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Caproic acid production through lactate-based chain elongation: Effect of lactate-to-acetate ratio and substrate loading

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ABSTRACT

Substrate properties play a key role in promoting the caproate yield through lactate-based carbon chain elongation pathway. In the present study, the effect of lactate-to-acetate (LA/AA) carbon ratio (from 0.5 to 5.0) and substrate loading (in terms of substrate/inoculum ratio within the range 20–180 mmol-C/g-VSS) on caproate fermentation was investigated. Results showed that both caproate content and yield increased by increasing the LA/AA ratio up to 3.0, then decreased at higher ratios due to activation of acrylate pathway and dispersion of carbon flux at elevated lactate content. At the optimal LA/AA carbon ratio of 3.0, substrate loading lower than 100 mmol-C/g-VSS was beneficial for efficient substrate utilization with low caproate selectivity, while higher substrate-to-inoculums (S/I) ratio led to incomplete substrate utilization and dispersed carbon flow, which finally reduced the caproate yield. Thus, the highest caproate yield of 0.42 g-COD/g-COD and selectivity of 49.5% were recorded at LA/AA and S/I ratio ratios of 3.0 and 100 mmol-C/g-VSS, respectively. The present study further depictures the novel approach for caproate production with lactate.

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1. Introduction

Recently, production of renewable chemicals from organic wastes via carboxylate platform has drawn increasing attentions (Agler et al., 2011; Tang et al., 2017b; Wu et al., 2020a). In such eco-friendly sustainable platform, complex organic wastes are converted by anaerobic microbes into short-chain carboxylic acids (SCCAs) and/or energy products (biomethane, biohydrogen and bioelectricity) (Duber et al., 2018; Tang et al., 2022). Biogas production through anaerobic digestion (AD) is a conventional method to recover energy from organic wastes (Deepanraj et al., 2017; Abomohra et al.,

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2022). However, the produced biogas has relatively low value that may subside the AD process into an economically unattractive technology (Xu et al., 2018), as it is rich in carbon dioxide and other impurities which reduce the calorific value (Zaks et al., 2011). In addition, biogas leakage during collection and transportation has raised many safety issues and serious negative environmental impact (Zaks et al., 2011). To overcome these limitations, production of other high-value chemicals such as short chain fatty acids (SCFAs), ethanol, and lactic acid via anaerobic fermentation has been widely studied in recent years (Xie et al., 2021; Zhang et al., 2019). However, their hydrophilic characteristics, complicates separation/purification processes, and energy intensiveness seriously restrict the practical large-scale applications.

As an alternative route, medium chain fatty acids (MCFAs) have been considered as a value-added anaerobic product (Xu et al., 2018). Due to their low solubilities, MCFAs exhibit excellent recovery properties, which simplifies separation and purification procedures with relatively low energy inputs (Lonkar et al., 2016; Xie et al., 2021). In addition, MCFAs have higher carbon-to-oxygen ratio and much higher energy densities than methane and SCCAs (Han et al., 2019; Wu et al., 2019). Due to their high demand in food and pharmaceutical industry, MCFAs produced via carboxylate pathways have become a hot research topic (Duber et al., 2018; Nzeteu et al., 2018; Wu et al., 2020a).

Carbon chain elongation (CE) via reverse β-oxidization (RBO) pathway is an emerging and efficient biotechnology to transform SCCAs into MCFAs. In this route, ethanol, lactate and methanol can be utilized as electron donors (ED), while SCFAs such as acetic acid, propionic acid and butyric acid act as electron acceptors (EA), resulting in elongation of the carbon chain length (Carvajal-Arroyo et al., 2019; Chwialkowska et al., 2019; Wu et al., 2019). Through RBO, acetate elongates to butyrate and then to caproate, while propionic acid elongates to valeric acid and/or heptoic acid (Liu et al., 2017; Xie et al., 2021). In lactate-based chain elongation, pyruvate and NADH are firstly produced from lactate oxidation, then pyruvate is further oxidized to acetyl-CoA and CO_2 with electrons released in the form of reduced ferredoxin (Wang et al., 2018; Wu et al., 2019). The derived acetyl-CoA takes part in RBO processes for *n*-butyrate and *n*-caproate generation. Lactate can be easily produced through acidogenic fermentation from organic wastes such as food waste, excess sludge, and agricultural waste (Tang et al., 2016, 2017a), which could significantly enhance the efficiency of caproate production. Thus, investigating caproate production from lactate could provide a promising route to combine organic wastes management with high-value products recovery.

In CE route, substrate content and composition significantly affect the microbial metabolism and product profile (Duber et al., 2020; Wu et al., 2020b). Increasing of lactic acid content can enhance caproate production, but high lactate load may activate the acrylate pathway and eventually reduces the caproate yield (Contreras-Dávila et al., 2020; Wu et al., 2019; Xie et al., 2021). Wu et al. (2019) also proved that if lactate is consumed through acrylate pathway, it is difficult to return it in the RBO process and propionate will become the dominant fermentation product. However, Zhu et al. (2015) utilized lactate as a sole substrate and obtained high caproate content and yield (approximately 70%) without propionate accumulation due to existence of particular microbial community (*Clostridium* cluster IV) in the sludge. Thus, substrate composition poses important impacts on the microbial communities and metabolism which affects the final products (Han et al., 2019).

Although the effect of ED and EA on caproate production has been previously evaluated (Wang et al., 2018; Wu et al., 2021; Xie et al., 2021), the conditions of lactate-based chain elongation are not clear yet and few studies evaluated the impact of ED/EA ratios on CE process. In that context, substrate content could influence the elongation process, leading to distinctive caproate yield and productivity (Cavalcante et al., 2020). So far, few studies investigated the effect of lactate content or substrate-to-inoculums (S/I) ratio on caproate production, making it a timely topic to further explore the role of these parameters and the optimal conditions for enhanced caproate production in order to set-up an efficient and robust industrial system. Therefore, the present study aimed to investigate the effect of LA/AA ratios on the performance of caproate fermentation in order to identify the optimal substrate composition for efficient CE route. Moreover, the properties of caproate fermentation at different substrate loading rates were evaluated to provide information on lactate-derived chain elongation.

