Supporting Information

Effect of n-Caproate Concentration on Chain Elongation and Competing Processes

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Profiles of product production

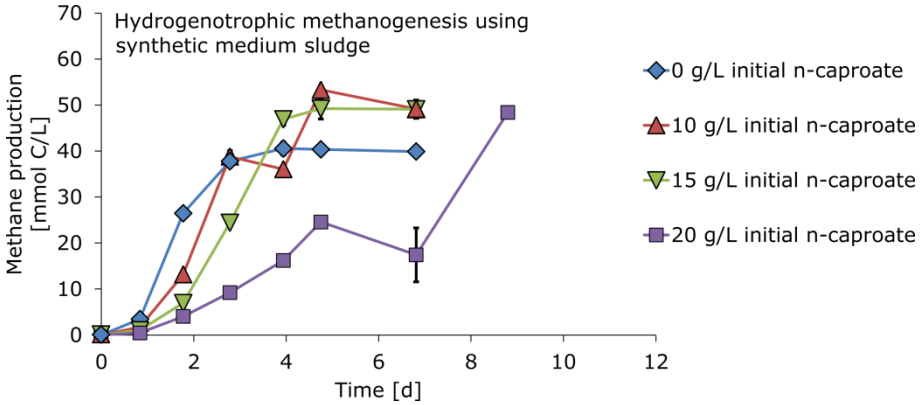

Figure S1. Methane production by hydrogenotrophic methanogenesis at different initial n-caproate concentrations using synthetic medium sludge. Values indicate averages of duplicates and bars indicate range of duplicates (often too small to be visual). T = 30 °C, pH = 6.8 (buffered).

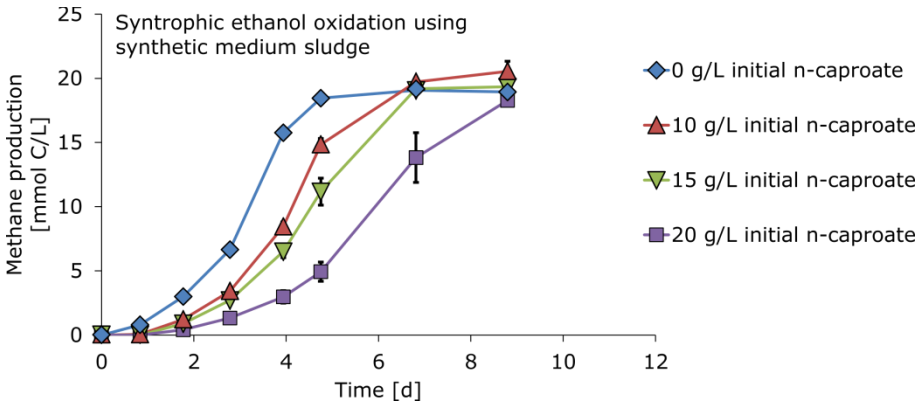

Figure S2. Methane production by syntrophic ethanol oxidation at different initial n-caproate concentrations using synthetic medium sludge. Values indicate averages of duplicates and bars indicate range of duplicates (often too small to be visual). T = 30 °C, pH = 6.8 (buffered).

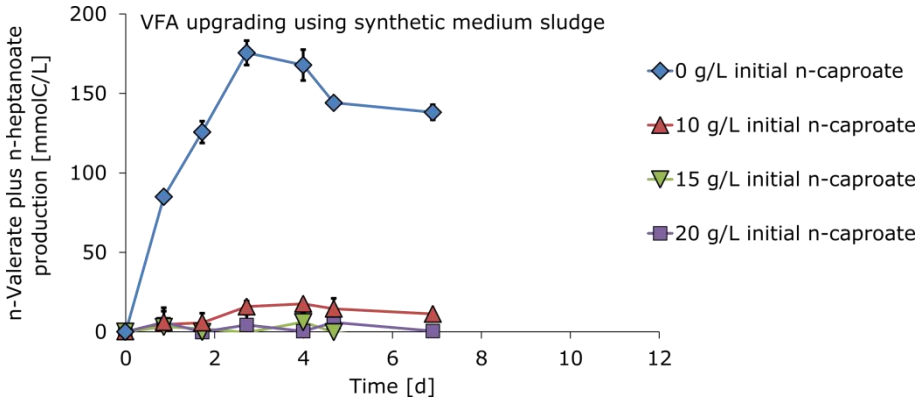

Figure S3. n-Valerate plus n-heptanoate production by VFA upgrading at different initial n-caproate concentrations using synthetic medium sludge. Values indicate averages of duplicates and bars indicate range of duplicates (often too small to be visual). T = 30 °C, pH = 6.8 (buffered).

