SUPPLEMENTARY NOTE 1

VFA PRODUCTION

1. StudyID: VFA_Liu2017

1.1. Reference information

- Case ID: From VFA_Liu_01 to VFA_Liu_09
- Reference: Liu, N., Wang, Q., Jiang, J., & Zhang, H. (2017). *Effects of salt and oil concentrations on volatile fatty acid generation in food waste fermentation*. Renewable Energy, 113, 1523–1528.
- DOI: https://doi.org/10.1016/j.renene.2017.07.042
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

1.2. Purpose of using this study. This study was used to quantify electron allocation to VFA production under salt and oil concentrations reported to inhibit food-waste fermentation. Nine cases were extracted: one control, four salt-amended conditions, and four oil-amended conditions, with maximum total VFA concentrations and corresponding acetate, propionate, and butyrate proportions.

1.3. Experimental system. Batch fermentation was conducted using diluted simulated food waste and anaerobic digester sludge food waste-to-sludge ratio of approximately of 20:1 (VS/VS), pH 6, 35 °C, and 180 rpm for 5 days under anaerobic conditions. Nine cases were extracted: one control, four salt-amended conditions (3–12 g/L), and four oil-amended conditions (5–35 g/L).

1.4. Analysis domain

- **Operational objective:** VFA production
- **System boundary**: The boundary covers the batch reactor, from the organic matter initially loaded with the food waste, together with added oil in the oil-amended cases, to the products present in the reactor at the selected analysis endpoint. No liquid outflow was considered.
- **Analysis window:** From t=0 to the case-specific maximum VFA concentration. This endpoint was selected because Tables 2 and 3 report maximum total VFA concentrations and VFA composition at the corresponding maximum VFA yield.

1.5. Electron map

- **Electron donor:** Organic matter initially loaded with the simulated food waste; added oil was included as an additional donor in oil-amended cases.
- **Electron pool:** Unhydrolyzed organic matter, soluble pre-VFA intermediates, and residual glycerol or long-chain fatty acids.
- **Target sink:** VFA formation.

- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Hydrogen production and biomass assimilation; methane production was also not quantified in this study.

1.6. Source data used

- **Section 2.2**: case definitions and added salt or oil concentrations.
- **Section 2.4 and Table 1**: reactor loading, food-waste dilution, and reported TCOD and VS of the food waste.
- **Table 2**: maximum total VFA concentrations and acetate, propionate, and butyrate proportions for the control and salt-amended cases.
- **Table 3**: corresponding data for the control and oil-amended cases.

1.7. Calculation decisions

- The quantified electron-input basis was defined according to $E_{\mathrm{in}} = E_{\mathrm{window\,input}} + E_{\mathrm{pool,start}}$. For this batch analysis, all materials were loaded at the beginning of the selected window; therefore, $E_{\mathrm{pool,start}} = 0$, and $E_{\mathrm{in}} = E_{\mathrm{window\,input}}$. $E_{\mathrm{window\,input}}$ comprised the organic matter initially loaded with the food waste, together with added oil in the oil-amended cases. The inoculum COD was not included in E_in because the digested sludge consists largely of residual, slowly biodegradable organic matter; including it would increase E_in by approximately 6%. Food-waste COD was calculated from the reported TCOD, adjusted from 19.95% to 15% VS and scaled to 2.96 L of diluted food waste in the 4 L working volume. No biodegradable-COD correction was applied. E_{in} is therefore based on total COD (E_{in} basis: Total COD for VFA_Liu_01–05; Mixed for VFA_Liu_06–09). Initial VFAs in the food waste are contained in the reported TCOD and were not subtracted from the measured VFA concentrations. Added oil was converted using a theoretical factor of 2.9 g COD/g oil.
- The theoretical COD factors, k (mg COD/mg VFA), were 1.066 for acetate, 1.512 for propionate, 1.816 for butyrate and iso-butyrate, and 2.037 for valerate and iso-valerate.
- The unreported VFA fraction was calculated as 100% minus the reported acetate, propionate, and butyrate fractions and converted using the mean COD factor of iso-butyrate, valerate, and iso-valerate, 1.963 mg COD/mg VFA, assuming equal contributions.
- Methane and other gaseous products were not quantified, and methanogenesis was not experimentally suppressed.

1.8. Assumptions and limitations

- **Assumption:** Food-waste COD scaled linearly with VS dilution; inoculum COD was excluded from E_{in}; oil COD used 2.9 g COD/g oil; the unreported VFA fraction was estimated using the mean COD factor of iso-butyrate, valerate, and iso-valerate.

- **Limitations:** Residual COD and gaseous products were not measured; therefore, $E_{\mathrm{pool,end}}$ and the complete competing-sink allocation could not be quantified, and the corresponding unquantified electron equivalents remain unresolved. Because E_competing could not be determined, $f_{\mathrm{expressed}}$ and A_{target} were not calculated (NC), and these cases were excluded from Figure 3.

2. StudyID: VFA_Sakar2020

2.1. Reference information

- Case ID: From VFA_Sakar_01 to VFA_Sakar_08
- Reference: Sarkar, O., Katari, J. K., Chatterjee, S., & Venkata Mohan, S. (2020). *Salinity induced acidogenic fermentation of food waste regulates biohydrogen production and volatile fatty acids profile*. Fuel, 276, 117794.
- DOI: https://doi.org/10.1016/j.fuel.2020.117794
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

2.2. Purpose of using this study. This study was used to quantify electron allocation to VFA production under increasing salinity during food-waste acidogenic fermentation. Eight cases were extracted, including one control and seven NaCl-amended conditions (1–50 g/L).

2.3. Experimental system. Semi-continuous (fed-batch) acidogenic fermentation was conducted with food waste at 15 g COD/L in 0.5-L reactors containing 10% (v/v) mixed inoculum, at initial pH 6, 28 ± 2 °C, and 100 rpm. Each cycle lasted 48 h, and the reactors were operated for 10 cycles.

2.4. Analysis domain

- **Operational objective:** VFA production
- **System boundary**: From food-waste COD supplied at the beginning of the selected cycle to VFA formation, methane formation, and residual or outgoing electron-pool components at the end of the cycle.
- **Analysis window:** 10th cycle, t=0–48h.

2.5. Electron map

- **Electron donor:** Organic matter supplied with food waste at an organic load of 15 g COD/L during the selected cycle. The 10% (v/v) inoculum was not included in E_{in}.
- **Electron pool:** Residual/unconverted food-waste COD and soluble non-VFA metabolites, including ethanol.
- **Target sink:** VFA formation (acetate, propionate, butyrate, and valerate).
- **Competing sink(s):** Hydrogen production and methanogenesis.

- **Potential unquantified pathways:** Biomass assimilation and other unmeasured soluble or gaseous products.

2.6. Source data used

- Section 2.1: food-waste characteristics, including COD and initial VFA concentration.
- Section 2.2: reactor configuration, inoculum fraction, salinity conditions, organic load, operating temperature, and 48-h cycle duration.
- Table 1: VFA concentrations, individual acetate/propionate/butyrate/valerate concentrations, acidification potential, COD removal, and hydrogen conversion efficiency for CTRL (VFA_Sakar_01) and S1–S50 (Case ID: From VFA_Sakar_02 to VFA_Sakar_08).
- Figures 1–2 and Section 3.1: cumulative H_2 production and biohythane/CH_4 information used to derive gaseous competing sinks. Section 3.1.3: initial VFA concentration at the start of the cycle (600–690 mg/L).

2.7. Calculation decisions

- The quantified electron input was based on the food-waste organic load of 15 g COD/L applied to the selected 10th cycle. E_{in} comprised only the food waste supplied in the 10th cycle ($E_{\mathrm{in}} = E_{\mathrm{window\,input}}$). VFAs carried over from the previous cycle (600–690 mg/L at t = 0) were already present as target products and were therefore subtracted from the VFA measured at 48 h, so that E_{target} represents VFA formed within the analysis window. The carried-over VFA was estimated as 905 mg COD/L (113.1 mmol e^-/L), the mean difference between the measured VFA-COD and the reported acidification potential × 15 g COD/L for VFA_Sakar_02–07. VFA_Sakar_01 and VFA_Sakar_08 were not used in this estimate because their reported values were internally inconsistent; the same value was applied to all eight cases because all reactors received the same food waste and inoculum. E_{in} basis: Total COD.
- Methane production for Control, S1 (1 g NaCl/L), and S5 (5 g NaCl/L) was reconstructed from the reported cumulative biohythane production and biohythane composition as $V_{CH4} = V_{\mathrm{biohythane}} \times [1 - H_2/(H_2 + CH_4)]$. This reconstruction was used because the H_2 volumes derived from the same biohythane data (0.39, 0.66, and 0.71 L) agree with the cumulative H_2 production in Table 1, whereas the biogas composition in Fig. 2 would imply substantially lower CH_4.
- The resulting CH_4 volume was normalized to the 0.5-L working volume and converted to electron equivalents using 24.71 mL/mmol at 28 °C and 1 atm and 8 mmol e^-/mmol CH_4. For the other samples, methane formation was reported as absent.
- Hydrogen production was taken from the cumulative H_2 production reported for the 10th cycle, normalized to the 0.5-L working volume, and converted using 24.71 mL/mmol at 28 °C and 1 atm and 2 mmol e^-/mmol H_2.

- $E_{\text{competing}}$ (mmol e^-/L) = $(V_{CH4}/24.71 \times 8) + (V_{H2}/24.71 \times 2)$, where V_{CH4} and V_{H2} are expressed in mL/L.

2.8. Assumptions and limitations

- **Assumption:** Gas volumes were assumed to correspond to 28 °C and 1 atm because no normalization condition was reported.
- **Limitations:** CH_4 for VFA_Sakar_01, VFA_Sakar_02, and VFA_Sakar_03 was reconstructed from rounded biohythane data. The biogas composition and biohythane composition reported in Fig. 2 are not mutually consistent; CH_4 for VFA_Sakar_01–03 is therefore uncertain. Ethanol (0.3–1.5 g/L) was reported as a pool component but not at 48 h for all cases and was left in E_unresolved. VFA_Sakar_01 remained above f_expressed = 1 after correction and was excluded from Figure 3.

3. StudyID: VFA_Sun2024

3.1. Reference information

- Case ID: From VFA_Sun_01 to VFA_Sun_03
- Reference: Sun, J., Zhang, X., Guan, J., & He, Z. (2024). *Volatile fatty acid production through arresting methanogenesis by electro-synthesized hydrogen peroxide in anaerobic digestion and subsequent recovery by electrodialysis*. ACS ES&T Engineering, 4, 2964–2973.
- DOI: https://doi.org/10.1021/acsestengg.4c00384
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

3.2. Purpose of using this study. This study was used to quantify electron allocation to VFA production during primary-sludge fermentation under increasing H_2O_2-mediated methanogenesis inhibition.

3.3. Experimental system. A 200-mL semi-continuous AD reactor was operated at ~37 °C and 150 rpm, with 40 mL digestate replaced by primary sludge every 3 days (SRT = 15 d). H_2O_2 was applied at 0, 40, and 80 mg/L during three successive operating stages. Primary sludge: TCOD 35,100 ± 8,833 mg/L, SCOD 4,780 ± 311 mg/L, VS 30 ± 1.65 g/L.

3.4. Analysis domain

- **Operational objective:** VFA production.
- **System boundary**: From primary-sludge organic matter supplied to the AD reactor during each 3-day feed-withdrawal cycle to its allocation to VFA formation, methanogenesis, and residual or outgoing electron-pool components. H_2O_2 electrosynthesis and downstream electrodialysis recovery are outside the electron-accounting boundary.
- **Analysis window**: A representative 3-day feed–withdrawal cycle was used as the electron-accounting basis for each operating condition. The selected conditions were days 0–30

(VFA_Sun_01; Control, 0 mg/L H_2O_2); days 30–51 (VFA_Sun_02; 40 mg/L H_2O_2); and days 93–150 (stabilized period; VFA_Sun_03; 80 mg/L H_2O_2).

3.5. Electron map

- **Electron donor:** Organic matter supplied with primary sludge.
- **Electron pool:** Residual/unconverted sludge organics and soluble non-VFA products.
- **Target sink:** VFA formation.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation and unmeasured soluble or gaseous products.

3.6. Source data used

- **Section 2.4:** reactor working volume, 40 mL sludge replacement every 3 days, SRT, operating temperature, and H_2O_2 dosing stages.
- **Table S1:** primary-sludge TCOD, SCOD, and VS concentrations used to calculate COD and VS input.
- **Section 3.2 and Figure 3:** CH_4 production for the control and 40 mg/L H_2O_2 stages and complete methanogenesis inhibition during the stabilized 80 mg/L H_2O_2 period.
- **Section 3.3 and Figure 4a:** Section 3.3: average effluent SCOD and COD_VFAs/SCOD ratios for stages I and II (801 and 840 mg/L; 8.66% and 7.18%), and the average VFA concentration for days 93–150 (10,585.5 mg COD/L).

3.7. Calculation decisions

- The electron-accounting basis was one representative 3-day feed-withdrawal cycle. The quantified electron input was the primary-sludge COD introduced during that cycle. With 40 mL of fresh sludge added to a 200-mL reactor, $E_{\text{window input}}$ corresponded to $35{,}100 \times (40/200) = 7{,}020$ mg COD/L-reactor per cycle. Retained reactor material from the preceding cycle was not added separately to E_{in}; its contribution was handled through the cycle-based net-production calculation. E_in basis: Total COD
- Total VFA concentrations were reported directly on a COD basis, so no compound-specific COD conversion was required. Because reactor VFA concentrations were approximately stable within each selected period, net VFA production per cycle was estimated at quasi-steady state as $C_{\text{VFA,end}} - 0.8C_{\text{VFA,previous}} = 0.2C_{\text{VFA}}$ where 0.8 is the fraction of reactor liquid retained after 40 mL of the 200-mL working volume was replaced. C_{VFA} was the period-average VFA concentration: 69.4 and 60.3 mg COD/L for VFA_Sun_01 and VFA_Sun_02, calculated from the reported average SCOD and COD_VFAs/SCOD ratios, and 10,585.5 mg COD/L for VFA_Sun_03.
- CH_4 production (mL/L per cycle) = reported CH_4 yield (mL/g VS) × 30 g VS/L × (40/200), giving 2,133 mL/L/cycle for the control, 1,773.6 mL/L/cycle for 40 mg/L H_2O_2, and

approximately 0 for the stabilized 80 mg/L H_2O_2 period because complete methane inhibition was reported after day 96. The reported CH_4 yield was interpreted as mL CH_4 per g VS fed per cycle (1.2 g VS per 40-mL feed).

- Gas volumes were converted to electron equivalents using the molar gas volume at the reported reactor temperature and 1 atm; for this study, 25.45 mL/mmol at 37 °C was used.

3.8. Assumptions and limitations

- **Assumption:** VFA concentration in the fresh primary-sludge feed was assumed negligible. Reactor VFA concentrations were assumed approximately constant between successive cycles within each selected period (quasi-steady state).
- **Limitations:** Net VFA production per cycle was not reported directly and was estimated from reactor VFA concentrations and the 20% feed-withdrawal fraction. Retained particulate and soluble electron-bearing material could not be fully quantified. Gas-volume reference conditions were not reported. The reported VFA production rate (~3,529 mg COD/L/day) was not used because it corresponds to the reactor concentration rather than to production per cycle.

4. StudyID: VFA_Xu2021

4.1. Reference information

- Case ID: From VFA_Xu_01 to VFA_Xu_05
- Reference: Xu, Y., & He, Z. (2021). *Enhanced volatile fatty acids accumulation in anaerobic digestion through arresting methanogenesis by using hydrogen peroxide*. Water Environment Research, 93, 2051–2059.
- DOI: https://doi.org/10.1002/wer.1575
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

4.2. Purpose of using this study. This study was used to quantify electron allocation to VFA production during glucose fermentation under increasing H_2O_2-mediated inhibition of methanogenesis.

4.3. Experimental system. Batch fermentation was conducted in 160-mL serum bottles with a 50-mL working volume, using glucose at 3 g COD/L as the substrate, at 35 ± 1 °C and 100 rpm under anaerobic conditions. H_2O_2 was added at 0, 0.03, 0.06, 0.10, or 0.13 wt.%.