2. Materials and methods

2.1. Inoculum and basal medium

The pit mud sludge was collected from a strong flavor alcohol industry at Chengdu city and used in the present study as inoculum. After being washed with buffer solution, the sludge was incubated in a semi-continuous reactor to enrich the functional microbiome, in which lactate and acetate were used under anaerobic conditions at pH 5.5 and 37 °C. Thereafter, the acclimated sludge was washed to remove the residual lactate and carboxylic acids, then utilized further as inoculum.

Components in basal medium used in the batch experiments was adjusted as previously described (Xie et al., 2021). After being prepared, the medium was sparged with N_2 (99.9%) for 5 min to remove the dissolved oxygen. Certain amounts of lactate and acetate were added to the basal medium according to the experimental design described in Section 2.2. To avoid substrates being converted to methane, 2-bromoethanesulphonate acid (2-BES) was supplemented to inhibit the methanogens.

2.2. Experimental design

2.2.1. Substrate optimization

Serum bottles with a working volume of 500 mL/each were used in the present study. Into each bottle, 100 mL of inoculum were added, then lactate and acetate were added at different LA/AA carbon ratios of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 with a final content of 300 mmol-C/L. Sole lactate and acetate were added into two bottles to investigate the chain elongation properties, in addition to a bottle without acetate nor lactate was used as a blank. The basal medium was then added into the reactors at a final volume of 500 mL. The pH in all serum bottles was adjusted to 5.5 using 5 M HCl or NaOH to reduce the methanogenesis and acrylate pathway (Kucek et al., 2016). Thereafter, all bottles were flushed with pure nitrogen gas for 5 min to ensure oxygen-free environment. Finally, bottles were incubated in a water bath shaker at 37 °C and 140 rpm. Liquid and gas samples were obtained periodically from each bottle to investigate the variations in carboxylic acids and biogas production. All experiments were performed in triplicates and data are presented as the mean ± standard deviation (SD).

2.2.2. Substrate to inoculum ratio

To investigate the effect of substrate loading on caproate production, another series of experiments were carried out. For that, 100 mL of sludge were used as inoculum, then the mixture of LA/AA at a ratio of 3 was added into each bottle to a final content of 100, 300, 500, 700 and 900 mmol-C/L based on preliminary experiments. Thereafter, the contents of each bottle were completed to 500 mL with basal medium. After adjusting the pH to 5.5, all bottles were flushed with nitrogen gas to remove the dissolved oxygen and incubated in a shaking water bath at 37 °C and 140 rpm. Liquid and gas samples from each bottle were analyzed periodically, and all tests were performed in triplicates.

2.3. Chemical analysis

The concentration of fatty acids (C2–C6) was measured by gas chromatograph (Agilent 7820 GC, USA) equipped with a flame ionization detector (FID) and 30 m × 0.32 mm × 0.25 μm column. The operating temperature of the GC oven was started and held at 60 °C for 2 min, increased to 210 °C at a rate of 25 °C/min, then maintained at 210 °C for 2 min. The injector and detector temperatures were maintained at 230 °C and 250 °C, respectively. Lactate was analyzed by high performance liquid chromatography (Shimadzu, LC20 A, Japan) equipped with 4.6 mm × 150 mm × 5 μm C_{18}-WP HPLC column and ultraviolet detector (210 nm). The temperature of the HPLC column was 40 °C and the flow rate of the mobile phase (10% acetonitrile in water) was 1.0 mL/min. Biogas volume was measured with a gas syringe, while its composition was analyzed using gas chromatography (Shimadzu, GC2010, Japan) equipped with a thermal conductivity detector (TCD) and 2-m stainless steel column packed with Porapak Q. The operating temperature of the GC column, injector and detector was 60 °C, 100 °C, and 150 °C, respectively.

2.4. Calculations

Electron efficiency and selectivity based on electron equivalent balance was used to indicate the fermentation effectiveness (Wu et al., 2019), which were calculated using Eqs. (1) and (2);

$$\text{Electron efficiency} = \frac{E_{products}}{E_{lactate}} \tag{1}$$

$$\text{Selectivity} = \frac{E_{products}}{E_{substrates}} \tag{2}$$

where $E_{products}$ represents the electron number of products, $E_{lactate}$ indicates the electron number of added lactate, and $E_{substrates}$ represents the electron number of consumed substrate. All acids and their electron numbers (mol electron/mol acid) are 12/lactate, 8/acetate, 14/propionate, 20/butyrate, 26/valerate, and 32/caproate.

3. Results and discussions

3.1. Effect of LA/AA ratio

3.1.1. Variations in caproate content

Fig. 1 shows the effect of LA/AA ratio on caproate fermentation. In the reactor with acetic acid as a sole feedstock, caproate was not detected, as a non-electron donor (lactate) was provided (Han et al., 2019; Wu et al., 2019). Increasing of LA/AA ratio to 1.0 slightly promoted the fermentation process, and caproate gradually increased to 0.07 mmol/L at the 12th day, confirming that lack of electron donors results in incomplete CE processes and longer chain carboxylate cannot be effectively synthesized (Contreras-Dávila et al., 2020). More caproate was obtained at higher LA/AA ratios due to enhancement of CE process and caproate production by providing more electrons and energy (Wang et al., 2018; Xie et al., 2021). The highest caproate content (11.02 mmol/L) was recorded at 3.0 LA/AA ratio, while it decreased to 6.11 and

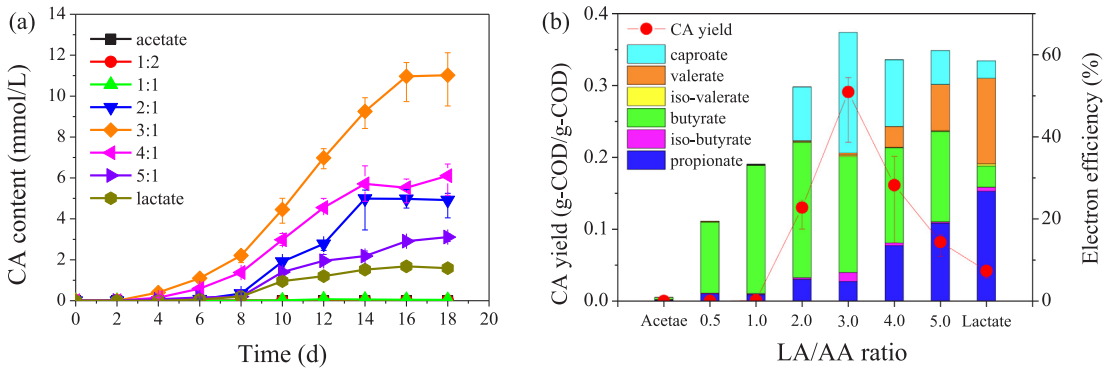

Fig. 1. Variations in caproate (CA) content during 18 days of batch experiment (a) and CA yield (b) under different lactate-to-acetate (LA/AA) ratios.

