Designing a Selective $n$-Caproate Adsorption–Recovery Process with Granular Activated Carbon and Screening of Conductive Materials in Chain Elongation

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Cite This: https://doi.org/10.1021/acsestengg.1c00214

ABSTRACT: Microbial chain elongation using biomass-derived lactate can be steered to produce a variety of medium-chain carboxylates (MCC), which then need to be separated before application. In this study, we evaluated the effects of adding conductive and/or adsorbing materials to batch and continuous open-culture lactate-based chain elongation. Incubation with granular activated carbon (GAC), nickel foam (NF), and stainless steel (SS) mesh improved lactate use for chain elongation due to a ∼30% reduction in propionate formation compared to the control (no material). Isobutyrate production was stimulated in the presence of GAC and NF (up to 1.2 g·L$^{-1}$, 9% electron selectivity). Adding GAC to a continuous reactor led to in situ adsorption of $n$-caproate. GAC showed a high affinity to $n$-caproate from real and artificial effluents, as well as from blends containing C2–C8 carboxylates, adsorbing 60–80% of the initial $n$-caproate with recoveries up to 42% after desorption. Adsorption isotherms showed that $n$-caproate adsorption increased with decreasing pH conditions (184–243 mg·g GAC$^{-1}$). In conclusion, conductive materials changed the product spectrum and steered to isobutyrate formation in batch open-culture chain elongation. Based on the promising adsorption properties of GAC, the first design of chain elongation with in-line adsorption–recovery is proposed as well as potential direct applications of MCC-loaded porous carbons.

KEYWORDS: microbial chain elongation, lactate, direct interspecies electron transfer, isobutyrate, adsorption.

1. INTRODUCTION

Resource recovery bioprocesses using chain elongation reactor microbiomes produce medium-chain carboxylates (MCC), which are saturated monocarboxylates with 6–12 carbon atoms that find applications in lubricants, biodegradable plastics, antimicrobials, feed additives, and biofuel production.$^1$ Herein, we use carboxylate to refer to both the undissociated and dissociated forms together, and we refer to each specific form when appropriate. Chain-elongating bacteria utilize energy-rich substrates e.g., ethanol, lactate, and glucose as electron donors to elongate short-chain carboxylates (SCC, one to five carbon units) to MCC such as $n$-caproate ($n$C6) or $n$-caprylate ($n$C8) through the reverse $β$-oxidation pathway.$^1$ Lactate is an interesting electron donor that can be easily obtained from residual biomass materials to produce $n$-caproate.$^2,3$ Alternatively, lactate may be converted to isobutyrate$^4$ or propionate.$^5$ To steer microbiome-based bioprocesses toward the effective production of a desired carboxylate, several operational conditions are applied such as gas supply,$^6$ pH control, or electron donor-to-acceptor ratio adjustment.$^7$ An alternative approach could be the use of conductive materials, which may promote electron transfer in chain elongation microbiomes. Conductive materials have been used to enhance anaerobic bioprocesses such as methanogenesis$^7$ and SCC production (acetate and $n$-butyrate) from hydrogen (H$_2$) and carbon dioxide (CO$_2$)$^8$ Porous carbon materials such as granular activated carbon (GAC) have also been shown to enhance syntrophic methane production through direct interspecies electron transfer (DIET) due to its high conductivity.$^9$ A similar effect has been observed with nickel foam (NF).$^10$ In chain elongation, biochar was suggested to facilitate electron transfer between ethanol oxidizers and chain-elongating bacteria with higher $n$-caproate and $n$-caprylate production.$^11$ However, the effect of

Received: June 10, 2021
Revised: November 26, 2021
Accepted: November 30, 2021
conductive materials in lactate-based chain elongation has not been studied.

In addition to their conductive properties, porous carbon materials may adsorb MCC, reducing their toxicity to chain-elongating bacteria and potentially facilitating MCC recovery. Since the produced MCC should be separated and recovered from the fermentation broth for further valorization, several methods have been applied in chain elongation, including liquid–liquid extraction and ion exchange. Carboxylate recovery with GAC has only been studied for SCC such as lactate, acetate, and n-butyrate from effluents of primary fermentation. Adsorption of n-caproate on GAC has been studied using single-acid aqueous solutions. More recently, n-caproate adsorption was observed in pure culture ethanol-based chain elongation incubation with added GAC or biochar. However, the recovery of MCC from chain elongation systems through adsorption and desorption with GAC has not been assessed. Adsorption with GAC may have the advantages of easy handling along with reduced space and energy demands.

The aim of this work was to evaluate the effect of conductive materials on lactate-based chain elongation and the potential of GAC to recover chain elongation products with a focus on n-caproate. First, conductive (GAC, nickel foam [NF], and stainless steel [SS]) and nonconductive (polyurethane [PU] foam) materials were tested in incubation to understand whether the addition of conductive materials can affect fermentation performance. Since GAC promoted chain elongation and may facilitate MCC recovery, GAC was chosen for further tests in a continuous chain elongation reactor converting lactate and acetate to n-caproate. Next, the feasibility of carboxylate adsorption and recovery with GAC was evaluated from real and artificial chain elongation effluents and from blends containing carboxylates with two to eight carbons. Finally, adsorption isotherms were recorded to determine the effect of pH on n-caproate adsorption capacity.