4.4. Analysis domain

- **Operational objective:** VFA production.
- **System boundary**: From glucose supplied at the beginning of the batch to its allocation to VFA formation, methanogenesis, and residual electron-carrying intermediates at the 5-day endpoint. H_2O_2 is treated as an intervention rather than part of the organic electron input.
- **Analysis window**: Days 0–5 for all cases.

4.5. Electron map

- **Electron donor:** Glucose.
- **Electron pool:** Residual/unconverted glucose and soluble non-VFA fermentation intermediates.
- **Target sink:** VFA formation.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** H_2 production, biomass assimilation, and H_2O_2-mediated oxidation of organic matter or VFAs, and other unmeasured products.

4.6. Source data used

- **Nutrient medium and inoculum section**: glucose substrate concentration of 3 g COD/L and inoculum preparation.
- **Experimental setup section**: 160-mL serum bottles, 50-mL working volume, 35 ± 1 °C, 100 rpm, anaerobic conditions, and H_2O_2 dosages of 0, 0.03, 0.06, 0.10, and 0.13 wt.%.
- **Figure 2a and corresponding text**: total VFA concentrations over the 5-day batch period and day-5 VFA concentrations for each H_2O_2 condition.
- **Figure 2c and corresponding text**: day-5 individual VFA distributions used to estimate acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate concentrations.
- **Figure 3 and corresponding text**: cumulative CH_4 production at day 5 for each H_2O_2 dosage.
- **Perspectives section**: hydrogen gas was not monitored and was therefore treated as an unquantified pathway.

4.7. Calculation decisions

- The quantified electron-input basis was the glucose supplied at the beginning of the batch at 3,000 mg COD/L. Because the analysis window begins with batch setup, no separate $E_{\mathrm{pool,start}}$ was assigned. Organic matter associated with the inoculum was not quantified and was therefore not included in the electron-input basis. Accordingly, $E_{\mathrm{in}} = E_{\mathrm{window\,input}}$ for the present analysis. E_in basis: Defined substrate.
- Day-5 VFA concentrations were used as the quantified target output because the initial VFA concentration at batch setup was not reported. Initial VFA contribution was therefore assumed to be negligible. Individual VFA concentrations were estimated by multiplying the day-5 total VFA concentration by the corresponding fractions in Figure 2c. Text-reported fractions were retained where available, while the remaining fractions were estimated from the figure. Total VFA concentrations at day 5 were 429.3, 1,326.3, 1,233.1, ~1,020, and 502.9 mg/L for the control, 0.03%, 0.06%, 0.10%, and 0.13% H_2O_2 cases, respectively. The 0.03% value was derived from the reported difference relative to the corresponding O_2 case, whereas the 0.10% value was estimated from Figure 2a.

- Cumulative CH_4 production at day 5 was taken from the text where reported and from Figure 3 otherwise: 20.7, 2.9, 2.0, ~1.0, and 0.7 mL for the control, 0.03%, 0.06%, 0.10%, and 0.13% H_2O_2 cases, respectively. These values were normalized to the 50-mL working volume and converted to electron equivalents using 25.29 mL/mmol at 35 °C and 1 atm and 8 mmol e^-/mmol CH_4.

4.8. Assumptions and limitations

- **Assumption:** Initial VFA concentration at batch setup was assumed negligible. Gas volumes were converted assuming 35 °C and 1 atm because gas-reference conditions were not reported. pH was not reported.
- **Limitation**: Organic matter associated with the inoculum was not quantified and was excluded from the defined electron-input basis. H_2 was not monitored. H_2O_2-mediated oxidation of organic matter or VFAs could not be quantified independently. The day-5 VFA concentration for the 0.10% H_2O_2 case, some individual VFA fractions, and the corresponding CH_4 value were estimated from figures.

5. StudyID: VFA_Jiang2025

5.1. Reference information

- Case ID: From VFA_Jiang_01 to VFA_Jiang_04
- Reference: Jiang, W., Jiang, Y., Tao, J., Luo, J., Xie, W., Zhou, X., Yang, L., Kang, J., & Ye, Y. (2025). *Enhanced volatile fatty acids production through anaerobic co-fermentation of dewatered sludge and food waste: Insights into performance, microbial dynamics and metabolic pathways*. Journal of Environmental Chemical Engineering, 13, 119189.
- DOI: https://doi.org/10.1016/j.jece.2025.119189
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

5.2. Purpose of using this study. This study was used to quantify electron allocation to VFA production during semi-continuous co-fermentation of food waste (F) and dewatered sludge (D) at different VS-based mixing ratios.

5.3. Experimental system. Anaerobic fermentation was conducted in a 20-L continuous stirred-tank reactor (CSTR) operated semi-continuously at 37 ± 1 °C and 70 rpm. Each day, 3 L of fermentation broth was withdrawn and replaced with 3 L of fresh substrate, corresponding to an HRT of approximately 6.67 days.

5.4. Analysis domain

- **Operational objective:** VFA production.
- **System boundary**: From the organic matter supplied as food waste and dewatered sludge to the semi-continuous reactor to its allocation to VFA formation and residual electron-carrying

organic matter remaining in the reactor or leaving with the daily withdrawn fermentation broth.

- **Analysis window**: A representative daily feed-withdrawal cycle was used as the electron-accounting basis under each operating condition: FD 100:0 (days 1–33), FD 19:1 (days 34–67), FD 9:1 (days 68–100), and FD 4:1 (days 101–133). FD 100:0 and FD 19:1 did not reach a stable state (acid collapse and VFA fluctuation, respectively).

5.5. Electron map

- **Electron donor:** Organic matter supplied with food waste and dewatered sludge. The inoculum was used only at start-up without re-inoculation and was not included in E_in.
- **Electron pool:** Residual/unconverted food-waste and dewatered-sludge organics and soluble non-VFA intermediates.
- **Target sink:** VFA formation.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** H_2 production, biomass assimilation, and other unmeasured soluble or gaseous products.

5.6. Source data used

- **Section 2.2 and Table 1:** reactor working volume (20 L), daily withdrawal/feed volume (3 L), semi-continuous operation, operating temperature, and stage-specific FW:DS ratios.
- **Table S2:** stage-specific feed TCOD concentrations used to calculate daily COD input.
- **Section 3.1 and Figure 1a–d:** total VFA concentrations and VFA distributions for FD 100:0, FD 19:1, FD 9:1, and FD 4:1. maximum total VFA concentrations of 4,200, 21,551, 25,381, and 37,421 mg COD/L.

5.7. Calculation decisions

- The quantified electron-input basis was the fresh substrate introduced during one daily feed–withdrawal cycle. COD input was calculated from the stage-specific feed TCOD reported in Table S2 and the daily replacement of 3 L in the 20-L reactor: $COD_{\mathrm{input}} = TCOD_{\mathrm{feed}} \times (3/20)$, giving 13,138.5, 11,506.5, 10,374, and 9,273 mg COD/L-reactor/d for FD 100:0, FD 19:1, FD 9:1, and FD 4:1, respectively. Retained electron-bearing material from the preceding cycle was not separately quantified and was therefore not included in the quantified electron-input basis. E_in basis: Total COD.
- Total VFA concentrations were reported directly on a COD basis. The daily VFA output associated with reactor withdrawal was estimated as $0.15 \times C_{\mathrm{VFA}}$, where 0.15 represents the fraction of the 20-L reactor volume withdrawn each day. Because stage-average VFA concentrations were not reported, the reported maximum concentrations were used; the resulting VFA output is therefore an upper estimate. VFA in the fresh feed was assumed

negligible. Under stable operating conditions, this daily output approximates net VFA production.

5.8. Assumptions and limitations

- **Assumption:** VFA in the fresh feed was assumed negligible. The reported reactor VFA concentration was used to estimate daily VFA output through the 3/20 daily withdrawal fraction.
- **Limitation**: Net VFA production was not reported directly. The calculated $0.15 \times C_{\mathrm{VFA}}$ represents daily VFA output and approximates net production only when changes in reactor VFA inventory are small. Retained electron-bearing material was not separately quantified. CH_4 and H_2 were not quantitatively reported; therefore, competing-sink allocation and the corresponding metrics could not be calculated.

6. StudyID: VFA_Li2022

6.1. Reference information

- Case ID: From VFA_Li_01 to VFA_Li_09
- Reference: Li, B.-Y., Xia, Z.-Y., Gou, M., Sun, Z.-Y., Huang, Y.-L., Jiao, S.-B., Dai, W.-Y., & Tang, Y.-Q. (2022). *Production of volatile fatty acid from fruit waste by anaerobic digestion at high organic loading rates: Performance and microbial community characteristics*. Bioresource Technology, 346, 126648
- DOI: https://doi.org/10.1016/j.biortech.2021.126648
- System family: VFA production
- Link to calculation file: Supplementary Data 1.xlsx (VFA_Formation sheet)

6.2. Purpose of using this study. This study was used to evaluate electron allocation to VFA production from fruit waste under different organic loading rates in a semi-continuous acidogenic reactor.

6.3. Experimental system. Anaerobic fermentation was conducted in 2.4-L working-volume CSTRs operated semi-continuously at 35 ± 1 °C, pH 6.0 ± 0.2, and 120 rpm. Synthetic fruit waste (watermelon:apple:banana = 4:3:1, wet-weight basis) was fed once daily by draw-and-fill. The OLR was increased stepwise from 8 to 24 g VTS/(L·d), with each OLR maintained for 1–3 HRTs to achieve steady state; the corresponding HRT decreased from 9.3 to 3.1 days

6.4. Analysis domain

- **Operational objective:** VFA production.
- **System boundary**: From fruit-waste organic matter supplied to the semi-continuous reactor to its allocation to VFA formation, residual electron-carrying organic matter leaving with the withdrawn fermentation liquid, and gaseous electron carriers leaving the reactor.
- **Analysis window**: Steady-state operation at each OLR (8, 10, 12, 14, 16, 18, 20, 22, and 24 g VTS/L/d.

6.5. Electron map

- **Electron donor:** Organic matter in the fruit-waste feed.
- **Electron pool:** Residual/unconverted fruit-waste organics and soluble non-VFA compounds remaining in the fermentation liquid/effluent.
- **Target sink:** VFA formation.
- **Competing sink(s):** Hydrogen production and methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation and other unmeasured soluble or gaseous products.

6.6. Source data used

- **Section 2.1:** fruit-waste characteristics, including VTS and SCOD.
- **Section 2.2:** reactor working volume, temperature, pH, mixing conditions, daily draw-and-fill operation, OLR range, and HRT.
- **Section 2.3 and Table 1:** VFA concentration, VFA composition, VFA yield, VFA productivity, and steady-state values at each OLR.
- **Figure 3B and Section 3.3:** biogas yield, H_2 fraction, and confirmation that no methane was detected.
- **Section 3.1.1:** confirmation that methanogenesis was arrested under the operating conditions.

6.7. Calculation decisions

- The quantified window input consisted of the fresh fruit-waste feed supplied during steady-state operation at each OLR. However, feed loading was reported on a VTS basis (g VTS/L/d), and feed TCOD was not reported. Therefore, E_{in}could not be quantified on an electron-equivalent basis, and f_{target} and $f_{\mathrm{expressed}}$ were recorded as NC. Under the steady-state flux basis, retained reactor inventory was not added separately as $E_{\mathrm{pool,start}}$. E_in basis: Total COD (not quantified).
- VFA production was taken directly from the steady-state VFA productivity reported in Table 1 (5.60–18.10 g COD/L/d). VFA productivity and H_2 production were both expressed per liter of reactor per day, so that E_target and E_competing are on the same temporal basis.
- Methane production was set to zero because no CH_4 was detected throughout the experiment as reported.
- H_2 production was estimated from Figure 3B using the stage-average digitized biogas yield and H_2 fraction. H_2 production (mL/L/d) was calculated as biogas yield (mL/g VTS) × OLR (g VTS/L/d) × H_2 fraction.
- H_2 production was converted to electron equivalents using a molar gas volume of 25.29 mL/mmol at the reported reactor temperature of 35 °C, assuming 1 atm.

6.8. Assumptions and limitations

- **Assumptions:** Stage-average biogas yield and H_2 fractions were digitized from Figure 3B. Gas volumes were converted at 35 °C and 1 atm because gas standardization conditions were not reported.
- **Limitations:** Feed TCOD was not reported; therefore, E_{in}, f_{target}, and $f_{\mathrm{expressed}}$ could not be quantified. H_2 production was estimated from figure digitization rather than tabulated data.

CHAIN ELONGATION

7. StudyID: CE_Roghair2018

7.1. Reference information

- Case ID: From CE_Roghair_01 to CE_Roghair_05
- Reference: Roghair, M., Hoogstad, T., Strik, D. P. B. T. B., Plugge, C. M., Timmers, P. H. A., Weusthuis, R. A., Bruins, M. E., & Buisman, C. J. N. (2018). Controlling ethanol use in chain elongation by CO2 loading rate. *Environmental Science & Technology, 52*, 1496–1505.
- DOI: https://doi.org/10.1021/acs.est.7b04904
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

7.2. Purpose of using this study. This study was used to evaluate electron allocation to medium-chain fatty acid production under different CO_2 loading rates in a continuous chain-elongation reactor supplied with ethanol and propionate. The study is particularly useful because CO_2 loading altered the relative importance of chain elongation, excessive ethanol oxidation, and hydrogenotrophic methanogenesis. The authors distinguished ethanol upgrading to even-numbered fatty acids from propionate upgrading to odd-numbered fatty acids.

7.3. Experimental system. Anaerobic chain elongation was conducted in a 1-L continuously stirred reactor containing granular and suspended chain-elongating sludge. The reactor was operated at 30 °C, 1 atm, pH 6.8, 100 rpm, and an HRT of 17 h. Synthetic medium containing propionate and ethanol was continuously supplied. Propionate loading was maintained at 10.9 g/L/d, while ethanol loading was 32.5 g/L/d at the high-CO_2 condition and 16.3 g/L/d at the subsequent lower-CO_2 conditions. CO_2 loading was varied between 2.5, 1.0, 0.5, 0, and subsequently 1.0 L CO2/L/d.

7.4. Analysis domain

- **Operational objective:** Production of medium-chain fatty acids by chain elongation.
- **System boundary**: From externally supplied ethanol and propionate entering the continuous reactor to their conversion into MCFAs, competing products such as methane, and residual electron-carrying intermediates or H_2 leaving the reactor.
- **Analysis window**: Five operational conditions were extracted.
  CE_Roghair_01: High CO_2, 2.5 L CO_2/L/d; steady-state intervals days 84–97 and 105–119.
  CE_Roghair_02: Medium CO_2, 1.0 L CO_2/L/d; steady-state interval days 155–187.
  CE_Roghair_03: Low CO_2, 0.5 L CO_2/L/d; steady-state interval days 188–201.
  CE_Roghair_04: No CO_2, 0 L CO_2/L/d; average value interval days 211–224;
  CE_Roghair_05: Medium CO_2 recovery, 1.0 L CO_2/L/d; steady-state interval day 229–240.
  The authors defined steady state as a period of at least 5 days during which net fatty-acid and ethanol production/consumption rates had a relative standard deviation ≤20%.

7.5. Electron map

- **Electron donor:** Ethanol.
  (Additional electron-bearing input: Propionate, supplied as the carbon-chain acceptor for VFA upgrading).
- **Electron pool:** Residual ethanol and propionate, butyrate and valerate formed as chain-elongation intermediates, H_2, and other electron-carrying intermediates remaining in or leaving the reactor.
- **Target sink:** MCFA-producing chain elongation, quantified by caproate and heptanoate formation.
- **Competing sink(s):** Methanogenesis. Propionate reduction to propanol was a further competing pathway but was not quantified (see Limitations).
- **Potential unquantified pathways:** Biomass assimilation, and other minor non-target product-forming pathways.

7.6. Source data used

- **Section 2.1:** Reactor configuration, operating conditions, CO_2 loading rates, ethanol loading rates, and constant propionate loading rate.
- **Section 2.2:** Carbon-flux calculation basis using net production and consumption rates under steady-state conditions.
- **Table 1:** Steady-state intervals, caproate and heptanoate production rates, methane production rates, and excessive ethanol oxidation at each CO_2 loading condition.

