0.56±0.05	0.93
L50	0.55±0.14	0.86	13.32±0.62	3.50±0.14	0.32±0.04	0.90

Table S4 Comparison of some PA fermentation approaches from the literature.

Strain	Fermentation Approach	Substrate(s)	Productivity (g PA/L·d)	Reference
P. acidipropionici	Fed-batch	Glycerol	4.80	(Zhu et al., 2010)
P. shermanii	Fibrous-bed bioreactor (Repeated-batch)	Glycerol/Glucose	6.00	(Wang and Yang, 2013)
P. acidipropionici	Sequential batch (with cell recycle)	Glycerol	5.28	(Dishisha et al., 2013)
P. acidipropionici	Batch	Glycerol/Glucose	2.94	(Zhang et al., 2015)
P. acidipropionici	Immobilized cell bioreactor	Glycerol	5.52	(Cavero-Olguin et al., 2021)
Veillonella and *norank_f_Propionibact-eriacea e*	Batch	FW/lipid	6.23	This study

Table S5. Pearson correlation between lipid and glycerol.

	Lipid	Glycerol
Lipid	1	0.786**
Glycerol	0.007	1

**：The correlation was significant at $p < 0.01$ (two-tailed).

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