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## Green synthesis of lactic acid and carbon dots using food waste and seashell waste†

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Waste valorisation plays a crucial role in the sustainable production of valuable chemicals and materials. This study investigates the feasibility of green technology utilising food waste as a renewable substrate for lactic acid and the resulting residue for subsequent carbon dot production through microbial fermentation and hydrothermal reaction, respectively, while evaluating seashell waste as a replacement for commercial neutralisation reagents. The results demonstrated that seashell waste exhibited effective neutralisation performance during lactic acid fermentation. Within the five types of seashell waste studied, apart from abalone seashells, which led to a significant decrease in lactic acid productivity, all resulted in similar lactic acid fermentations. Additionally, fine powders of seashells were found to be optimal when combined with food waste hydrolysate for lactic acid fermentation. The highest lactic acid productivity of  $1.48 \text{ g L}^{-1} \text{ h}^{-1}$  obtained in 2 L bioreactor batch fermentation using fine shell powder was 1.64-fold and 0.41-fold higher than those obtained using shell pieces and shell powder, respectively. The results of cell immobilisation fermentation exhibited a superior performance with  $2.90 \text{ g L}^{-1} \text{ h}^{-1}$  glucose consumption rate and  $1.89 \text{ g L}^{-1} \text{ h}^{-1}$  lactic acid productivity, which were 1.23-fold and 0.97-fold higher compared to those obtained using NaOH as the neutraliser, respectively. The results of life-cycle assessment also revealed lower environmental impacts associated with lactic acid production using food waste and seashell waste. Cell biomass derived from this study was further utilised to synthesise biomass-derived carbon quantum dots (Bio-CQDs), which demonstrated excellent water solubility, photophysical properties, and potential application as an antibiotic sensor. Overall, the study highlights the potential of seashell waste as an acid neutraliser in lactic acid fermentation and showcases the promising properties of fluorescent Bio-CQDs synthesised from cell biomass, providing valuable insights into the development and implementation of green and sustainable production from waste sources.

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### 1. Introduction

Lactic acid holds great promise as a versatile building block for the production of both commodity and specialty chemicals across diverse industries.<sup>1</sup> In 2023, the global lactic acid market was evaluated at USD 3.37 billion, and it is projected to show a compound annual growth rate of 8% from 2024 to 2030.<sup>2</sup> Microbial fermentations offer a cost-effective and sustain-

able approach for lactic acid production, utilising inexpensive and renewable substrates. This approach has advantages such as low energy consumption and mild production temperature compared to chemical synthesis routes.<sup>3–5</sup> A number of studies have explored the use of waste materials including food industry by-products, agro-industrial residues and waste biomass, for lactic acid production.<sup>6,7</sup> Additionally, food waste has been investigated as a reliable feedstock in lactic acid production, given its nutrient-rich nature. Based on our previously demonstrated techno-economic analysis, the use of waste-based substrates can reduce the significant contribution of glucose (up to 51%) to the operating cost in fermentation, and lactic acid production using food waste has economic viability under the designed scenarios.<sup>8,9</sup> This not only reduces organic waste but also contributes to biorefinery advancement by alleviating dependence on fossil-based resources.<sup>10–12</sup>

Control of pH in microbial fermentation is crucial for cell growth and efficient organic acid production.<sup>13</sup> However, the

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alkalis commonly used in fermentations such as sodium hydroxide and potassium hydroxide can lead to issues such as high osmotic stress on microorganisms and potential equipment corrosion.<sup>14</sup> The use of these alkalis also has environmental and economic implications. Kwan *et al.* (2018) pointed out that the massive amounts of sodium hydroxide required during lactic acid fermentation accounted for 5% of the annual operating cost, and the life cycle assessment (LCA) in a similar biorefinery practice (using sugarcane bagasse for lactic acid production) revealed a major contribution of 52% to the total climate change impact when alkali pretreatment was applied to the feedstock.<sup>9,15</sup> Calcium carbonate is generally used as a neutralising agent in lactic acid fermentation and can yield a high production, while commercial calcium carbonate needs tedious manufacturing procedures.<sup>16,17</sup> A study conducted by Li *et al.* (2021) has pointed out the noteworthy aspects that a 12–18% contribution to the material cost was associated with using lime during lactic acid fermentation, as well as a 14–18% contribution to the global warming potential and a 4–5% contribution to fossil energy consumption.<sup>18</sup> These findings pinpoint the need to find a suitable replacement for commercial alkalis for the sustainable production of organic acids. To address these challenges and align with the principles of green chemistry and production, it would be beneficial to explore the utilisation of sustainable resources of calcium carbonate as a replacement for commercial alkalis in organic acid production.

Shell waste represents an abundant waste stream, particularly in coastal areas and the fishing industry. The production of molluscs (mostly bivalves) reached 17.7 million tons in 2020.<sup>19</sup> Effective management strategies are required to address shell waste, as improper disposal or incomplete recycling processes would lead to both economic and environmental burdens. Previous studies have extensively focused on crab and shrimp waste in view of the potential separation and valorisation of valuable components such as chitin and protein.<sup>20,21</sup> Another important component found in shell waste is biogenic calcium carbonate mineral, which is synthesised by living organisms. Seashell waste comprises approximately 96% calcium carbonate, which can be converted into useful products such as mineral fillers in cement.<sup>22–24</sup> As a sustainable source of calcium carbonate, seashells have also been studied for calcium lactate production *via* chemical reactions.<sup>25</sup> The mild reaction conditions and short reaction time indicate that seashells have potential as a feasible acid neutraliser in lactic acid fermentation, on which research is still rarely conducted.

Upon microbial fermentation, the cell biomass is typically discarded, leaving a substantial content of carbonaceous compounds unharnessed. Such biomass alternatively offers a promising carbon source for the production of value-added carbon quantum dots (CQDs). The trace heteroatoms in the biomass could be converted into amine, hydroxyl, carboxyl or mercaptan functional groups on the surface of CQDs, which not only eliminate the subsequent surface passivation step, but also enhance the physicochemical properties, quantum yield and visible light absorption capacities of CQDs.<sup>26,27</sup> Additionally,

biomass-derived CQDs exhibited enhanced characteristics such as water solubility, biocompatibility and ecological friendliness compared to chemically derived CQDs.<sup>28</sup> In view of the several excellent properties possessed by CQDs including ease of functionalisation, quantum confinement effects, controllable photoluminescence properties, and high economic value, CQDs have attracted growing investigation in the fields of photocatalysis, biological imaging, and sensing.<sup>29–31</sup>

Herein, we present a green and sustainable production strategy for efficient lactic acid fermentation *via* *Lactobacillus casei* Shirota. Firstly, we investigated the feasibility of adopting seashell wastes as an acid neutraliser in lactic acid fermentation. Subsequently, the combination of food waste hydrolysate and cell immobilisation technique was demonstrated in lactic acid fermentation using seashell wastes. LCA was applied to estimate the environmental impacts of using different acid neutralisers. Furthermore, residual cell biomass was further upcycled into CQDs as the environmental pollutant sensor. This strategy not only utilises seashell waste and food waste as renewable substrates, but also valorises cell biomass into valuable CQDs, establishing an environmentally friendly approach for the production of valuable materials in the lactic acid production value chain.

## 2. Materials and methods

### 2.1 Strains and cultivation media

*Lactobacillus casei* Shirota was obtained from the School of Biological Sciences at The University of Hong Kong. It was cultivated in a synthetic De Man, Rogosa and Sharpe (MRS) broth with an initial glucose concentration of 20 g L<sup>-1</sup> in a shaking incubator at 200 rpm at 37 °C for 16 hours.<sup>11</sup> The seed culture was prepared using MRS broth (20 g L<sup>-1</sup> glucose) for 24 hours, and inoculum was applied to get an initial optical density at 600 nm (OD<sub>600</sub>) of 0.1 using a visible spectrophotometer (Jenway 7300, USA).

### 2.2 Preparation of food waste hydrolysate and synthetic media

Food waste was collected from a university student canteen with the main components of rice and noodles for the production of glucose-rich hydrolysates as feedstock for the lactic acid fermentation. Saccharification of food waste was carried out in a 2 L bioreactor (ESI†), and the resultant food waste hydrolysate (FWH) was used to prepare FWH media (with an initial glucose concentration of 100 g L<sup>-1</sup> and the addition of 10 g L<sup>-1</sup> yeast extract) which were filter-sterilised for subsequent use in lactic acid fermentation. Synthetic MRS media with different initial glucose concentrations (20–100 g L<sup>-1</sup>) were prepared accordingly and sterile-filtered through a 0.22 μm pore size membrane filter (Sartorius, Germany).

### 2.3 Collection, pretreatment, and characterisation of seashell wastes

Five types of seashell waste, namely clam, oyster, abalone, scallop, and razor clam shells were collected from local restaur-

rants in Hong Kong based on the local preferences for seafood. Residual tissues and impurities were removed manually, and waste shells were washed with soap and rinsed with distilled water thoroughly. The cleaned seashell wastes were oven-dried at 90 °C overnight and stored for further processing. Pictures of the different forms of seashell are shown in Fig. S1.† For seashell-waste pieces, the size of seashell wastes was reduced using an iron hammer to break them into small pieces. For seashell-waste powder, seashell wastes were ground using a pulveriser (GX-04, Gaoxin, China), and homogenised and sieved to get powders of particle size smaller than 150 µm. For seashell-waste fine powder, seashell wastes were ground, homogenised and sieved to get powders of particle size smaller than 80 µm.

Different types of fine seashell powder and calcium carbonate (Aladdin, AR 99%) were used for characterisation. The crystallography of samples was characterised using an X-ray diffractometer (XRD, Bruker D2 Phaser), and the diffraction database was applied to compare and confirm the diffraction patterns of samples. The thermal decomposition investigation of samples was conducted using a thermogravimetric analyser (TA SDT 650, Waters, USA). Thermal measurement was conducted under a N<sub>2</sub> atmosphere and thermogravimetric curves were recorded from room temperature to 950 °C using a constant heating rate of 10 °C min<sup>-1</sup>. The surface morphology and composition of samples were examined using a field emission scanning electron microscope (FE-SEM) with energy-dispersive X-ray spectroscopy (EDS) (ZEISS SIGMA 500, Germany).