7.7. Calculation decisions

- The quantified window input comprised the externally supplied electron-bearing organic inputs, ethanol and propionate. Their loading rates were taken directly from the study and converted to COD equivalents using theoretical COD factors of 2.084 g COD/g ethanol and 1.512 g COD/g propionate. Accordingly, $E_{\text{window input}} = E_{\text{ethanol}} + E_{\text{propionate}}$. For the steady-state cases, retained reactor inventory was not added separately as $E_{\text{pool,start}}$ because the analysis was performed on a steady-state flux basis. E_in basis: Defined substrate.
- Caproate and heptanoate production rates were taken directly from the reported net production rates and converted to COD equivalents using theoretical COD factors of 2.204 g COD/g caproate and 2.335 g COD/g heptanoate. Their sum was used to quantify electron allocation to the target sink. Caprylate production was reported as insignificant and was not included in the quantified target.
- CH_4 was taken directly from the reported mmol/L/d values and converted using 8 mmol e^-/mmol CH_4; no gas-volume correction was required.

7.8. Assumptions and limitations

- **Assumptions:** None.

- **Limitations:** The case CE_Roghair_04 (No CO_2 condition) did not reach steady state, so reported values for this condition are period averages. Propanol was produced at 0.5–1.2 g/L/d (Section 3.2), but condition-specific rates were not reported in the main text; A_target is therefore an upper estimate. Butyrate and valerate formed as intermediates were treated as pool components and are contained in E_unresolved.

8. StudyID: CE_Liu2017

8.1. Reference information

- Case ID: From CE_Liu_01 to CE_Liu_02
- Reference: Liu, Y., He, P., Shao, L., Zhang, H., & Lü, F. (2017). Significant enhancement by biochar of caproate production via chain elongation. *Water Research, 119*, 150–159.
- DOI: https://doi.org/10.1016/j.watres.2017.04.050
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

8.2. Purpose of using this study. This was used to evaluate electron allocation to medium-chain carboxylate production in chain-elongation systems with and without biochar. The study compared a control reactor with a biochar-amended reactor and quantified changes in caproate production and electron efficiency.

8.3. Experimental system. Anaerobic chain elongation was conducted in two upflow blanket filter reactors with working volumes of 17.3 L for the control and 18.2 L for the biochar reactor. The biochar reactor received 20 g/L biochar, while the control received none. Both reactors were operated at 35 ± 1 °C. 2-BES (10.5 g/L) was added to inhibit methanogenesis. Ethanol was used as the electron donor and acetate as the chain-elongation substrate.

8.4. Analysis domain

- **Operational objective:** Production of medium-chain fatty acids by chain elongation.
- **System boundary**: From ethanol supplied at the beginning of Phase IV and electron-bearing substrates carried over from Phase III to their conversion into MCFAs, competing products, and residual or gaseous electron-carrying component.
- **Analysis window**: Phase IV (days 78–117), comparing the control reactor and the biochar-amended reactor. At the start of Phase IV, ethanol was readjusted to 20 g/L, no acetate was added, and both reactors were operated under otherwise comparable conditions.

8.5. Electron map

- **Electron donor:** Ethanol.
- **Electron pool:** Residual ethanol and short-chain carboxylates available for further chain elongation, particularly acetate and butyrate.
- **Target sink:** MCFA-producing chain elongation, quantified by caproate and caprylate formation.

- **Competing sink(s):** Methanogenesis, hydrogen production, and butanol production.
- **Potential unquantified pathways:** Biomass assimilation, propanol formation, residual H_2, and other non-target products.

8.6. Source data used

- **Section 2.1:** Reactor configuration, working volumes, temperature, biochar addition, and methanogenesis inhibition by 2-BES**.**
- **Section 2.2 and Table 1:** Definition of Phase IV, ethanol readjustment to 20 g/L, absence of acetate feeding, and batch operation. pH was adjusted only at the start of Phase IV (day 79) and was not controlled thereafter.
- **Figure 1a:** Caproate concentrations at the start and end of Phase IV for the control and biochar reactors.
- **Figure 1b:** Acetate, butyrate, caprylate, and butanol concentrations used to estimate initial acceptor pools and net Phase-IV product changes.
- **Figure 1c:** Cumulative H_2 and CH_4 production for net gas production during Phase IV.

8.7. Calculation decisions

- At the beginning of Phase IV, the residual ethanol concentration was approximately 2 g/L and was readjusted to 20 g/L. The newly supplied ethanol was assigned to $E_{\text{window input}}$, whereas the residual ethanol together with acetate and butyrate carried over from Phase III constituted $E_{\text{pool,start}}$. Accordingly, the total E_{in} was represented by the electron equivalents contained in 20 g/L ethanol plus the quantified initial acetate and butyrate. Ethanol was converted to COD using 2.084 g COD/g ethanol. Initial acetate and butyrate concentrations were digitized immediately before Phase IV and were approximately 3.0 and 16.7 g/L, respectively, for the control reactor and 2.5 and 25.3 g/L for the biochar reactor. Acetate and butyrate were converted using 1.067 and 1.816 g COD/g, respectively. E_in basis: Defined substrate.
- Butanol formation was treated as a competing sink and quantified from the net concentration change during Phase IV because butanol was already present at the beginning of the analysis window. Digitized concentrations approximately 2.7 g/L at the start and 3.1 g/L at the end for the control reactor (net ≈ 0.4 g/L), and approximately 1.0 g/L at the start and 2.0 g/L at the end for the biochar reactor (net ≈ 1.0 g/L).
- CH_4 was set to zero because no additional methane production occurred during Phase IV. Net H_2 production was digitized from the increase in cumulative H_2 during Phase IV (~1.2 L control; ~1.1 L biochar) and converted to mmol/L at 35 °C and 1 atm using the respective reactor working volumes (17.3 and 18.2 L).
- Caproate and caprylate formation were used to quantify the target sink. Net production during Phase IV was calculated as the final minus initial concentration. Initial caproate concentrations were digitized from Figure 1a (~8.0 and ~13.8 g/L for the control and biochar reactors, respectively), whereas initial caprylate concentrations were digitized from Figure 1b (~0.10 and ~0.13 g/L). Final caproate concentrations of 14.4 and 21.1 g/L were reported directly, whereas final caprylate concentrations (~0.12 and ~0.21 g/L) were digitized. Caproate and caprylate were converted to COD equivalents using theoretical factors of 2.204 and 2.441 g COD/g, respectively.

8.8. Assumptions and limitations

- **Assumptions:** Initial Phase-IV acetate, butyrate, caproate, caprylate, and butanol concentrations were estimated by digitizing Figure 1 immediately before the Phase IV boundary.
- **Limitations**: Several Phase-IV values were derived from figure digitization.

9. StudyID: CE_Fu2021

9.1. Reference information

- Case ID: From CE_Fu_01 to CE_Fu_06
- Reference: Fu, X., Jin, X., Ye, R., & Lu, W. (2021). Nano zero-valent iron: A pH buffer, electron donor and activator for chain elongation. *Bioresource Technology, 329*, 124899.
- DOI: https://doi.org/10.1016/j.biortech.2021.124899
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

9.2. Purpose of using this study. This study was used to evaluate electron allocation to caproate production under different ethanol-to-acetate ratios, with and without nano zero-valent iron (NZVI). The selected cases compare matched control and 5 g/L NZVI treatments to assess how NZVI affected electron supply, substrate utilization, and chain elongation.

9.3. Experimental system. Anaerobic chain elongation was conducted in 500-mL batch reactors containing 400 mL culture medium and 100 mL inoculum, operated for 18 days at 35 °C. The medium contained 50 mmol/L acetate and 1 g/L 2-BES to inhibit methanogenesis. Ethanol was supplied at 200, 300, or 400 mmol/L, corresponding to ethanol-to-acetate ratios of 2:1, 3:1, and 4:1, respectively. For the selected comparisons, reactors without NZVI were paired with reactors receiving 5 g/L NZVI.

9.4. Analysis domain

- **Operational objective:** Production of caproate by chain elongation.
- **System boundary**: From externally supplied ethanol and acetate, the NZVI-derived reducing equivalents, and the electron-bearing components carried over with the inoculum, to their conversion into caproate, other carboxylates/alcohols, and residual or gaseous electron-carrying components remaining in the batch reactor at the end of fermentation.
- **Analysis window**: Days 0–18 of the batch fermentation; end-point values are averages of days 14–18 (Fig. 1).

9.5. Electron map

- **Electron donor:** Ethanol and NZVI-derived reducing equivalents.
- **Electron pool:** Residual ethanol, acetate, butyrate, H_2, and other electron-carrying intermediates. Butyrate (~9.4 mmol/L) carried over with the inoculum was present at t = 0.
- **Target sink:** Caproate-producing chain elongation.

- **Competing sink(s):** Hydrogen production and butanol production.
- **Potential unquantified pathways:** Biomass assimilation, and other unmeasured products.

9.6. Source data used

- **Section 2.1:** Initial medium composition, including 50 mmol/L acetate and 1 g/L 2-BES for methanogenesis inhibition.
- **Section 2.2 and Table 1:** Batch reactor conditions, ethanol concentrations, ethanol-to-acetate ratios, NZVI doses, and the selected treatment pairs.
- **Section 2.5:** Electron-equivalent factors used for caproate, butyrate, H_2, acetate, ethanol, and Fe^{2+}. (32 for caproate, 20 for butyrate, 2 for H_2, 8 for acetate, 12 for ethanol, 2 for Fe^{2+})
- **Figure 1 and Section 3.1:** Final caproate concentrations (average of days 14–18); initial butyrate concentration (9.4 mmol/L).
- **Figure 2**: Initial caproate (~2.0 mmol/L) and butyrate (~9 mmol/L) concentrations at day 0.
- **Figure 5 and Section 3.3:** Electron allocation to caproate, butanol, H_2, ethanol, and dissolved iron.

9.7. Calculation decisions

- E_window input comprised the initial ethanol and acetate, converted using 12 and 8 mol e^-/mol as in the source study, and the NZVI-derived electrons. E_pool,start comprised the butyrate carried over with the inoculum (9.4 mmol/L × 20 mol e^-/mol = 188 mmol e^-/L), applied to all six cases because all reactors received the same inoculum. NZVI-derived electrons were taken from the authors' electron balance (Section 3.3, Fig. 5): 75.0 mmol e^-/L for CE_Fu_02, 57.9 mmol e^-/L for CE_Fu_04 (26.5% of the 218.5 mmol e^-/L additional supply relative to B0), and ~50 mmol e^-/L for CE_Fu_06 (digitized). Dissolved Fe in Fig. 4 (<40 mg/L) represents only the iron remaining in solution and was not used.
- Final caproate concentrations were obtained from Figure 1 and Section 3.1 and converted to COD equivalents using 32 mol e^-/mol caproate, equivalent to 256 mg COD/mmol caproate. Caproate concentrations were approximately 16.0, 21.9, 13.6, 27.2, 15.2, and 22.4 mmol/L for A0, A5, B0, B5, C0, and C5. Heptanoate and other MCFAs were not reported. Caproate present at t = 0 (~2.0 mmol/L; Fig. 2) was already a target product and was therefore subtracted from the final caproate rather than assigned to E_pool,start, so that E_target represents net formation within the window.
- Hydrogen and butanol electron contributions were digitized from Figure 5 and converted to molar concentrations using 2 mmol e^-/mmol H_2 and 24 mmol e^-/mmol butanol, respectively. Methane was set to zero because methanogenesis was inhibited by 2-BES.
- E_pool,end (residual ethanol, acetate, and butyrate) and E_pool,out were not quantified and are contained in E_unresolved. E_in basis: Defined substrate.

9.8. Assumptions and limitations

- **Assumptions:** The initial butyrate reported for A10 (9.4 mmol/L) was applied to all cases, consistent with the similar day-0 values in Fig. 2. Initial concentrations were applied as reported per liter of the 400-mL medium; dilution by the 100-mL inoculum was not reported explicitly and was not applied.

- **Limitations**: The NZVI-derived electron input for CE_Fu_06, the initial caproate, and the H2 and butanol contributions were estimated from figures and carry graphical uncertainty. Residual butyrate at the end of fermentation (~35–45 mmol/L) accounts for a large share of E_unresolved.

10. StudyID: CE_Kucek2016

10.1. Reference information

- Case ID: From CE_Kucek_01 to CE_Kucek_02
- Reference: Kucek, L. A., Nguyen, M., & Angenent, L. T. (2016). Conversion of L-lactate into n-caproate by a continuously fed reactor microbiome. Water Research, 93, 163–171.
- DOI: https://doi.org/10.1016/j.watres.2016.02.018
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

10.2. Purpose of using this study. This study was used to evaluate electron allocation between n-caproate production and the competing propionate pathway during continuous lactate-based chain elongation. The selected cases represent a high n-caproate productivity condition and a subsequent lactate-overloading condition in which propionate production increased and n-caproate productivity declined.

10.3. Experimental system. Anaerobic chain elongation was conducted in a continuously fed upflow anaerobic filter with a working volume of 0.55 L, operated at 34 ± 1 °C. L-lactate and n-butyrate were continuously supplied in the feed, with HRTs of 1.9 and 1.3 d in Periods 12 and 13, respectively. The reactor was operated near pH 5.0 during the selected Phase III periods, and an in-line pertraction system was used for continuous recovery of medium-chain carboxylates.

10.4. Analysis domain

- **Operational objective:** Production of caproate by chain elongation.
- **System boundary**: From externally supplied L-lactate and n-butyrate entering the continuously fed reactor to their conversion into n-caproate and other carboxylates, competing propionate formation, and residual or gaseous electron-carrying components leaving via the effluent, gas outlet, or pertraction system.
- **Analysis window**: Two operating periods in Phase III were selected:
  CE_Kucek_01: Period 12, days 176–193 and **CE_Kucek_02:** Period 13, days 193–204

10.5. Electron map

- **Electron donor:** L-lactate.
- **Electron pool:** Residual/unconverted lactate and reduced intermediates within the reactor. n-Butyrate (fed and net-produced) and other short-chain carboxylates leaving with the effluent and pertraction stream.

- **Target sink:** n-Caproate formation.
- **Competing sink(s):** Propionate formation via the acrylate pathway and methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation and other unmeasured products.

10.6. Source data used

- **Section 2.1–2.2:** Reactor configuration, operating temperature, continuous feeding, L-lactate and n-butyrate feed, working volume, and HRT.
- **Section 2.4:** Definition of operating periods, calculation of loading rates and carboxylate productivities, and inclusion of pertraction recovery in total productivity.
- **Table S1:** Average residual concentrations of lactate and carboxylates during Periods 12 and 13.
- **Table S2:** Total OLR, L-lactate loading rates, n-caproate productivity, net n-butyrate productivity, propionate productivity, and HRT for Periods 12 and 13.
- **Figure S1:** Gas productivity and CH_4/H_2 concentrations used to estimate gaseous competing sinks.
- **Section 3.5–3.6:** Interpretation of the shift from n-caproate production to the competing propionate pathway under lactate overloading.

10.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input comprised the L-lactate and n-butyrate loading. Yeast extract (~1.1 g COD/L/d, ~6% of the total OLR) was supplied in excess as a nutrient and was not included in E_in. At steady state, E_pool,start and E_pool,end are equal and cancel (Section 2.3). Pool components leaving the reactor (E_pool,out), including n-butyrate (fed plus net production of 4.8 and 4.7 g COD/L/d) and other short-chain carboxylates (1.4 and 2.3 g COD/L/d), were reported but not assigned to sinks and are contained in E_unresolved. E_in basis: Defined substrate.
- L-lactate loading rates were taken directly from Table S2. n-Butyrate loading was calculated as total OLR minus L-lactate loading and the reported yeast-extract loading (~1.1 g COD/L/d), giving approximately 7.5 and 13.5 g COD/L/d for Periods 12 and 13, respectively. Acetate and propionate were not externally supplied.
- n-Caproate productivities were taken directly from Table S2 as 6.9 and 3.0 g COD/L/d for Periods 12 and 13, respectively.
- Propionate productivities were taken directly from Table S2 and converted from COD to molar units using 112 g COD/mol propionate. CH_4 and H_2 production rates were estimated from total gas productivity and gas composition digitized from Figure S1; both gas sinks were minor relative to propionate.