2. MATERIALS AND METHODS

2.1. Materials Used in Batch Chain Elongation. Three conductive (granular activated carbon [GAC], nickel foam [NF], and stainless steel mesh [SS]) and one nonconductive (polyurethane [PU] foam) materials were tested. The GAC used (Norit PK 1–3, Cabot Norit Nederland B.V., the Netherlands) is produced from peat through steam activation, has an alkaline pH, particle size between 0.71 and 3.15 mm (85 wt %), a total surface area (Brunauer–Emmett–Teller [BET]) of 875 m² g⁻¹, and a methylene blue adsorption capacity of 110 g kg⁻¹ (as reported by Cabot Norit Nederland B.V.). The same material has been reported to have a pore volume of 0.55 cm³ g⁻¹ and an average pore size of 2.7 nm. The other materials were stainless steel T304 mesh 40 (Salomon’s Metalen B.V., the Netherlands), nickel foam (Salomon’s Metalen B.V., the Netherlands), and PU foam (Recticel, Ltd., Brussels, Belgium) with a density of 30.4 kg m⁻³ and a porosity of 20 pores per inch (PPI) (pore diameter of ~13 μm).

2.2. Screening of Conductive Materials in Batch Chain Elongation. All of the materials were added at 15 g L⁻¹ in triplicate batch chain elongation experiments. A duplicate control experiment with no added material was included. Lactate (≥90% concentrated lactic acid, VWR, the Netherlands) was added as an electron donor and acetate (99–100% acetic acid, Merck KGaA, Germany) as an electron acceptor. Concentrated lactic acid was a mixture of lactate monomers (~64%) and oligomers (e.g., lactyl lactate) containing mainly l-lactic acid (~97%, as reported by VWR). Incubation was performed in 500 mL Scotch bottles (300 mL liquid volume) with the headspace connected directly to a gas flow measuring device (AMPTS II, Bioprocess Control). The feeding medium contained lactate (20 g L⁻¹), acetate (2.5 g L⁻¹), yeast extract (1 g L⁻¹), and BisTris buffer (100 mM), and supplemented with mineral medium, trace elements, and vitamins as described elsewhere. The medium had an adjusted pH of 5.0 (adjusted with 2 M KOH) and was bubbled for 5 min with N₂ gas before being added to the bottles. The inoculum was derived from a food waste fermentation reactor and added at 4% v/v. Once the bottles were closed, the headspace was flushed for 12 min with a mixture of 80% N₂ and 20% CO₂ to ensure anaerobic conditions. The liquid was stirred intermittently (40 s on; 20 s off) at 70 rpm and temperature was controlled at 30 °C in the water bath.

2.3. Continuous Chain Elongation with GAC Addition. GAC was selected for testing in continuous chain elongation to study its effects on the type of carboxylates produced and their possible adsorption. The reactor used was a double-walled glass upflow anaerobic reactor consisting of a vertical column (Dₘ = 5.7 cm; length = 90 cm), a settler, an inverted funnel for solid–gas–liquid separation, and a recirculation line (Figure S1) with a liquid working volume of 2.2 L. The fermentation broth was recirculated from the top to the bottom of the reactor where it mixed with the feeding solution before flowing upward into the reactor. A two-necked glass tube was connected in the recirculation line for pH control. Effluent overflowed at the top of the reactor and was collected in a reservoir. Temperature was controlled at 35 °C using a water bath. The reactor was filled with the feeding medium adjusted to pH 5.0 containing lactate (30 g L⁻¹), acetate (1.5 g L⁻¹), yeast extract (2.5 g L⁻¹), mineral medium and trace elements, and vitamins, bubbled with N₂ gas for 15 min and inoculated (4% v/v) with biomass derived from a food waste fermentation reactor. The reactor was operated in batch mode with pH control switched off until gas production and pH increase were observed (11 days). Then, continuous operation was started by feeding medium to an hydraulic retention time (HRT) of 2.2 days with automatic pH control at 5.1–5.2 with 1 M HCl. Chain elongation was first evaluated without GAC addition until stable conditions were observed (period I, days 55–77). Then, GAC was added at 20% v/v (~112 g) on day 77 with stable conditions observed on days 84–88 (period II). Before addition, GAC was sieved to remove fine particles (no. 18; 1 mm) and “pretreated” by submerging it for 4 days in the same feeding medium fed to the reactor containing lactate, acetate, and nutrients to discard possible adsorption of these substrates. To test whether lactate or acetate could still be adsorbed by the “pretreated” GAC, 20% v/v GAC (4.1 g) was mixed with 80 mL of feeding medium and shaken at 30 °C and 120 rpm for 2 days in duplicate serum bottles. The headspace in the bottles was exchanged with N₂ gas through vacuum and filling cycles to an overpressure of 0.2 atm.

2.4. Adsorption and Desorption Experiments with GAC. To evaluate the possibility of recovering fermentation products through adsorption with GAC, adsorption and desorption tests were conducted with four different media: real effluent, artificial effluent, and C2–nC6 and C2–nC8 carboxylates blends (Table 1). The real effluent was derived from...
Table 1. Carboxylate Concentrations Used in the Adsorption and Desorption Experiments

