Supporting Information

for

Green Synthesis of Lactic Acid and Carbon Dots using Food Waste and Seashell Waste

Jin-Hua Mou[a], Ling-Feng Ouyang[b], Zi-Hao Qin[a], Ya-Hui Miao[a], Xin-Tian Jiang[a], Mui-Choo Jong[b], Man-Chung Tang[c], Chenyu Du[d], Season Si Chen[b, *], Carol Sze Ki Lin[a, *]

[a] School of Energy and Environment, City University of Hong Kong, Hong Kong SAR, China

[b] Institute of Environment and Ecology, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen 518005, China

[c] Institute of Materials Research, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen 518005, China

[d] School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, United Kingdom

* Correspondence authors

E-mail address: carollin@cityu.edu.hk

season.chen@sz.tsinghua.edu.cn

1. Supplementary material and methods

1.1 Preparation of food waste hydrolysate

Briefly, 1.5 kg of food waste was blended with 400 mL of deionised water using a kitchen blender, and then subjected to enzymatic hydrolysis using 1% (v/m, enzyme/food waste) dosage of glucoamylase and cellulase (Novozymes, China). The hydrolysis broth was centrifuged at 8,000 ×g for 20 min, and the supernatant was collected, filtered using 1-μm pore size of filter paper, and stored at -20 °C until further processing. The final glucose concentrations are 197 ± 40 g/L.

1.2 Quantification of dry cell weight

The cells were washed with 1.5 mL of 0.9% NaCl solution and centrifuged again. After removing the supernatant, the residual pellets were washed with 1.5 mL deionized (DI) water and dried at 60 °C till constant weight. For the cultivations using seashell powders and fine powders, 1.5 mL of 7% HCl was first added to remove the powders collected during the sampling procedure, and then washed with NaCl solution and DI water for dry cell weight (DCW) testing.[1]

1.3 High-performance liquid chromatography conditions for glucose and lactic acid quantification

An Aminex HPX-87H column (Bio-Rad, USA) was used for HPLC testing. The injection volume was 10 μL and 5 mM H_2SO_4 was used as the mobile phase with 0.6 mL/min flow rate and column temperature at 60 °C. Detection was performed by a refractive index (RI) detector (Waters, UK) at 35 °C.

1.4 Ion chromatography conditions for Ca^{2+} quantification

The injection volume was 25 μL, and the chromatographic column was the IonPac CS12A (4 × 250 mm, Thermo Scientific, USA) using 20 mM methanesulfonic acid as the eluent with a flow rate of 1.0 mL/min. The electrolytically regenerated suppressor was CSRS 300 (4 mm, Thermo Scientific, USA) with the applied current of 59 mA. The column and cell temperature were controlled at 30 °C and 35 °C, respectively.

1.5 Simulation of industry-scale biorefinery models for lactic acid production

In total, three food waste biorefinery models (scenarios) for lactic acid production were simulated using the SuperPro Designer v13 software (Intelligen, USA). The detailed process description is presented below, and the corresponding flowsheets of Scenario Shell, Scenario Na, Scenario Ca were presented in **Fig. S8†**, **Fig. S9†**, and **Fig. S10†**, respectively. Each scenario consists of three sections: Section 1 of food waste hydrolysis, Section 2 of lactic acid fermentation, and Section 3 of lactic acid purification.

Based on the reported experimental results, Scenario Shell is the case where shell waste fine powders were adopted for lactic acid production in *in-situ* fibrous bed bioreactor (*is*FBB) fermentation mode using food waste hydrolysate (FWH) medium. The unit procedures were allocated into three sections. Section 1 covered food waste hydrolysis. First, the size of the food waste was reduced through grinding, and then mixed with water. A solid-to-liquid ratio of 30 w/v% has been noted to result in more effective hydrolysis in terms of the glucose yield.[2, 3] This ratio was applied accordingly in this simulation model. Subsequently, hydrolysis was performed at 55 °C for 24 h in a stirred reactor. Specifically, since this study focused on the utilisation of FWH, glucoamylase was applied in 0.01 v/w %. The hydrolysed slurry was subsequently centrifuged into three phases. The crude lipids (oil phase) can be combined with the remaining solids (solid phase) for the recovery of food waste lipids (which can be further converted into other products such as polyurethane rigid foams) and residual solids for animal feeds. However, since these were not within the scope, the utilization of these two phases was not simulated in the current model, and mass allocation was performed in order to obtain the LCI for the lactic acid solely. As the main focus of this study, the hydrolysate (aquatic phase) was sent to Section 2 for LA fermentation. The hydrolysate stream was mixed with water to obtain a final glucose concentration of 100 g/L. Yeast extract was supplemented on a 10 g/L basis. All ingredients of the fermentation medium were properly sterilised by pasteurization. The fermentation condition was set according to the settings described. A train of seed fermenters was adopted to inoculate the final fermenter in a stepwise manner. In addition, to mimic the final *is*FBB fermentation