#### 2.4 Utilisation of seashell wastes as the acid neutraliser for lactic acid production in shake flasks

Shake flask experiments were conducted in a shaking incubator with a temperature of 37 °C and an agitation speed of 200 rpm. Samples were frequently taken to determine the dry cell weight (DCW), glucose, lactic acid, and Ca<sup>2+</sup> concentrations. The pH value of the culture was measured at the end of the cultivation using a pH meter (Starter 3100, OHAUS, China).

The optimisation of glucose concentrations was carried out in triplicate in 250 mL flasks containing 100 mL of MRS medium with different initial glucose concentrations (20, 40, 60, 80, and 100 g L<sup>-1</sup>) in the scenario of clam shell addition.

The feasibility of adopting different types of seashell was evaluated in triplicate in 250 mL flasks containing 100 mL of MRS medium with an initial glucose concentration of 100 g L<sup>-1</sup>. The seashell pieces (12 g) were weighed and added to the flask at the beginning of the fermentation.

The combination of FWH medium and seashell wastes was conducted in triplicate using 250 mL flasks containing 100 mL of FWH medium with an initial glucose concentration of 100 g L<sup>-1</sup>. Control groups consisting of synthetic MRS medium with 100 g L<sup>-1</sup> glucose were also included. Calcium carbonate, seashell pieces, seashell powder and fine seashell powder (12 g for each) were individually applied to investigate their respective effects on lactic acid production.

#### 2.5 Batch fermentation and cell immobilisation fermentation in bench-top bioreactors

The batch fermentations were carried out in duplicate in 2 L bench-top bioreactors (Biostat, Sartorius stedim, Germany) using FWH medium. The fermentation temperature was set at 37 °C, with an airflow rate of 2 vvm and an agitation speed of 200 rpm. The pH of cultivation was either automatically controlled at 6.0 using 10 M NaOH solution, or the pH was monitored using an online monitoring software (MFCS\_DA, Sartorius) with 80 g of seashell waste (in the form of pieces, powders or fine powders) added as the acid neutraliser at the beginning of the fermentation. Samples were taken to quantify DCW, glucose, lactic acid, and Ca<sup>2+</sup> concentrations.

An *in situ* fibrous bed bioreactor (*isFBB*) was specifically designed for cell immobilisation fermentation using *L. casei* Shirota, with FWH as the cultivation medium, and either NaOH solution or fine seashell powder serving as the acid neutraliser. Sugarcane bagasse was utilised, as shown in the ESI,† in the *isFBB*, and the procedure for the preparation of the fibrous bed with a size of 8 cm × 10 cm using 2 g of sugarcane bagasse was described in our previous study.<sup>32</sup> The seed culture was inoculated at the beginning of the free-cell cultivation with a working volume of 1.5 L of FWH medium. After 24 hours of cultivation, the fermentation broth was transferred into a sterile *isFBB* for continuous cell growth and simultaneous cell immobilisation on the matrix. After another 24 hours, the fermentation broth was transferred out and 1.5 L of fresh FWH medium was added to start the *isFBB* fermentation. A repeat cycle was performed by replacing the fermentation broth with fresh FWH media. A total of five repeated cycles (C1–C5) were conducted for each *isFBB* fermentation. Samples were taken throughout the fermentation process to quantify DCW, glucose, lactic acid, and Ca<sup>2+</sup> concentrations. The pH was either controlled using a 10 M NaOH solution or monitored when using seashells as the acid neutraliser.

#### 2.6 Analytical methods of lactic acid fermentation

After centrifugation at 13 000g for 3 min, the supernatants of fermentation samples were collected for subsequent analysis. The testing methods for DCW are described in the ESI.† The supernatants were diluted properly and filtered through 0.22 µm Nylon membrane filters (Jinteng, China). Glucose and lactic acid concentrations were analysed using high-performance liquid chromatography (HPLC, Waters, UK) with detailed conditions given in the ESI.† The yield of lactic acid is defined as the resulting lactic acid per gram of glucose consumed (g lactic acid per g glucose). The lactic acid productivity is defined as the maximum lactic acid titre divided by the hours needed to get the maximum titre (g L<sup>-1</sup> h<sup>-1</sup>). The concentrations of Ca<sup>2+</sup> in samples were determined using an ion chromatography system (Dionex ICS-6000, Thermo Scientific, USA); the detailed conditions are given in the ESI.†

#### 2.7 Life-cycle assessment

This LCA study was conducted in the following four phases to comply with the International Organization for

Standardization (ISO): (1) goal and scope definition, (2) inventory analysis, (3) impact assessment, and (4) interpretation.<sup>33,34</sup>

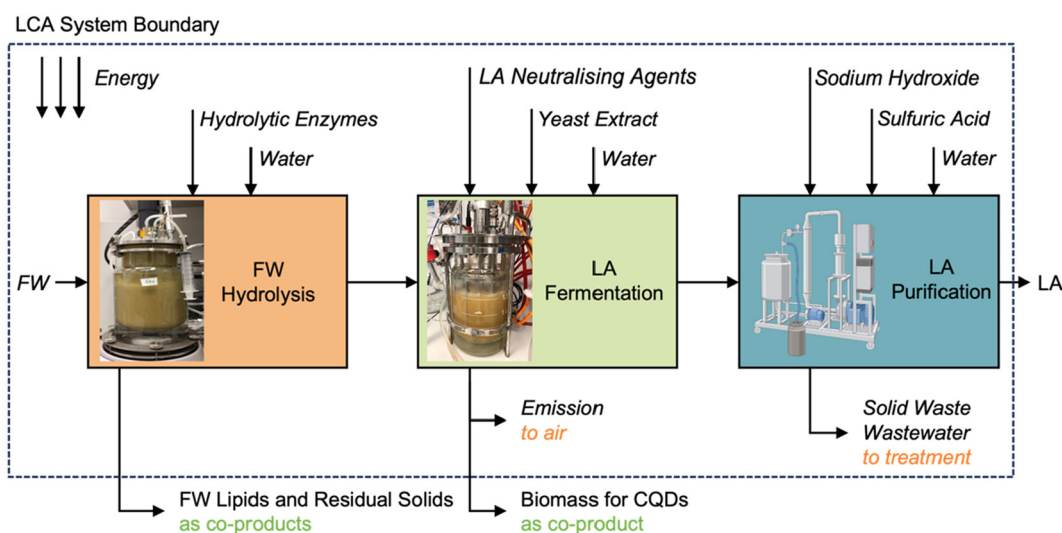
The goal of this LCA study is to assess the environmental impact associated with lactic acid production by *L. casei* Shirota using food waste and various neutralisers. There are three scenarios proposed in this LCA model, where Scenario Na and Scenario Shell refer to lactic acid production using sea-shells and NaOH (experimental results of this study), and Scenario Ca refers to lactic acid production using Ca(OH)<sub>2</sub>, which is usually applied in industrial lactic acid production (details are described in the ESI†). The system boundaries of this cradle-to-gate study are presented in Fig. 1. Transportation, uses, and the end-of-life of the product were excluded from this study. To facilitate an easy comparison with commercial lactic acid, the functional unit of this LCA was chosen to be 1 kg of lactic acid. The life cycle inventory (LCI) was obtained in SuperPro Designer v13 through the constructed models for different scenarios (details can be found in the ESI†). The detailed LCI for each scenario is presented in Table S1.† The background LCI databases include Ecoinvent 3, Agri-footprint 5, and US Life Cycle Inventory (USLCI). The choice of data from these databases was based on the geographical location preference in the order of China (Guangdong Province), global, and the rest of the world.

Life cycle impact assessment was performed using SimaPro v9.3. Assessment methods include the single issue methods of IPCC2021 GWP100 (Global Warming Potential on a 100-years horizon) and Cumulative Energy Demand (CED) v1.11. Interpretation was conducted by comparing the calculated characterisation results to the environmental impacts associated with commercial lactic acid. The relevant statistics for commercial lactic acid were extracted from the Ecoinvent 3 database using SimaPro v9.3. Sensitivity analysis was performed by determining the change in impact categories result-

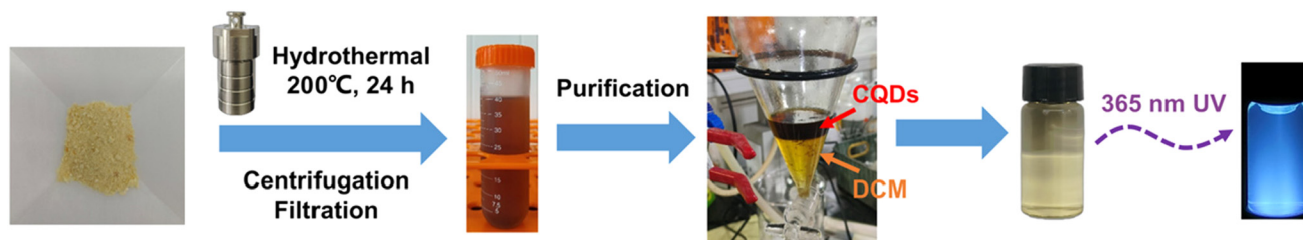
ing from a ±10% variation in the selected parameters. Uncertainty in this LCA study was determined through Monte Carlo simulation with 10 000 trials to randomly sample from the statistical distributions of GWP and CED characterisation factors using SimaPro software.

