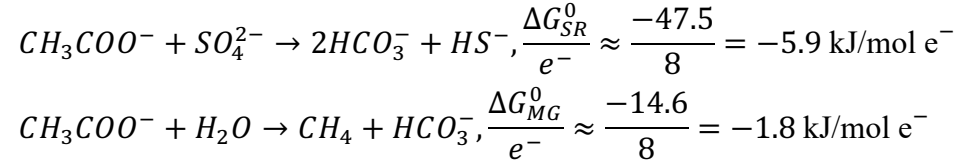


SUPPLEMENTARY NOTE 2

S2.1. Derivation of the crossover point

Reactions and standard free energies. The competition between sulfate reduction (SR) and methanogenesis (MG) is evaluated at the acetate node:



Standard free energies were calculated from the standard free energies of formation reported by Thauer et al. [1], in which CH_4 is given in the gaseous state; on this basis, methanogenesis from acetate yields $\Delta G^{\circ'} = -31.0$ kJ/mol. Because CH_4 is expressed below as a dissolved concentration, the standard free energy of formation of dissolved CH_4 (standard state 1 mol/L) was obtained from the gaseous value using the Henry constant of methane at 25 °C [2]. Either standard state gives the same crossover point, provided that CH_4 in the reaction quotient is expressed consistently, as a dissolved concentration or as a partial pressure, respectively.

Crossover condition. Because both reactions transfer 8 e^- per acetate, equality of ΔG per electron is equivalent to equality of ΔG per reaction: $\Delta G_{MG} = \Delta G_{SR}$ gives $\Delta G_{MG}^0 + RT \ln Q_{MG} = \Delta G_{SR}^0 + RT \ln Q_{SR}$ or equivalently:

$$\frac{Q_{MG}}{Q_{SR}} = \frac{[CH_4][SO_4^{2-}]}{[HCO_3^-][HS^-]} = \exp\left(\frac{\Delta G_{SR}^0 - \Delta G_{MG}^0}{RT}\right) = \exp(-3957/T)$$

$$\text{where } R = 8.314 \times 10^{-3} \frac{\text{kJ}}{\text{mol} \cdot \text{K}}$$

Concentrations as functions of sulfate reduced. Let y (mol/L) be the amount of sulfate reduced and C_{SR} be the initial sulfate concentration. Then: $[SO_4^{2-}] = C_{SR} - y$; $[HS^-] = \alpha_{HS^-} \times y$, where α_{HS^-} is the HS^- fraction at a given pH, calculated as $\alpha_{HS^-} = \frac{1}{1 + 10^{pK_{a1} - pH}}$ with $pK_{a1} = 7$; $[HCO_3^-] = B + 2y$ where B is the background bicarbonate concentration (mol/L); $[CH_4] = m = K_H p_{CH_4}$; where K_H is the Henry constant for methane at the operating temperature and p_{CH_4} is the methane partial pressure inferred from the methane fraction in the biogas. Substituting these expressions into the crossover condition gives:

$$\frac{m \times (C_{SR} - y)}{(B + 2y) \times \alpha_{HS^-} \times y} = \exp(-3957/T)$$

Quadratic equation and solution. With $K = \exp(-3957/T)$, substitution gives: $m(C_{SR} - y) = K \alpha_{HS^-} y(B + 2y)$ or $F(y) = 2K\alpha_{HS^-}y^2 + (K\alpha_{HS^-}B + m)y - mC_{SR}$ whose positive root is:

$$y^* = \frac{-(K\alpha_{HS^-}B + m) + \sqrt{(K\alpha_{HS^-}B + m)^2 + 8K\alpha_{HS^-}mC_{SR}}}{4K\alpha_{HS^-}}$$

Existence and location of the root. K , α_{HS^-} , and m are strictly positive, and B is non-negative. Then $F(0) = -mC_{SR} < 0$, whereas $F(C_{SR}) = K\alpha_{HS^-}C_{SR}(2C_{SR} + B) > 0$. Moreover, $F'(y) = 4K\alpha_{HS^-}y + K\alpha_{HS^-}B + m > 0$ for $y \geq 0$, so F is strictly increasing over the physically admissible interval. Exactly one positive root therefore exists, and it satisfies $0 < y^* < C_{SR}$: the SR-MG pair is formally crossover-limited under all operating conditions. The ratio y^*/C_{SR} quantifies the separation between crossover and depletion; values close to 1 indicate that the two effectively coincide, so that the nominal SR allocation is effectively limited by sulfate capacity.