process, although five fermentation unit procedures were shown in **Fig. S8†**, they all used the same set of fermenter. The fermentative lactic acid production yield was modelled based on the results reported in this study. Seashell waste fine powders, obtained via grinding of shell waste (95% calcium carbonate), were used to neutralise the lactic acid produced during the whole fermentation process, resulting in the formation of calcium lactate. At the end of fermentation, all broth was transferred to a storage tank which also works as a buffer tank between Section 2 and Section 3 of lactic acid purification. The first purification process involved the separation of biomass from the broth via a disk stack centrifuge. The separated biomass could be utilised for the production of carbon quantum dots (CQDs) as described. However, as this LCA focused on lactic acid, the relevant unit procedures required for the production of CQDs were not modelled in this scenario. The supernatant, on the other hand, is heated up to 85°C and entered a neutralising reactor where calcium lactate is converted back to lactic acid using concentrated sulfuric acid solutions. The generated calcium sulfate (gypsum), which has low solubility and precipitates out of solution, was separated using a belt filter and was disposed of as solid waste. The filtrate solution, which contains the lactic acid product, impurities, unfermented substrates, and some dissolved calcium sulfate, is cooled down to 52 °C and sent to the ion exchange columns and a granular activated carbon column for purification. The purified product solution is then sent to a multi-effect evaporator for concentration. The feed to the evaporator contains around 10.5% w/w lactic acid and 89.5% water, while the concentrated outlet solution contains around 87.7% lactic acid and 12% water. The concentrated lactic acid solution is further dewatered in a series of distillation columns, and the final product stream contains approximately 99.5% lactic acid.

Since the majority of the unit procedures were shared within the three scenarios, here we only point out the differences of Scenario Na and Scenario Ca in comparison to Scenario Shell. Scenario Na is the case where sodium hydroxide (NaOH) is used for the neutralisation of lactic acid during the fermentation process. This Scenario shared almost the same configurations in Section 1 and Section 2 in comparison to Scenario Shell with the only difference being NaOH as the lactic acid neutraliser. In

Section 3, the dewatered broth was also neutralised by strong sulfuric acids. However, since sodium sulphate does not precipitate out as $CaSO_4$, they were removed by the following ion exchange and granular activated carbon columns after a simulated ionisation process. Therefore, in this Scenario, the ion exchange resins, and water needed to regenerate these columns sharply increased compared to other two scenarios.

Scenario Ca is the case where $Ca(OH)_2$ is used for the neutralisation of lactic acid during the fermentation process. Since lactic acid is also converted into calcium lactate, similar to Scenario Shell, this scenario generally shared the same unit procedures in all three sections compared to Scenario Shell with the only difference being calcium hydroxide as the lactic acid neutraliser.