## 2.8 Preparation and characterization of biomass-derived carbon quantum dots

Biomass-derived carbon quantum dots (Bio-CQDs) were prepared from freeze-dried cell biomass *via* a one-pot hydrothermal method (Scheme 1). Typically, 0.5 g of the cell biomass and 30 mL of ultrapure water were mixed and then transferred into a 50 mL Teflon-lined autoclave. The mixture was heated at 200 °C for 24 h and then cooled to room temperature, where the resulting brown suspension was centrifuged at 12 000g for 10 min to separate the supernatant from the residual solids. Subsequently, the supernatant was filtered through a 0.22 μm filter membrane to remove large or agglomerated particles. Extraction with dichloromethane (DCM) further removed unreacted organic molecules to yield a higher-purity Bio-CQD solution. The final Bio-CQD yield was approximately 540 mg (g biomass)<sup>-1</sup>. The CHONS analysis of the cell biomass and the as-synthesized Bio-CQDs was conducted using an elemental analyzer (Elementar Vario EL Cube). Transmission electron microscopy (TEM, FEI spirit T12) was used to determine the morphology and size distribution of Bio-CQDs. The ultraviolet-visible (UV-vis) absorption spectra of Bio-CQDs were measured using a UV spectrophotometer (Shimadzu UV-2600). The photoluminescence (PL) properties and fluorescence quantum yield of Bio-CQDs were tested using a fluorescence spectrophotometer (Hitachi F-4700) and a steady-state fluorescence spectrometer (Edinburgh FLS1000), respectively. The characterization of the surface functional groups was carried out at room temperature using Fourier transform infrared spectroscopy (JASCO FT/IR-4100).



**Fig. 1** System boundary diagram. Italics indicate the connection to background LCI databases. FW: food waste. LA: lactic acid. LA neutralising agents: NaOH, shell, Ca(OH)<sub>2</sub>. Glucoamylase is used in FW hydrolysis in the simulation process.



Scheme 1 One-pot synthesis of Bio-CQDs from cell biomass.

## 2.9 Calculation of photoluminescence quantum yield

The photoluminescence quantum yield (QY) of Bio-CQDs was calculated based on the ratio of emitted photons to absorbed photons.<sup>35</sup> Based on this setup, the QY can be calculated using eqn (1):

$$\text{QY} = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}} = \frac{L_{\text{sample}}}{E_{\text{reference}} - E_{\text{sample}}} \quad (1)$$

where  $L_{\text{sample}}$  is the emission intensity, and  $E_{\text{reference}}$  and  $E_{\text{sample}}$  are the intensities of the excitation light not absorbed by the reference and the sample, respectively. The difference in integrated areas between the sample and the reference represents the number of absorbed photons. The photons emitted are determined by integrating the area of the emission band.

## 2.10 Detection of antibiotics using Bio-CQDs

The as-synthesized Bio-CQDs solution was diluted 50-fold using ultrapure water. The selectivity of the Bio-CQD solution was investigated. One mL of antibiotic solutions (*i.e.*, tetracycline [TC] and oxytetracycline [OTC]) ranging from 10 to 50  $\mu\text{M}$  was added to the Bio-CQD solution (4 mL) to evaluate the sensing capabilities. The PL spectra of the mixed solutions were acquired in the range of 250–550 nm using an excitation wavelength ( $\lambda_{\text{ex}}$ ) of 320 nm after incubation at room temperature for 10 min.

# 3. Results and discussion

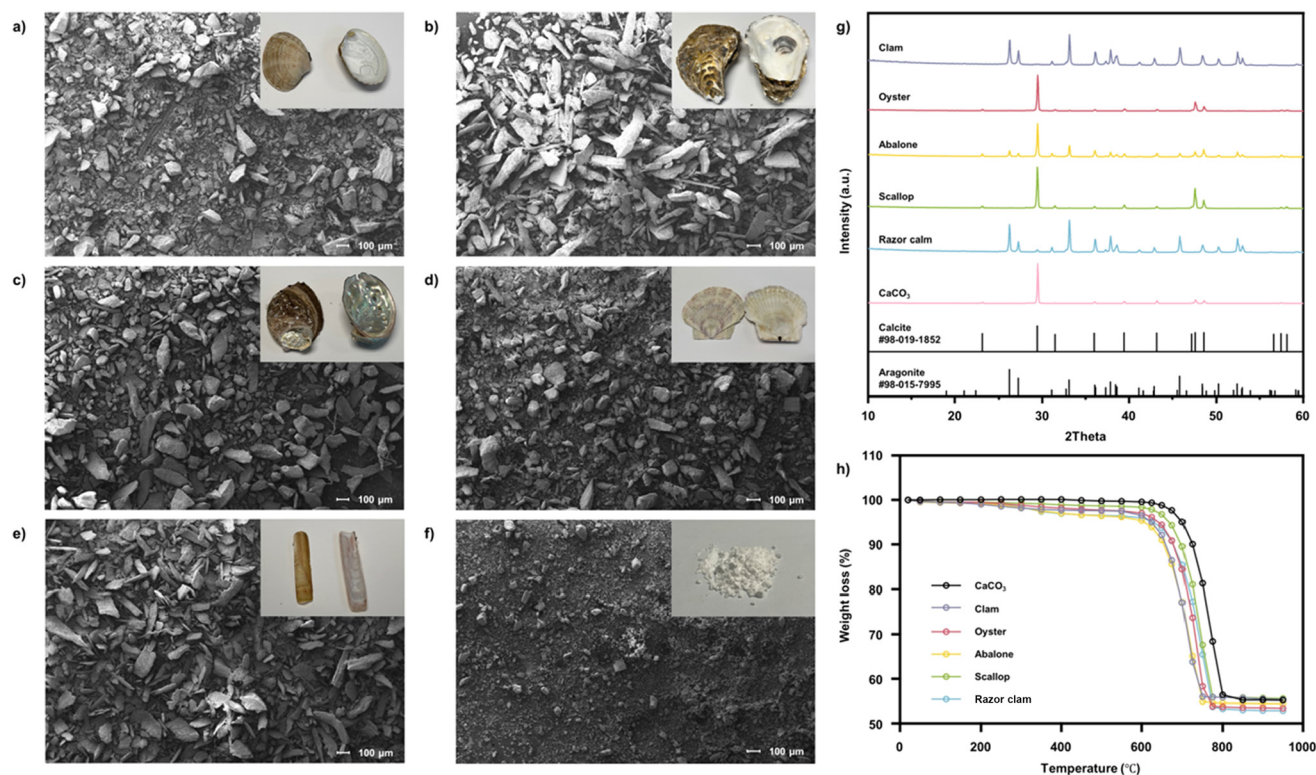
## 3.1 Clam seashell waste as the acid neutraliser in lactic acid fermentation by *L. casei* Shirota in shake flasks

In organic acid production, pH control is crucial as the acid accumulation causes a sharp decline in pH levels particularly in upscaling, while many microorganisms are not able to tolerate the low pH in most cases, resulting in reduced cell densities and lower production yields.<sup>36</sup> Instead of using alkali solutions, the feasibility of using seashell waste in lactic acid fermentation was investigated in the current study for its acid neutralisation ability. Clam seashell waste was chosen as a representative case as it is a major seafood waste in the local area. As shown in Fig. S2,† lactic acid fermentations were conducted using a synthetic medium with initial glucose concentrations ranging from 20 g L<sup>-1</sup> to 100 g L<sup>-1</sup>. The depletion of

glucose can be achieved within 3 days, with maximum biomass concentrations ranging from 4.05 to 4.85 g L<sup>-1</sup>. Lactic acid yields of over 0.91 g g<sup>-1</sup> and productivities of over 1.40 g L<sup>-1</sup> h<sup>-1</sup> were obtained (Table S2,† 0.74 g L<sup>-1</sup> h<sup>-1</sup> for the initial glucose concentration of 20 g L<sup>-1</sup>). The profiles of Ca<sup>2+</sup> concentration mirrored those of lactic acid production, and the pH at the end of cultivation was close to 6 (Fig. S2 and Table S2†). These results indicated that seashell waste was capable of lactic acid neutralisation under the designed fermentation conditions. A previous study investigated the impact of different glucose concentrations on *L. casei* bacterium fermentation under similar conditions while controlling the pH (6.0–6.5) using buffer solution and manual base addition. The lactic acid yields and productivities were 0.77–0.81 g g<sup>-1</sup> and 1.00–1.07 g L<sup>-1</sup> h<sup>-1</sup> (0.57 g L<sup>-1</sup> h<sup>-1</sup> for an initial glucose concentration of 20 g L<sup>-1</sup>), respectively.<sup>37</sup> In comparison, our results suggested that the seashell waste exhibits good neutralisation capacity during lactic acid fermentation and has the potential to improve both the yield and productivity of lactic acid by *L. casei* bacterium.

## 3.2 Different types of seashell waste as the acid neutraliser in lactic acid fermentation by *L. casei* Shirota in shake flasks

Besides clams, there are other types of seashells, and their wastes are also abundant in the local region (Fig. 2a–e). It is crucial to compare the effect of different types of seashells on lactic acid fermentation, to give insights into biorefinery practices, for example, if different types can be mixed for fermentation. Although seashells exhibit distinct appearances, their primary composition consists of calcium carbonate. The SEM-EDX results revealed a remarkable similarity in elemental composition, with calcium, carbon, and oxygen comprising over 99% atomic percentage (Table S3†). Trace amounts of magnesium, silica, and aluminium were also detected. There are also other elements of trace amounts such as lithium and strontium reported in seashells detected by high-resolution analysis.<sup>38,39</sup> The XRD results demonstrated that seashell wastes have two polymorphs of calcium carbonate, aragonite (clam, abalone, and razor clam) and calcite (oyster and scallop), which are similar in crystalline structures and are selectively formed by mineralising organisms (Fig. 2g).<sup>40</sup> The SEM images also revealed layer-like particles of powders from oyster and scallop shells, which were different from other rounded particles. The TGA results showed that there was



**Fig. 2** (a–f) Photos and SEM images (100×) of (a) clam; (b) oyster; (c) abalone; (d) scallop; (e) razor clam; and (f) calcium carbonate. (g) XRD patterns of different seashells and pure calcium carbonate. (h) TG curves of different seashells and pure calcium carbonate.