| Carboxylate    | Concentrations [mM (g·L⁻¹)] | real effluent | artificial effluent | C2–nC6 blend | C2–nC8 blend |
|----------------|-----------------------------|---------------|---------------------|--------------|--------------|
| lactate        | 200 (18)                    | 200 (18)      |                     |              |              |
| acetate (C2)   | 42 (2.5)                    | 49 (2.9)      | 57 (3.42)           | 57 (3.42)    |              |
| propionate (C3)| 3 (0.2)                     | 57 (4.22)     | 57 (4.22)           |              |              |
| n-butyrate (nC4)| 22 (1.9)                   | 2 (0.2)       | 57 (5.02)           | 57 (5.02)    |              |
| n-valerate (nC5)| 57 (5.85)                |               | 57 (5.85)           |              |              |
| n-caproate (nC6)| 57 (6.7)                  | 60 (7.0)      | 57 (6.65)           | 57 (6.65)    |              |
| n-heptylate (nC7)| 3.8 (0.5)                |               |                     |              |              |
| n-caprylate (nC8)| 3.5 (0.5)                |               |                     |              |              |

from a lab-scale continuous stirred tank reactor (CSTR) producing n-caproate from lactate and acetate and centrifuged two times at 10 000 rpm for 20 min to remove biomass. The artificial effluent and the two carboxylates blends contained the same mineral medium as in the real effluent except that (NH₄)HPO₄ was replaced at equimolar phosphate concentrations with KH₂PO₄ (4.26 g·L⁻¹) to avoid microbial growth by elimination of a nitrogen source. Trace elements, vitamins, and yeast extract were left out for the same purpose. Lactate was added as a sodium salt (50% sodium-(-)lactate, Merck) as added to the CSTR to mimic the real effluent, while the rest were added as carboxylic acids (≥99% purity, Sigma-Aldrich). Triplicate experiments were conducted in 125 mL serum bottles with 40 mL liquid volume and 40 g·L⁻¹ (1.6 g) of washed and dried GAC. A pH value of 5.0 was set by adding 1 M HCl to simulate the pH in the continuous fermentation effluent. The serum bottles were closed and the headspace was exchanged with N₂ gas through vacuum and filling cycles to an overpressure of 0.2 atm. The bottles were placed in a rotary shaker at 30 °C and 120 rpm. The adsorption period lasted 9 days, after which the bottles were opened to remove the liquid with the help of a syringe, refilled with 40 mL of alkaline sodium borate buffer (0.5 M borate, pH ~ 9.4), headspace-exchanged and returned to the rotary shaker for desorption. Desorption lasted at least 5 days at 30 °C and 120 rpm.

2.5. Adsorption Isotherms. Since n-caproate was the main chain elongation product in the continuous reactor, isotherm experiments with GAC were conducted to determine the n-caproate adsorption behavior at different pH values. The GAC used was the same as in the previous experiments (Norit PR 1–3). Isotherms were determined at three different pH values (4.5, 5.0, and 5.5). Each pH was established using acetate buffer with different proportions of sodium acetate and acetic acid. The total acetate concentration remains similar between buffers (6 g·L⁻¹) with calculated sodium concentrations being 0.92, 1.48, and 1.83 g·L⁻¹ at pH 4.5, 5.0, and 5.5, respectively. An additional isotherm at pH 5.0 was obtained in the artificial effluent containing mineral medium and lactate (18 g·L⁻¹) (50% sodium-(-)lactate, Merck). The (NH₄)₂HPO₄ in the mineral medium was replaced with equimolar phosphate concentrations of KH₂PO₄ (4.26 g·L⁻¹) to avoid microbial growth.

Experiments were carried out in triplicates in 125 mL serum bottles containing 40 mL of liquid, 10 g·L⁻¹ (0.4 g) GAC, and varying initial n-caproate concentrations (≥99% hexanoic acid, Sigma-Aldrich). Before use, GAC was rinsed several times with demi-water (a total of ~83 mL of demi-water g GAC⁻¹) to remove fine particles, and dried at 40 °C for 24 h. Seven initial n-caproate concentrations were set (0.1, 0.2, 0.5, 1, 3, 6, and 8 g·L⁻¹) for experiments at pH 5.0 and 5.5. Due to decreased n-caproate solubility in the acetate buffer at pH 4.5, GAC was...
reduced to 5 g L⁻¹ (0.2 g) and n-caproate concentrations used were 0.05, 0.1, 0.25, 0.5, 1.5, 3, and 4 g L⁻¹ to ensure all of the n-caproate was solubilized. After filling the bottles with GAC and the buffer containing n-caproate, pH was measured and adjusted to the initial value by adding a 1 M HCl or KOH when needed. The serum bottles were closed and the headspace was exchanged with N₂ gas through vacuum and filling cycles to an overpressure of 0.2 atm. The bottles were placed in a rotary shaker at 30 °C and 120 rpm. Equilibrium was measured on day 10, except for n-caproate concentrations of 0.5, 1.5, 3, and 4 g L⁻¹ at pH 4.5 for which samples were taken again on day 59. For the n-caproate concentrations of 3, 6, and 8 g L⁻¹ at pH 5.0 and 6 and 8 g L⁻¹ at pH 5.5, samples were taken again on day 72 to make sure equilibrium took place.

2.6. Calculations. Fermentation and adsorption results were evaluated as described in the Supporting Information. Substrate conversion, productivities, and selectivities of batch and continuous chain elongation fermentation were calculated on mass balances. Adsorption isotherms were evaluated using Gibbs free energy change calculations. See the Supporting Information for details on the calculations.

2.7. Analytical Methods. Liquid samples were centrifuged (15,000 rpm, 10 min) and stored at −20 °C before analyses. Lactate, succinate, and formate were measured by high-performance liquid chromatography (HPLC). Gas chromatography was used to quantify monocarboxylates (straight-chain C2–C8 and branched-chain isobutyrate, isovalerate, isocaprate) and alcohols (C2–C6), as well as gas headspace composition (O₂, N₂, CO₂, CH₄, H₂). Raw experimental data are available in the 4TU.ResearchData repository (https://doi.org/10.4121/17086010).