Fig. S1† Different forms of seashell waste.
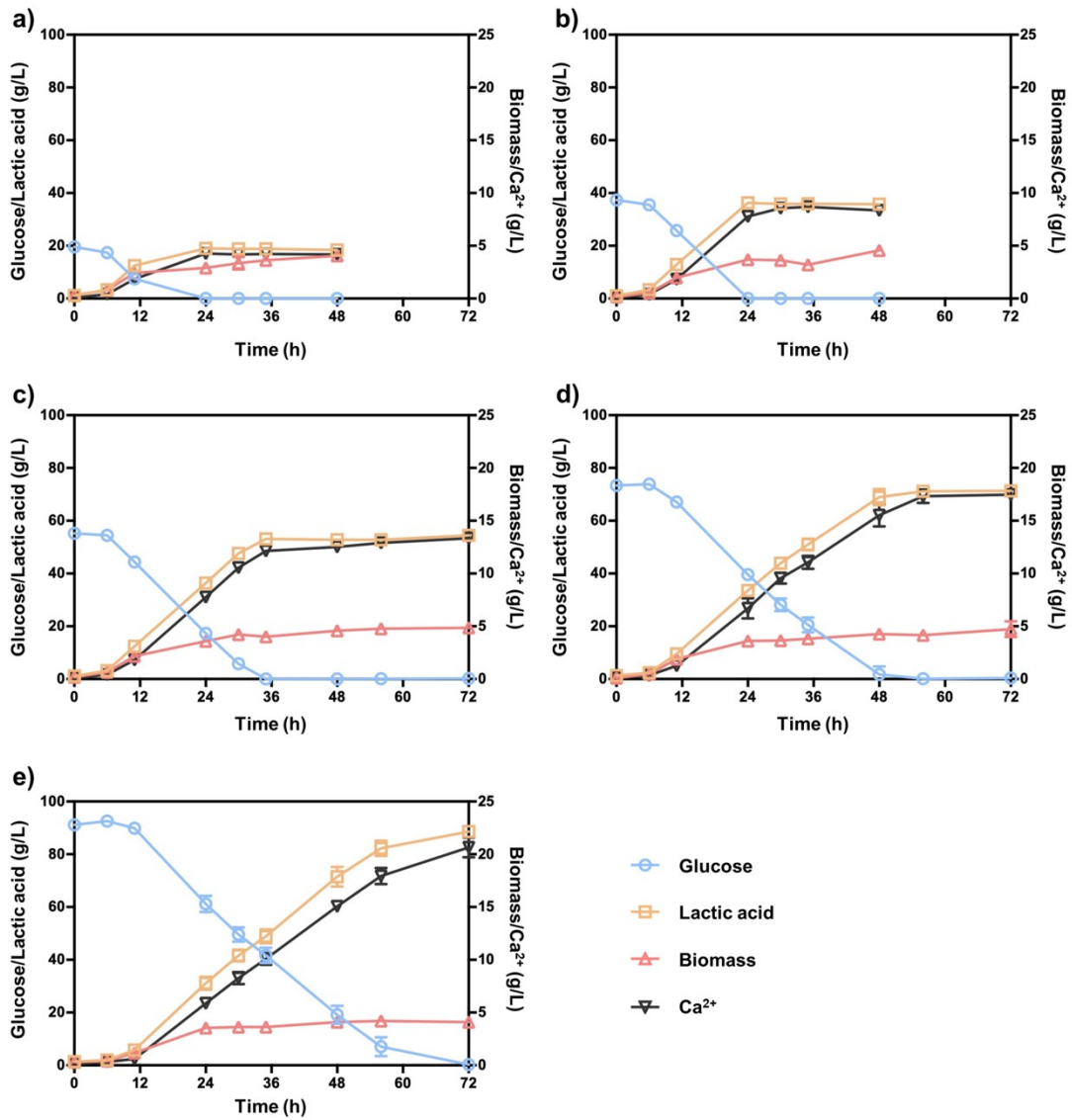
Fig. S2† Lactic acid fermentation in shake flasks using MRS medium with different glucose concentration and using seashell pieces as the acid neutraliser. **a)** 20 g/L; **b)** 40 g/L; **c)** 60 g/L; **d)** 80 g/L; **e)** 100 g/L.
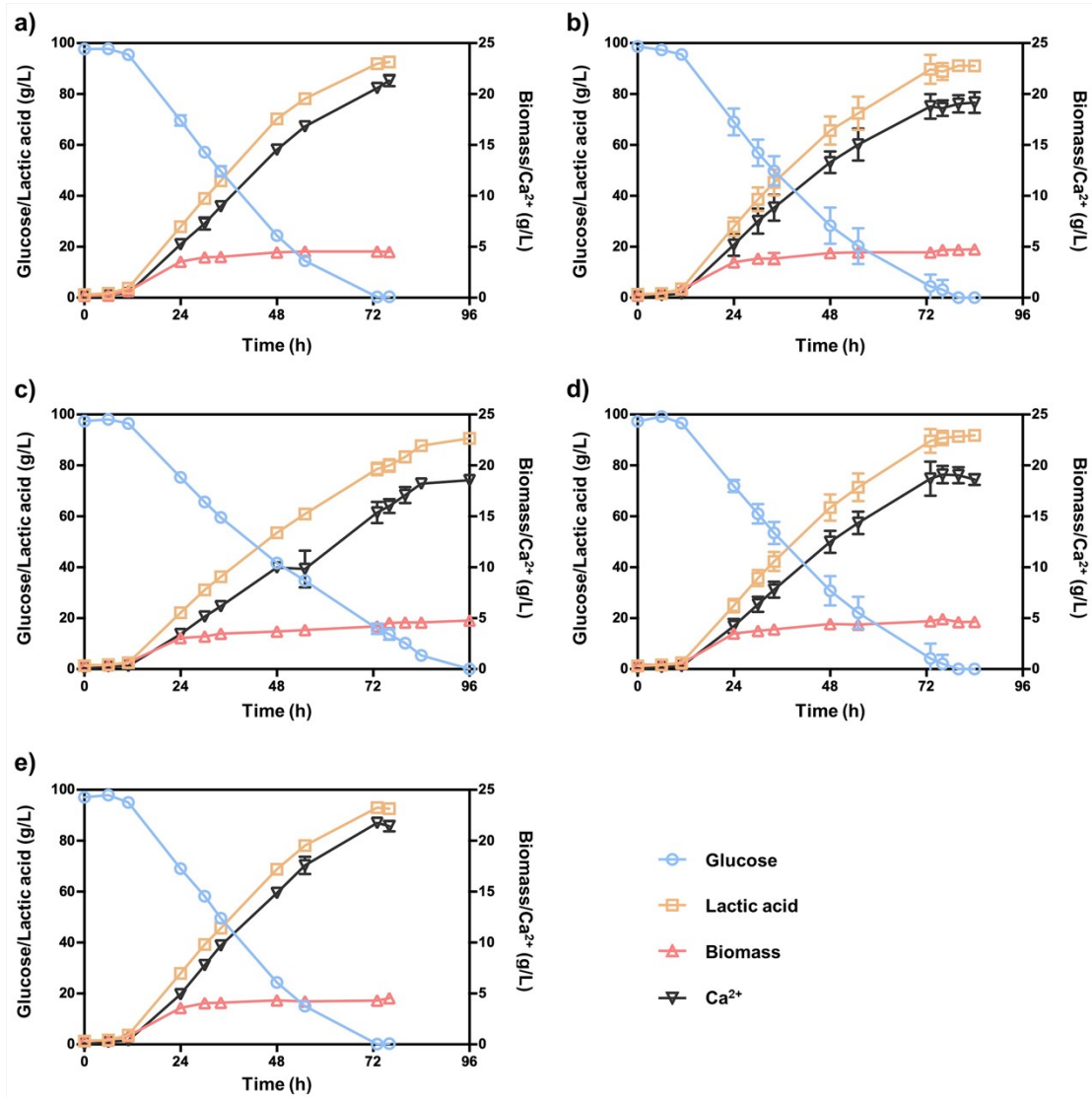
Fig. S3† Lactic acid fermentation in shake flasks using MRS medium with different seashell wastes (pieces) as the acid neutraliser. **a)** clam; **b)** oyster; **c)** abalone; **d)** scallop; **e)** razor clam.