Assumptions. Activities were approximated by concentrations. Standard free energies refer to 25 °C, and temperature enters only through RT , without van't Hoff correction of ΔG° ; this approximation has little effect within the 25–37 °C range of the dataset. A common pK_a of 7.0 was used for all cases. Where the methane fraction in biogas was not reported, it was assumed to be 50%; the sensitivity of y^*/C_{SR} to this and other parameters is evaluated in Section S2.4.

Choice of an analytical solution. Under these assumptions, the crossover condition reduces to a quadratic equation in y , which allows two results to be obtained that a numerical solution evaluated case by case cannot provide. First, the location of the root, $0 < y^* < C_{SR}$ is proven for all admissible values of the operating variables. Second, the closed-form expression shows how each variable shifts y^* ; for example, a lower dissolved CH_4 concentration (smaller m) moves the crossover to an earlier stage of sulfate consumption. When additional sinks, activity corrections, or temperature-corrected free energies are included, the crossover condition no longer reduces to a quadratic, and y^* must be obtained numerically.

S2.2. Study-specific data extraction and calculation decisions

This note documents the data sources, study-specific extraction and calculation decisions, assumptions, and standardized calculations used to construct **Supplementary Data 2 (Sheet Mechanistic level)**.

Research	Note
Shu et al., 2025 [3]	Temperature, influent COD, influent sulfate, and KHCO_3 concentrations were directly reported in Section 2.1. Effluent sulfate concentrations were directly reported as the averages over the last 20 days of operation in Section 3.1.1. pH values were digitized from Fig. 1d. CH_4 production was digitized from Fig. 1b as the mean over approximately the last 20 days (407, 471, and 439 mL/L for CFR-Control, CFR- Fe_3O_4 , and CFR-PAC, respectively). These values were interpreted as daily CH_4 production per liter of reactor (5-L working volume) and converted to mL per liter of influent by multiplying by 5 L/2.5 L/d (HRT = 48 h). The CH_4 fraction in biogas was not reported and was assumed to be 50%.
Cetecioglu et al., 2019 [4]	Temperature was directly reported as 35 °C in Section 2.2. Incubations lasted 7 days for acetate, propionate, and butyrate, and the values in Table 1 correspond to the end of incubation. Influent COD was calculated from the substrate amount added to each 30-mL microcosm, as reported in Table 1. Influent sulfate was calculated from the sulfate amount added in Table 1, while effluent sulfate was calculated as the difference between sulfate added and sulfate consumed. CH_4 production was calculated from the actual methane yield reported in Table 1 ($\mu\text{mol CH}_4/\text{bottle}$), converted to gas volume using $V_m = 22.414 \text{ L/mol}$ and normalized to the 30-mL liquid volume. The quantities in Table 1 are labelled mmol but correspond to μmol per bottle (e.g., 45 mM acetate $\times$ 30 mL = 1350 μmol). pH was not reported and was assumed to be 7.0. KHCO_3 was not reported to add to system and was assumed to be approximately zero. The CH_4 fraction in biogas was not reported and was assumed to be 50% for the thermodynamic crossover calculation. Seven of the nine cases had over-closed electron balances ($f_{\text{expressed_eff}} > 1$) and were excluded. This over-closure originates from the source data: CH_4 yields in the no-sulfate controls already reached 88–111% of the theoretical yield, indicating an additional electron contribution, most likely from the inoculum (1000 mg VSS/L), which could not be corrected because no substrate-free blanks were reported.