1.63–4.22% weight loss when seashells were subjected to a temperature of 600 °C, which was mainly caused by the release of water and oxidation/removal of volatile matter (Fig. 2h and Table S4†).<sup>41</sup> A dramatic weight loss of over 40% was observed in the temperature range between 600 and 950 °C, which was attributed to the decomposition of calcium carbonates and the formation of calcium oxide.<sup>42,43</sup>

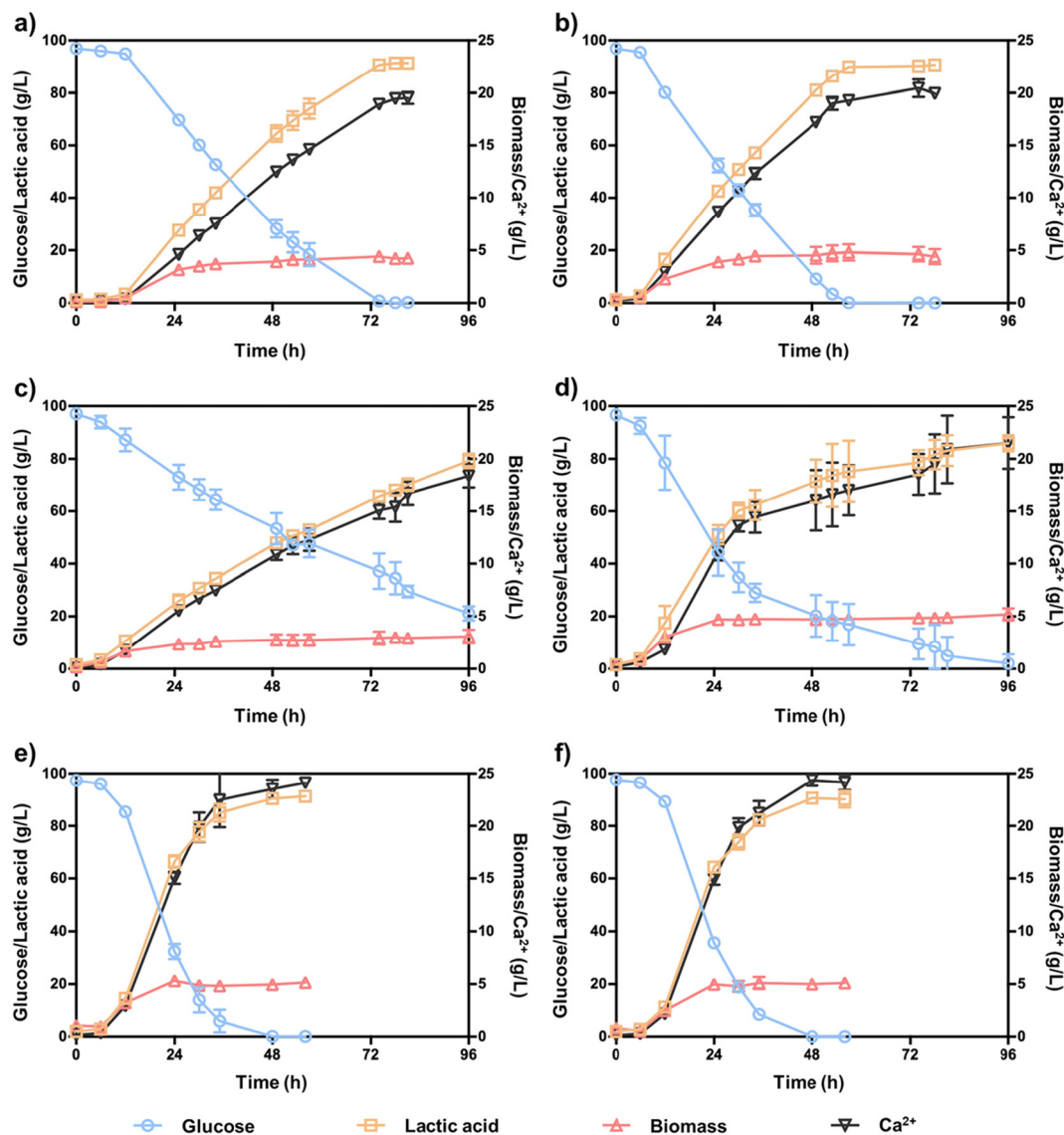
The fermentation profiles using different types of seashell waste are shown in Fig. S3.† In general, all the cultivations could achieve the resulting lactic acid concentration of 90 g L<sup>-1</sup> with a glucose consumption of 100 g L<sup>-1</sup>, and a lactic acid yield of over 0.90 g g<sup>-1</sup> (Table S5†). The profiles of Ca<sup>2+</sup> concentration for all of the types showed the same pattern with the production of lactic acid, which demonstrated the neutralisation abilities of seashells. The pH at the end of cultivation was close to 5, and the pH levels of cultivations using oyster, abalone, and scallop shells were significantly lower than those using clam and razor clam shells. Moreover, for abalone shells, the productivity was only 1.04 g L<sup>-1</sup> h<sup>-1</sup> which was significantly lower than the values for other shells (over 1.23 g L<sup>-1</sup> h<sup>-1</sup>), and the complete depletion of glucose was prolonged to 4 days which reflected the inefficient utilisation of substrates. Abalone shells contain a high content (4.22%) of impurities (components other than calcium carbonate) according to the TGA results, and the fermentation broth was green in colour as compared to the yellow colour for other seashells (Fig. S4†). There may be potential inhibitors released from

abalone shells that have a negative impact on *L. casei* bacterium, which requires future in-depth studies for identification.

In summary, the utilisation potential of different type of seashells for lactic acid fermentation by *L. casei* bacterium can vary considering the differences in their characterisation. In this study, clam shells were selected as the preferred seashell for further investigation.

### 3.3 Seashell waste as the acid neutraliser in lactic acid fermentation with food waste hydrolysate in shake flasks

To further validate the utilisation of seashell waste, lactic acid fermentation using FWH media was conducted in shake flasks to evaluate the feasibility of co-utilisation of seashell waste and FWH. As shown in Fig. 3a, fermentation using the MRS medium and seashell pieces ended after 72 hours with lactic acid production of 90 g L<sup>-1</sup> and complete depletion of glucose. The lactic acid yield and productivity were 0.94 g g<sup>-1</sup> and 1.21 g L<sup>-1</sup> h<sup>-1</sup>, respectively (Table S6†). However, fermentation using FWH medium was not complete after 96 hours of cultivation, with lactic acid production of 68 g L<sup>-1</sup> and 20 g L<sup>-1</sup> glucose remaining (Fig. 3c). Although the lactic acid conversion yield was similar, the lactic acid productivity was only 0.72 g L<sup>-1</sup> h<sup>-1</sup>, which was significantly lower than that using MRS medium. It was predicted that acid neutralisation was hindered when using large pieces of seashell in FWH medium (and further influenced the acid production), thus fermentations using seashell powders with increased surface area for



**Fig. 3** Lactic acid fermentation in shake flasks using MRS and FWH medium with different forms of seashell as the acid neutraliser. (a) MRS medium + shell piece; (b) MRS medium + shell powder; (c) FWH medium + shell piece; (d) FWH + shell powder; (e) FWH medium + fine shell powder; and (f) FWH medium +  $\text{CaCO}_3$ .

reaction were conducted (Fig. 3b & d). The fermentations using MRS medium and seashell powders ended within 60 hours, which was 12 hours faster than when using seashell pieces, with an increased productivity of  $1.58 \text{ g L}^{-1} \text{ h}^{-1}$ . Improved performance was also observed when using FWH medium, with a resulting lactic acid concentration of  $84 \text{ g L}^{-1}$  and complete depletion of glucose (Fig. 3d). Since the productivity was still low (*i.e.*  $1.06 \text{ g L}^{-1} \text{ h}^{-1}$ ), the further reduction of seashell size was investigated. As shown in Fig. 3e and Table S6,<sup>†</sup> a lactic acid productivity of  $2.43 \text{ g L}^{-1} \text{ h}^{-1}$  was obtained when using FWH medium and fine shell powders, which was 2.37-fold and 1.29-fold higher than those using shell pieces and shell powders, respectively. It is worth noting that the fermentation performance using fine shell powders was similar to that using pure calcium carbonate (Fig. 3f).

Regardless of the acid production ability when using different forms of shells, the  $\text{Ca}^{2+}$  profiles still showed the same patterns as those of lactic acid production, which again confirmed the neutralisation ability of seashell waste. To this end, the combination of FWH and seashell waste was believed to be feasible for lactic acid production by *L. casei* Shirota, and fine shell powder was the most suitable form compared with shell pieces and powders.