3. RESULTS

3.1. Conductive Materials Change Product Formation. In the incubations, n-butyrate (nC₄) was the main chain elongation product with no clear effect of the added materials on its production (60–70% electron selectivities) (Figure 1). However, propionate was produced in relatively lower amounts in the presence of conductive materials. Propionate concentrations were consistently lower compared to the control (Figure 1), although they were not significantly different when compared to experiments with PU (nonconductive) (Figure S2A and Table S1). Propionate concentration was the lowest with NF (0.36 ± 0.01 e⁻equiv L⁻¹; 14 ± 1%), followed by SS (0.41 ± 0.07 e⁻equiv L⁻¹; 15 ± 2%) and GAC (0.43 ± 0.03 e⁻equiv L⁻¹; 17 ± 2%), whereas it reached 0.64 ± 0.09 e⁻equiv L⁻¹ (25 ± 3%) in the control and 0.64 ± 0.20 e⁻equiv L⁻¹ (21 ± 6%) in the PU experiments (Figure 1). Assuming that part of propionate was stoichiometrically elongated to n-valerate (1 mol C₃mol nCS⁻¹), the total propionate production corrected for n-valerate formation would be 0.48 ± 0.02 e⁻equiv L⁻¹ with NF, 0.47 ± 0.09 e⁻equiv L⁻¹ with SS, 0.47 ± 0.05 e⁻equiv L⁻¹ with GAC, and 0.68 ± 0.18 e⁻equiv L⁻¹ with PU. This total propionate production was ~30% lower with conductive materials compared to the control (0.68 ± 0.07 e⁻equiv L⁻¹) although not significantly different from the PU experiment (Table S1). Chain elongation with GAC and NF yielded considerably high amounts of isobutyrate, which showed significant differences with the SS and PU materials (Figure S2B and Table S1). Isobutyrate (iC₄) was produced at 0.20 ± 0.03 e⁻equiv L⁻¹ (0.9 ± 0.14 g L⁻¹) and a selectivity of 7 ± 1% with the addition of GAC while NF showed increased formation of both isobutyrate (0.27 ± 0.11 e⁻equiv L⁻¹; 1.2 ± 0.57 g L⁻¹) and n-valerate (0.22 ± 0.04 e⁻equiv L⁻¹; 0.8 ± 0.16 g L⁻¹) with respective selectivities of 9 ± 4 and 8 ± 1% (Figure 1). n-Valerate (nCS) selectivities remained low (3–4%) under other conditions tested. With the addition of SS, n-caproate (nC₆) was observed at high concentrations (2.5 g L⁻¹) in one (SS1) but was <0.3 g L⁻¹ in the other two replicates. Although gas measurements showed high variations between replicates, gas production was observed earlier (Figure S3) in experiments with GAC and NF (on days 2 and 3) accompanied by n-butyrate production (Figure S4), compared to the control and PU experiments (4–6 days). Part of the substrates or produced n-butyrate seemed to be adsorbed on GAC in the initial days since n-butyrate concentrations were lower than the expected from lactate and acetate consumption. Additionally, the hydrogen partial pressure (P_H₂) at the end of the incubation was lower in experiments with GAC ((5 × 10⁻⁴) ± (1 × 10⁻⁴) atm) and NF ((4 × 10⁻³) ± (3 × 10⁻⁴) atm) compared to the control (0.19 ± 0.02 atm), SS (0.12 ± 0.13 atm), and PU (0.14 ± 0.09 atm) experiments (Figure S3). Acetate formation seemed to occur before lactate was depleted in experiments with GAC (0.27 ± 0.05 e⁻equiv L⁻¹; 2.03 ± 0.38 g L⁻¹) and NF (0.18 ± 0.09 e⁻equiv L⁻¹; 1.35 ± 0.67 g L⁻¹), while acetate was formed after lactate depletion in the control experiment (0.14 ± 0.09 e⁻equiv L⁻¹; 1.05 ± 0.67 g L⁻¹). Net acetate production was not clear with SS and PU (<0.06 e⁻equiv L⁻¹) (Figure S4).

The electron balance showed that part of the lactate oligomers present in the concentrated lactic acid solution were used for fermentation (Figure 1A). Lactate monomers and acetate comprised about 68% of the electrons in the added substrate (total lactate and acetate). Substrate conversion was 82 ± 3% in the control experiment increasing to 88–92% with conductive materials and reaching a maximum of 95 ± 4% in the PU experiment. Considering a composition of CH₁₂O₇N₀₂ (34.5 mM carbon) of the substrates (total lactate + acetate) and was, therefore, not considered in the electron balance.