10.8. Assumptions and limitations

- **Assumptions:** n-Butyrate loading was estimated from total OLR after subtracting L-lactate and yeast-extract loading.
- **Limitations**: CH_4 and H_2 production rates were estimated from Figure S1 and therefore carry digitization uncertainty. For Period 12, the sum of the reported outputs, including n-butyrate leaving the reactor (24.2 g COD/L/d), exceeds the total OLR (17.7 g COD/L/d), indicating

inconsistency among the reported period-average rates (HRT 1.9 ± 0.4 d). Because f_expressed is based on the quantified sinks only, this case remained within the valid range and was retained.

11. StudyID: CE_Xu2018

11.1. Reference information

- Case ID: From CE_Xu_01 to CE_Xu_03
- Reference: 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*, 280–295.
- DOI: https://doi.org/10.1016/j.joule.2017.11.008
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

11.2. Purpose of using this study. This study was used to evaluate electron allocation to the production of medium-chain carboxylic acids during continuous lactate-based chain elongation using a real waste-derived substrate without addition of an external electron donor. The selected cases represent three operating periods of the mesophilic chain-elongation reactor under different influent conditions and HRTs.

11.3. Experimental system. Anaerobic chain elongation was conducted in a 0.55-L continuously fed upflow anaerobic filter operated at 30 ± 1 °C and pH 5.0 ± 0.1. The reactor was fed with diluted, lactic-acid-rich effluent from a thermophilic first-stage reactor treating acid whey. An in-line pertraction system was used to continuously recover MCCAs. No external ethanol or lactic acid was added directly to the chain-elongation reactor.

11.4. Analysis domain

- **Operational objective:** Production of medium-chain carboxylic acids.
- **System boundary**: From the soluble organic matter in the Phase A effluent entering the mesophilic Phase B reactor to MCCA products, competing products, and residual electron-carrying compounds leaving via the effluent, gas outlet, or pertraction system. The thermophilic Phase A reactor is outside the boundary.
- **Analysis window**: Three operating periods in Phase B were selected: **CE_Xu_01:** Period B-I, days 0–57; **CE_Xu_02**: Period B-II, days 58–74; and **CE_Xu_03**: Period B-III, days 74–92.

11.5. Electron map

- **Electron donor:** Soluble organic matter in the Phase A effluent, dominated by lactic acid with residual lactose and galactose.

- **Electron pool:** Residual lactic acid and reduced intermediates within the Phase B reactor. Short-chain carboxylic acids (C2–C5) produced in Phase B (120.0, 100.7, and 57.4 mmol C/L/d)
- **Target sink:** n-Caproate, n-heptanoate, and other MCFA (n-caprylate and n-nonanoate) formation.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation and other unmeasured soluble products.

11.6. Source data used

- **Experimental Procedures / Bioreactor Systems:** Phase B reactor configuration, working volume, temperature, pH control, continuous feeding, and in-line pertraction system.
- **Outline of Experimental Periods:** Definition of Periods B-I, B-II, and B-III, including HRT and influent dilution conditions.
- **Table S1:** COD conversion factors for lactic acid and individual carboxylic acids.
- **Table S3:** Composition of Phase A effluent, including the observation that SCCA excluding lactic acid was below detection.
- **Table S4:** Lactic acid loading rate, SCOD loading rate, CH_4 production rate, SCCA production, and individual MCCA production rates for B-I, B-II, and B-III.

11.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the volumetric SCOD loading rate of Phase B (10.9, 6.91, and 7.77 g COD/L/d; Table S4). Lactic acid accounted for only 54–85% of this SCOD; when lactic acid alone was used, the reported products (MCCA + SCCA, ~83% of SCOD in Period B-I) exceeded the lactic acid input, indicating that residual sugars also contributed to the products. With SCOD as E_in, f_target reproduces the reported MCCA SCOD conversion efficiencies (40.9, 60.1, and 61.3%). At steady state, E_pool,start and E_pool,end cancel (Section 2.3). C2–C5 carboxylic acids leaving the reactor (E_pool,out; 42.0, 56.4, and 28.2% of the SCOD loading) were not assigned to sinks and are contained in E_unresolved.
- E_in basis: Total COD.
- Lactic acid loading rates (183, 176, and 207 mmol C/L/d; Table S4) were converted to COD using 3 C/mol and 0.096 g COD/mmol for comparison only and were not used as E_in. No external carbon-chain acceptor was supplied to Phase B; acetate, propionate, and butyrate were therefore recorded as N/A.
- MCCA production rates were taken from Table S4 and converted from mmol C/L/d to COD equivalents using the compound-specific carbon numbers and COD factors reported in Table S1. Total MCCA was calculated as the sum of caproate, heptanoate, caprylate, and nonanoate; caprylate and nonanoate were grouped as Other MCFA.

11.8. Assumptions and limitations

- **Assumptions:** The SCOD of the Phase A effluent was assumed to be the electron-bearing input to Phase B; no biodegradability correction was applied.
- **Limitations**: Individual SCCA production rates were not reported separately, so propionate could not be quantified as a competing sink.

12. StudyID: CE_Wu2018

12.1. Reference information

- Case ID: CE_Wu_01
- Reference: 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 Research, 145*, 650–659.
- DOI: https://doi.org/10.1016/j.watres.2018.08.046
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

12.2. Purpose of using this study. This study was used to evaluate electron allocation to medium-chain fatty acid production from a real complex wastewater containing co-electron donors and multiple short-chain carboxylates. The selected case represents the conversion of Chinese liquor-making wastewater (CLMW), in which ethanol and lactate were simultaneously consumed and substantial n-caproate, n-heptylate, and n-caprylate were produced.

12.3. Experimental system. Anaerobic chain elongation was conducted in 1-L serum-bottle batch reactors using a microbiome previously enriched in reactor R12. CLMW was sterilized and diluted tenfold before use. The reactors were operated at 35 ± 1 °C, pH 6.5 ± 0.1, and 120 rpm, and were purged with N_2. 2-Bromoethanesulfonic acid (10 g/L) was added to inhibit methanogenesis. Ethanol and lactate were the principal co-electron donors, while the wastewater also contained glucose and several short-chain carboxylates.

12.4. Analysis domain

- **Operational objective:** Production of medium-chain carboxylic acids.
- **System boundary**: From ethanol, lactate, and short-chain fatty acids supplied in the batch substrate or contained in CLMW to their conversion into MCFAs, competing propionate and other carboxylates, and residual or gaseous electron-carrying products remaining in the reactor at the end of the batch.
- **Analysis window**: Full batch period, approximately 36 days of fermentation.

12.5. Electron map

- **Electron donor:** Ethanol, lactate, and glucose contained in the diluted CLMW.

- **Electron pool:** Acetate, propionate, n-butyrate, n-valerate, and n-caproate supplied with the CLMW, and short-chain carboxylates remaining at the end of the batch.
- **Target sink:** n-Caproate, n-heptylate, and n-caprylate formation.
- **Competing sink(s):** Methanogenesis (inhibited by 2-BES), H2 production (not quantified), and propionate formation (net consumption over the batch).
- **Potential unquantified pathways:** Biomass assimilation and conversion of unmeasured organic matter (e.g., polysaccharides) in CLMW.

12.6. Source data used

- **Section 2.2–2.3:** Experimental design, batch operating conditions, use of R12-enriched microbiome, CLMW feeding, pH, temperature, and 2-BES addition.
- **Section 2.5:** Analytical methods for ethanol, lactate, SCFAs, MCFAs, H_2, CH_4, and CO_2.
- **Section 2.6:** Definition of MCFA selectivity based on electron equivalents from consumed substrates.
- **Table S2:** Composition of diluted CLMW, including lactate, ethanol, glucose, acetate, propionate, n-butyrate, n-valerate, and n-caproate.
- **Table S3:** Influent, effluent, consumption, and production values in mg COD/L for substrates, carbon-chain acceptors, and MCFA products.
- **Figure S1 and Figure 2:** Temporal changes in substrate consumption and MCFA formation during the CLMW batch.
- **Supplementary data analysis section:** COD conversion factors and electron-equivalent factors used for ethanol, lactate, glucose, carboxylates, H_2 and CH_4.

12.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input comprised the ethanol, lactate, and glucose in the diluted CLMW and E_pool,start comprised the acetate, propionate, n-butyrate, n-valerate, and n-caproate already present in the CLMW (Table S3). Influent n-caproate was a target product and was subtracted from the final n-caproate. E_pool,end (acetate, propionate, and n-butyrate remaining at the end; 226.7 mmol e^-/L) was quantified but not assigned to sinks and is contained in E_unresolved; the unassigned remainder was ~45 mmol e^-/L. E_in basis: Defined substrate (sum of quantified compounds).
- Substrate input was calculated as the sum of influent lactate, ethanol, and glucose COD. Acetate, propionate, and n-butyrate were entered in their respective carbon-chain acceptor columns, while influent n-valerate and n-caproate were grouped as Other carbon-chain acceptors.
- Net MCFA production was used. n-Caproate production was calculated as effluent minus influent COD because n-caproate was already present in CLMW, whereas n-heptylate and n-caprylate, reported as Other MCFA were absent from the influent and their effluent concentrations were therefore treated as net production. Total MCFA was calculated as the sum of n-caproate, n-heptylate, and n-caprylate production.
- Methanogenesis was inhibited using 10 g/L 2-BES; CH_4 was therefore treated as negligible and assigned a value of zero for electron-allocation calculations. Propionate showed net consumption and was also assigned zero as a competing sink.

12.8. Assumptions and limitations

- **Assumptions:** Methanogenesis was treated as negligible because 10 g/L 2-BES was added to inhibit methanogens, and propionate was treated as having no competing-sink formation because it showed net consumption over the batch.
- **Limitations**: H_2 could not be quantified in mmol/L because only headspace gas composition was reported without total gas production. TCOD of the CLMW was not reported, and the authors attributed early SCFA formation to acidification of polysaccharides in CLMW. Because these unmeasured organics are not included in E_in, f_target and f_expressed may be overestimated. A_target (1.00) is an upper estimate because H2 could not be quantified.

13. StudyID: CE _Grootscholten2013A

13.1. Reference information

- Case ID: From CE_GrootscholtenA_01 to CE_GrootscholtenA_03
- Reference: Grootscholten, T. I. M., Steinbusch, K. J. J., Hamelers, H. V. M., & Buisman, C. J. N. (2013). Chain elongation of acetate and ethanol in an upflow anaerobic filter for high rate MCFA production. *Bioresource Technology, 135*, 440–445.
- DOI: https://doi.org/10.1016/j.biortech.2012.10.165
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

13.2. Purpose of using this study. This study was used to evaluate electron allocation to MCFA production under increasing ethanol loading during continuous acetate-ethanol chain elongation. The selected cases represent three stable operating phases with a constant acetate loading rate and progressively increasing ethanol loading rates.

13.3. Experimental system. Anaerobic chain elongation was conducted in a 1-L upflow anaerobic filter packed with polyurethane cubes for biomass retention. The reactor was continuously operated at 30.0 ± 0.1 °C with a hydraulic retention time of 17 h and was fed a synthetic medium containing acetate and ethanol, with 1 g/L yeast extract from day 12; pH was maintained between 6.5 and 7.0 by NaOH addition to the medium. The acetate loading rate was maintained at 8.5 g/L/d, while the ethanol loading rate was increased from 13.0 to 26.0 g/L/d across the three stable phases.

13.4. Analysis domain

- **Operational objective:** Production of medium-chain carboxylic acids.
- **System boundary**: From externally supplied acetate and ethanol entering the continuous upflow anaerobic filter to their conversion into caproate/caprylate, butyrate and other residual products, with remaining electron-carrying compounds leaving via the effluent or gas outlet.
- **Analysis window**: Three stable operating phases were selected:
  CE_GrootscholtenA_01: Phase I, days 56–63; ethanol load = 13.0 g/L/d
  CE_GrootscholtenA_02: Phase II, days 64–70; ethanol load = 19.5 g/L/d

CE_GrootscholtenA_03: Phase III, days 71–80; ethanol load = 26.0 g/L/d

13.5. Electron map

- **Electron donor:** Ethanol.
- **Electron pool:** Acetate (supplied as carbon-chain acceptor), residual ethanol, and butyrate formed as a chain-elongation intermediate, leaving with the effluent.
- **Target sink:** Caproate and caprylate formation.
- **Competing sink(s):** Methanogenesis (<0.1% CH_4 in the headspace) and H_2 production (not quantified).
- **Potential unquantified pathways:** Biomass assimilation and conversion of unmeasured organic matter (e.g., polysaccharides) in CLMW.

13.6. Source data used

- **Section 2.1:** Reactor configuration, inoculum source, and operating temperature.
- **Section 2.2:** Synthetic medium composition, acetate and ethanol feeding, and pH control during reactor operation**.**
- **Section 2.3 and Table 1:** Definition of the three stable operating phases, analysis periods, HRT, acetate loading rate, and ethanol loading rates.
- **Section 2.5.1:** Electron-equivalent factors and definition of selectivity based on consumed acetate and ethanol.
- **Section 2.5.2:** Volumetric production-rate calculation used to derive caprylate production from steady-state concentration and the 17-h HRT.
- **Table 2:** Steady-state caproate and caprylate concentrations, caproate production rates, and MCFA selectivities for Phases I–III.
- **Figures 2–4 and Section 3.3:** Steady-state ethanol, acetate, butyrate, caproate, and caprylate behavior and electron distribution across the three phases.
- **Section 3.1:** Methane remained below 0.1% of the reactor headspace, supporting treatment of methanogenesis as negligible.

13.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input comprised the ethanol and acetate loading. Yeast extract (1 g/L in the medium from day 12, ~1.4 g/L/d) was supplied as a nutrient and was not included in E_in, consistent with CE_Kucek2016. At steady state, E_pool,start and E_pool,end cancel (Section 2.3). Pool components leaving with the effluent (E_pool,out), including butyrate (2.1, 1.3, and 1.1 g/L; ~5.4, 3.3, and 2.8 g COD/L/d) and residual ethanol, were not assigned to sinks and are contained in E_unresolved.
- E_in basis: Defined substrate.
- Ethanol was treated as the electron-donor substrate and acetate as the externally supplied carbon-chain acceptor. Reported mass loading rates were converted to COD equivalents using 2.084 g COD/g ethanol and 1.066 g COD/g acetate.
- Caproate production rates were taken directly from Table 2. Caprylate production rates were calculated from the reported steady-state concentrations using the 17-h HRT according to Eq. (2) in Section 2.5.2 (Volumetric production rate). Product mass rates were converted to COD

equivalents using 2.204 g COD/g caproate and 2.441 g COD/g caprylate. Heptanoate was not reported. Total MCFA was calculated as caproate plus caprylate.

- Methanogenesis was treated as negligible and assigned zero because methane remained below 0.1% of the reactor headspace throughout operation.

13.8. Assumptions and limitations

- **Assumptions:** Methanogenesis was treated as negligible because methane remained below 0.1% of the reactor headspace throughout operation.
- **Limitations**: H_2 production was not quantified, and no quantitative data were reported for butanol or propionate formation. Caprylate production rates were derived from steady-state concentrations and the 17-h HRT. A_target (1.00) is an upper estimate because H_2 was not quantified.

14. StudyID: CE _Grootscholten2013B

14.1. Reference information

- Case ID: From CE_GrootscholtenB_01 to CE_GrootscholtenB_04
- Reference: Grootscholten, T. I. M., Steinbusch, K. J. J., Hamelers, H. V. M., & Buisman, C. J. N. (2013). Improving medium chain fatty acid productivity using chain elongation by reducing the hydraulic retention time in an upflow anaerobic filter. *Bioresource Technology, 136*, 735–738.
- DOI: https://doi.org/10.1016/j.biortech.2013.02.114
- System family: Chain elongation
- Link to calculation file: Supplementary Data 1.xlsx (Chain_Elongation sheet)

14.2. Purpose of using this study. This study was used to evaluate electron allocation to MCFA production under progressively shorter hydraulic retention times and increasing substrate loading rates. The selected cases represent four stable operating phases in which acetate and ethanol loading were increased while HRT was reduced from 16 to 4 h.

