Supporting Information

Long-term *n*-caproic acid production from yeast-fermentation beer in an anaerobic bioreactor with continuous product extraction

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Summary:

This supporting information consists of seven pages, two figures, and one table, and includes: the cover sheet; the calculations of the total carboxylate production rate (**S1** and equation **1**); the thermodynamic calculations (**S2** and equations **2-8**); the composition of the yeast-fermentation beer substrate (**Table S-1**); the solids concentration in the bioreactor broth and in the effluent (**Figure S-1**); the ammonium concentrations during the operating period (**Figure S-2**); and the references.

S1. Calculations of the volumetric *n*-caproate production rate

The *n*-caproate production rate for the system was calculated from the concentrations in two streams: the effluent and the stripping solution.

$$\gamma_n = \left[\frac{C_{e,n}V_{ASBR}}{HRT} + \frac{(C_{b,n}-C_{b,n-1})V_b}{T_n}\right]\frac{M}{1000V_{ASBR}} \quad \textbf{(1)}$$

where

γ_n=production rate on day *n*, g COD l^{-1} d^{-1}

$C_{e,n}$=concentration of *n*-caproate in the bioreactor effluent on day *n*, mmol l^{-1}

V_{ASBR}=volume of ASBR, L

HRT=hydraulic retention time on day *n*, d

$C_{b,n}$, $C_{b,n-1}$=concentrations of *n*-caproate in the base extraction solution on day *n* and *n-1*, mmol l^{-1}

V_b=volume of the base extraction solution on day *n*, L

T_n=day *n*, d

M=substances chemical oxygen demand, g O_2 mol^{-1}; for example, acetic acid is 64 g O_2 mol^{-1}

S2. Thermodynamic calculations

We estimated the minimum hydrogen partial pressure required in the system to prevent carboxylate oxidation under anaerobic conditions. The oxidation of acetate to hydrogen and carbon dioxide, *n*-butyrate acid to acetate, and *n*-caproate to *n*-butyrate and acetate is expressed by equations (2-4):

$$C_2H_3O_2^- + H^+ + 2H_2O \leftrightarrow 2CO_2 + 4H_2 \quad \textbf{(2)}$$

$$C_4H_7O_2^- + 2H_2O \leftrightarrow 2C_2H_3O_2^- + H^+ + 2H_2 \quad \textbf{(3)}$$

$$C_6H_{11}O_2^- + 2H_2O \leftrightarrow C_4H_7O_2^- + C_2H_3O_2^- + H^+ + 2H_2 \quad \textbf{(4)}$$

The Gibbs free energy change ($\Delta G_r'$) available can be calculated with the standard ΔG_r^0 and the reaction quotient (Q):

$$\Delta G_r' = \Delta G_r^0 + RTln(Q) \quad \textbf{(5)}$$

where R is the universal gas constant (8.314 J K^{-1} mol^{-1}) and T is the absolute temperature in K. For equation (2) and equation (4), the energetics of the chemical reactions showed very little variation over a narrow temperature range near 25°C,[1] and therefore we assumed the ΔG_f^0 at 25°C to be the same compared to 30°C. For equation (3), the standard ΔG_r^0 of the components at 30°C was calculated by linear interpolation of the values from 25°C and 37°C given by Amend and Shock.[1] Moreover, a minimal energy quantity for a microbial reaction is reported to be in the range of -15 to -20 KJ mol^{-1}.[2, 3] Thus, with equation 6-8 and setting $\Delta G_r'$ to -20 KJ mol^{-1},[4, 5] the limits for the partial pressures of hydrogen for anaerobic oxidation of *n*-butyrate and *n*-caproate were calculated using the measured carboxylate concentrations of the bioreactor.

$$pH_2 \approx \sqrt[4]{e^{\frac{(\Delta G_r' - \Delta G_r^0)}{RT}} \frac{[C_2H_3O_2^-][H^+]}{[pCO_2]^2}} \quad \textbf{(6)}$$

$$pH_2 \approx \sqrt{e^{\frac{(\Delta G_r' - \Delta G_r^0)}{RT}} \frac{[C_4H_7O_2^-]}{[C_2H_3O_2^-]^2[H^+]}} \quad \textbf{(7)}$$

$$pH_2 \approx \sqrt{e^{\frac{(\Delta G_r' - \Delta G_r^0)}{RT}} \frac{[C_6H_{11}O_2^-]}{[C_4H_7O_2^-][C_2H_3O_2^-][H^+]}} \quad \textbf{(8)}$$

Table S-1. The composition of two batches of yeast-fermentation beer. The yeast-fermentation beer fed to the reactor was obtained from Western New York Energy in Medina, NY. During the first 443 days, we used batch 1 and for the remainder of the study we used batch 2.

Batch	TS (g l^{-1})	VS (g l^{-1})	TCOD (g l^{-1})	SCOD (g l^{-1})	Ethanol (g l^{-1})	Average ethanol COD (%)
1	126±0.1(n=8)	117±0.2(n=8)	388±13.6(n=8)	328±18.9(n=8)	148±4.1(n=8)	79.6
2	110±1.0(n=6)	102±1.0(n=6)	567±35.8(n=6)	440±16.3(n=6)	160±6.9 (n=6)	59.1