Fig. S4† Lactic acid fermentation using abalone shell (i.e. shake flask on the right with fermentation broth in green colour).
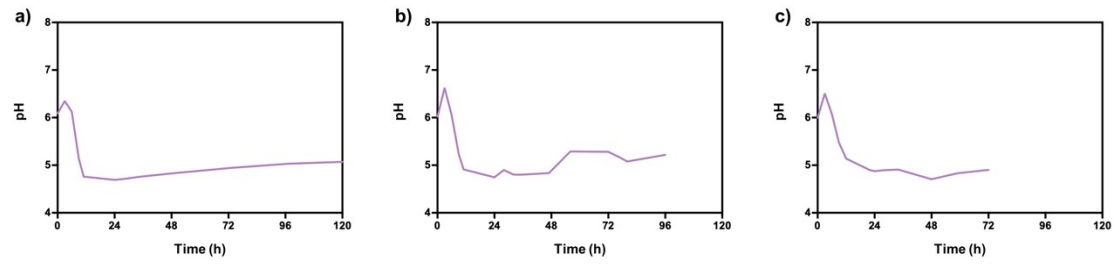
Fig. S5† The pH profiles of lactic acid fermentation in 2.5-L bioreactors using seashell waste as the acid neutraliser. **a)** seashell piece; **b)** seashell powder; **c)** seashell fine powder.
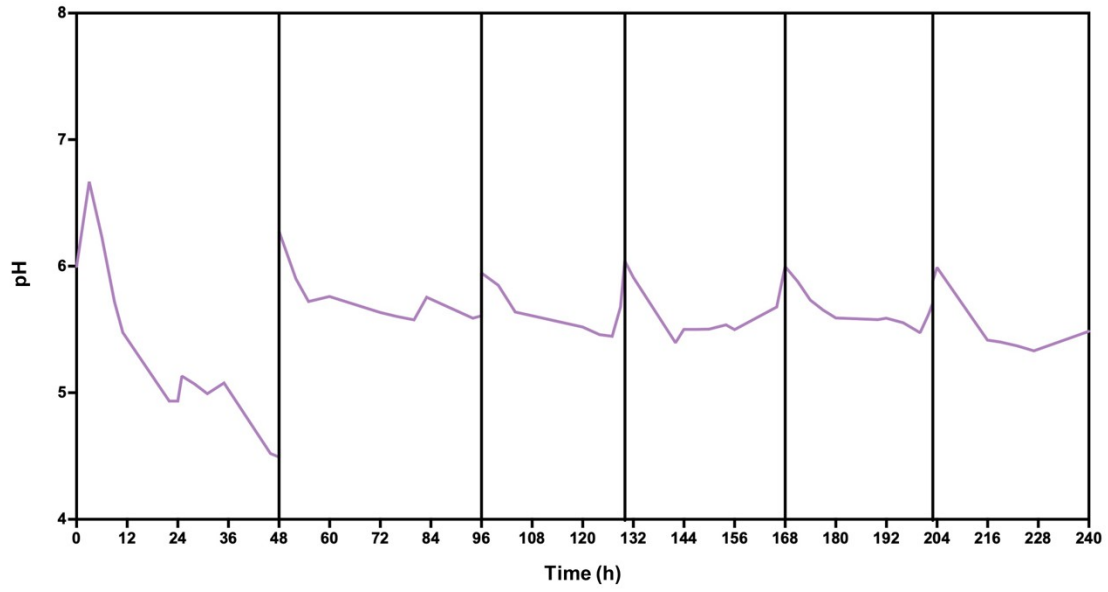
Fig. S6† The pH profile of lactic acid fermentation in *in-situ* fibrous bed bioreactors using food waste hydrolysate and seashell fine powders for pH control.