3.11 mmol/L at LA/AA ratios of 4.0 and 5.0, respectively (Fig. 1a). These results indicate that LA/AA ratio higher than 3.0 is not suitable for caproate fermentation, which is in consistent with previous studies (Wu et al., 2018).

Due to the lack of ED in substrate, caproate yield in reactors with LA/AA ratio lower than 1.0 was very low (Fig. 1b), while it gradually increased and reached the maximum value of 0.29 g-COD/g-COD at 3.0 LA/AA ratio. Further increase of LA/AA ratio resulted in lower caproate yield. It was reported that high LA/AA ratio would affect the microbial metabolic pathways and activate the competitive metabolic processes, which disperses lactate-carbon flow and finally reduces the caproate yield (Han et al., 2019; Wu et al., 2019).

Electron efficiency is an indicator to evaluate substrate utilization effectiveness during fermentation. Under theoretically optimal LA/AA ratio of 2.0, electron efficiency for caproate production was only 13.1%, which is much lower than that for butyrate generation (32.9%). This might be attributed to participation of a part of electron donors in other reactions, and cannot provide sufficient reducing equivalents for CE processes. However, the highest electron efficiency for caproate production (29.4%) was recorded at 3.0 LA/AA ratio (Fig. 1b), where propionate and valerate were the dominant electron pools at higher LA/AA ratios. Thus, LA/AA ratios less than 3.0 result in butyrate accumulation due to the shortage of valid electrons, while higher LA/AA ratios may augment the acrylate pathway and enrich propionate and valerate. Therefore, a 3.0 LA/AA ratio was considered as a proper value for caproate fermentation in the present study.

3.1.2. Variations in substrate content

Lactate content showed continuous reduction during the whole fermentation period (Fig. S1, Supplementary data). In the reactor with LA/AA ratio of 0.5, lactate content decreased sharply from 33.3 mmol/L to 0.7 mmol/L in 10 days. In the other reactors, lactate content also rapidly decreased to the lowest level in a very short fermentation period. Even in the reactor with only lactate, microbial activity was not inhibited by high lactate content, which is in consistence with other studies (Nzeteu et al., 2018; Zhu et al., 2015). The high degradation efficiency of lactate was beneficial for CE process by providing energy and electrons. Acetate can be produced through lactate degradation and also be utilized as EA during CE. In the reactor with sole acetate, substrate content remained almost constant (Fig. S1, Supplementary data). However, acetate content slightly increased at the earlier stages by adding lactate, and gradually decreased thereafter. In the reactor with sole lactate, acetate showed increase up to 14.3 mmol/L at 8th day, then gradually decreased to 10.57 mmol/L, which might be attributed to microbial conversion of lactate into acetate which was then utilized as EA for butyrate and caproate production. After 10 days of fermentation, acetate content in all reactors remained constant due to the exhaustion of lactate.

3.1.3. Variations of product content

In the reactor with LA/AA ratios lower than 3.0, propionate content was relatively lower (Fig. S1, Supplementary data), which increased to 11.56 mmol/L by increasing of LA/AA ratio to 4.0. Further increase in lactate concentration to 83.3 mmol/L (LA/AA = 5.0) resulted in obvious accumulation of propionate (19.8 mmol/L). It was previously reported that high concentration of lactate activates the acrylate pathway and promotes propionate generation (Prabhu et al., 2012; Wu et al., 2019). The highest propionate content of 22.9 mmol/L was recorded in the reactors with sole lactate, which can be explained by formation of lactyl-CoA and propionyl-CoA under lactate-rich conditions (Kucek et al., 2016; Wu et al., 2019), which further transformed into propionate to achieve electron balance (Candry et al., 2020). Since propionate can be utilized as EA for CE, the concentration of propionate gradually decreased over time when its consumption rate was higher than the generation rate.

Butyrate is an intermediate product of CE and can be utilized also as electron acceptor for caproate production. In the reactor with sole acetate, butyrate was not detected (Fig. S1, Supplementary data), which increased gradually in other reactors. The final butyrate content in the reactor with 0.5 LA/AA ratio was around 10.47 mmol/L, which increased to 18.91 mmol/L by increasing of LA/AA ratio to 1.0. Much higher butyrate content was detected in other reactors, indicating

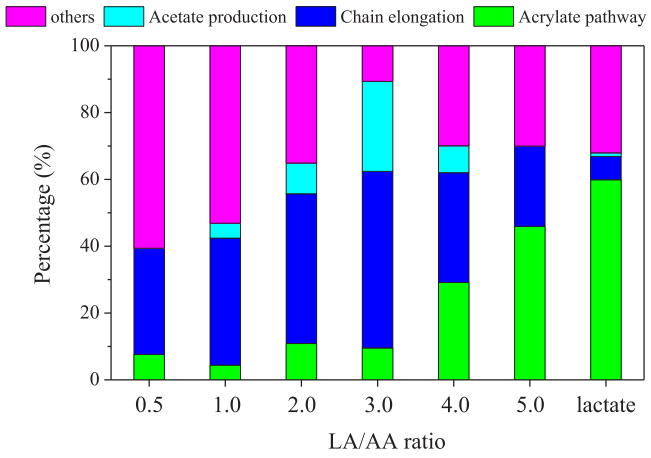

Fig. 2. Metabolic pathways under different lactate-to-acetate (LA/AA) ratios.

that high LA/AA ratio could enhance chain elongation and butyrate production. However, in the reactors with LA/AA ratio between 2.0 and 5.0, butyrate firstly accumulated and then gradually decreased. In the reactor with LA/AA ratio of 3.0, butyrate gradually increased to 31.81 mmol/L, while it decreased to 18.26 mmol/L, which might be attributed to microbial utilization of substrates to produce butyrate through chain elongation at the early stages of fermentation, then the generated butyrate was transformed into caproate after enough butyrate accumulation. Similar trend was observed in other reactors, particularly in the reactor with sole lactate where 3.71 mmol/L of butyrate were detected, confirming that lactate could simultaneously produce acetate as acceptor and also acts as electron donor for CE (Zhu et al., 2015).