14.3. Experimental system. Chain elongation was conducted in a 1-L continuous upflow anaerobic filter using the same synthetic acetate-ethanol medium and inoculum as the previous Grootscholten study. The reactor was operated at 30 °C and pH 6.5–7.2. HRT was progressively reduced from 16 to 8 and then 4 h. Carbon dioxide supply and yeast extract concentration were also modified during the experiment to improve MCFA production.

14.4. Analysis domain

- **Operational objective:** Production of medium-chain carboxylic acids.
- **System boundary**: From externally supplied acetate, ethanol, and yeast extract entering the continuous upflow anaerobic filter to their conversion into caproate/caprylate, butyrate and other residual products, with remaining electron-carrying compounds leaving via the effluent or gas outlet.
- **Analysis window**: Stable operating phases I–IV:

CE_GrootscholtenB_01: Phase I, days 32–36
CE_GrootscholtenB_02: Phase II, days 37–44
CE_GrootscholtenB_03: Phase III, days 47–50 and 53–55
CE_GrootscholtenB_04: Phase IV, days 66–69

14.5. Electron map

- **Electron donor:** Ethanol and yeast extract.
- **Electron pool:** Acetate (supplied as carbon-chain acceptor), residual ethanol, and butyrate formed as a chain-elongation intermediate, leaving with the effluent.
- **Target sink:** n-Caproate and n-caprylate formation.
- **Competing sink(s):** Methanogenesis (<0.1% CH4 in the headspace) and H2 production (not quantified).
- **Potential unquantified pathways:** Biomass assimilation, unconverted yeast extract, and other residual/unmeasured products.

14.6. Source data used

- **Table 1:** HRT, acetate loading rate, ethanol loading rate, and yeast extract concentration for Phases I–IV.
- **Section 2.3.2:** Electron-equivalent factors for ethanol, acetate, yeast extract, caproate, and caprylate; complete yeast extract conversion assumption.
- **Table 2:** Steady-state caproate and caprylate concentrations for Phases I–IV.
- **Section 2.3.1, Eq. (1):** Volumetric production rate calculated as product concentration divided by HRT.
- **Section 3.2:** Methane remained below 0.1% of the reactor headspace; used to treat methanogenesis as negligible.

14.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input comprised the ethanol, acetate, and yeast extract loading. Yeast extract ($CH_{1.7}O_{0.5}N_{0.24}$, 4.7 mol e^-/mol, 25.1 g/mol; Section 2.3.2) was included because the authors assumed its complete conversion. At steady state, E_pool,start and E_pool,end cancel (Section 2.3). Pool components leaving with the effluent (E_pool,out), including butyrate (1.3–1.7 g/L) and residual ethanol (2.2–5.1 g/L), were not assigned to sinks and are contained in E_unresolved.
- E_in basis: Defined substrate.
- Substrate input was calculated as the sum of ethanol and yeast extract loading in COD equivalents, consistent with the authors' selectivity calculation in Section 2.3.2. Yeast extract loading was derived from its influent concentration and the phase-specific HRT, assuming complete conversion as stated by the authors. Acetate was treated as the externally supplied carbon-chain acceptor.
- Caproate and caprylate production, reported as other MCFA, rates were calculated from the steady-state concentrations reported in Table 2 using the phase-specific HRT according to Eq. (1) in Section 2.3.1 (Volumetric production rate). Mass production rates were converted

to COD equivalents using 2.204 g COD/g caproate and 2.441 g COD/g caprylate. Heptanoate was not reported. Total MCFA was calculated as the sum of caproate and caprylate.

14.8. Assumptions and limitations

- **Assumptions:** Yeast extract was assumed to be completely converted, consistent with the authors' selectivity calculation. Methanogenesis was treated as negligible because methane remained below 0.1% of the reactor headspace.
- **Limitations**: A_target (1.00) is an upper estimate because H2 was not quantified. Yeast extract conversion was not measured; its complete conversion was assumed following the authors.

BIOGAS UPGRADING

15. StudyID: BU_Luo2013

15.1. Reference information

- Case ID: From BU_Luo_01 to BU_Luo_03
- Reference: Luo, G., & Angelidaki, I. (2013). Hollow fiber membrane based H_2 diffusion for efficient in situ biogas upgrading in an anaerobic reactor. *Applied Microbiology and Biotechnology, 97*, 3739–3744.
- DOI: https://doi.org/10.1007/s00253-013-4811-3
- System family: Biogas upgrading
- Link to calculation file: Supplementary Data 1.xlsx (Biogas_Upgrading sheet)

15.2. Purpose of using this study. This study was used to evaluate electron allocation during in situ biological biogas upgrading under increasing external H_2 supply. The selected cases represent three steady-state operating phases in which H_2 flow was increased from 930 to 1,440 and 1,760 mL/L/d.

15.3. Experimental system. Biogas upgrading was conducted in a 1-L continuously stirred anaerobic reactor with a 600-mL working volume under thermophilic conditions (55 °C) and an HRT of 15 d. The reactor was fed once daily with a cattle manure–whey mixture and mixed at 150 rpm. External H_2 was supplied to reactor A through a hollow fiber membrane module for bubbleless gas transfer. A parallel reactor B operated without H_2 addition and was used as the no-H_2 control.

15.4. Analysis domain

- **Operational objective:** Enrichment of CH_4 using externally supplied H_2.
- **System boundary**: From externally supplied H_2 entering reactor A to its allocation to CH_4 formation, acetate formation, or unreacted H_2 leaving the reactor boundary; organic-feed-derived electron flows were excluded as background.
- **Analysis window**: Steady-state operation within Phases I–III:
  BU_Luo_01: Phase I, days 1–45
  BU_Luo_02: Phase II, days 46–91
  BU_Luo_03: Phase III, days 92–134
  Steady state was defined by the authors as operation after two to three HRTs, when VFA and biogas production varied by ≤10% per day; the reported values are steady-state means within each phase.

15.5. Electron map

- **Electron donor:** Externally supplied H_2.
- **Electron pool:** Dissolved H_2 and intermediate electron carriers within the reactor. Propionate accumulated in reactor A relative to reactor B (+0.4, +1.3, and +1.4 mM), reflecting inhibited

propionate oxidation at elevated H_2 partial pressure rather than H_2 consumption; it was not assigned as a sink.

- **Target sink:** Hydrogenotrophic methanogenesis.
- **Competing sink(s):** Homoacetogenesis (Acetate production).
- **Potential unquantified pathways:** Biomass assimilation, dissolved or residual H_2 below detection, other unmeasured H_2-consuming pathways, and uncertainty associated with separating H_2-derived products from the background anaerobic digestion process.

15.6. Source data used

- **Table 1:** H_2 flow rate, biogas production rate, CH_4 and CO_2 fractions, pH, acetate, propionate, effluent VS, and total inorganic carbon for Phases I–III in reactors A and B.
- **Materials and Methods – Experimental setup:** Reactor configuration, 600-mL working volume, 55 °C, 15-d HRT, daily feeding, 150 rpm mixing, cattle manure–whey feed, and H_2 delivery through the hollow fiber membrane.
- **Table 1 footnote:** Authors' calculation of substrate-derived CH_4 assuming consumed H_2 was fully converted to CH_4; used only as supporting methodological information, not as the primary basis for E_{target}.
- **Results:** No detectable H_2 was observed in the produced biogas of reactor A throughout operation; used to treat gas-phase unreacted H_2 as not quantified above the detection limit.
- **Results/Discussion:** Phase III showed elevated pH, higher acetate accumulation, and reduced substrate-derived CH_4 production in reactor A; used to flag uncertainty in the control-based CH_4 and acetate background corrections.

15.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the externally supplied H_2 (Table 1). Organic-feed-derived electron flows were treated as background and excluded by subtracting reactor B from reactor A. At steady state, E_pool,start and E_pool,end cancel (Section 2.3); H_2 was not detected in the biogas of reactor A, so E_pool,out was negligible.
- E_in basis: Defined substrate (H_2).
- Gas volumes were converted to molar quantities using the reported gas reference conditions where available. When the reference condition was not reported, standard conditions of 0 °C and 1 atm were assumed, corresponding to a molar gas volume of 22.414 L/mol. Because H_2 and CH_4 volumes were measured on the same basis, f_target and A_target are independent of the assumed molar gas volume; only the acetate term is affected.
- Net acetate formation was calculated as the difference in steady-state acetate concentration between reactors A and B divided by the HRT (15 d), i.e., the acetate leaving the reactor per day: (3.5 − 1.2)/15, (10.2 − 1.4)/15, and (36 − 1.5)/15 mmol/L/d.
- CH_4 input/background and CH_4 output were calculated from the reported biogas production rate and CH_4 volumetric fraction. CH_4 production in the no-H_2 control reactor (Reactor B) at the corresponding steady-state phase was used as the background contribution. Net CH_4 formed was calculated as CH_4 output in the H_2-supplied reactor (Reactor A) minus the corresponding CH_4 background from Reactor B. This calculation assumes comparable background CH_4 production between the paired reactors in the absence of H_2 addition. This

assumption is less certain in Phase III, where elevated pH affected the digestion performance of Reactor A.

15.8. Assumptions and limitations

- **Assumptions:** No-H_2 reactor B represents background CH_4 and acetate production; unreported gas volumes were assumed at 0 °C and 1 atm; excess acetate was used as an estimate of homoacetogenesis.
- **Limitations**: Phase III background correction is less reliable because elevated pH affected digestion performance. In Phase III, the substrate-derived CH4 yield of reactor A was lower than that of reactor B (251 vs. 286 mL/g VS added), so the A − B difference may underestimate H2-derived CH4; f_target for BU_Luo_03 is therefore conservative.

16. StudyID: BU_Bassani2016

16.1. Reference information

- Case ID: From BU_Bassani_01 to BU_Bassani_07
- Reference: Bassani, I., Kougias, P. G., & Angelidaki, I. (2016). In-situ biogas upgrading in thermophilic granular UASB reactor: key factors affecting the hydrogen mass transfer rate. *Bioresource Technology, 221*, 485–491.
- DOI: https://doi.org/10.1016/j.biortech.2016.09.083
- System family: Biogas upgrading
- Link to calculation file: Supplementary Data 1.xlsx (Biogas_Upgrading sheet)

16.2. Purpose of using this study. This study was used to evaluate electron allocation during in situ biological biogas upgrading under different H_2-transfer conditions. The selected cases represent seven steady-state upgrading periods in which H_2 distribution device, liquid and gas recirculation, and H_2-injection chamber configuration were modified to improve H_2 utilization and CO_2 conversion to CH_4.

16.3. Experimental system. Biogas upgrading was conducted in a thermophilic granular UASB reactor with a 1.4-L working volume connected to a separate H_2-injection chamber. The chamber initially had a 0.2-L working volume and was increased to 0.4 L in Periods VII–VIII. The reactor was operated at 55 ± 1 °C and an HRT of 5 d and was fed with diluted potato-starch wastewater. External H_2 was continuously supplied to upgrading reactor R1 through the separate injection chamber, while parallel reactor R2 was operated without H_2 addition as the control.

16.4. Analysis domain

- **Operational objective:** Enrichment of CH_4 using externally supplied H_2.
- **System boundary**: From externally supplied H_2 entering upgrading reactor R1 to its allocation to CH_4 formation, acetate formation, or unreacted H_2 leaving the reactor boundary; organic-feed-derived electron flows were excluded as background.

- **Analysis window**: Steady-state operation within upgrading Periods II–VIII: BU_Bassani_01: Period II, Rashig rings; BU_Bassani_02: Period III, Rashig rings with increased liquid recirculation; BU_Bassani_03: Period IV, ceramic sponge; BU_Bassani_04: Period V, ceramic sponge with gas recirculation at 4 mL/min; BU_Bassani_05: Period VI, ceramic sponge with gas recirculation at 6 mL/min; BU_Bassani_06: Period VII, serial H_2-injection chambers; BU_Bassani_07: Period VIII, single extended H_2-injection chamber Exact day ranges used to calculate the reported steady-state values were not specified.

16.5. Electron map

- **Electron donor:** Externally supplied H_2.
- **Electron pool:** Dissolved H_2 and intermediate electron carriers within the reactor; unreacted H_2 leaving with the output gas is treated as pool-out.
- **Target sink:** Hydrogenotrophic methanogenesis.
- **Competing sink(s):** Homoacetogenesis (Acetate production).
- **Potential unquantified pathways:** Biomass assimilation, dissolved H_2 not quantified separately, other unmeasured H_2-consuming pathways, and uncertainty associated with separating H_2-derived CH_4 and acetate from the background anaerobic digestion process.

16.6. Source data used

- **Tables 1–2 and Section 1, Eq. (1):** H_2 flow rates for Periods II–VIII; gas volumes were expressed at STP using 22.4 L/mol. CH_4 production rates of upgrading reactor R1 and no-H_2 control R2; used to calculate net CH_4 formation as R1 − R2. Total VFA concentrations and acetate fractions in R1 and R2; used to reconstruct acetate concentrations for the upgrading and background conditions. H_2 consumption rates of R1, used to quantify unreacted H_2 leaving with the output gas.
- **Section 2.2:** Reactor configuration, R1/R2 assignment, thermophilic operation at 55 ± 1 °C, and HRT of 5 d during the upgrading periods.
- **Section 2.2 and Tables 1–2:** H_2 distribution devices, liquid/gas recirculation conditions, and chamber configurations defining Periods II–VIII.
- **Section 2.2, Eq. (3):** Authors' VFA-corrected approach for estimating CH_4 derived from CO_2 and H_2 conversion; used to identify the limitation of the present uncorrected R1–R2 CH_4 estimate because individual non-acetate VFA concentrations were not reported.

16.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the externally supplied H_2 (Tables 1–2; STP, 22.4 L/mol). Organic-feed-derived electron flows were treated as background and excluded by subtracting the no-H_2 control R2 from R1. At steady state, E_pool,start and E_pool,end cancel (Section 2.3). Unreacted H_2 leaving with the output gas (E_pool,out) was quantified as the reported H_2 flow rate minus the H_2 consumption rate (111–1708 mL/L/d, i.e., 6–49% of the H_2 supplied) and is contained in E_unresolved; it accounts for most of the low f_expressed in Periods II–IV.
- E_in basis: Defined substrate (H_2).

- CH_4 production rates were taken directly from Tables 1–2 and not recalculated from biogas production rate and CH_4 fraction, because the outlet gas composition of the H_2-supplied reactor included unreacted H_2 whereas the reported biogas production rate represented biologically produced CH_4 and CO_2. Net CH_4 formed was calculated as the reported CH_4 production rate in R1 minus that in the no-H_2 control R2.
- Acetate input/background and acetate output/upgrading were calculated from the reported total VFA concentration and acetate fraction in R2 and R1, respectively. Acetate concentration was calculated as: Acetate (mmol/L) = Total VFA (g/L) × acetate fraction / 100 × 1000 / 60.05, where 60.05 g/mol is the molecular weight of acetic acid. R2 was used as the no-H_2 background and R1 as the H_2-upgrading condition.

16.8. Assumptions and limitations

- **Assumptions:** R2 was assumed to represent the no-H_2 background for CH_4 and acetate production. Excess acetate in R1 relative to R2 was used as an operational estimate of homoacetogenesis. Excess acetate in R1 may also reflect inhibited acetoclastic methanogenesis at the elevated pH of R1 (7.8–8.4) rather than homoacetogenesis alone; A_target is therefore conservative.
- **Limitations**: The authors corrected the R1–R2 CH_4 difference for differences in residual VFA using Eq. (3). This correction could not be reproduced because concentrations of the individual non-acetate VFAs required for the calculation were not reported. Therefore, the present estimate uses the uncorrected R1–R2 CH_4 production-rate difference and may not fully separate H_2-derived CH_4 from changes in background substrate degradation. Non-acetate VFAs were also higher in R1 than in R2 (e.g., 1.52 vs. 0.68 g/L in Period II), indicating reduced background substrate degradation in R1; the uncorrected R1 − R2 difference may therefore underestimate H_2-derived CH_4.