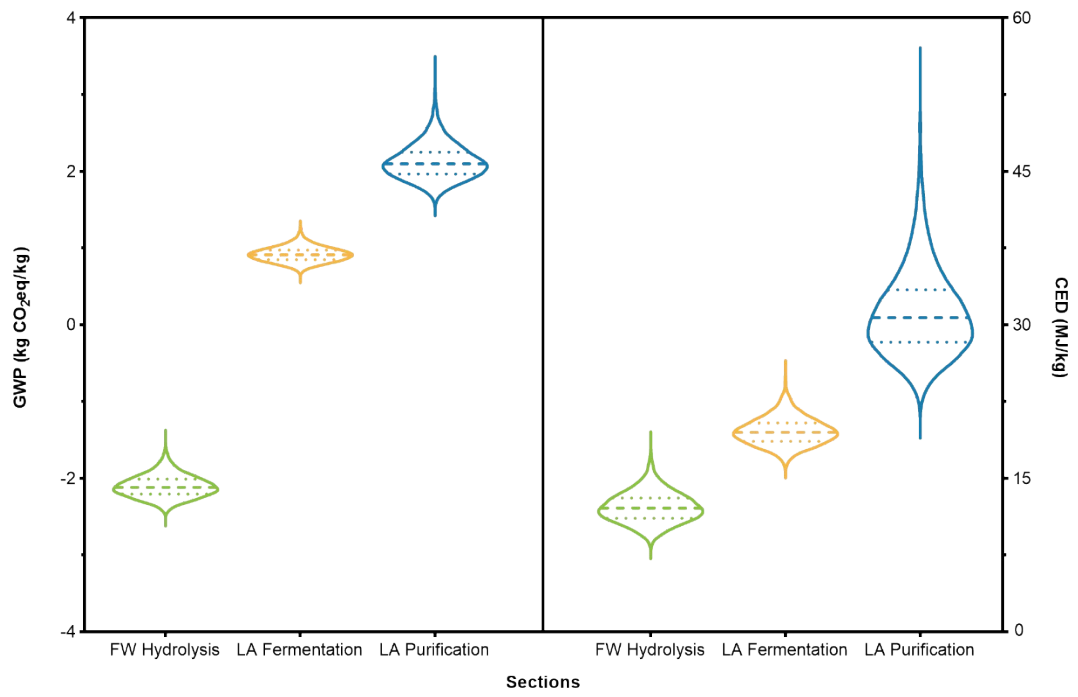
Fig. S7† Uncertainty analysis of lactic acid produced from Scenario Shell. FW: food waste. LA: lactic acid.
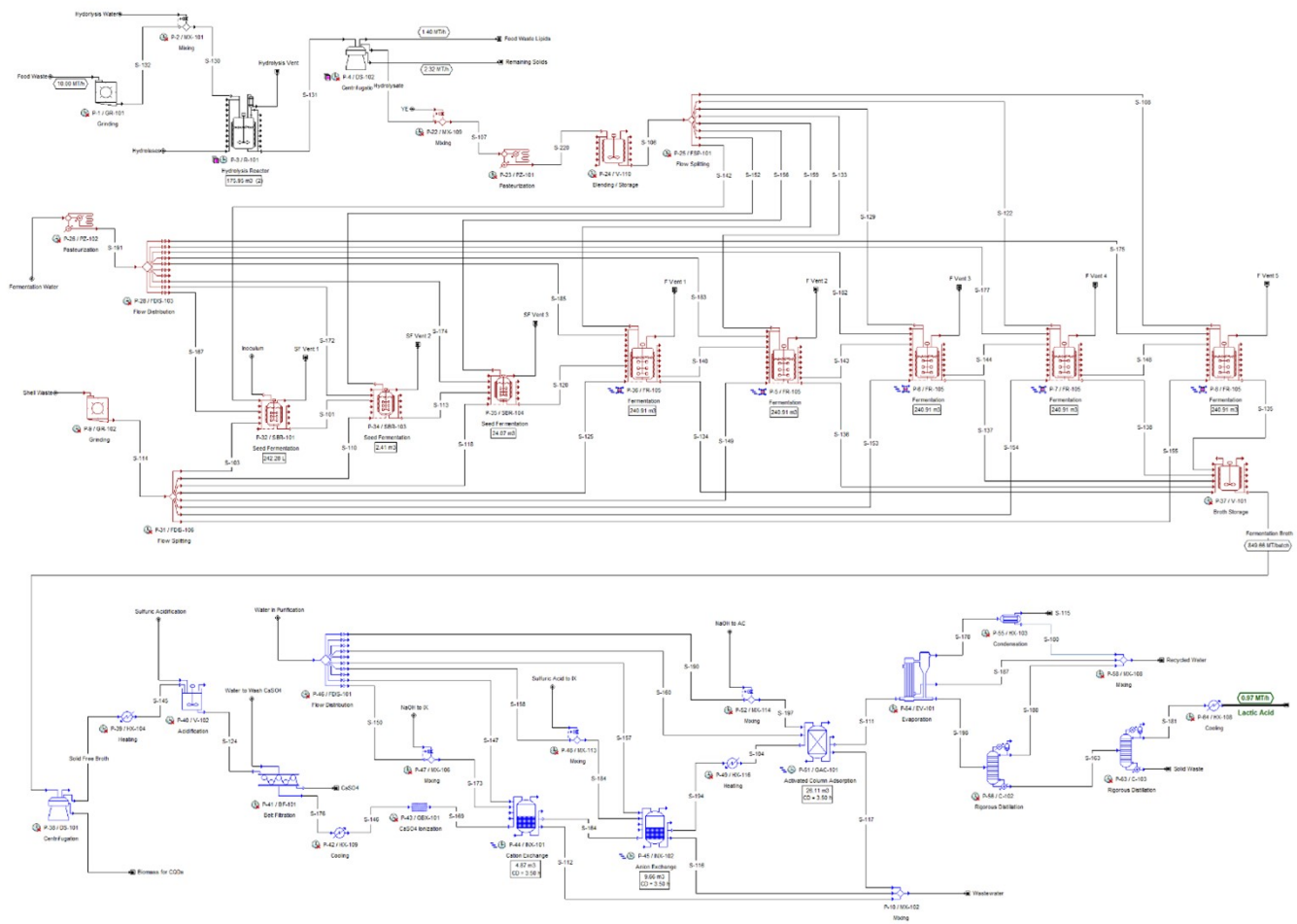
Fig. S8† Unit procedure flowsheet of Scenario Shell built in SuperPro Designer v13. Black icons indicate unit procedures in the food waste hydrolysis section; red icons indicate the lactic acid fermentation section; and blue icons indicate the lactic acid purification section.