3.2. GAC Increases Conversion Rates in Continuous Chain Elongation. From the conductive materials, GAC was further studied for its effect on continuous chain elongation. GAC was added (20% v/v) to an upflow anaerobic reactor that was converting lactate and acetate into mainly n-caproate (Figure 2). Before GAC addition (period 1), the reactor produced n-caproate at 0.61 ± 0.07 e⁻equiv L⁻¹·day⁻¹ (2.3 ± 0.4 g L⁻¹·day⁻¹) at 2 days HRT with a selectivity of 80 ± 3%. Other carbohydrates were produced at lower selectivities such as propionate (2 ± 1%), n-butyrate (9 ± 4%) and n-caprylate (3 ± 1%). n-Caproate and lactate concentrations were 1.35 ± 0.16 e⁻equiv L⁻¹ (4.9 ± 0.6 g L⁻¹) and 0.42 ± 0.21 e⁻equiv L⁻¹ (3.2 ± 1.5 g L⁻¹), respectively (Table S1). Electron recovery in the fermentation products was 96 ± 15%. When GAC was added, n-caproate concentrations dropped to ~0.8 g L⁻¹ after 2 days of continuous operation (day 79) and slowly
increase afterward and stabilized after 7 days of continuous operation (day 84 onward). Despite the drop in n-caproate from day 77 to 84, lactate and acetate were still consumed resulting in electron recoveries of 34% on day 79 and 60% on day 81. These observations suggest that a major fraction of fermentation products was adsorbed. Adsorption of substrates was most likely low since before being added to the reactor, GAC was soaked in the feed medium containing nutrients and substrates. Furthermore, “pretreated” GAC did not adsorb lactate or acetate in adsorption tests (Figure S5). This indicates that the lower electron recovery observed from day 79 onward in the reactor was related with the adsorption of fermentation products rather than substrates. Once productivities stabilized in period II, lactate was consumed at higher rates, although MCC production rates were comparable to period I (Table S3). Additionally, the electron recovery was 79 ± 11% and n-caprylate was not detected in the effluent suggesting that adsorption of carboxylates still occurred.

Figure 2. Conversion rates in continuous lactate-based chain elongation with GAC addition. Shaded areas show the start-up of the reactor (batch); stable operation before GAC addition (period I) and stable operation with GAC (period II). GAC was added on day 77.

Figure 3. Adsorption and desorption of carboxylates over time from (A) real effluent; (B) artificial effluent; (C) C2–nC6 blend; and (D) C2–nC8 blend.
Isobutyrate formation was, in contrast to the batch screening of conductive materials (Section 3.1), not observed with GAC addition to the continuous reactor.

3.3. MCC Are Preferentially Adsorbed and Recovered with GAC. Tests with real and artificial chain elongation effluents showed low adsorption of SCC while n-caproate was readily adsorbed within 2 days (Figure 3). Microbial activity was observed when using real effluent with net production of propionate and n-butyrate. n-Caprate concentrations also increased in the last days of the adsorption phase. After 1.7 days, 0.86 ± 0.02 mmol·g GAC⁻¹ (~100 mg·g GAC⁻¹) of n-caproate was adsorbed, equivalent to 60 ± 2% of the initial concentrations. These values were 0.9 ± 0.07 mmol·g GAC⁻¹ (~105 mg·g GAC⁻¹; 63 ± 5% adsorbed) on day 3.8 before substantial amounts of n-butyrate and n-caproate were produced (Table S2). Microbial activity was not apparent in the artificial effluent where n-caproate adsorption on day 1.7 was 1.1 ± 0.03 mmol·g GAC⁻¹ (~128 mg·g GAC⁻¹; 73 ± 2% adsorbed) reaching 1.22 ± 0.01 mmol·g GAC⁻¹ (~142 mg·g GAC⁻¹; 81 ± 1% adsorbed) by the end of the adsorption phase (Table S2). From the high lactate concentrations in the artificial effluent, 16 ± 3% was adsorbed (0.78 ± 0.014 mmol·g GAC⁻¹; ~70 mg·g GAC⁻¹). Adsorption of SCC could not be quantified in the real effluent but 8 ± 5% of acetate and 12 ± 5% of n-butyrate was adsorbed from the artificial effluent by the end of the adsorption phase.

From the adsorbed n-caproate, 64 ± 10 and 53 ± 4% was desorbed from real and artificial effluents, respectively, resulting in similar n-caproate recoveries (40–42%) with respect to the n-caproate initially present in the experiments (Table S2). About 2.6-times more n-butyrate was desorbed from the real effluent experiment (0.18 mmol·g GAC⁻¹) compared to artificial effluent (0.07 mmol·g GAC⁻¹) showing that n-butyrate was produced in situ and partly adsorbed onto GAC. Recovery of acetate and n-butyrate from the artificial effluent was ≤15% (Table S2).

Acetate, propionate, and n-butyrate showed low adsorption and recovery from the carboxylates blends, compared to carboxylates with ≥5 carbons (nC5–nC8) (Figure 3 and Table S2). For carboxylates with ≥5 carbons, higher fractions were adsorbed as the carbon chain length increased (Figure 4) with >90% of n-heptylate and n-caprylate adsorbed. n-Caprate adsorption was ~2.2-times higher than n-valerate, ~6-times higher than acetate and n-butyrate, and more than 12-times higher than propionate (Figure 4 and Table S2). Less than 13% of acetate, propionate, and n-butyrate was adsorbed in both C2–nC6 and C2–nC8 blends. Notably, the presence of n-heptylate and n-caprylate in the C2–nC8 blend seemed to decrease the adsorption of other carboxylates, especially n-valerate and n-caproate (about 13 and 8% lower, respectively), compared to the C2–nC6 blend.

The desorbed fraction negatively correlated with the carboxylates chain length (Figure 4). Only a fraction of the adsorbed n-valerate (55–60%), n-caproate (39–55%), and n-heptylate (37%) was desorbed while n-caproate did not seem to desorb into the alkaline solution. Despite adsorption of n-valerate and n-caproate was slightly reduced in the C2–nC8 blend, similar amounts of n-valerate were desorbed from both blends, whereas n-caproate desorption in the C2–nC8 blend was higher (55 ± 7%) than that in the C2–nC6 blend (39 ± 2%). n-Caprate recovery was also higher in the C2–nC8 blend (35 ± 5%) at similar levels to n-heptylate. n-Caprylate was not recovered.