Mahesh et al., 2018 [5]	<p>For the three cases at $F/M = 0.3$, influent COD, influent sulfate, effluent sulfate, and final pH were directly reported in Table 1. The final pH values at 108 h were used as the reaction-endpoint pH. Operating temperature was not reported; the bottles were incubated at ambient temperature and pressure, and 30 °C was assumed for the thermodynamic calculations. KHCO_3 was not reported and was assumed to be approximately zero because no bicarbonate addition was specified. The CH_4 fraction in biogas was not reported and was assumed to be 50% for the thermodynamic crossover calculation. For $\text{COD}/\text{SO}_4 = 0.62$, methane production was reported as nil and V_{CH_4} was therefore set to zero. For $\text{COD}/\text{SO}_4 = 0.69$ and 1.20, V_{CH_4} was calculated from the reported methane yield ($\text{L CH}_4/\text{g COD removed}$) and the corresponding measured COD removal. Gas volumes were measured by NaOH displacement at ambient temperature and were converted using 22.414 L/mol for consistency with the other studies, because the measurement temperature was not reported. Effluent COD accounted for 46%, 43%, and 37% of the influent COD, close to the unresolved fractions (0.52, 0.47, and 0.35), so the unresolved electrons were largely retained as undegraded organic matter in the effluent. E_{in} was based on the total COD of the real wastewater; effective and nominal allocations are therefore lower bounds.</p>
Prakash et al., 2023 [6]	<p>pH was reported to range from 7.8 to 8.0 in the reactor broth; a representative value of 7.9, calculated as the midpoint of this reported range, was used for all cases. Temperature was directly reported as 37 ± 1 °C in Section 2.2, and 37 °C was used in the calculations. Influent COD was calculated from the operating conditions in Table 1: all selected cases were operated at an OLR of 8 g COD/L/d and an HRT of 24 h, giving $\text{COD}_{\text{in}} = 8 \text{ g COD/L} = 8000 \text{ mgCOD/L}$. Influent sulfate concentration was calculated from the COD/SO_4 ratio using $\text{COD}_{\text{in}} = 8000 \text{ mgCOD/L}$ ($\text{SO}_4_{\text{in}} = \text{COD}_{\text{in}}/\text{CSR}$), consistent with the sulfate loading rates reported in Table 1. Each case corresponds to the operating period at the respective CSR in Table 1 (control: CSR 10.0, 7.5, 5.0, 3.0, 2.0, and 1.0 on days 84–107, 108–132, 133–150, 151–163, 174–177, and 178–194; EVR: days 61–83, 84–107, 108–120, 121–132, 133–150, and 151–164), and the values in Table 2 are averages of at least seven samples per CSR. The medium was buffered with NaHCO_3 at a reported $\text{COD}:\text{HCO}_3^-$ ratio of 2:1; this corresponds to 4000 mg HCO_3^-/L and was converted to an equivalent KHCO_3 concentration of approximately 6563 mg/L for calculation of background bicarbonate. The CH_4 fraction in biogas was not reported and was assumed to be 50% for the thermodynamic crossover calculation. V_{CH_4} was calculated from the $\text{COD}-\text{CH}_4$ fractions reported in Table 2, using 64 gCOD/mol CH_4 and $V_m = 22.414 \text{ L/mol}$. Effluent sulfate was calculated from the $\text{COD}-\Delta\text{SO}_4$ fractions in Table 2 using the</p>

	<p>authors' conversion factor of 1.5 g SO_4^{2-} per g COD consumed for sulfate reduction. Table 2 also reported effluent COD (4–20% of influent COD), giving overall COD recoveries of 84–94%; the unresolved fraction therefore consisted mainly of organic matter leaving with the effluent. Dissolved CH_4 was reported to be <1% of COD and was not included. The control at CSR 2.0 was operated for only four days, and the control at CSR 1.0 was reported to have failed; these two cases (Prakash_05 and Prakash_06) therefore represent transient or deteriorating states rather than steady state.</p>
Sarti & Zaiat et al., 2011 [7]	<p>pH was reported as a range for each period in Table 3 (effluent); the midpoint of each range was used as the representative reactor pH. Temperature was directly reported as $25 \pm 1 \text{ }^\circ\text{C}$ in Section 2.2, and $25 \text{ }^\circ\text{C}$ was used in the calculations. Influent COD concentrations were directly reported as mean COD_Total values in Table 2 for each operational period. Influent sulfate concentrations were directly reported as mean values in Table 2. KHCO_3 was not directly reported; influent bicarbonate alkalinity (BA, mg CaCO_3/L) reported in Table 2 was converted to an equivalent KHCO_3 concentration using $\text{KHCO}_3_{\text{eq}} = \text{BA} \times 100.115/50$. Methane concentrations in Fig. 4 refer to the generated biogas (Section 2.3) and correspond to approximately 0.01–0.04% CH_4; biogas volume was not reported. Because methanogenesis was strongly inhibited, CH_4 production was negligible regardless of biogas volume, and $f_{\text{MG_eff}}$ was effectively zero (the digitized values, interpreted per litre of liquid, give $V_{\text{CH}_4} < 0.4 \text{ mL/L}$). With an inactive methanogenic sink, the thermodynamic crossover does not apply (main text, Section 3.2.2.1), and the nominal SR allocation was taken as capacity-limited. The 50% CH_4 fraction entered only as a placeholder in the crossover calculation and does not affect $f_{\text{SR_nom}}$. Effluent COD accounted for 50–68% of the influent COD in periods III–V, mainly as VFAs, whereas in periods I–II 88–93% of the COD was removed but only 16–20% of E_{in} was recovered by sulfate reduction; this fraction remains unresolved. E_{in} comprised butanol and the industrial sulfonation wastewater (Mixed); effective and nominal allocations are therefore lower bounds.</p>
Paepatung et al., 2022 [8]	<p>pH in the reactor without ZVI was reported to fluctuate between 7.0 and 7.7 during Phase I; the midpoint, 7.35, was used as a representative value. For the ZVI-treated case, the average reactor pH during the stable third sub-phase was directly reported as 7.38 ± 0.22, and 7.38 was used. Operating temperature was reported as room temperature of 28–30 $^\circ\text{C}$; the midpoint, 29 $^\circ\text{C}$, was used for calculations. Influent COD was directly reported as 9000 mgCOD/L glucose in Section 2.1. Influent sulfate concentration was directly reported as 4500 mg/L in Section 2.1 for both</p>