17. StudyID: BU_Kougias2017

17.1. Reference information

- Case ID: From BU_Kougias_01 to BU_Kougias_06
- Reference: Kougias, P. G., Treu, L., Peñailillo Benavente, D., Boe, K., Campanaro, S., & Angelidaki, I. (2017). Ex-situ biogas upgrading and enhancement in different reactor systems. *Bioresource Technology, 225*, 429–437.
- DOI: https://doi.org/10.1016/j.biortech.2016.11.124
- System family: Biogas upgrading
- Link to calculation file: Supplementary Data 1.xlsx (Biogas_Upgrading sheet)

17.2. Purpose of using this study. This study was used to evaluate electron allocation during ex-situ biological biogas upgrading under different reactor configurations and gas recirculation rates. The selected cases represent three reactor configurations, serial upflow reactors, CSTR, and bubble column, operated at gas recirculation rates of 4 and 12 L/h.

17.3. Experimental system. Ex-situ biogas upgrading was evaluated using three thermophilic reactor configurations: two 1.4-L upflow reactors connected in series (R1 + R2), a 1.2-L CSTR (R3), and a 1.2-L bubble column reactor (R4). The reactors were operated at 52 ± 1 °C. A gas mixture containing 23% CH_4, 15% CO_2, and 62% H_2 was supplied through stainless-steel diffusers. Digested slurry was supplied daily at 80 mL/d as nutrient medium. Gas recirculation was operated at 4 L/h during Period I and increased to 12 L/h during Period II. Liquid HRT was 35 d for the serial upflow configuration and 15 d for the CSTR and bubble column reactors.

17.4. Analysis domain

- **Operational objective:** Enrichment of CH_4 through externally supplied H_2.
- **System boundary**: From externally supplied H_2 entering the upgrading reactor system to its allocation to CH_4 formation, acetate formation, or unreacted H_2 leaving the reactor boundary.
- **Analysis window**: Periods I–II for each reactor configuration:
  BU_Kougias_01: Serial upflow reactors (R1 + R2), Period I, days 0–18
  BU_Kougias_02: Serial upflow reactors (R1 + R2), Period II, days 19–51
  BU_Kougias_03: CSTR (R3), Period I, days 0–18
  BU_Kougias_04: CSTR (R3), Period II, days 19–51
  BU_Kougias_05: Bubble column reactor (R4), Period I, days 0–18
  BU_Kougias_06: Bubble column reactor (R4), Period II, days 19–51

17.5. Electron map

- **Electron donor:** Externally supplied H_2.
- **Electron pool:** Dissolved H_2 and intermediate electron carriers within the reactor.
- **Target sink:** Hydrogenotrophic methanogenesis.
- **Competing sink(s):** Homoacetogenesis (Acetate production).
- **Potential unquantified pathways:** Biomass assimilation, other H_2-consuming pathways including sulfate-reducing activity, residual or dissolved H_2 not fully quantified, and uncertainty associated with separating upgrading-derived products from methane and acetate originating from the digestate nutrient medium.

17.6. Source data used

- **Table 2:** reactor configuration, working volume, HRT, gas feed flow, gas composition, gas recirculation rate, and operating periods.
- **Methods / reactor operation:** digestate feeding rate of 80 mL/d and operating temperature of 52 ± 1 °C.
- **Residual methane potential assay:** 2.5 ± 0.3 mL CH_4/mL digestate, used to estimate methane potentially derived from the digestate background.
- **Figure 2a:** CH_4 fraction in the effluent gas, digitized for each reactor configuration and operating period.
- **Figure 2c:** H_2 fraction in the effluent gas, digitized for reconstruction of outlet gas flow.

- **Figure 2d:** H_2 utilization efficiency, digitized for calculation of residual H_2 and outlet gas flow.
- **Digestate characterization:** acetate concentration in the supplied digestate, 293 ± 11 mg/L, used as acetate input/background.
- **Figure 3:** acetate concentration profiles, digitized to estimate acetate output for each case. The authors report that VFA accumulation was dominated by acetate and quantify substantial acetate increases during Period II.

17.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the H_2 in the supplied gas mixture (Table 2). CH_4 in the feed gas and CH_4 derived from the digestate nutrient medium (80 mL/d × 2.5 mL CH_4/mL) were treated as background and subtracted. At steady state, E_pool,start and E_pool,end cancel (Section 2.3); unreacted H_2 leaving with the outlet gas (E_pool,out) is contained in E_unresolved.
- E_in basis: Defined substrate (H_2).
- H_2 input was calculated from the reported total gas feed flow and H_2 fraction in the feed gas: H_2 input = gas feed flow × 0.62. The reported gas mixture was CH_4:CO_2:H_2 = 23:15:62%.
- CH_4 input was calculated from the reported total gas feed flow and feed-gas CH_4 fraction: CH_4 input = gas feed flow × 0.23. The feed composition was CH_4:CO_2:H_2 = 23:15:62%.
- CH_4 output was reconstructed from H_2 input, digitized H_2 utilization efficiency, digitized outlet H_2 fraction, and digitized outlet CH_4 fraction. Residual H_2 was first calculated from H_2 input and utilization efficiency; total outlet gas volume was then reconstructed from residual H_2 and the outlet H_2 fraction, and CH_4 output was calculated from this outlet gas volume and the CH_4 fraction. Methane potentially generated from the digestate nutrient feed was treated separately from the gaseous CH_4 input and output. Digestate-derived CH_4 was estimated from the reported liquid feed rate (80 mL/d), residual methane potential (2.5 mL CH_4/mL digestate), and reactor working volume. Net CH_4 formation was calculated as CH_4 output minus gaseous CH_4 input and digestate-derived CH_4 background.
- Acetate input/background was calculated from the reported acetate concentration in the digestate nutrient feed (293 mg/L). Acetate output concentrations were digitized from Fig. 3 for each reactor configuration, using the last available measurement within Period I and the endpoint of Period II. Concentrations were converted to mmol/L using a molecular weight of 60.05 g/mol for acetic acid. Net acetate was calculated as reactor acetate minus influent digestate acetate, and net acetate formation rate was obtained by dividing by the corresponding liquid HRT

17.8. Assumptions and limitations

- **Assumptions:** Outlet gas flow was reconstructed from H_2 input, digitized H_2 utilization, and digitized outlet H_2 fraction; digitized endpoint values were treated as representative of each operating period. CH_4 derived from the digestate was estimated from the reported residual methane potential (2.5 ± 0.3 mL CH_4/mL digestate) and treated separately from gaseous CH_4 input. Excess acetate above the digestate-feed acetate concentration was used as an operational estimate of H_2-derived homoacetogenesis.

- **Limitations**: Absolute outlet gas flow and CH_4 production were not reported numerically and therefore depended on figure digitization and H_2 mass-balance reconstruction. This becomes highly uncertain when residual outlet H_2 approaches zero; For BU_Kougias_02 and BU_Kougias_06 (Period II, R1+R2 and R4), the reconstructed net CH_4 (592 and 948 mL/L/d) exceeded the stoichiometric maximum from the supplied H_2 (H2/4 = 200 and 465 mL/L/d), confirming that outlet gas flow could not be reconstructed when residual H_2 approached zero; these cases were recorded as NC. The authors' correction for methane potentially formed from residual VFAs could not be reproduced from the available numerical data; the full digestate residual methane potential was therefore subtracted as background. Acetate accumulation is also not pathway-specific, because the authors associated the observed accumulation with strong inhibition of acetoclastic methanogenesis while also identifying homoacetogenesis as a possible H_2-consuming route.

18. StudyID: BU_Omar2018

18.1. Reference information

- Case ID: From BU_Omar_01 to BU_Omar_04
- Reference: Omar, B., Abou-Shanab, R., El-Gammal, M., Fotidis, I. A., Kougias, P. G., Zhang, Y., & Angelidaki, I. (2018). *Simultaneous biogas upgrading and biochemicals production using anaerobic bacterial mixed cultures*. Water Research, 142, 86–95.
- DOI: https://doi.org/10.1016/j.watres.2018.05.049
- System family: Biogas upgrading
- Link to calculation file: Supplementary Data 1.xlsx (Biogas_Upgrading sheet)

18.2. Purpose of using this study. This study was used to evaluate electron allocation during batch ex-situ biogas upgrading in which externally supplied H_2 and CO_2 were converted predominantly to acetate by an acetogen-enriched mixed culture. The selected cases represent four H_2:CO_2 ratios used to evaluate how gas composition affected H_2 utilization, acetate production, and biogas upgrading performance.

18.3. Experimental system. Batch gas-fermentation experiments were conducted in 540-mL serum bottles with a 100-mL working volume using digested sewage-sludge inoculum chemically treated with 50 mM 2-bromoethanesulfonate (BES) to suppress methanogenesis. The inoculum represented 10% of the working volume. Bottles were operated at 37 ± 1 °C, 150 rpm, and an initial pH of 6.0 ± 0.1. Synthetic gas was prepared from biogas containing 40% CO_2 and 60% CH_4 together with pure H_2. Four gas ratios were evaluated: H_2:CO_2:CH_4 of 1:1:1.5, 2:1:1.5, 3:1:1.5, and 4:1:1.5. The batch incubation lasted 10 days.

18.4. Analysis domain

- **Operational objective:** Enrichment of CH_4 in biogas through H_2-driven conversion of CO_2, predominantly to acetate, or in other words, acetate formation.

- **System boundary**: From externally supplied H_2 present in the initial bottle headspace to its allocation to acetate and other fermentation products, biomass formation, methane formation, or residual H_2 remaining at the end of the batch.
- **Analysis window**: Entire 10-day batch incubation for each gas ratio:
  BU_Omar_01: GR(1x), H_2:CO_2:CH_4 = 1:1:1.5
  BU_Omar_02: GR(2x), H_2:CO_2:CH_4 = 2:1:1.5
  BU_Omar_03: GR(3x), H_2:CO_2:CH_4 = 3:1:1.5
  BU_Omar_04: GR(4x), H_2:CO_2:CH_4 = 4:1:1.5.

18.5. Electron map

- **Electron donor:** Externally supplied H_2.
- **Electron pool:** H_2 in the bottle headspace, dissolved H_2, and intermediate electron carriers during fermentation. Other CO_2-reduction products (propionate, butyrate, ethanol) were not classified as competing because they also contribute to CO_2 removal.
- **Target sink:** Homoacetogenesis (acetate production).
- **Competing sink(s):** Hydrogenotrophic methanogenesis (E_competing = 0; see Calculation decisions).
- **Potential unquantified pathways:** Biomass assimilation and other H_2-consuming pathways whose contribution to net CO_2 removal could not be individually resolved from the data.

18.6. Source data used

- **Section 2.2:** Batch reactor configuration and operating conditions: 540-mL serum bottles, 100-mL working volume, 10% inoculum, initial pH 6.0 ± 0.1, 37 ± 1 °C, and 150 rpm.
- **Sections 2.2.1–2.2.2:** BES treatment of the inoculum (50 mM) and selection of the chemically treated inoculum for the gas-ratio optimization experiment.
- **Table 2:** H_2:CO_2:CH_4 gas ratios for GR(1x)–GR(4x), used to determine H_2 fractions for each case.
- **Table 4**: CO2-to-methane conversion (0%) for the BES-treated inoculum. Conclusions: chemical treatment completely blocked methanogenic activity
- **Table 5:** Initial and final H_2 COD equivalents, used to quantify H_2 input and residual H_2; initial and final acetate COD equivalents, used to calculate net acetate formation.
- **Section 3.2.2:** Acetate identified as the major fermentation product; acetate accounted for approximately 89–93% of total VFA products.

18.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the initial H_2 (Table 5). No CH_4 formation was attributed to the BES-treated inoculum used in the gas-ratio experiments: the same treatment gave 0% CO_2-to-methane conversion (Table 4), and the authors concluded that chemical treatment completely blocked methanogenic activity. Because all other quantified products were CO_2-reduction products, E_competing was therefore set to zero; A_target (1.00) is an upper estimate. Residual H_2 at the end of the batch (E_pool,end; ~0, 12.6, 348.8, and 607.1 mg COD/L), the other organic products, and biomass

are contained in E_unresolved; residual H_2 accounts for the decline in f_expressed at higher H_2 ratios.
- E_in basis: Defined substrate (H_2).
- Initial H_2 quantities were calculated from the reported initial H_2 COD equivalents in Table 5 using 16 mg COD per mmol H_2. Values were multiplied by the 0.1-L working volume to obtain mmol H_2 per batch case. Gas volumes were subsequently calculated assuming 0 °C and 1 atm (22.414 mL/mmol), because gas reference conditions were not reported. H_2 fractions were calculated from the reported H_2:CO_2:CH_4 gas ratios in Table 2.
- CH_4 formation was not quantified as an electron sink because biogas upgrading in the selected BES-treated experiments was achieved primarily through H_2-driven CO_2 conversion/removal not hydrogenotrophic methane formation; therefore, the CH_4-production block was treated as not applicable.
- Acetate concentrations were taken directly from the initial and final acetate COD values reported in Table 5. Acetate COD was converted to molar concentration using 64 mg COD per mmol acetate. Net acetate was calculated as final minus initial acetate, and because the experiments were batch systems with a 0.1-L working volume, net acetate formation was expressed as mmol per case by multiplying the net concentration by 0.1 L.
- Net acetate formation was used as the major quantified electron sink associated with H_2-driven CO_2 conversion. Other fermentation products and biomass were not classified as competing sinks because their formation may also contribute to CO_2 conversion and hence to the defined biogas-upgrading objective. No H_2-consuming pathway that could be quantitatively separated and shown not to contribute to CO_2 removal was reported.

18.8. Assumptions and limitations

- **Assumptions:** Gas volumes were assumed at 0 °C and 1 atm when converted from mmol.
- **Limitations**: Acetate represents only the major quantified target pathway; other CO_2-converting products and biomass were not fully resolved. Biomass formation (8.6–14.7% of H_2; Section 3.2.2) was not quantified separately.

19. StudyID: BU_Mulat2017

19.1. Reference information

- Case ID: From BU_Mulat_01 to BU_Mulat_02
- Reference: Mulat, D. G., Mosbæk, F., Ward, A. J., Polag, D., Greule, M., Keppler, F., Nielsen, J. L., & Feilberg, A. (2017). *Exogenous addition of H_2 for an in situ biogas upgrading through biological reduction of carbon dioxide into methane*. Waste Management, 68, 146–156.
- DOI: https://doi.org/10.1016/j.wasman.2017.05.054
- System family: Biogas upgrading
- Link to calculation file: Supplementary Data 1.xlsx (Biogas_Upgrading sheet)

19.2. Purpose of using this study. This study was used to evaluate electron allocation during batch in-situ biogas upgrading through H_2-driven conversion of CO_2 to CH_4. The selected

cases represent H_2-supplemented reactors fed with unlabelled maize (UMH) and U-^{13}C-labelled maize (LMH) during the stable upgrading period on days 6–10.

19.3. Experimental system. Batch experiments were conducted in sealed 120-mL bottles inoculated with 75 g of pre-incubated thermophilic digestate and fed once with 1.33 g wet maize leaf. UMH received unlabelled maize and LMH received U-^{13}C-labelled maize; otherwise, operating conditions were identical. H_2 was periodically injected into the headspace. Bottles were incubated at 52 °C and 100 rpm for 24 days, and pH was periodically adjusted to 7.0–8.0. During the first 10 days, H_2 was supplied approximately according to the stoichiometric requirement for conversion of produced CO_2 to CH_4; excess H_2 was supplied after day 10 to investigate process inhibition.

19.4. Analysis domain

- **Operational objective:** Enrichment of CH_4 through externally supplied H_2.
- **System boundary**: From externally supplied H_2 entering the batch reactor to its allocation to CH_4 formation, homoacetogenic acetate formation, or other H_2-consuming pathways.
- **Analysis window**: Days 6–10, identified by the authors as a stable upgrading period with high H_2 conversion:
  BU_Mulat_01: UMH, unlabelled maize + H_2, days 6–10
  BU_Mulat_02: LMH, U-^{13}C-labelled maize + H_2, days 6–10.