Fig. S9† Unit procedure flowsheet of Scenario Na built in SuperPro Designer v13. Black icons indicate unit procedures in the food waste hydrolysis section; red icons indicate the lactic acid fermentation section; and blue icons indicate the lactic acid purification section.

Fig. S10† Unit procedure flowsheet of Scenario Ca built in SuperPro Designer v13. Black icons indicate unit procedures in the food waste hydrolysis section; red icons indicate the lactic acid fermentation section; and blue icons indicate the lactic acid purification section.

Table S1† Life cycle inventories of 1 MT lactic acid produced in different scenarios.

| Input	Scenarios			Unit
	Shell	Na	Ca	
Avoided Products				
Food Waste	5450	5239	5406	kg
Shell Waste	651.92	N.A.	N.A.	kg
Materials				
Calcium Hydroxide	N.A.	N.A.	452.22	kg
CIP-Acid	118.18	116.19	119.52	kg
CIP-Caustic	197.22	193.82	199.40	kg
Food Waste	163.51	157.16	162.19	tkm
Hydrolases	0.55	0.52	0.54	kg
Shell Waste	19.56	N.A.	N.A.	tkm
Sodium Hydroxide	70.85	1031.54	89.64	kg
Sulfuric Acid	596.91	1425.58	606.84	kg
Water	4.95	101.34	6.75	MT
WFI	1.35	1.34	1.38	MT
Yeast Extract	162.57	156.37	161.06	kg
Consumables				
INX Resin	0.08	2.93	0.13	kg
GAC Packing	0.05	0.05	0.05	kg
Utilities				
Std Power	1393.11	1287.75	1330.99	kWh
Steam	3.87	4.01	4.09	MT
Cooling Water	18650.99	18021.99	17324.74	MJ
Waste Management				
Solid Waste	1.44	3.01	1.48	MT
Wastewater	6.11	94.80	7.14	m³

Note. 1) Avoided products represent the avoided waste management of food waste or shell waste; 2) CIP means the operation of clean-in-place which requires both acidic solution (2 w/w % phosphoric acid solution in water) and caustic solution (1.96 w/w % sodium hydroxide solution in water) for the cleaning of bioreactors; 3) food waste and shell waste categorised in materials represents the transportation of these materials to the simulated factory, and therefore expressed in tkm, one MT of goods over one km by a given transport mode; 4) WFI represents water for injection, which is used in the operation of SIP, sterilise-in-place, for the sterilisation of fermenters; 5) INX resin represent ion exchange resin; 6) GAC packing represents granular activated carbon packing materials; 7) Std power means electricity; 8) cooling water is presented in the form of cooling energy required (expressed in megajoule, MJ), due to lack of 'cooling water' background data in the background LCI databases; 9) N.A. represents not applicable.

Table S2† Lactic acid fermentation results in shake flasks using MRS medium with different initial glucose concentrations and clam seashell pieces.

Glucose concentration (g/L)	Titre (g/L)	Yield (g/g)	Productivity (g/L/h)	Final pH
20	17.86 ± 0.12	0.91 ± 0.03	0.74 ± 0.00	5.78 ± 0.22
40	35.27 ± 0.74	0.91 ± 0.02	1.47 ± 0.03	5.83 ± 0.08
60	51.92 ± 0.28	0.94 ± 0.02	1.48 ± 0.01	5.57 ± 0.10
80	67.68 ± 2.94	0.92 ± 0.03	1.41 ± 0.06	5.54 ± 0.13
100	87.19 ± 0.85	0.96 ± 0.01	1.44 ± 0.05	5.33 ± 0.12

Table S3† Surface component analysis with atomic % of different type of seashells and $CaCO_3$.