	<p>cases. The basal medium contained 7.0 g/L NaHCO_3 and 1.0 g/L NH_4HCO_3; these bicarbonate sources were converted to a KHCO_3-equivalent concentration of approximately 9608 mg/L based on molar equivalence. For the reactor without ZVI, the CH_4 fraction was digitized from Fig. 1d near the end of Phase I (~2%), whereas for the ZVI-treated reactor, the CH_4 fraction was reported to increase up to 75%. Electron allocation was derived from the cumulative COD balance of each phase in Fig. 3, consistent with Supplementary Data 1. The cumulative values were expressed per litre of influent by dividing by the influent volume, calculated as the cumulative glucose-COD divided by 9 g COD/L (34.65 L in Phase I and 49.02 L in Phase II). V_{CH_4} was calculated from the cumulative CH_4-COD (7.57 and 226.84 g COD) using 64 g COD/mol CH_4 and $V_m = 22.414$ L/mol. SO_4_{out} was calculated from the cumulative sulfide-COD (39.92 and 127.41 g COD), converted to sulfate reduced using 1.5 g SO_4^{2-}/g COD. For Phase II, COD_{in} included the ZVI-derived electrons (28.85 g COD) inferred by the authors from the COD balance, so that the Phase II balance was closed by construction. Residual glucose and VFAs (78% of E_{in} in Phase I and 24% in Phase II) and biomass account for most of the unresolved fraction.</p>
Xu et al., 2023 [9]	<p>pH was reported to remain relatively stable at 7.4 ± 0.2 during anaerobic digestion; therefore, 7.4 was used as the representative operating pH for all cases. Temperature was directly reported as 35 ± 1 °C, and 35 °C was used in the calculations. The reactors (0.8-L working volume) contained synthetic sulfate wastewater (7.25 g COD/L) and digested sludge inoculum at a volume ratio of 7:3 and were incubated for 15 days. KHCO_3 addition was not reported and was therefore assumed to be approximately zero. The CH_4 fraction in biogas was not reported and was assumed to be 50% only for the thermodynamic crossover calculation. V_{CH_4} was calculated from the maximum cumulative methane production, P_{max}, reported in Table 1 for the control and Table 3 for the biochar treatments, normalized to the 0.8-L working volume ($V_{\text{CH}_4} = P_{\text{max}}/0.8$). Because the CH_4 produced (e.g., 2816 mL, ≈ 8.0 g COD, at $\text{SO}_4^{2-}/\text{COD} = 0.1$) exceeded the COD supplied with the wastewater (≈ 4.1 g COD), the inoculum contributed substantially to the electron input. COD_{in} was therefore based on the total COD of the wastewater–inoculum mixture, consistent with the authors' COD balance, in which the reported CH_4 conversion ratios (83.62% and 45.46% at $\text{SO}_4^{2-}/\text{COD} = 0.1$ and 2) equal $2.855 \times \text{methane yield} \times \text{COD removal efficiency}$. A common COD_{in} of 12,283 mg COD/L ($E_{\text{in}} = 1535$ mmol e^-/L) was derived from the four no-biochar cases and applied to all cases, because all reactors received the same wastewater and inoculum. Case-specific values were not used because the COD distributions in Figs. 3 and 6 are normalized to the total output, and biochar adsorbed part of the COD. E_{in} type: Mixed. Influent sulfate</p>