19.5. Electron map

- **Electron donor:** Externally supplied H_2.
- **Electron pool:** H_2 in the reactor headspace, dissolved H_2, and intermediate electron carriers.
- **Target sink:** Hydrogenotrophic methanogenesis.
- **Competing sink(s):** Homoacetogenesis (acetate production).
- **Potential unquantified pathways:** Other H_2-consuming microbial pathways and uncertainty in separating acetate produced through homoacetogenesis from changes in substrate-derived acetate production and degradation.

19.6. Source data used

- **Section 2.2:** Batch setup, inoculum and maize loading, periodic H_2 addition schedule, 52 °C, 100 rpm, 24-day incubation, and definition of H_2-supplemented and no-H_2 control treatments
- **Section 2.4:** Biogas-volume measurement at room temperature and atmospheric pressure, gas-composition analysis, pH control, and VFA measurements.
- **Figure 1:** Temporal profiles of cumulative/daily CH_4 production, acetate, propionate, and CH_4/CO_2/H_2 gas composition; used to assess the stable upgrading period and VFA behavior.
- **Table 3:** H_2 injection and consumption rates, total CH_4 production, CH_4 from H_2, CH_4/CO_2/H_2 fractions, and pH for days 6–10; primary source for H_2 and net CH_4 calculations.

19.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the H_2 injected during days 6–10 (507 mL/L/d; Table 3). Organic-substrate-derived CH_4 was excluded by subtracting the matched no-H2 control. H_2 consumption was 100%, so no H_2 left as pool.
- E_in basis: Defined substrate (H_2).
- H_2 input was taken directly from the H_2 injection rate reported in Table 3 for days 6–10 (507 ± 30 mL/L/d). Because H_2 was injected as pure gas, the H_2 fraction was 100%.
- CH_4 input/background was represented by the corresponding no- H_2 control (UM for UMH and LM for LMH). Total biogas volumes were reconstructed from the reported CH_4 production rates and CH_4 fractions. Net CH_4 formation was calculated as the measured CH_4 production in the H_2-supplemented reactor minus that in its matched no-H_2 control. The reported "CH_4 from H_2" value of 127 mL/L/d was not used as net CH_4 formation because it represents the stoichiometric CH_4 equivalent of H_2 consumption but not the observed increase relative to the control. Because both the injected H_2 and the produced gas were measured at room temperature (Section 2.4), all gas volumes were converted using 24.465 L/mol (25 °C, 1 atm); f_target is therefore independent of the assumed reference temperature.
- Homoacetogenesis was identified as a potential competing H_2-consuming pathway, but its electron allocation could not be quantified independently because acetate accumulation may reflect both homoacetogenic production and inhibition of acetate degradation.

19.8. Assumptions and limitations

- **Assumptions:** UM and LM were treated as matched no-H_2 controls for UMH and LMH, respectively. Reported room temperature was approximated as 25 °C at 1 atm for CH_4 volume-to-mole conversion.
- **Limitations**: Net CH_4 formation based on H_2-supplemented minus control reactors may still include differences in substrate degradation between treatments. Homoacetogenic electron allocation could not be quantified independently because acetate accumulation may reflect both acetate production and inhibited acetate degradation; therefore competing-allocation metrics were NC. Net CH4 accounted for only 34–38% of the H2 consumed; the remainder was associated with VFA accumulation, consistent with the authors' estimate of ~66 mL CH4-equivalent residual VFA.

REDUCTIVE TREATMENT

20. StudyID: RedAzo_Dai2018

20.1. Reference information

- Case ID: From RedAzo_Dai_01 to RedAzo_Dai_20
- Reference: Dai, R., Chen, X., Xiang, X., Wang, Y., & Wang, F. (2018). Understanding azo dye anaerobic bio-decolorization with artificial redox mediator supplement: Considering the methane production. *Bioresource Technology*, 249, 799–808.
- DOI: https://doi.org/10.1016/j.biortech.2017.10.072
- System family: Reductive treatment – azo dye reduction
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Azodye sheet)

20.2. Purpose of using this study. This study was used to evaluate electron allocation between anaerobic reduction of Acid Orange 7 (AO7) and methanogenesis under different electron-donor, azo-dye, and artificial-redox-mediator conditions. The selected cases include a matched pre-trial comparison with and without anthraquinone-2-sulfonate (AQS), together with 18 BBD/RSM runs covering different combinations of glucose, AQS, and AO7 concentrations.

20.3. Experimental system. Batch assays were conducted in 118-mL serum bottles with 2.0 g VSS/L anaerobic granular sludge at pH 7.3 ± 0.2, 38 °C, and 110 rpm. Glucose was used as the electron donor, AO7 as the target electron acceptor, and AQS as the redox mediator.

20.4. Analysis domain

- **Operational objective:** Decolorization of AO7.
- **System boundary**: From supplied glucose to AO7 reduction, methanogenesis, and remaining electron-carrying intermediates.
- **Analysis window**: 0–48 h for all cases; all experiments were terminated at 48 h (Section 3.1.1).

20.5. Electron map

- **Electron donor:** Glucose.
- **Electron pool:** Fermentation intermediates and electron carriers, including VFAs, H2, and AQS when supplied.
- **Target sink:** AO7 reduction/decolorization
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation, residual donor, and unmeasured reduced intermediates or other electron-consuming pathways.

20.6. Source data used

- **Section 2.1:** AO7 molecular weight (350.32 g/mol) and use of glucose as electron donor and AQS as redox mediator.

- **Sections 2.3.1–2.3.2**: Batch conditions, glucose/AO7/AQS concentrations, and experimental design.
- **Section 2.5**: COD conversion for AO7 reduction (64 mg COD/mmol AO7) and methane COD basis.
- **Sections 3.1.1–3.1.2 and Fig. 1d:** Pre-trial decolorization, methane production, AO7-reduction COD, and methane COD for cases 01–02. AO7-reduction COD (40.5 and 105.1 mg/L) and VFA COD (236.0 mg/L for RedAzo_Dai_01).
- **Table 2:** Glucose, AQS, AO7, actual decolorization rate, and actual COD proportion for methane production for cases 03–20.

20.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the glucose supplied at t = 0. The inoculum (2.0 g VSS/L) was not included in E_in. E_pool,end comprised VFAs, which were quantified only for RedAzo_Dai_01 (236.0 mg COD/L) and are contained in E_unresolved; AQS acts only as an electron shuttle and does not retain electrons when AO7 is not fully reduced (Section 2.5).
- E_in basis: Defined substrate.
- AO7 reduction was calculated from initial AO7 concentration × actual decolorization rate; conversion used the authors' value of 64 mg COD/mmol AO7.
- For cases 01–02, directly reported AO7-reduction COD values were used; methane COD was digitized from Fig. 1d.
- For cases 03–20, methane COD was calculated as COD_input × Y2 actual, where Y2 = COD_CH4/COD_glucose.

20.8. Assumptions and limitations

- **Assumptions:** For cases 03–20, the reported COD_CH_4/COD_glucose ratio was assumed to represent methane allocation over the selected batch endpoint.
- **Limitations**: Methane COD for cases 01–02 was digitized from Fig. 1d and is approximate. RedAzo_Dai_02 slightly exceeded closure (f_expressed = 1.004), within the uncertainty of the digitized CH_4, consistent with the authors' report of ~1000 mg/L COD recovery; it was excluded under the [0, 1] criterion.

21. StudyID: RedDen_Wu2022

21.1. Reference information

- Case ID: From RedDen_Wu_01 to RedDen_Wu_03
- Reference: Wu, X., Wang, C., Wang, D., Huang, Y.-X., Yuan, S., & Meng, F. (2022). Simultaneous methanogenesis and denitrification coupled with nitrifying biofilm for high-strength wastewater treatment: Performance and microbial mechanisms. *Water Research, 225*, 119163.
- DOI: https://doi.org/10.1016/j.watres.2022.119163
- System family: Reductive treatment – Denitrification
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Denitrification sheet)

21.2. Purpose of using this study. This study was used to evaluate electron allocation between denitrification and methanogenesis under three influent C/N conditions.

21.3. Experimental system. A continuous UBF-MBBR system was operated with approximately 2000 mg COD/L synthetic wastewater. The UBF operated at approximately 30 °C with a 24-h HRT and received glucose and sodium acetate at an 8:2 COD ratio.

21.4. Analysis domain

- **Operational objective:** Denitrification.
- **System boundary**: UBF reactor, from organic COD entering the UBF to its allocation to denitrification, methanogenesis, and remaining intermediates.
- **Analysis window**: Stage-level operation:
  RedDen_Wu_01: Stage I, COD/NH_4-N = 40
  RedDen_Wu_02: Stage II, COD/NH_4-N = 10
  RedDen_Wu_03: Stage III, COD/NH_4-N = 5

21.5. Electron map

- **Electron donor:** Glucose and sodium acetate.
- **Electron pool:** Fermentation intermediates and electron carriers, including acetate, H_2, and formate.
- **Target sink:** Denitrification.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation and other unmeasured intermediates or electron-consuming pathways.

21.6. Source data used

- **Section 2.1:** UBF-MBBR configuration, reactor volumes, HRT, recirculation ratios, temperature, influent COD, substrate composition, and stage-specific ammonium concentrations.
- **Section 2.3**: Definitions and calculation approach for COD removal by denitrification and methanogenesis, including the study-specific denitrification coefficient.

- **Section 3.1 and Fig. 2d**: Stage-specific CH_4 yields.
- **Fig. S5a**: Stage-specific organic loading rate (OLR) and organic removal rate (ORR).
- **Fig. S5b**: Stage-specific nitrate loading rate (NALR) and nitrate removal rate (NARR).

21.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the influent organic loading rate of the UBF (Fig. S5a). Nitrate entering the UBF via the 300% recirculation from the MBBR is an electron acceptor and carries no donor electrons. At steady state, E_pool,start and E_pool,end cancel (Section 2.3); organic COD leaving with the UBF effluent (E_pool,out; ~15% of the OLR), biomass, and dissolved CH_4 are contained in E_unresolved. E_in basis: Defined substrate.
- Stage-specific organic loading rate (OLR) from Fig. S5a was used and converted from kgCOD/m^3/d to mgCOD/L/d (×1000).
- Nitrate removal rate (NARR) was taken directly from Fig. S5b for each stage and converted from kg-N/m^3/d to mg-N/L/d.
- COD_target was calculated as NARR × 2.857 mg COD/mg N, the electron equivalent of $NO3^-$-N reduction to N_2. The study-specific coefficient of 3.5 g COD/g N (Eq. 2; Del Pozo and Diez, 2003) was not used because it includes COD for cell synthesis, which is not part of the target sink in the present framework.
- Stage-specific organic removal rate (ORR) from Fig. S5a and reported CH_4 yield were used to calculate CH_4 production rate as ORR × CH_4 yield.
- CH_4 production was converted to COD using 1 mol CH_4 = 64 gCOD. Because gas reference conditions were not reported, 0 °C and 1 atm (Vm = 22.414 L/mol) were assumed. The CH_4 yield was defined per unit of total COD removed in the UBF (Eq. 5), so CH_4 production was calculated as ORR × CH_4 yield.

21.8. Assumptions and limitations

- **Assumptions:** Nitrate removal was assumed to represent reduction to N_2. Gas conversion assumed 0 °C and 1 atm.
- **Limitations**: N_2 production was not directly measured, and stage-average values may mask temporal variation. Dissolved CH_4 in the UBF effluent was not quantified, so methanogenic allocation may be underestimated.

22. StudyID: RedDen_Xie2012

22.1. Reference information

- Case ID: From RedDen_Xie_01 to RedDen_Xie_10
- Reference: Xie, L., Chen, J., Wang, R., & Zhou, Q. (2012). Effect of carbon source and COD/NO3−-N ratio on anaerobic simultaneous denitrification and methanogenesis for high-strength wastewater treatment. *Journal of Bioscience and Bioengineering, 113*(6), 759–764.
- DOI: https://doi.org/10.1016/j.jbiosc.2012.01.007
- System family: Reductive treatment – Denitrification
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Denitrification sheet)

22.2. Purpose of using this study. This study was used to evaluate electron allocation between denitrification and methanogenesis under different COD/NO_3^--N ratios and carbon sources.

22.3. Experimental system. Batch assays were conducted at a 400-mL working volume with anaerobic granular sludge at 35 °C, 110 rpm, and initial pH 7.80 ± 0.05. Initial COD was maintained at 3800 ± 30 mg/L, using either anaerobic cassava-stillage effluent or glucose as the carbon source, with different $NaNO_3$ additions.

22.4. Analysis domain

- **Operational objective:** Denitrification.
- **System boundary**: From initial organic COD to denitrification, methanogenesis, and remaining organic/intermediate pools.
- **Analysis window**: 0–19 h for all selected cases.
  RedDen_Xie_01–05: Cassava-stillage effluent, COD/NO_3^--N = 3, 5, 8, 19, 55.
  RedDen_Xie_06–10: Glucose, COD/NO_3^--N = 3, 5, 7, 18, 50

22.5. Electron map

- **Electron donor:** Organic matter in anaerobic cassava-stillage effluent or glucose.
- **Electron pool:** Fermentation intermediates and VFAs, including acetate, propionate, and butyrate.
- **Target sink:** Denitrification.
- **Competing sink(s):** Methanogenesis.
- **Potential unquantified pathways:** Biomass assimilation, residual organic matter, and unmeasured intermediates or other electron-consuming pathways.

22.6. Source data used

- **Materials and Methods - Batch experiments:** Working volume, initial COD, COD/ NO_3^--N ratios, pH, temperature, and substrate conditions.
- **Table 2:** NOx^--N removal efficiencies at 19 h for all selected cases.
- **Figure 2:** CH_4 production at 19 h for the selected COD/ NO_3^--N conditions.
- **Table 1:** Characteristics of the cassava-stillage anaerobic effluent used as carbon source.

22.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the initial COD (3800 mg/L). The granular sludge inoculum (100 mL) was not included in E_in. E_pool,end (residual organic matter and VFAs at 19 h) was not quantified and is contained in E_unresolved. E_in basis: Total COD for the cassava-stillage effluent (RedDen_Xie_01–05) and Defined substrate for glucose (RedDen_Xie_06–10).
- Initial NO_3^--N was calculated from the reported initial COD (3800 mg/L) and COD/ NO_3^--N ratio.
- NOx-N reduced was calculated as initial NO_3^--N × NOx-N removal fraction using the 19-h removal efficiencies in Table 2.
- CH_4 production at 19 h was digitized from Figure 2 and converted to mL CH_4/L using the 0.4-L working volume. CH_4 at 19 h was zero for all cassava-stillage cases; the small CH_4 values at COD/$NO3^-$-N of 8–55 in Fig. 2A correspond to 48 h.
- CH_4 was converted to COD assuming 0 °C and 1 atm (Vm = 22.414 L/mol) and 1 mol CH_4 = 64 gCOD.

22.8. Assumptions and limitations

- **Assumptions:** Nitrate removal was assumed to represent denitrification to N_2. Gas conversion assumed 0 °C and 1 atm. DNRA was not significant (Fig. 3), so nitrate removal was attributed to denitrification.
- **Limitations**: CH_4 production at 19 h was digitized from Fig. 2 and is approximate. Initial $NO3^-$-N was calculated from the nominal COD/$NO3^-$-N ratios; Fig. 1 indicates higher measured initial nitrate at the lowest ratios (e.g., ~1380–1480 vs. 1267 mg N/L at a ratio of 3), so f_target may be underestimated for these cases. For the cassava-stillage effluent, part of the COD was not readily biodegradable, so f_target and f_expressed are lower bounds.

23. StudyID: RedFe_Wang2024

23.1. Reference information

- Case ID: From RedFe_Wang_01 to RedFe_Wang_05
- Reference: Wang, X., Gong, Y., Sun, C., Wang, Z., Sun, Y., Yu, Q., & Zhang, Y. (2024). New insights into inhibition of high Fe(III) content on anaerobic digestion of waste-activated sludge. *Science of the Total Environment, 916*, 170147.
- DOI: https://doi.org/10.1016/j.scitotenv.2024.170147
- System family: Reductive treatment – Fe(III) reduction
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Iron sheet)

23.2. Purpose of using this study. This study was used to evaluate electron allocation between dissimilatory Fe(III) reduction and methanogenesis under different Fe(III) concentrations.