Profiles of product production

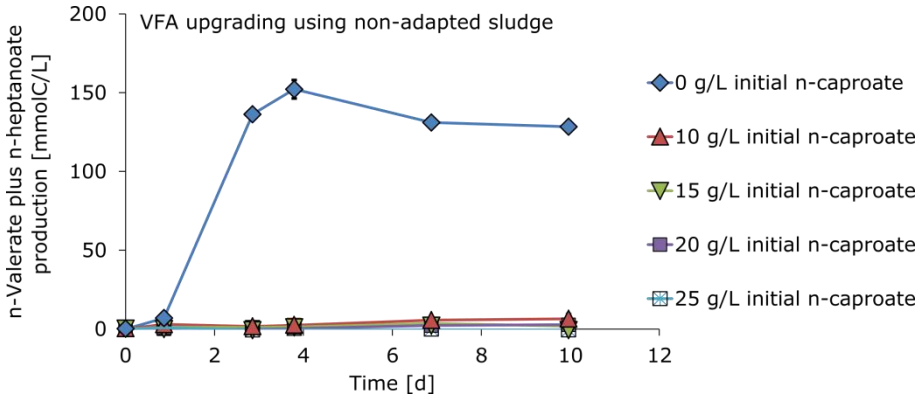

Figure S4. n-Valerate plus n-heptanoate production by VFA upgrading at different initial n-caproate concentrations using non-adapted sludge. Values indicate averages of duplicates and bars indicate range of duplicates (often too small to be visual). T = 30 °C, pH = 6.8 (buffered).

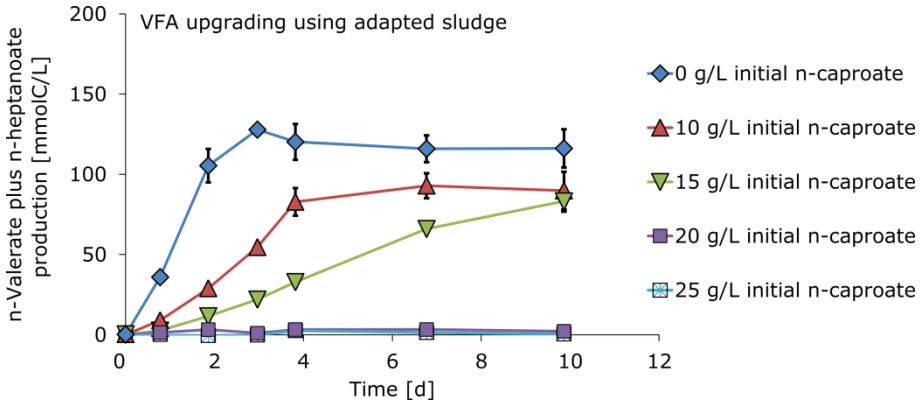

Figure S5. n-Valerate plus n-heptanoate production by VFA upgrading at different initial n-caproate concentrations using adapted sludge. Values indicate averages of duplicates and bars indicate range of duplicates (often too small to be visual). T = 30 °C, pH = 6.8 (buffered).

Specifications 'synthetic medium sludge'

Synthetic medium sludge was directly derived from a running continuous chain elongation process that converted synthetic substrates (propionate and ethanol) into MCFAs. The reactor configuration has been described previously [1]. Specifications on process conditions are reported in Table S1. Concentrations and rates of substrates and products are reported in Table S2.

Table S1. Specifications of process conditions that conditioned synthetic medium sludge

Specification	Unit	Value
Temperature	°C	30
pH	-	6.8
HRT	d	0.7
Substrates in influent	g/L	12.8 [Ethanol] 13.4 [Propionate]
CO_2 loading rate	L/L/d	2.5
Days after startup	d	377
VSS	g/L	0.61

Table S2. Concentrations and rates of substrates and products in the process from which synthetic medium sludge was derived