	<p>concentrations reported in Section 2.1 for each $\text{SO}_4^{2-}/\text{COD}$ ratio were corrected for the 7:3 dilution with inoculum ($\text{SO}_4_{\text{in}} = \text{reported concentration} \times 0.7$). Sulfate reduction was calculated from the CH_4 electrons and the SRB/MPB electron-flow ratios in Table 2, and SO_4_{out} was obtained as SO_4_{in} minus the sulfate reduced. This approach reproduces the SO_4^{2-}-COD fractions reported in the text (e.g., 3.95%, 29.97%, 20.94%, and 28.85%). For Xu_05 and Xu_06 (1 g biochar at $\text{SO}_4^{2-}/\text{COD} = 0.1$ and 0.2), the reported SRB electron-flow ratios imply that more sulfate was reduced than was supplied ($f_{\text{SR_eff}} > f_{\text{SR_nom}}$; calculated $\text{SO}_4_{\text{out}} = -44$ and -394 mg/L). Because the inconsistent value could not be identified, these two cases were excluded manually and are marked as Excluded in Supplementary Data 2.</p>
Li et al., 2017 [10]	<p>pH was not reported for the UASB operation and was therefore assumed to be 7.0 for the thermodynamic calculations. Temperature was directly reported as 37 ± 1 °C, and 37 °C was used. Influent COD and sulfate concentrations were directly reported as 1600 mgCOD/L and 1300 mg/L, respectively, for the artificial wastewater. KHCO_3 addition was not reported and was assumed to be approximately zero. The CH_4 fraction in biogas was not numerically reported and was assumed to be 50% only for the thermodynamic crossover calculation. V_{CH_4} was calculated from the reported methane production rates (39.4 and 159.9 mL/d for R1 and R2) using the reactor working volume of 0.7 L and HRT of 6 h, giving 14.1 and 57.1 mL/L, respectively. Effluent sulfate was calculated from the reported sulfate removal efficiencies of 83.1% and 80.3%, giving 219.7 and 256.1 mg/L for R1 and R2, respectively. Values are steady-state averages; the averaging period was not specified. COD removal efficiencies of 64.4% (R1) and 72.9% (R2) indicate that organic matter leaving with the effluent accounts for most of the unresolved fraction. Dissolved CH_4 was not measured; at an HRT of 6 h and 37 °C it may be comparable to the gaseous CH_4 of R1, so $f_{\text{MG_eff}}$ may be underestimated.</p>
Lu et al., 2016 [11]	<p>pH was reported to remain within 6.9–7.5 during phases I–V; therefore, the midpoint value of 7.2 was used as a representative pH for COD/SO_4 ratios of 10–1. At $\text{COD}/\text{SO}_4 = 0.5$, pH increased and a representative value of approximately 7.4 was digitized from Fig. 1a. Temperature was directly reported as 35 ± 1 °C, and 35 °C was used in the calculations. Influent COD concentrations were taken from the measured mean values reported in Table 2 for each COD/SO_4 condition rather than the nominal 1000 mg/L feed concentration. Influent sulfate concentrations were calculated from the nominal influent COD concentration of approximately 1000 mg/L and the imposed COD/SO_4</p>