3.4. Increased n-Caprate Adsorption Capacity at Decreasing pH. Adsorption isotherms with GAC and n-caprate in acetate buffer showed that n-caproate adsorption increased as pH decreased (Figure 5). The data from adsorption isotherms could be described by the Freundlich model ($R^2 ≥ 0.997$) with increasing $K_F$ and decreasing $n$ values as pH decreased (Table S4). The maximum adsorption capacity at pH 4.5 (2.1 ± 0.01 mmol·g GAC⁻¹; 243 ± 2 mg·g GAC⁻¹) was followed closely by that at pH 5.0 (2.06 ± 0.01 mmol·g GAC⁻¹; 239 ± 15 mg·g GAC⁻¹). At pH 5.5, n-caproate adsorption capacity decreased considerably to 1.58 ± 0.08 mmol·g GAC⁻¹ (184 ± 7 mg·g GAC⁻¹) and similar values were observed with the artificial effluent (containing mineral...
medium and lactate) at pH 5.0 (1.77 ± 0.11 mmol·g GAC⁻¹; 205 ± 13 mg·g GAC⁻¹). The lower buffer capacity of the artificial effluent compared to the pH 5.0 acetate buffer resulted in a higher acetic acid value (Figure S6), which may explain the lower adsorption observed with the artificial effluent.

4. DISCUSSION

4.1. Chain Elongation Is Favored with Conductive Materials. The presence of conductive materials has been shown to improve anaerobic microbial conversion processes. Carbon nanotubes, for instance, decreased the lag phase in pure cultures and improved methane production in both pure and co-cultures. Stainless steel increased acetic and n-butyrate production in microorganisms converting H₂ and CO₂. Nickel foam was recently shown to improve production by enriching organisms potentially involved in direct interspecies electron transfer (DIET). In the present work, conductive materials such as granular activated carbon (GAC) and nickel foam (NF) decreased the lag phase and changed the product spectrum of batch lactate-based chain elongation. The chain elongation lag phase was increased to <2 days with GAC and NF compared to experiments with stainless steel (SS) or without conductive materials (>4 days). In line with previous studies, n-butyrate was the main product of batch lactate fermentation, whereas mainly MCC are produced in continuous systems. However, total propionate production was consistently lower (>20%) than any of the conductive materials was present resulting in higher selectivities for chain elongation products (n-butyrate, isobutyrate, or n-caproate). Chain elongation to iso/n-butyrate in the presence of conductive materials may present some advantages over single-species fermentation.

Conductive materials such as GAC are known to promote DIET between ethanol-oxidizing bacteria and methanogens. Similar effects could be observed in open-culture chain elongation, where conductive materials may promote lactate oxidation to acetic, electrons, and protons (eq 1), which may be coupled to acetate and electron uptake by specialized chainelongating bacteria to produce iso/n-butyrate (eq 2) or propionate and acetate to n-valerate. An analogous scheme has been proposed for ethanol-based chain elongation where biochar was suggested to promote electron transfer between ethanol oxidizers and chain elongators with improved production of MCC.11 The thermodynamic analysis of this hypothetical syntrophic process under standardized conditions (1 M, 1 atm, pH 7.0) indicates that the reduction potential of electrons released from lactate oxidation (eq 1, E° = −0.44 V) is low enough to drive the elongation of acetate to iso/n-butyrate (eq 2, E° = −0.29 V). Figure S7 shows that when lactate is not depleted, the coupled reaction (eq 3) could occur during the start of the incubations with conductive materials, as the measured bulk substrates and product concentrations.

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lactate^- + H_2O \rightarrow \text{acetate}^- + 4e^- + 4H^+ + CO_2 \tag{1}
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\[
2 \text{acetate}^- + 4e^- + 5H^+ \rightarrow \text{iso/n-butyrate}^- + 2H_2O \tag{2}
\]

\[
lactate^- + \text{acetate}^- + H^+ \rightarrow \text{iso/n-butyrate}^- + CO_2 + H_2O \tag{3}
\]

Although factors like local pH or substrate adsorption on the materials surface may influence the energetics of DIET, these calculations suggest that thermodynamics alone would not give a clear advantage of DIET-driven chain elongation. One alternative potential advantage of DIET could be that electroactive syntrophic bacteria carry out chain elongation at higher rates compared to conventional single-species fermentation. This hypothetical division of labor between two organisms performing the oxidation and reduction reactions separately would benefit from shorter metabolic pathways for each syntrophic partner which, in other bioprocesses such as nitriﬁcation, results in increased overall conversion rates. Additionally, conductive materials may allow faster electron transfer rates through DIET, accelerating syntrophic processes. Thus, selecting syntrophic DIET-driven chain elongation could potentially be an alternative to improve chain elongation. Although the occurrence of DIET was not proven in the present study, future research could explore the development of related new chain elongation processes and gather evidence for DIET through a combination of microbiological and electrochemical methods.
Table 2. Adsorption Capacity and Total Acid Load for Different Systems