	Ca	C	O	Mg	Al	Si
Calm	16.8	30.9	52.3	--	--	--
Oyster	10.0	52.1	37.7	0.1	--	0.1
Abalone	9.3	50.7	39.8	0.1	0.1	0.1
Scallop	16.4	33.5	49.8	0.1	--	0.1
Razor calm	14.3	37.3	48.2	--	0.1	0.1
$CaCO_3$	21.3	23.5	54.9	0.2	--	--

Table S4† Weight loss of different type of seashells and $CaCO_3$ during thermogravimetric analysis.

Weight loss (%)	Room temperature to 600 °C	600 °C to 950 °C
Calm	2.61	44.42
Oyster	2.82	46.37
Abalone	4.22	45.23
Scallop	1.63	44.31
Razor calm	4.10	47.02
$CaCO_3$	0.72	44.77

	Room temperature to 600 °C	600 °C to 950 °C
Calm	2.61	44.42
Oyster	2.82	46.37
Abalone	4.22	45.23
Scallop	1.63	44.31
Razor calm	4.10	47.02
$CaCO_3$	0.72	44.77

Table S5† Lactic acid fermentation results in shake flasks using MRS medium and using different types of seashells.

Seashell type	Titre (g/L)	Yield (g/g)	Productivity (g/L/h)	Final pH
Calm	90.62 ±0.62	0.93 ±0.01	1.24 ±0.01[a]	5.15 ±0.07[a]
Oyster	89.68 ±0.84	0.91 ±0.00	1.23 ±0.01[a]	4.45 ±0.18[b]
Abalone	89.31 ±0.65	0.92 ±0.00	1.04 ±0.01[b]	4.48 ±0.10[b]
Scallop	90.59 ±0.75	0.93 ±0.01	1.23 ±0.06[a]	4.58 ±0.16[b]
Razor calm	91.76 ±0.76	0.94 ±0.01	1.27 ±0.01[a]	5.02 ±0.06[a]

(Different lowercase letters indicate significant differences at $P < 0.05$)

Table S6† Lactic acid fermentation results in shake flasks using MRS and FWH medium with different forms of the seashell and $CaCO_3$.

Medium	Seashell type	Titre (g/L)	Yield (g/g)	Productivity (g/L/h)
MRS	Piece	90.16 ±0.18[a]	0.93 ±0.00	1.22 ±0.01[a]
	Powder	89.45 ±0.14[a]	0.92 ±0.00	1.58 ±0.00[b]
FWH	Piece	68.82 ±2.37[b]	0.89 ±0.01	0.72 ±0.02[c]
	Powder	84.36 ±3.17[a]	0.87 ±0.04	1.06 ±0.06[d]
	Fine powder	89.38 ±1.62[a]	0.92 ±0.01	2.43 ±0.10[e]
	$CaCO_3$	88.26 ±3.13[a]	0.90 ±0.03	2.35 ±0.04[e]

(Different lowercase letters indicate significant differences at $P < 0.05$)

Table S7† Lactic acid fermentation results in 2-L bioreactors.

	Titre (g/L)	Yield (g/g)	Productivity (g/L/h)	Max. Biomass (g/L)
NaOH	77.63 ±1.28	0.73 ±0.02	1.55 ±0.02	3.72 ±0.26
Piece	51.80 ±0.15	0.54 ±0.00	0.56 ±0.00	1.67 ±0.67
Powder	83.80 ±1.84	0.86 ±0.02	1.05 ±0.01	3.59 ±0.57
Fine powder	85.91 ±4.68	0.88 ±0.01	1.48 ±0.06	4.91 ±0.48

Table S8† Environmental impacts associated with 1 kg commercial lactic acid. Data is extracted from Ecoinvent database using single issue methods of IPCC2021 GWP100 and Cumulative Energy Demand v1.11.

Category	Value	Unit
GWP100-fossil	4.37	kg CO_2eq/kg
GWP100-biogenic	0.01	kg CO_2eq/kg
GWP100-land transformation	0.0030	kg CO_2eq/kg
Total GWP	4.38	kg CO_2eq/kg
Nonrenewable, fossil	79.68	MJ/kg
Nonrenewable, nuclear	5.07	MJ/kg
Nonrenewable, biomass	0.0041	MJ/kg
Renewable, biomass	0.93	MJ/kg
Renewable, wind, solar, geothermal	0.51	MJ/kg
Renewable, water	1.54	MJ/kg
Total CED	87.74	MJ/kg

Table S9† Itemized breakdown of global warming potential (GWP) and cumulative energy demand (CED) results associated with the lactic acid produced from Scenario Shell. FW: food waste. LA: lactic acid.

Items	GWP (kg CO$_2$eq/kg)	CED (MJ/kg)
Section 1: FW Hydrolysis		
FW Offset	-3.34	-2.04
FW Delivery	0.21	3.03
Raw Materials	0.0055	0.084
Utilities	1.02	11.08
Waste Treatment	0.00045	0.0055
Section 2: LA Fermentation		
Shell Waste Offset	-0.40	-0.24
Shell Waste Delivery	0.025	0.36
Raw Materials	0.25	5.85
Utilities	1.03	13.59
Waste Treatment	0.0021	0.026
Section 3: LA Purification		
Raw Materials	0.19	3.51
Consumables	0.00041	0.0063
Utilities	1.88	27.28
Waste Treatment	0.055	0.41

Table S10† Operating cost and total production cost of 1 MT lactic acid in different scenarios.