	<p>ratios. NaHCO_3 dosage was reported to be maintained at 1500 mg/L throughout the experiment, and this value was used for all cases. CH_4 fractions of 67.2%, 64.0%, and 61.6% at COD/SO_4 ratios of 10, 5, and 3 were directly reported, whereas representative values for ratios 2, 1, and 0.5 were digitized from Fig. 1e. V_{CH_4} was calculated from the total methane-COD fractions reported in Table 2 ($\text{COD}_{\text{CH}_4\text{gas}} + \text{COD}_{\text{CH}_4\text{aq}}$) using the authors' conversion factor of 1 L $\text{CH}_4 = 2.857$ g COD. Effluent sulfate was calculated from influent sulfate and the reported sulfate-removal efficiencies: 80.9% at $\text{COD}/\text{SO}_4^{2-} = 10$ (end of phase I), 82.6% at ratios of 5, 3, and 2 (average for phases II–IV; phase-specific means were not provided), and 21.2% and 15.0% at ratios of 1 and 0.5. Sulfate removal rather than the measured gaseous and aqueous sulfide (Table 2) was used because 17.7–32.7% of the produced sulfide was estimated to precipitate as metal sulfides, and because the nominal SR allocation is defined on a sulfate basis; the same values were used in Supplementary Data 1. Because COD_{in} includes the inoculum COD and SO_4_{in} was corrected for dilution, the resulting $\text{SO}_4^{2-}/\text{COD}$ ratio (0.04–0.83) are lower than the nominal ratios reported by the authors (0.1–2)</p>
Chen et al., 2025 [12]	<p>pH values were digitized from Fig. 1C at day 7 and used as representative end-point pH values for the control, 2 μM AHL, and 20 μM AHL treatments. Temperature was directly reported as 35 °C, and the initial COD concentration was directly reported as 4000 mgCOD/L for all cases. Influent sulfate concentration was directly reported as 1000 mg/L. NaHCO_3 was added at 2 g/L and was converted to a KHCO_3-equivalent concentration of approximately 2383.5 mg/L based on molar bicarbonate equivalence. The CH_4 fraction in biogas was not numerically reported and was assumed to be 50% only for the thermodynamic crossover calculation. Cumulative CH_4 production at day 7 was digitized from Fig. 1B and normalized to the 0.2-L working volume to obtain V_{CH_4} in mL/L. Effluent sulfate concentrations were digitized directly from Fig. 1D at day 7 for each treatment. COD decreased to 300, ~130, and ~50 mg/L by day 7 (Fig. 1A), so organic matter remaining in the liquid accounts for only a small part of the unresolved fraction (32–41%); biomass growth, dissolved CH_4, and sulfide not captured by the sulfate balance are not quantified. The granular sludge inoculum (50 mL of 200 mL) was not included in E_{in}.</p>

S2.3. Common calculation framework

- **T (K):** Converted from the reported operating temperature: $T = \text{Temperature } (^{\circ}\text{C}) + 273.15$.
- **E_{in} (mmol e⁻/L):** Total electron input was calculated from influent biodegradable COD, using $1 \text{ mmol e}^{-} = 8 \text{ mg COD}$: $E_{\text{in}} = \text{COD}_{\text{in}} / 8$.
- **C_{SR} (mol/L):** Initial sulfate amount was calculated from influent sulfate concentration using a sulfate molecular weight of 96 g/mol: $C_{\text{SR}} = \text{SO}_4_{\text{in}} (\text{mg/L}) / 96,000$.
- **SO₄²⁻/COD:** The influent ratio reported in Table 1 and used throughout Section 3.2.2 was calculated as $\text{SO}_4_{\text{in}} / \text{COD}_{\text{in}}$ from the values used in the calculations. It may therefore differ from the nominal ratio reported in the original study when COD_{in} or SO_4_{in} was adjusted.
- **alpha_{HS}:** Fraction of total sulfide present as HS⁻ was calculated as $\alpha_{\text{HS}} = 1 / [1 + 10^{(\text{pK}_a - \text{pH})}]$, a common value of $\text{pK}_a = 7.0$ was used across all cases for consistency, unless otherwise specified, $\text{pK}_a = 7.0$.
- **B (mol/L):** Background bicarbonate concentration was calculated from the added KHCO₃ concentration, assuming 1 mol KHCO₃ provides 1 mol HCO₃⁻: $B = \text{KHCO}_3 (\text{g/L}) / 100.1 \text{ g/mol}$. B represents the initial bicarbonate concentration before sulfate reduction.
- **m_{CH₄} (mol/L):** Dissolved methane concentration was estimated using Henry's law: $m_{\text{CH}_4} = K_{\text{H}} \times p_{\text{CH}_4}$. The methane Henry constant was corrected to the selected operating temperature using $K_{\text{H}}(T) = 0.0013 \times \exp[1900 \times (1/T - 1/298.15)]$, using $K_{\text{H}}^{\circ} = 1.3 \times 10^{-3} \text{ mol}/(\text{kg} \cdot \text{bar})$ at 298.15 K, based on the NIST Chemistry WebBook Henry's law data for methane [2]. Assuming a total gas pressure of 1 atm, p_{CH_4} was calculated from the CH₄ mole fraction in the biogas.
- **K:** Thermodynamic equilibrium term used in the crossover, and calculated as $K = \exp(-3957/T)$, where T is in Kelvin.
- **y (mol/L)*:** The sulfate-reduction/methanogenesis crossover point was obtained as the positive root of: $2K \cdot \alpha_{\text{HS}} \cdot y^2 + (K \cdot \alpha_{\text{HS}} \cdot B + m_{\text{CH}_4}) \cdot y - m_{\text{CH}_4} \cdot C_{\text{SR}} = 0$. The resulting y* represents the amount of sulfate reduced when the thermodynamic driving forces of sulfate reduction and methanogenesis become equal.
- **Case:** Cases were classified according to the position of y* relative to the initial sulfate amount. Capacity-limited was assigned when $y^* \geq C_{\text{SR}}$, indicating sulfate depletion before crossover. Crossover-limited was assigned when $0 < y^* < C_{\text{SR}}$, indicating a formal crossover before complete sulfate depletion.