Accumulation of valerate has positive correlation with propionate generation and initial lactate concentration, as high content of LA was reported to promote propionate production (Wu et al., 2019). Therefore, in the reactor with LA/AA ratio less than 3.0, valerate content was very low (<0.5 mmol/L), which increased to 2.39 and 5.29 mmol/L at LA/AA ratio of 4.0 and 5.0, respectively (Fig. S1, Supplementary data). In the reactor with sole lactate, valerate sharply increased to 9.87 mmol/L, which is much higher than that of caproate (1.59 mmol/L), verifying that high lactate content is not suitable for caproate fermentation. Due to the competition of acrylate pathway, chain elongation for caproate generation would be seriously restricted (Wu et al., 2019). Although high caproate yield was observed with sole lactate (Zhu et al., 2015), obvious accumulation of propionate was previously reported at high lactate concentrations (Kucek et al., 2016), which might be attributed to different microbial community structures.

3.1.4. Metabolic pathways under different LA/AA ratios

The impacts of LA/AA ratio on microbial metabolic pathways were further analyzed (Fig. 2). It can be concluded that acrylate pathway was enhanced but CE process was weakened at high LA/AA ratios. In the reactors with LA/AA ratio lower than 1.0, lactate was mainly utilized in other metabolic activities (e.g. cell growth and energy production), indicating that low concentration of initial lactate was not proper for caproate production. Similarly, small amounts of lactate (7.7% at a LA/AA ratio of 0.5 and 4.4% at a LA/AA ratio of 1.0) participated in the acrylate pathway, confirming that low lactate content was beneficial to restrict propionate production. Lactate for CE increased from 31.7% (at LA/AA ratio 0.5) to 52.9% (at LA/AA ratio 3.0), indicating that increasing of LA/AA ratio could enhance CE and promote caproate production. Nevertheless, due to enhancement of acrylate pathway under lactate-rich conditions, more lactate will be transformed into propionate or valerate (Wu et al., 2019). As a result, lactate used for chain elongation sharply decreased and lower caproate content was detected. In the reactor with sole lactate, 59.9% of lactate was consumed in acrylate pathway and only 7.0% entered into CE pathway, which further explained the high content of propionate and low caproate yield under these conditions. Additionally, the reactor with LA/AA ratio of 3.0 showed the highest proportion of acetate production and lowest percentage of other pathways, verifying that more lactate was consumed for CE. It can be seen that different fermentation product showed similar variations (Fig. S2, Supplementary data). Thus, LA/AA ratio of 3.0 is favorable for caproate production, which might be related to enzymes activity and microbial communities in the reactors, which requires further validation studies.

3.2. Effect of substrate/inoculum (S/I) ratio

Based on the above discussions, LA/AA ratio of 3.0 was optimal for caproate production. In this section, the effect of substrate loading (S/I ratio) on caproate fermentation was further investigated.

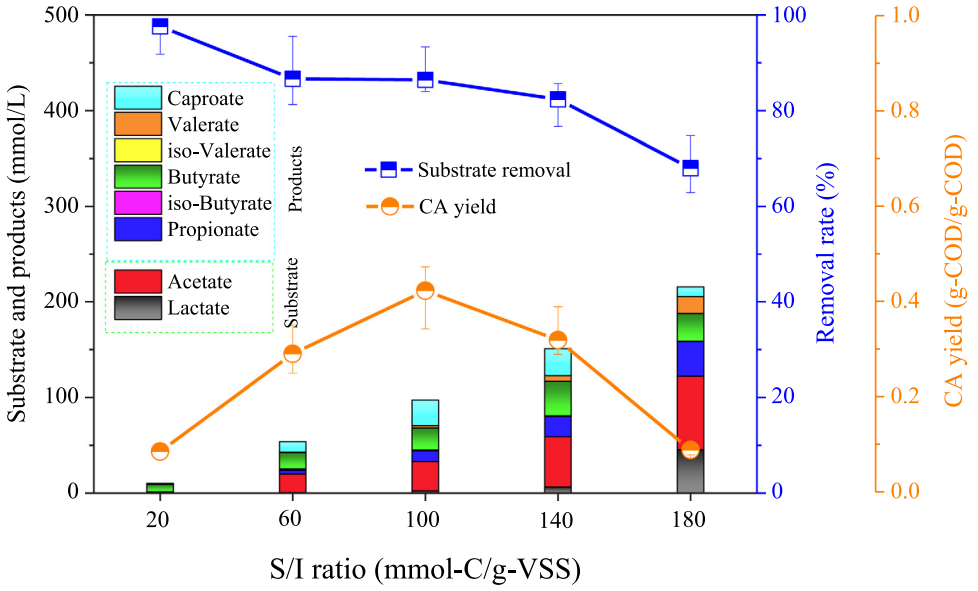

Fig. 3. Variations in acids content, substrate removal rate, and caproate yield at different substrate-to-inoculums (S/I) ratios, CA refers to caproate.

3.2.1. Effect of S/I ratio

The effect of S/I ratio (20–180 mmol-C/g-VSS) on CE process was further studied (Fig. 3). At 20 mmol-C/g-VSS, the final lactate and acetate contents were only 0.30 and 0.75 mmol/L, respectively. However, increasing of S/I ratio to 180 mmol-C/g-VSS resulted in sharp increase of lactate and acetate to 45.5 and 76.5 mmol/L, respectively. Accordingly, substrate removal rate was almost stable (between 93.7% and 82.4%) at S/I ratio lower than 140 mmol-C/g-VSS, while it sharply decreased to 68.0% by increasing S/I value to 180 mmol-C/g-VSS. It was reported that higher substrate concentration would increase the cellular osmotic pressure, damage the microbial cell membrane, limit the microbial enzyme activity, reduce the metabolic capability, and may be kill the microbes involved in fermentation process (Jarboe et al., 2013), which finally results in low substrate utilization rate and product yield.