### 3.4 Lactic acid fermentation by *L. casei* Shirota using seashell waste and food waste hydrolysate in 2 L bioreactors

Subsequent investigations of seashell waste in lactic acid production were conducted at a 2 L bioreactor scale. Firstly, lactic acid fermentation using FWH medium was conducted, and the pH was automatically maintained at 6 using 10 M NaOH

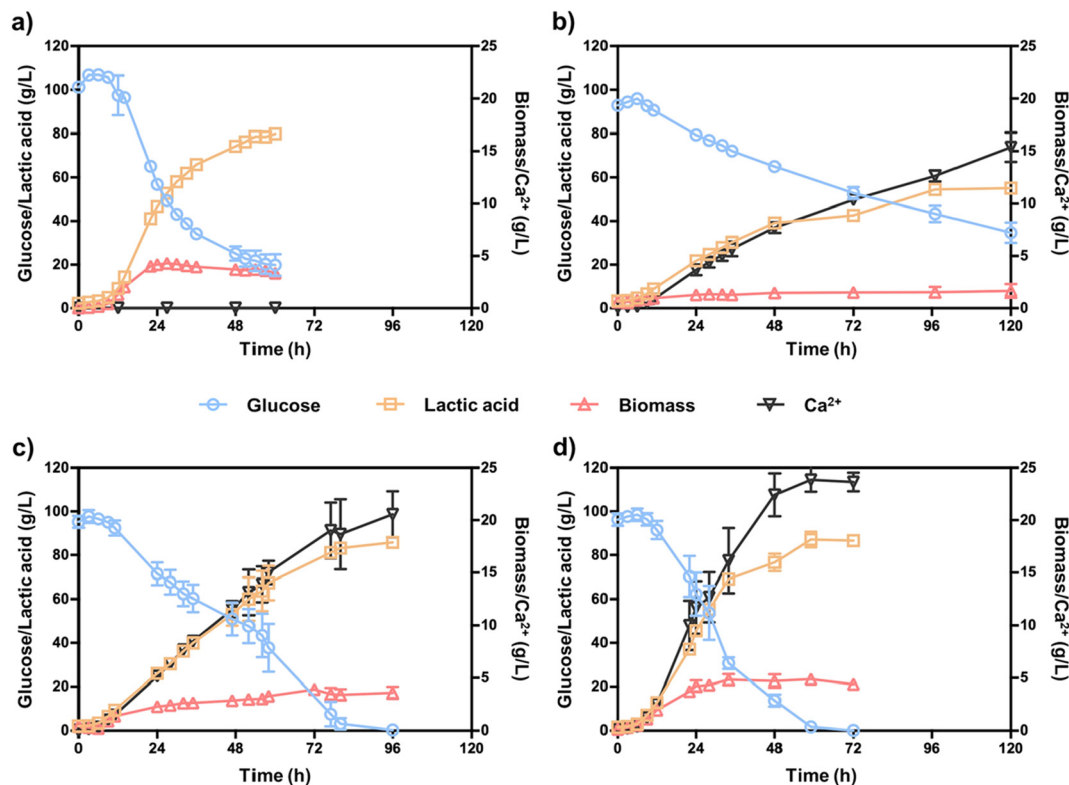


Fig. 4 Lactic acid fermentation using FWH medium in 2 L bioreactors using 10 M NaOH or different forms of seashell as the acid neutraliser. (a) 10 M NaOH; (b) seashell piece; (c) seashell powder; and (d) fine seashell powder.

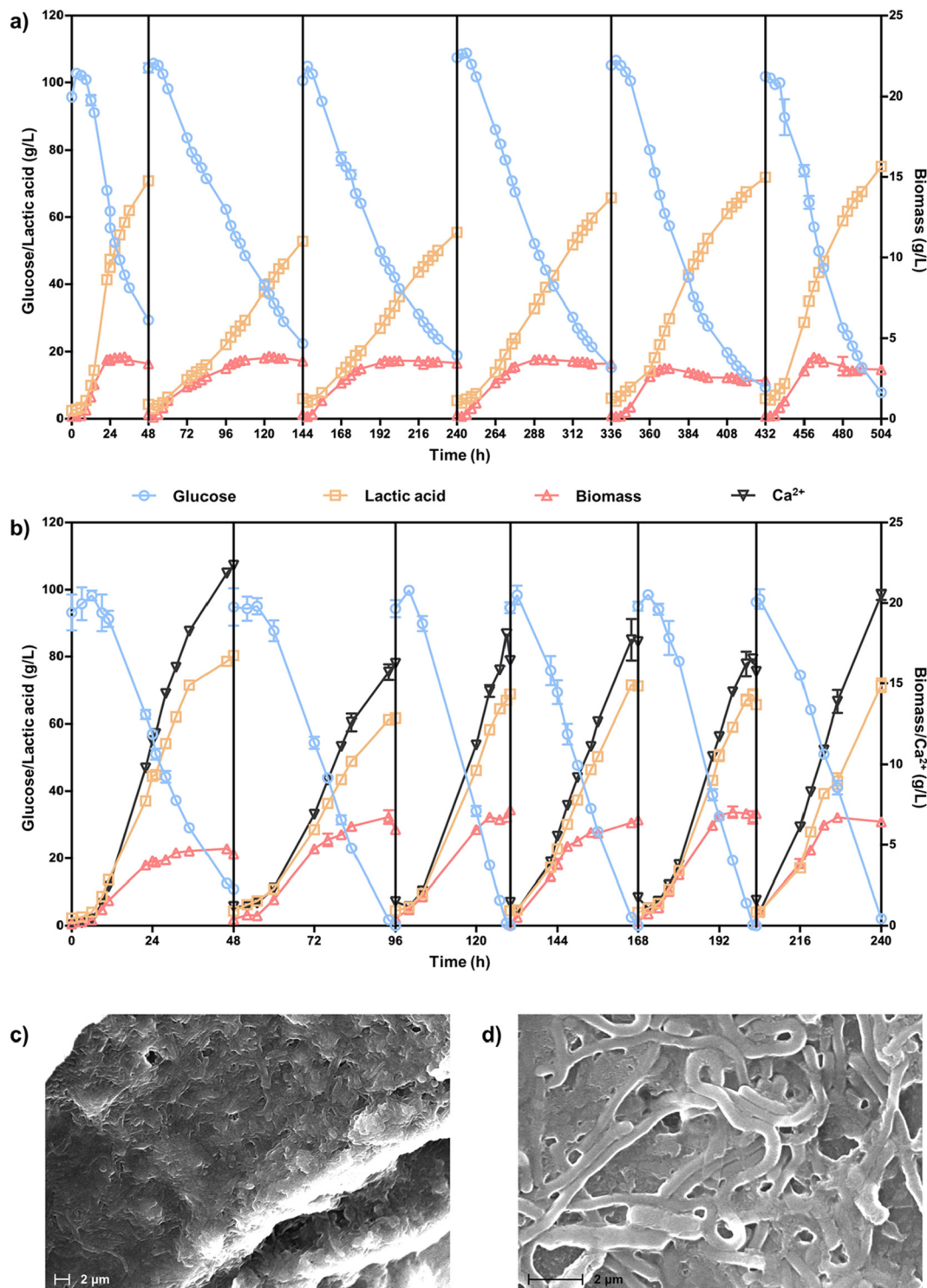
solution (Fig. 4a). After 60 hours, incomplete glucose consumption ( $19 \text{ g L}^{-1}$ ) was observed, with a resulting lactic acid concentration of  $77 \text{ g L}^{-1}$ . During the first 48 hours, the lactic acid production was rapid, achieving a productivity of  $1.55 \text{ g L}^{-1} \text{ h}^{-1}$ . Glucose utilisation was hindered thereafter, and so was acid production. When seashell pieces were employed in the fermentation process, inefficient substrate utilisation and product conversion were observed compared to shake flasks (Fig. 4b and Table S7<sup>†</sup>). After 120 hours of cultivation, the glucose utilisation was slow, and  $34 \text{ g L}^{-1}$  remained at the end of fermentation. The lactic acid production was  $51 \text{ g L}^{-1}$  with a yield of  $0.54 \text{ g g}^{-1}$ , and the overall productivity was  $0.56 \text{ g L}^{-1} \text{ h}^{-1}$  which was 36% of that achieved by using NaOH solution. By adopting seashell powders, the fermentation performance improved dramatically (Fig. 4c). Glucose was completely consumed within 80 hours, and lactic acid production was  $84 \text{ g L}^{-1}$ , with a yield of  $0.86 \text{ g g}^{-1}$  and a productivity of  $1.05 \text{ g L}^{-1} \text{ h}^{-1}$ . The results suggested that seashell powders were more efficient at neutralising acid, particularly in bioreactors where heavy shell pieces were submerged at the bottom while powders had better dispersion in the broth to facilitate the reaction. Also, the low cell density obtained using pieces ( $1.67 \text{ g L}^{-1}$  compared with  $3.59 \text{ g L}^{-1}$  obtained using powders) has limited ability to produce acid. To show the impact of the seashell particle size on the fermentation performance, fine powders were further applied in lactic acid production using FWH medium, and fermentation profiles are shown in Fig. 4d.

The glucose was consumed within 72 hours, and a lactic acid production of  $86 \text{ g L}^{-1}$  was obtained with a productivity of  $1.48 \text{ g L}^{-1} \text{ h}^{-1}$  which was 1.64-fold and 0.41-fold higher than those achieved using pieces and powders, respectively. Irrespective of the form of seashell utilised, the profiles of Ca<sup>2+</sup> concentration exhibited similar patterns to those of lactic acid production, with the pH levels being maintained around 5 (Fig. S5<sup>†</sup>).

The experimental results from this study suggested that the optimal form of seashell waste is fine powders when applied in lactic acid production using FWH medium at the bioreactor scale. In addition, applying seashell waste can reduce NaOH usage by approximately 40 g per litre of fermentation broth under the experimental conditions. Future studies can focus on optimisation work for fermentation upscaling or integration into a more complex system. It is also noted that the glucose uptake rate was enhanced when using seashell waste compared with the fermentation mediated by base solutions. In-depth investigation at the molecular or gene levels is warranted in future to understand the mechanisms for applications.