Input	Unit	Unit price (USD/unit)	Scenario Shell		Scenario Na		Scenario Ca	
			Quantity	Cost (USD)	Quantity	Cost (USD)	Quantity	Cost (USD)
Raw Materials								
Calcium hydroxide	kg	0.13	N.A.	N.A.	N.A.	N.A.	452.22	58.79
CIP-Acid	kg	0.19	118.18	22.45	116.19	22.08	119.52	22.71
CIP-Caustic	kg	0.30	197.22	59.17	193.82	58.15	199.40	59.82
Hydrolases	kg	4.00	0.55	2.20	0.52	2.08	0.54	2.16
Sodium hydroxide	kg	0.3	70.85	21.26	1031.54	309.46	89.64	26.89
Sulfuric acid	kg	0.19	596.91	113.41	1425.58	270.86	606.84	115.30
Water	MT	0.49	4.95	2.43	101.34	49.66	6.75	3.31
WFI	MT	0.49	1.35	1.82	1.34	1.81	1.38	1.86
Yeast extract	kg	7.30	162.57	1186.76	156.37	1141.50	161.06	1175.74
Total				1409.50		1855.59		1466.58
Consumables								
INX resin	kg	2.00	0.08	0.16	2.93	5.86	0.13	0.26
GAC packing	kg	2.00	0.05	0.10	0.05	0.10	0.05	0.10
Total				0.26		5.96		0.36
Utilities								
Std power	kWh	0.03	1393.11	41.79	1287.75	38.63	1330.99	39.93
Steam	MT	12.00	3.87	46.44	4.01	48.12	4.09	49.08
Cooling water	MJ	0.001	18650.99	18.65	18021.99	18.02	17324.74	17.32
Total				106.88		104.77		106.33
Waste Management								
Solid waste	MT	12.8	1.44	18.43	3.01	38.53	1.48	18.94

Item	Unit	Unit price						
Wastewater	m^3	0.20	6.11	1.22	94.80	18.96	7.14	1.43
Total				19.65		57.49		20.37
Logistics								
Transportation of food waste	kg	0.025	5450	136.25	5239	130.98	5406	135.15
Transportation of shell waste	kg	0.025	651.92	16.30	N.A.	N.A.	N.A.	N.A.
Total				152.55		130.98		135.15
Operating cost				1688.84		2154.79		1728.79
Credits for solid waste management								
Food waste treatment	kg	0.08	5450	436	5239	419.12	5406	432.48
Shell waste treatment	kg	0.08	651.92	52.15	N.A.	N.A.	N.A.	N.A.
Total				488.15		419.12		432.48
Total production cost (USD/MT)				1200.69		1735.67		1296.31

Note: 1) Unit prices are calculated according to reference 4-7; 2) Government subsidies for waste management are considered by avoiding waste management of food waste or shell waste in traditional landfilling; 3) Food waste and shell waste require transportation to the simulated factory that charged with USD 25/MT; 4) N.A. represents not applicable.

Table S11† Elemental analysis of cell biomass and biomass-derived carbon quantum dots (Bio-CQDs).

Elemental analysis (wt.%)	C	H	O	N	S
Cell biomass	45.95	6.86	37.20	9.20	0.79
Bio-CQDs	39.00	6.82	42.12	11.57	0.49

References

1. C.S.K. Lin, C. Du, A. Koutinas, R. Wang and C. Webb, *Biochemical Engineering Journal*, 2008, **41**, 128-135.

2. I.K.M. Yu, K. L. Ong, D.C.W. Tsang, M. A. Haque, T.H. Kwan, S.S. Chen, K. Uisan, S. Kulkarni and C.S.K. Lin, *Catalysis Today*, 2018, **314**, 70-77.

3. T.H. Kwan, Y. Hu and C.S.K. Lin, *Bioresource Technology*, 2016, **217**, 129-136.

4. T.H. Kwan, Y. Hu and C.S.K. Lin, *Journal of Cleaner Production*, 2018, **181**, 72-87.

5. Glucoamylase Market Price in China. Accessed on 2024/05/14. https://www.made-in-china.com/products-search/hot-china-products/Glucoamylase_Price.html.

6. Alibaba.com https://www.alibaba.com/?gad_source=1&gclid=CjwKCAjwo6GyBhBwEiwAzQTmc9_5JNNw34dTbPHN2-6JJnHUiUkpUX_mXB1caAyB6JYTTn9kpSWAAhoCotwQAvD_BwE

7. H. Wang, C.-W. Tsang, M.H. To, G. Kaur, S.L.K.W. Roelants, C.V. Stevens, W. Soetaert, C.S.K. Lin, Techno-economic evaluation of a biorefinery applying food waste for sophorolipid production – A case study for Hong Kong, *Bioresource Technology*, 2020, **303**, 12285.