Caproate content in fermentation broth increased by increasing S/I ratio from 20 to 140 mmol-C/g-VSS. Butyrate was largely accumulated (7.89 mmol/L) in the reactor with S/I ratio of 20 mmol-C/g-VSS, while only 1.1 mmol/L of caproate was detected, indicating that low substrate concentration was not suitable for CE. However, caproate content sharply increased to 11.02 mmol/L by increasing of S/I ratio to 60 mmol-C/g-VSS. Butyrate with a content of 18.26 mmol/L was the dominant intermediate, while a relatively small amount of propionate (4.06 mmol/L) was accumulated in the reactor. Further increase of S/I ratio to 100 and 140 mmol-C/g-VSS showed higher caproate and propionate contents. At S/I ratio of 180 mmol-C/g-VSS, lactate was mainly transformed into propionate and valerate, leading to a lower caproate content (10.25 mmol/L). Thus, low S/I ratio cannot provide enough substrates for CE and results in butyrate accumulation, while high S/I ratio would also reduce the caproate production due to enhancement of acrylate pathway which disperse lactate-carbon flow to propionate and valerate (Wang and Yin, 2022). Therefore, results confirmed that reactor with S/I ratio of 100 and 140 mmol-C/g-VSS showed the relatively higher caproate content (Fig. 3).

It was reported that high substrate content would damage the cell membrane of functional bacteria and restrict their biological activities or destroy the enzyme activity (Jarboe et al., 2013). The highest caproate yield (0.42 g-COD/g-COD) was obtained at S/I ratio of 100 g-COD/g-VSS. This can be mainly attributed to two reasons; firstly, higher substrate content may affect the microbial growth and metabolisms which results in lower substrates conversion. Secondly, higher S/I ratios may activate the competitive metabolic pathways and restrict the RBO pathway. Although fermentation conditions are varied, caproate yield and selectivity in this study are comparable to other studies (Table 1).

3.2.2. Product selectivity under different S/I ratios

The product selectivity exhibits electrons transferred from substrate to fermentation products. It can be clearly noted that when S/I ratio is lower than 100 mmol-C/g-VSS, the selectivity of caproate increased by increasing of S/I ratio (Table 2). At S/I ratio of 20 mmol-C/g-VSS, 40.4% of electrons flowed into butyrate, and only 9.0% electrons participated in the caproate fermentation. Increasing of S/I ratio to 60 mmol-C/g-VSS, electrons for butyrate production slightly decreased to 32.7%, while 34.0% of electrons were used to produce caproate, indicating that higher substrate content was beneficial for CE. Further increase of S/I ratio to 100 mmol-C/g-VSS resulted in the highest recorded caproate selectivity (49.5%), which explains the highest caproate yield. However, too high S/I ratio was not suitable for CE, due to increasing the selectivity for valerate and propionate. In addition, total selectivity sharply reduced as the S/I increased. It was reported

Table 1
The caproate yield and selectivity in different studies compared to the present study.

Substrate	Inoculum	ED/EA ratio	Substrate load	pH and temperature	CA content	CA yield	CA selectivity	References
Lactate (30 g/L)	Pit-mud	–	–	6.0–6.5, 30 °C	12.54 g/L	0.85 g-COD/g-COD	86.5%	Zhu et al. (2015)
Lactate (20 mM), butyrate (80 mM)	Excess activated sludge	0.25	–	5.0, 30 °C	3.2 mmol/L	0.056 g-COD/g-COD	5.6%	Xie et al. (2021)
Lactate (22.35 g/L), Butyrate (16.48 g/L) and sucrose (13.3 g/L)	Ruminococcaceae bacterium CPB6	1.0	–	5.5–5.8, 37 °C	16.73 g/L	0.68 g-COD/g-COD	69%	Wang et al. (2018)
Lactate (100 mM), acetate (33.3 mM)	Excess sludge and pit-mud	4.5	–	7.0, 35 °C	14.57 mM	0.31 g-COD/g-COD	31.8%	Wu et al. (2018)
Lactate (125 mM), acetate (62.5 mM)	Pit-mud	3.0	100 mmolC/g-VSS	5.5, 30 °C	26.71 mM	0.42 g-COD/g-COD	42.7%	This study

"–" not provided.

Table 2
Selectivity of the fermentation products (%) under different S/I ratios.

S/I ratios	Propionate	Iso-butyrate	Butyrate	Iso-valerate	Valerate	Caproate
20	0.50	0.00	40.42	0.00	0.00	9.02
60	5.47	2.51	32.65	0.41	0.62	33.96
100	9.14	0.99	26.59	0.25	3.39	49.51
140	12.93	0.47	31.12	0.19	6.68	39.26
180	20.79	0.44	23.60	0.17	18.70	13.43

that higher substrate concentrations would restrict microbial activity and reduce the product yield. Moreover, at higher S/I ratios, microorganisms would transform the substrate into other products such as propionate and valerate. Based on these findings, it can be concluded that lower substrate content is more beneficial for caproate production, while too low S/I ratio would also restrict the RBO process due to substrate shortage. Thus, 100 mmol-C/g-VSS was considered as the optimal S/I ratio for effective caproate fermentation.

3.2.3. Carbon fluxes at different S/I ratios

The suggested carbon flux of lactate at different substrate concentrations is shown in Fig. 4. It can be seen that lactate was utilized in similar fermentation pathway (RBO pathway, acrylate pathway, and other metabolic processes), but the flux was quite different. At S/I ratio of 100 mmol-C/g-VSS, approximately 72.6% of carbon in lactate was flowed into CE process and resulted in higher caproate content. Only 16.4% of carbon was utilized for propionate and valerate production. However, at higher S/I ratio, more lactate was utilized for propionate and valerate production (36%), which explains the lower caproate yield. Carbon flux analysis provides a way to investigate the effect of lactate load on caproate and SCCAs production. Obviously, by increasing of lactate loading, the carbon flux of acrylate pathway was enhanced while caproate production was suppressed, resulting in higher valerate production.