| Material | System | pH  | Lactate | Acetate | Propionate | nC4 | nC5 | nC6 | nC7 | nC8 | Acid Load (wt %) | Reference |
|----------|--------|-----|---------|---------|------------|-----|-----|-----|-----|-----|----------------|-----------|
| GAC      | Continuous reactor | 5   | 274     | 27.4    |            |     |     |     |     |     |                             | this study |
|          | Real effluent | 5   | 105     | 10.5    |            |     |     |     |     |     |                             |           |
|          | Artificial effluent | 5   | 142     | 22.4    |            |     |     |     |     |     |                             |           |
|          | Multi-acid | 5   | 116     | 19.3    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 4.5 | 107     | 19.8    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 5   | 117     | 24.3    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 5.5 | 13.0    | 18.4    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 4.5 | 119     | 18.4    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 5   | 159     | 23.9    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 5.5 | 194     | 19.4    |            |     |     |     |     |     |                             | 17        |
|          | Batch fermentation | 6.95 | 142 | 14.2 | 18        |     |     |     |     |     |                             |           |
|          | DSM S2 medium | 6.7 | 141     | 14.1    |            |     |     |     |     |     |                             |           |
|          | Single-acid | 2   | 121     | 12.1    |            |     |     |     |     |     |                             | 15        |
|          | Single-acid | 2   | 48.3    | 12.5    |            |     |     |     |     |     |                             | 16        |
|          | Multi-acid | 2   | 44.8    | 12.5    |            |     |     |     |     |     |                             |           |
|          | Multi-acid | 2   | 42.7    | 7.5     |            |     |     |     |     |     |                             |           |
| Biochar  | DSM S2 medium | 6.7 | 18.7    | 1.9     |            |     |     |     |     |     |                             | 18        |
|          | Artificial effluent | 7 | 62     | 6.2    |            |     |     |     |     |     |                             | 14        |
|          | Single-acid | 2   | 60      | 6.0     |            |     |     |     |     |     |                             | 15        |
|          | Single-acid | 5   | 48      | 4.8     |            |     |     |     |     |     |                             |           |
|          | Single-acid | 2   | 52.5    | 13.3    |            |     |     |     |     |     |                             | 16        |
|          | Multi-acid | 2   | 50.7    | 13.5    |            |     |     |     |     |     |                             |           |

*Anion-exchange resin. Estimated from electron balances. For n-caproate only.

other conditions (0.12–0.2 atm). Acetate formation was also the highest with these two materials, which could be produced from homoacetogenesis and then promote isobutyrate formation with H2 or electrons. Reactor microbiomes producing isobutyrate at high selectivities (~65%) have been developed with methanol as the electron donor26,36 where C. luticellarii was presumed to be responsible for isobutyrate production.16 This was confirmed for a wild-type strain of C. luticellarii shown to produce isobutyrate from methanol and H2/CO2 but not from lactate.34 Further research is needed to identify the key microorganisms and clarify the mechanisms of isobutyrate production from lactate in the presence of conductive materials.

4.3. n-Caproate Adsorption with GAC at High Selectivities and Loadings. Isobutyrate formation was not promoted when GAC was added to continuous chain elongation where n-caproate continued to be produced at high selectivities (~80%). Lactate consumption continued at apparent higher conversion rates (~40% higher) probably due to in situ adsorption of potentially toxic n-caproate12 and/or biomass retention on GAC. During this period II, a ~79% electron recovery was observed compared to ~96% in period I. Assuming that the missing electrons ended up in n-caproate, productivities in period II would be ~0.9 e− equiv nC6-L−1 day−1 (3.3 g nC6-L−1 day−1), ~54% higher than that without in situ adsorption (period I). The estimated n-caproate selectivity would also be increased to 85%. Then, a maximum of 274 mg g GAC−1 (13.9 g L−1) of n-caproate was adsorbed from the time when GAC was added to the reactor. This estimated amount is comparable to the adsorption capacity of GAC determined at pH 5.0 (239 mg g GAC−1), supporting the idea that n-caproate adsorption continued in period II. The GAC loaded with n-caproate could then be regenerated for n-caproate recovery or collected for other applications (Section 4.4). Additionally, n-caproate can also be recovered after fermentation. Within 2 days of adsorption, similar amounts of n-caproate were adsorbed from real (100 mg g GAC−1; 57 mg g GAC−1 day−1) and artificial (130 mg g GAC−1; 74 mg g GAC−1 day−1) effluents. Differences in performance may be related to initial n-caproate concentrations in the artificial (7 g nC6-L−1) and real (6.7 g nC6-L−1) effluents or potential bacterial cells adsorption on GAC, which may reduce the area available for carboxylates adsorption.

GAC showed increasing affinity for longer carboxylates with ≥5 carbon atoms. Particularly, n-caproate adsorption was 2-times higher than that for n-valerate. Shorter carboxylates such as acetate, propionate, and n-butrate were poorly adsorbed (<13%). The MCC n-heptylate and n-caprylate were effectively adsorbed (>90%), although they were present at ~10-times lower concentrations. Better adsorption for longer carboxylates is expected due to increased hydrophobic interactions with GAC (Traube’s rule) as reported in literature.17 The reduced n-caproate desorption and recovery performances at high concentrations of SCC in the C2−nC6 blend may require further research to improve n-caproate recovery when using alkaline solutions under these conditions. More acidic conditions increase the fraction of the hydrophobic undissociated n-caproic acid, which may explain the improved adsorption as pH was decreased for the different isotherms. The pH values (4.5–5.5) tested here are at comparable levels used in chain elongation processes from food waste,3 lactate,6,13,24,37 and ethanol with in-line extraction.38
affinity for lactate than for acetate or n-butyrate. Anion-exchange resins also show lower n-caproate loads (6.2 wt %) from artificial chain elongation effluent compared to the results obtained here (15.4 wt %). However, they are easily regenerated and can be reused several times whereas only a fraction of the adsorbed carboxylates could be desorbed from GAC in the present study (<60% for nC5–nC8). This may decrease the GAC adsorption capacity and reusability over time. Nevertheless, GAC reusability will probably depend on the regeneration method used such as thermal or solvent desorption.