- **f_SR_nom:** Nominal electron allocation to sulfate reduction was calculated from the sulfate electron-accepting capacity relative to the total electron input. Because the calculated crossover point was effectively coincident with sulfate depletion, sulfate reduction was treated as capacity-limited: $f_SR_nom = \min(8 \times C_SR \times 1000, E_in) / E_in$; where C_SR is in mol SO_4^{2-}/L and E_in is in mmol e^-/L . The factor 8 represents 8 mol e^- accepted per mol sulfate.
- **f_MG_nom:** Methanogenesis was treated as the residual terminal sink and therefore received the nominal electron fraction not allocated to sulfate reduction: $f_MG_nom = 1 - f_SR_nom$.
- **f_SR_eff:** Effective electron allocation to sulfate reduction was calculated from the measured sulfate removal: $SO_4_removed = SO_4_in - SO_4_out$; $E_SR_eff = (SO_4_removed / 96) \times 8$; $f_SR_eff = E_SR_eff / E_in$ where SO_4 concentrations are in mg/L; division by 96 converts mg SO_4^{2-}/L to mmol SO_4^{2-}/L , and each mmol sulfate reduced accepts 8 mmol e^- .
- **f_MG_eff:** Effective electron allocation to methanogenesis was calculated from the measured CH_4 production. Gas volume was converted to mmol CH_4 using the applicable molar gas volume, and each mole of CH_4 was assigned 8 mol e^- : $N_e_MG_eff = (V_CH_4 / V_m) \times 8$; $f_MG_eff = N_e_MG_eff / E_in$. When the gas reference condition was not reported, standard conditions of 0 °C and 1 atm were assumed, with $V_m = 22.414$ mL/mmol.
- **d_f_SR:** Deviation of effective sulfate-reduction allocation from its nominal allocation: $d_f_SR = f_SR_eff - f_SR_nom$
- **d_f_MG:** Deviation of effective methanogenic allocation from its nominal allocation: $d_f_MG = f_MG_eff - f_MG_nom$
- **sum_f_eff:** Total fraction of input electrons quantified at the two terminal sinks: $sum_f_eff = f_SR_eff + f_MG_eff$

S2.4. Sensitivity of the crossover position to operating conditions

Across the 61 cases calculated as described in Section S2.3, y^*/C_{SR} ranged from 0.989 to 1.000. Because several inputs required estimation or standardized assumptions, the robustness of this result was tested over a full-factorial grid. The standard free-energy difference between the two reactions is fixed, so variation in y^*/C_{SR} arises only from the reaction-quotient terms: sulfate supply, background bicarbonate concentration, pH, methane fraction in the biogas, and temperature. Temperature affects both the equilibrium term and the dissolved methane concentration through the temperature dependence of the Henry constant.

The selected levels spanned the conditions represented in the compiled cases and were extended beyond the observed ranges (Table S1). The five sets of levels generated $8 \times 6 \times 6 \times 8 \times 6 = 13,824$ combinations. Of these, 3,600 combinations ($6 \times 5 \times 5 \times 6 \times 4$) used only levels within the observed ranges (levels without an asterisk in Table S1). Each combination was evaluated with the same equations applied to the individual cases. The calculations were implemented independently in R and in a spreadsheet with live formulas, and the two implementations gave numerically identical results within the reported precision.

Table S1. Levels used in the sensitivity analysis. Values marked with an asterisk fall outside the observed dataset.