3.3. Metabolic pathways of lactate-based chain elongation

3.3.1. RBO pathway

Chain elongating microorganisms utilize lactate and short-chain fatty acids (e.g. acetate, propionate and butyrate) to produce butyrate, valerate and caproate through RBO pathways. Thus, lactate and SCFA are determining factors for the fermentation efficiencies and final products. Different lactate and acetate loadings would lead to discrepancy in the product structures (see Fig. 1). Results showed that caproate was almost not produced at early stages of fermentation, while it slowly increased until butyrate accumulated to a certain level, which confirms that butyrate is an important driving factor for caproate production (Wu et al., 2019). Based on the carbon flux (Fig. 4), caproate formation was strongly determined by butyrate. Therefore, it can be concluded that when lactate and acetate are used as substrates, butyrate is firstly generated and accumulated to a certain level before caproate synthesis.

Based on the reactions shown in supplementary data (Eqs. S1–S3), LA/AA ratio of 2.0 is the theoretically optimal ratio for caproate synthesis and the stoichiometric caproate yield should be 0.69 g-COD/g-COD. However, the present study showed caproate yield of 0.13 g-COD/g-COD, which might be because consumption of substrates in other metabolic activities (Kang et al., 2022). Additionally, microbial communities in the reactors may not be optimized, which also reduces the fermentation efficiency (Li et al., 2021). Interestingly, higher caproate content and yield were obtained at 3.0 LA/AA ratio, indicating that more lactate could enhance the CE processes and promote the caproate production (Wu et al., 2022; Zhu et al., 2017). Even though lactate is the main electron donor for chain elongation, high proportion of lactate is also not proper for caproate fermentation, and may results in high content of by-products. For example, increasing of LA/AA

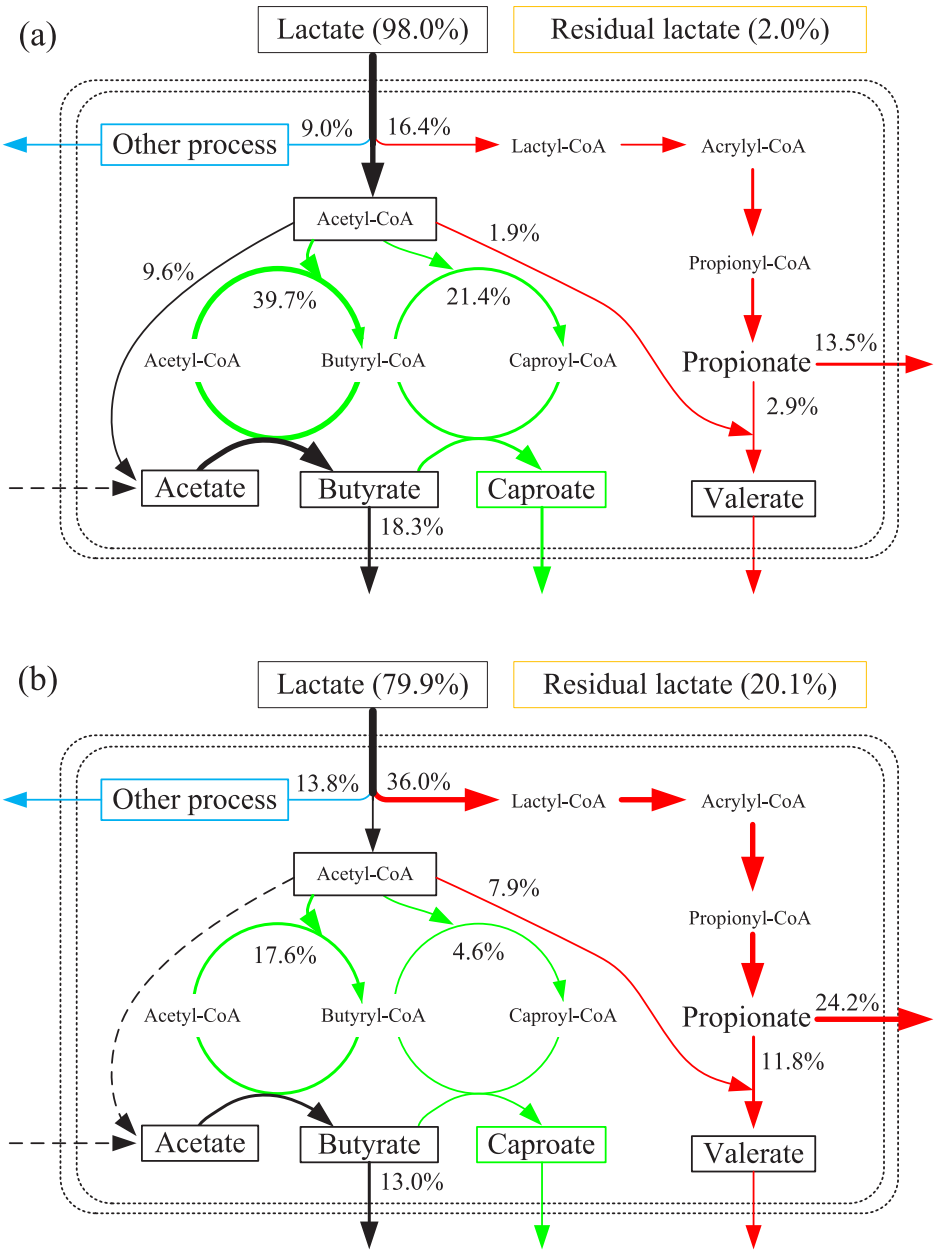

Fig. 4. Carbon flux at substrate-to-inoculums (S/I) ratio of 100 mmol-C/g-VSS (a) and 180 mmol-C/g-VSS (b).

ratio from 3.0 to 4.0 and 5.0, the caproate yield decreased by 44.8% and 72.4%, respectively, due to enhancement of other metabolic pathways (such as acrylate pathway and valerate production), which is in consistent with previous studies (Contreras-Dávila et al., 2020; Wu et al., 2019).

Except for LA/AA ratio, substrate concentration also showed obvious impacts on RBO pathway. Although caproate content increased with the increase of substrate content, the highest yield was observed at S/I ratio of 100 mmol-C/g-VSS. Higher substrate concentration resulted in lower caproate content and selectivity, while lower substrate concentrations led to reduction of electron efficiency. Therefore, the most advantageous substrate load was 100 mmol-C/g-VSS in this study.