### 3.5 Lactic acid fermentation by *L. casei* Shirota using *in situ* fibrous bed bioreactors

Cell immobilisation cultivation is widely used in microbial fermentations to improve productivity.<sup>32,44,45</sup> Herein, lactic acid fermentation in an *is*FBB was investigated. Fig. 5a shows the



**Fig. 5** (a) Lactic acid fermentation in *is*FBB using FWH medium and 10 M NaOH as the pH control. (b) Lactic acid fermentation in *is*FBB using FWH medium and fine shell powder as the pH control. (c) SEM image of the surface of sugarcane bagasse (5000 $\times$ ). (d) SEM image of immobilised cells (20 000 $\times$ ).

fermentation profiles using FWH medium in *is*FBB, and the pH was controlled at 6 using 10 M NaOH solution. After 48 hours of free-cell cultivation and immobilisation (C0), the glucose was not depleted (the same as the batch cultivation),

and the lactic acid concentration was 68 g L<sup>-1</sup>. From the first repeated cycle, the glucose consumption rate and lactic acid production sharply decreased (Table 1). The fermentation period lasted for 96 hours from C1 to C4 with a glucose con-

**Table 1** Fermentation results in *is*FBB using FWH medium with NaOH (C) or fine seashell powder (SP-C) as the acid neutraliser

	Titre (g L <sup>-1</sup> )	Yield (g g <sup>-1</sup> )	Productivity (g L <sup>-1</sup> h <sup>-1</sup> )	Glucose uptake rate (g L <sup>-1</sup> h <sup>-1</sup> )
C0	68.46 ± 0.13	0.93 ± 0.01	1.48 ± 0.02	1.53 ± 0.01
C1	48.47 ± 0.48	0.58 ± 0.00	0.50 ± 0.04	0.87 ± 0.01
C2	49.48 ± 0.30	0.58 ± 0.03	0.52 ± 0.03	0.90 ± 0.01
C3	60.37 ± 0.24	0.64 ± 0.01	0.63 ± 0.02	0.97 ± 0.06
C4	65.88 ± 0.83	0.68 ± 0.01	0.69 ± 0.01	1.01 ± 0.01
C5	69.13 ± 0.07	0.74 ± 0.02	0.96 ± 0.02	1.30 ± 0.02
SP-C0	78.02 ± 0.05	0.89 ± 0.01	1.62 ± 0.00	1.82 ± 0.03
SP-C1	57.30 ± 1.38	0.60 ± 0.05	1.24 ± 0.03	1.98 ± 0.12
SP-C2	64.31 ± 0.39	0.64 ± 0.01	1.89 ± 0.01	2.86 ± 0.08
SP-C3	66.99 ± 1.16	0.68 ± 0.02	1.76 ± 0.03	2.50 ± 0.03
SP-C4	64.62 ± 1.76	0.66 ± 0.02	1.85 ± 0.05	2.90 ± 0.02
SP-C5	67.39 ± 2.08	0.69 ± 0.01	1.82 ± 0.06	2.63 ± 0.08

sumption rate of around 1.0 g L<sup>-1</sup> h<sup>-1</sup> which was lower than that for C0 (1.53 g L<sup>-1</sup> h<sup>-1</sup>) indicating inefficient utilisation of the supplied glucose. In C1 and C2, the lactic acid production was reduced to 50 g L<sup>-1</sup>, and the production gradually increased to 70 g L<sup>-1</sup> in C3–C5. However, due to the prolonged fermentation, the lactic acid productivity decreased by a maximum of 0.66-fold (0.50 g L<sup>-1</sup> h<sup>-1</sup>, C1) and it was unable to reach a similar level to that for C0 after five repeated cycles. The lactic acid yield also dropped to a range of 0.58–0.74 g g<sup>-1</sup>, indicating a low conversion efficiency of glucose to lactic acid and potential production of byproducts such as acetic acid and ethanol.<sup>46</sup> The poor fermentation performance of the immobilisation cultivation might result from biofilm formation on the supporting material (Fig. 5c & d). Adsorption to the surfaces, particularly for rough surfaces, has been suggested to promote biofilm growth, and the biofilm can be further strengthened by the synthesis of extracellular polymeric substances (EPS).<sup>47</sup> The SEM images also indicated the high occupancy of bacteria in the surface area of sugarcane bagasse, and the close contact among cells could support the EPS connection and thus biofilm formation as per the proposed theory. This change in the cell physiological status of immobilised cells led to different micro-conditions during the fermentation compared with free cells, which could further redirect the carbon flux into biofilm formation or byproduct production. Nevertheless, improved performances were observed with the successive repeated cycles regarding acid productivity and glucose consumption.

Considering the superior results obtained using fine seashell powder in batch mode, fine shell powder was further applied as the acid neutraliser in the *is*FBB mode to investigate the performance. Fermentation profiles are shown in Fig. 5b and Fig. S6.† A resultant lactic acid concentration of 78 g L<sup>-1</sup> was obtained in SP-C0, while afterward the production was reduced to less than 70 g L<sup>-1</sup>. It is worth noting that the fermentation periods were shortened to 36 hours from SP-C2, with full depletion of glucose and enhanced glucose consumption rate to a maximum of 2.90 g L<sup>-1</sup> h<sup>-1</sup>, which was 1.23-fold higher than the highest value achieved in *is*FBB regulated by NaOH solution. Furthermore, although lactic acid production

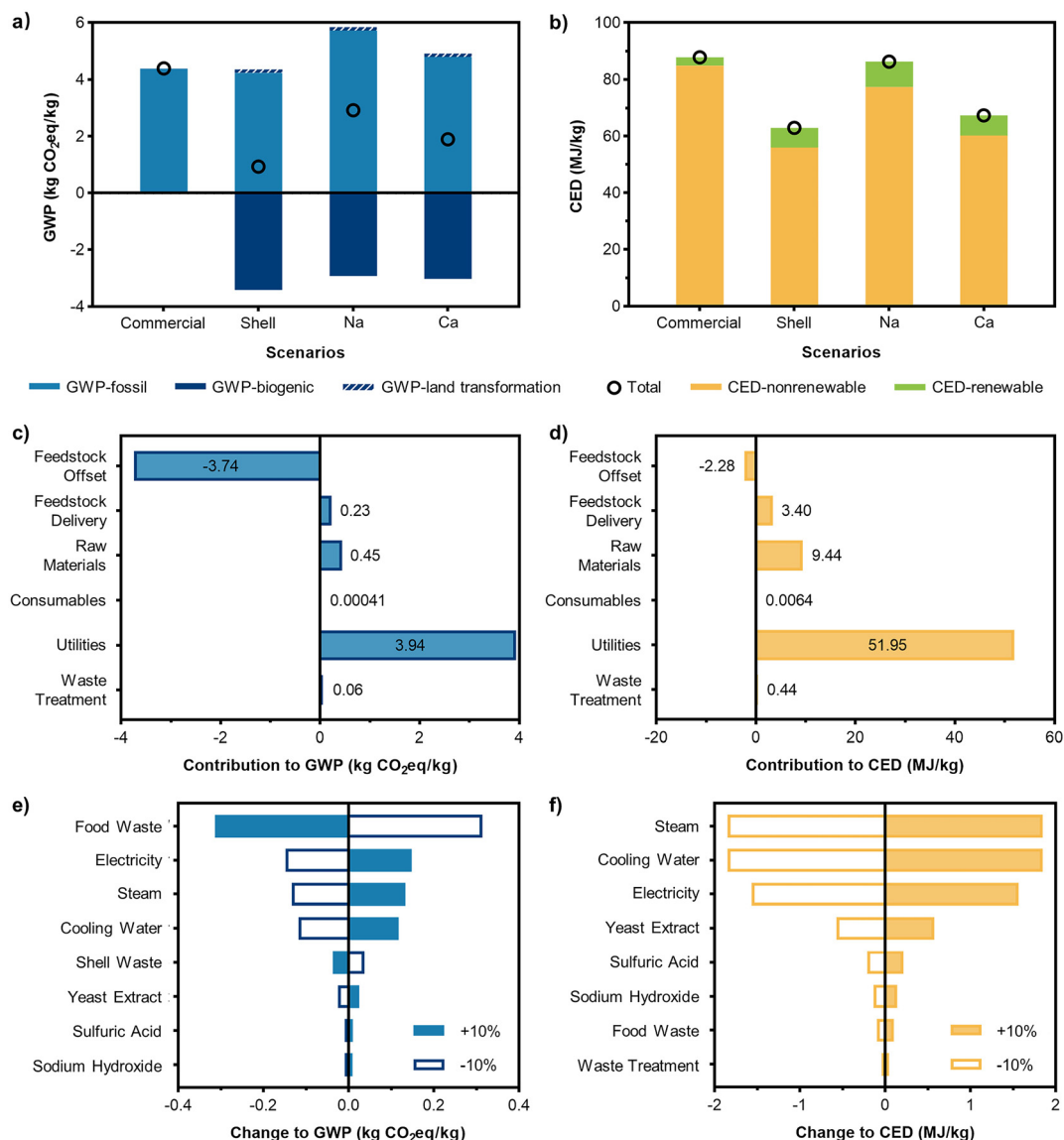
and yield were still undermined, the productivity was enhanced to 1.80 g L<sup>-1</sup> h<sup>-1</sup> from SP-C2, which was even higher than that obtained in batch fermentations (1.48 g L<sup>-1</sup> h<sup>-1</sup>). Cell densities (from SP-C2) higher than 6.42 g L<sup>-1</sup> were recorded which were significantly higher than those obtained in *is*FBB using NaOH (less than 4 g L<sup>-1</sup>), also supporting the improved fermentation performances as biomass was essential for lactic acid production. In conclusion, the utilisation of seashell waste, specifically in the form of fine powders, demonstrated superior *is*FBB fermentation compared to the use of NaOH, which would save approximately 250 g of NaOH by estimation.