Compound	Concentration [mmol·L^{-1}]	Rate [mmol·L^{-1}·d^{-1}]
Ethanol	6.8 ± 2.9	-400.7 ± 36.7
Propanol	3.5 ± 2.6	5.1 ± 3.5
Acetate	26.0 ± 4.8	38.3 ± 6.7
Propionate	59.1 ± 6.6	-179.9 ± 20.1
Isobutyrate	0.2 ± 0.2	0.3 ± 0.3
n-Butyrate	15.3 ± 1.4	22.6 ± 1.9
Isovalerate	0.7 ± 0.1	1.0 ± 0.1
n-Valerate	88.6 ± 3.1	131.0 ± 11.3
Isocaproate	0.1 ± 0.1	0.1 ± 0.2
n-Caproate	29.3 ± 2.6	43.3 ± 5.4
n-Heptanoate	16.8 ± 2.6	24.9 ± 4.5
n-Caprylate	0.6 ± 0.1	0.8 ± 0.2
CO_2	23.2 ± 12.4 % [i]	-91.3 ± 4.5
CH_4	69.0 ± 26.1 % [i]	35.7 ± 6.4
H_2	2.2 ± 3.7 % [i]	1.1 ± 1.7

[i] Concentrations of gaseous compounds (CO_2, CH_4, H_2) are shown as percentage in the headspace at 1 atm

Specifications ‘synthetic medium sludge’

Specific activity of syntrophic ethanol oxidation of synthetic medium sludge in reactor

- Specific syntrophic ethanol oxidation activity = $1 \cdot r_{CH4}$ / VSS = $1 \cdot 35.7$ mmol/L/d / 0.61 g/L = 59 mmolC/gVSS/d

Specific VFA upgrading activity of synthetic medium sludge in reactor

- Specific (total) VFA upgrading activity = specific odd-numbered VFA upgrading activity + specific even-numbered VFA upgrading activity = 1362 + 0 = 1362 mmol C/gVSS/d
    - Specific odd-numbered VFA upgrading activity = $(5 \cdot r_{n\text{-Valerate}} + 7 \cdot r_{n\text{-Heptanoate}})$ / VSS = $(5 \cdot 131.0$ mmol/L/d + $7 \cdot 24.9$ mmol/L/d) / 0.61 g/L = 1362 mmol C/gVSS/d
    - Specific even-numbered VFA upgrading activity = 0 mmol C/gVSS/d (no even-numbered VFAs were net consumed)

Specifications 'non-adapted sludge'

Non-adapted sludge was directly derived from a running continuous chain elongation process that converted acidified food waste and ethanol into MCFAs [2]. The reactor configuration has been described previously [1]. Specifications on process conditions are reported in Table S3. Concentrations and rates of substrates and products are reported in Table S4.

Table S3. Specifications of process conditions that conditioned non-adapted sludge

Specification	Unit	Value
Temperature	°C	30
pH	-	6.8
HRT	d	1.0
Substrates in influent	g/L	[Acidified Food Waste] 32.2 [Ethanol]
CO_2 loading rate	L/L/d	1.0
Days after startup	d	103
VSS	g/L	0.35

Table S4. Concentrations and rates of substrates and products in the process from which non-adapted sludge was derived

Compound	Concentration [mmol·L^{-1}]	Rate [mmol·L^{-1}·d^{-1}]
Ethanol	437.3 ± 34.0	-267.6 ± 23.5
Propanol	0.7 ± 0.9	0.7 ± 0.9
Acetate	122.1 ± 8.6	-13.1 ± 8.3
Propionate	15.5 ± 1.0	-9.2 ± 1.5
Isobutyrate	6.2 ± 0.4	-0.9 ± 0.4
n-Butyrate	122.8 ± 11.3	18.3 ± 12.4
Isovalerate	4.0 ± 0.3	-0.5 ± 0.3
n-Valerate	9.4 ± 1.1	4.3 ± 0.9
Isocaproate	0.4 ± 0.1	0.4 ± 0.1
n-Caproate	60.7 ± 7.8	48.1 ± 7.5
n-Heptanoate	0.6 ± 0.1	0.6 ± 0.1
n-Caprylate	0.5 ± 0.1	0.5 ± 0.1
CO_2	5.4 ± 3.0 % [i]	-37.2 ± 0.1
CH_4	77.0 ± 42.0 % [i]	43.8 ± 2.5
H_2	0.0 ± 0.1 % [i]	0.1 ± 0.0

[i] Concentrations of gaseous compounds (CO_2, CH_4, H_2) are shown as percentage in the headspace at 1 atm

Specifications ‘non-adapted sludge’

Specific VFA upgrading activity of non-adapted sludge in reactor

- Specific VFA upgrading activity = specific odd-numbered VFA upgrading activity + specific even-numbered VFA upgrading activity = 71.6 + 148.8 = 220 mmol C/gVSS/d