Caproate productivity was lower than that of acetate and butyrate at the early stages (Section 3.1), which might be due to the fact that microorganisms had an urgent need of energy for self-sustainability during this period (Wu et al., 2019). Lactate was firstly oxidized to produce acetyl-CoA, then part of acetyl-CoA was converted to acetate and ATP to maintain self-growth of microorganisms, while others will participate in the RBO cycle (Wang and Yin, 2022; Wu et al.,

2019). Accumulation of acetate indicates the enhancement of microbial activity at the initial stage. Although the elevated lactate content facilitates this process, acrylate pathway will also be augmented (Prabhu et al., 2012; Wu et al., 2019). The complex competitive reactions led to a low caproate production under lactate-rich conditions.

3.3.2. Acrylate pathway

It has been confirmed that acrylate pathway is the main competitive pathway in CE process induced by lactate, especially under high lactate content (Han et al., 2019; Wu et al., 2019). Similarly, in this study, more lactate participated in acrylate pathway when its concentration was higher (Section 3.2). Propionate is the dominant by-product from lactate transformation, which finally results in reduction of carbon flux participating in caproate fermentation. Through acrylate pathway, propionate was produced and then elongated to valerate with lactate as electron donor. Thus, a lot of lactate was consumed after acrylate pathway is activated, which reduced the utilizable substrate for caproate production and finally resulted in low caproate yield (Wu et al., 2019; Xie et al., 2021).

At LA/AA ratios over 3.0, propionate appeared and sharply increased in a short term (Section 3.1), and then the content of propionate gradually reduced with valerate generation, indicating that the generated propionate was further elongated to valerate by residual lactate. It is worth noting that accumulation of valerate was faster at higher lactate content, which might be due to the fact that high lactate is more suitable for acrylate pathway, and can provide more substrates for further CE (Candry et al., 2020; Prabhu et al., 2012). In the acrylate pathway, lactate is converted to lactyl-CoA as an intermediate. Once lactyl-CoA is generated, the microorganisms are committed to convert lactyl-CoA to propionyl-CoA (Prabhu et al., 2012). There is a driving force for lactate to be converted into its thioester via propionyl-CoA transferase with a relatively high concentration of lactate (Wu et al., 2019, 2018), which explains the easier conversion of lactate to propionate in a lactate-rich environment. Therefore, low lactate concentration is not conducive to the formation of propionate and increases the carbon flux toward caproate generation.

4. Conclusions

The effect of different LA/AA ratios and substrate loading (S/I ratios) on lactate-based caproate fermentation was investigated in the present study. It was found that LA/AA ratio lower than 3.0 could not provide enough electron donors for CE process and resulted in butyrate accumulation. However, higher LA/AA ratio would activate the acrylate pathway, leading to high propionate content and low caproate yield. In addition, low S/I ratios result in higher carbon loss in other metabolic activities, but high substrate loading rate leads to incomplete substrate utilization and carbon flow disperse. Thus, a LA/AA and S/I ratios of 3.0 and 100 mmol-C/g-VSS could be regarded as the preferable conditions for maximum caproate production (0.42 g-COD/g-COD).

CRediT authorship contribution statement

Jialing Tang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Funding acquisition. **Yunhui Pu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Funding acquisition. **Jin Huang:** Writing – original draft, Writing – review & editing. **Shengwang Pan:** Writing – original draft, Writing – review & editing. **Xiaochang C. Wang:** Writing – original draft, Writing – review & editing. **Yisong Hu:** Writing – original draft, Writing – review & editing. **Huu Hao Ngo:** Writing – original draft, Writing – review & editing. **Yuyou Li:** Writing – original draft, Writing – review & editing. **Abdelfatah Abomohra:** Funding acquisition, Investigation, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102918.

References

Abomohra, A., Faisal, S., Ebaid, R., Huang, J., Wang, Q., Elsayed, M., 2022. Recent advances in anaerobic digestion of lipid-rich waste: Challenges and potential of seaweeds to mitigate the inhibitory effect. Chem. Eng. J. 28, 137829.

Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends Biotechnol. 29 (2), 70–78.

Candry, P., Radić, L., Favere, J., Carvajal-Arroyo, J.M., Rabaey, K., Ganigué, R., 2020. Mildly acidic pH selects for chain elongation to caproic acid over alternative pathways during lactic acid fermentation. Water Res. 186, 116396.

Carvajal-Arroyo, J.M., Candry, P., Andersen, S.J., Props, R., Seviour, T., Ganigué, R., Rabaey, K., 2019. Granular fermentation enables high rate caproic acid production from solid-free thin stillage. Green Chem. 21 (6), 1330–1339.

Cavalcante, W.A., Gehring, T.A., Santaella, S.T., Freitas, I.B.F., Angenent, L.T., van Haandel, A.C., Leitão, R.C., 2020. Upgrading sugarcane biorefineries: Acetate addition allows for conversion of fermented sugarcane molasses into high-value medium chain carboxylic acids. J. Environ. Chem. Eng. 8 (2), 103649.

Chwialkowska, J., Duber, A., Zagrodnik, R., Walkiewicz, F., Łężyk, M., Oleskowicz-Popiel, P., 2019. Caproic acid production from acid whey via open culture fermentation-Evaluation of the role of electron donors and downstream processing. Bioresour. Technol. 279, 74–83.

Contreras-Dávila, C.A., Carrión, V.J., Vonk, V.R., Buisman, C.N.J., Strik, D.P.B.T.B., 2020. Consecutive lactate formation and chain elongation to reduce exogenous chemicals input in repeated-batch food waste fermentation. Water Res. 169, 115215.

Deepanraj, B., Sivasubramanian, V., Jayaraj, S., 2017. Effect of substrate pretreatment on biogas production through anaerobic digestion of food waste. Int. J. Hydrogen Energy 42 (42), 26522–26528.

Duber, A., Jaroszynski, L., Zagrodnik, R., Chwialkowska, J., Juzwa, W., Ciesielski, S., Oleskowicz-Popiel, P., 2018. Exploiting the real wastewater potential for resource recovery-n-caproate production from acid whey. Green Chem. 20 (16), 3790–3803.

Duber, A., Zagrodnik, R., Chwialkowska, J., Juzwa, W., Oleskowicz-Popiel, P., 2020. Evaluation of the feed composition for an effective medium chain carboxylic acid production in an open culture fermentation. Sci. Total Environ. 728, 138814.

Han, W., He, P., Shao, L., Lü, F., 2019. Road to full bioconversion of biowaste to biochemicals centering on chain elongation: A mini review. J. Environ. Sci. 86, 50–64.