Compared with the chemocatalysis of lactic acid production from food waste, it was found that both biochemical conversion and chemocatalysis demonstrated appealing and comparable results.<sup>48</sup> As there is no one good solution that fits all, it is beneficial to explore diverse technological approaches to address the pressing issue of food waste. To further enhance lactic acid production, future work should target improved metabolic performance through the regulation of metabolic pathways to minimise the formation of byproducts. Immobilisation methods and supporting material should be carefully chosen, as recent studies reported positive results of lactic acid production by immobilised cells using entrapments such as alginate and polyvinyl alcohol.<sup>49,50</sup>

### 3.6 Environmental and economic evaluation of lactic acid production using food waste and shell waste

Based on the experimental results, we developed corresponding evaluation models using SuperPro Designer® to simulate close-to-industry lactic acid production using the proposed food and seashell waste-based biorefinery scheme, which facilitates further benchmarking of environmental impacts against commercial lactic acid. The environmental impact of commercial lactic acid is presented in Table S8.† The GWP and CED of the lactic acid derived from the constructed scenarios are summarized in Fig. 6. Detailed statistics of lactic acid produced in Scenario Shell are tabulated in Table S9.†

While commercial lactic acid is embedded with a GWP of 4.38 kg CO<sub>2</sub>eq per kg, the lactic acid produced from Scenario Shell, Na, and Ca, achieved lower GWPs of 0.94, 2.93, and 1.90 kg CO<sub>2</sub>eq per kg, respectively, mostly due to the biogenic GWP offset resulting from the utilisation of feedstocks. In terms of CED, the constructed scenarios also produced lactic acid with lower CED, but with fewer variations. For instance, CED experienced a 23.3% reduction in Scenario Shell (63.0 MJ kg<sup>-1</sup>) compared to commercial lactic acid (87.7 MJ kg<sup>-1</sup>), but a 78.6% reduction was found in GWP between the same groups. The reason behind this difference is the different consequences associated with the feedstocks used in terms of GWP or CED. Taking Scenario Shell as an example (Fig. 6c & d), the same amounts of the feedstocks used (offset and delivery of food waste and shell waste) led to a cumulative GWP of -3.51 kg CO<sub>2</sub>eq per kg, but a cumulative CED of 1.12 MJ kg<sup>-1</sup>. As landfill was chosen as the original destination of utilised



**Fig. 6** Summary of global warming potential (GWP) and cumulative energy demand (CED) results. (a) GWP of three scenarios in comparison with commercial lactic acid. (b) CED of three scenarios in comparison with commercial lactic acid. (c) Breakdown of GWP in Scenario Shell. (d) Breakdown of CED in the Scenario Shell. (e) Sensitivity analysis of GWP in Scenario Shell. (f) Sensitivity analysis of CED in Scenario Shell.

food waste and shell waste, avoiding waste management of these feedstocks brought relatively more significant environmental benefits in terms of GWP than CED, due to the considerable emissions derived from landfilling.<sup>51</sup> Apart from the feedstocks, another key contributor is the utilities. In Scenario Shell, they contributed 3.94 kg CO<sub>2</sub>e per kg and 51.95 MJ kg<sup>-1</sup> in terms of GWP and CED, respectively. This demonstrated the importance of balancing the energy demand within the lactic acid factory to facilitate a more environmentally friendly production line, similar to the LCA study of lactic acid produced from glycerol using cascade bio- and chemocatalysis.<sup>52</sup> Raw materials slightly contributed to GWP and CED (0.45 kg CO<sub>2</sub>e per kg and 9.44 MJ kg<sup>-1</sup>, respectively). In Scenario Shell, waste management barely influences the

environmental impacts associated with the produced lactic acid.

A univariate sensitivity analysis of Scenario Shell was performed to identify the most impactful parameters regarding the environmental impacts associated with the overall process of lactic acid production. In terms of GWP, food waste played the most important role. The 10% change in food waste used as a feedstock (which related to the overall yield from food waste to lactic acid) led to the most significant change in GWP. Shell waste used as a feedstock was also shown to be a sensitive parameter. However, regarding CED, the change in the feedstocks became less important. In both environmental impact categories, utilities were demonstrated to be a dominant factor. This suggests that it is imperative that the lactic

acid production facilities need to achieve higher energy consumption efficiency to reduce the associated environmental burdens. Another important factor identified from the sensitivity study is the yeast extract supplemented during the lactic acid fermentation as the nitrogen source. It might be of interest to explore nitrogen sources with lower environmental burdens or adopt waste materials for this purpose. Moreover, Fig. S7† reveals that the purification processes of lactic acid contributed to the majority of the embedded uncertainties, resulting from the use of steam and cooling water within these energy-intensive processes.

To evaluate the economic feasibility of lactic acid production using food and seashell waste, a preliminary profitability analysis was performed based on Table S1.† The estimated operating cost is presented in Table S10.† Scenario Na resulted in the highest total production cost (*i.e.* USD 1735/MT), which mainly arises from the extensive use of acid and alkali during fermentation and product recovery. When compared with Scenario Ca (USD 1296/MT), Scenario Shell has a lower total production cost of USD 1200/MT due to the credit of using seashell waste, which enhances the overall profitability of the proposed approach. The grinding process of seashell waste is energy intensive (*i.e.* with an annual electricity consumption of 542 154 kW h for the grinding of 5422 MT of shell waste in this study), which accounts for a 4.5% increase in the total production cost as compared with Scenario Ca. Nevertheless, the additional cost of electricity inputs due to the grinding operation can be compensated for using seashell waste streams.<sup>53</sup> According to the local statistics in Hong Kong, shellfish consumption in 2022 was 85 984 tonnes, which would lead to around 40 000 tonnes of seashell waste (assuming 50% of the shellfish consumption resulted in seashell waste). Therefore, our proposed food and shell waste-based biorefinery focusing on the green and sustainable material recovery approach can resolve the food and fishery waste burdens, and meanwhile, facilitate the transition from a linear to a circular economy.<sup>54</sup>

It is noticed that yeast extract accounts for over 50% of the material cost, which should be replaced by cheaper alternative nitrogen supplements in future studies, such as sunflower meal and soybean cake produced as by-products of the biodiesel industry.<sup>55,56</sup> Moreover, the transportation of waste streams contributed up to 9% of the total operating cost, and the inefficient waste logistics in practical waste valorisation needs to be optimised and well designed. In this preliminary economic analysis, the capital costs are not included, while most biotechnological processes are capital-intensive which leads to unfavorable profitability and economic potential for upscaling (for example, high capital investments and low return on investment would lead to extended payback time). To realise a feasible and sustainable waste valorisation, more efforts should be devoted to addressing the technoeconomic barriers.<sup>57</sup> For example, co-products are essential for improving the overall economic potential of waste production systems. In this study, solid waste (*i.e.* gypsum) can be recycled for construction use instead of landfilling, and residual cell biomass

can be utilised in lipid extraction, as animal feeds, and in the production of promising advanced materials.<sup>9,58</sup>

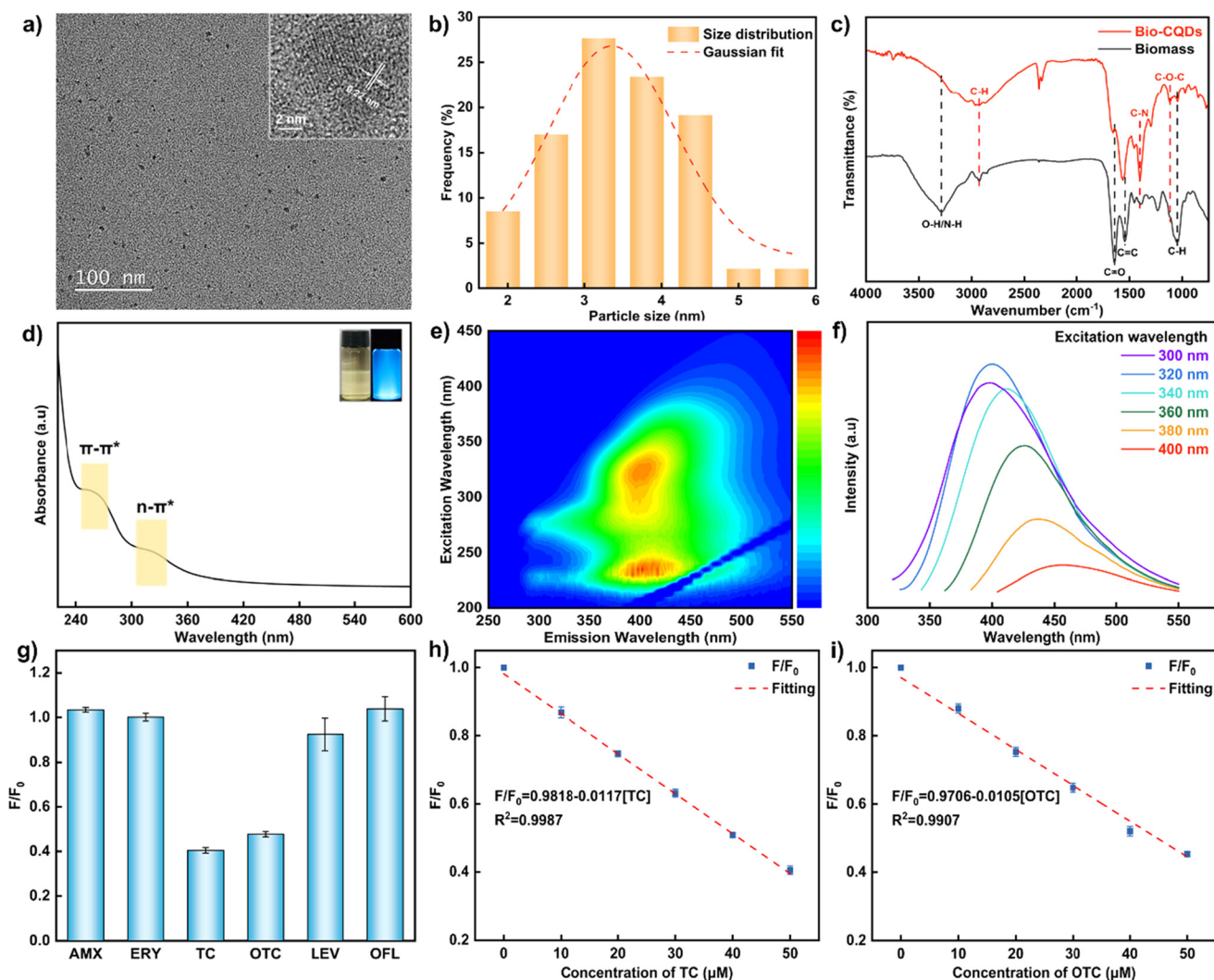
### 3.7 Biomass-derived CQD production from cell biomass

**3.7.1 Characterization of Bio-CQDs.** Cell biomass waste resulting from the fermentation process in this study was further valorised to Bio-CQDs through a bottom-up approach. The results of the elemental analysis of biomass and Bio-CQDs are summarised in Table S11.† The high carbon content (45.93%) of the cell biomass provided a green and suitable organic precursor for the synthesis of Bio-CQDs. In addition, the as-synthesized Bio-CQDs contained a higher percentage of nitrogen content (11.57%) than that of the cell biomass (9.20%), indicating a successful and efficient approach of doping nitrogen into carbon dots by valorising waste biomass.