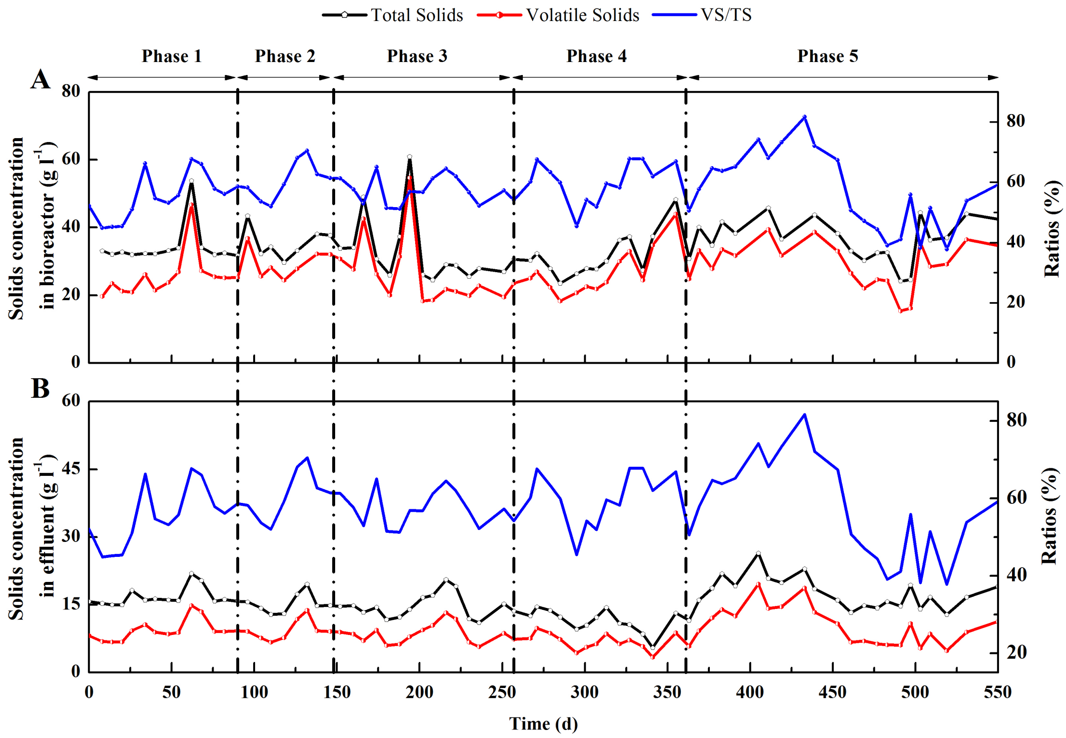

Figure S-1. Solids concentrations in the: (A) bioreactor broth; and (B) effluent during the operating period.

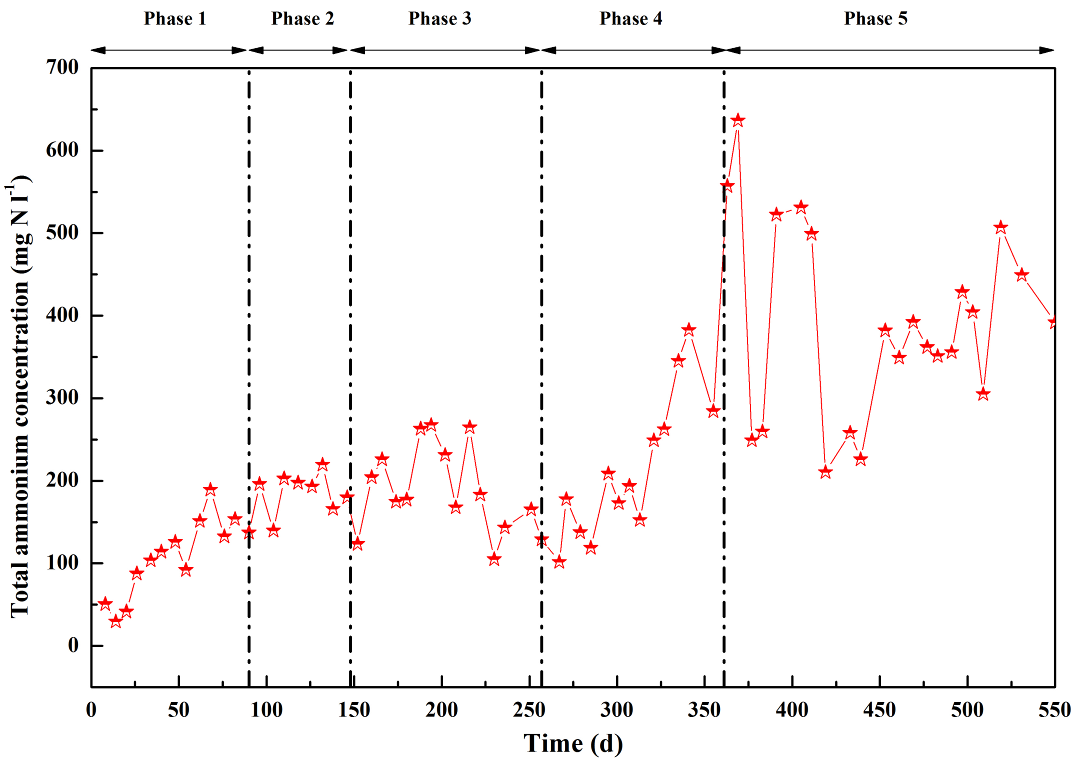

Figure S-2. Total ammonium concentration during the operating period.

References:

1. Amend, J. P.; Shock, E. L., Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. *FEMS Microbiol. Rev.* **2001,** *25*, (2), 175-243.
2. Thauer, R. K.; Jungermann, K.; Decker, K., Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. rev.* **1977,** *41*, (1), 81.
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4. Agler, M. T.; Wrenn, B. A.; Zinder, S. H.; Angenent, L. T., Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* **2011,** *29*, (2), 70-78.
5. Steinbusch, K. J. J.; Hamelers, H. V. M.; Buisman, C. J. N., Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed cultures. *Water Res.* **2008,** *42*, (15), 4059-4066.