    - Specific odd-numbered VFA upgrading activity = $(5 \cdot r_{n\text{-}Valerate} + 7 \cdot r_{n\text{-}Heptanoate}) / VSS$ = $(5 \cdot 4.3$ mmol/L/d + $7 \cdot 0.6$ mmol/L/d) / 0.35 g/L = 71.6 mmol C/gVSS/d

    - Specific even-numbered VFA upgrading activity = $(4 \cdot |r_{acetate}|) / VSS = (4 \cdot |-13.1|) / 0.35$ = 148.8 mmol C/gVSS/d (acetate upgrading to n-butyrate)

Specifications 'adapted sludge'

Adapted sludge was directly derived from a running continuous chain elongation process that converted acidified food waste and ethanol into MCFAs [2]. The reactor configuration has been described previously [1]. Specifications on process conditions are reported in Table S5. Concentrations and rates of substrates and products are reported in Table S6.

Table S5. Specifications of process conditions that conditioned adapted sludge

Specification	Unit	Value
Temperature	°C	30
pH	-	6.8
HRT	d	4.0
Substrates in influent	g/L	[Acidified Food Waste] 32.2 [Ethanol]
CO_2 loading rate	L/L/d	1.0
Days after startup	d	124
VSS	g/L	0.51

Table S6. Concentrations and rates of substrates and products in the process from which adapted sludge was derived

Compound	Concentration [mmol·L^{-1}]	Rate [mmol·L^{-1}·d^{-1}]
Ethanol	64.4 ± 35.5	-125.6 ± 26.0
Propanol	5.7 ± 3.7	1.4 ± 1.0
Acetate	31.2 ± 15.2	-26.4 ± 4.7
Propionate	6.4 ± 1.1	-4.6 ± 0.3
Isobutyrate	5.8 ± 0.3	-0.3 ± 0.1
n-Butyrate	90.6 ± 18.4	-3.9 ± 4.5
Isovalerate	4.1 ± 0.3	-0.1 ± 0.1
n-Valerate	13.6 ± 1.7	2.2 ± 0.4
Isocaproate	0.8 ± 0.1	0.1 ± 0.0
n-Caproate	199.9 ± 16.0	46.3 ± 2.8
n-Heptanoate	2.5 ± 0.7	0.5 ± 0.1
n-Caprylate	3.5 ± 1.1	0.8 ± 0.2
CO_2	54.6 ± 25.1 % [i]	-22.5 ± 2.9
CH_4	19.9 ± 21.5 % [i]	6.4 ± 5.8
H_2	11.1 ± 14.0 % [i]	3.7 ± 3.8

[i] Concentrations of gaseous compounds (CO_2, CH_4, H_2) are shown as percentage in the headspace at 1 atm

Specifications ‘adapted sludge’

Specific VFA upgrading activity of adapted sludge in reactor

- Specific VFA upgrading activity = specific odd-numbered VFA upgrading activity + specific even-numbered VFA upgrading activity = 29.0 + 357.5 = 386 mmol C/gVSS/d

    - Specific odd-numbered VFA upgrading activity = $(5 \cdot r_{n\text{-Valerate}} + 7 \cdot r_{n\text{-Heptanoate}}) / VSS$ = $(5 \cdot 2.2$ mmol/L/d + $7 \cdot 0.5$ mmol/L/d) / 0.51 g/L = 29.0 mmol C/gVSS/d

    - Specific even-numbered VFA upgrading activity = $(6 \cdot |r_{acetate}| + 6 \cdot |r_{n\text{-Butyrate}}|) / VSS$ = $(6 \cdot |-26.4$ mmol/L/d $| + 6 \cdot |-3.9$ mmol/L/d $|) / 0.51$ g/L = 357.5 mmol C/gVSS/d (acetate upgrading to n-caproate plus n-butyrate upgrading to n-caproate)

References

1. Roghair, M.; Strik, D. P. B. T. B.; Steinbusch, K. J. J.; Weusthuis, R. A.; Bruins, M. E.; Buisman, C. J. N., Granular sludge formation and characterization in a chain elongation process. *Process Biochem.* **2016,** *51* (10), 1594-1598.
2. Roghair, M.; Liu, Y.; Strik, D. P. B. T. B.; Weusthuis, R. A.; Bruins, M. E.; Buisman, C. J. N., Development of an Effective Chain Elongation Process From Acidified Food Waste and Ethanol Into n-Caproate. *Frontiers in Bioengineering and Biotechnology* **2018,** *6* (50).