The microstructure of Bio-CQDs extracted from cell biomass was characterized by transmission electron microscopy (TEM). The TEM image shown in Fig. 7a reveals that the Bio-CQD sample was spherical or elliptical in shape, with a narrow size distribution from about 1.94 to 5.68 nm, with an average diameter of  $3.36 \pm 0.14$  nm (Fig. 7b).<sup>59</sup> The high-resolution TEM (HRTEM) image (Fig. 6a, inset) clearly showed that the Bio-CQDs were highly crystallized with a lattice distance of 0.22 nm, corresponding to the (100) crystal planes of graphite carbon.<sup>60</sup> These results confirmed that Bio-CQDs with graphite-like structures can be synthesized from cell biomass waste *via* a facile hydrothermal process.

**3.7.2 Formation mechanism of Bio-CQDs.** To appropriately characterize the formation of Bio-CQDs, FT-IR spectroscopy was conducted to analyse the absorption behaviour of biomass and Bio-CQDs in the near-infrared region in the powder form, as shown in Fig. 7c. For the IR spectrum of biomass, broad absorption bands centred at  $3285\text{ cm}^{-1}$  were assigned to the stretching of O–H and N–H bonds, while the absorption bands near  $2925$  and  $1045\text{ cm}^{-1}$  could be attributed to the in-plane and out-of-plane bending of aromatic C–H bonds, possibly combined with stretching vibrations of the epoxide/ether arising from the C–O–C moiety. Such absorption was weakened from biomass to Bio-CQDs, while a new stretching peak of the C–N group emerged at  $1399\text{ cm}^{-1}$  in Bio-CQDs. This result suggests that some of the C–O–C bonds in the biomass were broken under hydrothermal treatment, accompanied by nitrogen-functional group formation in Bio-CQDs. Meanwhile, these polar functional groups (O–H, C=O, and C–N) present at the edges of the aromatic backbone endowed Bio-CQDs with high hydrophilicity and stability.<sup>61</sup> It is believed that the formation mechanism of biomass-derived CQDs in this study involves the hydrolysis, aromatization and condensation of biomass to form Bio-CQDs, and the C–N group was simultaneously enhanced in the process of carbonisation.<sup>62</sup>

**3.7.3 Optical properties of Bio-CQDs.** The photophysical properties of Bio-CQDs were explored by UV-vis absorption and emission spectroscopy. As shown in Fig. 7d–f, the UV-vis absorption spectrum of Bio-CQDs exhibited two adsorption shoulders at *ca.* 250 nm and 325 nm, with an optical absorption edge at about 580 nm. The high energy absorption peak



**Fig. 7** (a) The TEM and HRTEM images of Bio-CQDs. (b) Size distribution of Bio-CQDs. (c) FT-IR spectra of biomass and Bio-CQDs. (d) UV-vis absorption spectra of Bio-CQDs in aqueous solutions (inset: photographs of the Bio-CQDs under sunlight and 365 nm UV irradiation). (e) Three-dimensional fluorescence plots of Bio-CQDs. (f) Excitation–emission photoluminescence (PL) spectra of Bio-CQDs. (g) Influence of different antibiotics on the fluorescence of Bio-CQDs. (h) Fluorescence intensity ratios ( $F/F_0$ ) after the addition of different concentrations of tetracycline (TC). (i) Fluorescence intensity ratios ( $F/F_0$ ) after the addition of different concentrations of oxytetracycline (OTC).

below 300 nm was assigned tentatively as originating from the  $\pi$ - $\pi^*$  transition of the carbon core,<sup>63</sup> while the low-energy absorption shoulder was attributed to the  $n$ - $\pi^*$  transition of the Bio-CQDs.<sup>64,65</sup> Upon excitation, a Gaussian emission band with peak maxima ranging from 395 to 455 nm was recorded and assigned as originating from the  $n$ - $\pi^*$  transition, mixing with some  $\pi$ - $\pi^*$  transition of the carbon core.<sup>66</sup> Such emission bands are found to be excitation dependent and a similar observation has been reported for structurally related CQDs, possibly attributed to the quantum effect, surface edge defects,  $sp^2\pi$  domains, and size variation.<sup>67</sup> Nonetheless, such insignificant energy shifts of this Bio-CQDs indicate their small size distribution, as discussed and confirmed by the high-resolution TEM study in the previous section. In addition, the presence of heteroatomic functional groups or surface defects (e.g.,

C-N, C-O-C, and C=O groups) evidenced by FT-IR analysis (Fig. 7c) could increase the emission sites, leading to a red-shift in PL emission wavelengths.<sup>68,69</sup> The photoluminescence quantum yield (QY) of the Bio-CQDs was 11.62% in  $H_2O$ , which is comparable with values for other CQDs reported in the literature, suggesting that the facile route proposed in this study was efficient in producing quality CQDs from biomass waste.<sup>70,71</sup>

**3.7.4 Bio-CQDs as antibiotic sensors.** A sensitivity test of Bio-CQDs towards various antibiotics was performed using an excitation wavelength of 320 nm. A series of commonly found antibiotics in wastewater, such as amoxicillin (AMX), erythromycin (ERY), tetracycline (TC), oxytetracycline (OTC), levofloxacin (LEV), and ofloxacin (OFL), were added separately to investigate their quenching effects on the fluorescence of Bio-

CQDs. The fluorescence intensity of Bio-CQDs was significantly suppressed only upon the addition of TC or OTC (Fig. 7g), suggesting the potential use of Bio-CQDs as fluorescent sensors for TC and OTC. As shown in Fig. 7h & i, the fluorescence intensity of Bio-CQDs was linearly related to the TC and OTC concentrations in the range of 0–50  $\mu\text{M}$  with correlation coefficients ( $R^2$ ) of 0.9987 and 0.9907, respectively. The as-prepared Bio-CQDs exhibit excellent selectivity and linear response towards tetracycline compounds, showcasing promising prospects for the detection of a particular type of antibiotic.

**3.7.5 Future perspectives of economic and environmental impacts.** The results of life-cycle assessment indicate the energy-intense nature of the chemical synthesis of carbon dot production. It was found that electricity represented over 68% of the potential environmental impacts, making it a significant focal point.<sup>72</sup> Moreover, the choice of the carbon precursor is crucial when considering sustainability, for instance, xylose, in comparison with biomass liquor (*i.e.*, obtained from olive pits treatment), has a lesser impact on human health, ecosystems, and resources. In this study, the CQDs were synthesised from waste cell biomass which was the by-product/waste flux of lactic acid fermentation. In view of the zero-waste target, it is recommended that the remaining fraction after food waste hydrolysis could be investigated in future work as the precursors for CQDs or other value-added carbonaceous material production. For example, waste biomass could also be potentially converted to other valuable carbonaceous products such as (i) hydrochars *via* hydrothermal reaction, (ii) biochar and activated carbon *via* pyrolysis, or (iii) utilised as carbon source for biogas production in anaerobic digestion.<sup>73–76</sup> The valorisation of waste flux from this integrated biorefinery into high-value CQDs shows promise in terms of both economic and environmental performance, while further explorations are warranted to optimise the manufacturing process and mitigate the associated impacts.

## 4. Conclusions

This study showcased seashell waste as an alternative acid neutraliser in the replacement of commercial bases during lactic acid fermentation by *L. casei* Shirota. Among the different forms of seashell waste examined, fine powders resulted in the highest lactic acid productivity of 1.48  $\text{g L}^{-1} \text{h}^{-1}$  in batch fermentation. Furthermore, the results from cell immobilisation fermentation provided further evidence of the superior performance of fine seashell powders compared to NaOH solution, with the highest glucose uptake rate and lactic acid productivity at 2.90  $\text{g L}^{-1} \text{h}^{-1}$  and 1.89  $\text{g L}^{-1} \text{h}^{-1}$ , respectively. Further optimisations of immobilisation techniques and supporting material are warranted to improve the production yield of the proposed process. The LCA results demonstrated improved environmental performance regarding GWP and CED by adopting seashell waste as the acid neutraliser. Moreover, fluorescent Bio-CQDs were successfully synthesised

from waste cell biomass *via* a one-step green hydrothermal method presenting favourable water solubility and excellent photophysical properties. Notably, the particular traits of fluorescent sensitivity and selectivity towards tetracycline compounds may open up potential sensing applications in the field of green and environmental studies, which demonstrate the multifaceted applications of waste-based materials *via* a waste-to-wealth green approach. These advancements will foster the development of a circular waste-based biorefinery, driving sustainable and environmentally friendly practices in biomass valorisation.

## Author contributions

Jin-Hua Mou: conceptualisation, data curation, formal analysis, methodology, visualisation, and writing – original draft, review & editing. Ling-Feng Ouyang: data curation, formal analysis, methodology and writing – original draft. Zi-Hao Qin: data curation, methodology and writing – original draft. Ya-Hui Miao: data curation. Xin-Tian Jiang: data curation. Mui-Choo Jong: resources and writing – review & editing. Man-Chung Tang: resources and writing – review & editing. Chenyu Du: resources and writing – review & editing. Season Si Chen: conceptualisation, funding acquisition, methodology, project administration, supervision, and writing – review & editing. Carol Sze Ki Lin: funding acquisition, methodology, project administration, supervision and writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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