Variable	Observed range	Levels evaluated
C_{SR} (mol/L)	0.0010–0.106	0.001, 0.005, 0.010, 0.026, 0.052, 0.104, 0.151*, 0.30*
B (mol/L)	0–0.096	0, 0.015, 0.030, 0.066, 0.096, 0.20*
pH	6.05–8.98	6.2, 7.0, 7.4, 8.0, 9.0, 9.5*
CH ₄ in biogas (%)	2–75	0.1*, 1.0*, 2, 5, 13, 30, 50, 75
Temperature (°C)	25–37	25, 30, 35, 37, 55*, 70*

Of the 3,600 combinations within the observed range, none gave $y^*/C_{SR} < 0.95$. The lowest value, 0.960, was obtained by combining the lowest observed methane fraction (2%) with the highest sulfate (0.104 mol/L) and bicarbonate (0.096 mol/L) levels, pH 9.0, and 37 °C. These extremes originated from different studies (methane fraction and bicarbonate from Paepatung et al., sulfate from Xu et al., and pH from Mahesh et al.) and did not co-occur in any compiled case. Over the full grid, approximately 19% of the combinations gave $y^*/C_{SR} < 0.95$; 94% of these occurred below 5% methane, and none at 30% or above (Figure S1).

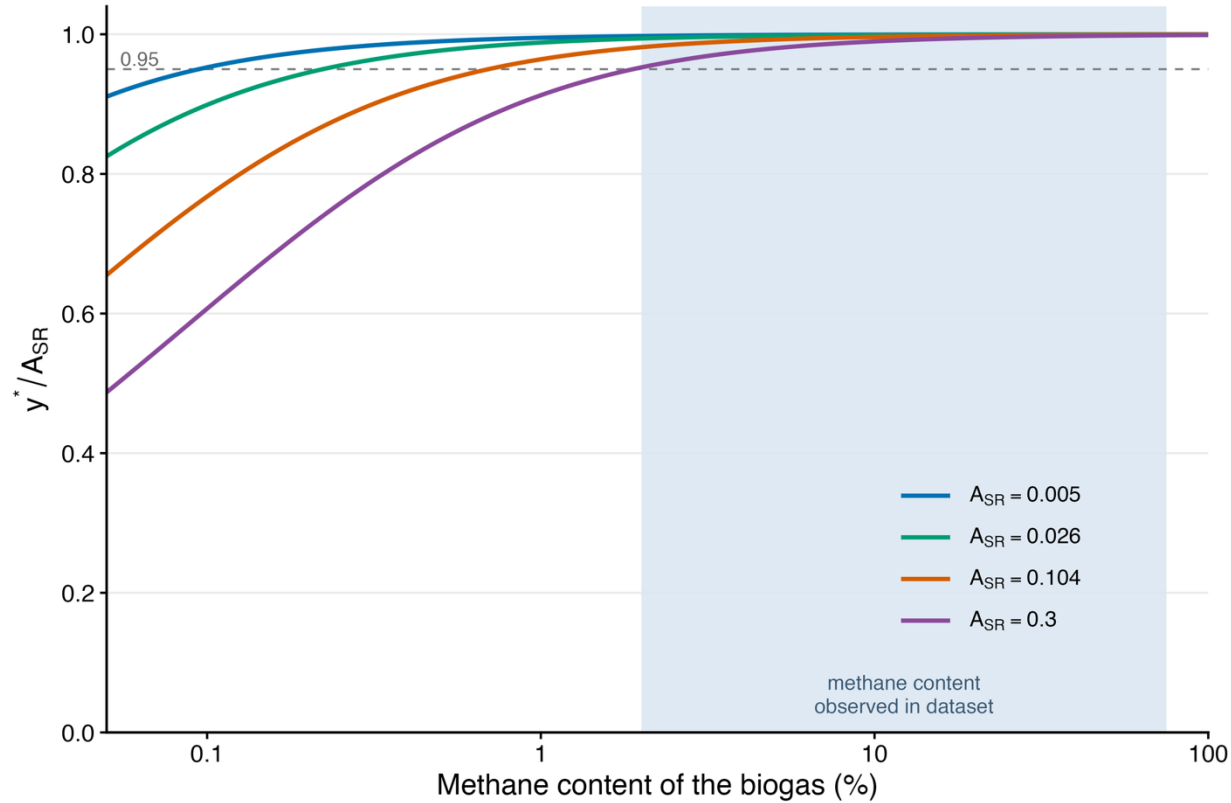


Figure S1. Position of the thermodynamic crossover relative to sulfate depletion as a function of methane content in the biogas.

Curves show y^*/C_{SR} at four sulfate concentrations ($C_{SR} = 0.005, 0.026, 0.104$, and 0.300 mol/L), with $B = 0.02$ mol/L, pH 7.4, and temperature fixed at 35 °C. The shaded region indicates the methane-content range observed in the compiled dataset (2–75%). The dashed line at $y^*/C_{SR} = 0.95$ marks the threshold below which more than 5% of the initial sulfate capacity remains at crossover.

Figure prepared in R using ggplot2.

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