4.4. Adsorptive Chain Elongation and Potential Uses of MCC-Loaded Porous Carbons. Based on the adsorption properties observed for GAC, in-line MCC separation with “adsorptive” chain elongation processes can be designed. The fermentation broth of chain elongation reactors can be recirculated to external packed columns filled with GAC to adsorb MCC while maintaining low concentrations in the effluent. Suspended biomass may be filtered out to avoid adsorption and operational problems related to GAC fouling. Assuming a maximum adsorption capacity of 243 mg nC6·g GAC⁻¹ (Section 3.4), an apparent GAC density of 290 kg·m⁻³ (Norit PK 1–3, Cabot Norit Nederland B.V.), and an n-caproate productivity of 4 g·L⁻¹·day⁻¹ as reported for an efficient lactate-fed chain elongation reactor, a reactor-to-adsorbent volumetric ratio of ~18:1 is estimated to adsorb the n-caproate produced during 1 day of operation. The volume of the adsorbent could be divided in at least two packed columns alternating adsorption and desorption to achieve in-line product separation. Thermal desorption may be ideal to obtain MCC-oils after spontaneous phase separation of the desorbed undissociated medium-chain carboxylic acids (Figure 6). A similar bioprocess with in situ adsorption has been proven effective in butanol fermentation where thermal regeneration of GAC produced concentrated butanol after phase separation. In the aforementioned study, the authors estimated an energy input for butanol desorption of 14.1 kJ·kg⁻¹ of which ~90% was required to evaporate the adsorbed water. Assuming similar values for water adsorption (~1.2 g·g GAC⁻¹) and heat capacity of GAC (0.84 J·g⁻¹·K⁻¹), the minimum energy input estimated to recover n-caproate is 13.8 kJ·g nC6⁻¹ (at boiling point of nC6 [205 °C]), equal to 3.8 kWh·kg nC6⁻¹ (Table S5). This estimated energy input is in the range of other MCC separation processes involving water electrolysis (9.9 kWh·kg MCC⁻¹), electrodeposition (1.1–15 kWh·kg MCC⁻¹), and solvent distillation (3.1 kWh·kg MCC⁻¹). However, further research is needed to evaluate MCC recovery efficiency, actual energy requirements, and overall feasibility of in-line adsorption in chain elongation processes.

GAC was experimentally determined to hold n-caproate mass contents up to 24.3 wt %, which are much higher values than reported for ion exchange resins (6.2 wt %) (Table 2). Such high loadings make GAC an attractive transport matrix when MCC production and upcycling processes are performed at two different geographic locations. Furthermore, direct use of MCC-loaded GAC may be preferred in some cases over conventional desorption and GAC regeneration steps or when GAC regeneration is not efficient anymore. For instance, activated carbon or biochar loaded with MCC may be used as feed additive for prolonged delivery of MCC in the animal gut. Both biochar and MCC have beneficial effects on animal welfare and methane emissions mitigation. MCC may desorb or be taken up by microorganisms. Bacteria have been shown to degrade adsorbed organic molecules and regenerate GAC and a similar mechanism may occur in soils. Biochar amendments show beneficial effects on soil properties and crop production, while MCC promotes plant growth. The addition of biochar with adsorbed plant growth promoters (nitrate) improved plant biomass yield compared to biochar alone. In polluted soils, slowly released MCC may sustain biodegradation of micropollutants by inhibiting methanogenesis and providing electron equivalents for reductive biodegradation.

5. CONCLUSIONS

Conductive materials such as GAC, NF, and SS are compatible with chain elongation microbiomes and influence the lag phase and product spectrum. In batch chain elongation, lactate conversion to propionate was reduced compared to fermentation without materials. Additionally, batch open-culture chain elongation was steered to isobutyrate formation with the addition of GAC and NF, materials that could be used to enrich and further study isobutyrate production with reactor microbiomes. MCC recovery with GAC shows promising features such as high n-caproate affinities (>2-times and ≥6-times higher than nC5 and C2–nC4, respectively) and loadings (184–243 mg nC6·g GAC⁻¹) under pH conditions used in chain elongation bioprocesses (pH 4.5–5.5). Furthermore, adsorption with GAC may be used to obtain final products such as MCC-oils or MCC-loaded materials. The hereby presented isobutyrate formation with conductive materials, the adsorptive chain elongation design, as well as the potential direct applications of MCC-loaded porous carbons may be developed into new chain elongation processes.
Calculations for fermentation, adsorption, desorption, and recovery performances; thermodynamic calculations; schematic of a continuous reactor; batch test time profiles; lactate and acetate adsorption with GAC; isotherms equilibrium pH values; statistical comparison for propionate and isobutyrate between treatments; adsorption and desorption values from effluents and carboxylates blends; continuous reactor average values; isotherm model parameters; and minimum energy input for thermal n-caproate desorption (PDF).

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**Funding**

This work was supported by the joint trust fund CONACYT-SENER Sustentabilidad Energética, Mexico [grant number 297027].

**Notes**

The authors declare no competing financial interest.

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