23.3. Experimental system. Batch digestion was conducted with 180 mL waste-activated sludge and 20 mL inoculum in 250-mL serum bottles at pH 7.0, 37 °C, and 100 rpm. Five Fe(III) concentrations were tested: 35, 750, 1470, 2180, and 2900 mg/L.

23.4. Analysis domain

- **Operational objective:** Methanogenesis/bioenergy recovery.
- **System boundary**: From organic matter in sludge to Fe(III) reduction, methanogenesis, and remaining organic/intermediate pools.
- **Analysis window**: 0–38 d (full batch digestion). Fe(II) reached a plateau by day 4 (Fig. 1C) and remained constant through day 20, when >96% of total Fe was Fe(II) (Fig. 5A); Fe(II) at day 38 was therefore taken as the plateau value. Cumulative CH_4 at day 38 was taken from Section 3.1.

23.5. Electron map

- **Electron donor:** Organic matter in waste-activated sludge (WAS).
- **Electron pool:** Solubilized organics and fermentation intermediates, including VFAs and H_2.
- **Target sink:** Methanogenesis.
- **Competing sink(s):** Fe(III) reduction to Fe(II).
- **Potential unquantified pathways:** Biomass assimilation, residual organic matter, and unmeasured intermediates or other electron-consuming pathways.

23.6. Source data used

- **Section 2.1 and Table 1:** TCOD and VSS of WAS and inoculum.
- **Section 2.2:** Batch setup, WAS/inoculum volumes, Fe(III) concentrations, pH, temperature, mixing speed, and digestion duration.
- **Fig. 1A:** Cumulative CH_4 production used for the 20-day competing sink.
- **Fig. 1C:** Fe(II) concentrations at day 0 and day 20 used to estimate Fe(III) reduction.
- **Section 3.1**: cumulative CH4 yields at the end of digestion (79.69, 83.25, and 75.28 mL/g VSS; mean 46.87 mL/g VSS for 2180–2900 mg/L).
- **Section 3.3 and Fig. 5A:** Confirmation that Fe(II) was the dominant iron species at day 20.

23.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the TCOD of the WAS–inoculum mixture (180:20 mL). Fe(II) already present at day 0 (~500 mg/L, mainly from the inoculum) was excluded by calculating Fe(III) reduction as the increase in Fe(II). E_pool,end (residual organic matter, VFAs) was not quantified and is contained in E_unresolved; TCOD removal was only 37.5–48% (Fig. 1D), so most of E_in remained unconverted.
- E_in basis: Total COD.
- COD_input was calculated from the reported TCOD of WAS and inoculum using their 180:20 mL mixing ratio.
- Fe(II) concentrations at day 0 and day 20 were digitized from Fig. 1C. Fe(III) reduced or Fe(II) formation was calculated as Fe(II)_day20 − Fe(II)_day0. E_competing was calculated as Fe(III) reduced / 55.845, assuming 1 mol Fe(III) reduced = 1 mol e^-.
- CH_4 yield at day 20 was digitized from Fig. 1A and converted to mL CH_4/L using the calculated initial VSS concentration of the 180:20 mL WAS-inoculum mixture.
- CH_4 was converted to COD assuming 0 °C and 1 atm (Vm = 22.414 L/mol) and 1 mol CH_4 = 64 gCOD.

23.8. Assumptions and limitations

- **Assumptions:** The increase in Fe(II) from day 0 to the plateau (reached by day 4 and maintained through day 20; Fig. 1C) was assumed to represent Fe(III) reduction (1 mol e^- per mol Fe), and Fe(II) was assumed to remain at this plateau until day 38. Gas volumes were converted assuming 0 °C and 1 atm.
- **Limitations**: Fe(II) and CH_4 values were digitized from figures and are approximate. CH_4 yields were normalized to the VSS added; this is consistent with the authors' equivalence of 79.69 mL/g VSS and 114.22 mL/g COD removed. CH_4 for 2180 and 2900 mg/L was digitized from Fig. 1A; their mean (46.85 mL/g VSS) agrees with the reported 46.87 mL/g VSS.

24. StudyID: RedSR_Paepatung2022

24.1. Reference information

- Case ID: From RedSR_Paepatung_01 to RedSR_Paepatung_02
- Reference: Paepatung, N., Boonapatcharoen, N., Songkasiri, W., Yasui, H., & Phalakornkule, C. (2022). Recovery of anaerobic system treating sulfate-rich wastewater using zero-valent iron. *Chemical Engineering Journal, 435*, 135175.
- DOI: https://doi.org/10.1016/j.cej.2022.135175
- System family: Reductive treatment – Sulfate reduction
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Sulfate sheet)

24.2. Purpose of using this study. This study was used to evaluate electron allocation between sulfate reduction and methanogenesis before and after ZVI addition in sulfate-rich wastewater treatment.

24.3. Experimental system. Anaerobic reactors were operated in fill-draw mode with synthetic wastewater containing 9000 mgCOD/L glucose and 4500 mg/L sulfate, at an HRT of 5 d. Phase I was operated without ZVI, whereas 210 g ZVI was added once at the beginning of Phase II.

24.4. Analysis domain

- **Operational objective:** Recovery of methanogenic performance.
- **System boundary**: From supplied glucose and, where applicable, ZVI-derived electrons to sulfate reduction, methanogenesis, and remaining organic/intermediate pools.
- **Analysis window**:
  RedSR_Paepatung_01: Phase I, day 0–67, without ZVI.
  RedSR_Paepatung_02: Phase II, day 108–223, with ZVI.

24.5. Electron map

- **Electron donor:** Glucose and ZVI-derived electrons additionally supplied in Phase II.
- **Electron pool:** VFAs, ethanol, H_2, and other fermentation intermediates.
- **Target sink:** Methanogenesis.
- **Competing sink(s):** Sulfate reduction to sulfide.
- **Potential unquantified pathways:** Biomass formation and unaccounted electron/COD pools.

24.6. Source data used

- Fig. 3: cumulative glucose-COD input, sulfide-COD, and methane-COD for Phase I and Phase II, including residual glucose (139.63 and 21.27 g COD), VFAs (103.96 and 91.55 g COD), biomass (13.23 and 2.96 g COD), and uncountable COD (7.54 g COD in Phase I).
- Section 2.2: reactor working volumes of 5.1 L for Phase I and 2.1 L for Phase II.
- Section 2.4/electron-flow analysis: COD conversion basis for sulfide and methane.

24.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the cumulative glucose-COD fed during each phase plus, in Phase II, the ZVI-derived electrons. Pool components were quantified in Fig. 3: residual glucose and VFAs (E_pool,end + E_pool,out) accounted for 78% of E_in in Phase I and 24% in Phase II, and biomass for 4% and 0.6%, respectively; these are contained in E_unresolved.
- E_in basis: Defined substrate.
- COD_input was based on the cumulative electron input over each analysis window and normalized to reactor working volume. Phase I used the reported cumulative glucose input of 311.85 gCOD. Phase II included 441.18 gCOD from glucose and 28.85 gCOD-equivalent from ZVI-derived electrons. The cumulative inputs were normalized to the respective working volumes (5.1 L for Phase I and 2.1 L for Phase II) to obtain COD_input in mgCOD/L.
- Sulfide-COD and CH_4-COD was calculated from the cumulative sulfide-COD and cumulative methane-COD reported in Fig. 3, normalized to reactor working volume.

24.8. Assumptions and limitations

- **Assumptions:** Cumulative COD values in Fig. 3 represent the full analysis window.
- **Limitations**: ZVI-derived electrons in Phase II were inferred from the COD balance, and subphase variation was not resolved. The cumulative glucose-COD for Phase I (311.85 g COD) corresponds to ~34 days of feeding at the nominal rate, shorter than the 67-day phase; because all allocation fractions were derived from the same COD balance, they are unaffected. The Phase II balance was closed by construction, since ZVI-derived electrons were inferred as the excess of product COD over influent COD.

25. StudyID: RedSR_ Li2017

25.1. Reference information

- Case ID: From RedSR_Li_01 to RedSR_Li_02
- Reference: Li, Y., Zhang, Y., Yang, Y., Quan, X., & Zhao, Z. (2017). Potentially direct interspecies electron transfer of methanogenesis for syntrophic metabolism under sulfate reducing conditions with stainless steel. *Bioresource Technology, 234*, 303–309.
- DOI: https://doi.org/10.1016/j.biortech.2017.03.054
- System family: Reductive treatment – Sulfate reduction
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Sulfate sheet)

25.2. Purpose of using this study. This study was used to evaluate electron allocation between methanogenesis and sulfate reduction with and without conductive stainless steel under sulfate-containing conditions.

25.3. Experimental system. Two parallel UASB reactors were fed synthetic wastewater containing 1600 mgCOD/L sodium lactate and 1300 mg/L sulfate at an HRT of 6 h and 37 ± 1 °C. R1 contained plastic threads, whereas R2 contained stainless steel.

25.4. Analysis domain

- **Operational objective:** Enhance methanogenesis and anaerobic treatment under sulfate-containing conditions.
- **System boundary**: From supplied lactate electrons to methanogenesis, sulfate reduction, and remaining organic/intermediate pools.
- **Analysis window**:
  RedSR_Li_01: UASB R1, plastic control.
  RedSR_Li_02: UASB R2, stainless steel.

25.5. Electron map

- **Electron donor:** Sodium lactate.
- **Electron pool:** VFAs, H_2, and other fermentation intermediates.
- **Target sink:** Methanogenesis.
- **Competing sink(s):** Sulfate reduction to sulfide.
- **Potential unquantified pathways:** Biomass formation, residual organic pools, and possible iron-oxide reduction associated with the stainless-steel surface.

25.6. Source data used

- **Section 2.1:** influent COD (1600 mgCOD/L) and sulfate concentration (1300 mg/L).
- **Section 2.2:** UASB working volume (0.7 L), HRT (6 h), and temperature (37 ± 1 °C).
- **Section 3.1 / Fig. 1:** average COD removal, methane production, sulfate removal, and effluent sulfide for R1 and R2. Average COD removal efficiencies (64.4% and 72.9%) and effluent sulfide (242.2 and 219.5 mg/L).

25.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the influent lactate COD (1600 mg/L). At steady state, E_pool,start and E_pool,end cancel (Section 2.3). Organic COD leaving with the effluent (E_pool,out; 35.6% and 27.1% of E_in, from the reported COD removal) accounts for most of E_unresolved; CH_4 and sulfate reduction together accounted for ~74% of the COD removed in both reactors.
- E_in basis: Defined substrate.
- Sulfate removed was calculated from influent sulfate concentration and reported sulfate removal efficiency, and converted to mgCOD/L using 64/96 gCOD per g SO_4^{2-} reduced, derived from the 8-electron reduction of SO_4^{2-} to sulfide (64 gCOD per mol SO_4^{2-}; molecular weight of SO_4^{2-} = 96 g/mol).
- CH_4-COD was calculated from the reported methane production by first normalizing CH_4 volume to the wastewater flow rate (Q = working volume/HRT), then converting CH_4 to COD assuming 0 °C and 1 atm (22.414 L/mol) and 64 gCOD/mol CH_4.

25.8. Assumptions and limitations

- **Assumptions:** CH_4 gas volume was assumed at 0 °C and 1 atm.
- **Limitations**: The exact averaging period for the reported UASB performance was not specified. Dissolved CH_4 in the effluent was not measured; at an HRT of 6 h and 37 °C it may be comparable to the gaseous CH_4 of R1, so methanogenic allocation, particularly for RedSR_Li_01, may be underestimated.

26. StudyID: RedSR_Lu2016

26.1. Reference information

- Case ID: From RedSR_Lu_01 to RedSR_Lu_06
- Reference: Lu, X., Zhen, G., Ni, J., Hojo, T., Kubota, K., & Li, Y.-Y. (2016). Effect of influent COD/SO_4^{2-} ratios on biodegradation behaviors of starch wastewater in an upflow anaerobic sludge blanket (UASB) reactor. *Bioresource Technology, 214*, 175–183.
- DOI: https://doi.org/10.1016/j.biortech.2016.04.100
- System family: Reductive treatment – Sulfate reduction
- Link to calculation file: Supplementary Data 1.xlsx (Reductive_Sulfate sheet)

26.2. Purpose of using this study. This study was used to evaluate electron allocation between methanogenesis and sulfate reduction across different influent COD/SO_4^{2-} ratios during anaerobic treatment of starch wastewater.

26.3. Experimental system. A 6-L UASB reactor was continuously operated with synthetic starch wastewater containing approximately 1000 mgCOD/L starch at 35 ± 1 °C. Sulfate loading was increased stepwise to obtain influent COD/SO_4^{2-} ratios of 10, 5, 3, 2, 1, and 0.5. After start-up, HRT was maintained at 6 h.

26.4. Analysis domain

- **Operational objective:** Methane/bioenergy recovery with simultaneous sulfate removal.
- **System boundary**: From supplied starch-COD to methanogenesis, sulfate reduction, effluent organic pools, and other unresolved COD sinks.
- **Analysis window**: Phases I (days 37–62, HRT 6 h) to VI (days 221–250) at COD/$SO4^{2-}$ ratios of 10, 5, 3, 2, 1, and 0.5 (Table 1); values are the phase averages in Table 2.

26.5. Electron map

- **Electron donor:** Starch.
- **Electron pool:** Glucose, ethanol, VFAs, H_2, and other fermentation intermediates.
- **Target sink:** Methanogenesis.
- **Competing sink(s):** Sulfate reduction to sulfide, quantified from sulfate removal.
- **Potential unquantified pathways:** Biomass formation, retained organics, and unrecovered COD.

26.6. Source data used

- **Table 1:** operational phases, HRT, COD/SO_4^{2-} ratios, OLR, and sulfate loading rate.
- **Table 2:** influent COD and COD fractions recovered as gaseous CH_4, dissolved CH_4, gaseous H_2S, and aqueous sulfide.
- **Section 2.2:** COD-equivalent conversion basis for methane and sulfate reduction.
- Section 3.2: sulfate removal efficiencies (80.9% in phase I; 82.6% averaged over phases II–IV; 21.2% and 15.0% in phases V and VI) and the estimated fraction of sulfide precipitated as metal sulfides (17.7–32.7%)

26.7. Calculation decisions

- E_in = E_window input + E_pool,start, where E_window input was the influent starch COD (Table 2). At steady state, E_pool,start and E_pool,end cancel (Section 2.3). Effluent suspended-solids COD and soluble COD (E_pool,out; 11–31% of E_in) and COD retained in the reactor (100% − reported recovery; 10–38%) were reported in Table 2 and are contained in E_unresolved.
- E_in basis: Defined substrate.
- Sulfate reduction was quantified from the influent sulfate concentration and the reported sulfate removal efficiency, converted using 64/96 g COD per g $SO4^{2-}$ (8 e^- per mol). Sulfate removal was used instead of the measured gaseous and aqueous sulfide (Table 2) because a substantial fraction of the produced sulfide (17.7–32.7%) was estimated to precipitate as metal sulfides, and because the nominal SR allocation in Section 3.2 is defined on a sulfate basis; this keeps the effective and nominal SR allocations on the same basis and ensures consistency with Supplementary Data 2.
- Total CH_4-COD (%) was calculated as the sum of the reported gaseous and dissolved CH_4-COD fractions, and CH_4-COD (mgCOD/L) was calculated by multiplying this total fraction by the reported influent COD concentration.

26.8. Assumptions and limitations

- **Assumptions:** The COD fractions in Table 2 and the sulfate removal efficiencies in Section 3.2 were assumed to represent the steady-state electron allocation for each COD/SO_4^{2-} condition.
- **Limitations**: The exact steady-state averaging periods underlying the COD balance in Table 2 were not explicitly specified. Dissolved CH4 at COD/SO_4^{2-} = 3 (18.3%) was markedly higher than in the other phases (1.5–5.0%); the reported value was used without adjustment. Sulfate removal was reported as a single average (82.6%) for phases II–IV and as the end-of-phase value (80.9%) for phase I.