Jarboe, L., Royce, L., Liu, P., 2013. Understanding biocatalyst inhibition by carboxylic acids. Front. Microbiol. 4, 272.

Kang, S., Kim, H., Jeon, B.S., Choi, O., Sang, B.I., 2022. Chain elongation process for caproate production using lactate as electron donor in Megasphaera hexanoica. Bioresour. Technol. 346, 126660.

Kucek, L.A., Nguyen, M., Angenent, L.T., 2016. Conversion of L -lactate into n-caproate by a continuously fed reactor microbiome. Water Res. 93, 163–171.

Li, J., Liu, H., Wu, P., Ding, P., Zhang, J., Zhang, C., Fu, B., Cui, M.H., 2021. Quorum sensing signals enhanced caproate production by changing microbial community in chain elongation enrichments. J. Environ. Chem. Eng. 9 (6), 106623.

Liu, Y., He, P., Shao, L., Zhang, H., Lü, F., 2017. Significant enhancement by biochar of caproate production via chain elongation. Water Res. 119, 150–159.

Lonkar, S., Fu, Z., Holtzapple, M., 2016. Optimum alcohol concentration for chain elongation in mixed-culture fermentation of cellulosic substrate. Biotechnol. Bioeng. 113 (12), 2597–2604.

Nzeteu, C.O., Trego, A.C., Abram, F., O'Flaherty, V., 2018. Reproducible, high-yielding, biological caproate production from food waste using a single-phase anaerobic reactor system. Biotechnol. Biofuels 11 (1), 108.

Prabhu, R., Altman, E., Eiteman, M.A., 2012. Lactate and acrylate metabolism by megasphaera elsdenii under batch and steady-state conditions. Appl. Environ. Microbiol. 78 (24), 8564–8570.

Tang, J., Pu, Y., Zeng, T., Hu, Y., Huang, J., Pan, S., Abomohra, A., 2022. Enhanced methane production coupled with livestock wastewater treatment using anaerobic membrane bioreactor: Performance and membrane filtration properties. Bioresour. Technol. 345, 126470.

Tang, J., Wang, X.C., Hu, Y., Ngo, H.H., Li, Y., 2017a. Dynamic membrane-assisted fermentation of food wastes for enhancing lactic acid production. Bioresour. Technol. 234, 40–47.

Tang, J., Wang, X., Hu, Y., Zhang, Y., Li, Y., 2016. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Manage. 52, 278–285.

Tang, J., Wang, X.C., Hu, Y., Zhang, Y., Li, Y., 2017b. Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula. Bioresource Technol. 224, 544–552.

Wang, H., Li, X., Wang, Y., Tao, Y., Lu, S., Zhu, X., Li, D., 2018. Improvement of n-caproic acid production with Ruminococcaceae bacterium CPB6: selection of electron acceptors and carbon sources and optimization of the culture medium. Microb. Cell Fact. 17 (1), 99.

Wang, J., Yin, Y., 2022. Biological production of medium-chain carboxylates through chain elongation: An overview. Biotechnol. Adv. 55, 107882.

Wu, Q., Bao, X., Guo, W., Wang, B., Li, Y., Luo, H., Wang, H., Ren, N., 2019. Medium chain carboxylic acids production from waste biomass: Current advances and perspectives. Biotechnol. Adv. 37 (5), 599–615.

Wu, Q., Feng, X., Guo, W., Bao, X., Ren, N., 2020a. Long-term medium chain carboxylic acids production from liquor-making wastewater: Parameters optimization and toxicity mitigation. Chem. Eng. J. 388, 124218.

Wu, Q., Guo, W., Bao, X., Meng, X., Yin, R., Du, J., Zheng, H., Feng, X., Luo, H., Ren, N., 2018. Upgrading liquor-making wastewater into medium chain fatty acid: Insights into co-electron donors, key microflora, and energy harvest. Water Res. 145, 650–659.

Wu, S.L., Luo, G., Sun, J., Wei, W., Song, L., Ni, B.J., 2021. Medium chain fatty acids production from anaerobic fermentation of waste activated sludge. J. Clean. Prod. 279, 123482.

Wu, Q., Ren, W., Guo, W., Ren, N., 2022. Effect of substrate structure on medium chain fatty acids production and reactor microbiome. Environ. Res. 204, 111947.

Wu, S.L., Sun, J., Chen, X., Wei, W., Song, L., Dai, X., Ni, B.J., 2020b. Unveiling the mechanisms of medium-chain fatty acid production from waste activated sludge alkaline fermentation liquor through physiological, thermodynamic and metagenomic investigations. Water Res. 169, 115218.

Xie, S., Ma, J., Li, L., He, Q., Xu, P., Ke, S., Shi, Z., 2021. Anaerobic caproate production on carbon chain elongation: Effect of lactate/butyrate ratio, concentration and operation mode. Bioresource Technol. 329, 124893.

Xu, J., Hao, J., Guzman, J.J.L., Spirito, C.M., Harroff, L.A., Angenent, L.T., 2018. Temperature-phased conversion of acid whey waste into medium-chain carboxylic acids via lactic acid: No external e-donor. Joule 2 (2), 280–295.

Zaks, D.P.M., Winchester, N., Kucharik, C.J., Barford, C.C., Paltsev, S., Reilly, J.M., 2011. Contribution of anaerobic digesters to emissions mitigation and electricity generation under U.S. climate policy. Environ. Sci. Technol. 45 (16), 6735–6742.

Zhang, C., Yang, L., Tsapekos, P., Zhang, Y., Angelidaki, I., 2019. Immobilization of Clostridium kluyveri on wheat straw to alleviate ammonia inhibition during chain elongation for n-caproate production. Environ. Int. 127, 134–141.

Zhu, X., Tao, Y., Liang, C., Li, X., Wei, N., Zhang, W., Zhou, Y., Yang, Y., Bo, T., 2015. The synthesis of n-caproate from lactate: a new efficient process for medium-chain carboxylates production. Sci. Rep. 5 (1), 14360.

Zhu, X., Zhou, Y., Wang, Y., Wu, T., Li, X., Li, D., Tao, Y., 2017. Production of high-concentration n-caproic acid from lactate through fermentation using a newly isolated Ruminococcaceae bacterium CPB6. Biotechnol. Biofuels 10 (1), 102.